PLoS MEDICINE

Table of Contents

PLoS MEDICINE

Review

A Research Agenda to Underpin Malaria Eradication

Pedro L. Alonso[1,2]*, Graham Brown[3], Myriam Arevalo-Herrera[4,5], Fred Binka[6], Chetan Chitnis[7], Frank Collins[8], Ogobara K. Doumbo[9], Brian Greenwood[10], B. Fenton Hall[11], Myron M. Levine[12], Kamini Mendis[13], Robert D. Newman[13], Christopher V. Plowe[14], Mario Henry Rodríguez[15], Robert Sinden[16], Laurence Slutsker[17], Marcel Tanner[18]

1 Barcelona Centre for International Health Research (Hospital Clínic, Universitat de Barcelona), Barcelona, Spain, 2 Centro de Investigaçao em Saude da Manhiça, Maputo, Mozambique, 3 Nossal Institute for Global Health, University of Melbourne, Melbourne, Australia, 4 Immunology Institute-Universidad del Valle, Cali, Colombia, 5 Centro de Investigación Caucaseco, Cali, Colombia, 6 School of Public Health, University of Ghana, Accra, Ghana, 7 International Center for Genetic Engineering and Biotechnology, Delhi, India, 8 University of Notre Dame, Notre Dame, Indiana, United States of America, 9 University of Bamako, Bamako, Mali, 10 London School of Hygiene & Tropical Medicine, London, United Kingdom, 11 National Institute of Allergy and Infectious Diseases (NIAID), Baltimore, Maryland, United States of America, 12 Center for Vaccine Development, University of Maryland, Baltimore, Maryland, United States of America, 13 Global Malaria Program, World Health Organization, Geneva, Switzerland, 14 Howard Hughes Medical Institute/University of Maryland School of Medicine, Baltimore, Maryland, United States of America, 15 Instituto Nacional de Salud Pública, Cuernavaca, Mexico, 16 Imperial College, London, United Kingdom, 17 US Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, 18 Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland

Abstract: The interruption of malaria transmission worldwide is one of the greatest challenges for international health and development communities. The current expert view suggests that, by aggressively scaling up control with currently available tools and strategies, much greater gains could be achieved against malaria, including elimination from a number of countries and regions; however, even with maximal effort we will fall short of global eradication. The Malaria Eradication Research Agenda (malERA) complements the current research agenda—primarily directed towards reducing morbidity and mortality—with one that aims to identify key knowledge gaps and define the strategies and tools that will result in reducing the basic reproduction rate to less than 1, with the ultimate aim of eradication of the parasite from the human population. Sustained commitment from local communities, civil society, policy leaders, and the scientific community, together with a massive effort to build a strong base of researchers from the endemic areas will be critical factors in the success of this new agenda.

Introduction

The unacceptable health burden of malaria, and its economic and social impacts on development, have made it a focal point of the international development agenda, and the world has embraced an ambitious plan for scaling up malaria control that progresses towards country-by-country and regional elimination and the ultimate goal of global eradication [1]. Over the past decade, resources and control efforts have intensified to a level not seen since the early days of the World Health Organization's Global Malaria Eradication Program (GMEP) in the late 1950s. Nonetheless, in 2009, with 3.28 billion people living in areas that have some risk of malaria transmission and about 1.2 billion people (one-fifth of the world's population) living in areas with a high risk of transmission (more than one reported case per 1,000 population per year), there were about 225 million cases of clinical malaria and 781,000 malaria-related deaths. Today, there is ongoing malaria transmission in 106 countries. Eighty-one of these countries are focusing on control, while 25 are in pre-elimination,

elimination, and prevention of reintroduction phases; Morocco, the United Arab Emirates, and Turkmenistan have recently been certified as malaria free [2–4].

These statistics emphasize the direness of the current malaria burden but also benchmark the accomplishments and progress that have been achieved in malaria control. Following declarations at the Malaria Forum in October 2007 convened by the Bill & Melinda Gates Foundation, and subsequent support voiced by the World Health Organization (WHO), the Roll Back Malaria (RBM) Partnership, and many other organizations and institutions, the paradigm of malaria control and elimination has been extended to encompass an ultimate goal of malaria eradication [1,2,5]. The question is no longer whether international agencies and national health authorities should be mobilized to pursue the goal of malaria eradication, but rather when and how.

A key question, however, is whether elimination from all regions of the world (eradication) is feasible with the current tools and state of knowledge. For a number of reasons, we believe that the answer is "no." First, malaria is not a single disease. The five *Plasmodium* species *(falciparum, vivax, ovale, malariae, knowlesi)* that cause human malaria are transmitted by more than 30 Anopheline mosquito species with diverse breeding and feeding habits, and result in different disease spectra in different population target groups and epidemiological settings. Second, current malaria control and elimination programs face remarkable heterogeneity of transmis-

Review articles synthesize in narrative form the best available evidence on a topic.

Summary Points

- Malaria remains a major global public health problem, but a recent paradigm shift has moved the emphasis from control of malaria to the interruption of malaria transmission and ultimately malaria eradication
- The Malaria Eradication Research Agenda (malERA) initiative was convened in 2008 to define the knowledge base, strategies, and tools required to eradicate malaria from the human population
- A two-year consultative process has resulted in the preparation of a detailed research and development agenda for malaria eradication, which is reported in this Supplement
- Implementation of this research agenda might enable the elimination of malaria, even in the most difficult areas
- However, to achieve the aim of malaria eradication in a timely manner, commitment to implementing this agenda must begin immediately

Box 1. Clarifying the Goals and Definitions

- **Control:** Reduction of disease incidence, prevalence, morbidity, or mortality to a locally acceptable level as a result of deliberate efforts; continued intervention measures are required to maintain the reduction.
- **Elimination:** Reduction to zero of the incidence of locally transmitted malaria infection in a defined geographical area as a result of deliberate efforts; continued intervention measures are required to prevent reestablishment of transmission.
- **Eradication:** Permanent reduction to zero of the global incidence of malaria as a result of deliberate efforts; intervention measures are no longer needed [1].
- **What species?** Although the eradication of *P. falciparum*, the most serious form of malaria, would constitute an historic public health achievement, the coexistence of transmission of *P. falciparum* and *P. vivax* in many areas of the world together with the fact that they are the species responsible for the major burden of disease, make it necessary to aim for the eradication of both.

sion dynamics of malaria in endemic areas, including differences in parasite, vector, human, social, and environmental factors. Third, operational limitations include underperforming health services, lack of political will, insufficient financial, social and human resources, and for some areas, inadequate tools to interrupt transmission given an exceedingly high force of transmission. Each country presents different combinations of these problems and their determinants. Thus, a widely held view suggests that with currently available tools, much greater gains could be achieved, including elimination from a number of countries and regions, but that even with maximal effort we will fall short of elimination in many areas and of global eradication [6]. For definitions of terms used regarding malaria eradication see Box 1.

Mixed Success and Failure of Past Malaria Control and Elimination Efforts

A detailed discussion of all the factors involved in the partial success of the past eradication campaign is beyond the scope of this introduction, but three critical elements can be highlighted. First, there was insufficient recognition of the heterogeneity of malaria transmission and disease. Much of the optimism that inspired WHO GMEP in 1955 was based on the successful outcomes of earlier control programs that benefited from a combination of biological, parasitological, social, and environmental factors that favoured success (e.g., the rarity of DDT-resistant Anophelines and of chloroquine-resistant parasites). Second, the first WHO GMEP (1955-1969) was predicated on an assumption that the available knowledge and tools were sufficient to achieve worldwide eradication. A single strategy that would work everywhere—"one size fits all"—proved to be ill-founded because it underestimated the challenges of dealing with the extremely efficient vectors in Africa (*An. gambiae)* and with transmission by outdoor-feeding mosquitoes that were not susceptible to attack by indoor residual insecticide. It also did not allow for the lack of safe drugs for mass administration to remove all infectious parasites from symptomatic and asymptomatic carriers, particularly from people carrying *P. vivax* or *P. ovale*, species that establish latent liver infections that are responsible for relapses months or years following initial infection. Third, insufficient research in biomedical and social sciences and inadequate local application of research findings across a wide

variety of settings are widely viewed to have contributed to demoralization and waning effort when tools proved ineffective or could not be adequately implemented. The neglect of malaria research during and after the campaign did long-term damage. These elements resulted in a lack of progress that in turn compromised continued financial support [7].

Current Malaria Control Efforts: The Goal of Eradication and Its Research and Development Implications

The past decade has witnessed renewed investment in malaria control and substantial increases in funding for malaria research. The Roll Back Malaria Global Malaria Action Plan (GMAP) and WHO have recently revised and updated the strategy and the steps for scaling up and sustaining malaria control (Figure 1). In addition, the Malaria Elimination Group (MEG), a group of scientists, public health decision makers, control program managers, and funders, has compiled a guide to policy makers for areas that embark or have embarked on elimination strategies [8].

Reductions in disease incidence are being documented, even in some areas of sub-Saharan Africa that constitute the heartland of malaria transmission [2]. There are, however, significant threats to current progress that cannot be ignored, and unmet needs that will continue to be central to the global research agenda for improving malaria control and eventually achieving eradication. Notable examples are the emergence of artemisinin resistance and the consequent need for improved strategies to contain dissemination of resistant parasite strains coupled with accelerated research into potential new drugs for first-line treatment [9,10]. Similarly, new insecticides are urgently needed to replace those threatened by increased mosquito resistance [11], and accelerated development of vaccines that can impact on malaria incidence, disease, and death remains a high priority [12].

Complementing the current research agenda—primarily directed towards improving malaria control and reducing morbidity and mortality—with research on developing tools, interventions, and strategies to interrupt transmission and ultimate eradication of the parasite from the human population constitutes a true paradigm shift.
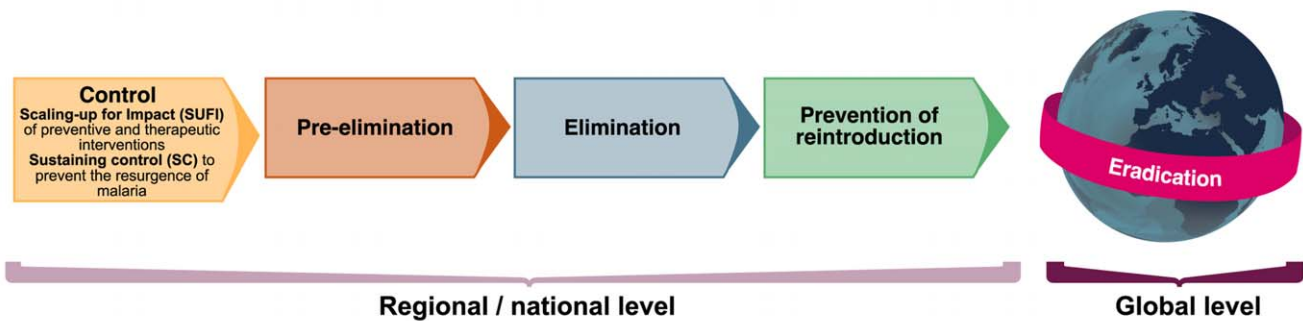
Figure 1. Epidemiological milestones [**1,23**]**.** Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000406.g001

The malERA Initiative

To catalyze this paradigm shift towards malaria elimination and eradication, it was necessary to design a process to bring together the best scientific minds in the malaria community. That process is the Malaria Eradication Research Agenda (malERA) initiative, which was established to complement GMAP and which aims to define the critical knowledge base, strategies, and tools required to reduce the basic reproduction rate (R_0 or the number of secondary cases arising from a single case) to less than one.

Scientists involved in malaria research were challenged to develop a multidisciplinary, global research and development agenda that would be actionable by research and public health agencies and funders/sponsors and available for discussion and debate through publication in a readily accessible format. The process engaged more than 250 scientists in a series of 20 consultations around the world (Figure 2) and was managed by a three-tier governance structure (Figure 3). The rest of this article briefly introduces the work undertaken by the various malERA Consultative Groups and presented in the other articles in this Supplement.

Tools to Interrupt Malaria Transmission

To reduce the basic reproduction rate to less than 1, and hence to interrupt transmission, interventions are needed to reduce the reservoir of infection, the time that a person or a mosquito is infectious, and the rate at which infections are spread. This goal can be achieved by drugs or vaccines directed against the parasite or by new tools that attack the vector, with the support of improved diagnostics and surveillance.

Drugs: Single Encounter Radical Cure and Prophylaxis

In the recent past, drug development efforts were guided by the need for first-line drugs to treat *P. falciparum* infections with an increasing emphasis on drugs with a short half-life that potentially minimize the risk of development of resistance rather than on drugs with a long half-life that have benefits for dosing and post-treatment prophylaxis [13]. Treatment of infected individuals with a variety of drug regiments has been used successfully in combination with intensive vector control to eliminate malaria from areas with relatively strong health systems and stable populations. However, interruption of malaria transmission is likely to require a new set of drugs and formulations.

As described in more detail in the article by the malERA Consultative Group on Drugs [14], such drugs will need to be used both in stable transmission areas and in complex urban or remote rural areas, with poorly functioning health systems where concerted campaigns may be the only way of achieving high coverage or

preventing reintroduction by migrants or travelers from endemic regions. For such campaigns to impact effectively on inaccessible populations, a single encounter between health providers and target populations is critical. Single Encounter Radical Cure and Prophylaxis (SERCaP) has a target product profile (TPP) that includes radical cure, defined as elimination of all parasites (including the long-lived hypnozoites of *P. vivax* or *P. ovale* in the liver), suitability for mass administration (including administration to healthy subjects 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. A drug with this profile would perform in a similar way to a highly efficacious pre-erythrocytic (infection-preventing) vaccine.

A drug with this TPP may take a long time to develop, but the development of new drugs that meet some of these essential requirements could dramatically improve chances of eradication. For example, development of new safe and effective drugs that block the infectivity of the mature sexual forms of *P. falciparum* gametocytes and/or the dormant hepatic forms (hypnozoites) of *P. vivax* could have a profound impact on transmission rates and would be valuable tools in the efforts to contain and eliminate parasite strains resistant to first-line treatment drugs. Presently, only the 8-aminoquinolines are known to be effective against both *P. vivax* hypnozoites and *P. falciparum* stage-five gametocytes. Unfortunately this class of drugs has significant side-effects in some individuals, particularly hemolysis in those with G6PD deficiency, that compromise their widespread use in mass administration for elimination [14].

Vaccines that Interrupt Malaria Transmission

Vaccines currently in clinical development have the primary aim of reducing morbidity and mortality from *P. falciparum* in young children living in highly endemic countries. However, with the new goal of elimination and eradication, vaccines that will reduce and contribute to interruption of transmission also need to be developed. The broader concept of "vaccines that interrupt malaria transmission (VIMT)" is introduced by the malERA Consultative Group on Vaccines to replace the term "transmission blocking vaccines" (TBVs), which has been used widely to refer to vaccines that target only the sexual and mosquito stages of the parasite [15]. VIMT could include antivector vaccines that target mosquito molecules essential for parasite development, highly effective pre-erythrocytic or erythrocytic stage vaccines, and vaccines targeting parasite antigens of sexual and mosquito stages of the infection. The desired TPP identified by the Consultative Group for VIMT indicates that they should be effective against both *P. falciparum* and *P. vivax*, suitable for administration to all age groups, and should impact transmission. Other issues discussed by the group in their article include the need
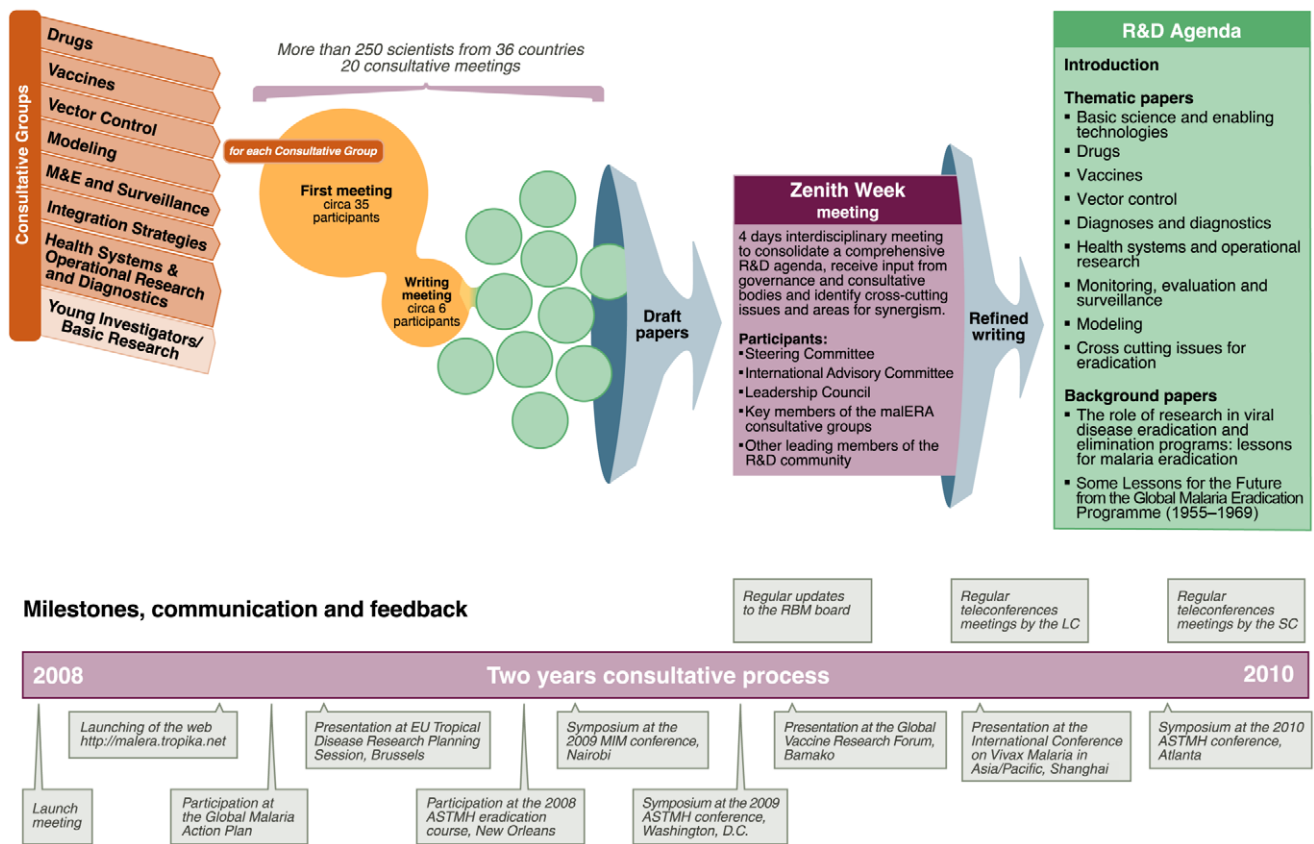
Figure 2. Consultative process towards a consolidated research and development agenda for malaria eradication. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000406.g002

for validated functional assays that measure the reduction in infectivity at the individual level after vaccination that could be used as surrogate measures to predict reductions in transmission rates at the community level. Such surrogate measures will be critical components of a regulatory pathway leading to licensure. Standardized, specific and sensitive methods for assessment of transmission rates, particularly when intensity is low, will be critical in the assessment of vaccine efficacy in interrupting transmission following large-scale deployment of vaccination as an elimination tool [15,16].

Vector Control

The overarching goal of vector control is to reduce the vectorial capacity of local vector populations below the critical threshold to prevent ongoing or epidemic transmission. Because it takes a relatively long time (days) after ingestion for *Plasmodia* to become infective to humans in its *Anopheles* vectors, the most effective vector control strategies currently in use rely on interventions like indoor residual insecticide spraying and insecticide treated bednets (ITNs) that reduce vector daily survival rates [17].

The malERA Consultative Group on Vector Control identifies three critical challenges in its article [18]. The most pressing challenge is the development of a coherent research agenda for discovering and developing a broader range of insecticides, with novel modes of action that can circumvent emerging resistance to existing insecticides, in particular, pyrethroid-based insecticides [11]. The second challenge is the development of interventions that affect vectors that do not rest or feed indoors and are therefore not susceptible to current tools. The final critical challenge is the development of novel approaches that permanently reduce the high

vectorial capacities of the dominant malaria vectors in sub-Saharan Africa. Genetic control programs based on permanent reduction of the vectorial capacities of natural vector populations have received the most attention to date [19,20], but the Consultative Group also considers the development of other novel approaches [18].

Diagnostics

Methods for measuring transmission are central to an elimination agenda. Current methods for measuring transmission that may be applied in endemic areas are time-consuming, expensive, and too insensitive for use in conditions of low and nonuniform infection [21,22]. Some years after regional elimination, as immunity declines, infection is likely to be symptomatic and may become the best marker of resumed transmission. However, during the early elimination phase in regions previously experiencing high transmission, populations will retain clinical immunity and will not experience symptomatic disease with every infection [23]. Thus, the main challenge identified by the malERA Consultative Group on Diagnoses and Diagnostics and discussed in detail in their article and in the article on Cross-cutting Issues for Eradication [24,25] is to find a robust, sensitive, and specific standardized method for assessment of transmission intensity in the intervening period when transmission continues at low and nonrandom levels. Improved serological tests have been suggested [26], but other minimally invasive biomarkers could be considered. This information will be essential for modeling potential effects of various interventions alone, or in combination, and for assessing efficacy of transmission–reducing vaccines and drugs. Other challenges for diagnostics discussed by the Consultative
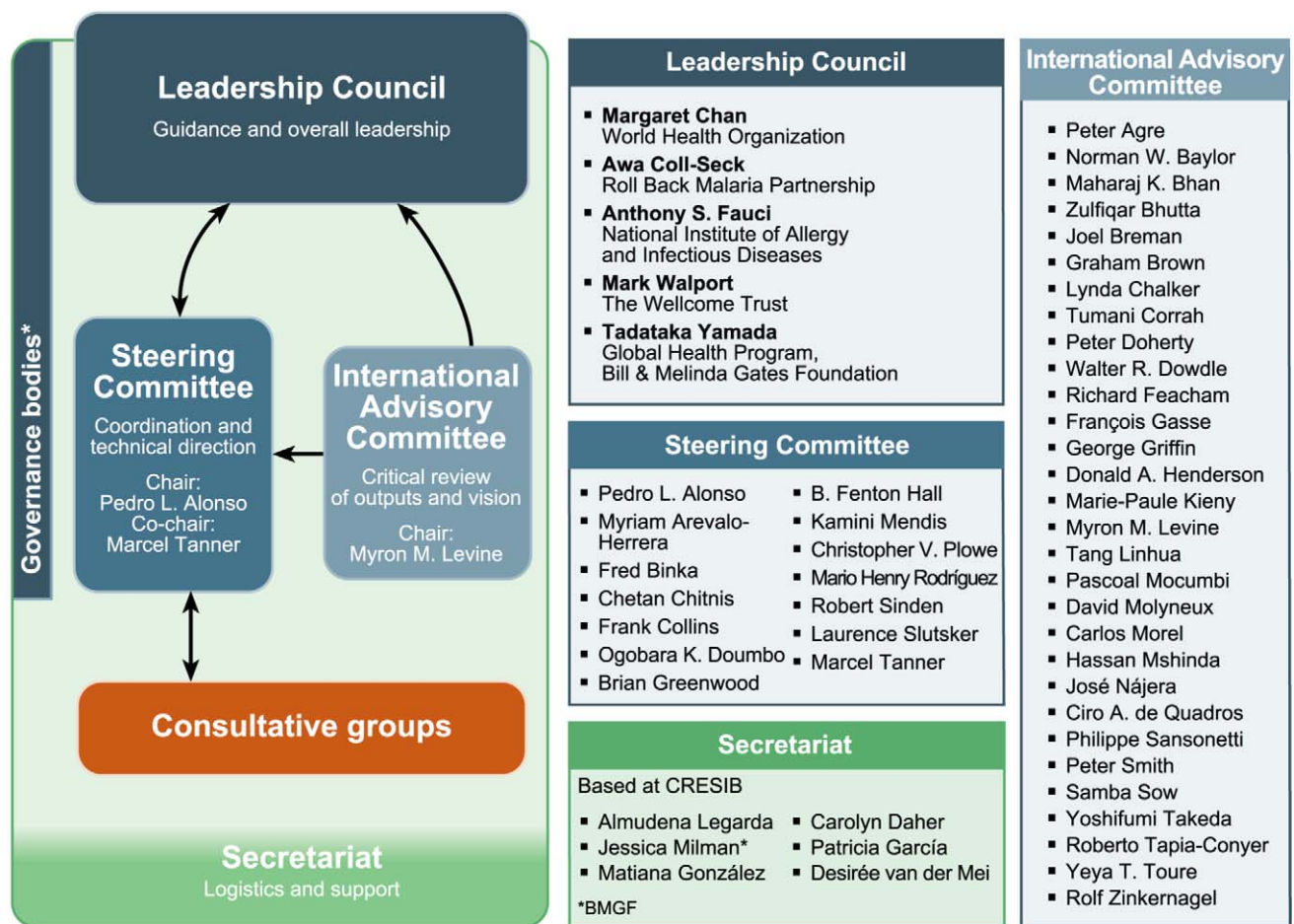
Figure 3. The malERA governance bodies. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000406.g003

Group include the need for tools that can rapidly detect and monitor unexpectedly high transmission that leads to outbreaks and that can identify reintroduction of infections that may be asymptomatic [16,24].

Beyond the Tools: Supporting Strategies and the Knowledge Base

Modeling and Harmonized Data Systems

Substantial advances have been made recently in computational approaches for modeling malaria epidemiology and in model-based approaches to economic evaluation [27–29]. As discussed by the malERA Consultative Group on Modeling [16], a significant research challenge for malaria eradication will be to integrate these new approaches into the planning of elimination, surveillance, monitoring, and evaluation, and to create appropriate interfaces for different user communities, including researchers, global and national policy makers, and local-level planners. Modeling can inform the definition of TPPs for new tools and intervention strategies and will be needed throughout a global eradication campaign to analyze the likely effects on malaria and of various elimination strategies and the costs of these strategies [30].

Importantly, a single unifying model will be insufficient to meet all these needs, so multiple modeling efforts need to be coordinated and made accessible to everyone. This harmonization and validation process will require close, iterative collaboration between software engineers, researchers, and malariologists to develop the necessary computer systems and connectivity (cyberinfrastructure). It will also necessitate the creation and maintenance of properly annotated and accessible malariometric databases that include all the research results needed to insert parameters into the models and the model outputs. How this can be achieved is considered in detail by the Consultative Group in their article [16].

Enabling Technologies and Platforms

The development of new tools for elimination is critically dependent on a vibrant and coherent agenda for basic sciences. We believe there are at least two potentially transformative developments that need to be pursued. First, continuous laboratory culture of *P. vivax*, *P. ovale*, and *P. malariae* needs to be developed to provide an essential platform for studying the biology of the liver stages and sexual forms of these parasites. These forms could be important targets of intervention strategies with drugs, vaccines, or other biological or chemical agents aimed at interrupting transmission. Second, systems analyses of transcrip-

tion, proteome, and metabolome libraries, rapid screening of drug libraries, high-throughput approaches to antigen identification, and the functional definition of gene products are all feasible but not yet fully exploited, but would bring important new tools to the bench scientist and to field operations. These and other aspects of enabling technologies and platforms are considered in detail in the articles prepared by the malERA Consultative Groups on Basic Science and Enabling Technologies and on Cross-cutting Issues for Eradication [25,31].

Health Systems Integration, Operational Research, and Effectiveness-Decay Analysis

The previous formal attempt at global eradication of malaria (1955–1969) depended largely on vertical operations that often bypassed health systems and their health services because it was assumed that eradication operations could be run most efficiently in this way. Many of the elimination efforts failed, because the health systems failed, leading to a pessimistic view that malaria can only be eliminated where economic progress, governance, and efficient health systems are in place to support maintenance of conditions necessary to block transmission [32,33].

It is now clear that the long-term solution to malaria elimination and eradication will require a systems approach in which malaria-specific interventions and actions are integrated into existing health systems [34]. To achieve this, research is needed into health systems, their readiness to optimize novel programs, systems, tests, or other interventions, and their continuing performance [35–37]. During their deliberations, the malERA Consultative Group on Health Systems and Operational Research identified the need for a substantial research approach to establish and validate a tool kit that allows effectiveness-decay analysis of health system impediments to effective and equitable coverage of malaria interventions and that allows decisions to be made on the degree of possible integration of interventions into an existing health system [16,38]. A further critical component of the research agenda identified by this Consultative Group is the development and validation of a decision-making framework to guide the move from control to elimination.

Finally, but equally importantly, the article by the malERA Consultative Group on Monitoring, Evaluation, and Surveillance considers the need to investigate the performance of surveillance, monitoring, and evaluation by new and old technologies [39,40] and to evaluate optimal strategies for implementation of surveillance as an active responsive intervention to further reduce transmission [41].

Training

The last time the world community tried to eliminate malaria, so the joke goes, the only thing that was eliminated was malariologists. For a renewed malaria eradication campaign to
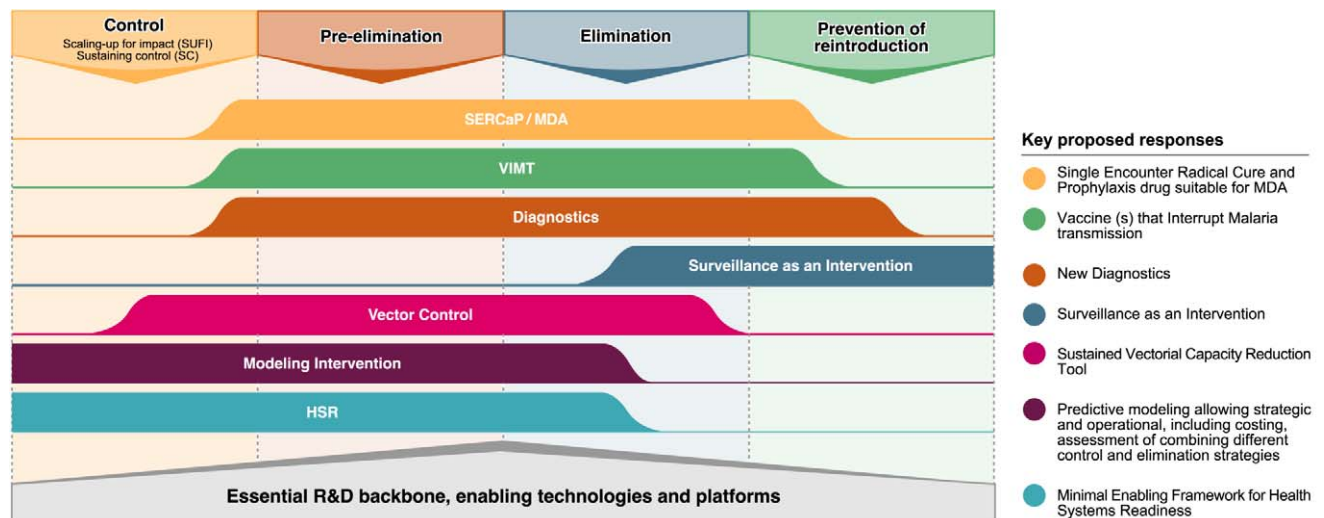
Figure 4. Key research and development issues and their position in relation to the different epidemiological phases towards eradication. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000406.g004

have a chance to succeed, it will be essential to train the malariologists and scientists in the multiple disciplines needed for an eradication campaign that might last 50 years, especially in endemic countries. This need cannot be overemphasized. The malaria research community remains small and often dominated by the views and strategies of scientists who sit far away from the problems. A massive effort to train, empower, and sustain research capacity in the endemic countries will be a critical factor for the success of improved control efforts and for the ultimate elimination and eradication of malaria.

Concluding Remarks

The past 2 years have reinvigorated an old malaria paradigm in which reduction of transmission is the driving strategy for malaria interventions. The malaria community has now used the malERA process to propose a research and development agenda that will be essential for regional elimination and eventual global eradication of malaria. Not every tool or strategy considered by the malERA Consultative Groups (see Box 2) will be essential in every situation (see Figure 4), but the complexity and heterogeneity, and in some places, the sheer intensity of transmission, demand that we start without delay to prepare for the most difficult challenges. This focus on the end goal of eradication must not displace our determination and efforts to continue to scale up ongoing efforts for control and to include a research agenda for reducing morbidity in areas of continuing moderate or high transmission. Rather, it must encourage us to supplement our efforts with a structured agenda that can realize the ultimate goal of eradication envisaged by the Global Malaria Action Plan and the Roll Back Malaria Partnership. An important lesson we can learn from other disease elimination efforts is that complacency is dangerous. The parasite and the vector are always evolving, and the human environment is always changing. Thus, new research questions will continually arise during the course of elimination [42], and active malaria research, particularly on the development of new tools, must continue up to the point when eradication is finally achieved. We anticipate that the results of research efforts proposed by our Consultative Groups for each stage of progression, from scaling up for improved control to the elimination phases, will have great synergy in design and application.

Past efforts at disease eradication, successful or otherwise, have highlighted the importance of sustained commitment from local communities, civil society, policy leaders, and the scientific community to implement research in the context of the desired integration of services, sector wide approaches, harmonisation of activities, and long-term funding commitment. Thus, research areas such as social science or research into direct and indirect economic benefits of malaria eradication also need to be strengthened. With these drivers in place, and the development of the new tools we describe briefly here and in the other articles in this Supplement, it may be possible to fulfil the dream that malaria eradication can be achieved within the lifetime of young scientists just embarking on their careers, even in the most difficult areas where current tools/strategies have proven to be insufficient. The time course may be long, but to have a chance of realizing that dream, the commitment to starting those research and development efforts must begin now.

Supporting Information

Text S1 malERA governance bodies
Found at: doi:10.1371/journal.pmed.1000406.s001 (0.05 MB DOC)

Text S2 malERA launch meeting participants
Found at: doi:10.1371/journal.pmed.1000406.s002 (0.06 MB DOC)

Author Contributions

ICMJE criteria for authorship read and met: PLA GVB MA FB CEC FHC OKD BG BFH MML KM RDN CVP MHR RES LS MT. Agree with the manuscript's results and conclusions: PLA GVB MA FB CEC FHC OKD BG BFH MML KM RDN CVP MHR RES LS MT. Wrote the first draft of the paper: PLA GVB MT. Contributed to the writing of the paper: PLA GVB MA FB CEC FHC OKD BG BFH MML KM RN CVP MHR RES LS MT. Contributed to the development of the research agenda: PLA GVB MA FB CEC FHC OKD BG BFH MML KM RDN CVP MHR RES LS MT. Members of the malERA steering committee which coordinated the malERA review process and guided the direction of the other papers in the series: PLA MA FB CEC FHC OKD BG BFH KM CVP MHR RES LS MT. Members of the malERA International Advisory Committee: GB MML. Contributed conceptually to the whole process leading to this paper: PLA GVB MA FB CEC FHC OKD BG BFH MML KM RDN CVP MHR RES LS MT.

References

1. Roll Back Malaria Partnership (2008) The global malaria action plan for a malaria free world. Geneva: RBM, Available: http://www.rollbackmalaria.org/gmap/gmap.pdf.
2. World Health Organization (2010) World malaria report. Geneva: World Health Organization, Available: http://whqlibdoc.who.int/publications/2010/9789241564106_eng.pdf.
3. World Health Organization (2010) Weekly epidemiological record 85, 229-236. Available: http://www.who.int/wer/2010/wer8524.pdf.
4. World Health Organization (2010) Weekly epidemiological record 85, 461-472. Available: http://www.who.int/wer/2010/wer8547/en/index.html.
5. Bill & Melinda Gates Foundation Malaria Forum (2007) Day-2 transcript. 17-10-2007. Available: http://www.gatesfoundation.org/speeches-commentary/Pages/melinda-french-gates-2007-malaria-forum.aspx.
6. Mendis K, Rietveld A, Warsame M, Bosman A, Greenwood B, et al. (2009) From malaria control to eradication: The WHO perspective. Trop Med Int Health 14: 802–809.
7. Nájera J, González-Silva M, Alonso, PL (2010) Some lessons for the future from the Global Malaria Eradication Programme (1955-1969). PLoS Med 8: e1000412. doi:10.1371/journal.pmed.1000412.
8. Feachem RGA, Phillips AA, Targett GA, eds (2009) Shrinking the malaria map: A prospectus on malaria elimination. San Francisco: The Global Health Group, Global Health Sciences University of California, San Francisco.
9. Plowe CV (2009) The evolution of drug-resistant malaria. Trans R Soc Trop Med Hyg 103: S11–S14.
10. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, et al. (2009) Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 361: 455–467.

11. Ranson H, Abdallah H, Badolo A, Guelbeogo WM, Kerah-Hinzoumbé C, et al. (2009) Insecticide resistance in *Anopheles gambiae*: data from the first year of a multi-country study highlight the extent of the problem. Malar J 8: 299.
12. Greenwood B, Fidock DA, Kyle DE, Kappe SH, Alonso PL, et al. (2008) Malaria: Progress, perils and prospects for eradication. J Clin Invest 118: 1266.
13. Nyunt MM, Plowe CV (2007) Pharmacologic advances in the global control and treatment of malaria: combination therapy and resistance. Clin Pharmacol Ther 82: 601–605.
14. The malERA Consultative Group on Drugs (2011) A research agenda for malaria eradication: Drugs. PLoS Med 8: e1000402. doi:10.1371/journal.pmed.1000402.
15. The malERA Consultative Group on Vaccines (2011) A research agenda for malaria eradication: Vaccines. PLoS Med 8: e1000398. doi:10.1371/journal.pmed.1000398.
16. The malERA Consultative Group on Modeling (2011) A research agenda for malaria eradication: Modeling. PLoS Med 8: e1000403. doi:10.1371/journal.pmed.1000403.
17. Enayati A, Hemingway J (2010) Malaria management: Past, present, and future. Ann Rev Entomol 55: 569–591.
18. The malERA Consultative Group on Vector Control (2011) A research agenda for malaria eradication: Vector control. PLoS Med 8: e1000401. doi:10.1371/journal.pmed.1000401.
19. Terenius O, Marinotti O, Sieglaff D, James AA (2008) Molecular genetic manipulation of vector mosquitoes. Cell Host Microbe 13: 417–423.
20. Sinkins SP, Gould F (2006) Gene drive systems for insect disease vectors. Nat Rev Genet 7: 427–35.
21. Bell D, Wongsrichanalai C, Barnwell JW (2006) Ensuring quality and access for malaria diagnosis: how can it be achieved? Nat Rev Microbiol. pp S7–S20.
22. Paris DH, Imwong M, Faiz AM, Hasan M, Yunus EB, et al. (2007) Loop-mediated isothermal PCR (LAMP) for the diagnosis of falciparum malaria. Am J Trop Med Hyg 77: 972–976.
23. World Health Organization (2007) Malaria elimination: a field manual for low and moderate endemic countries. Geneva: World Health Organization.
24. The malERA Consultative Group on Diagnoses and Diagnostics (2011) A research agenda for malaria eradication: Diagnosis and diagnostics. PLoS Med 8: e1000396. doi:10.1371/journal.pmed.1000396.
25. The malERA Consultative Group on Integration Strategies (2011) A research agenda for malaria eradication: Cross-cutting issues for eradication. PLoS Med 8: e1000404. doi:10.1371/journal.pmed.1000404.
26. Bousema T, Youssef RM, Cook J, Cox J, Alegana VA, et al. (2010) Serologic markers for detecting malaria in areas of low endemicity, Somalia 2008. Emerging Infect Dis 16: 392–399.
27. Ghani AC, Sutherland CJ, Riley EM, Drakeley CJ, Griffinet JT, et al. (2009) Loss of population levels of immunity to malaria as a result of exposure-reducing interventions: consequences for interpretation of disease trends. PLoS One 4: e4383. doi:10.1371/journal.pone.0004383.
28. McKenzie FE, Bossert WH (2005) An integrated model of *Plasmodium falciparum* dynamics. J Theor Biol 232: 411–426.
29. Smith T, Killeen GF, Maire N, Ross A, Molineaux L, et al. (2006) Mathematical modeling of the impact of malaria vaccines on the clinical epidemiology and natural history of *Plasmodium falciparum* malaria: Overview. Am J Trop Med Hyg 75: 1–10.
30. Smith DL, Smith T, Hay SI (2009) "Measuring malaria for elimination. Feachem RGA, Phillips AA, Targett GA, eds. Shrinking the malaria map: A prospectus on malaria elimination. San Francisco: The Global Health Group, Global Health Sciences University of California, San Francisco. pp 108–126.
31. The malERA Consultative Group on Basic Science and Enabling Technologies (2011) A research agenda for malaria eradication: Basic science and enabling technologies. PLoS Med 8: e1000399. doi:10.1371/journal.pmed.1000399.
32. Alilio MS, Bygbjerg IC, Breman JG (2004) Are multilateral malaria research and control programs the most successful? Lessons from the past 100 years in Africa. Am J Trop Med Hyg 71: 268–278.
33. Nájera JA (2001) Malaria control: Achievements, problems and strategies. Parassitologia 43: 1–89.
34. Tanner M, de Savigny D (2008) Malaria eradication back on the table. Bull World Health Organ 86: 82–83.
35. de Savigny D, Adam T (2009) Health systems thinking for health systems strengthening. Geneva: World Health Organization.
36. World Health Organization (2007) Everybody's business: Strengthening health systems to improve health outcomes: WHO's framework for action. Geneva: World Health Organization.
37. World Health Organization (2008) World health report 2008 – Primary health care: More than ever. Geneva: World Health Organization.
38. The malERA Consultative Group on Health Systems and Operational Research (2011) A research agenda for malaria eradication: Health systems and operational research. PLoS Med 8: e1000397. doi:10.1371/journal.pmed.1000397.
39. Hay SI, Smith DL, Snow RW (2008) Measuring malaria endemicity from intense to interrupted transmission. Lancet Infect Dis 8: 369–378.
40. Drakeley C, Cook J (2009) Potential contribution of sero-epidemiological analysis for monitoring malaria control and elimination: Historical and current perspectives. Adv Parasitol 69: 299–352.
41. The malERA Consultative Group on Monitoring, Evaluation, and Surveillance (2011) A research agenda for malaria eradication: Monitoring, evaluation, and surveillance. PLoS Med 8: e1000400. doi:10.1371/journal.pmed.1000400.
42. Breman JG, Quadros CA, Dowdle WR, Foege WH, Henderson DA, et al. (2011) The role of research in viral disease eradication and elimination programs: Lessons for malaria eradication. PLoS Med 8: e1000405. doi:10.1371/journal.pmed.1000405.

Review

A Research Agenda for Malaria Eradication: Basic Science and Enabling Technologies

The malERA Consultative Group on Basic Science and Enabling Technologies¶*

Abstract: Today's malaria control efforts are limited by our incomplete understanding of the biology of *Plasmodium* and of the complex relationships between human populations and the multiple species of mosquito and parasite. Research priorities include the development of in vitro culture systems for the complete life cycle of *P. falciparum* and *P. vivax* and the development of an appropriate liver culture system to study hepatic stages. In addition, genetic technologies for the manipulation of *Plasmodium* need to be improved, the entire parasite metabolome needs to be characterized to identify new druggable targets, and improved information systems for monitoring the changes in epidemiology, pathology, and host-parasite-vector interactions as a result of intensified control need to be established to bridge the gap between bench, preclinical, clinical, and population-based sciences.

Introduction

The current malaria control effort has focused on developing existing products and procedures (for example, drugs and the distribution of bednets) to reduce malaria morbidity and mortality. However, it is commonly accepted that eradication will not be achieved with current tools. Thus, we must now accelerate the development of a new generation of tools and knowledge aimed specifically at malaria eradication. As we look towards this ambitious goal, we must recognize that cutting-edge basic science, novel research strategies, and creative multidisciplinary approaches all need to be mobilized to bridge the gap between bench, preclinical, clinical, and population-based sciences. The malaria science community is now at a turning point where major advances are needed to move the field forward from control towards the goal of global malaria eradication.

The malERA Consultative Group on Basic Science and Enabling Technologies was convened to identify the major knowledge gaps in basic science and to prioritize basic/fundamental science approaches that might have an impact on malaria eradication, particularly with respect to the design of vaccines, drugs, and diagnostics. We recognize that there are likely to be many more research questions that merit equal importance in the broad field of malaria biology than we can cover in this paper, but herein we highlight only those approaches that were discussed by the consultative group and that may have a direct bearing on malaria eradication.

Leading the charge are new molecular, chemical, immunological, and epidemiological research tools that, whilst requiring adaptation to malaria, have realizable rewards in the near future. In particular, developments in systems biology, metabolomics, glycomics, and lipid metabolism and new high-throughput approaches involving chemical biology are likely to be of great

use in the field of malaria eradication. From these new avenues of investigation, it is reasonable to expect advances in vaccine development and the identification of novel drug targets. Moreover, the interlacing of high-throughput molecular technologies with population studies will greatly facilitate the rational application of interventions in diverse malaria-endemic environments and, we anticipate, will significantly increase our ability to shed light on complex and heterogeneous host-parasite-vector interactions.

At the basic science level, we specifically identified a deeper understanding of the whole parasitic life cycle and the interaction of the parasite with human and vector hosts at different stages as a high priority. Such knowledge will augment our ability to evaluate current and future interventions and allow us to determine the potential action of drugs or vaccines across all parasite developmental stages. In addition, a life cycle–based perspective will provide insights into the transitions from one host to another and highlight key points, triggers, decisions, and co-incident events as the parasite moves from one life stage to the next that could prove crucial in malaria eradication attempts.

Most importantly, the consultative group recognized that multidisciplinary approaches will be required to exploit and apply new knowledge and techniques in order to make significant and novel gains in combating malaria. The malaria community needs to involve experts who can bring technologies from other seemingly distant areas of basic and applied research such as physics, electronics, information technology, and engineering. By defining desired outcomes, free from the constraints of preconceived ideas based on current tools, an expansion of the malaria research community skill base will create opportunities for lateral thinking and bring with it new approaches not previously considered.

This paper considers the key research priorities identified by the malERA Consultative Group for basic sciences and enabling technologies. Some of these key priorities have also been identified

Review articles synthesize in narrative form the best available evidence on a topic.

Summary Points

- Creative and collaborative multidisciplinary basic science approaches are needed to address pressing questions about the biology of *Plasmodium*
- The research paradigm needs to shift from a focus on solely parasite or host to one that incorporates the triad of parasite, mosquito, and human host and their respective interactions
- Key research priorities include: the development of in vitro culture systems for all life stages of *P. falciparum* and *P. vivax*, in particular, hepatic stages; improved genetic technologies for the manipulation of *Plasmodium*; and systems-based approaches incorporating cutting-edge technologies such as metabolomics
- Parasite, human, and vector research needs to be backed up by in-depth population-based field studies
- Most importantly, eradication of malaria will require bridging of the artificial gap between bench-based research, preclinical research, clinical research, and population-based science

and discussed by the malERA Consultative Groups on vaccines, drugs, and diagnoses and diagnostics [1–3].

Plasmodium In Vitro Culture Systems

The development of *Plasmodium* in vitro culture systems that encompass the entire parasite life cycle of *P. falciparum* and *P. vivax* is critical for efforts to develop new vaccines, drugs, diagnostic tests, and challenge/test systems for clinical trials. The development of such systems will require a sustained community-wide collaborative effort and a long-term commitment. Specific stages of the life cycle for human malaria parasites that remain key priorities for in vitro culture development are sporogony, sustained blood-stage culture for *P. vivax*, and the pre-erythrocytic liver stage.

In Vitro Culture of Mosquito Stages

To date, in vitro culture of mosquito stage parasites and, in particular, in vitro development of sporozoites (sporogonic development) has only been achieved for rodent malaria parasite species; the reproduction of these achievements for *P. falciparum*—and even more so *P. vivax*—has met with limited success [4–10]. The need for an effective, in vitro sporogonic culture system is highlighted by the following example. In Brazil, the major local vector *Anopheles darlingi* cannot be reared in the lab and, as in other malaria endemic countries, the importation of laboratory colonies of nonindigenous vector species is understandably prohibited. Such a situation makes it difficult for endemic country scientists to address one of the key issues pertaining to eradication—parasite transmission through the mosquito. Moreover, despite several attempts in the past, an appropriate *Anopheles* midgut cell line model does not exist, which prevents an intimate analysis of *Plasmodium* ookinete invasion of this mosquito tissue [11]. Thus, to facilitate malaria control efforts in Brazil (and in other endemic areas), it is essential that researchers work towards the development of a simple, widely utilizable, and robust mosquito-free (axenic) sporogonic culture system and an in vitro midgut cell invasion assay for the major human malaria parasites.

P. vivax Blood-Stage Cultures

The availability of continuous in vitro blood-stage culture of *P. falciparum* has revolutionized our understanding of the parasite [12], but there is no analogous culture system for *P. vivax* research. In part, this is because *P. vivax* has a predilection for reticulocytes, a cell type that may need to be purified or generated from hematopoietic stem cell progenitor cells—a process that has met with only limited success [13–19]. The development of an efficient inexpensive, automated *P. vivax* blood-stage culture system would undoubtedly enhance the study of this parasite's biology. It would also enable in vitro drug susceptibility testing, the development of growth inhibitory assays to test humoral immunity, and, ultimately, the development of new genetic research methods. Importantly, both the asexual and sexual stages would become available for study, which would facilitate the generation of the other stages of the life cycle using defined parasite strains, without the requirement for primates.

Liver-Stage Cultures

Our current understanding of the biology of the parasite's liver stage (the hypnozoite stage) suggests this stage will be an important target in efforts to eradicate malaria [20]. Specifically, hepatic development occupies a critical position in mediating the establishment of blood-stage infection and, consequently, the transmission of malaria. Moreover, in the case of *P. vivax*, the dormant hypnozoite stages remain in the liver for a variable and protracted period before leading to relapse. Clearly, eradication of *P. vivax* (and *P. ovale*) is unlikely to be attained without developing effective hypnozoiticides.

The availability of a *Plasmodium* liver-stage model would allow the investigation of the host factors that are involved in primary and latent intrahepatic development and of the metabolic pathways that regulate development of this parasitic stage. In addition, the existence of such a model would allow the development of much needed drug screens for this stage that could, like the recently available drug screens for asexual blood-stage infections [21–27], take advantage of the unprecedented access to the three chemical compound libraries—GlaxoSmithKline's Tres Cantos Antimalarial TCAMS dataset [24], the Novartis-GNF Malaria Box Dataset, and the St. Jude Children's Hospital Malaria dataset [25]—that are hosted at ChEMBL-NTD (www.ebi.ac.uk/chemblntd), an Open Access repository of primary screening and medicinal chemistry data.

Finally, with the resurgence in interest in genetically attenuated or irradiated sporozoite-based, pre-erythrocytic vaccines [28,29], a liver-stage model would permit investigation of the molecular basis of their developmental arrest—an understanding that will be critical in both the licensing of such vaccines and in ensuring that breakthrough infections do not arise.

Thus, the development of in vitro systems to understand hypnozoite biology as it relates to liver-stage biology is a clear priority. However, the culture of parasites through the liver stage is likely to be a significant challenge given the intractability of this stage relative to other life stages. Such an endeavour will require a highly collaborative and interdisciplinary approach that includes specialists in the fields of hepatocyte and stem cell biology as well as biomedical engineering. The development of hepatocytes that maintain their polarity and normal trafficking properties is a necessary step towards this kind of model, as is development of primary or immortalized hepatocyte cultures with sufficient life span to allow hypnozoite formation and survival [30–34]. Cell lines that allow high infectivity and that can yield high parasite numbers would be especially valuable for generating more useful quantities of parasite material with which to work. Moreover, a single hepatocyte line may not be amenable or useful to all the different subdisciplines present in the malaria community. Some may be appropriate for immunological studies, while others may

be suited to drug studies against primary or relapse infection from hypnozoites [1,2]. Finally, it should be noted that the possibility of using humanized mouse models engrafted with functional human cells and tissues, including human hepatocytes or human hematolymphoid cells, presents a unique in vivo approach that could also facilitate our understanding of *Plasmodium* liver-stage biology [35].

Primate Models of Disease

Not every aspect of parasite biology can be studied using in vitro culture. In some cases, whole animal models will be needed. For example, validated biomarkers for intrahepatic development and markers of past infection that could help distinguish between new infection and relapse will be important during elimination and can only be identified in whole animal models [3,36]. Data from primate studies could provide an interim platform for developing novel diagnostics that could inform future work in parallel with in vitro models [37–39]. Mechanisms to support cross-institute/ laboratory collaborations and access to the few centres with expertise and resources in primate/malaria research would facilitate and enhance a wide range of essential research.

Development of Genetic Tools for *P. vivax* and Approaches for Systematic Mutagenesis in *Plasmodium*

Major advances towards understanding fundamental aspects of model organisms inherently follow technological innovations that move fields in new directions. Thus, the ability to manipulate the genomes of different *Plasmodium* species has revolutionized malaria research. Nevertheless, we are still a long way from the systematic use of reverse genetics seen in other model systems such as yeast. For example, although the *P. falciparum* genome was completed more than 5 years ago, as many as half of the annotated genes are still listed as having a hypothetical or unknown function; around 90% of the genes have little biological evidence for function. Furthermore, little is being done currently to coordinate the study of individual genes or gene families, with the exception of recent efforts to systematically define the function of proteins involved in erythrocyte remodeling and export [40].

Despite many recent improvements to genetic technologies in *Plasmodium*, many roadblocks that prevent scale-up of genetic manipulation and functional analysis of essential genes need to be overcome [41–44]. These roadblocks include the low frequency of homologous recombination in *Plasmodium*, difficulties associated with the manipulation of large fragments of AT-rich and repetitive genomic DNA, and the lack of robust and scalable systems for conditional gene expression. In addition, there are no practical strategies for achieving saturation mutagenesis. Technologies to tackle some of these roadblocks are available for other organisms [45,46] and need to be introduced to the malaria research agenda.

If these technical limitations can be overcome, systematic mutagenesis on a genome-wide scale will allow us to distinguish essential from redundant metabolic pathways and will be critical to obtaining a comprehensive picture of the stage-specific biology of the parasite that could be targeted with drugs or vaccines. Stable, conditional knock-out approaches for genes that are essential in one life stage but not in another would also identify potential drug targets. Improved genetic technologies will also enable the systematic production of large-scale repositories of gene knock-out or epitope-tagged versions for every plasmodial gene. Such community resources would avoid duplication and benefit from the economy of scale. More importantly, easy access to large

numbers of mutants would inspire new experimental approaches, as they have in the yeast field [47–49], and widen access to genetic technology.

Finally, the recent completion of several parasite and mosquito genomes [50–54] and new insights into the contribution of human and mosquito host genotype to transmission have radically changed how researchers approach malaria. This information, together with an internationally accessible repository of transgenic lines for every *Plasmodium* gene, will change the way that the research community approaches the most basic and relevant questions related to *Plasmodium* biology (of all species) and interactions of the various *Plasmodium* species with their hosts.

Metabolomics

As with genomic innovations, new technological platforms that permit the deep characterization of the metabolome (complete set of small-molecule metabolites) of *Plasmodium* will identify new potentially druggable targets [55,56]. Indeed, analysis of the parasite's metabolome is already revealing profound new insights into parasite biology that were not amenable to or that were missed by genomic approaches [57–59]. For metabolites that are readily identifiable, differences among parasite strains, under varying drug conditions, or in mutant backgrounds will enhance understanding of the known metabolic pathways present in *Plasmodium* spp. However, many of the measurable compounds are likely to derive from previously undetected novel metabolites (including the products of poorly understood lipid and carbohydrate metabolism). The identification of these compounds could yield key insights for the development of new antimalarial drugs or the control of drug resistance. Moreover, the identification of the metabolic similarities between different parasite stages could provide new approaches to the development of drugs with potential to kill the parasites at many points in their life cycle, possibly in both the human host and the mosquito vector [58–61].

Metabolomic approaches should also enable identification of metabolic differences between, for example, patients who are asymptomatic and those with advanced stage cerebral malaria (or other severe syndromes). Metabolomic studies of such samples may not only provide information about the state of the host, but also about the interaction between the host and the parasite. The Consultative Group felt that such studies, which bring together bench scientists and field clinicians, should be encouraged as the true picture of the diversity of metabolic effects can only be fully appreciated from field-derived samples. Finally, the group noted that the application of metabolomic technology will be particularly powerful in unraveling the biochemical strategies of parasites with no or poor genomic resources such as *P. ovale* or *P. malariae*.

The Importance of Relating Molecular Science to Field Science

The emergence of artemisinin resistance [62,63] and changes in the interrelationships of humans, mosquitoes, and parasites as elimination proceeds will produce unexpected new challenges. The Consultative Group, therefore, considered it a priority to establish information systems for monitoring the changes in epidemiology, pathology, and host-parasite-vector interactions that result from intensified control and burgeoning elimination efforts so that basic research can react in a timely manner to changing circumstances (see also [36,64]).

Indeed, a core theme in our discussions was that, throughout the eradication era, basic science and multidisciplinary approaches must be seen as integral components of a Malaria Eradication

Research Agenda that are valuable even in the absence of clear field application because one can never predict the impact of novel insights. Enabling technologies cut across many themes in this agenda (see Box 1), and the application of basic science in the field is especially important. For example, genomic, proteomic, and high-throughput immunological methods can now be applied to population studies, greatly increasing their ability to shed light on complex host-parasite-vector interactions [65,66].

Human Host Factors and Improving Epidemiological Models

No campaign for the control or elimination of malaria can proceed without a detailed appreciation of the epidemiology of the disease and of host-parasite-vector interactions. As increasing parts of the world move towards elimination, a deeper understanding of the basic science of host-parasite-vector population interactions in disease transmission and of the changes in these interactions that result from intensified control and elimination efforts will be increasingly important [64].

For example, mixed species and strain infections are common in natural malaria infections in both human and vector hosts [67,68]. Application of next generation high-throughput sequencing and genotyping of mixed infections in both obligate hosts will help to identify important target genes and phenotypes and will provide insights into whether and how parasites impact each other's behaviour in the context of the human host and transmission through the vector host that will be important as elimination proceeds.

Similarly, a better understanding of the human response to malaria will be increasingly important as elimination proceeds. Despite many decades of studies on immune responses to malaria, there is still no consensus on an immune correlate of protection [69,70]. Well designed, longitudinal studies in which the exposure to malaria and protection against uncomplicated or severe malaria are reliably assessed are required to remedy this shortcoming. Other modern technologies that offer new ap-

proaches to understanding the potential mechanism of action of compounds or antibodies on malaria will also need to be fully introduced into ongoing and planned longitudinal studies of human populations.

Vector-Host-Parasite Interactions

Strategies aimed at decreasing mosquito life span are predicted to impact upon transmission (see also [2,71]). Research that investigates the parasite stages that develop within the mosquito and their transmission through the vector is likely to be of great use, therefore, in malaria control and eradication. Focused research efforts designed to understand the epidemiology of the gametocyte and how it varies with species, with host, and with the environment are required. Insights arising from such research will be critical for determining the driving factors for new human and mosquito infections, and manipulation of these factors will open up new avenues for targeting the key parasite regulatory switches that occur when a parasite undergoes a transition event. Importantly, however, such a focus on transmission need not necessarily be aimed at finding a magic bullet—a compound that can work against all parasite stages in all hosts. Instead, there will be significant utility in developing several inhibitors with similar pharmacokinetic and pharmacodynamic profiles that affect different metabolic pathways and stages in a combined drug treatment regimen. For now, the absence of compounds that preferentially affect gametocytogenesis, gamete-ookinete, or ookinete-oocyst transition as well as the lack of understanding of the mechanism of action for those very few currently available compounds highlights the need for renewed efforts in this area [72].

