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DOI 10.1182/blood-2018-08-868117

IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Scarfò et al, page 1495

Getting the most from your CAR target

David M. Barrett | Children's Hospital of Philadelphia

In this issue of *Blood*, Scarfò et al describe a new target for chimeric antigen receptor (CAR) T cells, CD37, that reveals how the field of immunotherapy is adapting after the landmark success of CD19 CAR T cells.[1]

The US Food and Drug Administration approval of both tisagenlecleucel for pediatric leukemia and axicabtagene ciloleucel for adult lymphoma was based on the stunning efficacy of targeting CD19-positive malignancies.[2,3] I remember quite clearly the first 2 pediatric patients at the Children's Hospital of Philadelphia who were treated with tisagenlecleucel. The first patient experienced grade 4 cytokine release syndrome, received the first ever dose of tocilizumab for this event, and entered a deep remission that lasts to this day. The second patient also had a complete response but sadly became the first case of CD19-negative or escape-variant relapse only 2 months after infusion and later died of her leukemia.[4] The second patient illustrates a key problem in the field, how to treat or prevent relapse as a result of single antigen loss, which is addressed by the Scarfò et al article.

Antigen-loss variants make up a substantial portion of the relapses seen with CD19 CAR therapies.[5,6] Landmark work with CAR T cells targeting CD22 was the first to target these relapses, but low antigen density and the evolution of cancer to resist immune therapies have resulted in lower initial response rates than that seen with CD19 CARs, and antigen escape remains a problem.[7] Scarfò et al describe CD37, a surface antigen on some B-cell and T-cell malignancies, as a potential CAR target that can be used in combination with CD19 in the setting of first-line therapy or as a treatment for CD19 antigen escape variants. In the field of immunotherapy, the approach to validating a new CAR target is fairly standardized: (1) validate expression on the relevant malignancy, (2) construct a CAR with your favored costimulatory domain, (3) test for killing and cytokine release in vitro, and (4) test in a mouse model. Scarfò et al hit all these benchmarks, and their approach and controls are of high quality. What sets their article apart is an additional and highly impactful observation about their target as well as the combination of their CAR with a CD19 CAR to make a 2-antigen targeting version.

As we learned from CD22 and CD19, there is no guarantee that any single antigen is a perfect target, and this is likely true when considering combination CAR therapy. The concept of combinations can take many forms, including simply 2 CARs in 1 T cell or more complex single chain structures such as the TanCAR approach described by Ahmed and colleagues.[8] Scarfò et al take the latter approach, chaining together the anti-CD37 and anti-CD19 recognition domains into a single long CAR molecule with 1 set of signaling domains. They then validate that this construct works against CD37- and CD19-expressing targets, which gives hope that this kind of construct can suppress and treat antigen loss variants.

The novelty of CD37 as an antigen for CAR T cells extends beyond simply not being CD19. As an important adjunct, this antigen is also expressed on some T cells and in peripheral T-cell lymphomas. T-cell malignancies in pediatrics have high remission rates with chemotherapy, but relapsed T-cell disease has a poor prognosis.[9] The field of immunotherapy has struggled with how to target T-cell malignancies with T-cell therapies, because the issue of fratricide, on the surface, seems to be quite formidable. The advent of gene editing technologies such as TALEN and CRISPR has given cellular therapists tools to delete the target antigen from the CAR T cells, but this approach only kicks the can down the road.[10] If you have CAR T cells targeting a developmental T-cell antigen such as CD2 or CD5, how will the patient recover normal T-cell function? How long do you need to have CAR T cells present to be cured? How can you be sure you eliminate all the CAR T cells with a suicide gene? One of the attractive features of CD37 described by Scarfò et al is that it is present on peripheral T-cell malignancies but does not seem to result in fratricide in CD37 CAR T cells. Finding an antigen that discriminates a malignant T cell from a normal T cell, at least in a subset of T-cell diseases, challenges researchers to look harder for these targets.

