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MYELOID NEOPLASIA

Comment on Berger et al, page 1846

Ups and downs of CHIP

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In this issue of *Blood*, Berger et al present a longitudinal study of the clonal trajectory of preleukemic mutations in patients who developed therapy-related myeloid neoplasms (t-MNs) after autologous stem-cell transplantation. The authors show that t-MN driver mutations are often detectable as clonal hematopoiesis of indeterminate potential (CHIP) many years before patients develop t-MNs, and they demonstrate complicated patterns of clonal expansion and evolution over time, leading to the development of t-MNs.[1]

t-MNs are one of the most devastating complications from cytotoxic chemotherapy and ionizing radiation therapy. Although overall incidence of t-MNs is <10% among patients with cancer, t-MNs often end with fatal outcomes.[2] When a patient develops a t-MN, we as oncologists struggle to share the bad news with the patient, who has already fought or been fighting the primary malignancy. It is hard to imagine how difficult it must be for the patient. Because current treatment modalities are ineffective in curing t-MNs, there is a real-world unmet need to predict and prevent t-MNs before their occurrence.

Recently, several studies identified that preleukemic mutations or chromosomal copy number alterations were detectable in the blood samples of patients with cancer before treatment.[3-7] Detection of preleukemic clonal hematopoiesis was associated with an increased risk of t-MNs. Similarly, preleukemic mutations were also detectable in autologous stem-cell apheresis samples, which was associated with significantly increased risk of t-MNs and non–t-MN–related mortality in patients with lymphoma.[8] The study by Berger et al, which accompanies this commentary, provides data on how these preleukemic mutations evolve over time, particularly in response to cytotoxic chemotherapies or hematopoietic growth factors.

The authors describe clonal kinetics of preleukemic mutations in 7 patients who developed t-MNs by analyzing multiple sequential blood or marrow samples taken before the t-MN development. Although the number of patients studied here was too small to derive meaningful patterns, the heterogeneous clinical courses of the 7 patients generated interesting questions about how clonal hematopoiesis behaves in response to external agents. In 1 patient, clonal expansion of *SMC1A* along with an increase in mean corpuscular volume followed treatment with danazole and erythropoietin. What do we know about clonal hematopoiesis and response to hematopoietic growth factors? In another patient, a *TP53*-mutated clone gradually increased while acquiring another *TP53* mutation during thalidomide and cyclophosphamide treatments. What is the response of clonal hematopoiesis to immunomodulatory imide drugs? In some patients, the authors observed clear expansion of preleukemic mutations under the selective pressure of chemotherapy, whereas there were small independent clones that remained stable. Why did some clones remain stable or disappear while others expanded under the pressure of chemotherapy? Ultimately, this leads to a more clinically relevant question: which clones have a high risk of developing into t-MNs, and which will remain stable for a long time? Longitudinal analyses of clonal hematopoiesis in a large number of patients as well as biological studies that address mechanisms of transformation from clonal hematopoiesis to t-MNs are needed to answer these questions.

There were several other findings that were noteworthy in the report by Berger et al. First, preleukemic mutations were also detectable in a T-cell fraction in 1 patient. Although this needs to be verified in larger cohort, it suggests that CHIP originates at early hematopoietic stem-cell or progenitor stages, which has been previously suggested by other studies.[9,10] Second, the authors concluded that lymphoma did not arise from CHIP, because the mutational landscape was completely different. Third, p53-overexpressed cells were detectable in marrow by immunohistochemistry in patients with *TP53*-mutated CHIP. Because mutations are detected "digitally," if these cells truly represent *TP53*-mutated CHIP, immunohistochemistry may help visualization of CHIP in marrow. Lastly, the authors found that t-MNs had higher numbers of mutations compared with de novo MDS but did not have a specific mutation signature; however, interpretation of these findings requires caution, because the data were derived from whole-exome sequencing, and they contradict previous findings.[7] Nonetheless, the study by Berger et al has helped advance our understanding of how CHIP behaves during chemotherapy and contributes to the development of t-MNs.

