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Review

Progress and challenges in developing allogeneic cell therapies

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SUMMARY

The new era of cell therapeutics has started with autologous products to avoid immune rejection. However, therapeutics derived from allogeneic cells could be scaled and made available for a much larger patient population if immune rejection could reliably be overcome. In this review, we outline gene engineering concepts aimed at generating immune-evasive cells. First, we summarize the current state of allogeneic immune cell therapies, and second, we compile the still limited data for allogeneic cell replacement therapies. We emphasize the advances in this fast-developing field and provide an optimistic outlook for future allogeneic cell therapies.

INTRODUCTION

Cell therapeutics are living medicines that are hoped to bring increased efficacy for the treatment of a large variety of diseases spanning from cancer to autoimmunity and regenerative medicine. While some concepts rely on autologous cells as starting material, which include the patient's own primary cells or induced pluripotent stem cells (iPSCs) derived thereof, others use allogeneic cells from healthy donors. From a drug development and manufacturing standpoint, allogeneic cell sources have a myriad of advantages. Healthy donors can be selected rather than patients with chronic or malignant diseases, the manufacturing can be scaled with improved quality control and decreased run-to-run variability, multiplex editing can more easily be implemented, and the cost of goods can be reduced.[1–4] Ultimately, PSCs have the potential to become the most consistent source for allogeneic cell products and further facilitate multiplex engineering and scaled manufacturing.[5] Allogeneic cell therapies also provide important advantages for patients, including improved access for more people in need, immediate availability without the risk of production failures, and consistent quality of the products.[6] Kill switches have been proposed to improve the safety of PSC-derived products, and their utility and necessity will need to be determined.[7] The single most daunting obstacle currently defying the success of allogeneic cell therapy is immune rejection and our struggle to overcome this immune barrier. Long-term data of immune cell products in cancer patients suggest that persistence is necessary for durable responses.[8] Extensive research in this area in recent years has immensely advanced our understanding of the immunological principles involved and has generated concepts around the idea of immune evasion.[2,6,9,10] The goal is to engineer allogeneic cells in a way that they would lose their alloimmunogenicity and experience no more immune response than any autologous cell would. Immune evasion concepts for allogeneic immune cell therapy and allogeneic cell replacement therapy are discussed separately below.

Immune cell therapies fight diseases by attacking the cells constituting or causing the disease, which can be malignancies or aberrant autoimmune cells, and whose elimination provides disease control or cure. In which cases long-term persistence of the allogeneic immune cells is required for their success and in which repeated dosing is equivalent or even advantageous still needs to be determined. Several different allogeneic immune cell products have already been tested in clinical trials.[11,12] Since the adoptively transferred allogeneic cells are immune cells, they can attack healthy patient cells and tissues and cause graft-versus-host disease, while they themselves are susceptible to the patient's own immune system in a host-versus-graft response called rejection. Graft-versus-host disease occurs when the T cell receptor (TCR) in allogeneic chimeric antigen receptor (CAR) T cells remains active and is reactive with patient tissue. That is true for a subset of T cells called αβT cells because they express a TCR that is made up of variable TCR-α and TCR-β chains and recognizes fragments of alloantigen peptides bound to major histocompatibility complex (MHC) molecules. Most allogeneic αβT cell products rely on a knockout of the *TRAC* gene to prevent expression of the TCR-α chain and subsequent graft-versus-host disease,[13] while more innate-type allogeneic immune cells like γδT cells and natural killer (NK) cells do not have this alloreactivity and are naturally more suitable for allogeneic cell therapy. The γδ-TCR does not recognize MHC-bound allopeptides and instead is activated by molecules that signal cellular stress, phosphoantigens, and lipids. An emerging alternative for *ex vivo* manufactured CAR T cell products is *in vivo* gene therapy, where patients are injected with a vehicle delivering a CAR transgene and editing tools that together generate CAR T cells in the patient. This approach comes with its own challenges and advantages and is discussed below.

Cell replacement therapies restore specific physiologic organ functions that have been impaired or lost during the disease process because critical cell populations have perished. The replenishment of adequate cell numbers and their long-term survival is crucial for the success of cell replacement therapy. The alloimmune response is unidirectional against the allogeneic cell product, and the reliability of full immune evasion is imperative for lasting efficacy. Alternative strategies aim to physically protect allogeneic cells from a patient's immune system in encapsulation devices. Their main challenges include nutrient support and vascularization within their devices while still allowing the encapsulated cells to sense and release factors.

ALLOGENEIC IMMUNE CELL THERAPIES

So far, many allogeneic immune cell therapies for cancer have failed to deliver treatment results comparable to those of autologous products[11] and will probably continue to do so until allogeneic cells can be engineered to fully escape rejection and achieve the same persistence as autologous products. For autoimmune diseases, where persistence does not appear to be necessary, the first allogeneic anti-CD19 CAR T cells demonstrated effectiveness in reducing disease activity.[14] Once rejection has reliably been overcome, there is hope that advanced engineering could generate allogeneic, immune evasive immune cell therapeutics possessing additional features that boost fitness, prevent exhaustion, facilitate trafficking, and defy the immunosuppressive tumor microenvironment. More extensive engineering will not be manageable for individual autologous products, given the complexity of editing and quality control, and seems only reasonable for large batches of allogeneic cells for the treatment of larger patient populations. Specifically, PSC-derived products offer the best options for multi-step engineering and subsequent large-scale manufacturing. With this approach, immune-evasive cell therapeutics could become overall superior to autologous products. Therefore, the establishment of comprehensive immune evasion can be seen as a necessary step that will enable further progress. The different approaches taken and their levels of success toward true immune evasion are discussed below.

