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Highlights:

- Malarial burden can be demonstrably reduced by interventions that inhibit the transmission of *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.

1   **Transmission Blocking Interventions for malaria – where do we stand and what does the**

2   **future look like?**

3

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33 **Abstract:**

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35 Malaria remains a major global health challenge. Appropriate use of current anti-malarial

36 tools has reduced the disease burden, but morbidity and mortality remain unacceptably

37 high. It is widely accepted that to achieve long term control/eradication, it will be necessary

38 to use interventions that inhibit the transmission of parasites to mosquitoes – these tools

39 are termed Transmission Blocking Interventions (TBIs). This article aims to outline the

40 rationale for the development of TBIs, with a focus on transmission-blocking drugs and

41 transmission-blocking vaccines. We describe and summarise the current status of each of

42 these intervention classes and attempt to identify future requirements in development,

43 with a focus on the challenges of establishing each method within an integrated malarial

44 control programme in the future.

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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 anti-malarial 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 ~10^9 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

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97 mosquito (compared to ~30 seconds in humans during merozoite invasion [11]), resulting in

98 a larger window of opportunity to target the parasite for immune/pharmacological

99 destruction. Finally, the genes expressed in the sexual stages of the parasite life cycle are

100 genetically invariant compared to blood/liver-stage genes [12-14], with the comparative

101 reduction in polymorphism resulting in a conceptual reduction in resistance, and

102 subsequent pathogen escape.

103

104 Allied to these key biological concepts, clear evidence exists that targeting malarial

105 transmission is effective in a global context. Modelling studies clearly demonstrate the

106 potential utility of targeting transmission, from the early dynamical models of Ross and

107 Macdonald [15], followed by the development of cyclic feeding models [16,17], simulation

108 of both vector transmission dynamics and within-human parasite dynamics [18,19].

109 Specialist models to predict impact on transmission alone also show impact across multiple

110 vector ecologies and behaviours [20]. The benefits of targeting transmission and the

111 mosquito vector were elegantly demonstrated by Bhatt *et al*., [21], in a study linking a large

112 database of African field studies with detailed reconstructions of changing intervention

113 coverage to quantify the attributable effect of individual malaria control efforts. The authors

114 clearly demonstrate that broad interventions targeting the vector/ transmission - *i.e.* ITNs,

115 and indoor residual spraying (IRS) are by far the most important interventions in Africa,

116 responsible for an estimated 68% and 13% of the reduction in *P. falciparum* prevalence

117 (*Pf*PR$_{2-10}$) since 2000-2015 (Figure 1). This is a clear indication of the potential clinical global

118 value of targeting the transmission of *Plasmodium* through the mosquito host.

119

120 Examination, development and assessment of interventions specifically targeting malarial

121 transmission is timely. The current stall in efforts to control malaria [1], reliance on a

122 relatively narrow toolkit of clinical interventions, increasing risk of resistance to anti-

123 malarial drugs and insecticides [22,23], and the potential of transmission blocking

124 interventions to complement (or synergise with) other anti-malarial control methods

125 currently available, or in the later stages of a development pipeline (e.g. the pre-

126 erythrocytic vaccine RTS,S) [10] renders this approach particularly opportune. A potential

127 manner of interrupting parasitic transmission directly is by targeting *Plasmodium* using

128 transmission-blocking interventions (TBIs). These can be broadly classified as transmission

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129   blocking drugs (TBDs), or transmission blocking vaccines (TBVs), against the parasitic sexual

130   stages (e.g. gametocytes/gametes/ookinetes). Here, we describe and summarise the

131   current status of each of these intervention classes and attempt to identify future

132   requirements and trends in their development, with a focus on the potential implications

133   and challenges of establishing each method within an integrated malarial control

134   programme in the future.

