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1 ***Plasmodium vivax* in haematopoietic niches: hidden and dangerous**

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18
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20
21 **Abstract**

22 A series of recent studies have suggested the haematopoietic niche of the bone marrow as a
23 major reservoir for parasite replication and the development of transmission stages. However
24 significant knowledge gaps remain in our understanding in the host parasite interactions,
25 pathophysiology and implications for treatment and diagnosis of such reservoir. Here, we
26 discuss the current status of this emerging research field in the context of *Plasmodium vivax*.

28 **Introduction**

29 Outside of Sub-Saharan Africa, *Plasmodium vivax* dominates the malaria public health
30 burden. In these regions it accounts for 41% of all malaria cases resulting in 35% of the
31 global population living at risk of *P. vivax* infection [1, 2]. Even in Sub-Saharan Africa, an
32 increase of *P. vivax* cases has been observed despite high frequency of Duffy-negative alleles
33 [3]. In the Brazilian Amazon region, *P. vivax* is the main species causing malaria and
34 responsible for more than 85% of all cases [1]. In general, *P. vivax* persists in areas that
35 succeeded to eliminate *P. falciparum* by malaria control programs [4]. However, major
36 knowledge and tool gaps remain in *P. vivax* research as the focus so far has been on *P.*
37 *falciparum*.

38 For a long time, *P. vivax* research was neglected due to failure to establish an *in vitro*
39 culture system and apparently low prevalence of severe cases compared to *P. falciparum* [5-
40 7]. Presence of all stages in the blood circulation, and therefore assumed lack of sequestration,
41 has contributed to the long-standing misconception that *P. vivax* is a benign parasite [8].
42 However, recent data have demonstrated that late asexual blood stage *P. vivax* parasites are
43 capable of cytoadhering to endothelial host receptors [7, 9], and that they are less abundant in
44 blood circulation than younger stages in *P. vivax* patients [7, 10]. Estimation of parasite
45 biomass based on circulating biomarkers indicates existence of a predominant parasite
46 biomass outside of circulation that is not captured by peripheral *P. vivax* parasitemia, in
47 particular in patients with complicated outcomes [10]. Moreover, a series of recent
48 histological studies in *P. vivax* patients and experimentally infected non-human primates
49 (NHP) provides direct evidence for the existence of a major reservoir of *P. vivax* blood stage
50 parasites, both asexual and sexual (gametocytes) in the haematopoietic niche of bone marrow
51 and possibly spleen. These recent findings, together with more stringent diagnosis techniques
52 of *P. vivax* infection suggesting a similar risk of severe disease and death as *P. falciparum*
53 infection [6], strongly argue against the benign nature of *P. vivax* malaria, especially in
54 patients with other comorbidities.

55

56 ***P. vivax* biomass in peripheral circulation: the tip of the iceberg?**

57 *P. vivax* parasites exhibit a narrow tropism by strictly infecting young reticulocytes. In
58 contrast, *P. falciparum* can infect normocytes even though it also prefers to infect young
59 reticulocytes [11-14]. The restriction of *P. vivax* for young reticulocytes that are exceedingly
60 rare in circulation (<2% of all circulating red blood cells) means parasitaemia is greatly
61 limited by the abundance of available host cells [6, 15]. Low peripheral parasitaemia and

62 apparent presence of all parasite stages in the blood contradicts numerous reports of vivax
63 malaria with severe illness and deaths due to *P. vivax* infection in all endemic regions [16-20].
64 It has been suggested that *P. vivax* parasites have a lower pyrogenic threshold and hence
65 induce a stronger inflammatory response compared to other *Plasmodium* infections with
66 similar or greater parasitaemia [15]. Indeed, the host inflammatory response and endothelial
67 activation are greater in patients infected with *P. vivax* than with other malaria infections [19-
68 21].

