

iPSCs from CD34<sup>+</sup> cells obtained from healthy controls and MPD patients carrying the JAK2-V617F mutation. While MPD-derived iPSCs retained the JAK2-V617F mutation, they had a normal karyotype, embryonic stem cell–like phenotype, and pluripotent differentiation potential. When control and diseased iPSCs were differentiated back into CD34<sup>+</sup>CD45<sup>+</sup> hematopoietic progenitors, the progenitors derived from MPD-iPSCs recapitulated the features of somatic CD34<sup>+</sup> cells from which the iPSCs were originally derived. Similar to somatic MPD CD34<sup>+</sup> cells, iPSC-derived CD34<sup>+</sup>CD45<sup>+</sup> cells demonstrated enhanced erythropoiesis and up-regulation of genes known to be increased in PV.

This study clearly demonstrates how iPSC technology could be used to model acquired blood diseases. This technology would be of particular value for the study of blood disorders such as myelodysplastic syndromes, paroxysmal nocturnal hemoglobinuria, and others for which animal models are not available or difficult to create. In addition, iPSCs carrying leukemia-specific cytogenetic translocation could be used to analyze how cancer stem cells develop. Importantly, the iPSC-based approach would be helpful in addressing the role of genetic background in manifestation of neoplastic blood disorders. Because iPSCs are capable of indefinite self-renewal, diseased blood cells can be generated continuously in the laboratory, eliminating the need for a constant supply of hematopoietic progenitors from the patients. In particular, a continuous supply of genetically diverse diseased blood cells for drug screening and discovery could be created. Because multiple types of cells can be generated from iPSCs, interaction of diseased blood cells with endothelial or stromal cells could be modeled *in vitro*. However, several important issues related to iPSC models of blood diseases remain to be addressed. It is known that the hematopoietic differentiation potential of iPSC lines generated from the same starting material varies significantly.<sup>8</sup> If several clones were generated from iPSCs, which clones should be selected to make an appropriate conclusion regarding differences in differentiation potential? What would be an appropriate control for diseased versus non-diseased iPSCs? For studies of acquired blood diseases, iPSC lines can be generated from hematopoietic cells and fibroblasts or bone marrow mesenchymal stem cells (see figure). In this way, iPSCs with the same genetic back-

ground, but different in terms of presence or absence of acquired mutations, will be available for comparative analysis. The majority of disease-specific iPSCs have been made using retroviral vectors. Although the impact of exogenous expression is unclear, the possibility remains that retroviral integration and background expression of pluripotency genes may affect the behavior of iPSC-derived hematopoietic progenitors. Recently developed new reprogramming methods allowing for the generation of transgene-free iPSCs will be helpful to overcome this limitation.

*Conflict-of-interest disclosure:* The author declares no competing financial interests. ■

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## CLINICAL TRIALS

Comment on Pagel et al, page 5444

# Increasing the punch of reduced-intensity transplants

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In this issue of *Blood*, Pagel and colleagues describe their experience in the pioneering use of a RIT-enhanced reduced-intensity preparative regimen for hematopoietic cell transplantation for older patients with advanced AML.

**L**ike many malignant blood disorders, acute myeloid leukemia (AML) continues to be a highly lethal disease, striking with no apparent logic, but especially affecting the aging person. In fact, the incidence of AML increases 20-fold from the decades of 20 to 30 through 70 to 80 years. Most patients with AML are more than 60 years old. This is the age now found by many clinical trials as the upper age barrier to successful therapy. Patients over the age of 60 years do especially poorly with long-term survival (not necessarily adjusted for age and actuarial life expectancies), usually quoted in the 5% to 10% range. Hematopoietic stem cell transplantation has been shown to increase the odds of survival for younger patients, but its application to the older adult has been slow and tentative.<sup>1</sup>

The most significant recent change in the field of hematopoietic cell transplantation is the use of reduced-intensity conditioning regimens, especially designed for older patients or

those with significant medical comorbidities.<sup>2</sup> Because most patients with transplantable illnesses are “older” adults, this is quite important, as it has permitted the use of hematopoietic stem cell transplantation (HSCT) in patients who heretofore were considered “too old.” Yet, the trade-off for a less intense preparative regimen, although well tolerated, has been a higher relapse rate. The study by Pagel et al may light the way toward better outcomes.<sup>3</sup> They demonstrate in a phase 1 study a novel method to intensify the preparative regimen without increasing toxicity.