The immunogenicity of autologous cells

The notion that products derived from autologous cells are always immunologically inert and accepted as self deserves questioning. Autologous cells possess the exact same DNA as the cell donor and thus are 100% human leukocyte antigen (HLA) and minor antigen matched. During *ex vivo* engineering and expansion, however, neoantigens can occur from mutations or genetic drift that happen in the dish.[15,16] Immunogenic antigens can undergo unchecked amplification in the absence of immune surveillance and render the cell product immunogenic when transplanted back into the same cell donor. The immune system in immunocompetent mice[17] and humans[15] was shown to be able to detect and respond to neoantigens derived from non-synonymous single nucleotide variations or genetic drift of the mitochondrial DNA and lead to immune rejection of the autologous cell grafts. The longer the *ex vivo* manufacturing process and the higher the rate of expansion, the more likely it is that neoantigens appear and amplify. The introduction of transgenes into autologous cells can further render them susceptible to immune recognition of xenogeneic[18] or synthetic constructs[19] or viral products from the engineering process.[20,21]

The innate immune system and more specifically macrophages have been identified as barriers to the engraftment of cell therapeutics even in hosts incapable of recognizing allogeneic disparities.[22] A lack of persistence of CAR T cells was associated with diminished antitumor efficacy. During the generation of CAR T cells in culture, the cells were shown to upregulate signals stimulating macrophage phagocytosis while downregulating the "don't eat me" signal CD47.[22] Upon encounter with macrophages, this ligand imbalance increased the phagocytic activity of macrophages, and CAR T cells were more likely to getting killed.[22] In a clinical trial with autologous GD2 CAR T cells, myeloid signatures including Gli1 enrichment, a protein involved in the CD47-SIRPα (signal regulatory protein alpha) pathway,[23] were identified as immune determinants in poor expanders.[24] The density of CD47 expression on HLA-replete CAR T cells substantially impacted the survival and antitumor potency *in vivo*, and CD47 enhancement could improve autologous CAR T therapeutics.[22]

The fact that subsequent infusions of the same autologous CAR T cells have led to disappointing outcomes hints at an adaptive immune response diminishing cell survival. Although it had been speculated that the failure of reinfused CAR T cells could be related to tumor features of treatment resistance, the mere lack of expansion and persistence after a second dose[25] points more toward an inability of the reinfused CAR T cells to avoid expedited clearance. One study found a cellular immune response against a transgene that was used as a selection marker.[21] In another study using CD19 CAR T cells with the murine FMC63 scFv, five of 29 patients with B cell acute lymphoblastic leukemia (ALL) had persistent or relapsed leukemia and received a second infusion of CAR T cells. In all five patients, there was no expansion or persistence of CAR T cells, and in all these patients, a CAR-specific host $CD8^+$ T cell response was detected.[25] Epitope mapping in one patient identified immunogenic epitopes within the murine FMC63 scFv. These findings pointed toward an immunogenicity of the murine sequence of the CAR, and the humanization of constructs was postulated to circumvent this problem.[25] This was confirmed in a study treating young adults with relapsed or refractory ALL pretreated with murine CD19 CAR T cells. When a humanized CAR was used for the second treatment, the overall response was much better, and long-term persistence and durable remissions were achieved.[26] The weakening of the host immune system with lymphodepletion before the first CAR T cell therapy was also shown to reduce the risk for generating an immune response against the CAR transgene[27,28] and a higher dose of the second CAR T infusion to overwhelm the host immune system was further associated with higher overall response rates.[28] The examples provided illustrate that engineered and re-infused autologous cells can occasionally prompt adaptive or innate immune responses and cannot generally be considered non-immunogenic.

Non-immunological factors for the persistence of adoptively transferred immune cells

Transplanted allogeneic immune cells can vanish for multiple reasons (Figure 1). The most obvious is immune rejection by the host immune system, but the lifespan of the cell type, cellular

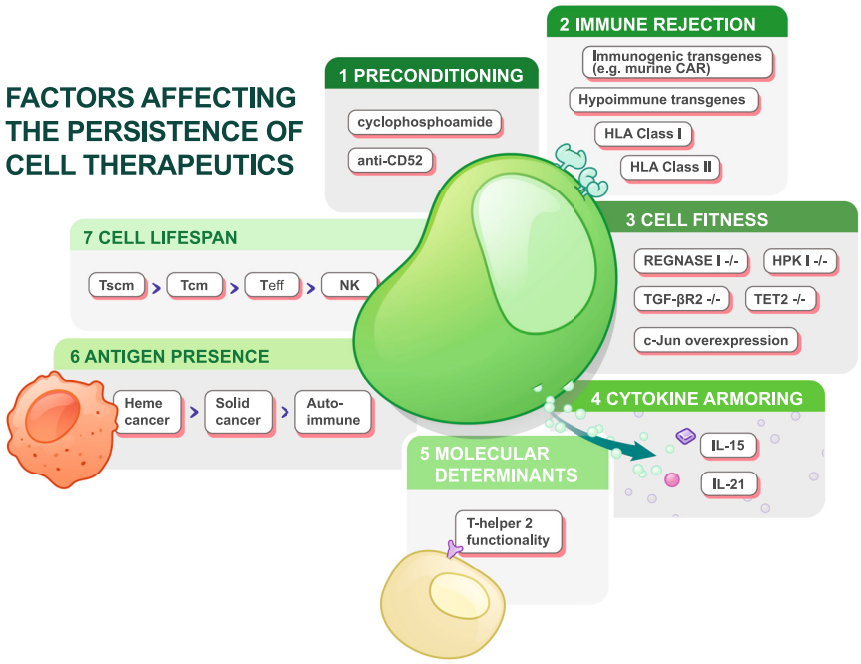

Figure 1. Reasons for the vanishing of cell therapeutics

Several factors affect the persistence of transplanted cell therapeutics, including the pre-treatment of the patient, the immunogenicity of the cells, the fitness of the cells, engineered cytokine armoring, molecular determinants, the presence of antigen, and the lifespan of the cells.

