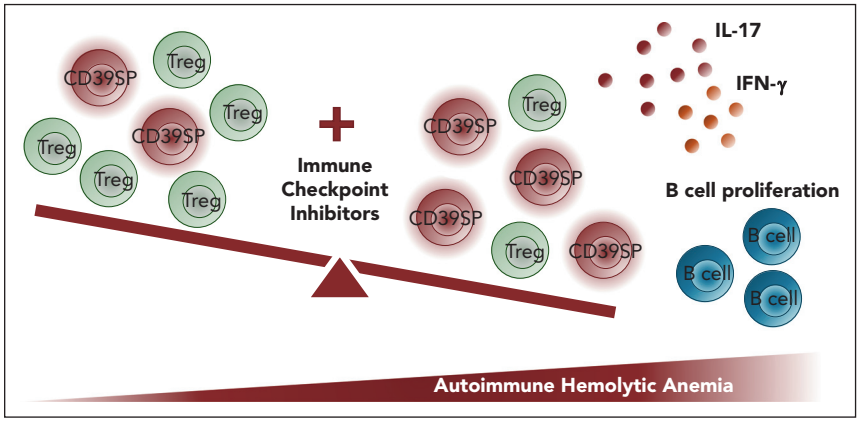
Check for updates



Combined immune checkpoint inhibitors disrupt T-cell tolerance, leading to the expansion of CD39SP T cells and the onset of autoimmune hemolytic anemia (AIHA). Combined ICPis (blocking antibodies against PD-1, Lag-3, CTLA-4, and IL-10R) promote the expansion of CD39SP, causing an imbalance between Treg and effector T cells. CD39SP cells release proinflammatory cytokines interleukin-17 (IL-17) and interferon-γ (IFN-γ), stimulate B-cell proliferation, and promote the development of AIHA.[1]

The study also demonstrated that injection of apyrase, an enzyme that degrades ATP, alleviated AIHA with reduced autoantibodies, improved anemia, and increased probability of survival.[1] This has significant implications for the future development of potential treatment options when CD39SP cells are increased and pathogenic during disease progression.

In conclusion, this study advances the understanding of underlying mechanisms of ICPi-induced AIHA, suggests that CD39SP may be an early predicator of potential ICPi-induced AIHA, and indicates that apyrase may be an attractive treatment where CD39SP is pathogenic. A critical next step is to validate the broader presence of increased CD39SP in idiopathic AIHA and other types of AIHA secondary to different conditions.

Conflict-of-interest disclosure: J.Y.C. reports no competing financial interests. ■

REFERENCES

1. Dei Zotti F, Qiu A, D'Agati VD, et al. Mitigation of checkpoint inhibitor–induced autoimmune hemolytic anemia through modulation of purinergic signaling. *Blood*. 2024;144(15):1581-1594.
2. Johnson PC, Gainor JF, Sullivan RJ, Longo DL, Chabner B. Immune checkpoint inhibitors—the need for innovation. *N Engl J Med*. 2023;388(16):1529-1532.
3. Tanios GE, Doley PB, Munker R. Autoimmune hemolytic anemia associated with the use of immune checkpoint inhibitors for cancer: 68 cases from the Food and Drug Administration database and review. *Eur J Haematol*. 2019;102(2):157-162.
4. Maquet J, Lafaurie M, Michel M, Lapeyre-Mestre M, Moulis G. Drug-induced immune hemolytic anemia: detection of new signals and risk assessment in a nationwide cohort study. *Blood Adv*. 2024;8(3):817-826.
5. Desmarets M, Cadwell CM, Peterson KR, Neades R, Zimring JC. Minor histocompatibility antigens on transfused leukoreduced units of red blood cells induce bone marrow transplant rejection in a mouse model. *Blood*. 2009;114(11):2315-2322.
6. Dei Zotti F, Qiu A, La Carpia F, Moriconi C, Hudson KE. A new murine model of primary autoimmune hemolytic anemia (AIHA). *Front Immunol*. 2021;12:752330.
7. Huuhtanen J, Kasanen H, Peltola K, et al. Single-cell characterization of anti-LAG-3 and anti-PD-1 combination treatment in patients with melanoma. *J Clin Invest*. 2023;133(6):e164809.
8. Wei SC, Anang NAAS, Sharma R, et al. Combination anti-CTLA-4 plus anti-PD-1 checkpoint blockade utilizes cellular mechanisms partially distinct from monotherapies. *Proc Natl Acad Sci U S A*. 2019;116(45):22699-22709.
9. Borsellino G, Kleinewietfeld M, Di Mitri D, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood*. 2007;110(4):1225-1232.
10. Dwyer KM, Hanidziar D, Putheti P, et al. Expression of CD39 by human peripheral blood CD4+ CD25+ T cells denotes a regulatory memory phenotype. *Am J Transplant*. 2010;10(11):2410-2420.

