maLERA - An Updated Research Agenda for Malaria Elimination and Eradication

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COLLECTION REVIEW

malERA: An updated research agenda for malaria elimination and eradication

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Abstract

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

Summary points

- The first malERA consultative process in 2011 identified a number of targets for investment and the scientific community has made progress across the research and
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**Abbreviations:** AIM, Action and Investment to defeat Malaria 2016–2030; CHMI, controlled human malaria infection; CRISPR, clustered regularly interspaced short palindromic repeats; DHIS 2, District Health Information Software 2; EIR, entomological inoculation rate; ERG, Evidence Review Group; GSPD, glucose-6-phosphate dehydrogenase; GTS, Global Technical Strategy for Malaria 2016–2030; malERA, Malaria Eradication Research Agenda; MDA, mass drug administration; MESA, Malaria Eradication Scientific Alliance; NTD, neglected tropical disease; RBM, Roll Back Malaria Partnership; RDT, rapid diagnostic test; SAG, Strategic Advisory Group; SERCAp, Single Encounter Radical Cure and Prophylaxis; VIMT, vaccines that interrupt malaria parasite transmission; WHO, World Health Organization.

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

**Introduction**

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

Global goals for a reduction in malaria burden and elimination were published in 2 complementary documents in 2015: the Global Technical Strategy for Malaria 2016–2030 (GTS) and development (R&D) continuum. Progress includes positive scientific opinion for a malaria vaccine, advanced development of 3 nonpyrethroid insecticides, new genetic technologies with the potential to alter malaria parasite transmission by the mosquito, identification of markers of drug resistance, and development of *Plasmodium vivax* liver stage assays as well as new collaborative approaches to mathematical modelling and screening for active ingredients for drugs and insecticides.

- **Scientific progress, however, has been matched with significant challenges. The expansion of both insecticide and drug resistance threatens progress in affected countries.** Gaps in the knowledge base persist, from epidemiological and entomological tools to guide programmes, particularly at low transmission levels, understanding the role of low-density infections in maintaining transmission and developing appropriate diagnostics for programmes, biomarkers, and tools to detect and clear hypnozoites, to tools to tackle residual transmission, receptivity, and prevention of reintroduction.

- **In some areas, progress has been too slow, particularly in the creation of a tool kit to tackle *P. vivax* malaria, investments in the development of new vector control tools, almost all aspects of entomology, and in systematically testing solutions in the context of the respective health and social systems.**

- **Malaria parasites and their infections continually evolve, creating new research and programme challenges.** In one region, human infections with *P. knowlesi* are rising, parasites with *hrp2/3* deletions are evading detection by current rapid diagnostic tests (RDTs), and current effective vector control tools are selecting for mosquitoes with both physiologic resistance and behavioural traits like outdoor biting and resting.

- **The malERA Refresh agenda proposes a broad agenda for transdisciplinary solutions to the problems faced.** It points to 3 areas in which innovation is critical: (i) iterative improvements in drugs and vector control; (ii) transformative improvements in tools and strategies to reduce, if not halt, the parasite’s capacity to transmit; and (iii) integrated approaches in which a robust elimination strategy responds to local variations in transmission dynamics, is tailored to the health and social system context, and draws strength from other sectors.
Action and Investment to defeat Malaria 2016–2030 (AIM), a global investment case for financing and coordinating these efforts [2,3]. Other groups have expressed a vision of global malaria eradication and underscored the need for R&D investments and country financing [4]. Building on the goals expressed in the GTS and AIM, the World Health Organization (WHO) has established a Strategic Advisory Group (SAG) to analyse future scenarios for malaria, including eradication. WHO SAG has affirmed WHO’s long-standing commitment to the goal of eradication, although it does not specify an end date for that goal [5,6].

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

Accelerating to elimination

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

Some key points emerge from experiences in elimination countries and are worth clarifying, because they frame the context for evaluation of new tools to accelerate progress. First,
Table 1. Glossary of terms. The meaning of the terms used in the malERA Refresh series are described here. The sources of the definitions are referenced; where no reference is cited, the authors of this paper provided the definition.

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

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

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

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

malERA Refresh process

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

The malERA Refresh process was overseen by a leadership group composed of Regina Rabinovich (chair, ISGlobal Barcelona Institute for Global Health and Harvard T.H. Chan School of Public Health), Pedro Alonso (WHO Global Malaria Programme), Marcel Tanner (Swiss TPH), and Dyann Wirth (Harvard T.H. Chan School of Public Health), and each consultative panel was led by a chair and 1 or 2 cochairs [25]. The process was managed by the MESA Secretariat (ISGlobal Barcelona Institute for Global Health). Diverse expert panels of scientists,
programme managers, and decision makers were convened for 6 thematic areas. The themes of the panels were adapted from the original malERA, reflecting the evolution of the knowledge base even since the first malERA process in 2011. One panel examined tools for elimination (vector control, vaccines, diagnostics, and drugs), one panel tackled the application of mathematical modelling to the challenges of combining interventions, and the health systems panel also addressed policy research. New panels were created, one to look at the infectious reservoir and one focussed on resistance to antimalarial drugs and insecticides (for the full list of panels, see Table 2). A systematic literature search was performed for each theme to identify papers published between 2010 and 2016. These papers were supplemented with suggestions from panelists and projects in the MESA Track database of active projects. Each panel had 1 in-person meeting to assess the progress since malERA 2011 and discussed whether there had been adequate efforts to address each area. Taking into consideration the major advances that have taken place since the first malERA consultations, the panels highlighted specific challenges and indicated key opportunities to generate knowledge, tools, and strategies for malaria
elimination (Box 1). Cross-links between the panels were ensured by cross-panel participation and an online consultation of main findings (Fig 3).

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

Cross-cutting priority research areas

Surveillance and towards surveillance–response approaches. Malaria programmes continuously need data to direct their actions and resources, to gauge their impact, and,
Table 2. Using the themes from the first malERA process as a starting point, malERA Refresh was organised by research themes that are relevant for malaria elimination and eradication and reflect current hypotheses and new thinking.

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

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

Surveillance platforms like the District Health Information Software 2 (DHIS 2) are being used to collect facility and community data across diseases. When fully functional, such platforms collect dynamic quality-assured information that can be analysed to track temporal and spatial changes in transmission [26,27]. High-quality information systems that collect real-time data from incoming cases can spot early warning signals of drug resistance, reintroduction, and resurgence. High-resolution platforms based on geographic information systems have been developed that collect, integrate, and share relevant data with various audiences [27]. These surveillance–response systems are particularly useful for the detection of and response to unevenly distributed transmission foci with sufficient detail as to depict the household or hamlet level and are key to targeting the operational response. In addition to collecting information on malaria infections, a quality malaria surveillance system should assess drug efficacy against the parasites and assess mosquito vector populations and insecticide
Box 1. Examples of challenges and opportunities to generate knowledge, tools, and strategies for malaria elimination

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

Biology

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

Tools and deployment strategies

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

Understanding transmission and tackling residual transmission

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

Malaria programmes and systems

- Questions remain around the best composition, phasing, and threshold triggers for intervention packages in different settings and as programmes advance along the elimination continuum.
• An area of promise for malaria programmes is testing and validating essential, collectable, and actionable data for programmatic decision-making.
• Advances in molecular technologies will help surveillance of resistance to insecticides and drugs.
• Strategies for deploying future tools in the field need to be tested and modelling can guide testing.
• Opportunities using systems-thinking approaches to identify where in the health system effectiveness of interventions is lost and can be recovered.

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

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

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

Each international Panel is led by a Chair and Co-chair(s). Rapporteur(s) support the writing process.

- Basic Science & Enabling Technologies
- Tools for Elimination
- Reservoir & Measuring Transmission
- Combination Interventions & Modelling
- Insecticide and Drug Resistance
- Health Systems & Policy Research

**CONSULTATIVE PROCESS**

Online consultation garners inputs from other experts.

Chairs and co-chairs meet to develop the main points of the research agenda as a whole.

Panels meet to review progress and generate ideas.

Writing groups from different Panels exchange ideas via telecom.

**FINDINGS ARE SHARED**

The monothematic series is published online and available to all. Findings are shared at international conferences.

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

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

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

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

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

Research to characterise the transmission reservoir has evolved to a focus on the role of low-density infections undetected by microscopy or current RDT in transmission [27]. Understanding determinants of the risk of infectiousness, understanding at what level of parasitaemia these are important for sustained transmission, and devising metrics and tools to measure and target transmission are proposed as key needs [26,27,29,34]. Recently, a highly sensitive
RDT has been launched and demonstration studies are being planned to test how and when to use this new tool [35].

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

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

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

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

Major neglected areas critical to elimination

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

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

The efficacy of available vector control tools is diminished by residual transmission and the enormous behavioural plasticity and biological variability of malaria vectors and is threatened by the capacity of the mosquito to develop resistance in the face of high pressure from
interventions. The papers in this malERA Refresh series offer potential solutions to be developed and tested [27–29,34].

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

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

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

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

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

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

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

**Looking forward**

Innovation and problem solving tailored to the local setting are critical to the long-term success of the global malaria programme. Three types of innovation need to be pursued: iterative,
breakthrough, and integrated. malERA Refresh is replete with examples: drugs to overcome resistance, gene drive as a transformative technology, and the acceleration hypothesis as a testable approach to elimination and its interaction with the health system in highly endemic countries. To pursue the opportunities proposed here for accelerating elimination, a diverse landscape of funders is needed to prioritise research objectives according to their strategic plans and stakeholders’ needs. A diligent monitoring of the uptake of the research questions in this agenda and the impact of the evolving evidence base will be essential to keep the malaria community on course.

Supporting information
S1 Translation. Spanish translation of abstract.
(DOCX)
S2 Translation. French translation of abstract.
(DOCX)

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

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References
COLLECTION REVIEW

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

The maLERA Refresh Consultative Panel on Basic Science and Enabling Technologies

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

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Abstract

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

Summary points

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

- Driven by the development and accessibility of large-scale research tools and technologies, the scientific community can systematically tackle key questions in malaria, such as the following. What are the genes that contribute to antimalarial drug resistance (thereby defining the full parasite “resistome”? What are the functions of key Plasmodium genes (providing much-needed annotation of key Plasmodium genes)? What are the genes and gene mutations that drive resistance in mosquito populations?

- Continued exploration of the potential of enabling technologies is needed. Important areas of future research include the use of gene-drive strategies and other gene-manipulation technologies; metabolomics-based approaches for biomarker discovery; structural vaccinology, novel technology platforms, and the use of novel adjuvants to improve vaccine design; and high-throughput approaches to facilitate drug discovery and screening.

**Background**

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

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

**Methods**

The findings presented in this paper result from an extensive literature review of published and unpublished materials and the deliberations of the 2015 Malaria Eradication Research Agenda (malERA) Refresh Consultative Panel on Basic Science and Enabling Technologies. Electronic databases were systematically searched for published literature between January 1, 2010, and July 2, 2016, without language limitations. Panelists were invited to recommend additional literature and additional ongoing research projects. A 2-day workshop was held with the majority of the panel members, including field researchers, specialists from basic science, malaria genomics and epigenomics, regenerative medicine, and National Institutes of Health representatives. The panel broke into 6 breakout sessions to identify the problems that
Table 1. A listing of the important research areas highlighted in malERA 2011, the progress made since then, and the remaining areas that require additional research.

<table>
<thead>
<tr>
<th>Research Area</th>
<th>Accomplishments in Past 5 years</th>
<th>References</th>
<th>Remaining Gaps</th>
</tr>
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<tbody>
<tr>
<td>Transmission Biology (Gametocytes to Mosquito)</td>
<td>Improved understanding of transcriptional and epigenetic control of sexual development</td>
<td>[2–6]</td>
<td>Limited work on <em>P. vivax</em> gametocytes due to lack of in vitro culture system</td>
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<td></td>
<td>Drug screens targeting transmission stages</td>
<td>[7–11]</td>
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<td></td>
<td>Improved understanding of mosquito host-seeking behavior and olfaction biology</td>
<td>[12–16]</td>
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<td></td>
<td>Improved understanding of mosquito–parasite interactions</td>
<td>[17–20]</td>
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<td></td>
<td>Anopheles midgut cell line model for in vitro ookinete production and invasion</td>
<td>[21–25]</td>
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<tr>
<td>Infection Biology (Mosquito to Liver)</td>
<td>Humanized mouse model for entire life cycle of <em>Plasmodium</em>, including <em>P. vivax</em> hypnozoites and liver stages</td>
<td>[26, 27]</td>
<td>Methods to increase sporozoite availability</td>
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<td></td>
<td>In vitro models for <em>Plasmodium</em> liver stages</td>
<td>[28–30]</td>
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<td>Genetic crosses in mouse model</td>
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<td></td>
<td>Primate models for <em>P. cynomolgi</em></td>
<td>[32]</td>
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<td></td>
<td>Controlled human malaria infections with sporozoites and blood-stage parasites</td>
<td>[33–40], reviewed in [41]</td>
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<td>Biology of Blood-stage Parasites</td>
<td>Improved production of continuous culture conditions, including identification of host cell environments necessary to support <em>P. vivax</em> invasion in culture and proof-of-principle that human hematopoietic stem cells can be immortalized, expanded, and differentiated into reticulocytes</td>
<td>[42–55]</td>
<td>No in vitro culture system for <em>P. vivax</em> asexual stages has been developed</td>
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<td></td>
<td><em>P. knowlesi</em> in vitro culture adaptation</td>
<td>[56, 57]</td>
<td>Poor functional annotation of genes</td>
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<td></td>
<td>Identification and spread of mutations associated with artemisinin resistance</td>
<td>[58–64], reviewed in [65–67]</td>
<td></td>
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<td></td>
<td>Comparison of mitochondrial and lipid metabolism of <em>P. falciparum</em> in sexual and asexual blood stages</td>
<td>[68, 69]</td>
<td></td>
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<tr>
<td>Persistence of Parasites and Mosquitoes</td>
<td><em>P. vivax</em> hypnozoites cultured in vitro</td>
<td>[26, 28]</td>
<td>Biomarkers for asymptomatic hosts</td>
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<td>Mosquito dry season estivation and long-distance migration observed in sub-Saharan populations</td>
<td>[70]</td>
<td>Ecology and migration rates of vector species</td>
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<td></td>
<td>Mechanisms of insecticide resistance identified</td>
<td>[71–73]</td>
<td>Long-term behavioral resistance studies</td>
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<tr>
<td>Additional Technological Developments</td>
<td>Mosquito genomic resources to identify population substructure and allow comparative genomic studies</td>
<td>[74–77]</td>
<td>Coordinated efforts to generate knockout or knockdown libraries to understand gene function, especially in human parasites</td>
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<td>Genome-editing systems (CRISPR/Cas9, Zinc-finger nuclease), posttranslational protein knockdown systems (DD tag, Riboswitch), conditional genome deletion systems (Cre-LoxP, FLP-frt, diCre), conditional gene expression system (TetR-aptamer)</td>
<td>[78–86]</td>
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<td>Proofs-of-principle for population suppression and population modification/replacement of Anopheles using gene drives</td>
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<td></td>
<td>Colonization of important mosquito vector species</td>
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<td>New techniques to improve antigen design and clinical evaluation of vaccine candidates</td>
<td>[94–100]</td>
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<td></td>
<td>Improved resolution in intravital imaging</td>
<td>[101, 102]</td>
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**Abbreviations:** Cre-LoxP, genetic recombination system involving the Cre (Causes recombination) protein and *loxP* (locus of X-over P); CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/associated protein-9 nuclease; diCre, dimerizable Cre recombinase; DD, destabilization domain; FLP-frt, Flippase used to recombine two frt domains; malERA, Malaria Eradication Research Agenda; TetR, tetracycline repressor.

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need to be solved in asexual blood stages, liver stage and mosquito, mosquito, *P. vivax*, population genetics and resistance, and transmission. The panel discussed what research is needed to address these problems and considered 6 crosscutting themes in CRISPR
technologies, immunology and malaria vaccines, genomics tools for malaria, metabolism and malaria, structural biology, and diagnostics for malaria. Each group fed back to plenary session, where further robust discussions and input occurred. This helped refine the opportunities and gap areas in which research is needed. The final findings were arrived at with inputs from all panelists and several iterations of the manuscript.

**Advances, challenges, and opportunities in transmission biology**

**Gametocytes**

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

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

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

Box 1. Opportunities for the next 5 years

1. Functional genomics

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

2. Advances in mosquito biology

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

3. New vaccine approaches

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

4. Biomarkers and diagnostics

- Indicators of transmissible gametocytes,
- Markers of liver-stage infection, in particular, hypnozoites,
• Markers/assays to identify asymptomatic carriers,
• Identification of metabolic signatures of different stages of the life cycle.

5. Greater understanding of resistance to antimalarials and insecticides

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

6. Greater accessibility to *P. vivax* gametocytes

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

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

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

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

**Mosquito biology and host seeking**

Transmission success also depends upon the interactions of the mosquito vector with both its human host and ingested parasites. Since 2011, there have been major advances in understanding the biology of olfaction and host-seeking behavior in mosquitoes via a combination of behavioral assays, electrophysiology, and functional genomic approaches [12–16]. High-throughput screens have identified new classes of attractants and repellents that are currently being tested in mosquito traps and spatial repellent trials ([107–110], also see MESA Track at http://www.malariaeradication.org/mesa-track). Moving forward, the identification of
oviposition cues and the role of olfaction and taste in larval stages could facilitate the development of additional tools for vector control. Comparative genomic analysis of odorant receptor pathways that differ between anthropophilic and zoophilic species will help to elucidate the molecular basis of host-seeking behavior. Recent studies have shown that the composition of the human skin microbiota influences host attractiveness to mosquitoes [111] and identified volatile substances produced by parasites in human hosts thought to preferentially attract mosquitoes to infected individuals [112]. Nevertheless, gaps remain in our knowledge regarding the potential for gametocyte-seeking behavior by the mosquito and parasite-induced changes to the human host that may influence mosquito behavior to enhance biting and transmission.

