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Genetic engineering and allogeneic optimization of Vδ1 γδ CAR-T cells (ADI-270) for cancer immunotherapy

Wenlong Liu ,[1] Branden S Moriarity,[1,2,3] Beau R Webber[1,2,3]

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[1]Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota, USA
[2]University of Minnesota Masonic Cancer Center, Minneapolis, Minnesota, USA
[3]University of Minnesota Center for Genome Engineering, Minneapolis, Minnesota, USA

Correspondence to
Dr Beau R Webber;
webb0178@umn.edu

ABSTRACT

Genetic engineering has fundamentally transformed T cell–based therapies by enabling tumor targeting capability, improving their functionality, and facilitating allogeneic use. These strategies—originally developed in αβ chimeric antigen receptor (CAR)-T cells—have become increasingly established as blueprints for enhancing the function of other immune effector cells, including gamma delta (γδ) T cells. A recent study by Nishimoto *et al* showcased the adaptation of these engineering approaches to Vδ1 γδ T cells (ADI-270) by coexpressing a CD70-targeted CAR and a dominant-negative TGFβRII receptor (dnTGFβRII) to target CD70$^+$ malignancies, addressing immunosuppression and host-versus-graft rejection. This commentary explores αβ T cell-derived engineering strategies applicable to γδ T cells, while also highlighting genome-editing innovations poised to advance next-generation γδ CAR-T development.

INTRODUCTION

Gamma delta (γδ) T cells have garnered increasing interest as an allogeneic platform due to their major histocompatibility complex (MHC)-independent T-cell receptor (TCR) recognition, broad tumor-recognition spectrum, and low risk of graft-versus-host disease (GvHD). As the field of engineered T cells for cancer immunotherapies moves beyond conventional αβ T platforms, the question is no longer whether γδ T cells can be modified, but how their engineering should be tailored to leverage their distinctive biology. Nishimoto *et al* demonstrated the feasibility of adapting genetic engineering approaches, originally established in the αβ chimeric antigen receptor (CAR)-T platform, to the development of a novel Vδ1 γδ T cell product. By coexpressing a CD70-targeted CAR and a dnTGFβRII, Nishimoto *et al* addressed two major challenges: the immunosuppressive tumor microenvironment (TME) and host-versus-graft (HvG) rejection. The latter leverages the "dual-action" of the CD70 CAR, targeting not only tumor cells but also graft-reactive host αβ T cells, thereby potentially improving persistence of the cell product in humans. Meanwhile, the dnTGFβRII armoring enhances functionality of the effector T cells under TGFβ-rich TME. While both approaches have been deployed previously in αβ CAR-T cells, the results provide additional insight on γδ T cell engineering approaches that may improve efficacy in both solid and hematological malignancies. As emphasized in this commentary, we outline αβ T-derived engineering strategies that can be tailored to leverage the distinctive features of γδ T cells, while also highlighting genome-editing innovations that promise to drive the development of the next generation of γδ CAR-T cell therapies.

Armoring γδ T cells to counteract the immunosuppressive tumor microenvironment

TGFβ is a central immunosuppressive cytokine in the TME of solid tumors, which inhibits the expression of many key cytotoxic gene products (e.g., perforin, granzyme) and cytokines (e.g., interferon-gamma) of cytotoxic T lymphocytes.[1] In αβ CAR-T cell therapies, resistance to TGFβ signaling has been achieved using either overexpression of a dnTGFβRII complementary DNA or CRISPR/Cas9-mediated knockout of TGFβRII. The dnTGFβRII-armoring approach has shown promise in enhancing resistance to exhaustion, persistence, cytokine secretion, and tumor clearance by antigen-targeting T cells in both preclinical and early clinical settings.[2]

Given their infiltration into solid tumors, Vδ1 γδ T cells are similarly exposed to high TGFβ levels, providing a strong rationale for equipping them with an engineered resistance mechanism. ADI-270 represents a pioneering development in this regard, incorporating dnTGFβRII into Vδ1 γδ T cells. In vitro, high-level TGFβ1 (20 ng/mL) significantly impaired the proliferation and cytotoxicity (20% inhibition in the second challenge, A498 cells) under "stress-test" of unarmored γδ T controls. In contrast, ADI-270 maintained both proliferation and persistent cytotoxicity

