During the last 2 decades, there has been substantial progress in identifying key cellular and molecular players in crosstalk between normal and malignant hematopoietic cells and the marrow microenvironment.[4] At least 2 hematopoietic stem cell (HSC) bone marrow niches have been described: one endosteal and one perivascular (see figure). Specialized osteoblasts lining the bone surface form endosteal niches. On the other hand, sinusoidal endothelial cells and a small population of perivascular reticular cells with long processes, expressing high amounts of CXCL12 (called CXCL12-abundant reticular or CAR cells), create vascular niches, which are scattered throughout the marrow cavity. Earlier groundbreaking in vivo studies from the same group revealed that malignant B cells not only interact with stromal cells and molecules that are described within HSC niches but specifically invade, exploit, and disrupt HSC niches during malignant progression, thereby negatively affecting benign hematopoiesis.[5,6]

How can we stop leukemia cells from parasitizing in these niches and give them a taste of their own medicine? CXCR4, a chemokine receptor that tethers hematopoietic cells to CXCL12-secreting stromal cells, is the first therapeutic target for disrupting crosstalk between leukemia cells and the marrow microenvironment.[7] Many clinical trials use CXCR4 antagonists (eg, plerixafor/AMD3100) for "chemosensitization" when given in combination with cytotoxic agents. These studies are either ongoing or are recently finished, and a first trial in acute myeloid leukemia has reported encouraging results.[8] Targeting OPN in ALL, as introduced in this study,[1] follows a similar concept: OPN neutralization releases ALL cells from their dormant state in the endosteal niches and makes them better accessible and receptive toward cytotoxic drugs that target cycling cells. As such, this study is in line with an ongoing important paradigm shift in cancer therapy, moving from cancer cells as the primary target of therapy toward new treatments that also interfere with protective tumor-microenvironment interactions.[9] It is also tempting to think about analogies between OPN and "The Sleeping Beauty": ALL cells are doomed to sleep in endosteal niches by OPN, but once this curse is lifted, life in the marrow awakens. The encouraging data about OPN neutralization in ALL in this study[1] stir hope that, unlike in "The Sleeping Beauty", it will take a much shorter time than 100 years until the reversion of leukemia cell dormancy in ALL can be clinically tested.

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● ● ● MYELOID NEOPLASIA

Comment on Gandre-Babbe et al, page 4925

JMML patient-derived iPSCs induce new hypotheses

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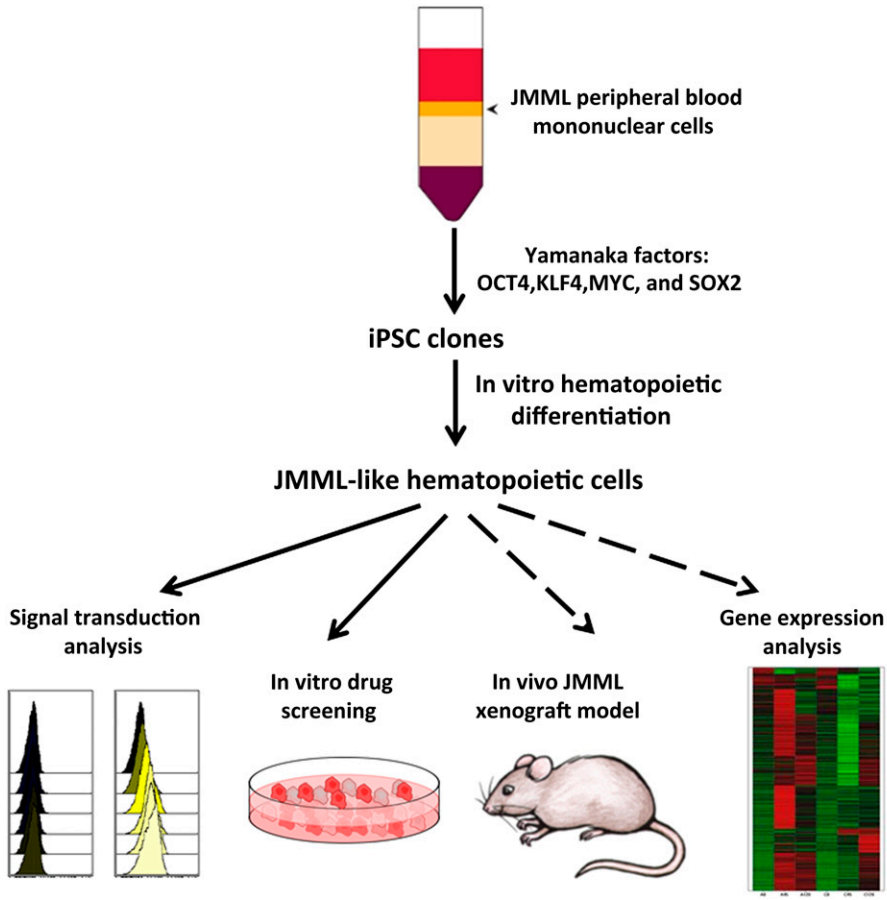
In this issue of *Blood*, Gandre-Babbe et al have, in part, overcome the obstacle of validating the molecular underpinnings of juvenile myelomonocytic leukemia (JMML) with the generation of induced pluripotent stem cells (iPSCs) from individuals with JMML.[1]