fitness, cytokine armoring, molecular determinants, the presence or absence of stimuli, and the preconditioning can also have a major impact. CAR NK cells and T cells, including subpopulations of the memory or effector phenotype, have vastly different longevities. Specifically, central memory and stem cell memory T cell clones contribute substantially to the circulating CAR T cell pools during both early expansion and long-term persistence.[29,30] In some patients, stem cell memory T cell clones have persisted and preserved their differentiation potential for over a decade.[31] The transcription factor FOXO1 was recently shown to promote memory and restrain exhaustion in human CAR T cells and might allow generalized memory reprogramming to optimize therapeutic T cell states.[32] By contrast, NK cells are usually short lived with a turnover time in blood of about 2 weeks.[33] However, interleukin-15 (IL-15) armoring,[34] IL-21 armoring,[35] or the expression of a membrane-bound IL-15/IL-15R fusion protein[36] can substantially increase their longevity.[37] Additional engineering to increase the fitness or cytokine support of CAR T and NK cells is discussed in detail elsewhere.[38] It has been suggested that TCR-deficient CD8 and CD4 T cells have a markedly reduced half-life, which is more significant for naive than for memory cells.[39] In a non-obese diabetic (NOD) severe combined immunodeficiency (SCID) gamma (NSG) mouse cancer model, CD19 CAR T cells with TCR-depletion showed reduced persistence, less efficacy to control cancer growth, and shorter animal survival than CD19 CAR T cells with endogenous TCR co-expression.[40] Homeostatic expansion of αβT cells appears to rely on the interaction of the TCR with self-peptide-MHC complexes and cytokines such as interleukin-7,[41] and it remains to be seen whether the requirement for TCR depletion in allogeneic T cell products comes to the detriment of their efficacy. When investigating molecular determinants for ultralong CAR T cell persistence, enriched T helper 2 functionality was observed in 5-year relapse-free responders.[42] Mechanistically, type 2 cells were then found to regulate a dysfunctional CAR T cell subset to maintain whole-population homeostasis and enhance fitness.[43] Strategies to enhance type 2 function in CAR T cell infusion products could further mitigate dysfunction and extend their durability of response. Further, it is believed that the stimulus pattern of target antigen affects the CAR T cell response, with transient antigen stimuli resulting in weak and increasing antigen stimuli yielding strong responses.[44] The limited abundance of benign CD19 cells in autoimmune disease provides a lower antigenic stimulus than in cancer and might accelerate contraction of the CAR T cell population[45] and allow for subsequent B cell reconstitution.[46,47] The renewed B cells feature a more naive character and display a broad range of light- and heavy-chain usage similar to that of healthy individuals.[46,47] Since most patients remained free of symptoms of autoimmune disease, the reconstituted B cells most likely lacked the autoreactive B cell clones.[47] This finding suggested that a "reset" of B cell homeostasis in patients with autoimmune diseases may be sufficient, and long-term persistence of CAR T cells might not be necessary.[45] However, whether a successful immune reset can be the singular event able to cure chronic diseases will need to be seen. Relapse in autoimmune patients after CAR T cell therapy has been reported (NCT05938725 and NCT06347718) and will most likely become more common with the rapid inclusion of more patients. The ability for redosing will become an advantage, and immune-evasive products that do not create any immune memory might be best suited for that. The level of lymphodepletion has been shown to correlate with CAR T cell success, and for allogeneic cell products, lymphodepletion additionally provides a window of opportunity with weakened host immune response and alleviated rejection response.[48] Effective immune evasion may allow the reduction or omission of lymphodepletion and reduce the side effect profile of allogeneic immune cell therapeutics. If allogeneic therapeutics can either achieve reliable persistence or allow repeated redosing without loss of efficacy, then they will most likely replace autologous immune cell therapies for cancer and autoimmune indications.

Transplantation tolerance

The aim for circumventing immune rejection of cell therapeutics should not be confused with the establishment of immune tolerance, despite the widespread and casual use of this term. Immune tolerance refers to a dynamic state of unresponsiveness

toward a specific antigen, which involves both innate and adaptive immune cells.[49] Tolerance is an active process and requires the presentation of antigen and depends on a minimum threshold affinity of binding between antigen and receptor.[50] Central tolerance involves clonal deletion during T and B cell development in the thymus and bone marrow[49,51] and selection of regulatory T cells (Tregs).[52] Although clonal deletion is efficient at removing high-affinity self-reactive clones, this process is imperfect, and many self-reactive cells develop into functional $CD4^+$ and $CD8^+$ T cells.[53] An additional layer called peripheral tolerance works through mechanisms of anergy, deletion, and suppression by regulatory immune cells.[49] Activation of T cells is inhibited through anti-inflammatory cytokines such as IL-10,[54] transforming growth factor β (TGF-β),[55] or IL-35[56] or through the activation of immune checkpoint molecules such as PD-1[57] or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4).[58] Several suppressive immune cell populations contribute to maintaining peripheral tolerance, including regulatory T cells, B cells, NK cells, and macrophages,[59] as well as tolerogenic dendritic cells.[60] Overall, immunological tolerance is an active and highly regulated collective unresponsiveness toward certain self or accepted antigens while maintaining responsiveness against other non-self antigens. This process, however, remains dynamic, and new tolerance can be induced, and established tolerance can be disrupted with changes in the immune environment. Vaccinations and infections can trigger the generation and expansion of novel T cell clones and subsequently increase the memory T cell repertoire. Simply by chance, any polyclonal T cell response can generate clones that will cross-react with other, completely unrelated antigens, including allogeneic HLA or alloantigens. This phenomenon of heterologous immunity can tilt the balance of tolerance and induce rejection of previously tolerated cells or organs.[61] Certain viral infections can induce an immune response in which nearly half of CD4 and CD8 virus-specific T cell clones cross-react with allogeneic HLA alleles.[62,63] Such cross-reactive virus-specific T cells can break tolerance and contribute to allograft rejection.[64] Viral infections can also activate Toll-like receptor signaling pathways[65] and further augment inflammation. The release of cytokines such as IFN-α and IL-6 facilitates the disruption of transplantation tolerance.[66] Overall, stable and persistent immune tolerance to allogeneic cells or organs is very difficult to induce and to maintain. The strength of the immune response varies from organ to organ, with kidney grafts being more permissive to tolerance induction than hearts or lungs.[67] So far, tolerance in the clinical setting can be induced reliably only for HLA-identical grafts and with a substantial effort. Reproducible tolerance to allogeneic, fully HLA-matched kidneys has been achieved with nonmyeloablative total lymphoid irradiation, anti-human thymocyte globulin (ATG)-preconditioning, HLA-matched hematopoietic cell transplantation, and temporary immunosuppression with steroids, calcineurin inhibitors, and MMF.[68] Maintenance immunosuppression could later be discontinued. With increasing HLA disparity, results have not been as good. In patients after haplotype-matched kidney and hematopoietic cell transplantation, mixed chimerism was more dependent on the continuation of the calcineurin inhibitor and could not establish tolerance.[68] Therefore, tolerance induction remains a major challenge, although there is clinical evidence of feasibility.

Immune evasion

Immune evasion is a fundamentally different approach in that the unresponsiveness of the immune system is not negotiated but attained through deception. That means so-called "hypoimmune" cells achieve immune evasion through a combination of avoiding T cell detection, which prevents priming and sensitization (the phase in which unprimed T cells interact with dendritic cells to become active lymphoblasts), and providing a dominant blocking signal to the innate immune system. The transplantation of hypoimmune cells will not induce an immune response and will not generate immunological memory. The immune system will not recognize transplanted hypoimmune cells as allogeneic. The lack of memory further permits unlimited redosing. Given the overall complexity and interconnectivity of the immune system with crosstalk between different immune cells and an armamentarium of cytotoxic factors and antibodies, a comprehensive hypoimmune coverage needs to be complete and gapless.

