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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Leung et al, page 1726

T cells take aim in AML: targeting IDH2

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In this issue of *Blood*, Leung et al[1] present preclinical findings demonstrating the potential of neoantigen-specific T cells to recognize acute myeloid leukemia (AML) driver mutations. The study assessed the immunogenicity of 14 recurrent driver mutations over 8 genes, confirming their ability to provoke an immune response in donors with varied HLA types. Notably, IDH2^{R140Q} was identified as an immunodominant neoantigen, discernible in both in vitro and in vivo models, thereby enabling selective targeting of malignant over normal hematopoietic cells. This research paves the way for AML-specific immunotherapy, leveraging T-cell-specific activity to target AML cells while sparing healthy counterparts.

Currently employed chimeric antigen receptor (CAR) T cells, but also bispecific antibodies, are engineered to bind to surface antigens presented on the neoplastic cells. In B-cell malignancies, CD19 has been commonly targeted by CAR T cells and bispecifics and has limited toxicity, partially due to the possibility of mitigating toxicity due to B-cell depletion through immunoglobulin replacement. However, in the context of AML, targeting of lineage-restricted antigens, like CD33, CD123, and CLEC12A, has been hampered by increased toxicity due to on-target, off-leukemia toxicity against normal healthy hematopoiesis.[2] In addition the ubiquitous expression of these myeloid antigens might contribute to antigen sink and T-cell exhaustion.[3]

For this reason, there is an increasing effort to target intracellular tumor antigens in AML and other hematologic malignancies. Intracellular tumor antigens are degraded into peptides that are presented on the cell surface by defined major histocompatibility complex (MHC) class I molecules and recognized by T cells though their T-cell receptor (TCR).[4] T cells recognizing overexpressed leukemia-associated antigens, such as WT1, PRAME, and NY-ESO-1, have been described and already successfully transferred into patients with AML. Early clinical trial data demonstrated safety and signs of efficacy.[5] Targeting neoantigens that arise from nonsynonymous mutations should enhance specificity and reduce the probability of the emergence of antigen escape variants, resulting in more precise AML targeting.

In the study, detecting neoantigen-specific T cells from healthy individuals required 25 days of ex vivo stimulation, indicating the low frequency of these T cells in the circulation and the need for amplification for detection. These T cells responded to a range of neoantigens including nonsynonymous mutations derived from KRAS, NRAS, IDH1/2 and FLT3, with IDH2^{R140Q} emerging as an immunodominant epitope. Further characterization of IDH2^{R140Q}-specific T cells led to the identification of a 15-mer peptide that elicited responses mainly in the CD8$^+$ T cells, but few donors also had responses in the CD4$^+$ T-cell subset. The unique immunogenic epitopes were found to be HLA-B*15:01* and *B*35:43 restricted. When primary AML samples harboring an IDH2 mutation were employed as targets, they confirmed the presentation of the neoepitope on the cell surface. Finally, in an NSG-SGM3 mouse model using HLA-modified AML cell lines (Kg1A), the specificity of the response was validated in vivo.

In this article, the authors successfully elicited T-cell responses against neoantigens that originate from mutations driving AML. Specifically, the study demonstrated the strong efficacy of IDH2-specific T cells in vitro and in vivo against AML cell lines and primary cells. This approach offers substantial hope for addressing several challenges in developing immunotherapy in AML. First, this strategy might minimize the risk of on-target, off-leukemia toxicity and might also circumvent adverse effects that arise from direct interactions with monocytes and macrophages that contribute to cytokine release syndrome, persistent antigen stimulation, and antigen sink. Moreover, the technique's potential could be enhanced by isolating and inserting the specific TCR into T cells, paving the way for the development of more potent, engineered T cells.[6]

