targets, but responses have been reported (reviewed in Mardiana and Gill [4]).

More complex strategies have been developed. "Logic gating" of CAR signaling can restrict recognition to a pattern of antigen expression, thus improving specificity.[5] For example, He et al[6] described CAR T cells that mediated cytolysis only after joint recognition of CD13 and TIM-3. Given that AML blasts express both targets, but HSCs lack TIM-3, HSCs were relatively spared. Because of the difficulties in sparing normal hematopoiesis, a radical solution of concomitant administration of donor HSCs, genetically edited so as not to express cognate target, has been proposed.[7] More recently, targeting CD70[8] and TIM3[9] has been suggested. These antigens are expressed by blasts and LSCs, but not on HSCs, although expression is found on NK cells and monocytes.

How does Siglec-6 fare against other targets and approaches? Jetani et al show that Siglec-6 is expressed in ∼60% of AML cases, expression is retained on LSCs, but there is little expression on HSCs. Further, expression within the hematopoietic system was restricted to memory B cell and basophil populations. Outside the hematopoietic system, no expression was identified other than the placenta. Furthermore, Jetani et al also demonstrate that Siglec-6 may be a useful target in B-Cell chronic lymphoblastic lymphoma (B-CLL). Siglecs are a large family of proteins that are thought to promote cell-cell interactions and regulate innate and adaptive immune systems through glycan recognition. It is worth noting that cancer immunotherapies targeting Siglecs are not new: CD22 (Siglec-2) and CD33 (Siglec-3) have been targeted with well-investigated immunoconjugates and CAR T cell therapies for ALL and AML, respectively.

The investigators next generated CARs based on a human monoclonal antibody (mAb) and demonstrated function in vitro, as well as in a range of AML models. Siglec-6 expression level is low but appears sufficient to direct CAR-mediated lysis against LSCs among other cells. HSCs were relatively spared after in vitro coincubation with Siglec-6 CAR T cells, although in vivo HSC engraftment studies were not described. One possible limitation of Siglec-6 is that proliferation and cytokine release was observed only when Siglec-6 CAR T cells were co-cultured with target cells expressing higher levels of target antigen.

In summary, Jetani et al expand the possibilities of CAR T-cell targeting in AML. Siglec-6 CAR T cell therapy could be useful for ∼60% of patients with AML and unlike many other targets, should spare normal hematopoiesis. It is hoped that clinical exploration of this and other strategies will bring CAR T cell therapy to patients with refractory AML.

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BETter insight into PRC2-mutated T-ALL

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In this issue of *Blood*, Andrieu et al studied mutations in polycomb repressor complex 2 (PRC2) in adult T-cell acute lymphoblastic leukemia (T-ALL) and correlated their findings with histone modification, gene expression, and sensitivity toward BET inhibitors.[1]

The organization of DNA in chromatin, with open and closed chromatin regions, is important for the regulation of cell-type–specific gene expression and maintenance of cell identity.[2] Chromatin organization is highly dynamic because of the exchange of histone variants, nucleosome remodeling, and reversible DNA and histone modifications. Among the large array of histone modifications, trimethylation and acetylation of lysine 27 of histone 3 are among the best studied and best understood modifications. They can be considered as the yin and yang of gene expression regulation. Trimethylation of histone 3 at position 27 (H3K27me3) is associated with gene suppression, whereas acetylation at the same position (H3K27ac) is associated with gene activation.[2] The H3K27me3 mark is written by the PRC2 complex, a multiprotein complex with EZH2, EED, and SUZ12 as the core components.

Gain-of-function *EZH2* mutations have been described in follicular lymphoma,

whereas loss-of-function mutations in the PCR2 complex are common in a variety of cancers.[2,3] Such loss-of-function mutations in *EZH2*, *EED*, or *SUZ12* are frequent in pediatric T-ALL and are positively associated with IL7R/JAK/STAT pathway mutations.[4] In their study, Andrieu et al evaluated 218 adult patients with T-ALL who were enrolled in the GRAALL-2003-2005 trials with targeted genomic sequencing (all samples), chromatin immunoprecipitation sequencing, and RNA sequencing (selected subset). The authors show that PRC2 mutations are also frequent (26%) in adult T-ALL and observe the association with NF1 inactivation (RAS pathway) and with IL7R/JAK/STAT pathway mutations. Cases involving PRC2 mutations were spread over almost all T-ALL subgroups, with a higher frequency in immature T-ALL and early T-cell precursor-ALL and a very low frequency in the TLX1 subgroup. There was a significant difference between the PRC2 mutation frequency in TLX1 vs the TLX3 subgroup, perhaps suggesting that the very similar TLX1 and TLX3 transcription factors use different transcriptional activities to transform T cells.

