Highlights:

- Malarial burden can be demonstrably reduced by interventions that inhibit the transmission of \textit{Plasmodium} through the mosquito. These interventions are termed transmission-blocking interventions (TBIs).
- Anti-parasitic forms of these interventions can be classified as transmission blocking drugs (TBDs), or transmission blocking vaccines (TBVs).
- In terms of TBDs, there are currently three clinically approved anti-malarials that show robust transmission-blocking efficacy; primaquine, methylene blue and atovaquone, with additional compounds in clinical development and trials ongoing.
- Although a wide range of proteins have been examined for TBV activity, there are only 5 immunogens that unquestionably demonstrate efficacy. Recent trials examining P230 and P25 and the development of a CMHI model to examine efficacy promise to give impetus to further development in the near future.
Transmission Blocking Interventions for malaria – where do we stand and what does the future look like?

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Abstract:

Malaria remains a major global health challenge. Appropriate use of current anti-malarial tools has reduced the disease burden, but morbidity and mortality remain unacceptably high. It is widely accepted that to achieve long term control/eradication, it will be necessary to use interventions that inhibit the transmission of parasites to mosquitoes – these tools are termed Transmission Blocking Interventions (TBIs). This article aims to outline the rationale for the development of TBIs, with a focus on transmission-blocking drugs and transmission-blocking vaccines. We describe and summarise the current status of each of these intervention classes and attempt to identify future requirements in development, with a focus on the challenges of establishing each method within an integrated malarial control programme in the future.
1). Targeting malarial transmission – why?

Malaria remains a major global health challenge with an estimated 216 million new cases and 445,000 deaths in 2016 [1]. Appropriate use of “historical”, currently existing antimalarial healthcare tools (e.g.; use of insecticide-treated bednets (ITN), artemisinin combination therapy (ACT) and increased access to higher quality healthcare) have substantially reduced the global burden of disease over the previous decade, however, progress has recently stalled and morbidity and mortality remain unacceptably high. It is obvious that new, innovative tools and approaches will be essential to achieve malaria control or elimination within the medium to long term. The causative agent of malaria, the protozoan parasite of the genus *Plasmodium*, is transmitted almost exclusively by *Anopheles* mosquitoes. Transmission of *Plasmodium* from humans to mosquitoes is entirely dependent on the presence of sexually committed, mature male and female gametocytes within the peripheral blood, which rapidly differentiate into flagellate male (micro) and sessile female (macro) gametes upon uptake by the mosquito within a blood meal. The subsequent process of parasitic fertilization is then initiated by gamete adhesion, followed by membrane, and nuclear fusion [2]. Following successful fertilization, the resulting parasitic zygotes develop into motile ookinetes, which migrate to and penetrate the midgut epithelium of the mosquito via the secretion of hydrolase (e.g. chitinase) and proteolytic (e.g. PPLP5) enzymes [3-5], enabling the progression of the lifecycle through development of oocysts and subsequent sporogony.

It is widely accepted that to achieve eradication, it will be necessary to use interventions that inhibit the transmission of parasites from humans to mosquitoes - and vice versa [6]. Targeting malaria transmission is a logical concept. There are multiple advantageous characteristics of the fundamental biology of plasmodial transmission that render this process an attractive point of intervention. Firstly, the process of transmission from human to mosquito in the field typically results in the presence of <5 parasites (oocyst-stage) per mosquito [7] (although this figure is widely variable [8,9]). Conversely, in malaria infected humans, there are typically ~10⁹ circulating parasites within the bloodstream, [10] resulting in an evident population bottleneck for the targeted killing of parasites within this stage of the lifecycle. Allied to this, sexually mature parasites are extracellular for ~24 hours in the
mosquito (compared to ~30 seconds in humans during merozoite invasion [11]), resulting in a larger window of opportunity to target the parasite for immune/pharmacological destruction. Finally, the genes expressed in the sexual stages of the parasite life cycle are genetically invariant compared to blood/liver-stage genes [12-14], with the comparative reduction in polymorphism resulting in a conceptual reduction in resistance, and subsequent pathogen escape.