under identical suppressive conditions, validating the efficacy of the dnTGFβRII construct in shielding γδ T cells from TGFβ-mediated dysfunction. Limited in vivo studies were conducted comparing dnTGFβRII-armored and non-armored CAR γδ T cells, with the only head-to-head comparison done in an A498 xenograft model. Although a minor benefit was achieved by armoring γδ T cells with dnTGFβRII, it remains to be seen whether this engineering approach is sufficient to benefit γδ T cells in TGFβ-rich conditions in vivo (A498 secreting only ~2 ng/mL TGFβ under examined in vitro culturing condition). It is important to consider that γδ T cells differ intrinsically from αβ T cells in their biology, which may shape their response to TGFβ in subset-dependent and context-dependent ways. Peters *et al* found that TGFβ enhanced cytotoxic function in short-term expanded Vδ2 T cells with the presence of interleukin (IL)-15.[3] Rafia *et al* demonstrated that TGFβ suppressed Vγ9Vδ2 activation and shifted transcriptional programs toward reduced effector potential.[4] These contrasting findings underscore the importance of activation context and raise questions about how "TGFβ imprinting" may vary across γδ T cell subsets—an area warranting further investigation.

Beyond dnTGFβRII, additional strategies adapted from αβ CAR-T platforms include Fas pathway modulation to prevent activation-induced cell death,[5] intracellular checkpoint gene knockout to overcome the immunosuppressive TME,[5] and cytokine armoring with IL-2 or IL-15 to enhance persistence.[6 7] However, directly translating these approaches from αβ T cells may yield suboptimal outcomes—or even unintended consequences—given the distinct activation pathways, exhaustion profiles, and tissue tropism of γδ T cells. In that case, focusing on gene-editing strategies biologically tailored to the unique properties of γδ T cells is essential.

Allogeneic strategies

Supporting their potential for development into "off-the-shelf" cancer immunotherapies, γδ T cells possess a unique MHC-independent TCR activation property that substantially lowers their GvHD risk. However, despite the low GvHD risk, HvG rejection remains a barrier to durable therapeutic activity and long-term persistence of allogeneic γδ T cell therapies. To eliminate the HvG responses, preconditioning-dependent approaches, partial HLA matching, and genetic immune-evasion strategies have been explored across αβ T and natural killer (NK) protocols. Within this framework, genetic engineering strategies can be broadly categorized into two complementary approaches: (1) passive cloaking to reduce immunogenicity, and (2) active cloaking/defense to resist or eliminate host rejection.

Passive cloaking

Immune rejection of allogeneic CAR-T cells is largely driven by recipient T-cell recognition of HLA molecules on donor cells. To reduce this immunogenicity, β2-microglobulin knockout disrupts HLA-I and reduces $CD8^+$ T-cell recognition, while CIITA knockout abrogates HLA-II expression to avoid $CD4^+$ T-cell engagement.[8] However, complete loss of HLA-I can trigger NK cell-mediated "missing-self" killing. To circumvent this, donor cells can be engineered to express non-classical HLA molecules (HLA-E or HLA-G) or with inhibitory ligands, such as CD47 (the "don't-eat-me" signal).[8] An emerging biomimetic strategy leverages viral immune evasion pathways by expression of HIV-1 Nef or viral inhibitors of transporter associated with antigen processing in donor CAR-T cells can down-modulate HLA-I, thereby improving in vivo survival.[9 10] Additional approaches continue to be developed, as highlighted by a recent report in *Cell* introducing a "glycan shielding" approach achieved by SPPL3 deletion, which achieves reduced allogeneic immunity without compromising the functionality of anti-CD19 CAR molecules.[11]

Active cloaking

Rather than only hiding, donor cells can be engineered to actively neutralize host effectors. One approach to enhance the survival of donor cells under hostile immunologic stress is to overexpress antiapoptotic genes (eg, BCL-XL).[12] A more direct, while aggressive strategy is to equip donor cells to recognize and eliminate activated host alloreactive immune cells. For example, CD19-targeted αβ CAR-T cells coexpressing a synthetic receptor targeting 4-1BB can selectively deplete 4-1BB$^+$ host $CD8^+$ T and NK cells—major HvG mediators—while preserving non-alloreactive populations and antitumor activity.[13]

Similarly, CD70-directed CAR-iNK and CAR-NKT products achieved dual benefit by killing $CD70^+$ tumor cells and concurrently clearing $CD70^+$ alloreactive host T cells, thereby improving persistence.[14 15] Adicet's CD70-targeted Vδ1 γδ CAR-T cell, ADI-270, embodies this dual-function concept, with its antitumor activity and resistance to alloreactive T cells independently validated by cytotoxicity and mixed lymphocyte reaction assays. However, one of the limitations of this study is the lack of in vivo validation. Given the variable clearance efficiency of alloreactive T cells observed among CD70-targeting products (CAR-T, CAR-NK, and CAR-NKT) in humanized mouse models,[14 15] confirming this activity in the γδ T-cell platform remains essential.