Parasite development in the mosquito

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

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

Advances, challenges, and opportunities in infection biology

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

In parallel, the development of humanized mouse models of P. vivax and P. falciparum infection have opened up the potential for surrogate in vivo models of human liver infection [26] and allowed the first genetic crosses of parasites (P. falciparum) outside of a primate [31]. Studies in primates continue to play an important role; the P. cynomolgi monkey model of liver infection is the only in vivo relapse model of the P. vivax hypnozoite [30, 32, 126]. Combined with controlled human malaria infections [34, 35, 38, 127, 128] and in vitro models,
these tools have highlighted key differences in the biology of different parasite species (specifically, *P. vivax* and *P. falciparum*) and paved the way for understanding the cellular biology of liver infection and the immune response and for performing high-throughput drug candidate screening.

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

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

**Advances, challenges, and opportunities in asexual-stage biology**

**Defining the parasite “resistome”**

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

**Systematic characterization of the asexual-stage parasite**

The systematic knockout of genes in *P. berghei* has led to numerous advances in our understanding of fundamental asexual biology [141, 142], including the *P. berghei* identification of
essential genes and pathways [143–146], greater understanding of merozoite invasion and egress [147–150], discovery of the parasite’s export machinery [145, 151, 152], and revealing how the red cell cytoplasm and membrane are remodelled [153, 154]. Such studies point to the critical nature of these processes and have opened the possibility of targeting them with drugs or vaccines.

Yet, major gaps remain in our knowledge of gene function in *P. falciparum* and, to an even greater extent, in other species (including *P. vivax*, *P. ovale*, and *P. malariae*) in which genetic diversity is also relatively uncharacterized. Although in many cases, genomic variants can be readily identified in sequencing data, poor annotations for predicted genes in the *P. falciparum* genome continue to slow progress. For example, we know little about the cellular function of the *pfkelch13* gene, a major contributor to artemisinin resistance ([62, 155, 156], reviewed in [67]). Given that it is more efficient and inexpensive for the community to work together to functionally annotate the *P. falciparum* genome systematically rather than in a 1-researcher-1-gene fashion, coordinated large-scale projects with a focus on the easily accessible *P. falciparum* asexual blood stage should be considered. Such systematic data would also help in the interpretation of whole genome sequencing data from drug- or vaccine-resistant parasites.

Desirable genomic annotations include the location of key transcription factor binding sites, transcriptional start and stop sites [157], epigenetic chromatin modifications, and the cellular localization of encoded proteins. These consortium-acquired data are critical to predict whether genetic variants discovered through genome sequencing of model organisms and humans are indeed functional and could also help prioritize antigens for vaccine development. In addition, if better in vitro culture systems can be developed for *P. vivax* (see “Advances, challenges, and opportunities in transmission biology”), these systematic approaches could be extended to this important species. A potential model for such a consortium-based effort is the human ENCODE project, which has identified functional elements in the human genome [158].

Using metabolomics to identify biomarkers and develop diagnostics

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

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

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

Persistence of malaria occurs in 2 modalities—asymptomatic carriers and latent liver stages. The asymptomatic carriers represent a significant threat to the reintroduction of malaria; thus,
the identification of such carriers requires a heightened level of awareness and detection. The absence of symptoms in an individual may reflect the presence of disease-prevention host responses in the absence of sterilizing immunity, thereby allowing persistent parasitemia or the sequestration of parasites in sites (e.g., the liver or bone marrow) in which they are “hidden” from the immune system. Understanding the relative contributions of both human immune responses and parasite biology will be essential to maximize the efficacy of antimalarial interventions, particularly vaccines.

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

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

**Mosquito vector persistence**

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

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

Mosquito persistence may also occur due to heritable changes in behavior selected for by control interventions, so-called behavioral resistance. Recent work has captured mosquito interactions with bednets using mosquito-tracking cameras [172] and could be extended to other interventions (e.g., traps, sprays, repellents). Consistent longitudinal studies are also needed to track changes in mosquito biting behavior (e.g., outdoor versus indoor, evening
versus night) after the use of interventions and to discriminate these changes from variation in species frequencies at specific sites. Subsequent genomic analyses could then reveal if there is a genetic component to these modified behaviors.

**Technology and its application to malaria biology**

**Fundamental technologies: Genomics and transcriptomics**

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

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

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

**Gene-manipulation technologies: Genome editing and transgenics**

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

With these powerful tools in place, we can now scale up the generation of conditional and/or complete knockout parasite libraries containing every single gene in the genome. Such an
effort would greatly enhance our understanding of the biology of the parasite at all stages of development, as well as identify the functions of many hypothetical genes.

Gene-manipulation technologies: Gene drives

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

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

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

Cell- and tissue-based technologies

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

**New technologies to support tool development: Biomarkers and novel diagnostics**

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

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

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

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

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

Controlled human challenge studies are potentially transformational in enabling our understanding of the human immune response to malaria. Coupling controlled infections with technical advances for interrogating human immune cells in real time can give us new insights into both the temporal response and the contributions from innate and acquired immunity. Additionally, deeper interrogation of the immune profile of naturally acquired
infections could also provide key insights. Providing access to them will require forethought in preparing future proposals, particularly with respect to human subject approvals, repository deposition, and community sharing. Harnessing available systems through existing networks as well as ongoing clinical trials could provide the necessary reagents and access to human samples.

**Drug design and screening**

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

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

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

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

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

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

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COLLECTION REVIEW

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

The malERA Refresh Consultative Panel on Insecticide and Drug Resistance†

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Abstract

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

Summary points

- Since 2011, significant progress has been made in understanding resistance. Surveillance has been expanded and improved in many malaria-endemic countries and there is a better understanding of the genetic basis of resistance, identifying some molecular markers that can be used to track its emergence and spread. Better tools to measure and manage the intensity of resistance are available.
Abbreviations: ACT, artemisinin-based combination therapy; DP, dihydroartemisinin-piperine; GMS, Greater Mekong Subregion; GPARC, Global Plan for Artemisinin Resistance Containment; GPIRM, Global Plan for Insecticide Resistance Management; IPTp, intermittent preventive treatment in pregnancy; IRS, indoor residual spraying; IVCC, Innovative Vector Control Consortium; K13, Kelch 13 (PF3D7_1343700); LLIN, long-lasting insecticidal net; malERA, Malaria Eradication Research Agenda; MESA, Malaria Eradication Scientific Alliance; MMV, Medicines for Malaria Venture; pfcr, P. falciparum chloroquine resistance transporter; pfdr, P. falciparum dihydrofolate reductase; pfdrps, P. falciparum dihydropteroate synthase; pfmdr1, P. falciparum multidrug resistance 1; PSA, piperine survival assay; SP, sulfadoxine-pyrimethamine; pvmdr1, P. vivax multidrug resistance 1.

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

Introduction and rationale

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

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

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

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

Methods

The findings presented in this paper result from an extensive literature review of published and unpublished materials and the deliberations of the 2015 malERA Refresh Consultative Panel on Insecticide and Drug Resistance. Electronic databases were systematically searched.
Box 1. Progress over the past 5 years in drug and insecticide resistance research

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

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

What do we know about resistance?

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

Despite ubiquitous pyrethroid resistance in some areas, millions of pyrethroid-impregnated nets are distributed annually. Once distributed, these nets can contribute to the selection of resistant vectors for the duration of their 3-year life. In Burkina Faso, the intensity of the pyrethroid resistance seen in *A. gambiae* increased 10-fold in a single year [8], and this trend is apparent in multiple locations throughout Africa [9]. *A. funestus* also exhibits resistance to multiple insecticides at increasing intensities [10–12]. Proactive defensive strategies are critical
Box 2. Research and development agenda for drug and insecticide resistance

Crosscutting issues for drug and insecticide resistance

**Applied research.**

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

**Policy and advocacy.**

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

**Insecticide resistance**

- Analyse the most cost-effective ways of slowing the spread and emergence of insecticide resistance (e.g., by using a combination of interventions, spatial mosaics, or mixtures of insecticides)
- Determine which spatial and temporal scale insecticide resistance management strategies should be carried out
• Study how much insecticide resistance has a negative impact on mosquito fitness survival or parasite development in the mosquito and investigate how this compares for different active ingredients

• Develop a method to assess the age of resistant mosquitoes

• Define the optimal use of bioassays and molecular markers to accurately predict the efficacy of vector control in relation to insecticide resistance

• Study the mechanisms of mosquito behavioural resistance and assess if this is sustained across generations

• Assess which novel, noninsecticidal tools for controlling mosquito populations would help to slow or prevent the emergence of resistance or restore susceptibility

Drug resistance

• Evaluate if the timing of community-based prevention, e.g., mass drug administration, can be optimised to reduce the risk of emerging drug resistance

• Investigate why drugs such as quinine are less likely to develop resistance and use this knowledge for future drug development

• Determine which approaches are most sensitive and specific to determine true drug treatment efficacy (e.g., molecular correction) in *P. falciparum* and *P. vivax* parasites

• Define what studies are required by policy makers to evaluate the use of multiple therapies

• Define the minimal criteria for inclusion of existing and new drugs in multiple agent regimes (e.g., efficacy, resistance, pharmacokinetic factors, and drug–drug interactions) and whether these criteria change in different programmatic modes

• Study the extent to which human immunity masks the presence of drug resistance, especially resistance to artemisinins

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

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

Identifying resistance

Two main mechanisms of insecticide resistance have been identified: target site mutations (such as *kdr* and *ace*) [24,25] and metabolic resistance involving mutation, duplication, or
altered regulation of enzymes and transporters that increase insecticide metabolism or excretion. Metabolic resistance has greater implications for malaria vector control because the efficacy of a range of insecticides is usually affected [5,26].

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

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

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

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

Chloroquine remains the recommended treatment for *P. vivax*, but resistance and declining efficacy has been noted in several populations, and ACTs are recommended in some areas [50,51]. There are no standardized molecular correlates of chloroquine resistance for *P. vivax*.
but *P. vivax* multidrug resistance 1 (*pvmdr1*) has been associated with resistance [52]. Beyond this, the understanding of resistance in nonfalciparum malaria is very limited.

**Public health impact of resistance**

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

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

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

**Managing resistance, moving toward elimination**

**Optimizing drug and insecticide use.** Avoiding parasite or mosquito population exposure to a single selective agent is the central principle of resistance management. Ideally, insecticidal compounds with different modes of action should be used simultaneously or in spatial or temporal rotation. These principles, which are identical to those used in the management of insecticides used for crop pests, have been outlined in the GPIRM in malaria vectors [63]. Unfortunately, implementation has been challenging; pyrethroid resistance is ubiquitous, non-pyrethroid LLINs are not currently available, and other forms of vector control can significantly increase costs [64]. New public health insecticides with different modes of action are on the horizon [65], but we lack information on the effectiveness of the proposed strategies to slow the emergence or spread of insecticide resistance, and there is no clear indication of how they should be integrated alongside existing tools. This includes those that are noninsecticidal
and products that work on different targets, e.g., spatial repellents and endocticides, whose efficacy may not be influenced by insecticide resistance [65]. Another confounder is the application of most insecticides for both agricultural and public health use. The impact of this on public health is highly variable depending on crop type and volume and timing of insecticide application.

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

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

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

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

Using data to support resistance management. Entomological data generated by countries vary in quantity and quality, and limited information flow between entomologists, programme managers, and research institutes has hindered advocacy efforts around improved resistance management. Linking entomological data to epidemiological outcomes is extremely complex [75] and by the time resistance has a demonstrable public health impact, it may be too late to intervene against it. However, South Africa [62], Zambia [76], and Equatorial Guinea [64] have resistance management plans in place. Similarly, molecular marker surveillance can inform which drug regimens are the most suitable for particular programmatic modes. This approach is now routine in some African countries [74,77] but is not universal. Drug-resistance monitoring in some countries also requires strengthening, and despite the tighter link to public health impact, the ability to respond rapidly may be lost if resistance monitoring is not well embedded. For both insecticides and drugs, defining the minimum essential data required for policy makers to manage resistance and ensuring that programs employ rigorous quality assurance in collecting and managing these data are critical.
Resistance surveillance is weak in many endemic countries. Inadequate attention and funding have been allocated to entomological monitoring and insecticide susceptibility research. Several countries in Africa have established sentinel sites for the longitudinal monitoring of insecticide resistance [5]. However, the methods, timing, and sampling are inconsistent, making meaningful inferences difficult [78]. Most of these sites use discriminating dose assays [79]. All bioassays are performed on 3–5-day-old mosquitoes under standard insectary conditions, so the effect of natural mosquito traits (e.g., age, blood-feeding status, circadian rhythm [80,81], and climatic variables [82]) on resistance is not assessed or reported [83]. Molecular species identification of mosquitoes undergoing resistance tests may also increase accuracy when compared to morphological identification. Techniques have been developed to measure the age distribution of mosquito populations in the laboratory [84], but a more precise, low-cost, field-applicable method is needed to allow malaria control programmes to evaluate the efficacy of vector control interventions is needed.

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

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

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

**Conclusion**

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

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COLLECTION REVIEW

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

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

Abstract

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

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

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

Since the 2011 malERA process, research has ranged from illuminating the basic biology of the development of sexual-stage parasites in humans and mosquitoes to evaluating operational approaches targeting infectious individuals in endemic communities. Additionally, a harmonised set of definitions relevant to malaria transmission and elimination has been developed.
Box 1. Terminology

Malaria typologies

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

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

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

Research agenda for characterising the reservoir of infection

Detecting malaria: Infection versus transmission

Malaria infection and transmission can be detected and measured with a variety of metrics (Tables 1 and 2). Their suitability and discriminatory power, however, can vary widely across settings and populations. To reliably confirm clinical malaria, a minimum diagnostic sensitivity of 200 parasites/μL. blood is required [6]. Microscopy and some rapid diagnostic tests (RDTs) meet this threshold [6]. In the absence of fever, some individuals will have parasitaemia levels detectable by microscopy and RDTs. These asymptomatic infections are particularly common in areas of high transmission (i.e., above 25 clinical cases per week per 1,000 persons) [7], where high levels of human immunity allow older individuals to carry relatively large parasite burdens chronically [8]. Such individuals would be detected within mass screen and treat (MSAT) programmes using currently available diagnostics. However, through the use of molecular amplification methods, it is now clear that many individuals harbour low-density malaria infections beneath the limit of detection of both microscopy and RDTs [9]. Meta-analyses indicate that molecular methods detect up to twice as many *P. falciparum* infections as RDT or microscopy [10], and approximately 5 times as many *P. vivax* infections [11,12]. This gap in sensitivity may be more pronounced when compared against ultra-sensitive molecular
Fig 1. Research needs and programmatic applications in measuring malaria transmission across the transmission spectrum. Range of malaria transmission intensity (grey line) from very high intensity to postelimination settings. Current metrics (navy blue line) used for routine measurement of malaria transmission at each level of transmission intensity. Knowledge gaps (orange line) in understanding the biology and epidemiology of malaria transmission and the infectious reservoir at all levels of transmission intensity. Technical gaps (light blue line) in the accurate measurement of transmission at each level of transmission intensity. Programmatic actions (yellow line) required for the interruption of transmission and the prevention of reintroduction at each level of transmission intensity.

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

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

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

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

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

*No WHO definition is available for this term.

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

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

### Low-density parasitaemia and transmission

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

Understanding the contribution of low-density parasitaemia to the infectious reservoir for a given malaria typology is critical to determine the diagnostic sensitivity required. It will also affect how much effort a programme should commit to detecting and treating these infections and when and where this effort is best deployed. As noted above, the proportion of low-density parasitaemia increases as transmission declines [9,10,19,20,24]. Recent findings from Senegal also suggest that the efficiency of human-to-mosquito transmission increases with decreasing transmission intensity [25].
Box 2. *P. vivax* and *P. ovale*

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

**Key advances**

**Relapses drive transmission**

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

**Research needs**

**Detection of hypnozoites to inform targeted drug or vaccination strategies**

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

**Improve understanding of parasite-vector bionomics**

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

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

**Detecting gametocytes**

All malaria infections have the capacity to produce gametocytes. Therefore, in the context of community chemotherapy programmes, treating any individuals who test positive for asexual parasites is a realistic programme aim. However, research tools that measure gametocytaemia are essential to further our understanding of transmission biology and to define the populations and individuals that drive transmission. Some studies have suggested that transmission efficiency may increase as malaria prevalence falls due to higher gametocyte densities. As the development of new transmission-blocking drugs and vaccines advances, understanding the factors that drive this transmission efficiency will be needed to determine in which settings interventions can be successfully trialled and/or implemented [25]. Although gametocytes can
be identified using microscopy, they often exist at low densities and may circulate only transiently in the blood. RDTs do not differentiate between gametocytes and asexual parasites. The limit of detection of microscopy is 8–16 gametocytes/μL of blood [30,31]. Predictably, molecular methods are more sensitive, with 0.3 mature females/μL of blood detected with Pf625 reverse transcription qPCR (RT-qPCR) and 1.8 mature males/μL of blood with Pf623op RT-qPCR [36]. As gametocyte densities are low, the increased sensitivity of molecular methods considerably increases gametocyte detection rates. For example, a recent study in Kenya found that Pf625 RT-qPCR detected gametocytes in 44% of the population compared with only 2.6% detected by microscopy [37].

