interwoven network of donor- and host-derived circulating factors and cellular interactions.[5] This begs the question: in such a complex network, how does our cell type of interest influence the rest of the inflammatory milieu and thus change overall outcome? To investigate, the polyclonal Tcons, which were cotransferred with each iNKT subset were analyzed with RNA-seq. The data suggest that early environmental changes induced by the adoptively transferred iNKTs alter the transcriptional programming of the polyclonal pool. The iNKT2 and iNKT17 subsets appear to drive differential expression of several functionally relevant genes in both the $CD4^+$ and $CD8^+$ Tcon populations (eg, *IL27ra* and *Stat1*). These transcriptomic changes were functionally associated with reduced GVHD lethality and less intestinal damage, as measured by traditional histopathologic analysis.

Unconventional T cells (the iNKT population studied here, as well as mucosa-associated invariant T cells [MAITs] and γδ T cells) are increasingly recognized as contributors to HCT outcome,[6] and the adoptive transfer of iNKTs in particular is the focus of several clinical trials of cancer-directed immunotherapy because of their antitumor properties.[7] Importantly, it is necessary that these cells undergo an in vitro expansion step before therapeutic transfer, and this study suggests that care should be taken to assess the subset composition of the product to be transferred, to maximize either regulatory or antitumor potential. As unconventional T cells are not donor-HLA restricted, but instead recognize antigens in the context of their particular HLA-like molecules (CD1d for NKT cells and MR1 for MAIT cells) and butyrophilins in the case of γδ T cells, they are likely to be more suitable for development into "off-the-shelf" cellular therapy products in the allogeneic HCT setting than therapies derived from Tcons. With respect to iNKT therapies, they are likely to be most successful if the subset composition is optimized for the desired clinical outcome in accordance with the subset-specific functions defined by Maas-Bauer and colleagues in their study.

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REFERENCES

1. Maas-Bauer K, Lohmeyer JK, Hirai T, et al. Invariant natural killer T cell subsets have diverse graft-versus-host-disease-preventing and antitumor effects. *Blood.* 2021;138(10):858-870.
2. Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB. The burgeoning family of unconventional T cells [published correction appears in Nat Immunol. 2016;17(2):214]. *Nat Immunol.* 2015;16(11):1114-1123.
3. Brennan PJ, Brigl M, Brenner MB. Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. *Nat Rev Immunol.* 2013;13(2):101-117.
4. Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat Methods.* 2013;10(12):1213-1218.
5. Koyama M, Hill GR. The primacy of gastrointestinal tract antigen-presenting cells in lethal graft-versus-host disease. *Blood.* 2019;134(24):2139-2148.
6. Andrlová H, van den Brink MRM, Markey KA. An unconventional view of T cell reconstitution after allogeneic hematopoietic cell transplantation. *Front Oncol.* 2021;10:608923.
7. Godfrey DI, Le Nours J, Andrews DM, Uldrich AP, Rossjohn J. Unconventional T cell targets for cancer immunotherapy. *Immunity.* 2018;48(3):453-473.

