elevation of miR-486 expression in CD34$^+$ cells. Unlike in ML-DS, wherein GATA1s can directly activate miR-486, modulation of BCR-ABL activity with either expression of a kinase-inactive BCR-ABL mutant or treatment with a tyrosine kinase inhibitor only partially restored miR-486 expression, suggesting that miR-486 may be regulated by BCR-ABL kinase-dependent and kinase-independent mechanisms in CML. Because malignant hematopoietic stem cells in chronic-phase CML maintain self-renewal and multilineage potential with clonally expanded granulocytic, megakaryocytic, and erythroid progenitor compartments,[6] the authors dissected miR-486 expression in the hematopoietic stem/progenitor populations of CML patient samples and found it most highly expressed in the megakaryocyte-erythroid progenitor (MEP) fraction.

Is there a role for miR-486 in hematopoiesis? Because GATA1s regulates megakaryocytic-erythroid differentiation, Shaham et al measured miR-486 steady-state levels in primary murine hematopoietic cells but found detectable expression only in the erythrocytes from both wild-type and Gata1s knockin mice. Underscoring this finding, miR-486 expression was robustly induced when human GATA1- and GATA1s-overexpressing MEP-like cell lines were differentiated to the erythroid lineage with cytokines. In agreement with this, Wang et al found that miR-486 expression is significantly induced upon erythroid, but not myeloid, cytokine-stimulated differentiation of human CD34$^+$ cells in vitro. Exogenous overexpression of miR-486 in human CD34$^+$ cells significantly enhances cytokine-stimulated erythroid differentiation in vitro. Conversely, knockdown of miR-486 in human CD34$^+$ cells significantly reduced cytokine-induced erythroid differentiation in vitro and significantly diminished the frequency of CD235$^+$ erythroid populations in mouse xenografts in vivo. Collectively, the results of these 2 independent studies solidify a role for miR-486 in erythroid differentiation, a phenomenon they show is conserved from mice to humans.

In building upon this new link between miR-486 and erythroid differentiation, both groups reveal implications that extend beyond normal hematopoiesis. Expression of miR-486 is tightly associated with expression of the erythroid marker glycophorin A (*GYPA* or CD235) in ML-DS patient blasts and BCR-ABL–expressing human CD34$^+$ cells. In ML-DS, Shaham et al find that knockdown of miR-486 significantly reduces the expression of *GYPA*, shifting ML-DS cells from a CD235$^+$CD61$^+$ erythromegakaryocytic immunophenotype to a CD235$^-$CD61$^+$ megakaryocytic phenotype. Although ML-DS cells exhibit slowed growth kinetics and undergo a significant increase in apoptosis upon miR-486 knockdown, the amount of cell death is not likely to account for the dramatic shift in CD235 expression. Wang et al knocked down miR-486 in an erythroleukemia cell line and in human CD34$^+$ cells expressing BCR-ABL, which caused a significant induction in cell death that was further enhanced with imatinib treatment. This was concomitant with decreased *GYPA* expression. Importantly, the authors show that knockdown of miR-486 does not cause cell death of normal human CD34$^+$ cells. The overall survival (OS) of patients with newly diagnosed chronic-phase CML is significantly increased by treatment with tyrosine kinase inhibitors such as imatinib. However, ~20% of patients are resistant or become treatment refractory. CML patients acquire additional mutations or epigenetic alterations causing the transition into the blast-crisis leukemia phase.[7] Children with ML-DS treated with standard low-dose chemotherapy do somewhat better, with a 3-year OS of 80%.[8] Data from these 2 reports strongly suggest that antagonism of miR-486 may work best as a combination therapy with the current standard of care.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● RED CELLS, IRON, & ERYTHROPOIESIS

Comment on Malleret et al, page 1314

Bone marrow reticulocytes: a *Plasmodium vivax* affair?

