Trends in Cancer

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Spotlight

Making normal hematopoiesis invisible to CAR T cells

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Two recent studies, by Casirati *et al.* and Wellhausen *et al.*, report genetically engineering normal hematopoietic stem and progenitor cells (HSPCs) to be resistant to chimeric antigen receptor (CAR)-T cells, by changing a single amino acid in the target protein that abrogates CAR binding, without compromising protein function. This allows for selective targeting of cancer cells without harming normal hematopoietic cells.

Therapy of B cell malignancies has made remarkable progress in recent years with the advent of targeted immunotherapy, such as monoclonal antibodies, antibody–drug conjugates, bispecific antibodies, and CAR-T cells. The key to this success has been the tolerability of normal B cell depletion, because this has permitted the use of increasingly potent methods of targeting malignant B cells with relative impunity. Normal B cells produce antibodies that are important for host defense, but these can be readily supplemented by immunoglobulin infusions in the absence of the B cells themselves.

However, targeted immunotherapy has been less successful for other malignancies, a major barrier being the inability to selectively target the disease. Targeting malignant cells leads to on-target, off-tumor toxicity to their normal counterparts, which is usually not well tolerated, making B cells the exception rather than the rule. For example, within the hematopoietic system, myeloid cells and T cells are important components of cellular immunity that cannot be exogenously replaced; thus, myeloid and T cell leukemias are difficult to treat and carry a high mortality rate.

A previous solution to this problem had been to delete a myeloid antigen from normal HSPCs, artificially creating an antigen specific to acute myeloid leukemia (AML) that can be targeted without undue hematopoietic toxicity. Three groups successfully applied this concept in preclinical studies to CD33, a pan-myeloid antigen that is expressed on AML and also on normal myeloid progenitors and mature myeloid cells [1–3]. CD33 can be genetically knocked out from normal HSPCs using CRISPR/Cas9, and these HSPCs can then be engrafted into patients with AML in the form of an allogeneic hematopoietic stem cell transplant (HSCT). The expectation is that patients will reconstitute a CD33-negative hematopoietic system and so be able to undergo aggressive CD33-targeting therapies while maintaining normal myeloid cell function. Clinical trials are ongoing to test the feasibility of this approach (NCT04849910 and NCT05945849).

However, one limitation of this strategy is that it only applies to cell surface proteins that are non-essential. While loss of CD33 has no discernable effects, loss of other targets with more essential roles in hematopoiesis would be less tolerable. Another problem is that the leukemic cells may easily lose non-essential proteins under selective pressure, leading to antigen-negative escape. Targeting multiple antigens with CAR-T cell therapy can improve efficacy, but to create corresponding multiplex gene knockouts in HSPCs is technically challenging and increases the potential for genotoxicity and functional consequences.

Two recently published papers, by Casirati *et al.* [4] and Wellhausen *et al.* [5], address these limitations by developing a method to target proteins with known important function (Figure 1). Casirati *et al.* developed CAR-T cells against FLT3, KIT, and CD123, which are cytokine receptors expressed by AML and normal HSPCs. Signaling through these receptors is important for HSPC survival and proliferation, therefore their deletion is not feasible. Instead, the authors identified the specific epitope on the target protein that is recognized by CAR-T cells. They showed that mutating this epitope can abrogate binding and recognition by CAR-T cells, but protein function remains intact, a strategy termed 'epitope editing'. Epitope-edited HSPCs could engraft and differentiate into hematopoietic cells that maintained their normal function even in the presence of CAR-T cells. Furthermore, combined editing of FLT3 and CD123 in HSPCs allowed for dual-targeting CAR-T cell therapy and achieved more effective elimination of AML.