69 It has also been suggested that the peripheral parasitaemia represents only a fraction
70 of the total *P. vivax* parasite biomass. Various indirect lines of evidence support this
71 hypothesis. First, several reports from *P. vivax* patients have shown that the total parasite
72 biomass, as defined by pvLDH levels in blood, is underestimated by microscopic analysis of
73 peripheral blood smears [6, 21]. Second, there is no clear correlation between the burden of
74 peripheral parasitaemia and disease severity. Accordingly, a wide range of clinical syndromes
75 occurs in *P. vivax* patients even with modest peripheral parasite counts, in contrast to *P.*
76 *falciparum*-infected individuals [6, 21-31]. Third, NHP models susceptible to *P. vivax*
77 infection have been very informative in inferring sequestered parasite biomass and
78 correlations with pathogenesis [31-34]. A computational model capable to quantify the
79 parasite biomass concealed in a tissue reservoir by measuring blood parasitaemia was
80 designed by observing the longitudinal dynamics of *P. cynomolgi* parasitaemia in infected
81 *Macaca mulatta*, a *P. vivax* simian malaria model [31]. Through the application of this model
82 and additional observations made in vivax malaria patients it was inferred that a large
83 fraction of parasites is withdrawn from the peripheral circulation early during blood stage
84 infection and hidden in a reservoir, with potential role in disease pathogenesis [31, 34].
85 Fourth, clinical studies in *P. vivax* patients and in NHP models demonstrate that this hidden
86 parasite population seemingly expands without detection and contributes to disease severity
87 [6, 21-34], systemic inflammation [15, 21, 28] and intravascular accumulation of immune
88 cells in pulmonary pathologies [28].

89 Finally, several studies have reported a biased distribution of asexual forms in blood
90 smears of *P. vivax* patients, with higher prevalence of ring stages compared to trophozoites
91 and schizonts in peripheral blood [7, 10]. Likewise, transcriptomic analysis from *P. vivax*
92 blood samples demonstrated a quantitative depletion of transcripts from late asexual and
93 immature sexual stages, or gametocytes, in the blood of *P. vivax*-infected patients [32],
94 similar to observations with *P. falciparum* [35]. At the same time these later asexual stages
95 display a higher adhesive capacity compared to young stages, indicating that the latter part of

96 the asexual *P. vivax* cycle could occur in deep tissues and outside of peripheral circulation [6,
97 7, 10]. Specifically, late asexual parasites are able to cytoadhere *in vitro* to endothelial
98 receptors, such as ICAM-1, CD36 and chondroitin sulfate A (CSA), receptors expressed in
99 cerebral, pulmonary and placental microvasculature, with a similar strength but lower
100 frequency than red blood cells (RBCs) infected with *P. falciparum* [7, 9, 36, 37].

101

102 **Emerging evidence for a *P. vivax* reservoir in the hematopoietic niche of the bone** 103 **marrow**

104 The reticulocyte population makes only 1–2% of all circulating RBCs [13, 14]. Immature
105 reticulocytes are largely confined to the bone marrow (BM, ~0.016% of all enucleated
106 erythroid cells in the circulation) and more cytoadhesive (higher expression of adhesion
107 molecules such as CD49d and CD44) than circulating reticulocytes. Reticulocytes are formed
108 from haematopoietic stem cells and released from the bone marrow niche for final maturation
109 in the spleen [11-14, 38, 39]. *P. vivax* preferentially invades BM resident immature
110 reticulocytes making this niche highly advantageous for the parasite. Multiple case reports
111 have detected *P. vivax* at higher parasite biomass in the BM compared to blood, or
112 exclusively in BM [12, 25, 40-43]. *P. vivax* infections after BM transplantation have also
113 been reported [27, 44-46], suggesting that BM may represent a pivotal tissue reservoir in *P.*
114 *vivax* infection.