Box 1. Enabling Technologies: Cross-Cutting Themes

- Collaborative approaches that bridge the gap between basic laboratory, preclinical, and clinical/population-based sciences
- Complete *P. falciparum* and *P. vivax* in vitro culture systems for the discovery of new targets, the characterization of the entire metabolome, and the evaluation of current and next generation interventions
- Development and wide distribution of several viable, easy to maintain polarized hepatocyte cell lines that support enhanced (>2% infection) *P. falciparum* and *P. vivax* intrahepatic development, and that are amenable to metabolomic, proteomic, glycomic and immunological studies, and the evaluation of new interventions
- Scalable genetic technologies that enable a shared resource containing genome-wide sets of genetically modified (knock-out/tagged) parasite lines (for *P. falciparum, P. vivax,* and the murine malaria parasites) to be maintained
- Novel classes of molecules that can function as chemical tools for probing the function of genes at transition stages; most especially the commitment to dormant liver stages and gametocytogenesis

---

Box 2. Summary of the Research and Development Agenda for Basic Science Research

- A research paradigm shift away from the "parasite-first" approach to an examination of what the human and mosquito host cells provide to the developing parasite is needed to complement on-going approaches
- A new approach is needed to support collaborative and truly cross-disciplinary arrangements among scientists to bridge the gap between basic laboratory and clinical/population-based sciences and to meet the scientific benchmarks outlined by malERA
- Desired target product profiles need to be defined without preferred technological approaches being suggested to create opportunities for lateral thinking by experts bringing new approaches from different fields
- Careful evaluation and appropriate use of today's technologies from the physical, chemical, and biomedical engineering sciences is needed to improve the molecular understanding of parasite developmental biology and of the mammalian host-parasite-vector interactions
- Mechanism of action studies for drugs and vaccines in the current pipeline are also needed to inform future strategies for the development of the next generation of interventions and therapeutics
- The study of human host and vector factors in large-scale, long-term population-based field studies and the use of appropriate technologies in translation applications is also essential.

Concluding Remarks

From our discussions, we propose a basic science research and development agenda for malaria eradication (Box 2) that will hopefully yield new interventions that are not hindered by the current drug resistance status of the parasites or by changes in environmental and host factors.

Central to this agenda is our contention that the establishment of a spectrum of creative and novel eradication interventions will require a strong commitment to collaborative work, wherein interdisciplinary teams of basic scientists, both within and outside of the malaria field, are organized and tasked with achieving well-defined research milestones. It is crucial that scientists have the possibility and flexibility to move between the field and the bench for collaborative translational research, an emerging specialty in its own right. Once individuals embrace the diversity of expertise necessary in the malaria research of the future, the promotion of a greater collaborative culture will inevitably make translation from bench to bedside more readily achievable. However, the 10–15-year timeline of translation from the bench to practical use in the clinic or field remains a significant barrier to progress that has to be recognized. Finally and importantly, our challenge to basic and applied scientists to engage in stronger partnership across projects and disciplines overrides some of the current guiding principles in science such as institutional and individual performance assessments and impact factors. These criteria may have helped to shape individual careers but they have rarely helped to answer major public health questions and should not be allowed to interfere with progress towards malaria eradication.

Author Contributions

RD is the corresponding author on behalf of the malERA Consultative Group on Basic Science and Enabling Technologies. Chaired the group: RD, MM. ICMJE criteria for authorship met: JB, OB, TB, RD, VMcG, MM, IM, RS. Participated in one or more discussion meetings resulting in the writing of this article: The malERA Consultative Group on Basic Science and Enabling Technologies.

References

1. The malERA Consultative Group on Vaccines (2011) A research agenda for malaria eradication: Vaccines. PLoS Med 8: e1000398. doi:10.1371/journal.pmed.1000398.
2. The malERA Consultative Group on Drugs (2011) A research agenda for malaria eradication: Drugs. PLoS Med 8: e1000402. doi:10.1371/journal.pmed.1000402.
3. The malERA Consultative Group on Diagnoses and Diagnostics (2011) A research agenda for malaria eradication: Diagnoses and diagnostics. PLoS Med 8: e1000396. doi:10.1371/journal.pmed.1000396.
4. Sinden RE, Hartley RH, Winger L (1985) The development of *Plasmodium* ookinetes in vitro: an ultrastructural study including a description of meiotic division. Parasitology 91: 227–244.
5. Carter EH, Suhrbier A, Beckers PJ, Sinden RE (1987) The in vitro cultivation of *P. falciparum* ookinetes, and their enrichment on nycodenz density gradients. Parasitology 95: 25–30.
6. Suwanabun N, Sattabongkot J, Tsuboi T, Torii M, Maneechai N, et al. (2001) Development of a method for the in vitro production of *Plasmodium vivax* ookinetes. J Parasitol 87: 928–930.
7. Hurd H, Al-Olayan E, Butcher GA (2003) In vitro methods for culturing vertebrate and mosquito stages of *Plasmodium*. Microbes Infect 5: 321–327.
8. Porter-Kelley JM, Dinglasan RR, Alam U, Ndeta GA, Sacci JB, Jr., et al. (2006) *Plasmodium yoelii*: Axenic development of the parasite mosquito stages. Exp Parasitol 112: 99–108.
9. Dinglasan RR, Alaganan A, Ghosh AK, Saito A, van Kuppevelt TH, et al. (2007) *Plasmodium falciparum* ookinetes require mosquito midgut chondroitin sulfate proteoglycans for cell invasion. Proc Natl Acad Sci U S A 104: 15882–15887.
10. Ghosh AK, Dinglasan RR, Ikadai H, Jacobs-Lorena M (2010) An improved method for the in vitro differentiation of *Plasmodium falciparum* gametocytes into ookinetes. Malar J 9: 194.
11. Wilkins S, Billingsley PF (2010) Mosquito cell line glycoproteins: An unsuitable model system for the plasmodium ookinete-mosquito midgut interaction? Parasit Vectors 3: 22.
12. Trager W, Jensen JB (2005) Human malaria parasites in continuous culture. 1976. J Parasitol 91: 484–486.
13. Giarratana MC, Kobari L, Lapillonne H, Chalmers D, Kiger L, et al. (2005) Ex vivo generation of fully mature human red blood cells from hematopoietic stem cells. Nat Biotechnol 23: 69–74.
14. Dorn I, Lazar-Karsten P, Boie S, Ribbat J, Hartwig D, et al. (2008) In vitro proliferation and differentiation of human CD34+ cells from peripheral blood into mature red blood cells with two different cell culture systems. Transfusion 48: 1122–1132.

15. Panichakul T, Sattabongkot J, Chotivanich K, Sirichaisinthop J, Cui L, et al. (2007) Production of erythropoietic cells *in vitro* for continuous culture of *Plasmodium vivax*. Int J Parasitol 37: 1551–1557.

16. Udomsangpetch R, Somsri S, Panichakul T, Chotivanich K, Sirichaisinthop J, et al. (2007) Short-term in vitro culture of field isolates of *Plasmodium vivax* using umbilical cord blood. Parasitol Int 56: 65–69.

17. Udomsangpetch R, Kaneko O, Chotivanich K, Sattabongkot J (2008) Cultivation of *Plasmodium vivax*. Trends Parasitol 24: 85–88.

18. Cui L, Trongnipatt N, Sattabongkot J, Udomsangpetch R (2009) Culture of exoerythrocytic stages of the malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*. Methods Mol Biol 470: 263–273.

19. Douay L, Lapillonne H, Turhan AG (2009) Stem cells–a source of adult red blood cells for transfusion purposes: Present and future. Crit Care Clin 25: 383–98, Table of Contents.

20. Prudencio M, Rodriguez A, Mota MM (2006) The silent path to thousands of merozoites: The *Plasmodium* liver stage. Nat Rev Microbiol 4: 849–856.

21. Chong CR, Chen X, Shi L, Liu JO, Sullivan DJ, Jr. (2006) A clinical drug library screen identifies astemizole as an antimalarial agent. Nat Chem Biol 2: 415–416.

22. Baniecki ML, Wirth DF, Clardy J (2007) High-throughput plasmodium falciparum growth assay for malaria drug discovery. Antimicrob Agents Chemother 51: 716–723.

23. Deu E, Leyva MJ, Albrow VE, Rice MJ, Ellman JA, et al. (2010) Functional studies of *Plasmodium falciparum* dipeptidyl aminopeptidase I using small molecule inhibitors and active site probes. Chem Biol 17: 808–819.

24. Gamo FJ, Sanz LM, Vidal J, de Cozar C, Alvarez E, et al. (2010) Thousands of chemical starting points for antimalarial lead identification. Nature 465: 305–310.

25. Guiguemde WA, Shelat AA, Bouck D, Duffy S, Crowther GJ, et al. (2010) Chemical genetics of *Plasmodium falciparum*. Nature 465: 311–315.

26. Kato N, Sakata T, Breton G, Le Roch KG, Nagle A, et al. (2008) Gene expression signatures and small-molecule compounds link a protein kinase to *Plasmodium falciparum* motility. Nat Chem Biol 4: 347–356.

27. Crowther GJ, Napuli AJ, Gilligan JH, Gagaring K, Borboa R, et al. (2010) Identification of inhibitors for putative malaria drug targets among novel antimalarial compounds. Mol Biochem Parasitol 175: 21–29.

28. VanBuskirk KM, O'Neill MT, De La Vega P, Maier AG, Krzych U, et al. (2009) Preerythrocytic, live-attenuated *Plasmodium falciparum* vaccine candidates by design. Proc Natl Acad Sci U S A 106: 13004–13009.

29. Mueller AK, Camargo N, Kaiser K, Andorfer C, Frevert U, et al. (2005) *Plasmodium* liver stage developmental arrest by depletion of a protein at the parasite-host interface. Proc Natl Acad Sci U S A 102: 3022–3027.

30. Snooks MJ, Bhat P, Mackenzie J, Counihan NA, Vaughan N, et al. (2008) Vectorial entry and release of hepatitis A virus in polarized human hepatocytes. J Virol 82: 8733–8742.

31. Mee CJ, Grove J, Harris HJ, Hu K, Balfe P, et al. (2008) Effect of cell polarization on hepatitis C virus entry. J Virol 82: 461–470.

32. Ploss A, Khetani SR, Jones CT, Syder AJ, Trehan K, et al. (2010) Persistent hepatitis C virus infection in microscale primary human hepatocyte cultures. Proc Natl Acad Sci U S A 107: 3141–3145.

33. de Jong YP, Rice CM, Ploss A (2010) New horizons for studying human hepatotropic infections. J Clin Invest 120: 650–653.

34. Khetani SR, Bhatia SN (2008) Microscale culture of human liver cells for drug development. Nat Biotechnol 26: 120–126.

35. de Jong YP, Rice CM, Ploss A (2010) New horizons for studying human hepatotropic infections. J Clin Invest 120: 650–653.

36. The malERA Consultative Group on Monitoring, Evaluation, and Surveillance (2011) A research agenda for malaria eradication: Monitoring, evaluation, and surveillance. PLoS Med 8: e1000400. doi:10.1371/journal.pmed.1000400.

37. Herrera S, Perlaza BL, Bonelo A, Arevalo-Herrera M (2002) *Aotus* monkeys: their great value for anti-malaria vaccines and drug testing. Int J Parasitol 32: 1625–1635.

38. Ntumngia FB, McHenry AM, Barnwell JW, Cole-Tobian J, King CL, et al. (2009) Genetic variation among *Plasmodium vivax* isolates adapted to non-human primates and the implication for vaccine development. Am J Trop Med Hyg 80: 218–227.

39. Collins WE, Sullivan JS, Jeffery GM, Williams A, Galland GG, et al. (2009) The chesson strain of *Plasmodium vivax* in humans and different species of *Aotus* monkeys. Am J Trop Med Hyg 80: 152–159.

40. Maier AG, Rug M, O'Neill MT, Brown M, Chakravorty S, et al. (2008) Exported proteins required for virulence and rigidity of *Plasmodium falciparum*-infected human erythrocytes. Cell 134: 48–61.

41. Janse CJ, Ramesar J, Waters AP (2006) High-efficiency transfection and drug selection of genetically transformed blood stages of the rodent malaria parasite *Plasmodium berghei*. Nat Protoc 1: 346–356.

42. Armstrong CM, Goldberg DE (2007) An FKBP destabilization domain modulates protein levels in *Plasmodium falciparum*. Nat Methods 4: 1007–1009.

43. Balu B, Chauhan C, Maher SP, Shoue DA, Kissinger JC, et al. (2009) piggyBac is an effective tool for functional analysis of the *Plasmodium falciparum* genome. BMC Microbiol 9: 83.

44. Combe A, Giovannini D, Carvalho TG, Spath S, Boisson B, et al. (2009) Clonal conditional mutagenesis in malaria parasites. Cell Host Microbe 5: 386–396.

45. Schnutgen F, De-Zolt S, Van Sloun P, Hollatz M, Floss T, et al. (2005) Genomewide production of multipurpose alleles for the functional analysis of the mouse genome. Proc Natl Acad Sci U S A 102: 7221–7226.

46. Langridge GC, Phan MD, Turner DJ, Perkins TT, Parts L, et al. (2009) Simultaneous assay of every *Salmonella typhi* gene using one million transposon mutants. Genome Res 19: 2308–2316.

47. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, et al. (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285: 901–906.

48. Giaever G, Chu AM, Ni L, Connelly C, Riles L, et al. (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418: 387–391.

49. Parsons AB, Lopez A, Givoni IE, Williams DE, Gray CA, et al. (2006) Exploring the mode-of-action of bioactive compounds by chemical-genetic profiling in yeast. Cell 126: 611–625.

50. Gardner MJ, Hall N, Fung E, White O, Berriman M, et al. (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 419: 498–511.

51. Pain A, Bohme U, Berry AE, Mungall K, Finn RD, et al. (2008) The genome of the simian and human malaria parasite *Plasmodium knowlesi*. Nature 455: 799–803.

52. Carlton JM, Adams JH, Silva JC, Bidwell SL, Lorenzi H, et al. (2008) Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. Nature 455: 757–763.

53. Aurrecoechea C, Heiges M, Wang H, Wang Z, Fischer S, et al. (2007) ApiDB: Integrated resources for the apicomplexan bioinformatics resource center. Nucleic Acids Res 35: D427–D430.

54. Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, et al. (2002) The genome sequence of the malaria mosquito *Anopheles gambiae*. Science 298: 129–149.

55. Gardiner DL, Skinner-Adams TS, Brown CL, Andrews KT, Stack CM, et al. (2009) *Plasmodium falciparum*: New molecular targets with potential for antimalarial drug development. Expert Rev Anti Infect Ther 7: 1087–1098.

56. Kafsack BF, Llinas M (2010) Eating at the table of another: Metabolomics of host-parasite interactions. Cell Host Microbe 7: 90–99.

57. Olszewski KL, Llinas M (2010) Central carbon metabolism of plasmodium parasites. Mol Biochem Parasitol. In press.

58. Olszewski KL, Mather MW, Morrisey JM, Garcia BA, Vaidya AB, et al. (2010) Branched tricarboxylic acid metabolism in *Plasmodium falciparum*. Nature 466: 774–778.

59. Olszewski KL, Morrisey JM, Wilinski D, Burns JM, Vaidya AB, et al. (2009) Host-parasite interactions revealed by *Plasmodium falciparum* metabolomics. Cell Host Microbe 5: 191–199.

60. Teng R, Junankar PR, Bubb WA, Rae C, Mercier P, et al. (2009) Metabolite profiling of the intraerythrocytic malaria parasite *Plasmodium falciparum* by (1)H NMR spectroscopy. NMR Biomed 22: 292–302.

61. Besteiro S, Vo Duy S, Perigaud C, Lefebvre-Tournier I, Vial HJ (2010) Exploring metabolomic approaches to analyse phospholipid biosynthetic pathways in *Plasmodium*. Parasitology 137: 1343–1356.

62. Hunt P, Martinelli A, Modrzynska K, Borges S, Creasey A, et al. (2010) Experimental evolution, genetic analysis and genome re-sequencing reveal the mutation conferring artemisinin resistance in an isogenic lineage of malaria parasites. BMC Genomics 11: 499.

63. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, et al. (2009) Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 361: 455–467.

64. The malERA Consultative Group on Modeling (2011) A research agenda for malaria eradication: Modeling. PLoS Med 8: e1000403. doi:10.1371/journal.pmed.1000403.

65. Daily JP, Scanfeld D, Pochet N, Le Roch K, Plouffe D, et al. (2007) Distinct physiological states of *Plasmodium falciparum* in malaria-infected patients. Nature 450: 1091–1095.

66. Arastu-Kapur S, Ponder EL, Fonovic UP, Yeoh S, Yuan F, et al. (2008) Identification of proteases that regulate erythrocyte rupture by the malaria parasite *Plasmodium falciparum*. Nat Chem Biol 4: 203–213.

67. Arez AP, Pinto J, Palsson K, Snounou G, Jaenson TG, et al. (2003) Transmission of mixed *Plasmodium* species and *Plasmodium falciparum* genotypes. Am J Trop Med Hyg 68: 161–168.

68. Bousema JT, Drakeley CJ, Mens PF, Arens T, Houben R, et al. (2008) Increased *Plasmodium falciparum* gametocyte production in mixed infections with *P. malariae*. Am J Trop Med Hyg 78: 442–448.

69. Osier FH, Fegan G, Polley SD, Murungi L, Verra F, et al. (2008) Breadth and magnitude of antibody responses to multiple *Plasmodium falciparum* merozoite antigens are associated with protection from clinical malaria. Infect Immun 76: 2240–2248.

70. Crompton PD, Kayala MA, Traore B, Kayentao K, Ongoiba A, et al. (2010) 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 107: 6958–6963.

71. The malERA Consultative Group on Vector Control (2011) A research agenda for malaria eradication: Vector control. PLoS Med 8: e1000401. doi:10.1371/journal.pmed.1000401.

72. Lawpoolsri S, Klein EY, Singhasivanon P, Yimsamran S, Thanyavanich N, et al. (2009) Optimally timing primaquine treatment to reduce *Plasmodium falciparum* transmission in low endemicity thai-myanmar border populations. Malar J 8: 159.

Review

A Research Agenda for Malaria Eradication: Drugs

The malERA Consultative Group on Drugs¶*

Abstract: Antimalarial drugs will be essential tools at all stages of malaria elimination along the path towards eradication, including the early control or "attack" phase to drive down transmission and the later stages of maintaining interruption of transmission, preventing reintroduction of malaria, and eliminating the last residual foci of infection. Drugs will continue to be used to treat acute malaria illness and prevent complications in vulnerable groups, but better drugs are needed for elimination-specific indications such as mass treatment, curing asymptomatic infections, curing relapsing liver stages, and preventing transmission. The ideal malaria eradication drug is a coformulated drug combination suitable for mass administration that can be administered in a single encounter at infrequent intervals and that results in radical cure of all life cycle stages of all five malaria species infecting humans. Short of this optimal goal, highly desirable drugs might have limitations such as targeting only one or two parasite species, the priorities being *Plasmodium falciparum* and *Plasmodium vivax*. The malaria research agenda for eradication should include research aimed at developing such drugs and research to develop situation-specific strategies for using both current and future drugs to interrupt malaria transmission.

Introduction

Antimalarial drugs are used to treat malaria illness, to prevent both infection and disease caused by *Plasmodia*, to eliminate dormant malaria parasites from the liver, and to prevent malaria transmission. In the context of malaria elimination or eradication, drugs have been used for both treatment and prevention in situations where intensive surveillance has been used to identify cases, and in mass drug administration (MDA) programmes without regard for the presence of infection.

The malERA Drugs Consultative Group brought together malaria biologists, drug developers, clinical investigators, and control officials, and consulted outside experts on drug development and disease eradication to identify and prioritize a preliminary set of knowledge gaps and research questions that need to be addressed to use drugs effectively along with other tools to eliminate and ultimately eradicate malaria. The consultative process was predicated on several key assumptions, and included a review of the role of drugs in past and recent elimination campaigns.

Several current research questions were identified that should be high priorities whether or not malaria eradication moves forward. However, the main work of the group was to draft a research and development agenda that focuses on those new research questions and knowledge gaps that arise specifically in response to the call for malaria eradication and that would not otherwise be at the top of the malaria research agenda. Thus, new

and better drugs for intermittent preventive treatment (IPT) of malaria in pregnancy and molecular markers that can be used as surveillance tools for monitoring artemisinin-resistant malaria are both critically important research priorities, but are not specific to the malaria eradication agenda, and are not discussed in this paper.

In this paper, "eradication" refers to the interruption of transmission and fall in disease incidence to zero worldwide, "elimination" refers to interruption of transmission and a fall in disease incidence to zero in a defined geographical area, and "control" refers to reduction of disease incidence and burden to the point where it is no longer a public health priority.

Starting Assumptions

The thinking of the malERA Drugs Consultative Group was based on the assumption that malaria eradication is not possible with existing tools, which include artemisinin-based combination treatments (ACTs), long-lasting insecticide-treated nets, and insecticide spraying. It is true that with this set of tools, dramatic reductions in malaria have been achieved recently in many countries, including some in Africa [1]. Malaria has even been completely eliminated from some areas with low levels of transmission and relatively sound health care infrastructure by the World Health Organization (WHO) Global Malaria Eradication Program and by more recent elimination efforts [2]. However, it is the view of the malERA Drugs Consultative Group that complete global malaria eradication will not be accomplished within most of our lifetimes, and that new tools, including new antimalarial drugs developed specifically for elimination indications, are essential to move towards and ultimately achieve this ambitious but eminently worthy goal. Our thinking was also predicated on the assumption that these new tools will need to be used in combinations with each other.

Review articles synthesize in narrative form the best available evidence on a topic.

The reasons for these first two starting assumptions include the critical fact that eradication entails the complete elimination of any latent or persistent parasite reservoir in the human population. The complex life cycles of the five malaria species infecting humans present different challenges. Malaria parasites can persist for years without causing symptoms, both in the liver (in the case of *P. vivax* and *Plasmodium ovale*), and in the blood (*Plasmodium malariae*), and low-level infections that cannot be detected by standard diagnostic methods can nevertheless propagate transmission. Eradication of targeted malaria species is therefore likely to require drugs that can accomplish complete "eradication" of every malaria parasite from the bodies of infected humans, including those who are carrying very low levels of parasites that cause no symptoms but that might be a source of transmission. Moreover, we anticipate that eradication tools are likely to become increasingly compromised by the emergence and spread of drug-resistant [3] and "vaccine-resistant" parasites [4] and of insecticide-resistant mosquitoes [5], and we recognize that tools and approaches that were successful in settings with reasonably intact health care systems, functioning governments, and accessible populations, will be inadequate for the elimination of malaria in the hardest-to-reach and most unstable corners of the malaria-endemic world.

Moreover, although insecticides are appropriately credited for much of the success of the first global eradication campaign carried out in the mid-20th century, careful review of malaria control and elimination efforts shows that treatment and prevention with drugs have also been essential components of all successful malaria elimination schemes. Similarly, although smallpox has been eradicated and polio nearly so primarily through the use of vaccines, for reasons elucidated elsewhere [6], including the partial and temporary nature of naturally acquired protective immunity to malaria, and the need to eliminate latent infections that persist in the face of natural immunity, it is very unlikely that malaria could be eradicated with even a highly efficacious vaccine without concomitant use of drugs and antivector methods. The notion that a single silver bullet in the form of one brilliant technological advance could spell the end of the single biggest killer of human beings for thousands of years is appealing, but borders on magical thinking. We fervently hope to be proven wrong on this point, and strongly encourage young scientists to pursue brilliant technological advances and silver bullets, but believe that investment in a variety of complementary tools is needed.

Another of our starting assumptions was that although the current scheme for malaria elimination described in the Global Malaria Action Plan [7] calls for the elimination stage to begin when control efforts result in a reduction in malaria incidence to <1 case/1,000 population at risk, malERA should consider research questions related to the possible role of new and old drugs at all stages of malaria control and elimination. In particular, the role of drugs in aggressive efforts to drive down high transmission rates during the control phase of eradication—formerly and perhaps more inspirationally called the "attack" phase—should be considered. For example, drugs might be used in mass screening and treatment or MDA campaigns, or as ongoing IPT intended to reduce both morbidity and transmission.

Finally, we assumed that incipient elimination and eradication efforts will likely focus initially chiefly on *P. falciparum* in Africa, but that *P. vivax* will be a major focus outside of Africa, where it is the most common form of malaria. *P. vivax* causes more morbidity, severe disease, and death than is often appreciated [8]. It also presents special challenges because of the relapsing liver-stage parasites (hypnozoites) that are refractory to treatment with most

antimalarial drugs, and it will increase in prominence as rates of falciparum malaria decrease. We therefore assumed that research and development will proceed in parallel to develop drugs that can be used to eliminate *P. falciparum* and *P. vivax*, ideally drugs that target both species, although better species-specific drugs are also likely to make a great contribution. Eventually, the other human malarias, *P. malariae*, *P. ovale*, and *Plasmodium knowlesi*, may have to be considered as specific targets for global eradication as their impact is modified by control or elimination of the other species, although it is hoped that these minority species will be eliminated in a collateral fashion by tools aimed at falciparum and vivax malaria.

What's New about the Approach to Drugs in the Context of Elimination?

In the first malaria eradication campaign, antimalarial drugs were considered for their role in eliminating infection in people and thus reducing the infectious reservoir. Subsequently, there was a reorientation towards thinking about controlling malaria as a disease rather than as an infection, with more emphasis on preventing clinical complications and death [9]. There was less concern about curing infection in settings where rapid re-infection was guaranteed. To prepare for a long-term approach to elimination, it is necessary to revive the earlier paradigm and again think about malaria drugs and other interventions in terms of their impact on malaria infection and transmission in addition to their use in the prevention and treatment of malaria disease. "Elimination thinking" also underlies the concept of adding anti-gametocytocidal drugs to the treatment of malaria in areas such as Cambodia [10] where resistance to ACT drugs is being observed [11,12].

Current Drug Indications

We identified several high priority research areas that need to be addressed urgently regardless of whether the world mobilizes for a renewed effort to eliminate malaria. The first such area is optimization of the use of ACTs and other currently available antimalarial drugs to maximize their useful lifespan. Approaches to achieve this optimization include the rational design of drug combinations with well-matched pharmacokinetic and pharmacodynamic profiles, operational research to increase uptake of coformulated ACTs while minimizing the use of artemisinin monotherapy and suboptimal dosing, and the evaluation of strategies to reduce relative pressure for emergence and dissemination of resistance [13,14].

The second high priority area is continued research and development to make new drugs available to replace current drugs (in particular, artemisinins) as resistance emerges. Specific priorities include first-line drugs for treating uncomplicated falciparum and vivax malaria, drugs to treat severe malaria, drugs for IPT of infants, pregnant women and children, drugs for travel chemoprophylaxis, and anti-relapse drugs to cure the liver stages of *P. vivax*.

Research is also needed to elucidate the pharmacokinetics and pharmacodynamics and optimal dosing of drugs used to treat and prevent malaria, especially in understudied vulnerable groups including pregnant women, young children and infants, as is operational research and research into improved diagnostics, and into monitoring to optimize drug deployment strategies and facilitate control efforts using currently available antimalarial drugs.

Although malERA's charge was to identify new research questions and knowledge gaps that arise in response to the call

for malaria eradication, several of these research areas—for example, maintaining the development pipeline of first-line drugs to treat uncomplicated falciparum malaria—will have to be addressed for eradication to succeed. But, while it is extremely important that this pipeline continues to flow whether or not global malaria eradication is being attempted, the malERA Drugs Consultative Group did not focus on defining the optimal characteristics of drugs for treating this or other clinical malaria syndromes. Instead, we focused on drugs that would be needed specifically for the purposes of eradication, noting, for example, the need for widespread use (possibly in whole asymptomatic populations) of drugs with better safety profiles than would be required for treatment of individuals with potentially life-threatening clinical malaria.

New Drug Indications in the Context of Eradication

Table 1 lists the current indications for antimalarial drugs, and considers the relevance of these for the specific goal of malaria eradication. For example, suppressive prophylaxis, which prevents malaria disease but that does not prevent and may even augment transmission, is not a relevant indication for malaria eradication.

It is reasonable to assume that drugs that target *P. falciparum* will generally be effective against *P. malariae*, and that those targeting *P. vivax* will be efficacious against *P. ovale* and *P. knowlesi*. This assumption is based on limited experience with current drugs, and should be tested by routinely including patients infected with these minority species in drug trials. No single trial would include enough cases of the minority species to provide a meaningful measure of efficacy, but pooling data from many trials using a global database such as the Worldwide Antimalarial Resistance Network (WWARN; www.wwarn.org) [15] would, over time, permit estimation of efficacy of commonly used drugs against these species. Importantly, the recent identification of the monkey malaria *P. knowlesi* as a widespread and potentially life-threatening human pathogen [16] suggests that vigilance for transfer of other nonhuman primate malarias to humans and the determination of

which drugs are effective against these emerging diseases may be necessary in the late stages of elimination.

Lessons Learned from Past Malaria Elimination Programmes and Efforts to Eradicate Other Diseases

As a matter of priority, experienced malariologists need to dedicate substantial time and effort to detailed analytical reviews of published and unpublished information on past elimination efforts. Here we briefly summarize a few of the insights gained from malERA reviews of some of the available material, including a dissection of the Global Malaria Eradication Program [17], and a broad overview of lessons learned from past malaria elimination efforts published by the Malaria Elimination Group [18]. In particular, we note the need for a much more comprehensive review of the use of drugs in past elimination efforts, which includes careful analysis of factors leading to success or failure (see Table 2).

Importance of Single-Encounter Therapy

For smallpox, the only infectious disease that has been eradicated, a single-dose vaccine was available that could emulate the lifelong protective immunity that results from natural infection. Similarly, the drugs that are presently being used in large-scale infectious disease control and elimination programmes such as those for onchocerciasis and trachoma can be administered in a single encounter once or twice a year. Discussions with leaders of these campaigns highlighted the notion that single-encounter interventions are an essential requirement for successful elimination campaigns. Notably, however, the antimalarial drug regimens that were used with varying success to eliminate malaria from Italy [19], the former Soviet Union [20], and various islands such as the Vanuatu island of Aneityum [21], have involved complex regimens of multiple administrations of at least two drugs usually repeated at frequent intervals for prolonged periods of time. We

---

Table 1. Indications for antimalarial drugs in the present control era and their relevance in the eradication era.

Indications for Antimalarial Drugs in the Control Era	Relevant to Malaria Eradication?
Prophylaxis	
Causal prophylaxis[a]	Yes, completely blocks infection and thus transmission
Suppressive prophylaxis[b]	No, does not prevent, and may augment, transmission
IPT of pregnant women, infants, or children	Maybe, but only if transmission-blocking drugs are used in a high proportion of the infected reservoir, essentially amounting to intermittent MDA
Treatment of disease	
Uncomplicated malaria	
P. falciparum and *P. malariae*	Maybe, treatment indications for specific clinical syndromes are not directly relevant to the goal of eradication unless treatment drugs have transmission-blocking efficacy; widespread use of treatment drugs with antiliver stage and gametocytocidal activity would contribute to transmission reduction.
P. vivax and *P. ovale*	As above
Severe malaria	As above
Antihypnozoite (liver-stage radical cure)	Yes, high priority
Transmission blocking	Yes, high priority

[a]Causal prophylaxis targets pre-erythrocytic liver stages and, if effective, prevents any parasites from reaching the blood state or being transmitted to mosquitoes.
[b]Suppressive prophylaxis is repeated subcurative dosing that suppresses blood-stage infection and prevents malaria illness but does not eradicate malaria infection or prevent transmission
doi:10.1371/journal.pmed.1000402.t001

Table 2. Past use of drugs in malaria elimination.

How Drugs Were Used in Elimination	General Assessment
Curative therapy for cases detected through surveillance	Essential component of all successful control and elimination programmes
Intensive, multidrug, multidose MDA used in conjunction with aggressive antivector interventions (nets, spraying, larvicides)	Contributed to several successful elimination programmes
Less intensive MDA as a complement to less aggressive or subsequent antivector interventions	Limited, transient, or no success at elimination
MDA as the main elimination measure	Successful only in a few cases of isolated, stable populations
Medicated salt	Mixed success and major drawbacks of danger of rapid selection for resistance and safety issues

concluded therefore that while multiple dosing regimens of multiple drugs have been used successfully to eliminate malaria from areas with relatively good health systems and stable populations, malaria eradication will require drugs that, ideally, can be administered in a single encounter at infrequent intervals (see Box 1).

Mass Drug Administration

MDA refers to the use of drugs to treat whole populations for malaria, irrespective of, and without knowledge of, who is infected [22]. Although this approach is not currently recommended, antimalarial drugs have been used in MDA campaigns since at least 1900, when subsidized and free quinine was distributed by the Italian government for both suppressive prophylaxis and curative treatment [19]. Suppressive prophylaxis reduces the risk of acute malaria illness by controlling the level of infection without ridding the body of parasites; curative treatment resolves an acute malaria illness episode by eliminating all asexual blood-stage malaria parasites and may or may not result in a fully sterilizing

cure; both approaches may either prevent, augment, or have no effect on transmission to mosquitoes. The Italian MDA campaign resulted in large decreases in malaria cases and mortality but not interruption of transmission [19]. Malaria was only finally eliminated in Italy when DDT spraying was aggressively deployed after World War II in combination with systematic diagnosis and quinine treatment and mass quinine prophylaxis.

In the former Soviet Union, mass chemoprophylaxis with blood schizonticides (drugs that kill the blood-stage malaria parasites that cause disease but that do not usually affect either liver-stage parasites or the sexual stage gametocytes that transmit malaria to the mosquito) was administered each year at the peak of the malaria season during the attack phase of elimination, then phased out during the "consolidation" phase as the last remaining foci of transmission were extinguished [20]. As malaria transmission risk coalesced into localized "islands" of risk, the entire local population was given both a blood schizonticide and an 8-aminoquinoline 2–3 weeks before the start of the malaria season. 8-aminoquinolines are active against gametocytes as well as against *P. vivax* and *P. ovale* relapsing liver forms; examples of 8-aminoquinolines include plasmocide and quinocide (now superseded drugs that were used in the USSR), the widely used and licensed primaquine, and tafenoquine, which is still investigational.

Other examples of MDA campaigns include the Garki Project in Nigeria, where simultaneous spraying and MDA significantly but only transiently reduced malaria parasite prevalence rates [23]. Similarly, mass chemoprophylaxis of a million soldiers used in conjunction with insecticide-treated nets and spraying resulted in the near-elimination of vivax malaria where it had reemerged 20 years after the Korean demilitarized zone had been declared malaria free [24]. Most recently, mass administration of artemisinin, piperaquine, and primaquine in Cambodia resulted in dramatic reductions in the prevalence of *P. falciparum*, *P. vivax*, and *P. malariae*, including a 10-fold reduction in the prevalence of falciparum gametocytes, but not the complete interruption of transmission [10]. Often, these schemes were implemented with no clear idea of what the MDA programme was trying to achieve, and in many cases political or economic factors were major drivers [22]. However, the main factors that are common to successful MDA schemes include a careful preparatory phase, social mobilization, improvement of the health care infrastructure and the inclusion of malaria control in comprehensive health care, and the concomitant use of antivector measures.

Another common success factor is that MDA (like other malaria elimination efforts) is more likely to work where malaria risk is circumscribed, such as on sea islands or in "islands" of malaria risk surrounded by areas with no malaria. Sustained interruption of falciparum and vivax malaria transmission was achieved in 1996

> ## Box 1. Single Encounter Radical Cure and Prophylaxis (SERCaP)
>
> Currently, the goal of antimalarial drug therapy is to reduce disease and death by targeting blood-stage parasites, with an emphasis on falciparum malaria in young children in Africa. This goal is accomplished by prompt diagnosis and treatment of fever with effective drugs such as ACTs. For eradication to be effective, drug therapy must eliminate the human reservoir of infection, an objective that is best achieved by Single Encounter Radical Cure and Prophylaxis (SERCaP). Achieving the objective in a single patient encounter is important for effectiveness. Radical cure is defined as eliminating all parasites in the patient; eradication of the disease on a population basis can only be achieved by "eradication" of the parasites in all individuals. For *P. falciparum*, this entails the elimination of all persistent asexual blood-stage forms, and the long-lived mature-stage V *P. falciparum* gametocytes that are responsible for transmission. For *P. vivax* malaria, radical cure includes elimination of all persistent asexual blood-stage forms, and the long-lived hypnozoites in the liver. Finally prophylaxis highlights the need to prevent reinfection of each individual treated for some defined period after treatment. This time should be at least for 1 month, to outlast the typical development period of *Plasmodium* parasites in Anopheline mosquitoes.

on the Vanuatu island of Aneityum with an intensive MDA regimen consisting of weekly chloroquine and primaquine for 9 weeks, sulfadoxine-pyrimethamine at weeks 1, 5, and 9, and concomitant use of insecticide-treated nets and larvicides [21]. A less intensive MDA regimen using three drugs at two-monthly intervals followed by DDT spraying at the end of the campaign had no measurable impact on overall malaria prevalence on the island of Zanzibar [25], highlighting the need to deploy multiple interventions aggressively and simultaneously to interrupt transmission. Where there are large areas of contiguous malaria risk, as in much of sub-Saharan Africa, the effectiveness of MDA has been transient at best. However, high transmission intensity does not necessarily preclude successful use of MDA; rather, high transmission often signifies contiguity with surrounding areas of malaria risk, with inevitable back-flow of infections unless MDA and other interventions are applied widely and simultaneously across the entire area of contiguous risk through transnational cooperation, another factor that is common to successful MDA programmes.

Finally, although MDA in the form of adding antimalarial drugs to salt used for cooking and flavoring food had some success in reducing malaria prevalence in large-scale pilot programmes in Asia, Africa, and South America [26], the inability to control dosage and the resulting rapid selection for drug-resistant parasites make this an unjustifiable approach [22].

Long-Acting Formulations

Another creative approach from the past that may hold promise for the future is the use of long-acting formulations. "Repository" formulations of malaria drugs to provide prolonged protection were extensively researched in the early 1960s [27], and oil-based depot injections of cycloguanil pamoate provided more than 1 year of protection against experimental challenge with *P. falciparum* sporozoites [28]. These injections were evaluated in at least 15,000 people, but never deployed as a tool for elimination because of the attendant pain and local abscesses.

Key Knowledge Gaps and Research Priorities

On the basis of this initial review of past and present malaria control and elimination efforts, the malERA Drugs Consultative Group concluded that antimalarial drugs will be essential components for elimination of malaria from endemic countries and eventually for worldwide eradication. In the next step of our discussions, we identified the key knowledge gaps about the role of drugs in malaria eradication and research priorities for developing and using drugs in malaria elimination and eradication programmes. We organized these knowledge gaps into three areas: (1) the optimization of the use of currently available drugs for elimination and eradication; (2) the development of new drugs for elimination and eradication; and (3) the development of drug

---

Box 2. A Draft Research and Development Agenda for Drugs for Malaria Eradication

KNOWLEDGE GAPS AND RESEARCH PRIORITIES FOR OPTIMIZING CURRENT DRUGS

- Pharmacology studies to optimize dosing regimens of 8-aminoquinolines for gametocytocidal and anti-relapse efficacy and safety
- Rapid and robust point-of-care glucose-6-phosphate dehydrogenase (G6PD) test to improve safety of 8-aminoquinoline use
- Tests that can detect resistance to artemisinins and ACT partner drugs
- Determine gametocytocidal and anti-relapse activity of current drugs and those in the pipeline

KNOWLEDGE GAPS AND RESEARCH PRIORITIES FOR DEVELOPING NEW DRUGS FOR MALARIA ERADICATION

Desired products

- Drugs that prevent transmission by killing or preventing development of gametocytes, or blocking sporozoite development in the mosquito
- Drugs that cure liver stages of vivax (and ovale) malaria
- Ideally, drugs that can be administered in a single encounter at infrequent intervals, and that result in radical cure of all parasite stages (Single Encounter Radical Cure and Prophylaxis, see Box 1)
- Sustained or pulsed release formulations
- Exceptionally safe schizonticidal drugs for curing asymptomatic falciparum infection

Fundamental research questions aimed towards developing desired drugs

- Fundamental studies of liver and sexual stage biology (in both host and mosquito)
- Mechanisms of resistance and pharmacological strategies to deter resistance
- In vitro culture of *P. vivax* to understand parasite biology

Tools and capacities

- Increased capacity for clinical pharmacology research including pharmacokinetics/pharmacodynamics studies in populations targeted for malaria elimination
- Increased capacity for human challenge studies for early go/no go decisions on drug candidates
- Assays to measure transmission-blocking activity
- Assays to measure activity against liver stages
- In vitro culture of *P. vivax* and other non-falciparum species for drug screening
- Genomic and proteomic approaches to identify transmission-blocking and liver-stage activity

KNOWLEDGE GAPS AND RESEARCH PRIORITIES FOR DRUG TREATMENT AND PREVENTION STRATEGIES FOR ERADICATION

- Field studies to evaluate new drugs and approaches in a variety of epidemiological settings
- Robust and highly sensitive malaria diagnostics for malaria infection and especially for carriage of infectious gametocytes
- Measures to monitor and improve adherence and safety
- How must drug treatment and prevention strategies change as elimination proceeds?
- Strategies to deter resistance

treatment and prevention strategies for elimination and eradication. The rest of this paper considers these areas, which together make up the draft research and development agenda that we propose in Box 2. Finally, we also briefly touch on cross-cutting issues that require coordination with the other malERA groups.

Optimization of the Use of Currently Available Drugs for Elimination

The time from lead identification of a new compound to a licensed drug is measured in decades. Thus, the optimization of existing tools for control and elimination must occur in parallel with development of new tools for elimination and eradication. As discussed earlier, one of the assumptions underlying the malERA process is that global eradication of malaria cannot be accomplished with existing tools, but that malaria is being eliminated from areas with relatively low transmission and relatively good health systems using these tools. Consequently, in parallel with the development of new drugs and other eradication tools, research is needed to optimize drugs that can be used now to reduce malaria transmission. The first section of Box 2 highlights priority knowledge gaps and research questions related to currently available antimalarial drugs. Most of these topics should already be research priorities irrespective of malaria eradication. They are highlighted here because they are essential for eradication but relatively neglected. The most important knowledge gaps relate to the use of 8-aminoquinolines and ACTs. 8-aminoquinolines are the only drugs available today that can kill dormant liver stages and gametocytes. Primaquine, the only currently licensed 8-aminoquinoline, is routinely used to prevent relapses of *P. vivax* and *P. ovale* and has played a prominent role in several successful elimination campaigns; the long-acting 8-aminoquinoline tafenoquine is not yet licensed. ACTs, which are presently the first-line treatment for both uncomplicated and severe falciparum malaria in most of the world, are threatened by the recent emergence of artemisinin resistance in Southeast Asia [12,29].

Research to optimize the successful use of these drugs to eliminate malaria from the approximately 30 countries now actively pursuing this goal represents "low-hanging fruit" that is likely to yield high gains at relatively low cost over the next 5–10 years. While recognizing that global eradication will require substantial investment in new tools, the large gains that can be made and consolidated by making the most of the tools now in hand should not be underestimated.

The paucity of information about pharmacokinetics, pharmacodynamics, and rational dosing of drugs represents a critical knowledge gap that needs to be addressed in order to use current drugs in conjunction with other tools to reduce malaria transmission, as well as to provide rationally designed treatment strategies. The other top priority is the development of robust and sensitive field diagnostics to guide drug interventions and to detect carriage of gametocytes that are infectious to mosquitoes. This type of research is also a priority for vaccine development [30].

Development of New Drugs for Elimination and Eradication

The second section of Box 2 summarizes key knowledge gaps and research priorities for the development of new drugs specifically for elimination and eradication indications. Below, we discuss some of these issues in more detail. Importantly, because antimalarial drugs have not previously been licensed for indications other than individual treatment, early and close consultation with regulatory authorities will be needed for any drugs used for elimination and eradication.

Targeting Liver and Sexual Stages and Greater Emphasis on Safety

In 1957 Wallace Peters wrote, "Development of an 8-aminoquinoline in depot form to give a safe and adequate blood level should be attempted as this would be an invaluable weapon against malaria if properly applied" [31]. More than 50 years later, the dream of a safe, long-acting drug that eliminates malaria infection by killing liver stages and that blocks transmission by killing gametocytes remains both unfulfilled and a top priority. As mentioned earlier, the only known antimalarial drugs that kill dormant liver stages and gametocytes are the 8-aminoquinolines primaquine and tafenoquine. Both of these drugs have a serious flaw for a drug that would be used to eliminate infection and block transmission in people who are not themselves acutely sick with malaria—they cause hemolysis (destruction of red blood cells leading to anemia) in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, a red cell polymorphism that is common in tropical populations because it is associated with some degree of protection against malaria illness [32]. Any drug used for malaria elimination in people who are not sick must have a low risk-to-benefit ratio akin to the low risk-to-benefit ratios of routine immunizations.

During its brainstorming sessions, the malERA drugs group developed draft target product profiles (TPPs) for new drugs that could be used for radical cure (including elimination of both liver stages and gametocytes) of *P. falciparum* and *P. vivax*. These TPPs (see Tables S1–S3) represent the ideal targets and a starting point for discussion with drug developers. Drugs that fall short of these ideals will still be of value for eradication, and adjudicating between the ideal and the acceptable will be a dynamic and continuous process. For example, the ideal drug would target all malaria species, but it would not be prudent to reject promising candidates that target only *P. falciparum* or *P. vivax*. Indeed, depending on leads and progress, it is likely to be necessary to pursue at least partly separate research agendas for these two key species.

Ideal Pharmacokinetic/Pharmacodynamic Characteristics

An ideal eradication drug would have a short half life with a sustained (depot-like) release followed by rapid elimination, and would be deployed in combination with other drugs with matching pharmacokinetic and pharmacodynamic profiles both to deter resistance and to improve efficacy [13]. Such characteristics would allow for rapid onset of action to exert quick killing, long duration of action to permit administration in a single encounter at infrequent intervals, and rapid clearance to avoid a long period of sublethal drug levels conducive to selection for resistant parasites. An intermediate goal may be to develop a safe product for delivery at a single encounter of a curative dose of a drug that also offers 4 or more weeks of post-treatment causal prophylactic efficacy.

Antimalarial drugs with half lives in the range of weeks are already available but do not offer the ideal pharmacokinetic/pharmacodynamic profile of sustained or intermittent pulsed killing levels followed by rapid drop-off to deter resistance. Previous research on polymers for pulsed release of malaria vaccines showed initial promise but was abandoned by WHO. Nanotechnology may offer another approach for developing drugs with the ideal pharmacokinetic/pharmacodynamic profile. Nanoparticle delivery of drugs and vaccines is in the early stages of development, and one challenge for this form of delivery is the limitation on the dose of drug that can be delivered. Highly potent

drugs that require a low dose would therefore be most attractive for this delivery method. Subcutaneous implants such as those used to deliver birth control drugs also warrant consideration.

The malERA drugs group felt strongly that concerns of impracticality and expense should not deter research into the ideal pharmacokinetic/pharmacodynamic profile. Seemingly risky approaches can and should be entertained in the quest for solutions and evaluated for their potential. Speculation about the ultimate cost of an intervention should not be the sole basis for its rejection from further consideration.

In some circumstances, these ideal pharmacokinetic/pharmacodynamic characteristics may be less critical, such as when drugs are administered in settings or at times when there is very low or no transmission. A specific example of this is the use of mass treatment to eliminate the infectious reservoir at the nadir of malaria transmission in settings with sharply seasonal malaria transmission. Because there is a very low probability of parasites encountering subtherapeutic drug levels in this setting, there may be little disadvantage to drug combinations with mismatched pharmacokinetic/pharmacodynamic profiles. This example highlights the importance of considering setting-specific epidemiology and indications when thinking about desired characteristics of drugs for elimination and supports the idea of tailoring TPPs to specific indications on the basis of the parasites, human populations, epidemiological settings, and stages of elimination and eradication that are to be targeted.

Drug Resistance

It has been suggested that concerns about drug resistance and strategies to deter it—for example, the obligatory use of combinations of drugs with different mechanisms—may not be a priority in the context of malaria eradication, because resistance is unlikely to emerge and spread when transmission is very low in the late stages of elimination. However, evidence suggests that drug resistance can spread rapidly and become fixed in populations in settings of low malaria transmission [33], and history amply demonstrates the folly of counting on the efficacy of drugs to endure in the face of widespread use [3]. Even at "the last mile," when eliminating the last few cases of malaria from an area, the risk of exporting malaria to, or reintroducing it from, other malarious areas will remain. This risk will only disappear during the final stages of global eradication when remaining foci are very few and far between. For these reasons, it is important that the development process for drugs and drug combinations for elimination and eradication indications should attempt to build in strategies for preventing and deterring resistance. These strategies include combining drugs with different mechanisms of action [34] or even drugs with opposing resistance mechanisms, and coformulation of drugs.

Clinical Research

The current capacity for conducting both laboratory and clinical malaria pharmacokinetics/pharmacodynamics studies is very limited. Consequently, most antimalarial drugs are used in risk groups and populations for whom there is little to no information on optimal dosing for efficacy and safety. Careful and rigorous clinical pharmacology studies will be needed for new drugs and drug combinations for eradication, and robust methods instituted for postlicensure marketing surveillance for side effects. This research will require expanded capacity for drug level measurements, pharmacokinetics analysis and clinical pharmacology studies, and surveillance. As malaria incidence falls at established malaria research sites, it is already becoming increasingly difficult to meet sample size requirements for drug

efficacy trials. It may, therefore, become necessary to establish mobile clinical trial networks or novel clinical trials designs (for example, field trials in malaria-exposed populations that measure gametocyte prevalence and infectivity) to assess the efficacy of drugs for blocking transmission and preventing relapse and/or to rely more on the use of experimental malaria challenge studies [35] to evaluate drugs (and vaccines) for eradication.

Drug Treatment and Prevention Strategies for Eradication

Although much can be learned from careful review of the role played by drugs in past elimination programmes, creatively designed prospective field research and pilot projects and operational research to assess interventions as they are implemented in different settings will be essential for the success of malaria eradication (see the final section of Box 2). That is, research is needed to understand when, where, and how to use drugs to eliminate and eradicate malaria. For example, current guidelines do not recommend MDA, and evidence from field studies of the efficacy of specific interventions in specific populations and epidemiological settings is needed to support a change in this recommendation. Thus, the effectiveness of mass screening and treatment of only infected individuals needs to be compared with treating all individuals irrespective of whether they are infected, as is done in MDA. Similarly, the effectiveness of "focal screening and treatment," (a variation on mass screening and treatment that uses molecular diagnostics to identify the individuals to be given curative treatment) that is now being used in an attempt to contain emerging artemisinin-resistant falciparum malaria in western Cambodia [36] needs to be properly evaluated.

Furthermore, research is needed into the different drug treatment and prevention strategies that will be needed for different epidemiological settings at different stages of the elimination process, and in settings with different levels of health care infrastructure. Drug treatment and prophylaxis schemes that are feasible and effective in stable rural populations with year-round malaria transmission may be completely ineffective if implemented in a setting with highly seasonal malaria, or impossible in mobile populations or in areas of civil unrest. Moreover, as transmission rates decline, so will levels of protective immunity, resulting in fewer cases of infection spread across a wider range of age groups. As reduced transmission is sustained for years, asymptomatic carriage will become increasingly uncommon, making MDA less attractive [37].

Research is also needed into robust and sensitive screening tests to guide drug treatment and prophylaxis both for asexual parasites and for infectious gametocytes and to evaluate the efficacy of drugs (and vaccines) that are intended to block transmission. The current gold standard, light microscopy, is insufficiently sensitive to detect low levels of gametocytes that are nevertheless capable of being transmitted, and current investigational assays that offer improved sensitivity are not robust enough for field surveillance in most settings, nor are they validated as predictive of infectivity.

Cross-Cutting Issues

Several important knowledge gaps and research priorities for drug strategies cut across one or more of the technical areas covered by malERA. For example, for any malaria research enterprise to succeed, it is essential to engage scientists in endemic countries to identify, prioritize, and refine research questions and to design the most appropriate approaches. This process is particularly important for research involving human-based interventions such as drugs, because issues like population

acceptance, adherence, and impact of epidemiological differences on drug efficacy and safety require knowledge of local cultural, political, ecological, and epidemiological factors. Other examples of cross-cutting research issues include research into health systems and how they deliver malaria interventions, operational research, malaria modeling and research into monitoring and surveillance, vaccines, and vector control. These cross-cutting issues are addressed in the other malERA papers in this series [30,37–40].

Concluding Remarks

The potential list of research priorities for developing and using drugs to eradicate malaria is as long as the list of research interests of the individuals who participated in the consultative process. To be useful in setting a research agenda for eradication, however, the list must be focused and prioritized. This report focuses on research goals that will be achieved largely in the longer term, and these suggestions are passed to the rest of the malaria community in the form of a draft research and development agenda (Box 2) with a sincere request that the whole of the malaria community uses its considerable wisdom and experience to improve this agenda in the spirit of a shared hope for a malaria-free future.

Supporting Information

Table S1 TPP for drugs used to treat and prevent infection (prophylaxis) in elimination programmes: Single Encounter Radical Cure and Prophylaxis against all parasitic species (SERCaP).
Found at: doi:10.1371/journal.pmed.1000402.s001 (0.05 MB DOC)

Table S2 Short of Single Encounter Radical Cure and Prophylaxis against all parasitic species (SERCaP), TPP for drugs used for radical cure of *P. falciparum* in elimination programmes.
Found at: doi:10.1371/journal.pmed.1000402.s002 (0.04 MB DOC)

Table S3 Short of Single Encounter Radical Cure and Prophylaxis against all parasitic species (SERCaP), TPP for drugs used for radical cure of *P. vivax* in elimination programmes.
Found at: doi:10.1371/journal.pmed.1000402.s003 (0.04 MB DOC)

Author Contributions

CP is the corresponding author on behalf of the malERA Consultative Group on Drugs. Chaired the group: CP. ICMJE criteria for authorship read and met: PLA, AD, AM, JM, JN, CP, TW, SY. Participated in one or more discussion meetings resulting in the writing of this article: the malERA Consultative Group on Drugs.

References

1. Chanda P, Hamainza B, Mulenga S, Chalwe V, Msiska C, et al. (2009) Early results of integrated malaria control and implications for the management of fever in under-five children at a peripheral health facility: A case study of Chongwe rural health centre in Zambia. Malar J 8: 49.

2. (2007) United Arab Emirates certified malaria-free. Wkly Epidemiol Rec 82: 30–32.

3. Plowe CV (2009) The evolution of drug-resistant malaria. Trans R Soc Trop Med Hyg 103: S11–S14.

4. Takala SL, Plowe CV (2009) Genetic diversity and malaria vaccine design, testing and efficacy: Preventing and overcoming 'vaccine resistant malaria'. Parasite Immunol 31: 560–573.

5. Kelly-Hope L, Ranson H, Hemingway J (2008) Lessons from the past: managing insecticide resistance in malaria control and eradication programmes. Lancet Infect Dis 8: 387–389.

6. Plowe CV, Alonso P, Hoffman SL (2009) The potential role of vaccines in the elimination of falciparum malaria and the eventual eradication of malaria. J Infect Dis 200: 1646–1649.

7. The Roll Back Malaria Partnership (2010) The global malaria action plan for a malaria-free world. Geneva: The Roll Back Malaria Partnership.

8. Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, et al. (2007) Vivax malaria: Neglected and not benign. Am J Trop Med Hyg 77: 79–87.

9. Marsh K (1992) Malaria–a neglected disease? Parasitology 104: S53–S69.

10. Song J, Socheat D, Tan B, Dara P, Deng C, et al. (2010) Rapid and effective malaria control in Cambodia through mass administration of artemisinin-piperaquine. Malar J 9: 57.

11. Alker AP, Lim P, Sem R, Shah NK, Yi P, et al. (2007) Pfmdr1 and in vivo resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border. Am J Trop Med Hyg 76: 641–647.

12. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, et al. (2009) Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 361: 455–467.

13. Nyunt MM, Plowe CV (2007) Pharmacologic advances in the global control and treatment of malaria: Combination therapy and resistance. Clin Pharmacol Ther 82: 601–605.

14. Boni MF, Smith DL, Laxminarayan R (2008) Benefits of using multiple first-line therapies against malaria. Proc Natl Acad Sci U S A 105: 14216–14221.

15. Price RN, Dorsey G, Ashley EA, Barnes KI, Baird JK, et al. (2007) World Antimalarial Resistance Network (WARN) I: Clinical efficacy of antimalarial therapy. Malar J 6: 119.

16. Cox-Singh J, Davis TM, Lee KS, Shamsul SS, Matusop A, et al. (2008) *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis 46: 165–171.

17. Najera JA (2001) Malaria control: Achievements, problems and strategies. Parassitologia 43: 1–89.

18. Feachem RGA (2010) Shrinking the malaria map: A guide on malaria elimination for policy makers. Chapter 6. San Francisco: The Global Health Group, UCSF Global Health Sciences.

19. Snowden FM (2005) The conquest of malaria. Italy 1900-1962. New Haven: Yale University Press.

20. Bruce-Chwatt LJ (1959) Malaria research and eradication in the USSR. A review of Soviet achievements in the field of malariology. Bull World Health Organ 21: 737–772.

21. Kaneko A, Taleo G, Kalkoa M, Yamar S, Kobayakawa T, et al. (2000) Malaria eradication on islands. Lancet 356: 1560–1564.

22. von Seidlein L, Greenwood BM (2003) Mass administrations of antimalarial drugs. Trends Parasitol 19: 452–460.

23. Molineaux L, Gramiccia G (1980) The Garki Project. Research on the epidemiology and control of malaria in the Sudan Savanna of West Africa. Geneva: World Health Organization.

24. Han ET, Lee DH, Park KD, Seok WS, Kim YS, et al. (2006) Reemerging vivax malaria: Changing patterns of annual incidence and control programs in the Republic of Korea. Korean J Parasitol 44: 285–294.

25. Dola SK (1974) Mass drug administration as a supplementary attack measure in malaria eradication programme. East Afr Med J 51: 529–531.

26. Pinotti M (1954) A new method of malaria prophylaxis: The addition of an antimalarial drug to cooking salt used in daily preparation of food. Rev Brasil Malariol 6: 5–12.

27. Peters W (1970) Chemotherapy and drug resistance in malaria. London: Academic Press.

28. Clyde DF (1965) Duration of antimalarial activity of cycloguanil pamoate amond semi-immune Africans in Tanzania. WHO/MAL/506.65. Geneva: World Health Organization.

29. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, et al. (2008) Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med 359: 2619–2620.

30. The malERA Consultative Group on Vaccines (2011) A research agenda for malaria eradication: Vaccines. PLoS Med. pp e1000398. doi:10.1371/journal.pmed.1000398.

31. Peters W (1957) A malaria survey in the Western District of Papua. P N G Med J 2: 25–38.

32. Ruwende C, Khoo SC, Snow RW, Yates SN, Kwiatkowski D, et al. (1995) Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. Nature 376: 246–249.

33. Cortese JF, Caraballo A, Contreras CE, Plowe CV (2002) Origin and dissemination of *Plasmodium falciparum* drug-resistance mutations in South America. J Infect Dis 186: 999–1006.

34. White NJ, Olliaro PL (1996) Strategies for the prevention of antimalarial drug resistance: Rationale for combination chemotherapy for malaria. Parasitol Today 12: 399–401.

35. Nyunt MM, Hendrix CW, Bakshi RP, Kumar N, Shapiro TA (2009) Phase I/II evaluation of the prophylactic antimalarial activity of pafuramidine in healthy volunteers challenged with *Plasmodium falciparum* sporozoites. Am J Trop Med Hyg 80: 528–535.

