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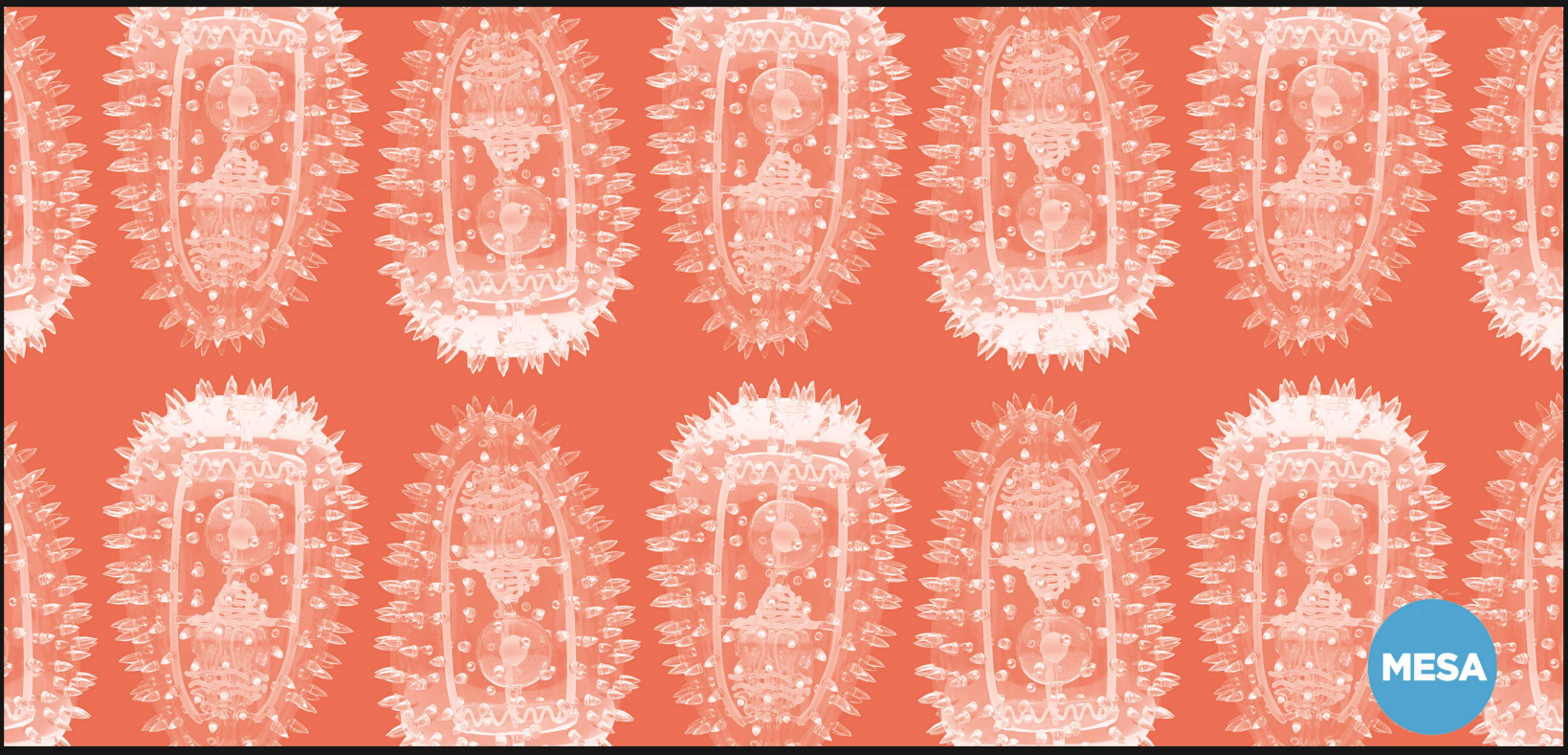

malERA – An Updated Research Agenda for Malaria Elimination and Eradication

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COLLECTION REVIEW

malERA: An updated research agenda for malaria elimination and eradication

Regina N. Rabinovich[1,2]*, Chris Drakeley[3], Abdoulaye A. Djimde[4], B. Fenton Hall[5], Simon I. Hay[6,7], Janet Hemingway[8], David C. Kaslow[9], Abdisalan Noor[10,11], Fredros Okumu[12,13,14], Richard Steketee[15], Marcel Tanner[16,17], Timothy N. C. Wells[18], Maxine A. Whittaker[19], Elizabeth A. Winzeler[20], Dyann F. Wirth[2], Kate Whitfield[1], Pedro L. Alonso[21]

1 ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic—Universitat de Barcelona, Barcelona, Spain, **2** Harvard T.H. Chan School of Public Health, Boston, Massachusetts, United States of America, **3** London School of Hygiene and Tropical Medicine, London, United Kingdom, **4** University of Science, Techniques and Technology of Bamako, Bamako, Mali, **5** National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH), Bethesda, Maryland, United States of America, **6** Institute for Health Metrics and Evaluation, University of Washington, Seattle, Washington, United States of America, **7** Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, United Kingdom, **8** Liverpool School of Tropical Medicine, Liverpool, United Kingdom, **9** PATH Essential Medicines and PATH Center for Vaccine Innovation and Access, Seattle, Washington, United States of America, **10** KEMRI Wellcome Trust Research Programme, Nairobi, Kenya, **11** Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom, **12** Ifakara Health Institute, Ifakara, Tanzania, **13** School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Parktown, South Africa, **14** Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, United Kingdom, **15** PATH Malaria Control and Elimination Partnership in Africa, Seattle, Washington, United States of America, **16** Swiss TPH, Basel, Switzerland, **17** University of Basel, Basel, Switzerland, **18** Medicines for Malaria Venture, Geneva, Switzerland, **19** College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Australia, **20** University of California, San Diego, School of Medicine, La Jolla, California, United States of America, **21** World Health Organization (WHO), Global Malaria Programme (GMP), Geneva, Switzerland

* rrabinov@hsph.harvard.edu, regina.rabinovich@isglobal.org

Citation: Rabinovich RN, Drakeley C, Djimde AA, Hall BF, Hay SI, Hemingway J, et al. (2017) malERA: An updated research agenda for malaria elimination and eradication. PLoS Med 14(11): e1002456. https://doi.org/10.1371/journal.pmed.1002456

Published: November 30, 2017

Funding: MESA received a grant from the Bill & Melinda Gates Foundation http://www.malariaeradication.org/mesa-track (OPP1034591). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: AD is a member of MPAC (Malaria Policy Advisory Committee). BFH is a member of the MESA (Malaria Eradication Scientific Alliance) Strategic Advisory Council. SIH is funded by grants from the BMGF (Bill & Melinda Gates Foundation), the Wellcome Trust, and the Fleming Fund. AMN is a member of the WHO malaria Surveillance, Monitoring and Evaluation Technical Experts Group and is co-chair of the Roll Back Malaria Monitoring and Evaluation Reference Group. AMN is also a member of the Editorial

Abstract

Achieving a malaria-free world presents exciting scientific challenges as well as overwhelming health, equity, and economic benefits. WHO and countries are setting ambitious goals for reducing the burden and eliminating malaria through the "Global Technical Strategy" and 21 countries are aiming to eliminate malaria by 2020. The commitment to achieve these targets should be celebrated. However, the need for innovation to achieve these goals, sustain elimination, and free the world of malaria is greater than ever. Over 180 experts across multiple disciplines are engaged in the Malaria Eradication Research Agenda (malERA) Refresh process to address problems that need to be solved. The result is a research and development agenda to accelerate malaria elimination and, in the longer term, transform the malaria community's ability to eradicate it globally.

Summary points

- The first malERA consultative process in 2011 identified a number of targets for investment and the scientific community has made progress across the research and

Board of PLOS Medicine. FOO is supported by a Wellcome Trust Intermediate Research Fellowship. RS receives funding from the BMGF and is a member of the MESA Strategic Advisory Council. MT declares that he is a member of the MESA Steering Committee, chair of the Scientific Advisory Group (SAG) of WHO/GMP on Malaria Eradication and was a member of MPAC until 2016. TW is a non-executive director of Kymab. EAW is on the Scientific Advisory Board of the Tres Cantos Open Lab Foundation. EAW receives funding from the BMGF, Medicines for Malaria Venture, NIH, and serves on the Scientific Advisory Board of the Tres Cantos Open Lab Foundation. DFW receives funding from the BMGF. PLA is Director of the WHO Global Malaria Programme, a member of the MESA Steering Committee and holds grants from the BMGF. KW is a member of the MESA Secretariat. RR is the Chair of the MESA Steering Committee and is Principal Investigator on the MESA grant from the BMGF. RR is the ExxonMobil Scholar in Residence at the Harvard T.H. Chan School of Public Health.

Abbreviations: AIM, Action and Investment to defeat Malaria 2016–2030; CHMI, controlled human malaria infection; CRISPR, clustered regularly interspaced short palindromic repeats; DHIS 2, District Health Information Software 2; EIR, entomological inoculation rate; ERG, Evidence Review Group; G6PD, glucose-6-phosphate dehydrogenase; GTS, Global Technical Strategy for Malaria 2016–2030; malERA, Malaria Eradication Research Agenda; MDA, mass drug administration; MESA, Malaria Eradication Scientific Alliance; NTD, neglected tropical disease; RBM, Roll Back Malaria Partnership; RDT, rapid diagnostic test; SAG, Strategic Advisory Group; SERCaP, Single Encounter Radical Cure and Prophylaxis; VIMT, vaccines that interrupt malaria parasite transmission; WHO, World Health Organization.

Provenance: Submitted as part of a Supplement; externally peer reviewed.

development (R&D) continuum. Progress includes positive scientific opinion for a malaria vaccine, advanced development of 3 nonpyrethroid insecticides, new genetic technologies with the potential to alter malaria parasite transmission by the mosquito, identification of markers of drug resistance, and development of *Plasmodium vivax* liver stage assays as well as new collaborative approaches to mathematical modelling and screening for active ingredients for drugs and insecticides.

- Scientific progress, however, has been matched with significant challenges. The expansion of both insecticide and drug resistance threatens progress in affected countries. Gaps in the knowledge base persist, from epidemiological and entomological tools to guide programmes, particularly at low transmission levels, understanding the role of low-density infections in maintaining transmission and developing appropriate diagnostics for programmes, biomarkers, and tools to detect and clear hypnozoites, to tools to tackle residual transmission, receptivity, and prevention of reintroduction.
- In some areas, progress has been too slow, particularly in the creation of a tool kit to tackle *P. vivax* malaria, investments in the development of new vector control tools, almost all aspects of entomology, and in systematically testing solutions in the context of the respective health and social systems.
- Malaria parasites and their infections continually evolve, creating new research and programme challenges. In one region, human infections with *P. knowlesi* are rising, parasites with hrp2/3 deletions are evading detection by current rapid diagnostic tests (RDTs), and current effective vector control tools are selecting for mosquitoes with both physiologic resistance and behavioural traits like outdoor biting and resting.
- The malERA Refresh agenda proposes a broad agenda for transdisciplinary solutions to the problems faced. It points to 3 areas in which innovation is critical: (i) iterative improvements in drugs and vector control; (ii) transformative improvements in tools and strategies to reduce, if not halt, the parasite's capacity to transmit; and (iii) integrated approaches in which a robust elimination strategy responds to local variations in transmission dynamics, is tailored to the health and social system context, and draws strength from other sectors.

Introduction

The 2011 malaria Eradication Research Agenda (malERA) was the first comprehensive analysis of the science needed to support national elimination of malaria and the long-term goal of its global eradication [1]. The 2011 malERA consultative process engaged a multidisciplinary group, involving members of the infectious disease and malaria research and implementation communities, and identified both emerging challenges and approaches to solving them. Five years later, the review of progress and emerging challenges, as well as a more nuanced understanding of the implementation problems that need to be solved, drove the 2016 'malERA Refresh', with the intent to assess progress and the emergence of new challenges, examine current hypotheses, and point to the key research and development areas that can advance the feasibility of malaria elimination in the most challenging areas of the world.

Global goals for a reduction in malaria burden and elimination were published in 2 complementary documents in 2015: the Global Technical Strategy for Malaria 2016–2030 (GTS) and

Action and Investment to defeat Malaria 2016–2030 (AIM), a global investment case for financing and coordinating these efforts [2,3]. Other groups have expressed a vision of global malaria eradication and underscored the need for R&D investments and country financing [4]. Building on the goals expressed in the GTS and AIM, the World Health Organization (WHO) has established a Strategic Advisory Group (SAG) to analyse future scenarios for malaria, including eradication. WHO SAG has affirmed WHO's long-standing commitment to the goal of eradication, although it does not specify an end date for that goal [5,6].

There is not an assumption that 1 single 'silver bullet' will solve all of the challenges, but—as was stated by Tachi Yamada in 2007—'imperfect tools applied imperfectly can still achieve remarkable impact', and a toolbox of solutions is needed that countries can draw upon and adapt to their health and social systems context [7,8]. A strong research base is a keystone for long-term progress in achieving the goals of the GTS. It is in this context that the malERA Refresh Panels propose a multidisciplinary research agenda for researchers, programme implementers, and research funders to accelerate problem solving and impact.

Accelerating to elimination

Elimination of malaria means the 'interruption of local transmission (reduction to zero incidence of indigenous cases) of a specified malaria parasite in a defined geographical area as a result of deliberate activities. Continued measures to prevent re-establishment of transmission are required' (see Glossary, Table 1). A number of countries have been able or are on their way to eliminating malaria by applying a combination of vector control, efficient case management, and active surveillance strategies, all with existing tools for prevention, diagnosis, and treatment. Between 2000 and 2015, 17 countries eliminated malaria [9]. A further 21 countries have been identified as having the potential to eliminate malaria by 2020, comprising the "E-2020" (Fig 1) [10,11]. There are key elements to the elimination strategy, reflected in high uptake of core interventions by programmes and communities: a robust surveillance, reporting, and response system; prevention with a variety of ways to deliver insecticides and barrier methods to stop infectious bites; and diagnosis and treatment with effective combination medications. For this reason, WHO now frames national elimination as a continuum rather than the achievement of milestones for specific phases [6]. The heterogeneous nature of malaria across geographies means that a single approach will not work in all settings with the same efficiency. According to the 'Acceleration Hypothesis', countries with high vectorial capacity, particularly in sub-Saharan Africa, may require measures to rapidly deplete the parasite population [6,12], after which, locally tailored vector control, case management, and surveillance strategies with active methods to investigate and clear infections can then more effectively reduce transmission [12]. Whilst currently being considered and tested, strategies to accelerate elimination (such as mass drug administration [MDA] with antimalarials, low dose primaquine, complementary tools to address residual transmission, etc.) have not yet, and may not be, proven to be widely effective in moving settings with high residual transmission towards sustainable elimination. Ongoing research testing these tools and strategies is curated in the open MESA Track database [13]. Across the malaria endemic world, there exist challenges, and it is here that innovation is required to achieve elimination and quicken its course. Those challenges include areas of high receptivity (where the ecosystems are favourable for malaria transmission), highly competent vectors, residual transmission, resistance to drugs and/or insecticides, and areas where there are human populations that are not adequately served by the health system.

Some key points emerge from experiences in elimination countries and are worth clarifying, because they frame the context for evaluation of new tools to accelerate progress. First,

Table 1. Glossary of terms. The meaning of the terms used in the malERA Refresh series are described here. The sources of the definitions are referenced; where no reference is cited, the authors of this paper provided the definition.

Term	Definition	Reference
Asymptomatic parasitaemia	The presence of asexual parasites in the blood without symptoms of illness.	[14]
CHMI, also called human blood-stage challenge model	An established malaria infection model in which a group of healthy volunteers are inoculated with *Plasmodium* sporozoites via the bite of laboratory-reared infected female *Anopheline* mosquitoes or via needle and syringe, followed by complete medical cure. Volunteers are closely monitored for safety and clinical trial end points. CHMI allows the assessment of malaria vaccines, drugs, diagnostics, and the study of immunological mechanisms.	[15–17]
CRISPR	Gene-editing technology allowing for highly specific DNA modification. The technique is based on a bacterially derived endonuclease, such as Cas9, which can cut DNA in any desired location given a synthetic RNA guide sequence, the CRISPR. A new DNA sequence can then be introduced in that position by DNA repair machinery.	
Dormancy	Any state of suppressed development (developmental arrest) that is adaptive (that is, ecologically or evolutionarily meaningful and not just artificially induced) and usually accompanied by metabolic suppression (can apply to the parasite or vector).	[18]
Efficacy	A measure of the beneficial effect of an intervention in a controlled setting, for example, a randomised controlled trial.	
Effectiveness	A measure of to what extent the efficacy of an intervention can be retained at the individual (clinical) or the community (systems) level.	[19]
Elimination (of malaria)	Interruption of local transmission (reduction to zero incidence of indigenous cases) of a specified malaria parasite in a defined geographical area as a result of deliberate activities. Continued measures to prevent reestablishment of transmission are required. Note that the certification of malaria elimination in a country will require that local transmission is interrupted for all human malaria parasites.	[14]
Eradication (of malaria)	Permanent reduction to zero of the worldwide incidence of infection caused by human malaria parasites as a result of deliberate activities. Interventions are no longer required once eradication has been achieved.	[14]
Operational research	Any research producing practically usable knowledge (evidence, findings, information, etc.) that can improve programme implementation regardless of the type of research (design, methodology, approach).	[20]
Persistence	The continued presence of malaria parasites (in humans or mosquitoes) for an extended period, generally after initial intervention has concluded.	
Receptivity	Receptivity of an ecosystem to transmission of malaria. Note that a receptive ecosystem should have, e.g., the presence of competent vectors, a suitable climate, and a susceptible population.	[14]
Recrudescence	Recurrence of asexual parasitaemia of the same genotype(s) that caused the original illness, due to incomplete clearance of asexual parasites after antimalarial treatment. Note that recrudescence is different than reinfection with a parasite of the same or different genotype(s) and relapse in *P. vivax* and *P. ovale* infections.	[14]
Reinfection	A new infection that follows a primary infection; it can be distinguished from recrudescence by the parasite genotype, which is often (but not always) different than the genotype that caused the initial infection.	[14]
Relapse	Recurrence of asexual parasitaemia in *P. vivax* or *P. ovale* infections arising from hypnozoites. Note that relapse occurs when the blood-stage infection has been eliminated but hypnozoites persist in the liver and mature to form hepatic schizonts. After an interval, generally from 3 weeks to 1 year, the hepatic schizonts rupture and liberate merozoites into the bloodstream.	[14]
Residual transmission	Persistence of transmission after good coverage has been achieved with high-quality vector control interventions, to which local vectors are fully susceptible. Note that both human and vector behaviour is responsible for such residual transmission, such as people staying outdoors at night or local mosquito vector species displaying behaviour that allows them to avoid core interventions.	[14]
SERCaP	A description of an ideal antimalarial drug therapy, which, in a single-patient encounter, both eliminates all parasites in the patient and provides individual protection from reinfection for at least 1 month after treatment.	[21]
Malaria stratification	Classification of geographical areas or localities according to epidemiological, ecological, social, and economic determinants for the purpose of guiding malaria interventions.	[14]

(*Continued*)

Table 1. (*Continued*)

Term	Definition	Reference
Subpatent infection	Low-density blood-stage malaria infection that is not detected by standard diagnostic tools.	
Submicroscopic infection	Low-density blood-stage malaria infection that is not detected by conventional microscopy.	[14]
Surveillance	Continuous, systematic collection, analysis, and interpretation of disease-specific data and use in planning, implementing, and evaluating public health practice. Note that surveillance can be done at different levels of the healthcare system (e.g., health facilities, the community) with different detection systems (e.g., case-based: active or passive) and sampling strategies (e.g., sentinel sites, surveys).	[14]
Vector competence	For malaria, the ability of the mosquito to support completion of malaria parasite development after zygote formation and oocyst formation and development and release of sporozoites that migrate to salivary glands, allowing transmission of viable sporozoites when the infective female mosquito feeds again. Note that human malarias are transmitted exclusively by competent species of *Anopheles* mosquitoes; various plasmodia are transmitted by competent species of mosquitoes of the genera *Aedes*, *Anopheles*, and *Culex* and other haematophagous *Diptera*.	[14]
VIMT	Vaccines that target the sexual- and mosquito-stage antigens, pre-erythrocytic vaccines that reduce asexual- and sexual-stage parasite prevalence rates, asexual erythrocytic-stage vaccines that inhibit multiplication of asexual stage parasites, or vaccines that target vector antigens to disrupt parasitic development in the mosquito.	[22]
Vulnerability	The frequency of influx of infected individuals or groups and/or infective anopheline mosquitoes. Note that vulnerability is also referred to as 'importation risk'. The term can also be applied to the introduction of drug resistance in a specific area.	[14]

Abbreviations: CHMI, controlled human malaria infection; CRISPR, clustered regularly interspaced short palindromic repeats; malERA, Malaria Eradication Research Agenda; SERCaP, Single-Encounter Radical Cure and Prophylaxis; VIMT, vaccines that interrupt malaria parasite transmission.

https://doi.org/10.1371/journal.pmed.1002456.t001

elimination has been progressing using current tools and strategies; second, transmission intensity varies widely between and within countries with different mosquitoes and parasite species as well as different health systems and a myriad of varying challenges to the scale-up of interventions; in addition, programmatic goals evolve as transmission changes (Fig 2). The reduction of transmission may progress in a highly variable fashion, affected by ecologic (e.g., climate and outbreaks), biologic (e.g., vector or parasite resistance), and operational (e.g., health delivery system, sociopolitical and -economic status) challenges. Moreover, while some countries have shown durable elimination [23], other countries have come close to but not achieved elimination and then experienced resurgences [24]. New approaches are needed to address vulnerability and receptivity so that elimination can be achieved and sustained in spite of predictable risk of importations.

malERA Refresh process

The malERA Refresh was undertaken against the background of WHO GTS that was unanimously adopted by the World Health Assembly in 2015 as well as the Roll Back Malaria (RBM) AIM framework [2,3]. Although focussed on malaria, the malERA process itself can be a useful model for defining the research needs, strategies, and portfolios to eliminate and eradicate neglected tropical diseases (NTDs).

The malERA Refresh process was overseen by a leadership group composed of Regina Rabinovich (chair, ISGlobal Barcelona Institute for Global Health and Harvard T.H. Chan School of Public Health), Pedro Alonso (WHO Global Malaria Programme), Marcel Tanner (Swiss TPH), and Dyann Wirth (Harvard T.H. Chan School of Public Health), and each consultative panel was led by a chair and 1 or 2 cochairs [25]. The process was managed by the MESA Secretariat (ISGlobal Barcelona Institute for Global Health). Diverse expert panels of scientists,

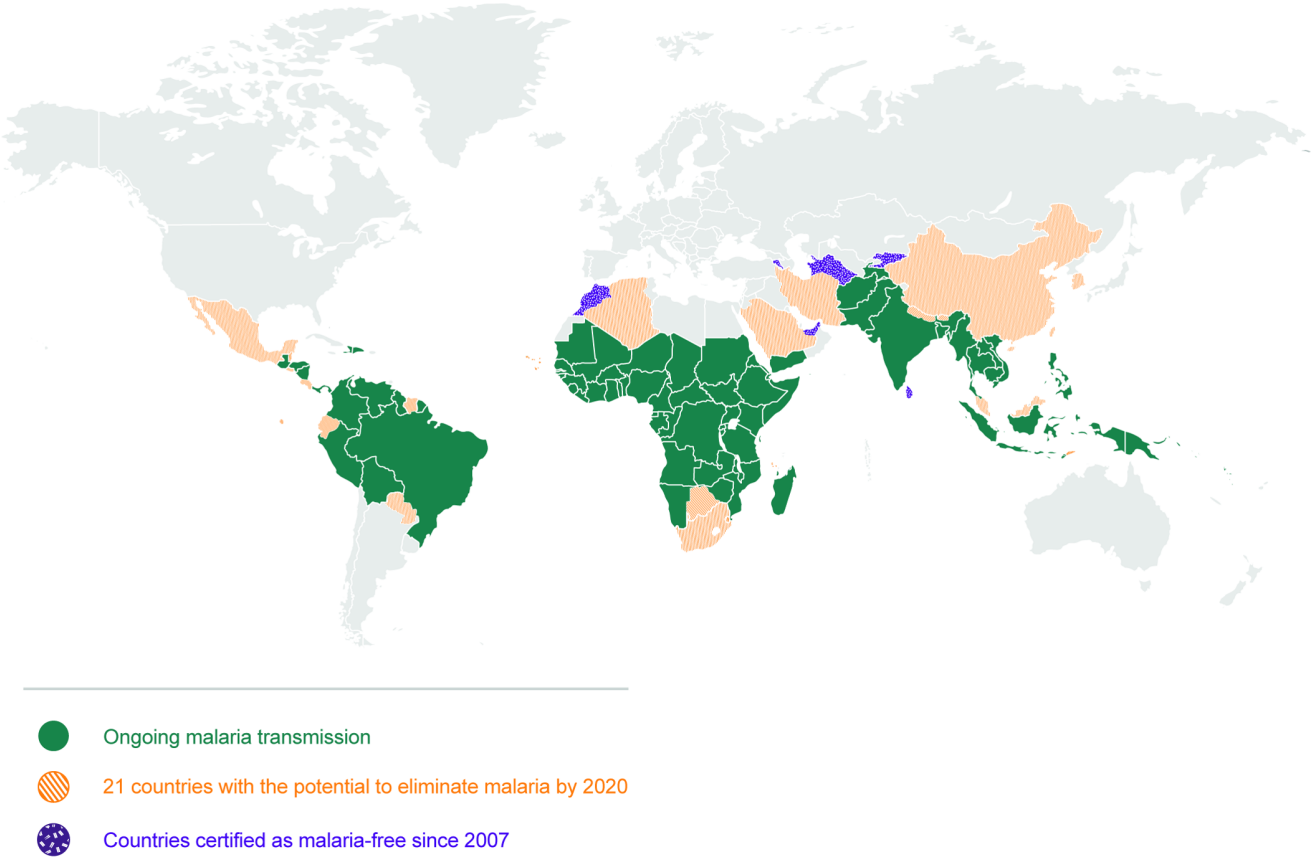

Fig 1. Map of 21 countries with the potential to eliminate malaria by 2020. There are 91 countries and territories with ongoing malaria transmission [9]. An analysis by WHO has identified 21 countries with the potential to eliminate by 2020: Algeria, Belize, Bhutan, Botswana, Cabo Verde, China, Comoros, Costa Rica, Ecuador, El Salvador, Iran (Islamic Republic of), Malaysia, Mexico, Nepal, Paraguay, Republic of Korea, Saudi Arabia, South Africa, Suriname, Swaziland, and Timor-Leste [10]. Countries and territories that have been certified malaria-free since 2007 are the United Arab Emirates (2007), Morocco (2010), Turkmenistan (2010), Armenia (2011), Maldives (2015), Sri Lanka (2016), and Kyrgyzstan (2016) [9,10]. Argentina and Paraguay have formally requested certification of malaria elimination and are in the process. Note that not all countries that have achieved zero indigenous cases for 3 consecutive years have sought certification from WHO. *Map base vector created by Freepik*.

https://doi.org/10.1371/journal.pmed.1002456.g001

programme managers, and decision makers were convened for 6 thematic areas. The themes of the panels were adapted from the original malERA, reflecting the evolution of the knowledge base even since the first malERA process in 2011. One panel examined tools for elimination (vector control, vaccines, diagnostics, and drugs), one panel tackled the application of mathematical modelling to the challenges of combining interventions, and the health systems panel also addressed policy research. New panels were created, one to look at the infectious reservoir and one focussed on resistance to antimalarial drugs and insecticides (for the full list of panels, see Table 2). A systematic literature search was performed for each theme to identify papers published between 2010 and 2016. These papers were supplemented with suggestions from panelists and projects in the MESA Track database of active projects. Each panel had 1 in-person meeting to assess the progress since malERA 2011 and discussed whether there had been adequate efforts to address each area. Taking into consideration the major advances that have taken place since the first malERA consultations, the panels highlighted specific challenges and indicated key opportunities to generate knowledge, tools, and strategies for malaria

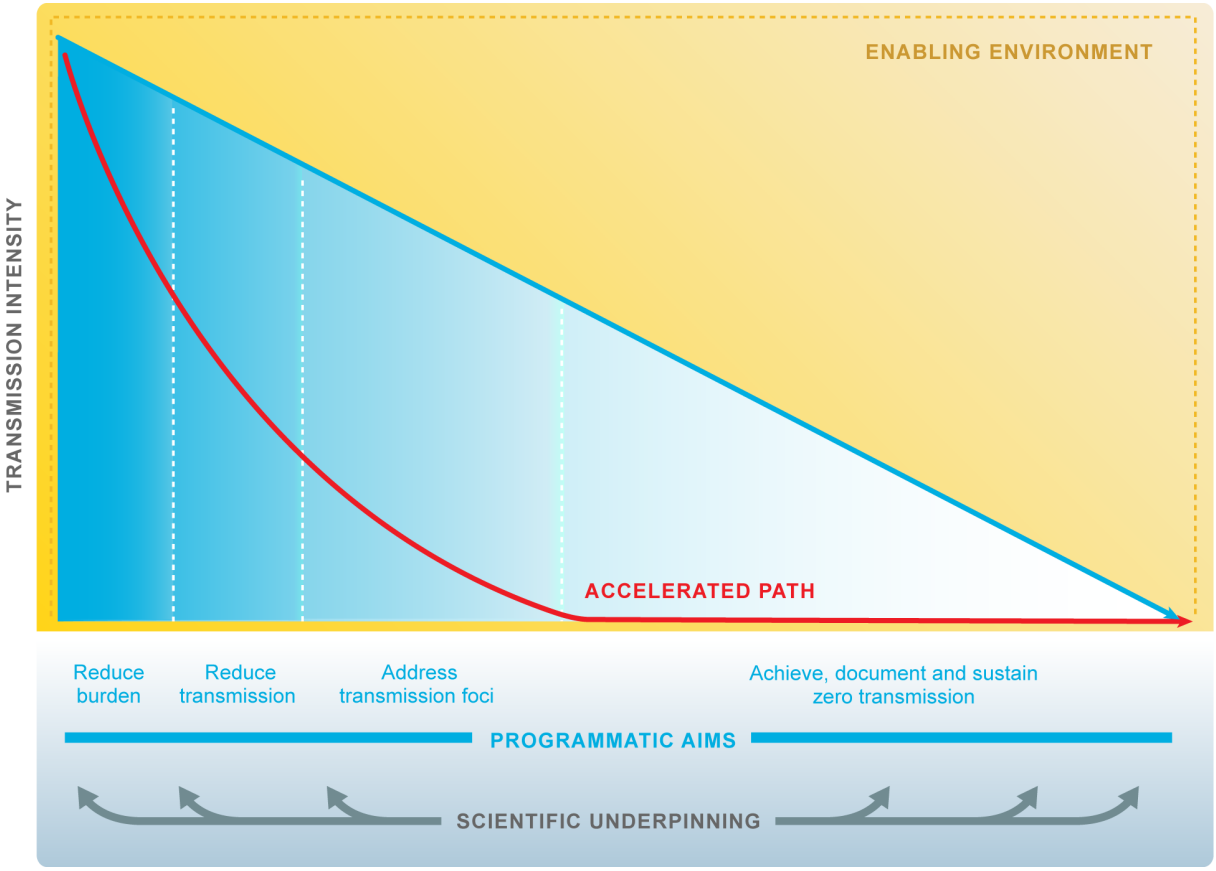

Fig 2. Research accelerates progress towards malaria elimination goals. Research and development responds to the diverse and evolving needs of malaria programmes to underpin elimination and eradication. 'Scientific underpinning': research agenda is multidisciplinary and includes all human *Plasmodium* species. Quality evidence informs policies and decision makers. 'Accelerated path': technical and operational innovations accelerate progress towards malaria elimination. 'Enabling environment': working partnerships between malaria programmes and research institutions. The malaria community and the research community respond effectively to opportunities afforded by other sectors, e.g., urbanisation, education. The malaria community and the research community respond effectively to threats, e.g., natural disasters, conflict, stimulation of career progression and scientific leadership from malaria endemic countries, and commitment to national malaria elimination goals by Ministries of Health, Finance, Science, Education, and Tourism.

https://doi.org/10.1371/journal.pmed.1002456.g002

elimination (Box 1). Cross-links between the panels were ensured by cross-panel participation and an online consultation of main findings (Fig 3).

A final meeting of all panel leaders reviewed results of this process and identified cross-cutting themes that arose across several panels. These are described further in this paper: surveillance, implementation science, and transmission and persistence. In addition, 2 areas—entomology and *P. vivax* malaria—were recognised as research areas that were consistently failing to garner adequate resources and thus scientific engagement. Rather than define specific areas for prioritisation, this research agenda lays out the rationale, context, and relevance for a range of interlinked areas.

Cross-cutting priority research areas

Surveillance and towards surveillance–response approaches. Malaria programmes continuously need data to direct their actions and resources, to gauge their impact, and,

Table 2. Using the themes from the first malERA process as a starting point, malERA Refresh was organised by research themes that are relevant for malaria elimination and eradication and reflect current hypotheses and new thinking.

malERA 2011	malERA Refresh 2017
• Introductory paper • Basic science and enabling technologies • Drugs • Vaccines • Vector control • Diagnosis and diagnostics • Monitoring, evaluation, and surveillance • Modelling • Cross-cutting issues for eradication • Lessons for the future • Role of research in viral disease eradication	• Overview and synthesis paper • Basic science and enabling technologies *• Basic science and enabling technologies remained a key theme.* • Insecticide and drug resistance *• Given the evolving threat and search for solutions to resistance, a panel was created to address resistance to insecticides and antimalaria drugs in the malaria elimination context.* • Characterising the reservoir and measuring transmission *• To reflect the evolving and nuanced questions around transmission, 1 panel examined the complexity of the parasite reservoir and the challenges of measuring transmission.* • Diagnostics, drugs, vaccines, and vector control *• A synthetic assessment of product development for malaria was included.* • Combination interventions and modelling *• A panel was created to tackle the power of combining tools and predicting and increasing their impact using mathematical modelling.* • Health systems and policy research *• A panel was dedicated to the operational challenges of malaria elimination in the context of existing health and social systems.*

Abbreviation: malERA, Malaria Eradication Research Agenda.

https://doi.org/10.1371/journal.pmed.1002456.t002

particularly in the elimination context, to reorient their tools and strategies to clear infections and stop transmission. The recent Ebola and Zika emergencies have highlighted the critical role of strong health systems with diligent surveillance to enable rapid responses. Surveillance is considered so fundamental to the malaria programme across the transmission spectrum that it represents 1 of the 3 pillars of the GTS [2]. Surveillance itself is an intervention and must be adapted to the respective epidemiological, health, and social system settings [12,26]. Information gleaned from surveillance also informs the rational incorporation of new interventions. In the context of elimination, however, surveillance must be both systematic and sufficiently robust to capture the diminishing number of cases of disease. As elimination nears, surveillance systems must be capable of correctly assessing the infection burden and direct actions; for example, if surveillance data show very few cases, then the programme action can pivot to a reactive approach to treatment around the index patient. Post elimination, surveillance systems must be capable of identifying cases that are reintroduced to prevent resumption of local transmission.

Surveillance platforms like the District Health Information Software 2 (DHIS 2) are being used to collect facility and community data across diseases. When fully functional, such platforms collect dynamic quality-assured information that can be analysed to track temporal and spatial changes in transmission [26,27]. High-quality information systems that collect real-time data from incoming cases can spot early warning signals of drug resistance, reintroduction, and resurgence. High-resolution platforms based on geographic information systems have been developed that collect, integrate, and share relevant data with various audiences [27]. These surveillance–response systems are particularly useful for the detection of and response to unevenly distributed transmission foci with sufficient detail as to depict the single-household or hamlet level and are key to targeting the operational response. In addition to collecting information on malaria infections, a quality malaria surveillance system should assess drug efficacy against the parasites and assess mosquito vector populations and insecticide

Box 1. Examples of challenges and opportunities to generate knowledge, tools, and strategies for malaria elimination

See the papers in this series for the full description of where science has and has not made progress since malERA and the considerations of the main challenges and exciting opportunities going forward.

Biology

- There are significant gaps in the knowledge base and ability to tackle the non-*falciparum Plasmodium* species (*P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi*).
- Applying new technologies including CRISPR-Cas9 mediated gene drives, high-throughput screening, metabolomics, and proteomics will help advance malaria biology.

Tools and deployment strategies

- Strategies to stop the expanding resistance to pyrethroids, artemisinins, and partner drugs are urgently needed.
- Tools to detect hypnozoites and *P. vivax* vaccine candidates remain to be developed.
- Deploying insecticides with novel modes of action.
- Two areas of promise for drug development are applying the controlled human malaria infection (CHMI) models as a bridge to field efficacy of transmission-blocking activity and high-throughput phenotypic screening for the 'neglected' product profiles, including hypnozoites and gametocytes.
- Novel approaches to vector control tools are beginning to be explored, including using drugs for vector control.
- Opportunities are emerging regarding monoclonal antibodies for passive immunity.

Understanding transmission and tackling residual transmission

- Major questions in understanding transmission remain, from gametocyte biology to characterising and detecting the infectious reservoir.
- Advances are needed in entomological sampling, analysis, and entomological surveillance systems.
- Innovation in genomics, serology, and geospatial tools can help sampling, validating the absence of malaria transmission, and measuring receptivity.

Malaria programmes and systems

- Questions remain around the best composition, phasing, and threshold triggers for intervention packages in different settings and as programmes advance along the elimination continuum.

- An area of promise for malaria programmes is testing and validating essential, collectable, and actionable data for programmatic decision-making.
- Advances in molecular technologies will help surveillance of resistance to insecticides and drugs.
- Strategies for deploying future tools in the field need to be tested and modelling can guide testing.
- Opportunities using systems-thinking approaches to identify where in the health system effectiveness of interventions is lost and can be recovered.

resistance phenotypes [27,28]. The metrics to best provide this information are still under evaluation.

Research is needed on 2 levels: to better understand low and zero transmission and to develop measures that can be used by programmes. As countries approach elimination, validated epidemiological and entomological markers and efficient sampling strategies will be required to detect transmission at low levels and to confirm the absence of transmission—i.e., the challenge of "measuring zero". Molecular and serological approaches are being evaluated. For example, identifying and responding to transmission foci would benefit from rapid and noninvasive diagnostic tools that can be applied in nonclinical settings [27]. The balance between predictive value and clinical or public health utility of diagnostic testing will differ in different epidemiologic settings, e.g., as incidence declines, more test-positive cases will be false positives. There are open questions regarding the programmatic impact of new tools to identify subpatent infections that might sustain malaria parasite transmission in some settings [27,29]. The critical balance is that the data collected need to be informative for the programme but also practical in terms of collection and interpretation. The concept of "minimal essential data" describes the balance between a collectable dataset and an informative one, such that programmes can respond to the data [26,28]. As a malaria programme progresses towards elimination, the data requirements will be continually changing and what is deemed "essential" data will also change. There is a need to build an evidence base for effective programmatic responses, e.g., analysis of the systems for data collection, analysis and response to minimise effectiveness decay, developing a portfolio of effective programmatic responses to surveillance data [26], and using modelling and operational research to test specific questions that could facilitate programme performance [12].

Implementation science. In contrast to the apparent simplicity of programmes that depend on a single intervention (e.g., vaccines), malaria programmes use a diverse set of tools in an integrated approach to prevent, detect, and treat infections. While the key elements (surveillance, diagnosis, treatment, and prevention) are constant, there are important nuances and evolution for each element as transmission declines. As new tools become available, they need to be integrated into the existing intervention package(s). A critical challenge in malaria elimination is finding the optimal combination of interventions to maximise impact and mitigate the risk of resistance and to modify this package in a timely fashion to respond to the increasingly focal and rapidly changing transmission environment. Interventions have to be introduced, altered, replaced, or possibly withdrawn through adaptive strategies responding to

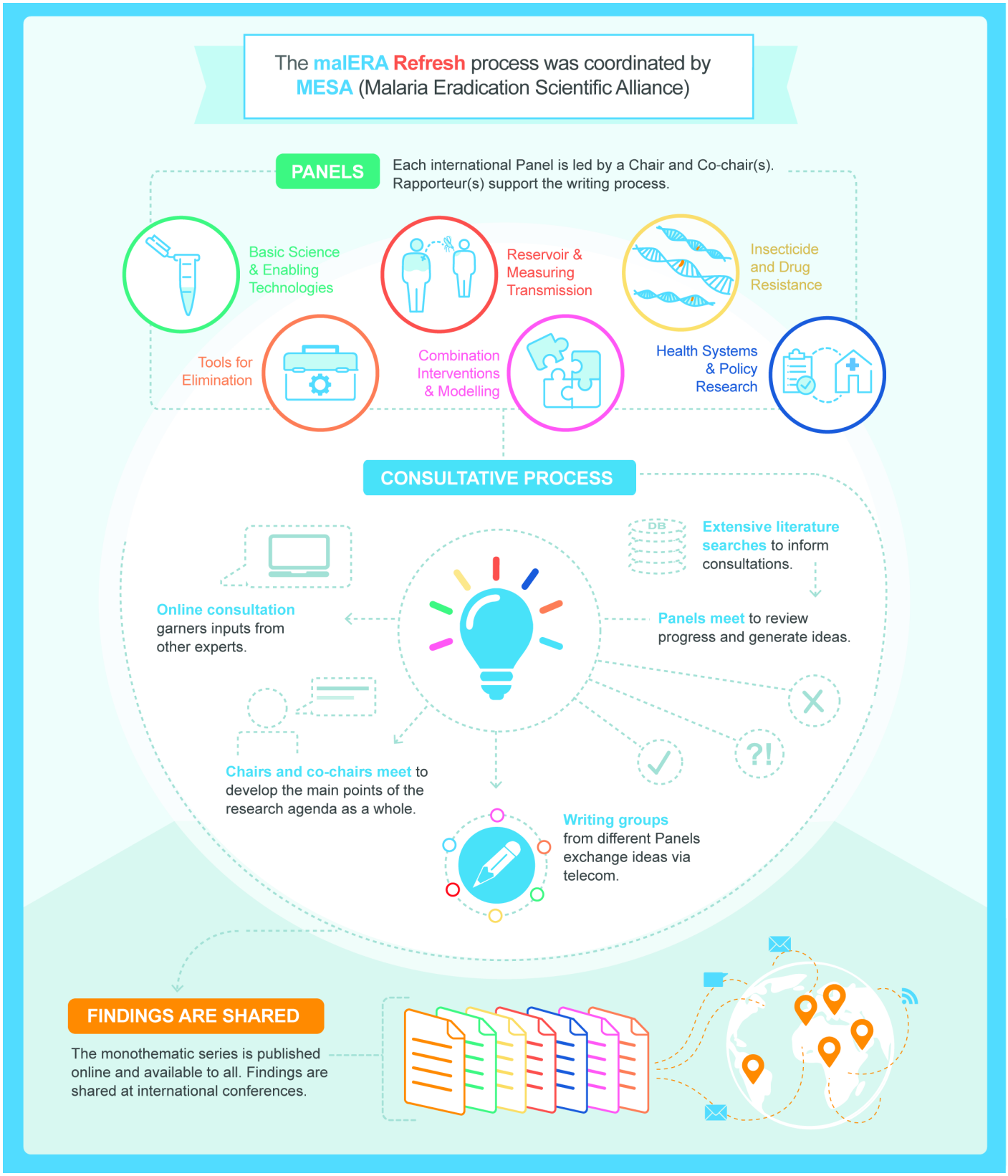

Fig 3. malERA Refresh process. malERA, Malaria Eradication Research Agenda; MESA, Malaria Eradication Scientific Alliance.

https://doi.org/10.1371/journal.pmed.1002456.g003

shifting transmission, emerging resistance, and response to unique community issues and needs.

Achieving universal coverage of preventive and curative interventions 'is one of the biggest opportunities to have a major impact on global mortality and morbidity' and is also on the critical pathway to elimination [30]. The programmes currently testing MDA approaches are providing evidence of the relevance of community engagement and the need for high uptake of interventions. Health systems and community engagement are both recognised as critical elements in achieving high coverage, but research to define the successful operational criteria is still needed; social science methods have not been fully applied to overcome these challenges [26].

The efficacy of individual interventions is determined through a rigorous set of well-powered comparative trials to answer very specific questions that quantify the potential for impact under controlled circumstances. Under these ideal conditions of very high coverage and adequate use, the efficacy of an intervention equals its effectiveness. Under real field conditions, measurable 'effectiveness decay' results from the impact of key elements of the health system, including challenges in financing, procurement, work force, supply chain, and adherence. However, the drivers of effectiveness decay vary and depend on the setting, i.e., unique cultural and/or health systems [26]. malERA 2011 underlined the need to establish a tool for analysing effectiveness decay within a health system, akin to a diagnostic tool for the system itself. It would allow the malaria programme to identify bottlenecks, test different approaches to overcome them, and thus minimise effectiveness decay [31]. Unfortunately, so far, too little investment and progress have been seen in this area and work to understand and mitigate effectiveness decay remains a priority [26].

Transmission and persistence. In elimination settings, the malaria programme takes on an added focus: understanding the nuances that contribute to continued transmission in scenarios of low parasitaemia and low incidence and to the parasite's persistence in host and vector. malERA 2011 stressed the importance of the infection and the transmission reservoir and catalysed a search for tools to identify and interrupt transmission [1,21,22,32,33]. Notably, the concept of a drug combination Single Encounter Radical Cure and Prophylaxis (SERCaP) was developed [21] (see Glossary in Table 1). Today, new chemical entities with a 'single encounter, radical cure' profile are undergoing early clinical development. The concept of SERCaP was that it could eliminate all parasites from the human (including the long-lived hypnozoites) in a single encounter suitable for mass administration (including administration to healthy people and the consequent need of a very good safety profile) and prophylaxis for at least 1 month after treatment, to outlast the typical development period of plasmodia parasites in anopheline mosquitoes. Today, new chemical entities with a 'single encounter, radical cure' profile are undergoing early clinical development [29]. malERA 2011 expanded the concept of transmission-blocking vaccines to the broader array of VIMT targets (vaccines that interrupt malaria parasite transmission), which can be achieved at several stages of the parasite life cycle, not just the sexual or mosquito stages, as in classical transmission-blocking vaccines [22]. Several VIMT candidates for *P. falciparum* are in the development pipeline. Although *P. vivax* is now included in the Malaria Vaccine Technology Roadmap strategic goals, VIMTs for *P. vivax* have not advanced [29].

Research to characterise the transmission reservoir has evolved to a focus on the role of low-density infections undetected by microscopy or current RDT in transmission [27]. Understanding determinants of the risk of infectiousness, understanding at what level of parasitaemia these are important for sustained transmission, and devising metrics and tools to measure and target transmission are proposed as key needs [26,27,29,34]. Recently, a highly sensitive

RDT has been launched and demonstration studies are being planned to test how and when to use this new tool [35].

Measuring zero transmission is a requisite for programmes that seek to eliminate malaria and for evaluating tools in the development pipeline that aim to interrupt or reduce transmission. Validated, measurable epidemiological and entomological indicators of transmission are needed. The papers in this series discuss the research agenda and potential solutions [12,26–29,34].

Transmission needs to be reliably measured both at the mosquito and human levels, but the tools available today only provide proxies for true transmission. Currently, vector control tools are not able to interrupt all malaria transmission, and 'residual transmission' can persist even in areas with good vector control coverage (see Glossary in Table 1). Residual transmission is now recognised as a target for investigation and intervention, but there is no consensus yet on how to quantify this concept. Novel tools to interrupt residual transmission as a complement to traditional vector control are under development and include toxic sugar-baited traps, endectocides, and targeted larviciding [27,29].

Gametocyes are the transmissible form of the parasite from humans and present a biological opportunity because they are relatively few in number compared to other parasite stages. Drug candidates with gametocytocidal properties are early in the pipeline and will need to be tested for their ability to arrest the transmission cycle, and the search for tractable vaccine targets that attack gametocytes in the human host needs to continue [29,34]. Knowledge of the drivers controlling gametocyte production is poor, e.g., understanding what environmental conditions might favour an increased production of gametocytes and facilitate transmission [27]. Moreover, there is a need to better define the relationship between gametocyte densities and transmission for both *P. falciparum* and *P. vivax*. Reliable biomarkers for both gametocytes and hypnozoites would enable this.

The key determinants for persistence and recrudescence remain to be established. In highly seasonal settings, it has been demonstrated that humans can act as the parasite reservoir by carrying gametocytes at levels beneath detection of current diagnostics, but the role of the mosquito as a reservoir during those months is still poorly understood [27,34].

Major neglected areas critical to elimination

Entomology. Despite the indisputable merit of vector control tools in the reductions of malaria morbidity and mortality and increasing vector resistance against insecticides, investment in this area has lagged [36]. This scenario extends from basic research through product development and training.

Currently, collecting entomological data is laborious and trained entomologists and staff are scarce. Programmes such as TDR and the US President's Malaria Initiative have recognised the need for improving national capacities for entomological monitoring and support training efforts in some countries [37,38]. The recently adopted 'Global Vector Control Response' report marks a significant commitment of WHO and member states to strengthen vector control within a collaborative framework [39]. Recent global outbreaks of other vector-borne diseases such as Zika and chikungunya highlight the need for countries to garner the necessary support for strengthening capacity in entomology and vector control that is also relevant for malaria. malERA Refresh panelists agreed that medical entomology must have a central role in the global health curriculum and in the training curriculum for Ministry of Health staff.

The efficacy of available vector control tools is diminished by residual transmission and the enormous behavioural plasticity and biological variability of malaria vectors and is threatened by the capacity of the mosquito to develop resistance in the face of high pressure from

interventions. The papers in this malERA Refresh series offer potential solutions to be developed and tested [27–29,34].

Novel entomological markers for transmission are needed because the traditional measure—entomological inoculation rate (EIR)—is not a practical or easily reproducible metric in lower-transmission settings [27]. The gap in data collection capacities needs to be addressed by testing and validating what constitute minimal essential, collectable, and actionable data. New technologies are needed to generate robust data on species distribution, temporal and spatial biting patterns, and spread of insecticide resistance, which would be actionable data from entomologic surveillance in the future [27,29].

Vivax malaria (and 3 other species). Five species of *Plasmodium* infect humans. *P. falciparum* has been a global priority due to its role as a driver of mortality and severe disease. However, *P. vivax* is geographically the most widely distributed form of human malaria, causes 13.8 million cases every year, and is associated with both significant morbidity and a risk for mortality [9]. The research agenda presented in the malERA Refresh series is relevant to *P. falciparum* and *P. vivax*; specific challenges posed by *P. vivax* are highlighted in the thematic papers and here.

There are important differences in the biology of *P. vivax*, particularly its ability to remain quiescent in the liver, different kinetics and appearance of infectious gametocytes, and significant differences in its clinical presentation and risk of recurrence. Unique drugs, diagnostics, and different targets for vaccine development and strategies are required beyond what is available today.

malERA 2011 acknowledged hypnozoites as a challenge to *P. vivax* elimination, and this remains the case, with a lack of diagnostics to identify carriers and safe efficacious treatments to clear them [1,29]. Proteomic and metabolomic techniques have been suggested as possible research tools to detect hypnozoites; additional in vitro studies are needed to expand current knowledge of their biology and metabolism [34].

Countries with *P. falciparum* and *P. vivax* malaria seek to eliminate the disease entirely rather than a single species. Thus, tackling *P. vivax* was considered critical in malERA 2011 and, while the biological and epidemiological knowledge base has significantly improved, there is still a relatively weak pipeline of drugs and vaccines [1,27,29,34].

Tafenoquine is in late-stage development. It is a candidate drug that results in radical cure of all circulating parasites and *P. vivax* hypnozoites in a single treatment and confers prophylaxis for several weeks posttreatment. Results from a Phase III clinical trial show that single-dose tafenoquine reduces risk of relapse in patients with *P. vivax* malaria [29,40]. When tafenoquine becomes available, it will not remove the need to test for glucose-6-phosphate dehydrogenase (G6PD) deficiency, which affects 350 million people at risk for malaria and remains a considerable obstacle to effective treatment [41]. Novel point-of-care diagnostic tests for G6PD deficiency are currently in late-stage development [29]. In the future, newly developed humanised mouse models could help predict the haemolytic potential of drugs in the pipeline [29,34].

P. knowlesi poses unique challenges among the 5 malaria species, owing to its zoonotic transmission. WHO convened an Evidence Review Group (ERG) to review existing data on *P. knowlesi*, including an upward trend in incidence documented in Malaysia, and identify knowledge gaps. The ERG articulated the need for evidence to better understand the likelihood of human to human transmission [11].

Looking forward

Innovation and problem solving tailored to the local setting are critical to the long-term success of the global malaria programme. Three types of innovation need to be pursued: iterative,

breakthrough, and integrated. malERA Refresh is replete with examples: drugs to overcome resistance, gene drive as a transformative technology, and the acceleration hypothesis as a testable approach to elimination and its interaction with the health system in highly endemic countries. To pursue the opportunities proposed here for accelerating elimination, a diverse landscape of funders is needed to prioritise research objectives according to their strategic plans and stakeholders' needs. A diligent monitoring of the uptake of the research questions in this agenda and the impact of the evolving evidence base will be essential to keep the malaria community on course.

Supporting information

S1 Translation. Spanish translation of abstract.
(DOCX)

S2 Translation. French translation of abstract.
(DOCX)

Acknowledgments

The malERA Refresh process was overseen by a leadership group and each consultative panel was led by a chair and 1 or 2 cochairs. The process was managed by the Malaria Eradication Scientific Alliance (MESA) Secretariat based at ISGlobal (Barcelona Institute for Global Health). For a description of the process, see the 'malERA Refresh Process' section of this paper and Table 2. For a full listing of all the chairs and panelists, see the individual papers in the malERA Refresh series. Vittoria Lutje prepared systematic literature searches for all 6 malERA Refresh panels and was funded by MESA. Desiree van der Mei managed the meeting logistics and travel for the process. Julie Chaccour provided writing support and Rachel Papernick developed the figures; both were funded by MESA. Authors reviewed several iterations of the manuscript to finalise it. All listed authors met the ICMJE criteria.

Disclaimer: The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the World Health Organization.

References

1. Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, Collins F, et al. A research agenda to underpin malaria eradication. PLoS Med. 2011; 8(1):e1000406. https://doi.org/10.1371/journal.pmed.1000406 PMID: 21311579
2. World Health Organization. Global technical strategy for malaria 2016–2030. Geneva: WHO; 2015. Contract No.: 17 March.
3. Roll Back Malaria Partnership. Action and investment to defeat malaria 2016–2030: for a malaria-free world Geneva: WHO; 2015 Available from:http://rollbackmalaria.com/. Date accessed 2017 Nov 1.
4. End malaria 2040. From Aspiration to Action: What Will It Take to End Malaria?; 2016.
5. World Health Organization. WHO Strategic advisory group (SAG) on Malaria. Eradication of malaria, Report by the Secretariat. Geneva; 2017 18.05.2017.
6. World Health Organization. A framework for malaria elimination. Geneva; 2017. Contract No.: ISBN: 978 92 4 151198 8.
7. Melinda French Gates. BMGF Malaria Forum Keynote Address: Bill & Melinda Gates Foundation; 2007 [Prepared remarks by Melinda French Gates, co-chair BMGF]. Available from: https://www.gatesfoundation.org/media-center/speeches/2007/10/melinda-french-gates-malaria-forum. Date accessed 2017 Oct 10.
8. Roberts L, Enserink M. Malaria. Did they really say. . . eradication? Science. 2007; 318(5856):1544–5. https://doi.org/10.1126/science.318.5856.1544 PMID: 18063766

9. World Health Organization. World Malaria Report. Geneva; 2016 13.12.2017. Report No.: ISBN: 978 92 4 151171 1.
10. World Health Organization. Eliminating malaria. Geneva: WHO; 2016, 20 May.
11. World Health Organization. Malaria Policy Advisory Committee meeting report Geneva; 2017, March. Report No.: WHO/HTM/GMP/MPAC/2017.8.
12. malERA Refresh Consultative Panel on Combination Interventions and Modelling. malERA: An updated research agenda on combination interventions and modelling for malaria elimination and eradication. PLoS Med. 2017; 14(11): e1002453. https://doi.org/10.1371.journal.pmed.1002453
13. MESA Track database [Internet]. [cited 2017]. Available from: http://www.malariaeradication.org/mesa-track. Date accessed 2017 Oct 10.
14. World Health Organization. WHO malaria terminology. Geneva: WHO; 2016. Contract No.: 1 October.
15. Epstein JE. Taking a bite out of malaria: controlled human malaria infection by needle and syringe. Am J Trop Med Hyg. 2013; 88(1):3–4. https://doi.org/10.4269/ajtmh.2013.12-0715 PMID: 23303797
16. Mordmuller B, Supan C, Sim KL, Gomez-Perez GP, Ospina Salazar CL, Held J, et al. Direct venous inoculation of *Plasmodium falciparum* sporozoites for controlled human malaria infection: a dose-finding trial in two centres. Malar J. 2015; 14:117. https://doi.org/10.1186/s12936-015-0628-0 PMID: 25889522
17. Gomez-Perez GP, Legarda A, Munoz J, Sim BK, Ballester MR, Dobano C, et al. Controlled human malaria infection by intramuscular and direct venous inoculation of cryopreserved *Plasmodium falciparum* sporozoites in malaria-naive volunteers: effect of injection volume and dose on infectivity rates. Malar J. 2015; 14:306. https://doi.org/10.1186/s12936-015-0817-x PMID: 26245196
18. Dan Strickman. Definition adapted from personal communication. 2017.
19. WHO Alliance for Health Policy and System Research. Systems thinking for health systems strengthening. 2009.
20. Zachariah R, Harries AD, Ishikawa N, Rieder HL, Bissell K, Laserson K, et al. Operational research in low-income countries: what, why, and how? Lancet Infect Dis. 2009; 9(11):711–7. https://doi.org/10.1016/S1473-3099(09)70229-4 PMID: 19850229
21. malERA Consultative Group on Drugs. A research agenda for malaria eradication: drugs. PLoS Med. 2011; 8(1):e1000402. https://doi.org/10.1371/journal.pmed.1000402 PMID: 21311580
22. malERA Consultative Group on Vaccines. A research agenda for malaria eradication: vaccines. PLoS Med. 2011; 8(1):e1000398. https://doi.org/10.1371/journal.pmed.1000398 PMID: 21311586
23. Smith DL, Cohen JM, Chiyaka C, Johnston G, Gething PW, Gosling R, et al. A sticky situation: the unexpected stability of malaria elimination. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2013; 368(1623):20120145. https://doi.org/10.1098/rstb.2012.0145 PMID: 23798693
24. Cohen JM, Smith DL, Cotter C, Ward A, Yamey G, Sabot OJ, et al. Malaria resurgence: a systematic review and assessment of its causes. Malar J. 2012; 11:122. https://doi.org/10.1186/1475-2875-11-122 PMID: 22531245
25. Malaria Eradication Scientific Alliance. MESA malERA Refresh [updated 2017. Available from: http://www.malariaeradication.org/. Date accessed 2017 Oct 10.
26. malERA Refresh Consultative Panel on Health Systems and Policy Research. malERA: An updated research agenda for health systems and policy research in malaria elimination and eradication. PLoS Med. 2017; 14(11): e1002454. https://doi.org/10.1371/journal.pmed.1002454
27. malERA Refresh Consultative Panel on Characterising the Reservoir and Measuring Transmission. malERA: An updated research agenda for characterising the reservoir and measuring transmission in malaria elimination and eradication. PLoS Med. 2017; 14(11): e1002452. https://doi.org/10.1371/journal.pmed.1002452
28. malERA Refresh Consultative Panel on Insecticide and Drug Resistance. malERA: An updated research agenda for insecticide and drug resistance in malaria elimination and eradication. PLoS Med. 2017; 14(11): e1002450. https://doi.org/10.1371/journal.pmed.1002450
29. malERA Refresh Consultative Panel on Tools for Elimination. malERA: An updated research agenda for diagnostics, drugs, vaccines and vector control in malaria elimination and eradication. PLoS Med. 2017; 14(11): e1002455. https://doi.org/10.1371/journal.pmed.1002455
30. Roll Back Malaria Partnership. Global Malaria Action Plan (GMAP) for a malaria-free world. Geneva; 2008.
31. malERA Consultative Group on Health Systems and Operational Research. A research agenda for malaria eradication: health systems and operational research. PLoS Med. 2011; 8(1):e1000397. https://doi.org/10.1371/journal.pmed.1000397 PMID: 21311588

32. malERA Consultative Group on Diagnoses and Diagnostics. A research agenda for malaria eradication: diagnoses and diagnostics. PLoS Med. 2011; 8(1):e1000396. https://doi.org/10.1371/journal.pmed.1000396 PMID: 21311583

33. malERA Consultative Group on Vector Control. A research agenda for malaria eradication: vector control. PLoS Med. 2011; 8(1):e1000401. https://doi.org/10.1371/journal.pmed.1000401 PMID: 21311587

34. malERA Refresh Consultative Panel on Basic Science and Enabling Technologies. malERA: An updated research agenda on basic science and enabling technologies in malaria elimination and eradication. PLoS Med. 2017; 14(11): e1002451. https://doi.org/10.1371/journal.pmed.1002451

35. Alere Launches the Alere Malaria Ag P.f, the First-Ever Rapid Test to Screen Malaria Infection in Asymptomatic Individuals [press release]. Alere Inc., 25.04.2017 2017.

36. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015; 526(7572):207–11. https://doi.org/10.1038/nature15535 PMID: 26375008

37. President's Malaria Initiative. Entomological Monitoring [Tools and training]. Available from: https://www.pmi.gov/how-we-work/technical-areas/entomological-monitoring. Date accessed 2017 Oct 10.

38. WHO TDR the Special Programme for Research and Training in Tropical Diseases. Capacity strengthening [Available from: http://www.who.int/tdr/capacity/en/. Date accessed 2017 Oct 10.

39. World Health Organization. Global vector control response 2017–2030. Geneva; 2017, May 31.

40. GSK and MMV announce positive headline phase III results showing single-dose tafenoquine reduces risk of relapse in patients with Plasmodium vivax malaria. [press release]. 12.06.2017 2017.

41. Thriemer K, Ley B, Bobogare A, Dysoley L, Alam MS, Pasaribu AP, et al. Challenges for achieving safe and effective radical cure of Plasmodium vivax: a round table discussion of the APMEN Vivax Working Group. Malar J. 2017; 16(1):141. https://doi.org/10.1186/s12936-017-1784-1 PMID: 28381261

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COLLECTION REVIEW

malERA: An updated research agenda for basic science and enabling technologies in malaria elimination and eradication

The malERA Refresh Consultative Panel on Basic Science and Enabling Technologies*¶

¶Membership of the malERA Refresh Consultative Panel on Basic Science and Enabling Technologies is listed in the Acknowledgments.

* dfwirth@hsph.harvard.edu, ewinzeler@ucsd.edu, LHALL@niaid.nih.gov

Citation: The malERA Refresh Consultative Panel on Basic Science and Enabling Technologies (2017) malERA: An updated research agenda for basic science and enabling technologies in malaria elimination and eradication. PLoS Med 14(11): e1002451. https://doi.org/10.1371/journal.pmed.1002451

Published: November 30, 2017

Funding: MESA received a grant from the Bill & Melinda Gates Foundation (OPP1034591). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: EAW, JHA, JB, SNB, FC, BSC, MTD, AAJ, AKL, ML, IM, RR, DFW, and SKV receive funding from the Bill & Melinda Gates Foundation. EAW additionally receives funding from the Medicines for Malaria Venture, NIH, and serves on the Scientific Advisory Board of the Tres Cantos Open Lab Foundation.

Abstract

Basic science holds enormous power for revealing the biological mechanisms of disease and, in turn, paving the way toward new, effective interventions. Recognizing this power, the 2011 Research Agenda for Malaria Eradication included key priorities in fundamental research that, if attained, could help accelerate progress toward disease elimination and eradication. The Malaria Eradication Research Agenda (malERA) Consultative Panel on Basic Science and Enabling Technologies reviewed the progress, continuing challenges, and major opportunities for future research. The recommendations come from a literature of published and unpublished materials and the deliberations of the malERA Refresh Consultative Panel. These areas span multiple aspects of the *Plasmodium* life cycle in both the human host and the *Anopheles* vector and include critical, unanswered questions about parasite transmission, human infection in the liver, asexual-stage biology, and malaria persistence. We believe an integrated approach encompassing human immunology, parasitology, and entomology, and harnessing new and emerging biomedical technologies offers the best path toward addressing these questions and, ultimately, lowering the worldwide burden of malaria.

Summary points

- The recent development of multiple in vitro systems for studying malaria biology has helped deepen our understanding of the disease. Nevertheless, research remains hampered by a lack of in vitro models that can probe key aspects of malaria (e.g., gametocyte development in *Plasmodium vivax*, fertilization, ookinete biology, parasite–midgut interactions, human hepatocyte infection) and generate biological materials (i.e., infectious sporozoites) for laboratory study. Developing the necessary cell lines and other in vitro culture tools to propel these studies represent important areas for future research.
- With the emergence of widespread insecticide resistance in mosquito populations, there is a strong need to bring basic research in mosquito biology back into the malaria

Abbreviations: ap2, activator protein 2; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/associated protein-9 nuclease; dsRNA, double-stranded ribonucleic acid; ENCODE, Encyclopedia Of DNA Elements Project; GWAS, genome-wide association study; IRS, indoor residual spraying; LLIN, long-lasting insecticide-treated net; malERA, Malaria Eradication Research Agenda; MMV, Medicines for Malaria Venture; TetR, tetracycline repressor.

¶Membership of the malERA Refresh Consultative Panel on Basic Science and Enabling Technologies is listed in the Acknowledgments.

eradication agenda to strengthen current insecticide-based control campaigns and generate alternate vector control strategies.

- Driven by the development and accessibility of large-scale research tools and technologies, the scientific community can systematically tackle key questions in malaria, such as the following. What are the genes that contribute to antimalarial drug resistance (thereby defining the full parasite "resistome")? What are the functions of key *Plasmodium* genes (providing much-needed annotation of key *Plasmodium* genes)? What are the genes and gene mutations that drive resistance in mosquito populations?
- Continued exploration of the potential of enabling technologies is needed. Important areas of future research include the use of gene-drive strategies and other gene-manipulation technologies; metabolomics-based approaches for biomarker discovery; structural vaccinology, novel technology platforms, and the use of novel adjuvants to improve vaccine design; and high-throughput approaches to facilitate drug discovery and screening.

Background

Since the first agenda for malaria eradication was published in 2011 [1], there have been many significant developments in basic science, including an enhanced understanding of parasite biology (both gametocyte and liver stages) as well as mosquito biology (Table 1). Some of these advances could not have been predicted 5 years ago, such as the use of mouse models engrafted with human liver to advance the biology of liver-stage parasites (including the quiescent *P. vivax* hypnozoite stage) and the development of powerful genome-editing capabilities based on clustered regularly interspaced short palindromic repeats/associated protein-9 nuclease (CRISPR/Cas9) technology. In contrast, little progress has been achieved in several key research areas that were previously prioritized and, as such, they remain important stumbling blocks on the road to eradication.

We focus here on these and other crucial areas—deficiencies in basic science research and the lack of enabling technologies—that currently limit our progress towards malaria elimination and eradication. Importantly, this analysis highlights specific aspects of the *Plasmodium* life cycle in both the human host and the *Anopheles* vector. Our integrated approach aims to combine research efforts and expertise across human immunology, parasitology, and entomology to introduce powerful new ideas and technologies from other fields, provide a multifaceted view of disease biology, and accelerate progress toward eradication.

Methods

The findings presented in this paper result from an extensive literature review of published and unpublished materials and the deliberations of the 2015 Malaria Eradication Research Agenda (malERA) Refresh Consultative Panel on Basic Science and Enabling Technologies. Electronic databases were systematically searched for published literature between January 1, 2010, and July 2, 2016, without language limitations. Panelists were invited to recommend additional literature and additional ongoing research projects. A 2-day workshop was held with the majority of the panel members, including field researchers, specialists from basic science, malaria genomics and epigenomics, regenerative medicine, and National Institutes of Health representatives. The panel broke into 6 breakout sessions to identify the problems that

Table 1. A listing of the important research areas highlighted in malERA 2011, the progress made since then, and the remaining areas that require additional research.

Research Area	Accomplishments in Past 5 years	References	Remaining Gaps
Transmission Biology (Gametocytes to Mosquito)	Improved understanding of transcriptional and epigenetic control of sexual development	[2–6]	Limited work on *P. vivax* gametocytes due to lack of in vitro culture system
	Drug screens targeting transmission stages	[7–11]	
	Improved understanding of mosquito host-seeking behavior and olfaction biology	[12–16]	
	Improved understanding of mosquito–parasite interactions	[17–20]	
	Anopheles midgut cell line model for in vitro ookinete production and invasion	[21–25]	
Infection Biology (Mosquito to Liver)	Humanized mouse model for entire life cycle of *Plasmodium*, including *P. vivax* hypnozoites and liver stages	[26, 27]	Methods to increase sporozoite availability
	In vitro models for *Plasmodium* liver stages	[28–30]	
	Genetic crosses in mouse model	[31]	
	Primate models for *P. cynomolgi*	[32]	
	Controlled human malaria infections with sporozoites and blood-stage parasites	[33–40], reviewed in [41]	
Biology of Blood-stage Parasites	Improved production of continuous culture conditions, including identification of host cell environments necessary to support *P. vivax* invasion in culture and proof-of-principle that human hematopoietic stem cells can be immortalized, expanded, and differentiated into reticulocytes	[42–55]	No in vitro culture system for *P. vivax* asexual stages has been developed Poor functional annotation of genes
	P. knowlesi in vitro culture adaptation	[56, 57]	
	Identification and spread of mutations associated with artemisinin resistance	[58–64] reviewed in [65–67]	
	Comparison of mitochondrial and lipid metabolism of *P. falciparum* in sexual and asexual blood stages	[68, 69]	
Persistence of Parasites and Mosquitoes	*P. vivax* hypnozoites cultured in vitro	[26, 28]	Biomarkers for asymptomatic hosts Ecology and migration rates of vector species Long-term behavioral resistance studies
	Mosquito dry season estivation and long-distance migration observed in sub-Sahelian populations	[70]	
	Mechanisms of insecticide resistance identified	[71–73]	
Additional Technological Developments	Mosquito genomic resources to identify population substructure and allow comparative genomic studies	[74–77]	Coordinated efforts to generate knockout or knockdown libraries to understand gene function, especially in human parasites
	Genome-editing systems (CRISPR/Cas9, Zinc-finger nuclease), posttranslational protein knockdown systems (DD tag, Riboswitch), conditional genome deletion systems (Cre-LoxP, FLP-frt, diCre), conditional gene expression system (TetR-aptamer)	[78–86]	
	Proofs-of-principle for population suppression and population modification/replacement of *Anopheles* using gene drives	[87–92]	
	Colonization of important mosquito vector species	[93]	
	New techniques to improve antigen design and clinical evaluation of vaccine candidates	[94–100]	
	Improved resolution in intravital imaging	[101, 102]	

Abbreviations: Cre-LoxP, genetic recombination system involving the Cre (Causes recombination) protein and *loxP* (locus of X-over P); CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/associated protein-9 nuclease; diCre, dimerizable Cre recombinase; DD, destabilization domain; FLP-frt, Flipase used to recombine two frt domains; malERA, Malaria Eradication Research Agenda; TetR, tetracycline repressor.

https://doi.org/10.1371/journal.pmed.1002451.t001

need to be solved in asexual blood stages, liver stage and mosquito, mosquito, *P. vivax*, population genetics and resistance, and transmission. The panel discussed what research is needed to address these problems and considered 6 crosscutting themes in CRISPR

technologies, immunology and malaria vaccines, genomics tools for malaria, metabolism and malaria, structural biology, and diagnostics for malaria. Each group fed back to plenary session, where further robust discussions and input occurred. This helped refine the opportunities and gap areas in which research is needed. The final findings were arrived at with inputs from all panelists and several iterations of the manuscript.

Advances, challenges, and opportunities in transmission biology

Gametocytes

Plasmodium transmission begins with the development of sexual forms of the parasite (known as gametocytes) in an infected human host and their subsequent transfer to an anopheline mosquito following a blood meal (Fig 1). This stage represents a key bottleneck in the parasite

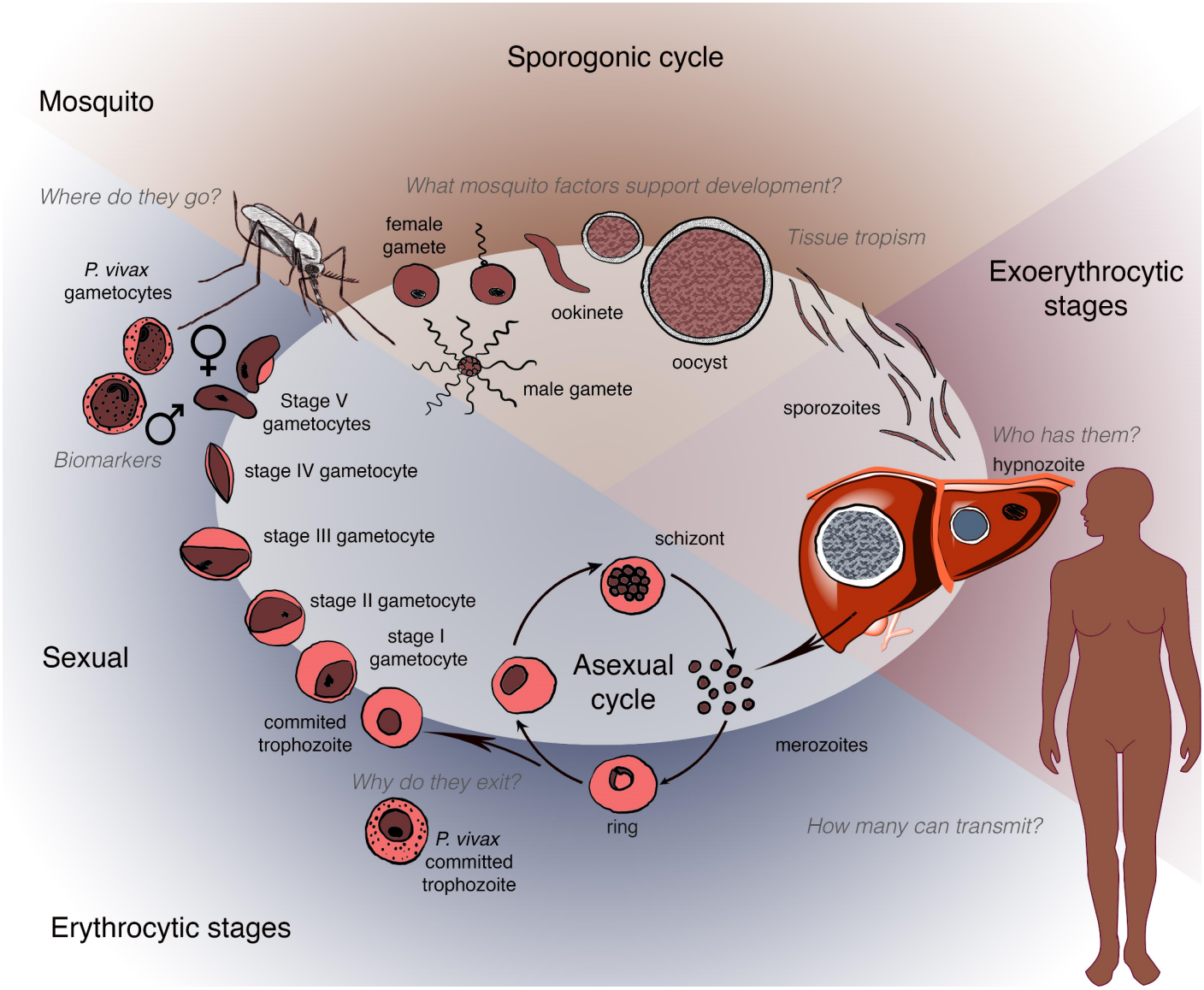

Fig 1. Schematic depicting the human and mosquito life cycles of *Plasmodium*, highlighting critical questions at specific points within the life cycle.

https://doi.org/10.1371/journal.pmed.1002451.g001

life cycle and thus is an attractive opportunity for disrupting disease transmission. As shown in Box 1, in the past 5 years significant and exciting progress has been made in understanding

Box 1. Opportunities for the next 5 years

1. **Functional genomics**

- Identification of regulatory sequences within the parasite genome, similar to the human Encyclopedia Of DNA Elements (ENCODE) project,
- Genome wide annotation of gene function in human parasites to identify sets of genes involved in discrete cellular processes, including drug resistance,
- Improved scalability of CRISPR/Cas9 technology in asexual parasites to allow for both pooled, genome-wide approaches (large scale) and single cell transformation (microscale),
- Greater collaboration between researchers to avoid overlapping gene annotation efforts.