Allied to these key biological concepts, clear evidence exists that targeting malarial transmission is effective in a global context. Modelling studies clearly demonstrate the potential utility of targeting transmission, from the early dynamical models of Ross and Macdonald [15], followed by the development of cyclic feeding models [16,17], simulation of both vector transmission dynamics and within-human parasite dynamics [18,19]. Specialist models to predict impact on transmission alone also show impact across multiple vector ecologies and behaviours [20]. The benefits of targeting transmission and the mosquito vector were elegantly demonstrated by Bhatt et al., [21], in a study linking a large database of African field studies with detailed reconstructions of changing intervention coverage to quantify the attributable effect of individual malaria control efforts. The authors clearly demonstrate that broad interventions targeting the vector/ transmission - i.e. ITNs, and indoor residual spraying (IRS) are by far the most important interventions in Africa, responsible for an estimated 68% and 13% of the reduction in P. falciparum prevalence (PFPR2-10) since 2000-2015 (Figure 1). This is a clear indication of the potential clinical global value of targeting the transmission of Plasmodium through the mosquito host.

Examination, development and assessment of interventions specifically targeting malarial transmission is timely. The current stall in efforts to control malaria [1], reliance on a relatively narrow toolkit of clinical interventions, increasing risk of resistance to anti-malarial drugs and insecticides [22,23], and the potential of transmission blocking interventions to complement (or synergise with) other anti-malarial control methods currently available, or in the later stages of a development pipeline (e.g. the pre-erythrocytic vaccine RTS,S) [10] renders this approach particularly opportune. A potential manner of interrupting parasitic transmission directly is by targeting Plasmodium using transmission-blocking interventions (TBIs). These can be broadly classified as transmission
blocking drugs (TBDs), or transmission blocking vaccines (TBVs), against the parasitic sexual
stages (e.g. gametocytes/gametes/ookinetes). Here, we describe and summarise the
current status of each of these intervention classes and attempt to identify future
requirements and trends in their development, with a focus on the potential implications
and challenges of establishing each method within an integrated malarial control
programme in the future.

2). Anti-malarial transmission blocking drugs (TBDs) – present and future?

Mature, mosquito-infectious gametocytes maintain an arrested state of cellular
development in peripheral circulation and show divergent transcriptomes and proteomes
from asexual stages [24-26]. As a consequence of this, they are insensitive to most
schizonticidal antimalarials [27-28]. To identify drugs and small molecules with the potential
to block transmission, numerous high throughput transmission screening assays have been
developed [28-34]. The updated Medicines for Malaria Venture target candidate profile for
a transmission-blocking drug (TCP-5) states that it should ideally “have activity against all
five differentiated forms of gametocytes (stages I–V), plus inhibition of oocyst or sporozoite
formation in the mosquito vector” [35]. There are three clinically approved antimalarials
that show well-supported transmission-blocking efficacy (Figure 2): primaquine (PQ),
methylene blue (MB) and atovaquone (ATQ).

PQ is an 8-aminoquinoline used predominantly in the cure of \textit{P.vivax} relapsing infections by
eliminating the dormant liver hypnozoite stage of the parasite. Its effectiveness additionally
against transmission stages has been known for over half a century, targeting mature
gametocytes by an unknown mechanism that manifests in accelerated gametocyte
clearance and cumulative impaired development of subsequent mosquito stages [36].
However, PQ causes haemolytic anaemia in G6PD deficient individuals – a mutation
widespread across Sub-Saharan Africa, thus limiting its use [37]. Nevertheless, low doses of
PQ are now recommended by the WHO for transmission-blocking
(www.who.int/malaria/publications/atoz/who_htm_gmp_2015.1.pdf), with positive field
trials in both safety and efficacy [38,39]. MB appears to perturb the redox balance within
the parasite and is effective against asexuals, gametocytes and mosquito stages [27,40],
with conflicting evidence against liver stages of the life cycle [27,41]. Clinical trials have found three days of 15mg/kg MB is similarly efficacious as a single low dose (0.25mg/kg) of PQ at preventing transmission to the mosquito [39]. ATQ targets the parasite cytochrome bc1 and interrupts mitochondrial function [42]. In combination with proguanil (Malarone®), it is primarily used for chemoprophylaxis to prevent the development of liver stage parasites. However, ATQ has potent activity against ookinete and oocyst formation in the mosquito when carried across in the bloodmeal [43]. Intriguingly, sera from ATQ-treated volunteers (n=3) has been found to block transmission for over 35 days after treatment [44]. Furthermore, although ATQ drug resistance in asexual parasites can arise within the patient rather rapidly, these mutations render the parasite sterile for transmission and so resistance is not heritable [45]. Although no large clinical trials have studied ATQ as a transmission-blocking agent, with the expiry of the patent for Malarone® in 2013, it is tempting to speculate that a similar atovaquone-combination therapy could provide an effective and long-lasting “chemical vaccine” to prevent transmission in a mass drug administration setting.

