REFERENCES

1. Bueno C, Barrera S, Bataller A, et al. $CD34^{+}CD19^{-}CD22^{+}$ B-cell progenitors might underlie phenotypic escape in patients treated with CD19-directed therapies. *Blood*. 2022;140(1):38-44.
2. Shah NN, Fry TJ. Mechanisms of resistance to CAR T cell therapy. *Nat Rev Clin Oncol*. 2019;16(6):372-385.
3. Nagel I, Bartels M, Duell J, et al. Hematopoietic stem cell involvement in *BCR-ABL1*-positive ALL as a potential mechanism of resistance to blinatumomab therapy. *Blood*. 2017;130(18):2027-2031.
4. Rabilloud T, Potier D, Pankaew S, Nozais M, Loosveld M, Payet-Bornet D. Single-cell profiling identifies pre-existing CD19-negative subclones in a B-ALL patient with CD19-negative relapse after CAR-T therapy. *Nat Commun*. 2021;12(1):865.
5. Myers RM, Taraseviciute A, Steinberg SM, et al. Blinatumomab nonresponse and high-disease burden are associated with inferior outcomes after CD19-CAR for B-ALL. *J Clin Oncol*. 2022;40(9):932-944.
6. Libert D, Yuan CM, Masih KE, et al. Serial evaluation of CD19 surface expression in pediatric B-cell malignancies following CD19-targeted therapy. *Leukemia*. 2020; 34(11):3064-3069.
7. Pillai V, Muralidharan K, Meng W, et al. CAR T-cell therapy is effective for CD19-dim B-lymphoblastic leukemia but is impacted by prior blinatumomab therapy. *Blood Adv*. 2019;3(22):3539-3549.
8. O'Brien MM, Ji L, Shah NN, et al. Phase II trial of inotuzumab ozogamicin in children and adolescents with relapsed or refractory B-cell acute lymphoblastic leukemia: Children's Oncology Group Protocol AALL1621. *J Clin Oncol*. 2022;40(9):956-967.
9. Fry TJ, Shah NN, Orentas RJ, et al. CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. *Nat Med*. 2018;24(1):20-28.
10. Fousek K, Watanabe J, Joseph SK, et al. CAR T-cells that target acute B-lineage leukemia irrespective of CD19 expression. *Leukemia*. 2021;35(1):75-89.

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LYMPHOID NEOPLASIA

Comment on Muller et al, page 45

Please eat me! Targeting CD47 and CD38 in T-ALL

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In this issue of *Blood*, Müller et al[1] demonstrate the efficacy of a new combinatorial strategy for targeting pediatric T-cell acute lymphoblastic leukemia (T-ALL).