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

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

Heterogeneity in the transmission reservoir

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

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

Surveillance systems at low-transmission settings will also need to be equipped to monitor emerging insecticide and drug resistance [55,56] that may threaten the success of existing
interventions [56]. Longitudinal monitoring of resistance markers via sentinel surveillance sites could prove invaluable for tracking risk of rebound or reintroduction. However, there are currently no field-based diagnostic tests for drug resistance, and more detailed information may be needed on local drug-resistance patterns in asymptomatic/low-density infections, particularly related to any changed infectiousness to mosquitoes.

**Research agenda for measuring transmission**

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

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

**Entomological metrics**

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

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

**Human metrics**

Current epidemiological metrics of malaria transmission in humans, diagnosed via passive and active systems, microscopy and RDTs, remain key for national malaria control
programmes in tracking progress in the reduction of malaria cases and identifying outbreaks and epidemics (Table 2). These data are complemented with large-scale surveys, such as the Demographic and Health Surveys (DHS), the Malaria Indicator Surveys (MIS) and UNICEF Multiple Indicator Cluster Surveys (MICS). However, as transmission declines to low
intensity, clinical cases become rare, slide and RDT positivity rates low, and transmission patterns increasingly heterogeneous.

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

Metrics to understand transmission

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

Antibody seroprevalence and the seroconversion rate (SCR) exploit human antibody responses to characterise previous parasite exposure and are specific to a particular antigen or combination of antigens [85]. Studies using enzyme-linked immunosorbant assays (ELISAs) have shown serological measures correlate well with parasitological and entomological measures in describing transmission levels and spatial and demographic risk [86,87].

Uniquely, serology, when combined with age, allows retrospective examination of exposure history, including the effects of interventions and the absence of recent exposure in elimination settings. High-throughput platforms, such as microarray and bead-based multiplex assays, allow screening of large numbers of potential antigenic targets with specific characteristics [87,88–91]. Targets of interest include stage- or species-specific biomarkers, particularly for *P. vivax* [88], serological signatures of hypnozoite carriage [92], and vector-specific antigenic targets in mosquito saliva [93,94]. The programmatic applications of serology have yet to be fully tested, though various approaches are being evaluated, including serological markers of incident infections [89,95–109]. Research is currently underway to identify a variety of biomarkers indicative of recent infection that are detectable for different durations following parasite infection, allowing finer-scale estimation of time since infection.
<table>
<thead>
<tr>
<th>Metric</th>
<th>Definition</th>
<th>Measure of transmission</th>
<th>Method</th>
<th>Discriminatory power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force of infection</td>
<td>Rate at which susceptible individuals contract malaria</td>
<td>• Probability of transmission</td>
<td>Time from birth to first malaria episode; microscopic detection of parasites following successful antimalarial treatment</td>
<td>• Difficult to measure&lt;br&gt;• Difficult to standardise&lt;br&gt;• Depends on diagnostic sensitivity&lt;br&gt;• Cannot differentiate superinfections</td>
</tr>
<tr>
<td>mFOI</td>
<td>The number of new parasite clones acquired by a host over time</td>
<td>• Population-level transmission intensity&lt;br&gt;• Transmission heterogeneity</td>
<td>Cohort study &gt;6 months with parasite genotyping</td>
<td>• Highly sensitive for monitoring changes in malaria exposure&lt;br&gt;• Superinfections can be differentiated</td>
</tr>
<tr>
<td>MOI</td>
<td>The number of different parasite strains coinfecting a single host</td>
<td>• Population-level transmission intensity&lt;br&gt;• Transmission heterogeneity</td>
<td>Parasite genotyping of positive samples</td>
<td>• Saturates at high transmission&lt;br&gt;• Restricted by age dependency&lt;br&gt;• Insensitive at low transmission&lt;br&gt;• Highly sensitive to spatial heterogeneity&lt;br&gt;• Highly sensitive to increases in imported infection&lt;br&gt;• Less sensitive to changes in seasonality</td>
</tr>
<tr>
<td>Genotyping: SNPs or amplicon sequencing</td>
<td>Genetic diversity, i.e., number of alleles in a population&lt;br&gt;Parasite signatures to map geographical relatedness of infection (i.e., spatial–temporal transmission)</td>
<td>• Population-level transmission intensity&lt;br&gt;• Transmission heterogeneity&lt;br&gt;• Geographical tracking of transmission patterns</td>
<td>• Haplotypes composed of &gt;12 informative SNPs from single clone infections&lt;br&gt;• Haplotype signatures from highly variable loci</td>
<td>• Sensitive to changes in malaria exposure and spatial–temporal flow of infection&lt;br&gt;• Standardisation of measures needed&lt;br&gt;• Methods for analysis and interpretation of data needed</td>
</tr>
<tr>
<td>Antibody seroprevalence</td>
<td>The percentage of seropositive individuals in a population</td>
<td>• Population-level transmission intensity</td>
<td>Seronegative or seropositive defined using appropriate cutoff points</td>
<td>• Dependent on antibody target tested&lt;br&gt;• Saturates at high transmission&lt;br&gt;• Sensitive at low transmission</td>
</tr>
<tr>
<td>SCR</td>
<td>The rate (typically annual) by which seronegative individuals become seropositive upon malaria exposure</td>
<td>• Population-level transmission intensity&lt;br&gt;• Temporal changes in transmission can be detected from a single sampling time point</td>
<td>Detection of antibodies in sera using serological assay (IFAT, ELISA, bead-based assays microarray)</td>
<td>• Dependent on antibody target tested&lt;br&gt;• Restricted by age dependency&lt;br&gt;• Saturates at high transmission&lt;br&gt;• Sensitive at low transmission&lt;br&gt;• Sensitive to risk of malaria in absence of transmission</td>
</tr>
</tbody>
</table>

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

https://doi.org/10.1371/journal.pmed.1002452.t003
For all these metrics, however, standardisation of methods is necessary, as well as a quantitative comparison to understand the relationship with existing and other new metrics. The development of operationally suitable platforms will ultimately be required to inform real-time or rapid response in programmatic settings. In relation to this, there needs to be a clearer understanding of what measures are needed to better define and monitor transmission, and what measures are useful for control programmes. New approaches to analyse metrics from different sources to improve estimates of transmission, or confirm its interruption, are needed. Looking to the veterinary world could be informative, where probability-based survey methods such as “freedom from infection” are used for animal disease surveillance in the food and agriculture industry [110]. These methods are based on defining the probability that a population is free of infection, allowing operational surveillance thresholds to be set based on the chosen sampling frame and the sensitivity of available diagnostics. Adapting these strategies for use in malaria surveillance will require tailoring the methods for specific malaria transmission measures.

Multimetrics to characterise transmission in time and space

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

Summary

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

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

The application of new and/or refined metrics for routine surveillance activities or research-specific contexts requires investigation. This needs to be done in the context of existing standard measures and the newer data collection platforms to understand the true utility. Metrics will also need to be optimised for the quality of the healthcare system in which they will be
Box 3. Research and development agenda

Characterising the reservoir

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

Research goals

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

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

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

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

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

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COLLECTION REVIEW

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

The maLERA Refresh Consultative Panel on Tools for Malaria Elimination

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

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Abstract

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

Summary points

- Achieving malaria elimination likely requires new interventions and strategies in some settings. In addition, the effectiveness of existing tools must be preserved and tools deployed to counter the numerous challenges, key among which are the emergence and spread of drug-resistant parasites and mosquitoes with resistance to vector control measures.
The key research goal for diagnostics is the detection of populations with subclinical infections and low parasite counts. Such diagnostics enable the development of effective surveillance systems directed at malaria parasite elimination.

The availability of new transmission-blocking drugs, vaccines, and vector control products would accelerate elimination where there is refractoriness to currently available interventions. New regulatory pathways and product development models are needed to efficiently develop and assess these new interventions.

In areas endemic for *Plasmodium vivax* and *P. ovale*, the hypnozoite reservoir must be targeted with more robust tools and strategies.

In areas of declining transmission, as cases become less frequent, the contribution to transmission of the subclinical parasite reservoir needs to be quantified and addressed with transmission-blocking interventions.

For vector control, addressing continuing escalation of insecticide resistance—including through the identification of new chemical classes and longer-lasting insecticide formulations—remains a priority. Changes in vector populations and behaviours must also be addressed to restore responsiveness to existing interventions. In some areas, new paradigms may be needed to understand how to design interventions that reduce vector populations and receptivity to sufficiently low levels.

Policy and decision makers, faced with chronic resource limitations, insufficient surveillance, spatial and temporal heterogeneity of malaria parasite transmission, and multiple intervention choices, need improved strategies and guidance on how, where, and when to best combine and deploy existing and new interventions to maximise their longevity and effectiveness.

**Introduction**

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

**Abbreviations:** ACT, artemisinin-based combination therapy; BS-VIMT, blood-stage vaccine that interrupts malaria parasite transmission; ChAd63, chimpanzee adenovirus 63; CHMI, controlled human malaria infection; GGD0, glucose-6-phosphate dehydrogenase; iPCR, insulated isothermal polymerase chain reaction; IRS, indoor residual spraying; LAMP, loop-mediated isothermal amplification; LDH, lactate dehydrogenase; LDR-FM, ligase detection reaction fluorescent microsphere; LLIN, long-lasting insecticidal net; malERA, Malaria Eradication Research Agenda; MDA, mass drug administration; MPT, multipurpose prevention technology; MVA, Modified Vaccinia Virus Ankara; NCE, new chemical entity; NINA-LAMP, noninstrumented nucleic acid amplification LAMP; PCR, polymerase chain reaction; PE-VIMT, preerythrocytic vaccine to interrupt malaria transmission; PPRP2, *Plasmodium falciparum* histidine-rich protein 2; OT-NASBA, quantitative nucleic acid sequence-based amplification; qPCR, quantitative PCR; R&D, research and development; RDT, rapid diagnostic test; SERCap, single encounter radical cure and prophylaxis; SMC, seasonal malaria chemoprevention; SNP, single nucleotide polymorphism; SPAQ, sulfadoxine-pyrimethamine + amodiaquine; SSM-VIMT, sexual-sporogonic-mosquito-stage vaccines to interrupt transmission; TPP, target product profile; WHO, World Health Organization.

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Progress on tools for malaria elimination

Based on literature reviews and panel consultations [11], the most significant advances in the development and deployment of malaria control and elimination tools between 2011 and 2015 were identified (S1–S4 Texts). For diagnostics, advances include widespread incorporation of *P. falciparum* rapid diagnostic tests (RDTs) into routine malaria case management [12], development of highly sensitive tools for detecting subclinical infections, and development and deployment of combined tests that differentiate *P. falciparum* from *P. vivax*. For drugs, advances include the deployment of hundreds of millions of artemisinin-based combination therapy (ACT) courses [12], publication of guidelines for mass drug administration (MDA) [15], the recommendation of low-dose primaquine for transmission interruption [16], progression of new antimalarial compound classes into clinical development [17–19], field trials to evaluate the potential role of medicines in killing mosquitoes (endectocides) [2], and the identification of *Kelch13* as a marker for artemisinin resistance, enabling mapping of its geographic distribution [20,21]. For vaccines, advances include the Article 58 positive opinion by the European Medicines Agency and recommendations by the World Health Organization (WHO) on the first vaccine targeting malaria, RTS,S-AS01e (Box 1) [22–33]; revision of the Malaria Vaccine Technology Roadmap [34]; and new vaccines that progressed to clinical trials [35,36]. For vector control, advances include registration of 2 additional long-lasting insecticide formulations for indoor residual spraying (IRS) [37,38], field trials of dual-insecticide bed nets [39–41], development programmes for new insecticides [42–44], and publication of the larval source management operational manual by WHO [45]. Advances have also been made in the ‘tools for developing tools’—for example, controlled human malaria infection (CHMI) blood-stage parasite inoculation (Box 2) [46–56]; the human blood-stage challenge model for early-stage determination of antimalarial drug pharmacokinetics/pharmacodynamics [57]; the development of human liver chimeric mice, human erythroid chimeric mice, and dually engrafted mice allowing replication of the entire *P. falciparum* life cycle [58]; and validation of phenotypic assays for gametocyte screening to identify compounds with transmission-blocking activity [59]. In addition, new technologies and scientific insights are emerging [10], with notable improvements in mapping and modelling [7,60–62].

Diagnostics research agenda

Diagnostics for malaria treatment and elimination

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

Beyond *P. falciparum*, improved RDTs are needed for other species. Available lactate dehydrogenase (LDH)-targeting RDTs are less sensitive for *P. vivax* compared to *P. falciparum*, because *P. vivax* parasite densities tend to be much lower [72]. There is a paucity of information on test performance against minor species.
Box 1. Malaria vaccine RTS,S/A101E

In 2015, the preerythrocytic candidate vaccine RTS,S/AS01E (RTS,S) received a positive scientific opinion by European regulators through the Article 58 procedure. This was a breakthrough in malaria vaccine development, identifying a regulatory pathway and demonstrating that the large clinical trials necessary for approval could be conducted in Africa [23–25].

- The target for RTS,S is the reduction of malaria incidence and severe disease in young children. A 3-dose regimen was shown to reduce the number of malaria cases by half in children 5–17 months of age during the first year following vaccination; efficacy waned over time but was prolonged by a fourth dose [25].

- Despite modest efficacy, RTS,S prevented about 1,700 cases for every 1,000 children vaccinated in a phase III study over a 4-year period, and modelling studies predict a considerable public health impact for RTS,S, with the greatest benefit expected in areas with the highest malaria burden [27].

- Following review of RTS,S data by the Strategic Advisory Group of Experts on Immunization and the Malaria Policy Advisory Committee, in 2016 the WHO adopted recommendations for RTS,S pilot implementations in 3–5 settings involving 100,000–200,000 children per setting (for a total of 400,000–800,000), in a staged manner to further evaluate safety (including meningitis [26]), feasibility of delivery, and impact on mortality.

- Phase IV studies with a primary objective of further evaluation of safety as part of the Risk Management Plan approved by European Medicines Agency are planned to be linked to the larger pilots, with complementary design and objectives [28,29].

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

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

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

  - A small study with RTS,S and an earlier adjuvant (AS02) found that fractional dosing, i.e., 2 full monthly doses plus a third low-dose at 7 months, resulted in apparent high efficacy against *P. falciparum* challenge (6/7 protected) [30].

  - A recent CHMI study in a larger number of volunteers using RTS,S/AS01 confirmed that a 0-, 1-, 7-month regimen that included a fractional third dose (Fx017M) was associated with higher efficacy (86.7% [95% confidence interval [CI], 66.8%–94.6%]; 26/30 protected) than the standard monthly full-dose regimen (62.5% [95% CI, 29.4%–80.1%]; 10/16 protected) against infection 3 weeks after the third dose [31].

- **Additional applications for RTS,S**

  Additional applications of RTS,S explored through modelling and, if indicated, evaluated in carefully designed field studies over the next 5-year period include the following [33]:
• Evaluating the contribution to elimination of artemisinin-resistant parasites in the Greater Mekong Subregion, although data supporting an adult indication (dose and regimen) would be needed [32];

• Combining RTS,S with other interventions or another malaria vaccine (mass drug administration [MDA] or a future VIMT, respectively), with the aim of enhancing or extending their effects;

• Combining RTS,S with seasonal malaria chemoprevention (SMC), a study of which is in progress in Burkina Faso and Mali.

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

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

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

Simplified molecular methods to detect low-level *P. falciparum* parasitaemia for use in low-resource settings are being developed, although improvements in throughput and cost are required [82]. Loop-mediated isothermal amplification (LAMP) is 1 promising approach, already validated in low-transmission settings [83] and as point-of-care detection of asymptomatic low-density malaria parasite carriers [84]. Further developments include noninstrumented nucleic acid amplification LAMP (NINA-LAMP) [85], achieving comparable sensitivity to *P. falciparum* polymerase chain reaction (PCR) detection in the field [85,86]. Another approach, using an insulated isothermal PCR (iPCR) in a commercially available portable device, for *Plasmodium* detection achieved an assay efficiency of 96.9% with a lower detection limit of ≥100 copies of plasmodial DNA [87]. Nucleic acid amplification techniques
Box 2. Tools for developing tools: Demonstrating transmission-blocking activity

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

**Mosquito feeding assays**

Three assays are available for assessing transmission-blocking activity:

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

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

**Controlled human malaria infection (CHMI) model**

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

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

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

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

can also be used for multiple pathogens in parallel, incorporating other infectious diseases (e.g., Ebola, dengue, and typhoid), depending on the setting and target population [88].
Fig 1. Tools for detecting and interrupting malaria transmission and their action in the malaria transmission cycle.

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Noninvasive testing

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

Detecting gametocytes

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

Detecting drug resistance

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

Detecting hypnozoites

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

Glucose-6-phosphate dehydrogenase (G6PD) testing

An affordable, easy-to-use, rapid, point-of-care, semiquantitative diagnostic test is needed to identify G6PD-deficient individuals at risk of haemolysis with use of 8-aminoquinolones (i.e.,
primaquine or tafenoquine) to prevent *P. vivax* relapses. Although several tests are available [105], further refinement is needed to support greater access to these medicines. Single administration of low-dose (0.25 mg/kg) primaquine as a gametocytocidal agent is recommended after treatment for *P. falciparum* malaria [106], but there is still a need for more data on the optimal dose and reassurance of safety in G6PD-deficient individuals and larger populations, if used in MDA, for example [107].