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LYMPHOID NEOPLASIA

Comment on Schmitt et al, page 871

Killing 2 birds with 1 stone in DLBCL

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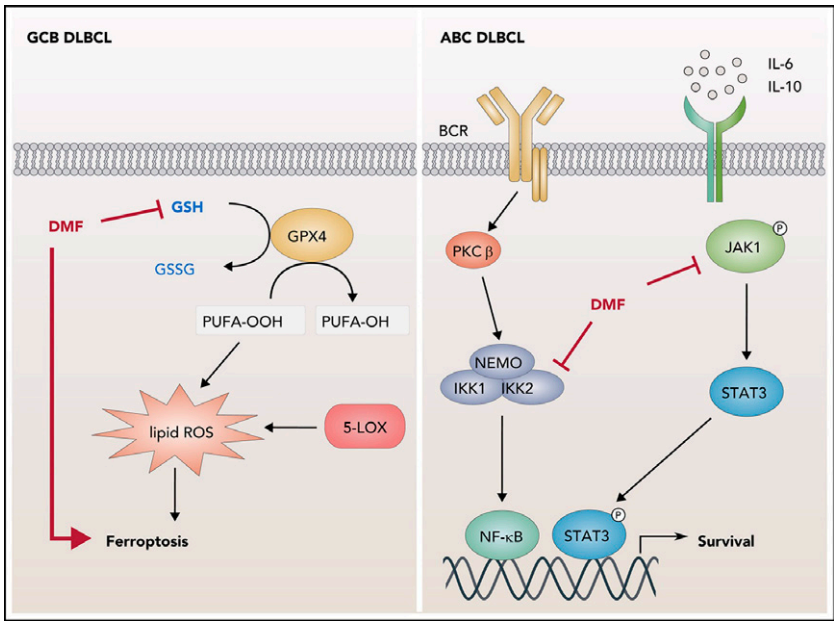
The success of small-molecule inhibitors targeting the BCR-ABL fusion kinase has been difficult to replicate in more common types of cancers with heterogeneous molecular abnormalities, such as diffuse large B-cell lymphoma (DLBCL). The original classification of DLBCL into germinal center B-cell (GCB) and activated B-cell (ABC) subtypes, based on gene expression profiling, still has a physiological basis but has been supplanted by newer schemes with more categories,[1] based on diverse but recurrent abnormalities. However, specific inhibitors targeting certain of these abnormalities or related pathways have not yet achieved Food and Drug Administration approval in DLBCL, even for patients displaying the targeted abnormality. Might an empiric approach be a complementary alternative for finding (or repurposing) effective drugs? Could 1 drug target different types of DLBCL in different ways? In this issue of *Blood*, Schmitt and colleagues[2] provide affirmative answers to both questions.

Dimethyl fumarate (DMF), a simple compound that is already approved for 2 diseases mediated by activated lymphocytes (multiple sclerosis and psoriasis), was tested in vitro against diverse cell lines. Efficacy was frequent against lines representing both ABC and GCB subtypes of DLBCL, and against mantle cell lymphoma (MCL), but not against myeloid, carcinoma, or melanoma types. The authors then did extensive work on the mechanism of action of DMF in DLBCL lines and found differences between the subtypes (see figure).

DMF profoundly depleted glutathione in GCB-DLBCL and MCL lines, apparently through direct succinylation. Because glutathione is critical for cellular redox balance and neutralization of ROS, its depletion led to ferroptosis, a caspase-independent form of programmed cell death in which iron-dependent lipid peroxidation causes loss of plasma membrane integrity.[3] Molecular features of ferroptosis were observed with DMF treatment, and multiple inhibitors of ferroptosis protected GCB-DLBCL lines from DMF, confirming its causal role. DMF-induced ferroptosis was facilitated by the ROS-generating, iron-containing enzyme arachidonate 5-LOX, expressed in the nucleus (as required for its activity) in GCB-DLBCL cell lines and 50% of primary tumors.



DMF has different mechanisms of action in different subtypes of DLBCL. In the GCB subtype (left), direct succinylation of glutathione (GSH) by DMF leads to GSH depletion, impairing the ability of glutathione peroxidase 4 (GPX4) to detoxify polyunsaturated fatty acids (PUFAs) that have undergone peroxidation. Such damage to plasma membrane lipids results from reactive oxygen species (ROS), produced by enzymes such as arachidonate 5-lipoxygenase (5-LOX), and causes ferroptosis, a caspase-independent form of programmed cell death. DMF does not cause ferroptosis in the ABC subtype of DLBCL (right), but instead inhibits 2 of its distinctive prosurvival signaling pathways. Transcription of target genes by canonical nuclear factor κB (NF-κB), one of the key consequences of "chronic active" signaling by the B-cell receptor (BCR) in ABC-DLBCL, depends on activation by the IKK2 kinase; similarly, activation of the STAT3 transcription factor depends on the kinase JAK1, downstream of autocrine signaling by interleukin-6 (IL-6) and IL-10. DMF inactivates both of these kinases by direct succinylation of critical cysteine residues. See the visual abstract in the article by Schmitt et al that begins on page 871.

In contrast, ABC-DLBCL lines were relatively resistant to DMF-induced ferroptosis, attributable to 2 effects of its "chronic active" form of B-cell receptor signaling: suppression of 5-LOX transcription and constitutive activity of the canonical NF-κB pathway. However, DMF was toxic to ABC-DLBCL lines by other mechanisms, including at least the inhibition of NF-κB and JAK/STAT3, another of the essential signaling pathways that are distinctive of ABC-DLBCL. Extensive analyses pinpointed the cause of these inhibitory effects to direct succinylation of critical cysteine residues of enzymes that activate these pathways, C179 of IKK-β and C257 of JAK1.