36. Dondorp AM, Yeung S, White L, Nguon C, Day NP, et al. (2010) Artemisinin resistance: current status and scenarios for containment. Nat Rev Microbiol 8: 272–280.

37. The malERA Consultative Group on Modeling (2011) A research agenda for malaria eradication: Modeling. PLoS Med. pp e1000403. doi:10.1371/journal.pmed.1000403.

38. The malERA Consultative Group on Health Systems and Operational Research (2011) A research agenda for malaria eradication: Health systems and operational research. PLoS Med. pp e1000397. doi:10.1371/journal.pmed.1000397.

39. The malERA Consultative Group on Monitoring, Evaluation, and Surveillance (2011) A research agenda for malaria eradication: Monitoring, evaluation, and surveillance. PLoS Med. pp e1000400. doi:10.1371/journal.pmed.1000400.

40. The malERA Consultative Group on Integration Strategies (2011) A research agenda for malaria eradication: Cross-cutting issues for eradication. PLoS Med. pp e1000404. doi:10.1371/journal.pmed.1000404.

Review

A Research Agenda for Malaria Eradication: Vaccines

The malERA Consultative Group on Vaccines¶*

Abstract: Vaccines could be a crucial component of efforts to eradicate malaria. Current attempts to develop malaria vaccines are primarily focused on *Plasmodium falciparum* and are directed towards reducing morbidity and mortality. Continued support for these efforts is essential, but if malaria vaccines are to be used as part of a repertoire of tools for elimination or eradication of malaria, they will need to have an impact on malaria transmission. We introduce the concept of "vaccines that interrupt malaria transmission" (VIMT), which includes not only "classical" transmission-blocking vaccines that target the sexual and mosquito stages but also pre-erythrocytic and asexual stage vaccines that have an effect on transmission. VIMT may also include vaccines that target the vector to disrupt parasite development in the mosquito. Importantly, if eradication is to be achieved, malaria vaccine development efforts will need to target other malaria parasite species, especially *Plasmodium vivax*, where novel therapeutic vaccines against hypnozoites or preventive vaccines with effect against multiple stages could have enormous impact. A target product profile (TPP) for VIMT is proposed and a research agenda to address current knowledge gaps and develop tools necessary for design and development of VIMT is presented.

Introduction

Vaccines are the most cost-effective tools for public health and have been instrumental in previous elimination campaigns against smallpox [1], polio [2], and measles [3,4]. Vaccines have also been useful for sustained control of diseases such as neonatal tetanus [5], and vaccines such as *Haemophilus influenzae* type b conjugate vaccine have the potential to lead to elimination in some settings [6].

Here, we discuss the research and development agenda for the development of vaccines that can serve as key components of a future arsenal of tools to eradicate malaria. Current efforts to develop malaria vaccines are primarily directed towards reducing the morbidity and mortality that are associated with malaria and focus on *P. falciparum*. For example, the Malaria Vaccine Roadmap [7] has a strategic goal of developing a vaccine with 80% protective efficacy against *P. falciparum* by 2020. However, if malaria vaccines are to contribute to programs for malaria elimination, they will need to have an impact on malaria transmission. The scientific and ethical basis for the development of vaccines referred to as transmission-blocking vaccines (TBVs) that specifically target malaria sexual stage antigens with the goal of having an impact on transmission has been described previously [8,9]. Here, we refocus attention on the development of vaccines that can be used in concert with other malaria control interventions to interrupt malaria transmission and eventually contribute to the eradication of this disease. We also recommend

Review articles synthesize in narrative form the best available evidence on a topic.

that vaccine development efforts need to pay attention to *Plasmodium* species other than *P. falciparum*, especially *Plasmodium vivax*, if malaria eradication is to be achieved.

Rationale of the Proposed malERA Approach to Development of Malaria Vaccines

First, we introduce the broad concept of VIMT. VIMT may be composed of one or more of the following components: classical TBVs that target sexual and mosquito stage parasite antigens; highly effective pre-erythrocytic vaccines that reduce asexual and sexual stage parasite prevalence rates; highly effective asexual erythrocytic stage vaccines that inhibit multiplication of asexual stage parasites efficiently to reduce blood-stage parasite densities and have an impact on malaria transmission; and vaccines that target vector antigens to disrupt parasite development in the vector. It seems obvious that a highly effective pre-erythrocytic vaccine that prevents erythrocytic stage infection will reduce transmission, but the effect of partially effective pre-erythrocytic or asexual blood-stage vaccines on individual infectivity needs investigation. A successful VIMT must primarily reduce malaria transmission. However, VIMTs that include pre-erythrocytic and/or asexual blood-stage vaccine components may also provide individuals with protection against malaria. Such VIMT would also protect the population against epidemic spread following reintroduction of malaria after elimination, an important characteristic given that the gains accrued through many years of elimination can be rapidly reversed if malaria is reintroduced to a population with no antimalarial immunity [10].

Second, the observed impact of concerted nonvaccine malaria control efforts on transmission dynamics in several malaria-endemic regions has shown that high-intensity transmission settings (entomological inoculation rate, EIR >50) can be

Summary Points

- Vaccines for malaria eradication need to have an impact on transmission rather than focusing on mortality and morbidity reduction alone

- Vaccines that interrupt malaria transmission (VIMT) may target many stages of the parasite's life cycle, not just the sexual and mosquito stages as in classical blocking vaccines and multiple plasmodium species, in particular *Plasmodium vivax*

- Novel vaccine delivery approaches and adjuvants need to be developed

- Other priority areas for research and development include the development of tools to measure transmission rates and the development of robust assays of functional immune responses in individuals, which could inform vaccine development

- A better understanding of the dynamics between the multiplication of parasites, gametocytogenesis, and malaria transmission rates in populations is also needed

converted to low-to-moderate intensity transmission settings (EIR <10) [11,12]. Implementation of VIMT together with such control efforts may successfully drive down transmission rates to reduce the effective reproduction rate ($R_{effective}$) to below 1.0.

Third, the consultative group introduces the concept of a detailed TPP for this class of vaccines and urges that novel clinical development methods and approaches be considered to shorten the time to VIMT registration and implementation.

Fourth, the consultative group lays out a detailed research agenda that must be developed, funded, and implemented in parallel with VIMT development efforts. This agenda includes development of critical tools that will be required to register and implement such a vaccine. In particular, we identify the need to develop robust assays to measure biologically relevant transmission-blocking activities at the individual level that are validated as surrogates of reductions in transmission rates at the population level. If this goal is achieved, such assays could become the key tool for measurement of primary vaccine efficacy endpoints in conditional registration trials, thereby simplifying the clinical development program.

Finally, the consultative group considers that interested industrial partners should be identified early on in development, because expertise in applied immunology, vaccinology, product development, manufacturing, and regulatory activities is concentrated within industry and will play an essential role in the successful development of VIMT. In addition, it will be important to engage with regulatory agencies to define efficient yet sound regulatory strategies to develop and register new tools that can meet the needs of global malaria elimination and eradication efforts.

TPP for VIMT

A TPP is an industry-standard tool that gives clear guidance on the critical characteristics of a candidate product under development. TPPs are developed early in the development process and ensure that research and development efforts are focused on those activities that are necessary to develop a product that will meet the needs of end users. Table 1 presents a TPP for VIMT. For each characteristic in this TPP, we propose a "desired target" (aspirational) and a "minimally acceptable target" (must achieve). A vaccine candidate that does not meet or exceed most, if not all,

of the minimally acceptable targets is likely to have a significantly reduced likelihood of successful introduction and uptake.

P. falciparum and *P. vivax* are the two most common *Plasmodium* species that cause human malaria. *P. falciparum* is responsible for most malaria-related deaths. As a result, previous efforts to develop vaccines for malaria have focused on *P. falciparum*, which causes ~500 million cases of malaria annually and is critically important for Africa. However, *P. vivax* causes significant morbidity in other regions of the world including South and Southeast Asia and Latin America with around 75–90 million cases of *P. vivax* malaria reported annually [13]. Recent clinical epidemiology studies have confirmed that *P. vivax* can cause severe disease and may also contribute to malaria-associated mortality [14–17]. Efforts to eliminate malaria outside Africa must therefore address both parasite species. Ideally, VIMT should reduce transmission rates so that $R_{effective}$ for both *P. falciparum* and *P. vivax* is driven to less than 1 and should provide protection against clinical malaria caused by both parasite species. At a minimum (and possibly more realistically), VIMT should achieve reduction of transmission rates ($R_{effective}$ <1) of at least all *P. falciparum* strains leading to elimination of *P. falciparum* when used in conjunction with other control measures in elimination/eradication campaigns.

As better control is achieved, exposure to malaria parasites will decrease and "naturally acquired" immunity may play a diminished role. The mechanisms of clinical immunity observed in populations under high exposure may have little relevance as, increasingly, most infections will occur in people with little previous exposure. Therefore, our TPP specifies that a vaccine intended to interrupt transmission should not presume an age-specific risk or preexisting state of immunity against malaria disease or transmission. It is likely that VIMT may need to be implemented in the entire population.

Other ideal as well as minimally acceptable parameters for VIMT include product presentation, dosage, storage, and coadministration with other immunizations. These parameters are detailed in Table 1.

Research in Support of Development of VIMT

Much of the ongoing work on malaria vaccine development has focused on the development of interventions that address disease manifestations and the work has been primarily focused on *P. falciparum*. To support the development of vaccines and other tools necessary for malaria eradication new dimensions need to be added to the fundamental research portfolio (see [18] also). For example, *P. vivax* needs to be added, and efforts need to be refocused on the development of vaccines that target sexual and mosquito stages of malaria parasites, which should interrupt transmission. The expanded portfolio also needs to include more research on vaccine delivery systems and adjuvants, the transmission dynamics and population biology of malaria parasites, and measurements of transmission rates.

Human Malaria Parasites beyond *P. falciparum*

VIMT that target *P. falciparum* alone are likely to be deployed only in regions where *P. falciparum* is the species predominantly responsible for malaria. Regions where *P. vivax* is responsible for a significant proportion of the malaria burden will require VIMT that target both species.

Control efforts in regions where *P. falciparum* and *P. vivax* both occur indicate that it is more difficult to reduce transmission of *P. vivax* than of *P. falciparum* This increased difficulty is attributed in part to the development of gametocytes earlier during blood-stage

Table 1. TPP VIMT.

Item	Desired Target	Minimally Acceptable Target
Indication	The candidate vaccine is indicated for active immunization of individuals for protection against *P. falciparum* and *P. vivax* malaria and to achieve reduction of transmission rates of all strains of *P. falciparum* and *P. vivax* so that $R_{effective}$ <1[a].	The candidate vaccine is indicated for active immunization of individuals to achieve reduction of transmission rates of all strains of *P. falciparum* so that $R_{effective}$ <1[a] in conjunction with other control measures.
Target populations	The vaccine can be administered to all age groups and populations, including pregnant women, persons with immunodeficiencies, malnourished individuals, or otherwise high risk populations.	The vaccine can be administered to otherwise healthy persons who may transmit malaria, including infants, children, adolescents, and adults in malaria-endemic regions.
Route of administration	The vaccine is administered orally or by intramuscular or subcutaneous injection or by other innovative device.	The vaccine is administered by intramuscular, intradermal subcutaneous injection, or an innovative device.
Product presentation	The vaccine is available in a single dose auto-disposable compact prefilled device. Low multidose presentations (ten doses/vial) are also needed.	The vaccine is provided as a lyophilized or liquid product in single dose vials or an auto-disposable compact prefilled device; or low-dosage (two doses) vials that may be accompanied by a separate paired vial containing adjuvant/diluents. A suitable preservative may be required for multidose vials. Reconstitution may be required prior to administration.
Dosage schedule	A single dose vaccine that can be administered by either mass administration or clinic-based programs. Booster dose may be required after 2 years.	A maximum of two to three doses of vaccine that can be administered according to a schedule feasible for both mass administration and clinical-based programs. A booster dose may be necessary 4–6 months after the second dose and after 2 years.
Warnings and precautions/ pregnancy and lactation	The vaccine has a safety and reactogenicity profile comparable to hepatitis B vaccine. The vaccine can be safely administered to pregnant women. There should be no increased risk of autoimmune or other chronic diseases related to vaccination.	In young children, the vaccine has a similar safety and reactogenicity profile to currently administered combination vaccines such as DTPwHepBHib administered through EPI. In adults, the vaccine has a similar safety and reactogenicity profile as hepatitis B vaccine or tetanus toxoid. The vaccine can be safely administered to pregnant women. There should be no increased risk of autoimmune or other chronic diseases related to vaccination.
Expected efficacy	Reduces $R_{effective}$ below 1.0 in a malaria-endemic population and provides protection against *P. falciparum* and *P. vivax* for at least 2 years.	When used in a malaria-endemic population that employs ITNs, IRS, or other malaria control tools, further reduces $R_{effective}$ to below 1.0 for at least 1 year.
Coadministration	The vaccine can be coadministered with any licensed vaccine without a clinically significant interaction in relation to safety or immunogenicity. For use in infants with other EPI vaccines, specific coadministration studies must be completed to demonstrate the noninferiority of responses to EPI vaccines given in coadministration.	The vaccine will be given as a stand-alone product not coadministered with other vaccines.
Shelf life	The product must have a minimum shelf life of 36 months and a Vaccine Vial Monitor should be attached (see [54]).	The product must have a shelf life of at least 24 months and a Vaccine Vial Monitor should be attached (see [54]).
Storage	The product must be stable at ambient temperature and withstand freeze thawing.	At a minimum, vaccines should be stable at refrigerated storage temperatures (2–8°C). New vaccines should be formulated to maximize heat stability to improve effectiveness in light of the challenges faced in distributing vaccines in developing countries. Vaccine vial monitors should be included on all vaccines in accordance with the WHO and UNICEF joint policy statement and the WHO prequalification standards for vaccines. In case of live, attenuated sporozoite vaccines, vaccine should be stable at −70°C.
	Vaccine vial monitors should be included on all vaccines in accordance with the WHO and UNICEF joint policy statement and the WHO prequalification standards for vaccines.	
Product registration and WHO prequalification	Product must be WHO prequalified (see [54]) and registered with EMEA and FDA.	Conditional registration or recommendation by WHO or competent NRA followed by a large impact study in phase IV.
		Product must be WHO prequalified (see [55]).

[a]$R_{effective}$, number of individuals who can be infected from a single untreated malaria case in an endemic area.
EMEA, European Medicines Agency; EPI Expanded Programme on Immunization; FDA, US Food and Drug Administration; NRA, National Regulatory Agency; IRS, indoor residual insecticide spraying; ITN, insecticide-treated net.
doi:10.1371/journal.pmed.1000398.t001

infections with *P. vivax* than is the case for *P. falciparum*, which allows transmission before clinical symptoms are apparent. Other factors contributing to the difficulty of reducing *P. vivax* transmission include: the development of hypnozoites that remain latent in hepatocytes and lead to blood-stage infections months or even years later; transmission by outdoor biting mosquitoes; and the ability of *P. vivax* to complete its life cycle in a wider range of climatic and ecological conditions than *P. falciparum*. Because of these unique features of *P. vivax*, traditional malaria control efforts such as vector control, bednets, and early detection and treatment

often fail to control *P. vivax* transmission. Vaccines that elicit long-lasting immune responses that prevent infection or inhibit gametocyte development or transmission of sexual stages are likely to be more effective tools for control of *P. vivax*. Given that latent hypnozoites can lead to blood-stage infections years after an infective bite, it may be necessary to continue deployment of VIMT that target *P. vivax* after elimination is achieved. An alternative would be to develop vaccine components that can target and eliminate hypnozoites. Design of such vaccines will require better understanding of the unique aspects of the biology of *P. vivax* hypnozoites at the molecular level.

Other *Plasmodium* species such as *Plasmodium ovale* and *Plasmodium malariae* account for less than 5% of malaria cases worldwide. Natural infection of humans by *Plasmodium knowlesi* has recently been reported [19,20]. Thus, we need to be prepared for the emergence of new *Plasmodium* species that can cause human malaria. It remains to be seen whether these parasite species will survive once efforts to eliminate *P. falciparum* and *P. vivax* are successful. For now, then, efforts should be focused on developing VIMT for *P. falciparum* and *P. vivax* malaria, but it will be important to monitor the epidemiology of *P. ovale*, *P. malariae*, and *P. knowlesi* as elimination of *P. falciparum* and *P. vivax* progresses. Decisions to support development of vaccines that block transmission of these parasite species may need to be made in the future.

Discovery Research

Malaria parasites have a complex life cycle during which they infect humans and are transmitted by Anopheline mosquitoes. The successful completion of the parasite life cycle requires specific molecular interactions between the parasite and various host and vector tissues. A clear understanding of the molecular interactions that mediate invasion of hepatocytes by Plasmodium sporozoites, invasion of erythrocytes by *Plasmodium* merozoites, and traversal of mosquito midgut epithelium by *Plasmodium* ookinetes may allow the development of strategies to target these key interactions and disrupt the parasite life cycle thereby reducing malaria transmission rates. It may be necessary to combine components that target different stages of malaria parasites to achieve synergistic effects that provide protection and reduce malaria transmission rates. For example, partially effective pre-erythrocytic and blood-stage components may not have any effect on transmission but the addition of such partially effective components to classical TBVs might allow the development of a multicomponent VIMT that can reduce malaria transmission as well as provide protection against malaria.

Targeting the Sexual and Mosquito Stages

Gametocytes are the source of the epidemiologically important transmission of all malaria parasites. In *P. falciparum*, recent work has demonstrated that the developmental switch from asexual replication to sexual stage development occurs at the ring stage and that all schizonts from that ring parasite are committed to form gametocytes upon invasion of new red blood cells [21]. *P. falciparum* then undergoes sequential development through five distinct morphological stages to form mature male and female gametocytes. Within the mosquito midgut, mature male and female gametes are released and fertilization occurs to form a zygote. The resultant motile ookinete passes through the midgut wall, undergoes reduction division, and forms an oocyst. Each step in this developmental pathway involves unique processes, including the transcription of specific genes, the expression of specific proteins, the upregulation of specific biochemical pathways, and the formation of new morphological structures.

Understanding the regulation of this developmental process could be the key to developing new interventions that target sexual and mosquito stages to interrupt transmission. For example, direct targeting of the developing gametocyte has the potential advantage of targeting a small subset of infected red blood cells that express proteins or pathways specific to parasite sexual development. A drug or a vaccine that could inhibit the initial switch to sexual development, coupled with a vaccine that targets gamete antigens might provide a powerful combinatorial approach to reduce transmission (also see [22]).

There is a large body of work on the key antigens on the surface of gametes of both *P. falciparum* and *P. vivax* [9]. Several of these antigens have been tested in animal models as transmission-blocking vaccines, at least two which have been tested in humans [23,24]. A phase I trial of the *P. vivax* ookinete surface antigen Pvs25 formulated with Alhydrogel demonstrated acceptable safety and reactogenicity with induction of anti-Pvs25 immunoglobulin G (IgG) with functional transmission-blocking activity in a membrane-feeding assay. However, these data suggest that a more immunogenic formulation would be desirable to achieve higher transmission-blocking activity [23]. More recently, a trial of ISA51 formulations of Pvs25 and Pfs25 was terminated because of unacceptable reactogenicity [24]. The expression of correctly folded Pfs48/45 gametocyte surface antigen has recently resulted in a demonstration of transmission-reducing activity in sera from immunized animals [25,26].

Targeting Pre-erythrocytic and Asexual Stages

Highly effective pre-erythrocytic stage vaccines can, in principle, reduce the prevalence of blood-stage parasites, including both the asexual stages and the gametocytes. Such vaccines can provide protection against malaria and reduce malaria transmission. Immunization with irradiated sporozoites has elicited complete protection against sporozoite challenge in experimental animal models and in humans. Thus, in principle, it should be possible to target pre-erythrocyte stage antigens to elicit complete protection against parasite infection. Protective immune mechanisms elicited by irradiated sporozoites are not well understood but are thought to include antibody responses against sporozoite antigens that prevent hepatocyte infection, and cellular responses that clear infected hepatocytes. Better understanding of the correlates of immunity elicited by immunization with irradiated sporozoites could guide the development of highly effective pre-erythrocytic subunit vaccines that both provide protection and reduce parasite transmission. A recombinant vaccine based on the circumsporozoite protein, RTS,S has been shown to elicit partial protection against *P. falciparum* infection [27,28]. It seems unlikely, however, that RTS,S will have significant impact on gametocyte prevalence or affect malaria transmission.

Other vaccines based on irradiated sporozoites or genetically modified attenuated sporozoites have provided protection in challenge models [29,30]. Such whole organism attenuated vaccines may provide effective protection against malaria and significantly reduce parasite transmission. However, considerable technological challenges in terms of manufacturing, formulation, and delivery of such attenuated sporozoite vaccines need to be overcome.

During *P. vivax* infections, some infected hepatocytes differentiate into latent hypnozoite stages that can yield merozoites after a long latency period. The biology of hypnozoites is very poorly understood but the development of drugs or vaccines that can clear hypnozoites is critical for success of efforts to eradicate *P. vivax* [22]. The development of methods for *in vitro* culture of hypnozoites could greatly help improve our understanding of this

latent stage. *In vitro* culture of hypnozoites would allow the application of whole genome approaches such as transcriptomics and proteomics to the identification of parasite proteins expressed in hypnozoites. It may be possible to elicit cellular immune responses against such hypnozoite specific proteins to clear these latent stages. Vaccines against pre-erythrocytic stages of *P. vivax* that are effective against both developing and resident hypnozoites would be of inestimable benefit in efforts to eliminate *P. vivax*.

Vaccines based on asexual blood-stage antigens may be effective at reducing parasite densities and provide protection against clinical disease but it is not clear whether such vaccines can reduce malaria transmission rates effectively. Basic research is needed to understand the dynamics of the relationship between asexual stage parasite growth, sexual stage parasite densities in blood, and individual infectivity or transmission efficiency. Recombinant vaccines based on asexual blood-stage antigens tested in human clinical trials have not yielded high rates of growth inhibition thus far and are unlikely to have significant impact on gametocyte prevalence or infectivity of individuals. Irrespective of whether vaccines based on asexual blood-stage antigens can reduce sexual stage parasite densities and reduce transmission, combinations of asexual blood-stage vaccines with classical TBVs will enable development of VIMT that provide direct benefit to vaccine recipients by providing protection against clinical disease in addition to reducing transmission.

Targeting the Vector to Reduce Malaria Transmission

As described earlier, *Plasmodium* parasites have an obligatory development stage in the mosquito during which zygotes transform into ookinetes that traverse the midgut epithelium to establish oocysts on the outer wall of the midgut. Attachment and invasion of the midgut epithelium requires specific interactions between ookinete surface proteins and midgut receptors. A set of conserved "invasion receptors" on the midgut of diverse Anopheline species are used by *Plasmodium* ookinetes to attach to the midgut epithelium [31]. Antibodies directed against such receptors have been shown to block development of oocysts in membrane-feeding transmission-blocking assays [31]. A vaccine based on such conserved vector antigens should be effective against all species of *Plasmodium* and obviate the need to develop separate vaccines for different *Plasmodium* species. Moreover, since such vaccines target vector antigens, parasite strain diversity, which has been a major problem for malaria vaccine development, will be overcome. Such novel strategies will require significant fundamental research to understand vector-parasite interactions [32].

Host-Parasite and Vector-Parasite Interactions

Plasmodium sporozoites invade human hepatocytes in a two-step process. In the first step, sporozoites pass through multiple hepatocytes by rupturing the plasma membrane of target hepatocytes [33]. After traversing multiple hepatocytes, sporozoites finally invade target hepatocytes by forming a parasitophorous vacuole where they multiply and differentiate into merozoites. Identification of key parasite proteins that mediate the two-step invasion process could provide functional targets for intervention. Sporozoite surface proteins such as the circumsporozoite protein (CSP) and thrombospondin-related protein (TRAP) have been shown to play a role in hepatocyte binding and invasion [34–37]. Both proteins contain functional cysteine-rich regions that share homology with thrombospondin and that mediate attachment to hepatocyte receptors. Antibodies targeting such functional regions can block hepatocyte invasion. Vaccines that elicit high-titer long-lasting antibodies against such functional domains might reduce

the prevalence of blood-stage infection effectively. Similarly, antibodies targeting merozoite antigens such as the 175-kD erythrocyte binding antigen (EBA175) [38–41], Duffy binding protein [42], or PfRH proteins [43], which mediate critical interactions with erythrocyte receptors, can inhibit multiplication of blood-stage parasites. Ookinete antigens that interact with the midgut wall to mediate traversal may also be useful as recombinant malaria vaccine candidates that block parasite transmission by mosquitoes.

Because processes such as host cell invasion involve multiple steps, some of the processes highlighted above may be mediated by multiple pathways that are redundant. As a result, effective inhibition of host invasion by parasites may require targeting of a combination of receptor-ligand interactions that mediate invasion. A clear understanding of the sequence of events and functional roles of different receptor-ligand interactions will be critical for the development of vaccines that target multiple steps to provide synergistic inhibition of invasion and parasite multiplication at different stages of the parasite life cycle.

It will also be important to develop functional assays that can be used to evaluate antibody responses against the parasite antigens that mediate host cell invasion and transmission to mosquitoes. These functional assays may directly test the inhibitory activity of antibodies elicited by vaccine candidates against the biological processes themselves or may be reduced to biophysical or biochemical assays in which antibodies are tested for inhibition of functions such as receptor binding or proteolytic cleavage that are known to mediate the biological processes. Harmonization of such assays is important so that results from different research groups are comparable and to facilitate decision making for down-selection of vaccine candidates during preclinical and clinical development. Currently, there are no clear correlates of immunity against pre-erythrocytic and blood-stage parasites. Immuno-assays can be validated only once a vaccine demonstrates efficacy in a clinical trial. Once an immune correlate for protection is identified, it can be used for decision making in clinical development.

Vaccine Delivery Systems and Adjuvants

The development of subunit vaccines will require the use of potent adjuvants and/or efficient vaccine delivery systems to elicit robust and sustainable immune responses. The unavailability of a wide range of potent adjuvants with a proven safety record in humans has been a bottleneck in the development of recombinant protein–based vaccines for malaria. Better understanding of mechanisms that activate the innate immune system might enable the design of adjuvants that elicit potent immune responses. Alternative methods to deliver antigens such as the use of virus-like particles or prime-boost strategies that use combinations of different viral vectors (e.g., recombinant adenovirus and modified vaccine virus–based vectors) or viral vectors and recombinant proteins have provided effective means to elicit potent immune responses [44], but further research on vaccine delivery systems is urgently required for development of effective malaria vaccines.

When the VIMT include multiple components, it will be important to develop formulations or delivery systems that are compatible with each component. A clear understanding of the correlates of protective immunity elicited by each component may allow the identification and development of a compatible delivery system or adjuvant formulation for the combination vaccine. Analysis of candidate vaccine–elicited immune responses in functional assays will allow optimization of compatible formulations. Importantly, development of multicomponent VIMT may require collaboration between researchers who have developed the

individual components. It will be important to develop innovative licensing arrangements that ensure accessibility of each component for commercial development of such multicomponent VIMT.

Understanding Transmission Dynamics and Population Biology of Malaria Parasites

As campaigns to reduce transmission of malaria are successful, it will be necessary to understand the changes in parasite population dynamics and population structure. In particular, it will be desirable to determine whether specific parasite strains dominate as the transmission pattern changes and whether this has implications with regard to antigenic diversity or parasite virulence. Field trials with *P. falciparum* blood-stage vaccines have provided evidence for allele-specific protection, which suggests that large-scale immunization may lead to the selection of "vaccine-resistant" parasites that can escape immune responses elicited by the vaccine [45]. A second important question is to determine whether reemergent parasites have been introduced from an outside source or whether they are parasites that have escaped control measures. These two options have very different implications for intervention strategies during the pre-elimination stage. Tools to track such parasites will be useful for surveillance as control efforts move towards eradication.

Measuring Malaria Transmission Rates

A key to the evaluation of vaccines that block transmission will be the measurement of transmission. The anticipated clinical outcome of vaccination will be the reduction of transmission in the community. It is therefore necessary to develop robust and readily usable tools to evaluate transmission levels in various epidemiological settings ranging from high transmission areas to areas of very low prevalence and transmission. In particular, as various malaria control measures are introduced, the transmission dynamics will change and robust evaluation of transmission will be challenging. Harmonization of existing tools for measurement of transmission rates is a high priority [46,47].

It is particularly important to be able to measure the effect on infectivity of an individual after vaccination with either a pre-erythrocytic or a blood-stage vaccine, and to understand the relation of this result to an effect on transmission in the community. Clinical efficacy trials of such vaccines have tended to focus on their impact on blood-stage infection or clinical disease; the impact of such vaccines on transmission remains to be determined. An important aspect of strategic thinking around malaria vaccines in years to come will be a greater emphasis on the evaluation of the impact of all classes of vaccines on transmission.

A second priority is the development of markers that define the infectivity of an individual for mosquitoes. These markers could include bioassays, serological parameters, or molecular markers. There is a need for robust models that predict the relationship of rates of individual infectivity to transmission at the community level in different epidemiological settings. Once this relationship is established, such markers could be used as surrogates of vaccine efficacy on transmission at the population level.

Strategies for Product and Clinical Development of VIMT
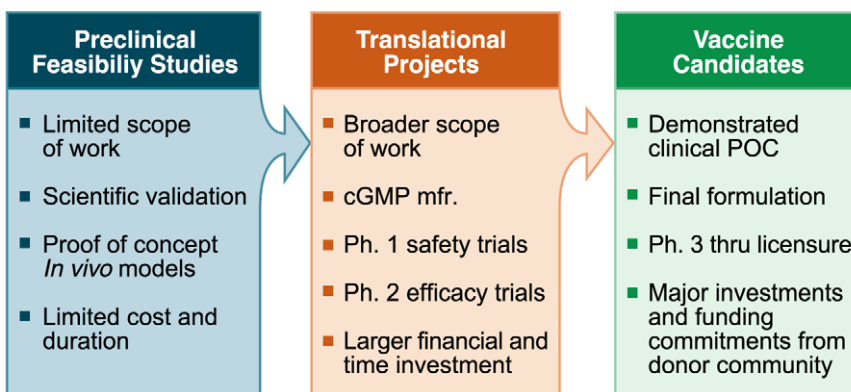
Product Development Based on TPP

Once TPPs are defined, they should be used to guide product development and evaluate the project in terms of achieving desired goals set for the vaccine candidates. It is important to understand where the project stands in terms of development. Terminology should be used appropriately and be in line with the development phase of the product (Figure 1).

Preclinical feasibility studies are conducted first to validate the scientific rationale for vaccine design. At this stage of the project, questions have to be asked that address issues such as whether the project is likely to achieve the final desired TPP. Numerous preclinical feasibility studies may be undertaken to assess a variety of antigens, adjuvants, and delivery systems. Importantly, immune responses with the experimental vaccine produced at pilot scale need to be evaluated in animal models, preferably using functional assays, to validate the concept and progress it to a translational project stage.

For the translational stage, a significant investment of resources is necessary, not least because the prototype vaccine must be produced under current good manufacturing practices. Thus, only the most promising approaches can be moved into this and later stages of development. The translational project, which will have a set of precise go/no-go milestones, drives a research program of relevance to public health from the preclinical phase, through phase I trials to evaluate safety, and into phase II trials to evaluate efficacy. A successful translational project will deliver a vaccine that should be ready for phase III trials.

A product can be considered as a vaccine candidate once its manufacturability has been established and it has undergone a

Classification of programs.

Preclinical Feasibiliy Studies	Translational Projects	Vaccine Candidates
■ Limited scope of work	■ Broader scope of work	■ Demonstrated clinical POC
■ Scientific validation	■ cGMP mfr.	■ Final formulation
■ Proof of concept *In vivo* models	■ Ph. 1 safety trials	■ Ph. 3 thru licensure
■ Limited cost and duration	■ Ph. 2 efficacy trials	■ Major investments and funding commitments from donor community
	■ Larger financial and time investment	

Figure 1. Classification of programs. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000398.g001

successful proof-of-concept phase II efficacy trial (Figure 1). For "classical" pre-erythrocytic or asexual stage vaccines, this typically requires either a phase IIa challenge trial or an efficacy trial in an endemic country. For VIMT, proof of concept may not need to be established in a malaria-endemic setting, provided that a robust read-out measurable at the level of the individual vaccinees has been shown to predict an effect on transmission at the population level. By this stage the product is fully characterized and will not change substantially. Major investments will be required, however, to complete the development program to deliver a viable vaccine for use in public health programs. Other considerations for a successful vaccine include the requirement for WHO prequalification of the vaccine for use in developing countries, an understanding in the affected communities of the ethical and practical issues associated with a long program of testing, and a significant commitment of the donor community to provide funds to support country-wide vaccine launches.

Clinical Development and Regulatory Strategy

A vaccine that has an effect on transmission alone may not provide direct benefit to the individual. Registration pathways for such a vaccine are therefore likely to be complex, and the licensure endpoints will require careful consideration and discussion with regulatory agencies early in the development program. If the vaccine also provides individual benefit, the regulatory pathway could well be simpler.

One approach to registration for VIMT is for phase I/II programs to focus on identification of well-tolerated and immunogenic vaccine doses and schedules across a wide age range of vaccine recipients using standard safety assessments and immunologic readouts tailored for the vaccine candidate being evaluated. Randomized, controlled phase IIb proof-of-concept studies should be designed to permit the identification of a suitable vaccine efficacy endpoint at the individual level that can be validated for use in phase III trials. This endpoint must be identified and agreed in advance with regulatory agencies. The possible endpoints might include: percent reduction in parasite prevalence, especially gametocyte prevalence; percent reduction in individual infectivity as measured by percent reduction in oocyst and sporozoite counts in membrane-feeding assays; and percent reduction in infected mosquitoes fed on vaccinated volunteers that can transmit malaria to susceptible volunteers. We recognize that such efficacy endpoints at the individual level will only be surrogates for effects on malaria transmission rates at the population level. Thus, a necessary stage after conditional registration based on surrogate efficacy data will be definitive community-scale phase IV trials, which will measure reductions in effective reproduction rate ($R_{effective}$) as a postmarketing commitment.

Alternatively, some experts have argued that it should be possible to design and conduct cluster-randomized trials to evaluate the efficacy of VIMT in terms of reductions in transmission rates in malaria-endemic settings. Measurement of surrogate efficacy parameters at the individual level using robust assays in such trials may allow the identification of correlates of efficacy at the population level. Such an approach would follow the more traditional route of registering a vaccine after collecting evidence for efficacy in phase IIb/III trials. Ultimately, it will be important to study the efficacy of combination of vaccines with other interventions aimed at reducing transmission.

Decision Making in Development of VIMT

Existing methods for measurement of transmission intensity need to be harmonized and optimized to ensure that good baseline estimates are available prior to introduction of a package of interventions such as drugs and vaccines. Thus, an essential step will be a consultation process that decides on the relative utility of assays that assess the infectiousness of individuals [48], that measure transmission-blocking activity of sera [49] raised against sexual stage or mosquito antigens, and that consider trial designs to measure the impact of vaccines targeting any life cycle stage on malaria transmission [50].

Possible trial designs include community-randomized trials that use measurement of the reduction in the proportion of gametocyte carriers, the reduction in the infectiousness of humans to mosquitoes in individually randomized controlled trials, and the reduction in infection of humans as endpoints. However, the development of an assay or trial design that could provide robust, reproducible data on vaccine impact on transmission without performing large-scale community-randomized trials would be a major step forward in increasing efficiencies and timelines.

Many questions will need to be addressed to aid decision making during development of VIMT. For example, can assays such as the membrane-feeding assay be validated to meet the requirements of the International Conference of Harmonization? If so, what level of reduced infectivity as demonstrated by this assay is likely to provide community-level reduction in infection? Questions like these need to be answered so that decisions can be made about the packages of interventions required to bring the $R_{effective}$ below 1 during elimination campaigns. An assessment of existing modeling work may provide information on this sort of issue [51,52]. Other questions that will need answering include: what population coverage and level of transmission-blocking efficacy should we require from a vaccine intervention before it is transitioned into elimination campaigns and are there assays other than the membrane-feeding assay that will be useful in measurement of infectiousness of humans (for example, nucleic acid amplification-based assays for gametocytaemia)? Ways will also need to be found to optimize mosquito-feeding experiments linked to clinical vaccine trials for decision-making purposes (see also [53]).

Importantly, every step of the vaccine development, clinical evaluation, regulatory, and implementation process for VIMT needs to focus on using the TPP for vaccines and targeting transmission rather than morbidity during decision making. In addition, it will be essential to make decisions about the need to include packages of interventions when evaluating vaccines that reduce transmission (see also [52]). Decisions will also have to be made about who should receive VIMT. In endemic regions, VIMT would be delivered to infants, preferably through the routine expanded program of immunization and through periodic campaigns to the rest of the population. In regions of low malaria transmission, it may not be necessary to immunize the entire population. Instead it may be more effective to identify and immunize individuals who are responsible for the majority of the transmission in the community.

Assessment of interruption of transmission presents novel challenges and large costs, hence every effort must be made to find and adopt the most efficient mechanism for assessing efficacy. For example, could a competent regulatory authority be provided with sufficiently compelling evidence of the biological interruption of transmission activity of a vaccine (either prevention of gametocyte production or effects of antisera on transmission to mosquitoes) to allow registration of a vaccine with an indication for interruption of transmission at the community level, without the requirement for large-scale community randomized trial data? As mentioned earlier, phase IV studies could then follow to provide the required safety database, and measures of community

effects on transmission for implementation. Industry involvement may be critical to successfully drive such a development pathway for VIMT. It will therefore be important to engage leaders of key vaccine industries as well as regulatory agencies and ethicists from affected countries in discussions early in the development pathway.

Conclusions

Vaccines can play a key role in multisectoral efforts to eliminate and eventually eradicate malaria. Current efforts to develop malaria vaccines are primarily focused on reducing infection rates, blocking replication of the parasite in the bloodstream, and the pathologic effects of the parasite in individuals, thereby reducing malaria morbidity and mortality in vaccinated individuals. Some of these vaccines, if highly effective, may also reduce transmission. These efforts need continued support.

For elimination, it is important to view vaccines for their potential contribution to reduction of transmission, and to support additional novel approaches to vaccines that directly target sexual and mosquito stages for use in malaria control programs. In this context, we propose the broader concept of VIMT and present an actionable research and development agenda to develop such vaccines (Box 1). We also propose that novel product development and regulatory strategies that reduce the time to market should be investigated to develop, license, and implement such vaccines.

Acknowledgments

Vaccine Initiative, Washington (D.C.), USA; Corinne Warren, PATH Malaria Vaccine Initiative, Washington (D.C.), USA; Colleen Woods, PATH Malaria Vaccine Initiative, Washington (D.C.), USA.

Author Contributions

References

1. Henderson DA (1987) Principles and lessons from the smallpox eradication programme. Bull World Health Organ 65: 535–546.
2. John J (2009) Role of injectable and oral polio vaccines in polio eradication. Expert Rev Vaccines Jan 8: 5–8.
3. Moss WJ, Griffin DE (2006) Global measles elimination. Nat Rev Microbiol 12: 900–908.
4. Breman JG, de Quadros CA, Dowdle WR, Foege WH, Henderson DA, et al. (2011) The role of research in viral disease eradication and elimination programs: Lessons for malaria eradication. PLoS Med 8: e1000405. doi:10.1371/journal.pmed.1000405.
5. Roper MH, Vandelaer JH, Gasse FL (2007) Maternal and neonatal tetanus. Lancet 370: 1947–1959.
6. Adegbola RA, Secka O, Lahai G, Lloyd-Evans N, Njie A, et al. (2005) Elimination of Haemophilus influenzae type b (Hib) disease from The Gambia after the introduction of routine immunisation with a Hib conjugate vaccine: A prospective study. Lancet 366: 144–150.
7. Malaria Vaccine Technology Roadmap Final Report (2006) Available: http://www.malariavaccineroadmap.net.
8. WHO (2000) Malaria transmission blocking vaccines: An ideal public good. Geneva: World Health Organization. Available: http://www.who.int/vaccine_research/feuille_1_4-1pdf.
9. Carter R (2001) Transmission blocking malaria vaccines. Vaccine 19: 2309–2314.
10. Roberts DR, Manguin S, Moudhet J (2000) DDT house spraying and re-emerging malaria. Lancet 356: 330–332.
11. O'Meara WP, Bejon P, Mwangi TW, Okiro EA, Peshu N, et al. (2008) Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. Lancet 372: 1555–1562.
12. Ceesay SJ, Casals-Pascual C, Erskine J, Anya SE, Duah NO, et al. (2008) Changes in malaria indices between 1999 and 2007 in The Gambia: A retrospective analysis. Lancet 372: 1545–1554.
13. Mendis K, Sina BJ, Marchesini P, Carter R (2001) The neglected burden of Plasmodium vivax malaria. Am J Trop Med Hyg 64: 97–106.
14. Kochar DK, Das A, Kochar SK, Saxena V, Sirohi P, et al. (2009) Severe Plasmodium vivax malaria: A report on serial cases from Bikaner in northwestern India. Am J Trop Med Hyg Feb 80: 194–198.
15. Kochar DK, Saxena V, Singh N, Kochar SK, Kumar SV, et al. (2005) Plasmodium vivax malaria. Emerg Infect Dis Jan 11: 132–134.
16. Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, et al. (2007) Vivax malaria: Neglected and not benign. Am J Trop Med Hyg 77: 79–87.
17. Price RN, Douglas NM, Anstey NM (2009) New developments in Plasmodium vivax malaria: Severe disease and the rise of chloroquine resistance. Curr Opin Infect Dis 22: 430–435.
18. The malERA Consultative Group on Basic Science and Enabling Technologies (2011) A research agenda for malaria eradication: Basic science and enabling technologies. PLoS Med 8: e1000399. doi:10.1371/journal.pmed.1000399.
19. Lee KS, Cox-Singh J, Brooke G, Matusop A, Singh B (2009) Plasmodium knowlesi from archival blood films: Further evidence that human infections are widely distributed and not newly emergent in Malaysian Borneo. Int J Parasitol 9: 1125–1128.
20. Cox-Singh J, Davis TM, Lee KS, Shamsul SS, Matusop A, et al. (2008) Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis 46: 165–171.
21. Dixon MW, Thompson J, Gardiner DL, Trenholme KR (2008) Sex in Plasmodium: A sign of commitment. Trends Parasitol 24: 168–175.
22. The malERA Consultative Group on Drugs (2011) A research agenda for malaria eradication: Drugs. PLoS Med 8: e1000402. doi:10.1371/journal.pmed.1000402.
23. Malkin EM, Durbin AP, Diemert DJ, Sattabongkot J, Wu Y, et al. (2005) Phase 1 vaccine trial of Pvs25H: A transmission blocking vaccine for Plasmodium vivax malaria. Vaccine 23: 3131–3138.
24. Wu Y, Ellis RD, Shaffer D, Fontes E, Malkin EM, et al. (2008) Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with montanide ISA 51. PLoS One 3: e2636. doi:10.1371/journal.pone.0002636.
25. Outchkourov NS, Roeffen W, Kaan A, Jansen J, Luty A, et al. (2008) Correctly folded Pfs48/45 protein of Plasmodium falciparum elicits malaria transmission-blocking immunity in mice. Proc Natl Acad Sci U S A 105: 4301–4305.
26. Chowdhury DR, Angov E, Kariuki T, Kumar N (2009) A potent malaria transmission blocking vaccine based on codon harmonized full length Pfs48/45 expressed in Escherichia coli. PLoS One 4: e6352. doi:10.1371/journal.pone.0006352.
27. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, et al. (2004) Efficacy of the RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African children: Randomised controlled trial. Lancet 364: 1411–1420.
28. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, et al. (2005) Duration of protection with RTS,S/AS02A malaria vaccine in prevention of Plasmodium falciparum disease in Mozambican children: Single-blind extended follow-up of a randomised controlled trial. Lancet 366: 2012–2018.
29. Hoffman SL, Billingsley PF, James E, Richman A, Loyevsky M, et al. (2010) Development of a metabolically active, non-replicating sporozoite vaccine to prevent Plasmodium falciparum malaria. Hum Vaccin 6: 97–106.
30. Pinder M, Moorthy VS, Akanmori BD, Genton B, Brown GV (2010) Development of a metabolically active, non-replicating sporozoite vaccine to prevent Plasmodium falciparum malaria. Vaccine 6: 97–106.
31. Dinglasan RR, Kalume DE, Kanzok SM, Ghosh AK, Muratova O, et al. (2007) Disruption of Plasmodium falciparum development by antibodies against a conserved mosquito midgut antigen. Proc Natl Acad Sci U S A 104: 13461–13466.
32. The malERA Consultative Group on Vector Control (2011) A research agenda for malaria eradication: Vector control. PLoS 8: e1000401. doi:10.1371/journal.pmed.1000401.
33. Mota MM, Hafalla JC, Rodriguez A (2002) Migration through host cells activates Plasmodium sporozoites for infection. Nat Med 8: 1318–1322.
34. Sultan AA, Thathy V, Frevert U, Robson KJ, Crisanti A, et al. (1997) TRAP is necessary for gliding motility and infectivity of Plasmodium sporozoites. Cell 90: 511–522.
35. Cerami C, Frevert U, Sinnis P, Takacs B, Clavijo P, et al. (1992) The basolateral domain of the hepatocyte plasma membrane bears receptors for the circumsporozoite protein of Plasmodium falciparum sporozoites. Cell 70: 1021–1033.
36. Tewari R, Spaccapelo R, Bistoni F, Holder AA, Crisanti A. (2002) Function of region I and II adhesive motifs of Plasmodium falciparum circumsporozoite protein in sporozoite motility and infectivity. J Biol Chem 277: 47613–47618.
37. Wengelnik K, Spaccapelo R, Naitza S, Robson KJ, Janse CJ, et al. (1999) The A-domain and the thrombospondin-related motif of Plasmodium falciparum TRAP are implicated in the invasion process of mosquito salivary glands. EMBO J 18: 5195–5204.
38. Narum DL, Haynes JD, Fuhrmann S, Moch K, Liang H, et al. (2000) Antibodies against the Plasmodium falciparum receptor binding domain of EBA-175 block invasion pathways that do not involve sialic acids. Infect Immun 68: 1964–1966.
39. Pandey KC, Singh S, Pattnaik P, Pillai CR, Pillai U, et al. (2002) Bacterially expressed and refolded receptor binding domain of Plasmodium falciparum EBA-175 elicits invasion inhibitory antibodies. Mol Biochem Parasitol 123: 23–33.
40. Pattnaik P, Shakri AR, Singh S, Goel S, Mukherjee P, et al. (2007) Immunogenicity of a recombinant malaria vaccine based on receptor binding domain of Plasmodium falciparum EBA-175. Vaccine 25: 806–813.
41. Sim BK, Chitnis CE, Wasniowska K, Hadley TJ, Miller LH (1994) Receptor and ligand domains for invasion of erythrocytes by Plasmodium falciparum. Science 264: 1941–1944.
42. Grimberg BT, Udomsangpetch R, Xainli J, McHenry A, Panichakul T, et al. (2007) Plasmodium vivax invasion of human erythrocytes inhibited by antibodies directed against the Duffy binding protein. PLoS Med 4: e337. doi:10.1371/journal.pmed.0040337.
43. Gao X, Yeo KP, Aw SS, Kuss C, Iyer JK, et al. (2008) Antibodies targeting the PfRH1 binding domain inhibit invasion of Plasmodium falciparum merozoites. PLoS Pathog 4: e1000104. doi:10.1371/journal.ppat.1000104.
44. Draper SJ, Moore AC, Goodman AL, Long CA, Holder AA, et al. (2008) Effective induction of high-titer antibodies by viral vector vaccines. Nat Med 14: 819–821.
45. Takala SL, Plowe CV (2009) Genetic diversity and malaria vaccine design, testing and efficacy: preventing and overcoming 'vaccine resistant malaria'. Parasite Immunol 31: 560–573.
46. The malERA Consultative Group on Cross Cutting Issues for Eradication (2011) A research agenda for malaria eradication: Cross cutting issues for eradication. PLoS 8: e1000404. doi:10.1371/journal.pmed.1000404.
47. The malERA Consultative Group on Basic Monitoring, Evaluation, and Surveillance (2011) A research agenda for malaria eradication: Monitoring, evaluation, and surveillance. PLoS 8: e1000400. doi:10.1371/journal.pmed.1000400.
48. Bousema JT, Schneider P, Gouagna LC, Drakeley CJ, Tostmann A, et al. (2006) Moderate effect of artemisinin-based combination therapy on transmission of Plasmodium falciparum. J Infect Dis 193: 1151–1159.
49. van der Kolk M, De Vlas SJ, Saul A, van de Vegte-Bolmer M, Eling WM, et al. (2005) Evaluation of the standard membrane feeding assay (SMFA) for the

determination of malaria transmission-reducing activity using empirical data. Parasitology 130: 13–22.

50. WHO (1997) Guidelines for the evaluation of *Plasmodium falciparum* vaccines in populations exposed to natural infection. Geneva: WHO.

51. Penny MA, Maire N, Studer A, Schapira A, Smith TA (2008) What should vaccine developers ask? Simulation of the effectiveness of malaria vaccines. PLoS One 3: e3193. doi:10.1371/journal.pone.0003193.

52. The malERA Consultative Group on Modeling (2011) A research agenda for malaria eradication: Modeling. PLoS 8: e1000403. doi:10.1371/journal.pmed.1000403.

53. The malERA Consultative Group on Diagnoses and Diagnostics (2011) A research agenda for malaria eradication: Diagnoses and diagnostics. PLoS 8: e1000396. doi:10.1371/journal.pmed.1000396.

54. World Health Organization (2000) Making use of vaccine vial monitors. Flexible vaccine management for polio. Geneva: World Health Organization Available: www.who.int/vaccines-documents/DocsPDF00/www516.pdf.

55. World Health Organization (2006) Procedures for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies. Geneva: World Health Organization Available: www.who.int/vaccines-documents/DocsPDF06/812.pdf.

A Research Agenda for Malaria Eradication: Vector Control

The malERA Consultative Group on Vector Control[¶]*

Abstract: Different challenges are presented by the variety of malaria transmission environments present in the world today. In each setting, improved control for reduction of morbidity is a necessary first step towards the long-range goal of malaria eradication and a priority for regions where the disease burden is high. For many geographic areas where transmission rates are low to moderate, sustained and well-managed application of currently available tools may be sufficient to achieve local elimination. The research needs for these areas will be to sustain and perhaps improve the effectiveness of currently available tools. For other low-to-moderate transmission regions, notably areas where the vectors exhibit behaviours such as outdoor feeding and resting that are not well targeted by current strategies, new interventions that target predictable features of the biology/ecologies of the local vectors will be required. To achieve elimination in areas where high levels of transmission are sustained by very efficient vector species, radically new interventions that significantly reduce the vectorial capacity of wild populations will be needed. Ideally, such interventions should be implemented with a one-time application with a long-lasting impact, such as genetic modification of the vectorial capacity of the wild vector population.

Introduction

The overarching goal of malaria vector control is to reduce the vectorial capacity of local vector populations below the critical threshold needed to achieve a malaria reproduction rate (R_0, the expected number of human cases that arise from each human case in a population) of less than 1. Because of the long extrinsic incubation time of *Plasmodium* in its *Anopheles* vectors, the most effective vector control strategies in use today rely on insecticide interventions like indoor residual insecticide sprays (IRSs) and long-lasting insecticide-treated nets (LLINs) that reduce vector daily survival rates [1]. For many malaria-endemic regions, these tools can make substantial contributions to malaria control and may be sufficient for local malaria elimination. These were the only regions considered by the recent Malaria Elimination Group (MEG). Regions where existing interventions will not be sufficiently effective include those where high rates of transmission occur. For example, in much of sub-Saharan Africa, where the entomological inoculation rates (EIRs) can reach levels approaching 1,000 infective bites per person per year [2,3], the best use of existing interventions can only help to reduce annual inoculation rates by approximately an order of magnitude. Additional interventions will clearly be required, however, both for regions with extremely high rates of transmission and for regions where the major vectors are not susceptible to current control tools [4].

To develop vector-targeted interventions in support of malaria eradication in all disease endemic settings that are unfettered by these limitations, three challenges need to be recognized and addressed with great urgency today. The first challenge, for which near-term product development is essential, is the preservation and improvement of the utility of existing insecticide-based interventions. This challenge will require a vibrant research agenda that develops a broader range of insecticides with novel modes of action that can circumvent emerging resistance to existing insecticides, particularly the pyrethroids. This agenda must include the creation of strategies for the use of new insecticides that minimize the emergence of resistance. A related and critical focus of the agenda will be the development of rapid and affordable methods for detecting the emergence of epidemiologically important levels of insecticide resistance. Because of the fundamental dependence of many current malaria control and elimination programs on pyrethroid insecticide–based LLINs and the emerging problem of pyrethroid insecticide resistance in many vector species, especially in sub-Saharan Africa, development of new insecticides that can be used in LLINs is the most immediate need [5].

The second challenge is development of interventions that affect vector species not effectively targeted by current tools. At least three dozen different species of *Anopheles* mosquitoes are important in malaria transmission worldwide. Many of these species are not susceptible to tools like IRS and LLINs, which target indoor feeding and/or resting vectors [6]. Control of malaria transmitted by these vectors will require new interventions that target other aspects of their biology, including outdoor feeding and resting, oviposition site preference, mating behaviour, or sugar meal selection. Major features of the agenda to tackle this challenge will be defining the vector species for which such new tools are most important and devising tools that will be effective for multiple important vector species.

Review articles synthesize in narrative form the best available evidence on a topic.

Summary Points

- Improved vector control is essential for the elimination/eradication of malaria

- In regions where transmission rates are low or moderate, existing tools may be sufficient to achieve elimination but in many malaria-endemic regions, new vector control interventions, including new insecticides and formulations, are needed

- Better understanding of vector biology is an essential prerequisite for the development of new control interventions

- Sustained commitment to the development of radically new approaches such as the genetic modification of mosquitoes is critical to reduce the high vectorial capacity in some malaria-endemic regions

- Innovative cross-disciplinary technologies are needed to control outdoor biting and resting mosquito vectors, to measure transmission, and to educate communities about vector control

The most difficult research challenge for vector control during all phases of malaria elimination/eradication but particularly during the final stages of eradication is development of novel approaches that will permanently reduce the very high vectorial capacities of the dominant malaria vectors in sub-Saharan Africa. Without such approaches, local elimination in Africa will be extremely challenging. Even when elimination is achieved, the residual vectorial capacities of local mosquitoes will pose a lingering threat of massive epidemics should malaria be reintroduced to a population that has lost partial immunity. Measures to reduce vectorial capacities will need to be either extremely cost-effective, if they are to be sustained until eradication is achieved, or able to effectively yield a long-term, sustained reduction of transmission following a one-time application. Genetic control programs (which could be achieved by a variety of genetic manipulation approaches) designed to permanently reduce the vectorial capacities of natural vector populations have received the most attention to date, and currently represent some of the most promising ideas in this area [7], but the development of other, novel approaches must be strongly encouraged.

It is these three challenges that the malERA Consultative Group on Vector Control concentrated on during its deliberations, the results of which are presented here.

Current Tools and Resource Gaps

The key goal of the malERA Consultative Group on Vector Control was to help define the research and development agenda that will be required to sustain and improve the effectiveness of currently available tools like LLINs and IRS and to develop new vector-targeted tools that can be used to interrupt transmission in environments or at intensities that these existing tools cannot reach. It is clear that new technology will be required in very high transmission areas to reduce vectorial capacity and achieve even effective control, let alone elimination. The main aim of this paper is to define a research and development agenda that focuses on those new research questions and knowledge gaps that arise specifically in response to the call for malaria eradication, and that would not otherwise be at the top of the agenda (Table 1). It is particularly important to recognize that this operationally specified goal significantly limits the scope of research and development under consideration, and this document should not be the basis for all vector research related to malaria. Our article does, however, describe the challenges for vector control methodology in the elimination phase, for detecting and monitoring areas of persistent transmission, and for detecting and monitoring nonrandom transmission leading to outbreaks. We also discuss the requirements for rapid and urgent intervention when outbreaks occur (see also [8]).

The Consultative Group identified four key components to successful vector control within an eradication agenda. First, the ecology of vectors responsible for malaria transmission in those regions of the world where current tools are insufficient for elimination needs to be understood. Second, sets of synergistic or complementary interventions tools need to be developed and applied through rationally designed programs that can be spatially and temporally combined into effective intervention programs. Third, appropriate monitoring and evaluation tools that can guide the application and evolution of control and elimination programs as malaria endemicity is pushed towards local elimination need to be developed and applied. Finally, there is a critical need for built-in flexibility in programs so that where initial efforts fail, they can adapt to circumstances by incorporating and implementing new approaches. Thus, as malaria programs are scaled up, vector control will have a major role in disease burden reduction but, as programs become increasingly successful in reducing transmission, accurate estimation of the point at which large-scale vector control activities can be relaxed will become critical. Premature removal of mainstream vector control, either through planned reductions in activities or through failure of long-lasting interventions like LLINs or IRS as resistance evolves, is likely in many instances to lead to a

Table 1. Vector control interventions required for sustained control and for eradication.

Sustained Control	Eradication
	Better vector monitoring and evaluation information to target interventions
Effective insecticides for LLINs and/or IRS	Effective insecticides for LLINs and/or IRS
Resistance monitoring and management	Resistance monitoring and management
Vector identification and incrimination	Vector identification and incrimination
Appropriate integrated vector management	Appropriate integrated vector management
	Targeted interventions for outdoor biting and resting mosquitoes
	Novel approaches to reduce permanently the high vectorial capacity of major vectors (e.g., genetic modification)
	Effective consumer products for vector control

doi:10.1371/journal.pmed.1000401.t001

catastrophic increase in morbidity and mortality because of resurgent malaria in a nonimmune population [8,9].

The exact role of vector control as countries enter the elimination phase of activities will be situation specific. However, valuable lessons can be drawn from the WHO Global Malaria Eradication Program (GMEP) of the 1950s and 1960s [10], in which vector control alone was considered to be enough in many situations to eliminate malaria. Although this approach was successful in some cases, success was often short-lived [11,12]. Another valuable lesson can be learned from current efforts to eradicate filariasis. For this vector-borne disease, multiple rounds of mass drug administration in many countries divorced from targeted vector control have not achieved the predicted interruption in transmission [13].

Indeed, there is now a consensus that malaria elimination with current tools is far more likely if the best available tools are used in combinations. In the past two decades, especially in an African context, the combination of drugs and vector control with impregnated nets has been highlighted for its role in the reduction of morbidity and mortality [14]. However as malERA sets out a research and development agenda for elimination/eradication and vector control, other interventions must be considered primarily in terms of their impact on malaria infection and transmission, not instead of, but in addition to, their role in prevention and modification of disease.

We highlight the research and development areas identified as priority areas by the Consultative Group before providing a summary research and development agenda that draws together the various strands of our discussions.

The Development of a Formal Analytical Framework

The malaria eradication agenda would clearly be advanced by the development of a formalized analytical framework that

facilitates the collection, analysis, and central presentation of relevant information (Figure 1). Such a framework could significantly help elimination/eradication programs optimize the use of current vector control tools. In addition, when available tools are properly deployed and transmission persists, such a framework could also highlight the knowledge gaps that currently limit accurate development of clear target product profiles (TPPs) for new tools. The generation and sharing of information from systematic assessments of the results of malaria elimination programs across different epidemiological settings will help drive the development of new technologies that will be needed to achieve elimination in more intransigent transmission settings.