**Pregnancy testing**

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

**Challenges**

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

**Drug research agenda**

**Drugs for malaria treatment, prevention, and transmission interruption**

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

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

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

Reducing duration of dosing regimens, ideally to a single dose, increases adherence, be it for prevention or treatment [8]. Although better adherence improves effectiveness, it must be achieved without significantly increasing the risk of selection for drug-resistant parasites as a result of creating long periods of subtherapeutic drug levels. As a country or area approaches elimination, the remaining parasites are likely to be those most resistant to treatment, and drug classes with a low propensity to select for parasite resistance should be prioritised [111,
112]. The temptation to combine new drugs with old drugs with preexisting resistance, whilst simpler from a regulatory perspective, must be avoided to prevent novel agents being exposed as functional monotherapies when used against strains resistant to the older partner drug.

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

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

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

**Antihypnozoite drugs**

In areas of high transmission, such as Papua New Guinea, relapses cause approximately 4 of every 5 *P. vivax* infections [128]. Modelling suggests that for rapidly relapsing tropical *P. vivax* strains, effective relapse prevention has the potential to significantly reduce transmission [104]. In areas of seasonal transmission, relapses allow parasites to rapidly reestablish transmission once vector populations recover [129]. The 8-aminoquinoline primaquine is the only antirelapse therapy currently available (aside from chloroquine, to which there is extensive resistance), but treatment courses are 7–14 days, and poor adherence undermines effectiveness [130]. Tafenoquine is a candidate single-dose 8-aminoquinoline, showing high antirelapse efficacy in *P. vivax* infections when given with chloroquine [18]. Phase III clinical trials were completed in 2016, with regulatory submission anticipated in 2017. The impact of tafenoquine on transmission
remains to be evaluated in post-registration CHMI and field trials. G6PD-deficient individuals cannot be given standard doses of 8-aminoquinolones; in addition, 8-aminoquinolones are considered contraindicated during pregnancy and lactation. As such, new antihypnozoite drug classes without these contraindications are needed. Discovery should be enhanced in the next 5 years through screening campaigns against *P. vivax* liver stages using stable human cell systems [131,132]. Additionally, humanised mouse models are facilitating drug development against this life cycle stage that thus far has been refractory to study [133].

**Seasonal malaria chemoprevention (SMC)**

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

**Endectocides**

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

**Novel formulations**

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

**Challenges**

Attrition rates in antimalarial drug development are comparable with those in other infectious diseases [126]. Thus, discovery momentum needs to be maintained at high levels if new drugs are to reach licensure. A major challenge in registering NCEs for malaria is assembling the substantial clinical safety data required for regulatory approval, particularly in the key target populations of infants and pregnant women. Thus, reproductive safety should be evaluated early in preclinical development to prioritise investment in NCEs with appropriate preclinical profiles.
With the introduction of NCEs during the next 5 years, pharmacovigilance needs strengthening in malaria-endemic areas. This is also a prerequisite for safe deployment of current ACTs and next-generation treatments during mass treatment programmes targeted at populations that include individuals with subclinical malaria or who are infection free.

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

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

**Vaccine research agenda**

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

**Vaccines to prevent clinical malaria and interrupt transmission**

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

Blood-stage vaccines are an alternative and complementary approach to PE-VIMT. Blood-stage vaccines that interrupt malaria parasite transmission (BS-VIMTs) by efficiently clearing blood-stage infections would limit gametocyte densities and the duration that a person is infectious, thus reducing human-to-mosquito malaria parasite transmission (Fig 1). Several promising *P. falciparum* vaccine candidates are in clinical development [150], including the unstructured peptide P27A, the well-studied PfRH5, and the 2 placental malaria vaccine...
candidates PAMVAC and PRIMVAC (S2 Table). Innovative new concepts in next-generation malaria vaccine protein subunit design are being explored to develop highly effective multi-component/multistage/multiantigen formulations [151].

Vaccines that only interrupt malaria parasite transmission

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

Vaccines for P. vivax/P. ovale

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

Adjuvants, delivery platforms, and desired human immune responses

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

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

Challenges

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

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

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

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

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

Insecticide-based interventions

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

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

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Product</th>
<th>Insecticide(s)</th>
</tr>
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<tbody>
<tr>
<td>IRS</td>
<td>Phantom</td>
<td>Chlorfenapyr (phase III)</td>
</tr>
<tr>
<td></td>
<td>SumiShield</td>
<td>Clothianidin (phase II)</td>
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<td></td>
<td>Fludora Fusion</td>
<td>Deltamethrin + clothianidin (phase II)</td>
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<tr>
<td>LLINs</td>
<td>DawaPlus 2.0</td>
<td>Deltamethrin coated on polyester</td>
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<td></td>
<td>LifeNet</td>
<td>Deltamethrin incorporated into polypropylene</td>
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<td></td>
<td>MiraNet</td>
<td>Alpha-cypermethrin incorporated into polyethylene</td>
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<td></td>
<td>Panda Net 2.0</td>
<td>Deltamethrin incorporated into polyethylene</td>
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<td></td>
<td>Yahe</td>
<td>Deltamethrin coated on polyester</td>
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<tr>
<td>LLINs + PBO</td>
<td>Olyset Plus</td>
<td>Permethrin + PBO incorporated into polyethylene</td>
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<tr>
<td></td>
<td>PermaNet 3.0</td>
<td>Deltamethrin coated on polyester side panels; deltamethrin + PBO incorporated into polyethylene (roof)</td>
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<tr>
<td></td>
<td>Veeratin</td>
<td>Alpha-cypermethrin and PBO incorporated into polyethylene</td>
</tr>
<tr>
<td>Combination LLINs</td>
<td>Olyset Duo</td>
<td>Pyriproxyfen and permethrin incorporated into polyethylene</td>
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<tr>
<td></td>
<td>Intercepter G2</td>
<td>Alpha-cypermethrin + chlorfenapyr coated on polyester</td>
</tr>
</tbody>
</table>

*March/April 2016.
PBO, piperonyl butoxide

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New ways of using insecticides require more extensive field evaluation, e.g., technological advances for improving spraying techniques [184], timing of insecticide deployment to coincide with seasonal transmission, slow-release polymer-based wall linings [185,186], insecticide-treated eave tubes or eave ‘bricks’ combined with house screening, and electrostatic coatings to enhance insecticide bioavailability [187].

Vector behaviour and outdoor targeting

Greater understanding of vector behaviour is needed, including the behavioural adaptations of vectors in response to control measures, such as changes in biting times, resting locations, and rates of zoophagy [188–194]. Improved targeting of specific vector behaviours—particularly sugar feeding, oviposition, mating, dry-season survival, and swarming behaviour—and zooproxyaxis are generating novel approaches to vector control, with potential application across transmission settings [195,196].

Long-standing evidence that malaria parasite transmission to humans occurs outdoors in Southeast Asia and South America and increasing evidence of outdoor transmission in sub-Saharan Africa [3,197–202] suggest a specific need for interventions that target mosquitoes outside dwellings. Attractants/traps are a potential new area of mosquito control that can be applied both indoors and outdoors. These include attractive toxic sugar baits [203,204] and sound traps, which lure male mosquitoes by broadcasting sounds similar to the wingbeats of female mosquitoes [195,203]. All major malaria parasite vectors in Africa mate in swarms [206], which are easily found and recognised, appear to be stable over time, and exist in a defined space [195]. This facilitates close targeting either with insecticides or traps [195]. Spatial repellents are another approach, releasing into the air volatile chemicals that prevent human–vector contact within the treated space (indoor or outdoor). Guidelines for efficacy testing are now available [205,207], and evaluation in outdoor settings is needed [208,209].

Environmental management and larval source management

Environmental management, such as improved housing and water management, can be highly effective in specific epidemiological and environmental settings [210]. The best of these environmental management approaches require further investigation in tropical climates and resource-poor settings to establish their epidemiological impact in these settings [210,211]. Mosquito larval source management is the management of water bodies that are potential larval habitats to prevent immature mosquitoes developing into adults, either by environmental management or application of larvicides [45]. Larval source management has been highly effective in certain situations [211], but as this is a resource-intensive activity, better definition of the appropriate requirements and approaches across a wider range of settings is needed.

Genetic approaches

There are 2 main strategies for genetically modifying mosquito populations: (1) population suppression, whereby mosquitoes are modified in such a way that upon mating with the wild type the resulting progeny are either sterile or dysfunctional, and (2) population alteration or replacement, in which the mosquitoes are modified in such a way that upon mating with the wild type, the resulting progeny are rendered refractory to malaria parasite infection. Genetic approaches now appear operationally feasible given recent advances in molecular biology, such as the efficient genome-editing techniques based on CRISPR/Cas9 and other approaches [10,212,213].

The sterile insect technique was the first attempt at genetic population suppression, whereby large numbers of irradiated sterile males are released with the hope that females mate
unsuccessfully [214]. A more recent development is the release of insects carrying a dominant lethality, with the progeny of females mating with genetically modified males inheriting a lethal gene [215,216].

Gene drive systems exploit ‘homing’ endonucleases. These induce the lateral transfer of an intervening DNA sequence to a homologous allele that lacks that sequence, thereby changing a heterozygote into a homozygote. Conventional homing endonucleases have been reengineered to recognise mosquito genes [217] and can rapidly increase the frequency of desirable traits in a mosquito population [218]. Technical feasibility has been demonstrated for a CRISPR/Cas9--based gene drive system with the potential to reduce mosquito populations [219] or make them less able to transmit malaria parasites [220].

There is also the potential for symbiont-mediated biocontrol in malaria Anopheles mos-quito populations, as suggested by recent successes achieved against Aedes aegypti (e.g., Wolbachia-mediated pathogen interference for dengue control). A further step is paratransgenesis, whereby a vector symbiont (virus, bacteria, or fungi) is engineered to express ‘effector’ molecules within the vector that are deleterious to the pathogen. Genetic modification of symbionts is easier than it is for mosquitoes and is independent of mosquito species, providing the symbi-ont can survive and colonise the host [221], and laboratory studies have shown promise [222].

There are environmental uncertainties associated with widespread distribution of technolo-gies involving genetic manipulation of pathogens, vectors, or their symbionts [10,212]. Phased testing starting at a small scale is recommended, though the parameters for ecological risk assessment are not well understood.

Challenges

The development of new insecticides will need to outpace the expansion of insecticide-resis-tant alleles in mosquito populations, and new products will need to be deployed to effectively combat behavioural resistance [8]. The imperfect correlation between entomological indica-tors and disease incidence complicates the accurate assessment of new vector control tools. Randomised controlled trials are expensive and time consuming, and new pathways should be explored for generating evidence for large-scale implementation of new interventions. Increasing fine-scale heterogeneity, in human and vector subpopulations and in geographic space, means that no single set of interventions will be effective across large areas or districts. Notwithstanding resource availability, the challenge is to understand which combinations of vec-tor control measures are appropriate in different settings and how their effects can be augmented with other interventions (e.g., endectocides, transmission-blocking drugs, and vac-cines) [7]. Targeting mosquito dormancy remains a challenge in large part because of the pau-city of mechanistic evidence by which vectors persist during the dry-season (e.g., diapause [ aestivation] and long-distance migration) [223]. Finally, it is important to note that there are very few trained entomologists in national malaria control programs, especially at the district level. To develop and implement vector-targeted interventions, greater entomology capacity building is required.

Conclusions

There are overarching areas in which greater knowledge is required to understand the utility of current interventions and define which products and strategies might be required going for-ward. Novel tools may allow further investigation of knowledge gaps, and some may be bridge-able (Table 2). The R&D agenda for tools for elimination is summarised in Box 3.

Transmission can remain high even with high coverage of good-quality case management and vector control. Thus, products and strategies directed specifically at accelerating
elimination by targeting transmission are needed. Interventions may only achieve transmission reduction when deployed in certain populations or settings. Conversely, some populations and settings may require specific measures for transmission reduction, for example, pregnant women and infants, migrant workers, subclinical parasitaemia, or addressing outdoor transmission. The availability of new interventions is expanding, but developing algorithms for their rational combination and deployment in packages to decrease transmission is a key research need that requires modelling support [7]. Cost-effectiveness is an important determinant of whether particular interventions are adopted in public health programmes [9].

New products and strategies are needed to overcome parasite drug resistance and vector resistance to insecticides [8]. Prevalence of vaccine escape mutants has been highlighted as a potential issue if vaccines become widely used [170-172]. Thus, product development must continue, and strategies for phased replacement are needed as effectiveness wanes. New product discovery and development requires investment in basic science [10], the alignment of regulatory structures to expedite product registration, and continued investment in pharmacovigilance and surveillance. Funding organisations and malaria programmes also need to be
Box 3. Research and development agenda for tools for elimination

**Diagnostics**

*Detecting transmission potential*
- Malaria diagnostic tools best suited for detection of low-density, subclinical infections
- Assay for detecting infectious gametocytes
- *P. vivax/P. ovale* hypnozoite detection methods
- Noninvasive diagnostic tests

*Directing treatment*
- Stable, valid, specific, and sensitive rapid diagnostic tests (RDTs) that do not depend on histidine-rich protein 2 (HRP2)
- Detection of drug-resistant parasites
- RDTs that detect and differentiate all relevant human *Plasmodium* ssp. pathogens
- Multiplexed point-of-care tests for acute febrile illness

*Special populations*
- Affordable, simple, and accurate point-of-care tests for glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals and pregnant women

**Drugs**

*Drugs for prevention and treatment*
- Drugs that overcome resistance to existing drugs, particularly artemisinin resistance
- A suite of combination drugs with different or competing resistance profiles
- New drugs for prophylaxis
- Simplifying therapy, with the potential objective of a single encounter radical cure and prophylaxis (SERCaP)
- New regimens for use in seasonal malaria chemoprevention (SMC) outside the Sahel and to potentially replace sulfadoxine-pyrimethamine + amodiaquine (SPAQ)

*Drugs to interrupt transmission*
- Investigation of the impact of low-dose primaquine in different settings
- New drugs with transmission-blocking potential
- Drug combinations incorporating both asexual and transmission-blocking activity
- Evaluate the impact of transmission-blocking drugs on pathogen resistance development and investigate optimal deployment strategies
- Endectocides for use in humans and animals

*Antihypnozoite drugs*
- Evaluation of *P. vivax* transmission reduction potential with tafenoquine via relapse prevention (draining of hypnozoite reservoir)
Special populations

- New small molecules or antibodies with a potential indication for use during pregnancy

Vaccines

RTS,S

- Further evaluation of RTS,S to determine the potential for increased efficacy with alternative dosing regimens
- Assessment of RTS,S in combination with other interventions (e.g., SMC) and in other epidemiological settings and populations

New vaccines

- Defining the required attributes of preerythrocytic or blood-stage vaccines to achieve transmission-blocking activity
- New preerythrocytic and or blood-stage vaccines, ideally with transmission-blocking potential
- A first sexual-sporogonic-mosquito-stage vaccine to interrupt transmission (SSM-VIMT)

Vaccines against P. vivax/P. ovale

- Vaccines that prevent infection and hypnozoite formation, target hypnozoites, or can interrupt transmission to eventually eliminate the hypnozoite reservoir

Adjuvants

- Access to a broader choice of adjuvants with improved risk–benefit profiles

Prophylactic biologics

- Development of monoclonal antibodies (mAbs) and combinations of recombinant multi-mAbs products

Vector control

Insecticides and long-lasting insecticidal nets (LLINs)

- New insecticides and combinations of insecticides to overcome vector resistance
- Nonpyrethroid insecticides for LLINs
- Investigation of new insecticide deployment strategies
- LLINs with improved durability

Environmental management

- Formal investigation of larval source management in a greater variety of settings
- Development of long-lasting safe larvicides
- Development of cost-effective and socially acceptable environmental management interventions

Genetic approaches
• Development of scalable genetic approaches
• Development of environmentally and socially responsible methods for field testing transgenic organisms

Exploiting vector behaviour
• Novel interventions to target populations and behaviours
• Increased entomological support for key decisions by national malaria programmes

Combination and mapping
• Modelling to suggest the most effective and efficient combinations of vector control for different settings
• Developing operationally relevant mapping tools to identify and target residual transmission

'Tools to develop tools'
• Validating outcomes from animal and human infection models that predict a reduction in transmission in real-life settings
• Robust mathematical and laboratory models of transmission and impact of combination interventions
• Increased understanding of parasite–host immunity and mechanisms of acquired and vaccine-induced protective and transmission-blocking immunity
• Development of high-throughput screening assays and evaluation assays for the identification and selection of compounds with neglected profiles (e.g., antihypnozoite activity)

convinced that tools are impactful and cost-effective [9]. However, measuring the efficacy of tools that potentially impact transmission is problematic, particularly at the extremes of transmission [154]. Thus, new diagnostics and screening methods are required to assess tool efficacy in low-transmission settings and determine their contribution to maintaining zero transmission [73]. Moreover, the development of new diagnostics with improved sensitivity, or for specific tasks such as resistance surveillance, may fundamentally change our perception of malaria parasite transmission and our understanding of the most appropriate interventions required to interrupt transmission.