115 A systematic analysis of *P. vivax* distribution in tissue samples from infected
116 splenectomized *Aotus* and *Saimiri* monkeys revealed enrichment of gametocytes and
117 schizonts in the BM and liver [32]. Together, these organs accounted for about 30% of the
118 total parasite burden. 70% of the gametocyte load and 90% of the schizont load was
119 accumulated in the BM and liver, suggesting that these tissues are major parasite reservoirs.
120 Importantly, in the BM the vast majority of parasites were located in the parenchyma, where
121 haematopoiesis takes place. Immunohistochemistry (IHC) analysis revealed that the majority
122 of parasites detected by the constitutive marker pLDH (*Plasmodium* lactate dehydrogenase)
123 were negative for antibodies against late sexual (PvLAP5) and asexual stages (PvAMA1)
124 markers, indicating the enrichment of early ring stages and immature gametocytes in the BM
125 parenchyma [32]. In agreement with these data a recent case report demonstrated enrichment
126 of rings, schizonts and gametocytes in BM compared to blood [47]. Together, these studies
127 suggest that the BM contributes significantly to the total *P. vivax* biomass, providing a niche
128 for asexual growth and development of gametocyte stages (Figure 1).

129 These findings are in line with similar observations in *P. falciparum* and the rodent
130 malaria parasite *P. berghei*. Autopsy case studies and analyses of biopsies and aspirates have
131 consistently revealed a significant enrichment of *P. falciparum* immature gametocytes in the
132 BM and spleen of infected patients [48-50]. In the BM parenchyma, gametocytes were
133 enriched at erythroblast islands before re-entering the circulation [49]. Quantitative imaging
134 experiments in the rodent malaria model *P. berghei* also demonstrated gametocyte
135 development in the extravascular niche of the BM and spleen, involving selective tissue
136 homing, transmigration across the endothelial barrier and mobile behaviour of mature
137 gametocytes [51]. In addition, asexual parasite stages were observed in the extravascular
138 environment both in *P. falciparum* (in human autopsies) and in *P. berghei* (in infected mice),
139 suggesting existence of a genuine extravascular replication cycle in both *Plasmodium* species
140 [51]. Altogether these observations establish infection of the BM haematopoietic niche as a
141 new paradigm in *Plasmodium* biology.

142

143 **What is the role of the spleen as a parasite reservoir?**

144 Experimental and clinical studies have demonstrated that *P. vivax* infection induces a marked
145 splenomegaly, with incidence of splenic rupture and death higher than in other malaria
146 infections [52-58]. In humans, the spleen contributes to the clearance of damaged and
147 infected RBCs, generation of immunity and it changes to parasite antigens expressed on the
148 surface of infected RBCs [54, 55]. Examinations of spleen samples from *P. vivax* patients
149 revealed extensive remodelling, enlargement of the white pulp, increased cellularity and large
150 numbers of intact *P. vivax*-infected reticulocytes in the red pulp [22, 54, 55]. In one case
151 report, confocal microscopy analysis showed macrophages containing large amounts of
152 parasite pigments, but no intact RBCs were detected in macrophages. Interestingly, intense
153 proliferation of B cells, plasma cells and plasmablasts in extrafollicular compartments, which
154 resembled a B-cell lymphoma phenotype was also observed [22], suggesting that alterations
155 in the spleen are linked to acquisition of anti-parasite immunity.

156 Initial investigation of *P. vivax* sequestration in spleen-intact common squirrel
157 monkeys (*Saimiri sciureus*) and night monkeys (*Aotus lemurinus lemurinus*) identified the
158 splenic vasculature as the primary site of *P. vivax* asexual development, with a high
159 proportion of schizont-infected RBCs [33]. The liver and BM appeared as secondary sites for
160 trophozoite and schizont accumulation [33] while gametocytes were not analysed. Although
161 these observations were based on organ crushes only and the organs were not perfused, a
162 recent study in *P. vivax*-infected *Saimiri* included one spleen-intact control animal that