The most immediate task of the analytical framework will be to focus research and development resources on the malaria transmission settings for which new or improved elimination tool development is most critical. These settings include much of sub-Saharan Africa and parts of Papua New Guinea, regions where vector populations are capable of sustaining transmission at high vectorial capacities that significantly exceed the possibility of elimination with current tools. In addition, however, there may be other malaria transmission regions of more modest vectorial capacity where important current tools such as IRS and LLINs have little impact because the important vectors do not enter houses to rest or to seek blood meals. Some information already exists that can be brought together to define these high-risk regions [15–17]. For other regions, however, problems may become obvious only when the application of current interventions proves insufficient.

The analytical framework should systematically coordinate available data from disparate multidisciplinary resources, including both peer-reviewed and "grey" literature, via a Web portal to facilitate access and analysis. The Consultative Group's recommendation is that disparate multidisciplinary resources are
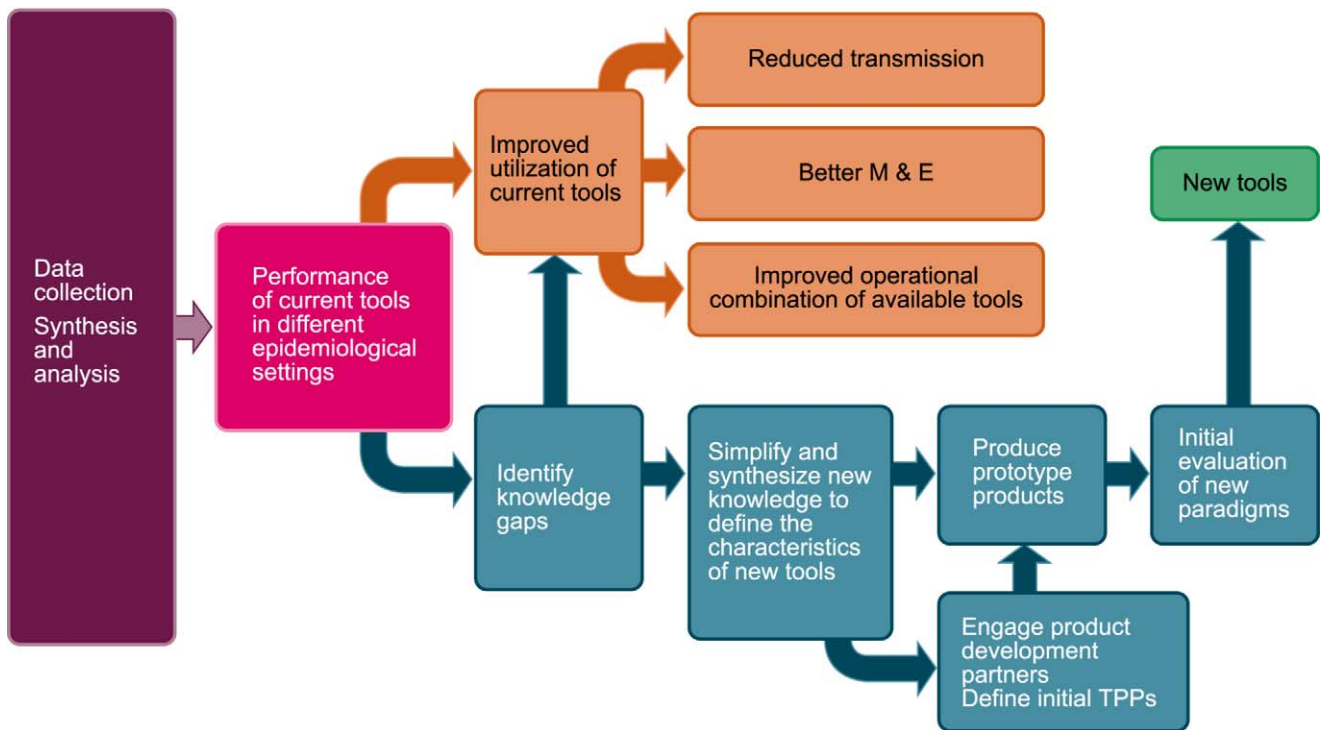
Figure 1. A formalized analytical framework for the collection, analysis, and central presentation of relevant information. M&E, monitoring and evaluation. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000401.g001

brought together in a coherent format that will allow the objective assessment of the knowledge base as it relates to the performance of current tools. The ideal format would allow the systematic assessment of issues arising in countries that have already eliminated malaria and in countries that are still in the first wave of malaria elimination, in isolation and in combination, and would allow comparisons to be made of tool performance in different epidemiological settings. Some of this information—for example, the worldwide distribution of malaria risk and information on the worldwide distribution of important malaria vectors—already exists in centralized resources and needs only be made more readily available. However, other kinds of important information will need to be assembled from disparate sources (for example, field data from major malaria research and control programs and the very significant but inaccessible literature that emerged from the first GMEP) or generated de novo (for example, the determination of the specific malaria transmission behaviours of vectors that have only recently been determined to be members of cryptic species complexes [18]).

As the elimination agenda progresses, this growing body of information can be used to develop and use models of vector biology and transmission and to test intervention hypotheses such as the effect of combining available control tools into integrated control programs. Further, modeling can be used to identify opportunities to develop new interventions and establish the settings where vector control–targeted interventions are inappropriate. It will be particularly important to invest in new interventions that are likely to impact additively or even synergistically with existing tools. Modeling can be an important first step in evaluating such potential interactions (also see [9]).

The Preservation and Improvement of Current Tools

The obvious major threat to current vector-targeted interventions is insecticide resistance, and addressing this problem will be both an important near-term research concern and a continuing, long-term concern as new insecticide formulations and ingredients are developed and used. Furthermore, this problem is critical for control efforts as well as for elimination and eradication efforts. Pyrethroids are the only insecticides currently used operationally on LLINs and are also the dominant insecticide class in IRS, but resistance to this insecticide class is now widespread, with multiple resistance mechanisms spreading in the two major African malaria vectors [19,20]. Although the operational impact of these resistance indicators remains to be established, multiple studies have demonstrated the direct association of resistance measures with entomological indicators such as mosquito mortality, biting rates, and blood feeding success.

Sporadic insecticide resistance monitoring is undertaken by control programs, predominantly using WHO bioassays, but the results from these bioassays are rarely linked to any assessment of control failure. Moreover, resistance-monitoring efforts are not typically used to provide formal guidance to control programs on the selection of alternative vector control strategies in the presence of resistance. Because of the very large number of vector species, the many insecticides in use, and the large numbers of potential resistance mechanisms, choosing the correct vector control strategy is clearly a complex and daunting problem. An essential first step towards developing a rational solution will be to develop and provide new tools for the quantitative monitoring of different forms of resistance in different vector species. Monitoring could be done through the provision of public protocols, through training and the provision of kits, or by establishing a regional service. The

potential complexity of meaningful data generation and interpretation suggests that the last option may be preferable. Indeed, we note that this type of activity could readily be combined in a monitoring and evaluation framework with a laboratory service that provides drug resistance or serology monitoring capability. In addition, data on the temporal and geographic distribution of insecticide resistance need to be efficiently assembled and made publicly available through a formal analytic framework to help guide both control program and research decisions.

LLINs, IRS, and larvicides attack different behaviours or life stages of the mosquito. There is some evidence that LLINs and IRS used in combination may be synergistic, although both target adult female mosquitoes indoors [21]. Within an eradication agenda, the cost-effectiveness and benefits of such combinations need to be assessed. The recommendation of the Consultative Group, therefore, is that potential combinations of present and new control tools be explored theoretically in a modeling framework [9], and that potentially optimal integrated vector management strategies be tested in large-scale field trials in different epidemiological settings to assess their ability to reduce transmission and the burden of disease. If insecticide resistance dramatically reduces our ability to reduce transmission, it becomes a major threat to eradication, and mitigating strategies must be tested in the field to contain resistance in the absence of new alternative insecticides. Finally, insecticide-resistance management technologies need to be developed for the future that use combinations of vector control tools that do not depend on the main classes of insecticide in current use. Such combinations might include repellents, larvicides, environmental management, and possibly pathogens.

Improvement of the Knowledge Base of Vector Ecology

Malaria is transmitted in diverse epidemiological situations by a wide range of potential combinations of "primary" and "secondary" vectors. Moreover, most widely recognized vector species are members of cryptic species complexes [18] and even within currently recognized complexes, further heterogeneities may exist in vector population structure that can limit the effectiveness of control tools [22]. The present vector control tools (LLINs and IRS) were developed to reduce transmission in areas where the primary vectors feed and/or rest indoors. When these interventions are implemented under optimal programmatic conditions, diligent monitoring will identify areas where there are limitations in their effectiveness.

Failure to achieve the expected level of control may result from a number of factors. Complex mixtures of vector species may be present, including vectors with outdoor biting and resting behaviours, or a more complex genetic structure within recognized vector taxa. Moreover, vector populations can develop behavioural as well as physiological forms of resistance to insecticides. To assess the possible impact of behavioural evolution on the effectiveness of vector control tools, and to better target vector species or populations escaping these tools, we have to understand both larval and adult ecologies and behaviours. At the present, we have only a limited understanding of the ecology and population structure of some of the major vectors, such as *An. gambiae* in Africa. Unfortunately, even less is known about where many of the other important vectors feed, rest, mate, and oviposit, or about their population structure, or even the extent of their geographic distributions. These deficiencies are due to both the lack of adequate sampling and monitoring methods and a historical lack of emphasis on the study of population biology of malaria vectors in many parts of the world.

Development of TPPs for new interventions that could supplement existing control tools will require knowledge of the critical points in the biology of different vector species. These points should be features of a vector's biology that are sufficiently predictable to constitute a target for the control tool, such as a predictable resting, blood- or sugar-feeding, oviposition, or mating site. Technologies (see later) that enable accurate tracking of mosquito movement in space and time are needed to establish these critical points in the biology of different vector species.

The Development of New Vector-Targeted Interventions

Near-Term Translation of Appropriate Interventions

Malaria vector control activities today are heavily reliant on the distribution of LLINs or IRS. In some instances, these are augmented with larval control or fortuitously complemented by social housing schemes or economic development that negatively impact on *Anopheles* mosquito breeding. This limited armamentarium is, in part, the legacy of a malaria control approach developed before and during the GMEP of the 1950s and 1960s that was followed by a shift in the 1970s through the 1990s away from the interruption of transmission to the control of morbidity and mortality based largely on chemotherapy [11,12]. Research on the control of malaria transmission was consequently very limited and poorly coordinated both during the time of the GMEP, which was characterized by overoptimistic expectations of the effectiveness of DDT, and in the years that followed when transmission was no longer the main concern. Nonetheless, a number of proposed alternative vector control methods have emerged recently, most of which have not yet been extensively evaluated and developed (see Table 2 for some examples). What is badly needed is a well-defined development pipeline to ensure that the more promising among these alternative methods are brought into mainstream operation and that the less appropriate are down-selected.

For example, the development of cost-effective longer lasting IRS formulations of different insecticide classes would remove the economic and logistical arguments that preclude the use of IRS in some settings. Today's heavy reliance on pyrethroids for both LLINs and IRS is driven both by a lack of new insecticides and by limited development in formulation technology. The latter problem is amenable to short-term resolution. Similarly, models suggest that interventions that act on older adult mosquitoes are less prone to resistance selection than traditional insecticides [23,24], but this has still to be demonstrated operationally. Other insecticides have failed to cross the translational gap because the short residual shelf life of the formulations under operational conditions is a major barrier to their commercialization. Until this element of the critical pathway to commercial uptake is resolved, many promising insecticides are unlikely to play an active role in operational control.

Novel tools need proper evaluation in field trials and, if their efficacy is demonstrated, they need testing in combination for their effect on infection and transmission. We recommend that in reviewing current and potential interventions within the analytical framework, a commercial-style analysis of the development status of the different interventions be undertaken (Figure 2) and the barriers on the critical pathway to implementation be identified. Once identified, the resources required to overcome these barriers can be established and an appropriate risk benefit analysis can be undertaken. This analysis will allow rapid movement away from long lists of potential vector control interventions and towards a better-defined list of actual interventions that can be coupled to clear guidance on appropriate deployment in the different stages of malaria elimination across a range of epidemiological settings. Analysis of the development status should also include modeling to guide selection and testing of combinations and settings where they should be introduced (see also [9]).

New vector control tools will be needed in the short and medium term as the current tools will be inadequate for malaria elimination in most settings. The strategy outlined above will allow researchers and developers to capitalize on information that is already in the public domain and to efficiently and cost-effectively develop the most appropriate new tools in the short term that

Table 2. Examples of novel tool development and intended objectives.

Sustained-Use Interventions		Time-Limited Interventions	
Category	**Objective**	**Category**	**Objective**
Insecticides and related chemical agents (synthetic and natural ["bio-prospecting"]) for environmental, dwelling, and systemic applications (humans or animals)	Control, elimination	Biological or chemical agents that affect age structure (decrease extrinsic incubation period, for example, *Wolbachia* spp.)	Control, elimination, eradication
House design to impede vector access and sustainability	Control, elimination	Genetic approaches to reduce adult longevity ("death-on infection" genes killing only those mosquitoes that become infected)	Control, elimination, eradication
Biological agents (plant, fungi, algae, predators, niche competitors, insect viruses, and other pathogens) for population suppression	Control, elimination	Biological agents targeting pathogens, for example, symbiotic organisms engineered to kill pathogens (paratransgenesis)	Control, elimination, eradication
Ecological/environmental modification (source reduction) targeting sites for breeding (oviposition), subadult development, and adult resting sites	Control, elimination	Genetic approaches targeting vector competence	Control, elimination, eradication
Chemical attractants/repellent agents (synthetic and natural ["bio-prospecting"]) for dwelling and personal applications that would target both indoor and outdoor biting	Control, elimination		

doi:10.1371/journal.pmed.1000401.t002
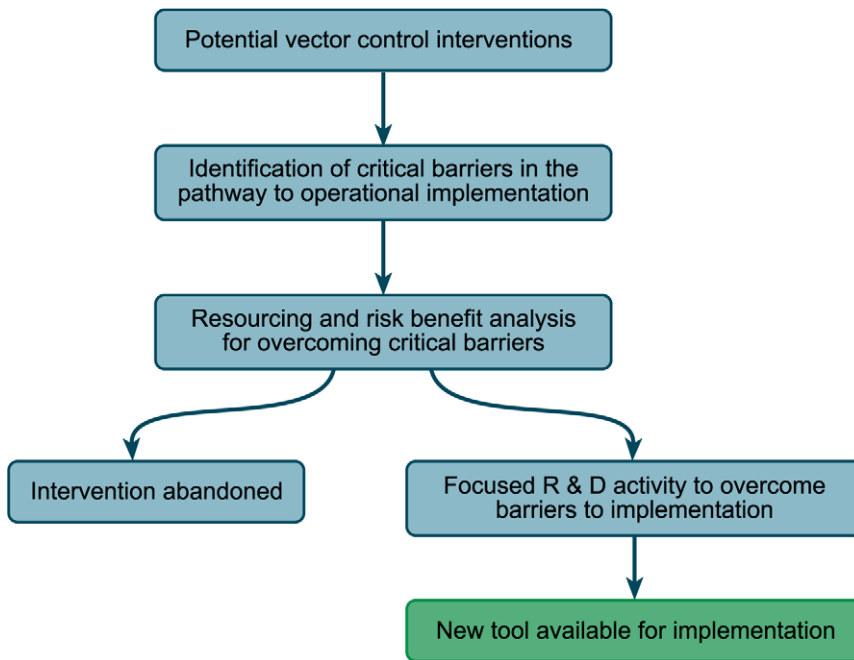
Figure 2. A scheme for the analysis of the development status of the different interventions; similar schemes are used in the commercial development of drugs, for example. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000401.g002

could be useful additions to the armamentarium for malaria eradication.

Longer-Term Development of Novel Sustained-Use Interventions

To achieve malaria eradication, we will need to reduce regional R_0 to less than 1 and sustain transmission rates below this critical threshold until global eradication can be achieved. Achieving this goal will require both augmentation of current control methods and the development of novel interventions to interrupt transmission in ways that address a broad range of potential impediments, including political instability, geographic isolation, and the development of chemical or behavioural resistance. Perhaps the biggest difficulties will be the economic and social challenges that will be associated with the need to sustain the impact of such interventions in some regions for very long periods, possibly decades, until the risk of parasite reintroduction is no longer a concern.

Insecticide-based interventions for sustained use should not be compromised by resistance. Models of resistance management developed using data from control of agricultural insect pests and from other large-scale vector control efforts indicate that stable, long-term resistance management strategies require a minimum of three active ingredients. These ingredients need different modes of action, distinct metabolic detoxification pathways, and no resistance to any of them should be present at the onset of the program. The levels of resistance currently circulating in many mosquito vectors to all registered public health pesticides precludes such a system being established today [25]. Hence, it is vital that we continue to develop new active ingredients to replace existing insecticides when vectors develop significant resistance. The ideal goal would be to do this in a time frame that allows multiple new insecticides to be introduced together.

It is also important that a broader set of tools for targeting adult female vectors be developed so that adult vector survival rates and the resulting population age structure can be reduced to levels where insufficient older female mosquitoes capable of supporting

parasite sporogonic development are present in the vector population. The most critical needs will be for vector control tools that complement existing methods by targeting aspects of the mosquito's life cycle that are not currently reached. New tools could potentially be developed to target outdoor blood-meal or sugar-meal feeding, for example, or to target mate-seeking or ovipositing females. Understanding the biology of these behaviours in the life cycle of important vectors could be the source of powerful new interventions. Even control approaches that achieve only a reduction in vector population density, such as interventions targeted at larvae, could prove valuable if they are sufficiently cost-effective and are complementary to existing tools.

Push-pull (repellent-attractant) technologies are well developed for some insect pests in the agricultural arena, but this technology has yet to be brought to bear on malaria control [26]. Our rapidly developing understanding of the mosquito sensory system, coupled to development of high-throughput screening technologies, should allow us to develop more effective attractants and repellents for mosquitoes within the next decade [27]. Modeling and experimental analysis of the impact of these compounds should allow us to develop new, targeted strategies for control. This technology also lends itself well to the extensive consumer market, which will undoubtedly play a major role in sustaining elimination efforts by reducing mosquito biting as mainstream vector control activities are reduced. Indeed, this is a situation that already exists in countries such as Sri Lanka and Mexico where the consumer market for products that reduce biting nuisance is high and national malaria control program vector control activities are minimal.

Longer-Term Development of Novel Time-Limited Interventions

The past decade has seen phenomenal advances in *Anopheles* genomics and proteomics [28]. These advances, coupled with the visionary but technically challenging development of mosquito transgenics and other genetic manipulation techniques, open up

mosquitoes, we need to establish the critical points in the life cycle of these mosquitoes where they congregate in numbers, are susceptible to attraction by external stimuli, or come into contact with their human hosts. Better sampling methods that continuously track mosquito movement in space and time, rather than current methods that sample at known fixed points of interaction, are therefore needed. Moreover, methods that generate representative samples of mosquitoes in areas with intensive vector control activity are needed for accurate monitoring and evaluation of insecticide resistance and infection rates.

Cross-disciplinary initiatives are also needed to achieve a defined research agenda for improving engagement and communication with communities and all other stakeholders in malaria elimination. Such an agenda is needed to avoid the mistakes of past efforts, which have all too often foundered because of community fatigue after long years of engagement. Better integration of epidemiological expertise into vector control evaluation initiatives is also needed to ensure accurate field evaluation in increasingly complex multi-initiative settings, and a more commercially oriented approach to the development and evaluation of vector control technologies is required to ensure that promising initiatives cross the translational gap to implementation and poor technologies are rapidly discarded. Finally, cross-disciplinary initiatives are needed to achieve the rapid definition of efficient regulatory pathways and frameworks for existing and new technologies.

Conclusions

On the basis of its deliberations, the malERA Consultative Group on Vector Controls proposes a research and development agenda for vector control (Box 1). The first of these agenda items—the development of an analytical framework to facilitate decision making—is achievable in the next 12–18 months. The other areas need to be rapidly progressed over the next decade. It will be up to everyone involved in malaria elimination/eradication to work together to ensure that all the needs and goals highlighted in this agenda are achieved as efficiently as possible. Importantly, however, our proposed agenda provides a starting point only for the research and development needs associated with vector control. This agenda must not be set in stone; it must continue to evolve as the elimination/eradication program progresses.

the possibility of developing novel technologies to suppress mosquito populations or to make parasite-refractory mosquitoes, and make mosquito-based transmission-blocking technologies possible (Table 2). Such innovative new technologies may be key tools in the eradication agenda in the highly malaria-endemic regions of the world, in particular, much of sub-Saharan Africa, because they can circumvent the extreme problems in control program application that will be posed by intractable logistical, technical, or political issues in many of these regions. Importantly in intractable settings—for example, dense forests or politically unstable areas—where the elimination of malaria may prove most difficult, these technologies have the advantage that the mosquito population itself acts as the distribution agent.

Fortunately, the number of different vector species for which such technologies will need to be considered is limited, probably to only a handful of species. Moreover, the highly technical research needed to develop such tools for one major vector species will likely be fairly easy to adapt or even transfer directly to others. We now need to progress to the exacting but exciting translational phase of this activity, which will involve selection of the most appropriate technically robust technologies for operational implementation. Development, analysis, and refinement of scale-up technology to move progressively from the laboratory, to cage trials, and ultimately to operational scale release of genetically modified mosquitoes, and the establishment of the regulatory pathways for commercialization and release are all needed. Finally and critically, stakeholders, particularly in disease endemic countries, must be persuaded to support the release of genetically modified mosquitoes.

Enabling Technologies

In order to establish TPPs for novel vector control products, particularly for products that target outdoor feeding or resting

and University of Basel, University of Basel, Basel, Switzerland; Larry Zwiebel, Vanderbilt University, Nashville, Tennessee, USA.

Participants in the malERA meetings and extra contributors were:

Washington (D.C.), USA (May 2009) Vector Control meeting: Kate Aultman, Bill & Melinda Gates Foundation, Seattle, Washington, USA; Thomas R. Burkot, US Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Fabrice Chandre, Institut de Recherche pour le Développement (IRD), Montpellier, France; Maureen Coetzee, National Institute for Communicable Diseases, Johannesburg, South Africa; Frank H. Collins, University of Notre Dame, Notre Dame, Indiana, USA; Vincent Corbel, Institut de Recherche pour le Développement (IRD/CREC), Cotonou, Benin; Vincent Dartigues, Bayer Environmental Science, Lyon, France; John Gimnig, US Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Bill Hawley, UNICEF, Jakarta, Indonesia; Janet Hemingway, Liverpool School of Tropical Medicine, Liverpool, UK; Marcelo Jacobs-Lorena, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; Anthony A. James, University of California, Irvine, California, USA; Parakrama Karunaratne, Zoology University of Peradeniya, Peradeniya, Sri Lanka; Gerry Killeen, Ifakara Health Institute, Ifakara, Tanzania; Christian Lengeler, Swiss Tropical and Public Health Institute, Basel, Switzerland; Jo Lines, World Health Organization, Geneva, Switzerland; Michael Macdonald, USAID, Washington (D.C.), USA; Jorge Mendez-Galván, Hospital Infantil de México "Federico Gómez," México DF, Mexico; Marc Muskavitch, Boston College, Boston, Massachusetts, USA; José Nájera, Independent, formerly WHO, Madrid, Spain; Daniel Neafsey, Microbial Analysis Group Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA; Ron Rosenberg, US Centers for Disease Control and Prevention, Fort Collins, Colorado, USA; Frederic Simard, Institut de Recherche pour le Développement (IRD), Ougadougou, Burkina Faso; Robert Sinden, Imperial College, London, UK; Sarala Subbarao, Independent, Formerly National Institute of Malaria Research (ICMR), New Delhi, India; Ned Walker, Michigan State University, East Lansing, Michigan, USA; Jacob Williams, RTI International, Research Triangle Park, North Carolina, USA; Robert Wirtz, US Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Sitges, Spain (January 2010) Vector Control writing meeting: Pedro L. Alonso, Barcelona Centre for International Health Research (Hospital Clínic, Universitat de Barcelona), Barcelona, Spain, and Centro de Investigaçao em Saude da Manhiça, Mozambique; Nora J. Besansky, University of Notre Dame, Notre Dame, Indiana, USA; Thomas R. Burkot, US Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Frank H. Collins, University of Notre Dame, Notre Dame, Indiana, USA; Janet Hemingway, Liverpool School of Tropical Medicine, Liverpool, UK; Anthony A. James, University of California, Irvine, California, USA; Christian Lengeler, Swiss Tropical and Public Health Institute, Basel, Switzerland; Steven Lindsay, London School of Hygiene & Tropical Medicine, London, UK; Qiyong Liu, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China; Neil F. Lobo, University of Notre Dame, Notre Dame, Indiana, USA; Abraham Mnzava, World Health Organization, Cairo, Egypt; Marcel Tanner, Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland; Larry Zwiebel, Vanderbilt University, Nashville, Tennessee, USA.

Author Contributions

References

1. Enayati A, Hemingway J (2010) Malaria management: Past, present, and future. Ann Rev Entomol 55: 569–591.
2. Hay SI, Rogers DJ, Toomer JF, Snow RW (2000) Annual *Plasmodium falciparum* entomological inoculation rates [EIR] across Africa: Literature survey, internet access and review. Trans R Soc Trop Med Hyg 94: 113–127.
3. Kelly-Hope LA, McKenzie FE (2009) The multiplicity of malaria transmission: A review of entomological inoculation rate measurements and methods across sub-Sahan Africa. Malar J 8: 19.
4. Shaukat AM, Breman JG, McKenzie FE (2010) Using the entomological inoculation rate to assess the impact of vector control on malaria parasite transmission and elimination. Malar J 9: 122.
5. Ranson H, Abdallah H, Badolo A, Guelbeogo WM, Kerah-Hinzoumbé C, et al. (2009) Insecticide resistance in *Anopheles gambiae*: Data from the first year of a multi-country study highlight the extent of the problem. Malar J 8: 299.
6. Terenius O, Marinotti O, Sieglaff D, James AA (2008) Molecular genetic manipulation of vector mosquitoes. Cell Host Microbe 13: 417–423.
7. Sinkins SP, Gould F (2006) Gene drive systems for insect disease vectors. Nat Rev Genet 7: 427–435.
8. The malERA Consultative Group on Monitoring, Evaluation, and Surveillance (2011) A research agenda for malaria eradication: Monitoring, evaluation, and surveillance. PLoS Med 8: e1000400. doi:10.1371/journal.pmed.1000400.
9. The malERA Consultative Group on Modeling (2011) A research agenda for malaria eradication: Modeling. PLoS Med 8: e1000403. doi:10.1371/journal.pmed.1000403.
10. Pampana EA (1969) A textbook of malaria eradication. London: Oxford University Press.
11. Bruce-Chwatt LJ (1987) Malaria and its control: present situation and future prospects. Ann Rev Public Health 8: 75–110.
12. Nájera J, González-Silva M, Alonso PL (2011) Some lessons from the previous Global Malaria Eradication Program (1955–1969). PLoS Med 8: e1000412. doi:10.1371/journal.pmed.1000412.
13. Bockarie MJ, Pedersen EM, White GB, Michael E (2009) Role of vector control in the global program to eliminate lymphatic filariasis. Ann Rev Entomol 54: 469–487.
14. Lengeler C (2004) Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database Syst Rev: CD000363.
15. Hay SI, Snow RW (2006) The malaria Atlas Project: Developing global maps of malaria risk. PLoS Med 3: e473. doi:10.1371/journal.pmed.0030473.
16. Hay SI, Guerra CA, Gething PW, Patil AP, Tatem AJ, et al. (2009) A world malaria map: *Plasmodium falciparum* endemicity in 2007. PLoS Med 6: e1000048. doi:10.1371/journal.pmed.100004.
17. Hay SI, Sinka ME, Okara RM, Kabaria CW, Mbithi PM, et al. (2010) Developing global maps of the dominant *Anopheles* vectors of human malaria. PLoS Med 7: e1000209. doi:10.1371/journal.pmed.1000209.
18. World Health Organization (2007) Anopheline species complexes in south and south-east Asia. SEARO Technical Publication No. 57. Geneva: World Health Organization.
19. Wondji CS, Irving H, Morgan J, Lobo NF, Collins FH, et al. (2009) Two duplicated P450 genes are associated with pyrethroid resistance in *Anopheles funestus*, a major malaria vector. Genome Res 19: 452–459.
20. Yadouleton AW, Padonou G, Asidi A, Moiroux N, Bio-Banganna S, et al. (2010) Insecticide resistance status of *Anopheles gambiae* in southern Benin. Malar J 9: 83.
21. Kleinschmidt I, Schwabe C, Shiva M, Segura JL, Sima V, et al. (2009) Combining indoor residual spraying and insecticide-treated bed net interventions. Am J Trop Med Hyg 81: 519–524.
22. Guelbeogo WM, Sagnon N, Grushko O, Yameogo MA, Boccolini D, et al. (2009) Seasonal distribution of *Anopheles funestus* chromosomal forms from Burkina Faso. Malar J 8: 239–246.
23. Thomas MB, Read AF (2007) Can fungal biopesticides control malaria? Nat Rev Microbiol 5: 377–383.
24. Farenhorst M, Mouatcho JC, Kikankie CK, Brooke BD, Hunt RH, et al. (2009) Fungal infection counters insecticide resistance in African malaria mosquitoes. Proc Natl Acad Sci USA 106: 17443–17447.
25. Penilla RP, Rodriguez AD, Hemingway J, Rarres JL, Arredondo-Jimenez JI, et al. (1998) Resistance management strategies in malaria vector control. Baseline data for a large-scale field trial against *Anopheles albimanus* in Mexico. Med Vet Ent 12: 217–233.
26. Khan ZR, Midega CAO, Bruce TJA, Hooper AM, Pickett JA (2010) Exploiting phytochemicals for developing a "push-pull" crop protection strategy for cereal farmers in Africa. J Exp Botany 6: 4185–4196.
27. Carey AF, Wang G, Su CY, Zwiebel LJ, Carlson JR (2010) Odorant reception in the mosquito *Anopheles gambiae*. Nature 464: 66–71.
28. Lawson D, Arensburger P, Atkinson P, Besansky NJ, Bruggner RV, et al. (2009) VectorBase: A data resource for invertebrate vector genomics. Nucleic Acids Res 37: D583–D587.

PLoS MEDICINE

Review

A Research Agenda for Malaria Eradication: Diagnoses and Diagnostics

The malERA Consultative Group on Diagnoses and Diagnostics¶*

Abstract: Many of malaria's signs and symptoms are indistinguishable from those of other febrile diseases. Detection of the presence of *Plasmodium* parasites is essential, therefore, to guide case management. Improved diagnostic tools are required to enable targeted treatment of infected individuals. In addition, field-ready diagnostic tools for mass screening and surveillance that can detect asymptomatic infections of very low parasite densities are needed to monitor transmission reduction and ensure elimination. Antibody-based tests for infection and novel methods based on biomarkers need further development and validation, as do methods for the detection and treatment of *Plasmodium vivax*. Current rapid diagnostic tests targeting *P. vivax* are generally less effective than those targeting *Plasmodium falciparum*. Moreover, because current drugs for radical cure may cause serious side effects in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, more information is needed on the distribution of G6PD-deficiency variants as well as tests to identify at-risk individuals. Finally, in an environment of very low or absent malaria transmission, sustaining interest in elimination and maintaining resources will become increasingly important. Thus, research is required into the context in which malaria diagnostic tests are used, into diagnostics for other febrile diseases, and into the integration of these tests into health systems.

Introduction

As malaria transmission declines across much of its range and the possibility of elimination (reduction of transmission to zero in a defined geographical area) is increasingly considered [1,2], accurate diagnosis and case identification through the demonstration of malaria parasites in sick patients presenting to health workers ("passive case detection") is ever more important. During case management in all settings, all symptomatic patients with demonstrated parasitemia should be considered to be malaria cases, and all parasitemic patients should be given definitive antimalarial treatment. Accurate diagnosis is essential both to target antimalarial drugs and to enable effective management of the frequently fatal nonmalarial febrile illnesses [3] that share signs and symptoms with malaria [4–13].

However, the very low levels of transmission now being attained in many countries present new challenges that will demand new diagnostic tools and strategies, in particular, a change from passive case detection to "active" case detection. That is, as the elimination agenda is increasingly followed [14], improvements in current field diagnostics (microscopy and rapid diagnostic tests [RDTs]) for case management and new diagnostics that can detect very low levels of *Plasmodium* in the blood of asymptomatic

individuals (and, in the case of *P. vivax*, in the blood of symptomatic individuals) who may contribute to continuing malaria transmission [15–21] will become essential. Furthermore, novel strategies will be needed to incorporate these new and improved diagnostics into routine health service activities.

More specifically, to avoid onward transmission, elimination programs for malaria will increasingly need to focus on detecting the highest possible fraction of infections in the general population through active rather than passive case detection. This change of focus will be essential because *Plasmodium* infections can persist at low densities for different lengths of time with no significant symptoms [16,22,23], and, in the case of *P. vivax* and *Plasmodium ovale*, as a latent stage in the liver that is not directly detectable. The contributions of these unseen reservoirs to the maintenance of transmission will depend on the success of detection and management of new cases and the coverage of vector and other control measures in the area [24,25]. Thus, the usefulness of active case detection will vary with the epidemiology and health resources in an area and is itself a subject requiring further research [26].

Countries with successful "sustained control," (the reduction of malaria transmission to a locally acceptable and sustained level through intensive use of vector control and effective case management) [14], will also need to adjust their diagnostic strategies as transmission declines to low levels and as they consider elimination. Importantly, until eradication of malaria (the reduction of transmission to zero worldwide) is achieved (and diagnostics therefore no longer required), efforts to eliminate malaria will continue to require diagnostics strategies as reintroduction will remain possible.

This article, which summarizes the deliberations of the malERA Consultative Group on Diagnoses and Diagnostics, proposes a research agenda for the tools required for this process; related articles address broader issues of health service requirements and case management that will arise from their use [26,27]. Figure 1 shows the position of different diagnostic approaches/tests in

Review articles synthesize in narrative form the best available evidence on a topic.

Summary Points

- New and improved screening tools and strategies are required for detection and management of very low-density parasitemia in the field

- Improved quality control is required for rapid diagnostic tests (RDTs) and microscopy in the field, to ensure confidence in diagnosis for case management

- More sensitive tests are required for *Plasmodium vivax* for case management

- Field-ready glucose-6-phosphate dehydrogenase (G6PD) deficiency tests and strategies for use to allow safe use of drugs against *P. vivax* liver stages are needed

- New strategies to manage parasite-negative individuals are needed to justify the continued inclusion of malaria diagnostics in febrile disease management in very low transmission areas.

relation to morbidity, parasite prevalence, and densities and the different stages towards malaria elimination. Given the changing priorities for diagnoses and diagnostics as transmission reduces, in our discussion of the research needs for diagnostics, we distinguish between the two broad but overlapping areas of case management and surveillance/screening. This distinction is reflected in the target product profiles presented in Table 1. In both areas, sustainability will require integration with the general health system, and as much commonality as possible between diagnostics for different diseases. Thus we discuss priority setting in the context of the approaches already in use, or in the pipeline, for other diseases managed at the same levels of the health system. Because *P. falciparum* and *P. vivax* are the most prevalent plasmodia, the following discussion concentrates on these species, which most commonly present as mono-species infections. However, as *P. falciparum* infections decline, *P. ovale* may become relatively more prominent in areas where it is endemic, with implications for

detection and management similar to those for *P. vivax*. Similarly, only time will tell whether transmission of *Plasmodium malariae*, which is transmitted across a broad geographical range, but at low prevalence, can be reduced using the measures applied to *P. falciparum*, or whether it will require specific strategies and tools. Notably, however, elimination of the zoonotic *Plasmodium knowlesi* is likely to require unique strategies (Figure 1).

Diagnostic Strategies for Programs in the Intensified Control Phase

Identification of parasitemia in febrile patients is essential in all of the programmatic phases of the continuum from malaria control to elimination, although the challenges for health systems in maintaining this activity in areas where malaria has become rare will be more prominent, as will the importance of detecting asymptomatic infections of low parasite density. The ongoing role of other routine interventions, such as intermittent preventive treatment in pregnancy, needs reevaluating as elimination is approached. Moreover, because the distribution of malaria transmission is often highly heterogeneous within a country, strategies may need to vary at a subnational level. Analyses of past experiences and operations research are required to guide decisions on when these changes in emphasis should take place as control progresses [27,28]. Although programs in areas of higher transmission will be less likely to engage in active case finding of individuals with low parasite densities, surveillance is nevertheless necessary to detect trends and the impact of interventions, and requires appropriate, high-throughput diagnostic tools. In addition to the diagnosis of malaria, it will be critical to have diagnostic capabilities for other causes of presenting illness, particularly fever. A sick adult or parent of a febrile child may not be satisfied with a diagnosis of "not malaria," and both patients and providers require guidance on the integrated management of childhood illnesses, to ensure that appropriate alternative and specific treatment is available and provided.
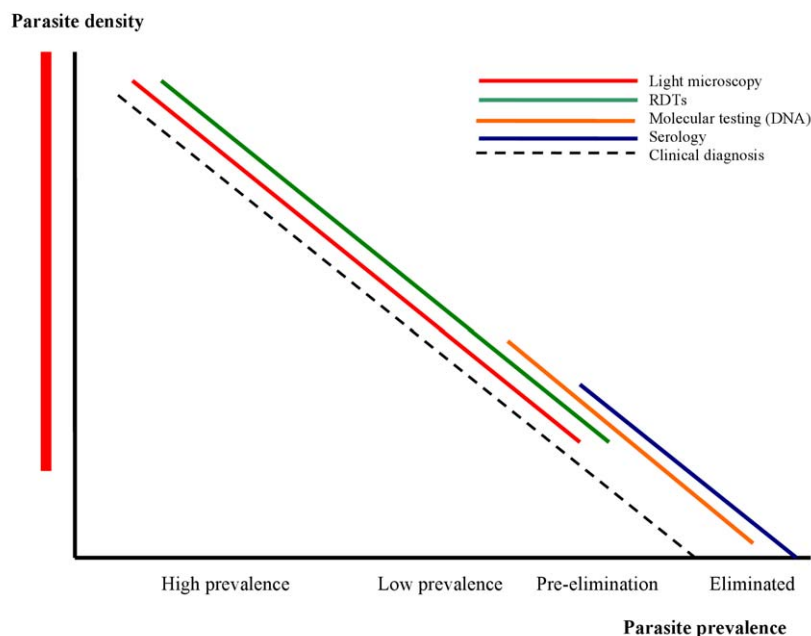
Figure 1. The position of different diagnostic approaches/tests in relation to morbidity, parasite prevalence, densities, and different stages towards malaria elimination. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000396.g001

Table 1. Target product profiles for malaria diagnostics.

Characteristic	Case Management in Elimination Settings	Screening/Surveillance (District Level or Below)
Technical specifications		
Analytic sensitivity (parasite/µl)[a]	E, 100–200, D<5[b]	E = 20, D≤5
Diagnostic sensitivity[a]	E>95%, D≥99%	E>95%, D≥99%
Analytic specificity	Negative all pathogens, common blood disorders	Negative all pathogens, common blood disorders
Diagnostic specificity	E>90%, D>95%	E>99% surveillance low-transmission areas, E>95% screening
Temperature stability	E>35°C, D>45°C[c] (2 y)	E, 30°C; D, 45°C for short periods
Integrity of packaging	E, Moisture proof	E, Moisture proof
Species detection/differentiation:		
Pf predominant areas[d]	E, Pf; D, Pf/pan	E, Pf; D, Pf/pan
Pf and non-Pf areas	E, Pf/pan	E, Pf/pan; D, differentiation all species
Genotyping	No	No/O[e]
Ability to detect gametocytes	No	O
Ability to detect hypnozoites	No	D
Health systems and technical specifications		
Packaging of tests or reagents[f]	D, individual; D, all required consumables enclosed; D, bulk packaging displays temperature violations	D, all required consumables enclosed; D, bulk packaging displays temperature violations
Field stability/shelf life of consumables[g]	E, 2 y from manufacture (≥18 mo in country)	E, 12 mo (6 mo since country); D, 2 y from manufacture (≥18 mo in country)
Training requirements	D, half-day of community-level health worker	D, <1 wk of pretrained medical technician
Reagent requirements	E, nontoxic, all nonroutine provided; D, all necessary consumable items to perform the test provided in the kit	E, nontoxic, all nonroutine provided; D, all necessary consumable items to perform the test provided in the kit
Invasiveness	E, finger prick or less; D, noninvasive	E, finger prick or less; D, noninvasive
Rapidity of results[h]	E≤30 min; D≤15 min	E≤2 d; D≤half-day
Ease of use	Community: E, simple, few steps; Clinic: E, within medical tech ability; D, simple, few steps	E, within medical tech ability; D, simple, few steps
Cost	D≤US$1 per test	D≤US$1 per test
Safety	E, high blood safety with basic universal precautions	E, high blood safety with basic universal precautions
Waste disposal	Village-level waste disposal	Basic health system waste disposal
Inter-reader reliability (clarity of result)	Kappa>0.9	Kappa>0.9
Instrumentation and laboratory infrastructure requirements	E, no external power source; D, all provided with test	D, all provided with test

D, desirable; E, essential; O, optional.

[a]Analytic sensitivity: detection threshold against the marker of the infective agent (target) in controlled conditions. Diagnostic sensitivity: proportion (percent) of target cases detected by the test in the setting of intended use. The sensitivity required for *P. vivax* is generally at least that required for *P. falciparum*, and the parameters here should be applied to both. To achieve the required diagnostic sensitivity in low-prevalence settings, a greater analytic sensitivity (lower threshold of detection) may be required in some cases.

[b]Not required for febrile case management, but in an elimination setting, it would be desirable to detect incidental parasitemia at this level.

[c]Essential where stored in the field in ambient temperatures that frequently reach this level. Ambient temperature of prolonged storage in place of use should be considered the essential temperature stability requirement for a particular product.

[d]Areas in which infections are almost exclusively monospecies or mixed species *P. falciparum* infections. It is likely that many such infections have subpatent coinfections with other species. Where this represents a minority of infections, treatment on the basis of *P. falciparum* alone is likely to be acceptable from a programmatic and public health point of view. Non-*P. falciparum* infections are likely to become relatively more prominent as *P. falciparum* infections decline in prevalence, making the detection of non-*P. falciparum* species more desirable.

[e]May be of importance in areas undergoing certification for elimination.

[f]All inner (individual test) packaging should display, at a minimum: manufacturer name, product name, expiry date, lot number, target use (malaria).

[g]Outcome of temperature stability and integrity of packaging (ability to exclude moisture).

[h]Rapidity of results: For case management, results must be available before a patient is likely to leave the clinic. For surveillance, result availability in time for finding and managing cases is highly desirable.

doi:10.1371/journal.pmed.1000396.t001

Experience in eliminating malaria and maintaining elimination (or very low transmission) in sub-Saharan Africa is lacking, but experience from other areas suggests that resource requirements may be prohibitive and long-term maintenance of very low transmission and prevention of rebound unachievable using conventional management [29,30]. Innovative approaches are therefore required. Diagnostic tools capable of detecting very low parasite densities (1 parasite/µl blood) in asymptomatic individuals

will increasingly be required for active case detection and population surveillance to obtain a true picture of the prevalence of parasitemia and probability of transmission (as distinct from symptomatic malaria) [16–21]. Active case detection and treatment will be required whenever ongoing transmission is suspected and in high-risk populations (including those crossing borders), if the likelihood of ongoing transmission is to be eliminated. In these circumstances, test specificity is of increased importance because the absence of false positive results is critical in understanding the presence or absence of transmission [26].

Diagnostic Strategies for Programs in Areas Where Elimination Has Taken Place

Once malaria is eliminated in a given area, considerable resources will be required to detect reintroduction through surveillance and to maintain capacity for rapid management and investigation of any cases found, as long as the risk factors that support transmission are still in place. Screening of migrant populations, screening of populations around detected cases, and case management tools for screening suspected patients, such as recent travelers or geographical associates of malaria cases may be needed. The tools to achieve these activities must be readily available in an environment where technicians are likely to be unskilled in the use of malaria diagnostic tests, particularly microscopy [27]. Thus, the requirements for surveillance and screening in areas where malaria has been eliminated, but risk of transmission is present, are similar to those of programs in an elimination phase. However, case management tools that are minimally dependent on previous technician experience in diagnosing malaria will be of particular importance.

Diagnostic Tools for Case Management in an Elimination Setting

In settings where there is risk of autochthonous or imported malaria, diagnostics must be capable of rapidly and accurately detecting and quantifying parasitemia in febrile patients, and identifying species. In addition, highly sensitive diagnostic tools are needed for passive case detection and case management at health care facilities (public or private) that report to the national health information or disease surveillance systems. The issues around diagnostics in both case management and surveillance and control settings have a large impact on, and are impacted by, monitoring and evaluation requirements and health systems implementation issues such as the development of improved supply lines and logistics management, reporting of results and commodity consumption, and adherence of health workers and patients to management consistent with diagnostic results. These are all important areas where pooling of knowledge and sometimes operational research is required to maximize the impact of the diagnostic tools discussed below [26,27].

Light Microscopy

When performed to a high standard, light microscopy is capable of accurately identifying and quantifying *Plasmodium* parasites with sufficient rapidity for case management in most settings. It remains the operational gold standard in both control and elimination settings. However, the quality of light microscopy in the field is often inadequate [31–36] and limited by factors such as the instability and difficult preparation of currently used Romanowsky-based stains [37–39], poorly maintained, low quality equipment, and inadequate training, supervision, and quality assurance. Additionally, as malaria transmission decreases, it is

likely that light microscopy technician skills may be redeployed elsewhere. Consequently, research into sustainable ways to maintain high-quality light microscopy in field settings, including innovative training, supervisory, and quality-assurance systems, is badly needed. More consistent and stable staining techniques are also required. This area of research has been ignored for the past 60 to 100 years, but has the potential to improve field accuracy significantly and may also improve the potential of the new reading techniques discussed below. Large volumes of slides pose particular challenges with respect to reading, especially in settings with low parasite prevalence where microscopist performance is hard to maintain [26].

Digital Microscopy

Computer-assisted analysis of Giemsa-stained slides (possibly combined with automated staining), or digitized image transfer (potentially via mobile telephone) to a reference centre for review by an expert microscopist may enable greater consistency in parasite detection [40–44]. Additional research is required to determine whether these techniques will detect lower parasite densities than can be obtained by traditional light microscopy. Related techniques under development use software analysis of the scatter of various wavelengths of light to identify *Plasmodium* parasites and other pathogens. Although these digital techniques have the potential to improve field detection of malaria parasites, field-ready versions are not yet available, and it is not known whether these tools will meet the requirements for use in resource-poor settings.

Fluorescent-Assisted Microscopy

Fluorescent-assisted microscopy (FAM)-based methods—for example, the quantitative buffy coat (QBC) method [45], incorporation of a fluorescent probe (fluorescence in situ hybridization [FISH]) or of parasite DNA [46], or antigen staining—has been used to a limited extent in various programs. FAM methods may eventually speed up slide reading and reduce operator error. High-throughput FAM may become possible if high specificity can be maintained by the absence of low artifactual staining. However, at present FAM cannot differentiate between species, a capability considered a major advantage of light microscopy over today's antigen-detection tests, although species-specific markers for FISH assays and fluorescent-tagged monoclonal antibodies are being developed. In addition, the applicability of FAM to parasite quantitation is not clear and FAM requires specialized equipment that will limit where it can be used.

Antigen-Detecting RDTs

RDTs based on the detection of specific parasite antigens that use a platform design of lateral immunochromatographic flow (dipsticks or plastic cassettes) have started to change the way malaria is diagnosed in endemic settings. RDTs are increasingly being used at the community level and in control programs for case management and in prevalence surveys. Good RDTs reliably detect parasitemia down to 100–200 parasites/µl, which is comparable to the sensitivity of routine well-performed light microscopy [47]. In general, RDTs are simple to use. With training and quality assurance, they can be used by peripheral facility and village health workers to determine whether malaria parasites are present in a patient. However, increasing use in field settings suggests that many commercial RDTs have variable detection thresholds and field stability [48]. Systems for monitoring performance and routine quality control of manufactured product lots are therefore required.

Three parasite antigen types are targeted by currently available RDTs. Histidine-rich protein 2 (HRP2)-detecting tests have high sensitivity and specificity for *P. falciparum* but detectable antigen frequently persists after parasite clearance. The presence of HRP2 deletions in areas of South America also limits the use of these tests [49]. Commercial tests for *Plasmodium* lactate dehydrogenase (pLDH) have yielded variable results and, in general, have less potential to detect low parasite densities and greater susceptibility to deterioration under storage at high temperature than HRP2-based tests [48,50]. However, species-specific (*P. falciparum* and *P. vivax*) and pan *Plasmodium* species-specific pLDH-based tests are available. Finally, tests targeting pan-specific parasite aldolase have shown inadequate detection thresholds in recent comparative trials, possibly because of the low concentrations of this target antigen in parasites [48].

The development of RDTs targeting other antigens may improve species identification (critical for elimination of *P. vivax*) and address some of the deficiencies of the current RDTs. In particular, current tests for *P. vivax*, which lack consistency in sensitivity and stability, might benefit from the use of monoclonal antibodies that target new antigens or improved manufacturing standards.

Quality-Control Methods for Malaria RDTs

Standardized quality-control methods for RDTs are important for confirming test quality and ensuring that health workers and patients trust results. As with microscopy [39], quality assurance of RDTs requires a comprehensive, organized program [47,51]. Such programs are absent in many countries. The development of standardized panels containing known concentrations of target antigens will greatly broaden the reach, applicability, and sustainability of RDT quality-control programs. Parasite-based panels that use cryo-preserved parasite preparations [52] are currently available at a centralized (regional) level, but panels that are easier to standardize and widely available are needed. Likewise, standardized regulatory approval and procurement in keeping with best practices will reduce the requirement for investment by individual procurement agencies in quality control and product evaluation programs. The development of low-cost tools for confirming quality at the national and field level (positive controls [53]) is also necessary to improve reach and sustainability. Finally, novel approaches that use PCR to confirm RDT results might eventually be useful.

Diagnostic Tools for Active Case Detection and Community Surveys

For use in active surveillance and case finding, a diagnostic tool must be suitable for use in resource-poor field settings. Diagnostic tests must therefore be supportable at the district level or below, be affordable and low-maintenance, require less operator training than current methods, and have a low requirement for consumables. They should also detect very low parasite densities and distinguish between all locally prevalent *Plasmodium* species, be minimally invasive, and provide sufficiently rapid results to facilitate effective case management when an infection is identified. For use in prevalence surveys, where immediate management of asymptomatic parasitemia is not the aim, testing at a more centralized level may be sufficient. But, even in this context, rapid feedback and case management are desirable.

Molecular (DNA) Detection

Current methods of detecting circulating parasites by demonstrating parasite DNA through amplification of ribosomal RNA (rRNA) genes by PCR assays represent the overall gold standard of malaria diagnostics. When sample concentration methods are used, 0.5 parasite/µl unconcentrated blood or lower can be detected. Quantitative PCR can be used to determine the concentration of circulating DNA and therefore estimate the density of circulating parasites. Survey and testing techniques, including pooling of samples, can reduce costs [54] but also reduce sensitivity to some extent by diluting samples.

At present, the application of PCR-based methods is restricted to well-equipped laboratories with specially trained technicians, partly because the need to avoid contamination (which leads to false-positive results) requires a very high standard of laboratory practice. PCR capacity is consequently limited in resource-poor malaria-endemic countries, where considerable investment would be required to establish and maintain it. PCR capacity-building programs are underway in several African countries through the Malaria Clinical Trials Alliance (MCTA). However, its restriction to well-equipped laboratories limits the applicability of PCR for surveillance and asymptomatic parasitemia case finding because timely feedback to allow the treatment of identified cases is impossible in most endemic areas. The development and field demonstration of high-throughput field-applicable PCR technologies is therefore needed to allow wider use of PCR in endemic settings.

Another molecular detection method based on DNA amplification is loop-attenuated isothermal amplification (LAMP). This method, which amplifies DNA (usually mitochondrial) with a single thermal cycle, has the potential to reduce the training and infrastructure requirements of molecular diagnosis [55–57], and would allow the timely feedback of results needed for case management. LAMP could also be used for surveillance, for detection of low-density parasitemia, and for monitoring parasite presence in antimalarial drug-efficacy monitoring and drug trials. However, LAMP has not yet been adequately field tested for wide-scale use or developed in a format suitable for the processes of high sample numbers.

Hemozoin Detection

Hemozoin, a by-product of *Plasmodium* metabolism, can be detected through refraction/absorbance of laser light of certain frequencies, and has been used to detect malaria and to determine species. Current field-ready technologies are based on flow cytometers. Their application is limited to screening, however, because of low sensitivity at low parasite densities [58–62]. Current research activities include the development of transcutaneous hemozoin detection. If sufficiently sensitive and specific, this approach might offer a noninvasive test for malaria for mass-population screening of, for example, individuals moving into a malaria elimination area. Hemozoin detection may find a place in routine case management if appropriate tools can be developed.

Antigen-Detection Tests

Current antigen-detecting RDTs (see earlier for details) are likely to miss a significant proportion of asymptomatic cases in low-transmission settings [16,22,23,39]. Thus, although the current generation of RDTs can indicate the presence of malaria in a community, they cannot determine the true prevalence of parasite carriage. Research aimed towards increasing the sensitivity of existing RDTs may not change this situation because of the limitations of the currently available technology. Some antigen-detecting ELISAs are more sensitive than RDTs. Furthermore, because they can also be used to quantify antigen, they have been used to monitor drug efficacy. Antigen-detecting ELISAs may also

facilitate high-throughput testing. However, their use is currently limited by laboratory and training requirements.

Antibody Detection

Antibody detection (see also [27]) is currently available in ELISA and RDT formats, and is a sensitive way to demonstrate past exposure to malaria parasites (past infection). Because antibodies may not be detectable in blood-stage infections of very recent onset, these tests are inappropriate for case management. However, they may be useful in detecting established *P. falciparum* infections in which the blood-stage parasite density has fallen below the limits of light microscopy or antigen-detecting RDTs [63]. Detection of antisporozoite antibodies (so-called anti-CSP antibodies) alone or in combination with antibodies to blood-stage parasites has also been suggested as a surrogate for detecting individuals with a high likelihood of carrying *P. vivax* hypnozoites (evidence of infection) [64–68]. However, anti-CSP antibody responses are usually low and transient, especially in areas of low and moderate transmission, which renders this test unreliable.

Because antibody-detecting tests can identify parasite-infected individuals who are undetectable by antigen detection or light microscopy because of low parasite density, they could be used to screen populations such as migrants or blood donors to identify asymptomatic individuals at risk of transmitting malaria. They could also be used for identifying foci of recent transmission in areas that are otherwise malaria free and to determine the presence or absence of recent malaria transmission in specific populations, such as young children. They therefore have potential applications in confirming areas free of transmission during a defined period, provided they are further refined and developed in terms of sensitivity and specificity.

Specific Issues for Reduction and Elimination of *P. vivax* Transmission

Detection of Hypnozoites

P. vivax detection and management will become increasingly important as control measures reduce *P. falciparum* transmission. In many programs, *P. vivax* already causes the majority of clinical malaria episodes. Because *P. vivax* can remain latent in the liver but produces relapse, its effective management normally requires the use of 8-aminoquinolones to clear hypnozoites from the liver. No current diagnostic technique is capable of detecting *P. vivax* hypnozoites, and none are in development, although tests that can detect the presence of hypnozoites are a key research and development need wherever and whenever elimination has a chance of becoming a realistic goal. While symptomatic cases of *P. vivax* can be assumed to harbor liver stages and managed accordingly, a method for detecting hypnozoites would enable populations in *P. vivax*-endemic areas to be screened during the nontransmission season for asymptomatic individuals likely to have relapses who could then be treated before they become symptomatic and transmit in the following transmission season. Screening could therefore reduce the use of 8-aminoquinolones in mass-treatment programs in *P. vivax*-endemic areas, which would reduce the probability of drug-related severe side effects in glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals (see next section). At present, compliance issues with the long course of primaquine (generally 14 days) have limited the broad application of this approach, and therefore the need for a diagnostic test for hypnozoites [24].

Potential biomarkers to detect hypnozoites include direct markers of metabolic activity, released antigens, markers of host immune response, and indirect serological markers of other stages (e.g., sporozoites). A lack of known markers of hypnozoite metabolic activity and markers of immunity limits the potential to assess the likely gains from investment in this area, and more knowledge of the biology of hypnozoites, perhaps through the development of liver-stage cultures, is required to determine whether such tests can be developed [69].

Detection of G6PD Deficiency

The only drug currently licensed for the radical cure of *P. vivax* infection is primaquine, and the only investigational drug showing promise is tafenoquine, Both these 8-aminoquinolones cause hemolysis in G6PD-deficient individuals, the clinical importance of which varies with the particular G6PD-deficiency phenotype, and the starting hemoglobin concentration, and may depend on how the drugs are administered [70].

Because eliminating *P. vivax* reservoirs will probably involve the use of a hypnozoiticidal drug [24], unless a non–8-aminoquinolone drug is developed, G6PD testing is likely to be required for wide-scale elimination of *P. vivax*. The requirements for such a test differ somewhat from those of parasite-detecting RDTs, because testing should only be required once in a lifetime and is not urgently required; the use of hypnozoiticidal drugs can be delayed if necessary. So, for example, a G6PD test does not have the stability requirements of an antigen-detecting RDT. Current tests for G6PD deficiency nevertheless have limitations regarding storage requirements and the complexity of the procedure, so research is needed to develop new tests. Importantly, addressing G6PD deficiency will also involve research into test implementation—how should samples be tested, where should tests be done, and how should results be recorded to facilitate retrieval? Moreover, to decide whether further development of field-applicable G6PD tests is needed also requires more data on the distribution of G6PD phenotypes and on the efficacy and safety of alternatives to the standard hypnozoiticidal primaquine regimen.

Other Research Priorities for Future Malaria Diagnostics

Noninvasive Sampling

Current RDTs detect antigen in peripheral blood samples obtained by finger prick. This method is generally acceptable for case management in the formal health care sector, but it presents some logistical challenges at the community level and in some private sector settings, particularly with regard to the potential risks of blood-borne infection. In addition, invasive tests may not be fully accepted in some settings, particularly when taking samples from asymptomatic individuals, which could diminish access to malaria diagnosis, treatment, and surveillance. Noninvasive sampling (for example, saliva or urine collection) has the potential to overcome these impediments but, at present, the limitations of sensitivity of nonblood sampling are even greater than the limitations of blood sampling combined with antigen-detecting RDTs for screening and surveillance [71–73]. Published trials of antigen sampling from saliva and urine, for example, have demonstrated inadequate sensitivity, probably because of the low concentration of available antigen in these samples [71,74]. Urine sampling may also present practical and cultural constraints. Techniques that concentrate antigen may have potential if they can be made practical for use in low-resource settings, but no such techniques are currently available. Additionally, if quantification is required, these methods would need to incorporate a standard to allow for variations in concentration of saliva or urine.

Multiplexing

Multiple diagnoses from one assay or "multiplexing" is made possible by, for example, the inclusion of multiple PCR-based nucleic acid probes in a single test or the inclusion of antibodies specific for nonmalarial diseases or of pathological markers of disease severity. The inclusion of antibodies targeting nonmalarial diseases in RDTs in their common format (visually read immunochromatographic tests) increases the technical challenge of achieving the stability needed for sufficient shelf life and makes interpretation of results more complex. The usefulness of such tests is also limited by the ability of the health system to provide appropriate management for each etiological agent that may be identified, and the highly variable prevalence of potential target differential diagnoses within malaria-endemic areas.