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

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138   Mature, mosquito-infectious gametocytes maintain an arrested state of cellular

139   development in peripheral circulation and show divergent transcriptomes and proteomes

140   from asexual stages [24-26]. As a consequence of this, they are insensitive to most

141   schizonticidal antimalarials [27-28]. To identify drugs and small molecules with the potential

142   to block transmission, numerous high throughput transmission screening assays have been

143   developed [28-34]. The updated Medicines for Malaria Venture target candidate profile for

144   a transmission-blocking drug (TCP-5) states that it should ideally "*have activity against all*

145   *five differentiated forms of gametocytes (stages I–V), plus inhibition of oocyst or sporozoite*

146   *formation in the mosquito vector*" [35]. There are three clinically approved antimalarials

147   that show well-supported transmission-blocking efficacy (Figure 2): primaquine (PQ),

148   methylene blue (MB) and atovaquone (ATQ).

149

150   PQ is an 8-aminoquinoline used predominantly in the cure of *P.vivax* relapsing infections by

151   eliminating the dormant liver hynozoite stage of the parasite. Its effectiveness additionally

152   against transmission stages has been known for over half a century, targeting mature

153   gametocytes by an unknown mechanism that manifests in accelerated gametocyte

154   clearance and cumulative impaired development of subsequent mosquito stages [36].

155   However. PQ causes haemolytic anaemia in G6PD deficient individuals – a mutation

156   widespread across Sub-Saharan Africa, thus limiting its use [37]. Nevertheless, low doses of

157   PQ are now recommended by the WHO for transmission-blocking

158   (*www.who.int/malaria/publications/atoz/who_htm_gmp_2015.1.pdf*), with positive field

159   trials in both safety and efficacy [38,39]. MB appears to perturb the redox balance within

160   the parasite and is effective against asexuals, gametocytes and mosquito stages [27,40],

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161 with conflicting evidence against liver stages of the life cycle [27,41]. Clinical trials have

162 found three days of 15mg/kg MB is similarly efficacious as a single low dose (0.25mg/kg) of

163 PQ at preventing transmission to the mosquito [39]. ATQ targets the parasite cytochrome

164 bc1 and interrupts mitochondrial function [42]. In combination with proguanil (Malarone®),

165 it is primarily used for chemoprophylaxis to prevent the development of liver stage

166 parasites. However, ATQ has potent activity against ookinete and oocyst formation in the

167 mosquito when carried across in the bloodmeal [43]. Intriguingly, sera from ATQ-treated

168 volunteers (n=3) has been found to block transmission for over 35 days after treatment [44].

169 Furthermore, although ATQ drug resistance in asexual parasites can arise within the patient

170 rather rapidly, these mutations render the parasite sterile for transmission and so resistance

171 is not heritable [45]. Although no large clinical trials have studied ATQ as a transmission-

172 blocking agent, with the expiry of the patent for Malarone® in 2013, it is tempting to

173 speculate that a similar atovaquone-combination therapy could provide an effective and

174 long-lasting "chemical vaccine" to prevent transmission in a mass drug administration

175 setting.

176

177 Looking to the future, antimalarials with transmission-blocking activity have been prioritised

178 with several in various stages of clinical development. Cipargamin® (KAE609/NITD609)

179 developed by Novartis has recently completed phase IIa clinical trials [46]. Cipargamin

180 inhibits PfATP4, a putative Na^+ efflux pump, causing an intracellular osmotic imbalance

181 within the cell [47]. Interestingly this causes the parasitized cell to swell and become rigid

182 and likely contributes to accelerated clearance by the spleen *in vivo* [48]. It also has *in vitro*

183 activity against early and late gametocytes, and oocyst development in the mosquito albeit

184 all at relatively high doses compared to asexual activity [49]. How this translates into

185 transmission-blocking efficacy *in vivo* remains to be determined. Similarly, MMV048 [50]

186 and SJ733 [51], also both PfATP4 inhibitors are entering phase IIa and first in human trials

187 respectively. KAF156, also developed by Novartis, is a rapid-acting antimalarial that is

188 effective gametocytes *in vitro* and mosquito transmission both *in vitro* and *in vivo* in a *P.*

189 *berghei* rodent model of infection [52]. The molecular target of KAF156 is unknown

190 although resistance has been generated though mutations in PfCARL, PfACT and PfUGT

191 [52,53]. Phase IIb trials in combination with lumefantrine are ongoing, and currently there is

192 no published clinical data on its transmission-blocking activity [54].