2. **Advances in mosquito biology**

- Generation of a mosquito consortium to evaluate promising gene drive-based strategies for efficacy at scale and/or over time and share knockout and/or transgenic strains,
- Greater understanding of mosquito behavior and ecology,
- Colonization of important vector species,
- Development of in vitro mosquito infection models.

3. **New vaccine approaches**

- Improved adjuvants and identification of new targets, including better structures for existing (and new) targets to improve structural approaches,
- Development of novel approaches with the potential to generate sterilizing immunity (i.e., cognate antigens),
- Coordinated functional annotation of asexual-stage parasites to enable prioritization of functional vaccine antigens,
- Greater access to samples and data from both human challenge studies and patient samples demonstrating natural immunity,
- Application of gene-editing technologies to systematically understand the function of hypothetical genes.

4. **Biomarkers and diagnostics**

- Indicators of transmissible gametocytes,
- Markers of liver-stage infection, in particular, hypnozoites,

- Markers/assays to identify asymptomatic carriers,
- Identification of metabolic signatures of different stages of the life cycle.

5. **Greater understanding of resistance to antimalarials and insecticides**

- Identification of genes and pathways (i.e., the "resistome") involved in resistance,
- Development of alternatives to insecticides,
- Use evolutionary approaches to prevent resistance.

6. **Greater accessibility to *P. vivax* gametocytes**

- Development of a *P. vivax* in vitro culture system (e.g., ookinetes to validate transmission-blocking vaccine targets),
- Greater collaboration between groups to improve access to existing sporozoite sources. This would be coupled with advances in cryopreservation to improve access to sporozoites globally.

gametocyte development, including insights into the transcriptional and epigenetic control of sexual differentiation and evidence for bone marrow sequestration [2–6, 103]. In the case of *P. falciparum*, newly available in vitro systems for gametocyte maturation have been used in small molecule screening, antibody reagent development, and transcriptional and metabolomics analyses [7–11].

In contrast, the mechanisms of *P. vivax* gametocyte development remain largely unknown. Gametocyte biology within this species is quite distinct—development takes just 2 to 3 days and unfolds prior to any clinical symptom. *P. vivax* gametocytes appear susceptible to existing antimalarial drugs that are not effective against *P. falciparum* gametocyte stages [104–106]. Progress in this area has been hampered by the absence of a comparable in vitro culture system for asexual *P. vivax* parasites, which is an urgent priority, as it would enable the generation of gametocytes for laboratory study, mosquito infections, and sporozoite production.

Another major area for discovery is the elucidation of the biological determinants of gametocyte transmissibility, especially in areas of low endemicity. Does the success of transmission depend on gametocyte quantity and/or quality? Are there mosquito-specific factors that actively recruit gametocytes to the biting site or do gametocytes preferentially sequester near the skin? What factors and mechanisms enable male and female gametes to find one another in the mosquito midgut? Biomarkers for transmission competency could enable a broader understanding of the heterogeneity in natural infections.

Mosquito biology and host seeking

Transmission success also depends upon the interactions of the mosquito vector with both its human host and ingested parasites. Since 2011, there have been major advances in understanding the biology of olfaction and host-seeking behavior in mosquitoes via a combination of behavioral assays, electrophysiology, and functional genomic approaches [12–16]. High-throughput screens have identified new classes of attractants and repellents that are currently being tested in mosquito traps and spatial repellent trials ([107–110], also see MESA Track at http://www.malariaeradication.org/mesa-track). Moving forward, the identification of

oviposition cues and the role of olfaction and taste in larval stages could facilitate the development of additional tools for vector control. Comparative genomic analysis of odorant receptor pathways that differ between anthropophilic and zoophilic species will help to elucidate the molecular basis of host-seeking behavior. Recent studies have shown that the composition of the human skin microbiota influences host attractiveness to mosquitoes [111] and identified volatile substances produced by parasites in human hosts thought to preferentially attract mosquitoes to infected individuals [112]. Nevertheless, gaps remain in our knowledge regarding the potential for gametocyte-seeking behavior by the mosquito and parasite-induced changes to the human host that may influence mosquito behavior to enhance biting and transmission.

Parasite development in the mosquito

Fertilized zygotes develop into the motile ookinete, which in turn crosses the midgut wall. Major advances have been made in understanding midgut invasion and early mosquito anti-*Plasmodium* immune responses that target the ookinete stage. Several parasite genes that interact with the vector to enable its invasion of epithelial cells have been identified [17–19], and new insights have emerged regarding the role of epithelial responses to invasion and the corresponding epithelial interactions with the complement-like system to limit ookinete survival [113–117]. There is increasing evidence that the oocyst stage is also a target of innate immunity in the mosquito [118, 119]. Genome-wide association study (GWAS) mapping of *Anopheles* populations displaying different vector competence has identified mosquito genes that influence parasite development [120]. This list of potential targets to disrupt malaria transmission could be extended through functional screens using double-stranded ribonucleic acid (dsRNA)-mediated gene silencing in mosquitoes and synthetic approaches such as single-chain antibodies to block *P. falciparum* from infecting salivary glands.

A particular challenge for developing new interventions is the lack of culture systems to study fertilization, ookinete biology, and parasite–midgut interactions in human malaria parasites. *Plasmodium* species of rodents and birds have provided rapid proof-of-principle for new transmission-blocking strategies [121–123] and will likely continue to be critical for revealing the basic biology of sexual and mosquito stages. The development of mosquito midgut-derived cell lines (or organoids) supporting the in vitro culture of ookinetes and oocyst of human malaria parasites would enable high-throughput transcriptomic and metabolomic studies as well as high-resolution functional analysis of the parasite's surface proteins and their interactions with mosquito cells. These assays could also be used to validate transmission-blocking drugs and vaccines.

Advances, challenges, and opportunities in infection biology

The past 5 years have seen rapid progress in understanding the biology of *Plasmodium* infection in the human liver. Increased availability of primary human hepatocytes has allowed the development of multiple in vitro platforms, all tailored toward the concept of a miniaturized experimental liver model [28, 29, 124]. Importantly, these innovations have allowed the liver stages of infection to be fully recapitulated outside the human host for the first time [26, 125]. They have also spurred the development of reagents to explore the biology of sporozoite infectivity and liver stage development and provided the first glimpse of the *P. vivax* hypnozoite [26, 28].

In parallel, the development of humanized mouse models of *P. vivax* and *P. falciparum* infection have opened up the potential for surrogate in vivo models of human liver infection [26] and allowed the first genetic crosses of parasites (*P. falciparum*) outside of a primate [31]. Studies in primates continue to play an important role; the *P. cynomolgi* monkey model of liver infection is the only in vivo relapse model of the *P. vivax* hypnozoite [30, 32, 126]. Combined with controlled human malaria infections [34, 35, 38, 127, 128] and in vitro models,

these tools have highlighted key differences in the biology of different parasite species (specifically, *P. vivax* and *P. falciparum*) and paved the way for understanding the cellular biology of liver infection and the immune response and for performing high-throughput drug candidate screening.

To facilitate efforts aimed at eradication, we have identified a number of transformative actions in the field of infection biology. A transformative innovation would be the in vitro cultivation of large numbers of infectious *P. falciparum* and *P. vivax* sporozoites, bypassing the mosquito vector. This would not only facilitate basic research but also contribute to whole-parasite vaccine development. Alternatively, advances in the preservation of sporozoite viability and infectivity after mosquito dissection and/or the engineering of mosquitoes to produce sporozoites at high levels would increase the availability and distribution of infectious material for research purposes.

Improved liver-stage cell lines could also have a transformative effect on the pace of novel drug and vaccine development, especially for *P. vivax* [28–30]. Cell lines provide readily available, immortal, and genetically identical cells, allowing researchers to reliably obtain the same sensitivity measurements for each compound or antibody. This development could enable high-throughput drug screening for discovery of liver stage-specific compounds targeting either parasite functions [129] or human targets necessary for parasite development. Moreover, the availability of robust and inexpensive in vitro hepatocyte infection models for *P. vivax* and *P. falciparum* may allow the development of better in vitro assays for antibody-dependent inhibition of invasion (akin to virus neutralization assays) and cell-mediated killing of infected cells. This could allow the discovery of human monoclonal antibodies with broadly neutralizing activity, whose cognate antigens could then be used to create vaccines that give sterilizing immunity. Recent advances in proteomics and mass spectrometry may also support the identification of biomarkers for exoerythrocytic stages that are relevant in vivo.

Advances, challenges, and opportunities in asexual-stage biology

Defining the parasite "resistome"

Notable advances in asexual biology over the past 5 years include improvements in functional genomics, such as more robust RNA sequencing methods [130–132], a deeper understanding of transcription factors such as activator protein 2 (ap2) transcription factors [133] or alternative RNA splicing [134], and whole genome sequencing and genotyping of both field isolates and evolved cultures (see Table 1). Due to its rapidly decreasing cost and increasing accuracy, sequencing has accelerated our understanding of the mechanisms and modes of action of current and new antimalarials through drug-resistant parasite selection in vitro (reviewed in [135]) as well as population genetics of the parasite in vivo [62, 136]. Although numerous studies have described using in vitro evolution and whole genome analysis to both find targets of new antimalarial compounds and identify genes conferring resistance [62, 137, 138], in most cases, only a handful of genes were identified. Now that single cell sequencing is becoming a reality [139], we are in a position to identify every gene (and potentially allele) that contributes to drug resistance, thus defining the parasite "resistome." The complete genetic basis of parasite drug resistance should provide better molecular markers of whether parasites have acquired resistance to drugs that may be used in elimination campaigns, informing drug or drug combination selections (See malERA Refresh paper on resistance [140]).

Systematic characterization of the asexual-stage parasite

The systematic knockout of genes in *P. berghei* has led to numerous advances in our understanding of fundamental asexual biology [141, 142], including the *P. berghei* identification of

essential genes and pathways [143–146], greater understanding of merozoite invasion and egress [147–150], discovery of the parasite's export machinery [145, 151, 152], and revealing how the red cell cytoplasm and membrane are remodelled [153, 154]. Such studies point to the critical nature of these processes and have opened the possibility of targeting them with drugs or vaccines.

Yet, major gaps remain in our knowledge of gene function in *P. falciparum* and, to an even greater extent, in other species (including *P. vivax*, *P. ovale*, and *P. malariae*) in which genetic diversity is also relatively uncharacterized. Although in many cases, genomic variants can be readily identified in sequencing data, poor annotations for predicted genes in the *P. falciparum* genome continue to slow progress. For example, we know little about the cellular function of the *pfkelch13* gene, a major contributor to artemisinin resistance ([62, 155, 156], reviewed in [67]). Given that it is more efficient and inexpensive for the community to work together to functionally annotate the *P. falciparum* genome systematically rather than in a 1-researcher-1-gene fashion, coordinated large-scale projects with a focus on the easily accessible *P. falciparum* asexual blood stage should be considered. Such systematic data would also help in the interpretation of whole genome sequencing data from drug- or vaccine-resistant parasites. Desirable genomic annotations include the location of key transcription factor binding sites, transcriptional start and stops sites [157], epigenetic chromatin modifications, and the cellular localization of encoded proteins. These consortium-acquired data are critical to predict whether genetic variants discovered through genome sequencing of model organisms and humans are indeed functional and could also help prioritize antigens for vaccine development. In addition, if better in vitro culture systems can be developed for *P. vivax* (see "Advances, challenges, and opportunities in transmission biology"), these systematic approaches could be extended to this important species. A potential model for such a consortium-based effort is the human ENCODE project, which has identified functional elements in the human genome [158].

Using metabolomics to identify biomarkers and develop diagnostics

There have been major advances in the use of modern mass spectrometry-based methods for identifying and profiling metabolites from parasite-infected cells [159–161] as well as determining the mode of action of drugs through the metabolic perturbations of exposed parasites [162–165]. Two key areas in which metabolomics-based approaches have yet to make a significant impact are biomarkers and diagnostics. Given the difficulty and cost associated with identifying infected individuals (particularly those who are asymptomatic—see malERA Refresh paper on reservoir and transmission [166]), the development of effective metabolomic biomarkers with significant correlation to infection would represent a critical advance. Furthermore, to determine host markers of infection, field samples across a broad range of infectivities, including asymptomatic carriers, should be studied using metabolomic methods. Such analyses should also aim to span all *Plasmodium* parasite species as well, particularly *P. vivax*.

The question of persistence: Where do parasites—And mosquitoes—Hide?

In the drive towards elimination and eradication, a key question is how and where malaria infection persists in both humans and mosquitoes, both in individuals as well as populations. Recent genomic studies indicate that parasites may also persist in an additional zoonotic reservoir in nonhuman primates [167–169], although how this contributes to disease transmission in humans is currently unclear.

Persistence of malaria occurs in 2 modalities—asymptomatic carriers and latent liver stages. The asymptomatic carriers represent a significant threat to the reintroduction of malaria; thus,

the identification of such carriers requires a heightened level of awareness and detection. The absence of symptoms in an individual may reflect the presence of disease-prevention host responses in the absence of sterilizing immunity, thereby allowing persistent parasitemia or the sequestration of parasites in sites (e.g., the liver or bone marrow) in which they are "hidden" from the immune system. Understanding the relative contributions of both human immune responses and parasite biology will be essential to maximize the efficacy of antimalarial interventions, particularly vaccines.

Parasite persistence in the liver is a major hurdle for elimination efforts, particularly for *P. vivax*, because of its rapid development of gametocytes in humans, enabling transmission before the onset of clinical symptoms. Insights have emerged from studies of nonhuman primate models and humanized mouse models [26] in which parasite forms resembling hypnozoites demonstrated some biologic activity. These findings imply that sensitive technologies, such as proteomics and metabolomics, may identify markers likely secreted at these stages. Such markers would require field validation but ultimately could be incorporated into point-of-care diagnostics, eliminating the need for primaquine or tafenoquine in mass drug administration campaigns and informing epidemiological studies of the load of hypnozoite infection in endemic regions.

The transmission of *Plasmodium* infections with low or submicroscopic levels of circulating gametocytes suggests the possibility of nonrandom sequestration of gametocytes at sites in peripheral skin that are accessible to mosquitoes. *P. falciparum* gametocytes have recently been found to have an extended maturation period in the bone marrow [103, 170]. A clear implication of this observation, however, is that gametocytes detected in the peripheral circulation may not accurately reflect overall or infectious gametocyte levels and that more sensitive assays are needed to identify potential sources of transmission.

Mosquito vector persistence

The aspects of vector biology that enable malaria persistence remain to be investigated and will be critical not only for informing and targeting current elimination and eradication strategies but also for the development and successful deployment of novel vector-based interventions. Recent data suggest that, in Africa, both mosquito estivation (dry season diapause) and long-distance migration contribute to the persistence of sub-Sahelian mosquito populations following a dry season, but in a species-specific manner [70]. New genomic resources have facilitated the understanding of fine-scale mosquito population structures [77, 171] suggesting large and stable populations [74–76]. The contribution of the observed genomic patterns to population persistence is unclear at this point, and a better understanding of the life history, ecology, and migration rates of vectors that result in the observed genomic patterns between populations is needed. Similar studies in non-African mosquito populations are needed.

Mosquitoes also persist through physiological resistance to insecticides (see malERA Refresh paper on resistance [140]), either through target site mutations, increased expression of detoxifying enzymes, or cuticular thickening. Genomic markers associated with resistance continue to be identified, yet together they do not adequately explain all the variation in insecticide resistance phenotypes observed in natural populations, and their relative functional impact in the field remains poorly understood.

Mosquito persistence may also occur due to heritable changes in behavior selected for by control interventions, so-called behavioral resistance. Recent work has captured mosquito interactions with bednets using mosquito-tracking cameras [172] and could be extended to other interventions (e.g., traps, sprays, repellents). Consistent longitudinal studies are also needed to track changes in mosquito biting behavior (e.g., outdoor versus indoor, evening

versus night) after the use of interventions and to discriminate these changes from variation in species frequencies at specific sites. Subsequent genomic analyses could then reveal if there is a genetic component to these modified behaviors.

Technology and its application to malaria biology

Fundamental technologies: Genomics and transcriptomics

Whole genome sequencing has already had a major impact on multiple areas of parasite and vector research. It has transformed our understanding of parasite biology and drug resistance (see "Advances, challenges, and opportunities in asexual-stage biology"). In addition, it has been widely used to study the population genetics of mosquito species in the field [74–76, 173], and the genomes of 19 *Anopheles* species spanning 3 subgenera and including major and minor malaria vectors from diverse geographical locations have now been sequenced [77, 171]. These genomic resources have improved our understanding of the patterns of gene flow within and among mosquito populations. These "big data" resources available to the research community allow for powerful comparative functional and evolutionary analyses that will help elucidate the common basis of vector competence and identify effective vector control targets across multiple species. Recent work using these datasets has identified a reproductive trait with consequences for vectoral capacity that has evolved within the *Anopheles* genus and presents new potential targets to induce sterility in field populations [174–176]. Additional targets may be identified as our understanding of the biological coordination of simultaneous egg development and parasite transmission is improved. The declining cost of sequencing will make such studies more feasible in the future, such that a mosquito resistome—similar to the parasite resistome—may be compiled.

Further advances in genomic technology will enable a detailed analysis of natural populations of *Plasmodium spp*. at a worldwide scale. These include single cell technologies for genome sequencing and transcriptomic analyses, genotyping, and whole genome sequencing from dried blood spot samples. In addition, further comparative genomics [177] among all *Plasmodium* species infecting humans as well as those infecting nonhuman primates should identify key pathways in host switching. Genomic analysis of longitudinal samples will allow for the identification of population structure changes associated with changing epidemiology and emerging drug resistance. Coupled with gene-editing technologies, hypotheses generated by comparative genomics can be functionally tested.

Technical advances in RNA sequencing now make it feasible to interrogate the dynamic gene expression profiles of both the human host and the parasite during infection. This will provide new insights into the host response during infection and the potential adaptation of parasites during the infective process.

Gene-manipulation technologies: Genome editing and transgenics

Genome engineering tools, such as CRISPR/Cas9 systems (see glossary in the malERA Refresh Introductory paper [178]), have transformed the ability to manipulate the genomes of *P. falciparum*, *P. berghei* (reviewed in [179]), and *Anopheles* and understand gene function. CRISPR/Cas9-based genetic engineering of *P. falciparum* asexual blood stages has allowed for more complex genetic modifications within the parasite; for example, the tetracycline repressor protein (TetR) aptamer system to control gene expression [84] utilized CRISPR/Cas9 as an initial step to introduce the aptameric cassette. Beyond CRISPR/Cas9, however, there have been several other successful gene-editing technologies (see Table 1).

With these powerful tools in place, we can now scale up the generation of conditional and/or complete knockout parasite libraries containing every single gene in the genome. Such an

effort would greatly enhance our understanding of the biology of the parasite at all stages of development, as well as identify the functions of many hypothetical genes.

Gene-manipulation technologies: Gene drives

Mirroring the advances in gene-editing capabilities in the parasite, *Anopheline spp*. genomes can also now be engineered with unprecedented precision (see Table 1). Recent reports show that CRISPR/Cas9 gene-editing tools can be used for the generation of gene-drive systems [91, 92] that manipulate genetic inheritance in mosquitoes to spread anti-*Plasmodium* transgenes (population modification/replacement strategies) or lethality-inducing transgenes (population suppression strategies) through natural mosquito populations. Mendelian inheritance predicts 50% of offspring will inherit a transgene carried on one of a parent's chromosomes. Genetic drive is the increased transmission of a genetic element to over 50% of offspring so that it increases in frequency in each generation. A gene drive typically refers to an artificial transgene that shows genetic drive by giving it the ability to trigger its own replication. A gene-drive transgene is copied from one chromosome to its homologous chromosome within germ line cells. With both chromosomes carrying a copy of the transgene (a homozygous germ line), all sperm or eggs derived from these cells will also carry the transgene, and if copying occurs in all germ cells, 100% of offspring will inherit the gene drive. This allows rapid spread of the gene drive (and its anti-*Plasmodium* cargo) into the mosquito population. A valuable debate on the safe use of gene drive systems has begun within the scientific community [180].

The feasibility of using gene drive strategies for mosquito control will need additional research efforts in 3 key areas. First, an understanding of mosquito mating biology and the determinants of male mating success and female mate choice will need to be developed. Colonization is likely to impact the mating ability of species that exhibit such a complicated mating behavior as swarming; mating competitiveness will be a key determinant of gene drive success. Second, effective, "evolution proof" gene-drive systems should be generated to preempt the selection of mosquitoes that are resistant to the drive mechanisms, which would otherwise reduce the efficiency of the drive. Gene drives will need to be optimized by testing different gene-drive architectures, especially if CRISPR/Cas9 mechanisms prove problematic. Third, effective antimalarial genes will need to be evaluated in a reliable and reproducible manner; many anti-*Plasmodium* factors have been identified and should be systematically tested in laboratory conditions for their ability to block parasite development within the mosquito host.

Consideration should be given to the formation of a consortium to evaluate and prioritize promising transgenic strategies and test these in multiple anopheline species and against a number of *Plasmodium* isolates. This represents an opportunity to avoid duplication of work; however, we would also argue for head-to-head comparison of transgenic strategies. Such a consortium could centralize resources, particularly in developing transgenic mosquitoes (e.g., injection service, mail-order mutants) and potentially a mutant library, but, currently, the space required for mosquito-line maintenance prevents this. As forward and reverse genetic screens become more realistic, we should develop methods to cryopreserve mosquito lines or, more realistically, store plasmids for injection to recreate lines as needed.

Cell- and tissue-based technologies

Since the discovery of malaria parasites by microscopy [181], imaging has played a central role in malaria research. However, recent advances in imaging techniques have allowed visualization of the parasite and its interactions with the mammalian host and insect vector at an unprecedented level of resolution [101] [182] [102]. We can expect that imaging will reveal

other novel insights into the biology of human malaria parasites and play a major role in the science of malaria eradication.

New technologies to support tool development: Biomarkers and novel diagnostics

As our understanding of parasite biology advances—including insights into sequestration and dormancy—the potential to leverage emerging technologies to support the discovery of biomarkers of infection (see above) increases. Such insights into parasite biology are laying the foundation for novel diagnostic approaches based on more sensitive techniques to detect parasite byproducts (e.g., hemozoin) [183] or volatile substances [184]. When noninvasive, rapid, and inexpensive, these diagnostic approaches are likely to facilitate the identification of infected individuals who may be asymptomatic and/or functioning as reservoirs (see malERA Refresh papers on Tools [185] and the Reservoir and Transmission [166]).

Exosomes are key new players implicated in intercellular communication without direct cellular contact [186] and have a potential role as biomarkers [187]. The release of microparticles is augmented in human malaria [188, 189], and exosomes containing parasite proteins have been shown to be produced by infected cells [190] as well as by parasites [191, 192].

New technologies in vaccine development and leveraging existing human volunteer sample datasets

Protective immunity requires that human hosts recognize and respond appropriately to parasite-derived antigens and epitopes. Such immunity is complex, however, requiring both innate and acquired responses and biological regulation of such responses as well as ensuring the responses' durability. Malaria parasites utilize a number of mechanisms to evade these immune responses, which infected hosts must then overcome. In this context, there is a fundamental gap in understanding the correlates of protective immunity in the human host that target exoerythrocytic-stage parasites in both *P. falciparum* and *P. vivax*. Multiple new technologies are now available to identify antigens and epitopes that are the targets of innate and acquired immune responses. Examples include high-throughput genomic sequencing, transcriptomics, and proteomics. Structural vaccinology [193–195] has proven immensely powerful in viral vaccine development through improved immunogen design and is now being applied to asexual blood stages [94–97]. Near-atomic resolution cryo-electron microscopy is now being used to inform antigen and drug target selection as well as the rational design of potent immunogens [196–198]. In addition, new technology platforms and novel adjuvants are being incorporated into vaccines to ensure appropriate immune responses are elicited. Approaches based on structural biology [98–100] and genomic sequencing [199] are now being introduced into the clinical evaluation of candidate malaria vaccines. These efforts provide an opportunity to further define the effective targets as well as the nature of protective immune responses.

An effective *P. vivax* vaccine strategy also needs to contend with the challenge of relapse infections. To prevent "relapse outbreaks," antirelapse vaccines will need to be multistage and multivalent, including components to suppress blood-stage parasites emerging from the dormant liver stages as well as block transmission. There are relatively few *P. vivax* vaccine candidates progressing currently through the global pipeline [200].

Controlled human challenge studies are potentially transformational in enabling our understanding of the human immune response to malaria. Coupling controlled infections with technical advances for interrogating human immune cells in real time can give us new insights into both the temporal response and the contributions from innate and acquired immunity. Additionally, deeper interrogation of the immune profile of naturally acquired

infections could also provide key insights. Providing access to them will require forethought in preparing future proposals, particularly with respect to human subject approvals, repository deposition, and community sharing. Harnessing available systems through existing networks as well as ongoing clinical trials could provide the necessary reagents and access to human samples.

Drug design and screening

The identification of potential targets through metabolomics and systems biology approaches coupled with advances in structural biology is now facilitating the design of compounds likely to interact with such targets. Moreover, high-throughput screening technologies are facilitating more rapid identification and prioritization of compounds for further investigation as potential leads, though corresponding techniques in high-throughput synthesis and characterization of small molecules require further development. In a reverse approach, high-throughput phenotypic screens are also enabling the selection of compounds whose structures can subsequently be used to inform the identification of potential molecular interactions and metabolic pathways for further analysis as targets for pharmacologic intervention (reviewed in [201]). It is important to note that because malaria primarily affects the developing world, the opportunity for profit is reduced. Malaria, with the assistance of the community and funders such as Medicines for Malaria Venture (MMV), has and will continue to function as a model for open source drug discovery [202–204].

Technologies targeting mosquito-based interventions: Paratransgenesis and genetically modified mosquitoes

Recent years have seen a focus toward the identification of microbial populations that can block parasite development in the mosquito vector [205–208]. Genetic modification of these bacterial populations (paratransgenesis) could be a key tool, particularly for the control of outdoor biting and resting mosquito populations that are not currently targeted by insecticide-based strategies. Advances in *Wolbachia* bacteria experiments in *Anopheles* mosquitoes are particularly promising. *Wolbachia* are intracellular endosymbiotic bacteria that, in some insects, spread through populations by maternal transmission and cytoplasmic incompatibility. These endosymbionts were shown to block malaria parasite development in artificial settings [209] and were negatively correlated with *Plasmodium* infections in natural *A*. *coluzzii* populations from Burkina Faso [210, 211]. Two key research priorities are the development of a method to transform *Wolbachia* to deliver effective antiplasmodial genes and understanding the role of natural *Wolbachia* infections in malaria transmission dynamics.

In light of widespread resistance to currently used insecticides, the identification of alternative, safe, active compounds that can extend the lifetime of long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) is imperative. The study of key pathways in mosquito reproduction, susceptibility to infection, blood feeding behavior, and longevity that can be effectively targeted to reduce vectoral capacity is therefore a priority. For example, new sterilizing compounds that interfere with key hormonal reproductive pathways, such as those regulated by juvenile hormone and 20-hydroxyecdysone, could be incorporated into mosquito nets to reduce mosquito fertility, including insecticide-resistant mosquitoes that may survive exposure to the net.

A key issue in applying these novel strategies will be achieving effective colonization of anopheline species, as the lack of mosquito colonies is preventing studies on the biology of important malaria vectors. An important breakthrough has been the recent colonization of *A*. *darlingi*, the most important American vector [93]. On the road to eradication, a deeper understanding of the biology and behavior of these species will be essential.

Conclusions

As illustrated above, recent advances in basic science are providing deeper insights into the biology of the parasite, the mosquito vector, and the human host as well as their interactions at molecular, cellular, and organismic levels. Coupling these insights with recent technologies that help pinpoint potential methods to intervene or disrupt essential interactions can spur the use of novel tools to help eliminate and, ultimately, eradicate malaria.

Acknowledgments

The Malaria Eradication Research Agenda (malERA) Refresh Consultative Panel on Basic Science and Enabling Technologies was chaired by Dyann F. Wirth, Harvard T.H. Chan School of Public Health, United States of America, and cochaired by Elizabeth A. Winzeler, University of California San Diego, United States of America, and B. Fenton ("Lee") Hall, National Institute of Allergy and Infectious Diseases at the National Institutes of Health, United States of America. The paper was written based on consultations during a malERA Refresh meeting held in Boston, United States of America, December 7–9, 2015, plus additional literature searches.

Members of the writing group who met the ICMJE criteria for authorship

Dyann F. Wirth (chair), Harvard T.H. Chan School of Public Health, United States of America; Elizabeth A. Winzeler (cochair), University of California San Diego, United States of America; B. Fenton ("Lee") Hall, National Institute of Allergy and Infectious Diseases at the National Institutes of Health, United States of America (cochair); Selina E. Bopp, Harvard T.H. Chan School of Public Health, United States of America; Gregory LaMonte, University of California San Diego, United States of America; N. Regina Rabinovich, Harvard T.H. School of Public Health, United States of America and ISGlobal Barcelona Institute for Global Health, Spain; W. Robert Shaw, Harvard T.H. Chan School of Public Health, United States of America.

Members of the panel

Dyann F. Wirth (chair), Harvard T.H. Chan School of Public Health, United States of America; Elizabeth A. Winzeler (cochair), University of California San Diego, United States of America; B. Fenton ("Lee") Hall (cochair), National Institutes of Health, United States of America; John H. Adams, University of South Florida, United States of America; Frédéric Ariey, Institut Cochin, Université Paris Descartes Sorbonne, France; Carolina V. Barillas-Mury, National Institutes of Health, United States of America; Jake Baum, Imperial College London, United Kingdom; Sangeeta N. Bhatia, Massachusetts Institute of Technology, United States of America; Oliver Billker, Wellcome Trust Sanger Institute, United Kingdom; Selina E. Bopp, Harvard T.H. Chan School of Public Health, United States of America; Flaminia Catteruccia, Harvard T.H. Chan School of Public Health, United States of America; Alan F. Cowman, The Walter and Eliza Hall Institute of Medical Research, Australia; Chetan E. Chitnis, Institut Pasteur, France; Brendan S. Crabb, Burnet Institute, Australia; Kirk W. Deitsch, Weill Cornell Medical College, United States of America; Hernando A. Del Portillo, Barcelona Institute of Global Health, Spain; Abdoulaye A. Djimde, University of Bamako, Mali; Carlota Dobaño, Barcelona Institute of Global Health, Spain; Patrick E. Duffy, National Institutes of Health, United States of America; Manoj T. Duraisingh, Harvard T.H. Chan School of Public Health, United States of America; Christian Happi, College of Natural Sciences and African Center of Excellence for Genomics of Infectious Diseases, Redeemer's University, Nigeria; Anthony A. James, University of California, United States of America; Gregory LaMonte, University of California San Diego, United States of America; Amanda K. Lukens, Broad Institute of MIT, United States of America; Manuel Llinas, Pennsylvania State University, United States of America; Prashant Mali, University of California San Diego, United States of America;

Matthias Marti, University of Glasgow, Glasgow, United Kingdom; Jesús Martinez-Barnetche, National Institute of Public Health, Mexico; Victoria McGovern, Burroughs Wellcome Fund, United States of America; Maria M. Mota, Instituto de Medicina Molecular, Portugal; Ivo Mueller, The Walter and Eliza Hall Institute of Medical Research, Australia and Institut Pasteur, France; Daouda Ndiaye, Cheikh Anta Diop University, Sénégal; Daniel E. Neafsey, Broad Institute of MIT and Harvard, United States of America; Francine Ntoumi, University of Tubingen, Germany; Jetsumon Prachumsri, Mahidol University, Thailand; Pushkar Sharma, National Institute of Immunology, India; W. Robert Shaw, Harvard T.H. Chan School of Public Health, United States of America; Photini Sinnis, Johns Hopkins Bloomberg School of Public Health, United States of America; Niraj H. Tolia, Washington University, United States of America; Sarah K. Volkman, Harvard T.H. Chan School of Public Health, United States of America; Edward A. Wenger, Institute for Disease Modeling, United States of America; Kim Williamson, Uniformed Services University of the Health Sciences, United States of America.

References

1. The malERA Consultative Group on Basic Science and Enabling Technologies. A Research Agenda for Malaria Eradication: Basic Science and Enabling Technologies. PLOS Medicine. 2011; 8(1): e1000399. https://doi.org/10.1371/journal.pmed.1000399 PMID: 21311584
2. Eksi S, Morahan BJ, Haile Y, Furuya T, Jiang H, Ali O, et al. Plasmodium falciparum gametocyte development 1 (Pfgdv1) and gametocytogenesis early gene identification and commitment to sexual development. PLoS pathogens. 2012; 8(10):e1002964. Epub 2012/10/25. https://doi.org/10.1371/journal.ppat.1002964 PPATHOGENS-D-12-01039 [pii]. PMID: 23093935; PubMed Central PMCID: PMC3475683.
3. Ikadai H, Shaw Saliba K, Kanzok SM, McLean KJ, Tanaka TQ, Cao J, et al. Transposon mutagenesis identifies genes essential for Plasmodium falciparum gametocytogenesis. Proc Natl Acad Sci U S A. 2013; 110(18):E1676–84. Epub 2013/04/11. https://doi.org/10.1073/pnas.1217712110 1217712110 [pii]. PMID: 23572579; PubMed Central PMCID: PMC3645567.
4. Brancucci NM, Bertschi NL, Zhu L, Niederwieser I, Chin WH, Wampfler R, et al. Heterochromatin protein 1 secures survival and transmission of malaria parasites. Cell host & microbe. 2014; 16(2):165–76. Epub 2014/08/15. https://doi.org/10.1016/j.chom.2014.07.004 S1931-3128(14)00258-3 [pii]. PMID: 25121746.
5. Kafsack BF, Rovira-Graells N, Clark TG, Bancells C, Crowley VM, Campino SG, et al. A transcriptional switch underlies commitment to sexual development in malaria parasites. Nature. 2014; 507 (7491):248–52. Epub 2014/02/28. https://doi.org/10.1038/nature12920 nature12920 [pii]. PMID: 24572369; PubMed Central PMCID: PMC4040541.
6. Sinha A, Hughes KR, Modrzynska KK, Otto TD, Pfander C, Dickens NJ, et al. A cascade of DNA-binding proteins for sexual commitment and development in Plasmodium. Nature. 2014; 507(7491):253–7. Epub 2014/02/28. https://doi.org/10.1038/nature12970 nature12970 [pii]. PMID: 24572359; PubMed Central PMCID: PMC4105895.
7. Duffy S, Avery VM. Identification of inhibitors of Plasmodium falciparum gametocyte development. Malaria journal. 2013; 12:408. Epub 2013/11/12. https://doi.org/10.1186/1475-2875-12-408 [pii]. PMID: 24206914; PubMed Central PMCID: PMC3842684.
8. Brancucci NM, Goldowitz I, Buchholz K, Werling K, Marti M. An assay to probe Plasmodium falciparum growth, transmission stage formation and early gametocyte development. Nature protocols. 2015; 10 (8):1131–42. https://doi.org/10.1038/nprot.2015.072 PMID: 26134953; PubMed Central PMCID: PMC4581880.
9. Duffy S, Loganathan S, Holleran JP, Avery VM. Large-scale production of Plasmodium falciparum gametocytes for malaria drug discovery. Nature protocols. 2016; 11(5):976–92. https://doi.org/10.1038/nprot.2016.056 PMID: 27123949.
10. Lucantoni L, Fidock DA, Avery VM. Luciferase-Based, High-Throughput Assay for Screening and Profiling Transmission-Blocking Compounds against Plasmodium falciparum Gametocytes. Antimicrobial agents and chemotherapy. 2016; 60(4):2097–107. https://doi.org/10.1128/AAC.01949-15 PMID: 26787698; PubMed Central PMCID: PMC4808229.
11. Plouffe DM, Wree M, Du AY, Meister S, Li F, Patra K, et al. High-Throughput Assay and Discovery of Small Molecules that Interrupt Malaria Transmission. Cell host & microbe. 2016; 19(1):114–26. https://doi.org/10.1016/j.chom.2015.12.001 PMID: 26749441; PubMed Central PMCID: PMC4723716.

12. Carey AF, Wang G, Su CY, Zwiebel LJ, Carlson JR. Odorant reception in the malaria mosquito Anopheles gambiae. Nature. 2010; 464(7285):66–71. Epub 2010/02/05. nature08834 [pii] https://doi.org/10.1038/nature08834 PMID: 20130575; PubMed Central PMCID: PMC2833235.

13. Wang G, Carey AF, Carlson JR, Zwiebel LJ. Molecular basis of odor coding in the malaria vector mosquito Anopheles gambiae. Proc Natl Acad Sci U S A. 2010; 107(9):4418–23. Epub 2010/02/18. https://doi.org/10.1073/pnas.0913392107 0913392107 [pii]. PMID: 20160092; PubMed Central PMCID: PMC2840125.

14. Rinker DC, Pitts RJ, Zhou X, Suh E, Rokas A, Zwiebel LJ. Blood meal-induced changes to antennal transcriptome profiles reveal shifts in odor sensitivities in Anopheles gambiae. Proc Natl Acad Sci U S A. 2013; 110(20):8260–5. https://doi.org/10.1073/pnas.1302562110 PMID: 23630291; PubMed Central PMCID: PMCPMC3657813.

15. Pellegrino M, Nakagawa T, Vosshall LB. Single sensillum recordings in the insects Drosophila melanogaster and Anopheles gambiae. J Vis Exp. 2010;(36):1–5. Epub 2010/02/19. https://doi.org/10.3791/1725 1725 [pii]. PMID: 20164822; PubMed Central PMCID: PMC2830253.

16. Tauxe GM, MacWilliam D, Boyle SM, Guda T, Ray A. Targeting a dual detector of skin and CO2 to modify mosquito host seeking. Cell. 2013; 155(6):1365–79. Epub 2013/12/10. https://doi.org/10.1016/j.cell.2013.11.013 S0092-8674(13)01426-8 [pii]. PMID: 24315103; PubMed Central PMCID: PMC3899525.

17. Molina-Cruz A, DeJong RJ, Ortega C, Haile A, Abban E, Rodrigues J, et al. Some strains of Plasmodium falciparum, a human malaria parasite, evade the complement-like system of Anopheles gambiae mosquitoes. Proc Natl Acad Sci U S A. 2012; 109(28):E1957–62. https://doi.org/10.1073/pnas.1121183109 PMID: 22623529; PubMed Central PMCID: PMC3396512.

18. Ghosh AK, Devenport M, Jethwaney D, Kalume DE, Pandey A, Anderson VE, et al. Malaria parasite invasion of the mosquito salivary gland requires interaction between the Plasmodium TRAP and the Anopheles saglin proteins. PLoS pathogens. 2009; 5(1):e1000265. https://doi.org/10.1371/journal.ppat.1000265 PMID: 19148273; PubMed Central PMCID: PMC2613030.

19. Ghosh AK, Coppens I, Gardsvoll H, Ploug M, Jacobs-Lorena M. Plasmodium ookinetes coopt mammalian plasminogen to invade the mosquito midgut. Proc Natl Acad Sci U S A. 2011; 108(41):17153–8. https://doi.org/10.1073/pnas.1103657108 PMID: 21949403; PubMed Central PMCID: PMC3193258.

20. Vega-Rodriguez J, Ghosh AK, Kanzok SM, Dinglasan RR, Wang S, Bongio NJ, et al. Multiple pathways for Plasmodium ookinete invasion of the mosquito midgut. Proc Natl Acad Sci U S A. 2014; 111(4):E492–500. Epub 2014/01/30. https://doi.org/10.1073/pnas.1315517111 1315517111 [pii]. PMID: 24474798; PubMed Central PMCID: PMC3910608.

21. Bounkeua V, Li F, Chuquiyauri R, Abeles SR, McClean CM, Neyra V, et al. Lack of molecular correlates of Plasmodium vivax ookinete development. Am J Trop Med Hyg. 2011; 85(2):207–13. Epub 2011/08/05. https://doi.org/10.4269/ajtmh.2011.10-0729 PMID: 21813836; PubMed Central PMCID: PMC3144814.

22. Bounkeua V, Li F, Vinetz JM. In vitro generation of Plasmodium falciparum ookinetes. Am J Trop Med Hyg. 2010; 83(6):1187–94. Epub 2010/12/02. https://doi.org/10.4269/ajtmh.2010.10-0433 PMID: 21118920; PubMed Central PMCID: PMC2990030.

23. Delves MJ, Sinden RE. A semi-automated method for counting fluorescent malaria oocysts increases the throughput of transmission blocking studies. Malaria journal. 2010; 9:35. Epub 2010/02/02. https://doi.org/10.1186/1475-2875-9-35 PMID: 20113492; PubMed Central PMCID: PMC2824803.

24. Ghosh AK, Dinglasan RR, Ikadai H, Jacobs-Lorena M. An improved method for the in vitro differentiation of Plasmodium falciparum gametocytes into ookinetes. Malaria journal. 2010; 9 (1)(194). http://dx.doi.org/10.1186/1475-2875-9-194. PMID: 2010408089.

25. Ghosh AK, Jacobs-Lorena M. In Vitro Differentiation of Plasmodium falciparum Gametocytes into Ookinetes. Methods in molecular biology. 2013; 923:27–33. Epub 2012/09/20. https://doi.org/10.1007/978-1-62703-026-7_3 PMID: 22990769.

26. Mikolajczak SA, Vaughan AM, Kangwanrangsan N, Roobsoong W, Fishbaugher M, Yimamnuaychok N, et al. Plasmodium vivax liver stage development and hypnozoite persistence in human liver-chimeric mice. Cell host & microbe. 2015; 17(4):526–35. https://doi.org/10.1016/j.chom.2015.02.011 PMID: 25800544.

27. Soulard V, Bosson-Vanga H, Lorthiois A, Roucher C, Franetich JF, Zanghi G, et al. Plasmodium falciparum full life cycle and Plasmodium ovale liver stages in humanized mice. Nat Commun. 2015; 6:7690. Epub 2015/07/25. https://doi.org/10.1038/ncomms8690 PMID: 26205537; PubMed Central PMCID: PMCPMC4525212.

28. March S, Ng S, Velmurugan S, Galstian A, Shan J, Logan DJ, et al. A microscale human liver platform that supports the hepatic stages of Plasmodium falciparum and vivax. Cell host & microbe. 2013; 14

(1):104–15. Epub 2013/07/23. https://doi.org/10.1016/j.chom.2013.06.005 PMID: 23870318; PubMed Central PMCID: PMC3780791.

29. Ng S, Schwartz RE, March S, Galstian A, Gural N, Shan J, et al. Human iPSC-derived hepatocyte-like cells support plasmodium liver-stage infection in vitro. Stem Cell Reports. 2015; 4 (3):348–59. http://dx.doi.org/10.1016/j.stemcr.2015.01.002. https://doi.org/10.1016/j.stemcr.2015.01.002 PMID: 25660406.

30. Dembele L, Franetich JF, Lorthiois A, Gego A, Zeeman AM, Kocken CH, et al. Persistence and activation of malaria hypnozoites in long-term primary hepatocyte cultures. Nat Med. 2014; 20(3):307–12. Epub 2014/02/11. https://doi.org/10.1038/nm.3461 PMID: 24509527.

31. Vaughan AM, Pinapati RS, Cheeseman IH, Camargo N, Fishbaugher M, Checkley LA, et al. Plasmodium falciparum genetic crosses in a humanized mouse model. Nature methods. 2015; 12(7):631–3. Epub 2015/06/02. https://doi.org/10.1038/nmeth.3432 PMID: 26030447; PubMed Central PMCID: PMC4547688.

32. Voorberg-van der Wel A, Zeeman AM, van Amsterdam SM, van den Berg A, Klooster EJ, Iwanaga S, et al. Transgenic fluorescent Plasmodium cynomolgi liver stages enable live imaging and purification of Malaria hypnozoite-forms. PloS one. 2013; 8(1):e54888. https://doi.org/10.1371/journal.pone.0054888 PMID: 23359816; PubMed Central PMCID: PMC3554669.

33. Herrera S, Solarte Y, Jordan-Villegas A, Echavarria JF, Rocha L, Palacios R, et al. Consistent safety and infectivity in sporozoite challenge model of Plasmodium vivax in malaria-naive human volunteers. Am J Trop Med Hyg. 2011; 84(2 Suppl):4–11. Epub 2011/02/15. https://doi.org/10.4269/ajtmh.2011.09-0498 84/2_Suppl/4 [pii]. PMID: 21292872; PubMed Central PMCID: PMC3032484.

34. Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science. 2013; 341 (6152):1359–65. Epub 2013/08/10. https://doi.org/10.1126/science.1241800 science.1241800 [pii]. PMID: 23929949.

35. Sheehy SH, Spencer AJ, Douglas AD, Sim BK, Longley RJ, Edwards NJ, et al. Optimising Controlled Human Malaria Infection Studies Using Cryopreserved P. falciparum Parasites Administered by Needle and Syringe. PloS one. 2013; 8(6):e65960. Epub 2013/07/05. https://doi.org/10.1371/journal.pone.0065960 PONE-D-12-35789 [pii]. PMID: 23823332; PubMed Central PMCID: PMC3688861.

36. Behet MC, Foquet L, van Gemert GJ, Bijker EM, Meuleman P, Leroux-Roels G, et al. Sporozoite immunization of human volunteers under chemoprophylaxis induces functional antibodies against pre-erythrocytic stages of Plasmodium falciparum. Malaria journal. 2014; 13:136. Epub 2014/04/09. https://doi.org/10.1186/1475-2875-13-136 1475-2875-13-136 [pii]. PMID: 24708526; PubMed Central PMCID: PMC4113136.

37. Talley AK, Healy SA, Finney OC, Murphy SC, Kublin J, Salas CJ, et al. Safety and comparability of controlled human Plasmodium falciparum infection by mosquito bite in malaria-naive subjects at a new facility for sporozoite challenge. PloS one. 2014; 9(11):e109654. Epub 2014/11/19. https://doi.org/10.1371/journal.pone.0109654 PONE-D-14-30972 [pii]. PMID: 25405724; PubMed Central PMCID: PMC4236046.

38. Gomez-Perez GP, Legarda A, Munoz J, Sim BK, Ballester MR, Dobano C, et al. Controlled human malaria infection by intramuscular and direct venous inoculation of cryopreserved Plasmodium falciparum sporozoites in malaria-naive volunteers: effect of injection volume and dose on infectivity rates. Malaria journal. 2015; 14:306. Epub 2015/08/08. https://doi.org/10.1186/s12936-015-0817-x 10.1186/s12936-015-0817-x [pii]. PMID: 26245196; PubMed Central PMCID: PMC4527105.

39. Ockenhouse CF, Regules J, Tosh D, Cowden J, Kathcart A, Cummings J, et al. Ad35.CS.01-RTS,S/AS01 Heterologous Prime Boost Vaccine Efficacy against Sporozoite Challenge in Healthy Malaria-Naive Adults. PloS one. 2015; 10(7):e0131571. Epub 2015/07/07. https://doi.org/10.1371/journal.pone.0131571 PONE-D-14-29774 [pii]. PMID: 26148007; PubMed Central PMCID: PMC4492580.

40. Schats R, Bijker EM, van Gemert GJ, Graumans W, van de Vegte-Bolmer M, van Lieshout L, et al. Heterologous Protection against Malaria after Immunization with Plasmodium falciparum Sporozoites. PloS one. 2015; 10(5):e0124243. Epub 2015/05/02. https://doi.org/10.1371/journal.pone.0124243 PONE-D-14-43731 [pii]. 25933168; PubMed Central PMCID: PMC4416703. PMID: 25933168

41. Spring M, Polhemus M, Ockenhouse C. Controlled human malaria infection. The Journal of infectious diseases. 2014; 209 Suppl 2:S40–5. Epub 2014/05/30. https://doi.org/10.1093/infdis/jiu063 jiu063 [pii]. PMID: 24872394.

42. Russell B, Suwanarusk R, Borlon C, Costa FT, Chu CS, Rijken MJ, et al. A reliable ex vivo invasion assay of human reticulocytes by Plasmodium vivax. Blood. 2011; 118(13):e74–81. https://doi.org/10.1182/blood-2011-04-348748 PMID: 21768300; PubMed Central PMCID: PMC3438884.

43. Russell B, Suwanarusk R, Malleret B, Costa FT, Snounou G, Kevin Baird J, et al. Human ex vivo studies on asexual Plasmodium vivax: the best way forward. International journal for parasitology. 2012; 42(12):1063–70. https://doi.org/10.1016/j.ijpara.2012.08.010 PMID: 23032102.

44. Borlon C, Russell B, Sriprawat K, Suwanarusk R, Erhart A, Renia L, et al. Cryopreserved Plasmodium vivax and cord blood reticulocytes can be used for invasion and short term culture. International journal for parasitology. 2012; 42(2):155–60. https://doi.org/10.1016/j.ijpara.2011.10.011 PMID: 22240310; PubMed Central PMCID: PMC3438882.

45. Noulin F, Borlon C, van den Eede P, Boel L, Verfaillie CM, D'Alessandro U, et al. Cryopreserved reticulocytes derived from hematopoietic stem cells can be invaded by cryopreserved Plasmodium vivax isolates. PloS one. 2012; 7(7):e40798. https://doi.org/10.1371/journal.pone.0040798 PMID: 22844411; PubMed Central PMCID: PMC3402485.

46. Tantular IS, Pusarawati S, Khin L, Kanbe T, Kimura M, Kido Y, et al. Preservation of wild isolates of human malaria parasites in wet ice and adaptation efficacy to in vitro culture. Tropical medicine and health. 2012; 40(2):37–45. https://doi.org/10.2149/tmh.2012-07o PMID: 23097618; PubMed Central PMCID: PMC3475313.

47. Noulin F, Borlon C, Van Den Abbeele J, D'Alessandro U, Erhart A. 1912–2012: a century of research on Plasmodium vivax in vitro culture. Trends in parasitology. 2013; 29(6):286–94. https://doi.org/10.1016/j.pt.2013.03.012 PMID: 23623759.

48. Galinski MR, Meyer EV, Barnwell JW. Plasmodium vivax: modern strategies to study a persistent parasite's life cycle. Advances in parasitology. 2013; 81:1–26. https://doi.org/10.1016/B978-0-12-407826-0.00001-1 PMID: 23384620.

49. Kurita R, Suda N, Sudo K, Miharada K, Hiroyama T, Miyoshi H, et al. Establishment of immortalized human erythroid progenitor cell lines able to produce enucleated red blood cells. PloS one. 2013; 8(3): e59890. https://doi.org/10.1371/journal.pone.0059890 PMID: 23533656; PubMed Central PMCID: PMC3606290.

50. Martin-Jaular L, Elizalde-Torrent A, Thomson-Luque R, Ferrer M, Segovia JC, Herreros-Aviles E, et al. Reticulocyte-prone malaria parasites predominantly invade CD71hi immature cells: implications for the development of an in vitro culture for Plasmodium vivax. Malaria journal. 2013; 12:434. https://doi.org/10.1186/1475-2875-12-434 PMID: 24289105; PubMed Central PMCID: PMC4220676.

51. Zeeman AM, der Wel AV, Kocken CH. Ex vivo culture of Plasmodium vivax and Plasmodium cynomolgi and in vitro culture of Plasmodium knowlesi blood stages. Methods in molecular biology. 2013; 923:35–49. https://doi.org/10.1007/978-1-62703-026-7_4 PMID: 22990770.

52. Furuya T, Sa JM, Chitnis CE, Wellems TE, Stedman TT. Reticulocytes from cryopreserved erythroblasts support Plasmodium vivax infection in vitro. Parasitology international. 2014; 63(2):278–84. https://doi.org/10.1016/j.parint.2013.11.011 PMID: 24291603; PubMed Central PMCID: PMC3943572.

53. Noulin F, Manesia JK, Rosanas-Urgell A, Erhart A, Borlon C, Van Den Abbeele J, et al. Hematopoietic stem/progenitor cell sources to generate reticulocytes for Plasmodium vivax culture. PloS one. 2014; 9(11):e112496. https://doi.org/10.1371/journal.pone.0112496 PMID: 25393299; PubMed Central PMCID: PMC4231068.

54. Roobsoong W, Tharinjaroen CS, Rachaphaew N, Chobson P, Schofield L, Cui L, et al. Improvement of culture conditions for long-term in vitro culture of Plasmodium vivax. Malaria journal. 2015; 14:297. https://doi.org/10.1186/s12936-015-0815-z PMID: 26243280; PubMed Central PMCID: PMC4524445.

55. Thomson-Luque R, Scopel KK. Immature reticulocytes as preferential host cells and the challenges for in vitro culture of Plasmodium vivax. Pathogens and global health. 2015; 109(3):91–2. https://doi.org/10.1179/2047772415Z.000000000264 PMID: 25943155; PubMed Central PMCID: PMC4455358.

56. Moon RW, Hall J, Rangkuti F, Ho YS, Almond N, Mitchell GH, et al. Adaptation of the genetically tractable malaria pathogen Plasmodium knowlesi to continuous culture in human erythrocytes. Proc Natl Acad Sci U S A. 2013; 110(2):531–6. Epub 2012/12/26. https://doi.org/10.1073/pnas.1216457110 1216457110 [pii]. PMID: 23267069; PubMed Central PMCID: PMC3545754.

57. Gruring C, Moon RW, Lim C, Holder AA, Blackman MJ, Duraisingh MT. Human red blood cell-adapted Plasmodium knowlesi parasites: a new model system for malaria research. Cellular microbiology. 2014; 16(5):612–20. https://doi.org/10.1111/cmi.12275 PMID: 24506567; PubMed Central PMCID: PMC4004062.

58. Cheeseman IH, Miller BA, Nair S, Nkhoma S, Tan A, Tan JC, et al. A major genome region underlying artemisinin resistance in malaria. Science. 2012; 336(6077):79–82. Epub 2012/04/12. https://doi.org/10.1126/science.1215966 336/6077/79 [pii]. PMID: 22491853; PubMed Central PMCID: PMC3355473.

59. Takala-Harrison S, Clark TG, Jacob CG, Cummings MP, Miotto O, Dondorp AM, et al. Genetic loci associated with delayed clearance of Plasmodium falciparum following artemisinin treatment in Southeast Asia. Proc Natl Acad Sci U S A. 2013; 110(1):240–5. Epub 2012/12/19. https://doi.org/10.1073/pnas.1211205110 1211205110 [pii]. PMID: 23248304; PubMed Central PMCID: PMC3538248.

60. Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, et al. Novel phenotypic assays for the detection of artemisinin-resistant Plasmodium falciparum malaria in Cambodia: in-vitro and ex-vivo drug-response studies. Lancet Infect Dis. 2013; 13(12):1043–9. Epub 2013/09/17. https://doi.org/10.1016/S1473-3099(13)70252-4 S1473-3099(13)70252-4 [pii]. PMID: 24035558.

61. Witkowski B, Khim N, Chim P, Kim S, Ke S, Kloeung N, et al. Reduced artemisinin susceptibility of Plasmodium falciparum ring stages in western Cambodia. Antimicrobial agents and chemotherapy. 2013; 57(2):914–23. Epub 2012/12/05. https://doi.org/10.1128/AAC.01868-12 AAC.01868-12 [pii]. PMID: 23208708; PubMed Central PMCID: PMC3553720.

62. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature. 2014; 505(7481):50–5. Epub 2013/12/20. https://doi.org/10.1038/nature12876 PMID: 24352242.

63. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med. 2014; 371(5):411–23. Epub 2014/07/31. https://doi.org/10.1056/NEJMoa1314981 PMID: 25075834; PubMed Central PMCID: PMC4143591.

64. Tun KM, Imwong M, Lwin KM, Win AA, Hlaing TM, Hlaing T, et al. Spread of artemisinin-resistant Plasmodium falciparum in Myanmar: a cross-sectional survey of the K13 molecular marker. Lancet Infect Dis. 2015; 15(4):415–21. Epub 2015/02/24. https://doi.org/10.1016/S1473-3099(15)70032-0 S1473-3099(15)70032-0 [pii]. PMID: 25704894; PubMed Central PMCID: PMC4374103.

65. Winzeler EA, Manary MJ. Drug resistance genomics of the antimalarial drug artemisinin. Genome Biol. 2014; 15(11):544. Epub 2014/12/04. https://doi.org/10.1186/s13059-014-0544-6 PMID: 25470531; PubMed Central PMCID: PMCPMC4283579.

66. Fairhurst RM, Dondorp AM. Artemisinin-Resistant Plasmodium falciparum Malaria. Microbiology spectrum. 2016; 4(3). Epub 2016/06/24. https://doi.org/10.1128/microbiolspec.EI10-0013-2016 PMID: 27337450; PubMed Central PMCID: PMCPMC4992992.

67. Tilley L, Straimer J, Gnadig NF, Ralph SA, Fidock DA. Artemisinin Action and Resistance in Plasmodium falciparum. Trends in parasitology. 2016; 32(9):682–96. Epub 2016/06/13. https://doi.org/10.1016/j.pt.2016.05.010 PMID: 27289273; PubMed Central PMCID: PMCPMC5007624.

68. MacRae JI, Dixon MW, Dearnley MK, Chua HH, Chambers JM, Kenny S, et al. Mitochondrial metabolism of sexual and asexual blood stages of the malaria parasite Plasmodium falciparum. BMC Biol. 2013; 11:67. Epub 2013/06/15. https://doi.org/10.1186/1741-7007-11-67 PMID: 23763941; PubMed Central PMCID: PMC3704724.

69. Gulati S, Ekland EH, Ruggles KV, Chan RB, Jayabalasingham B, Zhou B, et al. Profiling the Essential Nature of Lipid Metabolism in Asexual Blood and Gametocyte Stages of Plasmodium falciparum. Cell host & microbe. 2015; 18(3):371–81. https://doi.org/10.1016/j.chom.2015.08.003 PMID: 26355219; PubMed Central PMCID: PMC4567697.

70. Dao A, Yaro AS, Diallo M, Timbine S, Huestis DL, Kassogue Y, et al. Signatures of aestivation and migration in Sahelian malaria mosquito populations. Nature. 2014; 516(7531):387–90. https://doi.org/10.1038/nature13987 PMID: 25470038; PubMed Central PMCID: PMC4306333.

71. Mitchell SN, Stevenson BJ, Muller P, Wilding CS, Egyir-Yawson A, Field SG, et al. Identification and validation of a gene causing cross-resistance between insecticide classes in Anopheles gambiae from Ghana. Proc Natl Acad Sci U S A. 2012; 109(16):6147–52. Epub 2012/03/31. https://doi.org/10.1073/pnas.1203452109 1203452109 [pii]. PMID: 22460795; PubMed Central PMCID: PMC3341073.

72. Kwiatkowska RM, Platt N, Poupardin R, Irving H, Dabire RK, Mitchell S, et al. Dissecting the mechanisms responsible for the multiple insecticide resistance phenotype in Anopheles gambiae s.s., M form, from Vallee du Kou, Burkina Faso. Gene. 2013; 519(1):98–106. Epub 2013/02/06. https://doi.org/10.1016/j.gene.2013.01.036 S0378-1119(13)00077-2 [pii]. PMID: 23380570; PubMed Central PMCID: PMC3611593.

73. Riveron JM, Yunta C, Ibrahim SS, Djouaka R, Irving H, Menze BD, et al. A single mutation in the GSTe2 gene allows tracking of metabolically based insecticide resistance in a major malaria vector. Genome Biol. 2014; 15(2):R27. Epub 2014/02/26. https://doi.org/10.1186/gb-2014-15-2-r27 gb-2014-15-2-r27 [pii]. PMID: 24565444; PubMed Central PMCID: PMC4054843.

74. Reidenbach KR, Neafsey DE, Costantini C, Sagnon N, Simard F, Ragland GJ, et al. Patterns of genomic differentiation between ecologically differentiated M and S forms of Anopheles gambiae in West and Central Africa. Genome Biol Evol. 2012; 4(12):1202–12. Epub 2012/11/08. https://doi.org/10.1093/gbe/evs095 evs095 [pii]. PMID: 23132896; PubMed Central PMCID: PMC3542583.

75. Pinto J, Egyir-Yawson A, Vicente JI, Gomes B, Santolamazza F, Moreno M, et al. Geographic population structure of the African malaria vectorAnopheles gambiaesuggests a role for the forest-savannah biome transition as a barrier to gene flow. Evolutionary Applications. 2013; 6(6):910–24. https://doi.org/10.1111/eva.12075 PMID: 24062800

76. O'Loughlin SM, Magesa S, Mbogo C, Mosha F, Midega J, Lomas S, et al. Genomic analyses of three malaria vectors reveals extensive shared polymorphism but contrasting population histories. Mol Biol Evol. 2014; 31(4):889–902. Epub 2014/01/11. https://doi.org/10.1093/molbev/msu040 msu040 [pii]. PMID: 24408911; PubMed Central PMCID: PMC3969563.

77. Neafsey DE, Waterhouse RM, Abai MR, Aganezov SS, Alekseyev MA, Allen JE, et al. Mosquito genomics. Highly evolvable malaria vectors: the genomes of 16 Anopheles mosquitoes. Science. 2015; 347(6217):1258522. Epub 2015/01/03. https://doi.org/10.1126/science.1258522 1258522 [pii] science.1258522 [pii]. PMID: 25554792; PubMed Central PMCID: PMC4380271.

78. van Schaijk BC, Vos MW, Janse CJ, Sauerwein RW, Khan SM. Removal of heterologous sequences from Plasmodium falciparum mutants using FLPe-recombinase. PloS one. 2010; 5(11):e15121. https://doi.org/10.1371/journal.pone.0015121 PMID: 21152048; PubMed Central PMCID: PMC2994908.

79. Muralidharan V, Oksman A, Iwamoto M, Wandless TJ, Goldberg DE. Asparagine repeat function in a Plasmodium falciparum protein assessed via a regulatable fluorescent affinity tag. Proc Natl Acad Sci U S A. 2011; 108(11):4411–6. Epub 2011/03/04. https://doi.org/10.1073/pnas.1018449108 PMID: 21368162; PubMed Central PMCID: PMC3060247.

80. O'Neill MT, Phuong T, Healer J, Richard D, Cowman AF. Gene deletion from Plasmodium falciparum using FLP and Cre recombinases: implications for applied site-specific recombination. International journal for parasitology. 2011; 41(1):117–23. https://doi.org/10.1016/j.ijpara.2010.08.001 PMID: 20816845.

81. Straimer J, Lee MC, Lee AH, Zeitler B, Williams AE, Pearl JR, et al. Site-specific genome editing in Plasmodium falciparum using engineered zinc-finger nucleases. Nature methods. 2012; 9(10):993–8. https://doi.org/10.1038/nmeth.2143 PMID: 22922501; PubMed Central PMCID: PMC3697006.

82. Collins CR, Das S, Wong EH, Andenmatten N, Stallmach R, Hackett F, et al. Robust inducible Cre recombinase activity in the human malaria parasite Plasmodium falciparum enables efficient gene deletion within a single asexual erythrocytic growth cycle. Molecular microbiology. 2013; 88(4):687–701. https://doi.org/10.1111/mmi.12206 PMID: 23489321; PubMed Central PMCID: PMC3708112.

83. Prommana P, Uthaipibull C, Wongsombat C, Kamchonwongpaisan S, Yuthavong Y, Knuepfer E, et al. Inducible knockdown of Plasmodium gene expression using the glmS ribozyme. PloS one. 2013; 8(8): e73783. https://doi.org/10.1371/journal.pone.0073783 PMID: 24023691; PubMed Central PMCID: PMC3758297.

84. Goldfless SJ, Wagner JC, Niles JC. Versatile control of Plasmodium falciparum gene expression with an inducible protein-RNA interaction. Nat Commun. 2014; 5:5329. https://doi.org/10.1038/ncomms6329 PMID: 25370483; PubMed Central PMCID: PMC4223869.

85. Ghorbal M, Gorman M, Macpherson CR, Martins RM, Scherf A, Lopez-Rubio JJ. Genome editing in the human malaria parasite Plasmodium falciparum using the CRISPR-Cas9 system. Nature biotechnology. 2014; 32(8):819–21. https://doi.org/10.1038/nbt.2925 PMID: 24880488.

86. Wagner JC, Platt RJ, Goldfless SJ, Zhang F, Niles JC. Efficient CRISPR-Cas9-mediated genome editing in Plasmodium falciparum. Nature methods. 2014; 11(9):915–8. https://doi.org/10.1038/nmeth.3063 PMID: 25108687; PubMed Central PMCID: PMC4199390.

87. Windbichler N, Menichelli M, Papathanos PA, Thyme SB, Li H, Ulge UY, et al. A synthetic homing endonuclease-based gene drive system in the human malaria mosquito. Nature. 2011; 473 (7346):212–5. https://doi.org/10.1038/nature09937 PMID: 21508956; PubMed Central PMCID: PMC3093433.

88. Isaacs AT, Jasinskiene N, Tretiakov M, Thiery I, Zettor A, Bourgouin C, et al. Transgenic Anopheles stephensi coexpressing single-chain antibodies resist Plasmodium falciparum development. Proc Natl Acad Sci U S A. 2012; 109(28):E1922–30. https://doi.org/10.1073/pnas.1207738109 PMID: 22689959; PubMed Central PMCID: PMC3396534.

89. Bernardini F, Galizi R, Menichelli M, Papathanos PA, Dritsou V, Marois E, et al. Site-specific genetic engineering of the Anopheles gambiae Y chromosome. Proc Natl Acad Sci U S A. 2014; 111 (21):7600–5. https://doi.org/10.1073/pnas.1404996111 PMID: 24821795; PubMed Central PMCID: PMC4040617.

90. Galizi R, Doyle LA, Menichelli M, Bernardini F, Deredec A, Burt A, et al. A synthetic sex ratio distortion system for the control of the human malaria mosquito. Nat Commun. 2014; 5:3977. https://doi.org/10.1038/ncomms4977 PMID: 24915045; PubMed Central PMCID: PMC4057611.

91. Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E, et al. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. Proc Natl Acad Sci U S A. 2015; 112(49):E6736–43. https://doi.org/10.1073/pnas.1521077112 PMID: 26598698; PubMed Central PMCID: PMC4679060.

92. Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, et al. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nature biotechnology. 2016; 34(1):78–83. https://doi.org/10.1038/nbt.3439 PMID: 26641531.

93. Moreno M, Tong C, Guzman M, Chuquiyauri R, Llanos-Cuentas A, Rodriguez H, et al. Infection of laboratory-colonized Anopheles darlingi mosquitoes by Plasmodium vivax. Am J Trop Med Hyg. 2014; 90 (4):612–6. https://doi.org/10.4269/ajtmh.13-0708 PMID: 24534811; PubMed Central PMCID: PMC3973502.

94. Batchelor JD, Zahm JA, Tolia NH. Dimerization of Plasmodium vivax DBP is induced upon receptor binding and drives recognition of DARC. Nature structural & molecular biology. 2011; 18(8):908–14. https://doi.org/10.1038/nsmb.2088 PMID: 21743458; PubMed Central PMCID: PMC3150435.

95. Batchelor JD, Malpede BM, Omattage NS, DeKoster GT, Henzler-Wildman KA, Tolia NH. Red blood cell invasion by Plasmodium vivax: structural basis for DBP engagement of DARC. PLoS pathogens. 2014; 10(1):e1003869. https://doi.org/10.1371/journal.ppat.1003869 PMID: 24415938; PubMed Central PMCID: PMC3887093.

96. Chen E, Paing MM, Salinas N, Sim BK, Tolia NH. Structural and functional basis for inhibition of erythrocyte invasion by antibodies that target Plasmodium falciparum EBA-175. PLoS pathogens. 2013; 9 (5):e1003390. https://doi.org/10.1371/journal.ppat.1003390 PMID: 23717209; PubMed Central PMCID: PMC3662668.

97. Chen E, Salinas ND, Ntumngia FB, Adams JH, Tolia NH. Structural Analysis of the Synthetic Duffy Binding Protein (DBP) Antigen DEKnull Relevant for Plasmodium vivax Malaria Vaccine Design. PLOS Neglected Tropical Diseases. 2015; 9(3):e0003644. https://doi.org/10.1371/journal.pntd.0003644 PMID: 25793371

98. Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, Kone AK, et al. A field trial to assess a blood-stage malaria vaccine. N Engl J Med. 2011; 365(11):1004–13. Epub 2011/09/16. https://doi.org/10.1056/NEJMoa1008115 PMID: 21916638; PubMed Central PMCID: PMC3242358.

99. Ouattara A, Takala-Harrison S, Thera MA, Coulibaly D, Niangaly A, Saye R, et al. Molecular basis of allele-specific efficacy of a blood-stage malaria vaccine: vaccine development implications. The Journal of infectious diseases. 2013; 207(3):511–9. Epub 2012/12/04. https://doi.org/10.1093/infdis/jis709 jis709 [pii]. PMID: 23204168; PubMed Central PMCID: PMC3537449.

100. Graves SF, Kouriba B, Diarra I, Daou M, Niangaly A, Coulibaly D, et al. Strain-specific Plasmodium falciparum multifunctional CD4(+) T cell cytokine expression in Malian children immunized with the FMP2.1/AS02A vaccine candidate. Vaccine. 2016; 34(23):2546–55. Epub 2016/04/19. https://doi.org/10.1016/j.vaccine.2016.04.019 S0264-410X(16)30148-7 [pii]. PMID: 27087149.

101. Gueirard P, Tavares J, Thiberge S, Bernex F, Ishino T, Milon G, et al. Development of the malaria parasite in the skin of the mammalian host. Proc Natl Acad Sci U S A. 2010; 107(43):18640–5. https://doi.org/10.1073/pnas.1009346107 PMID: 20921402; PubMed Central PMCID: PMC2972976.

102. Gruring C, Heiber A, Kruse F, Ungefehr J, Gilberger TW, Spielmann T. Development and host cell modifications of Plasmodium falciparum blood stages in four dimensions. Nat Commun. 2011; 2:165. https://doi.org/10.1038/ncomms1169 PMID: 21266965.

103. Joice R, Nilsson SK, Montgomery J, Dankwa S, Egan E, Morahan B, et al. Plasmodium falciparum transmission stages accumulate in the human bone marrow. Science translational medicine. 2014; 6 (244):244re5. https://doi.org/10.1126/scitranslmed.3008882 PMID: 25009232; PubMed Central PMCID: PMC4175394.

104. Pukrittayakamee S, Imwong M, Singhasivanon P, Stepniewska K, Day NJ, White NJ. Effects of different antimalarial drugs on gametocyte carriage in P. vivax malaria. Am J Trop Med Hyg. 2008; 79 (3):378–84. Epub 2008/09/12. 79/3/378 [pii]. PMID: 18784229.

105. Douglas NM, Simpson JA, Phyo AP, Siswantoro H, Hasugian AR, Kenangalem E, et al. Gametocyte dynamics and the role of drugs in reducing the transmission potential of Plasmodium vivax. The Journal of infectious diseases. 2013; 208(5):801–12. Epub 2013/06/15. https://doi.org/10.1093/infdis/jit261 PMID: 23766527; PubMed Central PMCID: PMCPMC3733516.

106. Abdul-Ghani R, Basco LK, Beier JC, Mahdy MA. Inclusion of gametocyte parameters in anti-malarial drug efficacy studies: filling a neglected gap needed for malaria elimination. Malaria journal. 2015; 14:413. Epub 2015/10/21. https://doi.org/10.1186/s12936-015-0936-4 PMID: 26481312; PubMed Central PMCID: PMCPMC4617745.

107. Okumu FO, Killeen GF, Ogoma S, Biswaro L, Smallegange RC, Mbeyela E, et al. Development and field evaluation of a synthetic mosquito lure that is more attractive than humans. PloS one. 2010; 5(1): e8951. Epub 2010/02/04. https://doi.org/10.1371/journal.pone.0008951 PMID: 20126628; PubMed Central PMCID: PMC2812511.

108. Mukabana WR, Mweresa CK, Otieno B, Omusula P, Smallegange RC, van Loon JJ, et al. A novel synthetic odorant blend for trapping of malaria and other African mosquito species. J Chem Ecol. 2012; 38

(3):235–44. Epub 2012/03/20. https://doi.org/10.1007/s10886-012-0088-8 PMID: 22426893; PubMed Central PMCID: PMC3310138.

109. Menger DJ, Otieno B, de Rijk M, Mukabana WR, van Loon JJ, Takken W. A push-pull system to reduce house entry of malaria mosquitoes. Malaria journal. 2014; 13:119. Epub 2014/03/29. 1https://doi.org/10.1186/1475-2875-13-119 1475-2875-13-119 [pii]. PMID: 24674451; PubMed Central PMCID: PMC3986670.

110. Menger DJ, Van Loon JJ, Takken W. Assessing the efficacy of candidate mosquito repellents against the background of an attractive source that mimics a human host. Med Vet Entomol. 2014; 28(4):407–13. Epub 2014/05/07. https://doi.org/10.1111/mve.12061 PMID: 24797537.

