Jutzi et al have identified an entirely new pathway for precision oncology approaches.

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

REFERENCES

1. Jutzi JS, Marneth AE, Ciboddo M, et al. Whole-genome CRISPR screening identifies *N*-glycosylation as a genetic and therapeutic vulnerability in *CALR*-mutant MPNs. *Blood.* 2022;140(11):1291-1304.
2. Spivak JL. Myeloproliferative neoplasms. *N Engl J Med.* 2017;376(22):2168-2181.
3. How J, Hobbs GS, Mullally A. Mutant calreticulin in myeloproliferative neoplasms. *Blood.* 2019;134(25): 2242-2248.
4. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med.* 2013;369(25):2379-2390.
5. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med.* 2013; 369(25):2391-2405.
6. Elf S, Abdelfattah NS, Chen E, et al. Mutant calreticulin requires both its mutant C-terminus and the thrombopoietin receptor for oncogenic transformation. *Cancer Discov.* 2016;6(4):368-381.
7. Albu RI, Constantinescu SN. Extracellular domain N-glycosylation controls human thrombopoietin receptor cell surface levels. *Front Endocrinol (Lausanne).* 2011;2:71.
8. Elf S, Abdelfattah NS, Baral AJ, et al. Defining the requirements for the pathogenic interaction between mutant calreticulin and MPL in MPN. *Blood.* 2018; 131(7):782-786.
9. Raez LE, Papadopoulos K, Ricart AD, et al. A phase I dose-escalation trial of 2-deoxy-D-glucose alone or combined with docetaxel in patients with advanced solid tumors. *Cancer Chemother Pharmacol.* 2013;71(2): 523-530.
10. Tefferi A, Gangat N, Pardanani A, Crispino JD. Myelofibrosis: genetic characteristics and the emerging therapeutic landscape. *Cancer Res.* 2022;82(5):749-763.

DOI 10.1182/blood.2022017448

TRANSPLANTATION

Comment on Gournay et al, page 1305

AML relapse after a TIGIT race

Livius Penter[1-4] and Catherine J. Wu[1-3] | [1]Dana-Farber Cancer Institute; [2]Broad Institute of Massachusetts Institute of Technology and Harvard University; [3]Harvard Medical School; and [4]Charité–Universitätsmedizin Berlin

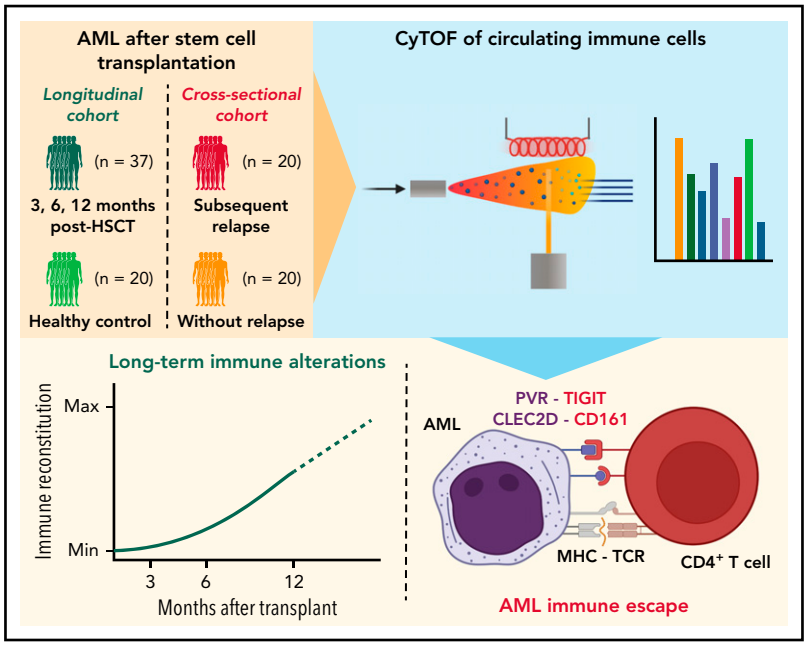
In this issue of *Blood*, Gournay et al[1] dissect donor immune reconstitution after allogeneic hematopoietic stem cell transplantation (HSCT) with mass cytometry and identify T-cell immunoreceptor with Ig and ITIM domains (TIGIT) and CD161-expressing CD4$^+$ T cells as early immune correlates of subsequent acute myelogenous leukemia (AML) relapse after HSCT.

