



Current developments in T-cell receptor therapy for acute myeloid leukemia

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T-cell receptor (TCR) therapies are a promising modality for the treatment of cancers, with significant efforts being directed toward acute myeloid leukemia (AML), a particularly challenging disease. Chimeric antigen receptor (CAR) T cells targeting single surface antigens have shown remarkable efficacy for B-cell lymphoblastic leukemia, lymphomas, and multiple myeloma. However, AML presents formidable obstacles to the effectiveness of CAR T cells because of the widespread expression of heterogenous leukemia immunophenotypes and surface antigen targets additionally present on normal myeloid cells. TCR therapies are an evolving field of cell therapies that allow targeting intracellular antigenic peptides presented via HLA molecules. The development of TCR therapy for AML is progressing rapidly through preclinical research and successful clinical trials. This review specifically explores the antigens targeted in AML, the diverse methodologies and strategies used in TCR identification, and preclinical TCR T-cell development. The review also discusses innovative molecular designs to improve functional efficacy, mitigate safety concerns, and overcome HLA restrictions. Specific outcomes of early clinical trials targeting important antigens Wilms tumor gene 1, preferentially expressed antigen in melanoma, and minor histocompatibility antigen HA-1 are also highlighted. Ultimately, this review underscores why TCR therapy is poised to become an indispensable component of AML immunotherapy.

Adoptive T-cell therapies for AML

Acute myeloid leukemia (AML) is a hematological malignancy arising from aberrant transformation of myeloid lineage cells. It can affect both young children, constituting 18% of pediatric leukemias, as well as adults, with a median age at diagnosis of 68 years.^{1,2} Younger, fit patients (aged ≤ 70 years) are typically treated with intensive chemotherapy, and if deemed at high risk of relapse, would undergo allogeneic hematopoietic stem cell (allo-HSCT) transplant while in remission. The long-term survival rates for pediatric cohorts are ~70%, reducing to just over 50% in patients aged between 18 and 60 years.^{3,4} Intensive treatments such as chemotherapy and allo-HSCT are not suitable for most older patients because of physiological frailty and inherent differences in genetic drivers of AML. Although hypomethylating agents and Bcl-2 inhibitors have improved outcomes in this group, the 5-year overall survival remains dismal at 27%.⁵ Overall, 10% to 45% of patients achieve sustained remission, with older patients performing worse than younger patients.⁶ In recent years, novel immunotherapeutic

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approaches such as adoptive T-cell therapies are being investigated for AML. This has led to promising advances in developing new treatment options, although key challenges remain, and no AML-directed cellular therapies aside from allo-HSCT are yet routinely available.

After the discovery of the role of immune cells in tumor suppression,⁷ the earliest form of adoptive cell therapy for malignant diseases were tumor-infiltrating lymphocytes or circulating cytotoxic lymphocytes (CTLs). Tumor-infiltrating lymphocytes and CTLs were isolated from patients or donors, expanded ex vivo, sometimes with additional antigenic stimulus to expand antitumor T-cell clones, and reinfused back into the patient.^{8,9} For hematological malignancies such as AML, there have been a handful of trials using HLA-matched ex vivo expanded CTLs, with favorable clinical responses observed.¹⁰⁻¹² Multiantigen-targeting CTLs to improve graft-versus-leukemia have shown minimal toxicity and favorable response¹³ and can even persist 1 year after infusion.¹⁴ However, generation of a sufficient polyclonal CTL product is not always possible. Naturally occurring AML tumor antigen-specific T cells (such as against Wilms tumor gene 1 [WT1] or preferentially expressed antigen in melanoma [PRAME]) in healthy donors are infrequent (<0.05% in circulation) and corresponding T-cell receptors vary in binding affinity, leading to variability in response. Furthermore, extended ex vivo stimulation could lead to exhaustion and further hinder efficacy in vivo, hindering their widespread use.

The development of chimeric antigen receptor (CAR) T cells presented a unique solution to these challenges, by modifying peripheral blood T cells to express a synthetic chimeric receptor (CAR), allowing for generation of a more uniform, reliable product with streamlined manufacture processes.¹⁵⁻¹⁸ The CAR molecule recognizes surface antigens in a HLA-independent manner, which results in T-cell activation, cytokine release, and targeted cytotoxicity. CAR T-cell therapy has had great clinical success in large B-cell lymphomas, mantle-cell lymphoma, B-cell acute

lymphoblastic leukemia, and multiple myeloma. There are currently 6 US Food and Drug Administration-approved products targeting either CD19 or B-cell maturation antigen.¹⁹ Unfortunately, despite extensive preclinical work,²⁰ these successes have yet to be translated to AML, because of, in part, lack of suitable surface antigens. Some promising targets (eg, CD33 and CD123) are shared with healthy hemopoietic cells, and require rescue allo-HSCT because of myelosuppression caused by treatment, increasing potential treatment toxicities.²¹ Moreover, there is heterogeneity in antigen expression between patients and also within the malignant T-cell population of each individual patient.²² One of the challenges for AML therapies remains in identifying the ideal targetable antigens.

TCR therapy is an alternative adoptive cell therapy that involves the introduction of TCR sequences into T cells to redirect specificity toward a target antigen. The TCR is a macromolecular multisubunit structure comprising of antigen-recognizing α and β subunits that combine with signaling subunits of the CD3 molecule (Figure 1). Binding of the antigen–HLA complex allows the recruitment of kinases, phosphorylation of key domains that result in downstream signaling, activation, and functional response.²³ TCRs differ from CARs in target recognition (requiring HLA-dependency), sensitivity, and signaling (Table 1). The nature and context of the antigen informs the choice of using either a CAR or TCR for targeting. For example, in contrast to CARs that target surface antigens, TCRs can recognize a larger pool of intracellular antigens through antigenic peptides that have been processed internally and presented on the HLA complex on the cell surface. Native TCRs undergo thymic selection, and are highly discriminatory with 100-fold higher antigen sensitivity than CARs.³³ Most TCRs are highly specific, recognizing antigenic peptides that are generally presented on only a single HLA subtype; HLA molecules are polymorphic, with over 1000 alleles reported. HLA-A*02:01 and HLA-A*24:02 alleles are present at higher frequencies across the global population.³⁵

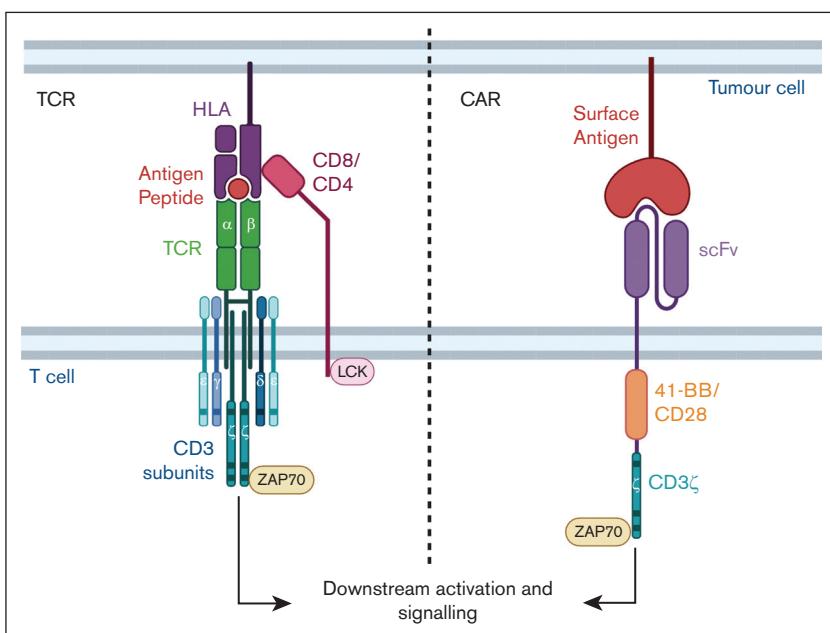


Figure 1. TCR and CAR. TCR $\alpha\beta$ subunits combine with γ , δ , ϵ , and ζ subunits of the CD3 to form the multisubunit TCR complex. Peptide-HLA binding to the TCR $\alpha\beta$ combined with the other subunits and with either CD4 or CD8 coreceptor initiates downstream signaling, T-cell activation, and effector functioning. Proteins involved in signaling such as LCK and ZAP70 are indicated and the activating motifs within the subunits are represented as horizontal bars. A typical CAR molecule comprises an antigen-specific scFv from a monoclonal antibody linked to a spacer, costimulatory domain (CD28 or 4-1BB shown in this example) and signaling CD3 ζ domain. Figure created with BioRender.com: Gore S. (2025); <https://BioRender.com/o91f055>

Table 1. Differences in TCRs and CARs

	TCR	CAR
Target	Intracellular antigen (recognition of peptide/HLA complex), HLA restricted	Surface antigen (conventionally, not always)
Intracellular signaling domains	Formation of CD3ελ, CD3εδ heterodimers, and CD3ζ homodimer, 10 ITAMs	Conventionally some combination of linked CD28 or 4-1BB and CD3ζ, 3 ITAMs
Coreceptor requirement	Yes (CD4, CD8)	Not required
Activation threshold	1-3 molecules on target cell ^{24,25}	>100 molecules on target cell ²⁶
Sensitivity	Higher antigen sensitivity ²⁷⁻²⁹	Lower antigen sensitivity, ²⁷⁻²⁹ up to 100-fold less at low-antigen levels ^{30,31}
Affinity	Generally lower (allowing for serial triggering), ³² in the mM range	Slightly higher, ³³ in the nM range
Immune synapse formation	Highly organized, 5-10 min formation time ³⁴	Disorganized, <2 min formation time ³⁴
Downstream activation/phosphorylation	Stronger activation ²⁸	Weaker activation ²⁸

ITAMs, immunoreceptor tyrosine-based activation motifs.