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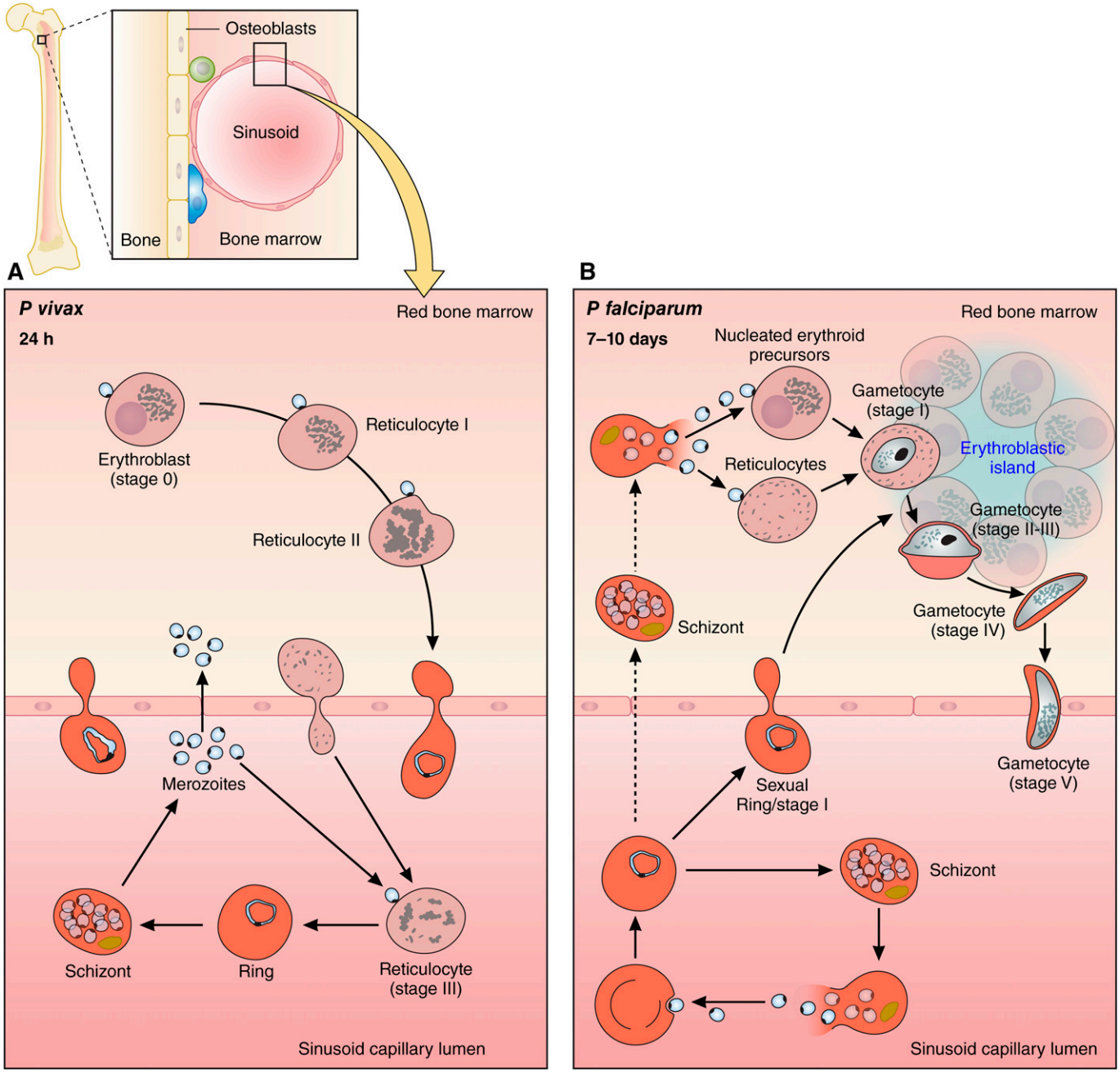
In this issue of *Blood*, Malleret and colleagues show the importance of the bone marrow in *Plasmodium vivax* biology by proving the preferential infection of young reticulocytes (generally restricted to the bone marrow), which then experience accelerated maturation postinvasion.[1]

Plasmodium vivax mainly invades reticulocytes, a heterogeneous population of red blood cell precursors characterized by a reticular network of residual RNA, whose maturation is indicated by the decreasing expression of the transferrin receptor CD71. This host cell specificity shapes *P vivax* pathobiology, and the strict requirement for reticulocytes has hampered the establishment of an in vitro culture system for this parasite. By sorting different developmental stages of cord blood reticulocytes for use in an ex vivo invasion assay, Malleret and colleagues show that *P vivax* merozoites prefer the youngest of the young erythrocytes. The immature CD71$^+$ reticulocytes, generally restricted to bone marrow, were more efficiently invaded than older CD71$^-$ reticulocytes principally



Proposed model for *P vivax* development in the bone marrow. (A) *P vivax* infects stage III reticulocytes that have egressed by diapedesis to the sinusoidal capillary lumen. Alternatively, *P vivax* merozoites or *P vivax*–infected erythrocytes might enter the red bone marrow compartment, leading to invasion of $CD71^+$ reticulocytes. Accelerated reticulocyte aging increases host cell deformability for subsequent endothelial crossing toward blood circulation. Adapted from Figure 5 in the article by Malleret et al that begins on page 1314. (B) Proposed model for *P falciparum* development in the bone marrow. Presence of immature gametocytes in the bone marrow extravascular space may be explained by erythrocytes infected by stage I gametocytes or sexually committed ring stages entering the bone marrow stroma through the endothelial lining. Alternatively, asexual schizonts, which develop in the extravascular compartment, may produce sexually committed merozoites that invade erythroid precursors, whose remodeling may share possible similarities with that of *P vivax*. Mature gametocyte-infected host cells cross the endothelial barrier to reenter the circulation. Adapted from supplemental Figure 7 in Joice et al.[7] Professional illustration by Patrick Lane, ScEYEnce Studios.

found in peripheral blood. This finding was puzzling, because *P vivax* ring-stage parasites from patients were instead predominantly found in $CD71^-$ reticulocytes. Analyzing the first steps of *P vivax* invasion of $CD71^+$ reticulocytes, Malleret and colleagues revealed a remarkably rapid remodeling of *P vivax*–invaded reticulocytes, leading to increased deformability, accelerated loss of CD71, and replacement of clathrin pits by distinctive caveolae nanostructures.