As ADI-270 enters clinical evaluation for relapsed/refractory clear cell renal cell carcinoma (NCT06480565),[16] key remaining questions include how concurrent targeting of $CD70^+$ tumor and $CD70^+$ alloreactive T cells may impact antitumor potency or immune defense activity, and whether sustained dual engagement accelerates γδ T exhaustion or metabolic stress, limiting their persistence. In addition, because activation markers (eg, CD70) are inducible across diverse immune contexts, there is the possibility that allogeneic donor-derived engineered cells may inadvertently harm non-alloreactive host immune populations, with potential impacts on antiviral/bacterial immunity or autoimmune-like pathology. These challenges highlight an opportunity to develop modalities

for temporal control and context-restricted targeting to ensure the safe application of γδ T cells in allogeneic settings. Rigorous in vivo animal studies are essential to evaluate the efficacy, durability, and safety of these approaches before clinical translation.

γδ T cell engineering approaches

While selecting the appropriate genetic modification is essential, equal attention must be given to manufacturing platforms used to deliver them. Given the substantial differences in activation pathways, tissue tropism, and functional plasticity between γδ T cell subsets—especially Vγ9Vδ2 and Vδ1—expansion and gene delivery strategies may also need to be adapted to the distinct biology of each subset.

Gene delivery approaches

γδ T cells are generally less permissive to conventional VSV-G-pseudotyped lentiviral transduction compared with αβ T cells. To overcome this, γ-retroviral vectors pseudotyped with the RD114 envelope—used in ADI-270—have been widely adopted for Vδ1-γδ T, which usually employ antibody-based stimulation approaches, achieving efficient delivery of constructs such as GPC3-CARs coexpressing soluble IL-15.[7] For Vγ9Vδ2 T cells, which are typically expanded using phosphoantigen stimulation in the presence of IL-2, baboon envelope-pseudotyped lentivirus has demonstrated superior performance over VSV-G, enabling higher transduction efficiency, robust CAR expression, and enhanced cytotoxicity across multiple targets.[17] To avoid random integration of CAR genes, the site-specific insertion of CD38-CAR in polyclonal γδ T cells was realized using a CRISPR/AAV-based knock-in platform.[18] As a non-viral alternative, transposon-based gene delivery offers a scalable and cost-effective approach with the added advantage of accommodating larger genetic payloads. For instance, the *TcBuster* system[19] has demonstrated approximately 50% stable CAR integration into pan-γδ T cells, alongside robust expansion exceeding 5,000-fold during manufacturing.[5]

Multiplex editing

Given the wide range of potential modifications, many of which may be complementary and/or synergistic, multiplex editing is particularly attractive for application to γδ T cell platforms. However, standard CRISPR–Cas9 approaches introduce double-strand breaks (DSBs), which can lead to genomic instability. To minimize DSB-associated genomic instability while enabling multiplex gene editing, DSB-free CRISPR base editors can disrupt targets by editing canonical splice donor/acceptor motifs.[20 21] In immune effector cells, base editing supports efficient single and multiplex editing while preserving viability and function, and edits at ≥6 loci have been achieved in a CAR-NK manufacturing platform.[22] Applied to γδ T platforms, this multiplex editing strategy enables installation of persistence/antisuppression modifications with a lower risk of genotoxicity and chromosomal alterations, aligning safety with scalable manufacturing.

While these platforms demonstrate promise, the impact of gene delivery method and activation protocol across different γδ T cell subsets remains incompletely defined. Notably, traditional lentiviral transduction of γδ T cells is