In this phase 1 clinical trial, they used an <sup>131</sup>I-coupled Bi-anti-CD45 antibody (BCA) administered along with a fludarabine and low-dose total body radiation (TBI) preparative regimen. They studied 58 patients with advanced AML or high-risk myelodysplastic syndrome, most of whom had persistent malignancy at the time of transplantation. Remarkably, all patients achieved a complete

response and had 100% donor-derived CD3- and CD33-positive cells in the blood by day 28. Seven of the 58 patients died of non-relapse-related causes by day 100. The estimated probability of recurrent AML was 40% at 1 year, and the 1-year survival rate estimate was 41%.

The use of reduced-intensity preparative regimens is now widely accepted and used for older and infirm patients.<sup>4-6</sup> The Seattle group has been in the vanguard of the development of reduced-intensity transplantation preparative regimens.<sup>7</sup> It is notable that the patients treated by Pagel et al were deemed ineligible for their standard reduced-intensity preparative regimen of fludarabine and TBI alone. The survival rate of 41% is quite remarkable, given that background. Toxicity to extramedullary organs was low, and treatment-related mortality was reasonable and low.

Targeting CD45+ hematopoietic cells is not the only way to target the marrow space with additional radiation. Other hematopoietic antigens can also be targeted.<sup>8</sup> Alternative methods include the use of external beam radiation using intensity-modulated radiotherapy techniques<sup>9</sup> and bone-seeking radionuclide therapy.<sup>10</sup> These approaches are promising but have not been studied in substantial numbers of patients. The advantage of these alternative approaches is their exportability, and potentially greater reliability, with higher dosing to the marrow compared with antibody-delivered therapy. However, there may be possible advantages to protocols combining radioimmunotherapy (RIT) and external beam therapy. RIT has the advantage of targeting extramedullary disease as well as medullary disease.

AML in the older patient is a pressing medical problem. Chemotherapy alone results in few cures. Approaches to augmenting the efficacy of hematopoietic cell transplantation without unduly increasing toxicity are urgently needed. Reducing the intensity of therapy to uninvolved organs while boosting treatments to the sites of disease is a promising approach to achieving more cures for patients with this lethal disease.

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

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## ● ● ● MYELOID NEOPLASIA

Comment on Luesink et al, page 5512

# CCL2/CCR2: push/pull for migration

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In this issue of *Blood*, Luesink and colleagues report that in APL, the induction of massive production CC-chemokines (CCLs) and their receptors (CCRs) in APL cells by differentiating therapy with ATRA or ATO may play an important role in the development of the DS,<sup>1</sup> formerly known as retinoic acid syndrome.<sup>2</sup>

**T**he full-blown differentiation syndrome (DS) is characterized by unexplained fever, weight gain, dyspnea with interstitial pulmonary infiltrates, pleural or pericardial effusions, hypotension, and acute renal failure.<sup>2</sup> However, the pathophysiology of DS is not fully understood, and detailed knowledge about its molecular mechanism remains largely unknown. Previously, it has been observed that differentiation therapy in acute promyelocytic leukemia (APL) (1) increases the release of inflammatory cytokines from differentiating APL cells,<sup>3</sup> (2) increases the expression of cellular adhesion molecules on leukocytes,<sup>4</sup> and (3) up-regulates specific chemokines.<sup>5</sup> The simultaneous production of these proteins after exposure to all-trans retinoic acid (ATRA) may exacerbate the hyperinflammation observed in DS. The incidence of DS in patients receiving ATRA/arsenic trioxide (ATO) treatment has been reported to range from 2% to 27% with an associated mortality of about 2%.

The paper by Luesink et al in this issue of *Blood* presents in vitro evidence that chemokines may have a role in the development of DS.<sup>1</sup> Chemokines, together with their receptors, play a crucial role in directing the movement of mononuclear cells throughout the body, contributing to the pathogenesis of a variety of diseases.<sup>6</sup> These investigators, using ATRA-stimulated of NB4 cells, were able to induce mRNA expression of multiple CC-chemokines (CCLs) and their receptors (CCRs), resulting in increased chemokine production in supernatant of these cells and increased chemotaxis. Two of these chemokines (CCL2 and CCL24) were up-regulated early and, despite the addition of cycloheximide (a protein translation inhibitor), up-regulation of the mRNA expression was confirmed even though protein levels were not increased. This indicated that their induction was directly mediated by retinoic acid receptors. The addition of dexamethasone to ATRA did not inhibit chemokine induction in