Although TCR therapy can be limiting because of its inherent HLA restriction, the potential to overcome constraints of CTL therapy is attractive, especially in the treatment of AML, which has numerous intracellular proteins with immunogenic peptide epitopes presented on various HLAs. Thus, this review will highlight recent developments in TCR therapy development for AML.

Preclinical development of TCR T-cell therapy in AML

Antigen selection

A range of AML associated antigens have been targeted with TCR T-cell therapy. Figure 2 provides an overview of the preclinical development methodologies. The studies outlined in Table 2 are limited to antigens, which, to date, have been targeted with TCR T cells in AML. Suitable antigens possess desirable characteristics, including immunogenicity and cancer-specific or cancer-associated expression.⁵⁷

Overexpressed antigens, known as leukemia-associated antigens can be used for generating broadly applicable TCR therapies. However, there is potential for on-target, off-tumor toxicities because of their expression on healthy cells. A subclass of overexpressed antigens known as cancer testes antigens have normal expression restricted to immune-privileged male gonadal tissue, making them particularly attractive targets with less risk of similar toxicities. Knowledge of antigen processing is essential because immunoproteasomes can be downregulated in AML, contributing to immune evasion. Peptides processed by alternative proteasomes were shown to be a more promising target.³⁸

Minor histocompatibility antigens (MiHA) are a large class of 9 to 20 amino acid-long peptides presented on the HLA complex, often arising from single nucleotide polymorphisms or other normal variants, some with selective expression on hemopoietic cells. In AML, they are involved in mediating the therapeutic graft-versus-leukemia effect of allo-HSCT. MiHA antigen therapies can circumvent on-target, off-tumor toxicities when correct donors are MiHA mismatched. Although MiHA restriction and post-allo-HSCT setting may limit applicability, allo-HSCT remains a standard of care in AML. Current clinical trials targeting minor histocompatibility

antigen HA-1 are still suitable for 10% to 15% of patients in the post-HCST setting.⁵⁸

Targeting cancer-specific neoantigens in AML, which arise often because of splicing defects,^{59,60} can also avoid off-tumor toxicities.⁵²⁻⁵⁶ However, given the limited pool of patients possessing both the targeted mutation and the correct HLA, broad application of a specific singular neoantigen-directed product will be limited. Banks of TCR products targeting a wide range of neoantigens may need to be developed. Neoantigen-specific TCR T cells are promising for AML with the initiation of the first clinical trials ([ClinicalTrials.gov](#) identifier: NCT06424340) likely to provide insights into the feasibility and efficacy of this approach.⁶¹

Ultimately, targeting multiple antigens simultaneously will likely improve outcomes to cell therapies and can also help overcome acquired resistance because of the emergence of single antigen-escape variants.

TCR identification

PRAME-specific TCRs have been isolated from peripheral blood mononuclear cells (PBMCs) of patients⁴⁴ that were not found in healthy donors⁴¹ and from CTLs generated from HLA-matched (autologous [auto]) or HLA-unmatched (allo) healthy donors^{36,37,52}

Some have argued that for overexpressed antigens survivin, PRAME, or WT1, auto-HLA-restricted T cells have undergone negative thymic selection with the target antigen considered as "self," and those donors would not have high affinity self-reactive or cross-reactive TCRs. TCRs from this pool theoretically should not be responsive to low levels of target antigen, which may be present in normal tissues, alleviating safety concerns for use. Indeed, a survivin-specific TCR identified from an allo-HLA-restricted donor was shown to exert fratricidal effects because of survivin expression on the T cells themselves,⁶² whereas another group was able to subsequently successfully identify a survivin TCR without the same effects from an auto-HLA-restricted donor.⁴⁰

Conversely, others have also argued that the same thymic selection processes in allo-HLA-restricted donor PBMCs would allow for higher-avidity TCRs because these have not undergone the negative selection pressures for the targeted HLA. Amir et al⁴⁴

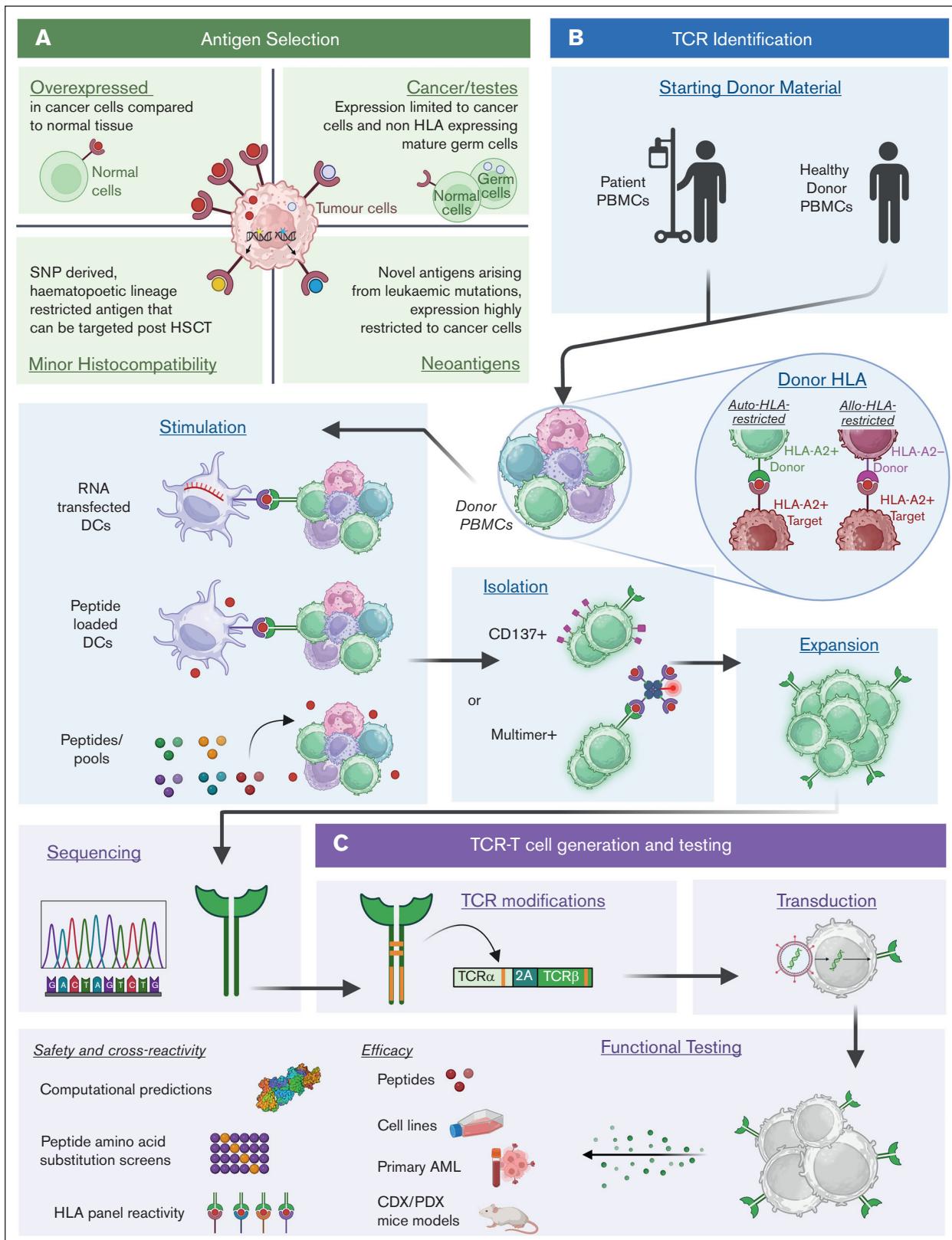


Figure 2. Pipeline of TCR T-cell preclinical development. (A) Antigens can either be overexpressed self-antigens seen on normal tissues or those that belong to the cancer testis antigen family with restricted expression; neo-antigens arising from mutations and gene rearrangements; or those belonging to the minor histocompatibility complex. (B) Donor PBMCs containing either auto- or allo-HLA-restricted TCR-expressing T cells can be stimulated with antigenic peptides; and activated or reactive T cells can be identified,

have demonstrated that PRAME-specific allo-HLA-restricted T clones were of higher avidity than auto-HLA-restricted clone but also responded to healthy cell subsets, exemplifying some of the safety concerns associated with allo-HLA repertoire-derived TCRs. Falkenberg et al⁶³ demonstrated similar results in which allo-HLA-restricted WT1 TCRs were more tumor reactive but also showed promiscuity against non-WT1 targets.

