

Review

Stealth designs to overcome allorejection in engineered cell therapy

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The rapid development of allogeneic engineered therapeutic cells has intensified the challenge of host immune-mediated rejection. Advances in molecular immunology, genetic engineering, and induced pluripotent stem cell–based multigene editing have enabled the creation of ‘stealth’ allogeneic cells designed to evade immune detection while maintaining function. Key strategies include the deletion of human leukocyte antigen class I and class II molecules to limit T cell recognition, the expression of natural killer (NK) cell inhibitory ligands to prevent NK cell-mediated killing, and the upregulation of CD47 to suppress phagocytosis. An expanding repertoire of immunomodulatory molecules, receptor–ligand interactions, and experimental assays is refining these approaches. Together, stealth designs are accelerating the translation of allogeneic cell therapies toward more durable and broadly applicable clinical use.

Immune-evasive designs enable allogeneic cells to overcome allorejection

Allogeneic cell therapies offer compelling solutions to the limitations inherent in autologous approaches, including high manufacturing costs, long production timelines, limited scalability, and patient-to-patient variability [1–3]. Advantages such as off-the-shelf availability, standardized manufacturing, and broad patient accessibility make allogeneic platforms particularly attractive. However, their clinical translation faces two major immunological barriers: **graft-versus-host (GvH) toxicity** (see [Glossary](#)) and **host-versus-graft (HvG) allorejection** [3,4]. Advances in cell selection strategies, such as the use of human leukocyte antigen (HLA)–matched donors, and genetic engineering approaches, including T cell receptor (TCR) knockout, have substantially reduced the risk of GvH toxicity [3,4]. Despite these improvements, immune-mediated rejection by the recipient remains a major unresolved challenge. This HvG allorejection is particularly pronounced in the setting of HLA mismatch and continues to limit the durability and efficacy of allogeneic therapies.

Recent advances in immunology and genetic engineering have enabled the development of immune-evasive (‘stealth’) allogeneic grafts through targeted modulation of T cell, natural killer (NK) cell, and myeloid cell responses. These strategies have been broadly applied to primary cells and, in particular, to induced pluripotent stem cells (iPSCs), which enable the integration of multiple genetic modifications [5]. This capability facilitates the generation of standardized cell products with defined genetic composition and functional properties, manufactured at large scale from renewable master cell banks and capable of maintaining stable phenotype and therapeutic activity after expansion and cryopreservation [6]. Here, we summarize key immune-evasion strategies, highlight their applications in disease modification, particularly cancer, review preclinical and clinical progress, and discuss future directions for stealth allogeneic therapies.

Highlights

Advances in genome editing and stem cell engineering have enabled ‘stealth’ allogeneic therapies that evade host T cell, natural killer cell, and myeloid immune responses while maintaining therapeutic function.

Multilayered immune-evasion strategies—combining human leukocyte antigen modulation, natural killer-inhibitory signaling, suppression of phagocytosis, and disruption of immune synapse formation—are emerging as a unifying principle for durable allogeneic graft persistence.

Induced pluripotent stem cell–based platforms uniquely support complex, multiplex genetic engineering, enabling scalable, off-the-shelf cell products that outperform single-edit approaches in preclinical and early clinical studies.

Early clinical data, including first-in-human trials of hypimmune islets and allogeneic chimeric antigen receptor cell therapies, provide proof-of-concept that immune cloaking can overcome both allogeneic and autoimmune barriers.

Significance

Immune-mediated rejection remains the principal barrier to scalable allogeneic cell and tissue therapies. Recent advances in stealth immune engineering demonstrate that coordinated modulation of T cell, natural killer cell, and myeloid immune pathways can enable durable graft persistence without systemic immunosuppression. These strategies redefine how immune tolerance can be achieved and have broad implications for cancer immunotherapy, autoimmune disease, and regenerative medicine.

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Stealth designs to reduce host T cell-mediated allorejection

HLA-I/II knockout

Immune rejection of HLA-mismatched allografts arises from complex and coordinated immune mechanisms. The response is initiated predominantly by naïve T cells that recognize donor cells expressing foreign HLA molecules and can be further intensified by crossreactive memory T cells generated through prior antigen exposure. Cytotoxic CD8⁺ T cells mediate direct destruction of allogeneic cells through recognition of HLA class I molecules, while CD4⁺ T cells respond primarily to HLA class II and orchestrate broader immune activation [7,8]. CD4⁺ T cell help promotes B cell differentiation and the generation of donor-specific antibodies, which exacerbate graft rejection via complement activation and antibody-dependent cellular cytotoxicity [7,8]. Consequently, suppression of host T cell-driven alloreactivity is a central and indispensable consideration in allogeneic graft design [5]. A foundational strategy to achieve this goal is the elimination of HLA class I and class II expression to attenuate both CD8⁺ and CD4⁺ T cell-mediated immune responses (Figure 1) [3,4].

A widely used strategy to eliminate HLA expression involves genetic disruption of key genes required for HLA molecule assembly, trafficking, and transcriptional regulation. These targets include β 2-microglobulin (B2M), which is essential for the stability and surface expression of HLA class I molecules, including HLA-A and HLA-B; transporter associated with antigen processing (TAP1 and TAP2), which are required for peptide loading onto HLA class I molecules; and class II transactivator

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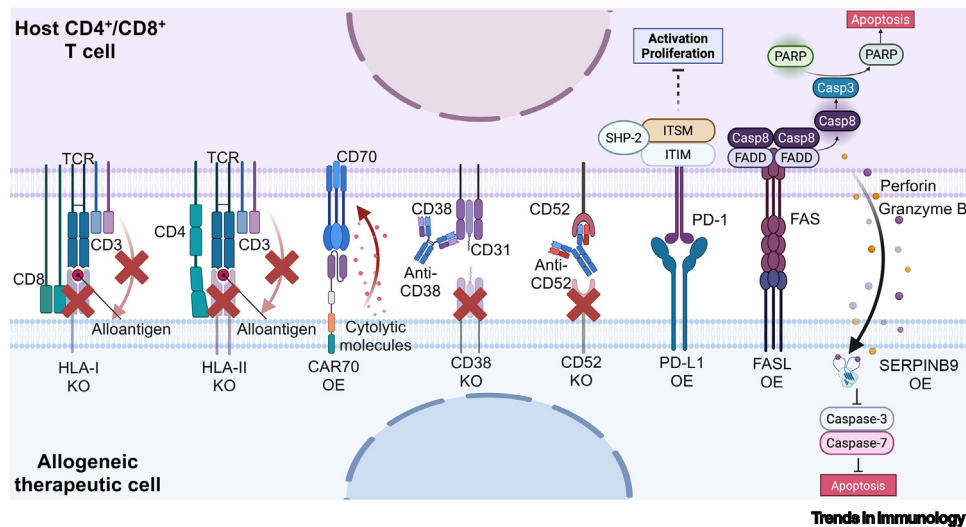


Figure 1. Stealth designs to reduce host T cell-mediated allorejection. This schematic illustrates the molecular interactions between host CD4⁺ and CD8⁺ T cells and an engineered allogeneic therapeutic cell, highlighting complementary genetic modifications that collectively suppress immune recognition and cytotoxicity. To prevent direct recognition by host T cell receptors (TCRs), HLA-I and HLA-II are knocked out (KO; red crosses), effectively limiting alloantigen presentation. Resistance to antibody-mediated depletion is achieved by knocking out CD38 and CD52, which prevents the binding of lymphodepleting antibodies (such as anti-CD38 and anti-CD52) and thus evades antibody-dependent cellular cytotoxicity (ADCC). Active counter-defense mechanisms include the overexpression (OE) of CAR70, which selectively targets and eliminates activated host T cells expressing CD70, and the overexpression of FAS ligand (FASL), which induces apoptosis in Fas (CD95)-expressing alloreactive T cells via the FADD–caspase-8 cascade. Immunosuppressive signaling is further enforced by PD-L1 OE, which engages PD-1 on T cells to recruit SHP-2 to ITIM/ITSM motifs, thereby inhibiting T cell activation and proliferation. Finally, intracellular SERPINB9 OE protects the therapeutic cell from perforin/granzyme B-mediated lysis by inhibiting the activation of downstream apoptotic cascades. Figure created using BioRender (<http://biorender.com/>). CAR: chimeric antigen receptor; FADD: Fas-associated protein with death domain; HLA: human leukocyte antigen; ITIM/ITSM: immunoreceptor tyrosine-based inhibitory/switch motif; PARP: poly (ADP-ribose) polymerase; PD-L1: programmed death-ligand 1; SHP: Src homology region 2 domain-containing phosphatase.

(CIITA), the master regulator of HLA class II gene transcription [9–12]. In addition, some studies have reported the deletion of CD74, the invariant chain required for proper assembly and intracellular trafficking of HLA class II molecules, as an effective means to suppress CD4⁺ T cell recognition [13]. Such modifications are typically achieved using Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated genome editing or short hairpin RNA (shRNA)-mediated gene silencing, thereby reducing T cell-mediated allogeneic immune responses [14–19].

To date, the majority of preclinical studies and clinical trials involving allogeneic grafts or therapeutic cell products have relied on HLA ablation strategies and have demonstrated reduced immunogenicity with effective evasion of host T cell-mediated rejection [18,20]. In support of this approach, deletion of both mouse MHC class I and class II molecules in mouse iPSCs has been shown to confer superior immune evasion compared with B2M knockout alone [21]. Rather than exhaustively cataloging all published studies, we highlight several representative and mechanistically informative examples below.