Looking to the future, antimalarials with transmission-blocking activity have been prioritised with several in various stages of clinical development. Cipargamin® (KAE609/NITD609) developed by Novartis has recently completed phase Ila clinical trials [46]. Cipargamin inhibits PfATP4, a putative Na\(^+\) efflux pump, causing an intracellular osmotic imbalance within the cell [47]. Interestingly this causes the parasitized cell to swell and become rigid and likely contributes to accelerated clearance by the spleen in vivo [48]. It also has in vitro activity against early and late gametocytes, and oocyst development in the mosquito albeit all at relatively high doses compared to asexual activity [49]. How this translates into transmission-blocking efficacy in vivo remains to be determined. Similarly, MMV048 [50] and SJ733 [51], also both PfATP4 inhibitors are entering phase Ila and first in human trials respectively. KAF156, also developed by Novartis, is a rapid-acting antimalarial that is effective gametocytes in vitro and mosquito transmission both in vitro and in vivo in a P. berghei rodent model of infection [52]. The molecular target of KAF156 is unknown although resistance has been generated though mutations in PfCARL, PfACT and PfUGT [52,53]. Phase Iib trials in combination with lumefantrine are ongoing, and currently there is no published clinical data on its transmission-blocking activity [54].
To date transmission-blocking activity has been regarded as giving added value to schizonticides. As a consequence, dosing regimens are designed with asexual treatment rather than transmission-blocking in mind. *In vitro* data of the most advanced transmission-blocking molecules shows that they require drug concentrations about an order of magnitude higher to be efficacious. This has several implications for clinical trials. Firstly, due to sub-effective dosing, there is the danger that expectations of efficacy will not be met, resulting in a drain in the scientific/political will to continue this approach. More worryingly, if resistance mechanisms in asexual parasites also translate to resistance in gametocytes which are already less sensitive to the particular drug, there likely will be preferential transmission of resistance alleles. An alternative approach increasingly being considered is the concept of a transmission-specific drug. This class of antimalarial would specifically target biological pathways specific to gametocytes and/or mosquito stages with no activity against asexual stages. When administered in combination with a schizonticidal therapy to cure the patient and clear residual asexuals (i.e. the source of new gametocytes), transmission would be completely abrogated, with the added benefits of minimising the chance of resistance selection to the transmission-blocking component (smaller target population = decreased probability of resistance) and protecting the “shelf-life” of the partner schizonticide(s) by preventing the propagation of any generated resistance alleles through transmission.