Wellhausen *et al.* used a similar strategy to perform epitope editing on CD45, a pan-hematopoietic antigen. Targeting CD45 could offer a universal therapy for all hematologic malignancies, rather than having to identify and validate a new target for each disease. However, CD45 has critical roles in lymphocyte signaling and development. An additional challenge in this study is the presence of the antigen on T cells, which can lead to fratricide when generating CAR-T cells targeting CD45. Deleting CD45 from CAR-T cells reduced fratricide but led to suboptimal CAR-T cell activity due to loss of CD45 protein function. Epitope editing of CD45 allowed the authors to avoid both fratricide and functional defects in the CAR-T cells. The same CD45 epitope edit in HSPCs then protected endogenous hematopoietic cells from CD45-targeting CAR-T cells and bispecific antibodies, permitting selective targeting of a wide variety of hematologic malignancies.

Both groups found that their CAR-T cells conveniently bound to an epitope on the

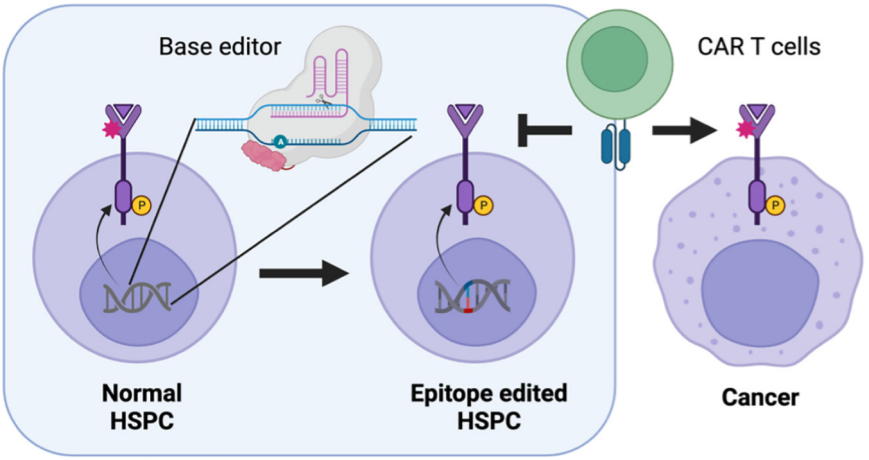

Figure 1. Epitope-edited hematopoietic stem and progenitor cells (HSPCs) enable tumor-specific chimeric antigen receptor (CAR)-T cells. Hematologic malignancies are indistinguishable from their normal cell counterparts in terms of cell surface protein expression, leading to on-target, off-tumor toxicity when using targeted immunotherapies, such as CAR-T cells. Two recently published studies [4,5] report a novel strategy to avoid hematopoietic toxicity by using base editing to modify the protein epitope targeted by CAR-T cells in HSPCs. They show that epitope editing of HSPCs can create an entire hematopoietic system that is resistant to CAR-T cells without compromising protein or cell function.

protein that did not interfere with function. This is because most antibodies against human antigens are generated in mice and bind to amino acids that are not conserved between mouse and human. Protein sequences that are conserved between species are more likely to be important for function; thus, mouse anti-human antibodies are more prone to bind to non-critical regions. This suggests that most cell surface receptors can be engineered in this manner to become invisible to targeting antibodies without compromising function.

To create the desired mutations in the target proteins, both studies used base editing [6]. Base editing uses the CRISPR/Cas9 machinery to bring in a deaminase enzyme that can directly modify specific DNA bases without causing double-stranded DNA breaks, thus increasing the precision of edits and decreasing the probability of genotoxic events. Base-edited CAR-T cells have already entered human clinical trials [7], and HSPCs are expected to follow soon [8]. Therefore, epitope-edited HSPCs can be developed as a viable therapy for patients with hematologic malignancies.

The concept of genetically modifying the normal hematopoietic system is somewhat foreign and may be concerning at first glance, because this may lead to unexpected and undesired consequences. However, advances in gene-editing technologies are allowing ever more precise DNA edits, and the single nucleotide changes in HSPCs reported in these studies could be considered equivalent to the single nucleotide variants that occur in the human population. The risk of changing one functionally inert amino acid in a protein is small compared with the benefit of being able to successfully treat a rapidly fatal malignancy. On this note, human clinical trials using these strategies are eagerly anticipated by the scientific and medical community.

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Declaration of interests

M.Y.K. is an inventor on patent US20200281975A1 relating to methods for gene editing in hematopoietic stem cells.

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