The high relapse rate of AML after stem cell transplant has remained one of the most tenacious problems in malignant hematology. With the graft-versus-leukemia (GVL) effect at the core of successful long-term disease control, the early posttransplant course can be thought of as a delicate race between nascent donor immune reconstitution and recovering malignant cell populations that seek to escape GVL. AML relapse after HSCT thus often associates with donor immune cell dysfunction through upregulated immune checkpoint molecules or reduced antigen presentation, which leads to leukemic relapse through impaired recognition and killing of malignant cells.[2]

Clinical studies with immune checkpoint blockade have demonstrated that relapsed AML after transplant can be successfully treated by closing loopholes in donor immunity, for example, using CTLA-4 blockade in cases of extramedullary AML, in which it induced long-term disease control through local CD8$^+$ T-cell infiltration.[3] However, such durable remissions following immune checkpoint blockade are the exception rather than the norm, and a deeper understanding is still lacking regarding the composition and interactions among donor immune cell populations that are needed to drive such effective GVL responses.

Mass cytometry is a systems immunology approach for the unbiased discovery of immune cell subpopulations and their dynamics at single-cell resolution that builds on the capability to capture dozens of surface markers. This permits extension of classical flow cytometry approaches to a much higher resolution and can identify rare, previously unknown cell subsets. The wealth of information provided per cell by mass cytometry requires dimensionality reduction techniques and unsupervised clustering for data analysis, which overcome the limitations of classical manual gating strategies to achieve the analytical depth and speed required for analysis of such data.

Gournay et al studied peripheral blood samples prospectively collected from 2 large cohorts of patients following allogeneic stem cell transplantation, which they analyzed using mass cytometry with a 45-antibody panel (see figure). The first cohort consisted of peripheral blood collected longitudinally at 3, 6, and 12 months after transplant from 37 patients with AML/MDS in remission, providing the opportunity to define immune cell kinetics during the crucial first year after transplant, when most cases of AML relapse occur. As comparator, the authors profiled peripheral blood from 20 healthy donors. As expected, based on long-standing characterizations in the field, immune reconstitution early after transplant (3 months) was dominated by innate cell types such as natural killer (NK) cells and monocytes, whereas adaptive immunity and professional antigen-presenting cells gradually recovered at later timepoints. Notably, however, they demonstrated that the circulating immune compartment after transplant, despite ongoing reconstitution, remained profoundly altered even after 12 months. Globally increased expression of immune checkpoint molecules, such as PD-1, LAG3, and TIGIT, were detected across T and NK cells. Moreover, higher TIGIT expression on several T-cell subsets was associated with subsequent AML relapse, suggesting that the immune checkpoint molecule is of high relevance for post-transplant relapse.



Using mass cytometry (cytometry by time-of-flight [CyTOF]) on serial peripheral blood samples obtained from patients with AML/MDS after hematopoietic allogeneic stem cell transplantation and healthy controls, Gournay et al documented immune reconstitution during the first 12 months after transplant (left). They further showed that TIGIT$^+$ and CD161$^+$ CD4$^+$ T cells were associated with subsequent disease recurrence through comparison of patients with AML who relapsed after transplant with patients who did not relapse (right). CLEC2D, C-type lectin domain family 2 member D; MHC, major histocompatibility complex; PVR, poliovirus receptor (CD155); TCR, T-cell receptor.

As follow-up to this finding, the authors evaluated a second cross-sectional cohort in which they compared circulating immune cells sampled 3 months after transplant from 20 patients with AML with sustained remission with cells from 20 patients who subsequently relapsed after stem cell transplantation. Through differential analysis of immune cell clusters associated with relapse, they again linked TIGIT$^+$ CD4$^+$ T cells with disease recurrence. TIGIT$^+$ CD4$^+$ T cells showed broad evidence of immune activation as they coexpressed other checkpoint molecules such as PD-1 or B- and T-lymphocyte attenuator and had high Ki-67 and human leukocyte antigen-DR isotype expression, suggesting that they represent T cells with ongoing antigen exposure. A second CD4$^+$ T-cell population with high expression of CD161 (KLRB1) was also associated with AML relapse and shorter disease-free survival after transplant. CD161 expressing T cells could be another high-priority target for immune modulation, especially because the ligand C-type lectin domain family 2 member D (CLEC2D) is widely expressed by AML blasts, and recent work with glioma-infiltrating T cells has demonstrated that inhibition of CD161 may increase activation and cytotoxicity of tumor-infiltrating T cells.[4]