TCR identification typically involves coculturing donor PBMCs with antigen-presenting cells such as dendritic cells modified to over-express the antigen of interest or directly loaded with antigenic peptides. Immunogenic epitopes can be predicted by algorithms such as NetMHC,⁶⁴ or by testing peptide pools of overlapping 9–15mers spanning the length of the entire antigen.^{65,66} Activated, antigen-specific T cells are then isolated either using activation marker selection, most commonly CD137 or, if the target peptide/HLA is known, using major histocompatibility complex (MHC) multimers. Activated T-cell culture or CTL clones are expanded through repetitive stimulations with feeder cells.

Single-cell sequencing platforms or Sanger sequencing after rapid amplification of complementary DNA-ends polymerase chain reaction are used to precisely identify the α and β TCR subunits from the CTL cultures. Improved technologies with DNA barcoding of multimers allow for high throughput screening of multiple epitopes/HLAs.⁶⁷ Alternatively, the inclusion of functional testing with high-throughput screening and sequencing pipelines can also shorten the developmental timeline.^{68,69}

Transgenic TCR T-cell generation, optimization, and functional testing

Although the introduction of full-length sequences of the TCR α and β subunits into nonspecific T cells is sufficient to confer antigen specificity, recent exciting developments in lymphocyte engineering has allowed for modifications that will ensure precise pairing of the introduced subunits. The expressed subunits from these transgenes can associate with the endogenous CD3 subunits to form a functional multisubunit TCR.

Retroviral, lentiviral, or adeno-associated viral transduction are frequently used to introduce the α and β subunit sequences. More recently, nonviral methods such as CRISPR-associated protein 9 (CRISPR-cas9)-mediated insertion and the transposon Sleeping Beauty system have also been used.^{36,51,70,71} Such DNA-based methods are being investigated to increase the affordability of cellular therapies, with benefits of larger payloads and/or targeted integration.

Specific TCR modifications include replacement of the human constant region with a murine constant region, which does not interfere with TCR recognition and also allows for antibody-based detection of the introduced TCR in preclinical studies.^{72,73} Complementary cysteine (Cys) residues introduced into the constant region enable Cys-Cys disulphide bonds further ensuring specific pairing of the exogenous $\alpha\beta$ to prevent risk of mispairing with endogenous TCR $\alpha\beta$ chains.^{74,75} Mispairing could reduce antigen-specific activity to

theoretically produce random new specificity with unpredictable toxicity. These design strategies highlight the potential advantages and evolving scope of synthetic immunology.

Antigen-specific TCR T cells are tested for functional response upon coculture with target cells by the release of effector cytokines such as interferon- γ and cytotoxicity assays. Often, TCR T cells are initially tested against peptide-loaded target cells to confirm specificity, and then against HLA-expressing cancer cell lines with either antigen overexpression or physiological antigen levels. A panel of HLA-expressing cell lines are used to rule out cross-reactivity.³⁶ Testing against primary tumor tissue and in vivo murine models are often used for final confirmation before clinical translation. Although usually extensive targeted testing is carried out to confirm specificity after the TCR T-cell generation, recent studies have also used a large-scale approach with testing of T-cell clones before TCR sequencing to identify promising candidates from hundreds of clones.^{36,37} More recently, novel fluidics-based platforms such as the Bruker Beacon system are being developed for efficient, high-throughput TCR functional testing using single cells.⁷⁶ This exciting technology can transform single-cell analysis because it has the advantage of screening thousands of cells for functional response, precisely capturing reactive cells for sequencing, and, thus, has the potential to revolutionize TCR identification and testing.

Affinity maturation of the TCR can be used to enhance efficacy. Amino acid substitutions in the CDR3a region of New York esophageal squamous cell carcinoma 1 (NY-ESO-1)-specific TCRs resulted in much greater reactivity against AML.⁷⁷ Other methods include phage display^{78,79} and somatic hypermutation.⁸⁰ Affinity-matured TCRs may require an additional focus on safety because an increased risk of toxicities has been seen in some studies. For instance, an affinity-matured TCR T-cell product targeting carcinoembryonic antigen led to transient severe colitis,⁸¹ and melanoma-associated antigens (MAGE)-A3-targeting TCRs with cross-reactivity to MAGE-A12 and titin^{82,83} resulted in neurotoxicity and cardiogenic shock in human trials. These cases demonstrate that although TCR modifications have their merit, extensive safety considerations are also required. However, Afamice, an affinity-matured TCR T-cell product targeting MAGE-A4, was recently US Food and Drug Administration approved for synovial sarcoma, paving the way for other TCRs to be approved in the future.

Cross-reactivity of the TCRs can be investigated using structural and predictive algorithms. Single amino acid substitutions using alanine (alanine scans) or other amino acids (X-scans) can be used for evaluating potential peptides and position of key amino acids that interact with the TCR.^{84,85} Combinatorial peptide libraries can also be used to test the TCR reactivity using *in vitro* assays, including cytokine release or target cell lysis. Although computational modeling to predict peptide-MHC binding such as EPIC-TRACE,⁸⁶ and ERGO (peptide-TCR matching prediction)^{87,88} are being developed, the precise rules for TCR-peptide binding are still not well established.²³ Empiric testing involving large peptide

Figure 2 (continued) isolated, and expanded in the laboratory. (C) Sequencing of reactive cells identifies the precise $\alpha\beta$ sequences of the TCR that are introduced into T cells after modifications to ensure proper pairing. Newly generated TCR T cells are tested for function and efficacy against tumor cells and tested for specificity and safety using *in silico* and experimental analyses. CDX, cell line-derived xenograft; DCs, dendritic cells; PDX, patient-derived xenograft. Figure created with BioRender.com: Gore S (2025); <https://BioRender.com/o91f055>

Table 2. Preclinical studies testing TCR T cells for AML

Reference	Target epitope	HLA	Donor pool	Pool type	Specific stimulation	Isolation	Sequencing	TCR modification	Additional elements	Transduction	Recipient cells	Testing methods	Testing against
Overexpressed antigens													
Ruggerio et al ³⁶	WT1 ₃₇₋₄₅ (VLDFAAPPGA)	A*02:01	Healthy	Not specified	Direct peptide loading	CD137	RACE PCR	-	CRISPR/Cas9 knockout of endogenous TCR	LV/AAV	Healthy donors Patient T cell	Flow cytometry cytokine release and cytotoxicity assays Live-cell imaging Luciferase assay	Cell lines, modified HLA expression (K562, 697, OCI-AML3) EBV-LCLs Peptide-loaded T2 Primary AML Healthy cells PDX NOD mouse model
Van Amerongen et al ³⁷	WT1 WT1 ₃₇₋₄₅ (VLDFAAPPGA) (ALLPAVPSL) (VLDFAAPPGASAY) (TPYSSDNL) (TPYSSDNL)	A*02:01 A*02:01 A*01:01 B*35:01	Healthy	Allo	-	Multimer single-cell clone	PCR	Murine constant Cysteine modified	-	RV	Healthy donors	ELISA Chromium 51	Cell lines, modified HLA/antigen expression, peptide loaded (Reji) EBV-LCLs Healthy cells
Lahman et al ³⁸	WT1 ₃₇₋₄₅ (VLDFAAPPGA)	A*02:01	Healthy	Not specified	Peptide-loaded DCs	Tetramer	RACE PCR	-	CD6 coreceptor	LV	Healthy HLA-A*02:01 donors Patient T cell	Flow cytometry intracellular cytokine release assay	Primary AML Cell lines, modified HLA/antigen expression (K562) PHA blasts, peptide loaded EBV-LCLs CDX NOD and NSG mouse model
Xue et al ³⁹	WT1 ₁₂₆₋₁₃₄ (RMFPNAPYL)	A*02:01	Healthy	Allo	Peptide-loaded T2 cells	V β 2 $^+$ and CD8 $^+$ (magnetic bead and FACS)	PCR	-	-	RV	HLA-A2 $^+$ Healthy Donor T cells Patient T cells	Chromium 51 IFN γ release	T2 peptide-loaded cells Cell lines (697, BV173, LAMA81, KYO-1) CDX NOD/SCID mouse model
Arber et al ⁴⁰	Survivin ₉₆₋₁₀₄ 97M (LMLGEFLKL)	A*02:01	Healthy	Auto	Peptide-loaded DCs	Single-cell clone testing	5' RACE PCR	Murine constant	-	RV	Healthy donor CD8 $^+$ cells	Chromium 51 ELISPOT CFU assay	Cell lines (BV173, U266, K562, HL-60) Primary AML CDX NSG mouse model
Sandri et al ^{41,42}	TERT ₈₆₅₋₈₇₃	A*02:01	Patients (B-CLL) Mouse CTLs	Auto	Peptide-loaded DCs	Single-cell clone testing	PCR	-	-	RV	Healthy donor PBMCs	ELISA flow cytometry cytotoxicity activity assay	Cell lines (THP1) Peptide-loaded PBMCs CDX NOG mouse model
Depreter et al ⁴³	TARP(P51) ₄₋₁₃	A*02:01	Healthy	Auto	Peptide-loaded DCs	Single-cell clone testing	5' RACE PCR	Murine constant	-	LV/RV	Healthy donor CD8 $^+$ cells	Flow cytometry cytokine release and cytotoxicity assays Chromium 51 Luciferase assay	Cell lines, modified HLA/antigen expression (THP1, LNCAp, KG1a, Molm13, OCI-AML3, HL60) Primary samples
Cancer testes antigens													
Amir et al ⁴⁴	PRAME ₄₂₅₋₄₃₃ (SLLQHHLGL)	A*02:01	Patient donors (after HSCT)	Auto- and allo-HSCT	-	Tetramer single-cell sorted	RT-PCR	Cysteine modified	-	RV	EBV-specific T cells	ELISA Chromium 51	Peptide-loaded T2 cell lines, modified HLA/antigen expression (K562, COS) EBV-LCLs Primary samples Healthy cell subsets
Kang et al ⁴⁵ Jager et al ⁴⁶	NY-ESO-1 ₁₅₇₋₁₆₅	A*02:01	Healthy	Auto	Primary tumor cell line	Not specified	Not specified	Cysteine-modified CDR3 α aa substitution	-	LV	Healthy donor PBMCs	ELISA, ELISPOT LDH cytotoxicity assay	Cell lines (U937, HL60, Kasumi-1) CDX NCG mouse model
Nagai et al ⁴⁷	AURKA ₂₀₇₋₂₁₅ (YLLEYAPL)	A*02:01	Healthy	Auto	Peptide-loaded DCs	Not specified	5' RACE PCR	-	-	RV	Healthy donor T cells	ELISA, ELISPOT Chromium 51 CSFE proliferation assay Luciferase reporter TCR signaling assay	Cell lines, modified HLA expression (GANMO-1, MEG01, KAZZ, OUN-1) Primary AML samples CDX NOG mouse model
Spranger et al ⁴⁸	HMMR (MSFPKAPL)	A*02:01	Healthy	Allo	RNA-loaded DCs	CD137 single-cell clones	PCR	Murine constant	-	RV	Healthy donor T cells	Chromium 51 CFU assay	Cell line, modified HLA expression (K562, THP1) Primary AML CDX NSG mouse model HSCs
MIHA													
Van Loenen et al ⁴⁹	HA-1 (VLHDDLLEA)	A*02:01	Healthy	Not specified	Not specified	Not specified	Not specified	Cysteine modifications	-	RV	Allo-restricted healthy donor T cells	ELISA Cytotoxicity assays	EBV-LCLs Primary AML
Dossa et al ⁵⁰	HA-1 (VLHDDLLEA)	A*02:01	Healthy	Auto	Peptide-loaded DCs	Single-cell clones	RACE PCR	Cysteine modifications	CD8 coreceptor iCasp9 safety switch	LV	Healthy donor T cells	Chromium 51 Intracellular cytokine release and CD107 α degranulation flow cytometry	T2 peptide-loaded cells Primary AML
Pilunov et al ⁵¹	HA-1	A*02:01	Healthy	Auto	Peptide-loaded DCs	CD137/multimer	RACE PCR	Murine constant Cysteine modifications	CRISPR-Cas9 knock down of endogenous TCR	LV	Healthy donor CD8 $^+$ cells	Fluorescent reporter J76 cell line assay ELISA Flow cytometry cytotoxicity assay	Primary AML Cell lines, modified HLA expressed, peptide loaded (K562) Healthy and patient PBMCs

