Twisted: Escape of epitope-edited healthy cells from immune attack

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Abstract: Hematopoietic stem and progenitor cell-derived neoplasia is challenging to target by cell surface-directed immunotherapy due to lack of tumor cell-specific antigen identification. Marone et al. (2023. J. Exp. Med.https://doi.org/10.1084/jem.20231235) provide a solution by target-epitope resistance editing in healthy hematopoietic stem cells.

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INSIGHTS

Twisted: Escape of epitope-edited healthy cells from immune attack

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Hematopoietic stem and progenitor cell–derived neoplasia is challenging to target by cell surface–directed immunotherapy due to lack of tumor cell–specific antigen identification. Marone et al. (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20231235) provide a solution by target-epitope resistance editing in healthy hematopoietic stem cells.

Targeting cell-surface proteins using antibodies (with or without cytotoxic payloads), multispecific antibody constructs (which lead to effector cell activation, such as T cell engagers and activators [TEAs]), and chimeric antigen receptor (CAR) T cells has become clinical standard of care for treating many cancers and some autoimmune diseases (Labanieh and Mackall, 2023; Schett et al., 2023; Labrijn et al., 2019). In most cases, targets are broadly expressed antigens on the healthy cell-of-origin (COO) and on the diseased cells themselves. Immune targeting is consequently limited and only possible if the COO is non-essential for the survival of the organism and/or its function can be rescued either by medical/pharmaceutical intervention or by regeneration of healthy tissue from non-targeted tissue stem cells after terminating the immunotherapies.

The most advanced clinical applications of such immune targeting are manifest in B cell and plasma cell neoplasia (see panel A of figure): engineered antibodies, TEAs, and CAR T cells are highly effective in targeting mainly B cell– and plasma cell–expressed antigens, such as e.g., CD19, CD20, CD22, BCMA, and GPRC5D, resulting in malignant and healthy B and/or plasma cell depletion. The consequent lack of healthy B and plasma cells can be compensated, if necessary at all, by immunoglobulin substitution. Once the therapeutic intervention is terminated, B and plasma cells re-grow from hematopoietic stem and progenitor cells (HSPCs). A considerably more profound challenge arises when applying a similar approach to malignancies originating from HSPCs. In diseases like acute myeloid leukemia (AML), myelodysplastic neoplasia (MDS), and myeloproliferative neoplasia (MPN), HSPC-derived leukemia-initiating and maintaining cells (LIMCs) rarely express not-dispensable surface molecules or antigens that distinguish them sufficiently from their COO HSPCs (see panel B of figure; Perna et al., 2017; Zeng et al., 2022). Targeting antigens shared by HSPCs and LIMCs results in elimination of the HSPC COO, leading to terminal aplasia incompatible with patient survival. Consequently, HSPCs need to be substituted by hematopoietic stem cell transplantation (HSCT). These HSPCs should be collected from an allogeneic HSPC donor instead of an autologous transplant due to the potential risk of co-transplanting residual LIMCs contained in the autologous graft. Further, it is crucial to terminate the immuno-targeting therapy before HSCT, as continuous targeting would immediately eliminate the incoming, new HSPCs. This necessary termination of immuno-targeting therapy carries the risk of leaving neoplastic cells behind. The allogeneic hematopoietic graft might not adequately control these remaining cells via a graft-versus-leukemia (GvL) effect, ultimately leading to neoplasia relapse. One possible solution to maintain immune targeting beyond HSCT is to eliminate the target antigen on the HSPCs within the incoming HSCT graft. A prominent example is the KO of CD33, which has been effectively demonstrated in pre-clinical models (Kim et al., 2018; Borot et al., 2019; Humbert et al., 2019) and is currently under investigation in a clinical study (http://clinicaltrials.gov NCT04849910). Another example is CD7KO, which also requires the corresponding CD7 deletion in CAR T cells to prevent fratricide (Kim et al., 2021; Chiesa et al., 2023). However, these KO approaches are only possible if the target KO antigen is non-essential for the proper hematopoietic function of HSPCs and their offspring, with the likely consequence that this holds true for LIMCs as well. Consequently, the probability of malignant cells emerging with non-essential target loss and immune escape