However, as malaria rates drop through successful control programs, the overall fever rate may not change significantly. Accordingly, it will be increasingly important to integrate management of malaria with that of other febrile diseases, at the point of diagnosis, if the program is to remain credible and sustainable (see also [27]). Nonmalarial fever will need to be diagnosed with sufficient accuracy to allow practitioners to manage the main causes of fever successfully and to at least distinguish major bacterial infections manageable with common antibiotics from nonbacterial infections.

Research and development needs for multiplexing include the development of field-ready multiplex tests for malaria and non-malarial diseases, which are not currently widely available, and research into the inclusion of markers for inflammation or severe disease in malaria tests, which would offer the potential to guide the referral of patients who require urgent management (see also [27]). Finally, the issue of complexity of interpretation in multidisease diagnostics needs to be addressed by the development of automated readers, particularly in combination with technology that allows multiple distinguishable markers to be captured in a single test line.

Pooling Samples for Surveillance, Gametocyte Detection, and Genotyping

Three other potential research priorities were discussed by the Consultative Group, but the consensus was that research into pooling samples, gametocyte detection, and genotyping was less

---

Box 1. Summary of the Research and Development Agenda for Diagnosis and Diagnostics

Overarching questions

- What proportion of effort should be directed to screening and surveillance versus early case detection at various time points in elimination? Question to be addressed by modeling and validated in different areas.
- Do we need microscopy for elimination, or can other tests replace it?

Programmatic issues

- Further data on thresholds of (i) parasite density likely to cause symptoms in low-transmission settings with variable or waning immunity, and (ii) transmission potential of cases with parasitemia below the threshold of microscopy and RDTs
- Diagnostic tests for nonmalarial febrile illness in malaria-endemic and malaria-elimination settings
- Distribution of severe G6PD variants

Technical issues: case-management tools
High priority
Stable tests for case management in low-training, low-technology settings with sensitivity sufficient for community-level case management, including:

- Antigen-detecting RDTs

  - Greater consistency in *P. falciparum* detection, particularly in the case of nonpersistent antigens
  - More sensitive and stable tests to detect non-*P. falciparum* parasites
  - Clarification of the programmatic/implementation requirements that will ensure good impact in the field
  - Standardized low-cost positive controls for antigen-detecting RDTs suitable for field use
  - Sustainable tools for quality control of RDTs at a country level.

- Further investigation of nonblood sampling to determine the potential for detecting recoverable antigen in these samples.

- More consistent, reliable staining methods for microscopy
- G6PD deficiency mapping and identification (if 8-aminoquinolones are to be used)

Medium priority

- Multiplexing: Other diseases, markers of severity
- Field G6PD detection (may be more important if tafenoquine approved), or raised priorities for *P. vivax* relapse prevention
- Tools to standardize and improve microscopy interpretation

Low priority

- Hypnozoite detection (becomes a high priority if feasibility can be demonstrated through further research on hypnozoite biology, identifying good biomarkers).

Technical issues: surveillance tools
High priority

- Field-applicable tools for detection of low-density parasitemia in a high-throughput manner, suitable for surveys and active detection of parasite carriage in time to allow management of positive cases
- Tools for minimally invasive, very rapid detection of low-density parasite infections suitable for screening of migrants/travelers

Innovation with potential for major operational impact

- Noninvasive, low-density parasite detection

Low-hanging fruit with immediate application for elimination

- High-throughput field molecular detection, capable of use at district level or below
- Positive control methods for RDTs

---

urgent. Thus, although the idea of pooling individual samples to detect parasitemia in very low transmission settings is intrinsically appealing and could result in cost savings using currently available tests, the Consultative Group felt that the limited quantity of antigen or DNA in pooled samples would severely limit the sensitivity of this approach. Similarly, the group decided that the development of a detection test for gametocytes should not be viewed as a high priority requirement. Finally, although WHO guidelines recommend genotyping of parasites during elimination phases [39], there is debate about whether research into methods for genotyping would be programmatically useful, particularly for *P. falciparum*. The resource needs to achieve genotyping are massive, and the long feedback time for results is likely to reduce the exercise to one of academic interest only. Genotyping could be useful for *P. vivax* infections to determine whether a blood-stage infection is new or a relapse. However, it has not yet been possible to develop methods that will reliably distinguish between relapse, recrudescence, and reinfection because of the multiplicity of hypnozoite genotypes present in *P. vivax*-infected individuals. Genotyping might, however, be useful in suspected outbreak or in new foci of transmission to determine the source of parasites, particularly when elimination in an area is being confirmed [26].

Sustaining the Effort

The central importance of active case detection in each programmatic stage towards elimination has been comprehensively dealt with by several of the other malERA Consultative Groups [24–27]. However, whether active case detection can be achieved at sufficiently high and sustainable levels will depend to a great extent on the field utility and costs of the diagnostic and other tools eventually adopted for this role and on how these tests are used.

Importantly, when malaria is rare and no longer perceived by local health services and the community to be of significant public health concern, ways must be found to maintain the resources needed to test febrile cases for parasitemia to prevent resurgence of infection. Because malaria parasite detection will be competing for resources with other disease priorities with higher mortality, it will be necessary to target diagnostics to those cases more likely to be malaria rather than necessarily screening whole populations (although some form of screening, and the ability to respond rapidly to reintroduction, will continue to be necessary [26–28]. It will also be important to integrate malaria detection more fully with other health service activities and, as nonmalarial causes of fever become predominant, it will be critical to provide appropriate diagnosis and management of alternative causes so that compliance is maintained through confidence in the ability of the health system to provide solutions to clinical problems.

Conclusions

Malaria elimination in the most challenging settings will require improvements in point-of-care tests for case management, and the development of new tests capable of identifying very low parasite densities in asymptomatic individuals in field settings for mass screening and treatment. As a result of our discussions, we propose a research and development agenda for diagnoses and diagnostics that should stimulate and facilitate the development, validation, and use of such tests (see Box 1).

Because malaria generally occurs in low-resource settings, the profits likely to be made from malaria diagnostic development and manufacture, particularly in the face of low mortality, are limited. The current market place for malaria rapid tests is dominated by small to medium-sized manufacturers, who are unlikely to be able to make the major investments needed to address these priorities alone. Thus, the role of donor agencies and product development partnerships and research institutions in enabling research and development and in providing the expertise and field access necessary to shape products to meet program needs will be an essential element of diagnostics development. Critically strong and focused, mainly public-private, partnerships will need to built and nurtured.

Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; Kara Hanson, London School of Hygiene & Tropical Medicine, London, UK; Kamini Mendis, World Health Organization, Geneva, Switzerland; Robert D. Newman, World Health Organization, Geneva, Switzerland; Don de Savigny, Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland; Allan Schapira, Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland; Laurence Slutsker, US Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Marcel Tanner (chair), Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland; Thomas Teuscher, Roll Back Malaria Partnership, Geneva, Switzerland.

Basel, Switzerland (October 2009) meeting split up Diagnosis/ Diagnostics: David Bell, Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; Robert D. Newman, World Health Organization, Geneva, Switzerland; Laurence Slutsker, US Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Marcel Tanner (chair), Swiss Tropical and Public Health Institute Basel and University of Basel, Basel, Switzerland.

Geneva, Switzerland (February 2010) Diagnoses/Diagnostic writing meeting: John W. Barnwell, US Centers for Disease Control and Prevention, Atlanta, Georgia, USA; David Bell, Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; Bruno Moonen, Clinton Health Access Initiative, Nairobi, Kenya; Robert D. Newman, World Health Organization, Geneva, Switzerland.

Extra contributor: Graham Brown, Nossal Institute for Global Health, Melbourne, Australia.

Author Contributions

References

1. O'Meara WP, Mangeni JN, Steketee R, Greenwood B (2010) Changes in the burden of malaria in sub-Saharan Africa. Lancet Infect Dis 10: 545–555.
2. WHO (2009) World malaria report 2009. Geneva: World Health Organization.
3. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, et al. (2010) Global, regional, and national causes of child mortality in 2008: A systematic analysis. Lancet 375: 1969–1987.
4. WHO (2006) Guidelines for the treatment of malaria. Geneva: World Health Organization.
5. Armstrong-Schellenberg JR, Smith T, Alonso PL, Hayes RJ (1994) What is clinical malaria? Finding case definitions for field research in highly endemic areas. Parasitol Today 10: 439–442.
6. Bojang KA, Obaro S, Morison LA, Greenwood BM (2000) A prospective evaluation of a clinical algorithm for the diagnosis of malaria in Gambian children. Trop Med Int Health 5: 231–236.
7. Chandramohan D, Carneiro I, Kavishwar A, Brugha R, Desai V, et al. (2001) A clinical algorithm for the diagnosis of malaria: Results of an evaluation in an area of low endemicity. Trop Med Int Health 6: 505–510.
8. Chandramohan D, Jaffar S, Greenwood B (2002) Use of clinical algorithms for diagnosing malaria. Trop Med Int Health 7: 45–52.
9. Font F, Alonso González M, Nathan R, Kimario J, Lwilla F, et al. (2001) Diagnostic accuracy and case management of clinical malaria in the primary health services of a rural area in south-eastern Tanzania. Trop Med Int Health 6: 423–428.
10. Kallander K, Nsungwa-Sabiiti J, Peterson S (2004) Symptom overlap for malaria and pneumonia: Policy implications for home management strategies. Acta Trop 90: 211–214.
11. Luxemburger C, Nosten F, Kyle DE, Kiricharoen L, Chongsuphajaisiddhi T, et al. (1998) Clinical features cannot predict a diagnosis of malaria or differentiate the infecting species in children living in an area of low transmission. Trans R Soc Trop Med Hyg 92: 45–49.
12. Reyburn H, Mbatia R, Drakeley C, Carneiro I, Mwakasungula E, et al. (2004) Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: A prospective study. BMJ 329: 1212.
13. Smith T, Hurt N, Teuscher T, Tanner M (1995) Is fever a good sign for clinical malaria in surveys of endemic communities? Am J Trop Med Hyg 52: 306–310.
14. RBM (2008) Global malaria action plan. Geneva: Roll Back Malaria Partnership.
15. WHO (2007) Malaria elimination: A field manual for low and moderate endemic countries. Geneva: World Health Organization.
16. Roper C, Elhassan IM, Hviid L, Giha H, Richardson W, et al. (1996) Detection of very low level Plasmodium falciparum infections using the nested polymerase chain reaction and a reassessment of the epidemiology of unstable malaria in Sudan. Am J Trop Med Hyg 54: 325–331.
17. Collins WE, Jeffery GM (2003) A retrospective examination of mosquito infection on humans infected with Plasmodium falciparum. Am J Trop Med Hyg 68: 366–371.
18. Collins WE, Jeffery GM, Roberts JM (2004) A retrospective examination of the effect of fever and microgametocyte count on mosquito infection on humans infected with Plasmodium vivax. Am J Trop Med Hyg 70: 638–641.
19. Harris I, Sharrock WW, Bain LM, Gray KA, Bobogare A, et al. (2010) A large proportion of asymptomatic Plasmodium infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: Challenges for malaria diagnostics in an elimination setting. Malar J 9: 254.
20. Ouedraogo AL, Bousema T, Schneider P, de Vlas SJ, Ilboudo-Sanogo E, et al. (2009) Substantial contribution of submicroscopical Plasmodium falciparum gametocyte carriage to the infectious reservoir in an area of seasonal transmission. PLoS One 4: e8410. doi:10.1371/journal.pone.0008410.
21. Shekalaghe SA, Bousema JT, Kunei KK, Lushino P, Masokoto A, et al. (2007) Submicroscopic Plasmodium falciparum gametocyte carriage is common in an area of low and seasonal transmission in Tanzania. Trop Med Int Health 12: 547–553.
22. Collins WE, Jeffery GM (1999) A retrospective examination of the patterns of recrudescence in patients infected with Plasmodium falciparum. Am J Trop Med Hyg 61: 44–48.
23. Boyd MF (1949) Malariology: A comprehensive survey of all aspects of this group of diseases from a global standpoint. Volumes 1 and 2 London: Saunders.
24. The malERA Consultative Group on Drugs (2011) A research agenda for malaria eradication: Drugs. PLoS Med 8: e1000402. doi:10.1371/journal.pmed.1000402.
25. The malERA Consultative Group on Vector Control (2011) A research agenda for malaria eradication: Vector control. PLoS Med 8: e1000401. doi:10.1371/journal.pmed.1000401.
26. The malERA Consultative Group on Monitoring, Evaluation, and Surveillance (2011) A research agenda for malaria eradication: Monitoring, evaluation, and surveillance. PLoS Med 8: e1000400. doi:10.1371/journal.pmed.1000400.
27. The malERA Consultative Group on Health Systems and Operational Research (2011) A research agenda for malaria eradication: Health systems and operational research. PLoS Med 8: e1000397. doi:10.1371/journal.pmed.1000397.
28. The malERA Consultative Group on Modeling (2011) A research agenda for malaria eradication: Modeling. PLoS Med 8: e1000403. doi:10.1371/journal.pmed.1000403.
29. Kidson C, Indaratna K (1998) Ecology, economics and political will: the vicissitudes of malaria strategies in Asia. Parassitologia 40: 39–46.
30. Spencer M (1992) The history of malaria control in the southwest Pacific region, with particular reference to Papua New Guinea and the Solomon Islands. P N G Med J 35: 33–66.
31. Durrheim DN, Becker PJ, Billinghurst K (1997) Diagnostic disagreement - the lessons learnt from malaria diagnosis in Mpumalanga. S Afr Med J 87: 1016.
32. Kachur SP, Nicolas E, Jean-Francois V, Benitez A, Bloland PB, et al. (1998) Prevalence of malaria parasitemia and accuracy of microscopic diagnosis in Haiti, October 1995. Rev Panam Salud Publica 3: 35–39.
33. Kain KC, Harrington MA, Tennyson S, Keystone JS (1998) Imported malaria: Prospective analysis of problems in diagnosis and management. Clin Infect Dis 27: 142–149.
34. Kilian AH, Metzger WG, Mutschelknauss EJ, Kabagambe G, Langi P, et al. (2000) Reliability of malaria microscopy in epidemiological studies: Results of quality control. Trop Med Int Health 5: 3–8.
35. Coleman RE, Maneechai N, Rachaphaew N, Kumpitak C, Miller RS, et al. (2002) Comparison of field and expert laboratory microscopy for active surveillance for asymptomatic Plasmodium falciparum and Plasmodium vivax in western Thailand. Am J Trop Med Hyg 67: 141–144.
36. O'Meara WP, McKenzie FE, Magill AJ, Forney JR, Permpanich B, et al. (2005) Sources of variability in determining malaria parasite density by microscopy. Am J Trop Med Hyg 73: 593–598.
37. Giemsa G (1904) Eine Vereinfachung und Vervollkommnung meiner Methylenazur-Methylenblau-Eosin-Färbenmethode zur Erzielung der Romanowsky-Nocht'schen Chromatinfärbung [A simplification and improvement of my methylenazur-methylenblue-eosin staining method to achieve a Romanowsky-Nocht chromatin stain]. Zentabl Bakteriol Parasitenkd Infectkrankh 37: 308.
38. Power KT (1982) The Romanowsky stains: A review. Am J Med Technol 48: 519–523.
39. WHO (2009) Malaria microscopy quality assurance manual - version 1. Manila: World Health Organization, Regional Office for the Western Pacific.
40. Murray CK, Mody RM, Dooley DP, Hospenthal DR, Horvath LL, et al. (2006) The remote diagnosis of malaria using telemedicine or e-mailed images. Mil Med 171: 1167–1171.
41. Shapiro HM, Perlmutter NG (2008) Killer applications: Toward affordable rapid cell-based diagnostics for malaria and tuberculosis. Cytometry B Clin Cytom 74: S152–S164.

42. Frean JA (2009) Reliable enumeration of malaria parasites in thick blood films using digital image analysis. Malar J 8: 218.
43. Suhanic W, Crandall I, Pennefather P (2009) An informatics model for guiding assembly of telemicrobiology workstations for malaria collaborative diagnostics using commodity products and open-source software. Malar J 8: 164.
44. Proudfoot O, Drew N, Scholzen A, Xiang S, Plebanski M (2008) Investigation of a novel approach to scoring Giemsa-stained malaria-infected thin blood films. Malar J 7: 62.
45. Adeoye GO, Nga IC (2007) Comparison of Quantitative Buffy Coat technique (QBC) with Giemsa-stained Thick Film (GTF) for diagnosis of malaria. Parasitol Int 56: 308–312.
46. Guy R, Liu P, Pennefather P, Crandall I (2007) The use of fluorescence enhancement to improve the microscopic diagnosis of falciparum malaria. Malaria J 6: 89.
47. Bell D, Wongsrichanalai C, Barnwell JW (2006) Ensuring quality and access for malaria diagnosis: how can it be achieved? Nat Rev Microbiol 4: S7–S20.
48. WHO-FIND-CDC-TDR (2009) Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: Round 1(2008). Geneva: World Health Organization.
49. Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, et al. (2010) 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 5: e8091. doi:10.1371/journal.pone.0008091.
50. Chiodini PL, Bowers K, Jorgensen P, Barnwell JW, Grady KK, et al. (2007) The heat stability of Plasmodium lactate dehydrogenase-based and histidine-rich protein 2-based malaria rapid diagnostic tests. Trans R Soc Trop Med Hyg 101: 331–337.
51. WHO (2003) Malaria rapid diagnosis: Making it work. Manila: World Health Organization.
52. WHO (2008) Rapid diagnostic tests for malaria: Methods manual for laboratory quality control testing. Manila: World Health Organization.
53. Lon CT, Alcantara S, Luchavez J, Tsuyuoka R, Bell D (2005) Positive control wells: A potential answer to remote-area quality assurance of malaria rapid diagnostic tests. Trans R Soc Trop Med Hyg 99: 493–498.
54. Taylor SM, Juliano JJ, Trottman PA, Griffin JB, Landis SH, et al. (2010) High-throughput pooling and real-time PCR-based strategy for malaria detection. J Clin Microbiol 48: 512–519.
55. Poon LL, Wong BW, Ma EH, Chan KH, Chow LM, et al. (2006) Sensitive and inexpensive molecular test for falciparum malaria: Detecting Plasmodium falciparum DNA directly from heat-treated blood by loop-mediated isothermal amplification. Clin Chem 52: 303–306.
56. Han ET, Watanabe R, Sattabongkot J, Khuntirat B, Sirichaisinthop J, et al. (2007) Detection of four Plasmodium species by genus- and species-specific loop-mediated isothermal amplification for clinical diagnosis. J Clin Microbiol 45: 2521–2528.
57. Paris DH, Imwong M, Faiz AM, Hasan M, Yunus EB, et al. (2007) Loop-mediated isothermal PCR (LAMP) for the diagnosis of falciparum malaria. Am J Trop Med Hyg 77: 972–976.
58. Scott CS, Van Zyl D, Ho E, Ruivo L, Mendelow B, et al. (2002) Thrombocytopenia in patients with malaria: Automated analysis of optical platelet counts and platelet clumps with the Cell Dyn CD4000 analyser. Clin Lab Haematol 24: 295–302.
59. Fawzi ZO, Fakhro NA, Nabhan RA, Alloueche A, Scott CS (2003) Differences in automated depolarization patterns of Plasmodium falciparum and P. vivax malaria infections defined by the Cell-Dyn CD4000 haematology analyser. Trans R Soc Trop Med Hyg 97: 71–79.
60. Scott CS, Van Zyl D, Ho E, Meyersfeld D, Ruivo L, et al. (2003) Automated detection of malaria-associated intraleucocytic haemozoin by Cell-Dyn CD4000 depolarization analysis. Clin Lab Haematol 25: 77–86.
61. Duffy P, Fried M (2005) Malaria: New diagnostics for an old problem. Am J Trop Med Hyg 73: 482–483.
62. Nyunt M, Pisciotta J, Feldman AB, Thuma P, Scholl PF, et al. (2005) Detection of Plasmodium falciparum in pregnancy by laser desorption mass spectrometry. Am J Trop Med Hyg 73: 485–490.
63. Bousema T, Youssef RM, Cook J, Cox J, Alegana VA, et al. (2010) Serologic markers for detecting malaria in areas of low endemicity, Somalia, 2008. Emerg Infect Dis 16: 392–399.
64. Cho D, Kim KH, Park SC, Kim YK, Lee KN, et al. (2001) Evaluation of rapid immunocapture assays for diagnosis of Plasmodium vivax in Korea. Parasitol Res 87: 445–448.
65. Kim S, Ahn HJ, Kim TS, Nam HW (2003) ELISA detection of vivax malaria with recombinant multiple stage-specific antigens and its application to survey of residents in endemic areas. Korean J Parasitol 41: 203–207.
66. Lee KN, Suh IB, Chang EA, Kim SD, Cho NS, et al. (2003) Prevalence of antibodies to the circumsporozite protein of Plasmodium vivax in five different regions of Korea. Trop Med Int Health 8: 1062–1067.
67. Park SK, Lee KW, Hong SH, Kim DS, Lee JH, et al. (2003) Development and evaluation of an immuno-chromatographic kit for the detection of antibody to Plasmodium vivax infection in South Korea. Yonsei Med J 44: 747–750.
68. Suh IB, Lee KH, Kim YR, Woo SK, Kang HY, et al. (2004) Comparison of immunological responses to the various types circumsporozoite proteins of Plasmodium vivax in malaria patients of Korea. Microbiol Immunol 48: 119–123.
69. The malERA Consultative Group on Basic Science and Enabling Technologies (2011) A research agenda for malaria eradication: Basic science and enabling technologies. PLoS Med 8: e1000399. doi:10.1371/journal.pmed.1000399.
70. Shekalaghe SA, ter Braak R, Daou M, Kavishe R, van den Bijllaardt W, et al. (2010) In Tanzania, hemolysis after a single dose of primaquine coadministered with an artemisinin is not restricted to glucose-6-phosphate dehydrogenase-deficient (G6PD A-) individuals. Antimicrob Agents Chemother 54: 1762–1780.
71. Mharakurwa S, Simoloka C, Thuma PE, Shiff CJ, Sullivan DJ (2006) PCR detection of Plasmodium falciparum in human urine and saliva samples. Malar J 5: 103.
72. Wilson NO, Adjei AA, Anderson W, Baidoo S, Stiles JK (2008) Detection of Plasmodium falciparum histidine-rich protein II in saliva of malaria patients. Am J Trop Med Hyg 78: 733–735.
73. Nwakanma DC, Gomez-Escobar N, Walther M, Crozier S, Dubovsky F, et al. (2009) Quantitative detection of Plasmodium falciparum DNA in saliva, blood and urine. J Infect Dis 199: 1567–1574.
74. Genton B, Paget S, Beck HP, Gibson N, Alpers MP, et al. (1998) Diagnosis of Plasmodium falciparum infection using ParaSight(R)-F test in blood and urine of Papua New Guinean children. Southeast Asian J Trop Med Public Health 29: 35–40.

PLoS MEDICINE

Review

A Research Agenda for Malaria Eradication: Health Systems and Operational Research

The malERA Consultative Group on Health Systems and Operational Research[¶][*]

Abstract: Health systems research and development is needed to support the global malaria eradication agenda. In this paper, we (the malERA Consultative Group on Health Systems and Operational Research) focus on the health systems needs of the elimination phase of malaria eradication and consider groupings of countries at different stages along the pathway to elimination. We examine the difference between the last attempt at eradication of malaria and more recent initiatives, and consider the changing health system challenges as countries make progress towards elimination. We review recent technological and theoretical developments related to health systems and the renewed commitment to strengthening health systems for universal access and greater equity. Finally, we identify a number of needs for research and development, including tools for analyzing and improving effective coverage and strengthening decision making and discuss the relevance of these needs at all levels of the health system from the community to the international level.

Introduction

The last attempt at (global) eradication of malaria, which lasted from 1955 to approximately 1969, depended on vertical operations (centrally organized activities not linked to subnational administrative levels and/or communities). These operations—largely indoor residual spraying—often bypassed health systems, because it was assumed that they could be run most efficiently with minimal collaboration with general health services, which were often poorly developed in endemic areas. In the later phases of the first eradication era, it became clear that some form of chemotherapy was needed to reduce transmission, and that good surveillance was essential for achieving and maintaining malaria-free status in a given area. Increased attention was then given to integration with existing health services and to using malaria eradication strategically to build rudimentary health services in remote areas [1,2].

Here, we examine the health systems research and development that is necessary to support a global malaria eradication agenda. We do not address broader macroeconomic and health system development needs, even though addressing them would be beneficial to all agendas. We focus on the elimination phase of the eradication agenda and considers groupings of countries at different stages along the pathway to elimination.

On the basis of previous experiences with malaria and other diseases for which eradication has been attempted, we use standard definitions for control, elimination, and eradication throughout this article (Box 1) [3]. Importantly, these definitions emphasize the need for continued interventions for both malaria control and elimination.

Review articles synthesize in narrative form the best available evidence on a topic.

The Health System

In 2000, The World Health Organization (WHO) articulated a comprehensive definition of health systems that is now widely adopted. A health system "consists of all organizations, people and actions whose primary intent is to promote, restore or maintain health" [4] with goals of "improving health and health equity in ways that are responsive, financially fair and make the best, or most efficient, use of available resources." In 2007, WHO developed a conceptual framework comprising six "health system building blocks" that has also been widely adopted (Box 2) [5].

This framework has now been further elaborated [6] to include the role of people, not just at the centre of the system as mediators and beneficiaries, but as key actors in driving the system itself. Thus, the framework includes the participation of people as individuals and in civil society organizations and stakeholder networks, which influence each of the building blocks. Placing people and their institutions at the centre of this framework emphasizes WHO's renewed commitment to the principles and values of primary health care—fairness, social justice, participation, and intersectoral collaboration (see Figure 1).

Currently, three revolutions are under way that will transform health systems: the biotechnology revolution, the communications and information technology revolution, and the systems thinking. Systems thinking is a holistic approach to analysis that focuses on the emergent behaviour of complex systems. It analyzes how a system's constituent parts interrelate and how systems work over time and within the context of larger systems. Applied to problem solving, systems thinking addresses the dynamic, mainly nonlinear linkages, interactions, and behaviours among the elements of the entire system. Systems thinking as developed and used for other complex systems is now being applied in health systems [7] and is essential for understanding what works, for whom, to what extent, and under what circumstances. It also helps predict and mitigate possible unintended consequences of particular actions and to exploit synergies from concerted action in the system.

Figure 1. Health system building blocks [**7**]**.** Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000397.g001

The promotion and application of systems thinking will be very timely as the malaria eradication agenda develops.

Health Systems for Malaria Control, Elimination, and Eradication

The Global Malaria Action Plan (GMAP) was launched in 2008 by the Roll Back Malaria partnership against a background of greatly increased investment in research and development for malaria-control technologies since 1999 and extraordinary increases in funding for malaria control through national and global financing mechanisms since 2002 [8]. The GMAP includes three phases. The first phase—scaling-up for impact—aims to rapidly reach universal coverage for all populations at risk with locally appropriate malaria-control interventions, supported by strengthened health systems. The second phase—sustained control—aims to prevent the resurgence of malaria by maintaining universal intervention coverage until countries enter the elimination stage. In the final phase—elimination and eradication—it is estimated that more than 20 lower burden countries around the world will be poised to eliminate malaria.

There is currently a broad global consensus on malaria-control strategies, and almost all malaria-endemic countries now have national malaria programmes in line with GMAP. Malaria indicators (both for coverage and health impact) are moving in the right direction in many countries [9]. However, progress in most endemic countries is slower than it could be, given the available financial resources. Among the main reasons for the suboptimal pace are constraints to the delivery of essential malaria interventions at effective coverage levels and quality to populations in need [9–11]. There is no doubt that success in moving towards eradication will be heavily dependent on health systems [12,13].

Some of the health system challenges in a country facing a huge malaria burden and in a country on the brink of phasing out the disease are similar, but such countries also pose different health system challenges. For example, quality case management is needed in all phases. In contrast to most other diseases for which elimination is being considered, the symptoms of malaria are nonspecific. Furthermore, treatment needs to start soon after symptoms appear both to prevent the development of severe disease and death and, particularly in areas where malaria prevalence is low, to help reduce transmission. The capacity to diagnose and provide early and effective treatment is therefore needed wherever there is a malaria risk. Achieving this capacity requires quality coverage of general health services and is an important systemic challenge for any antimalaria programme.

By contrast, although survey data can be useful for gauging progress in highly endemic areas, disease surveillance becomes increasingly important as the disease burden is lowered. Highly sensitive and dynamic surveillance becomes the crucial element in the pre-elimination phase and after [14]. Again, this capacity can only be achieved by a solid articulation between a specialized programme and functional general health services.

Finally, although the integration (or at least coordination) of malaria vector control and other preventive interventions with other health programmes can be synergistic and efficient in many settings, such integration becomes less efficient as progress makes malaria an increasingly focal and epidemic disease. Thus, malaria preventive interventions can sometimes be managed independently from general

health services but these operations nevertheless depend on fundamental health system elements such as policy and governance, human resources, financing, supplies, and monitoring.

Much progress has been made in recent years towards understanding health systems better and the importance of strengthening them. The result is that global health initiatives are providing increased funding for national health systems to accelerate progress on universal access to essential health interventions, including malaria interventions. New initiatives such as the Task Force on Innovative Financing for Health Systems [15], and initiatives from the Global Fund to Fight Aids, Tuberculosis and Malaria (GFATM), Global Alliance for Vaccines and Immunization (GAVI), WHO, World Bank Joint Platform for Health Systems Strengthening, and President Obama's Global Health Initiative are evidence of the growing momentum in favour of health system strengthening. At the same time, there is also an increased emphasis on health systems research. During the last attempt at malaria eradication, research, including health systems research, was neglected because it was assumed that rapid, uniform spraying operations would lead to eradication. More recent successful malaria disease and control programmes have been notable for including research as a critical element [2,11,16].

Health Systems Effectiveness

As an original approach to understanding health system impediments to sustaining malaria interventions at coverage levels sufficient to reduce malaria morbidity and mortality to very low levels, and to achieve and maintain malaria-free status, we introduce the concept of health systems effectiveness. We used this concept and a framework for analyzing constraints to scale-up (see below) as "stepping stones" during our development of a health systems research and development agenda.

Malaria control and elimination depend in equal measure on high-performance health systems that can deliver malaria interventions at high and equitable levels of quality and with effective coverage. In this context, effective coverage goes beyond the usual notion of population access to include provider compliant delivery, patient adherence, and individual benefit from the intervention [17]. Effective coverage requires the concerted strength of all the health system building blocks. When effective coverage levels are inadequate or inequitable, the reasons are nearly always interacting failures across the building blocks. To pinpoint where

system interventions and strengthening will be effective and efficient, programme managers need to be able to diagnose those problems and their determinants and interactions.

Figure 2 provides a graphical representation of the systems effectiveness framework and shows how an initially high intervention efficacy translates into low effectiveness in the real world because of system-specific issues of suboptimal intervention access, inadequate programme targeting because of diagnostic shortcomings, incomplete provider compliance, and client adherence.

District health system observatories are being established in Burkina Faso, Ghana, Mozambique, and Tanzania to determine their respective health systems' effectiveness in delivering artemisinin-based combination therapies (ACTs) [18], and research projects are starting to use the health systems effectiveness framework to analyze the determinants of coverage [19]. However, the results of these research projects have yet to be translated into strategically targeted health system–strengthening interventions and programme corrections.

A final stepping stone we used to develop the research agenda outlined in this paper is the framework for analyzing constraints to scale-up, developed for the Commission for Macroeconomics and Health [20]. This framework illustrates how barriers to expanding coverage of essential health services operate at all levels of the health system, from communities and households, through to cross-sectoral and sociopolitical levels, and thus suggests that interventions to address these barriers may need to operate at multiple levels.

Towards a Systems Research and Development Agenda

The health systems research and development agenda that our group has developed derives from the ideas and concepts discussed above and proposes the creation of a set of tools for applying the systems effectiveness framework for malaria elimination and control in different health system settings. The agenda is organized both across health system levels (community, facility, district, national, regional/global, and intersectoral; more details of these levels are given later) and health system building blocks (see Box 2), but, importantly it also takes account of "country groupings." These groupings are relevant to the phases defined in the GMAP and we discuss them here in some detail before presenting our research and development agenda in full.
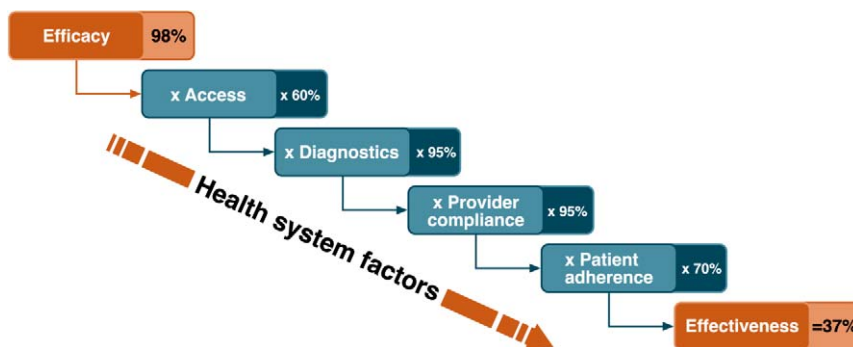
Figure 2. Graphical representation of the systems effectiveness framework. How interventions lose traction in health systems: example of artemisinin-based combination therapy (ACT) antimalarial treatment in Rufiji Demographic Surveillance Area Tanzania in 2006. Source: INDEPTH INESS Project. Systems Effectiveness Module, Swiss Tropical and Public Health Institute with data from Ifakara Health Institute and US Centers for Disease Control and Prevention based on [45–47]. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000397.g002

We suggest that the following grouping of countries is used to identify the most relevant health system research agendas for individual countries.

Group 1 includes countries where most of the population lives in areas where malaria elimination is considered impossible with existing tools. Currently, most of these countries are scaling up malaria-control efforts and some are entering the sustained control phase. This group includes most countries of sub-Saharan Africa and Papua New Guinea. In these countries, which have large areas with very intense malaria transmission, it is generally assumed that malaria elimination will only be possible though the large-scale application of new tools, which are still to be developed. Most likely such new tools will need to be applied in combination with existing ones, and the health system requirements for the effective delivery of these tools will probably be similar to those of current malaria-control interventions. Therefore, although undertaking systems research from the perspective of elimination is likely to be unproductive in group 1 countries, addressing current health system constraints on malaria control will almost certainly prove crucial for any future elimination efforts.

Group 2 includes countries with focal malaria, where a large part of the population lives in malaria-free areas, and where research aimed at health system strengthening is likely to play a crucial role in interrupting transmission in many of the existing foci. Many of these countries have diverse and complex health system challenges. This group includes most of the malaria-endemic countries in South and Central America, middle South Asia, and Southeast Asia. In sub-Saharan Africa, it includes southern Africa, the Horn, and the northern part of Sudan. It corresponds closely to the GMAP group designated as "control: low contribution to global deaths" [9], but includes additional countries such as Bangladesh, India, Indonesia, and Nepal.

Group 3 includes countries that are elimination ready. This group is almost identical to the "pre-elimination and elimination" countries in the World Malaria Report [9] and includes Argentina, Mexico, most of the countries of the Middle East and Central Asia, Central China, and possibly Sri Lanka, Vanuatu, and the Solomon Islands. In these countries, health system issues are generally not a crucial impediment to elimination, not necessarily because the health systems are exceptionally strong but often because the country's malaria problem has always been relatively easy to tackle with existing technologies due to intrinsic biologic (e.g., vectorial capacity or efficiency) or socioeconomic and development (e.g., improved housing) factors. However, review of the road to elimination in these countries with the identification of crucial health system determinants could provide valuable lessons, so international collaborations/global initiatives should focus on learning from past experiences rather than undertaking direct support or capacity strengthening.

This grouping of countries is intended to be specific to the malaria eradication health systems research agenda. A comprehensive listing of countries by these groupings has been avoided because many countries have areas belonging to more than one of these categories; this heterogeneity by itself presents policy and implementation challenges. Furthermore, the boundaries between groups are imprecise, and some countries could move from one group to another within few years.

What Goals and Needs Should the malERA Health Systems Research and Development Agenda Include?

From our discussions, we propose that the malERA health systems research and development agenda should consider the critical/transformational and conditional/situation goals and needs described in detail in Table 1. Some of these goals and needs are also partly covered in other papers in this series. For example, the need for tools to reduce unacceptably and avoidably low effective coverage of essential malaria interventions and malaria surveillance is also partly covered by the Monitoring and Evaluation and Surveillance malERA consultative group [21], the need for decision support tools to remove policy decision uncertainty for when to commit to transitioning from control to elimination is also covered in part by the malERA Consultative Groups on Modeling and Cross-Cutting Issues [22,23], and the need for a tool to determine the kind and mix of integrated interventions that are cost-effective in differing epidemiologic and health system contexts is covered in part by the malERA Consultative Group on Modeling [22].

What Research Questions Must Be Asked to Satisfy Health Systems Needs and Goals?

The research questions that emerge from this above analysis are presented in Table 2 in a matrix of health system levels and health system building blocks. Below, we discuss these questions in greater detail arranged by health system organizational level. As in Table 2, when no country grouping is specified, the discussion refers to both group 1 and group 2 countries.

Community Level

Past experience indicates that fixed health facilities cannot reach all those in need, and that extending the reach of services is essential to achieve universal and equitable coverage with interventions for malaria and other diseases. Community health workers (CHWs) and home management of fevers (which has been well documented in Africa) offer possible approaches. Several examples of CHW initiatives are emerging from countries as varied as India, Sri Lanka, Ethiopia, and Uganda [24,25]. More needs to be done to capture and share the experience gained from these programmes, and to ensure that opportunities are taken to evaluate the effectiveness of different approaches to designing and implementing CHW programmes.

A range of community-level factors affects the ability of health systems to reach the population effectively, particularly groups that are located far from formal health facilities and/or are mobile. Some of these factors reflect the conventional barriers to access—financial, physical/geographic, and social [26], but a better understanding is needed of how community-level factors influence use in particular settings, and how they can be addressed in the context of malaria-control and elimination measures.

In the past, some community health programmes failed because they did not recognize the need to compensate CHWs for time spent delivering services, and because they were not sufficiently linked into and supported by the health systems' "higher" levels [27,28]. There is a rich literature on CHW systems that should be exploited, but given rapid changes—such as the greatly improved levels of education and the proliferation of private providers in many areas—continued experimentation with different approaches is needed to sustain CHW performance and motivation, including different forms of health facility support (for example, supervision). Better ways of integrating CHWs' results into health information and surveillance systems and ensuring that they receive information from these systems also need investigating. Furthermore, as malaria transmission falls and countries enter the elimination phase, it will become critical that malaria surveillance systems improve their coverage to include data from whichever services are used by people at risk [21]. Finally, diagnostic and

Table 1. Categorization of the malERA health systems research goals and approaches.

Categories	Goals/Problems	Means/Approaches	Cross-Cutting	Stage of Elimination/Eradication
Critical/transformational	Reduce unacceptable and avoidably low effective coverage of essential malaria interventions.	Develop/validate toolkit for owning, analyzing, and responding to system-level bottlenecks in intervention delivery and use.	Yes, drugs, vaccines, vector control, diagnostics.	Scaling-up, sustained control, preelimination, and elimination.
	Harness a community of health systems analysts into the malaria elimination community.	Assess other models of global disease elimination enterprises to develop an optimal approach to an appropriately widened community.	Yes.	Scaling-up, sustained control, preelimination and elimination, prevention of reintroduction
	Understand how and facilitate strengthening of health systems by disease-specific global health initiatives in malaria.	Assist global health initiatives to apply systems science to guide health system strengthening investments.	Yes, concerns all agendas.	Scaling-up, sustained control, preelimination, and elimination.
Conditional/situational	Facilitate policy decision uncertainty for when to commit to transitioning from control to elimination phase and understand how disease-specific global health investments in malaria strengthen health systems and facilitate it.	Develop systems dynamic modeling, tools and case studies to understand determinants for elimination go-no/go policy decisions.	Yes, concerns all agendas.	Scaling-up, sustained control and preelimination, elimination.
	Determine whether the kind and mix of integrated interventions are cost-effective in differing epidemiologic and health system contexts.	Develop system dynamic modeling and respective tools as well as case studies to assess synergies.	Yes, drugs, vaccines, vector control.	Control, preelimination, elimination.
Increased emphasis	Communicate determinants of successful regional and inter-country collaboration for disease elimination	Critical review and analysis.	No.	Elimination.
	Major increase in community and district engagement and ownership of the malaria-control and elimination agenda.	Develop means to engage communities more effectively in case management, vector control, and surveillance.	Yes, drugs, vaccines, vector control, surveillance.	Control, preelimination, elimination.

doi:10.1371/journal.pmed.1000397.t001

other tools for use at the community level that are implemented as part of integrated strategies for managing illness, such as the Integrated Management of Childhood Illness (IMCI) and Integrated Management of Adult and Adolescent Illness (IMAI) strategies, have the potential to create quantum leaps in service and need to be adapted through research to the changing malaria epidemiological context. Unfortunately, few, if any, of these strategies are being systematically promoted in malaria risk areas in category 2 countries.

Facility Level

The health facility is the main point of contact with the health system for many people with fever, although private and informal providers are also important in many settings. It is also the focal point for collection, and ideally, use of data gathered through routine health management information systems. Many health systems face the challenge of ensuring that health workers are present in health facilities, have the required training and

knowledge, are equipped with the relevant drugs and other supplies, and are motivated to use these resources to provide high-quality and responsive care that follows national policies and standards.

New research is needed on how best to improve health worker performance [29,30]. A range of potential policy interventions has been suggested, including the traditional approaches of training and supervision, performance-based pay, bottom-up approaches using community accountability structures, and interventions addressing the mindset of health workers [31]. Other than training, the evidence about what works best and in what contexts is very limited, and deserves urgent attention.

Critically, interventions to improve health worker performance need to recognize the interconnectedness of the different health system building blocks. The design of pay-for-performance schemes, for example, involves questions of how best to govern such arrangements and the role of the community in these schemes, what the form and level of payments to health workers

Table 2. Matrix of health systems research and development needs.

Level/Building Block	Governance	Human Resources	Financing	Information[a]	Service Delivery, Medicines, and Technology
Community level	How can lay boards (community leader councils) strengthen local health service delivery? (Group 1 countries)	What is the role of CHWs and private sector providers in treatment of malaria and nonmalaria fevers, and in what settings are they crucial?	What are the main financial (and other) barriers to health services use and how can these be overcome?	What is the best approach to community-based monitoring of malaria and other communicable diseases building on existing and past efforts?	How can the community components of integrated approaches (IMCI and IMAI) be strengthened and adapted to different epidemiological and system settings?
	What is the role of communities in active efforts at transmission reduction (as opposed to reducing morbidity and mortality from malaria)?	How can they be incentivized and integrated with the health system to support and sustain their performance?	—	How can health information systems include information from and to CHWs? (Group 1 countries)	—
Facility level	Tools for assessing illicit payment for services	What are the most effective and appropriate methods for monitoring health worker performance?	—	How can modeling and evaluation innovations for malaria eradication strengthen health systems?	Development of IMCI and IMAI updated with new diagnostic tools and adapted to the malaria elimination context
	—	What types of financial and nonfinancial incentives can best support and sustain improved health worker performance?	—	Tools for assessing local coverage, quality, and equity to apply to systems effectiveness framework	Development of appropriate multidisease diagnostic tools
	—	—	—	—	Tools for drugs and supplies stock management
District level	What model(s) for district management of malaria-control programmes are effective in achieving and maintaining near zero malaria burden en route to elimination?	What are the appropriate organization and management, skill mix, human resource structure, and enabling factors to support effective service delivery?	Tools for developing efficient decentralized decision making and administration	How do we engage private providers and capture their data?	How can private provider involvement in case management, surveillance and vector control be harnessed?
	—	—	—	How can district managers be supported to use the systems effectiveness framework and tools to remove bottlenecks in service delivery?	How can data for decision-making skills be taught such that responses to resurgences in malaria burden are swiftly responded to? [17] Tools for systems effectiveness framework
National level	What investment and tools will ensure the quality of governance and accountability required for malaria elimination? (Groups 2 and 3).	What experience is there of strengthening health worker motivation and performance through disease-specific programmes, especially looking at global elimination initiatives (positive synergies)?	What financial resources will be required to manage the certification process at subnational and national levels? (Groups 2 and 3).	What experience is there of strengthening health management information systems through disease-specific programmes, especially looking at global elimination initiatives (positive synergies)?	What is the cost-effectiveness of different delivery modes in different national/subnational settings (e.g., community strategy versus facility, integrated curative services versus specialized, integrated vector management) malaria vector control; operations research on effect of scale on optimal organizational structures?

Table 2. Cont.

Level/Building Block	Governance	Human Resources	Financing	Information[a]	Service Delivery, Medicines, and Technology
	What governance structures are required to manage the elimination certification process?	—	What financing mechanisms are optimal at the national level to ensure a predictable and sustained flow of resources for malaria elimination? (Group 2)	—	What experience is there of strengthening service delivery and logistics/distribution chains through disease-specific programmes, especially looking at global elimination initiatives (positive synergies)?
	What experience is there of strengthening health system governance through disease-specific programmes, especially looking at global elimination initiatives (positive synergies).	—	—	—	—
	Tools to identify and evaluate possible interventions required in health system governance	—	—	—	—
Regional and global level	What are the determinants of successful intercountry collaboration on shared public health targets?	Tools: development of better regional training	—	—	What are the strengths and weaknesses of current malaria surveillance and case-management practices in endemic countries belonging to group 2?
Intersectoral level	Does the formulation of time-specific malaria elimination targets strengthen the participation of public and private stakeholders?	—	What are the macroeconomic benefits of malaria elimination? (Group 3)	—	What are the local geographic, economic, ecological, cultural determinants of malaria, and community and health system response? Includes operations research on service provision for mobile and marginalized populations
	—	—	—	—	What architecture and dynamics of complex intersectoral intervention strategies are required to achieve a major, sustainable, and cost-effective city-wide impact on persistent urban malaria?

Group 1, countries that are scaling up and entering the sustained control phase, where most of the population lives in areas where malaria elimination is considered impossible with existing tools; group 2, countries with focal malaria, where a large part of the population lives in malaria-free areas, and where health systems strengthening could play a crucial role in interrupting transmission in many but not necessarily all of the existing foci. These are often countries with very diverse and complex health systems challenges; group 3, elimination-ready countries. When a group of countries is not indicated, the text applies to group 1 and group 2 countries alike.

[a]Responsibility for these issues shared with malERA Monitoring, Evaluation, and Surveillance group.

IMAI, integrated management of adult and adolescent illness; IMCI, integrated management of childhood illness.

doi:10.1371/journal.pmed.1000397.t002

should be, and the source of funding, how to use the health information system to measure performance, how to ensure that complementary inputs such as training and supplies are coordinated and sustained, and how to avoid the risk that incentives may distort targets and divert attention from nontargeted services. Research on health worker performance should be multidisciplin-ary, therefore, and needs to recognize the complexity of possible interventions.

Research that focuses on developing new tools for assessing coverage, quality, and equity at the facility level that can be used to monitor health facility performance and analyze system effectiveness is also needed. Such tools are essential to identify

bottlenecks that may impede malaria elimination efforts. In the context of malaria elimination activities, the development of surveillance systems and the development of ways to monitor their performance require highest attention.

In some settings, a significant amount of treatment seeking for fever takes place outside public sector facilities, through private health providers, pharmacies, and shops. The engagement of such providers has mainly been done in limited malaria intervention projects rather than in programmes, and most of the literature concerns the pre-artemisinin-based combination therapy–rapid diagnostic test (ACT-RDT) era [32]. More research is therefore needed on approaches to quality assurance that will ensure that these facilities/providers adhere to guidelines [33], and are covered by systems for gathering surveillance data.

District Level

The district is the initial coordination hub for delivering services and commodities to people (through health facilities and community programmes). The district is therefore the focal point for priority setting, resource allocation, financial administration, supply chain management, accountability for health worker performance, engagement of the private sector, surveillance and response, and monitoring, evaluation, and information management.

Some of the critical bottlenecks in malaria-control operations currently stem from weaknesses at the district level for the above operations. These bottlenecks result in inequitable or irrational financial distribution, frequent stock-outs, poor-quality services, and inefficient disease-control operations. The enhancement of district-level system operations will therefore contribute significantly to reducing effectiveness losses for interventions, and increase the cost-effectiveness of programmes. Although there has been substantial investment in district-strengthening approaches and tools, these have not been as productive as they could be for a variety of reasons, such as insufficient decentralization and lack of information feedback. Innovations in information, communication, and decision-support tools (biometrics, bar coding, mobile phones and texting, computerized logistics systems, server-based data systems, among others) have the potential to improve district health systems in a cost-effective manner.

The systems challenges at the district level are common to group 1 and 2 countries. However, in group 1 countries they may be almost universal in rural areas, whereas in group 2 countries, systems challenges may only be considerable in the most malaria-endemic areas where deep poverty, difficult terrain, and various social barriers converge. Thus, district-strengthening efforts need to be more targeted in group 2 than in group 1 countries. Furthermore, in group 1 countries, the primary challenge is to enable the health system to achieve universal coverage of malaria-control interventions and to optimize their use (thereby reducing effectiveness losses), whereas in group 2 countries and in group 1 countries where the malaria burden has decreased, the challenge at the district level is increasingly to enable the system to respond to the technical demands of elimination. This challenge requires a greater focus on real-time information management and response and so, in these settings, research data that is relevant to developing decision-making skills will be critical.

National Level

Ultimately, the decision to embark on malaria elimination rests at the national level even if malaria elimination applies to only a region of the country. Such decisions must be based on operational and technical feasibility, as well as regional economic and political considerations [34].

Group 2 countries are more likely to have had some historical or more recent regional experiences with malaria elimination, and may see elimination as a "completeness exercise" or as an entry point to strengthening the systems response to address the health needs of neglected areas or population groups [35]. In some group 1 countries, elimination may be possible in specific areas. Generally, elimination targets for provinces, regions, or other administrative units that are highly developed and already close to malaria-free status may be sensible and justified from a regional viewpoint but of little importance from a national health perspective (e.g., Zanzibar in Tanzania, Goa in India). By contrast, national elimination targets—provided they are realistic—may provide strategic leverage for improving health equity at the national level.

The overriding research questions at the national level must be directed towards defining the best possible arrangements for governance, structural and functional organization between the system and malaria-specific programmes, and must be directed towards determining the implications of malaria elimination for cross-border political dialogue and arrangements with neighbouring countries. Models for financial sustainability also need to be established. These issues will be relevant in group 1, 2, and 3 countries, and through all the phases of disease control, elimination, certification, and prevention of reintroduction. An analytical review of past elimination programmes for other diseases—both successes and failures—with these questions in mind would be helpful. A recently published review provides useful information on interactions between global health initiatives and country health systems [36], but there are obvious differences between initiatives for reducing major disease burdens and elimination activities, which aim at small burdens.

Regional/Global Level

WHO recently revised its guidelines on malaria elimination and certification, emphasizing the need for regional intercountry collaboration [37]. In recent years, cross-border collaboration for malaria control has been inefficient in contrast to, for example, collaboration on polio elimination. Therefore, experiences from these successful intercountry collaborations and malaria-control initiatives should be mapped to provide a better evidence base for strengthening the intercountry collaboration needed to achieve national elimination targets.

An issue that will and should be addressed is subnational elimination. While any country is free to certify any area as malaria-free, WHO needs data on the achievement and maintenance of subnational areas of malaria elimination to develop guidance so that countries are spared the embarrassment of declaring an area malaria-free only to have transmission be detected soon after.

In addition, the current malaria surveillance and case-management practices of a sample of countries should be investigated and mapped by health systems research groups that are external to and independent of the malaria-control/elimination programme (see [21]).

Intersectoral Collaboration

The engagement of sectors other than health is sometimes but not always important for malaria control and elimination. The importance of intersectoral collaboration is determined by the extent to which other sectors are responsible for causing a local malaria problem through environmental change or population movement, and by whether a particular sector, such as education, plays a crucial role in achieving elimination. There is an extensive literature on the influence of development projects on malaria

Box 3. Summary of the Research and Development Agenda for Health Systems and Operational Research

Overarching issue: Development and validation of a tool kit for the national and subnational level, comparable to rapid assessment procedures, allowing (i) effectiveness decay analysis for identifying bottlenecks for effective coverage of malaria interventions and (ii) decisions on the degree of integration of interventions into existing and strengthened health systems.

Priority health systems research questions:

- At the health facility level, how can health worker performance and compliance with best practice be monitored, enhanced, and sustained?
- At the district level, what are the factors impeding greater application of existing tools and approaches to district health system strengthening including surveillance?
- At the national level, what experience is there of strengthening health system components using disease-specific programmes?
- At the regional/global level, what are the strengths and weaknesses of current malaria surveillance and patient management practices in malaria-endemic countries and what are the likely determinants of success of inter-country collaboration for disease elimination?

These research questions need to be defined locally but are of relevance to all programmes engaged in control or elimination

(e.g., [38]) and on integrating health considerations in programme planning (e.g., [39,40]). Serious difficulties can be expected where population movements related to natural and man-made disasters and conflict occur, in situations where "spontaneous" population movements related to traditional economies (for example, nomadism, transhumance) occur, and in urban areas where a multitude of actors make it difficult to identify the most important partners, where there is often less social cohesion, and where indoor residual spraying is often not possible.

Situations such as these have proven resilient to malaria control and elimination efforts over several decades. Mobile populations that are exposed to malaria, especially in or near forested areas in Latin America and South and Southeast Asia, often belong to ethnic minority groups and are subjected to various political and economic pressures. Interdisciplinary research (geographical, ecological, economic, social) and trials of different service delivery modes have proven useful in, for example, the Amazon, Thailand, and Vietnam [41–43]. Such research is needed in many more areas to validate for local adaptations of approaches in specific settings.

Urban malaria is a specific problem on the Indian subcontinent [44] where it needs to be investigated in all its dimensions from entomology to basic human ecology, and from household and industrial politics to local, municipal, and national level politics.

Concluding Remarks

In our discussions and in this article we have identified and characterized the major health systems needs relevant to the elimination of malaria and have articulated key research questions that need to be addressed at various health systems levels. In Box

3, we present the summary of the research and development agenda for health systems and operational research that resulted from our discussions. With malaria elimination on the agenda, one important, generic question needs to be addressed through health systems research. To what extent does an explicit target of malaria elimination motivate other sectors to participate in malaria control? If research evidence shows that such an explicit target is a potent motivator of other sectors, then ministries of health might be more inclined to be highly vocal and explicit about elimination targets and about the possible consequences of not meeting these targets.

Author Contributions

MT is the corresponding author on behalf of the malERA Consultative Group on Health Systems and Operational Research. Chaired the group: MT. ICMJE criteria for authorship read and met: PA, DB, KH, KM, RDN, DdS, AS, LS, MT, TT. Participated in one or more discussion meetings resulting in the writing of this article: the malERA Consultative Group on Health Systems and Operational Research.

References

1. Gramicci G, Beales PF (1988) The recent history of malaria control and eradication. In: Wernsdorfer WH, McGregor SI, eds. Principles and practice of malariology. Edinburgh: Churchill Livingstone. pp 1335–1420.
2. Nájera JA (2001) Malaria control: Achievements, problems and strategies. Parassitologia 43: 1–89.
3. Dowdle WR, Hopkins DR, eds (1997) The eradication of infectious diseases, Dahlem Workshop Report. Chichester: John Wiley & Sons.
4. WHO (2000) World health report 2000 – Health systems: Improving performance. Geneva: World Health Organization.
5. WHO (2007) Everybody's business: Strengthening health systems to improve health outcomes: WHO's Framework for Action. Geneva: World Health Organization.
6. WHO (2008) World health report 2008 – Primary health care: More than ever. Geneva: World Health Organization.
7. de Savigny D, Adam T (2009) Health systems thinking for health systems strengthening. Geneva: World Health Organization.
8. Roll Back Malaria Partnership (2008) The Global Malaria Action Plan. For a Malaria Free World. RBM: Geneva. Available: http://www.rollbackmalaria.org/gmap/.
9. WHO (2009) World malaria report 2009. Geneva: World Health Organization.
10. Bryce J, Terreri N, Victora CG, Mason E, Daelmans B, et al. (2006) Countdown to 2015: Tracking intervention coverage for child survival. Lancet 368: 1067–1076.
11. Webster J, Hill J, Lines J, Hanson K (2007) Delivery systems for insecticide treated and untreated mosquito nets in Africa: Categorization and outcomes achieved. Health Pol Plan 22: 277–293.
12. Tanner M, de Savigny D (2008) Malaria eradication back on the table. Bull WHO 86: 82–83.
13. Mills A, Rasheed F, Tollman S (2006) Health system strengthening. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Alleyne G, et al. (2006) Disease control priorities in developing countries, 2nd edition. New York: Oxford University Press and World Bank.
14. Hay S, Smith D, Snow R (2008) Measuring malaria endemicity from intense to interrupted transmission. Lancet Infect Dis 8: 369–378.
15. International Health Partnership (2010) Leaders commit new finance to tackle women's and children's health in the developing world. Available: http://www.internationalhealthpartnership.net/en/taskforce. 25 June 2010.
16. Alilio MS, Bygbjerg IC, Breman JG (2004) Are multilateral malaria research and control programs the most successful? Lessons from the past 100 years in Africa. Am J Trop Med Hyg 71: 268–278.
17. Lozano R, Soliz P, Gakidou E, Abbott-Klafter J, Feehan DM, et al. (2006) Benchmarking of performance of Mexican states with effective coverage. Lancet 368: 1729–1741.
18. INDEPTH Network Effectiveness and Safety Studies Project (INESS). Available: http://www.indepth-network.org/iness/index.php.
19. Beer N, Ali AS, de Savigny D, Al-Mafazy AW, Ramsan M, et al. (2010) System effectiveness of a targeted free mass distribution of long lasting insecticidal nets in Zanzibar, Tanzania. Malar J 9: 173.
20. WHO (2001) Commission on Macroeconomics and Health. Geneva: World Health Organization.
21. The malERA Consultative Group on Monitoring, Evaluation, and Surveillance (2011) A research agenda for malaria eradication: Monitoring, evaluation and surveillance. PLoS Med 8: e1000400. doi:10.1371/journal.pmed.1000400.
22. The malERA Consultative Group on Modeling (2011) A research agenda for malaria eradication: Modeling. PLoS Med 8: e1000403. doi:10.1371/journal.pmed.1000403.
23. The malERA Consultative Group on Integration Strategies (2011) A research agenda for malaria eradication: cross cutting issues for eradication. PLoS Med 8: e1000404. doi:10.1371/journal.pmed.1000404.
24. Haines A, Sanders D, Lehmann U, Rowe AK, Lawn JE, et al. (2007) Achieving child survival goals: Potential contribution of community health workers. Lancet 369: 2121–2131.
25. Staedke SG, Mwebaza N, Kamya MR, Clark TD, Dorsey G, et al. (2009) Home management of malaria with artemether-lumefantrine compared with standard care in urban Ugandan children: A randomised controlled trial. Lancet 373: 1623–1631.
26. Hetzel MW, Obrist B, Lengeler C, Msechu JJ, Nathan R, et al. (2008) Obstacles to prompt and effective malaria treatment lead to low community-coverage in two rural districts of Tanzania. BMC Public Health 8: 317.
27. Segall M (2003) District health systems in a neoliberal world: A review of five key policy areas. Int J Health Plan Manag 18: S5–S26.
28. Walt G (2005) Commentary: lay health workers in primary and community health care. Int J Epidemiol 34: 1251.
29. Rowe AK, de Savigny D, Lanata CF, Victora CG (2006) How can we achieve and maintain high-quality performance of health workers in low-resource settings? The Lancet 366: 1026–1035.
30. Rowe AK, Onikpo F, Lama M, Deming MS (2010) The rise and fall of supervision in a project designed to strengthen supervision of Integrated Management of Childhood Illness in Benin. Health Pol Plan 25: 125–134.
31. Maestad O, Schellenberg J, Hanson K, et al. (2009) A research agenda for improving health worker performance. London: London School of Hygiene & Tropical Medicine.
32. Goodman C, Brieger W, Unwin A, Mills A, Meek S, et al. (2007) Medicine sellers and malaria treatment in sub-Saharan Africa: What do they do and how can their practice be improved? Am J Trop Med Hyg 77: 203–218.
33. Adeyi O, Atun R (2009) Innovating for impact: The Affordable Medicines Facility-malaria (AMFm). Nat Med 15: 991.
34. Feachem RGA, The Malaria Elimination Group (2009) Shrinking the malaria map: a guide on malaria elimination for policy makers. San Francisco: University of California, San Francisco.
35. WHO EMRO (2007) Malaria in Morocco: Relentless efforts towards the goal of elimination. Geneva: WHO EMRO. Available: http://www.emro.who.int/publications/Book_Details.asp?ID=813. Accessed 25 June 2010.
36. WHO Maximizing Positive Synergies Collaborative Group (2009) An assessment of interactions between global health initiatives and country health systems. Lancet 373: 2137–2169.
37. WHO GMP (2007) Malaria elimination. A field manual for low and moderate endemic countries. Geneva: World Health Organization.
38. Service MW (1991) Agricultural development and arthropod-borne diseases: A review. Revista de Saúde Pública 25: 165–178.
39. Birley MH, Gomes M, Davy A (1997) Health aspects of environmental assessment. Environmental Assessment Sourcebook Update 18. Washington (D.C.): Environmental Division, World Bank.
40. WHO (1982) Manual on environmental management for mosquito control, with special emphasis on malaria vectors. WHO Offset Publication number 66. Geneva: World Health Organization.
41. Peeters GK, Xuan XN, Van BW, Duc TN, Ribera JM, et al. (2010) Low perception of malaria risk among the Ra-glai ethnic minority in south-central Vietnam: Implications for forest malaria control. Malar J 9: 23.
42. Sawyer D (1993) Economic and social consequences of malaria in new colonization projects in Brazil. Soc Sci Med 37: 1131–1136.
43. Singhanetra-Renard A (1986) Population movement, socio-economic behavior and the transmission of malaria in northern Thailand. Southeast Asian J Trop Med Public Health 17: 396–405.
44. Rao R (1984) The anophelines of India. New Delhi: Malaria Research Centre, India Council of Medical Research.
45. Tanner M (1990) Von der Tropenmedizin zur Medizin in den Tropen - Prioritäten bei der Bekämpfung übertragbarer Erkrankungen [From tropical medicine to medicine in the tropics – priorities in the control of communicable diseases]. Therapeut Umschau 47: 856–863.
46. Vlassoff C, Tanner M (1992) The relevance of rapid assessment to health research and interventions. Health Pol Plan 7: 1–9.
47. Tugwell P, de Savigny D, Hawker G, Robinson V (2006) Applying clinical epidemiological methods to health equity: The equity effectiveness loop. Br Med J 332: 358–361.