Finally, developing new tools can be expensive. When the malaria burden is significant, the economic case for innovation is clear. However, as the malaria burden decreases, the economic argument for continued development becomes more nuanced. Public–private partnerships, which first emerged 15 years ago, have demonstrated the ability to partner and drive development for a variety of tools, including diagnostics (e.g., PATH and Foundation for Innovative New Diagnostics), drugs (e.g., Medicine for Malaria Venture and formerly Drugs for Neglected Diseases Initiative), vaccines (e.g., PATH Malaria Vaccine Initiative and European Vaccine initiative), and vectors (e.g., Innovative Vector Control Consortium and more broadly Malaria No More). New business models to attract and engage industry in developing tools for
the elimination should be considered as well. Interventions will be directed at increasingly smaller populations, but these populations often represent the most difficult contexts in which to achieve elimination, and multiple interventions may be required. Once a country achieves elimination, there is the temptation to scale back infrastructure and interventions for malaria. This risks triggering a potentially lethal outbreak that could be difficult to reeliminate or even contain. There are numerous examples from earlier malaria elimination campaigns in the 1950s and 1960s of initial successes that were followed by resurgence as campaigns were deprioritized or discontinued administratively, financially, and technically. Unless malaria can be completely eradicated, interventions to maintain malaria elimination and a reserve of effective measures to counter malaria outbreaks will always be needed. However, if the right products and strategies are developed, and if they are used efficiently, effectively, and consistently, malaria eradication is an achievable goal.

**Supporting information**

S1 Table. Antimalarial drugs in preclinical and early clinical development.
(PDF)

S2 Table. Vaccines against *P. falciparum* in clinical development.
(PDF)

S3 Table. Vaccines against *P. vivax* in clinical development.
(PDF)

S1 Text. Summary of progress in malaria diagnostics since the initial Malaria Eradication Research Agenda (malERA) initiative and remaining gaps.
(DOCX)

S2 Text. Summary of progress in malaria drugs since the initial Malaria Eradication Research Agenda (malERA) initiative and remaining gaps.
(DOCX)

S3 Text. Summary of progress in malaria vaccines since the initial Malaria Eradication Research Agenda (malERA) initiative and remaining gaps.
(DOCX)

S4 Text. Summary of progress in malaria vector control since the initial Malaria Eradication Research Agenda (malERA) initiative and remaining gaps.
(DOCX)

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Members of the MESA malERA Refresh Tools for Malaria Elimination Panel were as follows:

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COLLECTION REVIEW

maLERA: An updated research agenda for combination interventions and modelling in malaria elimination and eradication

The maLERA Refresh Consultative Panel on Combination Interventions and Modelling

†Membership of the maLERA Refresh Consultative Panel on Combination Interventions and Modelling is listed in the Acknowledgments.

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Abstract

This paper summarises key advances and priorities since the 2011 presentation of the Malaria Eradication Research Agenda (maLERA), with a focus on the combinations of intervention tools and strategies for elimination and their evaluation using modelling approaches. With an increasing number of countries embarking on malaria elimination programmes, national and local decisions to select combinations of tools and deployment strategies directed at malaria elimination must address rapidly changing transmission patterns across diverse geographic areas. However, not all of these approaches can be systematically evaluated in the field. Thus, there is potential for modelling to investigate appropriate ‘packages’ of combined interventions that include various forms of vector control, case management, surveillance, and population-based approaches for different settings, particularly at lower transmission levels. Modelling can help prioritise which intervention packages should be tested in field studies, suggest which intervention package should be used at a particular level or stratum of transmission intensity, estimate the risk of resurgence when scaling down specific interventions after local transmission is interrupted, and evaluate the risk and impact of parasite drug resistance and vector insecticide resistance. However, modelling intervention package deployment against a heterogeneous transmission background is a challenge. Further validation of malaria models should be pursued through an iterative process, whereby field data collected with the deployment of intervention packages is used to refine models and make them progressively more relevant for assessing and predicting elimination outcomes.

Summary points

- Since 2011, there have been significant improvements in the development, organisation, and infrastructure of country programmes for malaria control and elimination globally. This has included the increasing use of combinations of interventions against the mosquito vector and the parasite in humans to reduce transmission in large and expanding
and the Wellcome-Trust Major Overseas Programme in SE Asia.

Abbreviations: ACT, artemisinin-based combination therapy; ESP, Elimination Scenario Planning; IMDA, focal mass drug administration; IRS, indoor residual spraying; LLIN, long-lasting insecticidal bed net; malERA, Malaria Eradication Research Agenda; MDA, mass drug administration; MSAT, mass screening and treatment; NMCP, National Malarial Control Programme; WHO, World Health Organization.

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

geographies and populations and an adaptation of these interventions as transmission is progressively reduced.

- Similarly, there has been substantial improvement in the sophistication and field validation of malaria transmission models and their ability to describe and predict the effects of ecologic changes and the impact of specific interventions. These advances permit the investigation and comparison of multiple complementary interventions in elimination settings.

- There is an increasing need to combine interventions into ‘packages’ that can be tailored to specific settings based on the characteristics of their transmission dynamics and epidemiology (landscape stratification). The challenge is to identify the complementary components of each intervention package and establish the triggers and thresholds for their deployment (or withdrawal) throughout the elimination process, including maintaining elimination once transmission has been interrupted.

Introduction

In 2011, the Malaria Elimination Research Agenda (malERA) made recommendations for how mathematical modelling efforts could best inform policy and guide research for specific intervention tools for elimination—diagnostics, drugs, vector control, and vaccines [1]. Since then, experience with malaria intervention tools has grown, and the toolbox has expanded with new drugs, new insecticides, better diagnostics, and a first vaccine [2]. As more countries seek elimination, grouping tools to best address diverse and changing transmission intensity has become a central issue. Some tools are oriented primarily towards reducing disease burden, e.g., seasonal malaria chemoprevention; others are dedicated to reducing transmission, e.g., drug-based population-wide parasite clearance; and some meet both of these objectives, e.g., vector control. Thus, not all tools will contribute equally to malaria elimination, and the timing and duration of their use must adapt as programmes progress.

This paper summarises progress since the initial malERA publication regarding transmission-aligned ‘elimination tool packages’ and deployment strategies and opportunities for models to help inform and prioritise intervention choices. The findings come from an extensive literature review of published and unpublished materials and the deliberations of the 2015 malERA Refresh Consultative Panel on Combination Interventions and Modelling, which includes specialists from malaria modelling, field researchers, and National Malarial Control Programme (NMCP) representatives [3].

Methods

The findings presented in this paper result from an extensive literature review of published and unpublished materials and the deliberations of the 2015 malERA Refresh Consultative Panel on Combination Interventions and Modelling. Electronic databases were systematically searched for published literature from 1 January 2010 until 1 August 2015, without language limitations. The websites of the institutions that apply modelling techniques to malaria research questions and the MESA Track database of current research projects relevant to malaria elimination were systematically searched to identify pertinent ongoing research.
Panellists were invited to recommend additional literature and additional ongoing research projects. The comprehensive search for literature and ongoing research provided the basis for launching the second step.

A 2-day workshop was held with the majority of the panel members, including specialists from malaria modelling, field researchers, and NMCP representatives. The panel broke into 2 working groups to identify the issues in combining interventions and how mathematical modelling could be applied to these problems. Each group fed back to a plenary session in which further robust discussions and input occurred. This helped refine the opportunities and gap areas in which research is needed. The final findings were arrived at with inputs from all panellists and several iterations of the manuscript.

**Intervention packages to achieve elimination**

Over the past 5 years, regardless of initial local transmission levels, most countries have continued to reduce the clinical burden of malaria and transmission [4]. The World Health Organization (WHO) recently published its Global Technical Strategy (GTS) for Malaria 2016–2030 (Fig 1) [5]. This builds on the core activities of vector control, case management, and surveillance, with additional interventions to accelerate progress to elimination. In the GTS, for the first time, modelling studies were used to support goal setting [5].

The malERA Refresh Consultative Panel on Combination Interventions and Modelling approach encompassed the full spectrum of malaria transmission—addressing emerging programmatic aims and combining into ‘packages’ the available tools and strategies directed towards malaria elimination (Fig 2). As transmission is reduced to very low levels, the intervention packages must adapt to increasingly focal and heterogeneous populations, in which infections are rare. Given the extensive range of available tools and the diversity/heterogeneity of transmission settings, it becomes difficult to field test all possible intervention packages. Models can assist the prioritisation and design of clinical trials and in the choice of an intervention package to achieve their desired goals.

**Progress in combination interventions and modelling**

Initial malERA recommendations for a research and development agenda in mathematical modelling are shown in Box 1 [1]. Subsequently, the scope and depth of research has expanded to include diverse vector control strategies, complex diagnostics, drug and vaccine dynamics, and deployment strategies. Additionally, infection models have advanced following incorporation of new field trial data, particularly regarding mass drug administration (MDA) and specific aspects of vector control, providing greater plausibility to model predictions.

The interface between modelling and implementation has not developed as was perhaps envisaged, in terms of appropriate portals to allow ‘end users’ access to relevant software and explore the effect of varying conditions on the ideal choice of control measures. However, the development, organisation, and infrastructure of malaria modelling has improved (Box 2), and recent efforts include an expansion of open-access data and software [6–13]. Also, modelling has been incorporated at the policy level within WHO [5] and included in planning tools for malaria elimination [14]. Wider implementation is possibly now dependent upon the development of next-generation models that sufficiently address combination interventions against a background of heterogeneity and low transmission as more countries move towards elimination.

These advances are complemented by discoveries in basic science, large field trials of new and existing interventions, and substantial data gathering efforts that provide the raw evidence to further validate models. A number of recent reports used models to address the role of...
multiple complementary interventions (Table 1) [15–35], and additional field trials are ongoing (Table 2) [29].

Consensus modelling

In consensus modelling, independent modelling groups examine the same research question, sometimes using the same source dataset to parameterise their model. Through objective comparison and critique, modelling groups have reached a degree of consensus on important issues, such as the relationship between health burden and transmission intensity [6], and have undertaken an in-depth analysis for the RTS,S vaccine [36]. Such efforts are resource intensive but may give robust answers incorporating the breadth of uncertainty in our understanding. There is also value in less intensive forms of model comparison in which common findings from work conducted independently are assessed (Table 3) [9,18,21,23,24,28,30–32,35,37–58]. This approach can also be particularly useful for identifying areas in which there is a lack of consensus, as this can focus efforts on further model development, basic science, and field data collection needs.
Fig 2. An example of the role of modelling across the spectrum of malaria elimination. Note that the measures of transmission are based on sub-Saharan Africa, and other constructs and transmission levels may be relevant in different geographical areas. Malaria transmission intensity measures and the relationship entomologic inoculation rate for *Plasmodium falciparum* from very high to zero transmission are adapted from data presented in [6]; personal communication from D. Smith and P. Gething. Zero refers to no locally transmitted cases of malaria infection; imported infections may be identified. Intervention package components and sequencing will depend on transmission intensity at the start of the elimination programme, the speed at which transmission declines, and the underlying typology (i.e., malaria epidemiology, species, vector ecology, and health.
Next steps for combination interventions and modelling in malaria elimination

Fig 2 provides an example of how transmission strata, programmatic aims, the choices of intervention packages, and the iterative development between modelling and programme choices change together as malaria transmission intensity is progressively reduced towards zero, summarizing key opportunities and identifying challenges. Note that not all countries will start from high transmission levels and that the measures of transmission used in Fig 2 are based on sub-Saharan Africa. Thus, other constructs and transmission levels may be relevant in different geographical areas.

Opportunities
Combination intervention modelling
There has been considerable progress in modelling combination interventions. Models have been developed to examine the overall expected impact of diagnostic, drug, vaccine, and vector
Box 2. Recent advances in malaria modelling.

Communications:

- A growing number of modelling groups are working in a collaborative fashion
- Greater engagement between modellers, country programmes, and operational research partners has helped refine the paramount research questions

Models:

- The development of model systems that are diverse but much improved in terms of their incorporation of malaria biology and natural history, as well as validated estimates for intervention effects, drug pharmacokinetics/pharmacodynamics, and vaccine dynamics
- The development of models that allow the investigation of target product profiles for new tools—for example, diagnostics, surveillance systems, and drugs

Infrastructure:

- Greater dissemination of malaria models at different levels of user-interface complexity, through online hosting and open-source code repositories leading to wider access to modelling information for programme implementers, planners, and policy decision makers
- Improved means of compiling data and using common ontologies, frameworks, and metadata standards with growing international databases of some measures of malaria transmission, e.g., parasite rate surveys

control intervention combinations, including cost-effectiveness [16,18–20,24,48,57,59–62], and comparing interventions added to the backbone of standard measures [21–23,25–27,30,36,63,64]. Modelling studies have investigated the applications of several new potential interventions such as the RTS,S vaccine [36], ivermectin [19,54], mosquito traps [17], and next-generation diagnostics [25,33,65,66] and have highlighted critical attributes of new products, such as a preerythrocytic vaccine [20,67–69], genetically modified mosquitoes [70–72], and combinations of future interventions [73].

Models are designed to allow scale-up and scale-down of interventions over time. The next step is to define the epidemiological information that would be most informative for making such dynamic changes and the triggers for switching or scaling. The aim is to develop a set of rules that define the characteristics of transmission that can direct specific changes in the composition and phasing of intervention packages and their targeting to specific locations and populations. These predictions can then be evaluated with further evidence from specific field trials. If reliable, such measures could be used in the subnational stratification of intervention packages.

Accelerating community clearance of malaria parasites. One hypothesis being tested in various settings is the potential to accelerate elimination by targeting the human parasite reservoir (symptomatic and asymptomatic) with time-limited deployment of community-based interventions such as MDA or mass screening and treatment (MSAT) [74]. If the intervention is justified, a wealth of modelling studies provides guidance on optimizing its deployment
Table 1. Key modelling studies on combination interventions quarter 4 2010–quarter 1 2016, with the main outcome indicated.

<table>
<thead>
<tr>
<th>Multi-intervention combined</th>
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<tbody>
<tr>
<td>• Mass campaigns with antimalarial drugs are highly effective at interrupting transmission if deployed shortly after ITN campaigns [15].</td>
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<tr>
<td>• Compared with untargeted approaches, selective targeting of hot spots with drug campaigns is an ineffective tool for elimination because of limited sensitivity of available field diagnostics [16].</td>
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<tr>
<td>• High coverage with a combination of LLINs and attractive toxic sugar baits could result in substantial reductions in malaria transmission [17].</td>
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<tr>
<td>• Mass treatment needs to be repeated or combined with other interventions for long-term impact in many endemic settings [18].</td>
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<tr>
<td>• Including ivermectin in mass treatment strategies could be a useful adjunct to reduce and interrupt malaria transmission [19].</td>
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<tr>
<td>• Preerythrocytic vaccines will have a maximum impact where bed net coverage has saturated, vector feeding is primarily outdoors, and transmission is moderate to low [20].</td>
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<th>Multi-intervention compared</th>
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<tr>
<td>• While adult killing methods can be highly effective under many circumstances, other vector control methods are frequently required to fill effective coverage gaps [21].</td>
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<tr>
<td>• Adding vaccines to existing vector control efforts extends the ability to achieve elimination starting from higher baseline transmission levels and with less favourable vector behaviour [22].</td>
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<tr>
<td>• Decreases in malaria transmission and burden can be accelerated over the next 15 years if the coverage of key interventions is increased [23].</td>
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<tr>
<td>• Vector control plans should consider the spatial arrangement of any intervention package to ensure effectiveness is maximised [24].</td>
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<tr>
<td>• The sensitivity of the diagnostic can play a part in increasing the chance of interrupting transmission [25].</td>
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<tr>
<td>• A failing partner drug will result in greater increases in malaria cases and morbidity than would be observed from artemisinin resistance only [26].</td>
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<tr>
<td>• Selecting combinations of interventions that target different stages in the vector’s life cycle will result in maximum reductions in mosquito density [27].</td>
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<th>Multi-intervention: Cost-effectiveness</th>
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<tr>
<td>• In all the transmission settings considered, achieving a minimal level of ITN coverage is a ‘best buy’. At low transmission, MSAT probably is not worth considering. Instead, MSAT may be suitable at medium to high levels of transmission and at moderate ITN coverage [28].</td>
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ITN, insecticide-treated bed net; LLIN, long-lasting insecticidal bed net; MSAT, mass screening and treatment.

https://doi.org/10.1371/journal.pmed.1002453.t001

[15,18,28,32,33,41,53–55,57,75–79]. However, estimating the level of coverage required for successful MDA is critical [53], and for MSAT, the sensitivity of the diagnostic tool is an additional key determinant of efficacy as the current tests may fail to detect low-level infections [16,25].

Current models of MDA all include the parameters whereby immediately following MDA, there is a dramatic drop in malaria prevalence, but in the absence of elimination, prevalence returns to preintervention levels (albeit at different rates depending on the model) [53]. Country malaria programs are increasingly aware of this potential and have learned not to rely solely on MDA to eliminate transmission; thus, MDA is an accelerator used to move to a next set of interventions and strategies to find and clear the remaining transmission foci. The models must now be adapted to include a next set of actions with the potential to end transmission, i.e., MDA moving to focal MDA (fMDA) and other reactive strategies in households and neighbourhoods with rare but remaining transmission [33,79,80]. In the field, these increasingly infrequent actions will require robust local information systems as part of the intervention, rather than models.

