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This CAR won't start: predicting nonresponse in ALL

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Comment on Masih et al, page 4218

In this issue of *Blood Advances*, the article by Masih et al[1] presents a novel, multiomic approach to predict which patients with relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL) will not respond to CD19-targeted chimeric antigen receptor (CAR) T cells. The authors defined primary nonresponse (PNR), which occurs in ~10% to 20% of cases, as patients who failed to achieve or maintain a measurable residual disease (MRD)-negative remission 63 days after CAR T-cell infusion in spite of CAR T-cell expansion and persistent leukemic expression of CD19. By identifying these patients before treatment with a high degree of specificity, it may be possible to spare them from the toxicities of CAR T-cell therapy, including its extraordinary cost,[2] and pre-emptively select alternative therapies to overcome identified mechanisms of resistance.

In contrast to PNR, most CAR T-cell failures are due to relapse after an initial MRD-negative response, in which consolidative allogeneic blood or bone marrow transplantation mitigates relapse risk.[3] In addition, CD19 antigen loss is a common mechanism of relapse,[4] and dual antigen targeting (ie, CD19 and CD22) may prevent such relapses in the future.[5] Thus, although relapse is more common than PNR, the latter represents a more pressing need because of the current lack of mitigation strategies. After assessing several factors, including cytogenetics, prior therapy, T-cell factors, and the presence of CD19 isoforms, the authors posit that a stem cell epigenome within the leukemia cells underlies PNR to CD19 CAR T cells in B-ALL.

The analysis by Masih et al is limited by small numbers in detecting associations between PNR and prior therapy, disease burden, or cytogenetics; but larger analyses have previously interrogated these variables (see table). The response rate to CD19-targeted CAR T cells is lower among patients with a history of nonresponse to the CD19-targeted bispecific T-cell engager blinatumomab (64.5%) than among patients who previously responded (92.9%) or were unexposed to blinatumomab (93.5%).[6] Because most blinatumomab nonresponders will respond to CD19 CAR T-cell therapy, it remains a good option to treat such patients. Similarly, a high disease burden (HB), defined as >5% blasts on the most recent bone marrow assessment, is a risk factor for nonresponse to CAR T cells, with 73% of HB patients responding compared with response rates of 98% and 100% in patients with a low disease burden or undetectable disease, respectively.[7] Notably, among the 14 patients analyzed by Masih et al, the median bone marrow blasts before CAR T-cell therapy were higher among patients with PNR (92%) than among responding patients (59%; $P = .08$), which included 2 patients with <5% bone marrow blasts. It remains unclear whether HB is a modifiable risk factor that can be ameliorated through additional pre–CAR T-cell treatment or merely a marker of leukemias that are inherently more aggressive and treatment-resistant. Although conventional cytogenetic risk groups have prognostic relevance in relapsed ALL treated with cytotoxic chemotherapy,[8] they do not correlate with PNR or long-term outcomes with CD19 CAR T cells.[9] Further studies are needed to better define outcomes in specific subtypes (ie, KMT2A-rearranged, Philadelphia chromosome–positive, etc), but even small subsets suggest these are not risk factors for PNR. Prior nonresponse to blinatumomab and HB are clear risk factors for PNR, so although patients with these characteristics are still likely to respond to CD19 CAR T cells, they should be considered for clinical trials that aim to augment their efficacy.

The identity and function of the native T cells before transformation to create autologous $CD19^+$ CAR T-cell products may play an important role in PNR. In one study, patients with PNR could be sensitively and specifically distinguished from responders based on their $CD8^+$ T cells at the time of pheresis, which showed an increased expression of LAG-3 and a decreased capacity to secrete tumor necrosis factor α.[10] Interestingly, these same attributes were not observed in the infused CAR T-cell products, but the CAR T cells of patients with PNR had subsequent rapid increases in LAG-3 and TIM-3

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Factors associated with PNR to CD19 CAR T cells

Risk factor	Response rates
Prior blinatumomab	64.5% in blinatumomab nonresponders vs 92.9% in blinatumomab responders vs 93.5% in blinatumomab-untreated patients[6]
Bone marrow disease burden	73% in HB (>5% blasts) vs 98% in low disease burden vs 100% in undetectable[7]
Cytogenetics	93% in high risk vs 86% in intermediate risk vs 98% in favorable risk[9]
Apheresed peripheral blood T cells	28.6% when CD8 cells express LAG3 ≥0.745% AND TNF-α <25.283% vs 100% for all others[10]
CD19 isoforms	The presence of increased transcripts with CD19 isoforms skipping exon 2 yield nonresponse[1,11]
Bone marrow epigenetics	Hypermethylation at genes known to be targets of PRC2 repression in embryonic stem cells yields nonresponse[1]

TNF-α, tumor necrosis factor α.

expression after engraftment, consistent with exhaustion. Because LAG-3 inhibitors are now commercially approved based on combination immunotherapy studies in melanoma,[12] there is an intervention that could potentially augment responses in patients identified to be at risk of PNR. Masih et al observe a downregulation of genes involved in T-cell cytotoxicity and peripheral homeostasis in PNR, which they conclude points to the underlying leukemias being less immunogenic and coexisting with a differential phenotype of T cells. The implication is that the differences in T-cell populations between patients with PNR and responders are driven by the underlying leukemia, which does seem consistent with differences in the markers of exhaustion observed on T cells at pheresis, before infusion and after engraftment because the T cells are differentially exposed to the leukemia at these time points. These findings would also be consistent with the relative prevalence of PNR among patients with HB, in whom the persistence of leukemic cells at CAR T-cell infusion may quickly lead to the re-expression of markers of exhaustion.