Targets for immune evasion

Mechanisms employed for immune evasion include those avoiding immune cell activation and those actively engaging with inhibitory immune checkpoints. Since allorejection is mainly a T cell-driven adaptive immune response, the foundation for most immune evasion strategies rests on the prevention of alloantigen presentation via HLA molecules (Figure 2A). The disruption of *B2M*,[69–76] which is necessary for all HLA class I molecule expression, or of transporter associated with antigen processing 2 (*TAP2*),[77] a transporter molecule required for the processing of antigen for presentation via HLA class I, has been successfully used by multiple groups to prevent alloantigen presentation through class I HLA. Many cell types also constitutively express HLA class II, and those include immune cell subsets of T cells, B cells, and myeloid cells. Numerous somatic cells, such as endothelial cells[72] and pancreatic beta cells,[78] can upregulate HLA class II during inflammation. To circumvent alloantigen presentation through class II HLA, the disruption of the critical transcriptional coactivator class II major histocompatibility complex transactivator (CIITA) has shown great efficacy.[72,73,76] Complete disruption of alloantigen presentation through HLA class I and II depletion prevents the activation of T cells and reliably averts an adaptive immune response.

The definitive avoidance of T cell activation through complete HLA depletion comes at the cost of an induced susceptibility for innate immunity through the "missing self" response.[79,80] Both NK cells and macrophages can independently sense HLA deficiency and exert cytotoxic responses.[72] NK cells express a multitude of activating and inhibitory receptors that transmit signals intracellularly, and their net input determines between activation or quiescence. Soluble mediators modulate the activation threshold and can enhance and suppress NK cell responses.[81] The interaction of an NK cell with a target through the formation of immune synapses is brief and allows the NK cell to detach and quickly engage other targets.[82] The sparing of target cells expressing sufficient inhibitory signals seems not to affect the ability of the same NK cell to kill subsequent targets with more unfavorable NK ligand pools. SIRPα has been identified as a very potent inhibitory immune checkpoint for NK cells, and overexpression of its natural ligand CD47 results in effective NK cell inhibition even in IL-15 or IL-2 environments[83] (Figure 2B). The

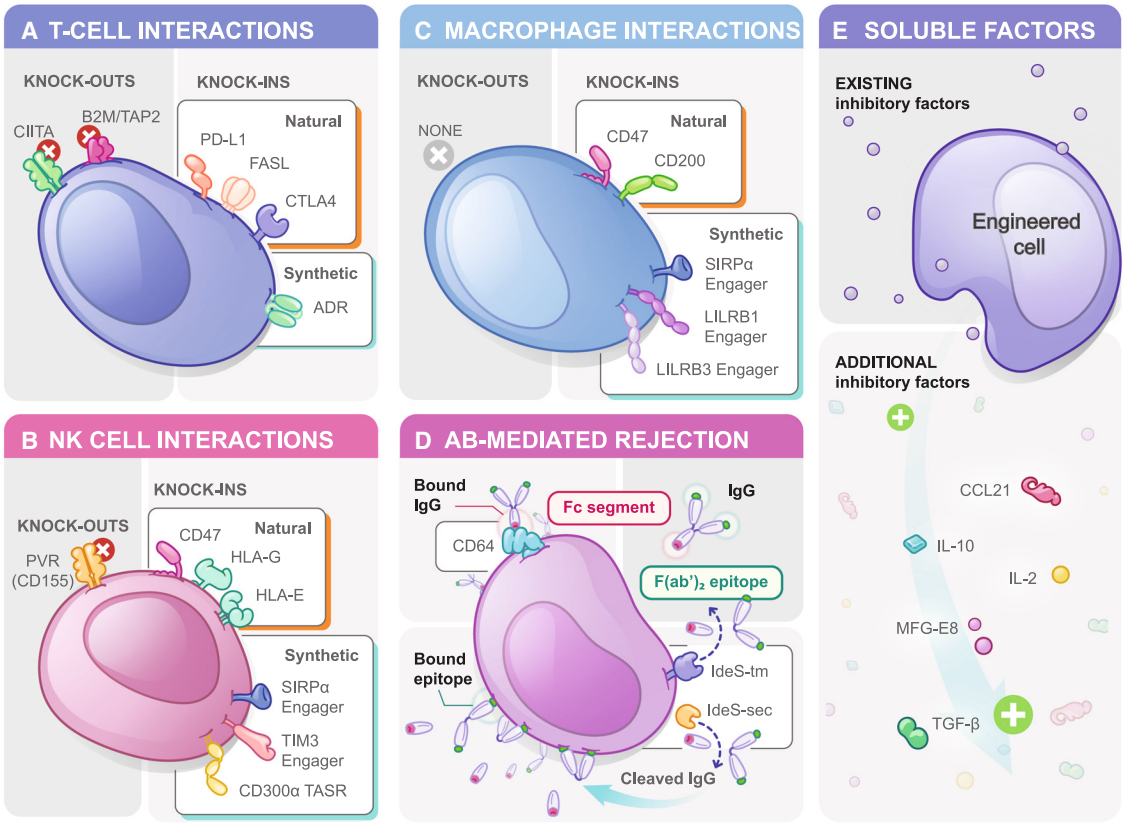

Figure 2. Gene engineering strategies for immune evasion

(A–C) Gene edits for T cell interactions (A), NK cell interactions (B), and macrophage interactions (C) are summarized. Gene knockouts (left) and transgene knockins (right) are shown separately, and natural ligands and synthetic ligands are distinguished.

(D) The two strategies to protect cells from antibody-mediated rejection are displayed.

(E) Additional soluble factors have been proposed to create a more permissible environment for immune evasion.(ADR, alloimmune defense receptor; TASR, trans antigen signaling receptor; IdeS-tm, immunoglobulin G protease tethered to the membrane; IdeS-sec, immunoglobulin G protease that is secreted).