PLoS MEDICINE

Review

A Research Agenda for Malaria Eradication: Monitoring, Evaluation, and Surveillance

The malERA Consultative Group on Monitoring, Evaluation, and Surveillance[¶]*

Abstract

Monitoring, evaluation, and surveillance measure how well public health programs operate over time and achieve their goals. As countries approach malaria elimination, these activities will need to shift from measuring reductions in morbidity and mortality, to detecting infections (with or without symptoms) and measuring transmission. Thus, the monitoring and evaluation and surveillance research and development agenda needs to develop the tools and strategies that will replace passive surveillance of morbidity with active and prompt detection of infection, including confirmation of interruption of transmission by detecting present and past infections, particularly in mobile populations. The capacity to assess trends and respond without delay will need to be developed, so that surveillance itself becomes an intervention. Research is also needed to develop sensitive field tests that can detect low levels of parasitaemia, together with strategies for their implementation. Other areas to explore include the rigorous evaluation of the utility of more detailed maps of disease and infection incidence and prevalence, the development of new maps to inform programmatic responses and the use of surveillance technologies based on cell phone or real-time internet Web-based reporting. Because any new strategies for monitoring and evaluation and surveillance for eradication have major implications for program implementation, research is also needed to test systems of delivery for acceptability, feasibility, efficiency, cost-effectiveness, and community engagement. Finally, there is a clear need to systematically review the information from past elimination efforts for malaria and other infectious diseases.

Introduction

Monitoring (the systematic tracking of program actions over time) and evaluation (the examination of progress and its determinants) activities measure how well public health programs operate over time and whether they are achieving their program milestones (markers of progress within and transition between phases) and ultimate goals. In the context of malaria program scale-up, monitoring and evaluation focuses on the evaluation of burden reduction, specifically morbidity and mortality [1]. However, as programs successfully reduce transmission to near-elimination levels, the measurement of malaria-associated morbidity and mortality burden becomes increasingly difficult and insensitive, particularly since a substantial proportion of infections will be asymptomatic in countries that experienced high infection rates in the recent past. Thus, burden measures that only detect clinical illness will not provide good estimates of ongoing

transmission as countries approach elimination, and malaria program monitoring and evaluation and surveillance methods will need to focus on detecting infections (with or without symptoms) and measuring transmission dynamics as the primary indicators of interest.

The malERA Consultative Group on Monitoring, Evaluation, and Surveillance focused on defining the monitoring and evaluation and surveillance research and development needs as malaria elimination efforts unfold over the next 5–20 years. Information gaps and research needs were identified by the group by considering several broad thematic areas: lessons learned from countries that have recently achieved malaria elimination [2] or elimination of other diseases; the required evolution of the malaria monitoring and evaluation framework and indicators; surveillance as an intervention to reduce transmission; measurement of transmission interruption and maintenance of zero transmission; the tools (currently available and in the pipeline) needed, including diagnostics (screening, confirmation, and transmission measurement), mapping, and communication; and implementation issues. Information and research needs that were identified include: systematic reviews of existing information and experience, and assembly of that work into guidance; protocol or standards development for conduct of certain activities; and research and development activities to produce new information where guidance or experience does not exist, and new tools where these will enhance capabilities.

The World Health Organization (WHO) and the Roll Back Malaria (RBM) Global Malaria Action Plan (GMAP) characterize different "phases" of malaria control as programs progressively reduce transmission, though it is understood that these phases are part of a continuum rather than abrupt shifts [3,4]. At high levels of transmission, initial efforts are focused on scaling up for impact (SUFI). Sustained control efforts subsequently lead to further transmission reduction. As very low levels of transmission are reached, programs move from a focus on control to a focus on pre-

Review articles synthesize in narrative form the best available evidence on a topic.

elimination and elimination, and finally prevention of reintroduction. Where appropriate, we shall indicate where proposed research and development activities would fit into this malaria elimination framework.

Lessons Learned from Other Diseases or Current Malaria Elimination Programs

Several diseases other than malaria have been proposed for eradication or elimination. General lessons learned from these other disease elimination efforts have been summarized and underscore the critical role that monitoring and evaluation and surveillance play in these efforts [5–9]. The essential role of monitoring and evaluation and surveillance in informing elimination program efforts is particularly clear in past smallpox efforts and ongoing polio activities. Many countries have either eliminated or are in the process of pursuing malaria elimination. There is, therefore, a clear need to systematically review and summarize the monitoring and evaluation and surveillance lessons learned from both successful and unsuccessful disease elimination programs. In the context of malaria elimination, efforts are underway to summarize and disseminate recently accrued experience [2,10]. This review work should be done even before the elimination phase.

General needs for monitoring and evaluation and surveillance that have already emerged from experience with elimination efforts for malaria and for other diseases include the need for: improved management of systems; improved identification of infected individuals; enhanced methods for engaging and developing community support; improved information sharing for advocacy (at the community level and involving high level leaders); and improved ways of conducting surveillance activities in the private sector. Past experience also indicates that current and future tools and strategies for monitoring and evaluation and surveillance will need to be tailored to the individual epidemiological, entomological, and socio-cultural situation.

Monitoring and Evaluation Framework and Indicators

The current Monitoring and Evaluation Framework for malaria comprises a series of activities, namely, Assessments and Planning,

Inputs, Processes, Outputs, Outcomes (intermediate effects), and Impact (long-term effects; Figure 1A) [1]. Each part of this schema can be monitored with a specific set of indicators that tracks progress in program implementation. Historically, the malaria community has focused on illness and mortality reduction as indicators of impact, but will these and the other current indicators shown in Figure 1A serve us well for elimination efforts?

There is general consensus that these coverage indicators will continue to be useful because high intervention coverage will need to be maintained en route to elimination, especially in Africa where transmission is intense. However, as elimination is approached, other indicators will need to be adapted and new ones will need to be introduced. For example, indicators that track the proportion of cases with parasitological confirmation or that focus on coverage of individuals in specific geographic areas where foci of transmission are located will be needed. Similarly, if transmission blocking vaccines are deployed, coverage with the vaccine will need to be tracked. The utility of indicators and databases for parasite strain information that could differentiate indigenous from imported cases may need to be evaluated. In addition, methods and indicators for tracking population movements within and between countries and quantifying their contribution to the risk of malaria transmission may be useful. Furthermore, greatly reduced malaria morbidity and mortality levels (achieved through intervention scale-up and sustained control) will need to be monitored, although ultimately, as elimination approaches, the measure of impact will need to be infection and transmission (sometimes from introduced cases), and programs will need to include active case detection and case-based investigation and response within a revised Monitoring and Evaluation Framework (Figure 1B) (also see [11,12]).

Surveillance as an Intervention

As noted in the Introduction, monitoring and evaluation are critically required for measurement of malaria control program success. Over time, the term "surveillance" has become somewhat synonymous to some with monitoring and evaluation, but the WHO Global Malaria Eradication Program (GMEP), which lasted from 1955 to 1969, defined surveillance quite specifically as an integral action or intervention within that eradication program (Box 1) [13].

Malaria programs contemplating an elimination strategy must be prepared to change their strategies of monitoring and evaluation and surveillance as transmission is reduced [14,15]. Thus, many countries begin scale-up of malaria control interventions with relatively high levels of malaria transmission and develop monitoring and evaluation programs that rely on the collection of routine information (often from health facilities and health management information systems) and on periodic population-based surveys. Together, these approaches collect information on intervention coverage and use as well as changes in malaria burden, but, as transmission intensity drops to near elimination levels, surveillance as defined by GMEP needs to increase (Table 1).

In the context of malaria elimination programs, the goal is to achieve complete reporting of each case of infection to health authorities, regardless of whether symptoms of fever or illness are present. Critically, malaria control programs usually identify individuals with fever/symptoms and laboratory-confirmed malaria parasite infection as "malaria cases," but do not systematically assess the extent of asymptomatic malaria infection. As transmission decreases and individuals have less exposure to malaria, they lose acquired immunity and a higher proportion of
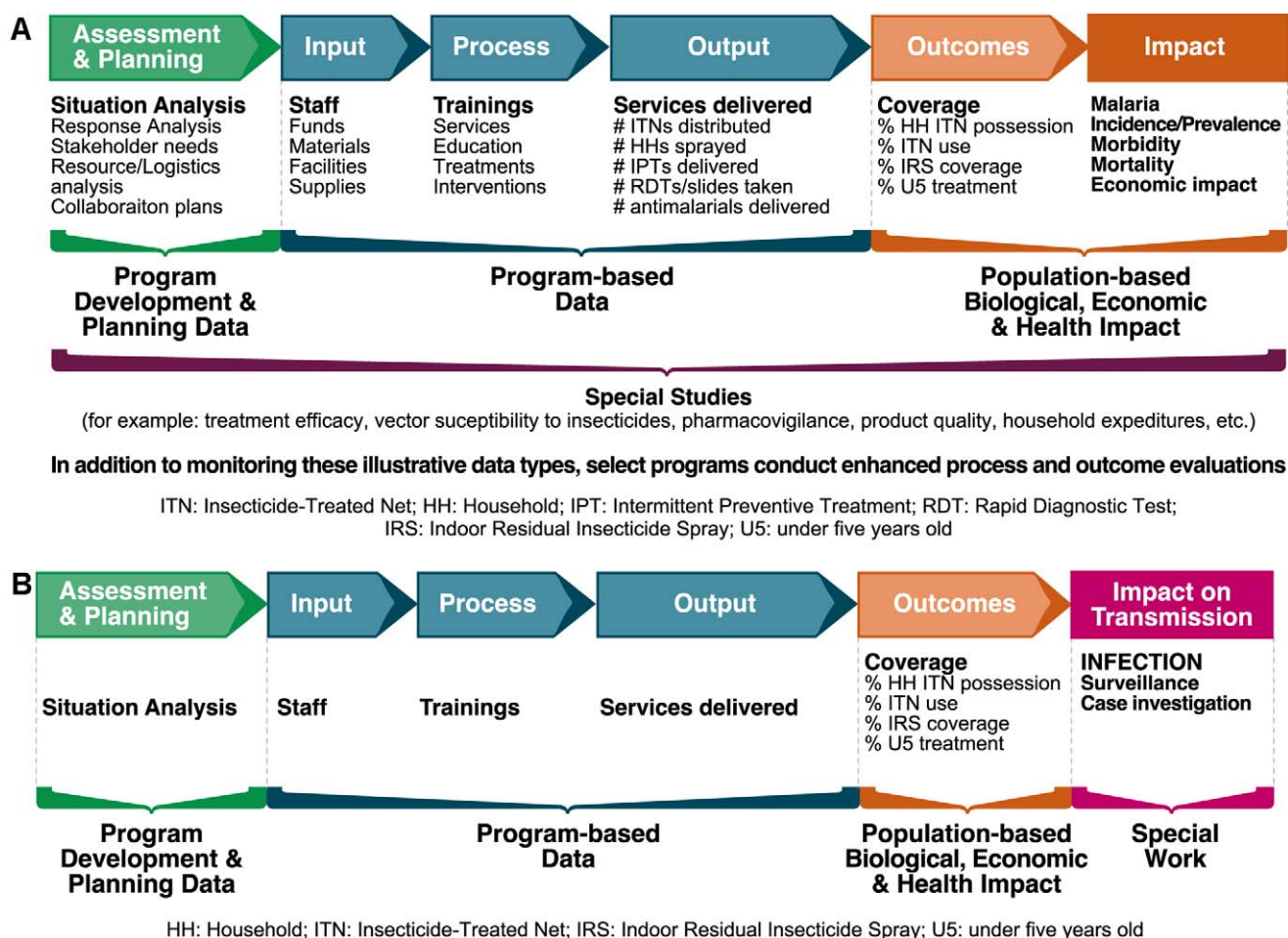
Figure 1. (A) Malaria monitoring and evaluation framework and illustrative data types. Source: adapted from [3]. (B) Evolving malaria monitoring and evaluation framework with emphasis on transmission. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000400.g001

infections present with symptoms. However, in populations in rapid transition from high exposure to low exposure, the proportion of persons with enough acquired immunity to harbour asymptomatic infections may remain substantial [16]. For example, in a low transmission setting in the western Pacific,

---

Box 1. Definitions of Surveillance

Per conventional use: Surveillance is the ongoing, systematic collection, analysis, and interpretation of data, often incidence of cases of disease or infection. Surveillance data are used to plan, implement, and evaluate the progress in public health programs.

Per the WHO Global Malaria Eradication Program: In malaria eradication terminology, surveillance was that part of the program aimed at the discovery, investigation, and elimination of continuing transmission, the prevention and cure of infections, and the final substantiation of claimed eradication. The individual functions of surveillance are case detection, parasitological examination, antimalarial drug treatment, epidemiological investigation, entomological investigation, elimination of foci by either residual spraying or mass drug administration, case follow-up, and community follow-up. In this definition, surveillance is seen as an intervention [16].

>80% of infections identified in a recent cross-sectional population-based survey were afebrile [17]. Because asymptomatic infections are a reservoir of transmission to others, it is critical to seek all infections rather than just symptomatic cases as a method to reduce transmission.

For surveillance, standardized definitions for case/infection reporting are needed, along with a strong mandate for notification to health authorities of all malaria cases/infections in both public and private settings [18]. An important area for further research, therefore, is to investigate how tools such as legal requirements, financial inducements, and other novel approaches can be used to improve the coordination of detection and reporting of infections from the private sector to public health authorities. Importantly, all malaria cases/infections must be epidemiologically investigated, and linked to geographic and laboratory data (species and genotyping) so that the source and potential spread of infection can be quickly addressed.

Furthermore, reporting systems must be able to analyze reported data rapidly to assess trends over time and place, particularly as transmission drops and cases of infection become increasingly focal in distribution [19]. Although some control programs in endemic areas have malaria early warning systems, these systems need better performance characteristics (for example, better linkages with local information systems) before they can be truly useful in malaria elimination.

Table 1. Program activities and methods for transmission reduction in populations.

Potential Activity	Description and Purpose
Prevalence surveys	Usually population-based surveys to stratify risk, evaluate impact of interventions, and track progress towards elimination
Active case detection	Regular efforts to ascertain fever and infection in the community
Focused screening for infections ("active infection detection")	Targeted search for main sources of rare cases (of Pf, Pv, drug-resistant Pf) and eliminating them
Case investigation	Detecting infections/cases around index cases for response
Mass screening and treatment	Screening large segments of the population to find and treat cases
Mass drug administration[a]	Administration of treatment to large segments of the populations regardless of infection status to reduce infections in a population with a relatively high infection rate
Surveillance for drug-resistant parasites	Enrollment of cases and follow-up of presence, density, or absence of parasites for in vivo resistance surveillance to assess treatment efficacy
Detection of gametocytaemia[b]	Find infections that contribute to ongoing transmission so that they can be treated to reduce transmission
Confirmation of elimination/detection of reintroduction[c]	Measurement of ongoing infection and transmission through sampling and use of biomarkers such as DNA or serology
Border screening/transit screening[d]	Rapid diagnostic testing of people crossing borders to allow immediate treatment of positives

[a]Note that mass drug administration is controversial for a variety of reasons but is presented here for completeness sake as it has been used to some benefit in the past (see also [25]).
[b]See also [11] and [25].
[c]See also [11] and [44].
[d]See also [12].
Pf, P. falciparum; Pv, P. vivax.
doi:10.1371/journal.pmed.1000400.t001

Assuming that effective infection detection and prompt and timely reporting exist, it is crucial that surveillance systems respond effectively to detected foci of infections and ultimately to individual infections in order to reduce transmission to a reproduction rate (R_0) of <1. Although many programmatic responses to detected infections exist, there is neither a systematic description of such responses nor a well-defined evidence base to suggest the optimal strategic approach. For surveillance to be effective as an intervention, research on useful and efficient modes of both detection and response must be undertaken [20]. At the most basic level, it is currently unclear when programs transitioning to very low transmission conditions should add active case and infection detection to their response strategies, and whether additional vector control interventions are needed [21]. The evolution of these actions and the optimal sequence and mix needs further evaluation as is also discussed in the malERA paper on modeling [22].

Finally, countries embarking on malaria elimination must establish a system for continuous data validation to identify problems and to prepare for the process of certification of elimination [23,24]. The concept of "good surveillance practices" should be implemented early to facilitate evaluation of the quality of the surveillance programs in the process of certification. Any system needs to be responsive and iterative to improve surveillance.

Tools to Improve the Efficacy and Efficiency of Malaria Elimination

The overall strategic approach and mix of actions to address transmission is critical, but the identification and development of key tools and actions to optimize these strategic actions is equally important. Improved diagnostics for screening and surveillance, optimal use of drugs to reduce transmission [25], better mapping and use of mapping to track foci of infections, and improved

communications for timely sharing of information and response are all important.

Diagnostics

Tests that are sensitive enough to detect asymptomatic infections (as opposed to symptomatic infections or cases) are needed for elimination [26]. Ultimately, for simplicity and efficiency, it will be preferable to have the same test for both surveillance and case management. Elimination has already been achieved in some areas of low endemicity using currently available diagnostic tools (principally microscopy), but future efforts will include areas of previously high transmission that have achieved significant reductions through intervention scale-up. Existing diagnostic tools will need to be improved to achieve elimination in these more challenging transmission areas. Microscopy has some limitations in human resource capacity needs, sensitivity and ease of widespread use at the community level. Similarly, currently available rapid diagnostic tests (RDTs) have limited sensitivity compared with PCR, and need to be improved in terms of specificity, ease of use, cost, shelf stability under tropical conditions, *Plasmodium vivax* detection, ability to return to negative after treatment, and multispecies detection capacity where this is an issue [27]. As discussed in the malERA paper on Diagnoses and Diagnostics [11], rapid techniques not requiring blood sampling could provide major breakthroughs.

There is also a need to address issues around effective supervision and support. In particular, as transmission decreases, residual foci of infection may cluster in difficult-to-access populations that are underserved and less likely to access the health system. Strategies need to be developed and tested for improving access to and tracking of these populations for screening and surveillance of infection.

Finally, for eradication, diagnostic tools to measure transmission and its interruption will be critical. There is considerable interest

in refining current serological tests (ELISA) to assist in the diagnosis of recent infection (incidence). Serology and other potential biomarkers are discussed in more detail below.

Mapping and Stratification

Maps of the global distribution of *P. vivax* and *Plasmodium falciparum* that were generated by the Malaria Atlas Project have recently been published, but there is little research on how best to use these maps in the context of elimination [28,29], and current mapping initiatives are limited by data availability, especially for scenarios that require high resolution. Maps can help define which low transmission areas are possible elimination targets, and can define the limits of adverse conditions for transmission, such as aridity and temperature. Maps can also help to determine where additional survey work is necessary for better spatial resolution of endemicity.

On a global scale, mapping malaria distribution will allow stratification to inform decision making and allow for interventions to be targeted or prioritized [29,30]. When allied with modeling, such maps can indicate which combinations of interventions may be most appropriate and how much these will cost [22,31]. However, for optimal utility, maps will need to be sensitive to different ecological scenarios and should provide enough detail of the principal factors governing transmission. From a technical point of view, more detailed maps are feasible, and linking mapping databases with other technologies such as Google will increase ease of access to mapping information.

For maps at regional or national levels, the spatial resolution of the information required is greater than that required for global scale risk mapping. Integration of mapping activities with the outputs of surveillance systems and other data sources (for example, intervention coverage and vector distribution) can provide the level of detail required to support effective elimination efforts. However, the incorporation of existing techniques for rapid mapping and the development of methods for optimal information dissemination to all levels of the malaria control programme remain major research challenges, as does the need to update protocols that do not currently incorporate our ability to image, map, and display information remotely, technologies that have been revolutionized since the Global Malaria Eradication Program.

As we progress closer to the goal of elimination, finer scale mapping will be required to identify residual foci [32]. Geographical reconnaissance remains part of control and elimination attempts in many countries and relies on local knowledge to make largely hand-drawn maps of potential foci and known vector breeding sites. This approach needs to be modernized to include a simple, user-friendly, and consistent methodology for micro-mapping. High resolution satellite imagery can detect households and water bodies at unprecedented spatial resolutions and thus replace some of the logistic burden in reconnaissance required to support elimination activities [33]. The use of maps to help find rare events such as individual cases of malaria is also a very poorly developed area that needs further research. Efficient signatures of transmission hotspots or disease foci (environmental, entomological, and human) are also not well known, so a final challenge will be to integrate novel monitoring and evaluation metrics with the existing mapping suite.

Communication Technologies

Technological advances in communications and reporting systems (data collection, aggregation, and dissemination) offer potential improvements for surveillance in the context of elimination and eradication. Other prerequisites for good communication and reporting include basic health systems, and the capacity to analyze and use data to improve program performance. Most importantly, it is only the relevant and useful surveillance information that is required for prompt and timely communication.

Examples of potential enhancements to improve timely reporting include widespread implementation of cell phone technology [34], which has been used with considerable success in some areas such as Zanzibar and Madagascar to provide cluster detection and response [35]. Systems such as real-time internet Web-based reporting are also being explored. As noted above, the development of methods to integrate surveillance reporting technology with mapping tools is a priority. Critically, systems developed for collection, reporting, analysis, and dissemination of information must be structured so that they enhance decision-making and programmatic direction at the local (district) level. In addition, these systems must enhance the capacity of the program to provide useful and timely information to policy makers so that program status and progress towards elimination is clear and well explained [36].

Resistance and High-Risk Populations

Tracking antimalarial drug resistance is an important activity in the context of malaria control, but it becomes less important in situations where there are relatively few cases who must all receive curative treatment. Thus, as elimination is approached, all outpatient therapy might be better administered as "directly observed therapy" as with tuberculosis. Because of inconsistent and inadequate access to health systems, difficult-to-access populations may be at increased risk of harbouring individuals with drug-resistant parasites. Strategies to improve access to these populations were discussed earlier (see also [25]).

As elimination is approached, declining transmission and thus fewer cases pose considerable challenges to monitoring for drug resistance because recruitment of sufficient numbers of patients is difficult and thus studies are prolonged and expensive. Simple drug efficacy protocols worked into routine surveillance activities at sentinel sites may be of some use; follow-up of all treated cases may be another approach to ensure that individuals have cleared parasites [37]. Molecular markers for resistance could be useful for population-level screening, although new assays relevant to current treatment drugs, particularly the artemisinins, need to be developed. Simple field PCR-based tools would be of use, both for resistance testing and to differentiate recrudescence from new infections [11].

Although no vaccine is currently available, it is likely that vaccines may be in use in the next decade. A challenge will be to monitor vaccines for efficacy against antigenically diverse parasites in the population, for their preventive effects against severe disease, and for their effects in settings with changing transmission, as well as for their effects on transmission itself (see also [38]). Newer molecular biology approaches may be useful in which human genes are used to predict immunological responses. Case control methodology can also be used to evaluate vaccine performance [39].

Tools for Transmission Measurement: Metrics

Accurate measurement of malaria transmission is essential for monitoring and evaluation of malaria control programs that are approaching interruption of transmission and elimination. Past and present metrics for measuring malaria transmission in humans in endemic regions were recently systematically reviewed [14] and include: the proportion of individuals in a population with a palpable spleen (spleen rate); the proportion of individuals in a

population with a laboratory-confirmed parasite infection per unit time (parasite rate [PR]); and the annual parasite incidence ([API], the product of the annual blood examination rate and slide positivity rate) [13,14]. The entomological inoculation rate ([EIR], the number of infective bites per person per unit time) remains the gold standard measure of transmission.

A valid metric, or a combination of metrics, for measuring the interruption of transmission nationally or subnationally is critical as elimination is approached; but the existing metrics all have serious limitations when transmission is approaching zero, including the EIR, which is difficult, expensive, and virtually impossible to measure when there is very low transmission.

For example, API (or alternatively annual case incidence) is an important metric of transmission that can be obtained from routine surveillance reporting even when the PR falls below 5%. However, to ascertain API accurately, all cases in the population must be identified through comprehensive and complete surveillance of the target population, ideally using both passive and active detection. API ascertained through passive detection alone only records those symptomatic individuals who are captured through the routine surveillance system and would, therefore, provide a biased (too low) estimate of transmission for the entire target population. Additionally, its failure to detect individuals with asymptomatic infections in the population would critically hinder the clearance of parasites from human reservoirs when working towards elimination.

Similarly, to obtain an unbiased estimate of PR for a target population where the combination of passive and active detection is incomplete, probability sampling of the population is required (see next section also), but this is problematic when transmission is reduced to nonrandom residual foci of cases. Furthermore, using PR ascertained from probability biomarker surveys for validation of freedom from disease is challenging, with sample size and resultant uncertainty dependent on the probability of committing a type 1 and 2 error, the size of the population being sampled, and the sensitivity and specificity of the diagnostic test [40]. Thus, unless extremely large sample sizes are used, PR will provide imprecise measures at near zero transmission. Research is needed, therefore, to develop new metrics for transmission and to improve or modify data systems for these kinds of measurements.

Tools for Transmission Measurement: Sampling and Surveys

To assess progress in intervention scale-up, nationally representative household surveys, such as the Malaria Indicator Survey (MIS), Demographic and Health Survey (DHS), and the UNICEF Multiple Indicator Cluster Survey (MICS), are recommended data collection instruments, Such surveys can provide population-based, relatively accurate, estimates of malaria intervention coverage, and parasite infection prevalence in the population, and should be useful in assessing sustained coverage of malaria interventions on a periodic basis, typically every 3–5 years.

However, once scale-up has been achieved and infection prevalence is approaching zero, or has been disrupted, such national surveys, with sample sizes typically of at least 2,000 households, would not be feasible for routine monitoring of low and/or focal malaria transmission. Alternative sampling methods for ascertaining population-based measures of malaria transmission are therefore needed. Ideally, such novel sampling strategies would approximate a "probability survey" (a survey having a known, nonzero probability of selection of all individuals for which it is desired to obtain estimates), while remaining logistically feasible to implement on a routine basis.

Once transmission has been interrupted, population-based collection of biological samples for detection of present infections,

or serology for detection of past exposure and infection, could prove important for routine monitoring of populations, although improved assays will be required. Such approaches might include routine sampling of populations through antenatal clinics, immunization programs, and schools. Assessment of the validity of these new approaches for obtaining relatively unbiased population estimates will be needed.

To maintain interrupted transmission or elimination, malaria control programs need to be able to obtain representative and precise estimates of parasite exposure and present infections among mobile populations, especially those that frequently cross national borders. Although respondent-driven sampling (a sampling approach in which existing study subjects recruit future subjects from among their acquaintances) has been used for ascertaining point estimates among hidden populations, this approach would likely be inappropriate for monitoring malaria transmission among mobile populations. One approach that should be tested for routine

---

Box 2. Summary of the Research and Development Agenda for Monitoring, Evaluation, and Surveillance

- Update the malaria monitoring and evaluation Framework to include transmission reduction, and develop key data elements for a surveillance system from a systematic review of previous elimination attempts
- Systematically review lessons learned from experiences with surveillance as an intervention to determine how it can be tailored to various programmatic settings
- Identify appropriate program time points for introduction of malaria infection detection in active or passive modes
- Develop improved diagnostic tools for use in monitoring and evaluation and surveillance, focusing on practical field-ready tools for detection of asymptomatic infection
- Develop information systems to monitor malaria infections, facilitate timely local program decisions and responses to reduce transmission
- Develop methods, indicators, and shareable databases for parasite strain information to better track transmission
- Develop methods for accessing and tracking population movements and quantifying their contribution and risk of malaria transmission
- Explore how maps can be constructed to: show the probability of a threshold of transmission being exceeded; incorporate a wider range of metrics such as serological and entomological data; assess cost-effectiveness of national stratification initiatives based on remotely sensed satellite data
- Perform a systematic review to assess and compare metrics of malaria transmission at near zero transmission levels; research the validity of novel metrics to measure transmission at near zero levels, and to measure transmission potential within areas where transmission has been eliminated
- Assess the precision, bias, feasibility, and cost-effectiveness of novel sampling methods for routine monitoring of present and past infections in target populations, including mobile populations
- Conduct research to develop biomarkers such as DNA-based methods or serology as monitoring and evaluation and surveillance tools

---

monitoring of mobile populations is time location sampling (TLS), a variation of traditional two-stage cluster sampling in which the primary sampling units are time-location settings where mobile and/or hidden populations are known to congregate. Assessment of the accuracy of TLS estimates of parasite infection prevalence among mobile populations is needed, as well as cost-effectiveness in relation to other sampling methods.

Biomarkers for Transmission Measurement

Serologic methods are currently an area of renewed interest as a potentially valuable tool for robust transmission measurement. Serology has been used to measure malaria exposure in humans for many years and was prominent in early elimination attempts [41,42]. But, as these elimination attempts were scaled back, so was the use of serological characterization. With little use over several decades, these serologic assays lacked standardized, reproducible, and objective methods [43]. Recent technological improvements (for example, techniques that facilitate the production of antigens) mean that serology has now become a much more robust tool for transmission measurement [44]. However, there is a need to standardize protocols and antigens; currently there are many different methodologies with associated variation in results. Fundamental issues relating to the generation and maintenance of antibody responses in children and adults also need to be addressed.

Other research and development needs include the development of serological assays that are sensitive and specific for different *Plasmodium* species. Assays also need to be developed that show cumulative exposure to the parasite, as well as recent changes in transmission intensity by measuring both the prevalence and the magnitude of the antibody response. Serological methods might also be developed that distinguish between relapse and new infection with *P. vivax* by measuring exposure to mosquito saliva through the detection of antisaliva antibodies.

PCR or similar molecular amplification–based methods may also prove useful for the measurement of transmission reduction/interruption, especially if pooled sampling and high-throughput automated techniques are used to handle large numbers of samples [45]. There is limited experience to date with these methods as tools to measure transmission; further research may help to elucidate their potential.

For all biomarkers, the most desirable assays would not require blood sampling so research into biomarkers in saliva or other bodily fluids is needed. Finally, for all biomarkers, there is a need to develop criteria that define an area as "malaria free."

Concluding Remarks

The new strategies proposed in this paper by the malERA Consultative Group on Monitoring, Evaluation, and Surveillance for eradication have major implications for implementation, and research is needed to test best systems of delivery for acceptability, feasibility, efficiency, and cost-effectiveness. Box 2 draws our discussions together in the form of a research and development agenda for monitoring, evaluation, and surveillance.

Acknowledgments

Author Contributions

References

1. Roll Back Malaria Partnership (2010) Framework for monitoring progress and evaluating outcomes and impact. Available: http://www.rollbackmalaria.org/cmc_upload/0/000/012/168/m_e_en.pdf.

2. Feachem RGA, Phillips AA, Targett GA, eds (2010) Shrinking the malaria map: a prospectus on malaria elimination. Available: http://www.malariaeliminationgroup.org.

3. Roll Back Malaria Partnership (2010) The global malaria action plan. Available: http://www.rollbackmalaria.org/gmap/.
4. WHO (2008) Global malaria control and elimination: report of a technical review. Geneva: World Health Organization Press.
5. Hopkins DR, Ruiz-Tiben E, Downs P, Withers PC, Jr., Roy S (2008) Dracunculiasis eradication: neglected no longer. Am J Trop Med Hyg 79: 474–479.
6. Henderson DA (1999) Lessons from the eradication campaigns. Vaccine 17: S53–S55.
7. Cochi SL, Kew O (2008) Polio today: are we on the verge of global eradication? JAMA 300: 839–841.
8. Breman JG, Quadros CA, Dowdle WR, Foege WH, Henderson DA, et al. (2011) The role of research in viral disease eradication and elimination programs: Lessons for malaria eradication. PLoS Med 8: e1000405. doi:10.1371/journal.pmed.1000405.
9. Nájera J, González-Silva M, Alonso PL (2011) Some lessons for the future from the Global Malaria Eradication Programme (1955–1969). PLoS Med 8: e1000412. doi:10.1371/journal.pmed.1000412.
10. Atta H, Zamani G (2008) The progress of Roll Back Malaria in the Eastern Mediterranean Region over the past decade. East Mediterr Health J 14: S82–S89.
11. The malERA Consultative Group on Diagnoses and Diagnostics (2011) A research agenda for malaria eradication: Diagnoses and diagnostics. PLoS Med 8: e1000396. doi:10.1371/journal.pmed.1000396.
12. The malERA Consultative Group on Cross-cutting Issues for Eradication (2011) A research agenda for malaria eradication: Cross-cutting issues for eradication. PLoS Med 8: e1000404. doi:10.1371/journal.pmed.1000404.
13. Pampana E (1969) A Texbook of malaria eradication. London: Oxford University Press.
14. Hay SI, Smith DL, Snow RW (2008) Measuring malaria endemicity from intense to interrupted transmission. Lancet Infect Dis 8: 369–378.
15. WHO (1963) Terminology of malaria and malaria eradication: Report of a drafting committee. Geneva: WHO.
16. Yekutiel P (1960) Problems of epidemiology in malaria eradication. Bull WHO 22: 669–683.
17. Harris I, Sharrock WW, Bain LM, Gray KA, Bobogare A, et al. (2010) A large proportion of asymptomatic *Plasmodium* infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: Challenges for malaria diagnostics in an elimination setting. Malar J 9: 254.
18. Centers for Disease Control and Prevention, Filler SJ, MacArthur JR, Parise M, Wirtz R, et al. (2006) Locally acquired mosquito-transmitted malaria: A guide for investigations in the United States. MMWR Recomm Rep 55: 1–9.
19. Ceccato P, Connor SJ, Jeanne I, Thomson MC (2005) Application of geographical information systems and remote sensing technologies for assessing and monitoring malaria risk. Parassitologia 47: 81–96.
20. Macauley C (2005) Aggressive active case detection: A malaria control strategy based on the Brazilian model. Soc Sci Med 60: 563–573.
21. Betanzos-Reyes AF, Rodríguez MH, Duran-Arenas LG, Hernández-Avila JE, Méndez-Galván JF, et al. (2007) Comparative analysis of two alternative models for epidemiological surveillance in the Mexican Malaria Control program. Health Policy 80: 465–482.
22. The malERA Consultative Group on Modeling (2011) A research agenda for malaria eradication: Modeling. PLoS Med 8: e1000403. doi:10.1371/journal.pmed.1000403.
23. WHO (2007) United Arab Emirates certified malaria-free. Wkly Epidemiol Rec 4: 25–32.
24. WHO (2007) Guidelines on the elimination of residual foci of malaria transmission. Cairo: World Health Organization Regional Office for the Eastern Mediterranean (EMRO). Technical Publications Series 33, Available: http://www.emro.who.int/dsaf/dsa742.pdf.
25. The malERA Consultative Group on Drugs (2011) A research agenda for malaria eradication: Drugs. PLoS Med 8: e1000402. doi:10.1371/journal.pmed.1000402.
26. Perkins MD, Bell DR (2008) Working without a blindfold: The critical role of diagnostics in malaria control. Malaria J 11: S5.
27. Bell D, Wongsrichanalai C, Barnwell JW (2006) Ensuring quality and access for malaria diagnosis: how can it be achieved? Nat Rev Microbiol 4: 682–695.
28. Hay SI, Guerra CA, Gething PW, Patil AP, Tatem AJ, et al. (2009) A world malaria map: *Plasmodium falciparum* endemicity in 2007. PLoS Med 6: e1000048. doi:10.1371/journal.pmed.1000048.
29. Hay SI, Snow RW (2006) The Malaria Atlas Project: Developing global maps of malaria risk. PLoS Med 3: e473. doi:10.1371/journal.pmed.0030473.
30. Noor AM, Mutheu JJ, Tatem AJ, Hay SI, Snow RW (2009) Insecticide-treated net coverage in Africa: Mapping progress in 2000–07. Lancet 373: 58–67.
31. Smith DL, Hay SI, Noor AM, Snow RW (2009) Predicting changing malaria risk after expanded insecticide-treated net coverage in Africa. Trends Parasitol 25: 511–516.
32. Gaudart J, Poudiougou B, Dicko A, Ranque S, Toure O, et al. (2006) Space-time clustering of childhood malaria at the household level: A dynamic cohort in a Mali village. BMC Public Health 6: 286.
33. Rogers DJ, Randolph SE, Snow RW, Hay SI (2002) Satellite imagery in the study and forecast of malaria. Nature 7: 710–715.
34. Blaya JA, Fraser HS, Holt B (2010) E-health technologies show promise in developing countries. Health Affairs 29: 244–251.
35. Randrianasolo L, Raoelina Y, Ratsitorahina M, Ravolomanana L, Andriamandimby S, et al. (2010) Sentinel surveillance system for early outbreak detection in Madagascar. BMC Public Health 10: 31.
36. The malERA Consultative Group on Health Systems and Operational Research (2011) A research agenda for malaria eradication: Health systems and operational research. PLoS Med 8: e1000397. doi:10.1371/journal.pmed.1000397.
37. WHO (2009) Methods for surveillance of antimalarial drug efficacy. Geneva: World Health Organization.
38. The malERA Consultative Group on Vaccines (2011) A research agenda for malaria eradication: Vaccines. PLoS Med 8: e1000398. doi:10.1371/journal.pmed.1000398.
39. Butler JC, Breiman RF, Campbell JF, Lipman HB, Broome CV, et al. (1993) Pneumococcal polysaccharide vaccine efficacy. An evaluation of current recommendations. JAMA 270: 1826–1831.
40. Cameron AR, Baldock FC (1998) A new probability formula for surveys to substantiate freedom from disease. Prev Vet Med 34: 1–17.
41. Bruce-Chwatt LJ, Dodge JS, Draper CC, Topley E, Voller A (1972) Sero-epidemiological studies on population groups previously exposed to malaria. Lancet 1: 512–515.
42. Bruce-Chwatt LJ, Draper CC, Avramidis D, Kazandzoglou O, et al. (1975) Sero-epidemiological surveillance of disappearing malaria in Greece. J Trop Med Hyg 78: 194–200.
43. Drakeley C, Cook C (2009) Potential contribution of sero-epidemiological analysis for monitoring malaria control and elimination: historical and current perspectives. Adv Parasitol 69: 299–352.
44. Corran P, Coleman P, Riley E, Drakeley C (2007) Serology: A robust indicator of malaria transmission intensity? Trends Parasitol 23: 575–582.
45. Taylor SM, Juliano JJ, Trottman PA, Griffin JB, Landis SH, et al. (2010) High-throughput pooling and real-time PCR-based strategy for malaria detection. J Clin Microbiol 48: 512–519.

PLoS MEDICINE

Review

A Research Agenda for Malaria Eradication: Modeling

The malERA Consultative Group on Modeling[¶]*

Abstract: Malaria modeling can inform policy and guide research for malaria elimination and eradication from local implementation to global policy. A research and development agenda for malaria modeling is proposed, to support operations and to enhance the broader eradication research agenda. Models are envisioned as an integral part of research, planning, and evaluation, and modelers should ideally be integrated into multidisciplinary teams to update the models iteratively, communicate their appropriate use, and serve the needs of other research scientists, public health specialists, and government officials. A competitive and collaborative framework will result in policy recommendations from multiple, independently derived models and model systems that share harmonized databases. As planned, modeling results will be produced in five priority areas: (1) strategic planning to determine where and when resources should be optimally allocated to achieve eradication; (2) management plans to minimize the evolution of drug and pesticide resistance; (3) impact assessments of new and needed tools to interrupt transmission; (4) technical feasibility assessments to determine appropriate combinations of tools, an associated set of target intervention coverage levels, and the expected timelines for achieving a set of goals in different socio-ecological settings and different health systems; and (5) operational feasibility assessments to weigh the economic costs, capital investments, and human resource capacities required.

Introduction

A global malaria eradication effort will require massive changes to a complex web of interconnected biological systems. The optimal path to eradication is intrinsically unpredictable because of the potential for parasites and vectors to evolve, the waxing and waning of human immunity, and behavioural changes in human and vector populations. The range of conditions that favour malaria transmission are so varied and diverse that decisions and plans cannot be based solely on the evidence that has been acquired in randomized control trials conducted in only a few settings. To succeed, eradication will require a strategic plan that is constantly updated with the latest surveillance, monitoring, and evaluation data. Moreover, planning processes involve some sort of conceptual model, and this model will necessarily consider many potential sources of uncertainty. Rational quantitative mathematical models provide the best way to synthesize information, quantify uncertainty, and extrapolate current knowledge. Such models can provide critical quantitative insights that are not otherwise possible.

The unique contributions that malaria modeling could potentially make to research and policy for malaria eradication led to the formation of a malERA Consultative Group on modeling tasked with defining a research and development agenda

Review articles synthesize in narrative form the best available evidence on a topic.

for modeling within a comprehensive malaria eradication research agenda. Our discussion about the proper use of models focused on balancing the need to provide robust policy recommendations while maintaining the energy and creativity of competitive science. The following document describes the history of malaria modeling, discusses the framework we developed for reaching consensus on the basis of independently derived models, provides an agenda to improve the science of modeling with supporting curated databases and digital interfaces, and identifies priority tasks within the broader agenda.

Historical Background

Malaria transmission models originated with Ronald Ross during a trip to organize malaria control in Mauritius (1907–1908) [1], but the models of George Macdonald [2] were applied more systematically during the Global Malaria Eradication Program (GMEP) from 1955 to 1969 [3]. Macdonald emphasized the importance of measuring quantities that were relevant for eradication planning, such as the stability index (the expected number of human bites by a mosquito over its lifetime) and the basic reproduction ratio, R_0 (the expected number of human cases that would arise from each human case in a population with no previous exposure to malaria and no malaria control) [4]. Mathematical analysis helped to explain why indoor residual spraying with DDT was such a potent malaria control strategy [5]. Later, mathematical modeling played a key role in the design and analysis of the Garki project in Nigeria [6], as well as the introduction of new indices to measure transmission, including vectorial capacity and the human blood index [7,8].

Despite its important contributions, the overall role of mathematical modeling in the GMEP was limited. Modeling informed the design of the "attack phase" of malaria eradication [3], but not the design or implementation of other phases, and

Summary Points

- Mathematical modeling can guide all stages of malaria elimination and eradication by synthesizing information, quantifying uncertainty, and extrapolating current knowledge

- Modelers and users/stakeholders need to work closely with each other to ensure that models meet user needs and end users understand the current limitations of malaria transmission models

- A framework for modeling is being established that is both collaborative and competitive

- Models must be closely tied to all the available data, and databases and model outputs should be harmonized

- A single approach aiming at one, comprehensive model for malaria elimination/eradication has limited value; instead a variety of models and analytical approaches should be employed to guide effectively elimination efforts.

there were no provisions made to evaluate or update the design of the GMEP despite its obvious limitations. For example, the Pare-Taveta Malaria Scheme [9], which was implemented over 3.5 years in villages on the Tanzania-Kenya border, reduced malaria prevalence to less than 5%. Without sustained investments, however, malaria endemicity rebounded within 10 years of the program ending. The lessons of this and other schemes were that malaria control would require longer interventions and at a much larger scale in the African context; the implications for the broader program were never considered.

The GMEP also never considered what would happen if the initial attack phase failed. Moreover, application of the modeling was mainly limited to the Global Malaria Program in Geneva, which was not considered to be an intrinsic part of the research agenda for the GMEP. The failure to integrate and the neglect of research were partly due an emphasis on streamlining GMEP's operations and contributed to a "one-size-fits-all" mentality, with programmatic criteria based on early successes in Europe. Despite having identified DDT resistance in *Anopheles sacharovi* in 1951 [10], the only GMEP plans for dealing with resistance were to have a highly focused and time-limited program. By 1964, the GMEP had reached only approximately 3.3% of the malarious area in Africa, and the efforts were mainly concentrated at the margins of the continent [11]. After a WHO meeting in Brazzaville in 1972, formal plans for dealing with malaria in many African settings were devised [12] in which malaria elimination was not considered to be feasible, and "control" was presented as an alternative and defined as reduction of malaria to a point where it was no longer a major public health threat. A final failure of the GMEP was in not providing guidelines for establishing quantitative and operationally meaningful definitions and milestones for measuring progress towards control in a range of contexts.

The failure of the GMEP was due to many factors, including the collapse of funding [13]. However, better research with built-in monitoring, surveillance, and evaluation would have contributed to the long-term prospects for the success of the GMEP, and within that research-oriented framework, mathematical modeling could have played a pivotal role. Insufficient use of modeling was not the reason why the GMEP failed, but it could have played a stronger role in helping to anticipate, analyze, and adjust to some of the other problems that developed, such as the evolution of insecticide resistance.

Since the GMEP, substantial advances have been made in the theory and simulation modeling of malaria transmission (see Text S1), but the main research challenge for malaria eradication will be to integrate these models with surveillance, monitoring, evaluation, and with the revision of national and regional plans through every phase of eradication.

A Consultative Framework for Malaria Modeling

After reviewing the role of malaria modeling in past control and eradication programs, the Consultative Group on modeling discussed the best way of organizing modelers and modeling. A consensus emerged that a unified approach aimed at developing an all-encompassing model for malaria elimination or eradication would probably repeat the mistakes of the past, and would therefore be inadequate. Instead, we agreed that accomplishing the modeling research agenda for eradication, avoiding errors, and providing robust advice for the future would require a framework that facilitates competitive and collaborative interactions and active communication between modelers and other scientists, research activities, surveillance, monitoring and evaluation, and that is based on a shared set of data resources.

We therefore established and endorsed a framework, motivated by climate modeling under the Intergovernmental Panel on Climate Change, which is both collaborative and competitive (Figure 1). In this framework, the core modeling functions would be conducted by independent teams, working in isolation and then coming together to compare and harmonize their results. The teams would compete with one another to provide answers to questions, and yet they would be cooperatively engaged in the common goal of finding the best solution to a defined set of problems. An added advantage of this approach, which builds on and formalizes the successful way in which scientific research ordinarily takes place, is a rapid critique from other competent modeling teams that limits the excesses of particular models or modelers and emphasizes the limitations of each approach and of models overall.

Two important features of this framework are the interface between modelers and the users, and the development of curated databases that are shared among all modelers. The Consultative Group felt that direct contact between modelers and users was the best way for modelers to be aware of the needs of their users, to be aware of new developments and data, and for modelers to communicate the limitations of their models. However, some information could be usefully shared through digital interfaces. The Consultative Group also recognized that the needs of the users would evolve over time, and that the models must be iteratively updated (the dashed arrows in Figure 1). It also regarded databases and digital interfaces as essential to the development of modeling and prioritized them as part of the research and development agenda for modeling.

Importantly, because this type of framework has not been part of the culture of malaria modeling, one of the first tasks of the modeling research and development agenda will be to operationalize the framework in Figure 1, and formally establish a process for consultations on relevant policy matters.

The Potential Role of Modeling: Strategic Planning and Technical Feasibility Assessment

Malaria modeling should be used to inform strategic planning and malaria elimination assessments at a range of scales from global policy to local-level planning, and in guiding malaria control whether or not such activities are considered the first step towards malaria elimination. Strategic planning involves the assessment of where
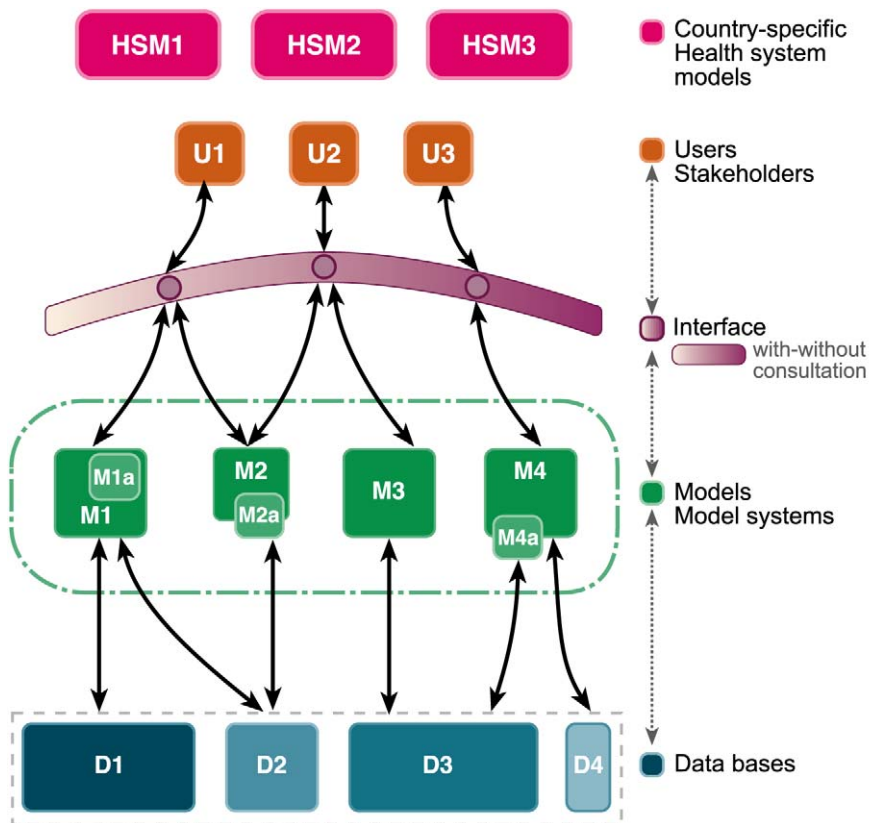
Figure 1. A comprehensive framework for malaria modeling. Consultations will allow policy makers, research scientists, and other stakeholders (U, users/stakeholders) from different country-specific health systems (HSM, country-specific health system models) to draw advice and analysis from multiple, independently derived models (M) grounded on data collected (D, data bases) from research on vector ecology, malaria epidemiology, and control through an interface that emphasizes direct engagement between modelers or modeling groups and end users. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000403.g001

and when resources should be allocated to achieve elimination/ eradication. Technical feasibility assessments define an appropriate combination of tools, an associated set of target intervention coverage levels, and the expected timelines for achieving reduction in burden, transmission interruption, and finally malaria elimination. Models can provide a rational and quantitative framework for integrating a range of implementation strategies, including optimizing the mix of interventions in a socio-ecological setting with different health systems to achieve a set of goals leading to malaria elimination. These results must be linked to operational assessments to describe the economic costs, capital investments, and human resource capacities required with explicit consideration of the long-term financing of malaria control and elimination.

To be of greatest benefit, models developed for malaria elimination support must specifically address changes in parasite, human, and animal hosts, and vector populations across a range of endemicities and health system conditions and capacities through the different phases of a malaria elimination program. These phases can be broadly categorized as: the initial planning phase (phase I); the introduction of interventions to interrupt transmission leading to zero incidence (phase II, which corresponds to the Global Malaria Action Plan [GMAP] "pre-elimination through elimination" phase); and "holding the line" (phase III or the GMAP "prevention of re-introduction" phase). Each phase has different goals and operational requirements, and will require different types of models. For each phase, models can be used to optimize the sequence and combinations of interventions, and for monitoring evaluation and surveillance. Although economic models and behaviour and

malaria transmission models have been developed in isolation, there is a great need for models that consider transmission within economic models, and vice versa, for all phases of elimination. Models can also be used to define and test phase-specific target product profiles (TPPs) of new tools. TPPs describe the ideal, desirable, or minimally sufficient properties of a new tool in formalized documents that facilitate discussion between funding agencies, product developers, and regulatory agencies. TPPs will remain relevant throughout the path towards global eradication as endemicity and health system requirements change, and as countries adapt to their own unique challenges.

We anticipate that strategic planning will also need to account for variation in the mix of parasite species across the geographical range of malaria. At present, models are mainly focused on single-species *Plasmodium falciparum* infections and require further development for *Plasmodium vivax*, other parasite species, and mixtures of species.

Phase I: Planning

Planning involves a technical assessment to determine whether elimination is feasible, based on the baseline distribution of malaria and current tools, and on what level of intervention coverage is required to reduce transmission intensity sufficiently to achieve elimination. A key variable here is the basic reproduction ratio R_0. At a country level, it may not be possible to provide direct estimates of R_0. However, several measures related to transmission intensity, including parasite prevalence, age-stratified seroprevalence, and entomological inoculation rate may be available. Mathematical models are required to translate these measures into

a single comparable quantity. A likely output of such an exercise would be a map of R_0 at an appropriate spatial resolution.

The technical requirements for elimination are also directly related to the operational and financial requirements for elimination, so these must be linked in models to assessments of health systems, economic costs and benefits of elimination, the risks of failure, and the likely funding.

The second aspect of an initial feasibility assessment is to define vulnerability, namely the risk that cases may be imported from surrounding malaria-endemic countries. Direct measurement of vulnerability is complicated in areas in which endemic transmission is ongoing and will only be achievable when imported cases become a substantial fraction of all cases. Preliminary assessments thus need to be estimated indirectly by taking into account patterns of endemicity in neighbouring countries and the level of cross-border movements. Spatially stratified mathematical models can aid these assessments, which are not considered in current strategic models. Each country's economic incentives to eliminate malaria may be strongly influenced by the decisions of their neighbours. The elimination of malaria from an entire region reduces the chances of re-introducing malaria and is likely to create a regional public good, which would make a strong economic case for coordinating elimination campaigns among countries.

Modeling also has a key role to play in selecting appropriate combinations of interventions to interrupt transmission and in setting response timelines and expectations of impact. Models can help to elucidate whether different interventions are likely to be synergistic, and when they can be deployed to best effect. Although insecticide-treated nets, indoor residual spraying, and artemisinin combination therapies have been used successfully in well-designed randomized control trials, these trials have been conducted in a limited number of settings and the results of applying the same control measures at the same intensity in different places may vary depending on such factors as the intensity and seasonality of transmission, the characteristics of the parasites, and the immunological status of human populations. There is no evidential basis for extending the results from existing randomized control trials to the whole range of conditions that exist in the real world and it is impossible to conduct randomized control trials that cover all the factorial combinations of those conditions. Using mathematical models, such experiments can be simulated with minimal expense on a computer to obtain immediate answers. Mathematical models are thus an indispensable tool for thinking carefully and quantitatively about the dynamics of malaria control and elimination. Although computer-based simulation studies are not a substitute for reality, they do provide a highly refined and structured way of synthesizing information and testing ideas. In particular, they provide a useful tool for testing how differences in transmission can lead to different results when the same interventions are applied in two different populations.

Finally, drug and pesticide resistance were blamed for slow progress during the GMEP and may have contributed to its failure [11]. Malaria elimination and global eradication must therefore anticipate that resistance will evolve and must incorporate this inevitability into the plans. The functional significance of drug and pesticide resistance on transmission has therefore been identified as an important research topic for modeling to facilitate effective strategic planning.

Phase II: Pre-elimination through Elimination

The context for transmission and the operational challenges inevitably change as transmission is reduced to low levels. Previous experience unambiguously demonstrates that low-level transmission presents protracted challenges that contribute to a loss of commitment of countries and donors. In particular, the biology of *P. vivax* poses unique challenges for malaria elimination during this phase because of the dormant liver stages. Experience during the previous malaria eradication campaign suggests that *P. falciparum* will be eradicated long before *P. vivax*. The patterns of species composition are therefore critical concerns for elimination, and changes in the patterns can be used as a measure of progress towards elimination of *P. falciparum*.

As exposure to malaria declines, malaria immunity begins to wane, so each new case is more likely to result in clinical disease. During these later phases, different strategies may be deployed to shorten the response timelines, such as mass drug administration, passive or active case detection, localized outbreak control, public relations campaigns, prophylaxis for citizens traveling in malaria-endemic areas, and possibly border controls. These strategies can be supplemented by well-timed vector control. The optimal and timely use of interventions may shorten the time until elimination by decades.

Modeling can serve several roles in this phase. The first is to help set expectations about the inevitable long response timelines, since these will place increasing challenges on public health officials to justify the expense. Setting unrealistic timelines can undermine support for an elimination campaign and contribute to failure.

As malaria becomes rare, the role of monitoring, evaluation, and surveillance becomes critical [14]. Thus, a second role for modeling is to help organize information about imported malaria, to characterize transmission foci, and to design interventions. Models can be used to simulate low-level transmission and control and thus to help design and establish efficient sampling schemes appropriate for the low and declining level of endemicity.

During this phase, new programmatic skills and capabilities need to be developed that will prevent re-introduction or "hold the line" in perpetuity. Modeling can help to establish the minimal essential intervention coverage levels needed in this new transmission setting, and models can help to fine tune the programs to minimize both costs and the risk that malaria will re-establish. Another important need at this stage will be to define specific timelines and optimal strategies for *P. vivax* elimination.

As transmission becomes less intense, it also becomes more sporadic and often highly focal. In many countries, a constant flow of imported malaria can generate small clusters of ongoing transmission without the re-establishment of endemic transmission. Consequently, this is likely to be a long phase for countries or geographical areas close to malaria-endemic areas. Moreover, the accomplishments of countries or geographical areas that have eliminated their endemic reservoir and limited onward transmission but continue to have sporadic outbreaks may not be recognized. Mathematical modeling can help to describe and interpret the patterns of endemic, low-level onward transmission or imported malaria, and provide important feedback to monitoring and evaluation programs.