193

194   To date transmission-blocking activity has been regarded as giving added value to

195   schizonticides. As a consequence, dosing regimens are designed with asexual treatment

196   rather than transmission-blocking in mind. *In vitro* data of the most advanced transmission-

197   blocking molecules shows that they require drug concentrations about an order of

198   magnitude higher to be efficacious. This has several implications for clinical trials. Firstly,

199   due to sub-effective dosing, there is the danger that expectations of efficacy will not be met,

200   resulting in a drain in the scientific/political will to continue this approach. More worryingly,

201   if resistance mechanisms in asexual parasites also translate to resistance in gametocytes

202   which are already less sensitive to the particular drug, there likely will be preferential

203   transmission of resistance alleles. An alternative approach increasingly being considered is

204   the concept of a transmission-specific drug. This class of antimalarial would specifically

205   target biological pathways specific to gametocytes and/or mosquito stages with no activity

206   against asexual stages. When administered in combination with a schizonticidal therapy to

207   cure the patient and clear residual asexuals (i.e. the source of new gametocytes),

208   transmission would be completely abrogated, with the added benefits of minimising the

209   chance of resistance selection to the transmission-blocking component (smaller target

210   population = decreased probability of resistance) and protecting the "shelf-life" of the

211   partner schizonticide(s) by preventing the propagation of any generated resistance alleles

212   through transmission.

213

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

215

216   The induction of transmission-blocking immunity as a potential tool in malarial control was

217   first demonstrated in the avian malaria parasite *P. gallinacium* [55,56]. Since then, the

218   feasibility of an anti-malarial TBV has been demonstrated in multiple species, with a wide

219   range of target antigens, expression systems and delivery methods assessed and examined.

220

221   Parasite-derived molecules of interest for transmission blocking purposes can be assigned to

222   one of two broad categories; 1). Proteins expressed in gametocytes and gametes, immunity

223   against which will be naturally boosted by infection; and 2). Proteins expressed solely in the

224   gamete, zygote and ookinete stages of the mosquito vector, which are therefore never

225 expressed within the human host. A perceived advantage to this is that these antigens are

226 never exposed to immune pressure in a vertebrate population and are therefore less likely

227 to exhibit extensive sequence variation [57] Conversely, the vast majority of gametocytes

228 are destined to die within the human host, and therefore all gametocyte antigens,

229 irrespective of cellular localisation, will be presented to the host immune system. Such

230 responses will "naturally boost" vaccine-induced immunity targeted to some gametocyte

231 antigens, but vaccines targeting ookinete-specific immunogens would not have this benefit

232 [58]. It is still unclear which of these contradictory concepts is more advantageous in

233 practical terms when deploying a TBV. A third class of TBV immunogen has been

234 characterised relatively recently – mosquito-derived antigens that can be targeting by

235 vaccination to inhibit penetration of the midgut epithelium (e.g. APN1, FREP1 [59,60].

236 Although undoubtedly a promising approach, this text is limited to descriptions of parasite-

237 derived TBVs only. Table 1 shows a range of parasitic molecules (both pre-and post-

238 fertilisation) that are considered to be potential candidate TBV antigens. Although a wide

239 range of parasite proteins have been examined for TBV activity over the previous decades,

240 there are still only 5 immunogens that unquestionably and reproducibly demonstrate

241 transmission blocking immunity and efficacy. These antigens are; 1). P230, 2). P48/45, 3).