CD47 expression level in HLA-depleted target cells that are required to protect the targets from NK cytotoxicity was clearly defined, several times above endogenous levels, and illustrated the threshold mechanism for a binary choice for NK cells. Agonistic synthetic ligands for SIRPα,[76] T-cell immunoglobulin and mucin domain-containing protein 3 (TIM3),[76] and CD300a[84] have similarly shown potent NK inhibitory activity, and those inhibitory receptors are expressed on most, if not all, NK cells and provide widespread coverage. HLA-E[71,73] or HLA-G[85] overexpression will contribute to NK cell inhibition in those subpopulations expressing their receptors CD94/NKG2A or ILT2, respectively. For cell types that strongly express activating NK cell ligands, their targeted knockout can diminish their stimulatory signal, elevate the activation threshold, and facilitate the inhibitory net effect of additional checkpoint ligands. The removal of stimulatory signals is thus more of a fine-tuning tool to support other inhibitory signals but does not by itself protect HLA-depleted cells from being killed. This has been shown for poliovirus receptor (PVR) knockout and HLA-E overexpression in HLA-depleted iPSC-derived T cells.[73]

For the inhibition of macrophage clearance, CD47 is similarly effective at similar threshold levels as shown for NK cells[83] (Figure 2C). Other approaches have used agonistic synthetic ligands for SIRPα,[76] LILRB1,[76] or LILRB3.[76] Overexpression of CD200, the natural inhibitory ligand for the CD200 receptor, has also been described.[86]

A variety of concepts restrict the knockout to certain HLA molecules and rely on HLA matching for those that are retained.[87,88] The aim is to minimize T cell activation while still providing enough resistance against innate immune cells.[6] Countries with more restricted HLA pools might require fewer cell lines for successful matching at retained HLA loci.[89,90] It remains to be seen, though, how reliable these concepts work in patients when no immunosuppression is being used. Certainly, in clinical kidney transplantation, HLA-matched transplants still require immunosuppression to prevent rejection, and nonadherence to maintenance immunosuppression is a common reason for rejection.[91] Also, these concepts of partially retained HLA are not fully aligned with the idea of universal cell products and require the creation of cell banks, have logistical challenges, quality control issues, and show product heterogeneity between lines.

Some groups have explored strategies in the context of fully retained HLA. The expression of individual ligands for the T cell immune checkpoints PD-1 or CD80/CD86 has led to different levels of success.[76,92,93] It seems that a total of 8 genetic modifications are required to prevent allorejection in the context of a

fully retained HLA pool. The described gene edits interfere with antigen presentation and activation signals of various immune cells and activate T cell apoptosis through the expression of Fas ligand (FasL).[86]

The secretion of immune inhibitory agents, including TGF-β, IL-10, and IL-2 mutein,[93] or milk fat globule-EGF factor 8 (MFG-E8) and CCL21,[86] was reported to further support immune evasiveness of engineered cells by dampening of alloimmune responses (Figure 2D). One approach described the targeted cytotoxicity against activated T cells through a defense receptor that selectively recognizes 4-1BB.[74] The latter strategy is reserved for cytotoxic effector cells and is not applicable to cell replacement products.

A number of patients had sensitizing events in their past and have pre-existing HLA antibodies that could jeopardize the engraftment of an allogeneic cell therapeutic if these antibodies recognize HLA on the cell product. Patients with multiple myeloma were screened for donor (product)-specific HLA antibodies before being enrolled to receive allogeneic B-cell maturation antigen (BCMA) CAR T cells in the UNIVERSAL trial.[94] Six patients with positive donor-specific antibody test had to be excluded from the study and were not eligible for this therapy. However, screening for product-specific HLA antibodies is not common in clinical trials, and the incidence of antibody-mediated failures of cell expansion and persistence remains unknown. Any administered HLA-replete cell product can be sensitizing itself, and *de novo* antibodies can expedite rejection when the same product is re-administered again at a later time point. HLA-depleted cell products are protected from pre-existing HLA antibodies and do not induce sensitization for HLA. However, even HLA-depleted products may still be susceptible to non-HLA antibodies, including blood type antibodies, transfusion antibodies, and autoantibodies. The pathogenesis of diseases such as Hashimoto's thyroiditis mechanistically involves organ damage through cytotoxic immunoglobulin G (IgG) autoantibodies. Any thyroid cell grafts transplanted into a patient with thyroid-targeting autoantibodies would undergo the same fate as the native organ, and regenerative thyroid organoids should be engineered to acquire protection from antibody killing. Two defense mechanisms against IgG-antibody-mediated cytotoxicity have been developed (Figure 2E). The overexpression of truncated or mutated CD64 captures the Fc segment of cytotoxic, monomeric IgG and thus functionally inactivates their cytotoxic potential.[95] Mechanistically, the data suggest that truncated CD64 captures IgG Fc and prevents effector cell or complement binding, while its Fab fragments still bind their target epitopes on the engineered cell, thus "masking" the epitopes from recognition by other free IgG antibodies. This phenomenon of *cis* binding and epitope masking by membrane-tethered binding moieties has recently been described in accidentally CAR-transduced B cell leukemia (CARB) cells.[96] Truncated CD64 on hypoimmune cells was shown to protect against both antibody-mediated cellular cytotoxicity and complement-dependent cytotoxicity. Expression of the IgG-degrading enzyme of S. pyogenes cleaves IgG bound to the engineered cells and covers those in a protective coating of non-functional $F(ab')_2$.[97] The protection of cells already resistant against all cellular cytotoxicity can be expanded to include IgG protection by adding a mutated CD64 transgene, without interfering with the protection from cellular cytotoxicity[76,95] (Figure 3).