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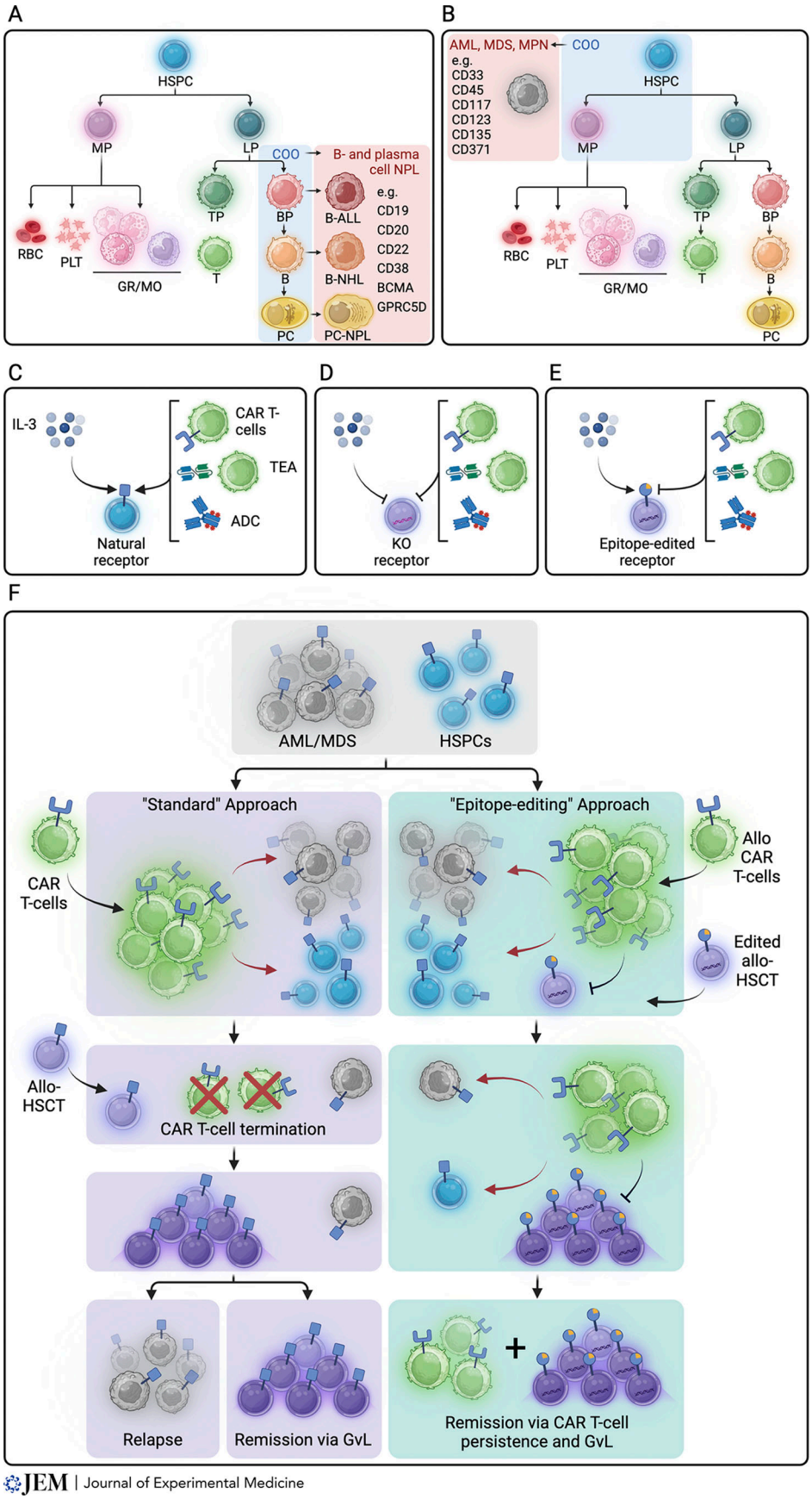

Immuno-targeting approaches in hematologic neoplasia. (A) Schematic hematopoietic hierarchy depicting B and plasma cell neoplasia, derived from their respective healthy COO. Examples of antigens currently used for immune targeting are depicted. Targeting of these antigens also leads to B and plasma cell COO depletion. The COO function can be replaced by immunoglobulin substitution. Upon termination of immunotherapy, COOs regrow from HSPCs. (B) Schematic hematopoietic hierarchy showing HSPC-derived (COO) myeloid neoplasms (AML/MDS/MPN). Examples of antigens currently used for immune targeting are also depicted. Targeting these antigens leads to HSPC depletion and the COO function can only be replaced by HSCT. Prior to HSCT, immune targeting needs to be terminated. (C–E) The sequence depicts the target cell displaying the different scenarios where the receptor (CD123) is unedited, knocked out, and base edited. While the unedited, natural receptor binds the cytokine (IL3) and is the target of immuno-therapy (C) and the KO of the receptor eliminates both functionalities (D), the base-edited receptor binds its natural ligand (IL-3) and signals adequately but is shielded from immune therapy (E). (F) Schematic representation of two pathways: a "standard approach" and a novel, "epitope-editing" approach, described in this issue of *JEM*, for the treatment of AML and other HSPC-derived diseases (MDS/MPN). The standard approach targets an HSPC and AML/MDS expressed antigen and requires immune-effector cell termination before allo-HSCT. Residual malignant cells (minimal residual disease) might lead to relapse if not controlled by GvL. The epitope-editing approach allows immune-effector cell persistence by excluding edited allo-HSCT from the immune attack. This allows for sustained remission and cure through immune-effector cells and transplanted adaptive immune cells via GvL. MP: myeloid progenitor; LP: lymphoid progenitors; TP: T cell progenitors; T: T cells; PLT: platelets; RBC: red blood cells; GR/MO: granulocytes, monocytes; BP: B cell progenitors; B: B cells; PC: plasma cells; B-ALL: B cell acute lymphoblastic leukemia; B-NHL: B cell lymphoma; PC-NPL: plasma cell neoplasia; ADC: antibody drug conjugates. Figure created using Biorender.com.

under continued therapeutic pressure is not negligible.