163 showed a similar pattern of parasite distribution [34]: spleen contained the highest parasite
164 counts followed by the liver, lung and BM. In agreement with the work by Obaldia *et al.* [32],
165 parasites were enriched in BM and liver in the splenectomised animals [34] (Figure 1).
166 Splenectomy before infection is an important limitation in these studies, as the significant
167 parasite load observed in BM and liver could mask a significant reservoir in the spleen. So far,
168 no systematic autopsy case or other tissue biopsy studies of *P. vivax*-infected patients
169 comparing the role of both spleen and BM as potential parasite reservoirs have been
170 conducted. In *P. falciparum*, high numbers of parasites were found in spleen samples from
171 autopsies cases [49], however it remains to be determined whether these are viable or present
172 within macrophages. In the rodent malaria model the spleen represents the major parasite
173 reservoir outside of circulation with significant levels of asexual and gametocyte stages [51].

174 In contrast to humans and primates, the adult murine spleen is haematopoietically
175 active. However, splenic extramedullary erythropoiesis can also occur in humans during
176 specific pathological conditions including malaria. Such mechanism has been suggested to be
177 stimulated during vivax malaria to compensate anaemia [59], and it would further support the
178 role of the spleen as a parasite reservoir [60-62]. In this scenario, haematopoiesis would take
179 place in the red pulp of the spleen before the release of reticulocytes into the circulation. In
180 addition, *P. vivax* infection may also induce a remodelling of uninfected RBCs, resulting in
181 their arrest in the spleen. In turn, this could generate a reservoir for parasite invasion. These *P.*
182 *vivax*-infected reticulocytes may remain trapped in the red pulp by interacting with
183 contractile fibroblasts, cells that proliferate during splenic erythropoiesis and surround
184 reticulocytes [59, 61].

185

186 **Sub-patent infection in the hematopoietic reservoir as a source of recurrences?**

187 The observed concealment of infected RBCs in the haematopoietic system is likely to be
188 critical for the parasite to evade immunity and drug pressure, and this reservoir may
189 contribute to the observed recurrence patterns in *P. vivax* infection. Proof-of-principle
190 support of this hypothesis comes from the *P. berghei* model, which – as *P. vivax* - has a
191 preference for young reticulocytes. Mice infected with *P. berghei* and treated with at least 10
192 mg/kg of artemisinin clear peripheral parasitaemia but maintain low level infection rates in
193 BM and spleen that initiate recurrence of peripheral parasitaemia [63].

194 Case reports also support the hypothesis of *P. vivax* recurrence from the
195 haematopoietic niche. For example, one report from Brazil documented a patient with
196 persistent thrombocytopenia and an enlarged spleen who was diagnosed with chronic *P. vivax*

197 malaria after discovering schizonts in BM aspirate [24]. In another case report, a patient
198 developed vigorous vivax malaria with relatively high parasitaemia (1%) 40 days after a
199 donor BM transplant [27]. Investigations revealed that the donor was diagnosed with malaria
200 11 months before BM collection, and had an asymptomatic recurrence following treatment of
201 the first infection [27]. The uncertain origin of homologous vivax recurrences [64], case
202 reports of *P. vivax* parasites detected only in BM aspirates without peripheral blood
203 parasitaemia [24, 27, 40-44, 65], reports of vivax infection following sibling allogeneic BM
204 transplants [45], and recurrence after autologous BM transplantation [46] further suggest
205 presence of sub-patent *P. vivax* infections in the BM that can lead to recurrence. Hence BM
206 could be an alternative source of parasite recurrence upon drug treatment, as opposed to liver
207 relapse from quiescent hypnozoite stages. Notably, primaquine and related 8-
208 aminoquinolines, the first line treatment against hypnozoites, are prodrugs that require
209 activation through an enzymatic pathway that is predominant in liver and BM tissue [66].