3. **Anti-malarial transmission blocking vaccines (TBVs) – present and future?**

The induction of transmission-blocking immunity as a potential tool in malarial control was first demonstrated in the avian malaria parasite *P. gallinacium* [55,56]. Since then, the feasibility of an anti-malarial TBV has been demonstrated in multiple species, with a wide range of target antigens, expression systems and delivery methods assessed and examined. Parasite-derived molecules of interest for transmission blocking purposes can be assigned to one of two broad categories; 1). Proteins expressed in gametocytes and gametes, immunity against which will be naturally boosted by infection; and 2). Proteins expressed solely in the gamete, zygote and ookinete stages of the mosquito vector, which are therefore never
expressed within the human host. A perceived advantage to this is that these antigens are never exposed to immune pressure in a vertebrate population and are therefore less likely to exhibit extensive sequence variation [57]. Conversely, the vast majority of gametocytes are destined to die within the human host, and therefore all gametocyte antigens, irrespective of cellular localisation, will be presented to the host immune system. Such responses will “naturally boost” vaccine-induced immunity targeted to some gametocyte antigens, but vaccines targeting ookinete-specific immunogens would not have this benefit [58]. It is still unclear which of these contradictory concepts is more advantageous in practical terms when deploying a TBV. A third class of TBV immunogen has been characterised relatively recently – mosquito-derived antigens that can be targeting by vaccination to inhibit penetration of the midgut epithelium (e.g. APN1, FREP1 [59,60]). Although undoubtedly a promising approach, this text is limited to descriptions of parasite-derived TBVs only. Table 1 shows a range of parasitic molecules (both pre- and post-fertilisation) that are considered to be potential candidate TBV antigens. Although a wide range of parasite proteins have been examined for TBV activity over the previous decades, there are still only 5 immunogens that unquestionably and reproducibly demonstrate transmission blocking immunity and efficacy. These antigens are; 1). P230, 2). P48/45, 3). HAP2, 4). P25, 5). P28. (Table 1).

P48/45, Pfs230 and HAP2 are all pre-fertilisation targets, expressed during gametocyte development, and all have a functional role in parasitic fertilisation. P48/45 and P230 are synthesised in the gametocyte, are co-expressed, and are essential for the adhesion of male (micro)-gametes to female (macro)-gametes. Antibodies against both of these antigens expressed in a range of heterologous systems have shown significant transmission-blocking activity in the Standard Membrane Feeding Assay (SMFA) and the Direct Membrane Feeding Assay (DMFA) (outlined in [61,62]). Clinical development of both of these immunogens is relatively advanced, with the development of the Pfs48/45-derived immunogen R0-6C [63] encompassing the optimisation of upstream immunogen production, downstream purification, and optimisation of immunogenicity currently underway [64,65]. Studies using Pfs230-derived antigen as a TBV are particularly advanced at present, with Pfs230D1 showing high levels of functional activity in non-human primates and in US-based clinical trials [66,67]. Studies of Pfs230 immunisation followed by Direct Skin Feeding (DSF) in Mali
have demonstrated immunogenicity and activity in the field, with acceptable toleration and reproducible antibody responses following vaccination [67,68]. It should be noted that anti-P230 TBV activity has been demonstrated to be entirely complement-dependent [69]. Studies of both of these candidate TBVs are at a particularly exciting phase, with impressive recent progress in terms of antigenic production and the generation of initial proof of concept data in humans. HAP2 is a male-specific class II fusogen first identified in plants and has been shown to be essential for post-adhesion membrane fusion of the male and female gametocyte during fertilization [70]. Polyclonal antibodies against DII and DIII of *P. berghei* and *P. falciparum* HAP2 expressed in *E. coli* and wheat-germ cell free system have also exhibited high levels of transmission-blocking activity in pre-clinical studies [71,72], whereas antibodies against the short “fusion loop” of the protein have also resulted in transmission blocking efficacy in the lab (SMFA) and the field (DMFA) [2]. Combination of these findings to facilitate the clinical examination of HAP2 as a candidate TBV are ongoing.