Further testing of DMF efficacy included showing that it reduced tumor formation by GCB-DLBCL lines in zebrafish embryos. In vitro combination testing showed synergism between DMF and an inhibitor of the glutathione-independent ferroptosis suppressor protein 1, as well as with the BCL2 inhibitor ABT-199 in ABC-DLBCL lines. Finally, DMF and ABT-199 were more effective when used together in vivo, substantially reducing the growth in mice of an ABC-DLBCL line (HBL-1) and a patient-derived xenograft (VFN-D1). However, whether the same molecular effects of DMF observed in vitro were also active in these in vivo settings was not examined.

This study challenges us to consider what "targeted therapy" really means. All drugs have targets, whether we know what they are or not; what is important is whether those targets are critical to tumor cells, more than to normal cells. DMF is not a targeted therapy in the usual sense; it is likely, although not investigated in this study, that numerous sulfhydryl-containing proteins and perhaps other molecules (like glutathione) are succinylated by DMF. In effect, empiric DMF treatment coupled with subsequent target identification in this study functioned as a screen; from a hypothetical "library" of succinylated molecular species that was created in cells, those that are critical targets, at least within the context of DMF treatment overall, were identified. Functional specificity of DMF's effects was shown by finding only a few critical targets in DLBCL lines, differing between subtypes, and apparently none in other cell types.

For 1 drug to have different mechanisms in different DLBCL subtypes has been seen before, with an inhibitor of the NEDD8-activating enzyme (MLN4924, Pevonedistat), a drug that has a precise direct molecular target that affects multiple proteins.[4] Similarly, lenalidomide and related immunomodulatory imide drugs have precise direct targets but pleiotropic and cell-specific effects on multiple proteins, affecting tumor cells (including ABC-DLBCL[5]) and immune cells in different but therapeutically beneficial ways.

The most important question is whether these findings can be successfully translated into therapy for DLBCL. At first glance, that DMF is already an approved and well-tolerated drug for certain nonneoplastic diseases should make this more plausible; however, there are many unknowns. Achieving sufficient DMF levels to treat DLBCL is likely to require injection, according to the authors, whereas DMF is currently only approved for oral use; therefore, dosing and administration will need to be determined. That bears on a second and larger concern: especially at levels needed for DLBCL, will the beneficial tumor cell-intrinsic effects of DMF be outweighed by its immunosuppressive effects,[6] which coincide with ones generally found in tumor microenvironments? This is countered by other, more encouraging evidence: ferroptosis induction is important for tumor-cell killing by T cells,[7] at least during treatment with checkpoint inhibitors, and could be enhanced by DMF; furthermore, ferroptosis makes tumor cells immunogenic,[8] which also suggests that short treatments with DMF might be more effective than longer ones. Uncertain for predicting DMF's efficacy is that it has been found in immune cells to suppress aerobic glycolysis,[9] essential for effector T-cell function, as well as in tumor cells.[10]

Further evaluation of these promising results, in immunocompetent models or clinical trials, will be necessary to determine whether "succinylation therapy" will become a new front in the war on DLBCL.

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REFERENCES

1. Wright GW, Huang DW, Phelan JD, et al. A probabilistic classification tool for genetic

subtypes of diffuse large B cell lymphoma with therapeutic implications. *Cancer Cell.* 2020;37(4):551-568.

2. Schmitt A, Xu W, Bucher P, et al. Dimethyl fumarate induces ferroptosis and impairs NF-κB/STAT3 signaling in DLBCL. *Blood.* 2021; 138(10):871-884.
3. Stockwell BR, Jiang X, Gu W. Emerging mechanisms and disease relevance of ferroptosis. *Trends Cell Biol.* 2020;30(6): 478-490.
4. Milhollen MA, Traore T, Adams-Duffy J, et al. MLN4924, a NEDD8-activating enzyme inhibitor, is active in diffuse large B-cell lymphoma models: rationale for treatment of NF-κB-dependent lymphoma. *Blood.* 2010; 116(9):1515-1523.
5. Yang Y, Shaffer AL III, Emre NC, et al. Exploiting synthetic lethality for the therapy of ABC diffuse large B cell lymphoma. *Cancer Cell.* 2012;21(6):723-737.
6. Mills EA, Ogrodnik MA, Plave A, Mao-Draayer Y. Emerging understanding of the mechanism of action for dimethyl fumarate in the treatment of multiple sclerosis. *Front Neurol.* 2018;9:5.
7. Wang W, Green M, Choi JE, et al. $CD8^+$ T cells regulate tumour ferroptosis during cancer immunotherapy. *Nature.* 2019; 569(7755):270-274.
8. Efimova I, Catanzaro E, Van der Meeren L, et al. Vaccination with early ferroptotic cancer cells induces efficient antitumor immunity. *J Immunother Cancer.* 2020;8(2):e001369.
9. Kornberg MD, Bhargava P, Kim PM, et al. Dimethyl fumarate targets GAPDH and aerobic glycolysis to modulate immunity. *Science.* 2018;360(6387):449-453.
10. Chen K, Wu S, Ye S, et al. Dimethyl fumarate induces metabolic crisie to suppress pancreatic carcinoma. *Front Pharmacol.* 2021;12:617714.