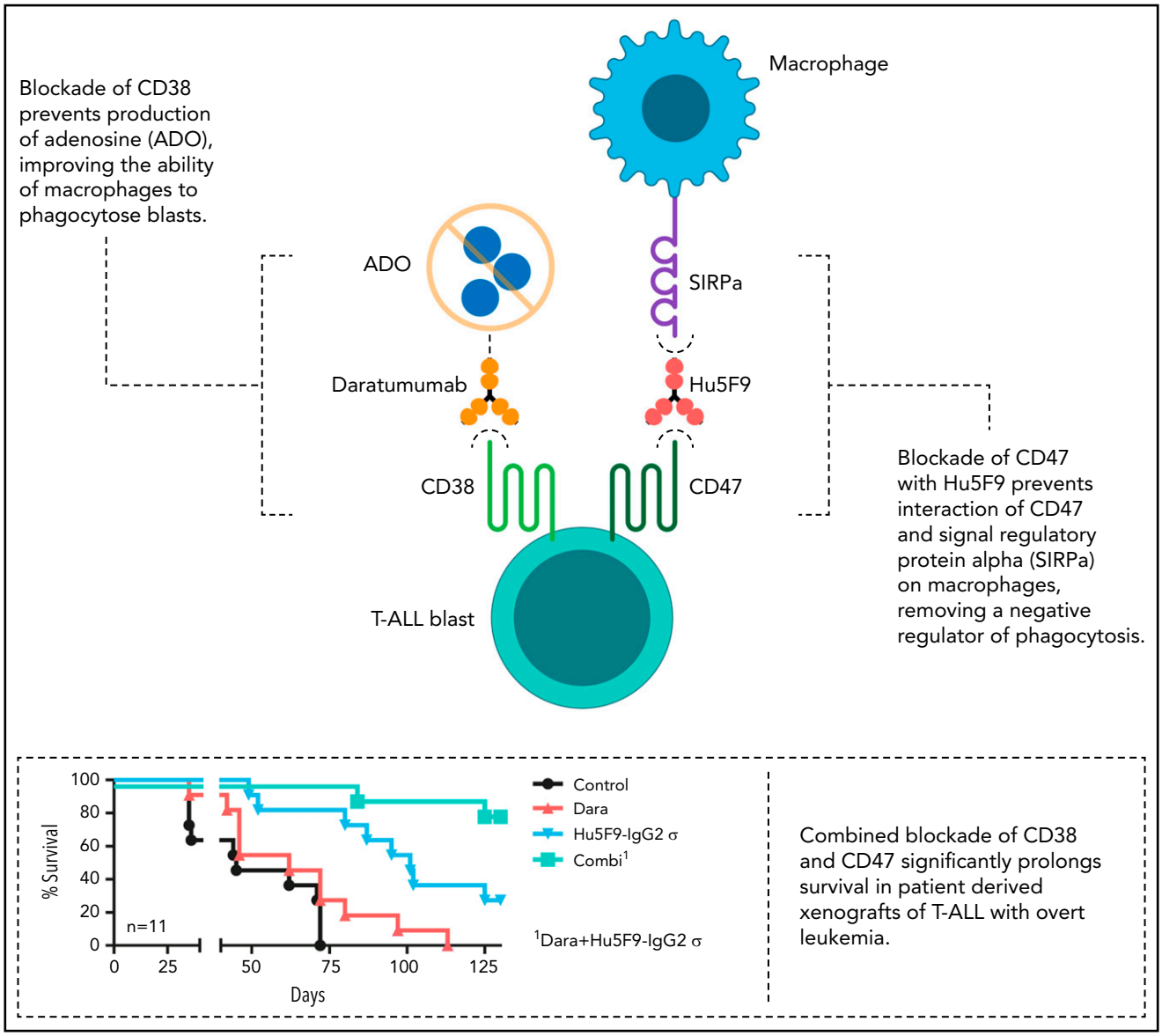
Survival rates in pediatric T-ALL, which makes up approximately 10% to 15% of pediatric ALLs, have improved steadily over the last decades.[2] Survival in adult patients has also improved but is significantly lower than that in children. In the setting of relapsed disease, survival in pediatric and adult T-ALLs remains poor, because salvage therapies are ineffective for most patients. New treatment strategies for this population are badly needed.

Immunotherapy for T-ALL has lagged behind that for B-cell ALL for several reasons, including the fundamental heterogeneity of T-ALL blasts. Moreover, antigens that are widely present on normal T cells risk significant toxicity with immune therapies. CD38 has previously been identified as an ideal target for immunotherapy in T-ALL. It has high surface expression on T-ALL blasts, and expression is durable during chemotherapy and after relapse.[3-5] CD38 is expressed on activated T cells and terminally differentiated B cells but is only expressed at low levels on normal lymphoid and myeloid cells. Expression is absent to low on most healthy tissues. The use of the CD38-targeting antibody daratumumab in combination with chemotherapy was investigated for relapsed pediatric T-ALL in a recently completed early-phase trial (registered at www.clinicaltrials.gov as #NCT03384654). The results of the trial will be presented at the American Society of Clinical Oncology Annual Meeting and European Hematology Association Annual Meeting in 2022. Preliminary results seem encouraging, with an overall response rate (complete remission and complete remission with incomplete count recovery) of 83.3% in children and 60% in young adults with relapsed T-ALL.[6,7]

Muller et al identify CD47 as an additional target that is highly expressed on pediatric T-ALL blasts, with CD47 and CD38 expression correlated ($r = 0.46$). CD47 acts as an inhibitor of phagocytosis, with blasts using CD47 expression to send a "don't eat me" signal to host macrophages via signal regulatory protein α (see figure). Blocking CD47 removes this negative signal, thereby allowing macrophages to phagocytose T-ALL blasts. Why targeting CD38 is effective is not fully understood; however, CD38 has a key role in the generation of extracellular adenosine, which also negatively regulates phagocytosis by macrophages (see figure). Thus, cotargeting CD38 and CD47 may have a synergistic effect that improves antibody-dependent cellular phagocytosis. Indeed, Muller et al demonstrate the efficacy of this strategy in multiple preclinical models and by targeting CD47 with both pharmacologic inhibition and antibody-based approaches.