The most extensively studied post-fertilisation candidates are P25 and P28, two GPI-anchored, EGF-domain containing, paralogous proteins with mutually redundant functions expressed on the surface of zygotes and ookinetes [73]. P25 is the most extensively studied TBV candidate, with a wide range of studies examining efficacy of P25-derived TBV immunogens previously reported. Although clear efficacy has been demonstrated in the lab with anti-P25 TBVs in many studies [74-47], and in the field, with serum derived from vaccination with anti-P25 TBVs followed by DMFA [76-78], direct demonstration of efficacy in humans following immunisation has been challenging. The first Phase Ia trials of recombinant Pfs25 in formulation with potent adjuvants (e.g. Pfs25-Montanide ISA21 [94]) lead to unacceptable levels of reactogenicity. More recently, a range of studies exploring different conjugates of Pfs25 (e.g. Pfs25-EPA, Pfs25-GPI), use of different adjuvants (e.g. AS01), use of transgenic parasites as expression systems, viral-vectored Pfs25 (e.g. ChAd63-Pfs25 and Pfs25-IMX313) have significantly advanced knowledge of this antigen [74,76,79]. Clinical of Pfs25-EPA in in clinical trials in the US and Phase Ib trials in Mali have demonstrated induction of functional antibody, but directly comparative data seems to show a lower efficacy with Pfs25-derived vaccine when compared to the use of Pfs230 as an immunogen [66-68].
The above five antigens are logically considered to be “priority” immunogens for vaccine development, although concerted efforts to broaden the repertoire of available antigens are ongoing. Surprisingly, discovery of the majority of these priority immunogens stems largely from historic studies where often crudely fractionated parasites were used to immunise mice to produce monoclonal antibodies, which were in turn validated by Western blot and laborious functional assays [80-83]. It is important to consider that these efforts are likely to identify only the most immunogenic of the natural antigens present in the whole-cell preparations used, and do therefore not preclude the discovery of new candidates for antigenic components of TBVs. Efforts to identify novel antigens using more advanced use of concerted ‘omics’ screens have only relatively recently started to yield the discovery of new TBV antigen candidates [84-87], although none have so far demonstrated reproducible efficacy or sufficient volume of data to a level where they are currently considered “priority” TBV candidates. This is unsurprising, considering the (well described) long period of time it takes to identify and validate malaria vaccine candidates, our gaps in knowledge about the effector arms of long-lasting anti-parasitic immunity, and the complex nature of the vaccine development pipeline, with no definitive targets for mode of action, assay threshold, or required efficacy in the lab or the field [88]. Information regarding some of these biologically fascinating “current non-priority” candidates is outlined in Table 1. Hopefully, these studies will yield a wider range of new and improved vaccine candidates to bolster the development pipeline in the near future. This is likely to be essential for the future utility of TBVs as a practical anti-malarial intervention.

4). Concluding remarks – what needs to be better?

Although undoubted progress has been made relatively recently in the efforts to control malaria, the disease remains a major issue in endemic areas, resulting in substantial impacts on morbidity/mortality and significant economic repercussions. The development of TBIs to contribute towards the drive to control/eliminate malaria has greatly accelerated relatively recently, to the extent that such interventions are already utilized as part of a clinical treatment pathway (in the case of PQ), or are tools that are likely to be integrated into clinical use in the near future. However, a large range of outstanding issues still need to be
resolved to optimise the use of these potentially powerful interventions. Some of these issues are discussed below (and see Outstanding Questions).

In terms of TBDs, although current field trials on an individual patient scale show efficacy, there is still a disappointing lack of trials showing the impact of TBD at the population level to reduce new cases of malaria [89]. It is known that asymptomatic submicroscopic gametocyte carriers contribute significantly to the infectious reservoir of malaria and so just treating symptomatic patients that present to the clinic is insufficient to impact transmission. “Test and treat” mass drug administration campaigns may be more effective; however, the current limiting factor is the lack of affordable and sufficiently rapid diagnostic tests for gametocytemia that can be used at the point of care to identify submicroscopic infections. Clearing this hurdle will facilitate the treatment coverage required for transmission-blocking, but overcoming the regulatory and psychological barriers of treating what to all purposes appear healthy individuals with a drug that helps the “next” patient need still to be addressed.

The practical development and use of a TBV within the field also requires a range of fundamental additional research. As discussed previously, there are only 5 “proven”/priority antigens for use as TBV components. It is exceptionally unlikely that this range of targets is sufficient to drive a long-term, robust development pipeline, thus the discovery of additional molecules/epitopes that can initiate a transmission-blocking response is essential and timely. Supplementary to this, the TBV development pipeline remains broadly opaque and undemocratic, with unclear go/no-go criteria for onward development from fundamental lab-based studies, and no clearly defined efficacy requirements for TBVs. This is likely due to the well-acknowledged disconnect between lab- and field-based assays to assess transmission-blockade [88,90]. Due to practical, concerted effort, this situation has improved in recent years, with in depth discussion and development of multiple assays/models to evaluate the biological efficacy of TBIs [90,91]. The development of a controlled human malaria infection (CHMI) model to facilitate the evaluation of TBIs within a controlled context [92,93] is exceptionally promising and has the promise to fill a critical gap within the development pipeline. The ability of TBVs to complement other, non-transmission-based interventions should also be examined carefully. Further down the
pipeline, it is essential for investigators and regulators to agree on future regulatory requirements and follow the most efficient acceptable clinical development plan. The design of Phase I, II, and large-scale population-based Phase III trials evaluating efficacy against infection and clinical endpoints promises to be challenging, but not insurmountable, and is likely to be vital to demonstrate the impact of a TBV and subsequently to achieve licensure.