A strategy has been developed to protect allogeneic chimeric antigen receptor-engineered T (CAR-T) cells from host immune attack by exploiting immune evasion mechanisms evolved by lymphotropic viruses. By expressing the HIV 1-derived viral immune evasin Nef, they achieved intermediate levels of HLA-I expression that reduced susceptibility to allogeneic CD8⁺ T cell-mediated killing without inducing NK cell activation [22]. This approach illustrates how fine-tuning HLA expression can balance evasion of both T cell- and NK cell-mediated alloreactivity.

CAR-engineered invariant natural killer T (NKT) cells have been generated from genetically modified human hematopoietic stem and progenitor cells (HSPCs) using clinically relevant feeder-free differentiation protocols [23]. These HSPC-derived NKT cells exhibited intrinsically low immunogenicity, characterized by markedly reduced HLA-I expression and nearly undetectable HLA-II levels [23]. Notably, this phenotype was specific to HSPC-derived NKT cells and was not observed in peripheral blood mononuclear cell-derived T cells or NKT cells. Mechanistic analyses suggested that epigenetic repression of HLA loci and impaired interferon receptor signaling contribute to this immune-evasive state [15,23,24]. These findings highlight HSPC engineering as a promising route for generating off-the-shelf cellular therapies with reduced alloreactivity risk.

Hypoimmunogenic regulatory T cells (Tregs), capable of evading both T cell- and NK cell-mediated immune responses, were also generated. Using nonviral CRISPR-based genome editing, they disrupted *B2M* and *CIITA* while simultaneously inserting an HLA-E/B2M fusion construct to inhibit NK cell activation [25]. The engineered Tregs maintained FOXP3 stability and potent suppressive function *in vitro* and significantly prolonged human skin graft survival in a humanized mouse model following a single low-dose infusion [25]. These results demonstrate the feasibility of off-the-shelf Treg therapy with efficacy comparable to autologous products.

In addition, allogeneic CD19-targeted CAR-T cells have been developed for the treatment of patients with severe **myositis and systemic sclerosis** using multiplex CRISPR–Cas9 gene editing [17]. The engineered cells harbored deletions of HLA-A, HLA-B, CIITA, TCR alpha constant (TRAC), and programmed cell death protein 1 (PD-1) [17]. Notably, HLA-C was retained, suggesting that selective ablation of HLA-A and HLA-B may be sufficient to confer resistance to host T cell-mediated rejection while preserving inhibitory signaling to NK cells. This study provides important clinical evidence supporting selective HLA editing strategies in allogeneic CAR-T cell therapy.

In summary, genetic modulation of HLA expression has emerged as a central strategy to mitigate host T cell-mediated alloreactivity in allogeneic therapies, often in combination with modifications

Glossary

Alemtuzumab: a monoclonal antibody directed against CD52 that induces profound lymphocyte depletion, commonly used for immunosuppression and to facilitate engraftment of allogeneic cell therapies.

C-peptide: a peptide released in equimolar amounts with insulin during proinsulin processing, commonly used as a biomarker of endogenous pancreatic β -cell function.

Cyclophosphamide: a nitrogen mustard alkylating agent that functions as a prodrug, undergoing hepatic activation to form phosphoramidate mustard, which creates DNA crosslinks to induce cell death and reduce the number of endogenous regulatory T cells prior to adoptive cell transfer.

Daratumumab: a monoclonal antibody targeting CD38, clinically used to deplete CD38-expressing immune cells and treat multiple myeloma, and leveraged in cell therapy to facilitate immune depletion and engraftment.

Fludarabine: a purine nucleoside analog and antimetabolite that inhibits DNA synthesis by interfering with DNA polymerase and ribonucleotide reductase, primarily used in lymphodepletion to deplete host T cells and create a favorable cytokine environment for allogeneic cell expansion.

Glycosylphosphatidylinositol: a glycolipid membrane anchor that tethers certain proteins to the cell surface, enabling their localization and function without transmembrane domains.

Graft-versus-host toxicity: an adverse immune reaction in which donor-derived immune cells recognize and attack host tissues, leading to inflammatory damage that limits the safety of allogeneic cell and tissue therapies.

Host-versus-graft alloreactivity: an immune response in which the recipient's immune system recognizes and eliminates transplanted allogeneic cells or tissues through coordinated adaptive and innate immune mechanisms.

Human papillomavirus: a group of DNA viruses that infect epithelial tissues, with certain high-risk types causing persistent infection and contributing to the development of cancers such as cervical and head and neck cancers.

Immunopeptidomic profiling: a mass spectrometry-based approach used to

to additional immune regulatory genes. Continued advances in multiplex genome editing, selective HLA targeting, and stem cell-based platforms are enabling the development of durable, scalable, and clinically translatable off-the-shelf immune cell products [6,26].

CD38/CD52 knockout combined with anti-CD38/CD52 antibodies

An alternative strategy to control host alloreactive immune responses involves combining genetic deletion of broadly expressed lymphocyte markers with antibody-mediated immune depletion. CD38 and CD52 are highly expressed on activated T cells, NK cells, and other immune subsets, and monoclonal antibodies targeting these molecules, such as **daratumumab** (anti-CD38) and **alemtuzumab** (anti-CD52), have been widely used for immune cell depletion in clinical settings [27,28]. By knocking out CD38 or CD52 in therapeutic allogeneic cells, these engineered cells become resistant to antibody-mediated depletion, while endogenous host immune cells are selectively eliminated following antibody administration (Figure 1) [29,30]. This approach enables transient but profound suppression of host alloreactive T and NK cells without permanently altering HLA expression on the graft. However, CD38 is also expressed on Tregs, and depletion with anti-CD38 antibodies may inadvertently reduce this immunosuppressive population, representing a potential complicating factor that could influence immune balance and tolerance [31]. Preclinical and early clinical studies have demonstrated that CD38 or CD52 knockout, combined with corresponding antibody treatment, enhances engraftment, persistence, and efficacy of allogeneic cell therapies.

PD-L1/FASL/SERPINB9 overexpression

Recent work describes the development of a cloaked human pluripotent stem cells (PSC)-derived neural graft that achieves both functional integration and immune evasion in xenograft and allogeneic transplantation models [32]. PSCs were engineered to overexpress a panel of eight immunomodulatory transgenes, including *PD-L1*, *CD200*, *CD47*, *HLA-G*, *FASLG*, *SERPINB9*, *CCL21*, and *MFGE8*, designed to suppress or evade immune responses mediated by distinct immune cell populations, including T lymphocytes, NK cells, dendritic cells (DCs), and macrophages [32].

In vitro coculture assays demonstrated that gene-engineered PSC-derived midbrain neurons resisted immune-mediated rejection by human T cells, NK cells, macrophages, and DCs [32]. In humanized mouse models, allogeneic cloaked neural grafts survived long term without signs of rejection, whereas control PSC-derived grafts elicited robust immune activation, characterized by infiltration of human immune cells, elevated inflammatory cytokines in blood and cerebrospinal fluid, and secondary lymphoid organ enlargement. Importantly, the immune-evasive grafts retained physiological functionality and were capable of reversing motor deficits in Parkinsonian rat models, indicating that immune cloaking did not compromise neuronal differentiation or therapeutic efficacy [32].

Mechanistic analyses revealed that programmed death-ligand 1 (PD-L1), FAS ligand (FASL), and SERPINB9 play central roles in protecting grafts from T cell-mediated allojection (Figure 1). PD-L1 suppresses adaptive immune responses through engagement of PD-1 on activated CD4⁺ and CD8⁺ T cells, leading to inhibition of TCR signaling, reduced proinflammatory cytokine production (including interferon gamma (IFN- γ) and tumor necrosis factor alpha (TNF- α)), and induction of T cell exhaustion or anergy [20,33]. In PSC-derived neural grafts, PD-L1 expression dampens local T cell activation and limits interferon-driven inflammatory amplification, a key driver of graft rejection. FASL provides an additional layer of immune protection by directly inducing apoptosis in activated alloreactive T cells. Binding of FASL to Fas (CD95) on antigen-stimulated T cells triggers caspase-dependent cell death, selectively eliminating pathogenic effector T cells while sparing

identify and quantify peptides presented by MHC molecules on the cell surface, providing insight into antigen presentation and immune recognition.

Myositis and systemic sclerosis:

autoimmune connective tissue diseases characterized by immune-mediated inflammation and fibrosis, affecting skeletal muscle in myositis and the skin and internal organs in systemic sclerosis.

Pharmacokinetics: the study of how a therapeutic agent is absorbed, distributed, metabolized, and eliminated in the body over time.

Pseudo-islets: three-dimensional aggregates of pancreatic endocrine cells, generated by reassembling dissociated islet cells or differentiated stem cell-derived cells, that recapitulate key structural and functional features of native pancreatic islets.

Transcription activatorlike effector nucleases: programmable genome-editing enzymes that combine customizable DNA-binding domains with a nuclease to introduce targeted double-strand breaks in specific genomic sequences.

naïve or resting lymphocytes [34]. SERPINB9 (also known as PI-9) acts intracellularly to protect graft cells from cytotoxic lymphocyte effector mechanisms [35]. By inhibiting granzyme B, which is delivered by cytotoxic CD8⁺ T cells and NK cells during immune synapse formation, SERPINB9 prevents activation of apoptotic cascades following perforin-mediated entry. This confers resistance to direct cytolytic killing even when immune recognition occurs.