242 HAP2, 4). P25, 5). P28. (Table 1).

243

244 P48/45, Pfs230 and HAP2 are all pre-fertilisation targets, expressed during gametocyte

245 development, and all have a functional role in parasitic fertilisation. P48/45 and P230 are

246 synthesised in the gametocyte, are co-expressed, and are essential for the adhesion of male

247 (micro)-gametes to female (macro)-gametes. Antibodies against both of these antigens

248 expressed in a range of heterologous systems have shown significant transmission-blocking

249 activity in the Standard Membrane Feeding Assay (SMFA) and the Direct Membrane Feeding

250 Assay (DMFA) (outlined in [61,62]). Clinical development of both of these immunogens is

251 relatively advanced, with the development of the Pfs48/45-derived immunogen R0-6C [63]

252 encompassing the optimisation of upstream immunogen production, downstream

253 purification, and optimisation of immunogenicity currently underway [64,65]. Studies using

254 Pfs230-derived antigen as a TBV are particularly advanced at present, with Pfs230D1

255 showing high levels of functional activity in non-human primates and in US-based clinical

256 trials [66,67]. Studies of Pfs230 immunisation followed by Direct Skin Feeding (DSF) in Mali

257 have demonstrated immunogenicity and activity in the field, with acceptable toleration and

258 reproducible antibody responses following vaccination [67,68]. It should be noted that anti-

259 P230 TBV activity has been demonstrated to be entirely complement-dependent [69].

260 Studies of both of these candidate TBVs are at a particularly exciting phase, with impressive

261 recent progress in terms of antigenic production and the generation of initial proof of

262 concept data in humans. HAP2 is a male-specific class II fusogen first identified in plants and

263 has been shown to be essential for post-adhesion membrane fusion of the male and female

264 gametocyte during fertilization [70]. Polyclonal antibodies against DII and DIII of *P. berghei*

265 and *P. falciparum* HAP2 expressed in *E. coli* and wheat-germ cell free system have also

266 exhibited high levels of transmission-blocking activity in pre-clinical studies [71,72], whereas

267 antibodies against the short "fusion loop" of the protein have also resulted in transmission

268 blocking efficacy in the lab (SMFA) and the field (DMFA) [2]. Combination of these findings

269 to facilitate the clinical examination of HAP2 as a candidate TBV are ongoing.

270

271 The most extensively studied post-fertilisation candidates are P25 and P28, two GPI-

272 anchored, EGF-domain containing, paralogous proteins with mutually redundant functions

273 expressed on the surface of zygotes and ookinetes [73]. P25 is the most extensively studied

274 TBV candidate, with a wide range of studies examining efficacy of P25-derived TBV

275 immunogens previously reported. Although clear efficacy has been demonstrated in the lab

276 with anti-P25 TBVs in many studies [74-47], and in the field, with serum derived from

277 vaccination with anti-P25 TBVs followed by DMFA [76-78], direct demonstration of efficacy

278 in humans following immunisation has been challenging. The first Phase Ia trials of

279 recombinant Pfs25 in formulation with potent adjuvants (e.g. Pfs25-Montanide ISA21 [94])

280 lead to unacceptable levels of reactogenicity. More recently, a range of studies exploring

281 different conjugates of Pfs25 (e.g. Pfs25-EPA, Pfs25-GPI), use of different adjuvants (e.g.

282 AS01), use of transgenic parasites as expression systems, viral-vectored Pfs25 (e.g. ChAd63-

283 Pfs25 and Pfs25-IMX313) have significantly advanced knowledge of this antigen [74,76,79].

284 Clinical of Pfs25-EPA in in clinical trials in the US and Phase Ib trials in Mali have

285 demonstrated induction of functional antibody, but directly comparative data seems to

286 show a lower efficacy with Pfs25-derived vaccine when compared to the use of Pfs230 as an

287 immunogen [66-68].