Overall, this study confirms and extends prior work to identify signatures of post-HSCT immune escape[5] and makes a strong case for the further investigation and clinical testing of additional immune checkpoint molecules beyond classical CTLA-4 and PD-1 in the context of AML relapse after allogeneic stem cell transplantation. Such efforts are currently already underway in studies that test inhibitors of TIM-3 and CD47 in transplant-naïve AML and MDS.[6,7] Although therapeutic inhibition of TIGIT has not yet reached AML/MDS, it is under investigation in several solid tumors such as non–small cell lung cancer, in which signs of clinical activity have been documented.[8] The observation by Gournay et al that TIGIT$^+$ CD4$^+$ T cells in peripheral blood of patients with AML after HSCT coexpress other immune checkpoint molecules suggests that combinatorial inhibition could be a relevant strategy. In fact, combined TIGIT and PD-1 inhibition has been documented to provide synergistic effects.[9] An important question for the posttransplant setting is the amount of immune toxicity induced by these novel immune checkpoint inhibitors because nivolumab monotherapy already has been shown to generate high rates of graft-versus-host disease.[10]

More broadly, Gournay et al provide a compelling testimony to the power of large-scale longitudinal analyses of thoughtfully curated biospecimen collections with systems biology approaches to deeply dissect the immunological race between donor and recipient after stem cell transplantation. Intriguingly, such an approach could be conceived as a future clinical tool for real-time monitoring of donor immune reconstitution in the early posttransplant setting. This approach may aid clinicians in preemptively intervening with immune checkpoint blockade as dysfunctional immune cells arise to ensure that donor immune cells come out ahead in the race against AML.

Conflict-of-interest disclosure: C.J.W. holds equity at BioNTech, Inc. and has received institutional research funding from Pharmacyclics outside the submitted work. L.P. declares no competing financial interests. ■

REFERENCES

1. Gournay V, Vallet N, Peux V, et al. Immune landscape after allo-HSCT: TIGIT- and CD161-expressing CD4 T cells are associated with subsequent leukemia relapse. *Blood.* 2022;140(11):1305-1321.
2. Zeiser R, Vago L. Mechanisms of immune escape after allogeneic hematopoietic cell transplantation. *Blood.* 2019;133(12):1290-1297.
3. Penter L, Zhang Y, Savell A, et al. Molecular and cellular features of CTLA-4 blockade for relapsed myeloid malignancies after transplantation. *Blood.* 2021;137(23):3212-3217.
4. Mathewson ND, Ashenberg O, Tirosh I, et al. Inhibitory CD161 receptor identified in glioma-infiltrating T cells by single-cell analysis. *Cell.* 2021;184(5):1281-1298.e26.
5. Toffalori C, Zito L, Gambacorta V, et al. Immune signature drives leukemia escape and relapse after hematopoietic cell transplantation. *Nat Med.* 2019;25(4):603-611.
6. Sallman D, Asch A, Kambhampati S, et al. AML-196: the first-in-class anti-CD47 antibody magrolimab in combination with azacitidine is well tolerated and effective in AML patients: phase 1b results. *Clin Lymphoma Myeloma Leuk.* 2021;21:S290.
7. Brunner AM, Traer E, Vey N, et al. Allogeneic hematopoietic cell transplantation outcomes of patients with R/R AML or higher-risk MDS treated with the TIM-3 inhibitor MBG453 (sabatolimab) and hypomethylating agents. *Blood.* 2021;138(suppl 1):3677.
8. Cho BC, Abreu DR, Hussein M, et al. Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell

lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study. *Lancet Oncol.* 2022; 23(6):781-792.

9. Banta KL, Xu X, Chitre AS, et al. Mechanistic convergence of the TIGIT and PD-1 inhibitory pathways necessitates co-blockade to optimize anti-tumor CD8$^+$ T cell responses. *Immunity.* 2022;55(3): 512-526.e9.

10. Davids MS, Kim HT, Costello C, et al. A multicenter phase 1 study of nivolumab for relapsed hematologic malignancies after allogeneic transplantation. *Blood.* 2020; 135(24):2182-2191.