Collectively, these findings demonstrate that coordinated overexpression of complementary immunomodulatory pathways can generate immune-evasive yet functionally competent neural grafts. Moreover, CD70-directed CAR (CAR70) overexpression represents a complementary immune-evasion strategy in allogeneic T cell therapy (Box 1). Together, these distinct stealth approaches provide a conceptual framework for achieving durable allogeneic cell survival without the need for systemic immunosuppression. However, such extensive genetic immune cloaking is currently feasible primarily in PSC-based and stem cell-derived grafts, as these platforms permit precise, stable, and multiplex genetic engineering at early developmental stages, followed by clonal selection and differentiation [26,46]. By contrast, primary somatic cells or mature tissues lack the proliferative capacity, genetic plasticity, and safety controls required for large-scale, multigene modification, limiting the applicability of this approach outside stem cell engineering contexts.

Stealth designs to reduce host NK cell-mediated allorejection

HLA-E/G overexpression

Host NK cells are key contributors to allogeneic graft rejection, with their activity governed by the integration of signals from activating receptors, such as NKG2D and DNAX accessory molecule-1 (DNAM-1), and inhibitory receptors, including killer cell immunoglobulinlike receptors (KIRs) and NKG2A/CD94, which recognize self-HLA class I molecules [47–49]. Disruption of this balance,

Box 1. CD70-directed CAR overexpression as another immune-evasion strategy in allogeneic T cell therapy

CD70-targeted CAR-T (CAR70-T) cells were initially developed to treat CD70-expressing malignancies, including acute myeloid leukemia and renal cell carcinoma [36–38]. The CAR construct typically incorporates the natural CD70 ligand CD27 as the extracellular recognition domain, fused to intracellular signaling motifs such as CD3 ζ , with or without co-stimulatory domains, to induce potent T cell activation upon antigen engagement. Unexpectedly, subsequent studies revealed a pronounced bystander effect in which CAR70-T cells selectively eliminated activated, alloreactive T cells, prompting interest in their application for controlling immune rejection.

Mechanistically, CD70 is minimally expressed on resting T cells but is rapidly upregulated following T cell activation, particularly downstream of TCR signaling and NF- κ B-dependent transcription [39,40]. Alloreactive T cells undergoing activation in the context of HLA mismatch exhibit sustained CD70 expression, rendering them susceptible to CAR70-mediated cytotoxicity. Engagement of CD70 by CD27-based CARs triggers T cell activation, cytokine secretion, and cytolytic effector functions, leading to selective depletion of pathogenic host T cells [41].

In xenograft mouse models enriched for alloreactive T cells, conventional CAR70-T cells, as well as stem cell-engineered CAR70-modified NK and NKT cells, demonstrated enhanced expansion and persistence compared with conditions lacking alloreactive targets [42,43]. This observation suggests that antigen-driven stimulation by CD70-expressing alloreactive T cells provides a proliferative and survival advantage to CAR70 effectors. In contrast to HLA ablation strategies that aim to evade host immunity, CD70-targeted CAR approaches actively eliminate host alloreactive T cells, thereby promoting long-term graft persistence and immune tolerance.

Despite these advantages, CD70-targeting strategies are associated with potential risks. Fratricide, resulting from CAR recognition of CD70 expressed on activated CAR-T cells themselves, can impair manufacturing efficiency and promote premature exhaustion. This limitation can be mitigated through genetic deletion of endogenous CD70 in CAR effector cells [44,45]. Additional concerns include chronic immune suppression due to prolonged depletion of activated T cell subsets and potential on-target, off-tumor effects in settings of sustained immune activation. Future development of CAR70-based approaches will likely focus on incorporating safety switches, transient CAR expression platforms, or inducible signaling systems to improve controllability. Collectively, CD70-targeted CAR cells represent a promising and mechanistically distinct strategy for controlling host alloreactivity and enabling long-term success of allogeneic cell therapies.

particularly through loss or alteration of HLA expression on allogeneic cells, can trigger NK cell-mediated cytotoxicity. Consequently, multiple immune-engineering strategies have been developed to modulate NK cell responses, including manipulation of activating ligand expression and reinforcement of inhibitory signaling pathways.

Overexpression of HLA-E and HLA-G represents two widely used strategies to enhance inhibitory ligand signaling to NK cells and thereby reduce NK cell-mediated cytotoxicity (Figure 2) [25,50–52]. HLA-E serves as a ligand for the inhibitory NK cell receptor CD94/NKG2A (and, in some contexts, NKG2C), whereas HLA-G engages inhibitory receptors such as LILRB1 (ILT2) and LILRB2 (ILT4) expressed on NK cells and other innate immune populations [53]. Elevated expression of these nonclassical HLA molecules reinforces inhibitory signaling, suppresses NK cell activation, and promotes immune tolerance toward allogeneic grafts [5].

However, complete ablation of B2M to prevent T cell-mediated allojection disrupts surface expression of HLA-E and HLA-G, inadvertently sensitizing graft cells to NK cell-mediated killing through ‘missing-self’ recognition. Consequently, B2M knockout strategies are often coupled with reconstitution or ectopic overexpression of HLA-E or HLA-G fused with B2M to restore NK cell inhibition [50,51,54–57]. iPSC-derived, hypoinmunogenic tissue-resident memorylike T cells were generated with potent antitumor activity against cervical cancer by creating HLA-A24/HLA-E dual-integrated, **human papillomavirus** (HPV)-specific cytotoxic T lymphocytes

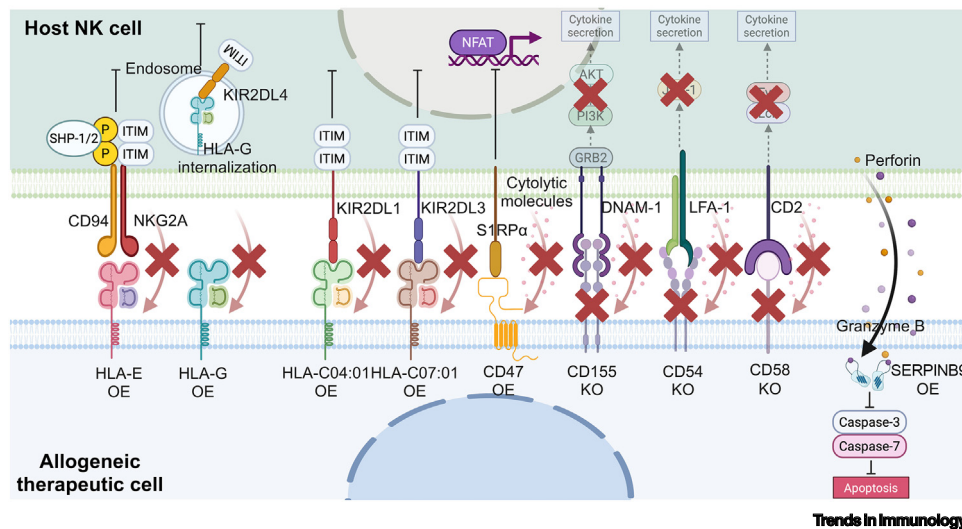


Figure 2. Stealth designs to reduce host NK cell-mediated allojection. Schematic depiction of the molecular interactions between host NK cells and an allogeneic, immune-engineered therapeutic cell, illustrating coordinated genetic modifications that collectively attenuate NK cell activation and effector function. The overexpression of nonclassical HLA molecules, HLA-E and HLA-G, engages the inhibitory receptors CD94/NKG2A and KIR2DL4, respectively, delivering ITIM-mediated inhibitory signals via SHP-1/2 recruitment and promoting receptor internalization. Inhibitory signaling is further reinforced by the retention or overexpression of selected HLA-C allotypes, which bind to inhibitory KIR2DL1/3 receptors. Concurrently, the deletion of activating ligands CD155 (Poliovirus receptor, PVR) and adhesion molecules CD54 (ICAM-1) and CD58 (LFA-3) disrupts DNAM-1-mediated signaling and prevents the formation of a functional immunological synapse. CD47 overexpression provides an additional ‘don’t eat me’ signal to limit macrophage-mediated clearance, while intracellular SERPINB9 expression protects the therapeutic cell from cytolysis by inhibiting granzyme B downstream of perforin delivery. Collectively, these complementary modifications suppress NK cell activation, cytokine secretion, and cytotoxic killing, thereby enhancing the long-term persistence of the allogeneic graft. Figure created using BioRender (<http://biorender.com>). DNAM-1: DNAX accessory molecule-1; HLA: human leukocyte antigen; ICAM-1: intercellular adhesion molecule 1; ITIM: immunoreceptor tyrosine-based inhibitory motif; KIR: killer cell immunoglobulin-like receptor; LFA-3: lymphocyte function-associated antigen 3; NK: natural killer; SHP: Src homology region 2 domain-containing phosphatase; NKG2A, Natural killer group 2A.

following broad ablation of endogenous HLA class I expression [50]. In this system, HLA-E provides dominant inhibitory signaling to NK cells through engagement of CD94/NKG2A, while HLA-A24 restores antigen presentation required for TCR recognition of HPV-derived epitopes, thereby preserving tumor-specific cytotoxicity [50].

As an alternative approach, selective deletion of HLA-A, HLA-B, and HLA-C, while preserving B2M, maintains HLA-E expression and its inhibitory function, generating cells resistant to both T cell- and NK cell-mediated cytotoxicity [58–60]. Other strategies involve targeted removal of specific HLA alleles (e.g., HLA-A and HLA-B), while retaining HLA-C, whose allotypes (HLA-C1 and HLA-C2) engage inhibitory KIR2DL2/3 and KIR2DL1 receptors, respectively, further constraining NK cell activation [17,61].

