CRISPR/Cas deletion of aberrant splice sites has also recently been described for severe retinal dystrophy.[2] The Xu et al article and the article by Maeder et al[2] demonstrate that this novel strategy may be close to clinical application, and again, this is great news for patients and their families.

*Conflict-of-interest disclosure: T.M.T. is a shareholder in HemEdits, LLC.* ■

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**IMMUNOBIOLOGY AND IMMUNOTHERAPY**

*Comment on Sánchez-Martínez et al, page 2291*

# Anti-CD1a CAR T cells to selectively target T-ALL

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**In this issue of *Blood*, Sánchez-Martínez and colleagues present a novel approach to the treatment of T-acute lymphoblastic leukemia (T-ALL) using chimeric antigen receptor (CAR) T cells redirected against CD1a[1] in in vitro and xenograft models.**

Recently, considerable progress has been made in the treatment of relapsed/refractory B-acute lymphoblastic leukemia (B-ALL) by application of antigen-directed immunotherapies such as blinatumomab, inotuzumab, and anti-CD19 CAR T cells.[2] Of these new approaches, perhaps the most promising is anti-CD19 CAR T cells, which can induce long-lasting remissions.[3]

Given the similarities between B- and T-ALL, development of CAR T-cell therapy against T-ALL seems a rational approach. However, equivalent progress has not been made,[4] mostly because CAR T-cell targeting of T-ALL is more difficult than that of B-ALL. CAR T-cell therapies against B-ALL target pan B-cell antigens. This strategy is feasible because the concomitant profound and prolonged B-cell aplasia is tolerable. However, the T-cell aplasia following CAR targeting of pan T-cell antigens would be prohibitively immunosuppressive. Furthermore, expression of the target antigen on the CAR T cells themselves may cause so-called "fratricide," where CAR T cells kill each other.

A recent approach has been described where 1 of 2 alleles at the T-cell receptor (TCR) β constant region is targeted, allowing preservation of approximately half of the normal T-cell compartment. However, this approach is mostly applicable to mature T-cell malignancies, because only a minority of cases of T-ALL express surface TCR.[5] An alternative approach is to generate CAR T cells targeting a pan T-cell antigen, from T cells in which expression of the pan T-cell antigen has been disrupted. Such disruption is typically achieved by using genome editing, and target antigens proposed for such a strategy include CD5[6] and CD7.[7,8] This approach prevents fratricide but will likely lead to T-cell aplasia, which would require rescue by allogeneic hematopoietic stem cell transplant. A simpler strategy would be possible if an antigen selectively expressed by T-ALL blasts but not by normal T cells was targeted.

In the present study, Sánchez-Martínez et al propose CD1a as a selective T-ALL target. CD1a is a transmembrane glycoprotein that presents self- or bacterial-derived lipids to specialized T cells. It is present on ~40% of cases of T-ALL, where its expression defines cortical T-ALL. CD1a expression on normal tissues is limited to a subset of skin-resident dendritic cells (Langerhans cells, LC) and a developmentally transient thymocyte population: crucially, it is not present on mature T cells. Sánchez-Martínez et al demonstrate that anti-CD1a CAR T cells could be generated without the use of genome editing, were not prone to fratricide or exhaustion, were able to efficiently lyse cortical T-ALL blasts in vitro and in multiple murine models, but spared normal T cells. Importantly, when anti-CD1a CAR T cells were incubated with fetal thymocytes, the majority of cells were preserved, indicating this therapy might not induce thymic ablation.

There are however limitations to CD1a as a target for T-ALL. First, only a minority of cases of T-ALL will express this antigen. CD1a has been associated with relatively good prognosis[4]; thus, the proportion of patients with CD1a$^+$ relapsed and refractory disease will be small. Furthermore, in those cases that do express CD1a, blast expression of the target may not be uniform, leading to the possibility of selection of preexisting CD1a negative blasts. Another mechanism for escape might be downregulation of target, which does not seem critical for blast survival. Furthermore, the clinical consequences of depletion of CD1a-expressing thymocytes and LC are yet to be completely elucidated. LCs are the predominant antigen-presenting cells in the skin and are also present at other mucosal surfaces: their absence could lead to localized immunodeficiency, autoimmunity, or keratosis.