DOI 10.1182/blood.2022017614

CLINICAL TRIALS AND OBSERVATIONS

Comment on Niss et al, page 1322

Diffuse myocardial fibrosis as an SCD biomarker

Swee Lay Thein and Vandana Sachdev | National Heart, Lung, and Blood Institute

In this issue of *Blood*, Niss et al[1] posit that early initiation of disease-modifying therapy can mitigate sickle myocardial fibrosis by using extracellular volume (ECV) fraction as a potential imaging biomarker.

Cardiovascular complications account for a third of the premature mortality rate in patients with sickle cell disease (SCD).[2] Underlying this cardiac risk is a progressive cardiomyopathy which is closely linked to the severity of anemia and impaired diastolic function.[3,4] In an earlier study,[5] Niss et al used cardiac magnetic resonance (CMR) imaging to measure ECV in a cohort of 25 patients with SCD and found markedly increased ECV in all of them, indicating diffuse myocardial fibrosis. In the current study,[1] the authors asked whether early intervention with disease-modifying therapy could mitigate cardiac complications of SCD. Twelve children and young adults who initiated hydroxyurea or blood transfusion at age younger than 6 years and who had at least 5 years of uninterrupted therapy,[1] were found to have ECV levels comparable to those of normal controls and significantly lower than a comparator group of 25 patients who did not have early therapy.

Diffuse myocardial fibrosis occurs from deposition of collagen in the myocardial interstitium, and its detrimental effects include systolic and diastolic dysfunction and the potential for arrhythmia. It can be detected in aging and in many chronic cardiac conditions, and its severity has been associated with an increased risk of adverse events.[6] Endomyocardial biopsies have shown direct correlations between ECV and collagen deposition in heart failure, but this has not been seen in other conditions, suggesting that patterns of deposition may vary significantly based on the underlying conditions.[6] Because ECV measures the total interstitial space, myocardial edema and/or inflammation are important potential confounders of an increased ECV, which is particularly relevant in patients with SCD.

In their letter to the editor, Niss et al addressed an important and interesting issue in sickle cardiomyopathy, and although their cohort was small, it is hypothesis generating and provides evidence for proceeding to larger studies that use CMR imaging. An important question is whether ECV provides incremental benefit over other parameters for the assessment of cardiac dysfunction. Echo studies have demonstrated that diastolic dysfunction is an independent risk factor for mortality in SCD, yet the diagnosis of diastolic dysfunction by echo is complex and varies from study to study.[3,7,8] There are no accepted methods for assessing diastolic function in SCD, and guidelines specifically mention that echo parameters may suggest chronically elevated left ventricular filling pressures in the *absence* of anemia. A heart failure scenario that is typically encountered in patients with SCD is one of high output with increased blood volume, and many imaging parameters (eg, left atrial volume) and Doppler parameters may not have the same normal thresholds as those in patients who do not have SCD. In this setting of diagnostic complexity, a new imaging marker of high risk in patients with SCD would be a welcome addition. As seen in this[1] and other studies, diastolic classifications are not always aligned. It has been suggested that myocardial fibrosis precedes diastolic dysfunction, and yet 1 of the 12 patients in the Niss et al study with a normal ECV had inconclusive diastolic function. Niss et al and others have previously demonstrated a link between ECV and diastolic echo parameters, but these measures are not provided in their current study, so it is difficult to discern whether ECV had greater value than commonly used echo parameters such as tricuspid regurgitant jet velocity. Unfortunately, there are few CMR imaging studies in patients with SCD, and until many more patients undergo ECV assessment, the relationship between ECV and outcomes will remain unknown. ECV measurement, which requires infusion of gadolinium contrast, provides additional information about cardiac morphology, but whether it adds incremental value to existing echo parameters or laboratory markers such as N-terminal prohormone brain natriuretic peptide remains unknown.

In this observational study,[1] ECV values in treated patients approached the normal range compared with high values in untreated patients. With this type of cross-sectional analysis, it is difficult to evaluate differences in disease severity between groups and no causal relationships can be determined. So far, CMR studies of ECV[1,5] have included highly selected patients and small numbers of patients, so the prevalence of diffuse myocardial fibrosis in patients with SCD is unknown. Early initiation of disease-modifying therapy would and should prevent the accumulation of damage to various organs, including the heart, but questions remain about how early to begin therapy, how the presence of high ECV might impact treatment decisions, and what markers can be used to monitor the response to an intervention.

Although there were no differences in cardiac parameters and hemoglobin