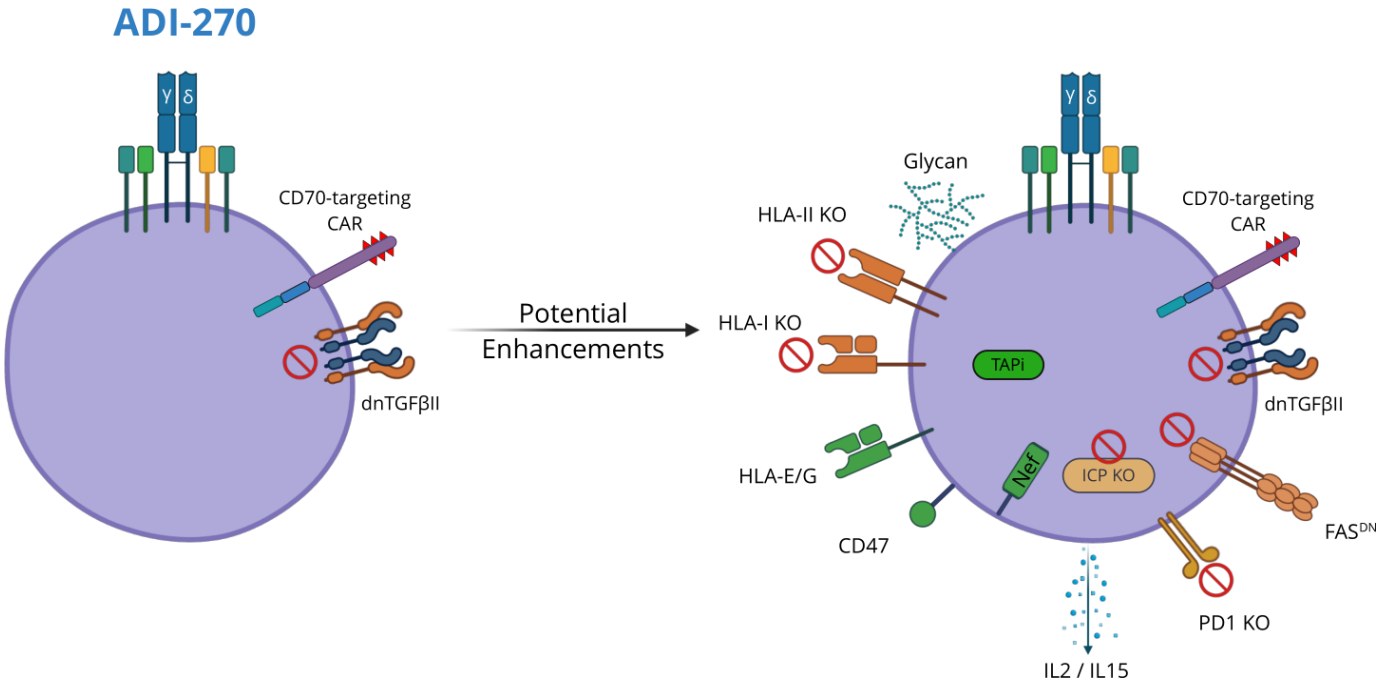

Figure 1 Schematic overview of gene-editing strategies for next-generation γδ CAR-T therapies to counter the immunosuppressive TME and minimize HvG rejection. CAR, chimeric antigen receptor; γδ, gamma delta; HvG, host-versus-graft; ICP, intracellular checkpoint gene; IL, interleukin; TAPi, transporter associated with antigen processing inhibition; TME, tumor microenvironment.

not inherently inefficient, as evidenced by the successful engineering of B7-H3 γδ CAR-T products armored with soluble IL-2.[6] Equally critical, rigorous safety evaluation is necessary for clinical translation. This includes quantification of CAR copy number in the final product and insertion-site mapping to minimize the risk of insertional mutagenesis. In addition, TCR repertoire profiling can address concerns regarding clonal dominance, while cytokine-independent growth assays—though often overlooked—remain essential to exclude uncontrolled expansion before clinical application.

CONCLUSION

Engineering γδ T cells for maximal activity has so far relied heavily on lessons learned from αβ CAR-T and CAR-NK therapies. The ADI-270 product exemplifies how conventional αβ T cell engineering approaches can be adapted to γδ T cells to advance off-the-shelf allogeneic cancer immunotherapy. The incorporation of dnTGFβRII demonstrates a rational approach to overcome TGFβ-mediated suppression within the hostile TME, and preclinical studies confirm potent in vitro cytotoxicity and in vivo tumor control across diverse cancer models. Nonetheless, key questions remain to be addressed. The benefit of armoring strategies like dnTGFβRII in γδ T cells under physiologic, TGFβ-rich conditions has not been fully validated, and the safety implications of dual-targeting designs such as ADI-270's "single-CAR, dual-function" approach require deeper investigation. Looking ahead, advances in armoring modalities, allogeneic immune-cloaking strategies, and scalable, multiplex gene-editing platforms are creating new opportunities to fully harness the antitumor therapeutic potential of γδ T cells (figure 1). With continued innovation, these therapies hold promise to deliver next-generation, durable, and broadly applicable immunotherapies capable of overcoming both the immunosuppressive TME and allogeneic immune barriers.

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ORCID iD

Wenlong Liu https://orcid.org/0000-0003-2988-7963

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