210 Analysis of recurrence patterns in neurosyphilis patients who underwent malaria
211 therapy either through inoculation by *P. vivax* blood stage or sporozoites provide interesting
212 information in that regard [67]. While there is wide variation in recurrence patterns across
213 individual patients, parasite dynamics are not significantly different during the first 2 months
214 post infection [67], whereas only sporozoite-inoculated infections seem to exhibit recurrent
215 parasitaemias weeks after absence of peripheral blood parasites, indicative of relapses from
216 liver hypnozoites (Figure 2). Data from other studies performed around the same time as the
217 malaria therapies confirm these observations. For example, a study covering around two
218 years of observations of general paralysis in patients inoculated with *P. vivax* trophozoites
219 and treated with quinine (30g, 2-4 days), revealed that 2% relapsed up to a month after end of
220 treatment [68, 69]. In patients who survived infection after mosquito bites, 18% recurred
221 between two to six months and 33% of these recurred more than once [68]. Another study
222 comparing *P. vivax* blood stage versus sporozoite infection reported that both inoculation
223 routes lead to 35-40% of recurrences within the first two months after termination of the
224 primary infection, while only sporozoite infections recurred beyond that point [70]. Similar to
225 the malaria therapy studies, a longitudinal study in rhesus monkeys infected with *P.*
226 *cynomolgi* showed recurrence in 48% of the monkeys inoculated with trophozoites while
227 79% of those infected with sporozoites recurred [71]. In this study, animals who were
228 negative by thick smear for 60 days or more underwent splenectomy: 25% of sporozoite-
229 infected animals relapsed up to two weeks after splenectomy, while none of the trophozoite-
230 infected monkeys did [71].

231 Taken together, comparative data from experimental infections in humans and
232 animals indicate that both the blood stage and sporozoite infection routes can exhibit similar
233 recurrence patterns, at least during the first 2 months post infection and following (sub-
234 curative) drug treatment. On the other hand, the limited data available indicates that
235 hypnozoite relapse have a much greater contribution during later phases of infection, which
236 together suggests that the BM reservoir and liver hypnozoites are distinct but synergistic
237 strategies by which the parasite prolongs infection and thus enhances its transmission success.

238

239 **What are the implications of *P. vivax* development in the BM for the host?**

240 Parasite infection in the haematopoietic niche has implications for malaria pathogenesis,
241 diagnosis and treatment. The BM parenchyma is a specialized and complex
242 microenvironment that provides a set of molecular, structural and physical cues to regulate
243 hematopoietic stem and progenitor cell (HSPC) production [72]. Haematopoiesis is a
244 dynamic biological process that can also be responsive and shaped by pathogens during
245 infection. HSPCs are capable of responding to pathogens by directly sensing pathogen-
246 associated molecule patterns (PAMPs) through their respective pathogen-recognition
247 receptors (PRRs). They also express a broad range of cytokines/chemokines receptors, which
248 allows them to detect pro-inflammatory signals (DAMPs).

249 Recent studies investigating the impact of parasite infection in the BM have focused
250 on potential changes in erythropoiesis to understand the pathophysiology of vivax malaria
251 anaemia [47, 73]. These changes include altered levels of miRNA transcripts [48] and
252 impaired activity of transcription factors, such as GATA1/GATA2 [74, 75], regulating
253 erythropoiesis. GATA1/GATA2 changes are mediated by intermediate and non-classical
254 monocytes and activation of IFN type I and II signalling pathways in the BM [74]. Similarly,
255 IFN- γ is implicated in malarial anaemia in rodent malaria models and can directly cause
256 apoptosis of erythroid progenitors *in vitro* [76]. It has also been suggested that inflammatory
257 cytokines produced by macrophages and monocytes in the BM in response to parasite by-
258 products promote the dyserythropoiesis observed during malarial anaemia [77]. In *P. vivax*-
259 infected patients lymphopenia and thrombocytopenia are common clinical signs of infection,
260 while myeloid cell count (e.g., monocytes and neutrophils) often remain unchanged in the
261 peripheral blood [6, 15, 21, 24, 73, 78-81]. Although the level of cytokines implicated in the
262 expansion of the megakaryocyte lineage and myelopoiesis in the BM remain to be
263 determined, these molecules (e.g., IL-1 α , IL-1 β , IL-6, IL-8, TNF- α , IFN- γ , thrombopoietin
264 [TPO] and G-CSF) are increased in the plasma of *P. vivax* patients [15, 21, 24, 73, 78, 82-84].