Interestingly, selective disruption of HLA-A and HLA-B, while preserving HLA-C expression in human iPSCs, effectively suppresses NK cell activation while maintaining sufficient antigen presentation [58]. These HLA-C-retained iPSCs were able to evade both T cell- and NK cell-mediated immune responses *in vitro* and *in vivo*. Furthermore, the authors estimated that a panel of 12 HLA-C-retained iPSC lines, when combined with HLA class II knockout, would be immunologically compatible with over 90% of the global population [58]. This finding highlights the potential of HLA-C-retained iPSCs as a broadly applicable and scalable platform for allogeneic iPSC-based regenerative medicine.

CD155 knockout

CD155 functions as a high-affinity ligand for the activating receptor DNAM-1, which is expressed on NK cells and subsets of CD8⁺ T cells. Engagement of CD155 by DNAM-1 enhances NK cell adhesion, stabilizes immune synapse formation, and promotes cytotoxic activation, thereby contributing to immune recognition and rejection of allogeneic grafts [62]. Targeted deletion of CD155 on donor cells mitigates NK cell-mediated alloreactivity by selectively removing a dominant activating cue while preserving inhibitory signaling pathways. Loss of CD155 markedly attenuates DNAM-1-dependent signaling, resulting in reduced NK cell degranulation, diminished production of proinflammatory cytokines such as IFN- γ , and impaired cytotoxic killing [63]. Notably, this effect persists even in the presence of other activating ligands, underscoring the nonredundant and central role of the CD155–DNAM-1 axis in NK cell activation [64].

Consistent with this mechanism, a recent study reported the generation of hypoimmunogenic, cancer antigen-specific T cells derived from iPSCs through combined knockout of B2M, CIITA, and CD155, together with enforced expression of HLA-E [54]. These iPSC-derived CAR-T cells effectively evaded recognition by NKG2A⁺ and DNAM-1⁺ NK cells, as well as by allogeneic CD8⁺ and CD4⁺ T cells, while retaining robust antitumor activity. Importantly, the study demonstrated that HLA-E overexpression alone was sufficient to suppress NKG2A⁺ NK cells but failed to inhibit NKG2A⁻ NK cell subsets [54]. Incorporation of CD155 ablation specifically reduced the activation of DNAM-1⁺ NK cells, thereby complementing HLA-E-mediated inhibitory signaling [54]. Collectively, these findings highlight CD155 knockout as a critical component of multilayered immune cloaking strategies designed to comprehensively suppress NK cell alloreactivity and enhance the persistence and efficacy of allogeneic cell therapies (Figure 2).

CD54/CD58 knockout

The formation of a cytolytic immune synapse represents a critical early step in the activation of both NK cells and T lymphocytes and is required for effective target cell killing [65]. This specialized cell–cell junction enables stable adhesion, signal integration, and polarized delivery of cytotoxic effector molecules. On target cells, the adhesion molecules CD54 (intercellular adhesion

molecule 1, ICAM-1) and CD58 (lymphocyte function-associated antigen 3, LFA-3) engage their cognate receptors LFA-1 (CD11a/CD18) and CD2 on effector lymphocytes, respectively, thereby stabilizing immune synapse formation and amplifying activation signals [66]. Consistent with their central role in immune recognition, loss or downregulation of CD54 and CD58 has been widely observed in virus-infected cells and tumors as a mechanism of immune evasion (Figure 2) [67,68].

Building on this principle, concurrent deletion of CD54 and CD58 on HLA class I-low target cells markedly reduces NK cell activation and cytotoxicity across all NK cell subsets, independent of their inhibitory receptor repertoires [69]. Extending these findings, genetic ablation of CD54 and CD58 in B2M-deficient CAR-T cells and iPSC-derived CAR-NK cells significantly diminishes host NK cell-mediated rejection while preserving potent antitumor activity [69]. Together, these results highlight immune synapse modulation as a powerful and broadly applicable strategy to decouple immune recognition from effector function, thereby enhancing the persistence, safety, and therapeutic efficacy of adoptively transferred allogeneic therapeutic cells.

Stealth designs to reduce host myeloid cell-mediated allorejection

CD47 overexpression

CD47 was originally identified as a ‘self’ recognition molecule on erythrocytes and was subsequently shown to function as a key regulator of innate immune tolerance by suppressing macrophage phagocytosis through engagement of its inhibitory receptor, signal regulatory protein α (SIRP α) [70–73]. This CD47–SIRP α axis is frequently exploited by malignant cells, which upregulate CD47 to evade immune clearance, and therapeutic disruption of this pathway has demonstrated robust antitumor activity in multiple preclinical models [74]. Building on this biology, recent studies have repurposed CD47 overexpression as an immune-evasion strategy in the context of cell-based therapies (Figure 3). Forced expression of CD47 in iPSC-derived grafts and CAR-T cells confers resistance to allogeneic rejection by inhibiting macrophage-mediated phagocytosis [5]. In addition, CD47 signaling has been shown to attenuate NK cell-mediated cytotoxicity, further enhancing the survival and persistence of engineered cells in allogeneic transplantation settings (Figure 2).

Hypoimmune (HIP) primary human pancreatic islets have been developed through targeted genetic engineering to eliminate immune recognition while preserving endocrine function [75]. In their initial study, primary human islet cells were edited to ablate HLA class I and class II expression via disruption of *B2M* and *CIITA*, respectively, while simultaneously overexpressing CD47 to inhibit innate immune clearance. These cells were subsequently reaggregated into human allogeneic, gene-engineered HIP **pseudo-islets** (p-islets) [75]. Upon allogeneic transplantation into immunocompetent, diabetic humanized mice, HIP p-islets demonstrated durable survival, successful engraftment, and effective glycemic control without evidence of immune rejection. Importantly, administration of a CD47-targeting antibody led to rapid and selective elimination of the transplanted HIP cells, establishing a built-in safety mechanism to ablate the graft in the event of adverse outcomes [75].

In a subsequent study, the same group extended this approach to a clinically relevant model, demonstrating that allogeneic transplantation of genetically engineered B2M^{-/-}, CIITA^{-/-}, CD47⁺ primary HIP p-islets into fully immunocompetent, diabetic nonhuman primates resulted in long-term engraftment and stable endocrine function [76]. Transplanted animals achieved sustained insulin independence without immunosuppression and showed no detectable cellular or humoral immune responses against the graft [76]. The same group also engineered rhesus macaque HIP PSCs and evaluated their performance in a fully immunocompetent allogeneic setting [77]. When these HIP PSCs were implanted intramuscularly into four allogeneic rhesus

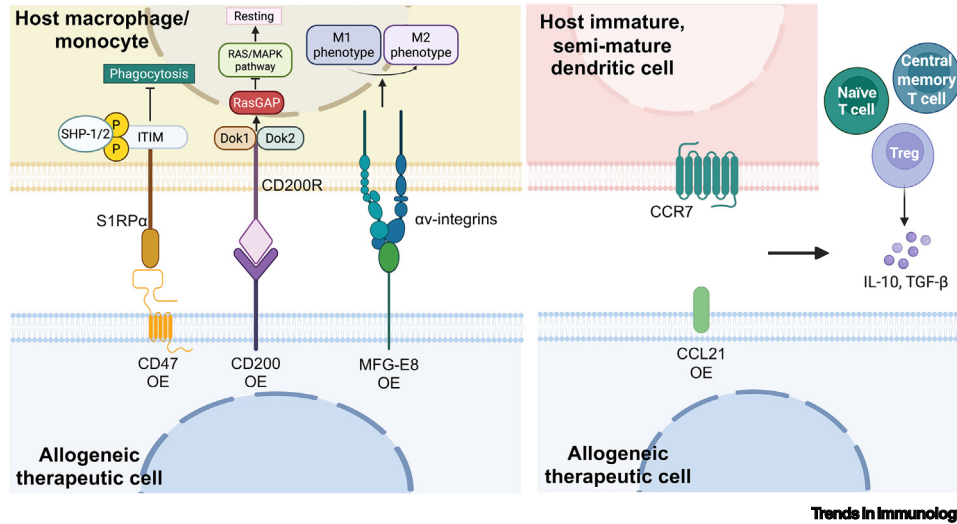


Figure 3. Stealth designs to reduce host myeloid cell-mediated allorejection. Schematic illustration of interactions between host macrophages/monocytes and dendritic cells (DCs) and an allogeneic, gene-engineered therapeutic cell, highlighting immune-cloaking strategies that suppress phagocytosis, inflammatory activation, and antigen presentation. Overexpression (OE) of CD47 on therapeutic cells engages signal regulatory protein α (SIRP α) on host macrophages, triggering phosphorylation of immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and recruitment of SHP-1/2 phosphatases, thereby suppressing cytoskeletal rearrangement and inhibiting phagocytosis. CD200 OE provides an additional inhibitory signal through binding to CD200 receptor (CD200R) on myeloid cells, leading to recruitment of adaptor proteins Dok1 and Dok2 and activation of RasGAP, which dampens RAS/MAPK signaling and proinflammatory activation. This signaling cascade promotes a tolerogenic macrophage phenotype and limits inflammatory cytokine production. Concurrently, MFG-E8 OE enhances noninflammatory efferocytosis by engaging α v integrins on macrophages, facilitating clearance of apoptotic material while reducing inflammatory signaling and promoting immune tolerance within the graft microenvironment. In parallel, C-C motif chemokine ligand 21 (CCL21) overexpression signals through CCR7 on immature and semi-mature DCs, altering their migration and functional state and promoting the induction of regulatory T cells and central memory T cells via increased IL-10 and TGF- β production. Figure created using BioRender (<http://biorender.com/>). CCR7: C-C chemokine receptor 7; IL-10: interleukin-10; MAPK: mitogen-activated protein kinase; MFG-E8: milk fat globule-epidermal growth factor 8; RasGAP: Ras GTPase-activating protein; SHP-1/2: Src homology region 2 domain-containing phosphatase-1/2; TGF- β : transforming growth factor- β ; Treg: regulatory T cell.

macaques, the engineered cells survived persistently for at least 16 weeks, differentiated into multiple lineages, and exhibited no signs of immune rejection, whereas wild-type allogeneic cells were rapidly eliminated [77]. Collectively, these studies extend the HIP engineering strategy across species and cell types, establishing genetically modified primary p-islets and PSC-derived grafts as promising immune-evasive therapies capable of durable engraftment and metabolic correction without immunosuppression.