111. Verhulst NO, Qiu YT, Beijleveld H, Maliepaard C, Knights D, Schulz S, et al. Composition of Human Skin Microbiota Affects Attractiveness to Malaria Mosquitoes. PLoS One. 2011; 6(12):e28991. https://doi.org/10.1371/journal.pone.0028991 PMID: 22216154

112. Kelly M, Su CY, Schaber C, Crowley JR, Hsu FF, Carlson JR, et al. Malaria parasites produce volatile mosquito attractants. MBio. 2015; 6(2). Epub 2015/03/26. https://doi.org/10.1128/mBio.00235-15 e00235-15 [pii] mBio.00235-15 [pii]. PMID: 25805727; PubMed Central PMCID: PMC4453533.

113. Kumar S, Molina-Cruz A, Gupta L, Rodrigues J, Barillas-Mury C. A peroxidase/dual oxidase system modulates midgut epithelial immunity in Anopheles gambiae. Science. 2010; 327(5973):1644–8. https://doi.org/10.1126/science.1184008 PMID: 20223948; PubMed Central PMCID: PMC3510679.

114. Molina-Cruz A, Garver LS, Alabaster A, Bangiolo L, Haile A, Winikor J, et al. The human malaria parasite Pfs47 gene mediates evasion of the mosquito immune system. Science. 2013; 340(6135):984–7. Epub 2013/05/11. https://doi.org/10.1126/science.1235264 science.1235264 [pii]. PMID: 23661646; PubMed Central PMCID: PMC3807741.

115. Oliveira Gde A, Lieberman J, Barillas-Mury C. Epithelial nitration by a peroxidase/NOX5 system mediates mosquito antiplasmodial immunity. Science. 2012; 335(6070):856–9. https://doi.org/10.1126/science.1209678 PMID: 22282475; PubMed Central PMCID: PMC3444286.

116. Ramphul UN, Garver LS, Molina-Cruz A, Canepa GE, Barillas-Mury C. Plasmodium falciparum evades mosquito immunity by disrupting JNK-mediated apoptosis of invaded midgut cells. Proc Natl Acad Sci U S A. 2015; 112(5):1273–80. Epub 2015/01/02. https://doi.org/10.1073/pnas.1423586112 1423586112 [pii]. PMID: 25552553; PubMed Central PMCID: PMC4321252.

117. Eldering M, Morlais I, van Gemert GJ, van de Vegte-Bolmer M, Graumans W, Siebelink-Stoter R, et al. Variation in susceptibility of African Plasmodium falciparum malaria parasites to TEP1 mediated killing in Anopheles gambiae mosquitoes. Sci Rep. 2016; 6:20440. Epub 2016/02/11. https://doi.org/10.1038/srep20440 srep20440 [pii]. PMID: 26861587; PubMed Central PMCID: PMC4748223.

118. Gupta L, Molina-Cruz A, Kumar S, Rodrigues J, Dixit R, Zamora RE, et al. The STAT pathway mediates late-phase immunity against Plasmodium in the mosquito Anopheles gambiae. Cell host & microbe. 2009; 5(5):498–507. https://doi.org/10.1016/j.chom.2009.04.003 PMID: 19454353; PubMed Central PMCID: PMC2701194.

119. Smith RC, Eappen AG, Radtke AJ, Jacobs-Lorena M. Regulation of anti-Plasmodium immunity by a LITAF-like transcription factor in the malaria vector Anopheles gambiae. PLoS pathogens. 2012; 8(10):e1002965. https://doi.org/10.1371/journal.ppat.1002965 PMID: 23093936; PubMed Central PMCID: PMC3475675.

120. Li J, Wang X, Zhang G, Githure JI, Yan G, James AA. Genome-block expression-assisted association studies discover malaria resistance genes in Anopheles gambiae. Proc Natl Acad Sci U S A. 2013; 110(51):20675–80. https://doi.org/10.1073/pnas.1321024110 PMID: 24297936; PubMed Central PMCID: PMC3870758.

121. Ojo KK, Pfander C, Mueller NR, Burstroem C, Larson ET, Bryan CM, et al. Transmission of malaria to mosquitoes blocked by bumped kinase inhibitors. The Journal of clinical investigation. 2012; 122(6):2301–5. https://doi.org/10.1172/JCI61822 PMID: 22565309; PubMed Central PMCID: PMC3366411.

122. Mathias DK, Pastrana-Mena R, Ranucci E, Tao D, Ferruti P, Ortega C, et al. A small molecule glycosaminoglycan mimetic blocks Plasmodium invasion of the mosquito midgut. PLoS pathogens. 2013; 9(11):e1003757. https://doi.org/10.1371/journal.ppat.1003757 PMID: 24278017; PubMed Central PMCID: PMC3836724.

123. Sala KA, Nishiura H, Upton LM, Zakutansky SE, Delves MJ, Iyori M, et al. The Plasmodium berghei sexual stage antigen PSOP12 induces anti-malarial transmission blocking immunity both in vivo and in vitro. Vaccine. 2015; 33(3):437–45. Epub 2014/12/03. https://doi.org/10.1016/j.vaccine.2014.11.038 PMID: 25454088.

124. Dembele L, Gego A, Zeeman AM, Franetich JF, Silvie O, Rametti A, et al. Towards an in vitro model of Plasmodium hypnozoites suitable for drug discovery. PloS one. 2011; 6(3):e18162. https://doi.org/10.1371/journal.pone.0018162 PMID: 21483865; PubMed Central PMCID: PMC3069045.

125. Vaughan AM, Mikolajczak SA, Wilson EM, Grompe M, Kaushansky A, Camargo N, et al. Complete Plasmodium falciparum liver-stage development in liver-chimeric mice. The Journal of clinical investigation. 2012; 122(10):3618–28. Epub 2012/09/22. https://doi.org/10.1172/JCI62684 PMID: 22996664; PubMed Central PMCID: PMC3461911.

126. Zeeman AM, van Amsterdam SM, McNamara CW, Voorberg-van der Wel A, Klooster EJ, van den Berg A, et al. KAI407, a potent non-8-aminoquinoline compound that kills Plasmodium cynomolgi early dormant liver stage parasites in vitro. Antimicrobial agents and chemotherapy. 2014; 58 (3):1586–95. https://doi.org/10.1128/AAC.01927-13 PMID: 24366744; PubMed Central PMCID: PMC3957848.

127. McCarthy JS, Griffin PM, Sekuloski S, Bright AT, Rockett R, Looke D, et al. Experimentally induced blood-stage Plasmodium vivax infection in healthy volunteers. The Journal of infectious diseases. 2013; 208(10):1688–94. Epub 2013/08/03. https://doi.org/10.1093/infdis/jit394 jit394 [pii]. PMID: 23908484; PubMed Central PMCID: PMC3888148.

128. McCarthy JS, Marquart L, Sekuloski S, Trenholme K, Elliott S, Griffin P, et al. Linking Murine and Human Plasmodium falciparum Challenge Models in a Translational Path for Antimalarial Drug Development. Antimicrobial agents and chemotherapy. 2016; 60(6):3669–75. Epub 2016/04/06. https://doi.org/10.1128/AAC.02883-15 AAC.02883-15 [pii]. PMID: 27044554; PubMed Central PMCID: PMC4879391.

129. Swann J, Corey V, Scherer CA, Kato N, Comer E, Maetani M, et al. High-Throughput Luciferase-Based Assay for the Discovery of Therapeutics That Prevent Malaria. ACS Infect Dis. 2016; 2(4):281–93. Epub 2016/06/09. https://doi.org/10.1021/acsinfecdis.5b00143 PMID: 27275010; PubMed Central PMCID: PMC4890880.

130. Hoeijmakers WA, Bartfai R, Stunnenberg HG. Transcriptome analysis using RNA-Seq. Methods in molecular biology. 2013; 923:221–39. Epub 2012/09/20. https://doi.org/10.1007/978-1-62703-026-7_15 PMID: 22990781.

131. Otto TD, Bohme U, Jackson AP, Hunt M, Franke-Fayard B, Hoeijmakers WA, et al. A comprehensive evaluation of rodent malaria parasite genomes and gene expression. BMC Biol. 2014; 12:86. Epub 2014/11/02. https://doi.org/10.1186/s12915-014-0086-0 s12915-014-0086-0 [pii]. PMID: 25359557; PubMed Central PMCID: PMC4242472.

132. Yamagishi J, Natori A, Tolba ME, Mongan AE, Sugimoto C, Katayama T, et al. Interactive transcriptome analysis of malaria patients and infecting Plasmodium falciparum. Genome Res. 2014; 24 (9):1433–44. Epub 2014/08/06. https://doi.org/10.1101/gr.158980.113 gr.158980.113 [pii]. PMID: 25091627; PubMed Central PMCID: PMC4158759.

133. Kaneko I, Iwanaga S, Kato T, Kobayashi I, Yuda M. Genome-Wide Identification of the Target Genes of AP2-O, a Plasmodium AP2-Family Transcription Factor. PLoS pathogens. 2015; 11(5):e1004905. Epub 2015/05/29. https://doi.org/10.1371/journal.ppat.1004905 PPATHOGENS-D-14-02008 [pii]. PMID: 26018192; PubMed Central PMCID: PMC4446032.

134. Eshar S, Altenhofen L, Rabner A, Ross P, Fastman Y, Mandel-Gutfreund Y, et al. PfSR1 controls alternative splicing and steady-state RNA levels in Plasmodium falciparum through preferential recognition of specific RNA motifs. Molecular microbiology. 2015; 96(6):1283–97. Epub 2015/03/27. https://doi.org/10.1111/mmi.13007 PMID: 25807998.

135. Flannery EL, Fidock DA, Winzeler EA. Using genetic methods to define the targets of compounds with antimalarial activity. J Med Chem. 2013; 56(20):7761–71. Epub 2013/08/10. https://doi.org/10.1021/jm400325j PMID: 23927658; PubMed Central PMCID: PMC3880619.

136. Miotto O, Amato R, Ashley EA, MacInnis B, Almagro-Garcia J, Amaratunga C, et al. Genetic architecture of artemisinin-resistant Plasmodium falciparum. Nat Genet. 2015; 47(3):226–34. Epub 2015/01/20. https://doi.org/10.1038/ng.3189 PMID: 25599401; PubMed Central PMCID: PMC4545236.

137. Baragana B, Hallyburton I, Lee MC, Norcross NR, Grimaldi R, Otto TD, et al. A novel multiple-stage antimalarial agent that inhibits protein synthesis. Nature. 2015; 522(7556):315–20. Epub 2015/06/19. https://doi.org/10.1038/nature14451 nature14451 [pii]. PMID: 26085270; PubMed Central PMCID: PMC4700930.

138. Bopp SE, Manary MJ, Bright AT, Johnston GL, Dharia NV, Luna FL, et al. Mitotic evolution of Plasmodium falciparum shows a stable core genome but recombination in antigen families. PLoS Genet. 2013; 9(2):e1003293. Epub 2013/02/15. https://doi.org/10.1371/journal.pgen.1003293 PGENETICS-D-12-01478 [pii]. PMID: 23408914; PubMed Central PMCID: PMC3567157.

139. Nair S, Nkhoma SC, Serre D, Zimmerman PA, Gorena K, Daniel BJ, et al. Single-cell genomics for dissection of complex malaria infections. Genome Res. 2014; 24(6):1028–38. Epub 2014/05/09. https://doi.org/10.1101/gr.168286.113 gr.168286.113 [pii]. PMID: 24812326; PubMed Central PMCID: PMC4032849.

140. The malERA Refresh Consultative Panel on Insecticide and Drug Resistance. malERA: An updated research agenda for insecticide and drug resistance in malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002450 https://doi.org/10.1371/journal.pmed.1002450

141. Gomes AR, Bushell E, Schwach F, Girling G, Anar B, Quail MA, et al. A genome-scale vector resource enables high-throughput reverse genetic screening in a malaria parasite. Cell host & microbe. 2015; 17(3):404–13. Epub 2015/03/04. https://doi.org/10.1016/j.chom.2015.01.014 S1931-3128(15)00034-7 [pii]. PMID: 25732065; PubMed Central PMCID: PMC4362957.

142. Schwach F, Bushell E, Gomes AR, Anar B, Girling G, Herd C, et al. PlasmoGEM, a database supporting a community resource for large-scale experimental genetics in malaria parasites. Nucleic Acids Research. 2015; 43(Database issue):D1176–D82. doi: 10.1093/nar/gku1143. PMC4383951. PMID: 25593348

143. Guttery DS, Poulin B, Ramaprasad A, Wall RJ, Ferguson DJ, Brady D, et al. Genome-wide functional analysis of Plasmodium protein phosphatases reveals key regulators of parasite development and differentiation. Cell host & microbe. 2014; 16(1):128–40. Epub 2014/07/11. https://doi.org/10.1016/j.chom.2014.05.020 S1931-3128(14)00219-4 [pii]. PMID: 25011111; PubMed Central PMCID: PMC4094981.

144. Lin JW, Meireles P, Prudencio M, Engelmann S, Annoura T, Sajid M, et al. Loss-of-function analyses defines vital and redundant functions of the Plasmodium rhomboid protease family. Molecular microbiology. 2013; 88(2):318–38. Epub 2013/03/16. https://doi.org/10.1111/mmi.12187 PMID: 23490234.

145. Matthews K, Kalanon M, Chisholm SA, Sturm A, Goodman CD, Dixon MW, et al. The Plasmodium translocon of exported proteins (PTEX) component thioredoxin-2 is important for maintaining normal blood-stage growth. Molecular microbiology. 2013; 89(6):1167–86. Epub 2013/07/23. https://doi.org/10.1111/mmi.12334 PMID: 23869529.

146. Philip N, Waters AP. Conditional Degradation of Plasmodium Calcineurin Reveals Functions in Parasite Colonization of both Host and Vector. Cell host & microbe. 2015; 18(1):122–31. Epub 2015/06/30. https://doi.org/10.1016/j.chom.2015.05.018 S1931-3128(15)00250-4 [pii]. PMID: 26118994; PubMed Central PMCID: PMC4509507.

147. Burda PC, Roelli MA, Schaffner M, Khan SM, Janse CJ, Heussler VT. A Plasmodium phospholipase is involved in disruption of the liver stage parasitophorous vacuole membrane. PLoS pathogens. 2015; 11(3):e1004760. Epub 2015/03/19. https://doi.org/10.1371/journal.ppat.1004760 PPATHOGENS-D-14-02170 [pii]. PMID: 25786000; PubMed Central PMCID: PMC4364735.

148. Falae A, Combe A, Amaladoss A, Carvalho T, Menard R, Bhanot P. Role of Plasmodium berghei cGMP-dependent protein kinase in late liver stage development. The Journal of biological chemistry. 2010; 285(5):3282–8. Epub 2009/11/27. https://doi.org/10.1074/jbc.M109.070367 M109.070367 [pii]. PMID: 19940133; PubMed Central PMCID: PMC2823412.

149. Lehmann C, Heitmann A, Mishra S, Burda PC, Singer M, Prado M, et al. A cysteine protease inhibitor of plasmodium berghei is essential for exo-erythrocytic development. PLoS pathogens. 2014; 10(8): e1004336. Epub 2014/08/29. https://doi.org/10.1371/journal.ppat.1004336 PMID: 25166051; PubMed Central PMCID: PMC4148452.

150. Rennenberg A, Lehmann C, Heitmann A, Witt T, Hansen G, Nagarajan K, et al. Exoerythrocytic Plasmodium parasites secrete a cysteine protease inhibitor involved in sporozoite invasion and capable of blocking cell death of host hepatocytes. PLoS pathogens. 2010; 6(3):e1000825. Epub 2010/04/03. https://doi.org/10.1371/journal.ppat.1000825 PMID: 20361051; PubMed Central PMCID: PMC2845656.

151. Elsworth B, Matthews K, Nie CQ, Kalanon M, Charnaud SC, Sanders PR, et al. PTEX is an essential nexus for protein export in malaria parasites. Nature. 2014; 511(7511):587–91. Epub 2014/07/22. https://doi.org/10.1038/nature13555 nature13555 [pii]. PMID: 25043043.

152. Pasini EM, Braks JA, Fonager J, Klop O, Aime E, Spaccapelo R, et al. Proteomic and genetic analyses demonstrate that Plasmodium berghei blood stages export a large and diverse repertoire of proteins. Mol Cell Proteomics. 2013; 12(2):426–48. Epub 2012/12/01. https://doi.org/10.1074/mcp.M112.021238 M112.021238 [pii]. PMID: 23197789; PubMed Central PMCID: PMC3567864.

153. Ingmundson A, Nahar C, Brinkmann V, Lehmann MJ, Matuschewski K. The exported Plasmodium berghei protein IBIS1 delineates membranous structures in infected red blood cells. Molecular microbiology. 2012; 83(6):1229–43. Epub 2012/02/15. https://doi.org/10.1111/j.1365-2958.2012.08004.x PMID: 22329949; PubMed Central PMCID: PMC3502748.

154. Matz JM, Goosmann C, Brinkmann V, Grutzke J, Ingmundson A, Matuschewski K, et al. The Plasmodium berghei translocon of exported proteins reveals spatiotemporal dynamics of tubular extensions. Sci Rep. 2015; 5:12532. Epub 2015/07/30. https://doi.org/10.1038/srep12532 srep12532 [pii]. PMID: 26219962; PubMed Central PMCID: PMC4518229.

155. Mbengue A, Bhattacharjee S, Pandharkar T, Liu H, Estiu G, Stahelin RV, et al. A molecular mechanism of artemisinin resistance in Plasmodium falciparum malaria. Nature. 2015; 520(7549):683–7.

Epub 2015/04/16. https://doi.org/10.1038/nature14412 PMID: 25874676; PubMed Central PMCID: PMCPMC4417027.

156. Straimer J, Gnadig NF, Witkowski B, Amaratunga C, Duru V, Ramadani AP, et al. Drug resistance. K13-propeller mutations confer artemisinin resistance in Plasmodium falciparum clinical isolates. Science. 2015; 347(6220):428–31. Epub 2014/12/17. https://doi.org/10.1126/science.1260867 science.1260867 [pii]. PMID: 25502314; PubMed Central PMCID: PMC4349400.

157. Adjalley SH, Chabbert CD, Klaus B, Pelechano V, Steinmetz LM. Landscape and Dynamics of Transcription Initiation in the Malaria Parasite Plasmodium falciparum. Cell Rep. 2016; 14(10):2463–75. Epub 2016/03/08. https://doi.org/10.1016/j.celrep.2016.02.025 S2211-1247(16)30128-0 [pii]. PMID: 26947071; PubMed Central PMCID: PMC4806524.

158. ENCODE Project Consortium, Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature. 2007; 447(7146):799–816. https://doi.org/10.1038/nature05874 PMID: 17571346; PubMed Central PMCID: PMC2212820.

159. Sana TR, Gordon DB, Fischer SM, Tichy SE, Kitagawa N, Lai C, et al. Global mass spectrometry based metabolomics profiling of erythrocytes infected with Plasmodium falciparum. PloS one. 2013; 8 (4):e60840. Epub 2013/04/18. https://doi.org/10.1371/journal.pone.0060840 PONE-D-12-01745 [pii]. PMID: 23593322; PubMed Central PMCID: PMC3621881.

160. Sengupta A, Ghosh S, Basant A, Malusare S, Johri P, Pathak S, et al. Global host metabolic response to Plasmodium vivax infection: a 1H NMR based urinary metabonomic study. Malaria journal. 2011; 10:384. Epub 2011/12/27. https://doi.org/10.1186/1475-2875-10-384 1475-2875-10-384 [pii]. PMID: 22196439; PubMed Central PMCID: PMC3298531.

161. Teng R, Lehane AM, Winterberg M, Shafik SH, Summers RL, Martin RE, et al. 1H-NMR metabolite profiles of different strains of Plasmodium falciparum. Biosci Rep. 2014; 34(6):e00150. Epub 2014/11/19. https://doi.org/10.1042/BSR20140134 e00150 [pii] BSR20140134 [pii]. PMID: 25405893; PubMed Central PMCID: PMC4240024.

162. Allman EL, Painter HJ, Samra J, Carrasquilla M, Llinas M. Metabolomic Profiling of the Malaria Box Reveals Antimalarial Target Pathways. Antimicrobial agents and chemotherapy. 2016; 60 (11):6635–49. Epub 2016/08/31. https://doi.org/10.1128/AAC.01224-16 PMID: 27572391; PubMed Central PMCID: PMCPMC5075069.

163. Creek DJ, Chua HH, Cobbold SA, Nijagal B, MacRae JI, Dickerman BK, et al. Metabolomics-Based Screening of the Malaria Box Reveals both Novel and Established Mechanisms of Action. Antimicrobial agents and chemotherapy. 2016; 60(11):6650–63. Epub 2016/08/31. https://doi.org/10.1128/AAC.01226-16 PMID: 27572396; PubMed Central PMCID: PMCPMC5075070.

164. Park YH, Shi YP, Liang B, Medriano CA, Jeon YH, Torres E, et al. High-resolution metabolomics to discover potential parasite-specific biomarkers in a Plasmodium falciparum erythrocytic stage culture system. Malaria journal. 2015; 14:122. Epub 2015/04/19. https://doi.org/10.1186/s12936-015-0651-1 10.1186/s12936-015-0651-1 [pii]. PMID: 25889340; PubMed Central PMCID: PMC4377044.

165. Tritten L, Keiser J, Godejohann M, Utzinger J, Vargas M, Beckonert O, et al. Metabolic profiling framework for discovery of candidate diagnostic markers of malaria. Sci Rep. 2013; 3:2769. Epub 2013/09/27. https://doi.org/10.1038/srep02769 srep02769 [pii]. PMID: 24067624.

166. The malERA Refresh Consultative Panel on Characterising the Reservoir and Measuring Transmission. malERA: An updated research agenda for characterizing the reservoir and measuring transmission in malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002452. https://doi.org/10.1371/journal.pmed.1002452

167. Liu W, Sundararaman SA, Loy DE, Learn GH, Li Y, Plenderleith LJ, et al. Multigenomic Delineation of Plasmodium Species of the Laverania Subgenus Infecting Wild-Living Chimpanzees and Gorillas. Genome Biol Evol. 2016; 8(6):1929–39. Epub 2016/06/12. https://doi.org/10.1093/gbe/evw128 evw128 [pii]. PMID: 27289102; PubMed Central PMCID: PMC4943199.

168. Loy DE, Liu W, Li Y, Learn GH, Plenderleith LJ, Sundararaman SA, et al. Out of Africa: origins and evolution of the human malaria parasites Plasmodium falciparum and Plasmodium vivax. International journal for parasitology. 2017; 47(2–3):87–97. Epub 2016/07/07. https://doi.org/10.1016/j.ijpara.2016.05.008 PMID: 27381764; PubMed Central PMCID: PMCPMC5205579.

169. Sundararaman SA, Plenderleith LJ, Liu W, Loy DE, Learn GH, Li Y, et al. Genomes of cryptic chimpanzee Plasmodium species reveal key evolutionary events leading to human malaria. Nat Commun. 2016; 7:11078. Epub 2016/03/24. https://doi.org/10.1038/ncomms11078 PMID: 27002652; PubMed Central PMCID: PMCPMC4804174.

170. Aguilar R, Magallon-Tejada A, Achtman AH, Moraleda C, Joice R, Cistero P, et al. Molecular evidence for the localization of Plasmodium falciparum immature gametocytes in bone marrow. Blood. 2014; 123(7):959–66. Epub 2013/12/18. https://doi.org/10.1182/blood-2013-08-520767 PMID: 24335496; PubMed Central PMCID: PMC4067503.

171. Fontaine MC, Pease JB, Steele A, Waterhouse RM, Neafsey DE, Sharakhov IV, et al. Mosquito genomics. Extensive introgression in a malaria vector species complex revealed by phylogenomics. Science. 2015; 347(6217):1258524. Epub 2014/11/29. https://doi.org/10.1126/science.1258524 1258524 [pii] science.1258524 [pii]. PMID: 25431491; PubMed Central PMCID: PMC4380269.

172. Parker JE, Angarita-Jaimes N, Abe M, Towers CE, Towers D, McCall PJ. Infrared video tracking of Anopheles gambiae at insecticide-treated bed nets reveals rapid decisive impact after brief localised net contact. Sci Rep. 2015; 5:13392. Epub 2015/09/02. https://doi.org/10.1038/srep13392 srep13392 [pii]. PMID: 26323965; PubMed Central PMCID: PMC4642575.

173. The Ag1000G Consortium. Anopheles gambiae 1000 Genomes Project: Ag1000G 2014 [updated 2016, Accessed Nov 1st, 2017]. Available from: https://www.malariagen.net/projects/ag1000g.

174. Baldini F, Gabrieli P, South A, Valim C, Mancini F, Catteruccia F. The interaction between a sexually transferred steroid hormone and a female protein regulates oogenesis in the malaria mosquito Anopheles gambiae. PLoS Biol. 2013; 11(10):e1001695. Epub 2013/11/10. https://doi.org/10.1371/journal.pbio.1001695 PMID: 24204210; PubMed Central PMCID: PMC3812110.

175. Gabrieli P, Kakani EG, Mitchell SN, Mameli E, Want EJ, Mariezcurrena Anton A, et al. Sexual transfer of the steroid hormone 20E induces the postmating switch in Anopheles gambiae. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111(46):16353–8. Epub 2014/11/05. 10.1073/pnas.1410488111. 25368171; PubMed Central PMCID: PMC4246312. https://doi.org/10.1073/pnas.1410488111 PMID: 25368171

176. Mitchell SN, Kakani EG, South A, Howell PI, Waterhouse RM, Catteruccia F. Mosquito biology. Evolution of sexual traits influencing vectorial capacity in anopheline mosquitoes. Science. 2015; 347 (6225):985–8. Epub 2015/02/28. 10.1126/science.1259435. 25722409; PubMed Central PMCID: PMC4373528. https://doi.org/10.1126/science.1259435 PMID: 25722409

177. Cai H, Zhou Z, Gu J, Wang Y. Comparative Genomics and Systems Biology of Malaria Parasites Plasmodium. Current bioinformatics. 2012; 7(4). Epub 2013/12/04. https://doi.org/10.2174/157489312803900965 PMID: 24298232; PubMed Central PMCID: PMCPMC3844129.

178. Rabinovich RN, Drakeley C, Djimde AA, Hall BF, Hay SI, Hemingway J, et al. malERA: An updated research agenda for malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002456. https://doi.org/10.1371/journal.pmed.1002456

179. de Koning-Ward TF, Gilson PR, Crabb BS. Advances in molecular genetic systems in malaria. Nature reviews Microbiology. 2015; 13(6):373–87. https://doi.org/10.1038/nrmicro3450 PMID: 25978707.

180. Oye KA, Esvelt K, Appleton E, Catteruccia F, Church G, Kuiken T, et al. Biotechnology. Regulating gene drives. Science. 2014; 345(6197):626–8. Epub 2014/07/19. https://doi.org/10.1126/science.1254287 science.1254287 [pii]. PMID: 25035410.

181. Laveran A. Note sur un nouveau parasite trouvé dans le sang de plusieurs malades atteints de fièvre palustres. Bull Acad Med. 1880; 9:1235–6.

182. Sturm A, Amino R, van de Sand C, Regen T, Retzlaff S, Rennenberg A, et al. Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids. Science. 2006; 313(5791):1287–90. https://doi.org/10.1126/science.1129720 PMID: 16888102.

183. Zimmerman PA, Howes RE. Malaria diagnosis for malaria elimination. Curr Opin Infect Dis. 2015; 28 (5):446–54. Epub 2015/07/24. https://doi.org/10.1097/QCO.0000000000000191 PMID: 26203855.

184. Berna AZ, McCarthy JS, Wang RX, Saliba KJ, Bravo FG, Cassells J, et al. Analysis of Breath Specimens for Biomarkers of Plasmodium falciparum Infection. The Journal of infectious diseases. 2015; 212(7):1120–8. Epub 2015/03/27. https://doi.org/10.1093/infdis/jiv176 jiv176 [pii]. PMID: 25810441; PubMed Central PMCID: PMC4559192.

185. The malERA Refresh Consultative Panel on Basic Science and Enabling Technologies. An updated research agenda for basic science and enabling technologies in malaria elimination and eradication. 2017. 10.1371/journal.pmed.1002451.

186. Yanez-Mo M, Siljander PR, Andreu Z, Zavec AB, Borras FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. Journal of extracellular vesicles. 2015; 4:27066. https://doi.org/10.3402/jev.v4.27066 PMID: 25979354; PubMed Central PMCID: PMC4433489.

187. Lin J, Li J, Huang B, Liu J, Chen X, Chen XM, et al. Exosomes: novel biomarkers for clinical diagnosis. TheScientificWorldJournal. 2015; 2015:657086. https://doi.org/10.1155/2015/657086 PMID: 25695100; PubMed Central PMCID: PMC4322857.

188. Campos FM, Franklin BS, Teixeira-Carvalho A, Filho AL, de Paula SC, Fontes CJ, et al. Augmented plasma microparticles during acute Plasmodium vivax infection. Malaria journal. 2010; 9:327. https://doi.org/10.1186/1475-2875-9-327 PMID: 21080932; PubMed Central PMCID: PMC2998527.

189. Nantakomol D, Dondorp AM, Krudsood S, Udomsangpetch R, Pattanapanyasat K, Combes V, et al. Circulating red cell-derived microparticles in human malaria. The Journal of infectious diseases. 2011;

203(5):700–6. https://doi.org/10.1093/infdis/jiq104 PMID: 21282195; PubMed Central PMCID: PMC3072726.

190. Martin-Jaular L, Nakayasu ES, Ferrer M, Almeida IC, Del Portillo HA. Exosomes from Plasmodium yoelii-infected reticulocytes protect mice from lethal infections. PloS one. 2011; 6(10):e26588. https://doi.org/10.1371/journal.pone.0026588 PMID: 22046311; PubMed Central PMCID: PMC3202549.

191. Mantel PY, Hoang AN, Goldowitz I, Potashnikova D, Hamza B, Vorobjev I, et al. Malaria-infected erythrocyte-derived microvesicles mediate cellular communication within the parasite population and with the host immune system. Cell host & microbe. 2013; 13(5):521–34. https://doi.org/10.1016/j.chom.2013.04.009 PMID: 23684304; PubMed Central PMCID: PMC3687518.

192. Regev-Rudzki N, Wilson DW, Carvalho TG, Sisquella X, Coleman BM, Rug M, et al. Cell-cell communication between malaria-infected red blood cells via exosome-like vesicles. Cell. 2013; 153(5):1120–33. https://doi.org/10.1016/j.cell.2013.04.029 PMID: 23683579.

193. Dormitzer PR, Grandi G, Rappuoli R. Structural vaccinology starts to deliver. Nature reviews Microbiology. 2012; 10(12):807–13. https://doi.org/10.1038/nrmicro2893 PMID: 23154260.

194. Kulp DW, Schief WR. Advances in structure-based vaccine design. Current opinion in virology. 2013; 3(3):322–31. https://doi.org/10.1016/j.coviro.2013.05.010 PMID: 23806515; PubMed Central PMCID: PMC4102719.

195. Welsh RM, Fujinami RS. Pathogenic epitopes, heterologous immunity and vaccine design. Nature reviews Microbiology. 2007; 5(7):555–63. https://doi.org/10.1038/nrmicro1709 PMID: 17558423.

196. Li H, O'Donoghue AJ, van der Linden WA, Xie SC, Yoo E, Foe IT, et al. Structure- and function-based design of Plasmodium-selective proteasome inhibitors. Nature. 2016; 530(7589):233–6. Epub 2016/02/13. https://doi.org/10.1038/nature16936 nature16936 [pii]. PMID: 26863983; PubMed Central PMCID: PMC4755332.

197. Sun M, Li W, Blomqvist K, Das S, Hashem Y, Dvorin JD, et al. Dynamical features of the Plasmodium falciparum ribosome during translation. Nucleic Acids Res. 2015; 43(21):10515–24. Epub 2015/10/04. https://doi.org/10.1093/nar/gkv991 gkv991 [pii]. PMID: 26432834; PubMed Central PMCID: PMC4666399.

198. Wong W, Bai XC, Brown A, Fernandez IS, Hanssen E, Condron M, et al. Cryo-EM structure of the Plasmodium falciparum 80S ribosome bound to the anti-protozoan drug emetine. Elife. 2014; 3. Epub 2014/06/11. https://doi.org/10.7554/eLife.03080 PMID: 24913268; PubMed Central PMCID: PMC4086275.

199. Neafsey DE, Juraska M, Bedford T, Benkeser D, Valim C, Griggs A, et al. Genetic Diversity and Protective Efficacy of the RTS,S/AS01 Malaria Vaccine. N Engl J Med. 2015; 373(21):2025–37. Epub 2015/10/22. https://doi.org/10.1056/NEJMoa1505819 PMID: 26488565; PubMed Central PMCID: PMCPMC4762279.

200. Mueller I, Shakri AR, Chitnis CE. Development of vaccines for Plasmodium vivax malaria. Vaccine. 2015; 33(52):7489–95. Epub 2015/10/03. https://doi.org/10.1016/j.vaccine.2015.09.060 S0264-410X(15)01336-5 [pii]. PMID: 26428453.

201. Hovlid ML, Winzeler EA. Phenotypic Screens in Antimalarial Drug Discovery. Trends in parasitology. 2016; 32(9):697–707. Epub 2016/06/02. https://doi.org/10.1016/j.pt.2016.04.014 PMID: 27247245; PubMed Central PMCID: PMCPMC5007148.

202. Williamson AE, Ylioja PM, Robertson MN, Antonova-Koch Y, Avery V, Baell JB, et al. Open Source Drug Discovery: Highly Potent Antimalarial Compounds Derived from the Tres Cantos Arylpyrroles. ACS central science. 2016; 2(10):687–701. Epub 2016/11/02. https://doi.org/10.1021/acscentsci.6b00086 PMID: 27800551; PubMed Central PMCID: PMCPMC5084075.

203. Van Voorhis WC, Adams JH, Adelfio R, Ahyong V, Akabas MH, Alano P, et al. Open Source Drug Discovery with the Malaria Box Compound Collection for Neglected Diseases and Beyond. PLoS pathogens. 2016; 12(7):e1005763. Epub 2016/07/29. https://doi.org/10.1371/journal.ppat.1005763 PMID: 27467575; PubMed Central PMCID: PMCPMC4965013.

204. Spangenberg T, Burrows JN, Kowalczyk P, McDonald S, Wells TN, Willis P. The open access malaria box: a drug discovery catalyst for neglected diseases. PloS one. 2013; 8(6):e62906. Epub 2013/06/27. https://doi.org/10.1371/journal.pone.0062906 PMID: 23798988; PubMed Central PMCID: PMCPMC3684613.

205. Bando H, Okado K, Guelbeogo WM, Badolo A, Aonuma H, Nelson B, et al. Intra-specific diversity of Serratia marcescens in Anopheles mosquito midgut defines Plasmodium transmission capacity. Sci Rep. 2013; 3:1641. Epub 2013/04/11. https://doi.org/10.1038/srep01641 PMID: 23571408; PubMed Central PMCID: PMC3622076.

206. Cirimotich CM, Dong Y, Clayton AM, Sandiford SL, Souza-Neto JA, Mulenga M, et al. Natural microbe-mediated refractoriness to Plasmodium infection in Anopheles gambiae. Science. 2011; 332

(6031):855–8. https://doi.org/10.1126/science.1201618 PMID: 21566196; PubMed Central PMCID: PMC4154605.

207. Dennison NJ, BenMarzouk-Hidalgo OJ, Dimopoulos G. MicroRNA-regulation of Anopheles gambiae immunity to Plasmodium falciparum infection and midgut microbiota. Dev Comp Immunol. 2015; 49 (1):170–8. Epub 2014/12/03. https://doi.org/10.1016/j.dci.2014.10.016 PMID: 25445902; PubMed Central PMCID: PMC4447300.

208. Ramirez JL, Short SM, Bahia AC, Saraiva RG, Dong Y, Kang S, et al. Chromobacterium Csp_P reduces malaria and dengue infection in vector mosquitoes and has entomopathogenic and in vitro anti-pathogen activities. PLoS pathogens. 2014; 10(10):e1004398. https://doi.org/10.1371/journal.ppat.1004398 PMID: 25340821; PubMed Central PMCID: PMC4207801.

209. Bian G, Zhou G, Lu P, Xi Z. Replacing a native Wolbachia with a novel strain results in an increase in endosymbiont load and resistance to dengue virus in a mosquito vector. PLoS Negl Trop Dis. 2013; 7 (6):e2250. Epub 2013/06/12. https://doi.org/10.1371/journal.pntd.0002250 PMID: 23755311; PubMed Central PMCID: PMC3675004.

210. Shaw WR, Marcenac P, Childs LM, Buckee CO, Baldini F, Sawadogo SP, et al. Wolbachia infections in natural Anopheles populations affect egg laying and negatively correlate with Plasmodium development. Nat Commun. 2016; 7:11772. https://doi.org/10.1038/ncomms11772 PMID: 27243367.

211. Baldini F, Segata N, Pompon J, Marcenac P, Shaw WR, Dabire RK, et al. Evidence of natural Wolbachia infections in field populations of Anopheles gambiae. Nat Commun. 2014; 5:3985. https://doi.org/10.1038/ncomms4985 PMID: 24905191; PubMed Central PMCID: PMC4059924.

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COLLECTION REVIEW

malERA: An updated research agenda for insecticide and drug resistance in malaria elimination and eradication

The malERA Refresh Consultative Panel on Insecticide and Drug Resistance[¶]*

¶ Membership of the malERA Refresh Consultative Panel on Insecticide and Drug Resistance is provided in the Acknowledgments.

* janet.hemingway@lstmed.ac.uk (JH); adjimde@icermali.org (AD)

Citation: The malERA Refresh Consultative Panel on Insecticide and Drug Resistance (2017) malERA: An updated research agenda for insecticide and drug resistance in malaria elimination and eradication. PLoS Med 14(11): e1002450. https://doi.org/10.1371/journal.pmed.1002450

Published: November 30, 2017

Funding: MESA received a grant from the Bill & Melinda Gates Foundation (http://www.malariaeradication.org/mesa-track/malaria-eradication-scientific-alliance-mesa, OPP1034591). MR (Bill & Melinda Gates Foundation) was a member of this Panel and participated in the Panel consultation and in the development of this paper.

Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: RR is Chair of the MESA (Malaria Eradication Scientific Alliance) Steering Committee, and SR is Portfolio Manager for IVCC (Innovative Vector Control Consortium).

Abstract

Resistance to first-line treatments for *Plasmodium falciparum* malaria and the insecticides used for *Anopheles* vector control are threatening malaria elimination efforts. Suboptimal responses to drugs and insecticides are both spreading geographically and emerging independently and are being seen at increasing intensities. Whilst resistance is unavoidable, its effects can be mitigated through resistance management practices, such as exposing the parasite or vector to more than one selective agent. Resistance contributed to the failure of the 20th century Global Malaria Eradication Programme, and yet the global response to this issue continues to be slow and poorly coordinated—too often, too little, too late. The Malaria Eradication Research Agenda (malERA) Refresh process convened a panel on resistance of both insecticides and antimalarial drugs. This paper outlines developments in the field over the past 5 years, highlights gaps in knowledge, and proposes a research agenda focused on managing resistance. A deeper understanding of the complex biological processes involved and how resistance is selected is needed, together with evidence of its public health impact. Resistance management will require improved use of entomological and parasitological data in decision making, and optimisation of the useful life of new and existing products through careful implementation, combination, and evaluation. A proactive, collaborative approach is needed from basic science and the development of new tools to programme and policy interventions that will ensure that the armamentarium of drugs and insecticides is sufficient to deal with the challenges of malaria control and its elimination.

Summary points

- Since 2011, significant progress has been made in understanding resistance. Surveillance has been expanded and improved in many malaria-endemic countries and there is a better understanding of the genetic basis of resistance, identifying some molecular markers that can be used to track its emergence and spread. Better tools to measure and manage the intensity of resistance are available.

Abbreviations: ACT, artemisinin-based combination therapy; DP, dihydroartemisinin-piperaquine; GMS, Greater Mekong Subregion; GPARC, Global Plan for Artemisinin Resistance Containment; GPIRM, Global Plan for Insecticide Resistance Management; IPTp, intermittent preventive treatment in pregnancy; IRS, indoor residual spraying; IVCC, Innovative Vector Control Consortium; K13, Kelch 13 (PF3D7_1343700); LLIN, long-lasting insecticidal net; malERA, Malaria Eradication Research Agenda; MESA, Malaria Eradication Scientific Alliance; MMV, Medicines for Malaria Venture; pfcrt, *P. falciparum* chloroquine resistance transporter; pfdhfr, *P. falciparum* dihydrofolate reductase; pfdhps, *P. falciparum* dihydropteroate synthase; pfmdr1, *P. falciparum* multidrug resistance 1; PSA, piperaquine survival assay; SP, sulfadoxine-pyrimethamine; pvmdr1, *P. vivax* multidrug resistance 1.

Provenance: Submitted as part of a Supplement; externally peer reviewed.

- However, our response to increases in the prevalence and intensity of resistance has been slow and reactive. A promising pipeline of new vector control tools and therapeutics is in development, but all actors in the malaria community need to plan proactively how to implement, integrate, and evaluate these products.
- Quantifying the public health impact of resistance has been difficult, particularly for insecticides. For both insecticides and drugs, defining the minimum essential evidence required for policy makers to manage resistance and ensuring that programs employ rigorous quality assurance in collecting and managing these data are critical.
- As malaria control increases, the selection pressure on the parasite or mosquito vector increases. Strategies for resistance management are therefore crucial for all stages of elimination. Countries need to allocate funding and human resources to effectively manage the threat of resistance and sustain the gains achieved to date.
- This paper reviews the current knowledge base and identifies research priorities addressing resistance to drugs and insecticides. It is a result of a unique collaborative effort of experts in drug and insecticide resistance brought together for the malERA Refresh process.

Introduction and rationale

Over the past decade, unprecedented progress has been made in reducing malaria morbidity and mortality [1]. However, growing resistance to the first-line treatment for *P. falciparum* malaria, artemisinin-based combination therapies (ACTs), and the insecticides used to suppress mosquito vectors threaten the sustainability of recent gains in malaria control and longer-term prospects for elimination.

Vector control and antimalarial treatment depend on a limited armamentarium, and when single drugs and insecticides are widely deployed, selection pressure is intense and the emergence of resistant parasites and mosquitoes is inevitable.

Drug and insecticide resistance were crosscutting issues in the original malERA (Malaria Eradication Research Agenda) series in 2011 [2]. However, the parasite and vector communities rarely interact. The increasing urgency of these issues and the contrasting operational responses warranted a dedicated panel in the malERA Refresh process. The failure of drug treatment has human consequences: recurrent parasitaemia, severe malaria, anaemia, and associated morbidity and mortality. In the early 2000s, resistance to single antimalarials led to policy changes recommending deployment of ACTs [3]. In contrast, resistance to the most widely used class of insecticide, pyrethroids, was first documented in the 1980s, but pyrethroid monotherapies still dominate current control efforts [4].

This paper aims to review developments in drug and insecticide resistance over the past 5 years (Box 1), discuss gaps in knowledge, and identify key research priorities (Box 2).

Methods

The findings presented in this paper result from an extensive literature review of published and unpublished materials and the deliberations of the 2015 malERA Refresh Consultative Panel on Insecticide and Drug Resistance. Electronic databases were systematically searched

Box 1. Progress over the past 5 years in drug and insecticide resistance research

- A promising pipeline of new therapeutics, insecticides, and noninsecticidal vector control tools is in development, largely due to the work of the Medicines for Malaria Venture (MMV) and the Innovative Vector Control Consortium (IVCC)
- Recognition of the impact and importance of drug and insecticide resistance with the creation of the WHO Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM) and WHO Global Plan for Artemisinin Resistance Containment (GPARC)
- Identification of genes and molecular markers associated with drug and insecticide resistance
- Improved understanding of resistance mechanisms in parasite and vector populations
- Global databases to monitor drug and insecticide resistance
- Development of new tools to study resistance in vivo and in vitro, e.g., ring-stage survival assay, parasite clearance estimator, human blood-stage challenge studies for drug resistance, and bioassays that measure the intensity of insecticide resistance

for published literature between January 1, 2010, and November 2, 2015, without language limitations. Panellists were invited to recommend additional literature. A 2-day workshop was held with the majority of the panel members, including specialists from basic science and product development, field researchers, and WHO representatives. The panel broke into 2 working groups to identify the problems that need to be solved in insecticide and drug resistance and what research is needed to address these problems. Each group fed back to the plenary session, in which further robust discussions and input occurred. This helped refine the opportunities and gap areas in which research is needed. The final findings were arrived at with input from all panellists and several iterations of the manuscript.

What do we know about resistance?

Insecticides for malaria vector control are limited to pyrethroids for long-lasting insecticidal nets (LLINs) and pyrethroids, organochlorines, organophosphates, and carbamates for indoor residual spraying (IRS). Vector resistance has been detected across Africa to all insecticide classes. However, resistance to the pyrethroids is the most widespread [5]. In Asia, insecticide resistance is common in some *Anopheles* species [6]. Sixty countries have reported resistance to at least 1 insecticide, but the scale of the problem is likely to be much greater [7].

Despite ubiquitous pyrethroid resistance in some areas, millions of pyrethroid-impregnated nets are distributed annually. Once distributed, these nets can contribute to the selection of resistant vectors for the duration of their 3-year life. In Burkina Faso, the intensity of the pyrethroid resistance seen in *A. gambiae* increased 10-fold in a single year [8], and this trend is apparent in multiple locations throughout Africa [9]. *A. funestus* also exhibits resistance to multiple insecticides at increasing intensities [10–12]. Proactive defensive strategies are critical

Box 2. Research and development agenda for drug and insecticide resistance

Crosscutting issues for drug and insecticide resistance

Applied research.

- Use in vitro, in vivo, and mathematical models to identify new combinations of drugs and insecticides, and understand how mechanisms of action and mechanisms of resistance inform this
- Determine which conditions are optimal for the emergence and spread of drug and insecticide resistance and how these can be minimised
- Evaluate whether resistance management strategies can restore susceptibility to drugs and insecticides
- Evaluate how new intervention types/paradigms should be introduced and assessed to limit the selection of resistant phenotypes
- Evaluate the optimal surveillance systems for resistance and determine the appropriate data that must be collected (including technical approach, frequency, geography, and temporal–spatial factors)
- Determine and validate the relationships between molecular markers and parasite/vector resistance phenotypes in different transmission settings

Policy and advocacy.

- Develop a framework to cost-elimination strategies that accounts for resistance management practices and increasing cost per case of malaria/malaria death averted and identify sources of funding for these strategies
- Agree on the process and minimum data required by the normative bodies to enable a new drug or insecticide product to complete the route to market
- Devise market strategies and incentives to ensure a mix of drug and insecticide products remains available and is used strategically to manage resistance
- Assess which decision-support systems can efficiently and rationally be adapted to drug and insecticide policies
- Determine the minimum dataset required to guide drug and insecticide resistance management and the level of evidence required to switch to new drug or insecticide strategies

Insecticide resistance

- Analyse the most cost-effective ways of slowing the spread and emergence of insecticide resistance (e.g., by using a combination of interventions, spatial mosaics, or mixtures of insecticides)
- Determine which spatial and temporal scale insecticide resistance management strategies should be carried out

- Study how much insecticide resistance has a negative impact on mosquito fitness survival or parasite development in the mosquito and investigate how this compares for different active ingredients
- Develop a method to assess the age of resistant mosquitoes
- Define the optimal use of bioassays and molecular markers to accurately predict the efficacy of vector control in relation to insecticide resistance
- Study the mechanisms of mosquito behavioural resistance and assess if this is sustained across generations
- Assess which novel, noninsecticidal tools for controlling mosquito populations would help to slow or prevent the emergence of resistance or restore susceptibility

Drug resistance

- Evaluate if the timing of community-based prevention, e.g., mass drug administration, can be optimised to reduce the risk of emerging drug resistance
- Investigate why drugs such as quinine are less likely to develop resistance and use this knowledge for future drug development
- Determine which approaches are most sensitive and specific to determine true drug treatment efficacy (e.g., molecular correction) in *P. falciparum* and *P. vivax* parasites
- Define what studies are required by policy makers to evaluate the use of multiple therapies
- Define the minimal criteria for inclusion of existing and new drugs in multiple agent regimes (e.g., efficacy, resistance, pharmacokinetic factors, and drug–drug interactions) and whether these criteria change in different programmatic modes
- Study the extent to which human immunity masks the presence of drug resistance, especially resistance to artemisinins

to reducing the spread and emergence of resistant phenotypes and preventing broad-spectrum cross resistance to multiple insecticides.

In the case of the antimalarials chloroquine and sulfadoxine-pyrimethamine (SP), resistant *P. falciparum* and *P. vivax* parasites evolved in the Greater Mekong Subregion (GMS) and the island of Papua and South America, respectively [13,14]. Retrospective analysis of molecular markers showed resistant *P. falciparum* parasites spread from Southeast Asia foci across Asia and throughout Africa over several decades [15–18]. ACTs were promoted to prevent or retard the selection of resistance by simultaneously administering 2 drug components with different modes of action [19]. However, resistance to artemisinins and their partner drugs is spreading and emerging independently among *P. falciparum* populations in the GMS [20–23].

Identifying resistance

Two main mechanisms of insecticide resistance have been identified: target site mutations (such as *kdr* and *ace)* [24,25] and metabolic resistance involving mutation, duplication, or

altered regulation of enzymes and transporters that increase insecticide metabolism or excretion. Metabolic resistance has greater implications for malaria vector control because the efficacy of a range of insecticides is usually affected [5,26].

Routine monitoring of insecticide susceptibility uses phenotypic bioassays that expose live mosquitoes to a single dose of a given insecticide over a fixed time period and measure mortality. The results are highly variable; hence, more laborious methods utilising a range of insecticide concentrations may be needed [27]. These assays have local utility but are often logistically challenging. Larger numbers of mosquitoes can be screened using molecular techniques, although it is unclear under what conditions validated molecular markers could serve as a replacement for phenotypic assays or if this might be appropriate for malaria control programmes [28].

The mechanisms of insecticide resistance can manifest as major changes in the insect nervous system or metabolome. Resistance may have an effect on insect longevity, mating competitiveness, and vectorial capacity [29,30]. Alongside physiological resistance, there is potentially also behavioural resistance, as increased mosquito numbers that bite or rest outdoors have been observed. There is limited evidence on the genetic basis of behavioural resistance, but determining whether vector control interventions are selecting a heritable trait warrants further research [31].

Resistance to artemisinins is assessed in clinical studies by measuring the parasite clearance in a patient in the first several days after treatment [32]. A lab-based assay that correlates with the in vivo parasite response to artemisinins has also been validated [33]. Mutations in the propeller domain of Kelch 13 (PF3D7_1343700) (K13) were identified as a major determinant of artemisinin resistance and may be reliable molecular markers in the GMS [34,35]. Outside the GMS, parasites with K13 mutant alleles are present in many areas at low levels; there is currently no molecular evidence to suggest that these alleles are being selected [22,36–38]. More than 100 K13 mutant alleles have been reported outside of Southeast Asia [22,38–40], but none have yet been associated with the slow-clearing phenotype [41]. One hypothesis is that artemisinin resistance may require additional genetic determinants in these locations to allow selection of K13 mutant parasites that exhibit the slow-clearing phenotype in vivo [20,42]. Nevertheless, the adoption of molecular markers to monitor drug resistance has been much faster than markers to assess insecticide resistance.

Molecular markers correlated with resistance to nonartemisinin antimalarials have also been identified. Polymorphisms or multicopy numbers in the *P. falciparum* chloroquine resistance transporter (*pfcrt*) and *P. falciparum* multidrug resistance 1 (*pfmdr1*) genes have been associated with resistance to chloroquine and mefloquine [43,44] and polymorphisms or multicopy numbers in the *P. falciparum* dihydrofolate reductase (*pfdhfr*) and *P. falciparum* dihydropteroate synthase (*pfdhps*) genes have been associated with resistance to SP [45]. Changes in the prevalence of *pfcrt* and *pfmdr1* alleles have been observed in many areas where ACTs including amodiaquine or lumefantrine have been intensively used [46,47]. However, clinical efficacy of leading ACTs that include lumefantrine, amodiaquine, piperaquine, or mefloquine appears to remain acceptable in areas outside the GMS. Recent research suggests that plasmepsin 2–3 is associated with clinical and in vitro piperaquine resistance (PSA, piperaquine survival assay) but other markers could also be involved [48]. In Southeast Asia, intensive use of dihydroartemisinin-piperaquine (DP) in parasites already resistant to piperaquine and artemisinin has selected parasites with multiple resistance mechanisms, and high levels of treatment failure to DP are now observed in Cambodia [49].

Chloroquine remains the recommended treatment for *P. vivax*, but resistance and declining efficacy has been noted in several populations, and ACTs are recommended in some areas [50,51]. There are no standardized molecular correlates of chloroquine resistance for *P. vivax*,

but *P. vivax* multidrug resistance 1 (*pvmdr1*) has been associated with resistance [52]. Beyond this, the understanding of resistance in nonfalciparum malaria is very limited.

Public health impact of resistance

While ecological studies have found broad evidence of dramatic health effects of spreading drug resistance [18], efficient assessment of the public health impact of antimalarial and insecticide resistance has been difficult. First, assessments of resistance prevalence are drawn from a few sentinel sites, but the heterogeneity of resistance in neighbouring populations can be enormous, making specific predictions difficult. Second, molecular markers are easier to measure at finer spatial and temporal scales, but the relationship with the drug or insecticide response is not direct [53,54]. Third, most policies on malaria treatment and vector control are implemented nationally, so recommending policies for regions within a country may be operationally unfeasible.

Drug resistance increases the risk of treatment failure and therefore transmission, but these relationships can be difficult to establish in the field. Human factors, especially immunity, affect treatment efficacy, so treatment failure in the whole population is not obvious until parasite resistance is well established [55]. However, in children there is a clear relationship between parasitaemia and anaemia, with associated morbidity and mortality [55,56]. Studies have correlated the prevalence of molecular markers with the risk of treatment failure, but no metric that works in all regions has been defined [57]. As a result, the prevalence of molecular markers has had a limited impact on policies for routine antimalarial use [58]. This disconnect is changing in the GMS, where ACT treatment failure has reached crisis levels [59], and rapid assessments of molecular markers for resistance to artemisinins and partner drugs are currently being used [47].

There are few published studies on the epidemiological impact of insecticide resistance, so decisions rely primarily on entomological end points. Evidence from a 5-country evaluation attempted to assess whether LLINs remain effective in the presence of pyrethroid resistance, although the studies were in areas with low to moderate resistance as measured in single-dose bioassays without assessment of resistance intensity [60]. This study was not able to quantify the effect on LLINs [59]. For IRS, the best evidence for an epidemiological impact of pyrethroid resistance comes from settings where pyrethroids were replaced in IRS campaigns with alternative insecticides and parasite prevalence rapidly declined [61, 62]. Similar evidence is available from a study in an area of Sudan with pyrethroid resistance but carbamate susceptibility, in which IRS with pyrethroids in addition to LLINs had no added impact, but changing to carbamate IRS halved the malaria incidence [60].

Managing resistance, moving toward elimination

Optimizing drug and insecticide use. Avoiding parasite or mosquito population exposure to a single selective agent is the central principle of resistance management. Ideally, insecticidal compounds with different modes of action should be used simultaneously or in spatial or temporal rotation. These principles, which are identical to those used in the management of insecticides used for crop pests, have been outlined in the GPIRM in malaria vectors [63]. Unfortunately, implementation has been challenging; pyrethroid resistance is ubiquitous, non-pyrethroid LLINs are not currently available, and other forms of vector control can significantly increase costs [64]. New public health insecticides with different modes of action are on the horizon [65], but we lack information on the effectiveness of the proposed strategies to slow the emergence or spread of insecticide resistance, and there is no clear indication of how they should be integrated alongside existing tools. This includes those that are noninsecticidal

and products that work on different targets, e.g., spatial repellents and endocticides, whose efficacy may not be influenced by insecticide resistance [65]. Another confounder is the application of most insecticides for both agricultural and public health use. The impact of this on public health is highly variable depending on crop type and volume and timing of insecticide application.

What are the benefits of insecticide rotations, mixtures, or spatial mosaics of different compounds? What is the impact of adding nonpyrethroid IRS where LLINs are already deployed at high coverage and quality? When should new insecticides be adopted? What is the ideal rotation period or mosaic configuration? How many insecticide classes are needed for effective rotation or mosaic strategies? Despite the absence of data to answer these questions, some countries have already developed operational frameworks for resistance management that could be adopted by other programmes [66].

ACTs are still effective in most regions outside the GMS. Optimisation of dose, duration of treatment, timing of treatment, and pharmacokinetic-dynamic profiles in specific subpopulations, e.g., children and pregnant women, should be systematically encouraged post-licensure to maximise efficacy and slow selection for resistance. Pooled analyses have assessed the effect of dosing strategies for the several currently used ACTs, but the uptake of this by malaria control programmes is limited [67]. Molecular markers are being used in addition to therapeutic efficacy studies in specific locations in the GMS to choose treatment policies more accurately [67], but far more complete information on all ACTs is needed.

Different published models diverged on the conclusion that implementation of multiple first-line therapies could more effectively prevent the emergence of drug resistance compared with the temporal rotation or sequential use of first-line treatments [68–71]. Multiple models need to be evaluated and studies to verify this must be defined [72]. We also need to better understand why parasites do not seem to have developed resistance to quinine and factor this into future drug development efforts. In Southeast Asia, the use of triple therapies using existing antimalarials is currently being tested and could be considered in the context of multidrug-resistant malaria [73].

Assessment of the selective pressures and emergence of resistance to antimalarials is difficult with small-scale studies, but large-scale public health interventions may provide evidence. For example, studies should be undertaken in countries using different drug combinations for treatment and mass chemoprevention campaigns, such as seasonal malaria chemoprevention, mass drug administration, or intermittent preventive treatment in pregnancy (IPTp). Coordination of these interventions in the same locality may provide one way to reduce or disrupt the selection pressure exerted on a single class of compound [74].

Using data to support resistance management. Entomological data generated by countries vary in quantity and quality, and limited information flow between entomologists, programme managers, and research institutes has hindered advocacy efforts around improved resistance management. Linking entomological data to epidemiological outcomes is extremely complex [75] and by the time resistance has a demonstrable public health impact, it may be too late to intervene against it. However, South Africa [62], Zambia [76], and Equatorial Guinea [64] have resistance management plans in place. Similarly, molecular marker surveillance can inform which drug regimens are the most suitable for particular programmatic modes. This approach is now routine in some African countries [74,77] but is not universal. Drug-resistance monitoring in some countries also requires strengthening, and despite the tighter link to public health impact, the ability to respond rapidly may be lost if resistance monitoring is not well embedded. For both insecticides and drugs, defining the minimum essential data required for policy makers to manage resistance and ensuring that programs employ rigorous quality assurance in collecting and managing these data are critical.

Resistance surveillance is weak in many endemic countries. Inadequate attention and funding have been allocated to entomological monitoring and insecticide susceptibility research. Several countries in Africa have established sentinel sites for the longitudinal monitoring of insecticide resistance [5]. However, the methods, timing, and sampling are inconsistent, making meaningful inferences difficult [78]. Most of these sites use discriminating dose assays [79]. All bioassays are performed on 3–5-day-old mosquitoes under standard insectary conditions, so the effect of natural mosquito traits (e.g., age, blood-feeding status, circadian rhythm [80,81], and climatic variables [82]) on resistance is not assessed or reported [83]. Molecular species identification of mosquitoes undergoing resistance tests may also increase accuracy when compared to morphological identification. Techniques have been developed to measure the age distribution of mosquito populations in the laboratory [84], but a more precise, low-cost, field-applicable method is needed to allow malaria control programmes to evaluate the efficacy of vector control interventions is needed.

Anticipating the challenges of lower transmission. High-level use of interventions can suppress malaria transmission but also increases the risk of selection of resistance, creating new challenges at the later stages of elimination. Resistance surveillance in low transmission regions is increasingly expensive, and maintaining human and material capacity in the context of many other public health needs is crucial. The minimal criteria for the inclusion of new or existing therapeutics or insecticides in a multi-agent regimen must be defined. For drugs, these criteria might depend on transmission levels and could include pharmacokinetic-dynamic profiles, mechanisms of resistance, cross resistance, and drug–drug interactions. The corresponding parameters for insecticides of persistence/residual efficacy, mechanisms of resistance/cross resistance, or compound interactions are equally relevant. If a robust resistant phenotype can be defined, whole genome sequencing of parasites and vectors can identify regions under selection very early in the process, giving clues to associated genetic changes [85].

Market strategies and getting products to market. Single first-line antimalarial treatments or insecticide monotherapies may be cheaper in the short term, but the long-term cost-effectiveness will be compromised by increasing levels of resistance [86]. Development of normative guidance on product use within a multiyear programme of interventions is essential if short-term decision-making is to change. The selection of products may be based on a number of epidemiological, entomological, logistic, and financial variables. It is critical to develop a framework that reliably costs the long-term elimination strategies, rather than short-term 'delivered units', and takes into account resistance management practices. As we head toward elimination, the increased cost of keeping drugs and insecticides available for a diminishing number of cases means incentives and market strategies for keeping the pipeline of products active are paramount.

Clarity is needed on the evidence required by normative bodies to approve new products and develop treatment guidelines. New tools are likely to have a higher unit price, so clear data requirements and paths to their use require definition. Without this, programme financial constraints; uncertainties around cost-effectiveness; and delays in recommendation, production, and procurement could mean products to overcome resistance are underutilised. If this situation becomes the norm, incentives for innovation will diminish and the pipeline of efficacious tools will soon be depleted.

Conclusion

Resistance is an inevitable consequence of drug and insecticide treatment, but the malaria community as a whole has repeatedly failed to respond to this issue in a proactive way.

Programme and policy decisions should be based on comprehensive resistance data, and this should be coupled with improved efforts to understand the complex biological processes that select for resistant phenotypes. The tools to surmount resistance are limited and little is known about the most effective resistance management measures, so new therapeutics and vector control products should have a clear route to market and be carefully implemented and evaluated to optimise the choice of interventions. Multidrug and insecticide regimens are not unique to malaria control and other disease systems such as HIV [87], tuberculosis [88], and agricultural pest control [89] offer important insights into the management of insecticide- and drug-based approaches. The malaria community must learn from other disease groups and industries and heed the lessons of the past or risk further erosion of the malaria elimination agenda as renewed efforts are undermined by resistance.

Acknowledgments

The Malaria Eradication Research Agenda (malERA) Refresh Consultative Panel on Insecticide and Drug Resistance was chaired by Janet Hemingway (Liverpool School of Tropical Medicine) and cochaired by Abdoulaye Djimdé (University of Science, Techniques and Technology of Bamako). The paper was written based on consultations during a malERA meeting held in Barcelona, Spain, on December 1–2, 2015. Vittoria Lutje prepared systematic literature searches for the panel. Kim West, independent consultant, London, UK, provided editorial and writing support and was funded by the Malaria Eradication Scientific Alliance (MESA). Panel members reviewed several iterations of the manuscript to finalise it.

The following members of the writing group met the ICMJE criteria for authorship:

Thomas Churcher, Imperial College London, London, UK; Abdoulaye Djimdé, University of Science, Techniques and Technology of Bamako, Bamako, Mali; Janet Hemingway, Liverpool School of Tropical Medicine, Liverpool, UK; Regina Rabinovich, ISGlobal, Barcelona Institute for Global Health, Barcelona, Spain, and Harvard T.H. Chan School of Public Health, Boston, USA; Carol Hopkins Sibley, Worldwide Antimalarial Resistance Network and Genome Sciences, University of Washington, Seattle, USA.

The following were members of the Panel:

Thomas Churcher, Imperial College London, London, UK; Emmanuel Chanda, WHO Regional Office for Africa, Brazzaville, Republic of Congo; Maureen Coetzee, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; Miles Davenport, University of New South Wales, Sydney, Australia; Abdoulaye Diabate, Institut de Recherche en Sciences de la Santé, Ouagadougou, Burkina Faso; Abdoulaye Djimdé, University of Science, Techniques and Technology of Bamako, Bamako, Mali; Arjen M. Dondorp, Mahidol-Oxford Tropical Medicine Research Unit in Bangkok, Thailand; Martin Donnelly, Liverpool School of Tropical Medicine, Liverpool, UK, and Wellcome Trust Sanger Institute, Cambridge, UK; Janet Hemingway, Liverpool School of Tropical Medicine, Liverpool, UK; Silvie Huijben, ISGlobal, Barcelona Institute for Global Health, Barcelona, Spain; Patrick Kachur, Centers for Disease Control and Prevention, Atlanta, USA; Edwin Kamau, United States Army Medical Research Unit Kenya, Nairobi, Kenya; Tessa B. Knox, WHO Global Malaria Programme, Geneva, Switzerland; Neil F. Lobo, University of Notre Dame, Indiana, USA; Alfredo Mayor, ISGlobal, Barcelona Institute for Global Health, Barcelona, Spain, and Centro de Investigação em Saúde da Manhiça (CISM), Maputo, Mozambique; Didier Menard, Institut Pasteur in Cambodia, Malaria Molecular Epidemiology Unit, Phnom Penh, Cambodia; Krijn Paaijmans, ISGlobal, Barcelona Institute for Global Health, Barcelona, Spain; Stephane Picot, University of Lyon, Lyon, France; Christopher V. Plowe, University of Maryland School of Medicine, Maryland, USA; Ric Price, Centre for Tropical Medicine and Global Health, Nuffield

Department of Clinical Medicine, University of Oxford, UK, and Global and Tropical Health Division, Menzies School of Health Research and Charles Darwin University, Darwin, NT, Australia; Regina Rabinovich, ISGlobal, Barcelona Institute for Global Health, Barcelona, Spain, and Harvard T.H. Chan School of Public Health, Boston, USA; Hilary Ranson, Liverpool School of Tropical Medicine, Liverpool, UK; Mike Reddy, Bill & Melinda Gates Foundation, Seattle, USA; Sarah Rees, Innovative Vector Control Consortium, Liverpool, UK; Pascal Ringwald, WHO Global Malaria Programme, Geneva, Switzerland; Regina Rabinovich, ISGlobal, Barcelona Institute for Global Health, Barcelona, Spain, and Harvard T.H. Chan School of Public Health, Boston, USA; Carol Hopkins Sibley, Worldwide Antimalarial Resistance Network and Genome Sciences, University of Washington, Seattle, USA; Frederic Simard, Institut de Recherche pour le Développement, Montpellier, France; Charles S. Wondji, Liverpool School of Tropical Medicine, Liverpool, UK.

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References

1. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature.; 2015; 526: 207–211. https://doi.org/10.1038/nature15535 PMID: 26375008
2. Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, Collins F, et al. A research agenda to underpin malaria eradication. PLoS Med. 2011; 8: e1000406. https://doi.org/10.1371/journal.pmed.1000406 PMID: 21311579
3. White N, Nosten F, Looareesuwan S, Watkins W, Marsh K, Snow R, et al. Averting a malaria disaster. Lancet. 1999; 353: 1965–1967. https://doi.org/10.1016/S0140-6736(98)07367-X PMID: 10371589
4. Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, et al. Averting a malaria disaster: will insecticide resistance derail malaria control? Lancet. 2016; https://doi.org/10.1016/S0140-6736(15)00417-1
5. Knox TB, Juma EO, Ochomo EO, Pates Jamet H, Ndungo L, Chege P, et al. An online tool for mapping insecticide resistance in major Anopheles vectors of human malaria parasites and review of resistance status for the Afrotropical region. Parasit Vectors. 2014; 7: 76. https://doi.org/10.1186/1756-3305-7-76 PMID: 24559061
6. Dai Y, Huang X, Cheng P, Liu L, Wang H, Wang H, et al. Development of insecticide resistance in malaria vector Anopheles sinensis populations from Shandong province in China. Malar J. 2015; 14: 62. https://doi.org/10.1186/s12936-015-0592-8 PMID: 25880316
7. WHO | World Malaria Report 2015. WHO. World Health Organization; 2016
8. Toé KH, Jones CM, N'Fale S, Ismail HM, Dabiré RK, Ranson H. Increased pyrethroid resistance in malaria vectors and decreased bed net effectiveness, Burkina Faso. Emerg Infect Dis. 2014; 20: 1691–6. https://doi.org/10.3201/eid2010.140619 PMID: 25279965
9. Ranson H, Lissenden N. Insecticide Resistance in African Anopheles Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. Trends Parasitol. 2015; 32: 187–196. https://doi.org/10.1016/j.pt.2015.11.010 PMID: 26826784
10. Glunt KD, Abílio AP, Bassat Q, Bulo H, Gilbert AE, Huijben S, et al. Long-lasting insecticidal nets no longer effectively kill the highly resistant Anopheles funestus of southern Mozambique. Malar J. 2015; 14: 298. https://doi.org/10.1186/s12936-015-0807-z PMID: 26242977
11. Riveron JM, Chiumia M, Menze BD, Barnes KG, Irving H, Ibrahim SS, et al. Rise of multiple insecticide resistance in Anopheles funestus in Malawi: a major concern for malaria vector control. Malar J. 2015; 14: 344. https://doi.org/10.1186/s12936-015-0877-y PMID: 26370361
12. Hargreaves K, Koekemoer LL, Brooke BD, Hunt RH, Mthembu J, Coetzee M. Anopheles funestus resistant to pyrethroid insecticides in South Africa. Med Vet Entomol. 2000; 14: 181–9. Available: http://www.ncbi.nlm.nih.gov/pubmed/10872862. Date accessed 17 Sept 2017 PMID: 10872862

13. Schuurkamp GJ, Spicer PE, Kereu RK, Bulungol PK, Rieckmann KH. Chloroquine-resistant Plasmodium vivax in Papua New Guinea. Trans R Soc Trop Med Hyg. 86: 121–2. Available: http://www.ncbi.nlm.nih.gov/pubmed/1440763. Date accessed 17 Sept 2017 PMID: 1440763

14. Mehlotra RK, Fujioka H, Roepe PD, Janneh O, Ursos LMB, Jacobs-Lorena V, et al. Evolution of a unique Plasmodium falciparum chloroquine-resistance phenotype in association with pfcrt polymorphism in Papua New Guinea and South America. Proc Natl Acad Sci. 2001; 98: 12689–12694. https://doi.org/10.1073/pnas.221440898 PMID: 11675500

15. Wootton JC, Feng X, Ferdig MT, Cooper RA, Mu J, Baruch DI, et al. Genetic diversity and chloroquine selective sweeps in Plasmodium falciparum. Nature. 2002; 418: 320–3. https://doi.org/10.1038/nature00813 PMID: 12124623

16. Ariey F, Fandeur T, Durand R, Randrianarivelojosia M, Jambou R, Legrand E, et al. Invasion of Africa by a single pfcrt allele of South East Asian type. Malar J. 2006; 5: 34. https://doi.org/10.1186/1475-2875-5-34 PMID: 16638153

17. Roper C, Pearce R, Bredenkamp B, Gumede J, Drakeley C, Mosha F, et al. Antifolate antimalarial resistance in southeast Africa: a population-based analysis. Lancet. 2003; 361: 1174–81. https://doi.org/10.1016/S0140-6736(03)12951-0 PMID: 12686039

18. Mita T, Venkatesan M, Ohashi J, Culleton R, Takahashi N, Tsukahara T, et al. Limited geographical origin and global spread of sulfadoxine-resistant dhps alleles in Plasmodium falciparum populations. J Infect Dis. 2011; 204: 1980–8. https://doi.org/10.1093/infdis/jir664 PMID: 22021623

19. White NJ. Delaying antimalarial drug resistance with combination chemotherapy. Parassitologia. 1999; 41: 301–8. Available: http://www.ncbi.nlm.nih.gov/pubmed/10697872. Date accessed 17 Sept 2017 PMID: 10697872

20. Miotto O, Amato R, Ashley EA, MacInnis B, Almagro-Garcia J, Amaratunga C, et al. Genetic architecture of artemisinin-resistant Plasmodium falciparum. Nat Genet.; 2015; 47: 226–34. https://doi.org/10.1038/ng.3189 PMID: 25599401

21. Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, et al. Independent emergence of artemisinin resistance mutations among Plasmodium falciparum in Southeast Asia. J Infect Dis. 2015; 211: 670–9. https://doi.org/10.1093/infdis/jiu491 PMID: 25180241

22. Ménard D, Khim N, Beghain J, Adegnika AA, Shafiul-Alam M, Amodu O, et al. A Worldwide Map of Plasmodium falciparum K13-Propeller Polymorphisms. http://dx.doi.org/101056/NEJMoa1513137. Massachusetts Medical Society; 2016;

23. Rosenthal PJ, The interplay between drug resistance and fitness in malaria parasites. Mol. Microbiol 89, 1025–38 2013 https://doi.org/10.1111/mmi.12349 PMID: 23899091

24. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Bergé JB, Devonshire AL, et al. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector Anopheles gambiae s.s. Insect Mol Biol. 1998; 7: 179–84. Available: http://www.ncbi.nlm.nih.gov/pubmed/9535162 Date accessed 17 Sept 2017 PMID: 9535162

25. Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids. Insect Mol Biol. 2000; 9: 491–497. https://doi.org/10.1046/j.1365-2583.2000.00209.x PMID: 11029667

26. Strode C, Donegan S, Garner P, Enayati AA, Hemingway J. The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: systematic review and meta-analysis. PLoS Med. 2014; 11: e1001619. https://doi.org/10.1371/journal.pmed.1001619 PMID: 24642791

27. Bagi J, Grisales N, Corkill R, Morgan JC, N'Falé S, Brogdon WG, et al. When a discriminating dose assay is not enough: measuring the intensity of insecticide resistance in malaria vectors. Malar J. 2015; 14: 210. https://doi.org/10.1186/s12936-015-0721-4 PMID: 25985896

28. Weetman D, Donnelly MJ. Evolution of insecticide resistance diagnostics in malaria vectors. Trans R Soc Trop Med Hyg. 2015; 109: 291–3. https://doi.org/10.1093/trstmh/trv017 PMID: 25740955

29. Platt N, Kwiatkowska RM, Irving H, Diabaté A, Dabire R, Wondji CS. Target-site resistance mutations (kdr and RDL), but not metabolic resistance, negatively impact male mating competiveness in the malaria vector Anopheles gambiae. Heredity; 2015; 115: 243–52. https://doi.org/10.1038/hdy.2015.33 PMID: 25899013

30. Alout H, Ndam NT, Sandeu MM, Djégbe I, Chandre F, Dabiré RK, et al. Insecticide resistance alleles affect vector competence of Anopheles gambiae s.s. for Plasmodium falciparum field isolates. PLoS ONE. 2013; 8: e63849. https://doi.org/10.1371/journal.pone.0063849 PMID: 23704944

31. Gatton ML, Chitnis N, Churcher T, Donnelly MJ, Ghani AC, Godfray HCJ, et al. The importance of mosquito behavioural adaptations to malaria control in Africa. Evolution. 2013; 67: 1218–30. https://doi.org/10.1111/evo.12063 PMID: 23550770

32. White LJ, Flegg JA, Phyo AP, Wiladpai-ngern JH, Bethell D, Plowe C, et al. Defining the in vivo phenotype of artemisinin-resistant falciparum malaria: a modelling approach. PLoS Med. 2015; 12: e1001823. https://doi.org/10.1371/journal.pmed.1001823 PMID: 25919029

33. Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, et al. Novel phenotypic assays for the detection of artemisinin-resistant Plasmodium falciparum malaria in Cambodia: in-vitro and ex-vivo drug-response studies. Lancet Infect Dis. 2013; 13: 1043–9. https://doi.org/10.1016/S1473-3099(13)70252-4 PMID: 24035558

34. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois A-C, Khim N, et al. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature. 2014; 505: 50–5. https://doi.org/10.1038/nature12876 PMID: 24352242

35. Straimer J, Gnädig NF, Witkowski B, Amaratunga C, Duru V, Ramadani AP, et al. Drug resistance. K13-propeller mutations confer artemisinin resistance in Plasmodium falciparum clinical isolates. Science. 2015; 347: 428–31. https://doi.org/10.1126/science.1260867 PMID: 25502314

36. Taylor SM, Parobek CM, DeConti DK, Kayentao K, Coulibaly SO, Greenwood BM, et al. Absence of putative artemisinin resistance mutations among Plasmodium falciparum in Sub-Saharan Africa: a molecular epidemiologic study. J Infect Dis. 2015; 211: 680–8. https://doi.org/10.1093/infdis/jiu467 PMID: 25180240

37. Kamau E, Campino S, Amenga-Etego L, Drury E, Ishengoma D, Johnson K, et al. K13-propeller polymorphisms in Plasmodium falciparum parasites from sub-Saharan Africa. J Infect Dis. 2014/11/05. KEMRI/United States Army Medical Research Unit-Kenya, Kisumu. Wellcome Trust Sanger Institute, Hinxton. Navrongo Health Research Centre. National Institute for Medical Research, Tanga, Tanzania. Wellcome Trust Centre for Human Genetics, University of Oxford; 2015; 211: 1352–1355. https://doi.org/10.1093/infdis/jiu608 PMID: 25367300

38. World Health Organization. Status report on artemisinin and ACT resistance. 2015.

39. Chenet SM, Akinyi Okoth S, Huber CS, Chandrabose J, Lucchi NW, Talundzic E, et al. Independent Emergence of the Plasmodium falciparum Kelch Propeller Domain Mutant Allele C580Y in Guyana. J Infect Dis. 2016; 213: 1472–5. https://doi.org/10.1093/infdis/jiv752 PMID: 26690347

40. Dondorp AM. New genetic markers for piperaquine resistance in Plasmodium falciparum. Lancet Infectious Diseases http://dx.doi.org/10.1016/S1473-3099(16)30212-5 Date accessed 17 Sept 2017

41. Maiga AW, Fofana B, Sagara I, Dembele D, Dara A, Traore OB, et al. No evidence of delayed parasite clearance after oral artesunate treatment of uncomplicated falciparum malaria in Mali. Am J Trop Med Hyg. 2012; 87: 23–8. https://doi.org/10.4269/ajtmh.2012.12-0058 PMID: 22764287

42. Mok S, Ashley EA, Ferreira PE, Zhu L, Lin Z, Yeo T, et al. Drug resistance. Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science. 2015; 347: 431–5. https://doi.org/10.1126/science.1260403 PMID: 25502316

43. Chaorattanakawee S, Saunders DL, Sea D, Chanarat N, Yingyuen K, Sundrakes S, et al. Ex Vivo Drug Susceptibility Testing and Molecular Profiling of Clinical Plasmodium falciparum Isolates from Cambodia from 2008 to 2013 Suggest Emerging Piperaquine Resistance. Antimicrob Agents Chemother. 2015; 59: 4631–43. https://doi.org/10.1128/AAC.00366-15 PMID: 26014942

44. Leang R, Taylor WRJ, Bouth DM, Song L, Tarning J, Char MC, et al. Evidence of Plasmodium falciparum Malaria Multidrug Resistance to Artemisinin and Piperaquine in Western Cambodia: Dihydroartemisinin-Piperaquine Open-Label Multicenter Clinical Assessment. Antimicrob Agents Chemother. 2015; 59: 4719–26. https://doi.org/10.1128/AAC.00835-15 PMID: 26014949

45. Plowe C V, Kublin JG, Doumbo OK, Peters W, Jones A, Radloff P, et al. P. falciparum dihydrofolate reductase and dihydropteroate synthase mutations: epidemiology and role in clinical resistance to antifolates. Drug Resist Updat. 1998; 1: 389–96. https://doi.org/10.1016/s1368-7646(98)80014-9 PMID: 17092820

46. Conrad MD, LeClair N, Arinaitwe E, Wanzira H, Kakuru A, Bigira V, et al. Comparative impacts over 5 years of artemisinin-based combination therapies on Plasmodium falciparum polymorphisms that modulate drug sensitivity in Ugandan children. J Infect Dis. 2014; 210: 344–53. https://doi.org/10.1093/infdis/jiu141 PMID: 24610872

47. Thomsen TT, Madsen LB, Hansson HH, Tomás EVE, Charlwood D, Bygbjerg IC, et al. Rapid selection of Plasmodium falciparum chloroquine resistance transporter gene and multidrug resistance gene-1 haplotypes associated with past chloroquine and present artemether-lumefantrine use in Inhambane District, southern Mozambique. Am J Trop Med Hyg. 2013; 88: 536–41. https://doi.org/10.4269/ajtmh.12-0525 PMID: 23382159

48. WHO Global Malaria Programme. Minutes of the Technical Expert Group on Drug Efficacy and Response. Available: http://www.who.int/malaria/mpac/mpac-oct2017-teg-drug-efficacy-response-session3.pdf?ua=1. Date accessed 14 Nov 2017.

49. Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, et al. Dihydroartemisinin–piperaquine resistance in Plasmodium falciparum malaria in Cambodia: a multisite prospective cohort study. Lancet Infect Dis. 2016; 16: 357–365. https://doi.org/10.1016/S1473-3099(15)00487-9 PMID: 26774243

50. Phyo AP, Lwin KM, Price RN, Ashley EA, Russell B, Sriprawat K, et al. Dihydroartemisinin-piperaquine versus chloroquine in the treatment of Plasmodium vivax malaria in Thailand: a randomized controlled trial. Clin Infect Dis. 2011; 53: 977–84. https://doi.org/10.1093/cid/cir631 PMID: 22002979

51. Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant Plasmodium vivax: a systematic review and meta-analysis. Lancet Infect Dis. 2014; 14: 982–91. https://doi.org/10.1016/S1473-3099(14)70855-2 PMID: 25213732

52. Schousboe ML, Ranjitkar S, Rajakaruna RS, Amerasinghe PH, Morales F, Pearce R, et al. Multiple Origins of Mutations in the mdr1 Gene—A Putative Marker of Chloroquine Resistance in P. vivax. PLoS Negl Trop Dis. 2015; 9: e0004196. https://doi.org/10.1371/journal.pntd.0004196 PMID: 26539821

53. Venkatesan M, Gadalla NB, Stepniewska K, Dahal P, Nsanzabana C, Moriera C, et al. Polymorphisms in Plasmodium falciparum chloroquine resistance transporter and multidrug resistance 1 genes: parasite risk factors that affect treatment outcomes for P. falciparum malaria after artemether-lumefantrine and artesunate-amodiaquine. Am J Trop Med Hyg. 2014; 91: 833–43. https://doi.org/10.4269/ajtmh.14-0031 PMID: 25048375

54. Briët OJ, Penny MA, Hardy D, Awolola TS, Van Bortel W, Corbel V, et al. Effects of pyrethroid resistance on the cost effectiveness of a mass distribution of long-lasting insecticidal nets: a modelling study. Malar J. 2013; 12: 77. https://doi.org/10.1186/1475-2875-12-77 PMID: 23442575

55. Trape J-F. The Public Health Impact of Chloroquine Resistance in Africa. American Society of Tropical Medicine and Hygiene; 2001.

56. Douglas NM, Lampah DA, Kenangalem E, Simpson JA, Poespoprodjo JR, Sugiarto P, et al. Major burden of severe anemia from non-falciparum malaria species in Southern Papua: a hospital-based surveillance study. PLoS Med. 2013; 10: e1001575; discussion e1001575. https://doi.org/10.1371/journal.pmed.1001575 PMID: 24358031

57. Djimde AA, Barger B, Kone A, Beavogui AH, Tekete M, Fofana B, et al. A molecular map of chloroquine resistance in Mali. FEMS Immunol Med Microbiol. 2010; 58: 113–8. https://doi.org/10.1111/j.1574-695X.2009.00641.x PMID: 20041947

58. Djimdé AA, Dolo A, Ouattara A, Diakité S, Plowe CV, Doumbo OK. Molecular Diagnosis of Resistance to Antimalarial Drugs during Epidemics and in War Zones. J Infect Dis. 2004; 190: 853–855. https://doi.org/10.1086/422758 PMID: 15272415

59. Lim P, Dek D, Try V, Sreng S, Suon S, Fairhurst RM. Decreasing pfmdr1 copy number suggests that Plasmodium falciparum in Western Cambodia is regaining in vitro susceptibility to mefloquine. Antimicrob Agents Chemother. 2015; 59: 2934–7. https://doi.org/10.1128/AAC.05163-14 PMID: 25712365

60. Hmooda Toto K, Bashir AI, Mnzava AP, Abdin MSE, Eltaher JS, Banaga AO, et al. The impact of insecticide resistance in *Anopheles arabiensis* on malaria incidence and prevalence in Sudan and the costs of mitigation. Proc. Natl Acad. Sci. In press.

61. Philip Ricks. The Importance of Insecticide Resistance Monitoring to Maintain IRS Program Effectiveness. 2015. Available: http://www.africairs.net/2015/11/the-pmi-airs-project-presents-at-astmh/ Date accessed 17 Sept 2017

62. Coetzee M, Kruger P, Hunt RH, Durrheim DN, Urbach J, Hansford CF. Malaria in South Africa: 110 years of learning to control the disease. S Afr Med J. 2013; 103: 770–8. Available: http://www.ncbi.nlm.nih.gov/pubmed/24079632 https://doi.org/10.7196/samj.7446 PMID: 24079632

63. WHO | Global plan for insecticide resistance management in malaria vectors. WHO. World Health Organization; 2015

64. Hemingway J, Vontas J, Poupardin R, Raman J, Lines J, Schwabe C, et al. Country-level operational implementation of the Global Plan for Insecticide Resistance Management. Proc Natl Acad Sci U S A. 2013; 110: 9397–402. https://doi.org/10.1073/pnas.1307656110 PMID: 23696658

65. Hemingway J, Beaty BJ, Rowland M, Scott TW, Sharp BL. The Innovative Vector Control Consortium: improved control of mosquito-borne diseases. Trends Parasitol. 2006; 22: 308–12. https://doi.org/10.1016/j.pt.2006.05.003 PMID: 16713358

66. Chanda E, Thomsen EK, Musapa M, Kamuliwo M, Brogdon WG, Norris DE, et al. An Operational Framework for Insecticide Resistance Management Planning. Emerg Infect Dis. Centers for Disease Control and Prevention; 2016; 22: 773–9. https://doi.org/10.3201/eid2205.150984 PMID: 27089119

67. Group TWARN (WWARN) DS. The effect of dosing regimens on the antimalarial efficacy of dihydroartemisinin-piperaquine: a pooled analysis of individual patient data. PLoS Med. 2013; 10: e1001564; discussion e1001564. https://doi.org/10.1371/journal.pmed.1001564 PMID: 24311989

68. Boni MF, Smith DL, Laxminarayan R. Benefits of using multiple first-line therapies against malaria. Proc Natl Acad Sci. 2008; 105: 14216–14221. https://doi.org/10.1073/pnas.0804628105 PMID: 18780786

69. Smith DL, Klein EY, McKenzie FE, Laxminarayan R. Prospective strategies to delay the evolution of anti-malarial drug resistance: weighing the uncertainty. Malar J. B 2010; 9: 217. https://doi.org/10.1186/1475-2875-9-217 PMID: 20653960

70. Nguyen TD, Olliaro P, Dondorp AM, Baird JK, Lam HM, Farrar J, et al. Optimum population-level use of artemisinin combination therapies: a modelling study. Lancet Glob Heal. 2015; 3: e758–e766. https://doi.org/10.1016/S2214-109X(15)00162-X PMID: 26545449

71. Pongtavornpinyo W1, Yeung S, Hastings IM, Dondorp AM, Day NP, White NJ. Spread of anti-malarial drug resistance: mathematical model with implications for ACT drug policies. Malar J. 2008 Nov 2; 7:229. https://doi.org/10.1186/1475-2875-7-229 PMID: 18976503

72. Boni MF, White NJ, Baird JK, Peters W, Dondorp A, Nosten F, et al. The Community As the Patient in Malaria-Endemic Areas: Preempting Drug Resistance with Multiple First-Line Therapies. PLoS Med. 2016; 13: e1001984. https://doi.org/10.1371/journal.pmed.1001984 PMID: 27022739

73. Maxmen A. Back on TRAC: New trial launched in bid to outpace multidrug-resistant malaria. Nat Med. Nature Research; 2016; 22: 220–221. https://doi.org/10.1038/nm0316-220 PMID: 26937610

74. Hamma M. Impact of seasonal malaria chemoprevention of sulphadoxinepyrimethamine plus amodiaquine on molecular markers resistance of Plasmodium falciparum malaria: A review in West Africa. Clin Rev Opin. A2016; 7: 1–10. https://doi.org/10.5897/CRO15.0098

75. Kleinschmidt I, Mnzava AP, Kafy HT, Mbogo C, Bashir AI, Bigoga J, et al. Design of a study to determine the impact of insecticide resistance on malaria vector control: a multi-country investigation. Malar J. 2015; 14: 282. https://doi.org/10.1186/s12936-015-0782-4 PMID: 26194648

76. Thomsen EK, Strode C, Hemmings K, Hughes AJ, Chanda E, Musapa M, et al. Underpinning sustainable vector control through informed insecticide resistance management. PLoS ONE. 2014; 9: e99822. https://doi.org/10.1371/journal.pone.0099822 PMID: 24932861

77. Mugittu K, Ndejembi M, Malisa A, Lemnge M, Premji Z, Mwita A, et al. Therapeutic efficacy of sulfadoxine-pyrimethamine and prevalence of resistance markers in Tanzania prior to revision of malaria treatment policy: Plasmodium falciparum dihydrofolate reductase and dihydropteroate synthase mutations in monitoring in vivo re. Am J Trop Med Hyg. 2004; 71: 696–702. Available: http://www.ncbi.nlm.nih.gov/pubmed/15642957 PMID: 15642957

78. Ranson H, Lissenden N. Insecticide Resistance in African Anopheles Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. Trends Parasitol. 2015; 32: 187–196. https://doi.org/10.1016/j.pt.2015.11.010 PMID: 26826784

79. Bagi J, Grisales N, Corkill R, Morgan JC, N'Falé S, Brogdon WG, et al. When a discriminating dose assay is not enough: measuring the intensity of insecticide resistance in malaria vectors. Malar J. 2015; 14: 210. https://doi.org/10.1186/s12936-015-0721-4 PMID: 25985896

80. Kulma K, Saddler A, Koella JC. Effects of age and larval nutrition on phenotypic expression of insecticide-resistance in Anopheles mosquitoes. PLoS ONE. 2013; 8: e58322. https://doi.org/10.1371/journal.pone.0058322 PMID: 23484017

81. Oliver S V, Brooke BD, Sinka M, Bangs M, Manguin S, Coetzee M, et al. The effect of multiple blood-feeding on the longevity and insecticide resistant phenotype in the major malaria vector Anopheles arabiensis (Diptera: Culicidae). Parasit Vectors. 2014; 7: 390. https://doi.org/10.1186/1756-3305-7-390 PMID: 25150975

82. Glunt KD, Paaijmans KP, Read AF, Thomas MB, Maxmen A, Kelly-Hope L, et al. Environmental temperatures significantly change the impact of insecticides measured using WHOPES protocols. Malar J. 2014; 13: 350. https://doi.org/10.1186/1475-2875-13-350 PMID: 25187231

83. Strode C, Steen K, Ortelli F, Ranson H. Differential expression of the detoxification genes in the different life stages of the malaria vector Anopheles gambiae. Insect Mol Biol. 2006; 15: 523–30. https://doi.org/10.1111/j.1365-2583.2006.00667.x PMID: 16907839

84. Sikulu MT, Majambere S, Khatib BO, Ali AS, Hugo LE, Dowell FE. Using a near-infrared spectrometer to estimate the age of anopheles mosquitoes exposed to pyrethroids. PLoS ONE. 2014; 9: e90657. https://doi.org/10.1371/journal.pone.0090657 PMID: 24594705

85. Donnelly MJ, Isaacs AT, Weetman D. Identification, Validation, and Application of Molecular Diagnostics for Insecticide Resistance in Malaria Vectors. Trends Parasitol. 2015; 32: 197–206. https://doi.org/10.1016/j.pt.2015.12.001 PMID: 26750864

86. Lubell Y, Dondorp A, Guérin PJ, Drake T, Meek S, Ashley E, et al. Artemisinin resistance—modelling the potential human and economic costs. Malar J. 2014; 13: 452. https://doi.org/10.1186/1475-2875-13-452 PMID: 25418416

87. Volberding PA, Deeks SG. Antiretroviral therapy and management of HIV infection. Lancet. 2010; 376: 49–62. https://doi.org/10.1016/S0140-6736(10)60676-9 PMID: 20609987

88. Goldberg DE, Siliciano RF, Jacobs WR. Outwitting evolution: fighting drug-resistant TB, malaria, and HIV. Cell. 2012; 148: 1271–83. https://doi.org/10.1016/j.cell.2012.02.021 PMID: 22424234

89. Mitchell PD, Onstad DW. Chapter 2 –Valuing Pest Susceptibility to Control. Insect Resistance Management. 2014. pp. 25–53. https://doi.org/10.1016/B978-0-12-396955-2.00002–3

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COLLECTION REVIEW

malERA: An updated research agenda for characterising the reservoir and measuring transmission in malaria elimination and eradication

The malERA Refresh Consultative Panel on Characterising the Reservoir and Measuring Transmission*¶

¶ Full listing of the members of the malERA Refresh Consultative Panel on Characterising the Reservoir and Measuring Transmission can be found in the Acknowledgements.
* Chris.Drakeley@lshtm.ac.uk; ANoor@kemri-wellcome.org

OPEN ACCESS

Citation: The malERA Refresh Consultative Panel on Characterising the Reservoir and Measuring Transmission (2017) malERA: An updated research agenda for characterising the reservoir and measuring transmission in malaria elimination and eradication. PLoS Med 14(11): e1002452. https://doi.org/10.1371/journal.pmed.1002452

Published: November 30, 2017

Funding: MESA received a grant from the Bill & Melinda Gates Foundation (OPP1034591 http://www.malariaeradication.org/mesa-track/malaria-eradication-scientific-alliance-mesa). BM (Bill & Melinda Gates Foundation) was a member of this Panel.

Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: JC receives funding from UNITAID. AMN is a member of the Editorial Board of *PLOS Medicine*.

Abbreviations: ABER, annual blood examination rate; DFA, direct feeding assay; DHS, Demographic

Abstract

This paper summarises key advances in defining the infectious reservoir for malaria and the measurement of transmission for research and programmatic use since the Malaria Eradication Research Agenda (malERA) publication in 2011. Rapid and effective progress towards elimination requires an improved understanding of the sources of transmission as well as those at risk of infection. Characterising the transmission reservoir in different settings will enable the most appropriate choice, delivery, and evaluation of interventions. Since 2011, progress has been made in a number of areas. The extent of submicroscopic and asymptomatic infections is better understood, as are the biological parameters governing transmission of sexual stage parasites. Limitations of existing transmission measures have been documented, and proof-of-concept has been established for new innovative serological and molecular methods to better characterise transmission. Finally, there now exists a concerted effort towards the use of ensemble datasets across the spectrum of metrics, from passive and active sources, to develop more accurate risk maps of transmission. These can be used to better target interventions and effectively monitor progress toward elimination. The success of interventions depends not only on the level of endemicity but also on how rapidly or recently an area has undergone changes in transmission. Improved understanding of the biology of mosquito–human and human–mosquito transmission is needed particularly in low-endemic settings, where heterogeneity of infection is pronounced and local vector ecology is variable. New and improved measures of transmission need to be operationally feasible for the malaria programmes. Outputs from these research priorities should allow the development of a set of approaches (applicable to both research and control programmes) that address the unique challenges of measuring and monitoring transmission in near-elimination settings and defining the absence of transmission.

and Health Surveys; DMFA, direct membrane feeding assay; ELISA, enzyme-linked immunosorbant assay; HLC, human landing collection; malERA, Malaria Eradication Research Agenda; MDA, mass drug administration; mFOI, molecular force of infection; MICS, Multiple Indicator Cluster Surveys; MIS, Malaria Indicator Surveys; MOI, multiplicity of infection; MSAT, mass screen and treat; NAAT, nucleic acid amplification test; RDT, rapid diagnostic test; RT-qPCR, reverse transcription qPCR; SCR, seroconversion rate.

Provenance: Submitted as part of a Supplement; externally peer reviewed.

Summary points

- Understanding the sources of transmission (the infectious reservoir) and those at risk of infection at the population level in order to inform programmatic decision-making can progress malaria elimination.
- There is considerable evidence for malaria infections at densities beneath the limit of conventional diagnostics. However, the contribution of these low-density infections to malaria transmission in different settings is not known.
- Characterising the spatial and temporal heterogeneity of the infectious reservoir becomes increasingly important as transmission declines if interventions are to be efficiently implemented to accelerate malaria elimination.
- The proportional contributions of low-density, asymptomatic, and symptomatic infections will differ by malaria typology and will determine the programmatic approach required to reduce transmission.
- *Plasmodium vivax* hypnozoites are undetectable with currently available diagnostics, representing a major barrier to both understanding the transmission reservoir for this parasite and its elimination.
- There is a need to standardise both existing transmission metrics and new metrics with greater sensitivity, particularly for their use in low-transmission settings.

Introduction

Transmission of malaria requires sexual-stage parasites, gametocytes, in humans to be taken up by female *Anopheles* mosquitoes when they feed. After a period of parasite development, mosquitoes can then infect humans. A break in this cycle at any point interrupts malaria transmission. Malaria control has historically focussed on the reduction of morbidity and mortality of the human host rather than on the interruption of transmission from human to mosquito. Understanding the variation in the relationship between infection (the presence of parasites in an individual or mosquito) and infectiousness (the ability to transmit parasites to a mosquito or human) at different transmission intensities and with different levels of intervention coverage is increasingly recognised as critical in the pursuit of malaria elimination.

In 2011, one of the main conclusions of the Malaria Eradication Research Agenda (malERA) process was the need to develop tools to measure transmission at low levels in elimination contexts. This article summarizes progress made since 2011 and for the first time develops a research agenda addressing the reservoir of transmissible parasites and measuring transmission [1,2]. Findings and recommendations presented here result from a systematic search of the literature and the deliberations of the 2015 malERA Refresh Consultative Panel on characterising the reservoir and measuring transmission, including specialists from field and implementation science, entomology, epidemiology, and basic science.

Since the 2011 malERA process, research has ranged from illuminating the basic biology of the development of sexual-stage parasites in humans and mosquitoes to evaluating operational approaches targeting infectious individuals in endemic communities. Additionally, a harmonised set of definitions relevant to malaria transmission and elimination has been developed

Box 1. Terminology

Malaria typologies

Malaria typology is the characterisation of malaria epidemiology according to ecology (climate and environment) and other determinants of transmission for the purpose of guiding malaria interventions. Relevant ecologies include (but are not limited to) savannah, lowland plains and valleys, highlands, desert and oasis, forest and jungle, coastal and marshland, and urban or peri-urban. The unique features of malaria transmission in each ecological area are also strongly driven by region-specific vectors and parasites (species, biology, behaviour, insecticide and antimalarial drug susceptibility), human biology and behaviour, and economic and health-system factors. These are discussed more comprehensively in [4] and [5].

(Box 1) [3]. However, there remains a need to further validate a 'toolkit' of metrics and associated surveillance activities to characterise the infectious reservoir and measure malaria transmission that can be applied programmatically to direct and evaluate interventions and to quantify progress towards malaria elimination. There are multiple factors that contribute to malaria epidemiology including ecology, vectors, parasites, human biology and behaviour, and economic and health-system factors (see Box 1), and these collectively make up a given 'typology' of malaria. The selection of appropriate surveillance activities and metrics from this toolkit will not only need to reflect variations in malaria 'typology' (Box 1) [3], but will need to be adapted as malaria transmission declines (Fig 1).

This paper discusses progress in the measurement and understanding of malaria transmission, highlighting the different malaria typologies in which transmission occurs (Box 1). This differentiation between typologies is needed to determine where existing strategies and systems can sufficiently achieve malaria elimination versus those where additional approaches or tools are required.

Research agenda for characterising the reservoir of infection

Detecting malaria: Infection versus transmission

Malaria infection and transmission can be detected and measured with a variety of metrics (Tables 1 and 2). Their suitability and discriminatory power, however, can vary widely across settings and populations. To reliably confirm clinical malaria, a minimum diagnostic sensitivity of 200 parasites/μL blood is required [6]. Microscopy and some rapid diagnostic tests (RDTs) meet this threshold [6]. In the absence of fever, some individuals will have parasitaemia levels detectable by microscopy and RDTs. These asymptomatic infections are particularly common in areas of high transmission (i.e., above 25 clinical cases per week per 1,000 persons) [7], where high levels of human immunity allow older individuals to carry relatively large parasite burdens chronically [8]. Such individuals would be detected within mass screen and treat (MSAT) programmes using currently available diagnostics. However, through the use of molecular amplification methods, it is now clear that many individuals harbour low-density malaria infections beneath the limit of detection of both microscopy and RDTs [9]. Meta-analyses indicate that molecular methods detect up to twice as many *P. falciparum* infections as RDT or microscopy [10], and approximately 5 times as many *P. vivax* infections [11,12]. This gap in sensitivity may be more pronounced when compared against ultra-sensitive molecular

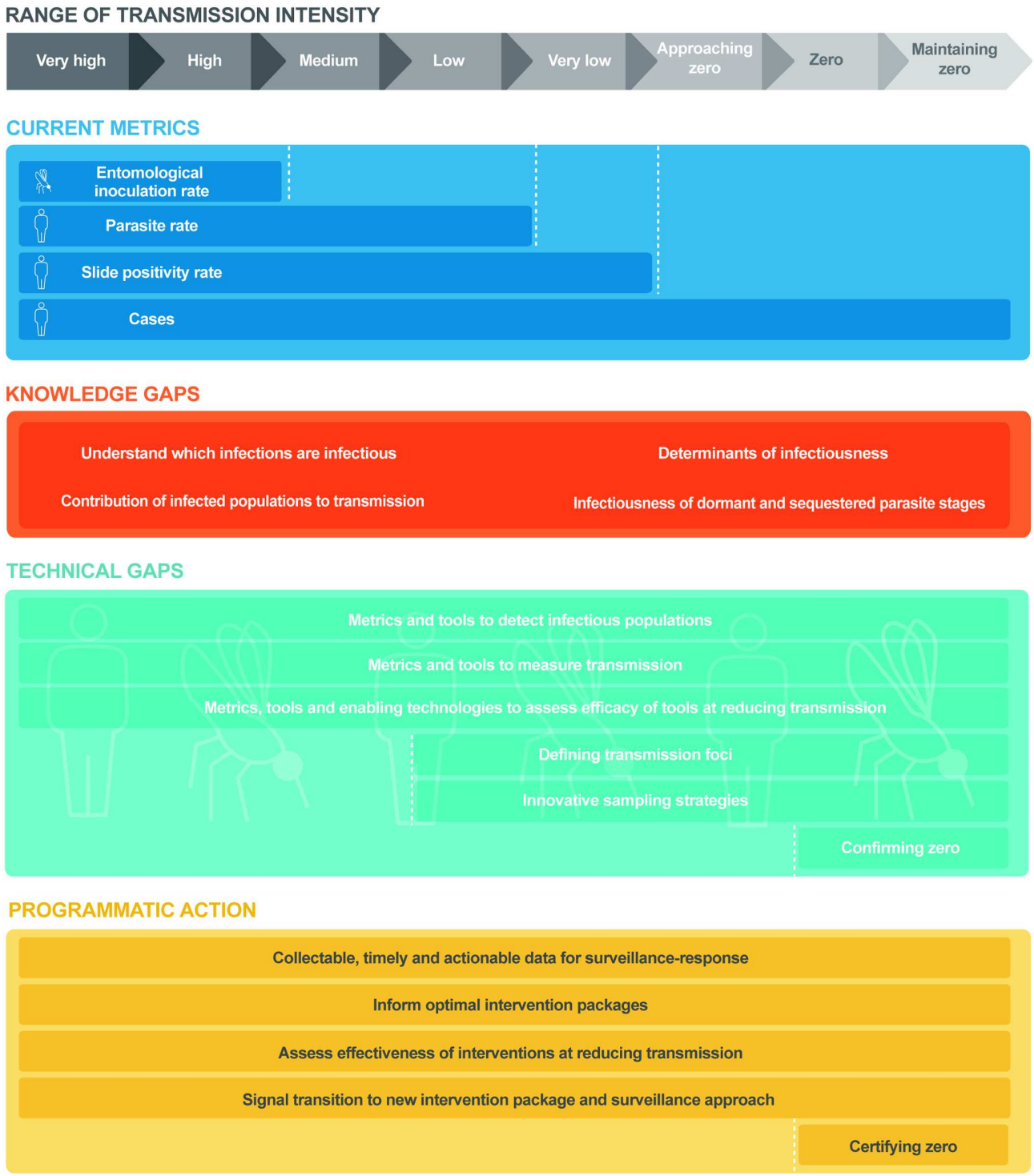

Fig 1. Research needs and programmatic applications in measuring malaria transmission across the transmission spectrum. Range of malaria transmission intensity (grey line) from very high intensity to postelimination settings. **Current metrics** (navy blue line) used for routine measurement of malaria transmission at each level of transmission intensity. **Knowledge gaps** (orange line) in understanding the biology and epidemiology of malaria transmission and the infectious reservoir at all levels of transmission intensity. **Technical gaps** (light blue line) in the accurate measurement of transmission at each level of transmission intensity. **Programmatic actions** (yellow line) required for the interruption of transmission and the prevention of reintroduction at each level of transmission intensity.

https://doi.org/10.1371/journal.pmed.1002452.g001

Table 1. Summary of currently available entomological malaria transmission metrics.

Metric	Definition [3]	Measure of transmission	Sampling method and resolution	Discriminatory power
Entomological inoculation rate (EIR)	Number of infective bites received per person in a given unit of time, in a human population	Transmission intensity	• Human landing collection; light traps • Resolution: Household or community level	• Insensitive at low transmission • Lack of standardised sampling design • Collected by malaria control programmes
Sporozoite rate (SR)	Percentage of female *Anopheles* mosquitoes with sporozoites in the salivary glands	Risk of infection	• Human landing catch; baited traps; gravid traps • Resolution: Community level	• Insensitive at low transmission
Human biting rate (HBR)	Average number of mosquito bites received by a host in a unit of time, specified according to host and mosquito species	Risk of exposure	• Human landing collection • Resolution: Person or community level	• Allows determination of the primary vector
Vectorial capacity	Rate at which given vector population generates new infections caused by a currently infectious human case	Efficiency of transmission	• Derived from human biting rate, parasite inoculation period, mosquito to human density and mosquito survival • Resolution: Community level	• Measures potential, not actual, rate of transmission—includes no parasitological information • Sensitive to changes in mosquito survival and biting behaviour but may not translate to significant change in human incidence • Can be useful when infection rates are low and mosquito sampling difficult

https://doi.org/10.1371/journal.pmed.1002452.t001

methods [13]. Lack of sensitivity of diagnostic detection is more acute for *P. vivax* infections, which circulate at lower parasite densities hampering accurate estimates of true prevalence. There are also other unique challenges presented by *P. vivax* that make characterising its transmission reservoir problematic (Box 2) [14–18].

Diagnosis and treatment of clinical malaria is vital for disease control, particularly if this can be rapidly implemented to reduce the likelihood of gametocyte production. There is also a good public health rationale for identifying and treating 'asymptomatic' malaria detectable with microscopy or RDTs, as it is increasingly recognised that this is associated with ongoing morbidity (e.g., anaemia, increased susceptibility to bacterial infections, and cognitive function; reviewed in [8]). If the aim is malaria elimination, the contribution of low-density infections to transmission needs to be considered given that, where data are available, low-density infections represent a significant proportion of malaria infections and can be the majority in low-endemic areas [9,10,19,20].

While the countries that have achieved malaria elimination to date have done so largely without specific attempts to detect and treat low-density parasitaemia, these may not be representative of malaria typologies in higher-transmission settings. In many areas, the persistence of malaria can occur despite high coverage of vector control measures and the availability of effective treatment, suggesting that novel approaches are needed for both surveillance and interventions that will accelerate the elimination process [19,21]. Furthermore, studies have documented the failure of strategies to reduce clinical malaria incidence and transmission, such as MSAT, when the transmission reservoir is not adequately identified and targeted with the currently available field diagnostics [22].

It follows that the cost-effectiveness of existing or novel surveillance methods and interventions in reducing malaria transmission cannot be predicted or evaluated unless the relative contribution to transmission of (1) clinical/symptomatic malaria, (2) asymptomatic

Table 2. Summary of currently available malaria transmission metrics in humans.

Metric	Definition [3]	Measure of transmission	Method	Discriminatory power
Annual blood examination rate (ABER)	The number of people receiving a parasitological test for malaria per unit population per year	Level of diagnostic monitoring activity	Microscopy or RDT	• Dependent on health-system provision
Case, confirmed	Malaria case (or infection) in which the parasite has been detected in a diagnostic test	Current transmission or incidence if data collection is repeated or routine	Microscopy or RDT positive	• Insensitive at low transmission; saturates at high transmission • Underestimates due to system inadequacies and poor health-seeking behaviour
Case, fever	The occurrence of fever (current or recent) in a person	Current transmission or incidence if data collection is repeated or routine	Reported or observed fever	• Overestimates malaria infection
Proportion of fevers parasitaemic (PFPf) *	Proportion of fever cases found to be positive for *Plasmodium*	Current transmission or incidence if data collection is repeated or routine	Microscopy; RDT; NAAT	• Depends on diagnostic sensitivity • Insensitive at low transmission
Slide positivity rate (SPR)	Proportion of blood smears found to be positive for *Plasmodium* among all blood smears examined	Current transmission or incidence if data collection is repeated or routine	Microscopy	• Depends on ABER • Insensitive at low transmission
RDT positivity rate (RDT-PR)	Proportion of positive results among all RDTs performed	Current transmission or incidence if data collection is repeated or routine	RDT	• Depends on RDT sensitivity • Insensitive at low transmission
Parasite rate (PR)	Proportion of the population found to carry asexual blood-stage parasites	Current transmission or incidence if data collection is repeated or routine	Microscopy; RDT; NAAT	• Depends on diagnostic sensitivity • Insensitive at low transmission
Gametocyte rate (GR)	Percentage of individuals in a defined population in whom sexual forms of malaria parasites have been detected	Potentially infectious human population	Microscopy; NAAT	• Depends on diagnostic sensitivity • Insensitive at low transmission

*No WHO definition is available for this term.

Abbreviations: ABER, annual blood examination rate; GR, gametocyte rate; NAAT, nucleic acid amplification test; PF*Pf*, proportion of fevers parasitaemic; PR, parasite rate; RDT, rapid diagnostic test; RDT-PR, RDT positivity rate; SPR, slide positivity rate.

https://doi.org/10.1371/journal.pmed.1002452.t002

parasitaemia (detectable by microscopy or RDT), and (3) low-density parasitaemia (not detectable by microscopy or RDT) are estimated for a particular setting. With an increasingly diverse array of potential approaches for malaria elimination [18], but with limited human and financial resources [23], characterising the contribution of low-density parasitaemia to transmission will help to focus elimination efforts.

Low-density parasitaemia and transmission

There are currently no field diagnostics with sufficient sensitivity to identify low-density submicroscopic parasitaemia, though various approaches are under evaluation for performance and scalability (discussed in the malERA Refresh 'Tools' paper) [18]. However, even if all infected individuals could be identified, there is a need to understand who is infectious to mosquitoes and for how long.

Understanding the contribution of low-density parasitaemia to the infectious reservoir for a given malaria typology is critical to determine the diagnostic sensitivity required. It will also affect how much effort a programme should commit to detecting and treating these infections and when and where this effort is best deployed. As noted above, the proportion of low-density parasitaemia increases as transmission declines [9,10,19,20,24]. Recent findings from Senegal also suggest that the efficiency of human-to-mosquito transmission increases with decreasing transmission intensity [25].

Box 2. *P. vivax* and *P. ovale*

P. vivax and *P. ovale* have a dormant liver stage, the hypnozoite, which is undetectable by currently available diagnostic methods. Periodic reactivation of hypnozoites results in repeated blood-stage infection (relapses) occurring weeks, or even years, following the initial infection. As control efforts reduce the incidence of *P. falciparum* cases, *P. vivax* cases can remain relatively stable and become a greater proportion of malaria cases overall [16]. *P. vivax* is refractory to traditional vector control methods: hypnozoites enable the parasite to evade conditions unfavourable to transmission and will survive in the host following schizonticidal anti-malarial therapy. Without new anti-hypnozoite drugs or vaccines that could be used safely across entire populations, the *P. vivax*/*ovale* transmission reservoir cannot be targeted, making elimination of these parasites challenging in any setting.

Key advances

Relapses drive transmission

- In children in Papua New Guinea, 4 of every 5 *P. vivax* infections and 3 of every 5 *P. ovale* infections were caused by relapses [14].
- Both primary and relapse *P. vivax* infections generate gametocytes, which typically appear before clinical symptoms, and promote onward 'silent' transmission of the parasite [15].
- Estimating transmission using the typical entomological measures is of limited relevance when clinical disease can emerge from an individual not recently infected by a mosquito bite.

Research needs

Detection of hypnozoites to inform targeted drug or vaccination strategies

- Access to existing anti-hypnozoite therapy needs to be expanded where possible in order to reduce the burden of disease and minimise the risk of human-to-mosquito transmission via relapse.
- However, several barriers to mass drug administration (MDA) for *P. vivax* exist. The 8-aminoquinolines primaquine and tafenoquine are the only known anti-hypnozoite drugs. Both drugs are contraindicated in pregnancy and individuals with glucose-6-phosphate dehydrogenase deficiency [17,18]. Even if rapid, accurate point-of-care tests were available to exclude these individuals from treatment, a significant proportion of the population (typically >10%) will remain untreated.
- Without being able to identify hypnozoites, MSAT is of no practical value in reducing *P. vivax* or *P. ovale* transmission [14].

- Compared to *P. falciparum*, *P. vivax* and *P. ovale* present as much lower parasite densities; therefore, determining the appropriate limit of detection for new diagnostics will be a major challenge.

Improve understanding of parasite-vector bionomics

Parasites can be transported undetected into areas where malaria has been eliminated, leading to outbreaks and the reestablishment of transmission where conditions are receptive. More effort needs to be directed at understanding specific parasite vector interactions to develop targeted vector control strategies for *P. vivax*/*ovale* to reduce the risk of mosquito-to-human transmission.

Currently, the only way to measure human infectiousness is by feeding colony-reared mosquitoes either on humans directly (direct feeding assay [DFA] [26,27]) or on infected human blood via a membrane (direct membrane feeding assay [DMFA] [28]). A number of studies have used these methods to estimate the contribution of low-density infections to malaria transmission [29–34]. For example, studies in Burkina Faso using DMFA found that 28.7% (25 out of 87) of infectious individuals were microscopy negative, causing 17.0% of mosquito infections [29]. Similarly, in Thailand, DFA studies found that 21% (13 out of 62) individuals submicroscopic for either *P. falciparum* or *P. vivax* were able to infect mosquitoes [34]. These preliminary studies suggest that surveillance systems could be modified in the future to detect submicroscopic infections and direct transmission reduction efforts. However, understanding the relationship between infectivity as measured in feeding assays and the infectivity in natural transmission settings to local mosquitoes is still a major research challenge. Furthermore, few empirical studies have quantified the proportion of the overall population that is both submicroscopic and infectious, particularly in low-transmission settings (i.e., less than 8 clinical cases per week per 1,000 persons) [7]. This is needed to determine when and where treating low-density parasitaemia is critical for interrupting transmission and the diagnostic sensitivity required to target them. Mathematical models suggest that conventional diagnostics can detect 55% of the infectious reservoir, but with a 100-fold increase in sensitivity of detection level, i.e., from 200 to 2 parasites/μL of blood, up to 95% of infectious individuals could be identified [35]. This level of diagnostic sensitivity could transform our understanding of the malaria transmission reservoir, allowing the development and delivery of better strategies to disrupt transmission toward malaria elimination.

Detecting gametocytes

All malaria infections have the capacity to produce gametocytes. Therefore, in the context of community chemotherapy programmes, treating any individuals who test positive for asexual parasites is a realistic programme aim. However, research tools that measure gametocytaemia are essential to further our understanding of transmission biology and to define the populations and individuals that drive transmission. Some studies have suggested that transmission efficiency may increase as malaria prevalence falls due to higher gametocyte densities. As the development of new transmission-blocking drugs and vaccines advances, understanding the factors that drive this transmission efficiency will be needed to determine in which settings interventions can be successfully trialled and/or implemented [25]. Although gametocytes can

be identified using microscopy, they often exist at low densities and may circulate only transiently in the blood. RDTs do not differentiate between gametocytes and asexual parasites. The limit of detection of microscopy is 8–16 gametocytes/μL of blood [30,31]. Predictably, molecular methods are more sensitive, with 0.3 mature females/μL of blood detected with Pfs25 reverse transcription qPCR (RT-qPCR) and 1.8 mature males/μL of blood with Pfs230p RT-qPCR [36]. As gametocyte densities are low, the increased sensitivity of molecular methods considerably increases gametocyte detection rates. For example, a recent study in Kenya found that Pfs25 RT-qPCR detected gametocytes in 44% of the population compared with only 2.6% detected by microscopy [37].

While there is an overall positive association between mosquito infection rates and gametocyte density, there is also evidence of infectiousness for individuals with very low gametocyte densities [27,38]. As the majority of malaria infections are submicroscopic, even if only a small proportion of these individuals are infectious, the contribution to the transmission reservoir is potentially significant enough to impact elimination programmes.

Where data are available, they suggest differences between high- and low-transmission settings in the gametocyte density needed for human infectivity to mosquitoes. In African populations, submicroscopic *P. falciparum* gametocytaemia is common, and studies in Kenya have found that the majority of infectious children (43 out of 62) had submicroscopic gametocytaemia [30,31]. In contrast, in Cambodia, *falciparum*-infected subjects with detectable gametocytes by microscopy were significantly more likely than gametocyte-negative individuals to infect mosquitoes, and those with microscopy-detectable gametocytaemia were the source of the majority of all mosquito infections [39].

Heterogeneity in the transmission reservoir

While data demonstrate an advance in our understanding of malaria transmission, they are limited and suggest the infectious reservoir differs across malaria typologies [24]. Most studies investigating human infectiousness have been conducted in high-transmission settings. There is a particular need for data from low-transmission and near-elimination settings, where temporal, spatial, and demographic heterogeneity in transmission can often be more pronounced. Longitudinal data characterising the transmission reservoir are also needed. These would not only allow more accurate assessments of the contributions of the different density infections but could also inform the sequence of intervention delivery needed to reduce transmission. Similarly, these data would inform the necessary intervention changes to most effectively transition countries from high to low transmission and ultimately elimination [40]. A key consideration is to advise when malaria control measures should be reoriented following elimination without the risk of reintroduction, particularly in the context of declining human immunity to malaria and the potential for outbreaks.

As transmission declines and heterogeneity increases, programmes need to adjust in order to respond to increasingly rare clinical cases. The persistence of residual transmission requires more aggressive and/or novel strategies, and targeting these areas will be key to local elimination. Significant progress has been made in approaches to identify transmission foci using a number of field-based, geo-spatial, and modelling approaches [41–53]. However, even where hotspots of malaria transmission can be identified, attempts to target these foci may fail against a background of low-level but widespread transmission [54]. Local implementation and high-coverage control interventions linked to surveillance information will be needed to adequately clear the reservoir at all levels of transmission.

Surveillance systems at low-transmission settings will also need to be equipped to monitor emerging insecticide and drug resistance [55,56] that may threaten the success of existing

interventions [56]. Longitudinal monitoring of resistance markers via sentinel surveillance sites could prove invaluable for tracking risk of rebound or reintroduction. However, there are currently no field-based diagnostic tests for drug resistance, and more detailed information may be needed on local drug-resistance patterns in asymptomatic/low-density infections, particularly related to any changed infectiousness to mosquitoes.

Research agenda for measuring transmission

Improved and validated metrics of transmission would enable the optimal design of control programmes and surveillance systems needed for malaria elimination [23]. This would include the ability to better track progress, confirm cases and foci, and identify and contain reintroduction of transmission, should it occur. Validated transmission metrics are also the key outcome to be measured in field trials evaluating the effectiveness of transmission-blocking interventions [18] and can be used to improve mathematical models assessing potential intervention combinations [7].

Measures of malaria transmission can be defined at different points in the transmission cycle (Fig 2). Since 2011, progress has been made in understanding the advantages and limitations of transmission metrics across epidemiological settings [57,58]. Further work is needed to better quantify the correlations between metrics, standardise their application for use in programmatic surveillance activities, and develop and validate new metrics. However, it is necessary that transmission metrics are reliable and reproducible on a consistent basis and can be assembled through existing national systems.

Entomological metrics

Between 30–40 species of *Anopheles* have been identified as vectors of human malaria, exhibiting varying feeding behaviours and preferences, habitats, and ecologies. Within this complexity, there is a need to standardise current metrics and develop more efficient sampling techniques [57] (Table 1). Whilst developments in sampling methods have been made to evaluate biting densities and infection rates [59–63], human landing collection (HLC) sampling remains the gold standard for providing epidemiologically relevant mosquito-to-human transmission metrics, despite inherent risks [64,65]. Alternative technologies to HLC are being tested that limit human exposure [66,67] and include traps with attractants that mimic a human host [68,69].

New approaches are particularly needed in settings where vector densities are low or heterogeneous. For example, reexamination of vectorial capacity using mathematical modelling to simulate settings with different baseline epidemiological and entomological characteristics has led to new insights into the effective deployment of vector control measures [70]. Technological advances in geolocation and mapping can precisely identify vector habitats that coincide with human activity and movement [71]. This information can be used to determine potential exposure points, enabling targeted sampling in these foci of transmission risk. Other innovative technologies include high-throughput technology, such as infrared spectrometry, to evaluate large samples of mosquitoes for vector age, species, and infection status [72–74], thus providing a measure of vector density and indicating the risk of malaria reintroduction. In this regard, as with parasite drug resistance, longitudinal monitoring of insecticide resistance via sentinel surveillance could prove invaluable.

Human metrics

Current epidemiological metrics of malaria transmission in humans, diagnosed via passive and active systems, microscopy and RDTs, remain key for national malaria control

Fig 2. Key programmatic and research metrics across the malaria parasite transmission cycle. NAAT, nucleic acid amplification test; RDT, rapid diagnostic test.

https://doi.org/10.1371/journal.pmed.1002452.g002

programmes in tracking progress in the reduction of malaria cases and identifying outbreaks and epidemics (Table 2). These data are complemented with large-scale surveys, such as the Demographic and Health Surveys (DHS), the Malaria Indicator Surveys (MIS) and UNICEF Multiple Indicator Cluster Surveys (MICS). However, as transmission declines to low

intensity, clinical cases become rare, slide and RDT positivity rates low, and transmission patterns increasingly heterogeneous.

To generate practical estimates of infection without excessive sampling, more sensitive diagnostics and/or combinations of diagnostic approaches are needed. While the utility of RDTs will need to be monitored in regions where deletions in the gene encoding HRP2 have been detected in the parasite population [75,76], research is currently underway to develop RDTs with detection thresholds corresponding to 10–20 parasites/μL or lower [77]. The development of highly sensitive nucleic acid–based tests for parasite detection [78,79], and hemozoin detection using nuclear magnetic resonance [80,81], is also ongoing and may be promising. While tests using molecular methods would increase the number of infections identified, their widespread deployment in low-transmission settings is probably not currently cost-effective for the identification of incident infections. Additionally, in recognition of heterogeneity, approaches should shift from tracking national or regional progress in malaria control towards targeted sampling and community-based surveys characterising transmission risk in key population groups. Once elimination has been achieved, maintaining 'zero' transmission will depend on the health system's ability to identify any emergent malaria cases, triggering case-based investigation to determine the origin (local or imported) and prevent onward transmission.

Metrics to understand transmission

Recent technical advances have produced a number of transmission metrics that are suitable for low-transmission settings (Table 3). Molecular force of infection (mFOI) and multiplicity of infection (MOI) both use parasite genotyping methods to assess the complexity of parasite infections [82]. mFOI can identify superinfected individuals that carry parasites from more than 1 infection, providing a more detailed measure of transmission compared to force of infection based on less sensitive methods (Table 2). Sequencing to determine parasite population structure can also be used to characterise transmission by measuring the genetic relatedness between infections in space and time. Other measures, such as allelic richness, can indicate the level of genetic diversity, which is expected to decline as transmission declines [83,84]. Even more refined sequencing approaches might be capable of assigning parasites as imported or local for monitoring the origin of infections.

Antibody seroprevalence and the seroconversion rate (SCR) exploit human antibody responses to characterise previous parasite exposure and are specific to a particular antigen or combination of antigens [85]. Studies using enzyme-linked immunosorbant assays (ELISAs) have shown serological measures correlate well with parasitological and entomological measures in describing transmission levels and spatial and demographic risk [86,87]. Uniquely, serology, when combined with age, allows retrospective examination of exposure history, including the effects of interventions and the absence of recent exposure in elimination settings. High-throughput platforms, such as microarray and bead-based multiplex assays, allow screening of large numbers of potential antigenic targets with specific characteristics [87,88–91]. Targets of interest include stage- or species-specific biomarkers, particularly for *P. vivax* [88], serological signatures of hypnozoite carriage [92], and vector-specific antigenic targets in mosquito saliva [93,94]. The programmatic applications of serology have yet to be fully tested, though various approaches are being evaluated, including serological markers of incident infections [89,95–109]. Research is currently underway to identify a variety of biomarkers indicative of recent infection that are detectable for different durations following parasite infection, allowing finer-scale estimation of time since infection.

Table 3. Advances in the development of metrics for measuring malaria transmission.

Metric	Definition	Measure of transmission	Method	Discriminatory power
Force of infection	Rate at which susceptible individuals contract malaria	• Probability of transmission	Time from birth to first malaria episode; microscopic detection of parasites following successful antimalarial treatment	• Difficult to measure • Difficult to standardise • Depends on diagnostic sensitivity • Cannot differentiate superinfections
mFOI	The number of new parasite clones acquired by a host over time	• Population-level transmission intensity • Transmission heterogeneity	Cohort study >6 months with parasite genotyping	• Highly sensitive for monitoring changes in malaria exposure • Superinfections can be differentiated
MOI	The number of different parasite strains coinfecting a single host	• Population-level transmission intensity • Transmission heterogeneity	Parasite genotyping of positive samples	• Saturates at high transmission • Restricted by age dependency • Insensitive at low transmission • Highly sensitive to spatial heterogeneity • Highly sensitive to increases in imported infection • Less sensitive to changes in seasonality
Genotyping: SNPs or amplicon sequencing	• Genetic diversity, i.e., number of alleles in a population • Parasite signatures to map geographical relatedness of infection (i.e., spatial–temporal transmission)	• Population-level transmission intensity • Transmission heterogeneity • Geographical tracking of transmission patterns	• Haplotypes composed of >12 informative SNPs from single clone infections • Haplotypic signatures from highly variable loci	• Sensitive to changes in malaria exposure and spatial–temporal flow of infection • Standardisation of measures needed • Methods for analysis and interpretation of data needed
Antibody seroprevalence	The percentage of seropositive individuals in a population	• Population-level transmission intensity	Seronegative or seropositive defined using appropriate cutoff points	• Dependent on antibody target tested • Saturates at high transmission • Sensitive at low transmission
SCR	The rate (typically annual) by which seronegative individuals become seropositive upon malaria exposure	• Population-level transmission intensity • Temporal changes in transmission can be detected from a single sampling time point	Detection of antibodies in sera using serological assay (IFAT, ELISA, bead-based assays microarray)	• Dependent on antibody target tested • Restricted by age dependency • Saturates at high transmission • Sensitive at low transmission • Sensitive to risk of malaria in absence of transmission

Abbreviations: ELISA, enzyme-linked immunosorbant assay; IFAT, Immunofluorescence Antibody Test; mFOI, molecular force of infection; MOI, multiplicity of infection; SCR, seroconversion rate.

https://doi.org/10.1371/journal.pmed.1002452.t003

For all these metrics, however, standardisation of methods is necessary, as well as a quantitative comparison to understand the relationship with existing and other new metrics. The development of operationally suitable platforms will ultimately be required to inform real-time or rapid response in programmatic settings. In relation to this, there needs to be a clearer understanding of what measures are needed to better define and monitor transmission, and what measures are useful for control programmes. New approaches to analyse metrics from different sources to improve estimates of transmission, or confirm its interruption, are needed. Looking to the veterinary world could be informative, where probability-based survey methods such as "freedom from infection" are used for animal disease surveillance in the food and agriculture industry [110]. These methods are based on defining the probability that a population is free of infection, allowing operational surveillance thresholds to be set based on the chosen sampling frame and the sensitivity of available diagnostics. Adapting these strategies for use in malaria surveillance will require tailoring the methods for specific malaria transmission measures.

Multimetrics to characterise transmission in time and space

The increasing availability of spatial databases on parasite rate [111,112], serology, vectors [113], malaria genetic epidemiology [114], and human population movements [115–118], together with the increased flexibility and computational efficiency of mathematical and statistical modelling methods [119,120], have led to substantial advances in the spatial–temporal characterisation of malaria transmission intensity. To date, most of these methods have focused on a single metric of endemicity or have relied on parameters derived from small studies. However, dynamic models are being developed that will capture the effect of human population movements, and could incorporate multimetric ensembles to allow self-consistent mapping across the entire spectrum of transmission settings [7]. For these technologies to achieve the greatest impact, they will need to be linked to and used by control programmes to inform operational decision-making in real time.

Summary

Considerable progress has been made not only in understanding the biology and epidemiology of malaria transmission but also in the development of new tools to more accurately quantify transmission; however, challenges remain and Box 3 summarises this Panel's research and development agenda. The foremost of these is an incomplete understanding of the infectious reservoir in low-transmission and elimination settings, particularly the relative infectiousness of (1) asymptomatic individuals and (2) susceptible vector species across a variety of malaria typologies. The spatial and temporal heterogeneity at which these factors interact will change as countries transition to lower transmission intensity.

The absolute and relative incidence of clinical and asymptomatic infections can vary widely between different low-transmission settings. Transmission can occur as focal outbreaks caused by human and vector migration. It can also persist for long periods despite aggressive control strategies or quickly rebound after reaching zero. These scenarios are caused by varying patterns of malaria risk across demographic groups, vectors, and parasite species in different ecological settings, which may not be easily captured by simple incidence and prevalence measures.

The application of new and/or refined metrics for routine surveillance activities or research-specific contexts requires investigation. This needs to be done in the context of existing standard measures and the newer data collection platforms to understand the true utility. Metrics will also need to be optimised for the quality of the healthcare system in which they will be

Box 3. Research and development agenda

Characterising the reservoir

Objective: Determine the relative contribution to transmission of symptomatic malaria, asymptomatic malaria detectable with microscopy or RDTs, and low-density infections detectable by molecular methods across different malaria typologies; data from low-transmission settings are particularly required.

Research goals

- Determine the kinetics of infectiousness of low-density parasitaemia.
- Determine the infectiousness of low-density gametocytaemia.
  - Refine mosquito feeding assays (DMFA or DFA) of human infectivity to mosquitoes and validate these against natural infectivity to local vector species.
- Determine the required sensitivity of field-based diagnostics to identify malaria infections contributing to transmission.
  - Continue to develop field-based molecular and serological diagnostics with sensitivities relevant for evaluation of infectious low-density parasitaemia and gametocytaemia.
  - Investigate non-invasive diagnostics of malaria infection and infectivity.
  - Develop hypnozoite diagnostics predictive of *P. vivax*/*P. ovale* relapse and subsequent infectivity.
  - Develop cost-effective programmatic triggers and protocols for the optimal deployment of transmission-based diagnostic tests and their incorporation within surveillance systems.
  - Evaluate the cost-effectiveness of programmatic actions and interventions directed by transmission-based diagnostics.
- Characterise changes in the transmission reservoir as transmission declines.
  - Conduct longitudinal studies in areas of declining transmission to investigate changes in the nature and distribution of the transmission reservoir.
  - Evaluate which surveillance activities and metrics are most informative and cost-effective for programmatic goals.
  - Develop operational methods to rapidly identify antimalarial drug-resistant parasites and insecticide-resistant vectors.
- Determine the relevance of spatial–temporal heterogeneity in the transmission reservoir to the acceleration of elimination.
  - Identify foci of residual transmission.

- Identify areas at risk for outbreaks and the reestablishment of malaria transmission following local elimination.

Measuring transmission

Objective: To develop a standardised and validated 'toolkit' of metrics and surveillance activities for characterising the infectious reservoir and measuring malaria transmission, which can be applied programmatically to direct interventions, evaluate interventions, and quantify progress towards malaria elimination.

Research goals

- Development of entomological as well as human measures and surveillance of transmission.
    - Continue to develop alternatives to HLC sampling for entomological measures of transmission risk.
- Continued quantification of the relationships between different metrics of transmission.
- Develop validated metrics for use in low-transmission settings and in the absence of transmission.
    - Continue to develop methods for evaluating transmission risk in low-transmission settings or in the absence of transmission.
    - Evaluate multimetric combinations for the efficient integration and analysis of low-intensity and/or heterogeneous transmission.
    - Evaluate the most cost-effective and informative metrics aligned to programmatic goals as transmission declines.
- Develop validated metrics for the evaluation of new tools directed at transmission interruption.

implemented. The same applies to the infectious reservoir. Whilst its characterisation across different transmission settings is important, translating this information into actionable programmatic decisions will be key to achieving zero malaria transmission.

Acknowledgments

We dedicate this paper to the memory of Alan Magill. Before Alan passed away in 2015, he generously accepted to play an active role in this work. We remember his extraordinary commitment to defeat malaria.

The Malaria Eradication Research Agenda (malERA) Refresh Consultative Panel on Characterising the Reservoir and Measuring Transmission was chaired by Chris Drakeley, London School of Hygiene & Tropical Medicine (LSHTM, UK) and co-chaired by Abdisalan M. Noor

(KEMRI Wellcome Trust Research Programme, Nairobi, Kenya). The paper was written based on consultations during a malERA meeting held in London, UK on 14–15th December 2015. A systematic literature search was performed by Vittoria Lutje. Panel members reviewed several iterations of the manuscript to finalise it. Laurence Slutsker (PATH Malaria and the Neglected Tropical Diseases (NTD) Program, Seattle, WA, USA) reviewed and commented on a draft of the manuscript. Figures were designed by Rachel Papernick. Additional writing and editorial support was provided by Naomi Richardson of Magenta Communications Ltd and was funded by MESA Alliance.

Disclaimer: The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the World Health Organization. MJH adds that the views expressed are those of the authors and do not necessarily reflect those of the US Centers for Disease Control and Prevention.

Members of the writing group met the ICMJE criteria for authorship and were:

Chris Drakeley (Chair), LSHTM, London, UK; Abdisalan M. Noor (Co-chair), WHO, Geneva, Switzerland; Nicole L. Achee, University of Notre Dame, USA; Teun Bousema, LSHTM, London, UK and Radboud University Nijmegen Medical Centre, the Netherlands; Ewan Cameron, Oxford Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, UK; Gonzalo Domingo, PATH, Diagnostics Program, Seattle, USA; Thomas P. Eisele, Center for Applied Malaria Research and Evaluation, Tulane School of Public Health and Tropical Medicine, New Orleans, USA; Ingrid Felger, Swiss Tropical and Public Health Institute, Basel, Switzerland and University of Basel, Switzerland; Peter Gething, Oxford Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, UK; Bryan Greenhouse, University of California, San Francisco (UCSF), USA; Ivo Mueller, ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic—Universitat de Barcelona, Barcelona, Spain, Institut Pasteur, Paris, France, and The Walter and Eliza Hall Institute of Medical Research (WEHI), Melbourne, Australia; Jetsumon Sattabongkot, Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand; Regina Rabinovich, Harvard University T.H. Chan School of Public Health, Boston, USA and ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic—Universitat de Barcelona, Barcelona, Spain; Sarah Volkman, Harvard University T.H. Chan School of Public Health, Boston, USA; Lotus van den Hoogen, LSHTM, London, UK; Lindsey Wu, LSHTM, London, UK.

malERA Refresh: Characterising the Reservoir and Measuring Transmission Panel Members: Chris Drakeley (Chair), London School of Hygiene & Tropical Medicine (LSHTM), UK; Abdisalan M. Noor (Co-chair), KEMRI Wellcome Trust Research Programme, Nairobi, Kenya; Nicole L. Achee, University of Notre Dame, USA; Maria Paz Ade, Pan American Health Organization (PAHO), Washington, DC, USA; Quique Bassat, ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic—Universitat de Barcelona, Barcelona, Spain, Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique, and ICREA, Barcelona, Spain; Adam Bennett, University of California, San Francisco (UCSF), USA; Teun Bousema, LSHTM, London, UK and Radboud University Nijmegen Medical Centre, the Netherlands; Ewan Cameron, Oxford Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, UK; Jun Cao, Jiangsu Institute of Parasitic Diseases (JIPD), China; Anna Cohuet, Institut de recherche pour le développement (IRD), MIVEGEC, Montpellier, France; Jonathan Cox, LSHTM, London, UK; Jane Cunningham, WHO, Geneva, Switzerland; Gunawardena Dissanayake, US Agency for International Development (USAID), Phnom Penh, Cambodia; Gonzalo Domingo, PATH, Diagnostics Program, Seattle, WA, USA; Thomas P. Eisele, Center for Applied Malaria Research and Evaluation, Tulane School of Public Health and Tropical Medicine, New Orleans, USA; Ingrid Felger, Swiss Tropical and Public Health Institute, Basel, Switzerland and University of Basel, Switzerland; Jaline Gerardin,

Institute for Disease Modeling, Intellectual Ventures, Bellevue, WA, USA; Peter Gething, Oxford Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, UK; Iveth Gonzalez, FIND, Geneva, Switzerland; Bryan Greenhouse, UCSF, USA; Mary J. Hamel, Centers for Disease Control and Prevention (CDC), Atlanta, USA; Melissa Kapulu, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya and Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK; Ouédraogo André Lin, Institute for Disease Modeling, Intellectual Ventures, Bellevue, WA, USA; Wendy Prudhomme O'Meara, Duke University and Duke Global Health Institute, Durham, NC, USA; Elfatih Mohamed Malik, Ministry of Health, Sudan; Alfredo Mayor, ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic—Universitat de Barcelona, Barcelona, Spain, Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique; Steve Meshnick, University of North Carolina (UNC) Gillings School of Global Public Health, USA; Bruno Moonen, BMGF, Seattle, USA; Ivo Mueller, ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic—Universitat de Barcelona, Barcelona, Spain, Institut Pasteur, Paris, France, and The Walter and Eliza Hall Institute of Medical Research (WEHI), Melbourne, Australia; Gao Qi, Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu Province, People's Republic of China; Jetsumon Sattabongkot Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand; Andre M. Siqueira, Instituto Nacional de Infectologia Evandro Chagas, Fiocruz, Brazil; Hannah Slater, Imperial College London, UK; Roger Tine, University Cheikh Anta DIOP, Department of Medical Parasitology, Senegal; Lucy Tusting, Oxford Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, UK; Sarah Volkman, Harvard University T. H. Chan School of Public Health, Boston, USA; Lotus van den Hoogen (Rapporteur), LSHTM, London, UK; Lindsey Wu (Rapporteur), LSHTM, London, UK.

References

1. malERA Consultative Group on Integration Strategies. A research agenda for malaria eradication: cross-cutting issues for eradication. PLoS Med. 2011; 8(1):e1000404. https://doi.org/10.1371/journal.pmed.1000404 PMID: 21283603
2. malERA Consultative Group on Monitoring ES. A research agenda for malaria eradication: monitoring, evaluation, and surveillance. PLoS Med. 2011; 8(1):e1000400. https://doi.org/10.1371/journal.pmed.1000400 PMID: 21311581
3. World Health Organization. WHO malaria terminology Geneva: WHO; 2016. http://apps.who.int/iris/bitstream/10665/208815/1/WHO_HTM_GMP_2016.6_eng.pdf?ua=1.
4. Schapira A, Boutsika K. Malaria ecotypes and stratification. Adv Parasitol. 2012; 78:97–167. https://doi.org/10.1016/B978-0-12-394303-3.00001-3 PMID: 22520442
5. Nájera J, Liese B, Hammer J. Malaria: new patterns and perspectives Washington, DC: The World Bank; 1992. http://documents.worldbank.org/curated/en/933151468740676854/pdf/multi-page.pdf.
6. World Health Organization. Malaria rapid diagnostic test performance Geneva: WHO; 2015. http://apps.who.int/iris/bitstream/10665/204118/1/9789241510035_eng.pdf?ua=1.
7. The malERA Refresh Consultative Panel on Combination Interventions and Modelling. malERA: An updated research agenda for combination interventions and modelling in malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002453. https://doi.org/10.1371/journal.pmed.1002453
8. Chen I, Clarke SE, Gosling R, Hamainza B, Killeen G, Magill A, et al. "Asymptomatic" malaria: A chronic and debilitating infection that should be treated. PLoS Med. 2016; 13(1):e1001942. https://doi.org/10.1371/journal.pmed.1001942 PMID: 26783752
9. Bousema T, Okell L, Felger I, Drakeley C. Asymptomatic malaria infections: detectability, transmissibility and public health relevance. Nat Rev Microbiol. 2014; 12(12):833–40. https://doi.org/10.1038/nrmicro3364 PMID: 25329408
10. Wu L, van den Hoogen LL, Slater H, Walker PG, Ghani AC, Drakeley CJ, et al. Comparison of diagnostics for the detection of asymptomatic *Plasmodium falciparum* infections to inform control and elimination strategies. Nature. 2015; 528(7580):S86–93. https://doi.org/10.1038/nature16039 PMID: 26633770

11. Cheng Q, Cunningham J, Gatton ML. Systematic review of sub-microscopic *P. vivax* infections: prevalence and determining factors. PLoS Negl Trop Dis. 2015; 9(1):e3413. https://doi.org/10.1371/journal.pntd.0003413 PMID: 25569135

12. Moreira CM, Abo-Shehada M, Price RN, Drakeley CJ. A systematic review of sub-microscopic *Plasmodium vivax* infection. Malar J. 2015; 14:360. https://doi.org/10.1186/s12936-015-0884-z PMID: 26390924

13. Imwong M, Hanchana S, Malleret B, Renia L, Day NP, Dondorp A, et al. High-throughput ultrasensitive molecular techniques for quantifying low-density malaria parasitemias. J Clin Microbiol. 2014; 52 (9):3303–9. https://doi.org/10.1128/JCM.01057-14 PMID: 24989601

14. Robinson LJ, Wampfler R, Betuela I, Karl S, White MT, Li Wai Suen CS, et al. Strategies for understanding and reducing the *Plasmodium vivax* and *Plasmodium ovale* hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model. PLoS Med. 2015; 12(10):e1001891. https://doi.org/10.1371/journal.pmed.1001891 PMID: 26505753

15. Douglas NM, Simpson JA, Phyo AP, Siswantoro H, Hasugian AR, Kenangalem E, et al. Gametocyte dynamics and the role of drugs in reducing the transmission potential of *Plasmodium vivax*. J Infect Dis. 2013; 208(5):801–12. https://doi.org/10.1093/infdis/jit261 PMID: 23766527

16. Anvikar AR, Shah N, Dhariwal AC, Sonal GS, Pradhan MM, Ghosh SK, et al. Epidemiology of *Plasmodium vivax* malaria in India. Am J Trop Med Hyg. 2016.

17. Llanos-Cuentas A, Lacerda MV, Rueangweerayut R, Krudsood S, Gupta SK, Kochar SK, et al. Tafenoquine plus chloroquine for the treatment and relapse prevention of *Plasmodium vivax* malaria (DETECTIVE): a multicentre, double-blind, randomised, phase 2b dose-selection study. Lancet. 2014; 383(9922):1049–58. https://doi.org/10.1016/S0140-6736(13)62568-4 PMID: 24360369

18. The malERA Refresh Consultative Panel on Tools for Malaria Elimination. malERA: An updated research agenda for diagnostics, drugs, vaccines, and vector control in malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002455. https://doi.org/10.1371/journal.pmed.1002455

19. Lindblade KA, Steinhardt L, Samuels A, Kachur SP, Slutsker L. The silent threat: asymptomatic parasitemia and malaria transmission. Expert Rev Anti Infect Ther. 2013; 11(6):623–39. https://doi.org/10.1586/eri.13.45 PMID: 23750733

20. Greenwood BM. Asymptomatic malaria infections—do they matter? Parasitol Today. 1987; 3(7):206–14. PMID: 15462957

21. World Health Organization. Global technical strategy for malaria 2016–2030 Geneva: WHO; 2015. http://apps.who.int/iris/bitstream/10665/176712/1/9789241564991_eng.pdf.

22. Tiono AB, Ouedraogo A, Ogutu B, Diarra A, Coulibaly S, Gansane A, et al. A controlled, parallel, cluster-randomized trial of community-wide screening and treatment of asymptomatic carriers of *Plasmodium falciparum* in Burkina Faso. Malar J. 2013; 12:79. https://doi.org/10.1186/1475-2875-12-79 PMID: 23442748

23. The malERA Refresh Consultative Panel on Health Systems and Policy Research. malERA: An updated research agenda for health systems and policy research in malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002454. https://doi.org/10.1371/journal.pmed.1002454

24. Lin JT, Saunders DL, Meshnick SR. The role of submicroscopic parasitemia in malaria transmission: what is the evidence? Trends Parasitol. 2014; 30(4):183–90. https://doi.org/10.1016/j.pt.2014.02.004 PMID: 24642035

25. Churcher TS, Trape JF, Cohuet A. Human-to-mosquito transmission efficiency increases as malaria is controlled. Nat Commun. 2015; 6:6054. https://doi.org/10.1038/ncomms7054 PMID: 25597498

26. Bousema T, Dinglasan RR, Morlais I, Gouagna LC, van Warmerdam T, Awono-Ambene PH, et al. Mosquito feeding assays to determine the infectiousness of naturally infected *Plasmodium falciparum* gametocyte carriers. PLoS ONE. 2012; 7(8):e42821. https://doi.org/10.1371/journal.pone.0042821 PMID: 22936993

27. Churcher TS, Bousema T, Walker M, Drakeley C, Schneider P, Ouedraogo AL, et al. Predicting mosquito infection from *Plasmodium falciparum* gametocyte density and estimating the reservoir of infection. Elife. 2013; 2:e00626. https://doi.org/10.7554/eLife.00626 PMID: 23705071

28. Ouédraogo AL, Guelbéogo WM, Cohuet A, Morlais I, King JG, Gonçalves BP, et al. A protocol for membrane feeding assays to determine the infectiousness of *P. falciparum* naturally infected individuals to *Anopheles gambiae*. MWJ. 2013; 4(16):1–4.

29. Ouedraogo AL, Goncalves BP, Gneme A, Wenger EA, Guelbeogo MW, Ouedraogo A, et al. Dynamics of the human infectious reservoir for malaria determined by mosquito feeding assays and ultrasensitive malaria diagnosis in Burkina Faso. J Infect Dis. 2016; 213(1):90–9. https://doi.org/10.1093/infdis/jiv370 PMID: 26142435

30. Drakeley C, Sutherland C, Bousema JT, Sauerwein RW, Targett GA. The epidemiology of *Plasmodium falciparum* gametocytes: weapons of mass dispersion. Trends Parasitol. 2006; 22(9):424–30. https://doi.org/10.1016/j.pt.2006.07.001 PMID: 16846756

31. Schneider P, Bousema JT, Gouagna LC, Otieno S, van de Vegte-Bolmer M, Omar SA, et al. Submicroscopic *Plasmodium falciparum* gametocyte densities frequently result in mosquito infection. Am J Trop Med Hyg. 2007; 76(3):470–4. PMID: 17360869

32. Graves PM, Burkot TR, Carter R, Cattani JA, Lagog M, Parker J, et al. Measurement of malarial infectivity of human populations to mosquitoes in the Madang area, Papua, New Guinea. Parasitology. 1988; 96 (Pt 2):251–63.