https://doi.org/10.1182/blood.2024026075

© 2024 American Society of Hematology. Published by Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

LYMPHOID NEOPLASIA

Comment on von Jan et al, page 1595

Targeting therapeutic vulnerabilities in T-PLL

Marwan Kwok[1,2] and Tatjana Stankovic[1] | [1]University of Birmingham and [2]Dana-Farber Cancer Institute

In this issue of *Blood*, von Jan and colleagues present results from an ex vivo drug screen for T-cell prolymphocytic leukemia (T-PLL), demonstrating selective sensitivity of T-PLL cells to idasanutlin and romidepsin, with subsequent validation of these agents in combination with cladribine in syngeneic and patient-derived xenograft mouse models of T-PLL.[1]

Over the past 2 decades, advances in our understanding of the molecular pathogenesis and tumor-specific dependencies of hematologic neoplasms have ushered in an era of rational targeted therapy. Notable successes include tyrosine kinase inhibitors for chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia, JAK/STAT pathway inhibitors for myeloid malignancies, as well as B-cell receptor signaling inhibitors and BCL2 inhibitors for chronic lymphocytic leukemia that have transformed therapeutic paradigms. Nevertheless, despite unprecedented technical advances afforded by next-generation sequencing and functional genomics, the development of novel therapeutics for rare tumor types is lagging, hampered by a paucity of patient biospecimens and animal models for preclinical research. Such is the case

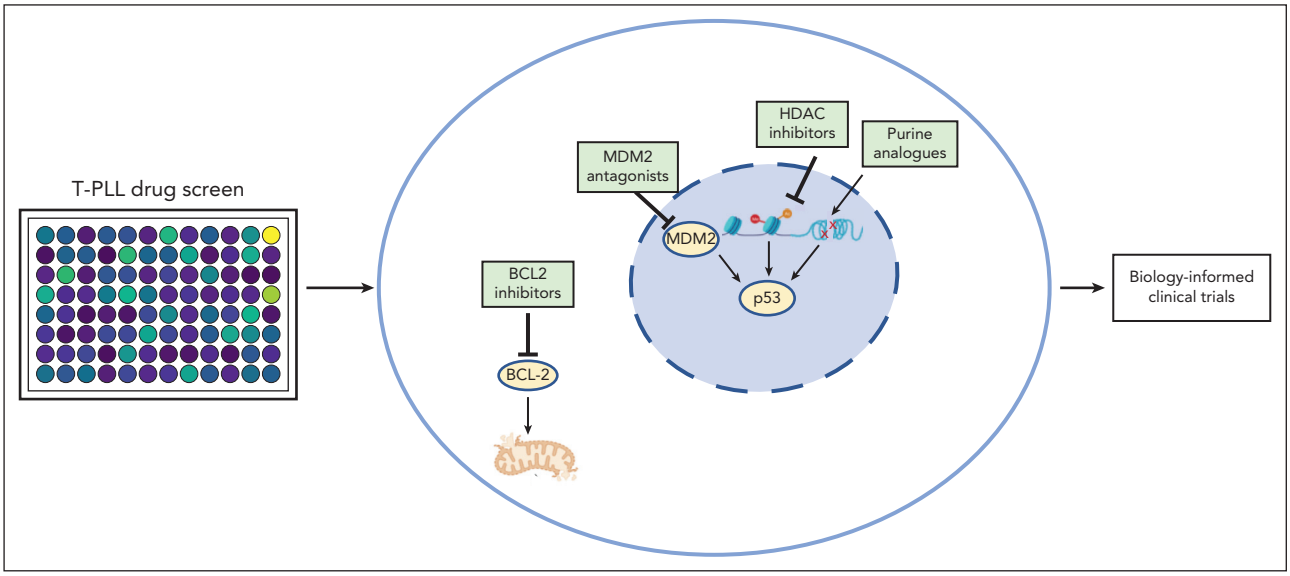
Downloaded from http://ashpublications.org/blood/article-pdf/144/15/1548/2246179/blood_bld-2024-025596-c-main.pdf by guest on 22 October 2024

with T-PLL, where the management of transplant-ineligible patients who inevitably relapse following frontline alemtuzumab clearly represents an unmet clinical need.[2] The collaborative effort by the authors to investigate targeted therapy for T-PLL based on actionable tumor-specific vulnerabilities is therefore important and timely.