Furthermore, the role of CD47 expression on adoptively transferred T cells has been investigated in the context of anti-CD47 antibody therapy [73]. Initial experiments revealed that coadministration of anti-CD47 antibodies with adoptively transferred CAR-T or TCR-engineered T cells resulted in rapid macrophage-mediated clearance of the therapeutic T cells, abrogating antitumor efficacy. Notably, this antibody-induced elimination of CAR-T cells was sufficiently potent to function as an effective safety switch. To overcome this limitation, the authors engineered a CD47 variant, CD47(Q31P) (47E), that retains engagement with the inhibitory receptor SIRP α but is not disrupted by anti-CD47 antibodies [73]. T cells expressing this engineered CD47 variant were resistant to macrophage clearance in the presence of anti-CD47 antibody and elicited robust, sustained macrophage recruitment within the tumor microenvironment [73]. This work demonstrates a strategy to simultaneously harness T cell and macrophage antitumor responses

and highlights the critical requirement for adequate CD47 engagement to support the survival, persistence, and therapeutic activity of adoptively transferred T cells, even in hosts incapable of recognizing allogeneic disparities.

CD200/MFGE8/CCL21 overexpression

CD200 is an immunoregulatory surface glycoprotein that maintains immune homeostasis through interaction with CD200R on myeloid cells, including macrophages and DCs [78]. CD200 overexpression delivers inhibitory signals that suppress mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathways, reducing proinflammatory cytokine production and co-stimulatory molecule expression while promoting a tolerogenic phenotype [79,80]. In allogeneic transplantation, CD200 overexpression limits innate immune activation, attenuates antigen presentation, and indirectly reduces T cell priming and expansion (Figure 3).

Milk fat globule–EGF factor 8 (MFGE8) facilitates the recognition and phagocytic clearance of apoptotic cells by macrophages through binding to phosphatidylserine and integrins (α v β 3/ α v β 5). Enhanced MFGE8 expression promotes noninflammatory efferocytosis, suppresses proinflammatory cytokine release, and supports the resolution of local inflammation [81]. Together, CD200 and MFGE8 overexpression reshape the graft microenvironment to favor immune tolerance, dampen inflammatory responses, and enhance the persistence of allogeneic cell therapies.

Overexpression of C–C motif chemokine ligand 21 (CCL21) also contributes to immune modulation through complementary effects on immune cell trafficking and clearance of apoptotic cells (Figure 3). CCL21 is a chemokine that binds to C–C chemokine receptor 7 (CCR7) and regulates the migration and spatial organization of T cells and DCs. When ectopically expressed by grafted cells, CCL21 can sequester CCR7⁺ immune cells in a nonactivating manner, disrupt effective immune synapse formation, and promote the accumulation of tolerogenic DCs, thereby limiting productive T cell priming [82]. Furthermore, ectopic expression of CCL21 by allogeneic graft cells is expected to interfere with the normal trafficking of local DCs to draining lymph nodes, thereby limiting the initiation of adaptive immune responses [83]. By altering CCR7-dependent migratory cues, CCL21 can retain DCs within the graft microenvironment or divert them into non-productive migratory pathways, reducing their capacity to prime naïve T cells. A similar immune-evasion strategy has been described in several malignancies, where aberrant CCL21 expression disrupts DC migration and effectively shields tumors from immune surveillance [84].

CD47, *CD200*, *CCL21*, and *MFGE8* are four additional immunomodulatory genes overexpressed in cloaked human PSC-derived neural grafts to further enhance immune evasion from macrophages and DCs [32]. To evaluate the capacity of gene-engineered immune-cloaked neurons to resist innate immune clearance, PSC-derived neurons overexpressing these four genes were cocultured with human macrophages and compared with nonengineered control neurons [32]. Immune-cloaked neuronal cultures exhibited significantly greater neuronal survival than controls, consistent with reduced neuronal death and diminished phagocytic uptake of apoptotic neurons by macrophages. Moreover, macrophages cocultured with immune-cloaked neurons secreted lower levels of inflammatory cytokines, indicating attenuated macrophage activation and a dampened inflammatory response [32].

In addition, DC migration was assessed using a transwell coculture system to evaluate the functional consequences of CCL21 secretion by immune-cloaked neurons. In response to elevated levels of CCL21 produced by cloaked PSC-derived neurons, DCs exhibited increased CCR7-dependent migration in the transwell assay, indicating active chemokine signaling [32].

Collectively, these results demonstrate that coordinated overexpression of *CD47*, *CD200*, *CCL21*, and *MFGE8* attenuates macrophage-mediated phagocytosis and inflammatory activation while modulating DC migration, thereby promoting enhanced neuronal survival and immune evasion in an allogeneic immune environment.

CD24 overexpression

CD24 is a **glycosylphosphatidylinositol**-anchored surface glycoprotein that acts as an important innate immune checkpoint. Its immunosuppressive function is mediated primarily through interaction with Siglec-10 in humans (and Siglec-G in mice), inhibitory receptors expressed on macrophages, DCs, and other myeloid populations [85].

Overexpression of CD24 on allogeneic cells dampens host innate immune activation by transmitting inhibitory signals through Siglec-10, resulting in reduced NF- κ B activation, decreased production of proinflammatory cytokines such as TNF- α and interleukin 6 (IL-6), and impaired macrophage phagocytic activity. Consistent with this role, genetically stable, multiplex-edited iPSC-derived NK cells were generated for cancer immunotherapy, incorporating CD24 overexpression alongside deletions of *B2M*, *CIITA*, *TRA*, *PDCD1*, and *CTLA4*, and the introduction of a CD19 CAR [86]. In this study, CD24 was identified as a previously underappreciated macrophage ‘don’t eat me’ signal [86]. Although comprehensive evaluation of allorejection was limited, these findings suggest that CD24 represents a promising additional target that could be integrated into multilayered immune cloaking strategies to further enhance the persistence and safety of allogeneic cell therapies.

Clinical trials examples of stealth-engineered allogeneic therapies

A growing number of clinical trials are evaluating allogeneic CAR-engineered cell therapies for the treatment of cancer and autoimmune diseases [18]. These approaches employ either primary donor-derived cells (e.g., peripheral blood mononuclear cell-derived T cells or NKT cells) or iPSC-based platforms, most notably iPSC-derived NK cells, to generate off-the-shelf therapeutic products. Owing to manufacturing and editing constraints in primary cells, current allogeneic therapies derived from donor lymphocytes primarily focus on mitigating host T cell- and NK cell-mediated rejection, with comparatively limited strategies targeting macrophage- and DC-driven innate immune responses. To prevent GvH disease (GvHD), all allogeneic CAR-T cell products incorporate TCR ablation to eliminate recognition of host HLA molecules [4,72,87,88].

The first allogeneic CAR-T product to enter clinical testing was UCART19, a genome-edited, donor-derived anti-CD19 CAR-T therapy developed for relapsed or refractory B-cell acute lymphoblastic leukemia. UCART19 cells undergo multiplex gene editing using **transcription activatorlike effector nucleases**-mediated disruption of *TRAC* and *CD52*, thereby eliminating TCR expression to prevent GvHD and conferring resistance to the lymphodepleting anti-CD52 antibody alemtuzumab [89,90]. A related strategy has been applied to ALLO-715, an allogeneic B cell maturation antigen (BCMA)-targeting CAR-T product currently under clinical evaluation for relapsed or refractory multiple myeloma (NCT04093596), which employs a similar immune-evasive design [91]. This study assessed safety and tolerability as primary endpoints, utilizing a conditioning regimen containing the anti-CD52 antibody ALLO-647 to permit engraftment. Interim results demonstrated that, while severe adverse events such as Grade 3+ cytokine release syndrome (CRS) and neurotoxicity were rare, infections were reported. Yet, importantly, no cases of GvHD were observed. Regarding the longevity of the cell therapy, peripheral blood analysis showed that ALLO-715 expansion peaked at days 7–14, with persistence detectable up to 6 months in some responders, though cell levels generally declined over time, consistent with allogeneic clearance kinetics.

More recently, a clinical study has evaluated a multiplex-edited, TCR-ablated allogeneic CAR-T therapy for the treatment of severe myositis and systemic sclerosis [17]. This product was generated using CRISPR–Cas9–based editing to disrupt HLA-A, HLA-B, CIITA, TRAC, and PD-1, thereby reducing both allogeneic immune recognition and intrinsic T cell exhaustion. Notably, the extensive genome editing did not compromise manufacturing efficiency, with individual production runs yielding sufficient cells to treat over 100 patients [17].