265 Increased cytokine levels inducing myeloid-biased HSC differentiation while reducing
266 lymphopoiesis could explain the normal counts of myeloid cells and decrease of lymphocytes
267 in the circulation in *P. vivax* patients [21, 24, 73, 78]. Indeed, less differentiated neutrophils
268 (band cells) in peripheral blood are increased in *P. vivax* patients during acute infection,
269 possibly as a result of rapid neutrophil production and/or their premature release from the
270 BM [78, 79]. Likewise, elevated levels of cytokines inducing megakaryocyte differentiation
271 indicates that the BM mounts a response to compensate reduced platelet counts in peripheral
272 blood. Analysis of a BM biopsy from a patient with chronic vivax malaria revealed
273 hyperplasia of myeloid and megakaryocytic cells [24], and a similar phenotype has been
274 described in *P. cynomolgi*-infected monkeys [74]. In the rodent malaria model *P. chabaudi* it
275 was demonstrated that IFN- γ signalling in hematopoietic progenitors induces myeloid-biased
276 differentiation and myeloid cell numbers, which appeared to be associated with parasite
277 clearance [85]. A similar mechanism was also observed in the *P. berghei* rodent malaria
278 model [86]. The analysis of *P. cynomolgi* infection in *Rhesus macaques* also demonstrated
279 transcriptomic changes in the BM including upregulation of *IFN- γ* and *IL-27*, as well as
280 pathways related to pathogen recognition, such as TLRs, NOD-like receptors and RIG-
281 1/MDA5 [74]. Of note, reticulocytes express parasite antigens via human leukocyte antigen
282 class I (HLA-1), which are recognized by antigen-specific CD8⁺ T cells, resulting in the
283 formation of immunological synapses and killing of the *P. vivax*-infected reticulocytes [87].

284 Collectively, these observations suggest that *P. vivax* antigens can be presented and
285 induce immune responses in the extravascular niches of the BM. Resident cells including
286 haematopoietic progenitor/stem cell (HPSCs) could adapt to these signals through
287 proliferation, mobilization from the BM and skewing toward the myeloid lineage, at the
288 expense of lymphopoiesis [82-84] (Figure 3, Key Figure). However, this infection-induced
289 adaptation toward enhanced myelopoiesis might also perpetuate inflammation in chronic or
290 repeated infections by generating a feed-forward loop between myeloid-biased HSPCs and
291 the inflammatory response. Indeed, chronic HSPC activation by infection and/or
292 inflammatory stimuli causes impairment of function and exhaustion, alters global patterns of
293 gene expression skewing hematopoietic potential and further perpetuates inflammation,
294 which leads to BM remodelling and potentially myelodysplastic syndromes [84]. It will be
295 important to investigate acute and long-term impacts of *P. vivax* infection in the BM, in
296 particular in patients continuously exposed to the parasite.

297

298 **Concluding remarks**

299 Recent studies have demonstrated that the haematopoietic niche represents a major reservoir
300 for *P. vivax* that is subject to significant changes during infection. These observations raise a
301 series of questions with regards to parasite biology (see Outstanding questions). In addition,
302 the host-parasite interactions established in the different reservoirs and their clinical
303 implications represent key knowledge gaps. Because *P. vivax* develops/accumulates in the
304 BM parenchyma, its antigens and parasite-induced cytokines can potentially be sensed by
305 HSPCs, MSCs, ECs and mature immune cells in the BM parenchyma and shape its function.
306 This raises questions about the underlying host-parasite interactions in hematopoietic stem
307 cell niche environments (see Outstanding questions). Understanding the acute and long-term
308 effects of the hidden parasite biomass in haematopoietic reservoirs is relevant to the study of
309 acute and chronic *P. vivax* infection. In addition, NHP models and human cohort studies
310 (autopsies, BM and/or spleen aspirates – see Box 1) will be of great value to further evaluate
311 the importance of the haematopoietic reservoirs for *P. vivax* survival, recurrence,
312 transmission and pathogenesis.