33. Ouedraogo AL, Bousema T, Schneider P, de Vlas SJ, Ilboudo-Sanogo E, Cuzin-Ouattara N, et al. Substantial contribution of submicroscopical *Plasmodium falciparum* gametocyte carriage to the infectious reservoir in an area of seasonal transmission. PLoS ONE. 2009; 4(12):e8410. https://doi.org/10.1371/journal.pone.0008410 PMID: 20027314

34. Pethleart A, Prajakwong S, Suwonkerd W, Corthong B, Webber R, Curtis C. Infectious reservoir of *Plasmodium* infection in Mae Hong Son Province, north-west Thailand. Malar J. 2004; 3:34. https://doi.org/10.1186/1475-2875-3-34 PMID: 15385050

35. Slater HC, Ross A, Ouedraogo AL, White LJ, Nguon C, Walker PG, et al. Assessing the impact of next-generation rapid diagnostic tests on *Plasmodium falciparum* malaria elimination strategies. Nature. 2015; 528(7580):S94–101. https://doi.org/10.1038/nature16040 PMID: 26633771

36. Schneider P, Reece SE, van Schaijk BC, Bousema T, Lanke KH, Meaden CS, et al. Quantification of female and male *Plasmodium falciparum* gametocytes by reverse transcriptase quantitative PCR. Mol Biochem Parasitol. 2015; 199(1–2):29–33. https://doi.org/10.1016/j.molbiopara.2015.03.006 PMID: 25827756

37. Zhou Z, Mitchell RM, Kariuki S, Odero C, Otieno P, Otieno K, et al. Assessment of submicroscopic infections and gametocyte carriage of *Plasmodium falciparum* during peak malaria transmission season in a community-based cross-sectional survey in western Kenya, 2012. Malar J. 2016; 15(1):421. https://doi.org/10.1186/s12936-016-1482-4 PMID: 27543112

38. Bousema T, Drakeley C. Epidemiology and infectivity of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes in relation to malaria control and elimination. Clin Microbiol Rev. 2011; 24(2):377–410. https://doi.org/10.1128/CMR.00051-10 PMID: 21482730

39. Lin JT, Ubalee R, Lon C, Balasubramanian S, Kuntawunginn W, Rahman R, et al. Microscopic *Plasmodium falciparum* gametocytemia and infectivity to mosquitoes in Cambodia. J Infect Dis. 2016; 213 (9):1491–4. https://doi.org/10.1093/infdis/jiv599 PMID: 26667316

40. Trape JF, Tall A, Sokhna C, Ly AB, Diagne N, Ndiath O, et al. The rise and fall of malaria in a West African rural community, Dielmo, Senegal, from 1990 to 2012: a 22 year longitudinal study. Lancet Infect Dis. 2014; 14(6):476–88. https://doi.org/10.1016/S1473-3099(14)70712-1 PMID: 24813159

41. Kangoye DT, Noor A, Midega J, Mwongeli J, Mkabili D, Mogeni P, et al. Malaria hotspots defined by clinical malaria, asymptomatic carriage, PCR and vector numbers in a low transmission area on the Kenyan Coast. Malar J. 2016; 15:213. https://doi.org/10.1186/s12936-016-1260-3 PMID: 27075879

42. Mwakalinga VM, Sartorius BK, Mlacha YP, Msellemu DF, Limwagu AJ, Mageni ZD, et al. Spatially aggregated clusters and scattered smaller loci of elevated malaria vector density and human infection prevalence in urban Dar es Salaam, Tanzania. Malar J. 2016; 15:135. https://doi.org/10.1186/s12936-016-1186-9 PMID: 26931372

43. Stresman GH, Baidjoe AY, Stevenson J, Grignard L, Odongo W, Owaga C, et al. Focal screening to identify the subpatent parasite reservoir in an area of low and heterogeneous transmission in the Kenya highlands. J Infect Dis. 2015; 212(11):1768–77. https://doi.org/10.1093/infdis/jiv302 PMID: 26019285

44. Bisanzio D, Mutuku F, LaBeaud AD, Mungai PL, Muinde J, Busaidy H, et al. Use of prospective hospital surveillance data to define spatiotemporal heterogeneity of malaria risk in coastal Kenya. Malar J. 2015; 14:482. https://doi.org/10.1186/s12936-015-1006-7 PMID: 26625721

45. Ndiath MM, Cisse B, Ndiaye JL, Gomis JF, Bathiery O, Dia AT, et al. Application of geographically-weighted regression analysis to assess risk factors for malaria hotspots in Keur Soce health and demographic surveillance site. Malar J. 2015; 14:463. https://doi.org/10.1186/s12936-015-0976-9 PMID: 26581562

46. Zhou G, Afrane YA, Malla S, Githeko AK, Yan G. Active case surveillance, passive case surveillance and asymptomatic malaria parasite screening illustrate different age distribution, spatial clustering and seasonality in western Kenya. Malar J. 2015; 14:41. https://doi.org/10.1186/s12936-015-0551-4 PMID: 25627802

47. Espie E, Diene Sarr F, Diop F, Faye J, Richard V, Tall A, et al. Spatio-temporal variations in malaria incidence in children less than 10 years old, health district of Sokone, Senegal, 2010–2013. PLoS ONE. 2015; 10(9):e0137737. https://doi.org/10.1371/journal.pone.0137737 PMID: 26381623

48. Ndiath M, Faye B, Cisse B, Ndiaye JL, Gomis JF, Dia AT, et al. Identifying malaria hotspots in Keur Soce health and demographic surveillance site in context of low transmission. Malar J. 2014; 13:453. https://doi.org/10.1186/1475-2875-13-453 PMID: 25418476

49. Bejon P, Williams TN, Nyundo C, Hay SI, Benz D, Gething PW, et al. A micro-epidemiological analysis of febrile malaria in Coastal Kenya showing hotspots within hotspots. Elife. 2014; 3:e02130. https://doi.org/10.7554/eLife.02130 PMID: 24843017

50. Mosha JF, Sturrock HJ, Greenwood B, Sutherland CJ, Gadalla NB, Atwal S, et al. Hot spot or not: a comparison of spatial statistical methods to predict prospective malaria infections. Malar J. 2014; 13:53. https://doi.org/10.1186/1475-2875-13-53 PMID: 24517452

51. Davis M, von Cavallar S, Wyres KL, Reumann M, Sepulveda MJ, Rogers P. Spatio-temporal information and knowledge representation of disease incidence and respective intervention strategies. Stud Health Technol Inform. 2014; 205:1173–7. PMID: 25160374

52. Yamana TK, Bomblies A, Laminou IM, Duchemin JB, Eltahir EA. Linking environmental variability to village-scale malaria transmission using a simple immunity model. Parasit Vectors. 2013; 6:226. https://doi.org/10.1186/1756-3305-6-226 PMID: 23919581

53. Valle D, Lima JM. Large-scale drivers of malaria and priority areas for prevention and control in the Brazilian Amazon region using a novel multi-pathogen geospatial model. Malar J. 2014; 13:443. https://doi.org/10.1186/1475-2875-13-443 PMID: 25412882

54. Bousema T, Stresman G, Baidjoe AY, Bradley J, Knight P, Stone W, et al. The impact of hotspot-targeted interventions on malaria transmission in rachuonyo south district in the estern Kenyan highlands: A cluster-randomized controlled trial. PLoS Med. 2016; 13(4):e1001993. https://doi.org/10.1371/journal.pmed.1001993 PMID: 27071072

55. Maude RJ, Pontavornpinyo W, Saralamba S, Aguas R, Yeung S, Dondorp AM, et al. The last man standing is the most resistant: eliminating artemisinin-resistant malaria in Cambodia. Malar J. 2009; 8:31. https://doi.org/10.1186/1475-2875-8-31 PMID: 19228438

56. The malERA Refresh Consultative Panel on Insecticide and Drug Resistance. malERA: An updated research agenda for insecticide and drug resistance in malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002450. https://doi.org/10.1371/journal.pmed.1002450

57. Pinder M, Moorthy V, Mendis K, Brown G, on behalf of the WHO MALVAC committee. MALVAC 2010: Measures of efficacy of anti-malarial interventions against malaria transmission, 15–16 November 2010 in Geneva, Switzerland Geneva: WHO; 2010. http://www.who.int/immunization/research/meetings_workshops/MALVAC_2010_meeting_report.pdf.

58. Tusting LS, Bousema T, Smith DL, Drakeley C. Measuring changes in *Plasmodium falciparum* transmission: precision, accuracy and costs of metrics. Adv Parasitol. 2014; 84:151–208. https://doi.org/10.1016/B978-0-12-800099-1.00003-X PMID: 24480314

59. Briet OJ, Huho BJ, Gimnig JE, Bayoh N, Seyoum A, Sikaala CH, et al. Applications and limitations of Centers for Disease Control and Prevention miniature light traps for measuring biting densities of African malaria vector populations: a pooled-analysis of 13 comparisons with human landing catches. Malar J. 2015; 14:247. https://doi.org/10.1186/s12936-015-0761-9 PMID: 26082036

60. Chaki PP, Mlacha Y, Msellemu D, Muhili A, Malishee AD, Mtema ZJ, et al. An affordable, quality-assured community-based system for high-resolution entomological surveillance of vector mosquitoes that reflects human malaria infection risk patterns. Malar J. 2012; 11:172. https://doi.org/10.1186/1475-2875-11-172 PMID: 22624853

61. Mathenge EM, Omweri GO, Irungu LW, Ndegwa PN, Walczak E, Smith TA, et al. Comparative field evaluation of the Mbita trap, the Centers for Disease Control light trap, and the human landing catch for sampling of malaria vectors in western Kenya. Am J Trop Med Hyg. 2004; 70(1):33–7. PMID: 14971695

62. Kilama M, Smith DL, Hutchinson R, Kigozi R, Yeka A, Lavoy G, et al. Estimating the annual entomological inoculation rate for *Plasmodium falciparum* transmitted by Anopheles gambiae s.l. using three sampling methods in three sites in Uganda. Malar J. 2014; 13:111. https://doi.org/10.1186/1475-2875-13-111 PMID: 24656206

63. Wong J, Bayoh N, Olang G, Killeen GF, Hamel MJ, Vulule JM, et al. Standardizing operational vector sampling techniques for measuring malaria transmission intensity: evaluation of six mosquito collection methods in western Kenya. Malar J. 2013; 12:143. https://doi.org/10.1186/1475-2875-12-143 PMID: 23631641

64. Sikaala CH, Killeen GF, Chanda J, Chinula D, Miller JM, Russell TL, et al. Evaluation of alternative mosquito sampling methods for malaria vectors in Lowland South-East Zambia. Parasit Vectors. 2013; 6:91. https://doi.org/10.1186/1756-3305-6-91 PMID: 23570257

65. Lima JB, Rosa-Freitas MG, Rodovalho CM, Santos F, Lourenco-de-Oliveira R. Is there an efficient trap or collection method for sampling *Anopheles darlingi* and other malaria vectors that can describe

the essential parameters affecting transmission dynamics as effectively as human landing catches? A Review. Mem Inst Oswaldo Cruz. 2014; 109(5):685–705. https://doi.org/10.1590/0074-0276140134 PMID: 25185008

66. Govella NJ, Maliti DF, Mlwale AT, Masallu JP, Mirzai N, Johnson PC, et al. An improved mosquito electrocuting trap that safely reproduces epidemiologically relevant metrics of mosquito human-feeding behaviours as determined by human landing catch. Malar J. 2016; 15:465. https://doi.org/10.1186/s12936-016-1513-1 PMID: 27618941

67. Maliti DV, Govella NJ, Killeen GF, Mirzai N, Johnson PC, Kreppel K, et al. Development and evaluation of mosquito-electrocuting traps as alternatives to the human landing catch technique for sampling host-seeking malaria vectors. Malar J. 2015; 14:502. https://doi.org/10.1186/s12936-015-1025-4 PMID: 26670881

68. Mweresa CK, Mukabana WR, Omusula P, Otieno B, Van Loon JJ, Takken W. Enhancing attraction of african malaria vectors to a synthetic odor blend. J Chem Ecol. 2016; 42(6):508–16. https://doi.org/10.1007/s10886-016-0711-1 PMID: 27349651

69. Pombi M, Jacobs F, Verhulst NO, Caputo B, della Torre A, Takken W. Field evaluation of a novel synthetic odour blend and of the synergistic role of carbon dioxide for sampling host-seeking *Aedes albopictus* adults in Rome, Italy. Parasit Vectors. 2014; 7:580. https://doi.org/10.1186/s13071-014-0580-9 PMID: 25499569

70. Brady OJ, Godfray HC, Tatem AJ, Gething PW, Cohen JM, McKenzie FE, et al. Vectorial capacity and vector control: reconsidering sensitivity to parameters for malaria elimination. Trans R Soc Trop Med Hyg. 2016; 110(2):107–17. https://doi.org/10.1093/trstmh/trv113 PMID: 26822603

71. Dewald JR, Fuller DO, Muller GC, Beier JC. A novel method for mapping village-scale outdoor resting microhabitats of the primary African malaria vector, *Anopheles gambiae*. Malar J. 2016; 15(1):489. https://doi.org/10.1186/s12936-016-1534-9 PMID: 27659918

72. Sikulu M, Dowell KM, Hugo LE, Wirtz RA, Michel K, Peiris KH, et al. Evaluating RNAlater(R) as a preservative for using near-infrared spectroscopy to predict *Anopheles gambiae* age and species. Malar J. 2011; 10:186. https://doi.org/10.1186/1475-2875-10-186 PMID: 21740582

73. Mayagaya VS, Michel K, Benedict MQ, Killeen GF, Wirtz RA, Ferguson HM, et al. Non-destructive determination of age and species of *Anopheles gambiae* s.l. using near-infrared spectroscopy. Am J Trop Med Hyg. 2009; 81(4):622–30. https://doi.org/10.4269/ajtmh.2009.09-0192 PMID: 19815877

74. Ntamatungiro AJ, Mayagaya VS, Rieben S, Moore SJ, Dowell FE, Maia MF. The influence of physiological status on age prediction of *Anopheles arabiensis* using near infra-red spectroscopy. Parasit Vectors. 2013; 6(1):298. https://doi.org/10.1186/1756-3305-6-298 PMID: 24499515

75. Amoah LE, Abankwa J, Oppong A. *Plasmodium falciparum* histidine rich protein-2 diversity and the implications for PfHRP 2: based malaria rapid diagnostic tests in Ghana. Malar J. 2016; 15:101. https://doi.org/10.1186/s12936-016-1159-z PMID: 26891848

76. Murillo Solano C, Akinyi Okoth S, Abdallah JF, Pava Z, Dorado E, Incardona S, et al. Deletion of *Plasmodium falciparum* histidine-rich protein 2 (pfhrp2) and histidine-rich protein 3 (pfhrp3) genes in Colombian parasites. PLoS ONE. 2015; 10(7):e0131576. https://doi.org/10.1371/journal.pone.0131576 PMID: 26151448

77. Tietje K, Hawkins K, Clerk C, Ebels K, McGray S, Crudder C, et al. The essential role of infection-detection technologies for malaria elimination and eradication. Trends Parasitol. 2014; 30(5):259–66. https://doi.org/10.1016/j.pt.2014.03.003 PMID: 24726857

78. Cordray MS, Richards-Kortum RR. Emerging nucleic acid-based tests for point-of-care detection of malaria. Am J Trop Med Hyg. 2012; 87(2):223–30. https://doi.org/10.4269/ajtmh.2012.11-0685 PMID: 22855751

79. Hofmann N, Mwingira F, Shekalaghe S, Robinson LJ, Mueller I, Felger I. Ultra-sensitive detection of *Plasmodium falciparum* by amplification of multi-copy subtelomeric targets. PLoS Med. 2015; 12(3):e1001788. https://doi.org/10.1371/journal.pmed.1001788 PMID: 25734259

80. Kong TF, Ye W, Peng WK, Hou HW, Marcos, Preiser PR, et al. Enhancing malaria diagnosis through microfluidic cell enrichment and magnetic resonance relaxometry detection. Sci Rep. 2015; 5:11425. https://doi.org/10.1038/srep11425 PMID: 26081638

81. Peng WK, Kong TF, Ng CS, Chen L, Huang Y, Bhagat AA, et al. Micromagnetic resonance relaxometry for rapid label-free malaria diagnosis. Nat Med. 2014; 20(9):1069–73. https://doi.org/10.1038/nm.3622 PMID: 25173428

82. Mueller I, Schoepflin S, Smith TA, Benton KL, Bretscher MT, Lin E, et al. Force of infection is key to understanding the epidemiology of *Plasmodium falciparum* malaria in Papua New Guinean children. Proc Natl Acad Sci U S A. 2012; 109(25):10030–5. https://doi.org/10.1073/pnas.1200841109 PMID: 22665809

83. Nkhoma SC, Nair S, Al-Saai S, Ashley E, McGready R, Phyo AP, et al. Population genetic correlates of declining transmission in a human pathogen. Mol Ecol. 2013; 22(2):273–85. https://doi.org/10.1111/mec.12099 PMID: 23121253

84. Daniels RF, Schaffner SF, Wenger EA, Proctor JL, Chang HH, Wong W, et al. Modeling malaria genomics reveals transmission decline and rebound in Senegal. Proc Natl Acad Sci U S A. 2015; 112 (22):7067–72. https://doi.org/10.1073/pnas.1505691112 PMID: 25941365

85. Ondigo BN, Hodges JS, Ireland KF, Magak NG, Lanar DE, Dutta S, et al. Estimation of recent and long-term malaria transmission in a population by antibody testing to multiple *Plasmodium falciparum* antigens. J Infect Dis. 2014; 210(7):1123–32. https://doi.org/10.1093/infdis/jiu225 PMID: 24737801

86. Drakeley CJ, Corran PH, Coleman PG, Tongren JE, McDonald SL, Carneiro I, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. Proc Natl Acad Sci U S A. 2005; 102(14):5108–13. https://doi.org/10.1073/pnas.0408725102 PMID: 15792998

87. Stewart L, Gosling R, Griffin J, Gesase S, Campo J, Hashim R, et al. Rapid assessment of malaria transmission using age-specific sero-conversion rates. PLoS ONE. 2009; 4(6):e6083. https://doi.org/10.1371/journal.pone.0006083 PMID: 19562032

88. Franca CT, Hostetler JB, Sharma S, White MT, Lin E, Kiniboro B, et al. An antibody screen of a *Plasmodium vivax* antigen library identifies novel merozoite proteins associated with clinical protection. PLoS Negl Trop Dis. 2016; 10(5):e0004639. https://doi.org/10.1371/journal.pntd.0004639 PMID: 27182597

89. Sarr JB, Orlandi-Pradines E, Fortin S, Sow C, Cornelie S, Rogerie F, et al. Assessment of exposure to *Plasmodium falciparum* transmission in a low endemicity area by using multiplex fluorescent microsphere-based serological assays. Parasit Vectors. 2011; 4:212. https://doi.org/10.1186/1756-3305-4-212 PMID: 22059951

90. Helb DA, Tetteh KK, Felgner PL, Skinner J, Hubbard A, Arinaitwe E, et al. Novel serologic biomarkers provide accurate estimates of recent *Plasmodium falciparum* exposure for individuals and communities. Proc Natl Acad Sci U S A. 2015; 112(32):E4438–47. https://doi.org/10.1073/pnas.1501705112 PMID: 26216993

91. Crompton PD, Kayala MA, Traore B, Kayentao K, Ongoiba A, Weiss GE, et al. A prospective analysis of the Ab response to *Plasmodium falciparum* before and after a malaria season by protein microarray. Proc Natl Acad Sci U S A. 2010; 107(15):6958–63. https://doi.org/10.1073/pnas.1001323107 PMID: 20351286

92. Goo YK, Seo EJ, Choi YK, Shin HI, Sattabongkot J, Ji SY, et al. First characterization of *Plasmodium vivax* liver stage antigen (PvLSA) using synthetic peptides. Parasit Vectors. 2014; 7:64. https://doi.org/10.1186/1756-3305-7-64 PMID: 24520895

93. Armiyanti Y, Nuryady MM, Arifianto RP, Nurmariana E, Senjarini K, Fitri LE, et al. Detection of immunogenic proteins from *Anopheles sundaicus* salivary glands in the human serum. Rev Soc Bras Med Trop. 2015; 48(4):410–6. https://doi.org/10.1590/0037-8682-0185-2015 PMID: 26312930

94. Londono-Renteria B, Drame PM, Weitzel T, Rosas R, Gripping C, Cardenas JC, et al. An. gambiae gSG6-P1 evaluation as a proxy for human-vector contact in the Americas: a pilot study. Parasit Vectors. 2015; 8:533. https://doi.org/10.1186/s13071-015-1160-3 PMID: 26464073

95. Yao MX, Sun XD, Gao YH, Cheng ZB, Deng WW, Zhang JJ, et al. Multi-epitope chimeric antigen used as a serological marker to estimate *Plasmodium falciparum* transmission intensity in the border area of China-Myanmar. Infect Dis Poverty. 2016; 5(1):98. https://doi.org/10.1186/s40249-016-0194-x PMID: 27604628

96. Yman V, White MT, Rono J, Arca B, Osier FH, Troye-Blomberg M, et al. Antibody acquisition models: A new tool for serological surveillance of malaria transmission intensity. Sci Rep. 2016; 6:19472. https://doi.org/10.1038/srep19472 PMID: 26846726

97. Baum E, Sattabongkot J, Sirichaisinthop J, Kiattibutr K, Jain A, Taghavian O, et al. Common asymptomatic and submicroscopic malaria infections in Western Thailand revealed in longitudinal molecular and serological studies: a challenge to malaria elimination. Malar J. 2016; 15:333. https://doi.org/10.1186/s12936-016-1393-4 PMID: 27333893

98. Baidjoe AY, Stevenson J, Knight P, Stone W, Stresman G, Osoti V, et al. Factors associated with high heterogeneity of malaria at fine spatial scale in the Western Kenyan highlands. Malar J. 2016; 15:307. https://doi.org/10.1186/s12936-016-1362-y PMID: 27259286

99. Yeka A, Nankabirwa J, Mpimbaza A, Kigozi R, Arinaitwe E, Drakeley C, et al. Factors associated with malaria parasitemia, anemia and serological responses in a spectrum of epidemiological settings in Uganda. PLoS ONE. 2015; 10(3):e0118901. https://doi.org/10.1371/journal.pone.0118901 PMID: 25768015

100. Cunha MG, Silva ES, Sepulveda N, Costa SP, Saboia TC, Guerreiro JF, et al. Serologically defined variations in malaria endemicity in Para state, Brazil. PLoS ONE. 2014; 9(11):e113357. https://doi.org/10.1371/journal.pone.0113357 PMID: 25419900

101. Hsiang MS, Hwang J, Kunene S, Drakeley C, Kandula D, Novotny J, et al. Surveillance for malaria elimination in Swaziland: a national cross-sectional study using pooled PCR and serology. PLoS ONE. 2012; 7(1):e29550. https://doi.org/10.1371/journal.pone.0029550 PMID: 22238621

102. Kobayashi T, Chishimba S, Shields T, Hamapumbu H, Mharakurwa S, Thuma PE, et al. Temporal and spatial patterns of serologic responses to *Plasmodium falciparum* antigens in a region of declining malaria transmission in southern Zambia. Malar J. 2012; 11:438. https://doi.org/10.1186/1475-2875-11-438 PMID: 23276228

103. Rosas-Aguirre A, Speybroeck N, Llanos-Cuentas A, Rosanas-Urgell A, Carrasco-Escobar G, Rodriguez H, et al. Hotspots of malaria transmission in the Peruvian Amazon: Rapid assessment through a parasitological and serological survey. PLoS ONE. 2015; 10(9):e0137458. https://doi.org/10.1371/journal.pone.0137458 PMID: 26356311

104. Sepulveda N, Paulino CD, Drakeley C. Sample size and power calculations for detecting changes in malaria transmission using antibody seroconversion rate. Malar J. 2015; 14:529. https://doi.org/10.1186/s12936-015-1050-3 PMID: 26715538

105. Stevenson JC, Stresman GH, Baidjoe A, Okoth A, Oriango R, Owaga C, et al. Use of different transmission metrics to describe malaria epidemiology in the highlands of western Kenya. Malar J. 2015; 14:418. https://doi.org/10.1186/s12936-015-0944-4 PMID: 26502920

106. van den Hoogen LL, Griffin JT, Cook J, Sepulveda N, Corran P, Conway DJ, et al. Serology describes a profile of declining malaria transmission in Farafenni, The Gambia. Malar J. 2015; 14:416. https://doi.org/10.1186/s12936-015-0939-1 PMID: 26492873

107. Zakeri S, van den Hoogen LL, Mehrizi AA, Karimi F, Raeisi A, Drakeley C. Anti-malarial seroprevalence assessment during an elimination programme in Chabahar District, south-eastern Iran. Malar J. 2016; 15(1):382. https://doi.org/10.1186/s12936-016-1432-1 PMID: 27448606

108. Weppelmann TA, von Fricken ME, Lam B, Telisma T, Existe A, Lemoine JF, et al. Sparse serological evidence of *Plasmodium vivax* transmission in the Ouest and Sud-Est departments of Haiti. Acta Trop. 2016; 162:27–34. https://doi.org/10.1016/j.actatropica.2016.05.011 PMID: 27230796

109. Cook J, Speybroeck N, Sochanta T, Somony H, Sokny M, Claes F, et al. Sero-epidemiological evaluation of changes in Plasmodium falciparum and *Plasmodium vivax* transmission patterns over the rainy season in Cambodia. Malar J. 2012; 11:86. https://doi.org/10.1186/1475-2875-11-86 PMID: 22443375

110. Cameron A, Njeumi F, Chibeu D, Martin T. Risk-based disease surveillance: A manual for vetinarians on the deisgn and analysis of surveillance for demonstration of freedom from disease Rome: Food and Agriculture Organization of the United Nations; 2014. http://www.fao.org/3/a-i4205e.pdf.

111. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015; 526(7572):207–11. https://doi.org/10.1038/nature15535 PMID: 26375008

112. Noor AM, Kinyoki DK, Mundia CW, Kabaria CW, Mutua JW, Alegana VA, et al. The changing risk of *Plasmodium falciparum* malaria infection in Africa: 2000–10: a spatial and temporal analysis of transmission intensity. Lancet. 2014; 383(9930):1739–47. https://doi.org/10.1016/S0140-6736(13)62566-0 PMID: 24559537

113. Tatem AJ. Mapping population and pathogen movements. Int Health. 2014; 6(1):5–11. https://doi.org/10.1093/inthealth/ihu006 PMID: 24480992

114. MalariaGEN. 2016. Cited 05 Sept 2016. https://www.malariagen.net/data.

115. Worldpop. 2016. Cited 05 Sept 2016. http://www.worldpop.org.uk/.

116. Pindolia DK, Garcia AJ, Wesolowski A, Smith DL, Buckee CO, Noor AM, et al. Human movement data for malaria control and elimination strategic planning. Malar J. 2012; 11:205. https://doi.org/10.1186/1475-2875-11-205 PMID: 22703541

117. Wesolowski A, Buckee CO, Pindolia DK, Eagle N, Smith DL, Garcia AJ, et al. The use of census migration data to approximate human movement patterns across temporal scales. PLoS ONE. 2013; 8(1):e52971. https://doi.org/10.1371/journal.pone.0052971 PMID: 23326367

118. Marshall JM, Toure M, Ouedraogo AL, Ndhlovu M, Kiware SS, Rezai A, et al. Key traveller groups of relevance to spatial malaria transmission: a survey of movement patterns in four sub-Saharan African countries. Malar J. 2016; 15:200. https://doi.org/10.1186/s12936-016-1252-3 PMID: 27068686

119. Blangiardo M, Cameletti M, Baio G, Rue H. Spatial and spatio-temporal models with R-INLA. Spat Spatiotemporal Epidemiol. 2013; 4:33–49. https://doi.org/10.1016/j.sste.2012.12.001 PMID: 23481252

120. Ruktanonchai NW, DeLeenheer P, Tatem AJ, Alegana VA, Caughlin TT, Zu Erbach-Schoenberg E, et al. Identifying Malaria Transmission Foci for Elimination Using Human Mobility Data. PLoS Comput Biol. 2016; 12(4):e1004846. https://doi.org/10.1371/journal.pcbi.1004846 PMID: 27043913

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COLLECTION REVIEW

malERA: An updated research agenda for diagnostics, drugs, vaccines, and vector control in malaria elimination and eradication

The malERA Refresh Consultative Panel on Tools for Malaria Elimination[¶]*

¶ Membership of the malERA Refresh Consultative Panel on Tools for Malaria Elimination is listed in the Acknowledgments.

* dkaslow@path.org (DCK), fredros@ihi.or.tz (FO), wellst@mmv.org (TW)

Citation: The malERA Refresh Consultative Panel on Tools for Malaria Elimination (2017) malERA: An updated research agenda for diagnostics, drugs, vaccines, and vector control in malaria elimination and eradication. PLoS Med 14(11): e1002455. https://doi.org/10.1371/journal.pmed.1002455

Published: November 30, 2017

Funding: MESA received a grant from the Bill & Melinda Gates Foundation (OPP1034591) http://www.malariaeradication.org/mesa-track/malaria-eradication-scientific-alliance-mesa. JC (Bill & Melinda Gates Foundation) was a member of this panel and participated in the panel consultation and in the development of this paper.

Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: FB is a full-time employee of Sanofi, a manufacturer of antimalarial medicines. JV was a full time employee of GlaxoSmithKline Biologicals at the time of the panel consultation.

Abstract

Since the turn of the century, a remarkable expansion has been achieved in the range and effectiveness of products and strategies available to prevent, treat, and control malaria, including advances in diagnostics, drugs, vaccines, and vector control. These advances have once again put malaria elimination on the agenda. However, it is clear that even with the means available today, malaria control and elimination pose a formidable challenge in many settings. Thus, currently available resources must be used more effectively, and new products and approaches likely to achieve these goals must be developed. This paper considers tools (both those available and others that may be required) to achieve and maintain malaria elimination. New diagnostics are needed to direct treatment and detect transmission potential; new drugs and vaccines to overcome existing resistance and protect against clinical and severe disease, as well as block transmission and prevent relapses; and new vector control measures to overcome insecticide resistance and more powerfully interrupt transmission. It is also essential that strategies for combining new and existing approaches are developed for different settings to maximise their longevity and effectiveness in areas with continuing transmission and receptivity. For areas where local elimination has been recently achieved, understanding which measures are needed to maintain elimination is necessary to prevent rebound and the reestablishment of transmission. This becomes increasingly important as more countries move towards elimination.

Summary points

- Achieving malaria elimination likely requires new interventions and strategies in some settings. In addition, the effectiveness of existing tools must be preserved and tools deployed to counter the numerous challenges, key among which are the emergence and spread of drug-resistant parasites and mosquitoes with resistance to vector control measures.

Abbreviations: ACT, artemisinin-based combination therapy; BS-VIMT, blood-stage vaccine that interrupts malaria parasite transmission; ChAd63, chimpanzee adenovirus 63; CHMI, controlled human malaria infection; G6PD, glucose-6-phosphate dehydrogenase; iiPCR, insulated isothermal polymerase chain reaction; IRS, indoor residual spraying; LAMP, loop-mediated isothermal amplification; LDH, lactate dehydrogenase; LDR-FM, ligase detection reaction fluorescent microsphere; LLIN, long-lasting insecticidal net; malERA, Malaria Eradication Research Agenda; MDA, mass drug administration; MPT, multipurpose prevention technology; MVA, Modified Vaccinia Virus Ankara; NCE, new chemical entity; NINA-LAMP, noninstrumented nucleic acid amplification LAMP; PCR, polymerase chain reaction; PE-VIMT, preerythrocytic vaccine to interrupt malaria transmission; PfHRP2, *Plasmodium falciparum* histidine-rich protein 2; QT-NASBA, quantitative nucleic acid sequence-based amplification; qPCR, quantitative PCR; R&D, research and development; RDT, rapid diagnostic test; SERCaP, single encounter radical cure and prophylaxis; SMC, seasonal malaria chemoprevention; SNP, single nucleotide polymorphism; SPAQ, sulfadoxine-pyrimethamine + amodiaquine; SSM-VIMT, sexual-sporogonic-mosquito-stage vaccines to interrupt transmission; TPP, target product profile; WHO, World Health Organization.

Provenance: Submitted as part of a Supplement; externally peer reviewed.

- The key research goal for diagnostics is the detection of populations with subclinical infections and low parasite counts. Such diagnostics enable the development of effective surveillance systems directed at malaria parasite elimination.
- The availability of new transmission-blocking drugs, vaccines, and vector control products would accelerate elimination where there is refractoriness to currently available interventions. New regulatory pathways and product development models are needed to efficiently develop and assess these new interventions.
- In areas endemic for *Plasmodium vivax* and *P. ovale*, the hypnozoite reservoir must be targeted with more robust tools and strategies.
- In areas of declining transmission, as cases become less frequent, the contribution to transmission of the subclinical parasite reservoir needs to be quantified and addressed with transmission-blocking interventions.
- For vector control, addressing continuing escalation of insecticide resistance—including through the identification of new chemical classes and longer-lasting insecticide formulations—remains a priority. Changes in vector populations and behaviours must also be addressed to restore responsiveness to existing interventions. In some areas, new paradigms may be needed to understand how to design interventions that reduce vector populations and receptivity to sufficiently low levels.
- Policy and decision makers, faced with chronic resource limitations, insufficient surveillance, spatial and temporal heterogeneity of malaria parasite transmission, and multiple intervention choices, need improved strategies and guidance on how, where, and when to best combine and deploy existing and new interventions to maximise their longevity and effectiveness.

Introduction

Achieving malaria parasite elimination across all countries (i.e., malaria eradication), especially for those with a high disease burden, likely requires new tools and strategies to complement existing interventions [1,2]. Given the inevitable uncertainties in product development and given that different sets of tools will be applicable in different settings, a broad and imaginative research and development agenda needs to be pursued. The research and development agenda presented in this paper is in support of the WHO Global Technical Strategy for malaria goals from 2016 to 2030, and tracking the progress of this research and development (R&D) agenda and reevaluating the research needs will be required over time [2]. In Malaria Eradication Research Agenda (malERA) 2011, diagnostics, drugs, vaccines, and vector control were considered separately [3–6]. However, for malERA Refresh, this paper considers together the research agenda for all existing and prospective tools to accelerate progress towards achieving and maintaining malaria elimination. In this case, the relationships between the different research agendas can be more easily recognised. Other papers in this malERA Refresh series consider the related discussions regarding the implementation and combination of tools [7], implications of insecticide and antimalarial drug resistance [8], health system and policy issues [9], and advances in basic science [10].

Progress on tools for malaria elimination

Based on literature reviews and panel consultations [11], the most significant advances in the development and deployment of malaria control and elimination tools between 2011 and 2015 were identified (S1–S4 Texts). For diagnostics, advances include widespread incorporation of *P. falciparum* rapid diagnostic tests (RDTs) into routine malaria case management [12], development of highly sensitive tools for detecting subclinical infections, and development and deployment of combined tests that differentiate *P. falciparum* from *P. vivax* [13,14]. For drugs, advances include the deployment of hundreds of millions of artemisinin-based combination therapy (ACT) courses [12], publication of guidelines for mass drug administration (MDA) [15], the recommendation of low-dose primaquine for transmission interruption [16], progression of new antimalarial compound classes into clinical development [17–19], field trials to evaluate the potential role of medicines in killing mosquitoes (endectocides) [2], and the identification of *Kelch13* as a marker for artemisinin resistance, enabling mapping of its geographic distribution [20,21]. For vaccines, advances include the Article 58 positive opinion by the European Medicines Agency and recommendations by the World Health Organization (WHO) on the first vaccine targeting malaria, RTS,S-AS01$_E$ (Box 1) [22–33]; revision of the Malaria Vaccine Technology Roadmap [34]; and new vaccines that progressed to clinical trials [35,36]. For vector control, advances include registration of 2 additional long-lasting insecticide formulations for indoor residual spraying (IRS) [37,38], field trials of dual-insecticide bed nets [39–41], development programmes for new insecticides [42–44], and publication of the larval source management operational manual by WHO [45]. Advances have also been made in the 'tools for developing tools'—for example, controlled human malaria infection (CHMI) blood-stage parasite inoculation (Box 2) [46–56]; the human blood-stage challenge model for early-stage determination of antimalarial drug pharmacokinetics/pharmacodynamics [57]; the development of human liver chimeric mice, human erythroid chimeric mice, and dually engrafted mice allowing replication of the entire *P. falciparum* life cycle [58]; and validation of phenotypic assays for gametocyte screening to identify compounds with transmission-blocking activity [59]. In addition, new technologies and scientific insights are emerging [10], with notable improvements in mapping and modelling [7,60–62].

Diagnostics research agenda

Diagnostics for malaria treatment and elimination

To direct malaria treatment, all cases should be confirmed with a diagnostic test, either RDT or light microscopy, even in low-transmission settings [63]. Current WHO criteria for RDT procurement recommend a false positive rate of $<10\%$ [14]. However, a test with 99% specificity, when used at the elimination threshold (prevalence of parasitaemia in the community of $\leq 0.1\%$), results in $\geq 90\%$ of positive tests coming from samples with no *Plasmodium* parasites [64]. In very low-transmission settings, addressing the challenge of false positive tests may require developing algorithms such as parallel or serial confirmation with a second test. Recently, false-negative results for *P. falciparum* histidine-rich protein 2 (PfHRP2)-based RDTs have been reported from several regions, caused by pfhrp2/pfhrp3 gene deletions [65–70]. Universal validity of these diagnostic tests cannot be assumed, and the WHO has issued guidance on PfHRP2-based RDT procurement [71].

Beyond *P. falciparum*, improved RDTs are needed for other species. Available lactate dehydrogenase (LDH)-targeting RDTs are less sensitive for *P. vivax* compared to *P. falciparum*, because *P. vivax* parasite densities tend to be much lower [72]. There is a paucity of information on test performance against minor species.

Box 1. Malaria vaccine RTS,S/A101$_E$

In 2015, the preerythrocytic candidate vaccine RTS,S/AS01$_E$ (RTS,S) received a positive scientific opinion by European regulators through the Article 58 procedure. This was a breakthrough in malaria vaccine development, identifying a regulatory pathway and demonstrating that the large clinical trials necessary for approval could be conducted in Africa [23–25].

- The target for RTS,S is the reduction of malaria incidence and severe disease in young children. A 3-dose regimen was shown to reduce the number of malaria cases by half in children 5–17 months of age during the first year following vaccination; efficacy waned over time but was prolonged by a fourth dose [25].
- Despite modest efficacy, RTS,S prevented about 1,700 cases for every 1,000 children vaccinated in a phase III study over a 4-year period, and modelling studies predict a considerable public health impact for RTS,S, with the greatest benefit expected in areas with the highest malaria burden [27].
- Following review of RTS,S data by the Strategic Advisory Group of Experts on Immunization and the Malaria Policy Advisory Committee, in 2016 the WHO adopted recommendations for RTS,S pilot implementations in 3–5 settings involving 100,000–200,000 children per setting (for a total of 400,000–800,000), in a staged manner to further evaluate safety (including meningitis [26]), feasibility of delivery, and impact on mortality.
- Phase IV studies with a primary objective of further evaluation of safety as part of the Risk Management Plan approved by European Medicines Agency are planned to be linked to the larger pilots, with complementary design and objectives [28,29].

Research to optimize the regimen and explore additional applications of RTS,S

- **Optimising the RTS,S dosing regimen**

Additional controlled human malaria infection (CHMI) and phase IIb studies are in progress to better define how to improve RTS,S/AS01 efficacy and how these data translate to the field, respectively.

- A small study with RTS,S and an earlier adjuvant (AS02) found that fractional dosing, i.e., 2 full monthly doses plus a third low-dose at 7 months, resulted in apparent high efficacy against *P. falciparum* challenge (6/7 protected) [30].
- A recent CHMI study in a larger number of volunteers using RTS,S/AS01 confirmed that a 0-, 1-, 7-month regimen that included a fractional third dose (Fx017M) was associated with higher efficacy (86.7% [95% confidence interval [CI], 66.8%–94.6%]; 26/30 protected) than the standard monthly full-dose regimen (62.5% [95% CI, 29.4%–80.1%]; 10/16 protected) against infection 3 weeks after the third dose [31].

- **Additional applications for RTS,S**

Additional applications of RTS,S explored through modelling and, if indicated, evaluated in carefully designed field studies over the next 5-year period include the following [33]:

- Evaluating the contribution to elimination of artemisinin-resistant parasites in the Greater Mekong Subregion, although data supporting an adult indication (dose and regimen) would be needed [32];
- Combining RTS,S with other interventions or another malaria vaccine (mass drug administration [MDA] or a future VIMT, respectively), with the aim of enhancing or extending their effects;
- Combining RTS,S with seasonal malaria chemoprevention (SMC), a study of which is in progress in Burkina Faso and Mali.

In the elimination context, a malaria diagnostic tool is needed for reactive or proactive detection of infectious parasite reservoirs residing in those individuals with subclinical infections and/or with parasite densities lower than those reliably detected with existing RDTs and microscopy (Fig 1) [73]. A target product profile (TPP) has been developed for a point-of-care malaria infection detection test for rapid detection of low-density, subclinical malaria infections [64]. Provided this was sufficiently sensitive, it would potentially enable targeting of populations harbouring reservoirs of parasite biomass with interventions interrupting transmission.

The most efficient uses for digital health are still being explored in malaria, but integrating diagnostic results generated from malaria case management into elimination programme surveillance efforts offer a near-term opportunity to fill critical data gaps in mapping malaria prevalence [2,7]. For example, 1 study combined a globally accessible database with mobile phone-based imaging of RDTs to provide an objective diagnostic readout and automated collection of surveillance data [74]. A similar approach in Kenya used digital RDT readers with upload to a cloud database [75]. However, lessons learned from digital health applied to the eradication programme for tuberculosis suggest that attaining a population-level impact are undermined by insufficient scale, coordination, and end-user engagement [76]. These issues are likely compounded in malaria given the higher prevalence of the disease globally.

Approaches to developing diagnostics. Several biomedical engineering approaches for malaria parasite detection have been investigated [77], including automated image processing [78], microfluidic systems [79], microarray chips [80], dielectrophoresis [81], and exploiting the bioelectrical properties of blood [77]. Further development of these techniques to increase sensitivity and specificity to detect clinically unapparent malaria parasite infections and allow field deployment continues.

Simplified molecular methods to detect low-level *P. falciparum* parasitaemia for use in low-resource settings are being developed, although improvements in throughput and cost are required [82]. Loop-mediated isothermal amplification (LAMP) is 1 promising approach, already validated in low-transmission settings [83] and as point-of-care detection of asymptomatic low-density malaria parasite carriers [84]. Further developments include noninstrumented nucleic acid amplification LAMP (NINA-LAMP) [85], achieving comparable sensitivity to *P. falciparum* polymerase chain reaction (PCR) detection in the field [85,86]. Another approach, using an insulated isothermal PCR (iiPCR) in a commercially available portable device, for *Plasmodium* detection achieved an assay efficiency of 96.9% with a lower detection limit of $\geq$100 copies of plasmodial DNA [87]. Nucleic acid amplification techniques

Box 2. Tools for developing tools: Demonstrating transmission-blocking activity

The validation of surrogate end points for transmission-blocking activity that translate into known effects in the field is necessary for the efficient development of new interventions aimed at this target.

Mosquito feeding assays

Three assays are available for assessing transmission-blocking activity:

- Direct feeding assay (DFA): allowing mosquitos to feed on parasitaemic hosts; the most 'physiologically relevant' method [46,47].
- Direct membrane feeding assay (DMFA): blood samples from parasitaemic hosts are fed to susceptible mosquitoes through an artificial membrane [48].
- Standard membrane feeding assay (SMFA): laboratory-reared mosquitoes are fed a controlled number of cultured gametocytes from a single parasite strain combined with uninfected human erythrocytes and serum from human volunteers or animals. SFMA is now available as a medium throughput, reproducible, standardised assay [49].

In the context of elimination, the relevant outcome from these assays is a reduction in the number of infected mosquitoes.

Controlled human malaria infection (CHMI) model

Three CHMI techniques have been developed to determine the ability of drugs and vaccines to prevent human infection:

- Sporozoite mosquito bites: infection of human volunteers via mosquito biting [50,51].
- Sporozoite direct venous inoculation (SDVI): injection of sporozoites into human volunteers [52,53].
- Induced blood-stage malaria parasite infection (IBSM): administration of *Plasmodium*-infected red blood cells to human volunteers [54,55].

Each of these techniques has advantages and disadvantages. Both sporozoite-based models allow evaluation of preerythrocytic and blood-stage drugs and vaccines, whereas IBSM can determine blood-stage efficacy only.

To evaluate transmission-blocking efficacy in preventing transmission from humans to mosquitoes, CHMI can be followed by a mosquito feeding assay using blood or serum from CHMI volunteers. Development of a regulatory pathway using mosquito feeding assays and CHMI with relevance to transmission-blocking activity in the field is ongoing. This effort would benefit from the development of new vaccines and drugs aimed specifically at this indication [56].

can also be used for multiple pathogens in parallel, incorporating other infectious diseases (e.g., Ebola, dengue, and typhoid), depending on the setting and target population [88].

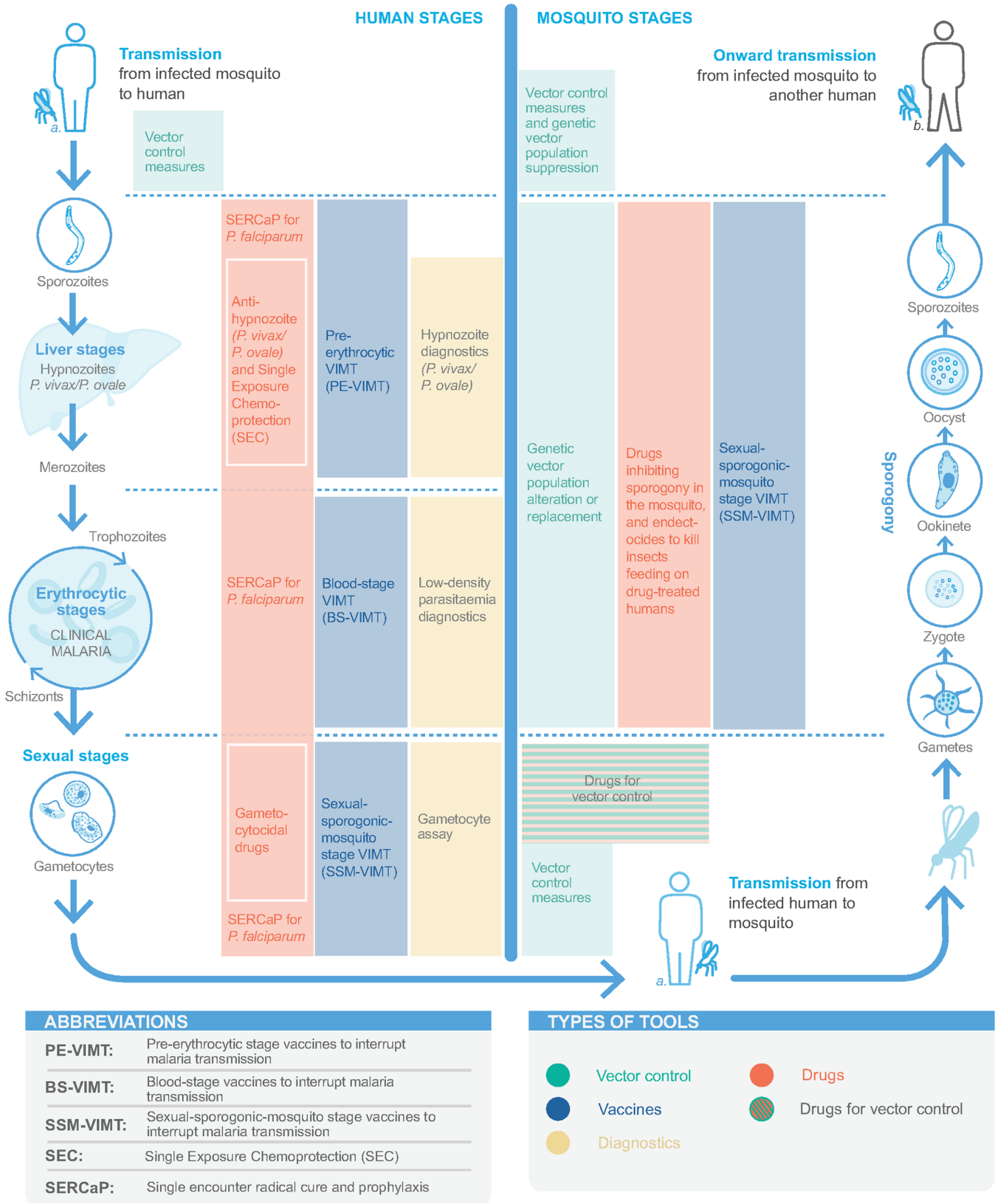

Fig 1. Tools for detecting and interrupting malaria transmission and their action in the malaria transmission cycle.

https://doi.org/10.1371/journal.pmed.1002455.g001

Noninvasive testing

Although a noninvasive technique is highly preferred, all currently available diagnostics require blood samples. PCR-based assays to detect *Plasmodium* parasites in saliva, although unsuitable for routine diagnosis, have been successfully developed [89]. Malaria detection in urine has been evaluated in field trials, but its sensitivity requires improvement [90]. Preliminary investigations indicate that malaria-specific volatile levels from breath samples correlate with parasite clearance [91], and studies are ongoing. A transdermal, noninvasive, reagent-free approach relying on the presence of iron-rich haemozoin to generate vapour nanobubbles is currently being field tested to detect parasites in skin blood vessels [92].

Detecting gametocytes

While gametocyte detection may indicate an individual's transmission potential, further definition is required as to the most appropriate clinical sample to collect and the relevant gametocyte levels reflecting infectiousness [47]; this is complicated by a lack of correlation between gametocyte density in the blood and infectiousness following antimalarial treatment [93]. Validation of relevant target sequences is a first step towards development of molecular methods amenable for routine gametocyte detection. Circulating *P. falciparum* female and male and *P. vivax* gametocytes can now be detected using quantitative nucleic acid sequence-based amplification (QT-NASBA) or quantitative PCR (qPCR) methods with pfs25-, pfs230p-, and pvs25-based primers, respectively [94–98].

Detecting drug resistance

Detection of Kelch-propeller polymorphisms conferring artemisinin-resistance is currently restricted to sentinel surveillance [21], though more granular information is needed with continuing efforts to eliminate artemisinin-resistant parasites [99]. For example, a next-generation amplicon sequencing method suitable for use in endemic countries enables high-throughput detection of genetic mutations in 6 *P. falciparum* genes associated with resistance to antimalarial drugs, including artemisinins, chloroquine, and sulfadoxine-pyrimethamine [100]. For detecting *P. falciparum* single nucleotide polymorphisms (SNPs) associated with antifolate drug resistance, the ligase detection reaction fluorescent microsphere (LDR-FM) assay has been validated in clinical trials in Uganda [101]. As noted elsewhere in the malERA Refresh series [8], continued research on identifying markers of resistance to the other antimalarial drugs in current use (e.g., lumefantrine and piperaquine) is critical, as tools are needed to detect and manage drug resistance inevitable in elimination efforts [102,103].

Detecting hypnozoites

Hypnozoites residing in the human host is one tactic used by *P. vivax* and *P. ovale* to sustain the parasite reservoir between transmission seasons and produce multiple clinical relapses over prolonged periods, each with the potential to maintain transmission [104]. Detecting *P. vivax*/*P. ovale* hypnozoites, however, is problematic because of their low density, metabolic inactivity, and sequestration within the liver. Biomarkers that detect hypnozoites would be breakthrough tools in both case management diagnostics and elimination surveillance for *P. vivax* and *P. ovale* infections.

Glucose-6-phosphate dehydrogenase (G6PD) testing

An affordable, easy-to-use, rapid, point-of-care, semiquantitative diagnostic test is needed to identify G6PD-deficient individuals at risk of haemolysis with use of 8-aminoquinolones (i.e.,

primaquine or tafenoquine) to prevent *P. vivax* relapses. Although several tests are available [105], further refinement is needed to support greater access to these medicines. Single administration of low-dose (0.25 mg/kg) primaquine as a gametocytocidal agent is recommended after treatment for *P. falciparum* malaria [106], but there is still a need for more data on the optimal dose and reassurance of safety in G6PD-deficient individuals and larger populations, if used in MDA, for example [107].

Pregnancy testing

Pregnant women are excluded from receiving certain drugs or interventions, and rapid, low-cost, low-complexity, point-of-use pregnancy tests are needed, particularly for populations receiving MDA drugs with contraindications for use during pregnancy.

Challenges

Diagnostics are needed to direct treatment, support surveillance, and identify transmission reservoirs [73] and for continued progress in the development and evaluation of other tools for elimination, e.g., in settings with low-density parasitaemia and low transmission and for interventions targeting hypnozoites/prevention of relapse. The relevance of low-density parasitaemia to transmission requires further investigation to enable the design of diagnostics appropriate for these needs. Longer term, development of noninvasive assays, and field assays for detecting drug-resistant parasites should be pursued. Detecting hypnozoites remains a more profound challenge, although proteomics and metabolomics are being explored [108].

Drug research agenda

Drugs for malaria treatment, prevention, and transmission interruption

A strong portfolio of combination medicines with different or competing resistance mechanisms is required to combat resistance. It is now possible to tune the development program to advance drugs that have high barriers to resistance development and a low potential for cross resistance with other agents. In addition to classic inhibitory experiments, the propensity of drugs to induce ring-stage dormancy, characteristic of artemisinin resistance, must also be evaluated [109].

Single encounter radical cure and prophylaxis (SERCaP). Proposed in malERA 2011, SERCaP remains a priority [5]. Radical cure means clearance of all asexual blood-stage forms, mature gametocytes, and *P. vivax*/*P. ovale* hypnozoites (Fig 1). Combination therapies of new chemical entities (NCEs) that are targeted to 'single encounter, radical cure' are now in phase II clinical trials, with potential regulatory submission dates circa 2021 (S1 Table) [17].

The post-treatment prophylactic component of the SERCaP will come from the long half-life of the active pharmaceutical ingredients. Malaria parasite elimination will require new generations of single-encounter chemoprotection, to protect migrating populations and protect against epidemics in the later stages of elimination. These products would include molecules that provide chemoprotection by targeting the preerythrocytic stages (see TCP-4 specific attributes in [110]).

Reducing duration of dosing regimens, ideally to a single dose, increases adherence, be it for prevention or treatment [8]. Although better adherence improves effectiveness, it must be achieved without significantly increasing the risk of selection for drug-resistant parasites as a result of creating long periods of subtherapeutic drug levels. As a country or area approaches elimination, the remaining parasites are likely to be those most resistant to treatment, and drug classes with a low propensity to select for parasite resistance should be prioritised [111,

112]. The temptation to combine new drugs with old drugs with preexisting resistance, whilst simpler from a regulatory perspective, must be avoided to prevent novel agents being exposed as functional monotherapies when used against strains resistant to the older partner drug.

Severe malaria. Intravenous and intramuscular artesunate are currently the most effective and well-tolerated treatments for severe malaria [113,114], with rectal artesunate recommended for pre-referral treatment of children who cannot quickly access hospital care [106]. The potential spread of artemisinin resistance threatens the effectiveness of artesunate for treatment of severe malaria. Thus, new compounds with rapid activity against asexual blood stage parasites, suitable for parenteral administration, are needed for this critical indication (see TCP-1 specific attributes in [110]). The decline in the incidence of severe malaria in adults will require alternative development approaches, including the development of surrogate end points [115], as not enough patients will be available for large mortality studies [116]. However, sufficient safety data in adults would still be required for a phase III trial in African children with severe malaria.

Interrupting transmission. Drugs with activity against gametocytes in humans or that impair sporogony in the mosquito could help to interrupt transmission (Fig 1) [117]. While low-dose (0.25 mg/kg single dose) primaquine is currently recommended as a gametocytocidal following ACT treatment for *P. falciparum* malaria in areas of low transmission [16,118,119], NCE combination therapies with both therapeutic and transmission-blocking activity would simplify drug administration. High-throughput screening and clinical evaluation of compounds with transmission-blocking activity are now possible (Box 2) [59,120–125] and have yielded new leads, including more than a dozen from the Medicines for Malaria Venture toolbox with activity in the standard membrane feeding assay [126].

Global antimalarial drug development portfolio. There are at least 15 active projects in preclinical development or phase I or II clinical trials (S1 Table) [17]. A range of new chemotypes targeting new parasite pathways are available, with antimalarial drug development accelerated using CHMI models (Box 2). Two pairs of NCE combinations are in phase II clinical studies: the long-lasting synthetic endoperoxide artefenomel (OZ439) combined with the next-generation 4-aminoquinoline, ferroquine; and the imidazolopiperazine KAF156 combined with a new once-per-day lumefantrine formulation. This latter combination is also being explored as a 3-day regimen for use as a frontline agent in areas with ACT resistance. Single-dose effectiveness with an appropriate safety profile may require triple combination therapy. Notably, KAF156, DSM265, and MMV390048 have activity against *P. falciparum* liver stages and could be given as a single-dose treatment or once weekly for chemoprotection (S1 Table) [127]. TPPs and target candidate profiles with minimal essential and ideal attributes for single-encounter chemoprotection have been published [110].

Antihypnozoite drugs

In areas of high transmission, such as Papua New Guinea, relapses cause approximately 4 of every 5 *P. vivax* infections [128]. Modelling suggests that for rapidly relapsing tropical *P. vivax* strains, effective relapse prevention has the potential to significantly reduce transmission [104]. In areas of seasonal transmission, relapses allow parasites to rapidly reestablish transmission once vector populations recover [129]. The 8-aminoquinoline primaquine is the only antirelapse therapy currently available (aside from chloroquine, to which there is extensive resistance), but treatment courses are 7–14 days, and poor adherence undermines effectiveness [130]. Tafenoquine is a candidate single-dose 8-aminoquinoline, showing high antirelapse efficacy in *P. vivax* infections when given with chloroquine [18]. Phase III clinical trials were completed in 2016, with regulatory submission anticipated in 2017. The impact of tafenoquine on transmission

remains to be evaluated in post-registration CHMI and field trials. G6PD-deficient individuals cannot be given standard doses of 8-aminoquinolones; in addition, 8-aminoquinolones are considered contraindicated during pregnancy and lactation. As such, new antihypnozoite drug classes without these contraindications are needed. Discovery should be enhanced in the next 5 years through screening campaigns against *P. vivax* liver stages using stable human cell systems [131,132]. Additionally, humanised mouse models are facilitating drug development against this life cycle stage that thus far has been refractory to study [133].

Seasonal malaria chemoprevention (SMC)

In areas where malaria is seasonal, providing SMC by monthly treatment with long-lasting antimalarial drugs greatly reduces malaria burden in children under 5 years of age [134,135]. Modelling studies indicate the potential for reducing transmission to very low levels if SMC is combined with long-lasting insecticidal nets (LLINs) at 80% coverage and expanded to children up to 10 years of age [136]. Sulfadoxine-pyrimethamine + amodiaquine (SPAQ) is used for SMC in the Sahel; drug resistance prevents SPAQ use in eastern and southern Africa, and there are concerns that resistance may spread to the Sahel. Thus, alternative drugs are required, ideally with simplified dosing regimens.

Endectocides

Endectocides are an alternative approach to malaria control whereby humans and/or livestock are given agents with insecticidal activity, resulting in reduced survival of the vector upon blood feeding and impairment of malaria parasite transmission [137]. Modelling studies suggest that the endectocide ivermectin could help achieve transmission interruption as an additional intervention in settings where mass treatment strategies with ACTs alone would be insufficient to accomplish elimination [138]. A research agenda was proposed in 2013 outlining the path for ivermectin use in malaria [139], with a number of studies in different settings underway. A WHO expert group recently examined this concept, with findings anticipated in 2017. The antimosquito properties of veterinary and other candidate endectocides are also being explored.

Novel formulations

An interesting possibility is the application of nanomilling and related technologies to develop long-acting drug formulations, which are being investigated for long-term HIV preexposure prophylaxis and in combination with contraception in so-called multipurpose prevention technologies (MPTs) [140–143]. Such long-acting drug formulations could potentially allow chemoprotection over several months from a single injection. Application to new generations of transmission-blocking molecules or endectocides could provide tools that reduce or prevent transmission over an entire transmission season.

Challenges

Attrition rates in antimalarial drug development are comparable with those in other infectious diseases [126]. Thus, discovery momentum needs to be maintained at high levels if new drugs are to reach licensure. A major challenge in registering NCEs for malaria is assembling the substantial clinical safety data required for regulatory approval, particularly in the key target populations of infants and pregnant women. Thus, reproductive safety should be evaluated early in preclinical development to prioritise investment in NCEs with appropriate preclinical profiles.

With the introduction of NCEs during the next 5 years, pharmacovigilance needs strengthening in malaria-endemic areas. This is also a prerequisite for safe deployment of current ACTs and next-generation treatments during mass treatment programmes targeted at populations that include individuals with subclinical malaria or who are infection free.

In the next 5–10 years, there is a need to enrich the early-stage portfolio with new antihypnozoite drugs beyond the current 8-aminoquinolones. Cell biology and the animal models supporting drug discovery for new antihypnozoite agents have progressed significantly but are still not amenable to high-throughput screening programmes [144,145]. Clinical trials for relapse prevention take 6–12 months, much longer than treatment trials. Additionally, relapses can be caused by hypnozoites that are homologous or heterologous to the initial infection and cannot, therefore, be distinguished from recrudescence or reinfection [146–148], except by the physical removal of treated patients from transmission areas, e.g., repatriated soldiers and travellers.

Although NCEs active against artemisinin-resistant isolates are in development, better strategies are needed to deploy drugs to delay or prevent the emergence of drug resistance, such as measures to tackle counterfeiting or manufacturing of poor-quality medicines, drug sequencing, multiple firstline therapies, and exploiting competing resistance mechanisms, as discussed elsewhere in the malERA Refresh series [8].

Vaccine research agenda

The Malaria Vaccines Technology Roadmap was updated in 2013 [34], with the goal of developing by 2030 vaccines for *P. falciparum* and *P. vivax* that have a protective efficacy of at least 75% against clinical malaria and/or reduce transmission of the parasite. The roadmap outlines key priorities in research, vaccine development, key capacities, policy, and commercialisation. The research issues in malaria vaccines are discussed below, but key to their success will be ensuring an efficient and cost-effective distribution system and redirection of the health system from delivering malaria treatment to prevention and transmission interruption [9].

Vaccines to prevent clinical malaria and interrupt transmission

A preerythrocytic vaccine to interrupt malaria transmission (PE-VIMT) that completely prevents liver-stage infection for a significant duration (e.g., at least 1 transmission season) would prevent parasitaemia and gametocyte generation and therefore interrupt onward transmission (Fig 1). Although RTS,S is a preerythrocytic vaccine, demonstrating modest efficacy in preventing clinical malaria, prevention of infection and transmission were not evaluated in the late-stage clinical trials (Box 1). More recently, a delayed fractional dose regimen of RTS,S with improved efficacy against a parasite transmission (mosquito-to-human) end point may be considered for transmission-blocking potential (Box 1) [31]. Several next-generation preerythrocytic candidates are in clinical development, including multistage (including asexual blood-stage and/or sexual/sporogonic/mosquito-stage targets) combinations and prime-boost strategies, as well as irradiated or genetically attenuated sporozoites (S2 Table) [35,149]. Future directions need to ensure a widely acceptable route of administration, optimised dose regimens, and lower inoculum sizes.

Blood-stage vaccines are an alternative and complementary approach to PE-VIMT. Blood-stage vaccines that interrupt malaria parasite transmission (BS-VIMTs) by efficiently clearing blood-stage infections would limit gametocyte densities and the duration that a person is infectious, thus reducing human-to-mosquito malaria parasite transmission (Fig 1). Several promising *P. falciparum* vaccine candidates are in clinical development [150], including the unstructured peptide P27A, the well-studied PfRH5, and the 2 placental malaria vaccine

candidates PAMVAC and PRIMVAC (S2 Table). Innovative new concepts in next-generation malaria vaccine protein subunit design are being explored to develop highly effective multi-component/multistage/multiantigen formulations [151].

Vaccines that only interrupt malaria parasite transmission

Sexual-sporogonic-mosquito-stage vaccines to interrupt transmission (SSM-VIMT) inhibit parasite transmission from human to mosquito, through reducing gametocytes' ability to infect mosquitoes or by interfering with parasite development (sporogony) within the mosquito (Fig 1). As the potential benefit to the recipient is both delayed and indirect, the PATH Malaria Vaccine Initiative and partners are exploring potential regulatory and policy approaches with the United States Food and Drug Administration and WHO, respectively [152,153]. Progress has been made, with a design proposed for a phase III study [154]. The Pfs25 antigen is expressed on the surface of zygotes and ookinetes in the mosquito midgut, and various attempts to improve immunogenicity and transmission-blocking activity have been undertaken (S2 Table) [36,153]. The most clinically advanced is Pfs25-EPA (a detoxified form of exotoxin A from *Pseudomonas aeruginosa*) conjugate [155]. Most recently, Pfs25 has been fused with IMX313, a molecular adjuvant, and expressed in chimpanzee adenovirus 63 (ChAd63) and Modified Vaccinia Virus Ankara (MVA) viral vectors and as a secreted protein nanoparticle [156]. The research agenda has broadened to include other SSM-VIMT antigens, including Pfs230 and Pfs48/45 (S2 Table).

Vaccines for *P. vivax/P. ovale*

A vaccine that could prevent *P. vivax/P. ovale* infection and hypnozoite formation, target hypnozoites, or prevent disease, thereby interrupting transmission and draining the hypnozoite reservoir, would be a significant step for accelerating malaria elimination (Fig 1). *P. vivax* is now included in the Malaria Vaccine Technology Roadmap strategic goals [34]. While basic research in *P. vivax* has increased in recent years, no vaccine candidate has progressed past early human studies (S3 Table) [35,36]. Three preerythrocytic vaccines have reached clinical trials [157–159]. A blood-stage vaccine targeting the Duffy-binding protein region II has progressed to early clinical trials [160], though combination with other blood-stage antigens is likely necessary to achieve high growth inhibition. The Pvs25 antigen is also being investigated as an SSM-VIMT. The recent development of *P. vivax* CHMI systems allows evaluation of vaccine efficacy [157,161]. Also, publication of the *P. ovale* and *P. malariae* genomes facilitates antigen discovery for these parasites [162].

Adjuvants, delivery platforms, and desired human immune responses

Most (but not all) malaria vaccines in development are based on *Plasmodium* protein subunits and have shown limited immunogenicity in humans. Suitable adjuvants and delivery platforms are therefore needed to elicit the desired immune response and induce significant protection from infection and disease without unacceptable collateral inflammation [163]. There are few adjuvants licensed for human use and there is a need to (1) better define the desired human immune response; (2) facilitate access to adjuvants in development and ensure downstream availability, affordability, and acceptability; (3) develop more specific targeted adjuvants that boost desired immune responses while maintaining acceptable safety; and (4) match individual adjuvants to individual vaccine candidates depending on the postulated mechanism of action while maintaining compatibility for combination vaccines.

Prophylactic biologics

Monoclonal antibodies are another potential tool. Recently, the major barriers of cost are being overcome through improvements in manufacturing and high-expressing cell lines [164]. A recent report estimated that the cost of goods for monoclonal antibodies had reduced 10-fold, from thousands of dollars per gram to around $100 per gram, with the costs of developing these agents comparable to other therapeutic drugs and vaccines [165]. Additionally, the volume and frequency of administration of monoclonal antibodies have been reduced by improvements in potency and pharmacokinetics [166]. There has been a significant increase in the number of validated vaccine targets, and now monoclonal antibodies can be studied early in clinical development for their ability to provide immediate protection in CHMI models, either singly or in combination [167]. Antibodies are less prone to the off-target safety and toxicity issues that often plague small molecule development and thus offer significant advantages for deployment in vulnerable populations, including the immunocompromised and pregnant women. In the context of elimination, monoclonal antibodies with suitable pharmacokinetics/pharmacodynamics could represent an alternative to active immunisation by VIMTs or transmission-blocking drugs. As with any tool, prophylactic biologicals will need to be designed to meet the needs and capabilities in target settings.

Challenges

To achieve malaria elimination, vaccines would ideally be able to prevent infection by all 5 species of human malaria parasites. While humans are the major (if not only) reservoir for 4 of 5 *Plasmodium* ssp., zoonotic *P. knowlesi* presents a unique challenge for elimination given continuous sylvatic transmission [168]. If a '*Plasmodium*' vaccine targeting all human-infecting species is not feasible, then vaccines are required against individual species. It remains to be determined whether experience gained in the development of *P. falciparum* vaccines can, in fact, inform approaches to other malaria species or whether new strategies are required.

Similar to drugs used in MDA, vaccines for mass inoculation need to be safe for use in pregnant women and children. Demonstrating safety across the target population is particularly important for vaccines that only prevent transmission and have an indirect benefit to the recipient.

For malaria vaccine candidates, there is limited information on immune correlates that may predict efficacy in the chosen indication. Antigenic diversity of many of the malaria vaccine targets [169,170] adds additional complexity to predicting efficacy and enables parasites to evade host immune responses, potentially leading to vaccine escape mutants [171,172].

There is also incomplete understanding of the development and maintenance of either naturally acquired or vaccine-induced human immunity to *Plasmodium*. A predictable 'age shift' in peak incidence of malaria associated with vaccines with modest and/or waning efficacy in children who have not acquired full natural immunity must be anticipated and appropriately managed [173]. The challenge of maintaining individual and population-based (herd) immunity may increase as circulating parasite prevalence declines during the later stages of elimination. Thus, rationally designing vaccines that induce long-lasting immunity in semi-immune adults and provide broad cross strain protection presents formidable challenges.

Finally, as with drugs, parasite genetic diversity and rich population structures, particularly in high-transmission settings, indicate the potential for differential parasite-specific efficacy and selection of resistant *Plasmodium*. The former has been observed in vaccine field studies, including a recent genetic analysis associated with a large phase III trial of RTS,S/AS01 [170]. However, there are no data regarding whether implementing malaria vaccination induces parasite resistance in the whole population of infected individuals.

Vector control research agenda

Insecticide-based interventions

LLINs are currently the single most important malaria control intervention, responsible for approximately 68% of malaria cases averted in Africa [174]. However, emerging resistance to insecticides among *Anopheles* mosquitoes threatens to reverse these gains [175,176]. New insecticides with different modes of action are urgently needed to deter resistance development. In response, 'Innovation to Impact' was initiated in 2013 with an aim to transform the process for developing and delivering life-saving vector control products for diseases caused by vector-borne pathogens. More than 30 different stakeholder groups are involved, including industry, global evaluation and regulatory bodies, procurers, local and national representatives, and donors [42,43].

Twelve insecticide products are currently available for vector control, confined to 4 chemical classes (pyrethroids, organochlorines, organophosphates, and carbamates), although only pyrethroids are widely used for LLINs. Several combination LLINs consisting of different insecticide classes or incorporating the synergist piperonyl butoxide are in late-stage development (Table 1) [39–41,177–181]. Similar to LLINs, long-lasting insecticide-treated hammocks could be effective in remote areas; however, the lifespan of these interventions has a significant impact on cost-effectiveness, and exploration of technologies to increase durability is needed [182].

After screening around 4 million compounds, 3 new insecticides have progressed to development, with registration typically taking 5–7 years [44,178]. These new insecticides are primarily pyrethroid alternatives for use in LLINs but also would be expected to have use in IRS. For IRS, 2 long-lasting formulations of existing compounds have become available: a microencapsulated formulation of the organophosphate insecticide pirimiphos methyl in 2012 [37] and a polymer-enhanced suspension of deltamethrin in 2013 [38]. The Next Generation IRS project is a market intervention to accelerate uptake and increase use of long-lasting IRS products [183]. Additional long-lasting insecticides suitable for IRS are in development (Table 1).

Table 1. Insecticides for indoor residual spraying (IRS) under World Health Organization Pesticide Evaluation Scheme (WHOPES) evaluation and long-lasting insecticidal nets (LLINs) in late-stage development [39–41,177,178]*.

Application	Product	Insecticide(s)
IRS	Phantom	Chlorfenapyr (phase III)
	SumiShield	Clothianidin (phase II)
	Fludora Fusion	Deltamethrin + clothianidin (phase II)
LLINs	DawaPlus 2.0	Deltamethrin coated on polyester
	LifeNet	Deltamethrin incorporated into polypropylene
	MiraNet	Alpha-cypermethrin incorporated into polyethylene
	Panda Net 2.0	Deltamethrin incorporated into polyethylene
	Yahe	Deltamethrin coated on polyester
LLINs + PBO	Olyset Plus	Permethrin + PBO incorporated into polyethylene
	PermaNet 3.0	Deltamethrin coated on polyester side panels; deltamethrin + PBO incorporated into polyethylene (roof)
	Veeralin	Alpha-cypermethrin and PBO incorporated into polyethylene
Combination LLINs	Olyset Duo	Pyriproxyfen and permethrin incorporated into polyethylene
	Interceptor G2	Alpha-cypermethrin + chlorfenapyr coated on polyester

*March/April 2016.

PBO, piperonyl butoxide

https://doi.org/10.1371/journal.pmed.1002455.t001

New ways of using insecticides require more extensive field evaluation, e.g., technological advances for improving spraying techniques [184], timing of insecticide deployment to coincide with seasonal transmission, slow-release polymer-based wall linings [185,186], insecticide-treated eave tubes or eave 'bricks' combined with house screening, and electrostatic coatings to enhance insecticide bioavailability [187].

Vector behaviour and outdoor targeting

Greater understanding of vector behaviour is needed, including the behavioural adaptations of vectors in response to control measures, such as changes in biting times, resting locations, and rates of zoophagy [188–194]. Improved targeting of specific vector behaviours—particularly sugar feeding, oviposition, mating, dry-season survival, and swarming behaviour—and zooprophylaxis are generating novel approaches to vector control, with potential application across transmission settings [195,196].

Long-standing evidence that malaria parasite transmission to humans occurs outdoors in Southeast Asia and South America and increasing evidence of outdoor transmission in sub-Saharan Africa [3,197–202] suggest a specific need for interventions that target mosquitoes outside dwellings. Attractants/traps are a potential new area of mosquito control that can be applied both indoors and outdoors. These include attractive toxic sugar baits [203,204] and sound traps, which lure male mosquitoes by broadcasting sounds similar to the wingbeats of female mosquitoes [195,203]. All major malaria parasite vectors in Africa mate in swarms [206], which are easily found and recognised, appear to be stable over time, and exist in a defined space [195]. This facilitates close targeting either with insecticides or traps [195]. Spatial repellents are another approach, releasing into the air volatile chemicals that prevent human–vector contact within the treated space (indoor or outdoor). Guidelines for efficacy testing are now available [205,207], and evaluation in outdoor settings is needed [208,209].

Environmental management and larval source management

Environmental management, such as improved housing and water management, can be highly effective in specific epidemiological and environmental settings [210]. The best of these environmental management approaches require further investigation in tropical climates and resource-poor settings to establish their epidemiological impact in these settings [210,211]. Mosquito larval source management is the management of water bodies that are potential larval habitats to prevent immature mosquitoes developing into adults, either by environmental management or application of larvicides [45]. Larval source management has been highly effective in certain situations [211], but as this is a resource-intensive activity, better definition of the appropriate requirements and approaches across a wider range of settings is needed.

Genetic approaches

There are 2 main strategies for genetically modifying mosquito populations: (1) population suppression, whereby mosquitoes are modified in such a way that upon mating with the wild type the resulting progeny are either sterile or dysfunctional, and (2) population alteration or replacement, in which the mosquitoes are modified in such a way that upon mating with the wild type, the resulting progeny are rendered refractory to malaria parasite infection. Genetic approaches now appear operationally feasible given recent advances in molecular biology, such as the efficient genome-editing techniques based on CRISPR/Cas9 and other approaches [10,212,213].

The sterile insect technique was the first attempt at genetic population suppression, whereby large numbers of irradiated sterile males are released with the hope that females mate

unsuccessfully [214]. A more recent development is the release of insects carrying a dominant lethality, with the progeny of females mating with genetically modified males inheriting a lethal gene [215,216].

Gene drive systems exploit 'homing' endonucleases. These induce the lateral transfer of an intervening DNA sequence to a homologous allele that lacks that sequence, thereby changing a heterozygote into a homozygote. Conventional homing endonucleases have been reengineered to recognise mosquito genes [217] and can rapidly increase the frequency of desirable traits in a mosquito population [218]. Technical feasibility has been demonstrated for a CRISPR/Cas9--based gene drive system with the potential to reduce mosquito populations [219] or make them less able to transmit malaria parasites [220].