Using patient-derived xenograft models of T-ALL, Muller et al show that targeting CD47 alone is an efficacious strategy in mice in a minimal residual disease–like state and with overt leukemia. Having data in both settings is important, because some immunotherapies may not be effective in the setting of bulk disease but may be highly effective at clearing minimal residual disease. Most importantly, in a relapsed model of leukemia, they demonstrate that targeting both CD47 and CD38 significantly prolongs survival, while targeting either alone is insufficient (see figure). This is of particular importance, because relapsed disease most closely recapitulates the clinical scenario where CD47 and CD38 dual targeting could be considered.

The optimal strategy for targeting CD47, whether in combination with daratumumab or alone, remains to be determined. Muller et al show the efficacy of both pharmacologic and antibody-based therapies in in vitro and in vivo models. As the authors acknowledge, some previous clinical studies of anti-CD47 antibodies were discontinued because of destruction of normal hematopoietic cells.[8] However, in the setting of relapsed or refractory T-ALL, this may be an



Blockade of CD38 prevents production of adenosine (ADO), improving the ability of macrophages to phagocytose blasts (top left). Blockade of CD47 with Hu5F9 prevents interaction of CD47 and signal regulatory protein α (SIRPa) on macrophages, removing a negative regulator of phagocytosis (middle right). Combined blockade of CD38 and CD47 significantly prolongs survival in patient-derived xenografts of T-ALL with overt leukemia (bottom right). Professional illustration by Somersault18:24.

acceptable risk, because these patients typically require hematopoietic stem cell transplantation for cure of their disease.

Several promising immunotherapeutic approaches are currently in preclinical and clinical development for T-ALL. Chimeric antigen receptor (CAR) T cells have shown particular promise in B-cell ALL, and the first results from clinical trials applying CAR T cells in T-ALL were recently published, using CD7 as a target.[9] In addition, several trials testing autologous and allogenic CAR T cells targeting CD2, CD5, CD7, and CD38 are in clinical development.[10] Anti-CD47 monoclonal antibodies are particularly attractive, because they could theoretically synergize with CAR T-cell therapies. Critically, preclinical data testing CAR T cells with anti-CD47 monoclonal antibodies are needed, and trials combining anti-CD47 monoclonal antibodies plus CAR T cells would need to be carefully designed, because activating macrophages could worsen cytokine release syndrome. The work by Muller et al highlights the critical importance of preclinical studies testing combinatorial immunotherapy approaches. Finally, although the current report is focused on T-ALL, it is important to highlight that these results could also affect other T-cell malignancies, including T-cell lymphoblastic lymphoma and Sezary syndrome.

With a rigorous series of experiments, Muller et al demonstrate the potential of dual targeting of both CD47 and CD38 as an efficacious strategy in relapsed or refractory T-ALL. This work represents an important foundation for future clinical studies and the promise of a new therapeutic avenue for a population of patients with few options.

Conflict-of-interest disclosure: D.T.T. has patents pending on chimeric antigen receptor T cells for acute lymphoblastic leukemia; receives research funding from BEAM

Therapeutics and NeoImmune Tech; and serves on advisory boards for Sobi, Janssen, and BEAM Therapeutics. C.D. declares no competing financial interests. ■

REFERENCES

1. Müller K, Vogiatzi F, Winterberg D, et al. Combining daratumumab with CD47 blockade prolongs survival in preclinical models of pediatric T-ALL. *Blood*. 2022; 140(1):45-57.
2. Teachey DT, Pui CH. Comparative features and outcome between pediatric T- and B-acute lymphoblastic leukemia. *Lancet Oncol*. 2019;20(3):142-154.
3. Bride KL, Vincent TL, Im SY, et al. Preclinical efficacy of daratumumab in T-cell acute lymphoblastic leukemia. *Blood*. 2018;131(9): 995-999.
4. Ofran Y, Ringelstein-Harlev S, Slouzkey I, et al. Daratumumab for eradication of minimal residual disease in high-risk advanced relapse of T-cell/CD19/CD22-negative acute lymphoblastic leukemia. *Leukemia*. 2020; 34(1):293-295.
5. Vogiatzi F, Winterberg D, Lenk L, et al. Daratumumab eradicates minimal residual disease in a preclinical model of pediatric T-cell acute lymphoblastic leukemia. *Blood*. 2019;134(8):713-716.
6. Hogan LE, Bhatla T, Teachey DT, et al. Efficacy and safety of daratumumab (DARA) in pediatric and young adult patients with relapsed/refractory T-cell acute lymphoblastic leukemia (ALL) or lymphoblastic lymphoma (LL): results from the phase 2 DELPHINUS study. Presented at the 58th Annual Meeting of the American Society of Clinical Oncology. 3-7 June 2022. Chicago, IL.
7. Vora A, Bhatla T, Teachey DT, et al. Efficacy and safety of daratumumab in pediatric and young adult patients with relapsed/refractory T-cell acute lymphoblastic leukemia or lymphoblastic lymphoma: results from phase 2 DELPHINUS study. Presented at the 27th Annual Congress of the European Hematology Association. 9-12 June 2022. Vienna, Austria.
8. Zhang W, Huang Q, Xiao W, et al. Advances in anti-tumor treatments targeting the CD47/SIRPα axis. *Front Immunol*. 2020; 11:18.
9. Pan J, Tan Y, Wang G, et al. Donor-derived CD7 chimeric antigen receptor T cells for T-cell acute lymphoblastic leukemia: first-in-human, phase I trial. *J Clin Oncol*. 2021;39(30):3340-3351.
10. Scherer LD, Brenner MK, Mamonkin M. Chimeric antigen receptors for T-cell malignancies. *Front Oncol*. 2019;9:126.