288

289 The above five antigens are logically considered to be "priority" immunogens for vaccine

290 development, although concerted efforts to broaden the repertoire of available antigens

291 are ongoing. Surprisingly, discovery of the majority of these priority immunogens stems

292 largely from historic studies where often crudely fractionated parasites were used to

293 immunise mice to produce monoclonal antibodies, which were in turn validated by Western

294 blot and laborious functional assays [80-83]. It is important to consider that these efforts are

295 likely to identify only the most immunogenic of the natural antigens present in the whole-

296 cell preparations used, and do therefore not preclude the discovery of new candidates for

297 antigenic components of TBVs. Efforts to identify novel antigens using more advanced use

298 of concerted 'omics' screens have only relatively recently started to yield the discovery of

299 new TBV antigen candidates [84-87], although none have so far demonstrated reproducible

300 efficacy or sufficient volume of data to a level where they are currently considered "priority"

301 TBV candidates. This is unsurprising, considering the (well described) long period of time it

302 takes to identify and validate malaria vaccine candidates, our gaps in knowledge about the

303 effector arms of long-lasting anti-parasitic immunity, and the complex nature of the vaccine

304 development pipeline, with no definitive targets for mode of action, assay threshold, or

305 required efficacy in the lab or the field [88]. Information regarding some of these

306 biologically fascinating "current non-priority" candidates is outlined in Table 1. Hopefully,

307 these studies will yield a wider range of new and improved vaccine candidates to bolster the

308 development pipeline in the near future. This is likely to be essential for the future utility of

309 TBVs as a practical anti-malarial intervention.

310

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

312

313 Although undoubted progress has been made relatively recently in the efforts to control

314 malaria, the disease remains a major issue in endemic areas, resulting in substantial impacts

315 on morbidity/mortality and significant economic repercussions. The development of TBIs to

316 contribute towards the drive to control/eliminate malaria has greatly accelerated relatively

317 recently, to the extent that such interventions are already utilized as part of a clinical

318 treatment pathway (in the case of PQ), or are tools that are likely to be integrated into

319 clinical use in the near future. However, a large range of outstanding issues still need to be

320   resolved to optimise the use of these potentially powerful interventions. Some of these

321   issues are discussed below (and see Outstanding Questions)..

322

323   In terms of TBDs, although current field trials on an individual patient scale show efficacy,

324   there is still a disappointing lack of trials showing the impact of TBD at the population level

325   to reduce new cases of malaria [89]. It is known that asymptomatic submicroscopic

326   gametocyte carriers contribute significantly to the infectious reservoir of malaria and so just

327   treating symptomatic patients that present to the clinic is insufficient to impact

328   transmission. "Test and treat" mass drug administration campaigns may be more effective;

329   however, the current limiting factor is the lack of affordable and sufficiently rapid diagnostic

330   tests for gametocytemia that can be used at the point of care to identify submicroscopic

331   infections. Clearing this hurdle will facilitate the treatment coverage required for

332   transmission-blocking, but overcoming the regulatory and psychological barriers of treating

333   what to all purposes appear healthy individuals with a drug that helps the "next" patient

334   need still to be addressed.

335

336   The practical development and use of a TBV within the field also requires a range of

337   fundamental additional research. As discussed previously, there are only 5 "proven"/priority

338   antigens for use as TBV components. It is exceptionally unlikely that this range of targets is

339   sufficient to drive a long-term, robust development pipeline, thus the discovery of

340   additional molecules/epitopes that can initiate a transmission-blocking response is essential

341   and timely. Supplementary to this, the TBV development pipeline remains broadly opaque

342   and undemocratic, with unclear go/no-go criteria for onward development from

343   fundamental lab-based studies, and no clearly defined efficacy requirements for TBVs. This

344   is likely due to the well-acknowledged disconnect between lab- and field-based assays to

345   assess transmission-blockade [88,90]. Due to practical, concerted effort, this situation has