There is also the potential for symbiont-mediated biocontrol in malaria *Anopheles* mosquito populations, as suggested by recent successes achieved against *Aedes aegypti* (e.g., *Wolbachia*-mediated pathogen interference for dengue control). A further step is paratransgenesis, whereby a vector symbiont (virus, bacteria, or fungi) is engineered to express 'effector' molecules within the vector that are deleterious to the pathogen. Genetic modification of symbionts is easier than it is for mosquitoes and is independent of mosquito species, providing the symbiont can survive and colonise the host [221], and laboratory studies have shown promise [222].

There are environmental uncertainties associated with widespread distribution of technologies involving genetic manipulation of pathogens, vectors, or their symbionts [10,212]. Phased testing starting at a small scale is recommended, though the parameters for ecological risk assessment are not well understood.

Challenges

The development of new insecticides will need to outpace the expansion of insecticide-resistant alleles in mosquito populations, and new products will need to be deployed to effectively combat behavioural resistance [8]. The imperfect correlation between entomological indicators and disease incidence complicates the accurate assessment of new vector control tools. Randomised controlled trials are expensive and time consuming, and new pathways should be explored for generating evidence for large-scale implementation of new interventions. Increasing fine-scale heterogeneity, in human and vector subpopulations and in geographic space, means that no single set of interventions will be effective across large areas or districts. Notwithstanding resource availability, the challenge is to understand which combinations of vector control measures are appropriate in different settings and how their effects can be augmented with other interventions (e.g., endectocides, transmission-blocking drugs, and vaccines) [7]. Targeting mosquito dormancy remains a challenge in large part because of the paucity of mechanistic evidence by which vectors persist during the dry-season (e.g., diapause [aestivation] and long-distance migration) [223]. Finally, it is important to note that there are very few trained entomologists in national malaria control programs, especially at the district level. To develop and implement vector-targeted interventions, greater entomology capacity building is required.

Conclusions

There are overarching areas in which greater knowledge is required to understand the utility of current interventions and define which products and strategies might be required going forward. Novel tools may allow further investigation of knowledge gaps, and some may be bridgeable (Table 2). The R&D agenda for tools for elimination is summarised in Box 3.

Transmission can remain high even with high coverage of good-quality case management and vector control. Thus, products and strategies directed specifically at accelerating

Table 2. Knowledge gaps and tools to potentially bridge the gaps.

Knowledge gaps	Tools to potentially bridge the gaps
High-to-low transmission	
• Why do transmission rates remain high even when case management and vector control have high coverage?	• Methods that are protective against infection and interrupt transmission (Fig 1).
• At which point should interventions specifically directed at reducing transmission be introduced?	• Robust mathematical and laboratory models of transmission and impact of combination interventions.
• What is the contribution of the subclinical reservoir to transmission in high-transmission settings?	• Sensitive point-of-care tests to detect transmission reservoirs and enable evaluation of interventions.
• In vector control, which factors drive changes in transmitting species?	• Prevention or control measures effective against all species.
Low-to-zero transmission	
• How best can the remaining sources of transmission be identified?	• Affordable, rapid, sensitive screening techniques to identify populations generating infectious gametocytes.
• What is the impact of nonhuman malaria parasite transmission on the effectiveness of vector control?	• Vector control measures with efficacy independent of nonhuman transmission.
• How can transmission be measured when it is low or zero?	• See Malaria Eradication Research Agenda (malERA) Refresh 'Characterising the reservoir and measuring transmission' [73].
• How can approaches to false-positive diagnostic tests be addressed?	• Development of highly sensitive and specific tests, along with combination testing algorithm/protocols to identify false positives.
• How can the *P. vivax*/*P. ovale* hypnozoite reservoir be identified and targeted?	• Development of hypnozoite diagnostics, and/or antihypnozoite drugs/vaccines that are safe enough for use in population-based administration.
• How can heterogeneity in transmission be managed?	• Interventions that are safe and cost-effective enough to be used across wider populations.
Maintaining zero transmission	
• How can the efficacy of tools be measured when transmission is zero?	• Development of validated surrogate end points of efficacy.
• What are the drivers of epidemic malaria?	• Modification of vector populations to decrease epidemic potential; tools for epidemic response, including for nonimmune populations.

https://doi.org/10.1371/journal.pmed.1002455.t002

elimination by targeting transmission are needed. Interventions may only achieve transmission reduction when deployed in certain populations or settings. Conversely, some populations and settings may require specific measures for transmission reduction, for example, pregnant women and infants, migrant workers, subclinical parasitaemia, or addressing outdoor transmission. The availability of new interventions is expanding, but developing algorithms for their rational combination and deployment in packages to decrease transmission is a key research need that requires modelling support [7]. Cost-effectiveness is an important determinant of whether particular interventions are adopted in public health programmes [9].

New products and strategies are needed to overcome parasite drug resistance and vector resistance to insecticides [8]. Prevalence of vaccine escape mutants has been highlighted as a potential issue if vaccines become widely used [170–172]. Thus, product development must continue, and strategies for phased replacement are needed as effectiveness wanes. New product discovery and development requires investment in basic science [10], the alignment of regulatory structures to expedite product registration, and continued investment in pharmacovigilance and surveillance. Funding organisations and malaria programmes also need to be

Box 3. Research and development agenda for tools for elimination

Diagnostics

Detecting transmission potential

- Malaria diagnostic tools best suited for detection of low-density, subclinical infections
- Assay for detecting infectious gametocytes
- *P. vivax/P. ovale* hypnozoite detection methods
- Noninvasive diagnostic tests

Directing treatment

- Stable, valid, specific, and sensitive rapid diagnostic tests (RDTs) that do not depend on histidine-rich protein 2 (HRP2)
- Detection of drug-resistant parasites
- RDTs that detect and differentiate all relevant human *Plasmodium* ssp. pathogens
- Multiplexed point-of-care tests for acute febrile illness

Special populations

- Affordable, simple, and accurate point-of-care tests for glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals and pregnant women

Drugs

Drugs for prevention and treatment

- Drugs that overcome resistance to existing drugs, particularly artemisinin resistance
- A suite of combination drugs with different or competing resistance profiles
- New drugs for prophylaxis
- Simplifying therapy, with the potential objective of a single encounter radical cure and prophylaxis (SERCaP)
- New regimens for use in seasonal malaria chemoprevention (SMC) outside the Sahel and to potentially replace sulfadoxine-pyrimethamine + amodiaquine (SPAQ)

Drugs to interrupt transmission

- Investigation of the impact of low-dose primaquine in different settings
- New drugs with transmission-blocking potential
- Drug combinations incorporating both asexual and transmission-blocking activity
- Evaluate the impact of transmission-blocking drugs on pathogen resistance development and investigate optimal deployment strategies
- Endectocides for use in humans and animals

Antihypnozoite drugs

- Evaluation of *P. vivax* transmission reduction potential with tafenoquine via relapse prevention (draining of hypnozoite reservoir)

Special populations

- New small molecules or antibodies with a potential indication for use during pregnancy

Vaccines

RTS,S

- Further evaluation of RTS,S to determine the potential for increased efficacy with alternative dosing regimens
- Assessment of RTS,S in combination with other interventions (e.g., SMC) and in other epidemiological settings and populations

New vaccines

- Defining the required attributes of preerythrocytic or blood-stage vaccines to achieve transmission-blocking activity
- New preerythrocytic and or blood-stage vaccines, ideally with transmission-blocking potential
- A first sexual-sporogonic-mosquito-stage vaccine to interrupt transmission (SSM-VIMT)

Vaccines against P. vivax/P. ovale

- Vaccines that prevent infection and hypnozoite formation, target hypnozoites, or can interrupt transmission to eventually eliminate the hypnozoite reservoir

Adjuvants

- Access to a broader choice of adjuvants with improved risk–benefit profiles

Prophylactic biologics

- Development of monoclonal antibodies (mAbs) and combinations of recombinant multi-mAbs products

Vector control

Insecticides and long-lasting insecticidal nets (LLINs)

- New insecticides and combinations of insecticides to overcome vector resistance
- Nonpyrethroid insecticides for LLINs
- Investigation of new insecticide deployment strategies
- LLINs with improved durability

Environmental management

- Formal investigation of larval source management in a greater variety of settings
- Development of long-lasting safe larvicides
- Development of cost-effective and socially acceptable environmental management interventions

Genetic approaches

- Development of scalable genetic approaches
- Development of environmentally and socially responsible methods for field testing transgenic organisms

Exploiting vector behaviour

- Novel interventions to target populations and behaviours
- Increased entomological support for key decisions by national malaria programmes

Combination and mapping

- Modelling to suggest the most effective and efficient combinations of vector control for different settings
- Developing operationally relevant mapping tools to identify and target residual transmission

'Tools to develop tools'

- Validating outcomes from animal and human infection models that predict a reduction in transmission in real-life settings
- Robust mathematical and laboratory models of transmission and impact of combination interventions
- Increased understanding of parasite–host immunity and mechanisms of acquired and vaccine-induced protective and transmission-blocking immunity
- Development of high-throughput screening assays and evaluation assays for the identification and selection of compounds with neglected profiles (e.g., antihypnozoite activity)

convinced that tools are impactful and cost-effective [9]. However, measuring the efficacy of tools that potentially impact transmission is problematic, particularly at the extremes of transmission [154]. Thus, new diagnostics and screening methods are required to assess tool efficacy in low-transmission settings and determine their contribution to maintaining zero transmission [73]. Moreover, the development of new diagnostics with improved sensitivity, or for specific tasks such as resistance surveillance, may fundamentally change our perception of malaria parasite transmission and our understanding of the most appropriate interventions required to interrupt transmission.

Finally, developing new tools can be expensive. When the malaria burden is significant, the economic case for innovation is clear. However, as the malaria burden decreases, the economic argument for continued development becomes more nuanced. Public–private partnerships, which first emerged 15 years ago, have demonstrated the ability to partner and drive development for a variety of tools, including diagnostics (e.g., PATH and Foundation for Innovative New Diagnostics), drugs (e.g., Medicine for Malaria Venture and formerly Drugs for Neglected Diseases Initiative), vaccines (e.g., PATH Malaria Vaccine Initiative and European Vaccine initiative), and vectors (e.g., Innovative Vector Control Consortium and more broadly Malaria No More). New business models to attract and engage industry in developing tools for

the elimination should be considered as well. Interventions will be directed at increasingly smaller populations, but these populations often represent the most difficult contexts in which to achieve elimination, and multiple interventions may be required. Once a country achieves elimination, there is the temptation to scale back infrastructure and interventions for malaria. This risks triggering a potentially lethal outbreak that could be difficult to reeliminate or even contain. There are numerous examples from earlier malaria elimination campaigns in the 1950s and 1960s of initial successes that were followed by resurgence as campaigns were deprioritized or discontinued administratively, financially, and technically. Unless malaria can be completely eradicated, interventions to maintain malaria elimination and a reserve of effective measures to counter malaria outbreaks will always be needed. However, if the right products and strategies are developed, and if they are used efficiently, effectively, and consistently, malaria eradication is an achievable goal.

Supporting information

S1 Table. Antimalarial drugs in preclinical and early clinical development.
(PDF)

S2 Table. Vaccines against *P. falciparum* in clinical development.
(PDF)

S3 Table. Vaccines against *P. vivax* in clinical development.
(PDF)

S1 Text. Summary of progress in malaria diagnostics since the initial Malaria Eradication Research Agenda (malERA) initiative and remaining gaps.
(DOCX)

S2 Text. Summary of progress in malaria drugs since the initial Malaria Eradication Research Agenda (malERA) initiative and remaining gaps.
(DOCX)

S3 Text. Summary of progress in malaria vaccines since the initial Malaria Eradication Research Agenda (malERA) initiative and remaining gaps.
(DOCX)

S4 Text. Summary of progress in malaria vector control since the initial Malaria Eradication Research Agenda (malERA) initiative and remaining gaps.
(DOCX)

Acknowledgments

The Malaria Eradication Research Agenda (malERA) Refresh Consultative Panel on Tools for Malaria Elimination was chaired by David C. Kaslow, PATH Essential Medicines and PATH Center for Vaccine Innovation and Access, Seattle, Washington, US; and co-chaired by Fredros Okumu, Ifakara Health Institute, Tanzania; and Timothy N. C. Wells, Medicines for Malaria Venture, Geneva, Switzerland. The paper was written based on consultations during a malERA meeting held in Washington, DC, US, on 12–13 October 2015 plus additional literature searches and methods detailed in the introduction paper to this series [11]. Mahamadou Ali Thera, European Vaccine Institute, participated in the panel discussion. Authors thank Dan Strickman (Bill & Melinda Gates Foundation) for providing expert information on dormancy and mosquitoes as parasitic reservoirs. Panel members reviewed several iterations of the manuscript to finalise it. Iveth Gonzalez from FIND, Switzerland, reviewed the

manuscript. Rob Hooft van Huijsduijnen of Hooft Pharma Services was rapporteur for the meeting, developed the initial meeting report, and was funded by the Malaria Eradication Scientific Alliance (MESA). Vittoria Lutje prepared systematic literature searches for the panel and was funded by MESA. Figure graphics were designed by Rachel Papernick and were funded by MESA. Naomi Richardson of Magenta Communications Limited provided writing services and editorial support for development of this paper and was funded by MESA.

Members of the writing group met the ICMJE criteria for authorship and were as follows:

David C. Kaslow (chair), PATH Essential Medicines and PATH Center for Vaccine Innovation and Access, Seattle, Washington, US; Fredros Okumu, Ifakara Health Institute, Ifakara, Tanzania, School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Parktown, Republic of South Africa, and Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, United Kingdom; Timothy N. C. Wells, Medicines for Malaria Venture, Geneva, Switzerland; Regina Rabinovich, ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clínic—Universitat de Barcelona, Barcelona, Spain, and Harvard T. H. Chan School of Public Health, Boston, Massachusetts, US.

Members of the MESA malERA Refresh Tools for Malaria Elimination Panel were as follows:

Quique Bassat, ISGlobal, Barcelona Centre for International Health Research (CRESIB, Hospital Clínic—Universitat de Barcelona, Barcelona, Spain, Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique, and ICREA, Barcelona, Spain; Ashley Birkett, PATH Malaria Vaccine Initiative, Washington, DC, US; François Bompart, Sanofi, Paris, France; Austin Burt, Imperial College London, London, United Kingdom; Carlos Chaccour, ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clinic - Universitat de Barcelona, Barcelona, Spain; Chetan Chitnis, Institut Pasteur, Paris, France; Janice Culpepper, Bill and Melinda Gates Foundation, Seattle, Washington, US; Gonzalo Domingo, PATH Diagnostics Program, Seattle, Washington, US; Patrick Duffy, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, US; Azra Ghani, Imperial College London, London, UK; Brian Greenwood, London School of Hygiene & Tropical Medicine, London, UK; B. Fenton Hall, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, US; Nick Hamon, Innovative Vector Control Consortium (IVCC), Liverpool School of Tropical Medicine, Liverpool, UK; Marcelo Jacobs-Lorena, Johns Hopkins Malaria Research Institute, Baltimore, Maryland, US; Stephanie James, Foundation for the National Institutes of Health, Bethesda, Maryland, US; David C. Kaslow, PATH Center for Vaccine Innovation and Access, Seattle, Washington, US; Kwadwo A. Koram, Noguchi Memorial Institute for Medical Research Koram, University of Ghana, Accra, Ghana; Peter Kremsner, Universitätsklinikum Tübingen, Germany and Centre de Recherches Medicales de Lambarene, Gabon; Ashwani Kumar, National Institute of Malaria Research, Goa, India; Didier Leroy, Medicines for Malaria Venture, Geneva, Switzerland; Odile Leroy, European Vaccine Initiative, UniversitätsKlinikum Heidelberg, Heidelberg, Germany; Steve Lindsay, University of Durham, Durham, UK; Silas Majambere, Innovative Vector Control Consortium (IVCC), Liverpool School of Tropical Medicine, Liverpool, UK; Charles Mbogo, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya; James McCarthy, QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia; Fredros Okumu, Ifakara Health Institute, Tanzania; Gao Qi, Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu Province, Peoples Republic of China; Jason Rasgon, The Huck Institutes of the Life Sciences, Penn State University, University Park, Pennsylvania, US; Regina Rabinovich, ISGlobal, Barcelona Centre for International Health Research (CRESIB),

Hospital Clinic - Universitat de Barcelona, Barcelona, Spain; Jason Richardson, Armed Forces Pest Management Board, Silver Spring, Maryland, US; Tom Richie, Sanaria, Rockville, Maryland, US; Robert Sauerwein, Radboud University Medical Center, Nijmegen, The Netherlands; Laurence Slutsker, PATH Malaria and the Neglected Tropical Diseases (NTD) Program, Seattle, Washington, US; Johan Vekemans, GlaxoSmithKline Biologicals, Rixensart, Belgium; Timothy N.C. Wells, Medicines for Malaria Venture, Geneva, Switzerland.

References

1. World Health Organization. Eliminating malaria Geneva: WHO; 2016 [Available from: http://apps.who.int/iris/bitstream/10665/205565/1/WHO_HTM_GMP_2016.3_eng.pdf?ua=1]
2. World Health Organization. Global technical strategy for malaria 2016–2030 Geneva: WHO; 2015 [Available from: http://apps.who.int/iris/bitstream/10665/176712/1/9789241564991_eng.pdf]
3. malERA Consultative Group on Vector Control. A research agenda for malaria eradication: vector control. PLoS Med. 2011; 8(1):e1000401. https://doi.org/10.1371/journal.pmed.1000401 PMID: 21311587
4. malERA Consultative Group on Vaccines. A research agenda for malaria eradication: vaccines. PLoS Med. 2011; 8(1):e1000398. https://doi.org/10.1371/journal.pmed.1000398 PMID: 21311586
5. malERA Consultative Group on Drugs. A research agenda for malaria eradication: drugs. PLoS Med. 2011; 8(1):e1000402. https://doi.org/10.1371/journal.pmed.1000402 PMID: 21311580
6. malERA Consultative Group on Diagnoses and Diagnostics. A research agenda for malaria eradication: diagnoses and diagnostics. PLoS Med. 2011; 8(1):e1000396. https://doi.org/10.1371/journal.pmed.1000396 PMID: 21311583
7. malERA Refresh Consultative Panel on Combination Interventions and Modelling. malERA: An updated research agenda on combination interventions and modelling for malaria elimination and eradication. PLoS Med. 2017;14(11):e1002453. https://doi.org/10.1371/journal.pmed.1002453
8. malERA Refresh Consultative Panel on Insecticide and Drug Resistance. malERA: An updated research agenda for insecticide and drug resistance in malaria elimination and eradication. PLoS Med. 2017;14(11):e1002450. https://doi.org/10.1371/journal.pmed.1002450
9. malERA Refresh Consultative Panel on Health Systems and Policy Research. malERA: An updated research agenda for health systems, policy and implementation research in malaria elimination and eradication. PLoS Med. 2017;14(11):e1002454. https://doi.org/10.1371/journal.pmed.1002454
10. malERA Refresh Consultative Panel on Basic Science and Enabling Technologies. malERA: An updated research agenda on basic science and enabling technologies in malaria elimination and eradication. PLoS Med. 2017;14(11):e1002451. https://doi.org/10.1371/journal.pmed.1002451
11. Rabinovich R, Drakeley C, Djimde AA, Hall BF, Hay S, Hemingway J, et al. malERA: An updated research agenda for malaria elimination and eradication. PLoS Med. 2017;14(11):e1002456. https://doi.org/10.1371/journal.pmed.1002456
12. World Health Organization. World malaria report Geneva: WHO; 2015 [Available from: http://apps.who.int/iris/bitstream/10665/200018/1/9789241565158_eng.pdf?ua=1]
13. Abba K, Kirkham AJ, Olliaro PL, Deeks JJ, Donegan S, Garner P, et al. Rapid diagnostic tests for diagnosing uncomplicated non-falciparum or *Plasmodium vivax* malaria in endemic countries. Cochrane Database Syst Rev. 2014; 12:CD011431.
14. World Health Organization. Malaria rapid diagnostic test performance Geneva: WHO; 2015 [Available from: http://apps.who.int/iris/bitstream/10665/204118/1/9789241510035_eng.pdf?ua=1]
15. World Health Organization. The role of mass drug administration, mass screening and treatment, and focal screening and treatment for malaria Geneva: WHO; 2015 [Available from: http://www.who.int/malaria/publications/atoz/role-of-mda-for-malaria.pdf?ua=1]
16. World Health Organization. Policy brief on single-dose primaquine as a gametocytocide in *Plasmodium falciparum* malaria Geneva: WHO; 2015 [Available from: http://www.who.int/malaria/publications/atoz/who_htm_gmp_2015.1.pdf]
17. Medicines for Malaria Venture. Interactive R&D portfolio Geneva: MMV; 2016 [Available from: http://www.mmv.org/research-development/interactive-rd-portfolio]
18. Llanos-Cuentas A, Lacerda MV, Rueangweerayut R, Krudsood S, Gupta SK, Kochar SK, et al. Tafenoquine plus chloroquine for the treatment and relapse prevention of *Plasmodium vivax* malaria (DETECTIVE): a multicentre, double-blind, randomised, phase 2b dose-selection study. Lancet. 2014; 383(9922):1049–58. https://doi.org/10.1016/S0140-6736(13)62568-4 PMID: 24360369

19. Leong FJ, Zhao R, Zeng S, Magnusson B, Diagana TT, Pertel P. A first-in-human randomized, double-blind, placebo-controlled, single- and multiple-ascending oral dose study of novel Imidazolopiperazine KAF156 to assess its safety, tolerability, and pharmacokinetics in healthy adult volunteers. Antimicrob Agents Chemother. 2014; 58(11):6437–43. https://doi.org/10.1128/AAC.03478-14 PMID: 25136017

20. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014; 505(7481):50–5. https://doi.org/10.1038/nature12876 PMID: 24352242

21. Menard D, Khim N, Beghain J, Adegnika AA, Shafiul-Alam M, Amodu O, et al. A worldwide map of *Plasmodium falciparum* K13-propeller polymorphisms. N Engl J Med. 2016; 374(25):2453–64. https://doi.org/10.1056/NEJMoa1513137 PMID: 27332904

22. European Medicines Agency. Assessment report: Mosquirix™ London: EMA; 2015 [Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Medicine_for_use_outside_EU/2015/10/WC500194577.pdf]

23. Agnandji ST, Fernandes JF, Bache EB, Ramharter M. Clinical development of RTS,S/AS malaria vaccine: a systematic review of clinical Phase I-III trials. Future Microbiol. 2015; 10(10):1553–78. https://doi.org/10.2217/fmb.15.90 PMID: 26437872

24. Kaslow DC, Biernaux S. RTS S: Toward a first landmark on the malaria vaccine technology roadmap. Vaccine. 2015; 33(52):7425–32. https://doi.org/10.1016/j.vaccine.2015.09.061 PMID: 26431982

25. RTS S, Clinical Trials Partnership,. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. Lancet. 2015; 386(9988):31–45. https://doi.org/10.1016/S0140-6736(15)60721-8 PMID: 25913272

26. World Health Organization. Global Advisory Committee on Vaccine Safety, 10–11 June 2015 Geneva: WHO; 2015 [Available from: http://www.who.int/vaccine_safety/committee/reports/Jun_2015/en/.]

27. Penny MA, Verity R, Bever CA, Sauboin C, Galactionova K, Flasche S, et al. Public health impact and cost-effectiveness of the RTS,S/AS01 malaria vaccine: a systematic comparison of predictions from four mathematical models. Lancet. 2016; 387(10016):367–75. https://doi.org/10.1016/S0140-6736(15)00725-4 PMID: 26549466

28. World Health Organization. Questions and answers on malaria vaccines Geneva: WHO; 2016 [Available from: http://www.who.int/immunization/research/development/malaria_vaccine_qa/en/.]

29. Greenwood B, Doumbo OK. Implementation of the malaria candidate vaccine RTS,S/AS01. Lancet. 2016; 387(10016):318–9. https://doi.org/10.1016/S0140-6736(15)00807-7 PMID: 26549465

30. Stoute JA, Slaoui M, Heppner DG, Momin P, Kester KE, Desmons P, et al. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. RTS,S Malaria Vaccine Evaluation Group. N Engl J Med. 1997; 336(2):86–91. https://doi.org/10.1056/NEJM199701093360202 PMID: 8988885

31. Regules JA, Cicatelli SB, Bennett JW, Paolino KM, Twomey PS, Moon JE, et al. Fractional third and fourth dose of RTS,S/AS01 malaria candidate vaccine: a phase 2a controlled human malaria infection and immunogenicity study. J Infect Dis. 2016; 214:762–71. https://doi.org/10.1093/infdis/jiw237 PMID: 27296848

32. Gosling R, von Seidlein L. The Future of the RTS,S/AS01 Malaria Vaccine: An Alternative Development Plan. PLoS Med. 2016; 13(4):e1001994. https://doi.org/10.1371/journal.pmed.1001994 PMID: 27070151

33. White MT, Verity R, Churcher TS, Ghani AC. Vaccine approaches to malaria control and elimination: Insights from mathematical models. Vaccine. 2015; 33(52):7544–50. https://doi.org/10.1016/j.vaccine.2015.09.099 PMID: 26476361

34. World Health Organization. Malaria vaccine technology roadmap: November 2013 Geneva: WHO; 2013 [Available from: http://www.who.int/immunization/topics/malaria/vaccine_roadmap/TRM_update_nov13.pdf?ua=1]

35. World Health Organization. Tables of malaria vaccine projects globally "The Rainbow Tables" Geneva: WHO; 2016 [Available from: http://www.who.int/immunization/research/development/Rainbow_tables/en/.]

36. Mueller I, Shakri AR, Chitnis CE. Development of vaccines for *Plasmodium vivax* malaria. Vaccine. 2015; 33(52):7489–95. https://doi.org/10.1016/j.vaccine.2015.09.060 PMID: 26428453

37. International Vector Control Consortium. WHO recommends Syngenta's new long-lasting insecticide formulation Liverpool: IVCC; 2013 [Available from: http://www.ivcc.com/news-and-media/news/who-recommends-syngentas-new-long-lasting-insecticide-formulation]

38. International Vector Control Consortium. K-Othrine Polyzone Liverpool: IVCC; 2016 [Available from: http://www.ivcc.com/news-and-media/news/bayer-cropscience-and-ivcc-offer-new-tool-for-malaria-control]

39. Djenontin A, Ahoua Alou LP, Koffi A, Zogo B, Duarte E, N'Guessan R, et al. Insecticidal and sterilizing effect of Olyset Duo(R), a permethrin and pyriproxyfen mixture net against pyrethroid-susceptible and -resistant strains of *Anopheles gambiae* s.s.: a release-recapture assay in experimental huts. Parasite. 2015; 22:27. https://doi.org/10.1051/parasite/2015027 PMID: 26489479

40. Koffi AA, Ahoua Alou LP, Djenontin A, Kabran JP, Dosso Y, Kone A, et al. Efficacy of Olyset(R) Duo, a permethrin and pyriproxyfen mixture net against wild pyrethroid-resistant *Anopheles gambiae* s.s. from Cote d'Ivoire: an experimental hut trial. Parasite. 2015; 22:28. https://doi.org/10.1051/parasite/2015028 PMID: 26489480

41. N'Guessan R, Ngufor C, Kudom AA, Boko P, Odjo A, Malone D, et al. Mosquito nets treated with a mixture of chlorfenapyr and alphacypermethrin control pyrethroid resistant Anopheles gambiae and Culex quinquefasciatus mosquitoes in West Africa. PLoS ONE. 2014; 9(2):e87710. https://doi.org/10.1371/journal.pone.0087710 PMID: 24498360

42. Innovation to Impact. [Available from: http://innovationtoimpact.org/.]

43. World Health Organization. Innovation to Impact–WHO change plan for strengthening innovation, quality and use of vector-control tools Geneva: WHO; 2015 [Available from: http://www.who.int/malaria/mpac/mpac-sept2015-vector-control-innovation.pdf]

44. International Vector Control Consortium. [Available from: http://www.ivcc.com/.]

45. World Health Organization. Larval source management—a supplementary measure for malaria vector control: an operational manual Geneva: WHO; 2013 [Available from: http://apps.who.int/iris/bitstream/10665/85379/1/9789241505604_eng.pdf?ua=1]

46. Bousema T, Dinglasan RR, Morlais I, Gouagna LC, van Warmerdam T, Awono-Ambene PH, et al. Mosquito feeding assays to determine the infectiousness of naturally infected *Plasmodium falciparum* gametocyte carriers. PLoS ONE. 2012; 7(8):e42821. https://doi.org/10.1371/journal.pone.0042821 PMID: 22936993

47. Churcher TS, Bousema T, Walker M, Drakeley C, Schneider P, Ouedraogo AL, et al. Predicting mosquito infection from *Plasmodium falciparum* gametocyte density and estimating the reservoir of infection. Elife. 2013; 2:e00626. https://doi.org/10.7554/eLife.00626 PMID: 23705071

48. Ouédraogo AL, Guelbéogo WM, Cohuet A, Morlais I, King JG, Gonçalves BP, et al. A protocol for membrane feeding assays to determine the infectiousness of *P. falciparum* naturally infected individuals to *Anopheles gambiae*. MWJ. 2013; 4(16):1–4.

49. Li T, Eappen AG, Richman AM, Billingsley PF, Abebe Y, Li M, et al. Robust, reproducible, industrialized, standard membrane feeding assay for assessing the transmission blocking activity of vaccines and drugs against *Plasmodium falciparum*. Malar J. 2015; 14:150. https://doi.org/10.1186/s12936-015-0665-8 PMID: 25890243

50. Laurens MB, Duncan CJ, Epstein JE, Hill AV, Komisar JL, Lyke KE, et al. A consultation on the optimization of controlled human malaria infection by mosquito bite for evaluation of candidate malaria vaccines. Vaccine. 2012; 30(36):5302–4. https://doi.org/10.1016/j.vaccine.2012.04.088 PMID: 22659449

51. Spring M, Polhemus M, Ockenhouse C. Controlled human malaria infection. J Infect Dis. 2014; 209 Suppl 2:S40–5.

52. Gomez-Perez GP, Legarda A, Munoz J, Sim BK, Ballester MR, Dobano C, et al. Controlled human malaria infection by intramuscular and direct venous inoculation of cryopreserved *Plasmodium falciparum* sporozoites in malaria-naive volunteers: effect of injection volume and dose on infectivity rates. Malar J. 2015; 14:306. https://doi.org/10.1186/s12936-015-0817-x PMID: 26245196

53. Mordmuller B, Supan C, Sim KL, Gomez-Perez GP, Ospina Salazar CL, Held J, et al. Direct venous inoculation of *Plasmodium falciparum* sporozoites for controlled human malaria infection: a dose-finding trial in two centres. Malar J. 2015; 14:117. https://doi.org/10.1186/s12936-015-0628-0 PMID: 25889522

54. Engwerda CR, Minigo G, Amante FH, McCarthy JS. Experimentally induced blood stage malaria infection as a tool for clinical research. Trends Parasitol. 2012; 28(11):515–21. https://doi.org/10.1016/j.pt.2012.09.001 PMID: 23041118

55. Krause A, Dingemanse J, Mathis A, Marquart L, Mohrle JJ, McCarthy JS. Pharmacokinetic/pharmacodynamic modelling of the antimalarial effect of Actelion-451840 in an induced blood stage malaria study in healthy subjects. Br J Clin Pharmacol. 2016; 82:412–21. https://doi.org/10.1111/bcp.12962 PMID: 27062080

56. Malaria Vaccine Initiative. Transmission-blocking vaccines (TBVs) against malaria Seattle: MVI; 2016 [Available from: http://www.malariavaccine.org/sites/www.malariavaccine.org/files/content/resource/files/MVI-TBVfactsheetFINAL_20160420.pdf]

57. McCarthy JS, Marquart L, Sekuloski S, Trenholme K, Elliott S, Griffin P, et al. Linking murine and human *Plasmodium falciparum* challenge models in a translational path for antimalarial drug development. Antimicrob Agents Chemother. 2016; 60:3669–75. https://doi.org/10.1128/AAC.02883-15 PMID: 27044554

58. Siu E, Ploss A. Modeling malaria in humanized mice: opportunities and challenges. Ann N Y Acad Sci. 2015; 1342:29–36. https://doi.org/10.1111/nyas.12618 PMID: 25678404

59. Miguel-Blanco C, Lelievre J, Delves MJ, Bardera AI, Presa JL, Lopez-Barragan MJ, et al. Imaging-based high-throughput screening assay to identify new molecules with transmission-blocking potential against *Plasmodium falciparum* female gamete formation. Antimicrob Agents Chemother. 2015; 59 (6):3298–305. https://doi.org/10.1128/AAC.04684-14 PMID: 25801574

60. Asare EO, Tompkins AM, Bomblies A. A regional model for malaria vector developmental habitats evaluated using explicit, pond-resolving surface hydrology simulations. PLoS ONE. 2016; 11(3): e0150626. https://doi.org/10.1371/journal.pone.0150626 PMID: 27003834

61. Chitnis N, Schapira A, Smith T, Steketee R. Comparing the effectiveness of malaria vector-control interventions through a mathematical model. Am J Trop Med Hyg. 2010; 83(2):230–40. https://doi.org/10.4269/ajtmh.2010.09-0179 PMID: 20682861

62. Ngwa GA, Wankah TT, Fomboh-Nforba MY, Ngonghala CN, Teboh-Ewungkem MI. On a reproductive stage-structured model for the population dynamics of the malaria vector. Bull Math Biol. 2014; 76 (10):2476–516. https://doi.org/10.1007/s11538-014-0021-0 PMID: 25234336

63. World Health Organization. Policy brief on malaria diagnostics in low-transmission settings Geneva: WHO; 2014 [Available from: http://www.who.int/malaria/publications/atoz/malaria-diagnosis-low-transmission-settings-sep2014.pdf]

64. DIAMETER (Diagnostics for Malaria Elimination Toward Eradication). Target product profile: point-of-care malaria infection detection test for rapid detection of low-density, subclinical malaria infections Seattle: PATH; 2014 [Available from: http://sites.path.org/dx/files/2012/11/DIAMETER_IDT_TPP_FINAL_forwebsite.pdf]

65. Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, Barnwell JW, et al. A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. PLoS ONE. 2010; 5(1):e8091. https://doi.org/10.1371/journal.pone.0008091 PMID: 20111602

66. Akinyi S, Hayden T, Gamboa D, Torres K, Bendezu J, Abdallah JF, et al. Multiple genetic origins of histidine-rich protein 2 gene deletion in *Plasmodium falciparum* parasites from Peru. Sci Rep. 2013; 3:2797. https://doi.org/10.1038/srep02797 PMID: 24077522

67. Cheng Q, Gatton ML, Barnwell J, Chiodini P, McCarthy J, Bell D, et al. *Plasmodium falciparum* parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting. Malar J. 2014; 13:283. https://doi.org/10.1186/1475-2875-13-283 PMID: 25052298

68. Bharti PK, Chandel HS, Ahmad A, Krishna S, Udhayakumar V, Singh N. Prevalence of pfhrp2 and/or pfhrp3 gene deletion in *Plasmodium falciparum* population in eight highly endemic states in india. PLoS ONE. 2016; 11(8):e0157949. https://doi.org/10.1371/journal.pone.0157949 PMID: 27518538

69. Deme AB, Park DJ, Bei AK, Sarr O, Badiane AS, Gueye Pel H, et al. Analysis of pfhrp2 genetic diversity in Senegal and implications for use of rapid diagnostic tests. Malar J. 2014; 13:34. https://doi.org/10.1186/1475-2875-13-34 PMID: 24472178

70. Parr JB, Belson C, Patel JC, Hoffman IF, Kamthunzi P, Martinson F, et al. Estimation of *Plasmodium falciparum* transmission intensity in lilongwe, malawi, by microscopy, rapid diagnostic testing, and nucleic acid detection. Am J Trop Med Hyg. 2016; 95(2):373–7. https://doi.org/10.4269/ajtmh.16-0156 PMID: 27325802

71. World Health Organization. False-negative RDT results and implications of new reports of *P. falciparum* histidine-rich protein 2/3 gene deletions Geneva: WHO; 2016 [Available from: http://www.who.int/malaria/publications/atoz/who-htm-gmp-2016.4.pdf]

72. Kobayashi T, Gamboa D, Ndiaye D, Cui L, Sutton PL, Vinetz JM. Malaria diagnosis across the International Centers of Excellence for Malaria Research: platforms, performance, and standardization. Am J Trop Med Hyg. 2015; 93(3 Suppl):99–109.

73. malERA Refresh Consultative Panel on Characterising the Reservoir and Measuring Transmission. malERA: An updated research agenda for characterising the reservoir and measuring transmission in malaria elimination and eradication. PLoS Med. 2017; 14: e1002452.

74. Scherr TF, Gupta S, Wright DW, Haselton FR. Mobile phone imaging and cloud-based analysis for standardized malaria detection and reporting. Sci Rep. 2016; 6:28645. https://doi.org/10.1038/srep28645 PMID: 27345590

75. Soti DO, Kinoti SN, Omar AH, Logedi J, Mwendwa TK, Hirji Z, et al. Feasibility of an innovative electronic mobile system to assist health workers to collect accurate, complete and timely data in a malaria

control programme in a remote setting in Kenya. Malar J. 2015; 14:430. https://doi.org/10.1186/s12936-015-0965-z PMID: 26530237

76. Falzon D, Timimi H, Kurosinski P, Migliori GB, Van Gemert W, Denkinger C, et al. Digital health for the End TB Strategy: developing priority products and making them work. Eur Respir J. 2016; 48(1):29–45. https://doi.org/10.1183/13993003.00424-2016 PMID: 27230443

77. Ibrahim F, Thio TH, Faisal T, Neuman M. The application of biomedical engineering techniques to the diagnosis and management of tropical diseases: a review. Sensors (Basel). 2015; 15(3):6947–95.

78. Das DK, Mukherjee R, Chakraborty C. Computational microscopic imaging for malaria parasite detection: a systematic review. J Microsc. 2015; 260(1):1–19. https://doi.org/10.1111/jmi.12270 PMID: 26047029

79. Tay A, Pavesi A, Yazdi SR, Lim CT, Warkiani ME. Advances in microfluidics in combating infectious diseases. Biotechnol Adv. 2016; 34(4):404–21. https://doi.org/10.1016/j.biotechadv.2016.02.002 PMID: 26854743

80. Taylor BJ, Howell A, Martin KA, Manage DP, Gordy W, Campbell SD, et al. A lab-on-chip for malaria diagnosis and surveillance. Malar J. 2014; 13:179. https://doi.org/10.1186/1475-2875-13-179 PMID: 24885206

81. Kasetsirikul S, Buranapong J, Srituravanich W, Kaewthamasorn M, Pimpin A. The development of malaria diagnostic techniques: a review of the approaches with focus on dielectrophoretic and magnetophoretic methods. Malar J. 2016; 15(1):358. https://doi.org/10.1186/s12936-016-1400-9 PMID: 27405995

82. Britton S, Cheng Q, McCarthy JS. Novel molecular diagnostic tools for malaria elimination: a review of options from the point of view of high-throughput and applicability in resource limited settings. Malar J. 2016; 15:88. https://doi.org/10.1186/s12936-016-1158-0 PMID: 26879936

83. Patel JC, Lucchi NW, Srivastava P, Lin JT, Sug-Aram R, Aruncharus S, et al. Field evaluation of a real-time fluorescence loop-mediated isothermal amplification assay, RealAmp, for the diagnosis of malaria in Thailand and India. J Infect Dis. 2014; 210(8):1180–7. https://doi.org/10.1093/infdis/jiu252 PMID: 24795480

84. Cook J, Aydin-Schmidt B, Gonzalez IJ, Bell D, Edlund E, Nassor MH, et al. Loop-mediated isothermal amplification (LAMP) for point-of-care detection of asymptomatic low-density malaria parasite carriers in Zanzibar. Malar J. 2015; 14:43. https://doi.org/10.1186/s12936-015-0573-y PMID: 25627037

85. Sema M, Alemu A, Bayih AG, Getie S, Getnet G, Guelig D, et al. Evaluation of non-instrumented nucleic acid amplification by loop-mediated isothermal amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia. Malar J. 2015; 14:44. https://doi.org/10.1186/s12936-015-0559-9 PMID: 25626339

86. Waggoner JJ, Abeynayake J, Balassiano I, Lefterova M, Sahoo MK, Liu Y, et al. Multiplex nucleic acid amplification test for diagnosis of dengue fever, malaria, and leptospirosis. J Clin Microbiol. 2014; 52(6):2011–8. https://doi.org/10.1128/JCM.00341-14 PMID: 24671788

87. Chua KH, Lee PC, Chai HC. Development of insulated isothermal PCR for rapid on-site malaria detection. Malar J. 2016; 15(1):134.

88. Foundation for Innovative New Diagnostics. Acute febrile syndrome strategy Geneva: FIND; 2012 [Available from: http://r4d.dfid.gov.uk/PDF/Outputs/FIND/0031-FIND-NMFI-document-print-inhouse.pdf]

89. Singh R, Singh DP, Gupta R, Savargaonkar D, Singh OP, Nanda N, et al. Comparison of three PCR-based assays for the non-invasive diagnosis of malaria: detection of *Plasmodium* parasites in blood and saliva. Eur J Clin Microbiol Infect Dis. 2014; 33(9):1631–9. https://doi.org/10.1007/s10096-014-2121-z PMID: 24792127

90. Oguonu T, Shu E, Ezeonwu BU, Lige B, Derrick A, Umeh RE, et al. The performance evaluation of a urine malaria test (UMT) kit for the diagnosis of malaria in individuals with fever in south-east Nigeria: cross-sectional analytical study. Malar J. 2014; 13:403. https://doi.org/10.1186/1475-2875-13-403 PMID: 25316216

91. Berna AZ, McCarthy JS, Wang RX, Saliba KJ, Bravo FG, Cassells J, et al. Analysis of breath specimens for biomarkers of *Plasmodium falciparum* infection. J Infect Dis. 2015; 212(7):1120–8. https://doi.org/10.1093/infdis/jiv176 PMID: 25810441

92. Lukianova-Hleb E, Bezek S, Szigeti R, Khodarev A, Kelley T, Hurrell A, et al. Transdermal diagnosis of malaria using vapor nanobubbles. Emerg Infect Dis. 2015; 21(7):1122–7. https://doi.org/10.3201/eid2107.150089 PMID: 26079141

93. Djimde AA, Maiga AW, Ouologuem D, Fofana B, Sagara I, Dembele D, et al. Gametocyte clearance dynamics following oral artesunate treatment of uncomplicated falciparum malaria in Malian children. Parasite. 2016; 23:3. https://doi.org/10.1051/parasite/2016003 PMID: 26839003

94. Mwingira F, Genton B, Kabanywanyi AN, Felger I. Comparison of detection methods to estimate asexual *Plasmodium falciparum* parasite prevalence and gametocyte carriage in a community survey in Tanzania. Malar J. 2014; 13:433. https://doi.org/10.1186/1475-2875-13-433 PMID: 25404207

95. Wampfler R, Mwingira F, Javati S, Robinson L, Betuela I, Siba P, et al. Strategies for detection of *Plasmodium* species gametocytes. PLoS ONE. 2013; 8(9):e76316. https://doi.org/10.1371/journal.pone.0076316 PMID: 24312682

96. Ouedraogo AL, Schneider P, de Kruijf M, Nebie I, Verhave JP, Cuzin-Ouattara N, et al. Age-dependent distribution of *Plasmodium falciparum* gametocytes quantified by Pfs25 real-time QT-NASBA in a cross-sectional study in Burkina Faso. Am J Trop Med Hyg. 2007; 76(4):626–30. PMID: 17426160

97. Schneider P, Reece SE, van Schaijk BC, Bousema T, Lanke KH, Meaden CS, et al. Quantification of female and male *Plasmodium falciparum* gametocytes by reverse transcriptase quantitative PCR. Mol Biochem Parasitol. 2015; 199(1–2):29–33. https://doi.org/10.1016/j.molbiopara.2015.03.006 PMID: 25827756

98. Hisaeda H, Stowers AW, Tsuboi T, Collins WE, Sattabongkot JS, Suwanabun N, et al. Antibodies to malaria vaccine candidates Pvs25 and Pvs28 completely block the ability of *Plasmodium vivax* to infect mosquitoes. Infect Immun. 2000; 68(12):6618–23. PMID: 11083773

99. World Health Organization. Emergency response to artemisinin resistance in the Greater Mekong subregion. Regional framework for action 2013–2015 Geneva: WHO; 2013 [Available from: http://www.who.int/malaria/publications/atoz/9789241505321/en/]

100. Rao PN, Uplekar S, Kayal S, Mallick PK, Bandyopadhyay N, Kale S, et al. A method for amplicon deep sequencing of drug resistance genes in *Plasmodium falciparum* clinical isolates from India. J Clin Microbiol. 2016; 54(6):1500–11. https://doi.org/10.1128/JCM.00235-16 PMID: 27008882

101. Nankoberanyi S, Mbogo GW, LeClair NP, Conrad MD, Tumwebaze P, Tukwasibwe S, et al. Validation of the ligase detection reaction fluorescent microsphere assay for the detection of *Plasmodium falciparum* resistance mediating polymorphisms in Uganda. Malar J. 2014; 13:95. https://doi.org/10.1186/1475-2875-13-95 PMID: 24629020

102. Amato R, Lim P, Miotto O, Amaratunga C, Dek D, Pearson RD, et al. Genetic markers associated with dihydroartemisinin-piperaquine failure in *Plasmodium falciparum* malaria in Cambodia: a genotype-phenotype association study. Lancet Infect Dis. 2017; 17:164–73. https://doi.org/10.1016/S1473-3099(16)30409-1 PMID: 27818095

103. Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, et al. A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: a phenotype-genotype association study. Lancet Infect Dis. 2017; 17:174–83. https://doi.org/10.1016/S1473-3099(16)30415-7 PMID: 27818097

104. White MT, Karl S, Battle KE, Hay SI, Mueller I, Ghani AC. Modelling the contribution of the hypnozoite reservoir to *Plasmodium vivax* transmission. Elife. 2014; 3:e04692.

105. Ley B, Luter N, Espino FE, Devine A, Kalnoky M, Lubell Y, et al. The challenges of introducing routine G6PD testing into radical cure: a workshop report. Malar J. 2015; 14:377. https://doi.org/10.1186/s12936-015-0896-8 PMID: 26416229

106. World Health Organization. Guidelines for the treatment of malaria, third edition Geneva: WHO; 2015 [Available from: http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf]

107. Graves PM, Gelband H, Garner P. Primaquine or other 8-aminoquinoline for reducing *Plasmodium falciparum* transmission. Cochrane Database Syst Rev. 2015(2):CD008152. https://doi.org/10.1002/14651858.CD008152.pub4 PMID: 25693791

108. Salinas JL, Kissinger JC, Jones DP, Galinski MR. Metabolomics in the fight against malaria. Mem Inst Oswaldo Cruz. 2014; 109(5):589–97. https://doi.org/10.1590/0074-0276140043 PMID: 25185001

109. Chavchich M, Van Breda K, Rowcliffe K, Diagana TT, Edstein MD. The spiroindolone KAE609 does not induce dormant ring stages in *Plasmodium falciparum* parasites. Antimicrob Agents Chemother. 2016; 60(9):5167–74. https://doi.org/10.1128/AAC.02838-15 PMID: 27297484

110. Burrows JN, Duparc S, Gutteridge WE, Hooft van Huijsduijnen R, Kaszubska W, Macintyre F, et al. New developments in anti-malarial target candidate and product profiles. Malar J. 2017; 16(1):26. https://doi.org/10.1186/s12936-016-1675-x PMID: 28086874

111. Artzy-Randrup Y, Alonso D, Pascual M. Transmission intensity and drug resistance in malaria population dynamics: implications for climate change. PLoS ONE. 2010; 5(10):e13588. https://doi.org/10.1371/journal.pone.0013588 PMID: 21060886

112. Hastings IM, Watkins WM. Intensity of malaria transmission and the evolution of drug resistance. Acta Trop. 2005; 94(3):218–29. https://doi.org/10.1016/j.actatropica.2005.04.003 PMID: 15847846

113. Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, Chhaganlal KD, et al. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. Lancet. 2010; 376(9753):1647–57. https://doi.org/10.1016/S0140-6736(10)61924-1 PMID: 21062666

114. Dondorp A, Nosten F, Stepniewska K, Day N, White N, South East Asian Quinine Artesunate Malaria Trial g. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. Lancet. 2005; 366(9487):717–25. https://doi.org/10.1016/S0140-6736(05)67176-0 PMID: 16125588

115. Jeeyapant A, Kingston HW, Plewes K, Maude RJ, Hanson J, Herdman MT, et al. Defining surrogate endpoints for clinical trials in severe falciparum malaria. PLoS ONE. 2017; 12(1):e0169307. https://doi.org/10.1371/journal.pone.0169307 PMID: 28052109

116. Cheah PY, Parker M, Dondorp AM. Development of drugs for severe malaria in children. Int Health. 2016; 8(5):313–6. https://doi.org/10.1093/inthealth/ihw038 PMID: 27620923

117. Abdul-Ghani R, Beier JC. Strategic use of antimalarial drugs that block falciparum malaria parasite transmission to mosquitoes to achieve local malaria elimination. Parasitol Res. 2014; 113(10):3535–46. https://doi.org/10.1007/s00436-014-4091-6 PMID: 25185662

118. Dicko A, Brown JM, Diawara H, Baber I, Mahamar A, Soumare HM, et al. Primaquine to reduce transmission of *Plasmodium falciparum* malaria in Mali: a single-blind, dose-ranging, adaptive randomised phase 2 trial. Lancet Infect Dis. 2016; 16:674–84. https://doi.org/10.1016/S1473-3099(15)00479-X PMID: 26906747

119. Goncalves BP, Tiono AB, Ouedraogo A, Guelbeogo WM, Bradley J, Nebie I, et al. Single low dose primaquine to reduce gametocyte carriage and *Plasmodium falciparum* transmission after artemether-lumefantrine in children with asymptomatic infection: a randomised, double-blind, placebo-controlled trial. BMC Med. 2016; 14:40. https://doi.org/10.1186/s12916-016-0581-y PMID: 26952094

120. Bolscher JM, Koolen KM, van Gemert GJ, van de Vegte-Bolmer MG, Bousema T, Leroy D, et al. A combination of new screening assays for prioritization of transmission-blocking antimalarials reveals distinct dynamics of marketed and experimental drugs. J Antimicrob Chemother. 2015; 70(5):1357–66. https://doi.org/10.1093/jac/dkv003 PMID: 25667405

121. Plouffe DM, Wree M, Du AY, Meister S, Li F, Patra K, et al. High-throughput assay and discovery of small molecules that interrupt malaria transmission. Cell Host Microbe. 2016; 19(1):114–26. https://doi.org/10.1016/j.chom.2015.12.001 PMID: 26749441

122. Lucantoni L, Fidock DA, Avery VM. Luciferase-based, high-throughput assay for screening and profiling transmission-blocking compounds against *Plasmodium falciparum* gametocytes. Antimicrob Agents Chemother. 2016; 60(4):2097–107. https://doi.org/10.1128/AAC.01949-15 PMID: 26787698

123. Almela MJ, Lozano S, Lelievre J, Colmenarejo G, Coteron JM, Rodrigues J, et al. A new set of chemical starting points with *Plasmodium falciparum* transmission-blocking potential for antimalarial drug discovery. PLoS ONE. 2015; 10(8):e0135139. https://doi.org/10.1371/journal.pone.0135139 PMID: 26317851

124. Lelievre J, Almela MJ, Lozano S, Miguel C, Franco V, Leroy D, et al. Activity of clinically relevant antimalarial drugs on *Plasmodium falciparum* mature gametocytes in an ATP bioluminescence "transmission blocking" assay. PLoS ONE. 2012; 7(4):e35019. https://doi.org/10.1371/journal.pone.0035019 PMID: 22514702

125. Roncales M, Vidal-Mas J, Leroy D, Herreros E. Comparison and optimization of different methods for the *in vitro* production of *Plasmodium falciparum* gametocytes. J Parasitol Res. 2012; 2012:927148. https://doi.org/10.1155/2012/927148 PMID: 22523643

126. Burrows JN, van Huijsduijnen RH, Mohrle JJ, Oeuvray C, Wells TN. Designing the next generation of medicines for malaria control and eradication. Malar J. 2013; 12:187. https://doi.org/10.1186/1475-2875-12-187 PMID: 23742293

127. Phillips MA, Lotharius J, Marsh K, White J, Dayan A, White KL, et al. A long-duration dihydroorotate dehydrogenase inhibitor (DSM265) for prevention and treatment of malaria. Sci Transl Med. 2015; 7 (296):296ra111. https://doi.org/10.1126/scitranslmed.aaa6645 PMID: 26180101

128. Robinson LJ, Wampfler R, Betuela I, Karl S, White MT, Li Wai Suen CS, et al. Strategies for understanding and reducing the *Plasmodium vivax* and *Plasmodium ovale* hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model. PLoS Med. 2015; 12(10):e1001891. https://doi.org/10.1371/journal.pmed.1001891 PMID: 26505753

129. White NJ. Why do some primate malarias relapse? Trends Parasitol. 2016; 32(12):918–20. https://doi.org/10.1016/j.pt.2016.08.014 PMID: 27743866

130. John GK, Douglas NM, von Seidlein L, Nosten F, Baird JK, White NJ, et al. Primaquine radical cure of *Plasmodium vivax*: a critical review of the literature. Malar J. 2012; 11:280. https://doi.org/10.1186/1475-2875-11-280 PMID: 22900786

131. Wells TN, Burrows JN, Baird JK. Targeting the hypnozoite reservoir of *Plasmodium vivax*: the hidden obstacle to malaria elimination. Trends Parasitol. 2010; 26(3):145–51. https://doi.org/10.1016/j.pt.2009.12.005 PMID: 20133198

132. March S, Ng S, Velmurugan S, Galstian A, Shan J, Logan DJ, et al. A microscale human liver platform that supports the hepatic stages of *Plasmodium falciparum* and *vivax*. Cell Host Microbe. 2013; 14 (1):104–15. https://doi.org/10.1016/j.chom.2013.06.005 PMID: 23870318

133. Vaughan AM, Kappe SH, Ploss A, Mikolajczak SA. Development of humanized mouse models to study human malaria parasite infection. Future Microbiol. 2012; 7(5):657–65. https://doi.org/10.2217/fmb.12.27 PMID: 22568719

134. Cairns M, Roca-Feltrer A, Garske T, Wilson AL, Diallo D, Milligan PJ, et al. Estimating the potential public health impact of seasonal malaria chemoprevention in African children. Nat Commun. 2012; 3:881. https://doi.org/10.1038/ncomms1879 PMID: 22673908

135. Zongo I, Milligan P, Compaore YD, Some AF, Greenwood B, Tarning J, et al. Randomized noninferiority trial of dihydroartemisinin-piperaquine compared with sulfadoxine-pyrimethamine plus amodiaquine for seasonal malaria chemoprevention in Burkina Faso. Antimicrob Agents Chemother. 2015; 59 (8):4387–96. https://doi.org/10.1128/AAC.04923-14 PMID: 25918149

136. Griffin JT, Bhatt S, Sinka ME, Gething PW, Lynch M, Patouillard E, et al. Potential for reduction of burden and local elimination of malaria by reducing *Plasmodium falciparum* malaria transmission: a mathematical modelling study. Lancet Infect Dis. 2016; 16(4):465–72. https://doi.org/10.1016/S1473-3099(15)00423-5 PMID: 26809816

137. Chaccour CJ, Rabinovich NR, Slater H, Canavati SE, Bousema T, Lacerda M, et al. Establishment of the Ivermectin Research for Malaria Elimination Network: updating the research agenda. Malar J. 2015; 14:243. https://doi.org/10.1186/s12936-015-0691-6 PMID: 26068560

138. Slater HC, Walker PG, Bousema T, Okell LC, Ghani AC. The potential impact of adding ivermectin to a mass treatment intervention to reduce malaria transmission: a modelling study. J Infect Dis. 2014; 210(12):1972–80. https://doi.org/10.1093/infdis/jiu351 PMID: 24951826

139. Chaccour CJ, Kobylinski KC, Bassat Q, Bousema T, Drakeley C, Alonso P, et al. Ivermectin to reduce malaria transmission: a research agenda for a promising new tool for elimination. Malar J. 2013; 12:153. https://doi.org/10.1186/1475-2875-12-153 PMID: 23647969

140. Bogers WM, Oostermeijer H, Mooij P, Koopman G, Verschoor EJ, Davis D, et al. Potent immune responses in rhesus macaques induced by nonviral delivery of a self-amplifying RNA vaccine expressing HIV type 1 envelope with a cationic nanoemulsion. J Infect Dis. 2015; 211(6):947–55. https://doi.org/10.1093/infdis/jiu522 PMID: 25234719

141. Kotta S, Khan AW, Ansari SH, Sharma RK, Ali J. Anti HIV nanoemulsion formulation: optimization and in vitro-in vivo evaluation. Int J Pharm. 2014; 462(1–2):129–34. https://doi.org/10.1016/j.ijpharm.2013.12.038 PMID: 24374067

142. Jain A, Thakur K, Kush P, Jain UK. Docetaxel loaded chitosan nanoparticles: formulation, characterization and cytotoxicity studies. Int J Biol Macromol. 2014; 69:546–53. https://doi.org/10.1016/j.ijbiomac.2014.06.029 PMID: 24971551

143. Mishra P, SS R, Jerobin J, Thomas J, Mukherjee A, Chandrasekaran N. Study on antimicrobial potential of neem oil nanoemulsion against *Pseudomonas aeruginosa* infection in Labeo rohita. Biotechnol Appl Biochem. 2014; 61(5):611–9. https://doi.org/10.1002/bab.1213 PMID: 24502533

144. Campo B, Vandal O, Wesche DL, Burrows JN. Killing the hypnozoite—drug discovery approaches to prevent relapse in *Plasmodium vivax*. Pathog Glob Health. 2015; 109(3):107–22. https://doi.org/10.1179/2047773215Y.0000000013 PMID: 25891812

145. Mikolajczak SA, Vaughan AM, Kangwanrangsan N, Roobsoong W, Fishbaugher M, Yimamnuaychok N, et al. *Plasmodium vivax* liver stage development and hypnozoite persistence in human liver-chimeric mice. Cell Host Microbe. 2015; 17(4):526–35. https://doi.org/10.1016/j.chom.2015.02.011 PMID: 25800544

146. Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, et al. Relapses of *Plasmodium vivax* infection usually result from activation of heterologous hypnozoites. J Infect Dis. 2007; 195(7):927–33. https://doi.org/10.1086/512241 PMID: 17330781

147. Chen N, Auliff A, Rieckmann K, Gatton M, Cheng Q. Relapses of *Plasmodium vivax* infection result from clonal hypnozoites activated at predetermined intervals. J Infect Dis. 2007; 195(7):934–41. https://doi.org/10.1086/512242 PMID: 17330782

148. Beck HP, Wampfler R, Carter N, Koh G, Osorio L, Rueangweerayut R, et al. Estimation of the antirelapse efficacy of tafenoquine, using *Plasmodium vivax* genotyping. J Infect Dis. 2016; 213(5):794–9. https://doi.org/10.1093/infdis/jiv508 PMID: 26500351

149. Richie TL, Billingsley PF, Sim BK, James ER, Chakravarty S, Epstein JE, et al. Progress with *Plasmodium falciparum* sporozoite (PfSPZ)-based malaria vaccines. Vaccine. 2015; 33(52):7452–61. https://doi.org/10.1016/j.vaccine.2015.09.096 PMID: 26469720

150. Miura K. Progress and prospects for blood-stage malaria vaccines. Expert Rev Vaccines. 2016; 15 (6):765–81. https://doi.org/10.1586/14760584.2016.1141680 PMID: 26760062

151. Draper SJ, Angov E, Horii T, Miller LH, Srinivasan P, Theisen M, et al. Recent advances in recombinant protein-based malaria vaccines. Vaccine. 2015; 33(52):7433–43. https://doi.org/10.1016/j.vaccine.2015.09.093 PMID: 26458807

152. World Health Organization. WHO Product Development for Vaccines Advisory Committee (PD-VAC) meeting– 2015 Geneva: WHO; 2015 [Available from: http://www.who.int/immunization/research/meetings_workshops/pdvac/en/.]

153. Nunes JK, Woods C, Carter T, Raphael T, Morin MJ, Diallo D, et al. Development of a transmission-blocking malaria vaccine: progress, challenges, and the path forward. Vaccine. 2014; 32(43):5531–9. https://doi.org/10.1016/j.vaccine.2014.07.030 PMID: 25077422

154. Delrieu I, Leboulleux D, Ivinson K, Gessner BD, Malaria Transmission Blocking Vaccine Technical Consultation G. Design of a Phase III cluster randomized trial to assess the efficacy and safety of a malaria transmission blocking vaccine. Vaccine. 2015; 33(13):1518–26. https://doi.org/10.1016/j.vaccine.2015.01.050 PMID: 25681064

155. Shimp RL Jr., Rowe C, Reiter K, Chen B, Nguyen V, Aebig J, et al. Development of a Pfs25-EPA malaria transmission blocking vaccine as a chemically conjugated nanoparticle. Vaccine. 2013; 31(28):2954–62. https://doi.org/10.1016/j.vaccine.2013.04.034 PMID: 23623858

156. Li Y, Leneghan DB, Miura K, Nikolaeva D, Brian IJ, Dicks MD, et al. Enhancing immunogenicity and transmission-blocking activity of malaria vaccines by fusing Pfs25 to IMX313 multimerization technology. Sci Rep. 2016; 6:18848. https://doi.org/10.1038/srep18848 PMID: 26743316

157. Bennett JW, Yadava A, Tosh D, Sattabongkot J, Komisar J, Ware LA, et al. Phase 1/2a Trial of *Plasmodium vivax* malaria vaccine candidate VMP001/AS01B in malaria-naive adults: safety, immunogenicity, and efficacy. PLoS Negl Trop Dis. 2016; 10(2):e0004423. https://doi.org/10.1371/journal.pntd.0004423 PMID: 26919472

158. Herrera S, Fernandez OL, Vera O, Cardenas W, Ramirez O, Palacios R, et al. Phase I safety and immunogenicity trial of *Plasmodium vivax* CS derived long synthetic peptides adjuvanted with montanide ISA 720 or montanide ISA 51. Am J Trop Med Hyg. 2011; 84(2 Suppl):12–20.

159. Bauza K, Malinauskas T, Pfander C, Anar B, Jones EY, Billker O, et al. Efficacy of a *Plasmodium vivax* malaria vaccine using ChAd63 and modified vaccinia Ankara expressing thrombospondin-related anonymous protein as assessed with transgenic *Plasmodium berghei* parasites. Infect Immun. 2014; 82(3):1277–86. https://doi.org/10.1128/IAI.01187-13 PMID: 24379295

160. de Cassan SC, Shakri AR, Llewellyn D, Elias SC, Cho JS, Goodman AL, et al. Preclinical assessment of viral vectored and protein vaccines targeting the duffy-binding protein region ii of *Plasmodium vivax*. Front Immunol. 2015; 6:348. https://doi.org/10.3389/fimmu.2015.00348 PMID: 26217340

161. McCarthy JS, Griffin PM, Sekuloski S, Bright AT, Rockett R, Looke D, et al. Experimentally induced blood-stage *Plasmodium vivax* infection in healthy volunteers. J Infect Dis. 2013; 208(10):1688–94. https://doi.org/10.1093/infdis/jit394 PMID: 23908484

162. Rutledge G, Boehme U, Sanders M, Reid A, Maiga-Ascofare O, Djimde A, et al. Elusive Plasmodium species complete the human malaria genome set bioRxiv: Cold Spring Harbor Laboratory; 2016 [Available from: http://biorxiv.org/content/early/2016/05/12/052696.full.pdf+html]

163. Lee S, Nguyen MT. Recent advances of vaccine adjuvants for infectious diseases. Immune Netw. 2015; 15(2):51–7. https://doi.org/10.4110/in.2015.15.2.51 PMID: 25922593

164. Kelley B. Industrialization of mAb production technology: the bioprocessing industry at a crossroads. MAbs. 2009; 1(5):443–52. PMID: 20065641

165. Centre for Biosecurity of UPMC. Next generation monoclonal antibodies: challenges and opportunities Baltimore, MD: Centre for Biosecurity of UPMC; 2013 [Available from: http://www.upmchealthsecurity.org/our-work/pubs_archive/pubs-pdfs/2013/2013-02-04-next-gen-monoclonal-antibodies.pdf]

166. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, et al. A novel investigational Fc-modified humanized monoclonal antibody, motavizumab-YTE, has an extended half-life in healthy adults. Antimicrob Agents Chemother. 2013; 57(12):6147–53. https://doi.org/10.1128/AAC.01285-13 PMID: 24080653

167. Rasmussen SK, Naested H, Muller C, Tolstrup AB, Frandsen TP. Recombinant antibody mixtures: production strategies and cost considerations. Arch Biochem Biophys. 2012; 526(2):139–45. https://doi.org/10.1016/j.abb.2012.07.001 PMID: 22820097

168. Brock PM, Fornace KM, Parmiter M, Cox J, Drakeley CJ, Ferguson HM, et al. *Plasmodium knowlesi* transmission: integrating quantitative approaches from epidemiology and ecology to understand malaria as a zoonosis. Parasitology. 2016; 143(4):389–400. https://doi.org/10.1017/S0031182015001821 PMID: 26817785

169. Li S, Plebanski M, Smooker P, Gowans EJ. Editorial: Why vaccines to HIV, HCV, and malaria have so far failed-challenges to developing vaccines against immunoregulating pathogens. Front Microbiol. 2015; 6:1318. https://doi.org/10.3389/fmicb.2015.01318 PMID: 26640461

170. Neafsey DE, Juraska M, Bedford T, Benkeser D, Valim C, Griggs A, et al. Genetic diversity and protective efficacy of the RTS,S/AS01 malaria vaccine. N Engl J Med. 2015; 373(21):2025–37. https://doi.org/10.1056/NEJMoa1505819 PMID: 26488565

171. Plowe CV. Vaccine-Resistant Malaria. N Engl J Med. 2015; 373(21):2082–3. https://doi.org/10.1056/NEJMe1511955 PMID: 26488465

172. Takala SL, Plowe CV. Genetic diversity and malaria vaccine design, testing and efficacy: preventing and overcoming 'vaccine resistant malaria'. Parasite Immunol. 2009; 31(9):560–73. https://doi.org/10.1111/j.1365-3024.2009.01138.x PMID: 19691559

173. Olotu A, Fegan G, Wambua J, Nyangweso G, Leach A, Lievens M, et al. Seven-year efficacy of RTS, S/AS01 malaria vaccine among young African children. N Engl J Med. 2016; 374(26):2519–29. https://doi.org/10.1056/NEJMoa1515257 PMID: 27355532

174. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015; 526(7572):207–11. https://doi.org/10.1038/nature15535 PMID: 26375008

175. World Health Organization. Insecticide resistance Geneva: WHO; 2015 [Available from: http://www.who.int/malaria/areas/vector_control/insecticide_resistance/en/.]

176. Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, et al. Averting a malaria disaster: will insecticide resistance derail malaria control? Lancet. 2016; 387(10029):1785–8. https://doi.org/10.1016/S0140-6736(15)00417-1 PMID: 26880124

177. World Health Organization Pesticide Evaluation Scheme. WHO recommended long-lasting insecticidal nets 2016 [Available from: http://www.who.int/whopes/Long-lasting_insecticidal_nets_April_2016.pdf?ua=1]

178. World Health Organization Pesticide Evaluation Scheme. Pesticide products under WHOPES laboratory and or field testing and evaluation Geneva: WHOPES; 2016 [Available from: http://www.who.int/whopes/Products_Under_WHOPES_Evaluation_March_2016.pdf?ua=1]

179. N'Guessan R, Asidi A, Boko P, Odjo A, Akogbeto M, Pigeon O, et al. An experimental hut evaluation of PermaNet((R)) 3.0, a deltamethrin-piperonyl butoxide combination net, against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes in southern Benin. Trans R Soc Trop Med Hyg. 2010; 104(12):758–65. https://doi.org/10.1016/j.trstmh.2010.08.008 PMID: 20956008

180. Pennetier C, Bouraima A, Chandre F, Piameu M, Etang J, Rossignol M, et al. Efficacy of Olyset(R) Plus, a new long-lasting insecticidal net incorporating permethrin and piperonyl-butoxide against multi-resistant malaria vectors [corrected]. PLoS ONE. 2013; 8(10):e75134. https://doi.org/10.1371/journal.pone.0075134 PMID: 24116029

181. Tungu P, Magesa S, Maxwell C, Malima R, Masue D, Sudi W, et al. Evaluation of PermaNet 3.0 a deltamethrin-PBO combination net against *Anopheles gambiae* and pyrethroid resistant *Culex quinquefasciatus* mosquitoes: an experimental hut trial in Tanzania. Malar J. 2010; 9:21. https://doi.org/10.1186/1475-2875-9-21 PMID: 20085631

182. Morel CM, Thang ND, Erhart A, Xa NX, Peeters Grietens K, Xuan Hung L, et al. Cost-effectiveness of long-lasting insecticide-treated hammocks in preventing malaria in South-central Vietnam. PLoS ONE. 2013; 8(3):e58205. https://doi.org/10.1371/journal.pone.0058205 PMID: 23536790

183. NgenIRS. 2016 [Available from: http://www.ngenirs.org/.]

184. Knapp J, Macdonald M, Malone D, Hamon N, Richardson JH. Disruptive technology for vector control: the Innovative Vector Control Consortium and the US Military join forces to explore transformative insecticide application technology for mosquito control programmes. Malar J. 2015; 14:371. https://doi.org/10.1186/s12936-015-0907-9 PMID: 26409879

185. Sibanda M, Focke W. Development of an insecticide impregnated polymer wall lining for malaria vector control. Malar J. 2014; 13 (Suppl 1):80.

186. Kruger T, Sibanda MM, Focke WW, Bornman MS, de Jager C. Acceptability and effectiveness of a monofilament, polyethylene insecticide-treated wall lining for malaria control after six months in dwellings in Vhembe District, Limpopo Province, South Africa. Malar J. 2015; 14:485. https://doi.org/10.1186/s12936-015-1005-8 PMID: 26628275

187. Andriessen R, Snetselaar J, Suer RA, Osinga AJ, Deschietere J, Lyimo IN, et al. Electrostatic coating enhances bioavailability of insecticides and breaks pyrethroid resistance in mosquitoes. Proc Natl Acad Sci U S A. 2015; 112(39):12081–6. https://doi.org/10.1073/pnas.1510801112 PMID: 26324912

188. Briet OJ, Chitnis N. Effects of changing mosquito host searching behaviour on the cost effectiveness of a mass distribution of long-lasting, insecticidal nets: a modelling study. Malar J. 2013; 12:215. https://doi.org/10.1186/1475-2875-12-215 PMID: 23802594

189. Gatton ML, Chitnis N, Churcher T, Donnelly MJ, Ghani AC, Godfray HC, et al. The importance of mosquito behavioural adaptations to malaria control in Africa. Evolution. 2013; 67(4):1218–30. https://doi.org/10.1111/evo.12063 PMID: 23550770

190. Govella NJ, Chaki PP, Killeen GF. Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations. Malar J. 2013; 12:124. https://doi.org/10.1186/1475-2875-12-124 PMID: 23577656

191. Killeen GF, Chitnis N. Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: a mathematical modelling analysis. Malar J. 2014; 13:97. https://doi.org/10.1186/1475-2875-13-97 PMID: 24629066

192. Russell TL, Beebe NW, Cooper RD, Lobo NF, Burkot TR. Successful malaria elimination strategies require interventions that target changing vector behaviours. Malar J. 2013; 12:56. https://doi.org/10.1186/1475-2875-12-56 PMID: 23388506

193. Sokhna C, Ndiath MO, Rogier C. The changes in mosquito vector behaviour and the emerging resistance to insecticides will challenge the decline of malaria. Clin Microbiol Infect. 2013; 19(10):902–7. https://doi.org/10.1111/1469-0691.12314 PMID: 23910459

194. Sougoufara S, Diedhiou SM, Doucoure S, Diagne N, Sembene PM, Harry M, et al. Biting by *Anopheles funestus* in broad daylight after use of long-lasting insecticidal nets: a new challenge to malaria elimination. Malar J. 2014; 13:125. https://doi.org/10.1186/1475-2875-13-125 PMID: 24678587

195. Diabate A, Tripet F. Targeting male mosquito mating behaviour for malaria control. Parasit Vectors. 2015; 8:347. https://doi.org/10.1186/s13071-015-0961-8 PMID: 26113015

196. Donnelly B, Berrang-Ford L, Ross NA, Michel P. A systematic, realist review of zooprophylaxis for malaria control. Malar J. 2015; 14:313. https://doi.org/10.1186/s12936-015-0822-0 PMID: 26264913

197. Durnez L, Coosemans M. Residual transmission of malaria: an old issue for new approaches Anopheles mosquitoes. In: Manguin S, editor. Anopheles mosquitoes, new insights into malaria vectors. Rijeka: InTech; 2013. p. 671–704.