313

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520

521

522 **Box 1: BM aspiration in the clinical routine**

523
524 BM biopsy is performed routinely as part of the clinical management of malaria patients with
525 anaemia ($Hb < 7g/dl$) to exclude other aetiologies, such as erythroid hyperplasia. The current
526 knowledge gaps reported in this review are due, in part, to the fact that BM biopsy is often
527 perceived as being associated with unnecessary risks for the patients and is therefore seldom
528 performed on conscious individuals with mild illness. A study conducted in 2001-2003 and
529 surveying about 20,000 BM aspiration/biopsy procedures across 63 hospitals reported only
530 sixteen adverse events, representing 0.08% of total reported procedures and suggesting that
531 risks are, in fact, minimal [88]. Larger studies with serial BM biopsies and/or aspirations
532 from patients infected with *P. vivax* (pre/post treatment, for example) should therefore be
533 considered in the future to shed more light on the BM “ecosystem” in vivax malaria.

534

535 **Figure 1: *P. vivax* tissue distribution in non-human primates (NHPs).** (A) Representative
536 images obtained by Obaldia et al. [32] of parasites in the immunohistochemistry (IHC)
537 analysis of bone marrow, liver, lung and brain. pLDH (total), PvLAP5 (gametocytes), and
538 PvAMA1 (schizonts) antibodies were used to detect stage-specific parasites; CD31
539 antibodies stained the endothelium. Black arrowheads mark parasites. (B) Representative
540 images obtained by Peterson et al. [34] of H&E-stained sections of bone marrow from a
541 splenectomized animal and the spleen from the intact animal indicating the distribution of
542 parasites (black arrows). (C-E) Heatmaps representing total, schizonts or gametocytes
543 distributions in similar organs analyzed in 3 different studies: (C) Freemont et al. [33], (D)
544 Obaldia et al. [32] and (E) Peterson et al. [34].

545

546 **Figure 2: Comparison blood- and sporozoite-inoculation on experimental *P. vivax***
547 **infection dynamics.** (A) Average parasitaemia curves of blood- (red line) and sporozoite-
548 inoculated (blue line) *P. vivax* infections (St. Elizabeth strain) are highly similar for the first
549 1-2 months (solid lines) before they start to diverge, partially driven by relapses in
550 sporozoite-inoculated individuals ($N_{blood}=92$, $N_{sporozoite}=88$). (B) Individual infection
551 timeseries of *P. vivax* (St. Elizabeth strain) infected individuals, illustrating recurrent
552 parasitaemias even in blood-inoculated infections (patient numbers S12 and S273, top and
553 middle graph), especially following sub-curative drug treatment (arrows), and liver relapse
554 following absence of peripheral parasites for ~ 2 weeks (patient number S484, bottom graph).
555 Data courtesy of G. M. Jeffery and W. E. Collins.

556

557 **Figure 3, Key Figure: Potential *P. vivax*-induced immune responses in the bone marrow.**
558 Resident bone marrow cells, including hematopoietic stem cells (HSCs), multi-potent
559 progenitors (MPPs) and endothelial cells express pathogen-recognition receptors (PRRs),
560 such as Toll-like receptors (TLRs). This allows them to directly sense parasite-derived
561 products presented by local antigen-presenting cells (APCs) or even infected immature
562 reticulocytes, which still retain the capacity to present antigens via human leukocyte antigen
563 class I. This would stimulate the release of cytokines such as IL-6 and G-CSF, which along
564 with other cytokines that are produced during the course of infection, such as IL-1, IFNs and
565 M-CSF, could act directly in the BM cells. This would promote HSC proliferation, myeloid-
566 biased differentiation and also act in the granulocyte-monocyte progenitors (GMPs) and
567 promote generation of myeloid cells.