**Non-falciparum species.** Recent progress has been made in models considering non-falciparum parasites and vectors, though further work is needed [76,81–95]. To address the public health and public engagement challenge of eliminating all human malaria species, multispecies
Table 2. Ongoing field studies in combination interventions as reported on the MESA Track database [29].

**Vector control**

<table>
<thead>
<tr>
<th>Study Description</th>
<th>Location</th>
<th>Duration</th>
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<tr>
<td>Combining indoor residual spraying and long-lasting insecticidal nets for malaria prevention: a cluster randomised controlled trial in Ethiopia (Malaria); Ethiopia (Sep 2014–Sep 2016); Addis Ababa University, Ethiopia</td>
<td>Ethiopia</td>
<td>Sep 2014–Sep 2016</td>
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<tr>
<td>Integrated vector management: Interaction of larval control and indoor residual spraying on Anopheles gambiae density and vectorial capacity for human malaria: Malaria Research and Training Center (MRTC), University of Bamako, Mali</td>
<td>Mali</td>
<td>Dec 2013–Dec 2014</td>
</tr>
<tr>
<td>IRS and LLIN: Integration of methods and insecticide mode of actions for control of African malaria vector mosquitoes: Tanzania, United Republic of; Ifakara Health Institute (IHI), Swiss Tropical and Public Health Institute (Swiss TPH)</td>
<td>Tanzania</td>
<td>Jan 2018–Dec 2018</td>
</tr>
<tr>
<td>Cluster randomised trial of the impact of dual-insecticide treated nets vs. traditional LLINs on malaria vectors and malaria epidemiology in 2 districts of Mali; Mali (Dec 2013–Dec 2014); Centers for Disease Control and Prevention (CDC), United States</td>
<td>Mali</td>
<td>Dec 2013–Dec 2014</td>
</tr>
<tr>
<td>The Majete Integrated Malaria Control Project (MMP): Community-based malaria control in the perimeter of Majete Wildlife Reserve in Chikhwawa district using a Scale-Up-For-Impact (SUFI) strategy, assessing complementary intervention options, including larval source management and house improvement; Malawi (Jan 2014–Dec 2018); Wageningen University, Netherlands; University of Amsterdam; College of medicine, University of Malawi; Liverpool School of Tropical Medicine</td>
<td>Malawi</td>
<td>Jan 2014–Dec 2018</td>
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**Case management and surveillance**

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<th>Study Description</th>
<th>Location</th>
<th>Duration</th>
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<tr>
<td>Routine case investigation and reactive case detection for malaria elimination in Richard-Toll District in northern Senegal; Senegal (2012–2017); PATH MACEPA, National Malaria Control Programme (NMCP) Senegal</td>
<td>Senegal</td>
<td>2012–2017</td>
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</table>

**Mass treatment**

<table>
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<tr>
<th>Study Description</th>
<th>Location</th>
<th>Duration</th>
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<tbody>
<tr>
<td>The Haiti Malaria Elimination Consortium (HaMEC); Dominican Republic, Haiti (Feb 2015–2020); Malaria Zero Consortium, US</td>
<td>Haiti</td>
<td>Feb 2015–2020</td>
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<tr>
<td>Assessing the effectiveness of household-level focal mass drug administration and community-wide mass drug administration with dihydroartemisinin + piperaquine for reducing malaria parasite infection prevalence and incidence in Southern Province Zambia; Zambia (2014–2016); PATH MACEPA, Tulane University, Zambian National Malaria Control Centre</td>
<td>Zambia</td>
<td>2014–2016</td>
</tr>
<tr>
<td>Community reactive case detection versus reactive drug administration in malaria elimination areas: a cluster randomised controlled trial; Zambia (2016–Dec 2017); Akros</td>
<td>Zambia</td>
<td>2016–Dec 2017</td>
</tr>
<tr>
<td>Assess the micro-epidemiology of resistant falciparum malaria in SE Asia and to perform and evaluate an intervention with targeted chemo-elimination through a modified mass drug administration approach (Cambodia, Myanmar, Thailand, Vietnam); Cambodia, Myanmar, Thailand, Vietnam (2014–Oct 2016); Mahidol Oxford Tropical Medicine Research Unit (MORU);</td>
<td>Cambodia, Myanmar, Thailand, Vietnam</td>
<td>2014–Oct 2016</td>
</tr>
<tr>
<td>Evaluation of the impact of seasonal malaria chemoprevention delivered by district health services in southern Senegal; Senegal (2013–2018); Cheikh Anta Diop University, Senegal</td>
<td>Senegal</td>
<td>2013–2018</td>
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IRS, indoor residual spraying; LLIN, long-lasting insecticidal net; SE, Southeast.

https://doi.org/10.1371/journal.pmed.1002453.t002

mathematical models that consider unified strategies and exploit the interactions between the species for improved cost-effectiveness should be used [96]. Notably, where P. vivax is present, the malaria programme might be sustained even as *P. falciparum* becomes rare and is eliminated. However, different approaches to both surveillance and malaria interventions would be required to reduce the *P. vivax* burden while detecting *P. falciparum* cases and preventing the reestablishment of *P. falciparum* transmission.

**Surveillance as an intervention**

Surveillance is an intervention tool. When honed for elimination purposes, surveillance must evolve to be able to discover evidence of transmission; establish its location, timing, nature,
Table 3. Consensus across multiple groups from modelling analyses conducted by each of the Malaria Modelling Consortium groups, which assessed impact on malaria transmission of combining multiple interventions or multiple methods of using a single intervention.

Vector control

- Achieving and maintaining high effective coverage of the population with LLINs is consistently predicted to result in the greatest reduction in transmission in a variety of settings and in many cases enables other interventions to become more effective and longer lasting [21,23,24,28,30,32,35,37–43,55].
- Other interventions such as IRS are also predicted to be effective and can even be more effective than LLINs in specific settings, particularly if sustained and optimised through seasonal or spatial targeting strategies [52,53,42].
- Vector control interventions that maximise killing of adult female mosquitoes are predicted to have the greatest transmission reducing effect (as opposed to repellents or killing juveniles); however, the optimal choice of intervention(s) will depend on both the specific biomics of local vectors and the costs required to reach high levels of effective coverage with each intervention [21,23,44–46].

Case management and surveillance

- Even before considering elimination, improving access to care has an important role to play in significantly reducing deaths and severe disease [9,41,47–49].
- While differing considerably in magnitude, all the models agree that levels of access to treatment of incident malaria cases and the delay in seeking treatment are 2 key measures that influence the endemicity at baseline (no interventions) and, as such, determine the following:
  - what scale of community-based programme will be required to achieve and maintain elimination [28,30,32].
  - what the risk will be of scaling back vector-based interventions post elimination [23,43,50,51].

Mass Treatment

- Short mass treatment campaigns will reduce the parasite reservoir—and consequently, transmission—in the short term but will have no long-term benefits unless other interventions are scaled up at the same time and then maintained [18,23,28,31,32,35,42,62–65].
- Treating a large proportion of the population in a single year in at least 1 round is a key determinant of MDA effectiveness whether it is achieved through high coverage in a single round or through follow-up rounds that reach new individuals [41,53,55–57].
- The addition of primaquine to MDA with long-lasting ACTs offers a small additional transmission reduction in the majority of epidemiological settings [18,30–32,42,53,54,57,58].
- Due to the prophylactic effect of treatment, MDA will always be more effective than MSAT or fMDA. If adherence or drug resistance is included in the model analysis, then this conclusion is more nuanced, and risk of drug resistance emergence and spread is an area with a lack of clear consensus among existing models [18,31,35,41].
- The longer-term effectiveness of MDA is highly sensitive to the population size of the trial area and its connectedness to other areas [18].

* Imperial College, London, United Kingdom; Institute for Disease Modelling, Seattle, Washington, US; Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand; Swiss Tropical and Public Health Institute, Basel, Switzerland; and University of Oxford, Oxford, UK.

* Compiled by Oliver Brady (University of Oxford) and Samantha Galvin (Bill & Melinda Gates Foundation). ACT, artemisinin-based combination therapy; fMDA, focal mass drug administration; IRS, indoor residual spraying; LLIN, long-lasting insecticidal net bed; MDA, mass drug administration; MSAT, mass screening and treatment.

https://doi.org/10.1371/journal.pmed.1002453.t003

and causes; identify and eliminate residual foci; prevent, detect, and contain imported malaria; and demonstrate the attainment and maintenance of zero malaria transmission [97]. As transmission declines, modification of data collection and reporting systems requires substantial investment and coordination across the malaria programmes and the surveillance management unit. Designing the necessary flexibility into a surveillance system to allow for adaptation to an elimination context will be critical.

There is an opportunity to use modelling to define the required components of surveillance systems depending on the stage of the elimination programme. This requires quantification of the detrimental effects of inaccurate, insufficient, or untimely surveillance and the beneficial effects of adding new measures to the surveillance system [25]. Modelling could also be used to
assess the level of hidden/unidentifiable cases/infections that would hinder (or would not hinder) elimination (e.g., asymptomatic or individuals with minor symptomology who would not seek treatment). As transmission declines, the addition of serological measures of past exposure [65,98–102] or active community-based transmission measurements and reactive case management [103–107] may be considered. Modelling can estimate the incremental benefit of adding specific surveillance activities to an already established surveillance system and could examine cost-effectiveness issues [48,108], specific epidemiologic aspects of contract tracing [109], and the target product profile of diagnostics [25,65,66] in case-investigation or foci-investigation settings.

Parasite and vector resistance

As efforts to reduce transmission are intensified, the risk and impact of parasite drug resistance and vector insecticide resistance becomes a key concern [110–112]. Modelling has been used to investigate the effects of resistance [25,26,30,32,113–116], and there have been some studies examining risk factors for resistance and drug failure [114,117–119]. Geostatistical models are also being developed to predict localities where resistance might be present in order to target surveillance activities, for example, mapping artemisinin-resistance in Southeast Asia [120]. The biology and natural history of mosquito vectors and malaria parasites tells us that the development and evolution of resistance will continue, given the pressure of insecticides and drugs. In terms of drug treatments, with artemisinin-based combination therapies (ACTs) globally recommended for malaria treatment, the focus must be on investigation of artemisinin and partner drug resistance, in terms of how this can be contained within the Greater Mekong subregion [111], and how its emergence or importation can be avoided in other regions [25,115]. Note that as transmission declines, the remaining parasites are those most likely to harbour resistance. Thus, even as malaria cases decline, continued field studies and modelling must be supported to address the efficacy and effectiveness of intervention tools critical for elimination programming. The next steps are to investigate how packages of interventions can be modified to mitigate the effects of resistance on existing interventions [30,121–123], how resistance can be contained [32], and how resistance can be avoided, particularly for new drugs and insecticides [124,125].

Human immunity

A gradual decline in human immunity to malaria across the population is an inevitable consequence of reducing malaria transmission and contracting parasite diversity [126,127]. The resulting delay in acquiring immunity likely will alter the age distribution and severity of malaria infections [126,128,129]. Understanding these changes is necessary to identify the most vulnerable populations or those most likely to need an intervention [128,130]. Models already include age-dependent immune factors and have dynamic modulation of immunity as a function of entomological inoculation rate [128,131], though additional temporal data could help reduce the uncertainty surrounding these functions. Gaps remain in our understanding of immunity in areas of long-standing low transmission (e.g., Haiti), where the level of asymptomatic infections is much higher than previously thought [132].

Modelling to inform policy

Strategic decisions are already being taken as part of elimination planning in a number of countries. There are numerous opportunities for modelling to inform these decisions—for example, scenario planning. An Elimination Scenario Planning (ESP) toolkit was published by WHO in 2014 following field testing using data from The Gambia and Senegal [14]. The
manual is linked to software that models malaria transmission (currently limited to \textit{P. falciparum} in Africa), which allows users to explore the effect of a range of combinations of malaria control interventions in order to achieve elimination. Such an approach has wide application and could be extended to \textit{P. falciparum} outside Africa or \textit{P. vivax} settings in the future. A key consideration is that malaria policy will need to respond to climate change. Historical data may become less reliable as seasonal patterns of rainfall and land use alter. Mapping climate change effects and possible scenarios following the varied consequences of climate change for human and vector population distributions has been investigated at continental and national levels, but incorporating this into policy is more challenging [133–151].

Mathematical models can provide a framework for exploring the relationship between population movement, heterogeneous transmission, and the deployment logistics of a national or regional elimination strategy. To carry out such analyses, new model frameworks should be developed that benefit from new field and genetic data characterising and measuring spatially and temporally dynamic transmission routes.

There is an increasing demand from NMCPs for pertinent and prompt mathematical modelling analyses to support their malaria elimination strategies. Established modelling groups have engaged in local capacity building. Also, malaria modelling research is being published by research groups from malaria-endemic countries [33,34,62,89,152–154], and this trend could be supported to the benefit of NMCPs.

**Modelling to maintain zero**

As noted above, when transmission becomes rare, models are increasingly challenged in informing policy and intervention choices; similarly, when there is no transmission, the evaluation of risk for the reintroduction of infection (vulnerability) and the risk of propagating local transmission given its reintroduction (receptivity) can present challenges to models designed to answer questions at high endemicity levels. A new class of highly heterogeneous, stochastic malaria models is being developed to inform the design of an elimination surveillance system.

**Vulnerability (risk of introduction or reintroduction).** Measuring vulnerability to malaria reintroduction requires pairing up-to-date maps of national and international parasite prevalence with human movement models. Both of these fields have advanced in recent years [33,34,117,155–160]. Human movement models, paired with travel survey and microcensus data, have improved their description of routine human movement (e.g., holiday season travel) [159,161]. Increasing use of mobile phones has enabled the tracking of human movement and permitted distribution advice on infection avoidance [159,162–164]. However, many national and international seasonal migrations remain difficult to predict, and their direct relationship to moving malaria infections requires additional investigation.

**Receptivity (risk of transmission given introduction).** In order to direct interventions, models must incorporate both the risk of importation and the risk for the reestablishment of local transmission [165–173]. The risk of malaria transmission reestablishment can be measured as a function of selected host, vector, and environmental data [156,170,171, 174]. For example, measures might include human use of insecticide-treated bed nets or indoor residual spraying, mosquito habitat suitability and its link to abundance, and climatic conditions (e.g., temperature, rainfall, and vegetation index measures) that support or accelerate vector and parasite development. If such data are collected widely enough, models can be validated using the occasional areas that do experience local transmission. Deciding which environmental and entomological data would be most valuable to collect could be iteratively informed by testing hypotheses based on longitudinal data from areas that have recently eliminated malaria, for example, Sri Lanka. The next step is to translate risk mapping into programmatic actions, such
as better allocation of human resources, and maintenance and targeting of vector control [56,178]. This will become increasingly important as more countries reach elimination.

**Challenges**

**Residual transmission**

Variable human and vector behaviours may enable sustained transmission in highly seasonal, heterogeneous environments, despite high intervention coverage [176]. The magnitude and importance of residual transmission in different settings require further field studies. In particular, human sociobehavioural data including human behaviour’s relevance for compliance and entomological data investigating the contribution of outdoor transmission are needed to develop models testing novel strategies and tools [102].

**Low transmission and incorporating heterogeneity**

Models have mostly been used to examine sub-Saharan Africa high transmission contexts with *P. falciparum* and relevant vector species, though they may be parameterised across the full spectrum of transmission. When modelling an isolated homogeneous population, it can be difficult to sustain transmission much below the 1% parasite prevalence level (though the precise level depends on the model), with the model becoming unstable, leading to ‘stochastic extinction’, i.e., the extinction of parasites based on random effects within the model, an effect that is compounded with increasing heterogeneity [177]. This suggests that importation of infections and local heterogeneities in host, vector, and parasite dynamics and in health service delivery systems are likely to play an important role in sustaining malaria in low transmission settings [178].

As a country progresses to very low levels of malaria transmission, the spatial and temporal heterogeneity of transmission increases in importance. In these contexts of varying historical transmission intensity, intervention coverage, human movement, and access to health system resources, malaria will tend to persist in the most remote regions and the poorest and most vulnerable populations [179,180]. While this issue may not require new models per se, heterogeneity will need to be better captured as transmission declines. Spatial heterogeneity is probably least well developed, and the required level of spatial granularity and relevant metrics for answering specific questions in low transmission settings requires definition [181,182]. However, at some point heterogeneity will exceed the ability of models to establish granularity, and decision making will require local health system and entomological data.

**Modelling malaria at borders**

When malaria transmission is moderate to high and similar on both sides of a border, often little attention is paid to border areas for specific disease interventions; however, this changes when one nation may be markedly reducing transmission and the other is not. Border areas present particular difficulties for malaria control and elimination efforts [183–187]. The complexity of human movements for trade, business, and visiting family, sometimes including vulnerable populations [188], and the coordination of efforts between different political and organisational frameworks increase the complexity of malaria control [184]. Some of the issues relate to spatial and temporal heterogeneity and could possibly be addressed with greater data on human cross-border movement and parasite genetics [189–191]. However, human factors, such as local conflicts, poverty, and the disenfranchisement of particular ethnic groups, can be highly variable in time and place and are more challenging to incorporate into transmission models [192,193]. Alternative complementary approaches include mapping malaria risk, for
better targeting of resources, plus goal setting by modelling what could potentially be achieved with coordinated versus independent elimination campaigns [33,185,194,195]. Once the potential benefits are understood, the barriers to reaching these goals can be researched and the feasibility of overcoming them explored.