Phase III: Prevention of Reintroduction

Mathematical modeling has two essential purposes once local elimination has been achieved. First, it can be used to assess the sustainability of elimination in the local area or country. Second, it provides a formal set of analytical tools to address the unique challenges of keeping malaria out of countries that have successfully eliminated the parasites.

After elimination, the basic approaches to holding the line are broadly similar to the strategies towards the end of the program for

"getting to zero." However, countries will face increasing pressure to shift resources away from malaria control to other, more pressing issues. Surveillance during this phase will remain critical, especially to identify where and when malaria is imported. In these circumstances, model development will play an important role in improving the criteria for and the process of certifying malaria elimination, and in determining when malaria elimination can be scaled back without risking re-emergence of the parasite.

The sustainability of malaria elimination is related to several factors. The evolution of drug and insecticide resistance, vaccine-escape variants, human and vector behavioural changes, and other kinds of "resistance" can threaten to undermine malaria elimination programs at every phase. Similarly, volatility in outside donor funding can threaten the viability of elimination efforts and country-level motivation. Modeling provides a realistic framework for setting donor expectations, as well as a way to anticipate the problems that might arise. Models can also be used to illustrate the consequences of stopping too early or failing to finish the job. Endgame planning is an integral part of strategic planning for regional elimination.

Research and Development Requirements for Model Improvement

To best support the specific goals of malaria elimination, a research and development agenda is required to improve modeling. Several topics are currently in need of additional model development and the acquisition of key pieces of evidence. Some of these topics have only recently been identified by research, others have not been addressed because they are considered to be of limited interest.

Biology and Natural History

As the complex life cycle of malaria parasites becomes better understood, new and improved models are needed to make use of this information in elimination programs. First, better models of the development of parasite species in their human and vector hosts need to be devised and the features of the parasite life cycle need to be quantified better. In particular, there is a need for better data and models to quantify the importance of relapse in *P. vivax* and the importance of other unique aspects of non-falciparum parasites, and to quantify the nature of interactions among all species [15].

Models are also needed to capture the human infectious reservoir across a range of transmission intensities. Ill-understood factors contribute to variability in the transition rates of parasites from the asexual stage onwards and through each subsequent stage of the transmission cycle in people and mosquitoes. Even if for operational purposes, individuals with measurable parasites are considered to be infected and therefore not distinguished from gametocyte carriers, it remains important to capture the relative infectiousness of different population groups in models.

The abiotic determinants of mosquito densities and the dynamics of larval stages are poorly understood. Thus, there is a need for models that consider the effects of, for instance, seasonality and dry season refuges. Such models can provide information about the potential of larval control and optimal larval control strategies. The effects of infection and environment on adult mosquito behaviour, infectivity, and survival also need to be considered in modeling efforts [16].

The existence of natural immunity to malaria that partially protects against disease or reduces transmission is a particularly challenging problem for epidemiological models. The stimulation, duration, and effects of acquired immunity need to be better

understood, and this understanding must be incorporated into models to determine, for example, how many years of zero transmission must pass before symptomatic disease can be used as a marker of re-introduction [14,15].

Another aspect of parasite natural history that is not comprehensively addressed in current malaria models is heterogeneity in hosts, parasites, and vectors. Substantive problems in measuring levels of heterogeneity need to be addressed and these effects need to be appropriately incorporated in models. Heterogeneity is likely to have a greater impact on model results as transmission is reduced.

Finally, as transmission is reduced, the effects of geographical movement of the parasite that occur because of both vector and human movements will dominate the dynamics. The relative role of movement versus dry-season refuge in maintaining the infectious reservoir in epidemic settings remains poorly understood but will be a major determinant of the required control strategy to achieve elimination and hold the line. Human movement in particular is difficult to quantify on the basis of current data. Spatially explicit models will need to be developed that can adequately capture parasite movement and the linking of spatially distinct populations [15].

Effects of Interventions

Models of the dynamics of drugs (pharmacokinetics and pharmacodynamics [PK/PD], dosing regimens) and of vaccines that interrupt transmission at various stages need to be developed. In addition, there is a need to develop models that describe the ecology of genetically modified mosquitoes and the potential impact of such insects on malaria transmission [2,16].

The scope of models needs to be expanded to consider the overall effects on and of health systems and to account for the capabilities of preexisting health system infrastructures. Modeling needs to include the effects of combinations of interventions/tools and the effects of scheduling of interventions. It also needs to support the optimization of TPPs and their alignment with the existing packages of interventions. All these components need to be supported by microeconomic appraisal [17].

Effects of Interventions on the Evolution of Resistance

Resistance to interventions is broadly defined to include any heritable changes that reduce the effectiveness of drugs, pesticides, vaccines, and other interventions. TPPs need to be considered prospectively with model-based analyses of the likely evolution of resistance. Modeling approaches need to be developed that integrate population genetics and direct intervention effects, such as PK/PD data for drug resistance, behavioural and physiological changes in response to vector control, and molecular epidemiology for vaccine escape variants. A critical feature for models is better characterization of the biological cost of resistance. As new tools are developed, it will be important to plan deployment strategies with an awareness of the effects they will have on the evolution of resistance [16,18,19].

Prerequisites for Achieving Modeling Objectives

To achieve these modeling objectives and to answer specific research and operational questions, there is a need to create, curate, and harmonize databases. An interface and a supporting infrastructure (see Figure 1) must also be created to facilitate combining databases and diverse datasets, including those that will be generated by mathematical modeling. Importantly, as much information as possible should be openly accessible from a single place to facilitate modeling and the dissemination of model outputs

to the broader community of users, stakeholders, and contributors. Below we discuss the perquisites for achieving modeling objectives.

Compilation and Curation of Databases and Harmonization of Model Outputs

The purpose of the databases will be to collect information for various users in one place. For modelers, this information is required to parameterize and validate malaria models, and to extend them geographically and temporally. The malaria community requires more general information for monitoring and evaluating progress towards control/elimination/eradication.

A Web site that links to relevant information already on the Web, that hosts databases and appropriate interfaces to databases that are not hosted elsewhere, and that provides technologies that allow other software applications to access the hosted information will facilitate Web-based information exchange. Such a Web site would also include automatically generated information summaries and post synthetic data, data summaries, and summary statistics.

Data to be included on such a Web site should comprise, among other things, disaggregated data on the natural history of different human malaria species, disaggregated malariological field data from published and unpublished field research studies, data aggregations from searches of published and unpublished literature, and data from model outputs. The results of basic laboratory research, data on nonhuman malarias, and genomic data should be excluded from the early stages of the structure, however, except through hyperlinks to major data repositories. There should be links to relevant nonmalaria databases (e.g., UN demographic data, Demographic and Health Surveys, climate, population, and remotely sensed environmental data), but the platform should not host these databases unless this is essential for the analysis of hosted core data. Table 1 in Text S2 represents an initial list of the databases that might be hosted or otherwise harmonized. The challenges and requirements for achieving this are outlined below.

Primary Databases and Key Models

The potential database resources in Text S2 are necessarily incomplete, but should be gradually extended and more effectively interlinked. Different modeling approaches have common data needs, many of which will be satisfied by the datasets listed in Text S2. Spatially specific data will be required by some types of models so many of these data need to be geolocated. An important subset of data is the results of malariological field studies, especially field trials of interventions; the results of observational studies (e.g., of drug action) are also important. Parasitological data that are specifically required include infectious durations and data from field studies that can be used to estimate clearance rates. Specific entomological data requirements include data on vector survival, behaviour, and biting rates (including heterogeneity in biting rates). There will be a need to include global databases of weather and climate data, in particular temperature, rainfall, humidity, and soil moisture. New databases will need to be developed to support tracking of larval habitats and prediction of vector emergence rates. Modeling will also need to be supported by access to human demographic databases, including those of population distribution, age structure, and migration rates. This information will require access to data on transport networks (e.g., roads) and communications networks such as cell phones.

The compendium of resources detailed in Text S2 contains information sources, at various levels of complexity and in various states of assembly, that are of variable use to the modeling teams. Text S1 describes the history of modeling and the range of models currently available and under development.

Minimal Reporting Standards

Databases without descriptors are a static resource. A traditional, if not widely used, way to audit data resources is to describe them in a peer-reviewed article and append the information as supplementary material. A new publication route for data, such as an entirely new journal or a new article style in existing journals, is perhaps required, with the intention of encouraging the release of preexisting unpublished data while solving the problem of suitable accreditation for data sources.

Data and Model Curation and Sustainability

The curation and improvement of large databases requires significant personnel capacity for correction and assembly of new information. Furthermore, this capacity needs to be sustained in the long term for its value to remain and agreement has to be reached on what constitutes acceptable information quality, how to define it, and how to moderate correction. All stakeholders, not just researchers, must be made aware of the limitations of models and the data on which they rely. Data and model curation needs to be inclusive while flagging and addressing known problems and using disclaimers to avoid excessive reliance on questionable information.

Common Ontologies, Frameworks, and Metadata Standards

An evolving way to audit database resources is to provide machine-readable metadata so that third parties can employ Web services to seamlessly harvest and/or integrate database information in downstream applications. This harmonization process requires that all databases be accessible to the extent that they can be shared at the human and machine level with any third party with as little administrative, technical, and logistical support as possible. This prerequisite is rooted in the concept of the semantic web, which provides the methods and technologies that allow machines to understand, share, and reuse data in real time across application, enterprise, and community boundaries. There will be many benefits in investing in a semantic web, not least the availability of resources that can be updated, minimizing human errors in translation for third-party applications.

To formalize minimum standards in databases, an ontology is often specified. An ontology is defined as relationships among a set of terms in an agreed nomenclature that describe a database resource. There are many examples of ontologies, all tailored to specific applications. To develop an ontology for our specific purposes (if it were considered valuable), the most relevant existing ones could be reviewed, a hybrid ontology of useful descriptors constructed, and an expert group established to fill the gaps. Ontologies are critical for translating minimum reporting requirements into machine-readable metadata. However, paradoxically, several metadata "standards" are under development. Advice should be solicited from the information technology community on which to adopt. Finally, candidate models may require some minor modifications to their outputs for harmonization with other similar models. This task could be done by the original authors of the model or they could provide the necessary information and a mandate for the modification to be performed by the curators.

Incentives for Data Sharing

Proper incentives are required to guarantee that the data-sharing tasks will be accomplished. Data provision and model integration are challenging tasks that do not achieve immediate recognition but facilitate exciting science and improve public health impact at some future point. To implement semantic enrichments to databases and make models more widely accessible will take time, thought, and

energy. Individuals and groups should be incentivized to do this on new and also, importantly, valuable old datasets. This process will require mechanisms for attribution, quality, and provenance control, and long-term curation and hosting obligations. There is a need to decide how resources will be partitioned between existing databases and downstream resources/portals.

Open-access data sharing is a collective benefit that outweighs individual concerns, even though most of the communities gathering relevant data do not yet operate a culture of open access. Accordingly, access to data needs to be negotiated carefully, with the general philosophy being to minimize restrictions and to gradually negotiate wider access for sensitive datasets as questions of ownership and attribution are resolved.

Accessing Software-Engineering Skills

At present the level of interaction between end users of model results and those developing and implementing the models is relatively limited. The creation of interfaces that allow user access depends on computer scientists and programmers and they must work closely with domain experts to ensure that interfaces meet the needs of all stakeholders. Most institutes carrying out malaria research have only limited capacity to develop Web database applications. Professional software teams with close links to malariologists are needed to set up and maintain such a system.

Interface for Users and Stakeholders

There are a wide range of potential end users of mathematical models and their outputs, including other researchers, funding bodies, program implementers, planners, and policymakers. All of these end users have different needs in regards to the models, and there are many ways in which they could potentially interact with them. The most common and effective interface is the modeler, who will ideally be embedded in a research or policy-making network with the research scientists, medical doctors, public health officials, or policy makers who will be using the models. Such an interface would facilitate active and direct communication about models and outputs, alert modelers to the availability of new data, and keep modelers current with a changing situation, which would lead to iterative updating of models. Direct contact with modelers can be supplemented in specific cases so that a researcher or policymaker is able to interact directly with a computer to obtain information ranging from specific queries about a predefined set of scenarios to more sophisticated outputs using decision-support systems. Regardless of the level of contact, it is important that stakeholders are engaged throughout the model development process so that model outputs and interfaces match user needs, and end users understand the current limitations of transmission models, in particular in terms of making quantitative predictions.

There is currently no readily available interface or "cyberinfrastructure" that brings together data, models, and stakeholders seamlessly at the required scale and scope, although prototype systems are being tested. The description below outlines what is feasible in the short term, assuming sufficient research and development support. Text S3 provides a more detailed design.

Given a (possibly distributed) annotated database, a set of software models with well-defined application programmer

Box 1. Research and Development Agenda for Modeling

Modeling approaches to guide elimination and eradication

- To provide practical tools to help planners and policymakers assess the technical, operational, and financial feasibility of malaria elimination.
- To assist in optimizing combined interventions for elimination in different transmission and health systems contexts.
- To assess and optimize TPPs for interventions and for monitoring and evaluation, and to determine the potential contribution of the products to the different phases of malaria elimination.
- To ensure flexible management in choosing and designing interventions, and for designing surveillance in collaboration with monitoring and evaluation programs to identify cost-effective strategies to shorten elimination timelines.

Further development of models and model systems

- Further basic modeling research is required on the 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, heterogeneities in hosts, vector, and parasite dynamics, and host and vector movements, to enable the models to better answer strategic questions for malaria elimination.
- Further development is required of models of drug dynamics, vaccines that interrupt malaria transmission, and the ecology of genetically modified mosquitoes. Health system attributes need to be integrated into

current models for packages of interventions and linked to microeconomic outputs.
- Models need to be further developed to consider the likely development and impact of drug and pesticide resistance at the various stages of elimination across different transmission settings.

Enabling technologies

- Harmonization of databases and model outputs, which entails:

○ Identifying key data needs and deciding whether existing information is of sufficient quality to inform the modeling.

○ Identifying technologies that support machine-level exchange of malariometric data.

○ Recognizing the importance of creating and maintaining thoroughly annotated databases and models, along with software tools and well-documented user interfaces with close collaboration between software engineers and malariologists to support model and data curation and access.

- Development of cyberinfrastructures to generate and execute efficient workflows for answering strategic questions. Cyberinfrastructures would identify and retrieve data from distributed databases; identify and execute appropriate models, compose data, and model results across multiple spatiotemporal scales and domains; and manage information about provenance, citations, and assumptions.

interfaces (APIs), and a semantic web or ontology, it may be possible to develop a cyberinfrastructure that automates much of the deductive reasoning required to answer common stakeholder-specified questions. Models can be fitted into well-established paradigms for data search and integration. The cyberinfrastructure would translate these questions into appropriate analyses on the model output. Missing data inputs could be replaced by data from other similar settings as extracted from the underlying databases with the appropriate caveats made clear to the end user. A question may trigger a cascade of data retrieval and model execution, all managed by the cyberinfrastructure. If the available data and models are insufficient to answer the question, the gaps would be noted to assist in research program development. Output of analyses performed by the structure would include a comprehensive list of citations of the source materials. A list of caveats to data inputs or model outputs (provided by the stakeholders) on the scope of appropriate use would also be included. The cyberinfrastructure therefore provides those contributing data and developing models with an incentive to include their information in the system with the assurance that results will not be misinterpreted.

Conclusions

On the basis of our discussions, we propose a research and development agenda for modeling that will effectively support operations and important research questions in attempts to achieve elimination and eradication of malaria and that lists the prerequisites and research questions for the development of modeling based on a comprehensive framework (Box 1). A single approach aiming at one, comprehensive model for malaria elimination/eradication has limited value. Rather, we will profit at the operational level as well as at the scientific level from answering the research questions and issues as outlined in this paper using a variety of models and analytical techniques, supported by direct interactions with modelers and common user interfaces, and linked to curated essential databases.

College, London, UK; Simon I. Hay, Spatial Ecology and Epidemiology Group, Department of Zoology, University of Oxford, Oxford, UK; Robert Sinden, Imperial College, London, UK; David Smith, University of Florida, Gainesville, Florida, USA; Thomas A. Smith, Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland; Marcel Tanner (chair), Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland; Lisa White, Wellcome Trust, Mahidol University, Bangkok, Thailand.

Extra contributor: Graham Brown, Nossal Institute for Global Health, Melbourne, Australia.

Author Contributions

References

1. Ross R (1908) Report on the prevention of malaria in Mauritius. London: Waterlow and Sons Limited.
2. Macdonald G (1957) The epidemiology and control of malaria. London; New York: Oxford University Press. 201 p.
3. WHO (1957) Expert Committee on Malaria, sixth report. Geneva: World Health Organization.
4. Macdonald G (1956) Theory of the eradication of malaria. Bull World Health Organ 15: 369–387.
5. Macdonald G (1956) Epidemiological basis of malaria control. Bull World Health Organ 15: 613–626.
6. Dietz K, Molineaux L, Thomas A (1974) A malaria model tested in the African savannah. Bull World Health Organ 50: 347–357.
7. Garrett-Jones C (1964) Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. Nature 204: 1173–1175.
8. Garrett-Jones C (1964) The human blood index of malaria vectors in relation to epidemiological assessment. Bull World Health Organ 30: 241–261.
9. Diseases EAIoV-B (1960) Report on the Pare-Taveta Malaria Scheme, 1954-59. Dar es Salaam: East Africa High Commission.
10. Garrett-Jones C, Gramiccia G (1954) Evidence of the development of resistance to DDT by *Anopheles sacharovi* in the Levant. Bull World Health Organ 11: 865–883.
11. Gramiccia G, Beales PF (1988) The recent history of malaria control and eradication. Wernsdorfer W, McGregor I, eds. Principles and practice of malaria. Edinburgh: Churchill Livingstone. pp 1335–1378.
12. WHO (1974) Malaria control in countries where time-limited eradication is impracticable at present. Report of a WHO interregional conference. Geneva: World Health Organization.
13. Najera J (1999) Malaria control: Achievements, problems, and strategies. Geneva: WHO.
14. The malERA Consultative Group on Monitoring, Evaluation, and Surveillance (2011) A research agenda for malaria eradication: Monitoring, evaluation and surveillance. PLoS Med: e1000400. doi:10.1371/journal.pmed.1000400.
15. The malERA Consultative Group on Basic Science and Enabling Technologies (2011) A research agenda for malaria eradication: Basic science and enabling technologies. PLoS Med: e1000399. doi: 10.371/journal.pmed.1000399.
16. The malERA Consultative Group on Vector Control (2011) A research agenda for malaria eradication: Vector control. PLoS Med: e1000401. doi:10.1371/journal.pmed.1000401.
17. The malERA Consultative Group on Health Systems and Operational Research (2011) A research agenda for malaria eradication: Health systems and operational research. PLoS Med: e1000397. doi:10.1371/journal.pmed.1000397.
18. The malERA Consultative Group on Vaccines (2011) A research agenda for malaria eradication: Vaccines. PLoS Med: e1000398. doi:10.1371/journal.pmed.1000398.
19. The malERA Consultative Group on Drugs (2011) A research agenda for malaria eradication: Drugs. PLoS Med: e1000402. doi:10.1371/journal.pmed.1000402.

Review

A Research Agenda for Malaria Eradication: Cross-Cutting Issues for Eradication

The malERA Consultative Group on Integration Strategies[¶]*

Abstract: Discipline-specific Malaria Eradication Research Agenda (malERA) Consultative Groups have recognized several cross-cutting issues that must be addressed to prevent repetition of some of the mistakes of past malaria elimination campaigns in future programs. Integrated research is required to develop a decision-making framework for the switch from malaria control to elimination. Similarly, a strong economic case is needed for the very long-term financial support that is essential for elimination. Another cross-cutting priority is the development of improved measures of intensity of transmission, especially at low and nonrandom levels. Because sustained malaria elimination is dependent on a functioning health system, a further key cross-cutting research question is to determine how inputs for malaria can strengthen health systems, information systems, and overall health outcomes. Implementation of elimination programs must also be accompanied by capacity building and training to allow the assessment of the impact of new combinations of interventions, new roles for different individuals, and the operational research that is needed to facilitate program expansion. Finally, because community engagement, knowledge management, communication, political, and multisectoral support are critical but poorly understood success factors for malaria elimination, integrated research into these issues is vital.

Introduction

During their deliberations, scientists in the various Consultative Groups contributing to the Malaria Eradication Research Agenda (malERA) concentrated on research questions relevant to their thematic areas. But, in addition, they also briefly noted many issues of relevance beyond their own domains. Some of these issues are likely to be critically important in malaria elimination/eradication programs. Consequently, they received special attention from the malERA Consultative Group on Integration Strategies. In this paper, we focus on the research and development needs of these important cross-cutting issues, especially in the context of historical reports of reasons for the failure of past campaigns. Consideration of these cross-cutting issues, we argue, is essential for regional elimination and, ultimately, global eradication of malaria, but is also relevant for scaled-up and improved control of disease.

The Historical Context

The Consultative Group identified many cross-cutting topics of special significance by examining reports of the failures and successes of earlier approaches to regional elimination of malaria. History reveals that political, social and human factors are likely to be just as important as, if not more important than, biological and

technological factors, and that a multidisciplinary approach to elimination/eradication is essential. Accordingly, special attention was given during the malERA consultations to finding synergies and strategies to prevent the "silo effects" that can occur when specialist groups work in isolation. It is important to identify critical partnerships between malaria elimination/eradication programs and programs in health or education, such as integrated management of childhood illness. Similarly, it is important to recognise the need to address social determinants of health for successful malaria eradication campaigns. Finally, ongoing critical analysis of the success or failure of current elimination efforts constitutes a research agenda in its own right, as exemplified in numerous campaigns against other diseases [1,2].

The Global Malaria Action Plan and Research for Eradication

The Global Malaria Action Plan (GMAP) [3] is focused predominantly on control, but nevertheless includes eradication as an ultimate goal. The malERA process, with its paradigm shift from control to elimination, has produced significant additions to GMAP by defining a research agenda that will assist in interruption of transmission. The malERA process emphasises the importance of clearly defining the essential research and development needed to achieve specific goals. That is, it focuses on the minimal essentials—what we "need to know"—rather than what would be maximally possible to know or even "nice to know."

Research for Readiness to Attempt Regional or National Elimination

The GMAP has identified the need to continue and scale up control of malaria in highly endemic areas for maximal reduction of

Review articles synthesize in narrative form the best available evidence on a topic.

Summary Points

- Several important cross-cutting issues must be addressed as the international community or an individual program moves from malaria control to malaria elimination/eradication: an integrated decision-making framework must be constructed for this paradigm shift

- Methods to measure transmission rapidly and cost-effectively in populations, particularly in low transmission settings, must be developed; very sensitive indicators of transmission are particularly important late in the elimination phase

- Elimination programs must be integrated for mutual benefit with strengthened health systems; better training and capacity building, better information systems, and modeling must also be developed

- New or improved tools alone will not be enough; community engagement and good communication between everyone involved in malaria elimination/eradication is essential

- A research and development agenda for cross-cutting issues is presented that should facilitate progress as programs aiming at malaria elimination/eradication supersede malaria control programs

morbidity and mortality, and recognises this as a priority for the foreseeable future [3]. As the malaria map shrinks and malaria incidence falls, some countries may consider attacking remaining foci with an elimination agenda. Many pre-elimination considerations are related directly to the competence and readiness of the health system, and are discussed in the malERA paper on health systems and operational research [4]. Decision-makers must also balance the consequences of diverting resources from urgent clinical needs to a problem that by definition is causing little morbidity.

Importantly, decision makers at national and regional levels may need to be reminded that successful elimination for a few years will inevitably lead to loss of the naturally acquired immunity that is a good defence against malaria. Attempts to eliminate malaria that are not sustained can therefore provide the grounds for serious epidemics in people of all ages, with rapid loss of the gains accrued during an elimination program if the program fails or is stopped prematurely [2,5].

The Malaria Elimination Group has recently highlighted the immediate needs of governments that are currently facing important decisions about malaria elimination/eradication [6]. Political commitment is essential; local research agendas for drug and insecticide resistance must be completed, health system readiness assessed, and cost-effectiveness analyses undertaken before deciding to make the long-term investment in elimination.

From its discussions, the malERA Consultative Group on Integration concluded that the cross-cutting research and development agenda in the context of the paradigm shift from control to eradication must take into account the research developments of the last few decades. Since the end of the Global Malaria Eradication Program (GMEP), innovations such as rapid diagnostic tests, insecticide-treated bednets, and improved information systems and communication systems have been developed, and a partially effective vaccine should be available in the foreseeable future. Thus, an algorithm needs to be defined and a tool developed for deciding the readiness of the system for elimination, or even for introduction of one of these innovations.

The Case for Long-Term Investment for Eradication

Cross-cutting research is needed to make the case for long-term investment in eradication for the global public good and to ensure that financial support is available for the "last mile" before elimination [7]. This case should align with, and complement, important and related development themes such as global security, migration, food security, and climate change. If research findings suggest that the case is strong, malaria eradication could be included in global policies for health that follow on from the Millennium Development Goals beyond 2015 [8]. Importantly, a development agenda consistent with the Paris Declaration on Aid Effectiveness and the Accra Agenda for Action [9,10] should be accompanied by strong harmonization with the GMAP and the goals of the Roll Back Malaria Partnership [3].

Cross-Cutting Research for a Good Measure of Transmission in the Later Stages of Elimination

Malaria elimination has a very different endpoint from malaria control and this change of paradigm demands the development of specific measures of progress. New infections are a direct measure of ongoing transmission but require labor-intensive, active surveillance studies, particularly during the elimination phase in regions previously experiencing high transmission where immune individuals are unlikely to experience symptomatic disease. After some years, as immunity declines, infection is more likely to be symptomatic and may then be a good surrogate marker for the detection of continued or resumed transmission during surveillance. Thus, at the end of the process, some years after elimination has been achieved and the population has lost clinical immunity, surveillance of clinical cases can become a guide to transmission. However, there are many years between the time when transmission can be measured in endemic areas (albeit with difficulty and high cost) and the time when active surveillance of occasional cases becomes a useful measure (see also [11,12]).

Accordingly, elimination programs need rapid, sensitive, standardised, and reproducible transmission measurement methods to monitor progress towards the desired goal [13], particularly when transmission continues at low and nonrandom levels. Research into and development of new measures that are simpler than surveillance for incident infections is a high priority in the cross-cutting research and development agenda. Such measures could potentially be based on serological or other biomarkers and used as indicators of readiness for elimination, progress towards that goal, and as markers of residual foci or reintroduced infection [12].

In particular, the new and improved measures of transmission could be used for measurement and certification of the absence of transmission. Such measures are essential to ensure that the decision to stop expensive entomological studies or indoor spraying that inconvenience communities is made at the appropriate time. Sustained funding is, of course, required to detect ongoing transmission or reintroduction of disease.

Integration with Strengthened Health Systems

Many past efforts at malaria elimination have failed because the health system failed during the implementation of stand-alone programs [2]. This failure, through neglect or at least under-resourcing during implementation of vertical programs, resulted in the pessimistic view that malaria can only be eliminated in regions where economic progress and stable governance are in place that

support well-functioning health systems. Even if a region initially opts for a purely vertical approach, when transmission declines, patient needs for appropriate diagnosis and treatment in the general health system become part of the surveillance system and need to be integrated with existing health system structures for local responses and central monitoring [2,4]. Moreover, diagnosis and appropriate treatment can contribute to reduction in transmission, and good health facilities are essential for management of other febrile illnesses. For these reasons, a malaria elimination program simply cannot succeed in the absence of an effective health system.

The importance of health systems thinking, the need for setting-specific and phase-specific integration, and the need for new approaches to replace the old separation into "horizontal" or "vertical" programs have been discussed by most of the other malERA Consultative Groups but particularly by the group that focused on health systems [4]. The consultative groups also highlighted relevant cross-cutting research and development agenda topics such as the need to measure synergies between malaria-specific programs and health systems strengthening, and the extent to which inputs for malaria elimination can be used to strengthen population health. Our group concluded that tailoring an approach to each setting is required, maximising synergy with the health system for mutual benefit, while maintaining the integrity of categorical program objectives, and the important activities of the health system.

Training

All of the consultative groups recognized the need for training and capacity building in the context of elimination, from discovery research in the laboratory, through social sciences research in communities, and on to operational research in the context of health systems thinking. Master's level research training that introduces the principles of a scientific approach, epidemiology, and evidence-based decision making would benefit anyone involved in deciding about resource allocation, timing, and refinement of the elimination approach before, during, and after any elimination/eradication program. Training for the eradication research agenda also needs to be accompanied by training of public health leaders and managers with substantial knowledge of malaria.

In addition, communities of health systems experts require research training to help them measure the impacts of an integrated approach to malaria elimination. "Elimination science" would assess the implementation of changed diagnostic or surveillance methods, or expanded roles of community health workers or reporters engaged in active surveillance ("learning in action"). The information gleaned through such assessments could be used for operational research or social science research relevant to community participation and engagement. It could also be used by a new cohort of experts in database development, management, or information technology.

For basic research, which has a longer time frame, academic expertise needs to be developed and sustained in fields relevant to technological development such as bioinformatics, genetics, drug and vaccine discovery, systems thinking, and mathematical modeling. It also needs to be developed in fields relevant to health promotion and communication and the enhancement of these fields by new technology.

Together, these training requirements, particularly those that focus on the needs of disease-endemic countries, are substantial and should be the subject of a later specific review.

Information Systems and Modeling for Assessing Combinations of Intervention Strategies

All the consultative groups acknowledged the importance of strong information systems that are reliable and responsive to local needs for rapid intervention, and that provide inputs to national and regional databases. The requirements for information systems will change over time with changes in transmission but an important attribute of these systems should be harmonization and the avoidance of unnecessary duplication to meet, for example, special or frequent requests from funding agencies. Importantly, additional sources of information have to be integrated into existing information systems to allow modeling of future interventions, to facilitate the analysis of system-wide effects for costing and implementation, and to provide a resource for researchers who are modeling transmission, as discussed in other malERA articles (also see [4,14]).

In common with surveillance systems, information systems need to be envisaged as tools for intervention (with a target product profile and standards to be developed and monitored), rather than as ends in themselves. The consideration of information systems as interventions (just as surveillance was defined as an intervention by the WHO Global Malaria Eradication Program), provides a useful perspective for the definition of the malERA research and development agenda and is well discussed elsewhere in this series.

Finally, because the costs and benefits, potential synergies, and operational assessments of combination strategies are likely to be different in different environments, modeling emerged as one of the key cross-cutting themes during the malERA consultation process. In particular, the use of modeling to assist discussions and decisions on intervention mixes in time and space emerged as a high priority cross-cutting theme that is discussed further in the relevant article in this Supplement [14].

Community Engagement

Successful public health programs are characterized by community engagement and good communication, but how to achieve these critical success factors is not well understood. Community case management and treatments such as piloted in Tigray [15], can be effective, but support from all sectors of society is critical, particularly where there is a requirement for behavioural change. Strategies are required to explain why efforts against malaria need to be maintained, even when malaria cases are extremely rare. Conversely, governments also have to choose the correct time, and explain the rationale for stopping certain interventions. We need to understand how public perception affects such decisions and provide guidance for countries on when certain interventions will no longer be cost-effective, and we have to communicate this information effectively.

Good communication is essential among malaria researchers. It is also essential that malaria researchers communicate well with people involved in health systems, malaria control specialists, health care workers, funders, stakeholders from public and private nongovernment sectors, communities, the general population, and the international community. Research should be undertaken on the range of factors that influence connectivity, from cultural aspects to technology, which could be revolutionised by the advent and availability of new means of communication.

Conclusions

An important part of the malERA process was to identify cross-cutting issues that could facilitate the achievement of the goal of elimination, particularly in the light of past failures, and build on the GMAP that already includes eradication as a long-term goal.

As recognized by the whole malaria community, integration is a prerequisite for success.

Tools alone are not enough, but need to be accompanied by excellent and ongoing coordination, operational research, information systems, and monitoring and evaluation supplemented by active surveillance. Integration with the health system and a multidisciplinary approach are also essential, providing new tools and approaches for modeling and for systems thinking about the concepts and strategy needed to achieve the ultimate goal. In addition, communication and research into its improvement and local adaptation are critical; without excellent communication and community and political engagement, elimination/eradication programs will not succeed. Moreover the community and the health system need to be ready with appropriate tools and trained personnel in place to take on new or specific tasks that need to be integrated into ongoing activities.

Before attempting elimination, a realistic feasibility assessment is required to determine readiness for this challenge. Some countries fall far short of readiness, having tools that are inadequate to complete the task where force of infection is very high, having health systems that are weak, or suffering from socio-political and civil disturbances that make public health practice nearly impossible. Other countries may simply lack one major prerequisite such as political will, or a drug to overcome resistance to available antimalarial therapy. Unrealistic promises about malaria elimination will inevitably lead to disappointment and disillusion with public health approaches and should be avoided.

We cannot provide estimates of the cost of the research and development agenda for cross-cutting issues that we present in Box 1, and recognise that further work will be required to delineate fully all the regulatory and ethical implications of new tools that have been envisaged or described here. Technology that may provide solutions may currently be beyond our imagination, but could be available within a short few years. Importantly, however, we recognize that very long-term investments will be needed for the research and development agenda that we have outlined. We also recognize that we need to build on public/private partnerships and connections with industry to facilitate new advances. Nevertheless, we emphasize that, even if elimination programs are decades away for some countries with very high transmission, now is the time to start work on the broad and integrated portfolio of long-term research that is essential if the goal of malaria eradication is to be achieved.

Author Contributions

GB is the corresponding author on behalf of the malERA consultative group on Cross-Cutting Issues. ICMJE criteria for authorship read and met: PLA, GB, MT. Participated in one or more discussion meetings resulting in the writing of this article: The malERA Consultative Group on Cross-Cutting Issues.

References

1. Breman JG, de Quadros CA, Dowdle WR, Foege WH, Henderson DA, et al. (2011) The role of research in viral disease eradication and elimination programs: Lessons for malaria eradication. PLoS Med 8: e1000405. 10.1371/journal.pmed.1000405.
2. Nájera J, González-Silva M, Alonso, PL (2011) Some lessons for the future from the Global Malaria Eradication Programme (1955–1969). PLoS Med 8: e1000412. doi:10.1371/journal.pmed.1000412.
3. Roll Back Malaria (2008) Global malaria action plan. Geneva: Roll Back Malaria Partnership. Available: http://rbm.who.int/gmap/gmap.pdf.
4. The malERA Consultative Group on Health Systems and Operational Research (2011) A research agenda for malaria eradication: Health systems and operational research. PLoS Med 8: e1000397. doi:10.1371/journal.pmed.1000397.
5. Roberts DR, Manguin S, Mouchet J (2000) DDT house spraying and re-emerging malaria. Lancet 356: 330–332.
6. Feachem RGA and the Malaria Elimination Group (2009) Shrinking the malaria map: a guide on malaria elimination for policy makers. San Francisco: The Global Health Group, Global Health Sciences, University of California, San Francisco.
7. Mills A, Lubell Y, Hanson K (2008) Malaria eradication: The economic, financial and institutional challenge. Malar J 7: S1–S11.
8. USAID (2010) Celebrate, innovate and sustain: toward 2015 and beyond. The United States' strategy for meeting the. Millennium Development. Available: http://www.usaid.gov/our_work/mdg/US_MDG_Strategy.pdf.
9. OECD (2005) Paris Declaration on Aid Effectiveness: Ownership, harmonization, alignment, results and mutual accountability. Paris: OECD.
10. Accra High Level Forum (2008) Accra agenda for action. 3rd high level forum on aid effectiveness. Available: http://siteresources.worldbank.org/ACCRAEXT/Resources/4700790-1217425866038/AAA-4-SEPTEMBER-FINAL-16h00.pdf.
11. The malERA Consultative Group on Monitoring, Evaluation, and Surveillance (2011) A research agenda for malaria eradication: Monitoring, evaluation, and surveillance. PLoS Med 8: e1000400. doi:10.1371/journal.pmed.1000400.
12. The malERA Consultative Group on Diagnoses and Diagnostics (2011) A research agenda for malaria eradication: Diagnoses and diagnostics. PLoS Med 8: e1000396. doi:10.1371/journal.pmed.1000396.
13. Hay SI, Smith DL, Snow RW (2008) Measuring malaria endemicity from intense to interrupted transmission. Lancet Infect Dis; 8: 369–378.
14. The malERA Consultative Group on Modeling (2011) A research agenda for malaria eradication: Modeling. PLoS Med 8: e1000403. doi:10.1371/journal.pmed.1000403.
15. World Health Organization (2009) Deployment of artemetherlumefantrine with rapid diagnostic tests at community level, Raya Valley, Tigray, Ethiopia. Geneva: World Health Organization. Available: http://www.who.int/malaria/publications/techreport_rdt_tigray.pdf.

Review

Some Lessons for the Future from the Global Malaria Eradication Programme (1955–1969)

José A. Nájera[1]*, **Matiana González-Silva**[2], **Pedro L. Alonso**[2,3]

1 Retired (formerly WHO), Crans-Celigny, Vaud, Switzerland, **2** Barcelona Centre for International Health Research (Hospital Clínic, Universitat de Barcelona), Barcelona, Spain, **3** Centro de Investigaçao em Saude da Manhiça, Maputo, Mozambique

Abstract: Encouraged by the early success of using dichloro-diphenyl-trichloroethane (DDT) against malaria, the World Health Organization (WHO) embarked on the Global Malaria Eradication Program (GMEP) in 1955. Fourteen years later, the campaign was discontinued when it was recognised that eradication was not achievable with the available means in many areas, although the long-term goal remained unchanged. During the GMEP, malaria was permanently eliminated from many regions. In other areas, however, substantial gains were lost in resurgences, sometimes of epidemic proportions. During the 1970s and 1980s, because of economic and financial crises, international support for malaria control declined rapidly, but in the past decade, following increasing demands from endemic countries and promising results from scaling up of control activities, interest in malaria elimination and the long-term goal of eradication has received international political and financial support. In 2007, there was a renewed call for malaria eradication and a consultative process to define a research and development agenda for malaria eradication (malERA) was established. Lessons learned from the GMEP (1955–1969) highlight the fact that no single strategy can be applicable everywhere and that a long-term commitment with a flexible strategy that includes community involvement, integration with health systems, and the development of agile surveillance systems is needed.

Introduction

The mechanisms of malaria transmission were first elucidated at the end of the 19th century. This research meant that malariologists could at last explain the observed effects of traditional control measures, such as drainage of marshes and mosquito nets, and develop better approaches to control malaria. Thanks to increasing public and political support, the early days of the 20th century witnessed the deployment of an increasing number of interventions against malaria. However, large-scale implementation of most of the proposed measures had severe operational and financial limitations, and some strategies were found to be suitable only in particular social, ecological, and epidemiological conditions.

The best approach to malaria control became the subject of intense debate during the first decades of the century. Experts were roughly divided into two major conceptual camps. Some (e.g., Ross, Gorgas, and Watson) favoured large-scale campaigns of vector control or mass drug administration to prevent and rapidly solve the problem. Others (the Malaria Commission of the

League of Nations and the so-called Italian and Dutch schools) advocated locally designed programs of progressive, albeit slow, development of case management facilities and environmental sanitation to stimulate health and economic development, and diminish malaria morbidity and mortality. While the first group achieved spectacular successes, such as the interruption of malaria and yellow fever transmission during the construction of the Panama Canal and the elimination of the introduced highly efficient African vector *Anopheles gambiae* in Brazil, sustainability seemed to require the solid public health foundations envisaged by the second approach. Thus, in 1939 Boyd summarised the prevailing public health point of view as: "malaria control should not be a campaign, it should be a policy, a long-term program. It cannot be accomplished or maintained by spasmodic effort. It requires the adoption of a practicable program, the reasonable continuity of which will be sustained for a long term of years" [1].

It is hoped that the following review of the history of the Global Malaria Eradication Program (GMEP) (1955–1969) will encourage current and future antimalarial programmes that are pursuing new goals to develop flexible strategies on the basis of analyses of their own history and to strengthen their existing expertise rather than relying on new cadres to adopt an imported strategy, as did the GMEP.

The Impact of DDT

The development of dichloro-diphenyl-trichloroethane (DDT) as the first residual insecticide in the early 1940s brought about a radical change in malaria control strategies. Killing indoor resting adult mosquitoes with insecticides sprayed on household walls had started in the 1930s using pyrethrum extracts, but had limited applicability because weekly applications were needed. DDT, which was first used against malaria by the US Army during World War II, required only semestrial or annual applications. This long residual effect meant that malaria control could be

Review articles synthesize in narrative form the best available evidence on a topic.

Summary Points

- An examination of the evolution, implementation, and outcome of the Global Malaria Eradication Programme provides useful lessons for current elimination/eradication attempts
- Programmes should develop flexible strategies, integrated into the national health infrastructure rather than only implementing vertical malaria elimination campaigns, in order to ensure sustainability
- Professional cadres that can adapt the strategy to the local epidemiology and that can develop an effective surveillance system deeply rooted in the communities should be strengthened
- To solve problems and to review strategies, close links should be established with field and laboratory research
- Communities should be encouraged and supported to adopt malaria elimination as their own goal, reporting abnormal situations and creating a demand for effectiveness

extended to large rural areas, although it needed a strong central organisation to handle the supply, transport, and distribution networks required for regular and correct application.

During the late 1940s and early 1950s, after numerous field trials, more and more national control programmes adopted DDT spraying. These programmes showed that transmission could be interrupted and that malaria did not necessarily return if spraying stopped [2,3]. DDT appeared to be effective everywhere, making eradication of malaria a feasible objective. However, DDT's effectiveness against agricultural pests and household insects made prices soar, and its widespread application rapidly led to the first appearance of vector resistance to DDT in Greece in 1951 [4].

In this context, it was felt that progress at a global level would require more than the slow recruitment of political support country by country. Rather, it would be necessary to mobilise political commitment at the UN level and gain the financial support of UN agencies and of the United States, where a strong lobby was formed to obtain funds for global malaria eradication [5]. Further support for a global eradication approach was

provided during the 1950s by Macdonald's mathematical model, which highlighted the great superiority of increasing adult vector mortality over mere reduction in density [6–8]. Malaria eradication was also advocated for with economic and political arguments that shifted from the impact of malaria on the local economies, to its influence on the price of imported goods and the risk that malaria could "predispose a community to infection with political germs that can delay and destroy freedom" as stated by Paul Russell, the Rockefeller malariologist who defended the WHO malaria eradication proposal at the 8th World Health Assembly (WHA) [9].

The GMEP was approved by the 8th WHA in Mexico in 1955 [10]. WHO was given the mandate to provide technical advice and coordinate resources, but not to act as "directing and coordinating authority" as proposed in the draft resolution submitted by 28 countries [11]. The 1955 WHA resolution also established a Malaria Eradication Special Account to channel public and private contributions [10], which opened the hope of general availability of funds.

Although approved by an overwhelming majority, the decision to launch the GMEP was not without controversy. Advocates of the eradication approach highlighted the emergence of mosquito resistance to DDT that, in their view, necessitated the launch of the GMEP before the world lost its most promising weapon. They also argued that eradication was, in the long term, financially more attractive than control. Conversely, critics of the campaign doubted the feasibility of eradication in vast areas that had poor communications and adverse environments and that lacked public health systems. They also emphasized the poor understanding of the implications of undertaking a malaria eradication campaign, both in terms of its cost and of the risk to the population posed by lost immunity if protection had to be interrupted [12].

In 1956, the WHO Expert Committee on Malaria was called to design the eradication campaign (Figure 1). The Committee felt that they were shaping a strong political force and that they had the opportunity of freeing malaria control from the frustrations of bureaucracy by prescribing autonomous organisations capable of achieving the precise execution of interventions. In contrast to control (measures of indefinite duration aimed at reducing the incidence of malaria), eradication was defined as "the ending of the transmission of malaria and the elimination of the reservoir of
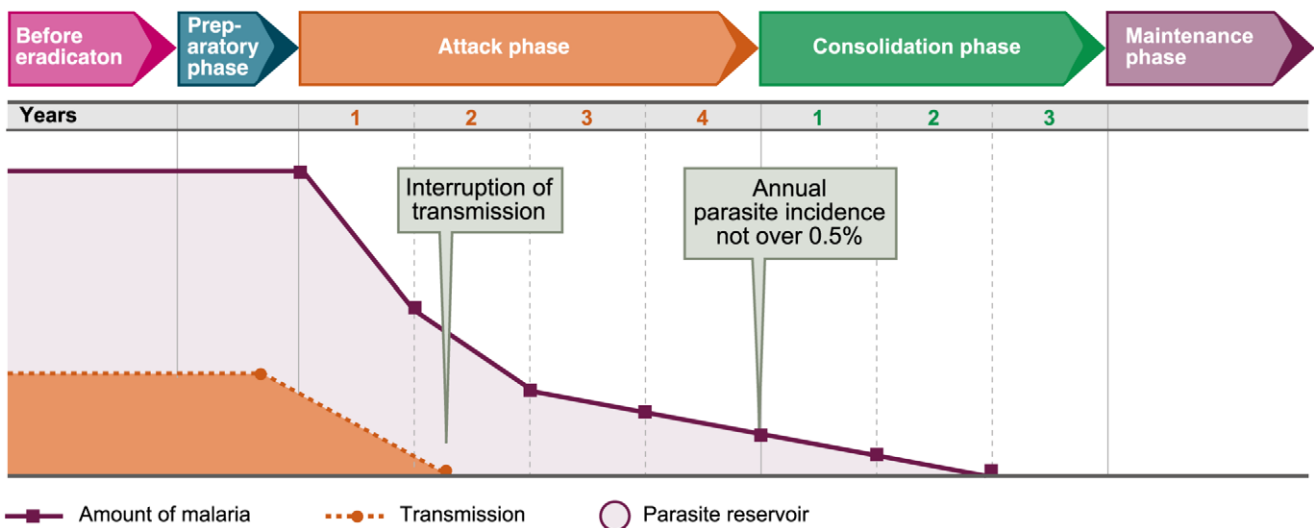
Figure 1. Phases of the Malaria Eradication Campaign as established by WHO in 1963. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000412.g001

infective cases in a campaign limited in time and carried out to such a degree of perfection that when it comes to an end, there is no resumption of transmission" [13].

The Expert Committee seems not to have realised that in creating such executive machinery, they were transforming the practice of malariology. The administration of such gigantic enterprises was a totally absorbing job; up to then, malariologists had been field scientists guiding governments and local authorities by trying to solve a problem. Now malariologists were forced to become managers trying to accomplish a complex task.

Moreover, the global eradication campaign was based on the assumption that all the necessary knowledge for eradication was available, that further research was superfluous, and that eradication required a rigid discipline in which local deviations from a centrally defined plan must be prevented. Thus, malaria eradication acquired the characteristics of an ideology and control was demonised. This attitude is clearly illustrated by the statement of the UNICEF Regional Director to the Executive Board: "Between malaria control and eradication there is as great a difference as that between night and day. Control … is a primitive technique. Now we know exactly … the schedule of an eradication campaign which will last four or five years, followed by three years of consolidation" [14].

This overoptimistic environment prevented the recognition of general problems in the conception of the campaign, which was based on an exaggerated extrapolation of early local experiences that, although successful, represented a very limited variety of epidemiological situations. Actually, it was obvious from the start that nobody knew how to deal with the problems of tropical Africa; this was one of the main objections to the GMEP in the 1955 WHA.

A serious consequence of that exaggerated confidence was the belief that the wide experience and knowledge of the old malariologists was superfluous and even counterproductive, particularly if they persisted in modifying the eradication strategy locally. Therefore, eradication campaigns were entrusted to new, preferably young "malariologists," trained in "Malaria Eradication Training Centres" established by WHO in several countries.

GMEP interventions consisted basically of indoor residual spraying with DDT or other approved insecticides. The Expert Committee developed standard guidelines for action on the basis of vertical, time-limited interventions clearly distinct from previous measures. Destruction of mosquito breeding marshes, prevention of mosquito bites, and other measures traditionally used in malaria control were abandoned, depicted not only as unnecessary but as antagonistic to the higher goal of eradication. Moreover, international funds became available only to countries adopting the goal and the means set by the WHO expert committee reports.

The fundamental principles of the campaign—total coverage and perfection in the execution of operations—served as a stimulus to those countries that already had, or could develop, the infrastructure to mobilize and use the new resources to eliminate malaria from their territories. Many other countries, following the Committee's directives, established new autonomous structures that favoured the delivery of services over the creation of a demand and the participation of local communities. These autonomous structures often became "self-perpetuating," dissociated from the general health services and incapable of adaptation to changes in the epidemiological situation.

Outcomes of the Campaign

It is not necessary to emphasize the positive contributions of the campaign to world health, which include: (1) achieving a considerable reduction in the geographical distribution of malaria although most of this reduction was in areas that already had well functioning control programmes; (2) being the first global health programme aimed at "total coverage"; (3) leading to the establishment, in some countries, of effective although partial contact with the communities, through networks of "voluntary collaborators" for diagnosis and treatment; (4) making a serious attempt to use local maps to guide its activities, even if that practice was later neglected; and (5) having an important influence on the subsequent planning of health programmes.

Nevertheless, as more and more countries joined the campaign and reported the achievement of total coverage with attack measures, often after strenuous efforts to reach remote areas, emerging problems were overlooked. Even the confirmation of chloroquine resistance in 1960, after treatment failures had been reported since the late 1950s from Venezuela and Thailand, was not given its full epidemiological importance because the campaign still hoped to interrupt transmission by spraying. In addition, it was assumed that the well-known periodic epidemic risk in certain areas would not return after local interruption of transmission. It was only in the mid-1960s that the existence of "problem areas" was recognised, after evidence of vector avoidance of contact with the insecticide in southern Mexico was confirmed.

As mentioned above, antimalarial interventions other than indoor residual spraying were abandoned. Even the use of antimalarial drugs as a complementary measure was considered redundant at the beginning. At the same time, there was a general disregard for social and cultural barriers, which often prevented the acceptance of the campaign activities in many of the "remote areas." Moreover, even though most country programmes established health education units, these were rarely given the recognition or the means needed to provide a useful contribution [5].

Malaria Resurgences after Interruption of Transmission

During the 1960s, not only did some areas fail to advance as expected, but other areas saw resurgences of malaria after relatively long periods of interruption of transmission. Some resurgences were surprisingly serious epidemics that required the reestablishment of spraying operations.

By 1962, it was already recognised that the consolidation phase required an infrastructure capable of supporting epidemiological surveillance. As a result, a new "pre-eradication programme" was established, mainly for Africa, with the aim of developing the required health infrastructure in parallel with the preparatory phase of the campaign. Unfortunately, there were no models of the minimum infrastructure required and the development of the "basic health services" continued to respond mainly to financial and political motivations.

Moreover, although by the mid-1950s, there was relatively wide experience in the use of DDT, nobody had a clear idea of how to organise a surveillance system capable of detecting the last cases of malaria. The sixth report of the Expert Committee [13] suggested the creation of surveillance systems involving direct—mainly house-to-house visits—and indirect means, such as engaging official or unofficial health services, of case detection. It also suggested that the search should be intensified as the number of cases decreased to manageable proportions.

However, the campaign managers considered terms like "manageable proportions" too vague and demanded clearer and more precise prescriptions. The Expert Committee obliged in its 8th and

10th reports by producing some indicators for when to stop total coverage with spraying (the end of the attack phase). These indicators were an annual parasite incidence of <0.5/1,000 and <0.1/1,000 (in the 8th and in the 10th reports, respectively), an annual blood examination rate of >10% of the population of the malarious areas, and a slide positivity rate of <5%. Although the committee insisted on the need to be guided by the experience and the capacity of the local services, campaign managers rapidly adopted these figures as thresholds for advancing through the phases of the campaign.

As problems became more widely recognised through the 1960s, there was some renewed interest in malaria research. WHO, for example, set up a programme for coordinating the development of new insecticides for public health and supported pilot projects to interrupt malaria transmission in Africa. Nevertheless, it was the spread of drug resistance in Southeast Asia and increased involvement of the US in the Vietnam war during the second half of the decade that led the US army to launch an intense malaria research programme aimed at the development of new antimalarials, but including studies on parasite biology, immune responses, in vitro culture, and the development of new animal models. McGregor described this development as: "throughout the world support for further research into malaria, even that concerned with insecticides and chemotherapeutics, contracted swiftly. Worse still, the apparent imminent demise of a once important disease removed the

necessity for training scientists in malariology. It took 10 years and a war to halt this tragic trend" [15].

After Global Eradication: A Return to Control

In 1967, as more areas reverted from consolidation to attack phase, the WHA requested a reexamination of the global strategy. The evaluation illustrated the slowing down of the global campaign [16], particularly after 1966 (Figure 2). GMEP also faced financial constraints during these years, as the US contributions to the WHO Malaria Special Account, which represented more than 85% of the total, were stopped in 1963, considerably reducing WHO's capacity to provide technical assistance [17].

An event that undoubtedly influenced the WHA was the 1968–1969 epidemic resurgence of malaria in Sri Lanka (then Ceylon), a country that had been considered a model for the training of malariologists. The surveillance system in this country had not reacted to 4 years of clear deterioration (1963–1967), nor had it taken into account 30 years of accumulated knowledge about the periodicity of epidemic risk in the country.

In 1969, 14 years after the launch of the GMEP, the 22nd World Health Assembly had to recognise that there were countries where eradication was not feasible in the short term, and that a strategy of control was an appropriate step towards future eradication in those areas. "In the regions where eradication does not yet seem feasible,
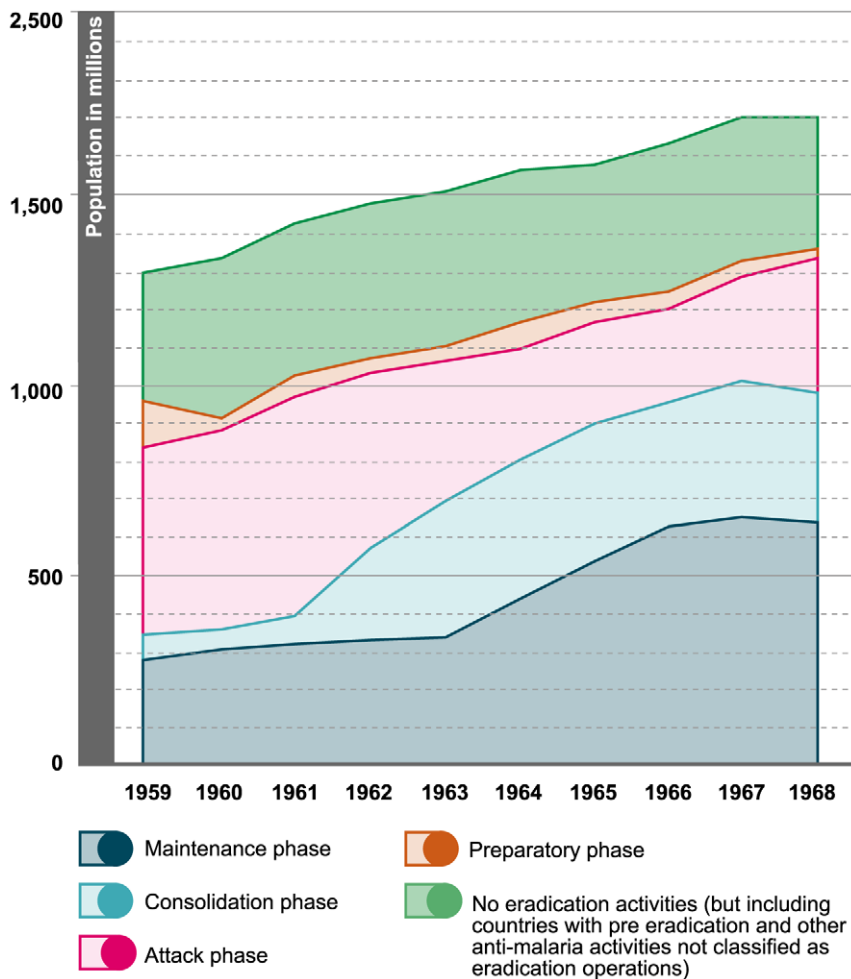
Figure 2. Progress of the campaign, presented to the 8th WHA [**16**]. Image credit: Fusión Creativa.
doi:10.1371/journal.pmed.1000412.g002

control of malaria with the means available should be encouraged and may be regarded as a necessary and valid step towards the ultimate goal of eradication," the Assembly stated, while reaffirming that eradication remained the ultimate objective [10].

Malaria Control during the 1970s and 1980s

Faced with the recognition that malaria eradication could not be conceived as a short-term programme, UNICEF and other major collaborating agencies withdrew their support to malaria programmes in favour of general health programmes. The economic crisis of the early 1970s also contributed to the accelerated contraction of funding for malaria control. Moreover, oil shortages caused considerable increases in insecticide prices that further deteriorated the financial situation of the campaigns. This reduction of programme resources, aided by a strong La Niña in 1975–1976, resulted in severe epidemics in several countries, particularly in the Indian subcontinent and Turkey.

Another problem that became evident during the 1970s was the attrition of professional staff. The lack of professional incentives as a result of the routine work imposed during the GMEP had reduced the professional cadres. At the same time, the organisation of spraymen into unions made it increasingly difficult to reduce this unqualified labour force.

All these factors combined such that the campaigns became less and less capable of reorienting their strategy. This lack of flexibility, together with the drastic reduction of their operational capacity, led to the so-called "fire-fighting" strategy. Paradoxically, in the name of maintaining previous achievements, operations were continued in the best protected areas, resulting in resources being concentrated in the areas with lesser problems.

To make matters worse, in response to the economic crisis, many countries encouraged the exploitation of their natural resources. Some, like Brazil or Indonesia, actively supported the colonisation of their extensive primary forests by agriculture and mining, a process supported by the construction of penetrating roads. These policies resulted in massive outbreaks of malaria that, because of the relative weakness of official malaria control, encouraged an intensive trade of all kinds of antimalarial drugs, thus contributing to the spread of drug resistance [18].

All these problems supported the view that progress required the development of new tools and strategies and, in the mid 1970s, WHO launched the Special Programme for Research and Training in Tropical Diseases (TDR) in collaboration with the United Nations Development Programme and the World Bank, in an effort to reestablish the role of research in malaria control. Since its establishment, the TDR has achieved important successes in the development of new tools and in laboratory and field research.

Nevertheless, the "problem solving" approach of field malariologists in the first half of the 20th century has not been recovered in most programmes and the rift between control and research, once described in India as "a curious rivalry between the malaria programme and outside research bodies," still persists. Most research projects have little operational bearing on the control programme and the latter lack "the capacity either to carry out research, to guide it, to generate issues for research based on analysis of incoming information, or to translate into operational use research carried out by other institutions" [18].

Lessons Learnt from the GMEP by Antimalarial and Other Health Programmes

Throughout the past decades, countries have tried to adapt to changing situations within the constraints of their financial and organizational limitations. These experiences show how antimalarial and other programmes tried to implement lessons learned from the GMEP, even though there were sometimes great gaps between the formulation of a lesson and its application. These lessons included:

(1) A public health service is needed to support malaria surveillance, even though there are still major disagreements among experts about when or how antimalarial programmes should be integrated with the health services. Relevant to this lesson, the WHO Registry of countries that have achieved local malaria eradication, elimination in present terminology, shows that a prerequisite for elimination may be the existence of a previous prolonged control programme that has contributed to the development of epidemiological services and a rural public health service (Table 1). Tourism-oriented, relatively rich islands maintain elimination through continuous expensive mosquito control programmes. It should also be recognised that countries included in the Registry were not highly malarious, although some of them had foci of high endemicity and areas subject to epidemic outbreaks.