346   improved in recent years, with in depth discussion and development of multiple

347   assays/models to evaluate the biological efficacy of TBIs [90,91]. The development of a

348   controlled human malaria infection (CHMI) model to facilitate the evaluation of TBIs within

349   a controlled context [92,93] is exceptionally promising and has the promise to fill a critical

350   gap within the development pipeline. The ability of TBVs to complement other, non-

351   transmission-based interventions should also be examined carefully. Further down the

352   pipeline, it is essential for investigators and regulators to agree on future regulatory

353   requirements and follow the most efficient acceptable clinical development plan. The

354   design of Phase I, II, and large-scale population-based Phase III trials evaluating efficacy

355   against infection and clinical endpoints promises to be challenging, but not insurmountable,

356   and is likely to be vital to demonstrate the impact of a TBV and subsequently to achieve

357   licensure.

358

359   Despite these ongoing issues, it is vital to acknowledge the considerable advances that have

360   been made in recent years in terms of reducing global malaria burden and the development

361   and assessment of multiple TBIs. Increased momentum and continued support for the

362   development of these logical interventions promises to generate a wider range of powerful

363   tools to continue our current progress, both in isolation, and in combination with a range of

364   other anti-malarial interventions.

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372   **Disclaimer Statement:**

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374   The authors declare no competing interests.

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729

730

731 **Figure Legends:**

732

733 <u>**Figure 1.**</u> **Changing endemicity and effect of interventions 2000–2015**. Predicted time series

734 of population-weighted mean $PfPR_{2-10}$ across endemic Africa. The red line shows the actual

735 prediction and the black line a 'counterfactual' prediction in a scenario without coverage by

736 ITNs, ACTs or IRS. The coloured regions indicate the relative contribution of each

737 intervention in reducing $PfPR_{2-10}$ throughout the period. Adapted from Bhatt *et al*., (2015),

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739

740 <u>**Figure 2.**</u> **A summary of the transmission-blocking effects of clinically approved**

741 **transmission-blocking drugs on the transmission stages of the *Plasmodium* life cycle.**

742

743 <u>**Table 1.**</u> **Parasitic molecules (both pre-and post-fertilisation) that are under consideration**

744 **as potential candidate TBV antigens.** Antigens are either classed as "priority", or "under

745 examination and consideration". Please note that "studies of interest" are not intended to

746 be an exhaustive list of relevant studies, but sensible starting points for further in-depth

747 reading.

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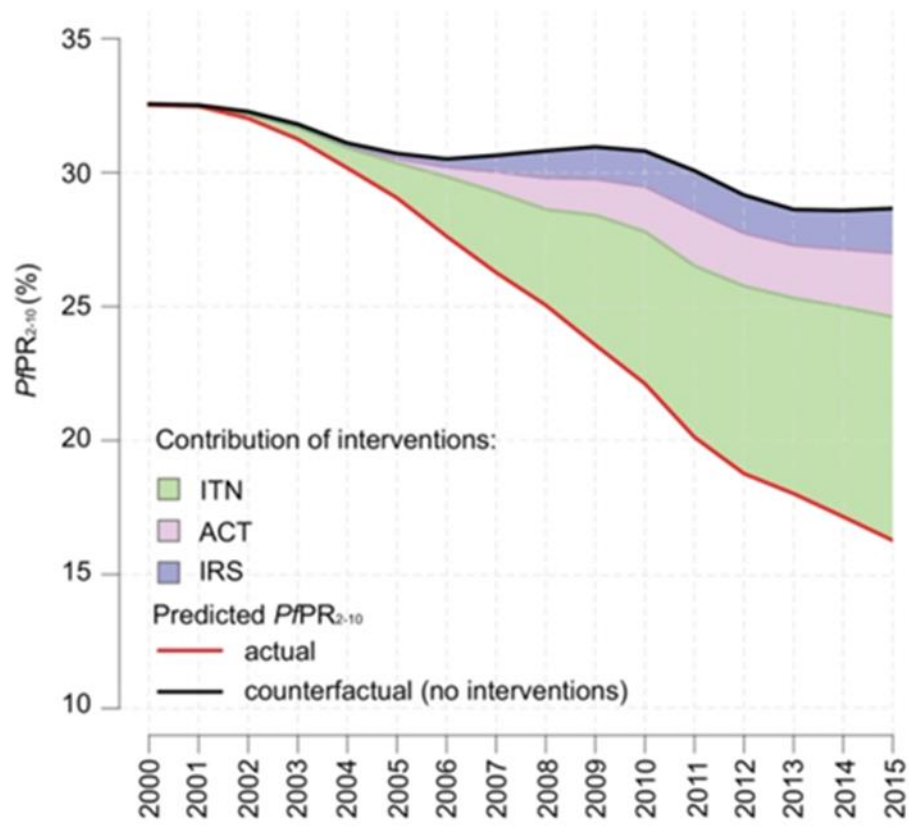750