In this issue of *JEM*, Marone and colleagues provide an elegant and innovative solution to the problem (Marone et al., 2023). They showed that non-viral gene editing of a cytokine receptor on donor HSPCs before HSCT confers selective resistance to immunotherapies while preserving receptor function, i.e., epitope engineering in HSPCs effectively prevented antigen recognition, thus "shielding" those cells from immune attack, while cytokine binding and respective receptor signaling remained intact. This study focused on the IL-3Ra chain (CD123), which plays a role in cellular expansion and is expressed in HSPCs, some mature immune cells, and most LICMs. The authors developed various immunotherapeutic strategies based on anti-CD123 targeting (see panels C–E of figure). In extensive studies, they rationally designed CD123 base-edited variants to shield them from multiple antibody-mediated immunotherapeutic approaches, including antibody-dependent cellular cytotoxicity, TCE, and CAR T cells. Also, they demonstrated that base-edited CD123 variants maintained IL3-mediated signal transduction while, as expected, CD123KO exhibited decreased cellular function. Finally, they provided evidence that wtCD123-directed immunotherapy efficiently targeted CD123-expressing leukemia cells and unedited HSPCs, whereas CD123 base-edited HSPCs and respective descendants were protected from immune attack. Collectively, an approach based on edited allo-HSCT enables the continuous immuno-targeting of a receptor relevant for AML/MDS/MPN survival beyond allo-HSCT, which stands in sharp contrast to an approach where the immune-effector function must be halted before standard allo-HSCT. In the edited HSCT scenario, sustained remission and eventual cure might be achieved through a combination of engineered immunotherapy (e.g., CAR T cells) and a GvL effect. In contrast, non-edited allo-HSCT relies solely on immune control of disease via a GvL effect, which, unfortunately, is insufficient in many cases (see panel F of figure).

As genetic engineering is becoming more feasible, it is not surprising that similar approaches to the one shown in this issue of *JEM* are being executed in parallel in various high-end laboratories, all focusing on the described problem. Indeed, two concurrent studies reported similar success: Wellhausen et al. (2023) demonstrated that epitope base-editing of the pan-leukocyte expressed receptor tyrosine phosphatase CD45 in HSPCs enables universal immune therapy for blood cancer (Wellhausen et al., 2023), and Casirati and colleagues showed that epitope editing in cytokine receptors CD135 (FLT3), CD123, and CD117 (KIT), individually or in multiplex combination, enabled targeted immunotherapy of AML, while protecting edited HSPC function (Casirati et al., 2023). These studies, in sum, increase confidence in feasibility of the approach for potential clinical translation.

What apparent problems and challenges might be associated with receptor epitope editing demonstrated by this study and others? First, the modified surface molecule might generate a neo-antigen or neo-epitope and thus could be immunogenic. Given that these alterations are relatively minor, the likelihood of this occurring might be quite low. In addition, if this editing is done in the context of allo-HSCT, there is the potential for developing immune tolerance as adaptive immunity re-emerges, due to adequate selection of newly emerging adaptive immune cells. Second, the engineered molecules might exhibit slight alterations in functionality, which will only become evident in the long run under the influence of hematologic and immunologic stressors that were not assessed and are challenging to test in preclinical settings. Even minor deviations from normal function could have substantial consequences when acting over years, possibly resulting in altered immune function, including the risk of immunodeficiency or autoimmunity. Third, editing-introduced alterations at target and potentially also off-target sites, in combination with selective pressure exerted through immunotherapy and proliferative stress following HSCT, might favor HSPC exhaustion or clonal evolution and finally malignancy. Fourth, the expression of some of the edited antigens is not exclusively restricted to hematopoiesis (e.g., multiple tissue expression of CD117 [Russkamp et al., 2021], CD123 expression on endothelial cells [Brizzi et al., 1993], CD135 expression on cardiac cells [Pfister et al., 2014]), and the endogenous unedited antigens remain targets of therapy (on-target, off-tumor), leading to possible side effects. Fifth, the approach seems currently limited to the editing of allo-HSPCs because autologous HSPC editing is at risk of co-editing contaminating LIMC. This, however, might

be solved in the future by editing either autologous HSPCs, which were stored before disease occurrence, or by editing autologous induced pluripotent stem cells, which then need to be directed to become HSPCs for transplantation.

While it's clear that these various challenges and limitations must be thoroughly addressed, the study conducted by Marone et al. (2023) in this issue of *JEM*, along with the related studies by Wellnausen et al. (2023) and Casirati et al. (2023), set the stage for translation of these findings into clinical studies for patients whom medicine currently has otherwise little to offer. Additionally, these studies might serve as a proof of concept for further advancements beyond the field of hematopoietic diseases.

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