Validation of the molecular underpinnings of JMML derived from mouse models,[2] has been limited in humans due to a scarcity of primary clinical samples. JMML is a rare childhood leukemia that has nonetheless played a central and highly informative role in clarifying the consequences of Ras hyperactivation in human malignancies as well as in human congenital disorders.[3] JMML also continues to be the recipient of intense investigation because its only curative therapy is allogeneic hematopoietic stem cell (HSC) transplantation, and yet 50% of children will succumb to leukemia relapse following this arduous therapy.[3]

JMML is renowned for displaying features of a disease based on well-defined and unambiguous genetic mutations, since most children diagnosed with JMML bear nonoverlapping loss-of-function mutations in *NF1* or *CBL* or gain-of-function mutations in *NRAS*, *KRAS*, or *PTPN11*. Each of these mutations yields RAS hyperactivation with a net output of excessive signaling through RAS effector pathways, including the canonical RAF-MEK-ERK and PI3K-AKT pathways. Functionally, the hallmark of hematopoietic progenitors from individuals with JMML is cytokine-independent growth in vitro and exquisite sensitivity to the growth factor granulocyte macrophage colony-stimulating factor (GM-CSF).[4]

The advent of iPSC technology has provided an opportunity to generate a renewable source of patient-derived reagents that may potentially produce authentic models of JMML in vitro and in vivo for deeper molecular analysis, straightforward and efficient screening of novel pharmacologic



Peripheral blood or bone marrow cells from patients with JMML were infected with a lentiviral vector expressing an inducible construct of all four Yamanaka factors. Induced iPSC clones were identified, and selected clones were differentiated into hematopoietic cells by using established protocols. Cells with JMML-like phenotypic and functional qualities were detected through several assays, including signal transduction analysis and responsiveness to growth factors. These cells were responsive to chemicals that inhibit certain signaling pathways. In future studies, these JMML-like cells can be tested for in vivo engraftment in immunodeficient mouse models and for more detailed molecular analysis.

agents, and refined genotype-dependent treatment recommendations (see figure). Thus, Gandre-Babbe et al[1] have successfully generated iPSC clones from two individual JMML patients bearing the somatic *PTPN11* mutation p.E76K (see figure). Multiple iPSC clones from each patient sample yielded increased myeloid cell ($CD45^+CD18^+$) production upon in vitro hematopoietic differentiation and produced increased myeloid colonies compared with wild-type controls. Consistent with the well-documented phenotype of primary JMML samples, the JMML iPSC-derived hematopoietic cells demonstrated cytokine-independent colony growth, hypersensitivity to GM-CSF, and signal transducer and activator of transcription 5 (STAT5) hyperphosphorylation in response to low GM-CSF concentrations. Initial pharmacologic studies demonstrated sensitivity of the JMML iPSC-derived hematopoietic cells to the MEK inhibitor PD0325901 (see figure), consistent with previous studies performed in loss-of-function *Nf1* and gain-of-function *Kras* murine models.[5] Collectively, these phenotypic qualities support the premise that these JMML iPSC clones can be used to generate hematopoietic progenitors that closely simulate primary JMML samples.

Although this work offers an exciting new tool in the armamentarium of JMML reagents, it also highlights unresolved questions in the field. First, it is notable that both published iPSC clones are derived from JMML samples harboring the *PTPN11* mutation p.E76K. JMML patients with *PTPN11* mutations are reported to have a significantly lower overall survival compared with patients with wild-type *PTPN11*. Furthermore, among *PTPN11* mutations, the p.E76K mutation produces a protein with the highest known basal and unregulated tyrosine phosphatase activity among *PTPN11* oncogenic protein products. In contrast, anecdotes of mild and spontaneously resolving disease have been reported for JMML patients with somatic *NRAS* and *KRAS* mutations.[3] Therefore, one wonders if the successful origination of JMML-derived iPSC clones is unique to mutations bearing the strongest oncogenic activity, or if this technology will be able to be applied more broadly to samples carrying less deleterious mutations.

