microenvironmental interdependency in CLL. *Blood*. 2020;135(6):411-428.

5. Khan S, Ince-Dunn G, Suomalainen A, Elo LL. Integrative omics approaches provide biological and clinical insights: examples from mitochondrial diseases. *J Clin Invest*. 2020;130(1):20-28.
6. Zhang F, Ge W, Ruan G, Cai X, Guo T. Data-independent acquisition mass spectrometry-based proteomics and software tools: a glimpse in 2020. *Proteomics*. 2020;20(17-18): e1900276.
7. Zhou W, Deiters A. Chemogenetic and optogenetic control of post-translational modifications through genetic code expansion. *Curr Opin Chem Biol*. 2021;63:123-131.
8. Wu Z, Huang R, Yuan L. Crosstalk of intracellular post-translational modifications in cancer. *Arch Biochem Biophys*. 2019;676: 108138.
9. Lu J, Böttcher M, Walther T, Mougiakakos D, Zenz T, Huber W. Energy metabolism is co-determined by genetic variants in chronic lymphocytic leukemia and influences drug sensitivity. *Haematologica*. 2019;104(9): 1830-1840.
10. Dietrich S, Oleś M, Lu J, et al. Drug-perturbation-based stratification of blood cancer. *J Clin Invest*. 2018;128(1):427-445.

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LYMPHOID NEOPLASIA

Comment on Najidh et al, page 2539

Blood will tell: profiling Sézary syndrome

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In this issue of *Blood*, Najidh and colleagues add important insights to the current understanding of immunophenotypic and transcriptional profiles in the circulating malignant cells of patients with Sézary syndrome.[1]

Sézary syndrome is a rare, aggressive leukemic variant of primary cutaneous T-cell lymphomas characterized by erythroderma (>80% body surface involvement), generalized lymphadenopathy, and circulating atypical lymphocytes called Sézary cells. Even though the circulating tumor clones are easily accessible for immunophenotyping in the blood, unequivocal identification and quantification of Sézary cells can be a challenge. Marker panels may fail to identify these cells, and there is considerable patient-to-patient variability. In the article by Najidh et al, blood cells from patients with Sézary syndrome were prospectively analyzed by both flow cytometric and RNA sequencing methods, which represent a highly sensitive and standardized approach based on the previously validated multiparameter EuroFlow marker panels. The authors demonstrate that conventional flow cytometry, generally used in clinical practice, either over- or underestimates the overall tumor burden when compared with EuroFlow. This finding adds a new aspect to the long-standing debate regarding whether percentages or absolute counts of circulating tumor cells are the best measure to use in diagnosis and monitoring.[2]

Currently, loss of CD26 or CD7 is used for the routine detection of Sézary cells. This study revealed an enormous range across patient samples: 1.13% to 100% and 0.05% to 100% antigen loss, respectively. Importantly, loss of CD26 or CD7 was not restricted to malignant cells; it was also found in reactive (ie, nonmalignant) $CD4^+$ T cells, thus limiting the specificity of conventional flow cytometry for assessing blood tumor burden. EuroFlow gating, aimed at a more comprehensive phenotyping, detected Sézary cells in all samples analyzed in the Najidh et al study. However, aberrant cell marker expression went beyond loss of CD26 and/or CD7 in 92% of the patients. Despite the advantage that this more precise methodology offers, identification of Sézary cells may remain an obstacle due to the complexity of multiparameter flow cytometry, because this tool is not widely available in routine clinical settings. This challenge could be overcome in the future with novel markers such as KIR3DL2, which has proven high diagnostic sensitivity and specificity.[3] Notably, initial reports of the clinical use of an anti-KIR3DL2 antibody are promising.[4]