aa, amino acid; AAV, adeno-associated virus; B-CLL, B-cell chronic lymphocytic leukaemia; Cas9, CRISPR-associated protein 9; iCasp9, inducible caspase 9; CDX, cell line-derived xenograft; CFU, colony-forming unit; CTLs, cytotoxic T lymphocytes; DCs, dendritic cells; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot; FACS, fluorescence-activated cell sorting; IFN γ , interferon- γ ; LCLs, lymphoblastoid cell lines; LDH, lactate dehydrogenase; LV, lentivirus; NSG, NOD scid gamma; NOD/SCID, nonobese diabetic/severe combined immunodeficiency; NOG, NOD/shi-scid IL2 γ r(null); PDX, patient-derived xenograft; PHA, phytohemagglutinin; RACE, rapid amplification of complementary DNA ends; RT, reverse transcription; RV, retrovirus; PCR, polymerase chain reaction.

Table 2 (continued)

Reference	Target epitope	HLA	Donor pool	Pool type	Specific stimulation	Isolation	Sequencing	TCR modification	Additional elements	Transduction	Recipient cells	Testing methods	Testing against
Neoadjuvants													
Van der Lee et al ⁵⁴	mutNPMT (CLAEVEVSL)	A*02:01	Healthy (unsuccessful with AML patients)	Auto	-	Tetramer single-cell clones	PCR	Murine constant Cysteine modification	-	RV	Healthy PBMCs, later CD4/8 purified	ELISA Chromium 51	Peptide-loaded T2 cells Primary AML CDX NSG mouse model
Van der Lee et al ⁵⁵	mutNPMT (AVEEVSLRK)	A*11:01	Healthy	Auto	-	Tetramer single-cell clones	PCR	Murine constant	-	RV	Healthy PBMCs, later CD4/8 purified	ELISA Chromium 51	Peptide-loaded T2 cells Cell lines, modified HLA expression (K562, OCI-AML2, OCHAM13) Primary AML CDX NSG mouse model
Biemacki et al ⁵⁶	CBFB/MYH11 fusion (REMEV/HEL)	B*40:01	Healthy	Auto	Peptide-loaded DCs	Single-cell clones	RACE PCR	Cysteine modifications	-	LV	Healthy donor T cells	Chromium 51 Flow cytometry-based cytotoxicity and CD107 degranulation assays	Peptide-loaded ICLs Cell lines, modified HLA antigen expression (NS4, ME-1) PDX MSTRo mouse model
Biemacki et al ⁵⁷	U2AF1 (137R) (DFREACCR)	A*38:01:03	Healthy	Auto	Peptide-loaded DCs	Single-cell clones	RACE PCR	Murine constant Cysteine modifications	CRISPR-Cas9 knock down of endogenous TCR	LV	Healthy donor T cells	Chromium 51 Flow cytometry-based cytotoxicity and CD107 degranulation assays	Peptide-loaded ICLs Cell lines, modified HLA expression (TF-1) Primary AML Healthy PBSCs/AMCs CDX NSG mouse model
Gianakopoulou et al ⁵⁸	mutET3-D88Y (MSESHYY)	A2	Healthy	Auto	RNA-loaded DCs	Multimer single-cell clones	RT-PCR	Murine constant Cysteine modifications	-	RV	Healthy donor PBMCs	ELISA Flow cytometry-based activity and cytotoxicity assays	Cell lines, modified HLA expression, peptide-loaded (K562) EBV-LCLs Primary AML Healthy A2 ⁺ cells CDX and PDX NSG mouse model

aa, amino acid; AAV, adeno-associated virus; B-CLL, B-cell chronic lymphocytic leukaemia; Cas9, CRISPR-associated protein 9; iCas9, inducible caspase 9; CTLS, cytotoxic T lymphocytes; DCs, dendritic cells; EBV, Epstein-Barr virus; ELISpot, enzyme-linked immunosorbent assay; IFN γ , interferon- γ ; LCLs, lymphoblastoid cell lines; LDH, lactate dehydrogenase; LV, lentivirus; NSG, NOD scid gamma NOD/scid, nonobese diabetic/severe combined immunodeficiency; NOG, NOD/shi-scid IL2r null ; PDX, patient-derived xenograft; PHA, phytohemagglutinin; RACE, rapid amplification of complementary DNA ends; RT, reverse transcription; RV, retrovirus; PCR, polymerase chain reaction.

libraries encoded in yeast or baculoviral expression systems or using PresentER in which peptides can be loaded on to endogenous MHC of mammalian cells through endoplasmic reticulum signaling sequences for in vitro and in vivo testing are being developed. Other library screening techniques described include SABR (signaling and antigen-presenting bifunctional receptors) and T-scan reporter systems, which mimic endogenous presentation.⁸⁹ Testing for off-target peptide binding is crucial to establish safety before clinical translation and we envision several new algorithms being described in the near future.

Novel designs

Advances in molecular biology and genetic engineering have seen the development of exciting novel receptors, that have extended the original chimeric designs described by Gross et al in 1989.⁹⁰ Some of these designs are likely to dictate the success of cell therapies. The reader is directed to Table 3 in which additional features and novel chimeras are highlighted.

Improving functional efficacy

Expression of coreceptors and costimulatory molecules.

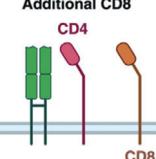
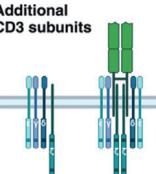
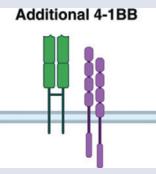
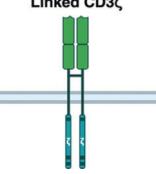
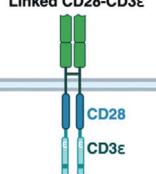
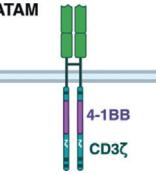
TCR activation relies on the association of coreceptors and signaling domains (Figure 1). The insertion of such additional CD3 subunits,⁹⁷ CD8 receptor,^{50,91,92,94,95} and 4-1BB costimulatory molecules^{98,133} was a logical approach to increase signaling efficacy and to boost TCR T-cell potency.