(2) Control has to be supported with research. This lesson has led to the considerable revival of malaria research since the 1970s, but the relations between control programmes and research institutions still need to be revived or strengthened.

(3) As highlighted by the Primary Health Care movement, active participation of communities in the understanding of and actions for the solution of their health problems needs to be incorporated into antimalarial programmes. Although there have been important local initiatives in the past, WHO has only recently formulated a strategy for Community-based Malaria Elimination. Conversely, it is worth recalling that the setbacks and general lack of progress of the GMEP were among the main stimuli for the generation of the primary health care movement in the 1970s.

(4) More specifically, the GMEP's "failure to achieve its objective" was taken into consideration in the design of the successful Intensified Smallpox Eradication Programme [19]. An important principle of this programme was that the administrative structure and pattern of operations of each national programme should be integrated into the health and socio-cultural setting of the country. Fenner and coauthors [20] noted that the programme's success depended on stating the strategic plan in terms of principles and illustrative methodologies rather than in terms of directives and on recognising that continuing field and laboratory research would be essential. Another important principle of the smallpox eradication programme was concentration on investigating all outbreaks or clustering of cases, before attempting to investigate each individual case. Although there are obviously great differences in the epidemiology and the response to control interventions between smallpox and malaria, these strategic considerations should now be taken into account in the malaria eradication programme where a lack of flexibility, an incapacity to adapt to changing situations, and a lack of coordination between control programmes and research institutions have all been identified as important obstacles to advancement in malaria control and elimination [17,21,22]. Noteworthy in this respect is China's experience. Although not included in the WHO Registry because only complete countries are included in this Registry, China has eliminated malaria from most of its territory by developing a control strategy on the basis of exhaustive attention to case detection and management by epidemiolog-

Table 1. Countries and regions certified malaria free up to 2010.

Countries and Regions with a Long History of Control	Islands with Tourism-Oriented Economy	Other
North Venezuela (1961), Hungary (1964), Spain (1964), Bulgaria (1965), Taiwan (1965), Cyprus (1967), Poland (1967), Romania (1967), Netherlands (1970), United States (1970), Italy (1970), Puerto Rico (1970), Cuba (1973), Portugal (1973), Yugoslavia (1973), Australia (1980), Turkmenistan (2010)	Grenada & Carriacou (1962), St. Lucia (1962) Trinidad & Tobago (1965), Dominica (1966), Jamaica (1966), U.S. Virgin Islands (1970), Mauritius (1973), Reunion (1979)	Singapore (1982), Brunei Darussalam (1987), United Arab Emirates (2007), Morocco (2010)

doi:10.1371/journal.pmed.1000412.t001

ical services deeply rooted in their communal organisation. These services are firmly supported by political will at all levels of society and deploy well-organised control measures when they were needed for elimination of foci, all "in sensible semi-defiance of WHO dictates," according to Kidson [23].

Conclusions and Recommendations

Although not a comprehensive coverage of the problems of malaria control, the authors' experience and the broad historical considerations presented above, suggest the following conclusions, which may be useful in planning new elimination programmes:

- It may be fair to say that there is no country that is still endemic today where the malaria problem is so simple and uniform that it can be solved by applying a single strategy.
- The GMEP generated heated debate that contrasted vertical and horizontal approaches to malaria elimination. Historical analysis suggests that, while sustainable elimination of an endemic problem from a wide geographical area requires the build up of a epidemiological services well rooted in the communities, a well-organised, disciplined campaign is required for the rapid solution of local problems, such as outbreaks.
- It is essential to identify and study the physical, social, and cultural barriers that have proved to be stumbling blocks to malaria control in the past, and make all necessary efforts to avoid them in future by encouraging better community involvement and ownership.
- Programmes should be adequately integrated into the national health infrastructure. Such integration will allow them to benefit from available epidemiological services for communication and analysis. Programmes should also benefit from the establishment of solid links with research and training institutions, including organisations studying ecology, anthropology, sociology, economic activities (e.g., agriculture, forestry, mining, fishing, etc.), production systems, labour relations, and population movements of endemic populations.

- Worryingly, the notion that problems can be solved before they are fully understood still seems widespread. This attitude is evidenced by the emphasis placed on scaling up control interventions rather than on developing an epidemiological infrastructure. While such scaling-up will most likely continue to reduce transmission in many areas, the timely identification and elimination of residual foci may not be possible unless programmes reestablish strong professional cadres capable of guiding flexible and adaptable action. That is, those involved in elimination efforts need to not only apply accepted control measures, but also to evaluate results and participate in problem solving.
- Surveillance should not only aim to detect the last case, it should be an essential instrument from the start, involved in the identification and study of problem areas, beyond the limits of administrative localities. As the elimination programme advances, epidemiological investigations should concentrate successively in the study of outbreaks or clustering of cases and finally of individual case investigations.

Finally, it is necessary to break the "quasi-cyclical" alternation between overoptimistic expectations and a "fire-fighting strategy." If malaria eradication is ever to succeed, the fate stated in 1927 by the Second Report of the Malaria Commission of the League of Nations—"The history of special antimalarial campaigns is chiefly a record of exaggerated expectations followed sooner or later by disappointment and abandonment of the work"—must be avoided.

Author Contributions

References

1. Boyd MF (1939) Malaria: Retrospect and prospect. Am J Trop Med Hyg 19: 1–6.
2. Livadas GA (1952) Is it necessary to continue indefinitely DDT residual spraying programmes? Relevant observations made in Greece. WHO document WHO/MAL/79. Geneva: WHO.
3. Pampana E (1969) A textbook of malaria eradication. 2nd edition. London, New York, Toronto: Oxford University Press. pp 5–6.
4. Livadas GA, Georgopoulos G (1953) Development of resistance to DDT by *Anopheles sacharovi* in Greece. Bull World Health Organ 8: 497–511.
5. Cueto M (2007) Cold war, deadly fevers. Malaria eradication in Mexico, 1955–1975. Washington (D.C.): Woodrow Wilson Center Press & Baltimore: The John Hopkins University Press.
6. Macdonald G (1957) The epidemiology and control of malaria. London: Oxford University Press.
7. MacDonald G (1956) Epidemiological basis of malaria control. Bull World Health Organ 15: 613–626.
8. The malERA Consultative Group on Modeling (2011) A research agenda for malaria eradication: Modeling. PLoS Med 8: e1000403. doi:10.1371/journal.pmed.1000403.
9. Russell PF (1955) Man's mastery of malaria. London, New York, Toronto: Oxford University Press. 221 p.
10. WHO (1973) Malaria. Handbook of resolutions and decisions of the World Health Assembly and the Executive Board. Volume I, 1948–1972, 1st to 25th WHA and 1st to 50th EB. Geneva: World Health Organization. pp 66–81.
11. Gramiccia G, Beales PF (1988) The recent history of malaria control and eradication. Wernsdorfer WH, McGregor I, eds. Malaria. Principles and practice of malariology. Edinburgh, London, Melbourne, New York: Churchill Livingston. pp 1335–1378.
12. WHO (1955) Eighth World Health Assembly (Mexico, D.F., 10–27 May 1955). Official records of the World Health Organization, N° 63. Geneva: World Health Organization. pp 236–240.
13. WHO (1957) Expert Committee on malaria, sixth report, WHO Technical Report Series, number 123. Geneva: WHO. 4 p.
14. UNICEF (1955) Statement read by the Regional Director before the Executive Board at its September Meeting. The Americas Regional Office Programme Progress Report, number 29 (March-August, 1955). New York: UNICEF.
15. McGregor IA (1982) Malaria: Introduction. Br Med Bull 38: 115–116.

16. WHO (1969) Re-examination of the global strategy of malaria eradication. Twenty-second World Health Assembly, Part I. WHO official records number 176, annex 13. Geneva: WHO. pp 106–26.
17. Nájera JA (2001) Malaria control: achievements, problems and strategies. Parassitologia 43: 1–89.
18. Evaluation Committee (1985) In-depth evaluation report of Modified Plan of Operation under National Malaria Eradication Programme of India. Delhi: National Malaria Eradication Programme. Government of India.
19. Henderson DA (1998) Eradication: Lessons from the past. Bulletin of the World Hlth Organization 76: 17–21.
20. Fenner F, Henderson DA, Arita I, Ježek Z, Ladnyi ID (1988) Smallpox and its eradication. Geneva: World Health Organization. 515 p.
21. Greenwood BM (2008) Control to elimination: Implications for malaria research. Trends Parasitol 24: 449–454.
22. Breman JG (2004) Conquering the intolerable burden of malaria: What's new, what's needed: a summary. Am J Trop Med Hyg 71: 1–15.
23. Kidson C, Indaratna K (1998) Ecology, economics and political will: The vicissitudes of malaria strategies in Asia. Parassitologia 40: 39–46.

PLoS MEDICINE

Review

The Role of Research in Viral Disease Eradication and Elimination Programs: Lessons for Malaria Eradication

Joel G. Breman[1], Ciro A. de Quadros[2], Walter R. Dowdle[3], William H. Foege[4], Donald A. Henderson[5], T. Jacob John[6], Myron M. Levine[7]*

1 Fogarty International Center, National Institutes of Health, Bethesda, Maryland, United States of America, 2 Sabin Vaccine Institute, Washington (D.C.), United States of America, 3 Task Force for Global Health, Decatur, Georgia, United States of America, 4 The Bill & Melinda Gates Foundation, Seattle, Washington, United States of America, 5 Center for Biosecurity, University of Pittsburgh Medical Center, Baltimore, Maryland, United States of America, 6 Christian Medical College, Vellore, India [retired], 7 Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland, United States of America

Abstract: By examining the role research has played in eradication or regional elimination initiatives for three viral diseases—smallpox, poliomyelitis, and measles—we derive nine cross-cutting lessons applicable to malaria eradication. In these initiatives, some types of research commenced as the programs began and proceeded in parallel. Basic laboratory, clinical, and field research all contributed notably to progress made in the viral programs. For each program, vaccine was the lynchpin intervention, but as the programs progressed, research was required to improve vaccine formulations, delivery methods, and immunization schedules. Surveillance was fundamental to all three programs, whilst polio eradication also required improved diagnostic methods to identify asymptomatic infections. Molecular characterization of pathogen isolates strengthened surveillance and allowed insights into the geographic source of infections and their spread. Anthropologic, sociologic, and behavioural research were needed to address cultural and religious beliefs to expand community acceptance. The last phases of elimination and eradication became increasingly difficult, as a nil incidence was approached. Any eradication initiative for malaria must incorporate flexible research agendas that can adapt to changing epidemiologic contingencies and allow planning for posteradication scenarios.

Introduction

Despite a previous global eradication campaign (1955–1969), malaria continues to be a major public health problem. Faced with hundreds of millions of malaria cases annually and nearly a million deaths, the international community is renewing efforts to eradicate this disease. But, initiatives for national or regional elimination or global eradication of any disease represent complex efforts that consume vast financial, health services, and infrastructural resources and require decades of commitment. Such programs demand sound scientific underpinnings and management structures that can adapt to changing epidemiologic scenes and can learn from the experiences of previous programs. Herein we describe three viral disease elimination/eradication efforts whose research agendas offer lessons for malaria scientists and public health program managers. The disease elimination programs we consider are smallpox (the one human infectious disease successfully eradicated), poliomyelitis (transmission of wild-

type 2 poliovirus was interrupted globally since 1999, although transmission of types 1 and 3 continues in several countries), and measles (whose transmission has been eliminated in the Americas and in several countries worldwide). Each author has participated in one or more of these eradication/elimination initiatives and some also have experience in malaria research.

Throughout this article we use the following terms to denote progressive decreases in the extent of human disease and transmission of agent, as a result of deliberate interventions [1]. "Control" is the reduction of incidence of a disease to an arbitrary level whereupon it is no longer a public health priority. "Elimination" is the interruption of transmission of the pathogen when disease incidence becomes zero in a population within a large defined geographic area (e.g., one or more countries). A caveat in measles and polio elimination initiatives is that imported cases may appear in a country without indigenous transmission, i.e., a country that has achieved elimination. Elimination is considered to remain intact, so long as the importations are contained and do not ignite anew extended indigenous transmission. Finally, "eradication" signifies the interruption of transmission of a pathogen worldwide and a reduction in disease incidence to zero; this assumes that surveillance systems could detect transmission, if any. Theoretically, eradication should obviate the need for further control measures other than surveillance (as with smallpox).

Aside from the common requirements for adequate resource commitment, broad advocacy and political will relevant to all disease eradication initiatives, there are biologic and epidemiologic factors that specifically affect the feasibility of eradication of smallpox, polio, measles, and malaria. Table 1 summarizes these

Review articles synthesize in narrative form the best available evidence on a topic.

Summary Points

- Lessons from the smallpox, poliomyelitis, and measles eradication/elimination initiatives (in particular, the importance of starting laboratory, clinical, and field research early in the program and continuing research in parallel) should be incorporated into any malaria eradication initiative from the onset

- Vaccines are likely to be the lynchpin interventions of elimination/eradication programs, but ongoing research will be needed to improve formulations, delivery, and immunization schedules

- Surveillance will be critical throughout any elimination/eradication initiative, coupled with improved diagnostic methods to detect asymptomatic infections and low rates of transmission

- Because socio-cultural, religious, and local politics can impede eradication efforts, it is prudent to support research into improving ways to communicate effectively with local populations about the disease and the interventions to eradicate it

- A cross-cutting theme among the viral disease programs is that interrupting the last vestiges of transmission is particularly problematic and requires allocation of many resources including support for focused "last kilometre" research activities

salient factors. Table 2 provides illustrative examples in which research played an important role in the eradication of smallpox, the near-eradication of polio, and the elimination of measles from the Americas and some other countries. From these experiences, lessons were learned that are applicable to the Malaria Eradication Program and that should, we believe, be incorporated in the

Malaria Eradication Research Agenda (malERA) described in this Supplement.

Lesson 1. Research Should Accompany Elimination/Eradication Efforts from the Outset

The foremost lesson learned from eradication/elimination efforts for viral diseases is that a flexible research agenda must be initiated early, prior to or concomitant with the launch of eradication interventions.

Smallpox

Since 1959, when the World Health Assembly (WHA) resolved to undertake global smallpox eradication, research played an integral role in every facet of its implementation [2]. Without the products of field and laboratory research and their incorporation into the program, eradication would not have been achieved. Research improved vaccine production methods to assure the universal availability of potent, heat-stable products [3,4] and provided improved instruments and methods for performing vaccination [5,6]. Field studies yielded new insights into the epidemiologic behaviour of smallpox under differing circumstances and identified optimal preventive and containment methods for control, elimination, and eradication [2,7–10].

Between 1959 and 1966, progress in smallpox eradication was limited. Then, in 1966 the WHA intensified the effort by allocating US$2.4 million for the program. An overall strategy was formulated that included vaccination of 80% of the population in each country using vaccines of assured potency and establishment in all countries of a weekly reporting system from all health units with plans to vaccinate contacts and neighbours of all cases to stop each outbreak rapidly—an approach termed "surveillance-containment" [11]. In 1967, 43 countries reported 132,000 cases

Table 1. A comparison of the inherent salient features of smallpox, polio, measles, and malaria infections that favour or impede elimination of the disease and the most effective past and current interventions.

Feature	Smallpox	Polio	Measles	Malaria
Disease syndrome is recognized by the public	Yes	Yes (paralytic form)	Yes	Variable
Extent of clinical expression	100%	<1% (many subclinical and nonparalytic cases)	~100%	Often low
Specificity of the clinical disease	High	High for paralytic disease; low for nonparalytic disease	Moderate	Often low
n serotypes or species	2: *V. major* (high case fatality) and *V. minor* (low case fatality)	3	1	5[a]
Reservoir	Humans	Humans	Humans	Humans (except for *P. knowlesi*)[a]
Transmissibility	Usually low to moderate	High	Very high	Variable
Seasonality	Yes (regional)	Yes (regional)	Yes (regional)	Often
Incubation period (d)	12–14	6–20	9–13	~12
Immunity follows a single clinical infection	Yes	Yes (type specific)	Yes	No[b]
Interventions	Vaccine (live)	Vaccines (live oral and killed parenteral)	Vaccine (live subcutaneous)	ITNs; ACTs; IRS; IPTp; IPTi

[a]*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* are restricted to human hosts. *P. knowlesi*, which mainly infects nonhuman primates, can also cause disease in humans following natural transmission.
[b]However, the development of immunity against clinical disease follows repeated infections.
ACT, artemisinin combination therapy; IPTi, intermittent preventive treatment in infants; IPTp, intermittent preventive treatment in pregnancy; IRS, indoor residual spraying; ITN, insecticide treated bednets.
doi:10.1371/journal.pmed.1000405.t001

Table 2. Research outputs that contributed to the eradication of smallpox and the regional elimination of polio and measles (or outputs that are still undergoing evaluation or development): Lessons for the rejuvenated Malaria Eradication Program.

Research Output	Smallpox	Polio	Measles	Malaria
Basic research	Heat-stable vaccine; Bifurcated needle; Differentiation of orthopoxviruses based on genomic sequence analysis; Search for candidate antiviral drugs with activity against *Variola* (disappointing results in clinical trials)	Identification of 3 serotypes; Development of live and killed virus vaccines; Modern monovalent (type 1 or 3) and bivalent (types 1 and 3) vaccines; Sequencing of viral isolates; Search for safe and effective antiviral drugs	Live measles vaccine strains; IgM measles antibody diagnostics; Oral fluid-based diagnostic assays; Sequencing of viral isolates to obtain epidemiologic insights; Measles H DNA vaccine (to prime very young infants immunologically so they can respond safely and effectively to current live vaccine)	Biology of liver stage parasites; *In vitro* culture of *P. vivax*; Sensitive, simple, point of care diagnostics to detect both symptomatic and asymptomatic infections; Single encounter radical cure and prophylaxis drug; Vaccines to interrupt transmission; New effective insecticides that are safe for humans
Clinical research	Immunogenicity of vaccine administered by new methods of delivery (e.g., Ped-O-Jet; bifurcated needle); Evaluation of antiviral agents (marboran, cytosine, adenine arabinoside)	Immunogenicity of tOPV in different settings; Immunogenicity of monovalent and bivalent vaccines; Duration of OPV excretion by immunocompromised subjects	Identification of a correlate of protection (serum plaque reduction neutralizing antibody); Immune responses following initial immunization and following booster dose; Respiratory tract administration of vaccine by small particle aerosol or by large droplet spray	Improved measures of immune response; Identify immunologic correlates of protection
Field research	Definition of transmission indices; Surveillance/containment strategy; Discovery of monkeypox	Impact of national and subnational mass immunizations; Identification of outbreaks due to circulating vaccine-derived polioviruses; Anthropological and sociological studies to enhance local support for vaccination	Identification of the "window of vulnerability" in infants; Impact of national and subnational mass immunizations; Coupling mass measles immunization with OPV and antihelminthic administration and bednet distribution	Improved methods to measure malaria transmission in different settings; Improved methods for measurement of malaria morbidity and mortality; Studies of local vectors to identify points of intervention

doi:10.1371/journal.pmed.1000405.t002

of smallpox, but studies revealed that only 1%–2% of cases were being reported at that time. [12]. The goal was to stop smallpox transmission in 10 years. The last case occurred 10 years, 11 months, and 26 days later.

Polio

Poliomyelitis (polio) was one of six childhood diseases targeted for control in 1974 by the World Health Organization (WHO) through the Expanded Program on Immunization (EPI). Research during the Smallpox Eradication Program confirmed the feasibility of coadministering multiple antigens and experience was acquired in managerial aspects of vaccine delivery and disease surveillance [13]. However, polio outbreaks continued in low- and middle-income countries, mostly tropical/subtropical, despite routine administration of trivalent oral polio vaccine (tOPV) [14].

In 1980, Brazil began coordinated mass administration of tOPV (supplementary immunization activity) twice annually to all children <5 years of age [15], and a dramatic reduction of paralytic polio incidence ensued. Encouraged by the success of this strategy, in 1985 the Pan American Health Organization resolved to eliminate polio in the Americas by 1990. In 1988, the WHA resolved to eradicate polio worldwide by 2000 [16] and the Global Polio Eradication Initiative (GPEI) was established in partnership with UNICEF, the US Centers for Disease Control and Prevention, and Rotary International; WHO was responsible for overall management, program implementation, and fundraising.

In GPEI's early years, with funding shortages and success of the program in the Americas, research was not a priority. Nevertheless, limited applied research, driven by emerging operational needs and gaps, led to advances in vaccine logistics, cold chains, monitoring and evaluations, laboratory methodology, and surveillance to detect cases of acute flaccid paralysis (AFP) [17].

Appreciation by GPEI of the need for intensified research grew as new programmatic challenges and findings about polio virology and epidemiology were encountered and posteradication questions emerged. With awareness that additional approaches would be essential if the target date for global interruption of transmission was to be met, a Global Technical Consultative Group was convened in 1996 to address challenges in eradication progress [18]. Although polio due to type 2 wild poliovirus was eradicated globally in 1999, cases and outbreaks due to types 1 and 3 continued. And, rather than marking global eradication, the year 2000 saw an unexpected outbreak of 21 polio cases in Hispaniola caused by circulating vaccine-derived poliovirus [19]. Recognition that vaccine-derived poliovirus could cause epidemics of AFP reinforced the need for flexible research efforts to respond expeditiously to emerging needs [19,20].

By 2004 [21], newer challenges to eradication in endemic regions were recognized, including low tOPV efficacy in certain populations, low herd immunity, and the participation of vaccinated children in wild poliovirus circulation—collectively considered as "failure-of-vaccine." More frequent supplementary immunization activity proved to be inadequate, highlighting the need for research to elucidate virus transmission better and to identify correlates of protection relevant at the population level. Consequently, the WHO Polio Research Committee was established in 2008 to provide a forum for addressing timely research questions [22]; the Advisory Committee on Poliomyelitis Eradication now provides oversight for research and application of findings in program implementation [23].

In retrospect, anticipating research questions was difficult when the path to polio eradication seemed straightforward. Today, 10 years past the original eradication goal, research has greatly expanded, including ongoing research in operations, evidence-based communication strategies to overcome socio-cultural or

religious belief-based resistance to vaccination [24], vaccines and immunity, molecular epidemiology, mathematical modeling [25], and a search for antivirals to curtail virus shedding.

The lesson learned from GPEI that research should accompany elimination/eradication efforts from the outset applies directly to the unsuccessful Malaria Eradication Program of 1955–1969. This Program relied heavily on indoor spraying with residual insecticides and detection of cases and treatment with chloroquine as the primary interventions. Without a strong ongoing research program within a flexible infrastructure, this program could not respond adequately to the emergence of widespread mosquito resistance to DDT and parasite resistance to chloroquine.

Measles

Measles, one of the most communicable of all infectious diseases, exhibits an extraordinary propensity to reach susceptible individuals even when they constitute only a small proportion of the population [26]. In the prevaccine era, all children experienced measles unless they lived in remote areas. [27]. The clinical expression of infection approached 100% and led to life-long protection against disease; the occasional individuals with subclinical infection did not, apparently, transmit virus.

The case fatality rate of measles in malnourished infants in developing countries exceeds 20% [26]. In 1999, measles was the third most common cause of death among children <5 years of age in developing countries and the most common vaccine-preventable cause. The gravity of measles disease and its complications and the magnitude of the human and economic tolls exacted are often insufficiently perceived by health professionals and the public: even in industrialized countries measles can be severe with at least one case among every 1,000 proving fatal [28].

In 1994, health ministers in the Americas committed to eliminating measles from the Western Hemisphere [29], using a triple pincer vaccination strategy consisting of a one-time "catch-up" campaign targeting children 9 months through 14 years of age (to interrupt wild-virus circulation), strengthened services to "keep-up" routine measles vaccination in infants, and "follow-up" campaigns to maintain immunity in the preschool age group. Indigenous transmission was interrupted by 2003, despite repeated importations of measles from Europe and Japan [29].

Since 2000, considerable progress has been made worldwide in diminishing mortality from measles through immunization campaigns, particularly in sub-Saharan Africa [30]. When high coverage is achieved, campaigns eliminate measles virus from the community and indirectly protect young infants by diminishing the force of infection. During field research in Togo in 2004 [31], a national campaign to administer measles vaccine to all children 9–59 months of age was coupled with giving oral polio vaccine (OPV), an oral antihelminthic, and insecticide-treated bednets; >90% vaccination coverage was achieved. However, in some African countries repetitive mass campaigns are proving difficult to sustain and measles mortality in young children remains problematic [32].

Research that helped interrupt transmission of measles in the Western Hemisphere and to diminish measles mortality in Africa includes studies of measles transmission in different populations, improved diagnostic tests and sero-epidemiologic methods, molecular finger printing to determine the geographic origin and relatedness of measles virus isolates [33,34], and improved methods of immunizing against measles using existing vaccines [35]. Other research focuses on developing new vaccines to immunize high-risk target groups (e.g., very young infants) who

cannot be effectively immunized with currently licensed measles vaccines [36].

Lesson 2. The Reservoirs of Infection and Degree and Specificity of Clinical Expression Influence the Eradication Program

A feature common to smallpox, poliomyelitis, measles, and *Plasmodium falciparum* and *Plasmodium vivax* malaria is that humans constitute the sole reservoir of these pathogens; one need not worry about animal or environmental reservoirs as sources of reintroduction into human populations.

Smallpox

The discovery of human monkeypox exemplifies the importance of research to confirm the absence of a nonhuman reservoir for diseases targeted for eradication, including malaria. The monkeypox exanthem in humans resembles smallpox, albeit with milder clinical symptoms and lower fatality rates. Accordingly, during and after smallpox eradication in Africa there was concern about the possible persistence of this orthopoxvirus virus in natural settings [37]. Epidemiologic and laboratory research on monkeypox in enzootic areas of Africa [38–40] confirmed that it did not spread easily in human populations and posed only a small threat for becoming an endemic human illness [39,40], even though some localized foci have been identified [37]. Recent reports of human infections with the nonhuman primate parasite *Plasmodium knowlesi* [41], raise concerns that, in certain ecologies, *P. knowlesi* may increase in humans as *P. falciparum* disappears.

Polio

Smallpox and measles have ~100% clinical expression in immunocompetent persons and asymptomatic chronic infections do not occur. By contrast, many asymptomatic or mild cases of poliovirus, *P. falciparum*, and *P. vivax* infection occur for every clinical case. Early epidemiologic field research of polio identified ~150 infections that did not progress to paralysis for each case of AFP [42]. Moreover, persons with B-cell immunodeficiencies can chronically excrete vaccine polioviruses. These hidden reservoirs make polio and malaria eradication fundamentally more difficult than smallpox. Improved diagnostic tests are needed to identify persons infected with polio and malaria, as cases become less common.

Measles

Akin to the clinical confusion of measles with rubella and other febrile exanthemata, clinical *P. falciparum* and *P. vivax* infections are easily confused with many other febrile disorders. In another parallel, immunocompromised individuals with measles giant cell pneumonia may shed virus without having a rash and malaria-immune individuals may have parasites in their blood in the absence of clinical symptoms and may act as infectious source for the mosquito vector. The most vexing issue in malaria elimination/eradication is *P. vivax* hypnozoites, a form of the parasite resident in the liver that creates persistent (for years), silent infection that is nonresponsive to standard treatment for clinical malaria. The only current drug effective against *P. vivax* hypnozoites is the 8-aminoquinoline primaquine.

To summarize, the lesson learned here is that malaria eradication will be facilitated by improved diagnostics that can detect mild and asymptomatic blood infections and that can identify asymptomatic persons harboring *P. vivax* hypnozoites. A corollary lesson is that high priority should be placed on developing new, well-tolerated drugs to treat persons with latent

P. vivax infection, particularly individuals genetically deficient in glucose 6-phosphate 1-dehydrogenase (who develop hemolytic anemia when treated with primaquine). malERA's concept of developing a Single Encounter Radical Cure and Prophylaxis ("SERCaP") drug, if successful, would accomplish that.

Lesson 3. The Critical Role of Surveillance

A theme common to the smallpox, polio, and measles eradication/elimination programs is the critical role that surveillance has played in every phase, including quantification of the burden at the onset of the program; monitoring progress of the program at local, national, and global levels; intensive searches for the last cases and infected persons; and documentation of the interruption of transmission. The critical role of surveillance necessitated research to develop new epidemiologic surveillance systems for all three diseases and, for measles and polio, sero-epidemiologic methods, tests to identify asymptomatic carriers, and molecular methods to establish the geographic source and relatedness of isolates from outbreaks and clusters over different time periods. This lesson is directly applicable to the Malaria Eradication Program, which will need to assure that adequate surveillance methods and techniques are in place to monitor the effectiveness of the program.

Smallpox

The magnitude of the smallpox problem was largely unknown in 1959, despite the International Health Regulation that all smallpox cases be reported. Finding and controlling outbreaks quickly was essential for the containment strategy. Accordingly, within each country, all health care facilities were asked to provide a weekly report about smallpox cases. Every 3 weeks, international surveillance reports were prepared and widely distributed that charted progress by country, informed new findings through research, and recommended changes in strategy. These reports and special national reports developed by some countries were invaluable in rapidly informing all concerned about progress in the program and in conveying new discoveries and new directions for the program.

Another aspect of smallpox eradication that might be relevant to the malaria elimination/eradication program is the rigorous program of certification of absence of smallpox that began in the 1970s and that was intensified until the WHA confirmed global eradication in 1980. Tens of thousands of specimens from persons with "fever and rash" were collected with well-publicized rewards being offered to persons reporting any patient with confirmed smallpox.

Polio

Pathogens other than polioviruses also cause AFP. A measure of the quality of polio surveillance is the adequacy of detection of AFP cases and the proportion of cases from whom stool specimens are obtained for virological analysis. Moreover, paralytic polio cases represent only the tip of the epidemiologic iceberg. Thus, polio shares with malaria the attribute that many persons harbouring infection will be clinically unsuspected. In the context of eradication, all infected individuals are epidemiologically important [43]. Consequently, malERA has rightly given high priority to the development of improved tests to detect clinically typical, mild and asymptomatic *Plasmodium* infections and to assess the extent of transmission.

Measles

Measles outbreaks must be detected and curtailed to limit transmission following importations. For outbreak detection, specific, practical, and rapid measles diagnostic tests are needed. Research developed such tests and the strategies to use them. Serum specimens and either urine or nasopharyngeal samples are obtained from suspect measles cases and, as appropriate, from contacts [44]. The serum is tested for measles-specific immunoglobulin M (IgM) antibodies indicative of acute infection. A noninvasive alternative involves collecting oral fluid [45]. Measles virus in urine or nasopharyngeal specimens is detected by culture or reverse transcriptase PCR. Unfortunately, these tests are not suitable for point-of-care diagnosis. A simple, rapid, inexpensive, sensitive, and specific point-of-care diagnostic for measles will facilitate eradication efforts. Similarly, malERA has identified the need for a sensitive, specific, and inexpensive diagnostic test amenable to use in the field.

Lesson 4. Molecular Epidemiology

Research fostered by the viral disease eradication/elimination programs has shown how molecular tools add precision to surveillance. The molecular epidemiologic evaluation of plasmodial parasites will be similarly helpful, particularly in the later stages of a Malaria Eradication Program; research in this area should be encouraged.

Smallpox

Genetic analysis of isolates of orthopoxviruses from patients and animals has shown the important differences among smallpox viruses (*Variola major* and *V. minor*), monkeypox, and vaccinia that are useful for surveillance [46,47].

Polio

Partial genomic sequencing of all wild poliovirus isolates is undertaken to determine genetic relatedness. Each 1% difference between two isolates correlates with approximately 1 year of undetected circulation between the specific chains of transmission. A difference of >1.5% suggests undetected past transmission, thereby identifying inefficient surveillance systems. In addition, timely genome sequencing and construction of phylogenic trees make it possible to assess eradication progress through genetic cluster elimination, to identify human reservoirs, to differentiate indigenous from imported viruses, to identify surveillance gaps (through isolates without recent parental strains), and to identify vaccine-derived polioviruses and quantify their period of circulation [20,48].

Measles

Genotyping of measles viruses allows identification of the geographic origin of imported viruses/cases and provides a means of tracking epidemiologic relationships among cases in the same or separate outbreaks [33,34].

Lesson 5. The Pivotal Role of Vaccines as a Tool for Disease Eradication

The eradication of smallpox and of type 2 poliovirus infection globally, and the elimination of polio and measles from various regions and countries was achieved using vaccines as the primary intervention tool. As malaria transmission diminishes, other interventions (e.g., vector control, insecticide-impregnated bednets, new drugs, etc.) will surely play critical roles, but the lesson from the viral disease programs is that vaccines that interrupt transmission could play a critical role in helping to eradicate malaria.

Smallpox

Until the 1960s, smallpox vaccine was typically a liquid product of suboptimal potency, readily inactivated by heat within a few days. Industrial process research developed a method for producing heat-stable, freeze-dried smallpox vaccine [4] that could withstand temperatures of 37°C for at least 1 month. With technical assistance from industrialized countries, >80% of lyophilized smallpox vaccine of acceptable quality was being manufactured in developing countries within 6 years of the eradication program starting. Having smallpox vaccine that did not require refrigeration was of immeasurable practical importance in the field [11].

At the onset of the eradication program, age-old, traditional techniques of scratching or pressing the vaccine into the skin frequently failed to immunize. New vaccination techniques were introduced that permitted more rapid and effective inoculations. Jet injectors were perfected and field tested that could vaccinate hundreds of people per hour. By 1971, the injectors were superseded by a simple two-pronged (bifurcated) needle [49]. WHO tested these needles for a unique multiple-puncture vaccination technique. Successful vaccination responses approached 100%, less vaccine was required for each vaccination, instruction in vaccination required only ~15 minutes, and the needles could be sterilized and reused repeatedly. In Africa and Asia, with a good working rapport with villagers and their leaders, a vaccinator with bifurcated needles could average 500 vaccinations per day. To measure vaccination coverage and vaccine "take" rates (vesicle or early crusting lesion on the skin 1 week after vaccination), a sample survey of villagers was routinely checked [50].

The impact of the bifurcated needle in improving the logistics of smallpox vaccination was immense. A possible analogous situation for malaria eradication may arise with the need to identify practical ways to deliver the promising attenuated sporozoite vaccines that are under development [51–53].

Smallpox field research may also provide lessons for malaria eradication efforts. For example, smallpox outbreak containment teams that were deployed to the field to determine how smallpox outbreaks spread and to vaccinate contacts and neighbours of patients discovered that smallpox did not spread as rapidly and widely as textbooks described. Chains of smallpox transmission could be broken in most areas by the surveillance-containment approach, and this approach was soon given priority over mass vaccination. Similarly, field research showed that smallpox vaccine protection lasted at least 10 years, not 3–5 years as traditionally thought. Recent research on immunologic memory has established the basis for the long-lived protection [54].

Polio

Research in the 1950s created two polio vaccines—an oral approach based on three live attenuated poliovirus strains (originally administered sequentially but subsequently licensed as a trivalent formulation) [43], and an intramuscular vaccine consisting of three formalin-inactivated polioviruses. Although both vaccines drastically diminished polio cases in industrialized countries, tOPV was selected as the lynchpin of the GPEI, being less expensive and easier to administer. Failure to achieve the goal of polio eradication by 2000 was attributed to inadequate vaccination coverage and research recommendations were primarily operational in nature. However, it has since become apparent that there are major gaps in our understanding of immune mechanisms. Current research priorities include the development of surrogate measures of mucosal immunity and interventions to boost and prolong immunity, and the determina-

tion of the relationship between waning immunity and virus circulation. Research is also addressing the observation that tOPV in infants appears to be less immunogenic in some areas in India than elsewhere [55–58].

Recognizing that type 2 polio has been eradicated since 1999 but that type 1 and 3 disease continues, an accelerated collaborative research and development effort undertaken with industry resulted in the licensure and use of monovalent type 1 and 3 vaccines and a novel bivalent (types 1 and 3) OPV formulation [23,59]. Deleting the more immunogenic and dominant type 2 virus that interferes with responses to the type 1 and 3 viruses allows enhanced serological responses to types 1 and 3. The bivalent vaccine has now become the preferred tool in supplemental immunization campaigns.

Measles

Cell culture propagation of measles virus in 1954 was followed by development of the first generation of parenteral live measles vaccines, which were protective but associated with unacceptably high rates of febrile reactions. Further research yielded the current well-tolerated vaccines. Inactivated measles-virus vaccines had also been licensed in 1963 based on safety, immunogenicity, and short-term efficacy data [60]. However, immunity was short-lived; postlicensure surveillance revealed that some vaccine recipients developed a syndrome of atypical measles when subsequently exposed to wild measles virus [61,62]. Accordingly, inactivated measles vaccine use was discontinued by 1967.

The fall in measles cases following introduction of the first generation measles vaccine in the United States in 1963 prompted epidemiologists to predict that measles could be eliminated country-wide by 1967, if vaccine could be administered routinely to infants and to susceptibles at school entry and if surveillance and epidemic control could be strengthened [63]. Although measles incidence fell by >90% by 1967, it took 26 more years until indigenous transmission was interrupted in the United States. This achievement required a routine second dose of vaccine before school entry and a reduction in imported infections consequent to enhanced measles control elsewhere [64].

By 1999, most measles deaths were occurring among children in the Indian sub-continent and sub-Saharan Africa, despite recommendations that measles vaccine should be given routinely to infants ~9 months of age. A notable proportion of these measles deaths clustered among young infants during their "window of vulnerability" (approximately 4–9 months of age) [65], when falling titres of maternally derived measles antibodies no longer protect against disease but nevertheless interfere with successful immunization. Reports that immunogenicity could be enhanced in infants <6 months of age by administering high-titre vaccine generated optimism that a solution to protecting young infants might be at hand [66]. However, this approach was soon abandoned when long-term follow-up revealed unexplained increased mortality in female children [67].

Three new research efforts are addressing ways to protect young infants in developing countries, to provide adjunct tools for measles elimination [68]. The first involves repetitive follow-up mass immunization of children with the existing vaccines to indirectly protect young infants [30]. The second involves clinical trials to allow licensed vaccine to be administered to the respiratory tract by small particle aerosol, thereby making mass immunization simpler and safer [35]; clinical research has shown that vaccine delivered to the nasal mucosa by large droplet spray is ineffective [69]. The third research effort has resulted in development of a candidate measles DNA vaccine encoding the hemagglutinin (H) antigen of measles virus [36].

Lesson 6. Modes of Transmission and Modeling

Smallpox and measles viruses are transmitted by the respiratory route (droplets/aerosol), while polio is mainly transmitted by the fecal-oral route in developing countries. Although modeling played no role in smallpox eradication, it has been extremely useful in the GPEI as a valuable epidemiologic research tool, for addressing economic issues, and for providing insight into future programmatic options [25]. Modeling research is currently addressing the risks of virulent vaccine-derived poliovirus that may be chronically shed by immunodeficient individuals and from circulating vaccine-derived poliovirus, after OPV is withdrawn posteradication [70]. Similarly, measles was one of the first infectious diseases studied with models, and models are now being used to elucidate better the epidemiologic behaviour of measles and predict the effect of interventions [71,72]. Although the ability to generalize from models is debated [73], there is consensus that the quality of input data is steadily improving, even as the epidemiology of measles is changing globally.

Malaria, spread by female *Anopheles* mosquitoes, has a more complex transmission than these viral infections, which allows transmission to be decreased by targeting to control the vector or vector-host contact, as well as by changing susceptibility of the human host. Modeling is therefore particularly important to predict the effect of various interventions used independently and in unison on the transmission of malaria. It can also identify ways to minimize and delay parasite resistance to drugs [74] and should be an integral part of any malaria elimination/eradication program, as recognized by malERA.

Lesson 7. Sociological, Anthropological, Cultural, and Religious Issues

Another lesson for malaria from the viral eradication/elimination programs is the important role that socio-cultural, religious, and local political factors play in public perception of the disease and of the main intervention tools of the eradication program; these factors can accelerate or impede eradication efforts. It is prudent to support research on these issues and on improving ways to communicate effectively with local populations. In this area of research, one size does not fit all.

Smallpox

Smallpox was a severe, commonly lethal infection that often left survivors scarred and occasionally blind. Thus, in most endemic areas smallpox was recognized and feared by the population. Aversion to vaccination was not, therefore, a major impediment during the Smallpox Eradication Program.

Polio

As paralytic polio (a relatively rare disease) diminished in incidence and became less of a threat, it became increasingly difficult to motivate populations to continue support for eradication activities. The GPEI and public health authorities worldwide became concerned by events in Nigeria in 2003–2004 that set polio eradication back there and in much of Africa. In late 2003, several states in northern Nigeria refused to participate in national mass immunization campaigns. Religious and political leaders in three states counseled parents against having their children immunized, preaching that the vaccine was contaminated with antifertility hormones, HIV, and cancer-inducing agents [75]. Only after a Nigerian team (including members from the affected states) visited a manufacturer of OPV in Indonesia, a Muslim country, did the state governments accept that OPV was safe [75].

Confidence was restored and progress in polio eradication has since been achieved in Nigeria and elsewhere in Africa [76]. The lesson here is that evidence-based communication strategies must be carefully planned and implemented to overcome resistance to vaccination that originates from socio-cultural or religious beliefs [24,77].

Measles

A potential barrier to global eradication of measles is the poor measles vaccine coverage in many industrialized countries (in Europe and Japan) where strong antivaccine movements specifically target the measles vaccine. Without supporting scientific evidence, these antivaccine groups indict measles vaccine as a cause of autism and other chronic disorders. Continuing measles transmission in such industrialized countries maintains a reservoir that imperils elimination efforts in other countries. Further research in communications, anthropology, and sociology must be undertaken to find ways to counteract the antivaccine propaganda and increase the acceptance of measles vaccine.

Lesson 8. The Concept of "The Last Kilometre"

A cross-cutting theme among the smallpox, polio, and measles eradication/elimination programs is that interruption of the last vestiges of transmission in a country or region is problematic and requires the allocation of as many resources as the early stages that achieved a 90%–99% reduction in incidence. Therefore, interventions often need to be modified, sometimes drastically, to complete the job of elimination.

Similarly, in the future, the final stages of the Malaria Eradication Program will likely confront barriers as complex, demanding, and refractory as ones encountered early in the program. Some will be resolvable only through directed, focused research. Thus, the rejuvenated Malaria Eradication Program should support a flexible research infrastructure that can adapt to the challenges.

Lesson 9. Posteradication Agendas

The final lesson learned from the viral disease eradication programs is that discussion of posteradication scenarios, problems, and potential solutions must begin at the onset of the programs. Focused research can find early solutions for some posteradication issues. In the case of smallpox, affirmation of the eradication of smallpox was followed by a discontinuation of routine vaccination globally. The only way that smallpox disease can occur anew is if nefarious individuals with access to virus undertake a deliberate bioterror release. In the case of polio, however, since 2005, GPEI has been grappling with posteradication questions of use of OPV, the quandary of vaccine-derived poliovirus persistence, laboratory destruction and containment of poliovirus stocks, surveillance needs, vaccine compositions, and response strategies. These questions have become the drivers of a research agenda [78]. For measles, the major posteradication dilemma will be whether to continue routine immunization with the live measles vaccine. Given that in some industrialized countries, certain groups in the population view measles vaccine with more suspicion than the wild virus, it might be necessary to develop and utilize an alternative nonliving type of measles vaccine [36].

Concluding Comments

Nine cross cutting lessons have been provided by these three vaccine-dependent eradication and elimination programs of viral diseases in which research was integral to guide program activities.

These lessons will be useful to the revitalized Malaria Eradication Initiative. Research played a critical role in the Smallpox Eradication Program and is still contributing critically to the GPEI and measles elimination and mortality control programs. Despite having tools for primary prevention, considerable research has been essential to address geographic variations in the force of transmission of smallpox, polio, and measles and to adjust the tactical use of the preventive tools.

The ecology and epidemiology of malaria are far more complex than the ecology and epidemiology of these viral infections. Thus, if a global Malaria Eradication Initiative is revived, from the outset the Malaria Eradication Research Agenda should be incorporated as an essential component. Malaria eradication proponents should

understand the importance of combining operational and research issues. Over time in successful elimination initiatives, the best researchers will see their ideas implemented and the best implementers will continue to ask what research could further improve operations.

Author Contributions

ICMJE criteria for authorship read and met: JGB CAdQ WRD WHF DAH TJJ MML. Agree with the manuscript's results and conclusions: JGB CAdQ WRD WHF DAH TJJ MML. Wrote the first draft of the paper: JGB DAH MML. Contributed to the writing of the paper: JGB CAdQ WRD WHF DAH TJJ MML. Wrote a section of the first draft of the paper; contributed to revisions and editing of the paper: TJJ.

References

1. Hinman A (1999) Eradication of vaccine-preventable diseases. Annu Rev Public Health 20: 211–229.
2. Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID (1988) Smallpox and its eradication. Geneva: World Health Organization.
3. Arita I (1972) The control of vaccine quality in the smallpox eradication programme. International Symposium on Smallpox Vaccine. 19. Basel: Karger. Symposium Series on Immunobiological Standardization.
4. Sparkes JD, Fenje P (1972) The effect of residual moisture in lyophilized smallpox vaccine on its stability at different temperatures. Bull World Health Organ 46: 729–734.
5. Roberto RR, Wulff H, Millar JD (1969) Smallpox vaccination by intradermal jet injection. 2. Cutaneous and serological responses to primary vaccination in children. Bull World Health Organ 41: 761–769.
6. Pattanayak S, Arora DD, Sehgal CL, Raghavan NG, Topa PK, et al. (1970) Comparative studies of smallpox vaccination by the bifurcated needle and rotary lancet techniques. Bull World Health Organ 42: 305–310.
7. Thomas DB, Arita I, McCormack WM, Khan MM, Islam S, et al. (1971) Endemic smallpox in rural East Pakistan. II. Intervillage transmission and infectiousness. Am J Epidemiol 93: 373–383.
8. Foege WH, Millar JD, Lane JM (1971) Selective epidemiologic control in smallpox eradication. Am J Epidemiol 94: 311–315.
9. Mack TM, Thomas DB, Muzaffar KM (1972) Epidemiology of smallpox in West Pakistan. II. Determinants of intravillage spread other than acquired immunity. Am J Epidemiol 95: 169–177.
10. Basu RN, Jezek Z, Ward NA (1979) The eradication of smallpox from India. 2. History of International Public Health. New Delhi: World Health Organization, Southeast Asia Regional Office.
11. Foege WH, Millar JD, Henderson DA (1975) Smallpox eradication in West and Central Africa. Bull World Health Organ 52: 209–222.
12. Hughes K, Foster SO, Tarantola D, Mehta H, Tulloch JL, et al. (1980) Smallpox surveillance in Bangladesh: II - Smallpox facial scar survey assessment of surveillance effectiveness. Int J Epidemiol 9: 335–340.
13. Ruben FL, Smith EA, Foster SO, Casey HL, Pifer JM, et al. (1973) Simultaneous administration of smallpox, measles, yellow fever, and diphtheria-pertussis-tetanus antigens to Nigerian children. Bull World Health Organ 48: 175–181.
14. Aylward RB, Cochi SL, Heymann DL (2009) Polio eradication: ongoing innovation to end an ancient scourge. Levine MM, Dougan G, Good MF, Liu MA, Nabel GJ, et al, eds. New generation vaccines. 4th edition. New York: Informa Healthcare.
15. Baptista RJ, Jr. (1984) The control of poliomyelitis in Brazil. Rev Infect Dis 6 Suppl 2: S400–S403.
16. Dowdle W, Cochi S (2002) Global eradication of poliovirus: history and rationale. Semler B, Wimmer E, eds. Molecular biology of picornaviruses. Washington (D.C.): American Society for Microbiology.
17. De Quadros CA, Hersh BS, Olive JM, Andrus JK, Da Silveira CM, et al. (1997) Eradication of wild poliovirus from the Americas: acute flaccid paralysis surveillance, 1988-1995. J Infect Dis 175 Suppl 1: S37–S42.
18. World Health Organization Expanded Programme on Immunization (1997) Expanded programme on immunization (EPI). Progress towards the global eradication of poliomyelitis, 1996. Wkly Epidemiol Rec 72: 189–194.
19. Kew O, Morris-Glasgow V, Landaverde M, Burns C, Shaw J, et al. (2002) Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. Science 296: 356–359.
20. Kew OM, Sutter RW, de Gourville EM, Dowdle WR, Pallansch MA (2005) Vaccine-derived polioviruses and the endgame strategy for global polio eradication. Annu Rev Microbiol 59: 587–635.
21. Ad Hoc Advisory Committee on Poliomyelitis Eradication (2004) Conclusions and recommendations of the Ad Hoc Advisory Committee on Poliomyelitis Eradication, Geneva, 21-22 September 2004. Wkly Epidemiol Rec 79: 401–407.
22. Polio Research Committee (2010) Polio Research Committee: conclusions and recommendations. The polio pipeline. Geneva: Global Polio Eradication Initiative, World Health Organization.
23. Advisory Committee on Poliomyelitis Eradication (2009) Advisory Committee on Poliomyelitis Eradication: recommendations on the use of bivalent oral poliovirus vaccine types 1 and 3. Wkly Epidemiol Rec 84: 289–290.
24. Obregon R, Chitnis K, Morry C, Feek W, Bates J, et al. (2009) Achieving polio eradication: a review of health communication evidence and lessons learned in India and Pakistan. Bull World Health Organ 87: 624–630.
25. Tebbens RJ, Pallansch MA, Kew OM, Caceres VM, Jafari H, et al. (2006) Risks of paralytic disease due to wild or vaccine-derived poliovirus after eradication. Risk Anal 26: 1471–1505.
26. Duke T, Mgone CS (2003) Measles: not just another viral exanthem. Lancet 361: 763–773.
27. Black FL (1966) Measles endemicity in insular populations: critical community size and its evolutionary implication. J Theor Biol 11: 207–211.
28. Gindler J, Tinker S, Markowitz L, Atkinson W, Dales L, et al. (2004) Acute measles mortality in the United States, 1987-2002. J Infect Dis 189 Suppl 1: S69–S77.
29. De Quadros CA (2004) Can measles be eradicated globally? Bull World Health Organ 82: 134–138.
30. World Health Organization (2007) Progress in global measles control and mortality reduction, 2000-2006. Wkly Epidemiol Rec 82: 418–424.
31. Takpa V, Morgah K, Gbedonou P, Eliades M (2005) Distribution of insecticide-treated bednets during an integrated nationwide immunization campaign–Togo, West Africa, December 2004. MMWR Morb Mortal Wkly Rep 54: 994–996.
32. Grais RF, Dubray C, Gerstl S, Guthmann JP, Djibo A, et al. (2007) Unacceptably high mortality related to measles epidemics in Niger, Nigeria, and Chad. PLoS Med 4: e16. doi:10.1371/journal.pmed.0040016.
33. Tischer A, Santibanez S, Siedler A, Heider A, Hengel H (2004) Laboratory investigations are indispensable to monitor the progress of measles elimination–results of the German Measles Sentinel 1999-2003. J Clin Virol 31: 165–178.
34. Mulders MN, Truong AT, Muller CP (2001) Monitoring of measles elimination using molecular epidemiology. Vaccine 19: 2245–2249.
35. Omer SB, Hiremath GS, Halsey NA (2010) Respiratory administration of measles vaccine. Lancet 375: 706–708.
36. Pasetti MF, Resendiz-Albor A, Ramirez K, Stout R, Papania M, et al. (2007) Heterologous prime-boost strategy to immunize very young infants against measles: pre-clinical studies in rhesus macaques. Clin Pharmacol Ther 82: 672–685.
37. Rimoin AW, Mulembakani PM, Johnston SC, Lloyd Smith JO, Kisalu NK, et al. (2010) Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. Proc Natl Acad Sci U S A 107: 16262–16267.
38. Ladnyj ID, Ziegler P, Kima E (1972) A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. Bull World Health Organ 46: 593–597.
39. Jezek Z, Fenner F (1988) Human monkeypox. Monographs in Virology. Basel: Karger.
40. Breman JG (2000) Monkeypox: an emerging infection for humans? Scheld WM, Craig WA, Hughes JM, eds. Emerging infections. Washington (D.C.): ASM Press.
41. Daneshvar C, Davis TM, Cox-Singh J, Rafa'ee MZ, Zakaria SK, et al. (2009) Clinical and laboratory features of human *Plasmodium knowlesi* infection. Clin Infect Dis 49: 852–860.
42. Nathanson N, Martin JR (1979) The epidemiology of poliomyelitis: enigmas surrounding its appearance, epidemicity, and disappearance. Am J Epidemiol 110: 672–692.
43. Nathanson N, Kew OM (2010) From emergence to eradication: the epidemiology of poliomyelitis deconstructed. Am J Epidemiol 172: 1213–1229.
44. Bellini WJ, Helfand RF (2003) The challenges and strategies for laboratory diagnosis of measles in an international setting. J Infect Dis 187 Suppl 1: S283–S290.
45. Ramsay ME, Jin L, White J, Litton P, Cohen B, et al. (2003) The elimination of indigenous measles transmission in England and Wales. J Infect Dis 187 Suppl 1: S198–S207.

46. Dumbell KR, Archard LC (1980) Comparison of white pock (h) mutants of monkeypox virus with parental monkeypox and with variola-like viruses isolated from animals. Nature 286: 29–32.

47. Nakano JH (1980) Smallpox, monkeypox, vaccinia, and whitepox viruses. Lennette EH, Truant JP, eds. Manual of clinical microbiology. 3rd edition. Washington (D.C.): American Society for Microbiology.

48. Kew OM, Mulders MN, Lipskaya GY, da Silva EE, Patlansch MA (1995) Molecular epidemiology of polioviruses. Nathanson N, ed. Seminars in virology. New York: Academic Press.

49. Rubin BA (1980) A note on the development of the bifurcated needle for smallpox vaccination. WHO Chron 34: 180–181.

50. Henderson RH, Davis H, Eddins DL, Foege WH (1973) Assessment of vaccination coverage, vaccination scar rates, and smallpox scarring in five areas of West Africa. Bull World Health Organ 48: 183–194.

51. Conteh S, Chattopadhyay R, Anderson C, Hoffman SL (2010) *Plasmodium yoelii*-infected *A. stephensi* inefficiently transmit malaria compared to intravenous route. PLoS ONE 5: e8947. doi:10.1371/journal.pone.0008947.

52. Van Buskirk KM, O'Neill MT, De La Vega P, V, Maier AG, Krzych U, et al. (2009) Preerythrocytic, live-attenuated *Plasmodium falciparum* vaccine candidates by design. Proc Natl Acad Sci U S A 106: 13004–13009.

53. Hoffman SL, Billingsley PF, James E, Richman A, Loyevsky M, et al. (2010) Development of a metabolically active, non-replicating sporozoite vaccine to prevent *Plasmodium falciparum* malaria. Hum Vaccin 6: 97–106.

54. Crotty S, Felgner P, Davies H, Glidewell J, Villarreal L, et al. (2003) Cutting edge: long-term B cell memory in humans after smallpox vaccination. J Immunol 171: 4969–4973.

55. Patriarca PA, Wright PF, John TJ (1991) Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. Rev of Inf Dis 13: 926–929.

56. Vashishtha VM, Kalra A, John TJ, Thacker N, Agarwal RK (2008) Recommendations of 2nd National Consultative Meeting of Indian Academy of Pediatrics (IAP) on polio eradication and improvement of routine immunization. Indian Pediatr 45: 367–378.

57. John TJ, Jayabal P (1972) Oral polio vaccination of children in the tropics. I. The poor seroconversion rates and the absence of viral interference. Am J Epidemiol 96: 263–269.

58. John TJ (1972) Problems with oral poliovaccine in India. Indian Pediatr 9: 252–256.

59. Sutter R, John TJ, Jain H, Agarkhedkar S, Ramanan P (2010) Randomized clinical trial of bivalent type 1 and 3 oral poliovirus vaccine. Lancet 376: 1682–1688.

60. Foege WH, Leland OS, Mollahan CS, Fulginiti VA, Henderson DA, et al. (1965) Inactivated measles-virus vaccine. Public Health Rep 80: 60–64.

61. Fulginiti VA, Eller JJ, Downie AW, Kempe CH (1967) Altered reactivity to measles virus. Atypical measles in children previously immunized with inactivated measles virus vaccines. JAMA 202: 1075–1080.

62. Rauh LW, Schmidt R (1965) Measles immunization with killed virus vaccine. Serum antibody titers and experience with exposure to measles epidemic. Am J Dis Child 109: 232–237.

63. Sencer DJ, Dull HB, Langmuir AD (1967) Epidemiologic basis for eradication of measles in 1967. Public Health Rep 82: 253–256.

64. Strebel PM, Henao-Restrepo AM, Hoekstra E, Olive JM, Papania MJ, et al. (2004) Global measles elimination efforts: the significance of measles elimination in the United States. J Infect Dis 189 Suppl 1: S251–S257.

65. Tapia M, Sow S, Medina-Moreno SM, Lim Y, Pasetti MF, et al. (2005) A serosurvey to identify the "window of vulnerability" to wild type measles among infants in rural Mali. Am J Trop Med Hyg 73: 26–31.

66. Whittle H, Hanlon P, O'Neill K, Hanlon L, Marsh V, et al. (1988) Trial of high-dose Edmonston-Zagreb measles vaccine in the Gambia: antibody response and side-effects. Lancet 2: 811–814.

67. World Health Organization (1992) Expanded programme on immunization (EPI). Safety of high titre measles vaccines. Wkly Epidemiol Rec 67: 357–361.

68. Taylor WR, Mambu RK, ma-Disu M, Weinman JM (1988) Measles control efforts in urban Africa complicated by high incidence of measles in the first year of life. Am J Epidemiol 127: 788–794.

69. Simon JK, Pasetti MF, Viret JF, Mischler R, Munoz A, et al. (2007) A clinical study to assess the safety and immunogenicity of attenuated measles vaccine administered intranasally to healthy adults. Hum Vaccin 3: 54–58.

70. Khetsuriani N, Prevots DR, Quick L, Elder ME, Pallansch M, et al. (2003) Persistence of vaccine-derived polioviruses among immunodeficient persons with vaccine-associated paralytic poliomyelitis. J Infect Dis 188: 1845–1852.

71. Agur Z, Cojocaru L, Mazor G, Anderson RM, Danon YL (1993) Pulse mass measles vaccination across age cohorts. Proc Natl Acad Sci U S A 90: 11698–11702.

72. Bauch CT, Szusz E, Garrison LP (2009) Scheduling of measles vaccination in low-income countries: projections of a dynamic model. Vaccine 27: 4090–4098.

73. Szusz EK, Garrison LP, Bauch CT (2010) A review of data needed to parameterize a dynamic model of measles in developing countries. BMC Res Notes 3: 75.

74. Pongtavornpinyo W, Hastings IM, Dondorp A, White LJ, Maude RJ, et al. (2009) Probability of emergence of antimalarial resistance in different stages of the parasite life cycle. Evol Appl 2: 52–61.

75. Jegede AS (2007) What led to the Nigerian boycott of the polio vaccination campaign? PLoS Med 4: e73. doi:10.1371/journal.pmed.0040073.

76. Centers for Disease Control and Prevention (2010) Progress toward poliomyelitis eradication - Nigeria, January 2009-June 2010. MMWR Morb Mortal Wkly Rep 59: 802–807.

77. Waisbord S, Shimp L, Ogden EW, Morry C (2010) Communication for polio eradication: improving the quality of communication programming through real-time monitoring and evaluation. J Health Commun 15 Suppl 1: 9–24.

78. Bruce AR, Sutter RW, Cochi SL, Thompson KM, Jafari H, et al. (2006) Risk management in a polio-free world. Risk Anal 26: 1441–1448.