Unprotected allogeneic immune cell therapeutics

There is a wide discrepancy between reports on the persistence of unprotected, allogeneic CAR T cells and CAR NK cells.[11,12] A number of different allogeneic CAR T cells with CD52 knockout were administered to patients after they were preconditioned with lymphodepletion and an anti-CD52 IgG1 antibody to more effectively deplete host immune cells and enable expansion and persistence. Expansion of CD19 CAR T cells negatively correlated with the occurrence of alloreactive CD8 host clones, and non-expansion was highly significantly correlated with disease progression.[98] Expansion of CD22 CAR T cells was only observed in patients with persistent anti-CD52 antibody levels and deeply depleted host CD3 cells for 28 days.[99] Despite this aggressive form of preconditioning, allorejection remained a critical issue, and re-dosing of the same product resulted in a dampened response.[100] A CD19/CD22 dual-targeting CAR T product achieved a median duration of persistence of 42 days.[101] In a trial with similarly preconditioned patients receiving unprotected allogeneic *CD52*$^{-/-}$ BCMA CAR T cells, the resulting immunosuppression was so effective that 100% of patients had at least one adverse event, 88% had an adverse event ≥ grade 3, and >50% of all patients had infectious complications.[94] Host NK cells recovered after 1–3 months, but T cells recovered more slowly, so the patients were immunocompromised for months. Despite that, by day 28, 67% of patients had no detectable CAR T cell levels. In a trial using unprotected allogeneic CD19 CAR T cells after lymphodepletion, peak expansion and area under the concentration time curve correlated strongly with the abundance of the infused minimally differentiated stem central memory cell population.[102] Whether this CCR7+ stem central memory T cell fraction also results in improved persistence remains to be seen, but better overall response to treatment has already been established. Allogeneic unprotected CD20 CAR γδT cells infused after lymphodepletion showed dose-dependent expansion kinetics, and CAR cells could be detected for 14 days in the highest dose group, which also showed the best response rate.[103] Another allogeneic unprotected BCMA CAR T product, administered after lymphodepletion, showed a correlation between the depth of lymphodepletion and engraftment.[104] A trial with unprotected, allogeneic CD19 CAR T cells administered after low-dose lymphodepletion appeared to gain efficacy through a PD-1 knockout that might protect from the immunosuppressive tumor microenvironment.[105] Interestingly, T and NK cells recovered within 3 weeks after lymphodepletion, but B cells remained below the limit of quantification beyond 3 months. Persistence of CAR T cells was not mentioned in this preliminary report. The first iPSC-derived CAR T cell products,[106] which are inherently allogeneic therapeutics, featured the depletion of the TCR and CD19 CAR expression.[107] Despite using standard-dose lymphodepletion, no transgene copies could be detected in peripheral blood DNA beyond 11 days. Whether iPSC-derived T cells show the same cellular fitness as peripheral blood T cells remains to be seen. Recent reports suggest that survival and trafficking of iPSC-derived T cells can be markedly improved with cytokine armoring,[108] but unprotected iPSC-derived T cells remain susceptible to rejection. In stark contrast, a trial testing unprotected, allogeneic cord-blood-derived CD19 CAR NK cells armed with IL-15 in patients with $CD19^+$ B cell tumors using low-dose lymphodepletion reported

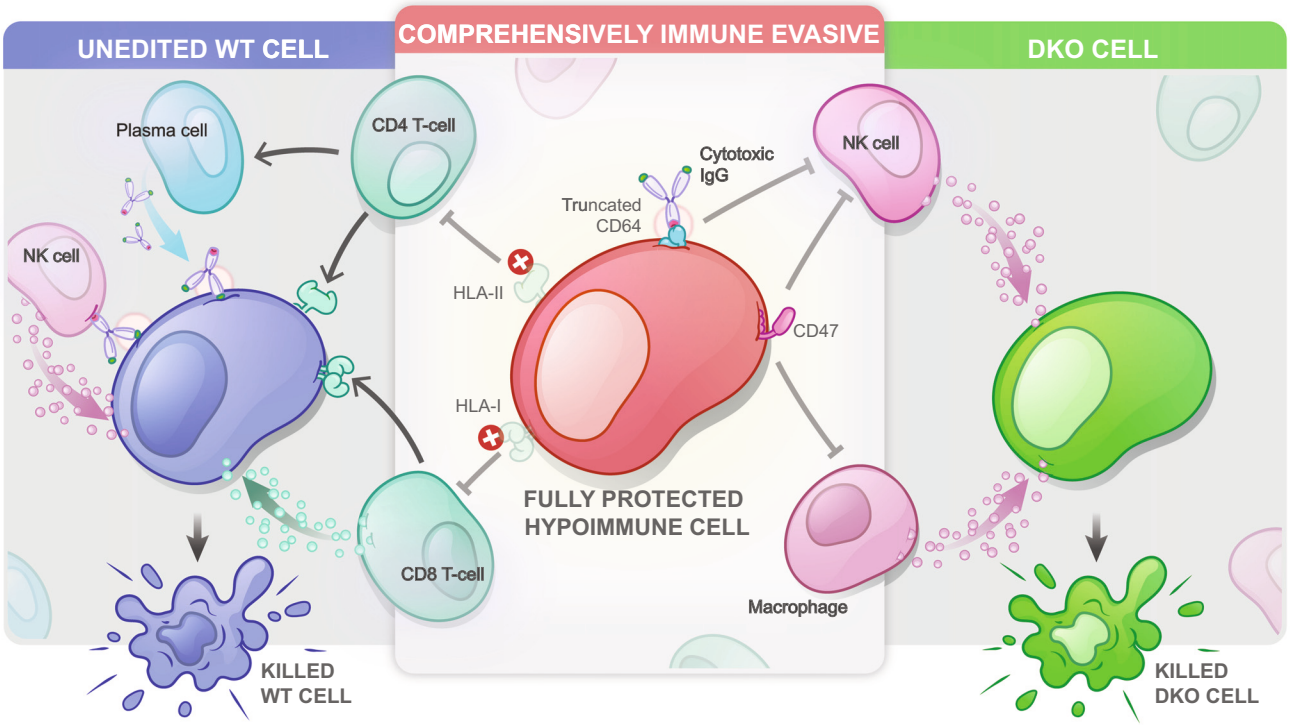

Figure 3. Comprehensively immune-evasive cells survive during ongoing rejection of wild-type (WT) and DKO cells
Mixed allogeneic cell therapeutics contain fully edited immune evasive cells as well as unedited WT cells and partially edited DKO cells. After transplantation into an immunocompetent recipient, DKO cells get killed by innate immune cells recognizing "missing self," and WT cells are killed through a concerted adaptive allorejection response mediated by T cells and involving an antibody response and innate immune cells. Hypoimmune cells escape all cellular and humoral cytotoxicity and are able to survive and persist within ongoing rejection.

routine persistence for 3 months even in non-responders and up to 1 year in responders.[34,109] The persistence of the allogeneic NK cells was independent of their degree of HLA mismatch with the recipient.[34] Another unprotected allogeneic NK cell product engineered to express an NKG2D activating receptor, used with standard lymphodepletion, was detectable for a week after infusion and became undetectable at 14 days.[110] A small study treated 5 children with relapsed or refractory neuroblastoma using unprotected but HLA-matched, allogeneic GD2 CAR T cells before or after allogeneic hematopoietic stem cell transplantation from the same donor.[111] Despite the full or haploidentical HLA match, all patients received standard fludarabine/cyclophosphamide lymphodepletion. Under these favorable conditions for engraftment, all CAR T products expanded well and persisted for 4–6 weeks.