Alternatively, new designs have investigated linking the TCR $\alpha\beta$ chains to downstream intracellular signaling domains of CD3 ζ , analogous to that of a CAR,¹⁰⁰ or to CD28-CD3 ϵ costimulatory domains.¹⁰³ Similarly, the 4-1BB costimulatory molecule inserted into the CD3 ζ , termed an artificial T-cell adaptor molecule was shown to have a higher proliferative capacity when highly expressed alongside the NY-ESO-1 TCR.^{104,105} These represent viable avenues for improving TCR T-cell efficacy, with particular application for low-avidity TCRs with a favorable safety profile but lacking the required potency.

Enhancing cytokine-mediated activation. Immune modulating cytokines exert either stimulatory or suppressive signaling and inclusion of transgenes encoding for cytokines within the T cells, overcomes the requirement for exogenous infusions. For example, transgenes encoding interleukin-12 (IL-12), T-cell stimulatory cytokine,¹⁰⁶ can be placed under an activation-inducible nuclear factor of activated T cells promoter¹⁰⁹ or a doxycycline-inducible Tet-On system, for efficient secretion from the modified T cells.¹¹⁰ Alternative approaches involve anchoring the IL-12 to the cell membrane, or the TCR itself, for a targeted approach, which showed efficacy in remodeling the TME in solid cancers.¹¹² IL-18 expression demonstrated similar efficacy as IL-12, without the toxicity seen in in vivo models.¹⁰⁷ These studies have been conducted for solid tumors and use in the context of AML may require further investigation because of the unique microenvironment of hematological malignancies.

Reversing immune suppression. AML cells can upregulate immune checkpoint ligands to induce T-cell exhaustion, limiting TCR T-cell efficacy and contributing to AML relapse. Switch receptors have been designed to convert the immune checkpoint

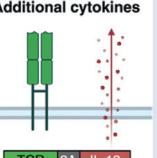
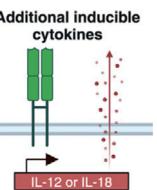
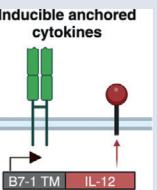
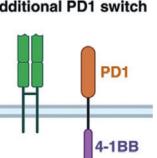
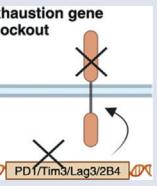
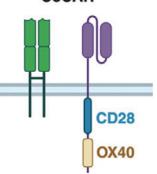
Table 3. Novel designs to enhance TCR T-cell functional efficacy and safety, and overcome HLA-restriction limitations

Novel feature		Additional functionality	Antigen sensitivity	Limitations	Cancer type tested	
Additional CD8		Additional CD8 coreceptor. Most commonly the CD8 $\alpha\beta$ dimer is inserted, with intracellular components not necessary. ⁹¹ CD8 α homodimer was effective in some studies ⁹² but suboptimal ⁵⁰ or insufficient in others. ^{91,93}	Conferring TCR T-cell-mediated antigen-specific IFN γ cytokine production, proliferative, and lytic capacity to CD4 $+$ cells both <i>in vitro</i> and <i>in vivo</i> , with a decreased exhaustion profile. ^{50,91,92,94,95} Also increased CD8 $+$ TCR T-cell function in some studies. ⁹⁵	10- to 100-fold increase in avidity of TCR T-cell CD4 $+$ cells. ^{91,94} TCR affinity of CD4 $+$ /CD8 coreceptor equivalent to CD8 $+$ cells. ⁹⁵ No increase in cross-reactivity. ⁹²	Need for CD8 coreceptor can be bypassed with the use of a CD4/8-independent TCR. ⁹⁶	AML ⁵⁰ CML ⁹⁵ CMV/EBV ^{91,94} Other tumours ⁹²
Additional CD3 subunits		Additional CD3 ζ , CD3 ϵ , CD3 δ , and CD3 γ subunits	Increased TCR expression corresponding with increased antigen-specific IFN γ cytokine production and T-cell proliferation. Also aided greater tumor clearance, infiltration, and persistence <i>in vivo</i> . ⁹⁷	Increased TCR avidity. ⁹⁷	Upregulation of TCR may increase risk of TCR mispairing toxicities. ⁹⁷	Lymphoma ⁹⁷
Additional 4-1BB		Additional 4-1BB costimulatory molecule	Increased antitumor function, expansion, and proliferative capacity of TCR T cells, dependent on tumor cell expression of 4-1BB ligand. ⁹⁸	Not tested	Dependent on tumor expression of 4-1BB ligand. ⁹⁸	Melanoma ⁹⁸
Linked CD3 ζ		Additional CD3 ζ linked to TCR α and β chains Extracellular, transmembrane, and intracellular domains are required for complete function. ⁹⁹	Enhanced TCR expression, activation, and decreased mispairing with other TCRs. Cytotoxic function was not compromised. ¹⁰⁰	Increased size of immune synapse formed. ¹⁰¹	Improvements in functional efficacy was not shown	Melanoma ^{100,102}
Linked CD28-CD3 ϵ		Additional linked CD28 and CD3 ϵ linked to TCR α and TCR β chains	Increased TCR expression, binding, T-cell activation, and peptide responsiveness. Increased tumor infiltration and clearance as well as delayed tumor recurrence <i>in vivo</i> . ¹⁰³	Decreased affinity of TCR to peptide-MHC complex. ¹⁰³	TCR affinity is compromised. ¹⁰³	Melanoma ¹⁰³
ATAM		Additional linked costimulatory molecule consisting of CD3 ζ with 4-1BB inserted after the transmembrane domain	Increased T-cell expansion and persistence without compromising cytotoxic ability, and <i>in vivo</i> tumor control. ¹⁰⁴	Not tested	Limited transduction efficiency. ¹⁰⁴ High ATAM expression is required. ¹⁰⁵	Myeloma ^{104,105}

ATAM, artificial T-cell adaptor molecule; CML, chronic myeloid leukemia; CMV, cytomegalovirus; GVHD, graft-versus-host disease; LAG-3, lymphocyte activation gene 3; NA, not applicable; NFAT, nuclear factor of activation T cells; PD1, programmed cell death 1; PDCD1, programmed cell death protein 1; PD-L1, programmed death ligand 1; TILs, tumor-infiltrating lymphocytes; TIM-3, T-cell immunoglobulin and mucin-domain containing 3; TNF α , tumor necrosis factor α ; siRNA, small interfering RNA; STAR, synthetic TCR and antigen receptor; TACs, T-cell antigen couplers; tEGFR, truncated epidermal growth factor receptor; TRAC, T-cell receptor α constant; TRBC, T-cell receptor β constant; TRUcs, T-cell receptor fusion constructs. Figures created with BioRender.com: Gore S. (2025); <https://BioRender.com/o91f055>

References 93, 96, 99, 101, 102, 108, 123, and 131 are cited in this table.

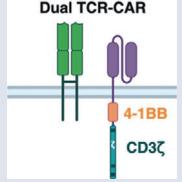
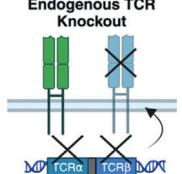
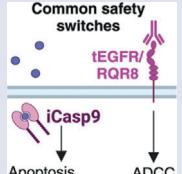
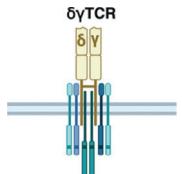
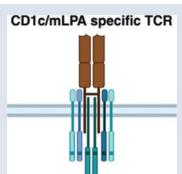
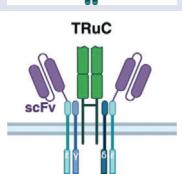
Table 3 (continued)