Second, generation of mutant hematopoietic progenitors with JMML-like features from differentiated iPSCs raises an intriguing question about the embryonic origin of the disease. Although the stem cell theory of hematopoiesis predicts that all blood cell lineages are derived via a stem cell precursor, recent information in the developing mouse suggests that erythromyeloid progenitor cells and B-1–cell and T-cell subsets arise during development prior to HSC emergence.[6] Furthermore, some long-lived resident macrophage populations in the adult murine liver, skin, and brain are derived from embryonic macrophage precursors and are maintained life-long, seemingly independent of HSC contributions.[7,8] Given the ongoing lack of proof for the generation of HSCs from differentiated human iPSCs[9] the production of mutant hyperproliferative JMML-like myeloid progenitor cells by Gandre-Babbe et al[1] suggests that these cells may have arisen from a non-HSC hemogenic endothelial-derived erythromyeloid progenitor cells. This is an intriguing hypothesis that serves as a new paradigm for understanding the ultimate origin of JMML disease in human patients. Since essentially half of the patients treated with allogeneic stem cell transplantation for JMML relapse, one wonders if the stem cells for this disease reside in tissue-resident monocyte-macrophage precursors and not in traditional HSC-derived myeloid progenitor cells. Essentially nothing is known about the location, composition, or function of the tissue macrophage niches that may exist. If some of the long-lived replenishing macrophage precursors are deeply quiescent, total body irradiation and/or chemotherapy may not penetrate and eliminate these cells.

Evidence for long-lived tissue-resident macrophage progenitor cells that are resistant to total body irradiation has been reported.[10] Development of methods to engraft the human iPSC-derived JMML-like cells in an optimized immunodeficient mouse model system (see figure) may assist in examining some of these exciting new questions.

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

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● ● ● PLATELETS & THROMBOPOIESIS

Comment on Morowski et al, page 4938

How low can you go?

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In this issue of *Blood*, Morowski et al use a mouse model of thrombocytopenia to determine the lowest platelet count needed to support thrombosis in a range of thrombosis challenges.[1]

How many platelets are needed for hemostasis? Are low platelet counts protective from thrombosis? Why do immune thrombocytopenia (ITP) patients have myocardial infarctions (MIs) and strokes? Why do some patients bleed and others do not at comparably low platelet counts? As anyone who has formulated guidelines or is in clinical practice knows, there is considerable empiricism but little actual data available to answer these questions.

The answer: relatively few platelets are necessary for thrombosis. The authors asked the seemingly simple question of how low can the platelet count fall in a mouse before interfering with common models of thrombosis. Thrombosis of small, medium, and large vessels remained intact at platelet counts down to 10% to 30% of normal. Mice ultimately need <2.5% of their normal platelet number to maintain the most basic level of hemostasis.

The authors used antibody-mediated clearance of platelets from circulation to induce thrombocytopenia. The anti-GPIb antibody they used binds specifically to platelets and induces their clearance through FcR-mediated uptake. They calibrated the dose of antibody needed to decrease the platelet count by a specific percentage and then induced different degrees of thrombocytopenia for the thrombosis challenges.

The models of thrombosis spanned the size and flow spectrum, including aortic and carotid artery injury, a small arteriole ischemia-reperfusion model of stroke, and the very low-flow tail-snip bleeding time. Aortic thrombosis remained intact at platelet counts down to 30% of normal. As the vessels tested became smaller, the platelet count needed for thrombosis decreased. The carotid artery injury model continued to thrombose normally down to a platelet count of 20% normal. A model of ischemia-reperfusion stroke injury that is known to be platelet dependent continued to thrombose normally down to platelet counts of only 10% of normal. Finally, the tail-snip bleeding time, roughly equivalent to the human bleeding time, remained normal until platelet counts fell to >97% below normal.

Although there are numerous factors contributing to thrombosis, it is notable that as shear stress increased from aorta to carotid to arteriole, the efficacy of the platelets to form thrombi increased as well (ie, fewer platelets or lower platelet density was required). In the rheological model of blood vessel flow, laminar flow with high shear rate pushes platelets into a marginal zone next to the endothelium, whereas red cells populate the center of the vessel.[2] In the current study, higher shear flow appeared to correlate with more efficient thrombus formation. Factors in addition to the platelets themselves appeared to play a larger role in the bigger arteries. Thus, the "flavor" of the thrombus may change depending on the size and flow characteristics of the blood vessel. These phenomena could potentially be tested in some of the newly developed ex vivo flow systems using endothelialized microfluidics devices.

Another interesting phenomenon was the appearance of a population of young platelets after destruction of the majority of circulating platelets. The authors showed that this population did not alter the overall platelet function. However, if at low platelet counts platelet function trumps number (as Karpatkin showed many years ago),[3] these young platelets may play a disproportionate role in maintaining hemostasis. An important factor that may impact this model is the likely large release of highly thrombogenic platelet microparticles on immune platelet destruction.[4] The authors did use a second immune model but did not explore thrombocytopenia induced by other means, ie, hypoplasia, in which the level of microparticles would be expected to be far less. These issues become important when comparing bleeding and clotting tendencies in ITP patients, who have elevated numbers of young platelets (and microparticles), with chemotherapy patients, who do not. At least 1 study supports the notion that ITP patients bleed less than chemotherapy patients with the same platelet counts presumably because of the increased function of young platelets.