198. Killeen GF. Characterizing, controlling and eliminating residual malaria transmission. Malar J. 2014; 13:330. https://doi.org/10.1186/1475-2875-13-330 PMID: 25149656

199. Reddy MR, Overgaard HJ, Abaga S, Reddy VP, Caccone A, Kiszewski AE, et al. Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. Malar J. 2011; 10:184. https://doi.org/10.1186/1475-2875-10-184 PMID: 21736750

200. Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, Killeen GF. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. Malar J. 2011; 10:80. https://doi.org/10.1186/1475-2875-10-80 PMID: 21477321

201. Derua YA, Alifrangis M, Hosea KM, Meyrowitsch DW, Magesa SM, Pedersen EM, et al. Change in composition of the *Anopheles gambiae* complex and its possible implications for the transmission of malaria and lymphatic filariasis in north-eastern Tanzania. Malar J. 2012; 11:188. https://doi.org/10.1186/1475-2875-11-188 PMID: 22681999

202. Mwangangi JM, Muturi EJ, Muriu SM, Nzovu J, Midega JT, Mbogo C. The role of *Anopheles arabiensis* and *Anopheles coustani* in indoor and outdoor malaria transmission in Taveta District, Kenya. Parasit Vectors. 2013; 6:114. https://doi.org/10.1186/1756-3305-6-114 PMID: 23601146

203. Revay EE, Schlein Y, Tsabari O, Kravchenko V, Qualls W, De-Xue R, et al. Formulation of attractive toxic sugar bait (ATSB) with safe EPA-exempt substance significantly diminishes the Anopheles sergentii population in a desert oasis. Acta Trop. 2015; 150:29–34. https://doi.org/10.1016/j.actatropica.2015.06.018 PMID: 26119042

204. Marshall JM, White MT, Ghani AC, Schlein Y, Muller GC, Beier JC. Quantifying the mosquito's sweet tooth: modelling the effectiveness of attractive toxic sugar baits (ATSB) for malaria vector control. Malar J. 2013; 12:291. https://doi.org/10.1186/1475-2875-12-291 PMID: 23968494

205. Johnson BJ, Ritchie SA. The siren's song: Exploitation of female flight tones to passively capture male *Aedes aegypti* (Diptera: Culicidae). J Med Entomol. 2016; 53(1):245–8. https://doi.org/10.1093/jme/tjv165 PMID: 26502754

206. Downes J. The swarming and mating flight of Diptera. Ann Rev Entomol. 1969; 14:271–98.

207. World Health Organization. Guidelines for efficacy testing of spatial repellents: WHO; 2013 [Available from: http://apps.who.int/iris/bitstream/10665/78142/1/9789241505024_eng.pdf?ua=1]

208. Syafruddin D, Bangs MJ, Sidik D, Elyazar I, Asih PB, Chan K, et al. Impact of a spatial repellent on malaria incidence in two villages in Sumba, Indonesia. Am J Trop Med Hyg. 2014; 91(6):1079–87. https://doi.org/10.4269/ajtmh.13-0735 PMID: 25311699

209. Hill N, Zhou HN, Wang P, Guo X, Carneiro I, Moore SJ. A household randomized, controlled trial of the efficacy of 0.03% transfluthrin coils alone and in combination with long-lasting insecticidal nets on the

incidence of *Plasmodium falciparum* and *Plasmodium vivax* malaria in Western Yunnan Province, China. Malar J. 2014; 13:208. https://doi.org/10.1186/1475-2875-13-208 PMID: 24885993

210. Tusting LS, Ippolito MM, Willey BA, Kleinschmidt I, Dorsey G, Gosling RD, et al. The evidence for improving housing to reduce malaria: a systematic review and meta-analysis. Malar J. 2015; 14:209. https://doi.org/10.1186/s12936-015-0724-1 PMID: 26055986

211. Tusting LS, Thwing J, Sinclair D, Fillinger U, Gimnig J, Bonner KE, et al. Mosquito larval source management for controlling malaria. Cochrane Database Syst Rev. 2013; 8:CD008923.

212. Committee on Gene Drive Research in Non-Human Organisms: Recommendations for Responsible Conduct. Gene drives on the horizon: advancing science, navigating uncertainty and aligning research with public values Washington DC: The National Academies Press; 2016 [Available from: http://www.nap.edu/catalog/23405/gene-drives-on-the-horizon-advancing-science-navigating-uncertainty-and]

213. Burt A. Heritable strategies for controlling insect vectors of disease. Philos Trans R Soc Lond B Biol Sci. 2014; 369(1645):20130432. https://doi.org/10.1098/rstb.2013.0432 PMID: 24821918

214. Oliva CF, Vreysen MJ, Dupe S, Lees RS, Gilles JR, Gouagna LC, et al. Current status and future challenges for controlling malaria with the sterile insect technique: technical and social perspectives. Acta Trop. 2014; 132 Suppl:S130–9. https://doi.org/10.1016/j.actatropica.2013.11.019 PMID: 24295892

215. Bourtzis K, Lees RS, Hendrichs J, Vreysen MJ. More than one rabbit out of the hat: Radiation, transgenic and symbiont-based approaches for sustainable management of mosquito and tsetse fly populations. Acta Trop. 2016; 157:115–30. https://doi.org/10.1016/j.actatropica.2016.01.009 PMID: 26774684

216. Black WCt, Alphey L, James AA. Why RIDL is not SIT. Trends Parasitol. 2011; 27(8):362–70. https://doi.org/10.1016/j.pt.2011.04.004 PMID: 21659002

217. Windbichler N, Menichelli M, Papathanos PA, Thyme SB, Li H, Ulge UY, et al. A synthetic homing endonuclease-based gene drive system in the human malaria mosquito. Nature. 2011; 473 (7346):212–5. https://doi.org/10.1038/nature09937 PMID: 21508956

218. Esvelt KM, Smidler AL, Catteruccia F, Church GM. Concerning RNA-guided gene drives for the alteration of wild populations. Elife. 2014:e03401. https://doi.org/10.7554/eLife.03401 PMID: 25035423

219. Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, et al. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. Nat Biotechnol. 2016; 34(1):78–83. https://doi.org/10.1038/nbt.3439 PMID: 26641531

220. Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E, et al. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. Proc Natl Acad Sci U S A. 2015; 112(49):E6736–43. https://doi.org/10.1073/pnas.1521077112 PMID: 26598698

221. Wilke AB, Marrelli MT. Paratransgenesis: a promising new strategy for mosquito vector control. Parasit Vectors. 2015; 8:342. https://doi.org/10.1186/s13071-015-0959-2 PMID: 26104575

222. Wang S, Ghosh AK, Bongio N, Stebbings KA, Lampe DJ, Jacobs-Lorena M. Fighting malaria with engineered symbiotic bacteria from vector mosquitoes. Proc Natl Acad Sci U S A. 2012; 109 (31):12734–9. https://doi.org/10.1073/pnas.1204158109 PMID: 22802646

223. Dao A, Yaro AS, Diallo M, Timbine S, Huestis DL, Kassogue Y, et al. Signatures of aestivation and migration in Sahelian malaria mosquito populations. Nature. 2014; 516(7531):387–90. https://doi.org/10.1038/nature13987 PMID: 25470038

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COLLECTION REVIEW

malERA: An updated research agenda for combination interventions and modelling in malaria elimination and eradication

The malERA Refresh Consultative Panel on Combination Interventions and Modelling¶*

¶ Membership of the malERA Refresh Consultative Panel on Combination Interventions and Modelling is listed in the Acknowledgments.

* rsteketee@path.org (RS); sihay@uw.edu (SIH)

Citation: The malERA Refresh Consultative Panel on Combination Interventions and Modelling (2017) malERA: An updated research agenda for combination interventions and modelling in malaria elimination and eradication. PLoS Med 14(11): e1002453. https://doi.org/10.1371/journal.pmed.1002453

Published: November 30, 2017

Funding: MESA received a grant from the Bill & Melinda Gates Foundation (OPP1034591 http://www.malariaeradication.org/mesa-track/malaria-eradication-scientific-alliance-mesa). KR, ES, and EO (Bill & Melinda Gates Foundation) were members of this Panel and participated in the Panel consultation and in the development of this paper.

Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: PG and TS receive funding from the Bill & Melinda Gates Foundation (BMGF) to study modelling. SIH is funded by grants from the BMGF, the Wellcome Trust and the Fleming Fund. LW receives funding from the BMGF

Abstract

This paper summarises key advances and priorities since the 2011 presentation of the Malaria Eradication Research Agenda (malERA), with a focus on the combinations of intervention tools and strategies for elimination and their evaluation using modelling approaches. With an increasing number of countries embarking on malaria elimination programmes, national and local decisions to select combinations of tools and deployment strategies directed at malaria elimination must address rapidly changing transmission patterns across diverse geographic areas. However, not all of these approaches can be systematically evaluated in the field. Thus, there is potential for modelling to investigate appropriate 'packages' of combined interventions that include various forms of vector control, case management, surveillance, and population-based approaches for different settings, particularly at lower transmission levels. Modelling can help prioritise which intervention packages should be tested in field studies, suggest which intervention package should be used at a particular level or stratum of transmission intensity, estimate the risk of resurgence when scaling down specific interventions after local transmission is interrupted, and evaluate the risk and impact of parasite drug resistance and vector insecticide resistance. However, modelling intervention package deployment against a heterogeneous transmission background is a challenge. Further validation of malaria models should be pursued through an iterative process, whereby field data collected with the deployment of intervention packages is used to refine models and make them progressively more relevant for assessing and predicting elimination outcomes.

Summary points

- Since 2011, there have been significant improvements in the development, organisation, and infrastructure of country programmes for malaria control and elimination globally. This has included the increasing use of combinations of interventions against the mosquito vector and the parasite in humans to reduce transmission in large and expanding

and the Wellcome-Trust Major Overseas Programme in SE Asia.

Abbreviations: ACT, artemisinin-based combination therapy; ESP, Elimination Scenario Planning; fMDA, focal mass drug administration; IRS, indoor residual spraying; LLIN, long-lasting insecticidal bed net; malERA, Malaria Eradication Research Agenda; MDA, mass drug administration; MSAT, mass screening and treatment; NMCP, National Malarial Control Programme; WHO, World Health Organization.

Provenance: Submitted as part of a Supplement; externally peer reviewed.

geographies and populations and an adaptation of these interventions as transmission is progressively reduced.

- Similarly, there has been substantial improvement in the sophistication and field validation of malaria transmission models and their ability to describe and predict the effects of ecologic changes and the impact of specific interventions. These advances permit the investigation and comparison of multiple complementary interventions in elimination settings.
- There is an increasing need to combine interventions into 'packages' that can be tailored to specific settings based on the characteristics of their transmission dynamics and epidemiology (landscape stratification). The challenge is to identify the complementary components of each intervention package and establish the triggers and thresholds for their deployment (or withdrawal) throughout the elimination process, including maintaining elimination once transmission has been interrupted.

Introduction

In 2011, the Malaria Elimination Research Agenda (malERA) made recommendations for how mathematical modelling efforts could best inform policy and guide research for specific intervention tools for elimination—diagnostics, drugs, vector control, and vaccines [1]. Since then, experience with malaria intervention tools has grown, and the toolbox has expanded with new drugs, new insecticides, better diagnostics, and a first vaccine [2]. As more countries seek elimination, grouping tools to best address diverse and changing transmission intensity has become a central issue. Some tools are oriented primarily towards reducing disease burden, e.g., seasonal malaria chemoprevention; others are dedicated to reducing transmission, e.g., drug-based population-wide parasite clearance; and some meet both of these objectives, e.g., vector control. Thus, not all tools will contribute equally to malaria elimination, and the timing and duration of their use must adapt as programmes progress.

This paper summarises progress since the initial malERA publication regarding transmission-aligned 'elimination tool packages' and deployment strategies and opportunities for models to help inform and prioritise intervention choices. The findings come from an extensive literature review of published and unpublished materials and the deliberations of the 2015 malERA Refresh Consultative Panel on Combination Interventions and Modelling, which includes specialists from malaria modelling, field researchers, and National Malarial Control Programme (NMCP) representatives [3].

Methods

The findings presented in this paper result from an extensive literature review of published and unpublished materials and the deliberations of the 2015 malERA Refresh Consultative Panel on Combination Interventions and Modelling. Electronic databases were systematically searched for published literature from 1 January 2010 until 1 August 2015, without language limitations. The websites of the institutions that apply modelling techniques to malaria research questions and the MESA Track database of current research projects relevant to malaria elimination were systematically searched to identify pertinent ongoing research.

Panellists were invited to recommend additional literature and additional ongoing research projects. The comprehensive search for literature and ongoing research provided the basis for launching the second step.

A 2-day workshop was held with the majority of the panel members, including specialists from malaria modelling, field researchers, and NMCP representatives. The panel broke into 2 working groups to identify the issues in combining interventions and how mathematical modelling could be applied to these problems. Each group fed back to a plenary session in which further robust discussions and input occurred. This helped refine the opportunities and gap areas in which research is needed. The final findings were arrived at with inputs from all panellists and several iterations of the manuscript.

Intervention packages to achieve elimination

Over the past 5 years, regardless of initial local transmission levels, most countries have continued to reduce the clinical burden of malaria and transmission [4]. The World Health Organization (WHO) recently published its Global Technical Strategy (GTS) for Malaria 2016–2030 (Fig 1) [5]. This builds on the core activities of vector control, case management, and surveillance, with additional interventions to accelerate progress to elimination. In the GTS, for the first time, modelling studies were used to support goal setting [5].

The malERA Refresh Consultative Panel on Combination Interventions and Modelling approach encompassed the full spectrum of malaria transmission—addressing emerging programmatic aims and combining into 'packages' the available tools and strategies directed towards malaria elimination (Fig 2). As transmission is reduced to very low levels, the intervention packages must adapt to increasingly focal and heterogeneous populations, in which infections are rare. Given the extensive range of available tools and the diversity/heterogeneity of transmission settings, it becomes difficult to field test all possible intervention packages. Models can assist the prioritisation and design of clinical trials and in the choice of an intervention package to achieve their desired goals.

Progress in combination interventions and modelling

Initial malERA recommendations for a research and development agenda in mathematical modelling are shown in Box 1 [1]. Subsequently, the scope and depth of research has expanded to include diverse vector control strategies, complex diagnostics, drug and vaccine dynamics, and deployment strategies. Additionally, infection models have advanced following incorporation of new field trial data, particularly regarding mass drug administration (MDA) and specific aspects of vector control, providing greater plausibility to model predictions.

The interface between modelling and implementation has not developed as was perhaps envisaged, in terms of appropriate portals to allow 'end users' access to relevant software and explore the effect of varying conditions on the ideal choice of control measures. However, the development, organisation, and infrastructure of malaria modelling has improved (Box 2), and recent efforts include an expansion of open-access data and software [6–13]. Also, modelling has been incorporated at the policy level within WHO [5] and included in planning tools for malaria elimination [14]. Wider implementation is possibly now dependent upon the development of next-generation models that sufficiently address combination interventions against a background of heterogeneity and low transmission as more countries move towards elimination.

These advances are complemented by discoveries in basic science, large field trials of new and existing interventions, and substantial data gathering efforts that provide the raw evidence to further validate models. A number of recent reports used models to address the role of

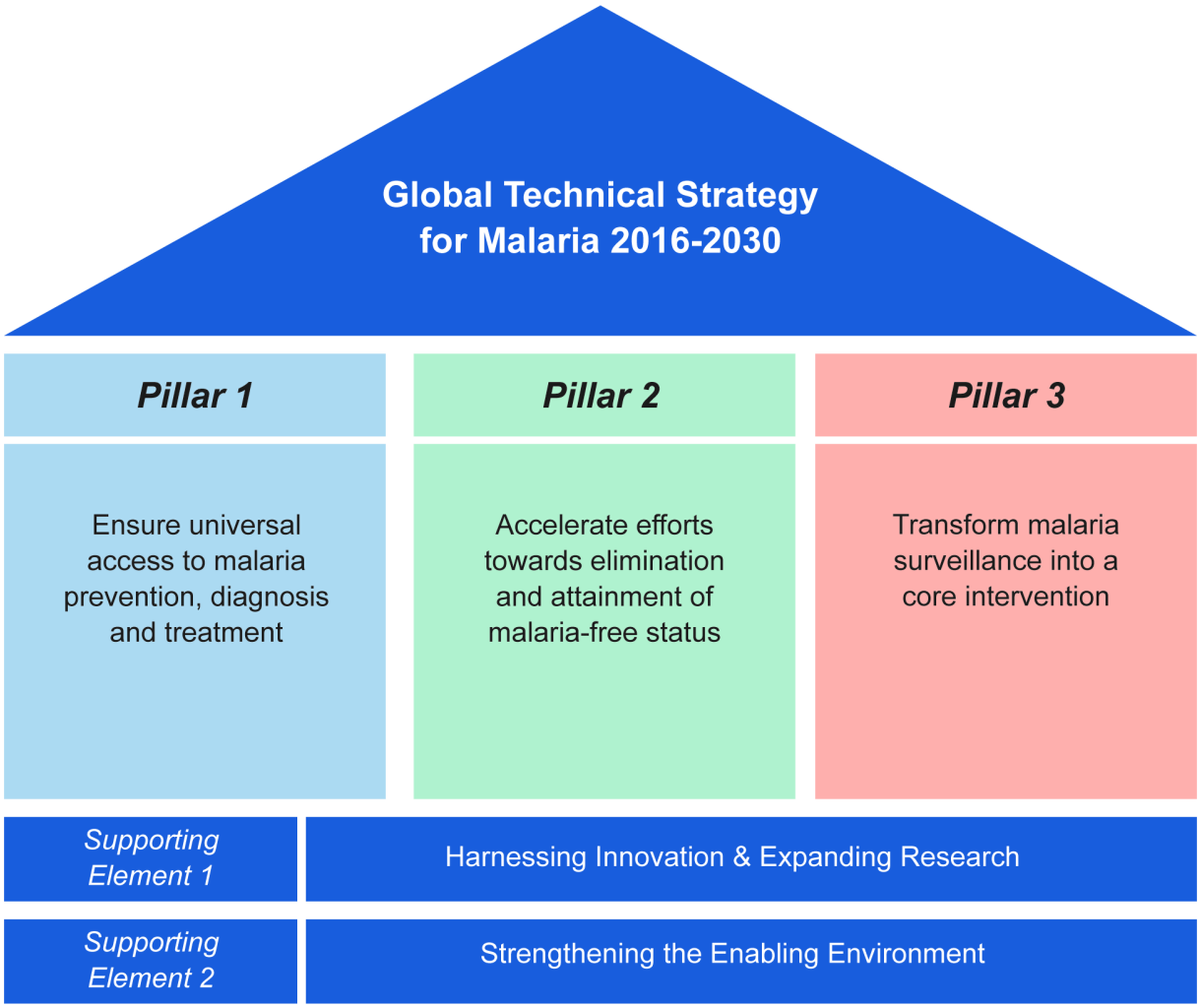

Fig 1. Schematic of the pillars and supporting elements of the World Health Organization (WHO) Global Technical Strategy for Malaria 2016–2030 (source: WHO, 2015) [5].

https://doi.org/10.1371/journal.pmed.1002453.g001

multiple complementary interventions (Table 1) [15–35], and additional field trials are ongoing (Table 2) [29].

Consensus modelling

In consensus modelling, independent modelling groups examine the same research question, sometimes using the same source dataset to parameterise their model. Through objective comparison and critique, modelling groups have reached a degree of consensus on important issues, such as the relationship between health burden and transmission intensity [6], and have undertaken an in-depth analysis for the RTS,S vaccine [36]. Such efforts are resource intensive but may give robust answers incorporating the breadth of uncertainty in our understanding. There is also value in less intensive forms of model comparison in which common findings from work conducted independently are assessed (Table 3) [9,18,21,23,24,28,30–32,35,37–58]. This approach can also be particularly useful for identifying areas in which there is a lack of consensus, as this can focus efforts on further model development, basic science, and field data collection needs.

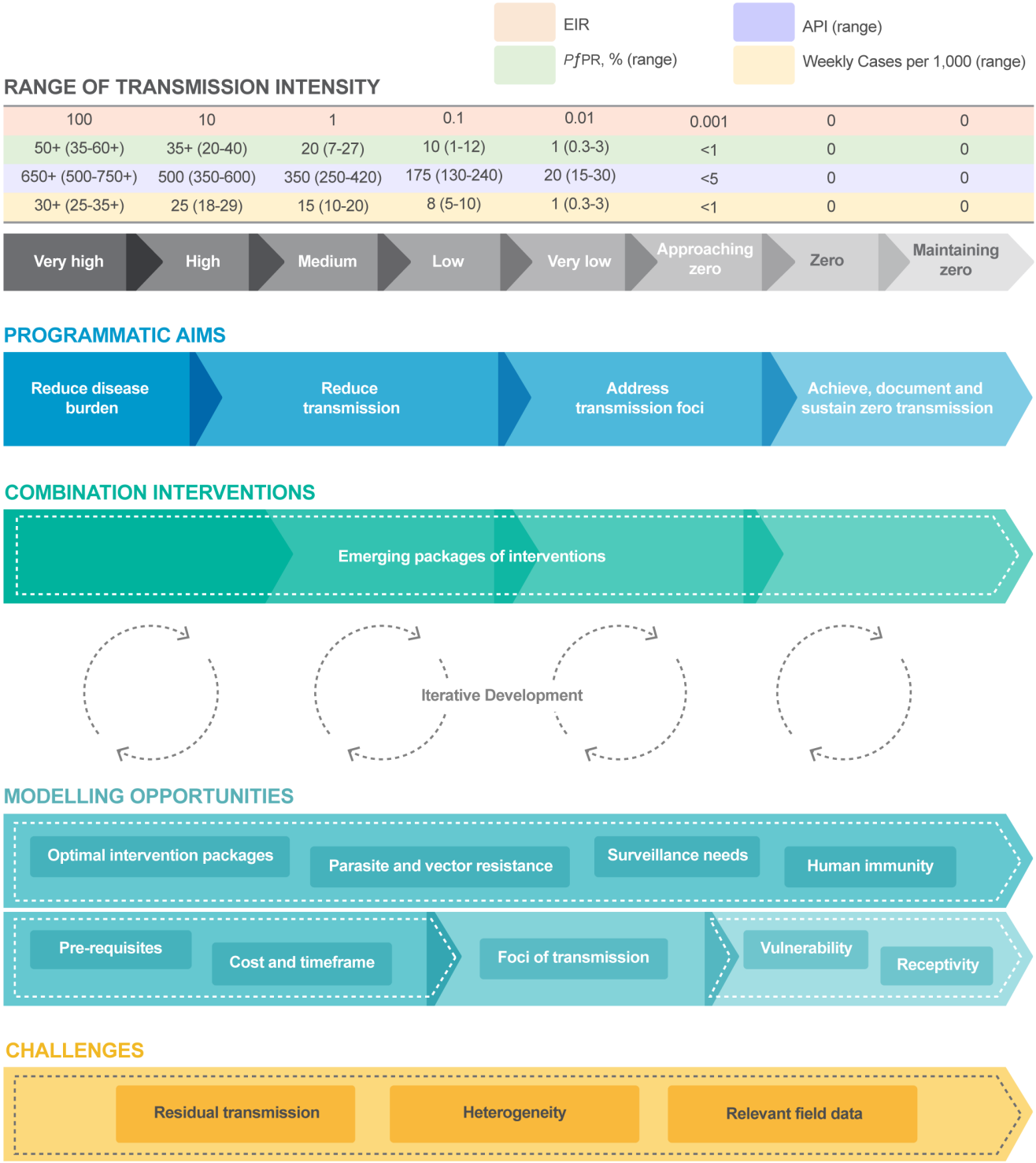

Fig 2. An example of the role of modelling across the spectrum of malaria elimination. Note that the measures of transmission are based on sub-Saharan Africa, and other constructs and transmission levels may be relevant in different geographical areas. Malaria transmission intensity measures and the relationship entomologic inoculation rate for *Plasmodium falciparum* from very high to zero transmission are adapted from data presented in [6]; personal communication from D. Smith and P. Gething. Zero refers to no locally transmitted cases of malaria infection; imported infections may be identified. Intervention package components and sequencing will depend on transmission intensity at the start of the elimination programme, the speed at which transmission declines, and the underlying typology (i.e., malaria epidemiology, species, vector ecology, and health

system factors). EIR, entomologic inoculation rate: average number of infectious mosquito bites per person per year; N.B. the table is organised by log differences in the EIR, and other measures are aligned (approximated) based on these entomologic measures. *PfPR*, *P. falciparum* parasite rate: proportion of people with a current infection with *P. falciparum*—typically determined by a population-based survey and often timed to a specific interval of the transmission season. API, annual parasite index: number of confirmed malaria cases per 1,000 population per year. Cases, cases per health facility per week: average number of confirmed malaria cases expected to present on an average week to a health facility serving a population of 5,000 people. Because many infections can be asymptomatic at any point in time (and thus not present to health services), the proportion of asymptomatic individuals varies with transmission intensity, and because most transmission is seasonal, these average estimates may vary substantially by location and season.

https://doi.org/10.1371/journal.pmed.1002453.g002

Next steps for combination interventions and modelling in malaria elimination

Fig 2 provides an example of how transmission strata, programmatic aims, the choices of intervention packages, and the iterative development between modelling and programme choices change together as malaria transmission intensity is progressively reduced towards zero, summarizing key opportunities and identifying challenges. Note that not all countries will start from high transmission levels and that the measures of transmission used in Fig 2 are based on sub-Saharan Africa. Thus, other constructs and transmission levels may be relevant in different geographical areas.

Opportunities

Combination intervention modelling

There has been considerable progress in modelling combination interventions. Models have been developed to examine the overall expected impact of diagnostic, drug, vaccine, and vector

Box 1. 2011 malERA research agenda for modelling to support malaria elimination.

Further development of models and model systems:

- Within-host dynamics of *Plasmodium* infections
- The human infectious reservoir
- Bionomics and ecology of the vectors
- Dynamics of the stimulation and decay of human immunity across a range of transmission settings
- Heterogeneities in host, vector, and parasite dynamics
- Heterogeneities in host and vector movements
- Drug pharmacokinetics/pharmacodynamics
- Vaccines that interrupt malaria transmission
- Ecology of genetically modified mosquitoes
- Development and impact of drug and pesticide resistance
- Integration of health system attributes and linking to microeconomic outputs

Box 2. Recent advances in malaria modelling.

Communications:

- A growing number of modelling groups are working in a collaborative fashion
- Greater engagement between modellers, country programmes, and operational research partners has helped refine the paramount research questions

Models:

- The development of model systems that are diverse but much improved in terms of their incorporation of malaria biology and natural history, as well as validated estimates for intervention effects, drug pharmacokinetics/ pharmacodynamics, and vaccine dynamics
- The development of models that allow the investigation of target product profiles for new tools—for example, diagnostics, surveillance systems, and drugs

Infrastructure:

- Greater dissemination of malaria models at different levels of user-interface complexity, through online hosting and open-source code repositories leading to wider access to modelling information for programme implementers, planners, and policy decision makers
- Improved means of compiling data and using common ontologies, frameworks, and metadata standards with growing international databases of some measures of malaria transmission, e.g., parasite rate surveys

control intervention combinations, including cost-effectiveness [16,18–20,24,48,57,59–62], and comparing interventions added to the backbone of standard measures [21–23,25–27,30,36,63,64]. Modelling studies have investigated the applications of several new potential interventions such as the RTS,S vaccine [36], ivermectin [19,54], mosquito traps [17], and next-generation diagnostics [25,33,65,66] and have highlighted critical attributes of new products, such as a preerythrocytic vaccine [20,67–69], genetically modified mosquitos [70–72], and combinations of future interventions [73].

Models are designed to allow scale-up and scale-down of interventions over time. The next step is to define the epidemiological information that would be most informative for making such dynamic changes and the triggers for switching or scaling. The aim is to develop a set of rules that define the characteristics of transmission that can direct specific changes in the composition and phasing of intervention packages and their targeting to specific locations and populations. These predictions can then be evaluated with further evidence from specific field trials. If reliable, such measures could be used in the subnational stratification of intervention packages.

Accelerating community clearance of malaria parasites. One hypothesis being tested in various settings is the potential to accelerate elimination by targeting the human parasite reservoir (symptomatic and asymptomatic) with time-limited deployment of community-based interventions such as MDA or mass screening and treatment (MSAT) [74]. If the intervention is justified, a wealth of modelling studies provides guidance on optimizing its deployment

Table 1. Key modelling studies on combination interventions quarter 4 2010–quarter 1 2016, with the main outcome indicated.

Multi-intervention combined
• Mass campaigns with antimalarial drugs are highly effective at interrupting transmission if deployed shortly after ITN campaigns [15].
• Compared with untargeted approaches, selective targeting of hot spots with drug campaigns is an ineffective tool for elimination because of limited sensitivity of available field diagnostics [16].
• High coverage with a combination of LLINs and attractive toxic sugar baits could result in substantial reductions in malaria transmission [17].
• Mass treatment needs to be repeated or combined with other interventions for long-term impact in many endemic settings [18].
• Including ivermectin in mass treatment strategies could be a useful adjunct to reduce and interrupt malaria transmission [19].
• Preerythrocytic vaccines will have a maximum impact where bed net coverage has saturated, vector feeding is primarily outdoors, and transmission is moderate to low [20].
Multi-intervention compared
• While adult killing methods can be highly effective under many circumstances, other vector control methods are frequently required to fill effective coverage gaps [21].
• Adding vaccines to existing vector control efforts extends the ability to achieve elimination starting from higher baseline transmission levels and with less favourable vector behaviour [22].
• Decreases in malaria transmission and burden can be accelerated over the next 15 years if the coverage of key interventions is increased [23].
• Vector control plans should consider the spatial arrangement of any intervention package to ensure effectiveness is maximised [24].
• The sensitivity of the diagnostic can play a part in increasing the chance of interrupting transmission [25].
• A failing partner drug will result in greater increases in malaria cases and morbidity than would be observed from artemisinin resistance only [26].
• Selecting combinations of interventions that target different stages in the vector's life cycle will result in maximum reductions in mosquito density [27]
Multi-intervention: Cost-effectiveness
• In all the transmission settings considered, achieving a minimal level of ITN coverage is a 'best buy'. At low transmission, MSAT probably is not worth considering. Instead, MSAT may be suitable at medium to high levels of transmission and at moderate ITN coverage [28].

ITN, insecticide-treated bed net; LLIN, long-lasting insecticidal bed net; MSAT, mass screening and treatment.

https://doi.org/10.1371/journal.pmed.1002453.t001

[15,18,28,32,33,41,53–55,57,75–79]. However, estimating the level of coverage required for successful MDA is critical [53], and for MSAT, the sensitivity of the diagnostic tool is an additional key determinant of efficacy as the current tests may fail to detect low-level infections [16,25].

Current models of MDA all include the parameters whereby immediately following MDA, there is a dramatic drop in malaria prevalence, but in the absence of elimination, prevalence returns to preintervention levels (albeit at different rates depending on the model) [53]. Country malaria programs are increasingly aware of this potential and have learned not to rely solely on MDA to eliminate transmission; thus, MDA is an accelerator used to move to a next set of interventions and strategies to find and clear the remaining transmission foci. The models must now be adapted to include a next set of actions with the potential to end transmission, i.e., MDA moving to focal MDA (fMDA) and other reactive strategies in households and neighbourhoods with rare but remaining transmission [33,79,80]. In the field, these increasingly infrequent actions will require robust local information systems as part of the intervention, rather than models.

Non-falciparum species. Recent progress has been made in models considering non-falciparum parasites and vectors, though further work is needed [76,81–95]. To address the public health and public engagement challenge of eliminating all human malaria species, multispecies

Table 2. Ongoing field studies in combination interventions as reported on the MESA Track database [29].

Vector control
• Combining indoor residual spraying and long-lasting insecticidal nets for malaria prevention: a cluster randomised controlled trial in Ethiopia (Maltrials); Ethiopia (Sep 2014–Sep 2016); *Addis Ababa University*, *Ethiopia*
• Integrated vector management: Interaction of larval control and indoor residual spraying on *Anopheles gambiae* density and vectorial capacity for human malaria; *Malaria Research and Training Center (MRTC)*, *University of Bamako*, *Mali*
• IRS and LLIN: Integration of methods and insecticide mode of actions for control of African malaria vector mosquitoes; Tanzania, United Republic of; *Ifakara Health Institute (IHI)*, *Swiss Tropical and Public Health Institute (Swiss TPH)*
• Cluster randomised trial of the impact of dual-insecticide treated nets vs. traditional LLINs on malaria vectors and malaria epidemiology in 2 districts of Mali; Mali (Dec 2013–Dec 2014); *Centers for Disease Control and Prevention (CDC)*, *United States*
• The Majete Integrated Malaria Control Project (MMP): Community-based malaria control in the perimeter of Majete Wildlife Reserve in Chikhwawa district using a Scale-Up-For-Impact (SUFI) strategy, assessing complementary intervention options, including larval source management and house improvement; Malawi (Jan 2014–Dec 2018); *Wageningen University*, *Netherlands; University of Amsterdam; College of medicine*, *University of Malawi; Liverpool School of Tropical Medicine*
Case management and surveillance
• Routine case investigation and reactive case detection for malaria elimination in Richard-Toll District in northern Senegal; Senegal (2012–2017); *PATH MACEPA*, *National Malaria Control Programme (NMCP) Senegal*
Mass treatment
• The Haiti Malaria Elimination Consortium (HaMEC); Dominican Republic, Haiti (Feb 2015–2020); *Malaria Zero Consortium*, *US*
• Assessing the effectiveness of household-level focal mass drug administration and community-wide mass drug administration with dihydroartemisinin + piperaquine for reducing malaria parasite infection prevalence and incidence in Southern Province Zambia; Zambia (2014–2016); *PATH MACEPA*, *Tulane University*, *Zambian National Malaria Control Centre*
• Population parasite clearance to decrease malaria transmission in Amhara Region, Ethiopia: a pilot study; Ethiopia (2014–2015); *PATH MACEPA*, *Ministry of Health (MOH) Ethiopia*
• Reduction of malaria parasitaemia and transmission in low to moderate seasonal transmission settings (Kanel, Ranérou and Linguère) in Senegal: a pilot study; Senegal (2014–2015); PATH MACEPA, National Malaria Control Programme (NMCP) Senegal
• Community reactive case detection versus reactive drug administration in malaria elimination areas: a cluster randomised controlled trial; Zambia (2016–Dec 2017); *Akros*
• Assess the micro-epidemiology of resistant falciparum malaria in SE Asia and to perform and evaluate an intervention with targeted chemo-elimination through a modified mass drug administration approach (Cambodia, Myanmar, Thailand, Vietnam); Cambodia, Myanmar, Thailand, Vietnam (2014–Oct 2016); *Mahidol Oxford Tropical Medicine Research Unit (MORU)*
• Evaluation of the impact of seasonal malaria chemoprevention delivered by district health services in southern Senegal; Senegal (2013–2018); *Cheikh Anta Diop University*, *Senegal*

IRS, indoor residual spraying; LLIN, long-lasting insecticidal net; SE, Southeast.

https://doi.org/10.1371/journal.pmed.1002453.t002

mathematical models that consider unified strategies and exploit the interactions between the species for improved cost-effectiveness should be used [96]. Notably, where *P. vivax* is present, the malaria programme might be sustained even as *P. falciparum* becomes rare and is eliminated. However, different approaches to both surveillance and malaria interventions would be required to reduce the *P. vivax* burden while detecting *P. falciparum* cases and preventing the reestablishment of *P. falciparum* transmission.

Surveillance as an intervention

Surveillance is an intervention tool. When honed for elimination purposes, surveillance must evolve to be able to discover evidence of transmission; establish its location, timing, nature,

Table 3. Consensus across multiple groups from modelling analyses conducted by each of the Malaria Modelling Consortium[a] groups, which assessed impact on malaria transmission of combining multiple interventions or multiple methods of using a single intervention[b].

Vector control
• Achieving and maintaining high effective coverage of the population with LLINs is consistently predicted to result in the greatest reduction in transmission in a variety of settings and in many cases enables other interventions to become more effective and longer lasting [21,23,24,28,30,32,35,37–43,55].
• Other interventions such as IRS are also predicted to be effective and can even be more effective than LLINs in specific settings, particularly if sustained and optimised through seasonal or spatial targeting strategies [32,39,42].
• Vector control interventions that maximise killing of adult female mosquitoes are predicted to have the greatest transmission reducing effect (as opposed to repellents or killing juveniles); however, the optimal choice of intervention(s) will depend on both the specific bionomics of local vectors and the costs required to reach high levels of effective coverage with each intervention [21,23,44–46].
Case management and surveillance
• Even before considering elimination, improving access to care has an important role to play in significantly reducing deaths and severe disease [9,41,47–49].
• While differing considerably in magnitude, all the models agree that levels of access to treatment of incident malaria cases and the delay in seeking treatment are 2 key measures that influence the endemicity at baseline (no interventions) and, as such, determine the following:
○ what scale of community-based programme will be required to achieve and maintain elimination [28,30,32]
○ what the risk will be of scaling back vector-based interventions post elimination [23,43,50,51]
Mass Treatment
• Short mass treatment campaigns will reduce the parasite reservoir—and consequently, transmission—in the short term but will have no long-term benefits unless other interventions are scaled up at the same time and then maintained [18,23,28,31,32,35,42,52–55].
• Treating a large proportion of the population in a single year in at least 1 round is a key determinant of MDA effectiveness whether it is achieved through high coverage in a single round or through follow-up rounds that reach new individuals [41,53,55–57]
• The addition of primaquine to MDA with long-lasting ACTs offers a small additional transmission reduction in the majority of epidemiological settings [18,30–32,42,53,54,57,58].
• Due to the prophylactic effect of treatment, MDA will always be more effective than MSAT or fMDA. If adherence or drug resistance is included in the model analysis, then this conclusion is more nuanced, and risk of drug resistance emergence and spread is an area with a lack of clear consensus among existing models [18,31,35,41].
• The longer-term effectiveness of MDA is highly sensitive to the population size of the trial area and its connectedness to other areas [18].

[a] Imperial College, London, United Kingdom; Institute for Disease Modelling, Seattle, Washington, US; Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand; Swiss Tropical and Public Health Institute, Basel, Switzerland; and University of Oxford, Oxford, UK.

[b] Compiled by Oliver Brady (University of Oxford) and Samantha Galvin (Bill & Melinda Gates Foundation). ACT, artemisinin-based combination therapy; fMDA, focal mass drug administration; IRS, indoor residual spraying; LLIN, long-lasting insecticidal bed net; MDA, mass drug administration; MSAT, mass screening and treatment.

https://doi.org/10.1371/journal.pmed.1002453.t003

and causes; identify and eliminate residual foci; prevent, detect, and contain imported malaria; and demonstrate the attainment and maintenance of zero malaria transmission [97]. As transmission declines, modification of data collection and reporting systems requires substantial investment and coordination across the malaria programmes and the surveillance management unit. Designing the necessary flexibility into a surveillance system to allow for adaptation to an elimination context will be critical.

There is an opportunity to use modelling to define the required components of surveillance systems depending on the stage of the elimination programme. This requires quantification of the detrimental effects of inaccurate, insufficient, or untimely surveillance and the beneficial effects of adding new measures to the surveillance system [25]. Modelling could also be used to

assess the level of hidden/unidentifiable cases/infections that would hinder (or would not hinder) elimination (e.g., asymptomatic or individuals with minor symptomology who would not seek treatment). As transmission declines, the addition of serological measures of past exposure [65,98–102] or active community-based transmission measurements and reactive case management [103–107] may be considered. Modelling can estimate the incremental benefit of adding specific surveillance activities to an already established surveillance system and could examine cost-effectiveness issues [48,108], specific epidemiologic aspects of contract tracing [109], and the target product profile of diagnostics [25,65,66] in case-investigation or foci-investigation settings.

Parasite and vector resistance

As efforts to reduce transmission are intensified, the risk and impact of parasite drug resistance and vector insecticide resistance becomes a key concern [110–112]. Modelling has been used to investigate the effects of resistance [25,26,30,32,113–116], and there have been some studies examining risk factors for resistance and drug failure [114,117–119]. Geostatistical models are also being developed to predict localities where resistance might be present in order to target surveillance activities, for example, mapping artemisinin-resistance in Southeast Asia [120]. The biology and natural history of mosquito vectors and malaria parasites tells us that the development and evolution of resistance will continue, given the pressure of insecticides and drugs. In terms of drug treatments, with artemisinin-based combination therapies (ACTs) globally recommended for malaria treatment, the focus must be on investigation of artemisinin and partner drug resistance, in terms of how this can be contained within the Greater Mekong subregion [111], and how its emergence or importation can be avoided in other regions [25,115]. Note that as transmission declines, the remaining parasites are those most likely to harbour resistance. Thus, even as malaria cases decline, continued field studies and modelling must be supported to address the efficacy and effectiveness of intervention tools critical for elimination programming. The next steps are to investigate how packages of interventions can be modified to mitigate the effects of resistance on existing interventions [30,121–123], how resistance can be contained [32], and how resistance can be avoided, particularly for new drugs and insecticides [124,125].

Human immunity

A gradual decline in human immunity to malaria across the population is an inevitable consequence of reducing malaria transmission and contracting parasite diversity [126,127]. The resulting delay in acquiring immunity likely will alter the age distribution and severity of malaria infections [126,128,129]. Understanding these changes is necessary to identify the most vulnerable populations or those most likely to need an intervention [128,130]. Models already include age-dependent immune factors and have dynamic modulation of immunity as a function of entomological inoculation rate [128,131], though additional temporal data could help reduce the uncertainty surrounding these functions. Gaps remain in our understanding of immunity in areas of long-standing low transmission (e.g., Haiti), where the level of asymptomatic infections is much higher than previously thought [132].

Modelling to inform policy

Strategic decisions are already being taken as part of elimination planning in a number of countries. There are numerous opportunities for modelling to inform these decisions—for example, scenario planning. An Elimination Scenario Planning (ESP) toolkit was published by WHO in 2014 following field testing using data from The Gambia and Senegal [14]. The

manual is linked to software that models malaria transmission (currently limited to *P. falciparum* in Africa), which allows users to explore the effect of a range of combinations of malaria control interventions in order to achieve elimination. Such an approach has wide application and could be extended to *P. falciparum* outside Africa or *P. vivax* settings in the future. A key consideration is that malaria policy will need to respond to climate change. Historical data may become less reliable as seasonal patterns of rainfall and land use alter. Mapping climate change effects and possible scenarios following the varied consequences of climate change for human and vector population distributions has been investigated at continental and national levels, but incorporating this into policy is more challenging [133–151].

Mathematical models can provide a framework for exploring the relationship between population movement, heterogeneous transmission, and the deployment logistics of a national or regional elimination strategy. To carry out such analyses, new model frameworks should be developed that benefit from new field and genetic data characterising and measuring spatially and temporally dynamic transmission routes.

There is an increasing demand from NMCPs for pertinent and prompt mathematical modelling analyses to support their malaria elimination strategies. Established modelling groups have engaged in local capacity building. Also, malaria modelling research is being published by research groups from malaria-endemic countries [33,34,62,89,152–154], and this trend could be supported to the benefit of NMCPs.

Modelling to maintain zero

As noted above, when transmission becomes rare, models are increasingly challenged in informing policy and intervention choices; similarly, when there is no transmission, the evaluation of risk for the reintroduction of infection (vulnerability) and the risk of propagating local transmission given its reintroduction (receptivity) can present challenges to models designed to answer questions at high endemicity levels. A new class of highly heterogeneous, stochastic malaria models is being developed to inform the design of an elimination surveillance system.

Vulnerability (risk of introduction or reintroduction). Measuring vulnerability to malaria reintroduction requires pairing up-to-date maps of national and international parasite prevalence with human movement models. Both of these fields have advanced in recent years [33,34,117,155–160]. Human movement models, paired with travel survey and microcensus data, have improved their description of routine human movement (e.g., holiday season travel) [159,161]. Increasing use of mobile phones has enabled the tracking of human movement and permitted distribution advice on infection avoidance [159,162–164]. However, many national and international seasonal migrations remain difficult to predict, and their direct relationship to moving malaria infections requires additional investigation.

Receptivity (risk of transmission given introduction). In order to direct interventions, models must incorporate both the risk of importation and the risk for the reestablishment of local transmission [165–173]. The risk of malaria transmission reestablishment can be measured as a function of selected host, vector, and environmental data [156,170,171, 174]. For example, measures might include human use of insecticide-treated bed nets or indoor residual spraying, mosquito habitat suitability and its link to abundance, and climatic conditions (e.g., temperature, rainfall, and vegetation index measures) that support or accelerate vector and parasite development. If such data are collected widely enough, models can be validated using the occasional areas that do experience local transmission. Deciding which environmental and entomological data would be most valuable to collect could be iteratively informed by testing hypotheses based on longitudinal data from areas that have recently eliminated malaria, for example, Sri Lanka. The next step is to translate risk mapping into programmatic actions, such

as better allocation of human resources, and maintenance and targeting of vector control [50,175]. This will become increasingly important as more countries reach elimination.

Challenges

Residual transmission

Variable human and vector behaviours may enable sustained transmission in highly seasonal, heterogeneous environments, despite high intervention coverage [176]. The magnitude and importance of residual transmission in different settings require further field studies. In particular, human sociobehavioural data including human behaviour's relevance for compliance and entomological data investigating the contribution of outdoor transmission are needed to develop models testing novel strategies and tools [102].

Low transmission and incorporating heterogeneity

Models have mostly been used to examine sub-Saharan Africa high transmission contexts with *P. falciparum* and relevant vector species, though they may be parameterised across the full spectrum of transmission. When modelling an isolated homogeneous population, it can be difficult to sustain transmission much below the 1% parasite prevalence level (though the precise level depends on the model), with the model becoming unstable, leading to 'stochastic extinction', i.e., the extinction of parasites based on random effects within the model, an effect that is compounded with increasing heterogeneity [177]. This suggests that importation of infections and local heterogeneities in host, vector, and parasite dynamics and in health service delivery systems are likely to play an important role in sustaining malaria in low transmission settings [178].

As a country progresses to very low levels of malaria transmission, the spatial and temporal heterogeneity of transmission increases in importance. In these contexts of varying historical transmission intensity, intervention coverage, human movement, and access to health system resources, malaria will tend to persist in the most remote regions and the poorest and most vulnerable populations [179,180]. While this issue may not require new models per se, heterogeneity will need to be better captured as transmission declines. Spatial heterogeneity is probably least well developed, and the required level of spatial granularity and relevant metrics for answering specific questions in low transmission settings requires definition [181,182]. However, at some point heterogeneity will exceed the ability of models to establish granularity, and decision making will require local health system and entomological data.

Modelling malaria at borders

When malaria transmission is moderate to high and similar on both sides of a border, often little attention is paid to border areas for specific disease interventions; however, this changes when one nation may be markedly reducing transmission and the other is not. Border areas present particular difficulties for malaria control and elimination efforts [183–187]. The complexity of human movements for trade, business, and visiting family, sometimes including vulnerable populations [188], and the coordination of efforts between different political and organisational frameworks increase the complexity of malaria control [184]. Some of the issues relate to spatial and temporal heterogeneity and could possibly be addressed with greater data on human cross-border movement and parasite genetics [189–191]. However, human factors, such as local conflicts, poverty, and the disenfranchisement of particular ethnic groups, can be highly variable in time and place and are more challenging to incorporate into transmission models [192,193]. Alternative complementary approaches include mapping malaria risk, for

better targeting of resources, plus goal setting by modelling what could potentially be achieved with coordinated versus independent elimination campaigns [33,185,194,195]. Once the potential benefits are understood, the barriers to reaching these goals can be researched and the feasibility of overcoming them explored.

Iteration and validation

Finally, models directed at assessing combination interventions must embrace a process of iteration with field data. In particular, data are needed from low to near-zero transmission settings. Such data needs might include high-resolution geographic information on cases, frequency and location of associated secondary cases, travel history identifying infection sources, vector-associated data, climate, and environmental parameters [109]. The requirement for field data to validate models remains problematic, as field data on intervention efficacy and the diverse parameters noted above can be difficult to assemble. When developing models, validation requirements should be clearly defined and data should be feasible to obtain. Amidst these challenges, modellers then need to consider how to best contribute to and bear responsibility for the assembly of required field data. Although capacity building and integration of modellers into NMCPs may address this at a local scale, there is a need for innovative mechanisms to allow increased exchanges in malaria elimination research, to allow better access to field empirical data for modellers.

Conclusions

Given the ongoing social and economic impact of malaria-related mortality and morbidity and the inevitable resource constraints for national malaria programmes, identifying the most timely and most cost-effective path to malaria elimination is a priority. Box 3 presents a research and development agenda for combination interventions and modelling in malaria elimination. Modelling affords a feasible and practical means of investigating rational combinations of interventions and the most appropriate setting for their deployment. Nevertheless, without a substantive dataset from operations research, the construction of meaningful models

Box 3. Research and development agenda for combination interventions and modelling.

- Determine which combinations of interventions to use in which sequence and in response to which triggers throughout elimination
- Identify the circumstances in which time-limited elimination acceleration interventions, such as mass drug administration (MDA), are appropriate and what needs to be done to retain the gains in transmission reduction following their withdrawal
- Model the effect of parasite drug and vector insecticide resistance on combination interventions and how resistance might be avoided or contained
- Understand human immunity in areas where transmission has always been low and parasite diversity very low and modelling the effect of changes in human immunity as transmission declines
- Identify which additional data would be most useful for validating or changing model predictions in order to drive iterative development and decision making

Surveillance as an intervention

- Model the target product profile of an elimination-specific surveillance system
- Determine the threshold at which reactive case strategies become feasible

Strategic modelling

- Estimate the long-term costs of elimination in different settings and with different intervention packages
- Assess the potential duration of an elimination campaign in various settings to help define the investment case and financing needs for elimination
- Estimate the maximal impact of currently available tools on elimination in various settings
- Determine the counterfactual to elimination, i.e., the effect of continuing current interventions in various settings
- Support capacity building of modellers embedded in National Malaria Control Programmes (NMCPs)

Modelling to maintain zero

- Investigate how vulnerability and receptivity measures can be translated into specific programme actions

Addressing transmission

- Apply models to low transmission settings, incorporating all relevant parasites/vectors
- Investigate the importance of residual transmission in different settings and what new strategies or novel tools are needed to overcome it

Incorporating heterogeneity

- Determine the relevance of spatial and temporal heterogeneity in transmission in different settings
- Investigate how much heterogeneity in transmission needs to be captured by models to make predictions in elimination settings

Iteration and validation

- Determine which measures of transmission or other metrics are most appropriate for guiding programmatic decisions in low transmission to maintaining-zero settings
- Define which new data need to be collected from low transmission to maintaining-zero settings in order to increase confidence in model predictions

is not possible. Models must also be continuously validated against field data, through programmatic experience and against clinical trials, with measures and outcomes data relevant to the transmission setting identified and collected for use in further model refinement. This is

especially the case as we increasingly encounter transmission settings that are shrinking in size and number and becoming more focal and heterogeneous and for which there are fewer field data. Thus, there is a codependency between modelling and field data, and the quality of both must be assured for findings to be valid and impactful. Since malERA 2011, there has been significant progress in aligning modelling with programmatic requirements and more effective communication with policy makers. This ongoing dialogue will ultimately determine the relevance of modelling to policy decision and its contribution towards achieving and maintaining malaria elimination.

Acknowledgments

The Malaria Eradication Research Agenda (malERA) Refresh Consultative Panel on Combination Interventions and Modelling was chaired by Richard Steketee, PATH Malaria Control and Elimination Partnership in Africa, Atlanta, Georgia, US, and cochaired by Simon I. Hay (University of Washington and University of Oxford). The paper was written based on consultations during a malERA meeting held in Basel, Switzerland on 30 September–1 October 2015. A systematic literature search was performed by Vittoria Lutje. Panel members reviewed several iterations of the manuscript to finalise it. Azra Ghani, Imperial College London, UK, commented on the manuscript. Fig 2 graphics were designed by Rachel Papernick, and this was funded by the Malaria Eradication Scientific Alliance (MESA). Naomi Richardson of Magenta Communications Limited provided editorial and writing support and was funded by MESA.

Members of the writing group met ICMJE criteria for authorship and were as follows:

Oliver Brady, London School of Hygiene and Tropical Medicine, UK; Timothy Finn, Tulane University, New Orleans, Louisiana, US; Simon I. Hay, Institute for Health Metrics and Evaluation, Seattle, Washington, US, and University of Oxford, Oxford, UK; Regina Rabinovich, ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clínic—Universitat de Barcelona, Barcelona, Spain, and Harvard T. H. Chan School of Public Health, Boston, Massachusetts, US; Richard Steketee (chair), PATH Malaria Control and Elimination Partnership in Africa, Atlanta, Georgia, US.

malERA Refresh: Combination Interventions and Modelling Panel members:

Oliver Brady, London School of Hygiene and Tropical Medicine, London, UK; Keith Carter, Pan American Health Organization, Washington, DC, US; Michelle Chang, Centers for Disease Control and Prevention, Atlanta, Georgia, US; Richard E. Cibulskis, Global Malaria Programme, World Health Organization; Phillip Eckhoff, Institute for Disease Modelling, Seattle, Washington, US; Thomas P. Eisele, Center for Applied Malaria Research and Evaluation, Tulane School of Public Health and Tropical Medicine, New Orleans, Louisiana, US; Iqbal Elyazar, Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia; Timothy Finn, Tulane University, New Orleans, Louisiana, US; Qi Gao, Jiangsu Institute of Parasitic Diseases, Meiyuan, Wuxi, People's Republic of China; Peter Gething, Oxford Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, UK; Dissanayake Gunawardena, US Agency for International Development (USAID), Phnom Penh, Cambodia; Busiku Hamainza, National Malaria Control Center, Lusaka, Zambia; Simon I. Hay, Institute for Health Metrics and Evaluation, Seattle, Washington, US, and Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, UK; Patrick Kachur, Centers for Disease Control and Prevention, Atlanta, Georgia, US; Rodrigo Marin, Ministerio de Salud, Costa Rica; Abdisalan M. Noor, KEMRI Wellcome Trust research Programme, Nairobi, Kenya; Emelda Okiro, Bill & Melinda Gates Foundation, Seattle, Washington, US; Regina Rabinovich, ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clínic—Universitat de Barcelona, Barcelona, Spain; Kathleen

Rankin, Bill & Melinda Gates Foundation, Seattle, Washington, US; Francisco Saute, Centro de Investigação em saúde da Manhiça, Mozambique; Thomas Smith, Swiss Tropical and Public Health Institute, Basel, Switzerland; David Smith, Institute for Health Metrics and Evaluation, Seattle, Washington, US; Richard Steketee, PATH Malaria Control and Elimination Partnership in Africa, Atlanta, Georgia, US; Erin Stuckey, Bill & Melinda Gates Foundation, Seattle, Washington, US; Chigozie J. Uneke, Ebonyi State University, Abakaliki, Nigeria; Patrick Walker, Imperial College London, London, UK; Lisa White, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

RC is a member of staff at WHO. The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the World Health Organization.

References

1. malERA Consultative Group on Modeling. A research agenda for malaria eradication: modeling. PLoS Med. 2011; 8(1):e1000403. https://doi.org/10.1371/journal.pmed.1000403 PMID: 21283605
2. The malERA Refresh Consultative Panel on Tools for Malaria Elimination. malERA: An updated research agenda for diagnostics, drugs, vaccines, and vector control in malaria elimination and eradication PLoS Med. 2017; 14(11):e1002455. https://doi.org/10.1371/journal.pmed.1002455
3. Rabinovich RN, Drakeley C, Djimde AA, Hall BF, Hay SI, Hemingway J, et al. malERA: An updated research agenda for malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002456. https://doi.org/10.1371/journal.pmed.1002456
4. World Health Organization. World malaria report 2015 Geneva: WHO; 2015. Available from: http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/.
5. World Health Organization. Global technical strategy for malaria 2016–2030 Geneva: WHO; 2015. Available from: http://apps.who.int/iris/bitstream/10665/176712/1/9789241564991_eng.pdf
6. Gething PW, Battle KE, Bhatt S, Smith DL, Eisele TP, Cibulskis RE, et al. Declining malaria in Africa: improving the measurement of progress. Malar J. 2014; 13:39. https://doi.org/10.1186/1475-2875-13-39 PMID: 24479555
7. Battle KE, Guerra CA, Golding N, Duda KA, Cameron E, Howes RE, et al. Global database of matched *Plasmodium falciparum* and *P. vivax* incidence and prevalence records from 1985–2013. Sci Data. 2015; 2:150012. https://doi.org/10.1038/sdata.2015.12 PMID: 26306203
8. Bejon P, White MT, Olotu A, Bojang K, Lusingu JP, Salim N, et al. Efficacy of RTS,S malaria vaccines: individual-participant pooled analysis of phase 2 data. Lancet Infect Dis. 2013; 13(4):319–27. https://doi.org/10.1016/S1473-3099(13)70005-7 PMID: 23454164
9. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015; 526(7572):207–11. https://doi.org/10.1038/nature15535 PMID: 26375008
10. Blagborough AM, Churcher TS, Upton LM, Ghani AC, Gething PW, Sinden RE. Transmission-blocking interventions eliminate malaria from laboratory populations. Nat Commun. 2013; 4:1812. https://doi.org/10.1038/ncomms2840 PMID: 23652000
11. Cameron E, Battle KE, Bhatt S, Weiss DJ, Bisanzio D, Mappin B, et al. Defining the relationship between infection prevalence and clinical incidence of *Plasmodium falciparum* malaria. Nat Commun. 2015; 6:8170. https://doi.org/10.1038/ncomms9170 PMID: 26348689
12. Moyes CL, Temperley WH, Henry AJ, Burgert CR, Hay SI. Providing open access data online to advance malaria research and control. Malar J. 2013; 12:161. https://doi.org/10.1186/1475-2875-12-161 PMID: 23680401
13. Wu L, van den Hoogen LL, Slater H, Walker PG, Ghani AC, Drakeley CJ, et al. Comparison of diagnostics for the detection of asymptomatic *Plasmodium falciparum* infections to inform control and elimination strategies. Nature. 2015; 528(7580):S86–93. https://doi.org/10.1038/nature16039 PMID: 26633770
14. World Health Organization. From malaria control to malaria elimination: a manual for elimination scenario planning Geneva: WHO; 2014. Available from: http://apps.who.int/iris/bitstream/10665/112485/1/9789241507028_eng.pdf
15. Gerardin J, Ouedraogo AL, McCarthy KA, Eckhoff PA, Wenger EA. Characterization of the infectious reservoir of malaria with an agent-based model calibrated to age-stratified parasite densities

and infectiousness. Malar J. 2015; 14:231. https://doi.org/10.1186/s12936-015-0751-y PMID: 26037226

16. Gerardin J, Bever CA, Hamainza B, Miller JM, Eckhoff PA, Wenger EA. Optimal population-level infection detection strategies for malaria control and elimination in a spatial model of malaria transmission. PLoS Comput Biol. 2016; 12(1):e1004707. https://doi.org/10.1371/journal.pcbi.1004707 PMID: 26764905

17. Marshall JM, White MT, Ghani AC, Schlein Y, Muller GC, Beier JC. Quantifying the mosquito's sweet tooth: modelling the effectiveness of attractive toxic sugar baits (ATSB) for malaria vector control. Malar J. 2013; 12:291. https://doi.org/10.1186/1475-2875-12-291 PMID: 23968494

18. Okell LC, Griffin JT, Kleinschmidt I, Hollingsworth TD, Churcher TS, White MJ, et al. The potential contribution of mass treatment to the control of *Plasmodium falciparum* malaria. PLoS ONE. 2011; 6(5): e20179. https://doi.org/10.1371/journal.pone.0020179 PMID: 21629651

19. Slater HC, Walker PG, Bousema T, Okell LC, Ghani AC. The potential impact of adding ivermectin to a mass treatment intervention to reduce malaria transmission: a modelling study. J Infect Dis. 2014; 210(12):1972–80. https://doi.org/10.1093/infdis/jiu351 PMID: 24951826

20. Wenger EA, Eckhoff PA. A mathematical model of the impact of present and future malaria vaccines. Malar J. 2013; 12:126. https://doi.org/10.1186/1475-2875-12-126 PMID: 23587051

21. Brady OJ, Godfray HC, Tatem AJ, Gething PW, Cohen JM, McKenzie FE, et al. Vectorial capacity and vector control: reconsidering sensitivity to parameters for malaria elimination. Trans R Soc Trop Med Hyg. 2016; 110(2):107–17. https://doi.org/10.1093/trstmh/trv113 PMID: 26822603

22. Eckhoff P. Mathematical models of within-host and transmission dynamics to determine effects of malaria interventions in a variety of transmission settings. Am J Trop Med Hyg. 2013; 88(5):817–27. https://doi.org/10.4269/ajtmh.12-0007 PMID: 23589530

23. Griffin JT, Bhatt S, Sinka ME, Gething PW, Lynch M, Patouillard E, et al. Potential for reduction of burden and local elimination of malaria by reducing *Plasmodium falciparum* malaria transmission: a mathematical modelling study. Lancet Infect Dis. 2016; 16(4):465–72. https://doi.org/10.1016/S1473-3099(15)00423-5 PMID: 26809816

24. Lutambi AM, Chitnis N, Briet OJ, Smith TA, Penny MA. Clustering of vector control interventions has important consequences for their effectiveness: a modelling study. PLoS ONE. 2014; 9(5):e97065. https://doi.org/10.1371/journal.pone.0097065 PMID: 24823656

25. Slater HC, Ross A, Ouedraogo AL, White LJ, Nguon C, Walker PG, et al. Assessing the impact of next-generation rapid diagnostic tests on *Plasmodium falciparum* malaria elimination strategies. Nature. 2015; 528(7580):S94–101. https://doi.org/10.1038/nature16040 PMID: 26633771

26. Slater HC, Griffin JT, Ghani AC, Okell LC. Assessing the potential impact of artemisinin and partner drug resistance in sub-Saharan Africa. Malar J. 2016; 15(1):10.

27. White MT, Griffin JT, Churcher TS, Ferguson NM, Basanez MG, Ghani AC. Modelling the impact of vector control interventions on *Anopheles gambiae* population dynamics. Parasit Vectors. 2011; 4:153. https://doi.org/10.1186/1756-3305-4-153 PMID: 21798055

28. Crowell V, Briet OJ, Hardy D, Chitnis N, Maire N, Di Pasquale A, et al. Modelling the cost-effectiveness of mass screening and treatment for reducing *Plasmodium falciparum* malaria burden. Malar J. 2013; 12:4. https://doi.org/10.1186/1475-2875-12-4 PMID: 23286228

29. MESA Track: Malaria Eradication Scientific Alliance; [updated 15 March 2016. Available from: http://www.malariaeradication.org/mesa-track

30. Maude RJ, Nguon C, Dondorp AM, White LJ, White NJ. The diminishing returns of atovaquone-proguanil for elimination of *Plasmodium falciparum* malaria: modelling mass drug administration and treatment. Malar J. 2014; 13:380. https://doi.org/10.1186/1475-2875-13-380 PMID: 25249272

31. Maude RJ, Pontavornpinyo W, Saralamba S, Aguas R, Yeung S, Dondorp AM, et al. The last man standing is the most resistant: eliminating artemisinin-resistant malaria in Cambodia. Malaria Journal. 2009; 8(1):31.

32. Maude RJ, Socheat D, Nguon C, Saroth P, Dara P, Li G, et al. Optimising strategies for *Plasmodium falciparum* malaria elimination in Cambodia: primaquine, mass drug administration and artemisinin resistance. PLoS ONE. 2012; 7(5):e37166. https://doi.org/10.1371/journal.pone.0037166 PMID: 22662135

33. Silal SP, Little F, Barnes KI, White LJ. Predicting the impact of border control on malaria transmission: a simulated focal screen and treat campaign. Malar J. 2015; 14:268. https://doi.org/10.1186/s12936-015-0776-2 PMID: 26164675

34. Silal SP, Little F, Barnes KI, White LJ. Hitting a moving target: A model for malaria elimination in the presence of population movement. PLoS ONE. 2015; 10(12):e0144990. https://doi.org/10.1371/journal.pone.0144990 PMID: 26689547

35. White LJ, Maude RJ, Pongtavornpinyo W, Saralamba S, Aguas R, Van Effelterre T, et al. The role of simple mathematical models in malaria elimination strategy design. Malar J. 2009; 8:212. https://doi.org/10.1186/1475-2875-8-212 PMID: 19747403

36. Penny MA, Verity R, Bever CA, Sauboin C, Galactionova K, Flasche S, et al. Public health impact and cost-effectiveness of the RTS,S/AS01 malaria vaccine: a systematic comparison of predictions from four mathematical models. Lancet. 2016; 387(10016):367–75. https://doi.org/10.1016/S0140-6736(15)00725-4 PMID: 26549466

37. Bhatt S, Weiss DJ, Mappin B, Dalrymple U, Cameron E, Bisanzio D, et al. Coverage and system efficiencies of insecticide-treated nets in Africa from 2000 to 2017. Elife. 2015; 4:e09672. https://doi.org/10.7554/eLife.09672 PMID: 26714109

38. Briet O, Hardy D, Smith TA. Importance of factors determining the effective lifetime of a mass, long-lasting, insecticidal net distribution: a sensitivity analysis. Malaria Journal. 2012; 11(1):20.

39. Chitnis N, Schapira A, Smith T, Steketee R. Comparing the effectiveness of malaria vector-control interventions through a mathematical model. Am J Trop Med Hyg. 2010; 83(2):230–40. https://doi.org/10.4269/ajtmh.2010.09-0179 PMID: 20682861

40. Griffin JT. The interaction between seasonality and pulsed interventions against malaria in their effects on the reproduction number. PLoS Comput Biol. 2015; 11(1):e1004057. https://doi.org/10.1371/journal.pcbi.1004057 PMID: 25590612

41. Griffin JT, Hollingsworth TD, Okell LC, Churcher TS, White M, Hinsley W, et al. Reducing *Plasmodium falciparum* malaria transmission in Africa: a model-based evaluation of intervention strategies. PLoS Med. 2010; 7(8):e1000324. https://doi.org/10.1371/journal.pmed.1000324 PMID: 20711482

42. Walker PG, Griffin JT, Ferguson NM, Ghani AC. Estimating the most efficient allocation of interventions to achieve reductions in *Plasmodium falciparum* malaria burden and transmission in Africa: a modelling study. Lancet Glob Health. 2016; 4(7):e474–84. https://doi.org/10.1016/S2214-109X(16)30073-0 PMID: 27269393

43. Briet OJ, Penny MA. Repeated mass distributions and continuous distribution of long-lasting insecticidal nets: modelling sustainability of health benefits from mosquito nets, depending on case management. Malar J. 2013; 12:401. https://doi.org/10.1186/1475-2875-12-401 PMID: 24200296

44. Brady OJ, Godfray HC, Tatem AJ, Gething PW, Cohen JM, McKenzie FE, et al. Adult vector control, mosquito ecology and malaria transmission. Int Health. 2015; 7(2):121–9. https://doi.org/10.1093/inthealth/ihv010 PMID: 25733562

45. Eckhoff PA. A malaria transmission-directed model of mosquito life cycle and ecology. Malar J. 2011; 10:303. https://doi.org/10.1186/1475-2875-10-303 PMID: 21999664

46. Killeen GF, Seyoum A, Gimnig JE, Stevenson JC, Drakeley CJ, Chitnis N. Made-to-measure malaria vector control strategies: rational design based on insecticide properties and coverage of blood resources for mosquitoes. Malaria Journal. 2014; 13:146. https://doi.org/10.1186/1475-2875-13-146 PMID: 24739261

47. Okell LC, Drakeley CJ, Bousema T, Whitty CJ, Ghani AC. Modelling the impact of artemisinin combination therapy and long-acting treatments on malaria transmission intensity. PLoS Med. 2008; 5(11):e226. https://doi.org/10.1371/journal.pmed.0050226 PMID: 19067479

48. Ross A, Maire N, Sicuri E, Smith T, Conteh L. Determinants of the cost-effectiveness of intermittent preventive treatment for malaria in infants and children. PLoS ONE. 2011; 6(4):e18391. https://doi.org/10.1371/journal.pone.0018391 PMID: 21490967

49. Ross A, Penny M, Maire N, Studer A, Carneiro I, Schellenberg D, et al. Modelling the epidemiological impact of intermittent preventive treatment against malaria in infants. PLoS ONE. 2008; 3(7):e2661. https://doi.org/10.1371/journal.pone.0002661 PMID: 18628828

50. Crowell V, Hardy D, Briet O, Chitnis N, Maire N, Smith T. Can we depend on case management to prevent re-establishment of *P. falciparum* malaria, after local interruption of transmission? Epidemics. 2012; 4(1):1–8. https://doi.org/10.1016/j.epidem.2011.10.003 PMID: 22325009

51. Smith DL, Cohen JM, Chiyaka C, Johnston G, Gething PW, Gosling R, et al. A sticky situation: the unexpected stability of malaria elimination. Philos Trans R Soc Lond B Biol Sci. 2013; 368(1623):20120145. https://doi.org/10.1098/rstb.2012.0145 PMID: 23798693

52. Cairns M, Ghani A, Okell L, Gosling R, Carneiro I, Anto F, et al. Modelling the protective efficacy of alternative delivery schedules for intermittent preventive treatment of malaria in infants and children. PLoS ONE. 2011; 6(4):e18947. https://doi.org/10.1371/journal.pone.0018947 PMID: 21533088

53. Okell L, Slater H, Ghani A, P P-R, Smith TA, Chitnis N, et al. Consensus modelling evidence to support the design of mass drug administration programmes Geneva: WHO; 2015 [updated 14th March 2016. Available from: http://www.who.int/malaria/mpac/mpac-sept2015-consensus-modelling-mda.pdf

54. Stuckey EM, Miller JM, Littrell M, Chitnis N, Steketee R. Operational strategies of anti-malarial drug campaigns for malaria elimination in Zambia's southern province: a simulation study. Malar J. 2016; 15(1):148.

55. Stuckey EM, Stevenson J, Galactionova K, Baidjoe AY, Bousema T, Odongo W, et al. Modeling the cost effectiveness of malaria control interventions in the highlands of western Kenya. PLoS ONE. 2014; 9(10):e107700. https://doi.org/10.1371/journal.pone.0107700 PMID: 25290939

56. Eckhoff PA, Bever CA, Gerardin J, Wenger EA. Fun with maths: exploring implications of mathematical models for malaria eradication. Malar J. 2014; 13:486. https://doi.org/10.1186/1475-2875-13-486 PMID: 25495423

57. Gerardin J, Eckhoff P, Wenger EA. Mass campaigns with antimalarial drugs: a modelling comparison of artemether-lumefantrine and DHA-piperaquine with and without primaquine as tools for malaria control and elimination. BMC Infect Dis. 2015; 15:144. https://doi.org/10.1186/s12879-015-0887-y PMID: 25887935

58. Global Health Group at the University of California, Malaria Centre at the London School of Hygiene & Tropical Medicine. Single low-dose primaquine to interrupt P. falciparum transmission in Africa: a roadmap update and meeting summary 2014. Available from: http://www.shrinkingthemalariamap.org/sites/www.shrinkingthemalariamap.org/files/content/resource/attachment/London%20PQ%202016%20summary%20final%20for%20internet%209-12-16%20%25282%2529.pdf

59. Rao VB, Schellenberg D, Ghani AC. The potential impact of improving appropriate treatment for fever on malaria and non-malarial febrile illness management in under-5s: a decision-tree modelling approach. PLoS ONE. 2013; 8(7):e69654. https://doi.org/10.1371/journal.pone.0069654 PMID: 23922770

60. Drake TL, Kyaw SS, Kyaw MP, Smithuis FM, Day NP, White LJ, et al. Cost effectiveness and resource allocation of *Plasmodium falciparum* malaria control in Myanmar: a modelling analysis of bed nets and community health workers. Malar J. 2015; 14:376. https://doi.org/10.1186/s12936-015-0886-x PMID: 26416075

61. Drake TL, Devine A, Yeung S, Day NP, White LJ, Lubell Y. Dynamic transmission economic evaluation of infectious disease interventions in low- and middle-income countries: A systematic literature review. Health Econ. 2016; 25 Suppl 1:124–39.

62. Kyaw SS, Drake T, Thi A, Kyaw MP, Hlaing T, Smithuis FM, et al. Malaria community health workers in Myanmar: a cost analysis. Malar J. 2016; 15(1):41.

63. Okell LC, Cairns M, Griffin JT, Ferguson NM, Tarning J, Jagoe G, et al. Contrasting benefits of different artemisinin combination therapies as first-line malaria treatments using model-based cost-effectiveness analysis. Nat Commun. 2014; 5:5606. https://doi.org/10.1038/ncomms6606 PMID: 25425081

64. Hodel EM, Kay K, Hayes DJ, Terlouw DJ, Hastings IM. Optimizing the programmatic deployment of the anti-malarials artemether-lumefantrine and dihydroartemisinin-piperaquine using pharmacological modelling. Malar J. 2014; 13:138. https://doi.org/10.1186/1475-2875-13-138 PMID: 24708571

65. Fernando SD, Navaratne CJ, Galappaththy GN, Abeyasinghe RR, Silva N, Wickermasinghe R. The importance of accuracy in diagnosis of positive malaria cases in a country progressing towards malaria elimination. J Glob Infect Dis. 2013; 5(4):127–30. https://doi.org/10.4103/0974-777X.121992 PMID: 24672172

66. Mavandadi S, Feng S, Yu F, Dimitrov S, Nielsen-Saines K, Prescott WR, et al. A mathematical framework for combining decisions of multiple experts toward accurate and remote diagnosis of malaria using tele-microscopy. PLoS ONE. 2012; 7(10):e46192. https://doi.org/10.1371/journal.pone.0046192 PMID: 23071544

67. McCarthy KA, Wenger EA, Huynh GH, Eckhoff PA. Calibration of an intrahost malaria model and parameter ensemble evaluation of a pre-erythrocytic vaccine. Malar J. 2015; 14:6. https://doi.org/10.1186/1475-2875-14-6 PMID: 25563798

68. Smith T, Ross A, Maire N, Chitnis N, Studer A, Hardy D, et al. Ensemble modeling of the likely public health impact of a pre-erythrocytic malaria vaccine. PLoS Med. 2012; 9(1):e1001157. https://doi.org/10.1371/journal.pmed.1001157 PMID: 22272189

69. White MT, Griffin JT, Riley EM, Drakeley CJ, Moorman AM, Sumba PO, et al. Efficacy model for antibody-mediated pre-erythrocytic malaria vaccines. Proc Biol Sci. 2011; 278(1710):1298–305. https://doi.org/10.1098/rspb.2010.1697 PMID: 20943696

70. Diaz H, Ramirez AA, Olarte A, Clavijo C. A model for the control of malaria using genetically modified vectors. J Theor Biol. 2011; 276(1):57–66. https://doi.org/10.1016/j.jtbi.2011.01.053 PMID: 21300074

71. Li J. Discrete-time models with mosquitoes carrying genetically-modified bacteria. Math Biosci. 2012; 240(1):35–44. https://doi.org/10.1016/j.mbs.2012.05.012 PMID: 22771952

72. Legros M, Xu C, Okamoto K, Scott TW, Morrison AC, Lloyd AL, et al. Assessing the feasibility of controlling *Aedes aegypti* with transgenic methods: a model-based evaluation. PLoS ONE. 2012; 7(12): e52235. https://doi.org/10.1371/journal.pone.0052235 PMID: 23284949

73. White MT, Smith DL. Synergism from combinations of infection-blocking malaria vaccines. Malar J. 2013; 12:280. https://doi.org/10.1186/1475-2875-12-280 PMID: 23927630

74. WHO Malaria Policy Advisory Committee Secretariat. Malaria Policy Advisory Committee to the WHO: conclusions and recommendations of eighth biannual meeting (September 2015). Malar J. 2016; 15 (1):117.

75. Guyant P, Corbel V, Guerin PJ, Lautissier A, Nosten F, Boyer S, et al. Past and new challenges for malaria control and elimination: the role of operational research for innovation in designing interventions. Malar J. 2015; 14:279. https://doi.org/10.1186/s12936-015-0802-4 PMID: 26185098

76. Hsiang MS, Hwang J, Tao AR, Liu Y, Bennett A, Shanks GD, et al. Mass drug administration for the control and elimination of *Plasmodium vivax* malaria: an ecological study from Jiangsu province, China. Malar J. 2013; 12:383. https://doi.org/10.1186/1475-2875-12-383 PMID: 24175930

77. Kiware SS, Chitnis N, Devine GJ, Moore SJ, Majambere S, Killeen GF. Biologically meaningful coverage indicators for eliminating malaria transmission. Biol Lett. 2012; 8(5):874–7. https://doi.org/10.1098/rsbl.2012.0352 PMID: 22647930

78. Poirot E, Skarbinski J, Sinclair D, Kachur SP, Slutsker L, Hwang J. Mass drug administration for malaria. Cochrane Database Syst Rev. 2013; 12:CD008846.