Although comparisons between these studies are difficult, the survival and persistence of unprotected allogeneic cell therapeutics seem to directly depend on the competence of the host immune system to reject them. Persistence depends largely on the depth of host immune cell depletion from pre-conditioning. However, not all allogeneic cell products seem to undergo rejection at the same pace. The presence of veto cells[112] in allogeneic cell products may account for some of the differences. Veto cells are immune cells with selective immunomodulation properties that can specifically delete T cells directed against the veto cells themselves but not against other third-party cells.[112,113] Various cell types have been shown to mediate veto activity, including T cells, NK cells, and dendritic cells.[114] Co-expression of CD8 and FasL was proposed to be required for the veto activity of CD8 T cells, with the Fas-FasL interaction mediating apoptosis of activated, engaged effector cells.[115] A mismatch between KIR on veto NK cells and KIR ligands on host cells was necessary for alloreactive NK cells to reduce graft-reactive T cells from the host.[116] Since NK cell alloreactivity was shown to enhance the engraftment of allogeneic hematopoietic transplantation, it may also contribute to improved persistence of allogeneic CAR NK cell therapeutics. However, veto cells are poorly described phenotypically, and their efficacy may vary depending on every patient's KIR repertoire.

Partial immune evasion

Partial immune evasion concepts are those that have shown gaps in their coverage in preclinical studies. Most commonly, only the *B2M* gene was deactivated in allogeneic CAR T cells to prevent CD8 T cell-mediated rejection without any strategy to protect the cells from the induced susceptibility against innate immunity. $B2M^{-/-}$ cells have repeatedly been shown to be lysed by activated NK cells.[70,71,87,117,118] *B2M* knockout through targeted gene disruption was achieved in CAR T cells against CD19 (NCT04035434 and NCT05643742), BCMA (NCT04244656 and NCT04960579), CD70 (NCT04502446 and NCT05795595), or MUC1C (NCT05239143), and all those products have entered clinical trials. Results from one allogeneic trial for relapsed or refractory large B-cell lymphoma (LBCL) showed that in patients

who received ≥ 300 million $B2M^{-/-}$ CD19 CAR T cells, the best overall response rate (ORR) and complete response (CR) rates were achieved.[119] Expansion peaked at 8 days, and cells were detectable for 28 days, in some cases for 3–4 weeks. Knocking out B2M as the sole strategy is a trade-off that shifts the susceptibility of the products from T cells to innate cells. The rationale rests on the premise that innate cells are much rarer in organs, especially lymphatic organs,[120] and cancer renders NK cells dysfunctional.[121] Although NK cells recover more quickly after lymphodepletion than host T cells,[122] all patients in allogeneic $B2M^{-/-}$ CAR T cell trials have received at least standard dose lymphodepletion. When comparing the cyclophosphamide doses necessary to achieve sufficiently low white blood cell (WBC) nadirs, patients with solid tumors receiving allogeneic $B2M^{-/-}$ T cells with MUC-1C CAR required substantially higher doses than patients with multiple myeloma receiving allogeneic $B2M^{-/-}$ T cells with BCMA CAR.[123] The success of allogeneic $B2M^{-/-}$ CAR T cells thus seems to depend on effective depletion of the patient's innate immune cells to create the window of functionality of the cell product.

Several products followed that included the HLA-E-peptide transgene to protect the cell products from NK cells. Prior *in vitro* testing confirmed protection from allogeneic CD8 T cells and partial protection from allogeneic NK cell killing.[124] The efficacy of HLA-E to protect from NKG2A-expressing NK cells while failing to protect from NKG2A-negative NK cells has been shown repeatedly.[71,125,126] In preclinical AML xenograft models, a single dose resulted in robust tumor control, but the CAR T cells exhibited limited tissue infiltration and expansion in treatment-naive, immunocompromised murine models.[127] No persistence data in humanized mice have been reported yet. A clinical trial with allogeneic primary CLL-1 CAR T cells ($B2M^{-/-}$ HLA-E^{+}) with TCR depletion and PD-1 knockout was recently started for patients with AML, but no data have been reported yet (NCT06128044).[128] The first partially hypoimmune iPSC-derived CD19 CAR NK cell product was depleted of HLA class I and II expression (double knockout [DKO] cells) with expression of HLA-E and was described to rapidly traffic out of the circulation with persistence in the extravascular space.[129] Transgene copies became undetectable in PBMCs 3 days after infusion but were observed in cell-free DNA up to 28 days. The use of lymphodepletion did not affect the persistence of the product, and functional ADCC- or CDC-mediating humoral immunogenicity was not observed within the first month.

Comprehensive immune evasion

The hypoimmune platform (HIP) concept is a specific hypoimmune strategy, comprising knockouts of the *B2M* and *CIITA* genes to achieve HLA class I and II depletion and overexpressing CD47, that has been shown to fully protect engineered cells from all allogeneic adaptive and innate immune cells[72] and is therefore a truly comprehensive immune evasion strategy. CD47 had long been known as a "don't eat me signal" for macrophages, but its inhibitory efficacy on NK cells via the CD47-SIRPα pathway has only recently been uncovered.[83] No NK cell populations could be identified that could circumvent SIRPα-induced inhibition.[93,126] Further support for the efficacy of the SIRPα pathway for the inhibition of NK cells has been provided by demonstrating that CD47 can be replaced with a synthetic cell surface molecule that similarly engages with SIRPα on NK cells.[76] In a recent study, HIP engineering of primary human CD19 CAR T cells without sorting created a heterogeneous pool of T cells with a variety of knockouts and transgene expression levels.[130] Injections of this pool of CAR T cells into allogeneic humanized mice triggered an allorejection response against HLA-replete subpopulations but not HIP CAR T cells. In Nalm6-baring humanized mice, all non-HIP subpopulations vanished over time, and after 95 days, only HIP CAR T cells remained. This means that HIP cells can survive and remain unaffected during an active allorejection response in the same host (Figure 3). Therefore, HIP cells not only avoid immune recognition but also avoid rejection in an ongoing, active immune response against non-HIP cells. This could be confirmed in rhesus monkeys that showed stable engraftment of allogeneic HIP iPSCs, while subsequently injected unedited allogeneic iPSCs were rejected in a fulminant immune response.[126] Since macrophages have been identified as a major inherent persistence barrier even in the absence of an adaptive alloimmune response,[22] the high CD47 expression of HIP CAR T cells is expected to subdue this innate survival obstacle.[130] From an immunological standpoint, allogeneic HIP products seem to not only be on par with autologous products but should outcompete them on both the adaptive and innate sides of the immune system.