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TRANSPLANTATION

Comment on Hülsdünker et al, page 1858

Pathogenic neutrophils in acute GVHD

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In this issue of *Blood*, Hülsdünker et al show how recipient neutrophils contribute to the pathogenesis of acute graft-versus-host disease (GVHD), a complication of allogeneic hematopoietic cell transplantation (HCT) caused by donor T cells that recognize recipient alloantigens.[1]

Previous studies of GVHD have shown that many types of hematopoietic cells can present recipient alloantigens to donor T cells.[2] These cell types include dendritic cells, plasmacytoid dendritic cells, macrophages, B cells, and Langerhans cells. In addition, certain recipient cells outside the hematopoietic lineage can present alloantigens that cause GVHD. Neutrophils are known to present antigens to T cells under pathological or inflammatory conditions,[3] but Hülsdünker et al are the first to investigate their unexpected role in the pathogenesis of acute GVHD.

As illustrated in the figure, they showed that intestinal bacterial preferentially invaded the ileal mucosa after damage caused by total body irradiation. As part of the inflammatory response to bacteria, neutrophils infiltrated the ileal mucosa and were activated to express MHC class II molecules. In the ileum, neutrophils expressing a transgenic photoconversion reporter were labeled by light exposure, and their subsequent migration was traced to draining mesenteric lymph nodes. In lymph nodes, the activated neutrophils presented alloantigen to donor T cells that contributed to the development of acute GVHD. Consistent with this hypothesis, antibody-mediated depletion of neutrophils in the recipient on the day before HCT decreased the severity of acute GVHD.

Based on the rationale that granulocyte colony-stimulating factor (G-CSF) signals through Janus kinase 1 (JAK1) to stimulate neutrophil differentiation, Hülsdünker et al extended their study to evaluate the effects of the JAK1/2 inhibitor ruxolitinib on antigen presentation by neutrophils after HCT. Ruxolitinib inhibited the activation-induced expression of MHC class II molecules by neutrophils and prevented their migration from the ileum to mesenteric lymph nodes. Ironically, neutrophils are induced to express MHC class II molecules by interferon γ (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-3 (IL-3), but not by G-CSF,[3] and while IFN-γ signals through JAK1, GM-CSF and IL-3 signal through JAK2.

Drug-based approaches for controlling GVHD after allogeneic HCT in humans have focused primarily on inhibition of T-cell responses with the use of antimetabolites such as methotrexate or mycophenolate mofetil, calcineurin inhibitors such as cyclosporine and tacrolimus, mechanistic target of rapamycin inhibitors such as sirolimus, and more recently, the alkylating agent cyclophosphamide.[4] Observations that type II cytokine receptors activate adaptive T-cell responses through JAK-mediated phosphorylation of signal transducers of activation and transcription (STAT) have prompted preclinical studies testing whether JAK inhibitors could prevent GVHD. One such study showed that administration of ruxolitinib beginning on the day before HCT and continuing until day 20 after HCT decreased the severity of acute GVHD in mice.[5]

The current study showing that neutrophils are involved in the pathogenesis of GVHD adds to evidence that the path to GVHD begins with inflammatory innate immune responses caused by the conditioning regimen before HCT.[4] These innate immune responses facilitate and enhance adaptive donor T-cell immune responses stimulated by recipient alloantigens that are presented redundantly by a wide variety of cell types. The current study also adds to preclinical evidence that inhibition of JAK-STAT signaling offers promise as a way to prevent GVHD in humans not only through effects on adaptive T-cell responses,[6] but also through effects on innate immune responses. For example, ruxolitinib inhibits antigen presentation not only by neutrophils, but also by dendritic cells and monocyte-derived dendritic cells.[7,8] Additional studies are needed to determine whether JAK inhibitors have similar effects on other types of cells involved in antigen presentation. Even