Although the wide variety of immunophenotypic aberrancies were not linked to previous treatment in this study, heterogeneous phenotypic shifts occurred in patients over time. The authors confirmed that Sézary cells also showed a wide range of T-helper cell phenotypes, encompassing almost all maturation stages investigated, with central memory cells being the most frequent, supporting the current dogma that Sézary cells derive from central memory T cells.[5] Interestingly, recent data suggest a skin-resident T-cell origin for Sézary syndrome similar to that in mycosis fungoides, with an ultraviolet (UV) signature of potential driver gene mutations in both entities.[6] Of note, data from T-cell biology suggest that $CD4^+$ T-cell clones can exist simultaneously as skin resident and circulating memory cells, even under physiological conditions,[7] further supporting the remarkable plasticity of lymphoma cells.[8] Najidh et al confirmed the presence of the skin-homing receptor CCR4 at high frequencies in Sézary cells, which is supported by the efficacy data for mogamulizumab, an approved anti-CCR4 therapeutic antibody.

In daily practice, opportunistic infections caused by profound overall immune dysfunction in patients with primary cutaneous lymphoma can be a challenge. Although infections contribute to overall mortality per se in Sézary syndrome, microbial dysbiosis may directly drive disease progression, as shown by immunostimulatory toxins derived from colonization by *Staphylococcus aureus*.[5] Najidh et al postulated that there is an overall imbalanced immune cell homeostasis in patients with Sézary syndrome. They showed that there are altered levels of innate and adaptive circulating leukocytes with a significant reduction of absolute cell counts of $CD8^+$ cytotoxic T cells, B cells, natural killer cells, and others when compared with healthy controls. By contrast, neutrophils, dendritic cells, and monocytes were significantly increased. Transcriptional profiles of monocytes from patients with Sézary syndrome have previously been shown to differ remarkably from those in healthy controls. In vitro, monocyte profiles could be reshaped after interferon-γ (IFN-γ) stimulation, which might explain the clinical efficacy of IFN-γ in cutaneous lymphomas.[9]

Interestingly, differentiation of monocytes into dendritic cells is thought to be relevant for the immunomodulatory

effects of extracorporeal photopheresis, an established immunotherapy for Sézary syndrome.[10]

The gene expression data from Nadijh et al demonstrated that phenotypically distinct Sézary cell populations within individual patients were still clonally related, further demonstrating the phenotypic plasticity of Sézary cells. On a transcriptomic level, the authors found strong downregulation of *THEMIS* and *LAIR1*, markers involved in T-cell receptor function. Importantly, these genes were recently linked to the pathogenesis of adult T-cell leukemia, which is clinically closely related to Sézary syndrome, suggesting some common pathogenetic mechanism.

In sum, the presented strategy of integrating EuroFlow-based multiparameter flow cytometry, fluorescence-activated cell sorting (FACS) and RNA sequencing provided a comprehensive exploration of the heterogeneity of Sézary cells and offers a precise method to quantify the overall tumor burden. These explorative results may initiate a revision of standardized international criteria for diagnosing blood involvement and responses for informed treatment decisions in Sézary syndrome.