Novel feature	Additional functionality	Antigen sensitivity	Limitations	Cancer type tested
Additional cytokines 	Additional IL-12 (p35 and p40 subunits joined by linker) Increased target-specific release of IFNγ, TNFα, but decrease in IL-2. Slight decrease in cytotoxic ability. Potent antitumor response in vivo with increased infiltration, but not persistence. ¹⁰⁶	NA	Some toxicities seen in vivo and in clinical trials with TILs. ^{107,108} Did not persist long term. ¹⁰⁶ Limited expansion ability	Melanoma ¹⁰⁶
Additional inducible cytokines 	Additional IL-12 ¹⁰⁹ (p35 and p40 subunits) or IL-18 ¹⁰⁷ have been tested under an activation-dependent inducible promoter (NFAT). The Tet-On inducible system exerts more effective control of IL-12 than NFAT. ¹¹⁰ IL-12 has also been placed under <i>PDCD1</i> locus, alongside PD1 knockout ¹¹¹ Some have showed inducible IL-12 resulted in increased antigen-specific cytokine release, without compromising expansion. Enhanced tumor infiltration, regression, and prolonged survival in vivo. ^{109,110} However, others showed that IL-12 resulted in toxicities in vivo with decreased persistence, unlike inducible IL-18, which increased survival and infiltration without toxicities. ¹⁰⁷	NA	Some toxicities seen in vivo and in clinical trials with TILs. ^{107,108} Some designs depended on expression of inducing ligand present on tumor. Further testing required for AML	Melanoma ^{107,109,110} Multiple myeloma ¹¹¹
Inducible anchored cytokines 	Additional IL-12 anchored to cell membrane with B7-1 transmembrane (TM) domain, under activation-dependent inducible promoter NFAT Enhanced tumor control in vivo, without toxicities. ¹¹²	NA	Basal IL-12 expression still present. ¹¹² Further testing required for AML	Melanoma ¹¹²
Additional PD1 switch 	Additional PD1 switch with extracellular and transmembrane PD1 subunits linked to intracellular 4-1BB subunit. Recognition of PD1 ligand converts inhibitory signals to activation signals through 4-1BB Increased cytokine release, without compromising safety profile. Increased tumor control in vivo. ¹¹³	Avidity was not altered ¹¹³	Dependent on PD-L1 expression of tumor. Further testing required for AML	Melanoma ¹¹³
Exhaustion gene knockout 	CRISPR-based genome editing to knock out exhaustion genes <i>PD1</i> , ¹¹¹ <i>TIM-3</i> , <i>LAG-3</i> and <i>2B4</i> ¹¹⁴ to prevent exhaustion and improve persistence Increased target-specific release of some cytokines, but not all. Cytotoxic ability is not compromised. Increased effector function upon repeated antigen stimulations. Increased tumor control and infiltration in vivo. ^{111,114}	NA	Dependent on exhaustion marker ligand profile of tumor. Further testing required for AML	Multiple myeloma ¹¹⁴
CoCAR 	Additional costimulatory CAR with antigen recognition and costimulatory domains but lacking the CD3ζ domain found in conventional CARs. Allows for dual-antigen targeting Increased specific IFNγ release and cytotoxic ability upon repeated antigen stimulations. Increased tumor control in vivo. ¹¹⁵	Not tested	Further testing required for AML ¹¹⁵	Acute lymphoblastic leukemia ¹¹⁵

ATAM, artificial T-cell adaptor molecule; CML, chronic myeloid leukemia; CMV, cytomegalovirus; GVHD, graft-versus-host disease; LAG-3, lymphocyte activation gene 3; NA, not applicable; NFAT, nuclear factor of activation T cells; PD1, programmed cell death 1; PDCD1, programmed cell death protein 1; PD-L1, programmed death ligand 1; TILs, tumor-infiltrating lymphocytes; TIM-3, T-cell immunoglobulin and mucin-domain containing 3; TNFα, tumor necrosis factor α; siRNA, small interfering RNA; STAR, synthetic TCR and antigen receptor; TACs, T-cell antigen couplers; tEGFR, truncated epidermal growth factor receptor; TRAC, T-cell receptor α constant; TRBC, T-cell receptor β constant; TRUCs, T-cell receptor fusion constructs. Figures created with BioRender.com: Gore S. (2025); <https://BioRender.com/o91f055>

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Table 3 (continued)

Novel feature	Additional functionality	Antigen sensitivity	Limitations	Cancer type tested
Dual TCR-CAR 	Additional conventional CAR expressed alongside TCR to allow for dual-antigen targeting	Increased antigen-specific cytotoxic ability to target cells expressing both antigens upon repeated stimulations. Increased tumor elimination at some TCR/CAR cellular combination ratios but not all ¹¹⁶	Not tested	Not all TCR/CAR combination ratios were effective and further work is required ¹¹⁶ AML ¹¹⁶
Endogenous TCR Knockout 	Zinc-finger nuclease, ¹¹⁷ siRNA ¹¹⁸ and CRISPR-mediated knockout of endogenous <i>TRAC</i> and <i>TRBC</i> loci, preventing mispairing of inserted antigen-specific TCR with endogenous TCR chains. Insertion of antigen-specific TCR can be targeted or random ^{36,119}	Increased antigen-specific TCR expression, and cytotoxic effect. Abrogated GVHD seen in vivo. ¹¹⁷ Increased tumor control and exhaustive phenotype seen in vivo ³⁶	Increased avidity with CRISPR <i>TRAC</i> and <i>TRBC</i> combined knockout/knockin as compared with <i>TRAC</i> only ³⁶	Multiple steps of genome editing required in some cases ¹¹⁷ AML ^{36,117}
Common safety switches 	Additional safety switches, including iCasp9, tEGFR, RQR8, Myc tag, and CRASH-IT. ¹²⁰ Various inducible mechanisms can control elimination of transduced cells	Antigen-specific target cell recognition was not compromised. iCasp9 had most effective inducible elimination of transduced T cells in comparison with tEGFR, RQR8, and Myc tag. ⁵⁰ CRASH-IT switch allowed for reversible elimination of transduced T cells ¹²⁰	Slight decrease in functional avidity of T cells with certain iterations of CRASH-IT ¹²⁰	Further testing required for AML for some switches AML ⁵⁰ Melanoma ¹²⁰
δγTCR 	Alternate γδ TCR chains, which do not require HLA for recognition	Reduced alloreactivity. Functional activity including both cytokine release and cytotoxicity is redirected in both CD8 ⁺ and CD4 ⁺ subsets. Functional activity was shown against primary AML blasts and in vivo, ¹²¹ with a favorable safety profile. ¹²² This product is currently in clinical trials ¹²³	NA	Limited knowledge of antigen targets available AML and other leukemias ¹²¹
CD1c/mLPA specific TCR 	Alternate TCRα and β, which mediates non-HLA-restricted recognition of self-lipids	Functional, non-HLA-restricted response to leukemic cell lines and primary blasts and in vivo ^{124,125}	NA	Limited knowledge of antigen targets available AML and other leukemias ^{124,125}
TRuC 	TCR fusion constructs (TRuCs) consist of antigen-specific scFvs linked to CD3ε subunits. Antigen specificity is redirected by the scFv, with downstream TCR activation	Antigen-specific TCR recognition does not compromise T-cell activation. ¹²⁶ TRuC functionality was enhanced in comparison with CARs. ^{126,127} TRuCs with a scFv recognizing the same antigen as the TCR had comparable cytotoxicity in vitro but decreased tumor control in vivo. ²⁷	TRuCs with a scFv recognizing the same antigen as the TCR had decreased avidity ^{27,31}	Reliant on the availability of an antigen-specific scFv. Further characterization and testing required for AML Breast cancer, leukemia ³¹

ATM, artificial T-cell adaptor molecule; CML, chronic myeloid leukemia; CMV, cytomegalovirus; GVHD, graft-versus-host disease; LAG-3, lymphocyte activation gene 3; NA, not applicable; NFAT, nuclear factor of activation T cells; PD1, programmed cell death 1; PDCD1, programmed cell death protein 1; PD-L1, programmed death ligand 1; TILs, tumor-infiltrating lymphocytes; TIM-3, T-cell immunoglobulin and mucin-domain containing 3; TNFα, tumor necrosis factor α; siRNA, small interfering RNA; STAR, synthetic TCR and antigen receptor; TACs, T-cell antigen couplers; tEGFR, truncated epidermal growth factor receptor; TRAC, T-cell receptor α constant; TRBC, T-cell receptor β constant; TRuC, T-cell receptor fusion constructs. Figures created with BioRender.com: Gore S. (2025); <https://BioRender.com/o91f055>

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Table 3 (continued)

Novel feature		Additional functionality	Antigen sensitivity	Limitations	Cancer type tested	
TAC		TACs consist of antigen-specific scFv linked to an anti-CD3 antibody and CD4 transmembrane domain	Increased functional response in comparison to CARs, but not tested in comparison with TCRs ¹²⁸	Not tested	Further safety assessments required. Testing required for AML	Ovarian cancer and leukemia ¹²⁸
STAR/HIT		V _H and V _L antibody domains are attached separately to TCR constant regions of α and β resp. ¹²⁹ Antigen recognition is antibody mediated, but activation is mediated by TCR signaling. Co-STAR designs include the addition of another costimulatory MyD88-CD40 molecule. ²⁷ This has also been tested in conjunction with CRISPR-based endogenous TCR knockout ¹³⁰	Increased functional response in comparison with CARs. Increased signaling seen in STARs but with similar transcriptional patterns as TCRs, without tonic signaling seen in CARs. ¹²⁹ Although some have showed that in vitro and in vivo functional response was comparable with or superior to that of TCRs, ³¹ others found that this increased efficacy was only seen after the addition of another MyD88-CD40 costimulatory domain ²⁷	STARs recognizing the same antigen as TCR had comparable avidity ³¹	Further safety assessments required. Testing required for AML	Solid tumors ^{31,129} AML ¹³¹ Other leukemias ²⁷
abTCR		V _H and V _L antibody domains are attached separately to TCR δ and TCR γ chains respectively	Increased functional response in comparison with CARs but not tested in comparison with TCRs ¹³²	Not tested	Further safety assessments required. Testing required for AML	Leukemias ¹³²