The narrow tropism toward $CD71^+$ immature reticulocytes, produced and largely residing in the bone marrow,[2] raises the intriguing possibility that *P vivax* invasion may mainly take place in this organ rather than in the circulating blood. The authors accordingly suggest that increased deformability associated with the accelerated reticulocyte aging might contribute to the trafficking of mature parasites into this organ and the egress of infected reticulocytes into the peripheral blood system. As pointed out in the article, the accumulation

of *P vivax* infections in the bone marrow extravascular spaces may explain early observations of patients scored negative for *P vivax* in peripheral blood but positive in bone marrow biopsy specimens.[3] Several case reports described recurrences of *P vivax*, as well as *Plasmodium falciparum*, after bone marrow transplants.[4] Together with the evidence provided by Malleret and colleagues, these observations suggest that bone marrow can be a site where *P vivax* parasites may hide and develop.

Recent reports have placed the host-parasite interplay in the bone marrow at the center of pathophysiology and transmissibility of the other main human parasite *P falciparum* by demonstrating the enrichment of late parasite stages and immature gametocytes in this organ, with the latter readily found in the bone marrow extravascular compartment.[5-7] The current study shows the potential of the bone marrow as a niche where *P vivax* invasion can occur before reentering the circulation (see figure). Intriguing similarities may deserve further attention. Most of the erythroid precursors containing *P falciparum* immature gametocytes in the marrow stroma in autopsy specimens were $CD71^-$,[7] suggesting a similar parasite-mediated acceleration of host cell differentiation. Moreover, the rapid increase in deformability of the *P vivax*–infected immature reticulocytes, proposed to facilitate migration through the bone marrow sinusoidal lining, parallels the analogous reduction in the rigidity of erythrocytes infected with *P falciparum* mature sexual stages, which may help their release from the bone marrow sequestration sites to peripheral circulation.[8]

The study by Malleret and colleagues raises several questions with far-reaching implications for *P vivax* biology that may have clinical impact. First, what is the role of bone marrow as a reservoir of *P vivax* parasites and what are the implications for strategies to eliminate malaria? The bone marrow is emerging as a site where both *P vivax* and *P falciparum* can hide and sustain infection and transmission, posing new challenges for malaria elimination initiatives directed at identifying and treating all *Plasmodium* infections. Second, through which mechanisms do infected reticulocytes transverse the bone marrow sinusoidal capillaries to the primary hematopoietic sinus? The authors argue that only young reticulocytes that have migrated out of the red bone marrow would be available for *P vivax* invasion, unless invasion principally occurs in the extravascular space of the red bone marrow. The latter scenario would imply a 2-way journey of *P vivax* merozoites and freshly invaded reticulocytes through the bone marrow sinusoidal capillaries, similarly to what has been proposed for *P falciparum*.[7] Third, to what extent are anemia or inflammatory reactions—common during *P vivax* infection—triggered by the presence of malaria parasites in the bone marrow, and might they disrupt the sinusoidal lining, permitting cellular transit? A relationship has been suggested between hematologic disturbances and *P falciparum* development in bone marrow,[5] which raises further questions on how this interplay in bone marrow is modified in the 2 parasite species after clinical malaria ceases in asymptomatic infected individuals, because these will stand as the last, most challenging parasite reservoirs to be attacked to achieve malaria eradication. Fourth, how does *P vivax* accelerate reticulocyte aging? Do loss of the CD71 clathrin pits and the formation of microvesicles contribute to the rapid host cell remodeling, and are there common mechanisms to affect deformability of reticulocytes by *P vivax* and of erythrocytes by *P falciparum* gametocytes?[8] Finally, do parasites in the bone marrow reach some degree of refractoriness to treatment, as shown for *Salmonella typhi*,[9] malignant hematopoietic cells, and epithelial tumor cells that metastasize to bone?[10] Investigating these topics will allow us to begin to dissect the role of bone marrow in the *P vivax* biology and the manifestation of disease.

Bone marrow, which accounts for approximately 5% of the body weight in humans, generates all hematopoietic cells circulating in peripheral blood. Although functional alterations of the bone marrow under pathogen attacks are largely unknown, it is tempting to speculate that even small changes in this delicate environment may lead to significant modification in the cellular constituents in peripheral blood and tissues. An important role for bone marrow is emerging in the pathobiology of *P falciparum* malaria. Malleret and colleagues expand a possibly similar role in *P vivax*, requiring the assessment of how this organ contributes to the parasite biomass and the pathogenesis in this parasite. As for *P falciparum*,[7] investigations of postmortem samples from *P vivax* malaria cases would be of great value.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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