**Iteration and validation**

Finally, models directed at assessing combination interventions must embrace a process of iteration with field data. In particular, data are needed from low to near-zero transmission settings. Such data needs might include high-resolution geographic information on cases, frequency and location of associated secondary cases, travel history identifying infection sources, vector-associated data, climate, and environmental parameters [109]. The requirement for field data to validate models remains problematic, as field data on intervention efficacy and the diverse parameters noted above can be difficult to assemble. When developing models, validation requirements should be clearly defined and data should be feasible to obtain. Amidst these challenges, modellers then need to consider how to best contribute to and bear responsibility for the assembly of required field data. Although capacity building and integration of modellers into NMCPs may address this at a local scale, there is a need for innovative mechanisms to allow increased exchanges in malaria elimination research, to allow better access to field empirical data for modellers.

**Conclusions**

Given the ongoing social and economic impact of malaria-related mortality and morbidity and the inevitable resource constraints for national malaria programmes, identifying the most timely and most cost-effective path to malaria elimination is a priority. Box 3 presents a research and development agenda for combination interventions and modelling in malaria elimination. Modelling affords a feasible and practical means of investigating rational combinations of interventions and the most appropriate setting for their deployment. Nevertheless, without a substantive dataset from operations research, the construction of meaningful models

**Box 3. Research and development agenda for combination interventions and modelling.**

- Determine which combinations of interventions to use in which sequence and in response to which triggers throughout elimination
- Identify the circumstances in which time-limited elimination acceleration interventions, such as mass drug administration (MDA), are appropriate and what needs to be done to retain the gains in transmission reduction following their withdrawal
- Model the effect of parasite drug and vector insecticide resistance on combination interventions and how resistance might be avoided or contained
- Understand human immunity in areas where transmission has always been low and parasite diversity very low and modelling the effect of changes in human immunity as transmission declines
- Identify which additional data would be most useful for validating or changing model predictions in order to drive iterative development and decision making
Surveillance as an intervention

- Model the target product profile of an elimination-specific surveillance system
- Determine the threshold at which reactive case strategies become feasible

Strategic modelling

- Estimate the long-term costs of elimination in different settings and with different intervention packages
- Assess the potential duration of an elimination campaign in various settings to help define the investment case and financing needs for elimination
- Estimate the maximal impact of currently available tools on elimination in various settings
- Determine the counterfactual to elimination, i.e., the effect of continuing current interventions in various settings
- Support capacity building of modellers embedded in National Malaria Control Programmes (NMCPs)

Modelling to maintain zero

- Investigate how vulnerability and receptivity measures can be translated into specific programme actions

Addressing transmission

- Apply models to low transmission settings, incorporating all relevant parasites/vectors
- Investigate the importance of residual transmission in different settings and what new strategies or novel tools are needed to overcome it

Incorporating heterogeneity

- Determine the relevance of spatial and temporal heterogeneity in transmission in different settings
- Investigate how much heterogeneity in transmission needs to be captured by models to make predictions in elimination settings

Iteration and validation

- Determine which measures of transmission or other metrics are most appropriate for guiding programmatic decisions in low transmission to maintaining-zero settings
- Define which new data need to be collected from low transmission to maintaining-zero settings in order to increase confidence in model predictions

is not possible. Models must also be continuously validated against field data, through programmatic experience and against clinical trials, with measures and outcomes data relevant to the transmission setting identified and collected for use in further model refinement. This is
especially the case as we increasingly encounter transmission settings that are shrinking in size and number and becoming more focal and heterogeneous and for which there are fewer field data. Thus, there is a codependency between modelling and field data, and the quality of both must be assured for findings to be valid and impactful. Since malERA 2011, there has been significant progress in aligning modelling with programmatic requirements and more effective communication with policy makers. This ongoing dialogue will ultimately determine the relevance of modelling to policy decision and its contribution towards achieving and maintaining malaria elimination.

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COLLECTION REVIEW

malERA: An updated research agenda for health systems and policy research in malaria elimination and eradication

The malERA Refresh Consultative Panel on Health Systems and Policy Research

Abstract

Health systems underpin disease elimination and eradication programmes. In an elimination and eradication context, innovative research approaches are needed across health systems to assess readiness for programme reorientation, mitigate any decreases in effectiveness of interventions (‘effectiveness decay’), and respond to dynamic and changing needs. The malaria eradication research agenda (malERA) Refresh consultative process for the Panel on Health Systems and Policy Research identifies opportunities to build health systems evidence and the tools needed to eliminate malaria from different zones, countries, and regions and to eradicate it globally. The research questions are organised as a portfolio that global health practitioners, researchers, and funders can identify with and support. This supports the promotion of an actionable and more cohesive approach to building the evidence base for scaled-up implementation of findings. Gaps and opportunities discussed in the paper include delivery strategies to meet the changing dynamics of needs of individuals, environments, and malaria programme successes; mechanisms and approaches to best support accelerated policy and financial responsiveness at national and global level to ensure timely response to evidence and needs, including in crisis situations; and systems’ readiness tools and decision-support systems.

Summary points

- Since 2011, few research questions identified in malERA Health Systems and Operational Research agenda have been addressed, or only addressed in a fragmented way by scientists and implementation researchers at national and international levels. Multiple factors, including funding, dependency upon existing national and provincial health systems to deliver, and limited buy-in by a range of disciplines into the malaria agenda, have contributed to this limited uptake.
- To address the complexity of health systems and any changes or adaptations within them requires a systemic and transdisciplinary approach in the conceptualization and conduct of the research. Transdisciplinary research requires the inclusion of various
sectors and agencies, communities, and civil society, as well as inclusion and integration of a range of research disciplines including policy, management, and social sciences.

- Presenting the research and development agenda here as a portfolio ensures the trans-disciplinary and multi-stakeholder approach and can therefore help funders and researchers take action and engage in the pursuit of this agenda according to their own diverse priorities.

**Introduction**

Between 2000 and 2015, a major expansion of WHO-recommended interventions have contributed to a 58% reduction in the global malaria mortality rate (69% among children under 5 years old in Africa), resulting in an estimated 6.2 million lives being saved from a malaria-related death [1].

As part of the initial malaria eradication research agenda (maLERA) process, published in 2011, a consultative group on health systems and operational research established a list of research priorities presented in a matrix system organized by the different levels (community, facility, district, and national levels) and building blocks of the health system [2]. Health-system building blocks are described in Box 1. The health systems concept and framework was based on the guiding summary on ‘health system thinking’ perspective formulated by the Alliance for Health Policy and System Research [3]. One key gap that was identified in 2011 was a

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**Box 1. Health system building blocks that together constitute a complete system.**

- Governance: Ensuring strategic policy frameworks combined with effective oversight, coalition building, accountability, regulations, incentives, and attention to system design.
- Human resources: Responsive, fair, and efficient, given available resources and circumstances, and available in sufficient numbers.
- Financing: Raising adequate funds for health in ways that ensure people can use needed services and are protected from financial catastrophe or impoverishment associated with having to pay for them.
- Health information: Ensuring the production, analysis, dissemination, and use of reliable and timely information on health determinants, health systems performance, and health status.
- Service delivery: Including effective, safe, and quality personal and nonpersonal health interventions that are provided to those in need, when and where needed (including infrastructure), with a minimal waste of resources.
- Commodities: Including medical products, vaccines, and other technologies of assured quality, safety, efficacy, and cost-effectiveness, and their scientifically sound and cost-effective use.
tool to diagnose impediments in a given health system that limited the effective and equitable impact of malaria interventions [2].

The momentum created by the successes since the turn of the century has led to reiterated commitment and partnership and 2 complementary key documents, (i) the Global Technical Strategy (GTS) for malaria 2016–2030 [4] and (ii) the Action and Investment to Defeat Malaria (AIM) 2016–2030 [5], which were launched respectively by the Global Malaria Programme (GMP) of WHO and the Roll Back Malaria (RBM) Partnership in 2015. The GTS and the investment and advocacy framework partnership document, AIM, pave the way for intensifying malaria control and elimination efforts and set ambitious yet realistic targets and goals. The GTS clearly outlines a common technical strategy while AIM provides the investment framework to reach these technical targets. Moving forward, GTS and AIM need to be brought to the country level and be clearly reflected in the national malaria control and elimination strategic plans, as well as in the overall national health policy and strategy.

In view of these advances, many challenges need to be coherently addressed in order to not threaten continued progress. These challenges are as reflected in GTS and AIM:

1. Emerging parasite resistance to antimalarial medicines and mosquito resistance to insecticides.
2. Systemic and technical obstacles, such as the inherent weakness of health systems, including poor disease surveillance and limited pharmaceutical regulation.
3. Lack of adequate technical and human resource capacities including community engagement.
4. High prevalence of asymptomatic infections and unknown dimension of the existing asymptomatic reservoir.
5. Diversity of vectors and their behaviour.

To complement GTS and AIM, a systematic review of progress in research and development (R&D) and a consultative process to update the research agenda, ‘malERA Refresh’, was launched [6]. malERA Refresh can be seen as the third pillar, defining research priorities to support the GTS targets, while the business case for achieving the GTS targets is provided by AIM.

Scope of this report

In the refresh of malERA presented here, an expert panel focussed on health systems and policy research reviewed the progress made since 2011 and set out an updated agenda and research portfolio necessary to support the global malaria elimination agenda. While addressing broader health system development needs would be beneficial to this as well as other public health agendas, it was not the focus of this piece of work, and others have addressed these in detail. A particular emphasis was placed on the elimination phase in order to best accompany the GTS and the AIM framework, as well as to assist the national strategies and operational plans. The main differences between the 2011 and 2017 health system agendas are that this 2017 agenda:

- Focusses on malaria, in particular malaria elimination. The 2011 agenda was a broader view on health systems and all phases of control to elimination.
- Recognizes that 1 country can have various phases of control to elimination occurring within its programme, so does not, as was done in 2011, group countries into phases.
- Frames the agenda as a portfolio from hypothesis driven (research), development of tools or interventions (R&D), or synthesis of existing information and evidence (evaluation science).
Recognizes the need to tailor questions to different settings and therefore has left the questions deliberately broad to allow general application to specific settings.

Context and rationale

In spite of increased financial and commodity resources, progress in malaria control and elimination in most countries has been slower than expected. Among the main reasons for the slow pace are constraints on the delivery of essential health interventions, including malaria interventions, at sufficient levels of coverage and quality to populations in need. At the same time, the attainment of GTS targets and goals, within the AIM investment framework, will rely heavily on well-performing health systems for the sustained control and elimination phases.

Progress has been made in recent years towards better understanding health systems and how to strengthen them. The previous malERA health systems working group recommendations have contributed to that change, which is clearly reflected in AIM as well as health-systems thinking and is acting as an integral part of WHO GMP Malaria Policy Advisory Committee (MPAC) recommendations [7]. Moreover, global health initiatives have certainly increased funding for strengthening national health systems to accelerate progress on universal access to essential health interventions, particularly for HIV/AIDS, tuberculosis, malaria, and immunisation. Initiatives such as the Task Force on Innovative Financing for Health Systems [8] testify to the increased commitment to funding health systems and momentum in favour of health system strengthening, minimising the negative impacts of vertical programmes on health systems, and leveraging malaria activities through other health programmes.

However, significant work and questions remain. By systematically reviewing the literature, as well as ongoing research projects in health systems in the Malaria Eradication Scientific Alliance (MESA) Track database [9] (see Methods and approaches section), the panel concluded that many research priorities identified in the 2011 Panel on Health Systems and Operational Research have hardly—or only in a fragmented way—been picked up by scientists and implementation researchers at national and international levels.

The malERA portfolio dealing with health systems attracted insufficient funding and interest over the last few years. One possibility for this is that funding for malaria is more vertically targeted on parasites, diagnostics, treatment, and vectors than on broad health systems and in part because the agenda was more accessed by malaria scientists and not the broader health system research community and public health practitioners. Additionally, many of the disciplines required to engage in the health system agenda are not presently majorly involved, and the existing malaria scientific community are not well equipped to implement the health system agenda. There is a need to reiterate the agenda as established in 2011, but more importantly—by building on it—add new dimensions to the research and R&D needs in the field of health systems and policy research. This paper presents the agenda as a portfolio ranging from priorities in evaluation science to specific research questions to R&D issues (see Box 2 for definitions).

Funding partners and implementation groups have clearly prioritized the quick delivery of malaria interventions and a system of monitoring and evaluation (M&E) linked to their funding and not necessarily integrated into routine health systems M&E, including health systems research. Contrasting this to governments in countries affected by malaria, which are tasked with responding to ambitious global targets and goals, reveals that these countries have long recognised the need for health system thinking and research in order to meet the healthcare needs of their populations. As noted above, addressing the more challenging but also more sustainable issue of health system strengthening to support malaria eradication tends to be postponed as it is still seen, conceptually and operationally, as a daunting issue. This difficulty can be overcome by presenting the research and R&D priorities within a portfolio approach.
Box 2. malERA Refresh research categories to support a portfolio approach to health systems and policy research.

- Evaluation science: Where information from the field and across several sites, contexts, and case studies already exists (mainly WHO/University of California San Francisco [UCSF] elimination case studies, RBM Progress and Impact reports, etc.) and an answer to the research question could be obtained by comparative, synthetic analysis.
- Research: Where new information is needed to answer a research question based on an underlying hypothesis or where new hypotheses need to be tested.
- R&D: Where an R&D process is required, based on underlying hypotheses, to develop a tool, a tool kit, or new approaches (e.g., surveillance as an intervention, ‘surveillance-response’). These tools or approaches would be created based on available data and information to serve implementers in carrying out their work. The development of this tool, tool kit, or approach becomes the activity pertinent to this portfolio.

tailored to the different levels and building blocks of a given health and social system (Fig 1). Addressing the list of key research questions outlined in this paper would go a long way in strengthening malaria control and elimination and inscribing it into the fundamental health infrastructure of endemic countries (full portfolio of questions in Table 1).
### Table 1. Priority questions for evaluation science, research, and R&D in health systems and policy research.

<table>
<thead>
<tr>
<th>Evaluation science</th>
<th>Health system building block</th>
<th>Health system level</th>
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</thead>
<tbody>
<tr>
<td><strong>1st Priority:</strong> What are the social &amp; political drivers that influence malaria elimination success within and across regions at national and regional levels?</td>
<td>Governance</td>
<td>National</td>
</tr>
<tr>
<td><strong>2nd Priority:</strong> How do optimized delivery strategies meet changing and dynamic needs of the system requirements and/or community perceived needs?</td>
<td>Service delivery</td>
<td>Community</td>
</tr>
<tr>
<td><strong>2nd Priority:</strong> What are effective strategies and tools to sustain community workers’ engagement in malaria activities during intensive control and elimination?</td>
<td>Human resources</td>
<td>Community</td>
</tr>
<tr>
<td>Additional Emphasis: Evaluation of case studies (including the malaria elimination case study series) regarding the determinants for successful scale-up of district management, financing, and human resource models in different contexts and settings.</td>
<td>Cross-cutting</td>
<td>Facility</td>
</tr>
<tr>
<td>What mechanisms support effective integration of communicable disease surveillance?</td>
<td>Information</td>
<td>All</td>
</tr>
<tr>
<td>With an emphasis on decentralization, what are the management strengths required, and how can the readiness of the different health systems structures be assessed for malaria elimination in different settings?</td>
<td>Governance</td>
<td>All</td>
</tr>
<tr>
<td>What is the range of effective HIS and tools to capture and use information at the community level?</td>
<td>Information</td>
<td>Community</td>
</tr>
<tr>
<td>What mechanisms, tools, and strategies can be utilized to sustain active community engagement in intensive malaria control and elimination?</td>
<td>Governance</td>
<td>Community</td>
</tr>
<tr>
<td>What is the range of HIS and tools to effectively capture and use information at the community level?</td>
<td>Information</td>
<td>Community</td>
</tr>
<tr>
<td>How to scale up measures to ensure quality and quantity of health commodities at the community level in both public and private sectors, especially to remote and vulnerable populations?</td>
<td>Information</td>
<td>Community</td>
</tr>
<tr>
<td>What are the key essential service delivery tools implemented at PHC level to ensure quality of malaria elimination activities (prevention, treatment, and surveillance-response) at facility and community levels in different system contexts?</td>
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<td>Community</td>
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<tr>
<td>What is the range of effective HIS and tools to capture and use information at the national level?</td>
<td>Information</td>
<td>National</td>
</tr>
<tr>
<td>Comparatively assess across countries and settings which mechanisms best support accelerated policy and financial responsiveness at the national level to ensure timely response to evidence and needs, including in crisis situations.</td>
<td>Governance</td>
<td>National</td>
</tr>
<tr>
<td>Comparatively assess across various countries and subnational settings which are the effective approaches and their determinants to transition funding to sustainable financing sources.</td>
<td>Financing</td>
<td>National</td>
</tr>
<tr>
<td>What are efficient and ethical approaches to health security issues that can be applied to managing malaria in epidemics, reintroduction, and resurgence?</td>
<td>Governance</td>
<td>National</td>
</tr>
<tr>
<td>Comparatively assess which mechanisms best support accelerated policy and financial responsiveness at the global level to ensure a timely response to evidence and needs, including in crisis situations.</td>
<td>Governance</td>
<td>Regional/Global</td>
</tr>
<tr>
<td>What are the social and political drivers to influence malaria elimination within and across regions at country and regional levels?</td>
<td>Governance</td>
<td>Regional/Global</td>
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<th>Research</th>
<th>Health system building block</th>
<th>Health system level</th>
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<tbody>
<tr>
<td><strong>1st Priority:</strong> What are the decision-making frameworks required to eliminate and prevent reestablishment of malaria?</td>
<td>Governance</td>
<td>National</td>
</tr>
<tr>
<td><strong>2nd Priority:</strong> What is the best way to optimize malaria elimination delivery strategies to meet the changing dynamics of needs of individuals, environments, and malaria programme successes?</td>
<td>Service delivery</td>
<td>Community</td>
</tr>
<tr>
<td>What are the determinants of efficiency of community-level health service delivery and of community systems, with an emphasis on malaria elimination outcomes?</td>
<td>Financing</td>
<td>Community</td>
</tr>
<tr>
<td>Which innovative measures would improve quality and quantity of malaria commodities at the community level in both public and private, especially to remote and vulnerable populations for malaria elimination?</td>
<td>Commodities</td>
<td>Community</td>
</tr>
<tr>
<td>What management tools and structures can improve transparency, accountability, and effectiveness of health facilities for malaria elimination activities (coverage, equity, and quality) in different contexts and systems?</td>
<td>Governance</td>
<td>District</td>
</tr>
<tr>
<td>What mechanisms and approaches best support accelerated policy and financial responsiveness at the national level to ensure timely response to evidence and needs, including in crisis situations?</td>
<td>Governance</td>
<td>National</td>
</tr>
<tr>
<td>In the context of a shared public health target of malaria elimination, what are the determinants and the effective modes &amp; models of intercountry &amp; cross-border collaboration for policy &amp; implementation?</td>
<td>Governance</td>
<td>National</td>
</tr>
</tbody>
</table>