79. Rosas-Aguirre A, Erhart A, Llanos-Cuentas A, Branch O, Berkvens D, Abatih E, et al. Modelling the potential of focal screening and treatment as elimination strategy for *Plasmodium falciparum* malaria in the Peruvian Amazon Region. Parasit Vectors. 2015; 8:261. https://doi.org/10.1186/s13071-015-0868-4 PMID: 25948081

80. Eisele TP, Silumbe K, Finn T, Chalwe V, Kamuliwo M, Hamainza B, et al. Assessing the effectiveness of household-level focal mass drug administration and community-wide mass drug administration for reducing malaria parasite infection prevalence and incidence in Southern Province, Zambia: study protocol for a community randomized controlled trial. Trials. 2015; 16:347. https://doi.org/10.1186/s13063-015-0862-3 PMID: 26268804

81. Alegana VA, Wright JA, Nahzat SM, Butt W, Sediqi AW, Habib N, et al. Modelling the incidence of *Plasmodium vivax* and *Plasmodium falciparum* malaria in Afghanistan 2006–2009. PLoS ONE. 2014; 9(7).

82. Chen Z, Shi L, Zhou XN, Xia ZG, Bergquist R, Jiang QW. Elimination of malaria due to *Plasmodium vivax* in central part of the People's Republic of China: analysis and prediction based on modelling. Geospatial Health. 2014; 9(1):169–77. https://doi.org/10.4081/gh.2014.14 PMID: 25545934

83. Galappaththy Gawrie NL, Tharyan P, Kirubakaran R. Primaquine for preventing relapse in people with *Plasmodium vivax* malaria treated with chloroquine. Cochrane Database of Systematic Reviews [Internet]. 2013; (10). Available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD004389.pub3/abstract

84. Lindsay SW, Hole DG, Hutchinson RA, Richards SA, Willis SG. Assessing the future threat from vivax malaria in the United Kingdom using two markedly different modelling approaches. Malaria Journal. 2010; 9.

85. Roy M, Bouma MJ, Ionides EL, Dhiman RC, Pascual M. The potential elimination of *Plasmodium vivax* malaria by relapse treatment: insights from a transmission model and surveillance data from NW India. PLoS Negl Trop Dis. 2013; 7(1):e1979. https://doi.org/10.1371/journal.pntd.0001979 PMID: 23326611

86. Shi B, Liu J, Zhou XN, Yang GJ. Inferring *Plasmodium vivax* transmission networks from tempo-spatial surveillance data. PLoS Negl Trop Dis. 2014; 8(2):e2682. https://doi.org/10.1371/journal.pntd.0002682 PMID: 24516684

87. Robinson LJ, Wampfler R, Betuela I, Karl S, White MT, Li Wai Suen CS, et al. Strategies for understanding and reducing the *Plasmodium vivax* and *Plasmodium ovale* hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model. PLoS Med. 2015; 12(10):e1001891. https://doi.org/10.1371/journal.pmed.1001891 PMID: 26505753

88. White MT, Karl S, Battle KE, Hay SI, Mueller I, Ghani AC. Modelling the contribution of the hypnozoite reservoir to *Plasmodium vivax* transmission. Elife. 2014; 3:e04692.

89. Abdullahi MB, Hasan YA, Abdullah FA. Optimal control of *Plasmodium knowlesi* malaria in human and macaques. British Journal of Mathematics & Computer Science. 2014; 4(2):271–87.

90. Abdullahi MB, Hasan YA, Abdullah FA. Optimal strategy for controlling the spread of *Plasmodium knowlesi* malaria: Treatment and culling. AIP Conference Proceedings. 2015; 1660(1):050005.

91. Adekunle AI, Pinkevych M, McGready R, Luxemburger C, White LJ, Nosten F, et al. Modeling the dynamics of *Plasmodium vivax* infection and hypnozoite reactivation *in vivo*. PLoS Negl Trop Dis. 2015; 9(3):e0003595. https://doi.org/10.1371/journal.pntd.0003595 PMID: 25780913

92. Qi Q, Guerra CA, Moyes CL, Elyazar IR, Gething PW, Hay SI, et al. The effects of urbanization on global *Plasmodium vivax* malaria transmission. Malar J. 2012; 11:403. https://doi.org/10.1186/1475-2875-11-403 PMID: 23217010

93. White MT, Shirreff G, Karl S, Ghani AC, Mueller I. Variation in relapse frequency and the transmission potential of *Plasmodium vivax* malaria. Proc Biol Sci. 2016; 283(1827).

94. Ross A, Koepfli C, Schoepflin S, Timinao L, Siba P, Smith T, et al. The incidence and differential seasonal patterns of *Plasmodium vivax* primary infections and relapses in a cohort of children in Papua New Guinea. PLoS Negl Trop Dis. 2016; 10(5):e0004582. https://doi.org/10.1371/journal.pntd.0004582 PMID: 27144482

95. Imai N, White MT, Ghani AC, Drakeley CJ. Transmission and control of *Plasmodium knowlesi*: a mathematical modelling study. PLoS Negl Trop Dis. 2014; 8(7):e2978. https://doi.org/10.1371/journal.pntd.0002978 PMID: 25058400

96. Aguas R, Ferreira MU, Gomes MG. Modeling the effects of relapse in the transmission dynamics of malaria parasites. J Parasitol Res. 2012; 2012:921715. https://doi.org/10.1155/2012/921715 PMID: 21966590

97. Pampana E. Textbook of Malaria Eradication. Oxford: Oxford University Press; 1969.

98. Ashton RA, Kefyalew T, Rand A, Sime H, Assefa A, Mekasha A, et al. Geostatistical modeling of malaria endemicity using serological indicators of exposure collected through school surveys. American Journal of Tropical Medicine and Hygiene. 2015; 93 (1):168–77. https://doi.org/10.4269/ajtmh.14-0620 PMID: 25962770

99. Bousema T, Drakeley C, Gesase S, Hashim R, Magesa S, Mosha F, et al. Identification of hot spots of malaria transmission for targeted malaria control. J Infect Dis. 2010; 201(11):1764–74. https://doi.org/10.1086/652456 PMID: 20415536

100. Mosha JF, Sturrock HJ, Greenwood B, Sutherland CJ, Gadalla NB, Atwal S, et al. Hot spot or not: a comparison of spatial statistical methods to predict prospective malaria infections. Malar J. 2014; 13:53. https://doi.org/10.1186/1475-2875-13-53 PMID: 24517452

101. Pothin E, Ferguson NM, Drakeley CJ, Ghani AC. Estimating malaria transmission intensity from *Plasmodium falciparum* serological data using antibody density models. Malar J. 2016; 15(1):79.

102. malERA Refresh Consultative Panel on Characterising the Reservoir and Measuring Transmission. malERA: An updated research agenda for characterising the reservoir and measuring transmission in malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002452. https://doi.org/10.1371/journal.pmed.1002452

103. Hustedt J, Canavati SE, Rang C, Ashton RA, Khim N, Berne L, et al. Reactive case-detection of malaria in Pailin Province, Western Cambodia: lessons from a year-long evaluation in a pre-elimination setting. Malar J. 2016; 15(1):132.

104. Larsen DA, Chisha Z, Winters B, Mwanza M, Kamuliwo M, Mbwili C, et al. Malaria surveillance in low-transmission areas of Zambia using reactive case detection. Malar J. 2015; 14(1):465.

105. Littrell M, Sow GD, Ngom A, Ba M, Mboup BM, Dieye Y, et al. Case investigation and reactive case detection for malaria elimination in northern Senegal. Malar J. 2013; 12:331. https://doi.org/10.1186/1475-2875-12-331 PMID: 24044506

106. van Eijk AM, Ramanathapuram L, Sutton PL, Kanagaraj D, Sri Lakshmi Priya G, Ravishankaran S, et al. What is the value of reactive case detection in malaria control? A case-study in India and a systematic review. Malar J. 2016; 15(1):67.

107. Zhou SS, Zhang SS, Zhang L, Rietveld AE, Ramsay AR, Zachariah R, et al. China's 1-3-7 surveillance and response strategy for malaria elimination: Is case reporting, investigation and foci response happening according to plan? Infect Dis Poverty. 2015; 4:55. https://doi.org/10.1186/s40249-015-0089-2 PMID: 26654106

108. Cohen JM, Smith DL, Cotter C, Ward A, Yamey G, Sabot OJ, et al. Malaria resurgence: a systematic review and assessment of its causes. Malar J. 2012; 11:122. https://doi.org/10.1186/1475-2875-11-122 PMID: 22531245

109. Reiner RC, Le Menach A, Kunene S, Ntshalintshali N, Hsiang MS, Perkins TA, et al. Mapping residual transmission for malaria elimination. Elife. 2015; 4:e09520. https://doi.org/10.7554/eLife.09520 PMID: 26714110

110. Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, et al. Averting a malaria disaster: will insecticide resistance derail malaria control? Lancet. 2016; 387:1785–8. https://doi.org/10.1016/S0140-6736(15)00417-1 PMID: 26880124

111. World Health Organization. Emergency response to artemisinin resistance in the Greater Mekong subregion. Regional framework for action 2013–2015 Geneva: WHO; 2013. Available from: http://www.who.int/malaria/publications/atoz/9789241505321/en/.

112. malERA Refresh Consultative Panel on Insecticide and Drug Resistance. malERA: An updated research agenda for insecticide and drug resistance in malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002450. https://doi.org/10.1371/journal.pmed.1002450

113. Briet OJ, Penny MA, Hardy D, Awolola TS, Van Bortel W, Corbel V, et al. Effects of pyrethroid resistance on the cost effectiveness of a mass distribution of long-lasting insecticidal nets: a modelling study. Malar J. 2013; 12:77. https://doi.org/10.1186/1475-2875-12-77 PMID: 23442575

114. Killeen GF, Chitnis N. Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: a mathematical modelling analysis. Malar J. 2014; 13:97. https://doi.org/10.1186/1475-2875-13-97 PMID: 24629066

115. Lubell Y, Dondorp A, Guerin PJ, Drake T, Meek S, Ashley E, et al. Artemisinin resistance—modelling the potential human and economic costs. Malar J. 2014; 13:452. https://doi.org/10.1186/1475-2875-13-452 PMID: 25418416

116. Griffin JT, Cairns M, Ghani AC, Roper C, Schellenberg D, Carneiro I, et al. Protective efficacy of intermittent preventive treatment of malaria in infants (IPTi) using sulfadoxine-pyrimethamine and parasite resistance. PLoS ONE. 2010; 5(9):e12618. https://doi.org/10.1371/journal.pone.0012618 PMID: 20838642

117. Hlaing T, Wai KT, Oo T, Sint N, Min T, Myar S, et al. Mobility dynamics of migrant workers and their socio-behavioral parameters related to malaria in Tier II, Artemisinin Resistance Containment Zone, Myanmar. BMC Public Health. 2015; 15:886. https://doi.org/10.1186/s12889-015-2241-0 PMID: 26370297

118. Malisa AL, Pearce RJ, Abdulla S, Mshinda H, Kachur PS, Bloland P, et al. Drug coverage in treatment of malaria and the consequences for resistance evolution—evidence from the use of sulphadoxine/pyrimethamine. Malar J. 2010; 9:190. https://doi.org/10.1186/1475-2875-9-190 PMID: 20602754

119. Winter K, Hastings IM. Development, evaluation, and application of an *in silico* model for antimalarial drug treatment and failure. Antimicrob Agents Chemother. 2011; 55(7):3380–92. https://doi.org/10.1128/AAC.01712-10 PMID: 21537019

120. Grist EP, Flegg JA, Humphreys G, Mas IS, Anderson TJ, Ashley EA, et al. Optimal health and disease management using spatial uncertainty: a geographic characterization of emergent artemisinin-resistant Plasmodium falciparum distributions in Southeast Asia. Int J Health Geogr. 2016; 15(1):37. https://doi.org/10.1186/s12942-016-0064-6 PMID: 27776514

121. Corbel V, Akogbeto M, Damien GB, Djenontin A, Chandre F, Rogier C, et al. Combination of malaria vector control interventions in pyrethroid resistance area in Benin: a cluster randomised controlled trial. Lancet Infect Dis. 2012; 12(8):617–26. https://doi.org/10.1016/S1473-3099(12)70081-6 PMID: 22682536

122. Menger DJ, Omusula P, Holdinga M, Homan T, Carreira AS, Vandendaele P, et al. Field evaluation of a push-pull system to reduce malaria transmission. PLoS ONE. 2015; 10(4):e0123415. https://doi.org/10.1371/journal.pone.0123415 PMID: 25923114

123. Tchuenche JM, Chiyaka C, Chan D, Matthews A, Mayer G. A mathematical model for antimalarial drug resistance. Math Med Biol. 2011; 28(4):335–55. https://doi.org/10.1093/imammb/dqq017 PMID: 20884768

124. Kunkel A, Colijn C, Lipsitch M, Cohen T. How could preventive therapy affect the prevalence of drug resistance? Causes and consequences. Philos Trans R Soc Lond B Biol Sci. 2015; 370 (1670):20140306. https://doi.org/10.1098/rstb.2014.0306 PMID: 25918446

125. Barbosa S, Hastings IM. The importance of modelling the spread of insecticide resistance in a heterogeneous environment: the example of adding synergists to bed nets. Malar J. 2012; 11:258. https://doi.org/10.1186/1475-2875-11-258 PMID: 22856525

126. Eckhoff PA. Malaria parasite diversity and transmission intensity affect development of parasitological immunity in a mathematical model. Malar J. 2012; 11:419. https://doi.org/10.1186/1475-2875-11-419 PMID: 23241282

127. Fowkes FJ, Boeuf P, Beeson JG. Immunity to malaria in an era of declining malaria transmission. Parasitology. 2016; 143(2):139–53. https://doi.org/10.1017/S0031182015001249 PMID: 26741253

128. Griffin JT, Ferguson NM, Ghani AC. Estimates of the changing age-burden of *Plasmodium falciparum* malaria disease in sub-Saharan Africa. Nat Commun. 2014; 5:3136. https://doi.org/10.1038/ncomms4136 PMID: 24518518

129. Griffin JT, Hollingsworth TD, Reyburn H, Drakeley CJ, Riley EM, Ghani AC. Gradual acquisition of immunity to severe malaria with increasing exposure. Proc Biol Sci. 2015; 282(1801):20142657. https://doi.org/10.1098/rspb.2014.2657 PMID: 25567652

130. Eckhoff P. *P. falciparum* infection durations and infectiousness are shaped by antigenic variation and innate and adaptive host immunity in a mathematical model. PLoS ONE. 2012; 7(9):e44950. https://doi.org/10.1371/journal.pone.0044950 PMID: 23028698

131. Smith T, Ross A, Maire N, Rogier C, Trape JF, Molineaux L. An epidemiologic model of the incidence of acute illness in *Plasmodium falciparum* malaria. Am J Trop Med Hyg. 2006; 75(2 Suppl):56–62.

132. Elbadry MA, Al-Khedery B, Tagliamonte MS, Yowell CA, Raccurt CP, Existe A, et al. High prevalence of asymptomatic malaria infections: a cross-sectional study in rural areas in six departments in Haiti. Malar J. 2015; 14:510. https://doi.org/10.1186/s12936-015-1051-2 PMID: 26689195

133. Alimi TO, Fuller DO, Qualls WA, Herrera SV, Arevalo-Herrera M, Quinones ML, et al. Predicting potential ranges of primary malaria vectors and malaria in northern South America based on projected changes in climate, land cover and human population. Parasit Vectors. 2015; 8:431. https://doi.org/10.1186/s13071-015-1033-9 PMID: 26289677

134. Baeza A, Bouma MJ, Dhiman R, Pascual M. Malaria control under unstable dynamics: reactive vs. climate-based strategies. Acta Trop. 2014; 129:42–51. https://doi.org/10.1016/j.actatropica.2013.04.001 PMID: 23567551

135. Caminade C, Kovats S, Rocklov J, Tompkins AM, Morse AP, Colon-Gonzalez FJ, et al. Impact of climate change on global malaria distribution. Proc Natl Acad Sci U S A. 2014; 111(9):3286–91. https://doi.org/10.1073/pnas.1302089111 PMID: 24596427

136. Caruana CM. A new breed of model: estimating the impact of climate change on malaria transmission. Environ Health Perspect. 2013; 121(10):A310. https://doi.org/10.1289/ehp.121-A310 PMID: 24218662

137. Christiansen-Jucht C, Erguler K, Shek CY, Basanez MG, Parham PE. Modelling *Anopheles gambiae* s.s. population dynamics with temperature- and age-dependent survival. Int J Environ Res Public Health. 2015; 12(6):5975–6005. https://doi.org/10.3390/ijerph120605975 PMID: 26030468

138. Khormi HM, Kumar L. Future malaria spatial pattern based on the potential global warming impact in South and Southeast Asia. Geospat Health. 2016; 11(3):416. https://doi.org/10.4081/gh.2016.416 PMID: 27903054

139. Laporta GZ, Linton YM, Wilkerson RC, Bergo ES, Nagaki SS, Sant'Ana DC, et al. Malaria vectors in South America: current and future scenarios. Parasit Vectors. 2015; 8:426. https://doi.org/10.1186/s13071-015-1038-4 PMID: 26283539

140. Leedale J, Tompkins AM, Caminade C, Jones AE, Nikulin G, Morse AP. Projecting malaria hazard from climate change in eastern Africa using large ensembles to estimate uncertainty. Geospat Health. 2016; 11(1 Suppl):393.

141. Mweya CN, Kimera SI, Stanley G, Misinzo G, Mboera LE. Climate change influences potential distribution of infected *Aedes aegypti* co-occurrence with dengue epidemics risk areas in tanzania. PLoS ONE. 2016; 11(9):e0162649. https://doi.org/10.1371/journal.pone.0162649 PMID: 27681327

142. Ngarakana-Gwasira ET, Bhunu CP, Masocha M, Mashonjowa E. Assessing the role of climate change in malaria transmission in Africa. Malar Res Treat. 2016; 2016:7104291. https://doi.org/10.1155/2016/7104291 PMID: 27066290

143. Onyango EA, Sahin O, Awiti A, Chu C, Mackey B. An integrated risk and vulnerability assessment framework for climate change and malaria transmission in East Africa. Malar J. 2016; 15(1):551. https://doi.org/10.1186/s12936-016-1600-3 PMID: 27835976

144. Pascual M. Climate and population immunity in malaria dynamics: Harnessing information from endemicity gradients. Trends Parasitol. 2015; 31(11):532–4. https://doi.org/10.1016/j.pt.2015.08.009 PMID: 26422773

145. Ryan SJ, McNally A, Johnson LR, Mordecai EA, Ben-Horin T, Paaijmans K, et al. Mapping physiological suitability limits for malaria in Africa under climate change. Vector Borne Zoonotic Dis. 2015; 15(12):718–25. https://doi.org/10.1089/vbz.2015.1822 PMID: 26579951

146. Salahi-Moghaddam A, Khoshdel A, Dalaei H, Pakdad K, Nutifafa GG, Sedaghat MM. Spatial changes in the distribution of malaria vectors during the past 5 decades in Iran. Acta Trop. 2017; 166:45–53. https://doi.org/10.1016/j.actatropica.2016.11.001 PMID: 27826012

147. Song Y, Ge Y, Wang J, Ren Z, Liao Y, Peng J. Spatial distribution estimation of malaria in northern China and its scenarios in 2020, 2030, 2040 and 2050. Malar J. 2016; 15(1):345. https://doi.org/10.1186/s12936-016-1395-2 PMID: 27387921

148. Tompkins AM, Caporaso L. Assessment of malaria transmission changes in Africa, due to the climate impact of land use change using Coupled Model Intercomparison Project Phase 5 earth system models. Geospat Health. 2016; 11(1 Suppl):380.

149. Tonnang HE, Tchouassi DP, Juarez HS, Igweta LK, Djouaka RF. Zoom in at African country level: potential climate induced changes in areas of suitability for survival of malaria vectors. Int J Health Geogr. 2014; 13:12. https://doi.org/10.1186/1476-072X-13-12 PMID: 24885061

150. Warszawski L, Frieler K, Huber V, Piontek F, Serdeczny O, Schewe J. The Inter-Sectoral Impact Model Intercomparison Project (ISI-MIP): project framework. Proc Natl Acad Sci U S A. 2014; 111 (9):3228–32. https://doi.org/10.1073/pnas.1312330110 PMID: 24344316

151. Yamana TK, Eltahir EA. Projected impacts of climate change on environmental suitability for malaria transmission in West Africa. Environ Health Perspect. 2013; 121(10):1179–86. https://doi.org/10.1289/ehp.1206174 PMID: 24043443

152. Bakare E, Nwozo C. On the mathematical analysis of the influence of chemoprophylaxis on the malaria epidemic model. International Journal of Contemporary Mathematical Sciences. 2016; 11:45–63.

153. Kyaw SS, Drake T, Ruangveerayuth R, Chierakul W, White NJ, Newton PN, et al. Cost of treating inpatient falciparum malaria on the Thai-Myanmar border. Malar J. 2014; 13:416. https://doi.org/10.1186/1475-2875-13-416 PMID: 25351915

154. Labadin J, Kon M, Juan S. Deterministic malaria transmission model with acquired immunity WCECS 2009, October 20–22, 2009, San Francisco, USA2009. Available from: http://www.iaeng.org/publication/WCECS2009/WCECS2009_pp779-784.pdf

155. Hagenlocher M, Castro MC. Mapping malaria risk and vulnerability in the United Republic of Tanzania: a spatial explicit model. Popul Health Metr. 2015; 13(1):2. https://doi.org/10.1186/s12963-015-0036-2 PMID: 25674040

156. Ranjbar M, Shoghli A, Kolifarhood G, Tabatabaei SM, Amlashi M, Mohammadi M. Predicting factors for malaria re-introduction: an applied model in an elimination setting to prevent malaria outbreaks. Malar J. 2016; 15(1):138.

157. Acevedo MA, Prosper O, Lopiano K, Ruktanonchai N, Caughlin TT, Martcheva M, et al. Spatial heterogeneity, host movement and mosquito-borne disease transmission. PLoS ONE. 2015; 10(6): e0127552. https://doi.org/10.1371/journal.pone.0127552 PMID: 26030769

158. Bomblies A. Agent-based modeling of malaria vectors: the importance of spatial simulation. Parasit Vectors. 2014; 7:308. https://doi.org/10.1186/1756-3305-7-308 PMID: 24992942

159. Pindolia DK, Garcia AJ, Huang Z, Fik T, Smith DL, Tatem AJ. Quantifying cross-border movements and migrations for guiding the strategic planning of malaria control and elimination. Malar J. 2014; 13:169. https://doi.org/10.1186/1475-2875-13-169 PMID: 24886389

160. Smith DL, Perkins TA, Reiner RC Jr., Barker CM, Niu T, Chaves LF, et al. Recasting the theory of mosquito-borne pathogen transmission dynamics and control. Trans R Soc Trop Med Hyg. 2014; 108 (4):185–97. https://doi.org/10.1093/trstmh/tru026 PMID: 24591453

161. Tatem AJ, Huang Z, Narib C, Kumar U, Kandula D, Pindolia DK, et al. Integrating rapid risk mapping and mobile phone call record data for strategic malaria elimination planning. Malar J. 2014; 13:52. https://doi.org/10.1186/1475-2875-13-52 PMID: 24512144

162. Blanas DA, Ndiaye Y, MacFarlane M, Manga I, Siddiqui A, Velez O, et al. Health worker perceptions of integrating mobile phones into community case management of malaria in Saraya, Senegal. Int Health. 2015; 7(3):176–82. https://doi.org/10.1093/inthealth/ihu075 PMID: 25316707

163. Ruktanonchai NW, Bhavnani D, Sorichetta A, Bengtsson L, Carter KH, Cordoba RC, et al. Census-derived migration data as a tool for informing malaria elimination policy. Malar J. 2016; 15(1):273. https://doi.org/10.1186/s12936-016-1315-5 PMID: 27169470

164. Tompkins AM, McCreesh N. Migration statistics relevant for malaria transmission in Senegal derived from mobile phone data and used in an agent-based migration model. Geospat Health. 2016; 11(1 Suppl):408.

165. Danis K, Lenglet A, Tseroni M, Baka A, Tsiodras S, Bonovas S. Malaria in Greece: historical and current reflections on a re-emerging vector borne disease. Travel Med Infect Dis. 2013; 11(1):8–14. https://doi.org/10.1016/j.tmaid.2013.01.001 PMID: 23434287

166. Miguel RB, Peiter PC, de Albuquerque H, Coura JR, Moza PG, Costa Ade P, et al. Malaria in the state of Rio de Janeiro, Brazil, an Atlantic Forest area: an assessment using the health surveillance service. Mem Inst Oswaldo Cruz. 2014; 109(5):634–40. https://doi.org/10.1590/0074-0276130558 PMID: 25185004

167. Dharmawardena P, Premaratne RG, Gunasekera WM, Hewawitarane M, Mendis K, Fernando D. Characterization of imported malaria, the largest threat to sustained malaria elimination from Sri Lanka. Malar J. 2015; 14:177. https://doi.org/10.1186/s12936-015-0697-0 PMID: 25902716

168. Wang D, Li S, Cheng Z, Xiao N, Cotter C, Hwang J, et al. Transmission risk from imported *Plasmodium vivax* malaria in the China-Myanmar border region. Emerg Infect Dis. 2015; 21(10):1861–4. https://doi.org/10.3201/eid2110.150679 PMID: 26401843

169. Ren Z, Wang D, Ma A, Hwang J, Bennett A, Sturrock HJ, et al. Predicting malaria vector distribution under climate change scenarios in China: Challenges for malaria elimination. Sci Rep. 2016; 6:20604. https://doi.org/10.1038/srep20604 PMID: 26868185

170. Noor AM, Uusiku P, Kamwi RN, Katokele S, Ntomwa B, Alegana VA, et al. The receptive versus current risks of *Plasmodium falciparum* transmission in northern Namibia: implications for elimination. BMC Infect Dis. 2013; 13:184. https://doi.org/10.1186/1471-2334-13-184 PMID: 23617955

171. Shahandeh K, Basseri HR, Sharifzadeh Y. An application of cultural model to assess and compare malaria prevention among Afghani migrant and Baluchi resident in the endemic area, southeastern Iran. J Immigr Minor Health. 2014; 16(1):102–10. https://doi.org/10.1007/s10903-013-9850-4 PMID: 23775110

172. Gomes E, Capinha C, Rocha J, Sousa C. Mapping risk of malaria transmission in mainland Portugal using a mathematical modelling approach. PLoS ONE. 2016; 11(11):e0164788. https://doi.org/10.1371/journal.pone.0164788 PMID: 27814371

173. Ranjbar M, Shoghli A, Kolifarhood G, Tabatabaei SM, Amlashi M, Mohammadi M. Predicting factors for malaria re-introduction: an applied model in an elimination setting to prevent malaria outbreaks. Malar J. 2016; 15:138. https://doi.org/10.1186/s12936-016-1192-y PMID: 26935846

174. Cohen JM, Ernst KC, Lindblade KA, Vulule JM, John CC, Wilson ML. Local topographic wetness indices predict household malaria risk better than land-use and land-cover in the western Kenya highlands. Malar J. 2010; 9:328. https://doi.org/10.1186/1475-2875-9-328 PMID: 21080943

175. Tatarsky A, Aboobakar S, Cohen JM, Gopee N, Bheecarry A, Moonasar D, et al. Preventing the reintroduction of malaria in Mauritius: a programmatic and financial assessment. PLoS ONE. 2011; 6(9): e23832. https://doi.org/10.1371/journal.pone.0023832 PMID: 21912645

176. Durnez L, Coosemans M. Residual transmission of malaria: an old issue for new approaches: InTech; 2013. Available from: http://www.intechopen.com/books/anopheles-mosquitoes-new-insights-into-malaria-vectors/residual-transmission-of-malaria-an-old-issue-for-new-approaches

177. Lloyd AL, Zhang J, Root AM. Stochasticity and heterogeneity in host-vector models. J R Soc Interface. 2007; 4(16):851–63. https://doi.org/10.1098/rsif.2007.1064 PMID: 17580290

178. Vazquez-Prokopec GM, Perkins TA, Waller LA, Lloyd AL, Reiner RC Jr., Scott TW, et al. Coupled heterogeneities and their impact on parasite transmission and control. Trends Parasitol. 2016; 32(5):356–67. https://doi.org/10.1016/j.pt.2016.01.001 PMID: 26850821

179. Rao VB, Schellenberg D, Ghani AC. Overcoming health systems barriers to successful malaria treatment. Trends Parasitol. 2013; 29(4):164–80. https://doi.org/10.1016/j.pt.2013.01.005 PMID: 23415933

180. Cohen JM, Sabot O, Sabot K, Gordon M, Gross I, Bishop D, et al. A pharmacy too far? Equity and spatial distribution of outcomes in the delivery of subsidized artemisinin-based combination therapies through private drug shops. BMC Health Serv Res. 2010; 10 Suppl 1:S6.

181. Perkins TA, Scott TW, Le Menach A, Smith DL. Heterogeneity, mixing, and the spatial scales of mosquito-borne pathogen transmission. PLoS Comput Biol. 2013; 9(12):e1003327. https://doi.org/10.1371/journal.pcbi.1003327 PMID: 24348223

182. Eckhoff P, Bever CA, Gerardin J, Wenger E, Smith D. From puddles to planet: Modeling approaches to vector-borne diseases at varying resolution and scale. Current Opinion in Insect Science. 2015; 10:118–23.

183. Moonasar D, Maharaj R, Kunene S, Candrinho B, Saute F, Ntshalintshali N, et al. Towards malaria elimination in the MOSASWA (Mozambique, South Africa and Swaziland) region. Malar J. 2016; 15 (1):419. https://doi.org/10.1186/s12936-016-1470-8 PMID: 27538990

184. Raman J, Morris N, Frean J, Brooke B, Blumberg L, Kruger P, et al. Reviewing South Africa's malaria elimination strategy (2012–2018): progress, challenges and priorities. Malar J. 2016; 15(1):438. https://doi.org/10.1186/s12936-016-1497-x PMID: 27567642

185. Sturrock HJ, Roberts KW, Wegbreit J, Ohrt C, Gosling RD. Tackling imported malaria: an elimination endgame. Am J Trop Med Hyg. 2015; 93(1):139–44. https://doi.org/10.4269/ajtmh.14-0256 PMID: 26013369

186. Wangdi K, Gatton ML, Kelly GC, Clements AC. Cross-border malaria: a major obstacle for malaria elimination. Adv Parasitol. 2015; 89:79–107. https://doi.org/10.1016/bs.apar.2015.04.002 PMID: 26003036

187. Ferreira MU, Castro MC. Challenges for malaria elimination in Brazil. Malar J. 2016; 15(1):284. https://doi.org/10.1186/s12936-016-1335-1 PMID: 27206924

188. Gryseels C, Peeters Grietens K, Dierickx S, Xuan XN, Uk S, Bannister-Tyrrell M, et al. High mobility and low use of malaria preventive measures among the Jarai male youth along the Cambodia-Vietnam border. Am J Trop Med Hyg. 2015; 93(4):810–8. https://doi.org/10.4269/ajtmh.15-0259 PMID: 26283747

189. Hu Y, Zhou G, Ruan Y, Lee MC, Xu X, Deng S, et al. Seasonal dynamics and microgeographical spatial heterogeneity of malaria along the China-Myanmar border. Acta Trop. 2016; 157:12–9. https://doi.org/10.1016/j.actatropica.2016.01.022 PMID: 26812008

190. Wangdi K, Banwell C, Gatton ML, Kelly GC, Namgay R, Clements AC. Development and evaluation of a spatial decision support system for malaria elimination in Bhutan. Malar J. 2016; 15(1):180.

191. Bi Y, Hu W, Yang H, Zhou XN, Yu W, Guo Y, et al. Spatial patterns of malaria reported deaths in Yunnan Province, China. Am J Trop Med Hyg. 2013; 88(3):526–35. https://doi.org/10.4269/ajtmh.2012.12-0217 PMID: 23269660

192. Lyttleton C. Deviance and resistance: Malaria elimination in the greater Mekong subregion. Soc Sci Med. 2016; 150:144–52. https://doi.org/10.1016/j.socscimed.2015.12.033 PMID: 26751710

193. Wang RB, Dong JQ, Xia ZG, Cai T, Zhang QF, Zhang Y, et al. Lessons on malaria control in the ethnic minority regions in Northern Myanmar along the China border, 2007–2014. Infect Dis Poverty. 2016; 5(1):95. https://doi.org/10.1186/s40249-016-0191-0 PMID: 27716435

194. Kanyangarara M, Mamini E, Mharakurwa S, Munyati S, Gwanzura L, Kobayashi T, et al. Individual- and household-level risk factors associated with malaria in Mutasa district, Zimbabwe: a serial cross-sectional study. Am J Trop Med Hyg. 2016; 95(1):133–40. https://doi.org/10.4269/ajtmh.15-0847 PMID: 27114289

195. Zhang Q, Sun J, Zhang Z, Geng Q, Lai S, Hu W, et al. Risk assessment of malaria in land border regions of China in the context of malaria elimination. Malar J. 2016; 15(1):546. https://doi.org/10.1186/s12936-016-1590-1 PMID: 27825379

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COLLECTION REVIEW

malERA: An updated research agenda for health systems and policy research in malaria elimination and eradication

The malERA Refresh Consultative Panel on Health Systems and Policy Research[¶]*

¶ Full listing of the members of the malERA Refresh Consultative Panel on Health Systems and Policy Research can be found in the Acknowledgments.
* marcel.tanner@unibas.ch, maxine.whittaker@jcu.edu.au

OPEN ACCESS

Citation: The malERA Refresh Consultative Panel on Health Systems and Policy Research (2017) malERA: An updated research agenda for health systems and policy research in malaria elimination and eradication. PLoS Med 14(11): e1002454. https://doi.org/10.1371/journal.pmed.1002454

Published: November 30, 2017

Funding: MESA received a grant from the Bill & Melinda Gates Foundation (OPP1034591 http://www.malariaeradication.org/mesa-track). KR (Bill & Melinda Gates Foundation) was a member of this panel and participated in the panel consultation and in the development of this paper.

Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: MT is a member of the MESA (Malaria Eradication Scientific Alliance) Steering Committee and was a member of MPAC (Malaria Policy Advisory Committee) until 2016. RG and MW were the co-coordinators of APMEN (Asia Pacific Malaria Elimination Network) Secretariat until February 2016. BR is the Executive

Abstract

Health systems underpin disease elimination and eradication programmes. In an elimination and eradication context, innovative research approaches are needed across health systems to assess readiness for programme reorientation, mitigate any decreases in effectiveness of interventions ('effectiveness decay'), and respond to dynamic and changing needs. The malaria eradication research agenda (malERA) Refresh consultative process for the Panel on Health Systems and Policy Research identifies opportunities to build health systems evidence and the tools needed to eliminate malaria from different zones, countries, and regions and to eradicate it globally. The research questions are organised as a portfolio that global health practitioners, researchers, and funders can identify with and support. This supports the promotion of an actionable and more cohesive approach to building the evidence base for scaled-up implementation of findings. Gaps and opportunities discussed in the paper include delivery strategies to meet the changing dynamics of needs of individuals, environments, and malaria programme successes; mechanisms and approaches to best support accelerated policy and financial responsiveness at national and global level to ensure timely response to evidence and needs, including in crisis situations; and systems' readiness tools and decision-support systems.

Summary points

- Since 2011, few research questions identified in malERA Health Systems and Operational Research agenda have been addressed, or only addressed in a fragmented way by scientists and implementation researchers at national and international levels. Multiple factors, including funding, dependency upon existing national and provincial health systems to deliver, and limited buy-in by a range of disciplines into the malaria agenda, have contributed to this limited uptake.
- To address the complexity of health systems and any changes or adaptations within them requires a systemic and transdisciplinary approach in the conceptualization and conduct of the research. Transdisciplinary research requires the inclusion of various

Director of APLMA (Asia Pacific Leaders' Malaria Alliance).

Abbreviations: AIM, Action and Investment to Defeat Malaria; APMEN, Asia Pacific Malaria Elimination Network; E8, Elimination 8; GMP, Global Malaria Programme; GTS, Global Technical Strategy; HIS, Health Information System; IMAI, integrated management of adolescent and adult illnesses; IMCI, integrated management of childhood illnesses; malERA, malaria eradication research agenda; M&E, monitoring and evaluation; MESA, Malaria Eradication Scientific Alliance; MPAC, Malaria Policy Advisory Committee; RBM, Roll Back Malaria; R&D, research and development; SORT-IT, Structured Operational Research and Training IniTiative.

Provenance: Submitted as part of a Supplement; externally peer reviewed.

sectors and agencies, communities, and civil society, as well as inclusion and integration of a range of research disciplines including policy, management, and social sciences.

- Presenting the research and development agenda here as a portfolio ensures the transdisciplinary and multi-stakeholder approach and can therefore help funders and researchers take action and engage in the pursuit of this agenda according to their own diverse priorities.

Introduction

Between 2000 and 2015, a major expansion of WHO-recommended interventions have contributed to a 58% reduction in the global malaria mortality rate (69% among children under 5 years old in Africa), resulting in an estimated 6.2 million lives being saved from a malaria-related death [1].

As part of the initial malaria eradication research agenda (malERA) process, published in 2011, a consultative group on health systems and operational research established a list of research priorities presented in a matrix system organized by the different levels (community, facility, district, and national levels) and building blocks of the health system [2]. Health-system building blocks are described in Box 1. The health systems concept and framework was based on the guiding summary on 'health system thinking' perspective formulated by the Alliance for Health Policy and System Research [3]. One key gap that was identified in 2011 was a

Box 1. Health system building blocks that together constitute a complete system.

- Governance: Ensuring strategic policy frameworks combined with effective oversight, coalition building, accountability, regulations, incentives, and attention to system design.
- Human resources: Responsive, fair, and efficient, given available resources and circumstances, and available in sufficient numbers.
- Financing: Raising adequate funds for health in ways that ensure people can use needed services and are protected from financial catastrophe or impoverishment associated with having to pay for them.
- Health information: Ensuring the production, analysis, dissemination, and use of reliable and timely information on health determinants, health systems performance, and health status.
- Service delivery: Including effective, safe, and quality personal and nonpersonal health interventions that are provided to those in need, when and where needed (including infrastructure), with a minimal waste of resources.
- Commodities: Including medical products, vaccines, and other technologies of assured quality, safety, efficacy, and cost-effectiveness, and their scientifically sound and cost-effective use.

tool to diagnose impediments in a given health system that limited the effective and equitable impact of malaria interventions [2].

The momentum created by the successes since the turn of the century has led to reiterated commitment and partnership and 2 complementary key documents, (i) the Global Technical Strategy (GTS) for malaria 2016–2030 [4] and (ii) the Action and Investment to Defeat Malaria (AIM) 2016–2030 [5], which were launched respectively by the Global Malaria Programme (GMP) of WHO and the Roll Back Malaria (RBM) Partnership in 2015. The GTS and the investment and advocacy framework partnership document, AIM, pave the way for intensifying malaria control and elimination efforts and set ambitious yet realistic targets and goals. The GTS clearly outlines a common technical strategy while AIM provides the investment framework to reach these technical targets. Moving forward, GTS and AIM need to be brought to the country level and be clearly reflected in the national malaria control and elimination strategic plans, as well as in the overall national health policy and strategy.

In view of these advances, many challenges need to be coherently addressed in order to not threaten continued progress. These challenges are as reflected in GTS and AIM:

1. Emerging parasite resistance to antimalarial medicines and mosquito resistance to insecticides.
2. Systemic and technical obstacles, such as the inherent weakness of health systems, including poor disease surveillance and limited pharmaceutical regulation.
3. Lack of adequate technical and human resource capacities including community engagement.
4. High prevalence of asymptomatic infections and unknown dimension of the existing asymptomatic reservoir.
5. Diversity of vectors and their behaviour.

To complement GTS and AIM, a systematic review of progress in research and development (R&D) and a consultative process to update the research agenda, 'malERA Refresh', was launched [6]. malERA Refresh can be seen as the third pillar, defining research priorities to support the GTS targets, while the business case for achieving the GTS targets is provided by AIM.

Scope of this report

In the refresh of malERA presented here, an expert panel focussed on health systems and policy research reviewed the progress made since 2011 and set out an updated agenda and research portfolio necessary to support the global malaria elimination agenda. While addressing broader health system development needs would be beneficial to this as well as other public health agendas, it was not the focus of this piece of work, and others have addressed these in detail. A particular emphasis was placed on the elimination phase in order to best accompany the GTS and the AIM framework, as well as to assist the national strategies and operational plans. The main differences between the 2011 and 2017 health system agendas are that this 2017 agenda:

- Focusses on malaria, in particular malaria elimination. The 2011 agenda was a broader view on health systems and all phases of control to elimination.
- Recognizes that 1 country can have various phases of control to elimination occurring within its programme, so does not, as was done in 2011, group countries into phases.
- Frames the agenda as a portfolio from hypothesis driven (research), development of tools or interventions (R&D), or synthesis of existing information and evidence (evaluation science).

- Recognizes the need to tailor questions to different settings and therefore has left the questions deliberately broad to allow general application to specific settings.

Context and rationale

In spite of increased financial and commodity resources, progress in malaria control and elimination in most countries has been slower than expected. Among the main reasons for the slow pace are constraints on the delivery of essential health interventions, including malaria interventions, at sufficient levels of coverage and quality to populations in need. At the same time, the attainment of GTS targets and goals, within the AIM investment framework, will rely heavily on well-performing health systems for the sustained control and elimination phases.

Progress has been made in recent years towards better understanding health systems and how to strengthen them. The previous malERA health systems working group recommendations have contributed to that change, which is clearly reflected in AIM as well as health-systems thinking and is acting as an integral part of WHO GMP Malaria Policy Advisory Committee (MPAC) recommendations [7]. Moreover, global health initiatives have certainly increased funding for strengthening national health systems to accelerate progress on universal access to essential health interventions, particularly for HIV/AIDS, tuberculosis, malaria, and immunisation. Initiatives such as the Task Force on Innovative Financing for Health Systems [8] testify to the increased commitment to funding health systems and momentum in favour of health system strengthening, minimising the negative impacts of vertical programmes on health systems, and leveraging malaria activities through other health programmes.

However, significant work and questions remain. By systematically reviewing the literature, as well as ongoing research projects in health systems in the Malaria Eradication Scientific Alliance (MESA) Track database [9] (see Methods and approaches section), the panel concluded that many research priorities identified in the 2011 Panel on Health Systems and Operational Research have hardly—or only in a fragmented way—been picked up by scientists and implementation researchers at national and international levels.

The malERA portfolio dealing with health systems attracted insufficient funding and interest over the last few years. One possibility for this is that funding for malaria is more vertically targeted on parasites, diagnostics, treatment, and vectors than on broad health systems and in part because the agenda was more accessed by malaria scientists and not the broader health system research community and public health practitioners. Additionally, many of the disciplines required to engage in the health system agenda are not presently majorly involved, and the existing malaria scientific community are not well equipped to implement the health system agenda. There is a need to reiterate the agenda as established in 2011, but more importantly—by building on it—add new dimensions to the research and R&D needs in the field of health systems and policy research. This paper presents the agenda as a portfolio ranging from priorities in evaluation science to specific research questions to R&D issues (see Box 2 for definitions).

Funding partners and implementation groups have clearly prioritized the quick delivery of malaria interventions and a system of monitoring and evaluation (M&E) linked to their funding and not necessarily integrated into routine health systems M&E, including health systems research. Contrasting this to governments in countries affected by malaria, which are tasked with responding to ambitious global targets and goals, reveals that these countries have long recognised the need for health system thinking and research in order to meet the healthcare needs of their populations. As noted above, addressing the more challenging but also more sustainable issue of health system strengthening to support malaria eradication tends to be postponed as it is still seen, conceptually and operationally, as a daunting issue. This difficulty can be overcome by presenting the research and R&D priorities within a portfolio approach

Box 2. malERA Refresh research categories to support a portfolio approach to health systems and policy research.

- Evaluation science: Where information from the field and across several sites, contexts, and case studies already exists (mainly WHO/University of California San Francisco [UCSF] elimination case studies, RBM Progress and Impact reports, etc.) and an answer to the research question could be obtained by comparative, synthetic analysis.
- Research: Where new information is needed to answer a research question based on an underlying hypothesis or where new hypotheses need to be tested.
- R&D: Where an R&D process is required, based on underlying hypotheses, to develop a tool, a tool kit, or new approaches (e.g., surveillance as an intervention, 'surveillance-response'). These tools or approaches would be created based on available data and information to serve implementers in carrying out their work. The development of this tool, tool kit, or approach becomes the activity pertinent to this portfolio.

tailored to the different levels and building blocks of a given health and social system (Fig 1). Addressing the list of key research questions outlined in this paper would go a long way in strengthening malaria control and elimination and inscribing it into the fundamental health infrastructure of endemic countries (full portfolio of questions in Table 1).

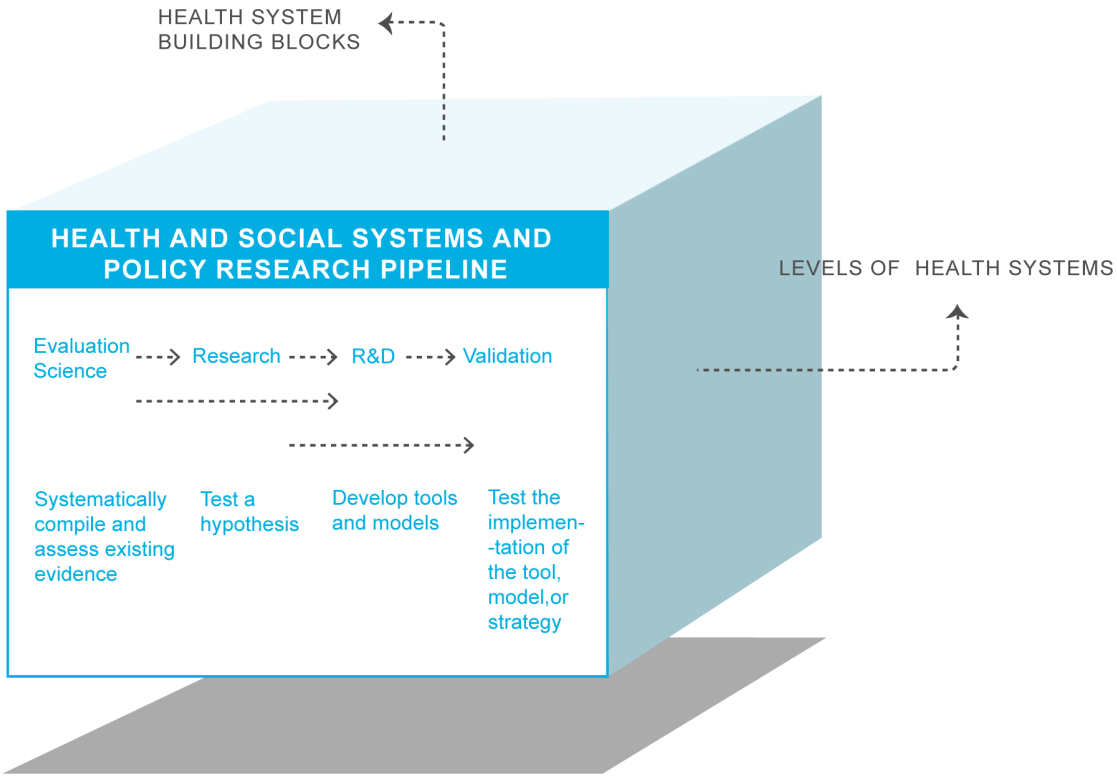

Fig 1. Research agenda cube for health systems and policy research. R&D, research and development.

https://doi.org/10.1371/journal.pmed.1002454.g001

Table 1. Priority questions for evaluation science, research, and R&D in health systems and policy research.

Evaluation science	Health system building block	Health system level
1st Priority: **What are the social & political drivers that influence malaria elimination success within and across regions at national and regional levels?**	Governance	National
2nd Priority: **How do optimized delivery strategies meet changing and dynamic needs of the system requirements and/or community perceived needs?**	Service delivery	Community
2nd Priority: **What are effective strategies and tools to sustain community workers' engagement in malaria activities during intensive control and elimination?**	Human resources	Community
Additional Emphasis: **Evaluation of case studies (including the malaria elimination case study series) regarding the determinants for successful scale-up of district management, financing, and human resource models in different contexts and settings.**	Cross-cutting	Facility
What mechanisms support effective integration of communicable disease surveillance?	Information	All
With an emphasis on decentralization, what are the management strengths required, and how can the readiness of the different health systems structures be assessed for malaria elimination in different settings?	Governance	All
What is the range of effective HIS and tools to capture and use information at the community level?	Information	Community
What mechanisms, tools, and strategies can be utilized to sustain active community engagement in intensive malaria control and elimination?	Governance	Community
What is the range of HIS and tools to effectively capture and use information at the community level?	Information	Community
How to scale up measures to ensure quality and quantity of health commodities at the community level in both public and private sectors, especially to remote and vulnerable populations?	Information	Community
What are the key essential service delivery tools implemented at PHC level to ensure quality of malaria elimination activities (prevention, treatment, and surveillance-response) at facility and community levels in different system contexts?	Service delivery	Community
What are the key essential service delivery tools implemented at PHC level to ensure quality of malaria elimination activities (prevention, treatment, and surveillance-response) at facility and community levels in different system contexts?	Service delivery	Facility
What is the range of effective HIS and tools to capture and use information at the national level?	Information	National
Comparatively assess across countries and settings which mechanisms best support accelerated policy and financial responsiveness at the national level to ensure timely response to evidence and needs, including in crisis situations.	Governance	National
Comparatively assess across various countries and subnational settings which are the effective approaches and their determinants to transition funding to sustainable financing sources.	Financing	National
What are efficient and ethical approaches to health security issues that can be applied to managing malaria in epidemics, reintroduction, and resurgence?	Governance	National
Comparatively assess which mechanisms best support accelerated policy and financial responsiveness at the global level to ensure a timely response to evidence and needs, including in crisis situations.	Governance	Regional/Global
What are the social and political drivers to influence malaria elimination within and across regions at country and regional levels?	Governance	Regional/Global
Research	**Health system building block**	**Health system level**
1st Priority: **What are the decision-making frameworks required to eliminate and prevent reestablishment of malaria?**	Governance	National
2nd Priority: **What is the best way to optimize malaria elimination delivery strategies to meet the changing dynamics of needs of individuals, environments, and malaria programme successes?**	Service delivery	Community
What are the determinants of efficiency of community-level health service delivery and of community systems, with an emphasis on malaria elimination outcomes?	Financing	Community
Which innovative measures would improve quality and quantity of malaria commodities at the community level in both public and private, especially to remote and vulnerable populations for malaria elimination?	Commodities	Community
What management tools and structures can improve transparency, accountability, and effectiveness of health facilities for malaria elimination activities (coverage, equity, and quality) in different contexts and systems?	Governance	District
What mechanisms and approaches best support accelerated policy and financial responsiveness at the national level to ensure timely response to evidence and needs, including in crisis situations?	Governance	National
In the context of a shared public health target of malaria elimination, what are the determinants and the effective modes & models of intercountry & cross-border collaboration for policy & implementation?	Governance	National

(*Continued*)

Table 1. (*Continued*)

What adaptive changes are needed in operations (management, financing, human resources, and responsibilities) of health systems to move to and support malaria elimination?	Cross-cutting	National
What are effective models of government leadership at leveraging integrated activities cross-sectorially for malaria elimination?	Governance	National
What ensures effective governance and accountability to support elimination?	Governance	National
What enables ownership of elimination at national and regional levels?	Governance	National
What are effective mechanisms to leverage financing for malaria prevention from health insurance schemes?	Financing	National
What are effective mechanisms and approaches to transition from external funding to sustainable financing sources?	Financing	National
What mechanisms and approaches best support accelerated policy and financial responsiveness at the global level to ensure timely response to evidence and needs, including in crisis situations?	Governance	Regional/Global
In the context of a shared public health target of malaria elimination, what are the determinants and the effective modes and models of intercountry and cross-border collaboration for policy and implementation?	Governance	Regional/Global
What are the decision-making frameworks required to eliminate and prevent reestablishment?	Governance	Regional/Global
What adaptive changes are needed in operations (management, financing, human resources, and responsibilities) of health systems to move to and support malaria elimination?	Cross-cutting	Regional/Global
What are effective models of government leadership at leveraging integrated activities cross-sectorially for malaria elimination?	Governance	Regional/Global
What ensures effective governance and accountability to support elimination?	Governance	Regional/Global
What enables ownership of elimination at national and regional levels?	Governance	Regional/Global
R&D	**Health system building block**	**Health system level**
1st Priority: **What are the health planning and funding models and tools required to eliminate and prevent reestablishment of malaria?**	Cross-cutting	National
2nd Priority: **What tools (existing, new, or a combination of both) can measure systems' readiness at community, facility, and district levels in an integrated way to support elimination and prevention of reintroduction?**	Cross-cutting	All
Develop the tools and SOPs for effectiveness decay analyses.	Cross-cutting	All levels
Development of tools and strategies to strengthen and sustain active community engagement in intensive control and malaria elimination.	Cross-cutting	Community
How can community components of integrated service delivery approaches (IMCI, IMAI, and ICCM) be adapted to malaria elimination and prevention of reintroduction?	Governance	Community
Develop a broad system readiness tool to include stop/start decisions, appropriate economic tool and approaches (e.g., CEA and CBA), and link the tool to decision-support systems.	Service delivery	National
What are the health planning and funding models and tools required to eliminate and prevent reestablishment of malaria?	Cross-cutting	Regional/Global
Develop a broad system readiness tool to include stop/start decisions, appropriate economic tool and approaches (e.g., CEA and CBA), and link the tool to decision-support systems at the regional level.	Cross-cutting	Regional/Global

CBA, cost-benefit analysis; CEA, cost-effectiveness analysis; HIS, health information system; ICCM, intergrated community case management; IMAI, integrated management of adolescent and adult illnesses; IMCI, integrated management of childhood illnesses; PHC, primary health care; R&D, research and development; SOP, standard operating procedure

https://doi.org/10.1371/journal.pmed.1002454.t001

Methods and approaches

In 2009, WHO [3] defined health systems research as 'the purposeful generation of knowledge that enables societies to organize themselves to improve health outcomes and services.' It is concerned with how health services are financed, delivered, and organised and how these functions are linked within an overall health and social system with its associated policies and institutions.

To understand and address the complexity of health systems and implementation of change into those systems requires not only a systemic but also clearly a transdisciplinary approach.

Transdisciplinary research entails approaching a complex problem in 'a way that can (a) grasp the complexity of problems, (b) take into account the diversity of life-world and scientific perceptions of problems, (c) link abstract and case-specific knowledge, and (d) constitute knowledge and practices that promote what is perceived to be the common good' [10]. This approach to research requires the inclusion of various sectors and agencies, community, and civil society, as well as inclusion and integration of a range of research disciplines including policy and political science, management, economics, and social sciences (including anthropology, sociology, and development sciences).

Based on this context, the review of the research agenda was pursued in 2 steps, each step engaging the panel members as described in S1 Text, to organise the health systems research needs in malaria elimination as a portfolio of evaluation science, research, and R&D. This kind of portfolio approach should make it easier for researchers and funding agencies to identify their subjects of interest. The panel used the modified Nominal Group Approach to identify the priority questions in each category [11] (see S1 Text).

Results: Fundamentals and prerequisites

Changing focus from pilot studies to scale-up

Based on analysis of MESA Track and extensive literature search (S1 Text) and taking into account the time it takes for the first malERA process to gain traction, it emerged that research taken up after the first malERA process led to a few interesting pilot studies. Even when conclusive, these projects remained fragmented and rarely led to or indicated initiation of scaled-up implementation of the key findings and recommendations. This malERA Refresh process aims to avoid fragmentation and move beyond pilot studies.

When research brings forward conclusive results and recommendations, a strong effort should be placed on scaling them up in that context. When results have not been internally and externally validated (e.g., because of the small size of pilot studies or fragmentation of research), then more research and evaluation efforts should focus on gathering the information pertinent to inform the scale-up for impact of interventions [8].

Effectiveness decay

Effectiveness of interventions is lost at almost all (if not all) levels of the health system. This means that at 1 or several points in a health system, efficacious interventions may lose some of their effect because they cannot be applied or implemented effectively; this is called effectiveness decay. By analysing effectiveness decay within a specific health and social system one can elucidate at which levels the greatest loss of effectiveness are occurring (Fig 2) and then test strategies with which to intervene and recover effectiveness of the intervention. For malaria control and elimination, understanding the equity effectiveness of interventions, that is whether all who need to access and use the intervention can do so equally, is of particular importance.

The effective execution of the elimination agenda would benefit from high performance of health systems that can deliver the optimal combination of malaria interventions at high and equitable levels of quality and population coverage. This requires the concerted and combined strength of all the health system building blocks. Program managers need to be able to detect the reasons why coverage levels are inadequate or inequitable and, based upon that, develop appropriate ways to address these reasons within their health and/or social system. And for system interventions and strengthening to be effective and efficient, they need to be able to diagnose those problems and their determinants and interactions (decay at each level is not the same in all health systems). The system effectiveness analytic framework has proven very useful

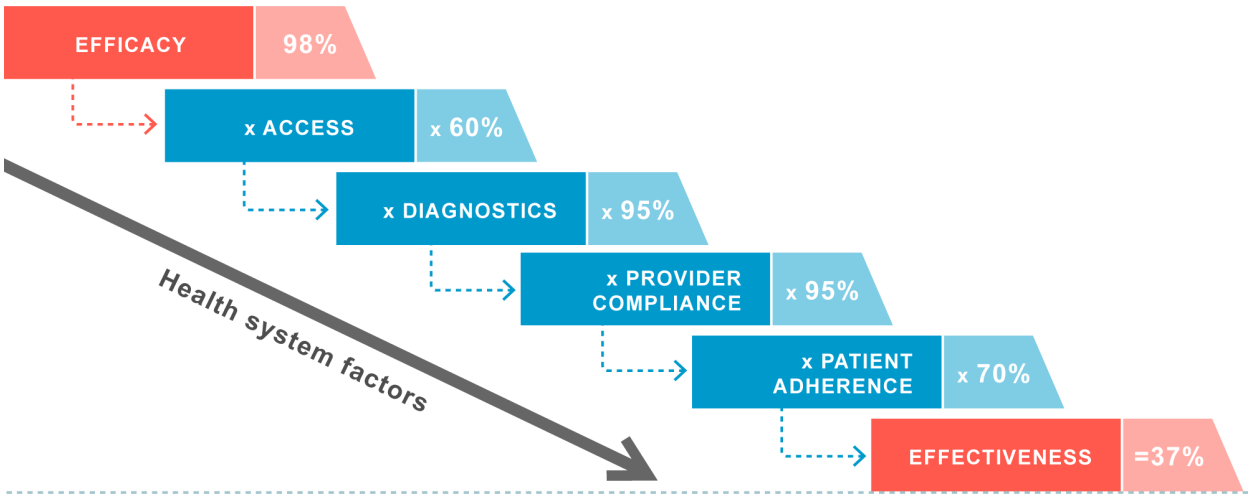

Fig 2. Effectiveness decay. Loss of effectiveness of interventions within the health system is depicted here by steps. The pattern of effectiveness decay (how much is lost and at what step) varies and depends on the specifics of a given health system [12]. The percentages of decay/loss are hypothetical.

https://doi.org/10.1371/journal.pmed.1002454.g002

as a health system diagnostic tool to identify areas where and how system factors need to be and can be strengthened. This framework sees a cascade of progressive loss of intervention efficacy (Fig 2) and addresses it through system-specific issues of access, targeting, provider compliance, and client adherence as the system tries to deliver an intervention at effective coverage levels in real-world settings. Many programmes have used it to analyse the determinants of coverage but results are often yet to be translated into targeted health systems. Such an analysis will need to be complemented with an analysis of allocation efficiency, which will help to optimize a portfolio of multiple interventions in a given context to maximize health impact.

Programme efficiency and health system readiness

After the effectiveness decay analysis, programmes at various levels within a country (local, subnational, and national) can establish the most efficient operational mix to meet their targets and objectives. They need to precisely define when and where interventions need to start and stop and what time frame is involved for certain operations. Based on surveillance data and prevalence and transmission intensity, and assisted by modelling, interventions or mixes of interventions to be applied in an integrated way should be clearly defined [13]. Starting from the initial decay analysis at the national and subnational levels, it becomes a health systems surveillance tool to be part of the national programme and the surveillance-response approaches. By ignoring the continued M&E of the health systems effectiveness, control and elimination programmes are at risk of inefficiencies and unrecognized loss of effectiveness.

Results: Research and R&D portfolio

Examples of the research portfolio discussed by the consultative panel are presented in Box 3, starting with cross-cutting priorities relevant to all health systems and policy research topics and then going over the research agenda in evaluation science, research, and R&D of new tools categories. For each research question in each category, the health system levels and the building blocks are mentioned. The top 2 priority questions and the question on which the panel chose to place special emphasis are always listed first in each category. A rationale is provided in the portfolio to offer some background and context on the issues raised. Then follows a list

Box 3. Examples of research questions in the research agenda for health systems and policy research.

Evaluation science (synthetic, comparative evaluation of existing interventions and case studies)

- What were the social, regulatory, and political drivers that supported or affected the ability of malaria elimination systems requirements to be integrated into existing health system in the country/countries that have eliminated malaria?
- What approaches to engaging community health workers in malaria elimination activities support sustaining their active engagement in service delivery in the country/countries that have eliminated malaria?
- What were the cost-effective strategies for optimal delivery of various components of a malaria elimination programme to targeted populations/locations developed in the country/countries that have eliminated malaria?
- What role has housing and rural/urban environmental improvements played in supporting malaria elimination and prevention of reintroduction in countries that have achieved malaria elimination?

Research (test hypotheses)

- That the integration of malaria elimination surveillance and response approaches can be cost-effectively integrated into other infectious disease surveillance systems.
- That communities can play an effective role in active efforts at transmission reduction (as opposed to reducing morbidity and mortality from malaria)?
- That routine primary healthcare services including maternal and child health services can sustain access to and utilization of individual and household-level malaria interventions to sustain malaria elimination and prevent reintroduction of malaria.
- That improved methods of data collection, synthesis, visualization, and real-time availability will increase the timeliness and effectiveness of health workers' responses to malaria cases in elimination settings.

R&D (develop and test tools, approaches/strategies, and models)

- Development of IMCI and IMAI updated with new diagnostic tools and adapted to the malaria elimination context.
- Development and scaling-up of tools to measure systems readiness for malaria elimination and prevention of reintroduction at local and subnational levels.
- Development of mechanisms to support and maintain financial and political responsiveness to malaria elimination and prevention of reintroduction.
- What are the messages and best means of conveying these to various communities/at risk populations to support access, acceptability, and utilisation of malaria interventions nearing elimination?

of additional questions linked to the priority questions to suggest key questions also to be answered.

A synthetic presentation of the full research portfolio is presented in this paper (Fig 3), color-coded for areas of activities: cross-cutting, governance, human resources, financing, information, service delivery, and commodities. Questions are ranked according to the health system level to which they pertain: all levels, community, facility, district, national, and regional/global. Keywords are used to refer to questions detailed later in the document. Following the synthetic table, the list of research questions is presented for evaluation science, research, and R&D, starting with priority questions (Table 1).

Capacity building and training

Capacity building and training is a priority cross-cutting all areas emphasized in this document. No positive outcome can be anticipated if an appropriately trained and competent work force cannot be relied upon. This is indeed an absolute prerequisite for well-functioning health systems and delivery of malaria control and elimination interventions.

Surveillance and M&E

A theme common to all programmes and systems areas is the critical role that surveillance and M&E play in every phase. Adequate surveillance and M&E methods must be in place to monitor effectiveness and inform the decision-making process. Surveillance and M&E fall under the 'surveillance-response' umbrella, where essential data are collected in space and time to inform well-tailored, integrated-response actions.

Supply-chain strengthening

A very common cause of loss of malaria programme effectiveness is the disruption in appropriate stocks of life-saving commodities for at-risk populations. No malaria control or elimination programme can hope to achieve its objectives without addressing the issue of stock outs of commodities and strengthening the supply chain so that an uninterrupted flow of necessary quality tools can be available at all times for people who most need them.

Community involvement and engagement

A critical element of success and of sustainability of any intervention, and particularly malaria elimination interventions, is community ownership and engagement. The RBM AIM (2015) noted as countries move along the path to elimination, resource requirements, processes, and services change, requiring national systems to adapt and improve, and to deepen their level of community engagement and in Chapter 6 discussed this in more detail, especially the role of people-centred and participatory research design and approaches [5].

Keeping a dynamic portfolio: Monitoring progress and dissemination of results

Multiple factors have slowed progress in the health systems and operations research agenda since the first malERA initiative, including funding, dependency upon existing national and provincial health systems to deliver, and limited buy-in by a range of disciplines into the malaria agenda. All of these factors have contributed to this limited uptake. The panel felt that in order to keep a dynamic portfolio and not lose traction again, monitoring the uptake of research priorities established during the malERA Refresh process was essential.

Fig 3. Overview of health systems and policy research portfolio. HIS, health information system; SOP, standard operating procedure.

https://doi.org/10.1371/journal.pmed.1002454.g003

The following processes could ensure proper monitoring of research activities: First, MESA, through the established tool of MESA Track and linking up with all research and implementation institutions involved, could be tasked with producing regular updates on progress accomplished [9]. The second important feedback would be from countries (national malaria control programmes level) reporting on evaluation science, research, and R&D tools development. A third approach is to use networks emerging throughout regions such as Asia Pacific Malaria Elimination Network (APMEN) [14] and the 8 eliminating southern African countries (Elimination 8 [E8]) [15] to both socialise and support the implementation of this research agenda, as well as provide platforms for dissemination of lessons learned from the research. This can use innovative platforms like case studies, focussed study tours, peer-to-peer mentoring, workshops, and health system fellowships.

Information collected through these 2 tracks would be regularly reviewed by the malERA Refresh committee and compared across the whole malERA spectrum. The results, regularly published, in combination with updates from national programmes and their partners on status of national research and implementation, would be included each year in the WHO World Malaria Report. Other channels for dissemination and garnering of scale-up support would be through platforms such as the political alliances of African Leaders Malaria Alliance and the Asia Pacific and Leaders Malaria Alliance [16,17]. The malERA Refresh has given and will continue to give the malaria community an opportunity to highlight perhaps the most important gaps in getting the world to the malaria-free goal; namely, the continuous and timely improvement in the operational delivery of interventions through health systems improvement.

Discussion

Setting health research priorities does not automatically guarantee uptake and funding, though there are examples of how it does [18]. And these health systems issues are 'wicked' problems for which no one solution will be found; solutions are not discrete from other systems' issues, and solutions cannot be divorced from socio-historical-political environments [19]. This agenda will also need a broad range of disciplines to engage in the implementation of the agenda beyond the 'malaria research community' to include social and behavioural sciences; political, management, and organisation science; health economics; and health systems specialists. Clearly, this health systems agenda to facilitate malaria eradication entails a transdisciplinary and multi-stakeholder approach and therefore needs to be more broadly disseminated and socialised in these scientific communities, and the findings from this research need to be more broadly disseminated to inform other health system interventions and their implementation, as well as health system strengthening in general.

As discussed in the section 'Keeping a dynamic portfolio', there is a need to rigorously provide evidence of the outcomes of the research against the overall eradication agenda. This evidence can attract more attention to and focus on the prioritised agenda for other researchers and funders. The research agenda links to GTS, the strategy, and AIM, the investment framework for action, and creates the essential third pillar on our journey to achieve elimination and eradication. Building the capacity of health and other staff and researchers in malarious countries to undertake health systems research, such as through the WHO TDR Structured Operational Research and Training IniTiative (SORT-IT) approach, is an important step, as many of these research topics need the 'localisation' required to understand the impact of different settings and health and social systems upon interventions and their effective implementation. Funding for these sorts of localised studies will likely need to be sourced from national budgets and/or small research grant schemes; be embedded into the M&E budgets of projects, donors, and governments; and/or be part of the university training and research agendas. The

larger number of 'case studies' can then allow some comparative analyses and synthesis through evaluation science to be undertaken at a larger scale to better identify enablers, barriers, approaches, and tools for others to 'trial'.

Conclusion

This remains an ambitious but essential research agenda for malaria eradication. Every tool or intervention developed, for vectors, parasites, drug administration, surveillance and response, etc., needs a health and social system operating in a socio-political system to deliver the intervention effectively. To address challenges relating to health systems, transdisciplinary and multi-stakeholder approaches need to be tested. Results from evaluation science, research and R&D in this field will likely generate multiple nuanced answers. This process will ensure that the research questions put forward in a portfolio approach can be tailored to any given setting, as one size will not fit all, and finally that the maximum efficacy of interventions is maintained universally across the different social, political, and economic differentials for populations to achieve malaria elimination and, finally, eradication.

Supporting information

S1 Text. Further details on panel methodology and prioritization process.
(DOCX)

Acknowledgments

The Malaria Eradication Research Agenda (malERA) Refresh Consultative Panel on Health Systems and Policy Research was chaired by Marcel Tanner, Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland, and University of Basel, Switzerland and co-chaired by Maxine Whittaker, James Cook University, Townsville, Australia. The paper was written based on consultations during a malERA meeting held in Boston, USA 20th June–22nd June 2015. Vittoria Lutje prepared literature searches for the panel and was funded by MESA. Eric Mouzin, Roll Back Malaria, Geneva, Switzerland, developed the original meeting report. Panel members reviewed several iterations of the paper to finalise it. Figures were designed by Rachel Papernick.

Members of the writing group met ICMJE criteria for authorship and were:

Marcel Tanner, Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland, and University of Basel, Basel, Switzerland, and Maxine Whittaker, James Cook University, Townsville, Australia.

malERA Refresh: Health Systems and Policy Research Panel Members:

Omari Kimbute Abdallah, National institute of Medical Research, Tanzania; Martin Alilio, Presidents Malaria Initiative, Washington, DC, USA; Andrea Bosman, WHO Global Malaria Programme, Geneva, Switzerland; Lesong Conteh, Imperial College London, UK; Seydou Doumbia, Malaria Research and Training Centre, University of Bamako-USTTB, Bamako, Mali; David Evans, Swiss TPH, Basel, Switzerland and University of Basel, Basel, Switzerland; Victoria Fan, University of Hawai'i at Mānoa, USA; Amanda Glassman, Center for Global Development, Washington, DC, USA; Roly Gosling, Global Health Group, University of California San Francisco, USA; Yusuf Lawal, University of Oxford, UK; Martin Madu Meremikwu, Cochrane Nigeria, Calabar, Nigeria; Eric Mouzin (Rapporteur), Roll Back Malaria, Geneva, Switzerland; Antoni Plasencia, ISGlobal, Barcelona Institute for Global Health, Spain; Franco Pagnoni, WHO Global Malaria Programme, Geneva, Switzerland; Risintha Premaratne, Ministry of Health, Sri Lanka; Ben Rolfe, Asia Pacific Leaders Malaria Alliance, Singapore;

Kathleen Rankin, Bill & Melinda Gates Foundation, Seattle, USA; Allan Schapira, Independent Consultant; David Schellenberg, London School of Hygiene & Tropical Medicine, UK; David Sintasath, President's Malaria Initiative, Bangkok, Thailand; Don de Savigny, Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland and University of Basel, Basel, Switzerland; Marcel Tanner (Chair), Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland and University of Basel, Basel, Switzerland; Fabrizio Tediosi, Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland and University of Basel, Basel, Switzerland; Kate Whitfield, ISGlobal, Barcelona Institute for Global Health, Spain; Maxine Whittaker (co-Chair), James Cook University, Townsville, Australia; Eve Worrall, Liverpool School of Tropical Medicine, UK.

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References

1. WHO. WHO World Malaria Report 2015. Geneva, Switzerland; 2015.
2. The malERA Consultative Group on Health Systems and Operational Research. A research agenda for malaria eradication: health systems and operational research. PLoS Med. 2011; 8(1):e1000397. https://doi.org/10.1371/journal.pmed.1000397 PMID: 21311588
3. Research WAfHPaS. Systems Thinking for Health Systems Strengthening. 2009.
4. WHO. Global Technical Strategy for malaria 2016–2030 WHO. Geneva, Switzerland; 2015.
5. WHO. Action and Investment to Defeat Malaria 2016–2030. 2015.
6. Malaria Eradication Scientific MESA. Available from: http://www.malariaeradication.org/. Cited 29 Sept 2017.
7. WHO. Malaria Policy Advisory Committee Available from: http://www.who.int/malaria/mpac/en/. Cited 29 Sept 2017.
8. WHO. Scaling up research and learning for health systems: now is the time. WHO, Geneva; 2008.
9. MESA Track database: Malaria Eradication Scientific Alliance (MESA); [Database]. Available from: http://www.malariaeradication.org/mesa-track. Cited 29 Sept 2017.
10. Hadorn GH, Pohl C. Principles for Designing Transdisciplinary Research. Munic, Germany: oekom verlag; 2007.
11. Delbecq AL, Van deVEn AH. A Group Process Model for Problem Identification and Program Planning. Journal of Applied Behavioral Science. 1971; 7:466–92.
12. Vlassoff C, Tanner M. The relevance of rapid assessment to health research and interventions. Health Policy and Planning. 1992; 7(1):1–9.
13. The malERA Refresh Consultative Panel on Combination Interventions and Modelling. malERA: An updated research agenda for combination interventions and modelling in malaria elimination and eradication. PLoS Med. 2017; 14(11):e1002453. https://doi.org/10.1371/journal.pmed.1002453
14. Whittaker M, Smith C, Mouzin E. The Asia Pacific Malaria Elimination Network: Supporting the common goal of a malaria free Asia Pacific. Geneva, Switzerland; 2014.
15. E8, the eight eliminating southern African countries. "Malaria Elimination 8 (E8)". Available from: https://malariaelimination8.org/. Cited 29 Sept 2017.
16. African Leaders Malaria Alliance. Available from:http://alma2030.org/. Cited 29 Sept 2017.
17. Asia Pacific Leaders Malaria Alliance. Available from: http://www.aplma.org. Cited 29 Sept 2017.
18. Evans DB, Etienne C. Health systems financing and the path to universal coverage. Bull World Health Organ. 2010; 88(6):402. https://doi.org/10.2471/BLT.10.078741 PMID: 20539847
19. Rittel H, Webber M. Dilemmas in a General Theory of Planning. Policy Science. 1973; 4:155–69.