The role of both genetic and epigenetic changes that underlie CD19 antigen loss, which is frequent at relapse after CD19 CAR T-cell therapy, has previously been characterized,[4] but there is little existing evidence supporting that such changes occur in PNR. In both responding patients and patients with PNR, Masih et al found that >70% of the *CD19* transcripts were of the wild-type isoform before therapy, except for 1 patient with PNR who had an alternative *CD19* isoform with exon 2 spliced out, representing 47% of transcripts. The authors suggest that this explains the lack of response to CD19 therapy in this patient, because exon 2 skipping yields a CD19 variant protein that fails to trigger CAR T-cell killing,[11] whereas the patient's persistent CD19 expression is explained by the fact that this splicing event does not interfere with the binding of the antibody used for flow cytometry. This argument would be most compelling if the authors had also shown subsequent changes in the expressed *CD19* transcripts after selective pressure from CD19 CAR T cells and could explain how other leukemias that harbored these same variants at lower frequencies before treatment were protected from that selective pressure. Notably, a previous study examining 2 CD19$^-$ relapses that occurred within 2 months of treatment, similar to the timeframe that has been defined for PNR, showed either a substantial increase (2.5-fold) from pretreatment or overwhelming predominance (97%) of the alternatively spliced CD19 transcript that omits exon 2 after CD19 CAR T-cell therapy. However, both patients also had mutations in the CD19 gene that may better explain the loss of CD19 expression after therapy.[11] Ultimately, more data are needed to understand the importance of genetic and epigenetic changes involving the CD19 gene in CAR T-cell responses and whether the resistance to CD19 CAR T cells that such changes confer can be overcome by targeting other antigens (ie, CD22) or by targeting multiple CD19 epitopes.

Because lineage switch from ALL to acute myeloid leukemia (AML) represents another form of acquired resistance to CD19 CAR T cells, Masih et al interrogated the epigenome of patients with PNR. DNA methylation studies of unsorted bone marrow cells demonstrated differential methylation patterns in PNRs compared with those in responding patients with enrichment of promoter hypermethylation at targets of PRC2 repression in embryonic stem cells, which was subsequently corroborated by chromatin accessibility assays and the differential expression of certain transcription factors. Presented in figures 3 and 4 in the article by Masih et al,[1] these findings represent the study's most compelling data to suggest that PNR leukemias can be specifically differentiated from responders using epigenetic analysis of pretreatment samples. The authors then compare DNA accessibility in PNR and responding leukemias with that in healthy hematopoietic stem cells, from which they conclude that PNR leukemias are more correlated with primitive cell types, suggesting they are less differentiated and have increased plasticity. These findings were subsequently corroborated using single-cell RNA sequencing, in which the B-ALL cells were bioinformatically isolated and demonstrated 5 transcriptionally distinct clusters, with PNR leukemias overrepresented in 2 clusters, with a velocity analysis suggesting these were the more primitive clusters, which had increased expression of genes associated with leukemic stem cells and myeloid phenotypes.[1] Notably, a correlation between these signatures and poorer outcomes in AML has previously been identified after treatment with both cytotoxic chemotherapy and immune checkpoint inhibitors,[13,14] but, to our knowledge, this is the first report of such a signature affecting the therapeutic response in ALL. Ultimately, the authors emphasize that their approach was focused on clinical feasibility, but the aspects that are immediately translatable to facilitate clinical decision-making in real time remain unclear.

The ability to specifically identify patients with PNR in advance of CAR T-cell infusion using clinical data and clinically available laboratory tests would represent an important advance for the field, allowing such patients to enroll on clinical trials to circumvent identified mechanisms of nonresponse. This manuscript and prior publications highlight that there is a complex interplay between leukemic cells and T cells in PNR, and further studies that better integrate findings in these distinct cell populations will certainly be illuminating. Although the findings of T-cell exhaustion in patients with PNR suggest a readily available remedy in immune checkpoint inhibition, the ability to target the stem cell epigenome of the leukemic cells is less clear. Furthermore, much work is needed to facilitate the creation of a clinically available test that can specifically distinguish patients with PNR from responders using pretreatment samples, although this manuscript raises the tantalizing possibility that such tests could be developed using leukemic cells

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or T cells. For now, HB and a history of nonresponse to blinatumomab remain the 2 most clinically available risk factors for PNR, which may prompt referral for clinical trials aimed at augmenting CAR T-cell efficacy.

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