In parallel, iPSC-derived CAR-NK cell therapies have advanced into the clinic. FT596, an iPSC-engineered CD19-targeting CAR-NK product (NCT04245722), integrates three synergistic anti-tumor components: a CD19 CAR, a high-affinity noncleavable CD16 Fc receptor to enhance antibody-dependent cellular cytotoxicity, and an interleukin 15 (IL-15)/IL-15 receptor fusion to promote *in vivo* persistence and functional durability [92]. This Phase 1, open-label, dose-escalation study evaluated safety and tolerability as primary endpoints in patients with relapsed or refractory B-cell lymphoma. Results indicated that FT596 was well tolerated, with severe adverse events such as Grade 3+ CRS and neurotoxicity being rare, and, importantly, no cases of GvHD were observed. Regarding longevity, while the IL-15 receptor fusion was designed to enhance survival, clinical data showed that FT596 persistence was generally limited, with cells typically becoming undetectable in peripheral blood within a few weeks, highlighting a challenge for future stealth iterations to address [93].

NKT cells offer an alternative allogeneic platform with an inherently favorable safety profile. Because NKT TCRs recognize the nonpolymorphic antigen-presenting molecule CD1d, the risk of GvHD is markedly reduced compared with conventional T cells [24,94,95]. Accordingly, CAR-NKT cells are well suited for allogeneic use. A clinical trial evaluating CD19-targeting CAR-NKT cells in patients with relapsed or refractory B-cell malignancies (NCT00840853) utilized NKT cells derived from a single HLA-mismatched healthy donor. These cells were engineered to express a CD19 CAR and soluble IL-15 and to downregulate HLA class I and II expression via shRNA-mediated targeting of *B2M* and *CD74*, respectively, thereby reducing host immune recognition [96].

Across these clinical studies, allogeneic CAR-based therapies have been administered following standard lymphodepleting conditioning regimens, typically incorporating **fludarabine** and **cyclophosphamide**, to transiently suppress host immunity and facilitate cell engraftment [97,98]. Collectively, early-phase trials have demonstrated favorable safety profiles and encouraging antitumor activity, with the off-the-shelf nature of allogeneic products enabling repeat dosing—a feature that may further enhance therapeutic efficacy. While these studies provide compelling early clinical validation, key questions remain regarding the *in vivo* **pharmacokinetics**, persistence, and immune dynamics of allogeneic CAR therapies, particularly in comparison with autologous CAR cells [99]. Continued clinical evaluation and integration of advanced stealth engineering strategies will be critical to fully realizing the potential of allogeneic cell therapies.

Concluding remarks

Driven by rapid advances in genome editing, synthetic immunology, and stem cell differentiation technologies, the development of allogeneic therapies incorporating stealth immune-evasion designs has accelerated markedly. A broad array of genetic and molecular strategies has now been deployed to mitigate host immune surveillance, including suppression of T cell-mediated alloreactivity through HLA modulation (Figure 1), inhibition of NK cell cytotoxicity via reinforcement of inhibitory ligand signaling or removal of activating ligands (Figure 2), and attenuation of innate myeloid responses by blocking phagocytosis and inflammatory sensing by macrophages and

Outstanding questions

What is the minimal, indication-specific combination of immune-evasion edits required to achieve durable allogeneic graft persistence without compromising therapeutic function?

How do different stealth strategies interact across immune compartments, and are certain combinations synergistic or redundant when suppressing T cell, natural killer cell, and myeloid responses?

How do the differentiation state, tissue context, and inflammatory microenvironment of an allogeneic graft influence the effectiveness and durability of immune cloaking?

To what extent does broad immune evasion alter host immune surveillance against infection or malignancy, and how can these risks be quantitatively assessed preclinically?

What safety-control mechanisms (e.g., antibody-mediated depletion, inducible suicide switches, or small-molecule regulators) are most reliable for stealth-engineered grafts in long-term clinical use?

How can standardized *in vitro* and *in vivo* immunomonitoring platforms be developed to reliably predict clinical persistence, efficacy, and rejection risk?

What pharmacokinetic and pharmacodynamic parameters best correlate with clinical outcomes for allogeneic stealth therapies, and how do these compare with autologous counterparts?

How should regulatory frameworks evolve to accommodate multiplex-edited, hypoimmune cell products while ensuring long-term patient safety?

Can universal or semiuniversal donor cell banks realistically achieve broad population coverage without increasing immune escape-related risks?

How can manufacturing scalability, genetic stability, and cost-effectiveness be balanced with the increasing complexity of stealth engineering design?

Box 2. Hypoimmune stem cell-derived beta cells for treating T1D

T1D is a chronic autoimmune disease characterized by immune-mediated destruction of pancreatic β cells, resulting in absolute insulin deficiency and persistent hyperglycemia [100]. Immune-evasive strategies, including HIP engineering of donor cells via *B2M* and *CIITA* knockout combined with *CD47* overexpression, have shown promising preclinical results and are now being evaluated in ongoing clinical trials [77,100]. VCTX210A is currently under investigation in a Phase 1, open-label study in individuals with T1D (NCT05210530). The primary objective is to assess safety and tolerability, measured by adverse events related to the implanted cell units or surgical procedures. Secondary endpoints evaluate host immune responses through histological analyses of innate and adaptive immune markers, the development of alloreactive or autoreactive antibodies, and graft longevity and differentiation, including the proportion of viable cells differentiated into endocrine/ β cells. Building on this platform, the next-generation candidate VCTX211 is being tested in a separate Phase 1, open-label study (NCT05565248). This trial evaluates safety and preliminary efficacy, including adverse events and changes in **C-peptide** levels from baseline. Additional endpoints assess functional and durability outcomes, including exogenous insulin use, hemoglobin A1C, hypoglycemic events, and time in glycemic range, along with histological assessment of immune responses, graft viability, and differentiation. These advances have led to the first-in-human trial of genetically modified allogeneic islets. At 6-month follow-up, data indicate that HIP-modified pancreatic islet cells are safe, well tolerated, and capable of long-term survival and insulin production without systemic immunosuppression, as demonstrated by sustained circulating C-peptide levels and imaging consistent with graft persistence [100]. These results provide the first clinical proof-of-concept that immune-evasive cell therapy can overcome both allogeneic and autoimmune barriers in T1D and support the development of scalable, curative treatments that eliminate the need for lifelong immunosuppression.

The optimal duration of graft persistence is context dependent and should reflect the therapeutic objective. For tissue-replacement approaches such as β -cell therapy in T1D, long-term engraftment is critical to ensure durable insulin production and sustained metabolic control [100]. By contrast, for NK cell- or T cell-based therapies, indefinite persistence may not be necessary; extending *in vivo* survival by several weeks may be sufficient to enhance the therapeutic window and enable repeat dosing without additional conditioning [1,2]. In some settings, early expansion and functional potency correlate more strongly with clinical response than long-term persistence, which may also increase toxicity risks. Thus, stealth strategies should aim for application-specific, optimized persistence rather than maximal durability.

DCs (Figure 3). Collectively, these approaches aim to enable long-term *in vivo* persistence, functional stability, and enhanced antitumor or regenerative efficacy of allogeneic grafts without reliance on chronic systemic immunosuppression. The convergence of multiplex genome editing

Box 3. Scientific and translational challenges in stealth immune-evasion strategies for allogeneic cell therapies

It is now evident that no single genetic modification is sufficient to confer durable immune tolerance across diverse host immune compartments [5,22]. Instead, long-term engraftment appears to require coordinated suppression of adaptive immunity, innate lymphoid responses, and myeloid-mediated inflammatory sensing. This recognition raises two interdependent questions that will shape future research: first, what constitutes the minimal, indication-specific combination of immune-evasion edits necessary to achieve durable and functional persistence; and second, how these edits interact with cell lineage, differentiation state, tissue context, and inflammatory milieu. Addressing these questions will require systematic, comparative studies that directly evaluate alternative design architectures, such as selective HLA retention versus B2M knockout combined with HLA-E or HLA-G reconstitution, incorporation of macrophage inhibitory signals (e.g., CD47 or CD24), and ablation of NK cell-activating or immune synapse-forming ligands (e.g., CD155, CD54, and CD58), across relevant disease models and cell types.

Safety and controllability must remain foundational principles in stealth graft design. Broad immune evasion carries inherent risks, including uncontrolled graft persistence, impaired immune surveillance against infection or malignancy, and unanticipated tissue-specific effects [101]. Consequently, stealth-engineered therapies should be systematically paired with robust and validated safety mechanisms, such as antibody-mediated depletion, inducible suicide switches, or small-molecule-regulated control circuits. These safeguards must be complemented by rigorous preclinical testing, including challenge studies for opportunistic infection, tumorigenicity assays, longitudinal clonal tracking, and large-animal surveillance, to establish acceptable safety margins for clinical translation.

Finally, successful clinical deployment will depend on the development of predictive immunomonitoring platforms and translationally relevant assays. Standardized humanized *in vivo* models, advanced *ex vivo* coculture systems incorporating adaptive and innate immune components, **immunopeptidomic profiling** to assess residual antigen presentation, and high-resolution single-cell and spatial immune analyses should be adopted as community benchmarks. These tools will enable the derivation of quantitative pharmacokinetic and pharmacodynamic metrics, including persistence, expansion, immune engagement, and functional output, that can be directly correlated with clinical efficacy and safety outcomes in early-phase trials.

with scalable iPSC platforms has further expanded the design space, enabling precise, multilayered immune cloaking strategies that were previously infeasible in primary cell products.

Despite substantial preclinical efficacy and promising early clinical signals, including the use of allogeneic CAR-engineered cells for the treatment of malignancies and autoimmune disorders, as well as HIP stem cell-derived β cells for type 1 diabetes (T1D) (Box 2), considerable scientific and translational challenges must still be addressed before stealth-engineered allogeneic cells or grafts can achieve their full therapeutic potential (Box 3) (see Outstanding questions). The integration of comparative immune-evasion benchmarking, built-in control mechanisms, rigorous immunomonitoring, and scalable manufacturing frameworks will be essential to translating stealth engineering from a powerful conceptual strategy into durable, safe, and broadly accessible allogeneic therapies across oncology, autoimmunity, and regenerative medicine.