Early insights into the genetic basis of T-PLL stem from the observation that T-PLL frequently develops in patients with ataxia telangiectasia, an inherited syndrome characterized by inactivating germ line mutations of *ATM* that functions as a master regulator of cellular response to DNA double-strand breaks. This implicates DNA damage response defect as an important component underlying T-PLL pathogenesis. The latter is substantiated by the genomic landscape of sporadic T-PLL, characterized by predominantly clonal *ATM* somatic mutations or deletions observed in >80% of patients that give rise to monoallelic or biallelic *ATM* loss. Beyond *ATM* alterations, the vast majority of T-PLLs harbor concurrent *TCL1* gene alterations that promote prosurvival signaling.[3] Previous work by the authors demonstrated cooperativity between *ATM* and *TCL1* gene alterations, wherein defective ATM fails to ameliorate DNA double-stranded breaks or induce p53-dependent apoptosis in response to cellular oxidative stress and telomere shortening potentiated by *TCL1* dysregulation, with detrimental consequence for genome stability.[4] *ATM* and *TCL1* aberrations may thus represent synergistic initiating events in T-PLL leukemogenesis, facilitating the subsequent acquisition of additional, predominantly subclonal genomic alterations involving *MYC*, epigenetic modifiers (eg, *EZH2*), microRNA processing (eg, *AGO2*) and JAK/STAT signaling during the course of tumor progression.[5]

Building on this work, the authors postulated that pathogenic mechanisms arising from these genetic defects constitute tumor-specific vulnerabilities in T-PLL that could be therapeutically targeted. Specifically, given that apoptotic resistance in T-PLL emerges predominantly from a failure of p53 induction secondary to ATM dysfunction rather than inactivating *TP53* mutations (a rarer event seen in <5%-15% of T-PLLs), the investigators proposed p53 reactivation as a rational strategy to therapeutically target T-PLL. This could be achieved through inhibition of MDM2 that targets p53 for proteasomal degradation or histone deacetylases (HDAC) that epigenetically repress p53 activity. Additional complementary strategies to promote p53-dependent apoptosis include the induction of DNA damage, inhibition of the antiapoptotic protein BCL2, or suppression of the JAK/STAT pathway as well as cyclin-dependent kinases that promote tumor survival and proliferation. These strategies were tested across a panel of primary T-PLL tumor samples and healthy peripheral blood mononuclear cells, in which MDM2 inhibition with idasanutlin emerged as the most potent and specific treatment against T-PLL cells, followed by HDAC inhibition with romidepsin. Although other strategies were effective in some or most T-PLL tested, their activity was less selective, highlighting functional defects of the ATM-Chk2-p53 axis as an important T-PLL-specific dependency (see figure).

These results corroborated earlier data from a screen of 301 compounds showing that p53 activators were among the most pharmacologically active agents against T-PLL cells and from CRISPR/Cas9 screens on *TP53* wild-type T-cell leukemia/lymphoma lines that identified MDM2 as a cellular vulnerability.[6,7] On the contrary, the more heterogenous response to BCL2 and JAK1/2 inhibitors could be attributed partly to variability in BCL2 dependence, which the authors demonstrated, and to the subclonal nature of activating JAK/STAT mutations in T-PLL. The investigators further showed that cladribine, although being among the most potent DNA damaging agents against T-PLL cells, was insufficient to fully liberate p53 from its inactive MDM2-bound state. The latter was achieved through cotreatment with idasanutlin, underpinning the synergism between cladribine and idasanutlin, which was confirmed in syngeneic and patient-derived xenograft



A comprehensive drug screen identifies the most potent therapeutic combinations in T-PLL. Different combinations of MDM2 antagonists, HDAC inhibitors, BCL-2 inhibitors, and purine analogues exert a potent cytotoxic effect in T-PLL with the potential to improve patients' outcomes.

Downloaded from http://ashpublications.org/blood/article-pdf/144/15/1548/2246179/blood_bld-2024-025596-c-main.pdf by guest on 22 October 2024

mouse models of T-PLL, alongside cladribine and idasanutlin combinations with romidepsin. Cotreatment with cladribine and idasanutlin also augmented promoter accessibility of p53 targets, providing a tantalizing window into the epigenetic regulation of genome stability in T-PLL that could yield additional therapeutic targets and thus warrants further investigation.

Collectively, the data presented by von Jan and colleagues provide a strong foundation for the clinical study of idasanutlin and romidepsin combinations with cladribine in T-PLL. In this regard, several potential caveats should be considered. First, although no major hematologic toxicity was reported in mice treated with idasanutlin in the current study, spontaneous p53 activity can occur in healthy cells in the absence of MDM2 activity,[8] with myelosuppression and infections being frequent adverse events in clinical trials of MDM2 inhibitors.[9] This justifies caution in the assessment and monitoring of treatment-related toxicities, particularly in view of the elderly T-PLL patient demographics. Second, given that intact p53 function is a prerequisite for therapeutic response to MDM2 inhibitor, assessment of baseline *TP53* status is of critical importance to ensure that patients with *TP53* mutations receive alternative treatments. Moreover, the acquisition of inactivating *TP53* mutations during treatment should be anticipated as a potential mechanism of therapeutic resistance.[10] Looking into the future, studies to better understand longitudinal clonal dynamics and tumor-immune microenvironmental interactions in T-PLL may open up additional possibilities for the treatment of this devastating disease.