ATAM, artificial T-cell adaptor molecule; CML, chronic myeloid leukemia; CMV, cytomegalovirus; GVHD, graft-versus-host disease; LAG-3, lymphocyte activation gene 3; NA, not applicable; NFAT, nuclear factor of activation T cells; PD1, programmed cell death 1; PDCD1, programmed cell death protein 1; PD-L1, programmed death ligand 1; TILs, tumor-infiltrating lymphocytes; TIM-3, T-cell immunoglobulin and mucin-domain containing 3; TNF α , tumor necrosis factor α ; siRNA, small interfering RNA; STAR, synthetic TCR and antigen receptor; TACs, T-cell antigen couplers; tEGFR, truncated epidermal growth factor receptor; TRAC, T-cell receptor α constant; TRBC, T-cell receptor β constant; TRUCs, T-cell receptor fusion constructs. Figures created with BioRender.com: Gore S. (2025); <https://BioRender.com/o91f055>

References 93, 96, 99, 101, 102, 108, 123, and 131 are cited in this table.

inhibitory signals into activation signals.^{92,134,135} Most commonly, programmed cell death protein 1 immune checkpoint can be linked to an intracellular 4-1BB activation domain, facilitating an activation signal instead of the usual negative signal.¹¹³ CRISPR-mediated programmed cell death protein 1 knockout has also been combined with an IL-2 knockin to abrogate exhaustion and boost efficacy.¹¹¹ Knock out of other exhaustion related genes such as T-cell immunoglobulin and mucin domain 3, lymphocyte activation gene 3, and 2B4 (also known as cluster of differentiation 244) results in improved persistence and response upon rechallenge.¹¹⁴ Such approaches have shown promise when paired with CAR T cells in early-phase clinical trials,¹³⁶ with potential for similar application in TCR T cells.

Dual-antigen targeting. A well-documented driver of relapse in AML is the expansion of clones with downregulated target antigen expression. Dual-antigen–targeting strategies are being rapidly developed. Increasing precision targeting can be achieved by costimulatory CAR designs coexpressing an antigen-specific single-chain variable fragment (scFv) with a costimulatory domain but lacking the CD3 ζ signaling. A surviving-targeting TCR, combined with such a scFv + costimulatory combination showed efficacy, without excessive CD3 activation.¹¹⁵ Alternatively, dual-expressing TCR CAR T cells have shown functional efficacy in other malignancies, with initial studies showing promise for application in AML.¹¹⁶ Further studies will be required to confirm whether such dual-targeting approaches are successful in improving responses and overcoming therapy resistance induced by emergence of antigen-escape variants.

Addressing safety concerns

Removal of endogenous TCR. One potential safety risk is mispairing of the introduced TCR $\alpha\beta$ with endogenous TCR $\alpha\beta$ chains, resulting in unexpected specificity and toxicity. The deletion of endogenous TCR using zinc-finger nucleases,¹¹⁷ or knockout using small interfering RNA or CRISPR-CRISPR-associated protein 9 as shown in WT1- and NY-ESO-1-directed TCR T cells for AML, can abrogate the risk.^{36,118,119} The CRISPR-mediated knock out can be accompanied by the insertion of the antigen-specific TCR into the natural *TRAC* and *TRBC* locus, not only limiting risk of mispairing but also preventing excessive activation because the antigen-specific TCR is now expressed at physiological levels under endogenous promoter controls.^{137,138}

Safety switches for unexpected toxicities. In the event of unexpected toxicity, safety switches to induce apoptosis of the infused T-cell product to abrogate further deleterious effects have been designed. Commonly used is the inducible caspase 9, with which infusion of a dimerizing agent allows for controllable dimerization of caspase 9 to activate the downstream apoptosis pathway.¹³⁹ Others include truncated epidermal growth factor receptor, which causes antibody-dependent cellular cytotoxicity upon interaction with cetuximab antibody,¹⁴⁰ a chimeric molecule called RQR8 comprising CD20/CD34 epitopes to be eliminated with rituximab via antibody-dependent cellular cytotoxicity,¹⁴¹ and Myc-tag.¹⁴² Each of these safety switches was coexpressed with HA-1 TCR, with inducible caspase 9 the most effective in eliminating TCR T cells.⁵⁰ More recently, the “CRASH-IT” switch explores a reversible safety switch wherein the addition of various

drugs resulted in a dose-dependent, reversible proteasomal degradation of the TCR.¹²⁰

Overcoming HLA restriction

Downregulation of the HLA molecule on AML blasts results in immune evasion, because TCR T cells are dependent on HLA presentation of targets.

HLA-independent TCRs. $\gamma\delta$ T cells that make up ~10% of T cells recognize cancer-specific phosphoantigens, in a HLA-independent manner.¹⁴³ $\gamma\delta$ TCRs can be inserted into $\alpha\beta$ T cells for antigen recognition and cytotoxic response without HLA restriction.¹²¹ Because the $\gamma\delta$ TCRs do not pair with $\alpha\beta$ chains, any risk of mispairing-mediated toxicity is removed. One such product, TEG1001, is moving into early-phase clinical trials.¹²²

Some alternate natural TCRs can recognize HLA-independent lipid molecules such as CD1c.^{124,144} One such TCR recognizing methyl-lysophosphatidic acid, which is selectively upregulated on AML cells, was shown to delay AML progression in xenograft mice models.¹²⁵ Such a product allows for a broader use in patients but is also greatly limited by the type of antigens that can be targeted.

Antibody-based antigen recognition. Other novel designs have combined antibody-based antigen recognition, with downstream TCR clustering and signaling. scFvs have been linked to CD3 ϵ subunits (TRuCs),^{126,127} or to an anti-CD3 antibody and CD4 transmembrane domain (T-cell antigen couplers) to overcome HLA restriction while maintaining a sensitive functional response.¹²⁸ Alternatively, separate light and heavy chains can be attached independently to TCR α and β chain constant domains. These formats, named synthetic TCR and antigen receptors¹²⁹ or HLA-independent TCRs¹³⁰ have shown equivalent or greater responsiveness and tumor control over TCR formats, as well as TRuCs.^{27,31} In a similar concept, antibody TCRs link the separate antibody chains to TCR γ and δ chains.¹³² These designs combine the superior sensitivity of TCR signaling with conventional CAR-like antigen recognition.

Furthermore, CAR-like designs centered around TCR-like antibodies can recognize peptide-MHC complexes. Such peptide-centric CARs¹⁴⁵ and TCR-mimic CARs¹⁴⁶ have shown promise for AML but are still HLA restricted and use CAR-like downstream signaling. Direct comparisons indicate that TCR-based designs convey superior sensitivity and functionality in low –antigen density contexts over CARs, which are ideal for AML neoantigens.^{27,31}

These technologies hold great promise in contributing to further detailed understanding of TCR and CAR signaling mechanisms, which will lead to fine-tuning of customized designs for the AML context.

Clinical trials with TCR T cells

The clinical trials of antigen-specific TCR T-cell therapy for AML are summarized in Table 4. WT1 was the target in 6 of 12 trials, with other trials targeting PRAME (2/12), HA-1/HA-2 (3/12), or mutated nucleophosmin 1 (dNPM1) (1/12). The source of production of TCR T-cell products were either autologous ($n = 8$) or allogeneic ($n = 4$), and 11 studies targeted HLA-A*02:01–restricted antigens.

Table 4. TCR T-cell therapy for clinical trials for AML

Trial reference	Phase/ status	Product details			Disease	Disease type	Patients treated	Response	Persistence	Significant adverse events
UMIN000011519 ¹⁴⁷ PMID: 28860210	Phase 1 Completed	Retroviral vector with siRNA knock down of endogenous TCR, WT1 peptide vaccine	Auto	WT1	A*24:02	Refractory AML, MDS	Active	8 (2 AML)	2/8 showed decreased blast counts in the BM (predicted leukemia regression). 2 patients with AML did not respond.	Yes, at 5 mo after treatment (4/5 patients survived for ≥12 mo).
NCT01621724 EudraCT-2006-004950-25	Phase 1/2 Completed	Retroviral vector, IL-2 standard conditioning	Auto	WT1	A*02:01	AML, CML	Active	7	4 patients showed disease responsiveness. No response in 3 patients.	Yes, for 4/7 patients at 1 y after treatment. Anemia (1).
NCT01640301 ¹⁴⁸ PMID: 31235963	Phase 1/2 Terminated	EBV-specific CD8 ⁺ T cells, Additional IL-2 injection	Allo	WT1	A*02:01	AML (recurrent/secondary) having undergone allo-HSCT (with no evidence of disease)	In remission	12	Maintenance of remission at median of 44 mo for 12 patients.	Yes, for 4/12 patients at 1 y after treatment. CRS grade 3 (2). Neutropenia (2). Thrombocytopenia (2). Lymphopenia (12). Anemia (7).
NCT02550535 ¹¹⁵ EudraCT-2014-003111-10	Phase 1/2 Completed	Retroviral vector, Additional IL-2 injection	Auto	WT1	A*02:01	AML, MDS	In remission	10 (AML)	Median survival of 12 mo in 6 patients with AML.	Yes, for 7/10 patients over 12 mo.
NCT05066165	Phase 1/2 Terminated	CRISPR/Cas9	Auto	WT1	A*02:01	AML	Active	2	Disease progression in both patients.	Not reported. Febrile neutropenia (1).
NCT02770820	Phase 1/2 Terminated	EBV-specific CD8 ⁺ TCM/TN T cells, Additional IL-n2 injection	Auto	WT1	A*02:01	High-risk non-M3 AML (with prior consolidation chemotherapy)	In remission	7	Not reported.	Not reported. No serious adverse events reported.
NCT03503968 ¹⁴⁹ EudraCT-2017-000440-18	Phase 1/2 Terminated	-	Auto	PRAME	A*02:01	AML, MDS, MM	Active	9	No disease progression for 1 patient. Remission at 4 wk followed by progression at 3 mo for 1 patient. Disease progression in remaining patients (7/9).	Yes, for 6/8 patients at 4 wk. CRS grades 1-2 (2). Other SAE (not specified) (5).
NCT02743611	Phase 2/2 Terminated	Includes safety switch activated with rimiducid	Auto	PRAME	A*02:01	Relapsed AML, MDS, uveal melanoma	Active	4	Not reported.	Not reported. Neutropenic fever, tachypnea, CRS, pseudomonas bacteremia infection, neurotoxicity, orthostatic hypotension (1).
NCT03326921 ¹⁵⁰ PMID: 38683966	Phase 1 Suspended	CD8 ⁺ CD4 ⁺ TM T cells	Allo	HA-1 HA-1 (H) genotype (RS_1801284: A/G, A/A)	A*02:01	Pediatric and adult leukemias after allo-HCT	Active/in remission	9	Reduction of marrow blasts lasting >30 days (2/9). Sustained remission (2/9). Disease progression in remaining patients (5/9).	Yes, for 8/9 patients beyond 1 y after treatment. Neutropenia (5). Fever (3). Infection (2). Infusion reaction (1).