The exact reason for the positive associations or mutual exclusivity between PRC2 mutations and other mutations remains to be determined, but they are most likely the result of Darwinian selection for the fittest cell during development of leukemia. Indeed, we recently demonstrated cooperation between mutant *JAK3* and loss of *Suz12* in a mouse T-ALL model,[4] and this combination of mutations is indeed frequent in both pediatric and adult T-ALL. Likewise, strong cooperation between mutant *JAK3* and *HOXA9* expression has been reported,[5] and, in Andrieu et al, *HOXA9* was found to be upregulated by PRC2 loss-of-function mutations.

It remains difficult to pinpoint the exact oncogenic consequences of PRC2 mutation, because this is a general regulator of gene expression, and many genes are affected in leukemia cells with PRC2 mutations. Andrieu and colleagues found that T-ALL cells with PRC2 mutations show lower H3K27me3 levels around transcription start sites and reciprocal higher H3K27ac levels compared with PRC2 wild-type leukemia cells. As a consequence, T-ALL cases with PRC2 mutation had deregulated gene expression with reactivation of a hematopoietic stem cell program, with upregulation of *HOXA* and *HOXC* genes, JAK/STAT pathway genes and genes regulated by BMI1, GATA1, GATA2, and SMAD4.

Adult cases of T-ALL with mutated PRC2 showed a poorer response to treatment with corticosteroids, but showed similar complete remission rates and overall survival compared with the other T-ALL cases. These data suggest that there is no direct prognostic value in the detection of PRC2 mutations, but there could be a therapeutic value. Because these are loss-of-function mutations, there may be no direct role for EZH2 inhibitors. However, what is intriguing from the current study is that the genetic mutations found in members of the PRC2 complex are more often within the structural proteins EED and SUZ12 compared with the enzymatic EZH2 that catalyzes the addition of the methyl groups at H3K27. Although EZH2 inhibition using GSK343 decreased H3K27 methylation in T-ALL cases with wild-type PRC2, there is increasing evidence in other cancers that EZH2 has functions outside of the PRC2 complex and is directly involved in gene activation.[6] Whether any remaining EZH2 expression in PRC2-altered T-ALL also plays a role in driving gene expression remains to be determined, but opens the possibility that observed synthetic lethality with BET inhibitors may still occur when using the newly developed selective degraders of EZH2.[7]

Indeed, T-ALL cells with PRC2 mutations showed a higher sensitivity toward the BET inhibitor JQ1. Interestingly, PRC2 inhibition with the EZH2 inhibitor GSK343 also sensitized T-ALL cells with wild-type PRC2 to BET inhibitor treatment ex vivo and in vivo. Further improvements in synergy may also be seen by moving away from pan-BET inhibitors (eg, JQ1 and OTX015) to the next generation of selective BET inhibitors that can target either the first bromodomain (BD1), responsible for binding chromatin and maintaining gene expression programs, or the second bromodomain (BD2), responsible for regulating inflammation.[8] Together, these data nicely illustrate the rationale for further investigation of BET inhibitors for targeted treatment in T-ALL cases with PRC2 mutations.

The underlying mechanisms of why certain combinatorial chemotherapy, especially for children, is so effective is now being understood using genetics. For example, the combination of 6-mercaptopurine and methotrexate avoids the emergence of clonal resistance to either drug through competing selection of loss and gain of HPRT, leaving no opportunity for leukemia cells to escape therapy.[9] Also in other cancers, insight in cancer genetics and biology have led to the development of novel targeted treatment, and combinations of such drugs are now showing impressive clinical responses, such as the combination of BTK and BCL2 inhibitors in chronic lymphocytic leukemia.[10] What the study by Andrieu and colleagues highlights is how sequencing data continue to help us identify these combinatorial approaches in a more efficient manner over historical empirical methods to cause synthetic lethality in adults with T-ALL.

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