(Continued)
Table 1. (Continued)

| What adaptive changes are needed in operations (management, financing, human resources, and responsibilities) of health systems to move to and support malaria elimination? | Cross-cutting | National |
| What are effective models of government leadership at leveraging integrated activities cross-sectorially for malaria elimination? | Governance | National |
| What ensures effective governance and accountability to support elimination? | Governance | National |
| What enables ownership of elimination at national and regional levels? | Governance | National |
| What are effective mechanisms to leverage financing for malaria prevention from health insurance schemes? | Financing | National |
| What are effective mechanisms and approaches to transition from external funding to sustainable financing sources? | Financing | National |
| What mechanisms and approaches best support accelerated policy and financial responsiveness at the global level to ensure timely response to evidence and needs, including in crisis situations? | Governance | Regional/Global |
| In the context of a shared public health target of malaria elimination, what are the determinants and the effective modes and models of intercountry and cross-border collaboration for policy and implementation? | Governance | Regional/Global |
| What are the decision-making frameworks required to eliminate and prevent reestablishment? | Governance | Regional/Global |
| What adaptive changes are needed in operations (management, financing, human resources, and responsibilities) of health systems to move to and support malaria elimination? | Cross-cutting | Regional/Global |
| What are effective models of government leadership at leveraging integrated activities cross-sectorially for malaria elimination? | Governance | Regional/Global |
| What ensures effective governance and accountability to support elimination? | Governance | Regional/Global |
| What enables ownership of elimination at national and regional levels? | Governance | Regional/Global |

| R&D | Health system building block | Health system level |
| 1st Priority: What are the health planning and funding models and tools required to eliminate and prevent reestablishment of malaria? | Cross-cutting | National |
| 2nd Priority: What tools (existing, new, or a combination of both) can measure systems’ readiness at community, facility, and district levels in an integrated way to support elimination and prevention of reintroduction? | Cross-cutting | All |
| Develop the tools and SOPs for effectiveness decay analyses. | Cross-cutting | All levels |
| Development of tools and strategies to strengthen and sustain active community engagement in intensive control and malaria elimination. | Cross-cutting | Community |
| How can community components of integrated service delivery approaches (IMCI, IMAI, and ICCM) be adapted to malaria elimination and prevention of reintroduction? | Governance | Community |
| Develop a broad system readiness tool to include stop/start decisions, appropriate economic tool and approaches (e.g., CEA and CBA), and link the tool to decision-support systems. | Service delivery | National |
| What are the health planning and funding models and tools required to eliminate and prevent reestablishment of malaria? | Cross-cutting | Regional/Global |
| Develop a broad system readiness tool to include stop/start decisions, appropriate economic tool and approaches (e.g., CEA and CBA), and link the tool to decision-support systems at the regional level. | Cross-cutting | Regional/Global |

CBA, cost-benefit analysis; CEA, cost-effectiveness analysis; HIS, health information system; ICCM, integrated community case management; IMAI, integrated management of adolescent and adult illnesses; IMCI, integrated management of childhood illnesses; PHC, primary health care; R&D, research and development; SOP, standard operating procedure

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Methods and approaches

In 2009, WHO [3] defined health systems research as ‘the purposeful generation of knowledge that enables societies to organize themselves to improve health outcomes and services.’ It is concerned with how health services are financed, delivered, and organised and how these functions are linked within an overall health and social system with its associated policies and institutions. To understand and address the complexity of health systems and implementation of change into those systems requires not only a systemic but also clearly a transdisciplinary approach.
Transdisciplinary research entails approaching a complex problem in a way that can (a) grasp the complexity of problems, (b) take into account the diversity of life-world and scientific perceptions of problems, (c) link abstract and case-specific knowledge, and (d) constitute knowledge and practices that promote what is perceived to be the common good [10]. This approach to research requires the inclusion of various sectors and agencies, community, and civil society, as well as inclusion and integration of a range of research disciplines including policy and political science, management, economics, and social sciences (including anthropology, sociology, and development sciences).

Based on this context, the review of the research agenda was pursued in 2 steps, each step engaging the panel members as described in S1 Text, to organise the health systems research needs in malaria elimination as a portfolio of evaluation science, research, and R&D. This kind of portfolio approach should make it easier for researchers and funding agencies to identify their subjects of interest. The panel used the modified Nominal Group Approach to identify the priority questions in each category [11] (see S1 Text).

**Results: Fundamentals and prerequisites**

**Changing focus from pilot studies to scale-up**

Based on analysis of MESA Track and extensive literature search (S1 Text) and taking into account the time it takes for the first malERA process to gain traction, it emerged that research taken up after the first malERA process led to a few interesting pilot studies. Even when conclusive, these projects remained fragmented and rarely led to or indicated initiation of scale-up implementation of the key findings and recommendations. This malERA Refresh process aims to avoid fragmentation and move beyond pilot studies.

When research brings forward conclusive results and recommendations, a strong effort should be placed on scaling them up in that context. When results have not been internally and externally validated (e.g., because of the small size of pilot studies or fragmentation of research), then more research and evaluation efforts should focus on gathering the information pertinent to inform the scale-up for impact of interventions [8].

**Effectiveness decay**

Effectiveness of interventions is lost at almost all (if not all) levels of the health system. This means that at 1 or several points in a health system, efficacious interventions may lose some of their effect because they cannot be applied or implemented effectively; this is called effectiveness decay. By analysing effectiveness decay within a specific health and social system one can elucidate at which levels the greatest loss of effectiveness are occurring (Fig 2) and then test strategies with which to intervene and recover effectiveness of the intervention. For malaria control and elimination, understanding the equity effectiveness of interventions, that is whether all who need to access and use the intervention can do so equally, is of particular importance.

The effective execution of the elimination agenda would benefit from high performance of health systems that can deliver the optimal combination of malaria interventions at high and equitable levels of quality and population coverage. This requires the concerted and combined strength of all the health system building blocks. Program managers need to be able to detect the reasons why coverage levels are inadequate or inequitable and, based upon that, develop appropriate ways to address these reasons within their health and/or social system. And for system interventions and strengthening to be effective and efficient, they need to be able to diagnose those problems and their determinants and interactions (decay at each level is not the same in all health systems). The system effectiveness analytic framework has proven very useful
as a health system diagnostic tool to identify areas where and how system factors need to be and can be strengthened. This framework sees a cascade of progressive loss of intervention efficacy (Fig 2) and addresses it through system-specific issues of access, targeting, provider compliance, and client adherence as the system tries to deliver an intervention at effective coverage levels in real-world settings. Many programmes have used it to analyse the determinants of coverage but results are often yet to be translated into targeted health systems. Such an analysis will need to be complemented with an analysis of allocation efficiency, which will help to optimize a portfolio of multiple interventions in a given context to maximize health impact.

Programme efficiency and health system readiness

After the effectiveness decay analysis, programmes at various levels within a country (local, subnational, and national) can establish the most efficient operational mix to meet their targets and objectives. They need to precisely define when and where interventions need to start and stop and what time frame is involved for certain operations. Based on surveillance data and prevalence and transmission intensity, and assisted by modelling, interventions or mixes of interventions to be applied in an integrated way should be clearly defined [13]. Starting from the initial decay analysis at the national and subnational levels, it becomes a health systems surveillance tool to be part of the national programme and the surveillance-response approaches. By ignoring the continued M&E of the health systems effectiveness, control and elimination programmes are at risk of inefficiencies and unrecognized loss of effectiveness.

Results: Research and R&D portfolio

Examples of the research portfolio discussed by the consultative panel are presented in Box 3, starting with cross-cutting priorities relevant to all health systems and policy research topics and then going over the research agenda in evaluation science, research, and R&D of new tools categories. For each research question in each category, the health system levels and the building blocks are mentioned. The top 2 priority questions and the question on which the panel chose to place special emphasis are always listed first in each category. A rationale is provided in the portfolio to offer some background and context on the issues raised. Then follows a list
Box 3. Examples of research questions in the research agenda for health systems and policy research.

**Evaluation science (synthetic, comparative evaluation of existing interventions and case studies)**

- What were the social, regulatory, and political drivers that supported or affected the ability of malaria elimination systems requirements to be integrated into existing health system in the country/countries that have eliminated malaria?
- What approaches to engaging community health workers in malaria elimination activities support sustaining their active engagement in service delivery in the country/countries that have eliminated malaria?
- What were the cost-effective strategies for optimal delivery of various components of a malaria elimination programme to targeted populations/locations developed in the country/countries that have eliminated malaria?
- What role has housing and rural/urban environmental improvements played in supporting malaria elimination and prevention of reintroduction in countries that have achieved malaria elimination?

**Research (test hypotheses)**

- That the integration of malaria elimination surveillance and response approaches can be cost-effectively integrated into other infectious disease surveillance systems.
- That communities can play an effective role in active efforts at transmission reduction (as opposed to reducing morbidity and mortality from malaria)?
- That routine primary healthcare services including maternal and child health services can sustain access to and utilization of individual and household-level malaria interventions to sustain malaria elimination and prevent reintroduction of malaria.
- That improved methods of data collection, synthesis, visualization, and real-time availability will increase the timeliness and effectiveness of health workers’ responses to malaria cases in elimination settings.

**R&D (develop and test tools, approaches/strategies, and models)**

- Development of IMCI and IMAI updated with new diagnostic tools and adapted to the malaria elimination context.
- Development and scaling-up of tools to measure systems readiness for malaria elimination and prevention of reintroduction at local and subnational levels.
- Development of mechanisms to support and maintain financial and political responsiveness to malaria elimination and prevention of reintroduction.
- What are the messages and best means of conveying these to various communities/at risk populations to support access, acceptability, and utilisation of malaria interventions nearing elimination?
of additional questions linked to the priority questions to suggest key questions also to be answered.

A synthetic presentation of the full research portfolio is presented in this paper (Fig 3), color-coded for areas of activities: cross-cutting, governance, human resources, financing, information, service delivery, and commodities. Questions are ranked according to the health system level to which they pertain: all levels, community, facility, district, national, and regional/global. Keywords are used to refer to questions detailed later in the document. Following the synthetic table, the list of research questions is presented for evaluation science, research, and R&D, starting with priority questions (Table 1).

**Capacity building and training**

Capacity building and training is a priority cross-cutting all areas emphasized in this document. No positive outcome can be anticipated if an appropriately trained and competent workforce cannot be relied upon. This is indeed an absolute prerequisite for well-functioning health systems and delivery of malaria control and elimination interventions.

**Surveillance and M&E**

A theme common to all programmes and systems areas is the critical role that surveillance and M&E play in every phase. Adequate surveillance and M&E methods must be in place to monitor effectiveness and inform the decision-making process. Surveillance and M&E fall under the ‘surveillance-response’ umbrella, where essential data are collected in space and time to inform well-tailored, integrated-response actions.

**Supply-chain strengthening**

A very common cause of loss of malaria programme effectiveness is the disruption in appropriate stocks of life-saving commodities for at-risk populations. No malaria control or elimination programme can hope to achieve its objectives without addressing the issue of stockouts of commodities and strengthening the supply chain so that an uninterrupted flow of necessary quality tools can be available at all times for people who most need them.

**Community involvement and engagement**

A critical element of success and of sustainability of any intervention, and particularly malaria elimination interventions, is community ownership and engagement. The RBM AIM (2015) noted as countries move along the path to elimination, resource requirements, processes, and services change, requiring national systems to adapt and improve, and to deepen their level of community engagement and in Chapter 6 discussed this in more detail, especially the role of people-centred and participatory research design and approaches [5].

**Keeping a dynamic portfolio: Monitoring progress and dissemination of results**

Multiple factors have slowed progress in the health systems and operations research agenda since the first malERA initiative, including funding, dependency upon existing national and provincial health systems to deliver, and limited buy-in by a range of disciplines into the malaria agenda. All of these factors have contributed to this limited uptake. The panel felt that in order to keep a dynamic portfolio and not lose traction again, monitoring the uptake of research priorities established during the malERA Refresh process was essential.
**Fig 3. Overview of health systems and policy research portfolio.** HIS, health information system; SOP, standard operating procedure.

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The following processes could ensure proper monitoring of research activities: First, MESA, through the established tool of MESA Track and linking up with all research and implementation institutions involved, could be tasked with producing regular updates on progress accomplished [9]. The second important feedback would be from countries (national malaria control programmes level) reporting on evaluation science, research, and R&D tools development. A third approach is to use networks emerging throughout regions such as Asia Pacific Malaria Elimination Network (APMEN) [14] and the 8 eliminating southern African countries (Elimination 8 [E8]) [15] to both socialise and support the implementation of this research agenda, as well as provide platforms for dissemination of lessons learned from the research. This can use innovative platforms like case studies, focussed study tours, peer-to-peer mentoring, workshops, and health system fellowships.

Information collected through these 2 tracks would be regularly reviewed by the malERA Refresh committee and compared across the whole malERA spectrum. The results, regularly published, in combination with updates from national programmes and their partners on status of national research and implementation, would be included each year in the WHO World Malaria Report. Other channels for dissemination and garnering of scale-up support would be through platforms such as the political alliances of African Leaders Malaria Alliance and the Asia Pacific and Leaders Malaria Alliance [16,17]. The malERA Refresh has given and will continue to give the malaria community an opportunity to highlight perhaps the most important gaps in getting the world to the malaria-free goal; namely, the continuous and timely improvement in the operational delivery of interventions through health systems improvement.

Discussion

Setting health research priorities does not automatically guarantee uptake and funding, though there are examples of how it does [18]. And these health systems issues are ‘wicked’ problems for which no one solution will be found; solutions are not discrete from other systems’ issues, and solutions cannot be divorced from socio-historical-political environments [19]. This agenda will also need a broad range of disciplines to engage in the implementation of the agenda beyond the ‘malaria research community’ to include social and behavioural sciences; political, management, and organisation science; health economics; and health systems specialists. Clearly, this health systems agenda to facilitate malaria eradication entails a transdisciplinary and multi-stakeholder approach and therefore needs to be more broadly disseminated and socialised in these scientific communities, and the findings from this research need to be more broadly disseminated to inform other health system interventions and their implementation, as well as health system strengthening in general.

As discussed in the section 'Keeping a dynamic portfolio’, there is a need to rigorously provide evidence of the outcomes of the research against the overall eradication agenda. This evidence can attract more attention to and focus on the prioritised agenda for other researchers and funders. The research agenda links to GTS, the strategy, and AIM, the investment framework for action, and creates the essential third pillar on our journey to achieve elimination and eradication. Building the capacity of health and other staff and researchers in malarious countries to undertake health systems research, such as through the WHO TDR Structured Operational Research and Training IniTiative (SORT-IT) approach, is an important step, as many of these research topics need the ‘localisation’ required to understand the impact of different settings and health and social systems upon interventions and their effective implementation. Funding for these sorts of localised studies will likely need to be sourced from national budgets and/or small research grant schemes; be embedded into the M&E budgets of projects, donors, and governments; and/or be part of the university training and research agendas. The
larger number of ‘case studies’ can then allow some comparative analyses and synthesis through evaluation science to be undertaken at a larger scale to better identify enablers, barriers, approaches, and tools for others to ‘trial’.

Conclusion
This remains an ambitious but essential research agenda for malaria eradication. Every tool or intervention developed, for vectors, parasites, drug administration, surveillance and response, etc., needs a health and social system operating in a socio-political system to deliver the intervention effectively. To address challenges relating to health systems, transdisciplinary and multi-stakeholder approaches need to be tested. Results from evaluation science, research and R&D in this field will likely generate multiple nuanced answers. This process will ensure that the research questions put forward in a portfolio approach can be tailored to any given setting, as one size will not fit all, and finally that the maximum efficacy of interventions is maintained universally across the different social, political, and economic differentials for populations to achieve malaria elimination and, finally, eradication.

Supporting information
S1 Text. Further details on panel methodology and prioritization process. (DOCX)

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