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Declaration of interests

L.Y. is a scientific advisor to AlzChem and Amberstone Biosciences, and a cofounder, stockholder, and advisory board member of Appia Bio. None of the declared companies contributed to this study. The other authors declare no competing interests.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to assist with improving the clarity, organization, and scientific language of the text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

References

- Depil, S. *et al.* (2020) 'Off-the-shelf' allogeneic CAR T cells: development and challenges. *Nat. Rev. Drug Discov.* 19, 185–199
- Diorio, C. *et al.* (2025) Allogeneic chimeric antigen receptor cell therapies for cancer: progress made and remaining roadblocks. *Nat. Rev. Clin. Oncol.* 22, 10–27
- Li, Y.-R. *et al.* (2025) Managing alloreactivity in off-the-shelf CAR-engineered cell therapies. *Mol. Ther.* 33, 2368–2390
- Lyu, Z. *et al.* (2025) Addressing graft-versus-host disease in allogeneic cell-based immunotherapy for cancer. *Exp. Hematol. Oncol.* 14, 66
- Martin, K.E. *et al.* (2024) Engineering immune-evasive allogeneic cellular immunotherapies. *Nat. Rev. Immunol.* 24, 680–693
- Fang, Y. *et al.* (2025) Engineering the next generation of allogeneic CAR cells: iPSCs as a scalable and editable platform. *Stem Cell Rep.* 20, 102515
- Carnel, N. *et al.* (2023) Pathways of antigen recognition by T cells in allograft rejection. *Transplantation* 107, 827–837
- Marino, J. *et al.* (2016) Allorecognition by T lymphocytes and allograft rejection. *Front. Immunol.* 7, 582
- Han, X. *et al.* (2025) The role of B2M in cancer immunotherapy resistance: function, resistance mechanism, and reversal strategies. *Front. Immunol.* 16, 1512509
- Mastemak, K. *et al.* (2000) CIITA is a transcriptional coactivator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex. *Genes Dev.* 14, 1156–1166
- Zhao, W. *et al.* (2020) Strategies for genetically engineering hypoinmunogenic universal pluripotent stem cells. *iScience* 23, 101162
- Lee, J. *et al.* (2024) Principles of peptide selection by the transporter associated with antigen processing. *Proc. Natl. Acad. Sci. U. S. A.* 121, e2320879121
- Moldenhauer, G. *et al.* (1999) Surface-expressed invariant chain (CD74) is required for internalization of human leucocyte antigen-DR molecules to early endosomal compartments. *Immunology* 96, 473–484
- Li, Y.-R. *et al.* (2021) Development of allogeneic HSC-engineered iNKT cells for off-the-shelf cancer immunotherapy. *Cell Rep. Med.* 2, 100449
- Li, Y.-R. *et al.* (2024) Engineering alloreactivity-resistant CAR-NKT cells from hematopoietic stem cells for off-the-shelf cancer immunotherapy. *Mol. Ther.* 32, 1849–1874
- Ramos, C.A. *et al.* (2024) Off-the-shelf CD19-specific CAR-NKT cells in patients with relapsed or refractory B-cell malignancies. *Transplant. Cell Ther.* 30, S41–S42
- Wang, X. *et al.* (2024) Allogeneic CD19-targeted CAR-T therapy in patients with severe myositis and systemic sclerosis. *Cell* 187, 4890–4904.e9
- Li, Y.-R. *et al.* (2025) Emerging trends in clinical allogeneic CAR cell therapy. *Med. (N. Y.)* 6, 100677
- Crivello, P. *et al.* (2019) Multiple knockout of classical HLA class II β -chains by CRISPR/Cas9 genome editing driven by a single guide RNA. *J. Immunol.* 202, 1895–1903
- Lanza, R. *et al.* (2019) Engineering universal cells that evade immune detection. *Nat. Rev. Immunol.* 19, 723–733
- Deuse, T. *et al.* (2019) Hypoinmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat. Biotechnol.* 37, 252–258
- Perica, K. *et al.* (2025) HIV immune evasion Nef enhances allogeneic CAR T cell potency. *Nature* 640, 793–801
- Li, Y.-R. *et al.* (2025) Generation of allogeneic CAR-NKT cells from hematopoietic stem and progenitor cells using a clinically guided culture method. *Nat. Biotechnol.* 43, 329–344

24. Li, Y.-R. *et al.* (2025) Overcoming ovarian cancer resistance and evasion to CAR-T cell therapy by harnessing allogeneic CAR-NKT cells. *Med N. Y.* 6, 100804
25. McCallion, O. *et al.* (2025) HLA matching or CRISPR editing of HLA class I/II enables engraftment and effective function of allogeneic human regulatory T cell therapy in a humanized mouse transplantation model. *Nat. Commun.* 16, 9090
26. Li, Y.-R. *et al.* (2023) Advancing cell-based cancer immunotherapy through stem cell engineering. *Cell Stem Cell* 30, 592–610
27. Panaampon, J. *et al.* (2022) Efficacy and mechanism of the anti-CD38 monoclonal antibody Daratumumab against primary effusion lymphoma. *Cancer Immunol. Immunother.* 71, 1017–1031
28. Jiang, L. *et al.* (2009) Variable CD52 expression in mature T cell and NK cell malignancies: implications for alemtuzumab therapy. *Br. J. Haematol.* 145, 173–179
29. Mbofung, R.M. *et al.* (2021) Off-the-shelf, iPSC-derived CAR-NK cells multiplexed-engineered for the avoidance of allogeneic host immune cell rejection. *Blood* 138, 2795
30. Mbofung, R.M. *et al.* (2022) iPSC-derived CD38-null NK cells in combination with CD38-targeted antibody: a dual therapeutic strategy to enable ADCC and eliminate host immune cells in multiple myeloma. *Blood* 140, 7388–7389
31. Feng, X. *et al.* (2017) Targeting CD38 suppresses induction and function of T regulatory cells to mitigate immunosuppression in multiple myeloma. *Clin. Cancer Res.* 23, 4290–4300
32. Pavan, C. *et al.* (2025) A cloaked human stem-cell-derived neural graft capable of functional integration and immune evasion in rodent models. *Cell Stem Cell* 32, 710–726.e8
33. Goodman, A. *et al.* (2017) PD-1–PD-L1 immune-checkpoint blockade in B-cell lymphomas. *Nat. Rev. Clin. Oncol.* 14, 203–220
34. Daniel, P.T. *et al.* (1999) CD95/Fas-triggered apoptosis of activated T lymphocytes is prevented by dendritic cells through a CD58-dependent mechanism. *Exp. Hematol.* 27, 1402–1408
35. Huang, H. *et al.* (2024) The biological function of Serpinb9 and Serpinb9-based therapy. *Front. Immunol.* 15, 1422113
36. Wei, W. *et al.* (2025) CD70-targeted cancer therapeutics: progress and challenges. *Med (N. Y.)* 6, 100671
37. Cheng, J. *et al.* (2023) Revealing the impact of CD70 expression on the manufacture and functions of CAR-70 T-cells based on single-cell transcriptomics. *Cancer Immunol. Immunother.* 72, 3163–3174
38. Pal, S.K. *et al.* (2024) CD70-targeted allogeneic CAR T-cell therapy for advanced clear cell renal cell carcinoma. *Cancer Discov.* 14, 1176–1189
39. O'Neill, R.E. *et al.* (2017) T cell-derived CD70 delivers an immune checkpoint function in inflammatory T cell responses. *J. Immunol.* 199, 3700–3710
40. Hintzen, R.Q. *et al.* (1995) Engagement of CD27 with its ligand CD70 provides a second signal for T cell activation. *J. Immunol.* 154, 2612–2623
41. Nolte, M.A. *et al.* (2009) Timing and tuning of CD27–CD70 interactions: the impact of signal strength in setting the balance between adaptive responses and immunopathology. *Immunol. Rev.* 229, 216–231
42. Li, Y.-R. *et al.* (2025) Multimodal targeting of metastatic renal cell carcinoma via CD70-directed allogeneic CAR-NKT cells. *Cell Rep. Med.* 6, 102321
43. Wang, L. *et al.* (2025) CD70-targeted iPSC-derived CAR-NK cells display potent function against tumors and alloreactive T cells. *Cell Rep. Med.* 6, 101889
44. Srinivasan, S. *et al.* (2020) Investigation of ALLO-316: a fratricide-resistant allogeneic CAR T targeting CD70 as a potential therapy for the treatment of AML. *Blood* 136, 23
45. De Munter, S. *et al.* (2024) Knocking out CD70 rescues CD70-specific NanoCAR T cells from antigen-induced exhaustion. *Cancer Immunol. Res.* 12, 1236–1251
46. Li, Y.-R. *et al.* (2021) Development of stem cell-derived immune cells for off-the-shelf cancer immunotherapies. *Cells* 10, 3497
47. Fang, F. *et al.* (2017) NK cell-based immunotherapy for cancer. *Semin. Immunol.* 31, 37–54
48. Middleton, D. and Gonzalez, F. (2010) The extensive polymorphism of KIR genes. *Immunology* 129, 8–19
49. Guillerrey, C. *et al.* (2016) Targeting natural killer cells in cancer immunotherapy. *Nat. Immunol.* 17, 1025–1036
50. Furukawa, Y. *et al.* (2023) iPSC-derived hypoinmunogenic tissue resident memory T cells mediate robust anti-tumor activity against cervical cancer. *Cell Rep. Med.* 4, 101327
51. Gornalusse, G.G. *et al.* (2017) HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. *Nat. Biotechnol.* 35, 765–772
52. Sætersmoen, M. *et al.* (2025) Targeting HLA-E-overexpressing cancers with a NKG2A/C switch receptor. *Med (N. Y.)* 6, 100521
53. Anfossi, N. *et al.* (2006) Human NK cell education by inhibitory receptors for MHC class I. *Immunity* 25, 331–342
54. Wang, B. *et al.* (2021) Generation of hypoinmunogenic T cells from genetically engineered allogeneic human induced pluripotent stem cells. *Nat. Biomed. Eng.* 5, 429–440
55. Kitano, Y. *et al.* (2022) Generation of hypoinmunogenic induced pluripotent stem cells by CRISPR-Cas9 system and detailed evaluation for clinical application. *Mol. Ther. Methods Clin. Dev.* 26, 15–25
56. Han, X. *et al.* (2019) Generation of hypoinmunogenic human pluripotent stem cells. *Proc. Natl. Acad. Sci. U. S. A.* 116, 10441–10446
57. Jo, S. *et al.* (2022) Endowing universal CAR T-cell with immune-evasive properties using TALEN-gene editing. *Nat. Commun.* 13, 3453
58. Xu, H. *et al.* (2019) Targeted disruption of HLA genes via CRISPR-Cas9 generates iPSCs with enhanced immune compatibility. *Cell Stem Cell* 24, 566–578.e7
59. Torikai, H. *et al.* (2013) Toward eliminating HLA class I expression to generate universal cells from allogeneic donors. *Blood* 122, 1341–1349
60. Liu, F. *et al.* (2025) Selective HLA knockdown and PD-L1 expression prevent allogeneic CAR-NK cell rejection and enhance safety and anti-tumor responses in xenograft mice. *Nat. Commun.* 16, 8809
61. Pende, D. *et al.* (2019) Killer Ig-like receptors (KIRs): their role in NK cell modulation and developments leading to their clinical exploitation. *Front. Immunol.* 10, 1179
62. Molfetta, R. *et al.* (2020) CD155: a multi-functional molecule in tumor progression. *Int. J. Mol. Sci.* 21, 922
63. Saunders, P.M. *et al.* (2025) CD155 density on target cells drives divergent natural killer cell responses owing to DNAM-1 loss. *J. Immunol.* 215, vkaf293
64. Kearney, C.J. *et al.* (2016) Loss of DNAM-1 ligand expression by acute myeloid leukemia cells renders them resistant to NK cell killing. *Oncoimmunology* 5, e1196308
65. Orange, J.S. (2008) Formation and function of the lytic NK-cell immunological synapse. *Nat. Rev. Immunol.* 8, 713–725
66. Davis, D.M. (2002) Assembly of the immunological synapse for T cells and NK cells. *Trends Immunol.* 23, 356–363
67. Challa-Malladi, M. *et al.* (2011) Combined genetic inactivation of β 2-microglobulin and CD58 reveals frequent escape from immune recognition in diffuse large B cell lymphoma. *Cancer Cell* 20, 728–740
68. Wang, E.C.Y. *et al.* (2018) Suppression of costimulation by human cytomegalovirus promotes evasion of cellular immune defenses. *Proc. Natl. Acad. Sci. U. S. A.* 115, 4998–5003
69. Hammer, Q. *et al.* (2024) Genetic ablation of adhesion ligands mitigates rejection of allogeneic cellular immunotherapies. *Cell Stem Cell* 31, 1376–1386.e8
70. Veillette, A. and Chen, J. (2018) SIRP α -CD47 immune checkpoint blockade in anticancer therapy. *Trends Immunol.* 39, 173–184
71. Logtenberg, M.E.W. *et al.* (2020) The CD47-SIRP α immune checkpoint. *Immunity* 52, 742–752
72. Hu, X. *et al.* (2023) Hypoimmune anti-CD19 chimeric antigen receptor T cells provide lasting tumor control in fully immunocompetent allogeneic humanized mice. *Nat. Commun.* 14, 2020
73. Yamada-Hunter, S.A. *et al.* (2024) Engineered CD47 protects T cells for enhanced antitumor immunity. *Nature* 630, 457–465
74. Willingham, S.B. *et al.* (2012) The CD47-signal regulatory protein alpha (SIRP α) interaction is a therapeutic target for human solid tumors. *Proc. Natl. Acad. Sci. U. S. A.* 109, 6662–6667
75. Hu, X. *et al.* (2023) Human hypoimmune primary pancreatic islets avoid rejection and autoimmunity and alleviate diabetes in allogeneic humanized mice. *Sci. Transl. Med.* 15, eadg5794