Despite these ongoing issues, it is vital to acknowledge the considerable advances that have been made in recent years in terms of reducing global malaria burden and the development and assessment of multiple TBIs. Increased momentum and continued support for the development of these logical interventions promises to generate a wider range of powerful tools to continue our current progress, both in isolation, and in combination with a range of other anti-malarial interventions.

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Disclaimer Statement:

The authors declare no competing interests.

References:


**Figure Legends:**

**Figure 1.** Changing endemicity and effect of interventions 2000–2015. Predicted time series of population-weighted mean PfPR$_{2-10}$ across endemic Africa. The red line shows the actual prediction and the black line a ‘counterfactual’ prediction in a scenario without coverage by ITNs, ACTs or IRS. The coloured regions indicate the relative contribution of each intervention in reducing PfPR$_{2-10}$ throughout the period. Adapted from Bhatt *et al.*, (2015), *Nature*; 526(7572):207-211.

**Figure 2.** A summary of the transmission-blocking effects of clinically approved transmission-blocking drugs on the transmission stages of the *Plasmodium* life cycle.

**Table 1.** Parasitic molecules (both pre-and post-fertilisation) that are under consideration as potential candidate TBV antigens. Antigens are either classed as “priority”, or “under examination and consideration”. Please note that “studies of interest” are not intended to be an exhaustive list of relevant studies, but sensible starting points for further in-depth reading.
## "Priority antigens"

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## "Antigens under examination and consideration"

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Outstanding Questions:

- When considering the use of transmission-blocking drugs, is it viable to, and can we effectively implement “test and treat” mass drug administration campaigns? Asymptomatic submicroscopic gametocyte carriers contribute significantly to the infectious reservoir of malaria and only treating symptomatic patients that present to the clinic is insufficient. Mass drug administration campaigns maybe effective; but confounding factors to this approach are costs, and lack of rapid diagnostic tests for gametocytemia that can be used at the point of care. Even if these technical issues are overcome to facilitate the treatment coverages required for effective transmission-blocking, is it possible to overcome the regulatory and psychological issues of treating what to all purposes appear “healthy” individuals with a drug that helps the “next” patient?

- Is it viable to utilize transmission-specific drugs, with no activity against asexual stages, within a clinical pathway in the future?

- There is a potentially insufficient number of TBV immunogens currently available, with are only 5 “proven”/priority antigens for use as TBV components. How do we effectively boost this number of available targets in the future, balancing the desire to increase the number of molecules within a robust development pipeline, whilst maintaining (or increasing) current immunogenicity and efficacy?

- How do we accelerate and democratize the TBV development pipeline? What desirable go/no-go criteria do we set for the triage and development of TBVs, and how do we reconcile lab and field-based assays? What do Phase III trials look like, and what licencing pathway is the most practical to follow?

- What level of TBV coverage is acceptable to maintain effectiveness in the field, and how does this relate to “standard” measures of efficacy?
Figure 1.
Figure 2.

**HUMAN**
Gametocyte development

Gametocyte development stages:
- I: Early stage
- II: Early stage
- III: Early stage
- IV: Late stage
- V: Mature

**MOSQUITO**
Sporogony

Fertilisation

Gametocytes

Oocyst

Sporozoites

Primaquine
- CYP2D6 metabolite in vertebrate host liver required for activity

Transmission blocking effect escalates through mosquito stage development

Methylene blue
- Active against all gametocyte stages

2hr pre-feed will prevent transmission in mice

Conflicting evidence for infectivity of sporozoites

Atovaquone
- Minimal effect on gametocytes

Long serum half-life permits carry-over into bloodstream and potentiates inhibition of early mosquito stages

No effect on sporozoites but potentially inhibits liver stage development in subsequent host