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

REFERENCES

1. von Jan J, Timonen S, Braun T, et al. Optimizing drug combinations for T-PLL: restoring DNA damage and P53-mediated apoptotic responses. *Blood*. 2024;144(15):1595-1610.
2. Staber PB, Herling M, Bellido M, et al. Consensus criteria for diagnosis, staging, and treatment response assessment of T-cell prolymphocytic leukemia. *Blood*. 2019;134(14):1132-1143.
3. Kiel MJ, Velusamy T, Rolland D, et al. Integrated genomic sequencing reveals mutational landscape of T-cell prolymphocytic leukemia. *Blood*. 2014;124(9):1460-1472.
4. Schrader A, Crispatzu G, Oberbeck S, et al. Actionable perturbations of damage responses by TCL1/ATM and epigenetic lesions form the basis of T-PLL. *Nat Commun*. 2018;9(1):697-719.
5. Braun T, Dechow A, Friedrich G, Seifert M, Stachelscheid J, Herling M. Advanced pathogenetic concepts in T-cell prolymphocytic leukemia and their translational impact. *Front Oncol*. 2021;11:775363-775371.
6. Andersson EI, Pützer S, Yadav B, et al. Discovery of novel drug sensitivities in T-PLL by high-throughput ex vivo drug testing and mutation profiling. *Leukemia*. 2018;32(3):774-787.
7. Ng SY, Yoshida N, Christie AL, et al. Targetable vulnerabilities in T- and NK-cell lymphomas identified through preclinical models. *Nat Commun*. 2018;9(1):2024.
8. Ringshausen I, O'Shea CC, Finch AJ, et al. Mdm2 is critically and continuously required to suppress lethal p53 activity in vivo. *Cancer Cell*. 2006;10(6):501-514.
9. Andreeff M, Kelly KR, Yee KW, et al. Results of the Phase I Trial of RG7112, a Small-Molecule MDM2 Antagonist in Leukemia. *Clin. Cancer Res*. 2015;22:868-876.
10. Maslah N, Verger E, Giraudier S, et al. Single-cell analysis reveals selection of TP53-mutated clones after MDM2 inhibition. *Blood Adv*. 2022;10:2813-2823.

https://doi.org/10.1182/blood.2024025596

© 2024 American Society of Hematology. Published by Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

LYMPHOID NEOPLASIA

Comment on Collinge et al, page 1611

FISHing for clarity in double-hit lymphomas

Stefan K. Alig | Ludwig Maximilian University Hospital

In this issue of *Blood*, Collinge and colleagues[1] explore the biological basis of unbalanced *MYC* fluorescence in situ hybridization (FISH) loss patterns (see figure, bottom) in diffuse large/high-grade B-cell lymphomas with *MYC* and *BCL2* rearrangements (HGBCL-DH-*BCL2*). They convincingly demonstrate that these rearrangements typically preserve *MYC* and result in its upregulation, similar to balanced rearrangements. These findings support the recommendation that unbalanced *MYC* break-apart FISH patterns be regarded as positive for *MYC* rearrangement when diagnosing HGBCL-DH-*BCL2*.

HGBCL-DH-*BCL2* are aggressive non-Hodgkin lymphomas, which are recognized as a distinct entity in both the current World Health Organization (WHO) Classification of Hematolymphoid Tumors and the International Consensus Classification (ICC). Due to the adverse prognosis associated with HGBCL-DH-*BCL2*, intensified treatment regimens, such as dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (DA-EPOCH-R), are used in many parts of the world.[2]

Molecular studies support the clinical distinction of HGBCL-DH-*BCL2*. Almost all cases belong to the germinal center B-cell (GCB) DLBCL subtype and are enriched within the C3/EZB genetic classes.[3-6] Recent studies further demonstrate that most HGBCL-DH-*BCL2* exhibit a centroblast-like gene expression signature reflective of the dark zone of the germinal center, similar to Burkitt lymphomas.[6-8] Interestingly, these signatures are not confined to HGBCL-DH-*BCL2*, but they identify nearly twice as many aggressive GCB cases lacking double-hit status by FISH, with a dismal prognosis. This suggests that *BCL2* and *MYC* translocations do not fully explain the distinct underlying biology of these lymphomas.

Clinically, identifying HGBCL-DH-*BCL2* relies on FISH using break-apart probes to detect the disease-defining translocations. Occasionally, unbalanced *MYC* break-apart patterns, where the red or

Downloaded from http://ashpublications.org/blood/article-pdf/144/15/1548/2246179/blood_bld-2024-025596-c-main.pdf by guest on 22 October 2024