The work by Sánchez-Martínez et al forms part of a growing literature describing CAR T-cell strategies for T-ALL. In addition to the approach where fratricide is prevented by genome editing, we anticipate continuing efforts to develop alternative targeting approaches that avoid T-cell aplasia. These may include CARs against combinatorial "logic-gated" antigen pairs to target pan T-cell antigens without inducing T-cell aplasia. Alternatively, multiplexed targeting of selective antigens such as CD1a with overlapping expression profiles may prevent antigen-negative escape and broaden the pool of potential recipients. This study by Sánchez-Martínez et al is an important first step in practicable CAR T-cell therapy for T-ALL and offers hope that the considerable success of CAR T cells in B-ALL can soon be extended to patients with T-ALL.

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**MYELOID NEOPLASIA**

*Comment on Sasca et al, page 2305*

# NCAM1 supports therapy resistance and LSC function in AML

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**In this issue of *Blood*, Sasca et al present intriguing data that the cell surface marker NCAM1 (neural cell adhesion molecule 1, also known as CD56), which is expressed in approximately 15% to 20% of acute myeloid leukemia (AML) cases, promotes chemotherapy resistance and leukemia stem cell (LSC, also referred to as a leukemia initiating cell) function, in part through activation of the MAPK signaling cascade (see figure). Notably, they provide data that pharmacologic inhibitors of the MAPK pathway synergistically cooperate with cytarabine (Ara-C) in eliminating NCAM1-expressing AML cells, suggesting that NCAM1 may represent both a potential targetable vulnerability and a biomarker for guiding treatment decisions in AML.[1-4]**

AML arises from the clonal outgrowth of mutated hematopoietic stem and progenitor cells that display uncharacteristic properties such as self-renewal, augmented growth, and an inability to differentiate into mature progeny.[5,6] The need for more-effective therapies is clear, as many patients are refractory to first-line chemotherapies, and chemotherapy-resistant relapse is a major contributor to treatment failure. However, AML is a complex and dynamic malignancy that can arise from myriad combinations of infrequent genetic mutations in which patients frequently display multiple, mutationally diverse coexisting clones.[6,7] Although efforts to target certain, more commonly mutated genes (such as *FLT3* or *IDH2*) have shown clinical efficacy, strategies targeting nonmutated molecules or pathways that are aberrantly expressed/activated irrespective of genetic subtype are highly desirable.

NCAM1 expression is largely associated with healthy neural tissue and lymphocytic lineage cells such as NK cells, but has also been observed in numerous solid cancers and hematologic tumors. In AML, its expression is found in 15% to 20% of patients analyzed, particularly in t(8;21) and acute promyelocytic leukemia, where it identifies a subgroup of patients with a more unfavorable prognosis, extramedullary leukemia, and shorter remissions.[2-4] However, before the study put forth in this issue by Sasca et al, the molecular mechanisms underlying these associations were uninvestigated.

Sasca et al show that *NCAM1* expression is heterogeneous across different genetic subtypes of AML, with the exception of complex-karyotype AML (CK-AML) as well as AMLs bearing 11q23 rearrangements or t(8;21), which display significantly higher *NCAM1* levels relative to other genetic subtypes.[1] Sasca et al also present data that NCAM1 expression is driven by several transcription factors, such as MEIS1, MEF2C, and STAT1, but do so in a cell context manner (see figure).[1]

To investigate the functional role of NCAM1 in human AML cell biology, Sasca et al employ an inducible shRNA system and observe that inhibition of NCAM1 expression selectively inhibits the growth of NCAM1-positive (NCAM1$^+$) human AML cells, both in vitro and in vivo. Notably, Sasca et al also show that NCAM1 inhibition renders NCAM1$^+$ AML cells more sensitive to the first-line AML chemotherapy Ara-C in vivo, and that enforced NCAM1 expression partially protects NCAM1-negative (NCAM1$^-$) AML cells from Ara-C or daunorubicin treatment in vitro.

Given that LSCs are purported to be the architects of disease relapse and chemotherapy evasion,[8] Sasca et al explored the role of NCAM1 in LSC biology in a mouse model of AML driven by the 11q23 rearrangement, MLL-AF9. A comparison of various malignant and healthy murine hematopoietic stem and progenitor cell populations revealed that MLL-AF9-expressing LSCs display significantly higher levels of Ncam1 compared with all other populations. Importantly, Sasca et al also show that deletion of *Ncam1* significantly extended the time of disease onset and drastically reduced the frequency of LSCs in this model.

Using a combination of phospho-proteomic and RNA-seq analyses, Sasca et al found that inhibition of NCAM1 diminished several signal transduction pathways, including MAPK signaling, as well as transcriptional