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MYELOID NEOPLASIA

Comment on Tashakori and colleagues, page 58

Revealing the dark secrets of *TP53*-mutated AML

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In this issue of *Blood*, Tashakori et al[1] elucidated the genomic and proteomic features of TP53-mutated acute myeloid leukemia (AML), a disease with a dismal prognosis that is currently spread across several AML disease categories.

TP53 mutation confers a poor prognosis for multiple neoplasms, and AML is no exception. *TP53* mutation is considered in the adverse prognostic group of the 2017 ELN (European LeukemiaNet) Classification of AML,[2] although recent data suggest that *TP53*-mutated AML confers a particularly poor prognosis compared with other ELN adverse cases, with a 2-year median overall survival of only 12.8% even when intensively treated.[3] Moreover, the dismal effect of *TP53* on patient outcome appears to transcend both blast count and disease ontogeny, with equally poor outcomes whether patients present as myelodysplastic syndrome (MDS) or AML, and whether the disease is therapy-related or clinically de novo.[4] However, several nagging questions remain as to how to best categorize AML patients in which *TP53* mutations are detected. Are all somatic *TP53* mutations equally pathogenic? Does the variant allele frequency (VAF) of the mutation matter? Although mostly associated with complex karyotypes, do cases with noncomplex karyotypes differ in their behavior? Is the loss of the wild-type allele (located at chromosome 17p) required for mutated *TP53* mutation to exert its effects, promoting genetic instability and an aggressive disease phenotype?

In a comprehensive analysis of 442 AML patients with mutated *TP53*, Takashori and colleagues have begun to answer these questions, shedding light on the heterogeneity of *TP53*-mutated AML and underscoring the importance of nuanced interpretation of individual *TP53* mutations. The authors found a diverse spectrum of mutations in the gene, with missense mutations in the DNA-binding domain being most common, followed by nonsense, frameshift, deletion, and splice-site mutations (the latter also mainly distributed around the DNA binding domain). They found a wide range of *TP53* mutation VAFs, which correlated only loosely with the bone marrow blast percentage, underscoring the fact that *TP53* mutations are usually carried not only with the blasts but also in nonblast hematopoietic cells.[5]

Mutant p53 protein, largely by virtue of its resistance to degradation, accumulates in affected cells and can be detected by immunohistochemistry in bone biopsy sections.[6] Tashakori and colleagues applied p53 immunohistochemistry to a series of 211 AML patients and confirmed that the staining pattern correlated very strongly with the presence of *TP53* mutation. Using a digital image analysis algorithm, they arrived at an optimal cutoff of 7.2% strong p53-positive cells (or completely absent staining due to a truncated protein), achieving a positive predictive value of 93.75% and negative predictive value of 91.57% in their cohort. Unlike *TP53* mutation analysis by next-generation sequencing (NGS), which typically has a turnaround time of 1 to 2 weeks, immunohistochemistry can usually be performed in 24 hours and thus has the potential to identify these ultra high-risk AML patients more rapidly than waiting for NGS results. Moreover, immunostaining revealed several discordant cases in which a *TP53* mutation was overcalled on the initial NGS report, suggesting potential utility as an orthogonal method to confirm a biologically significant mutation.

The authors also examined factors influencing outcomes in their *TP53*-mutated AML cohort. As expected, an adverse (usually complex) karyotype was associated with shorter overall survival and was seen in the vast majority of patients. In concordance with the data for MDS, multihit *TP53* mutation (due to copy number loss or multiple mutations) was also associated with shorter survival. An important aspect is whether an intact wild-type TP53 allele can partly abrogate the effect of a single mutated TP53 gene. In MDS, single (monoallelic) TP53-mutated