A barrier to the clinical development of neoantigen-specific T cells is the restriction of genetic mutations within the HLA system. The IDH2^{R140Q} mutation, for instance, appears in roughly 10% of AML cases and is specific to HLA types B15:01 and B35:43, effectively narrowing the

potential patient population for this treatment to under 2% of those with AML. Moreover, therapies based on TCRs are potentially vulnerable to immune evasion tactics, such as the reduction of target antigens or the genes of MHC classes I and II. Despite these obstacles, T cells that target the IDH2^{R140Q} mutation hold substantial promise as an innovative treatment strategy for AML, potentially revolutionizing the way the disease is combated.[7]

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LYMPHOID NEOPLASIA

Comment on Abdelbaky et al, page 1752

New signature predicts MBL-to-CLL progression

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In this issue of *Blood*, Abdelbaky and colleagues report the development of a combined epigenetic and immunogenetic signature to study individuals with high-count monoclonal B-cell lymphocytosis (HC-MBL).[1] Using this signature, they define a new high-risk group with significantly increased likelihood of progression to chronic lymphocytic leukemia (CLL) compared with low-risk individuals.

Twenty-five years ago, 2 seminal articles were published in *Blood*, fundamentally altering the landscape of CLL research.[2,3] These studies demonstrated that patients with CLL with somatically hypermutated immunoglobulin heavy variable (IGHV) genes (M-CLL; 60%-70% of patients) experienced better outcomes than patients with unmutated IGHV genes (U-CLL; 30%-40% of patients). Over time, the IGHV gene mutational status has been established as a robust prognostic and predictive marker. As a result, contemporary guidelines recommend IGHV gene analysis for all patients before initiating treatment.

DNA methylation arrays revealed early on that U-CLL and M-CLL display distinct methylation profiles. In a pivotal study from 2012, Kulis et al compared the methylation signature in CLL with different B-cell differentiation stages.[4] This led to the identification of 2 major "epitypes," one resembling naive B cells, largely corresponding to U-CLL (termed naive B-cell-like CLL), and the other more similar to memory B cells, overlapping with M-CLL (designated memory B-cell-like CLL). An intermediate epitype (i-CLL) with an intermediate methylation pattern and a corresponding intermediate outcome was also identified. In a similar study, based on the level of DNA methylation programming, Oakes et al also identified 3 prognostic subgroups, which were named the high-programmed (HP), intermediate-programmed (IP), and low-programmed (LP) epitypes.[5]

Further characterization of the intermediate epitype revealed an enrichment of CLL cases with borderline IGHV mutational status (97%-97.9% identity) or *SF3B1* mutations or belonging to stereotyped subset 2.[6,7] Subsequently, Maity et al identified a critical somatic mutation (G>C) in the IGLV3-21 gene at position R110 in subset 2, crucial for homeotypic B-cell receptor interaction.[8] Extended analysis showed that nonsubset 2 cases with the IGLV3-21^{R110} mutation had a similarly poor prognosis as subset 2. Furthermore, a recent study suggested that intermediate patients can be regrouped depending on IGLV3-21^{R110} status; patients with IGLV3-21^{R110}-mutated i-CLL demonstrated a poor outcome similar to patients with naive B-cell-like CLL, and patients with IGLV3-21^{R110}-wildtype i-CLL had a similar prognosis as patients with memory B-cell-like CLL.[7]

Healthy individuals may harbor clonal B-cell expansions of varying sizes, termed monoclonal B-cell lymphocytosis, a precursor condition always preceding CLL.[9] The likelihood of MBL increases with age and in patients with first-degree relatives with CLL. MBL can be categorized into low-count MBL (<0.5 × 10^9 clonal B cells per L) and HC-MBL (>0.5 × 10^9 to <5 × 10^9 per L). Although the majority of HC-MBL cases exhibit favorable-prognostic features (IGHV-mutated and low-risk genomics), an estimated 1%-2% of individuals with HC-MBL will develop CLL requiring treatment.[1,9] Considering the relatively low proportion of HC-MBL cases developing high-risk disease, identifying new biomarkers is crucial for early-stage identification of those at high risk of progression.

In the study by Abdelbaky et al, information from DNA methylation profiling was