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"Priority antigens"			
Pre-fertilisation		**Post-fertilisation**	
Antigen	*Studies of interest*	*Antigen*	*Studies of interest*
P48/45	Outchkourov *et al.* 2008; van Djik *et al.* 2001/2008; Theisen *et al.* 2014; Singh *et al.* 2015	P25	Kaslow *et al.* 1994, Radtke *et al.* 2017; Talaat *et al.* 2016; Scally *et al.*, 2017
P230	Williamson *et al.* 1995; Tachibana *et al.*2012; Farrance *et al.* 2011; MacDonald et al., 2016	P28	Quian *et al.* 2009; Kim *et al.* 2011
HAP2	Blagborough & Sinden 2009; Miura *et al.* 2013; Angrisano *et al.* 2017		

"Antigens under examination and consideration"			
Pre-fertilisation		**Post-fertilisation**	
Antigen	*Studies of interest*	*Antigen*	*Studies of interest*
Pfg27	Lobo Konings & Kumar 1994; Lobo *et al.* 1999; Ploton *et al.* 1995	CelTOS	Kariu *et al.* 2006;
PfMR5	Eksi & Williamson 2002	Chitinase	Shahabuddin 1995; Langer *et al.* 2002; Li *et al.* 2005; Takeo *et al.* 2009
Pfs16	Lobo Konings & Kumar 1994; Moelans *et al.* 1995	Enolase	Ghosh *et al.* 2011
Pfs2400/Pf11-1	Feng *et al.* 1993	PfGAP50	Beiss *et al.* 2015; Simon *et al.* 2013
Plasmepsin 4	Li , Patra *et al.* 2010	PSOP12	Sala *et al.* 2015
PfCCP/LAP proteins	Scholz *et al.* 2008; Carter *et al.*2008; Saeed *et al.* 2010	SOAP	Dessens *et al.* 2003
GEST	Talman *et al.*, 2011	Plasmepsin 7	Li *et al.* 2016
Pfs47	van Schaijk *et al.* 2006; Tachibana *et al.* 2015; Molina-Cruz *et al.*, 2015	Plasmepsin 10	Li *et al.* 2016
PSOP12	Sala *et al.*, 2015	CTRP	Trottein 1995; Ramakrishnan *et al.* 2011
		MAOP/PPLP3	Kadota *et al.* 2004; Kaiser *et al.* 2004, Ecker 2007
		PPLP5	Ecker 2007; Kadota 2004
		PSOP25	Zheng *et al.*, 2016
		PbPH	Xou *et al.*, 2016
		PSOP7	Zheng *et al.*, 2016
		PSOP26	Zheng *et al.*, 2016

Outstanding Questions:

- When considering the use of transmission-blocking drugs, its 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

<u>Figure 1.</u>

Figure

Figure 2.