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REFERENCES

1. Najidh S, Tensen CP, van der Sluijs-Gelling AJ, et al. Improved Sézary cell detection and novel insights into immunophenotypic and molecular heterogeneity in Sézary syndrome. *Blood*. 2021;138(24):2571-2583.
2. Vermeer MH, Nicolay JP, Scarisbrick JJ, Zinzani PL. The importance of assessing blood tumour burden in cutaneous T-cell lymphoma. *Br J Dermatol*. 2021;185(1):19-25.
3. Roelens M, de Masson A, Ram-Wolff C, et al. Revisiting the initial diagnosis and blood staging of mycosis fungoides and Sézary syndrome with the KIR3DL2 marker. *Br J Dermatol*. 2020;182(6):1415-1422.
4. Bagot M, Porcu P, Marie-Cardine A, et al. IPH4102, a first-in-class anti-KIR3DL2 monoclonal antibody, in patients with relapsed or refractory cutaneous T-cell lymphoma: an international, first-in-human, open-label, phase 1 trial. *Lancet Oncol*. 2019;20(8):1160-1170.
5. Durgin JS, Weiner DM, Wysocka M, Rook AH. The immunopathogenesis and immunotherapy of cutaneous T cell lymphoma: Pathways and targets for immune restoration and tumor eradication. *J Am Acad Dermatol*. 2021;84(3):587-595.
6. Park J, Daniels J, Wartewig T, et al. Integrated genomic analyses of cutaneous T cell lymphomas reveal the molecular bases for disease heterogeneity [published online ahead of print on 11 June 2021]. *Blood*.
7. Klicznik MM, Morawski PA, Hollbacher B, et al. Human CD4(+)CD103(+) cutaneous resident memory T cells are found in the circulation of healthy individuals. *Sci Immunol*. 2019 Jul5;4(37):eaav8995.
8. Rindler K, Bauer WM, Jonak C, et al. Single-cell RNA sequencing reveals tissue compartment-specific plasticity of mycosis fungoides tumor cells. *Front Immunol*. 2021;12:666935.
9. Torrealba MP, Manfrere KCG, Yoshikawa FSY, et al. IFN-γ reshapes monocyte responsiveness in Sezary syndrome. *Int J Dermatol*. 2021;60(1):e3-e6.
10. Vieyra-Garcia PA, Wolf P. Extracorporeal photopheresis: A case of immunotherapy ahead of its time. *Transfus Med Hemother*. 2020;47(3):226-235.

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TRANSFUSION MEDICINE

Comment on Tang et al, page 2570

HSC engraftment in SCD: a MiSCing piece of the puzzle?

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In this issue of *Blood*, Tang et al,[1] using sickle cell disease (SCD; Townes transgenic) mice, report defects in bone marrow (BM) mesenchymal stromal cells (MSCs) and hematopoietic stem cells (HSCs) related to chronic heme exposure, which can be reversed through treatment with *N*-acetylcysteine (NAC) or inhibition with Toll-like receptor 4 (TLR4).

MSCs are known to be important in supporting the BM stroma, including HSCs.[2] Tang et al provide an in-depth evaluation of murine SCD MSCs, demonstrating many defects in the SCD BM space. Although the pathophysiology is certainly multifactorial, these results may help explain the difficulty in mobilizing autologous HSCs in patients[3] with SCD and higher rates of graft rejection after allogeneic hematopoietic cell transplantation (HCT).[4,5]

Tang et al hypothesized that free heme (released via hemolyzed SCD red blood cells [RBCs]) induces oxidative stress, altering the SCD BM microenvironment, most notably MSCs (see figure). Previous work examining human SCD MSCs (median age, 8.3 years) demonstrated comparable basic phenotype and function of MSCs, compared with those of healthy controls.[6] They performed a more detailed in vitro and in vivo evaluation of murine SCD MSCs. They found defects in MSCs from Townes mice, including decreased frequency in the BM, increased reactive oxygen species (ROS), decreased ability to form secondary mesenspheres, and decreased adipogenic and osteogenic differentiation potential. Decreased BM HSCs, increased HSC ROS, and increased peripheral blood (PB) hematopoietic stem and progenitor cells (HSPCs) were also documented. Further, expression of key genes involved in HSC maintenance was downregulated in SCD MSCs, with upregulation of TLR4 and related genes. Notably, preceding coculture with SCD MSCs (compared with wild-type [WT]) led to decreased engraftment of WT HSCs, with increased HSC ROS found 16 weeks after transplant. Many of these findings were confirmed in WT mice treated with hemin, implicating free heme in the induction of these downstream effects.

Most important, the defects in MSCs and HSCs exposed to free heme were reversible. First, treatment with NAC, in vitro or in vivo, reversed these findings, implicating oxidative stress downstream of exposure to free heme. Second, TLR4 inhibition reversed findings in hemin-treated (WT mice receiving the TLR4 inhibitor TAK-242 or TLR4 knockout [KO] mice) and SCD mice (TAK-242 treated). This effect