In summary, the article by Scarfò et al exemplifies 2 areas at the heart of innovation in cellular therapy for cancer: multiantigen targeting and smart antigen selection. The next phase is testing the CD37 CAR for safety and hopefully moving the combination CD37 and CD19 CAR to the clinic soon thereafter. The family of the second patient we treated generously agreed to let us study her leukemia in the laboratory so we could learn why antigen escape happens and work to prevent it from happening to other children. After reading the article by Scarfò et al, I want to determine whether her leukemia had CD37, even though CD37 is rare in immature B-cell malignancies. Maybe it did and maybe it did not, but there is now a reason to hope that we can target CD19 escape variants or better yet, prevent them in the first place.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood-2018-08-869552

MYELOID NEOPLASIA

Comment on Sanders et al, page 1526

MBD4: guardian of the epigenetic galaxy

Lambert Busque[1] and Lucy A. Godley[2] | [1]Hôpital Maisonneuve-Rosemont; [2]The University of Chicago

In this issue of *Blood*, Sanders et al[1] made a very insightful observation about mutational burden in 3 early-onset leukemias that led to the identification of methyl-binding domain 4 (*MBD4*) as a candidate gene for a new acute myeloid leukemia (AML) predisposition syndrome.

CG dinucleotides serve as sites for cytosine modifications, beginning with the addition of a methyl group by DNA methyltransferase (DNMT) enzymes to form 5-methylcytosine (m^5C). Spontaneous deamination of m^5C results in a mispaired thymine, which if left within the DNA, causes a C>T transition mutation. C>T transitions that occur by this mechanism are a common cause of age- and cancer-associated mutations, because they occur at a rate 10 to 50 higher than other transitions.[2] At least 2 proteins are responsible for repairing these unpaired thymine bases and protecting our genome integrity: thymine DNA glycosylate[3] and *MBD4* (see figure).[4] In this paper, the authors measured tumor mutational burden and found 3 cases with a 33-fold higher level of mutations than in sporadic AML, with >95% being C>T transitions that occurred within a CG dinucleotide, suggesting a failure to detect a deaminated m^5C base. Importantly, 2 of these cases occurred in sisters who developed AML 4 years apart, and for which the second sister was the apparently healthy peripheral stem cell donor for the first. Deep sequencing studies identified a germline homozygous deletion of *MBD4* in 1 patient and compound heterozygous *MBD4* mutations in the 2 sisters.

In addition to the high C>T transition mutation burden, the 3 leukemia cases also shared acquired biallelic *DNMT3A* mutations and either *IDH1* or *IDH2* hot spot mutations, a relatively rare combination in de novo AML. Interestingly, all of these mutations arose from C>T transition mutations. Analysis of sequential samples in treatment and remission using single-cell sequencing methods demonstrated that (1) *DNMT3A* mutations occurred first and were present in remission, (2) several *DNMT3A* clones coexisted, and (3) mutations were also present in other genes associated with clonal hematopoiesis of indeterminate potential (CHIP),[5] such as *TET2*, *ASXL1*, and *TP53*. The authors were able to reproduce this signature in an *mbd4*-deficient mouse model.

Germline predisposition to myeloid neoplasms has recently been recognized by the World Health Organization (WHO).[6] This includes clinical bone marrow failure syndromes (eg, Fanconi anemia, Diamond-Blackfan anemia, etc) and mutations in a specific genes (eg, *ANKRD26*, *CEBPA*, *DDX41*, *ELANE*, *ETV6*, *GATA2*, *HAX1*, *MECOM/EVI1*, *RTEL1*, *RUNX1*, *SAMD9*, *SAMD9L*, and *SRP72*) reviewed in Godley and Shimamura.[7] Does *MBD4* have all the prerequisites to join this infamous club? Perhaps not yet, given that the WHO requires 2 independent publications to establish a new syndrome. What is the prevalence of *MBD4* germline mutation? This should be determined in the normal population of young individuals. It would also be crucial to determine its prevalence in AML across ages, which would allow determining the penetrance of the mutation. However, given that only 9/10 683 (0.8%) of The Cancer Genome Atlas subjects carried monoalleleic germline *MBD4* mutations, we can extrapolate that homozygous germline mutations or double heterozygous mutations will be exceedingly rare.

MBD4 deficiency seems to recapitulate, accelerate, and aggravate the CHIP phenotype. The most frequently mutated genes in CHIP are *DNMT3A* and *TET2*,[8] both of which are associated with increased hematopoietic stem cell self-renewal capacity. The relative risk of transformation to hematological cancers is estimated to be increased by 2.3- to 10-fold. Risk factors for transformation have been identified and include the specific gene, the variant allele frequency of the mutation, and the number of different mutations.[9] The 3 *MBD4*-deficient patients had a high frequency of different *DNMT3A* mutations, acquisition of new mutations, and rapid transformation, indicating that acquired mutation in