76. Hu, X. *et al.* (2024) Hypoimmune islets achieve insulin independence after allogeneic transplantation in a fully immunocompetent non-human primate. *Cell Stem Cell* 31, 334–340.e5
77. Hu, X. *et al.* (2024) Hypoimmune induced pluripotent stem cells survive long term in fully immunocompetent, allogeneic rhesus macaques. *Nat. Biotechnol.* 42, 413–423
78. Minas, K. and Liversidge, J. (2006) Is the CD200/CD200 receptor interaction more than just a myeloid cell inhibitory signal? *Crit. Rev. Immunol.* 26, 213–230
79. Shao, A. and Owens, D.M. (2023) The immunoregulatory protein CD200 as a potentially lucrative yet elusive target for cancer therapy. *Oncotarget* 14, 96–103
80. Rygiel, T.P. and Meygaard, L. (2012) CD200R signaling in tumor tolerance and inflammation: a tricky balance. *Curr. Opin. Immunol.* 24, 233–238
81. Lauber, K. *et al.* (2013) Milk fat globule-EGF factor 8 mediates the enhancement of apoptotic cell clearance by glucocorticoids. *Cell Death Differ.* 20, 1230–1240
82. Takeuchi, H. *et al.* (2004) CCL21 chemokine regulates chemokine receptor CCR7 bearing malignant melanoma cells. *Clin. Cancer Res.* 10, 2351–2358
83. Förster, R. *et al.* (2008) CCR7 and its ligands: balancing immunity and tolerance. *Nat. Rev. Immunol.* 8, 362–371
84. Shields, J.D. *et al.* (2010) Induction of lymphoidlike stroma and immune escape by tumors that express the chemokine CCL21. *Science* 328, 749–752
85. Wang, X. *et al.* (2022) CD24-Siglec axis is an innate immune checkpoint against metaflammation and metabolic disorder. *Cell Metab.* 34, 1088–1103.e6
86. Kwon, D. *et al.* (2024) Genetically stable multi-gene edited iPSCs-derived NK cells for enhanced cancer immunotherapy. *Mol. Ther. Oncolytics* 32, 200885
87. Kagoya, Y. *et al.* (2020) Genetic ablation of HLA class I, class II, and the T-cell receptor enables allogeneic T cells to be used for adoptive T-cell therapy. *Cancer Immunol. Res.* 8, 926–936
88. Georgiadis, C. *et al.* (2018) Long terminal repeat CRISPR-CAR-coupled “universal” T cells mediate potent anti-leukemic effects. *Mol. Ther.* 26, 1215–1227
89. Qasim, W. *et al.* (2017) Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Sci. Transl. Med.* 9, eaaj2013
90. Benjamin, R. *et al.* (2020) Genome-edited, donor-derived allogeneic anti-CD19 chimeric antigen receptor T cells in paediatric and adult B-cell acute lymphoblastic leukaemia: results of two phase 1 studies. *Lancet* 396, 1885–1894
91. Mailankody, S. *et al.* (2023) Allogeneic BCMA-targeting CAR T cells in relapsed/refractory multiple myeloma: phase 1 UNIVERSAL trial interim results. *Nat. Med.* 29, 422–429
92. Ghobadi, A. *et al.* (2025) Induced pluripotent stem-cell-derived CD19-directed chimeric antigen receptor natural killer cells in B-cell lymphoma: a phase 1, first-in-human trial. *Lancet* 405, 127–136
93. Li, Y.-R. *et al.* (2025) IL-15 in CAR engineering: striking an efficacy-safety balance. *Trends Mol. Med.* 31, 977–981
94. Li, Y.-R. *et al.* (2025) The clinical landscape of CAR-engineered unconventional T cells. *Trends Cancer* 11, 520–539
95. Li, Y.-R. *et al.* (2024) Breaking the mold: unconventional T cells in cancer therapy. *Cancer Cell* 43, 317–322
96. Haraguchi, K. *et al.* (2004) Recovery of V α 24⁺ NKT cells after hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 34, 595–602
97. Muranski, P. *et al.* (2006) Increased intensity lymphodepletion and adoptive immunotherapy—how far can we go? *Nat. Clin. Pract. Oncol.* 3, 668–681
98. Lickefett, B. *et al.* (2023) Lymphodepletion—an essential but undervalued part of the chimeric antigen receptor T-cell therapy cycle. *Front. Immunol.* 14, 1303935
99. Lyu, Z. *et al.* (2025) Protocol for assessing pharmacokinetics and pharmacodynamics of human CAR-NKT cells in humanized mouse models using bioluminescence imaging. *STAR Protoc.* 6, 103957
100. Licht, B.J.M. *et al.* (2025) Engineering hypoimmune stem cell-derived beta cells. *Stem Cell Res. Ther.* 16, 610
101. Moore, M.A. *et al.* (2018) Allograft tissue safety and technology. *Biologics Orthop. Surg.* 26, 49–62