BM, bone marrow; CRS, cytokine release syndrome; MDS, myelodysplastic syndrome; MM, myelodysplastic syndrome; SAE, severe adverse event; siRNA, small interfering RNA; T_{CM}, central memory T cell; T_M, memory T cell; T_N, naive T cell.

Table 4 (continued)

Trial reference	Phase/ status	Product details			Disease type	Patients treated	Response	Persistence	Significant adverse events
EudraCT-2010-024625-20 ¹⁵¹ PMID: 32973796	Phase 1 Terminated	EBV- or CMV-specific T cells retroviral vector	Allo	HA-1	A*02:01 allo-HCT	High-risk leukemia, after allo-HCT	Active (1)/in remission (4)	Maintenance of relapse-free survival at follow-up in 2 patients. Disease progression in 3 patients.	Yes, for 2 patients up to 21 wk. Neutropenia. Thrombocytopenia (1).
NCT05473910 ^{152,153}	Phase 1 Recruiting	-	Allo	HA-1 or HA-2	A*02:01	AML, MDS, ALL undergoing haplo-identical allo-HCT	In remission	Maintenance of remission at a median follow-up of 162 d in all patients (at time of reporting).	Yes, ongoing persistence at longest follow-up of 203 d.
NCT06424340	Phase I/II Recruiting	-	Auto	dNPM1	A*02:01	AML (relapsed or refractory)	Active	-	GVHD (4).

BM, bone marrow; CRS, cytokine release syndrome; MDS, myelodysplastic syndrome; MM, myelodysplastic syndrome; RNA, small interfering RNA; T_{CM}, central memory T cell; T_{Na}, naive T cell.

Following from promising WT1-directed CTLs, in trial NCT01640301, 12 patients with AML were treated prophylactically with allo-HCT donor-derived Epstein-Barr virus-specific CD8⁺ WT1 TCR T cells after allo-HSCT. All patients achieved relapse-free survival at a median follow-up of 44 months. In a comparative cohort, there was a relapse-free survival rate of only 54%, indicating that the treatment likely has some efficacy in preventing relapse.¹⁴⁸ Trial NCT01621724 treated 7 patients with AML or chronic myeloid leukemia, disease status not specified, with autologous WT1–directed TCR T cells, 4 of whom showed disease responsiveness.¹⁵⁴ A similar product was used in NCT02550535, in which all 6 prophylactically-treated patients with AML remained in remission at follow-up (median of 12 months).¹⁴⁹ In these 3 trials, persistence of T cells was shown in 33% to 70% of patients at 1 year. Generally, the treatment was well tolerated, with no on-target, off-tumor toxicities. Adverse events included cytokine release syndrome, and, some instances of neutropenia, anemia, thrombocytopenia, and lymphopenia that resolved in all cases.

It is important to note that in 2 of the aforementioned trials, patients enrolled were in remission at the time of the treatment. Thus, direct contribution of the TCR T cells in preventing relapse is not clear. Indeed, 1 trial treated 8 patients with AML or refractory myelodysplastic syndrome with an autologous WT1 TCR T cell, which had small interfering RNA-mediated endogenous TCR knockdown and an additional WT1 peptide vaccine treatment (UMIN000011519). Of 8 patients, 2 showed decreased blast counts the remaining 6 patients did not respond (including 2 patients with AML). Still, T cells persisted for 5 months in 5 of patients (of whom 4 survived past 12 months), and no treatment-related toxicities or adverse events were detected.¹⁴⁷ Similarly, 2 patients with AML with detectable disease were treated in trial NCT05066165, with both patients experiencing disease progression without severe adverse events after treatment. These 2 trials spoke to the safety of TCR T-cell therapies, and indicated their potential for preventing relapse, which is a significant challenge in AML. Undeniably, further study is required in this area.

There have also been 2 TCR T-cell trials with a small number of participants (ClinicalTrials.gov identifier: NCT03503968 and NCT02743611) targeting the cancer testes antigen HLA -A*02:01/ PRAME with AML, myelodysplastic syndrome, and uveal melanoma, which have been completed and results awaited. In addition, a recent trial has been initiated targeting a neomutation in dNPM1 (ClinicalTrials.gov identifier: NCT06424340).

Three phase 1 clinical trials for patients with AML or other leukemias, undergoing allo-HSCT have been initiated using HA-1–specific T cells. Although 1 trial is still open (ClinicalTrials.gov identifier: NCT05473910¹⁵⁵), results for the EudraCT-2010-024625-20 and NCT03326921 trials have been reported. In the former, 5 patients positive for HA-1 were treated prophylactically with Epstein-Barr virus- or cytomegalovirus-specific CD8⁺ HA-1 TCR T cells generated from allo-HA-1-negative donors.¹⁵¹ Of these, 2 patients remained in remission throughout the study duration and HA-1 T cells persisted but did not appear to expand *in vivo*. No graft-versus-host disease or toxicity was observed. Trial NCT03326921, a CD8 and CD4 TCR T-cell product incorporating a CD8 coreceptor with the HA-1 TCR, treated 9 patients who had relapsed early after allo-HSCT, some of whom had achieved

another remission. T cells persisted in 8 patients for up to a year, and disease responsiveness was seen in 4 patients, with 1 maintaining complete remission for >27 months.

This is a rapidly evolving field, and the handful of trials completed thus far have provided key insights. The paucity of severe adverse events seen in most trials, and sustained remission accompanied by long-term persistence of the therapeutic T cells, even with low doses, seen in some patients has been very promising. One common limitation resulting in early termination of trials was slow accrual, likely because of the strict HLA and antigen-specificity requirements for this sort of therapy. Furthermore, inability to generate products for all enrolled patients is another limitation. In the EudraCT-2010-024625-20 trial almost half (4/9 patients) were not treated for this reason. In autologous programs, this is made more difficult because patient T-cell quality may be affected by previous chemotherapy and/or other treatments. Indeed, in the NCT05066165 trial, only 2 patients were treated before the trial was terminated to move to an allogeneic version of the same WT1 TCR therapy. Thus, larger trials with increased patient recruitment are required to be able to draw more reliable conclusions. Platform trials using TCR T cells to multiple antigens, HLAs, and/or multiple cancers may be an option. Upcoming phase 2 trials will provide further information regarding the efficacy of these products for AML.

Conclusion

TCR therapy is poised to revolutionize treatment for AML as evidenced by the substantial preclinical development of TCR T cells and promising early-phase clinical trials. Although it is premature to predict which 1 of the developed options will become an approved treatment, the favorable safety profiles encourage further testing. Novel designs to enhance efficacy and safety are being fine-tuned. It is expected that combining engineering strategies with high throughput identification and testing, along with the development of algorithms and in silico analyses will facilitate rapid development of TCR therapies. The technologies will also likely enable improving responses and reducing off-target toxicities. Thus, TCR therapy is expected to become a crucial component in the T-cell therapeutic arsenal for AML treatment, and given AML's complex immunophenotype, combinatorial targeting of multiple antigens will likely be necessary.

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Conflict-of-interest disclosure: M.B. is an inventor on a patent describing HA-1 TCR T cells that was previously licensed to Elevate Bio and has recently been licensed to Promicell Inc; received research funding from HighPass Bio, an Elevate Bio portfolio company; and has financial interests in HighPass Bio and Promicell Inc. E.B. and K.M. hold patents in adoptive cell therapy for opportunistic infection and malignancy. E.B. reports advisory board membership for IQVIA, AbbVie, MSD, Astellas, Novartis, Gilead, and Bristol Myers Squibb, and research funding from MSD. The remaining authors declare no competing financial interests.

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