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TRANSPLANTATION

Comment on Reilly et al, page 898

The journey of a thousand miles begins with 1 step

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In this issue of *Blood*, Reilly et al analyze *TERT* rare variants in a large cohort of patients with myelodysplastic syndrome (MDS) enrolled in the Center for International Blood and Marrow Transplant Research (CIBMTR) database. They cloned all variants, quantified their impact on telomere elongation in cell-based assays, and proved their association with inferior posttransplant outcome.[1]

This study has implications for both clinical and translational research. The pathophysiology of MDS has remained largely obscure for a long time. High-throughput DNA sequencing has identified the key somatic mutation drivers of this type of malignancy, including splicing factors, DNA methylation, chromatin modification, transcription regulation, DNA repair, and signal transduction.[2] In 2016, the World Health Organization classification added germline predisposition to myeloid neoplasms.[3] This change has helped reveal the relatively well-hidden world of germline mutations in apparently sporadic hematologic cancers associated with a spectrum of underlying conditions. However, the relative contribution of the germline genetic component to the pathophysiology of MDS, as well as its clinical implications, still needs to be elucidated. Indeed, some disorders seem to have high penetrance and strong clinical expressivity that raise clinical suspicion, whereas others, such as germline *DDX41* mutations, do not.[4] In addition, even within those mutations associated with organ dysfunction, clinical expression may be extremely variable, which results in late onset or incidental diagnosis in adulthood.[5]

Reilly et al analyzed a large cohort of 1514 MDS patients in the CIBMTR repository and research database who had banked whole peripheral blood DNA, as well as a separate cohort of 401 adult patients with non-Hodgkin lymphoma who had been treated with autologous stem cell transplantation as a control group. In a previous study on the same CIBMTR cohort, the authors reported compound heterozygous mutations in the Shwachman-Diamond syndrome–associated *SBDS* gene in young adults with MDS that were associated with a remarkably shorter posttransplant survival.[6] More recently, using the same cohort, the authors measured relative telomere length in blood samples from pretransplant recipients and found a significant association with inferior survival because of a high risk of nonrelapse mortality (NRM).[7] On the basis of this information, Reilly et al analyzed *TERT* rare variants in the CIBMTR cohort and found a non-negligible prevalence of 2.7% in patients with no clinical diagnosis of telomere biology disorder. In line with their previous findings, functionally relevant *TERT* mutations proved to be clinically relevant, significantly affecting posttransplant outcome, mainly as a consequence of an increased incidence of NRM. Overall, these results concur to sustain systematic genetic screening of germline variants in patients who are candidates for allogeneic stem cell transplantation to guide risk-adapted approaches and inform donor selection.

Although next-generation sequencing (NGS) technologies have helped pave the way to integrating somatic mutation analysis into the diagnosis process, disease classification, and risk assessment, they have also fostered a harmful feeling of having a solution to every (diagnostic) problem within arm's reach. Indeed, not all that glitters is gold, and the study by Reilly et al nicely illustrates the huge effort and the translational research methodology that must be applied to generate a robust basis of evidence for interpreting NGS test results in our clinical practice. In fact, high-throughput genome sequencing outputs a plethora of data (variants) of uncertain significance, of which we do not yet know the actual relevance for protein function and, consequently, their clinical value.

Standards and guidelines for interpreting sequence variants have been developed to provide guidance for interpreting genetic tests by integrating a spectrum of information sources, including population data, computational data, functional data, and segregation data, and by adopting of standard terminology.[8] In addition, the Clinical Genome Resource (ClinGen; https://clinicalgenome.org), a large collaborative effort funded by the National Institutes of Health, has been promoted