HIP CD19 CAR T cells have entered clinical trials for patients with B cell malignancies.[131] Since the engineering is done using primary donor T cells, the product is heterogeneous, containing fully HIP-edited, partially edited HLA I/II negative cells without CD47 overexpression (DKO), and HLA-replete non-HIP-edited populations. Early patient immune data replicate the results from preclinical iPSC transplant studies in non-human primates. There was no host T cell activation or killing, no NK cell killing, and no antibody production against fully HIP-edited CAR T cells. DKO subpopulations were killed by NK cells and HLA-replete cells induced a cellular and humoral response. HIP CAR T cells remained unaffected by this immune response, and the patient showed a deep B cell depletion. The fact that comprehensively immune-evasive cells do not induce immune memory should set them up for repeated redosing without loss of efficacy of subsequent infusions. HIP products do not have to outcompete autologous products in persistence because they are not limited to a "one and done" strategy that has governed autologous therapeutics but might be more successful in a multi-dose regimen. Comprehensively immune-evasive products thus maximize the efficacy of a single dose by avoiding immune rejection and additionally allow for redosing, which could enable more titrated dosing regimens.

Potential risks of immune-evasive cells

Whether persistence of allogeneic HLA-depleted hypoimmune cells carries long-term risks is yet unknown. Studies with knockout mice devoid of MHC class I showed impaired clearance of certain virus strains.[132,133] However, these animals had an associated profound deficiency in the development of mature CD8 T cells, which impaired their overall immune response against infections. Fully immunocompetent patients receiving hypoimmune cell replacement therapies will not have any immune deficiencies, and the overall number of HLA-depleted cells is low. It is yet to be determined whether viruses may remain undetected and persist in hypoimmune cells, as many viruses

persist in other specific cellular reservoirs.[134] A HIP mouse was recently generated that is MHC class I and II deficient and has CD47 overexpressed in all cells and tissues.[135] It was viable, fertile, showed normal life expectancy, and had no abnormal risks for viral infections or tumors. While transplants of its cells or organs did not induce any immune response in allogeneic recipients, the HIP mouse itself had an immune system able to vigorously reject allogeneic cells.

In vivo CAR T cells as an alternative strategy

Instead of *ex vivo* CAR T cell production, *in vivo* strategies are currently being developed where T cell-targeting viral or non-viral vehicles are infused that deliver a CAR into circulating T cells of the patient.[136] Potential advantages of this approach include their scalable manufacturing, reduced costs, and easier logistics for national and international distribution.[137] Also, *in vivo* therapy may generate T cells with various differentiation states and properties that could be therapeutically favorable but difficult to capture with an *ex vivo* manufacturing process.[138] Current challenges of *in vivo* CAR T cell therapy include specificity for targeting T cells and sufficient gene transduction efficiency.[139] Viral vectors have high editing efficiency but pose the risk of off-target transfection of non-T cells.[140] The use of lentiviruses also raises safety concerns due to their potential to trigger immune responses, causing tissue inflammation.[141] Nanocarriers are cheaper to manufacture than viral particles but have limited transfection efficiency,[139] which may improve with advanced nanoparticle designs.[142,143] The immunogenicity of *in vivo* CAR T cells is also concerning.[144] Patient conditioning or lymphodepleting is obsolete as it would weaken or diminish the T cell population that needs to be modified. However, a fully functional immune system might reject either the vector or the CAR it encodes more vigorously, and anti-CAR antibodies may become a problem for redosing. A few companies have already started clinical trials for *in vivo* CAR T cell therapy, and multiple more are close to phase 1, which means half a dozen trials will have readouts by 2026.[144]

ALLOGENEIC CELL REPLACEMENT THERAPIES

Cell replacement therapies for regenerative medicine are still in their infancy and years behind immune cell therapy. There are currently several clinical trials ongoing that inject allogeneic PSC-derived cardiomyocytes[106] into the myocardium of heart failure patients (NCT05068674, NCT04945018, NCT03763136, NCT04982081, and NCT05566600) and two trials using iPSC-derived cardiomyocyte patches (jRCT2053190081 and NCT04396899). All of them immunosuppress the recipients, and hypoimmune cells have not yet been used clinically.[145] Early data of 3 patients that received cardiomyocyte patched showed no transplanted-cell-related adverse events, but all patients showed an immune response with increase in transplant-cell-specific antibody titers.[146] Preclinically, allogeneic HIP cardiomyocytes injected into infarcted mouse hearts were shown to survive and engraft and improve hemodynamic heart failure parameters.[147] Based on these proof-of-concept data, second-generation, hypoimmune products for heart failure are under development. Similarly, there are various ongoing clinical trials using human embryonic-stem-cell-derived dopaminergic neurons (NCT04802733, NCT05635409, NCT05887466, NCT03119636, and NCT02452723) or allogeneic iPSC-derived dopaminergic progenitors (UMIN000033564) for Parkinson's disease.[106] No hypoimmune therapeutics have been used in patients yet. Instead, autologous iPSC-derived precursors are also being tested to avoid immune rejection (NCT06145711 and NCT06344026). Also, the successful manufacturing of 4 iPSC-derived midbrain dopaminergic cells for an upcoming autologous cell therapy trial in Parkinson's disease has recently been reported.[148] A considerable inter-individual variability of cell product characteristics has been observed and will require comprehensive quality control guidelines. The enrichment of neoantigens during *ex vivo* manufacturing is a concern for autologous cell replacement therapies, but successful long-term engraftment and maturation of autologous iPSC-derived products have been shown preclinically and suggest that autologous products would similarly engraft in humans.[149] A multitude of additional cell replacement trials for other neurological diseases are underway.[150,151] Although the blood-brain barrier provides the brain with some immune privilege,[152] immune rejection of allogeneic grafts has been reported.[153,154] Thus, most trials of allogeneic cell replacement therapy products in the central nervous system use some level of immunosuppression.[150] Cell replacement therapies with pancreatic islet cells for patients with diabetes mellitus are probably most advanced preclinically with early clinical data, including both encapsulation strategies and hypoimmune editing. This landscape will be described in more detail below.

Pancreatic islet cells

Pioneering work with autologous iPSC-derived islets has very recently been published. Autologous iPSCs were generated from one patient with 25-year history of type 2 diabetes mellitus who already had a kidney transplant for diabetic nephropathy. Autologous iPSC-derived islets were administered via transhepatic portal vein transplantation, and the authors reported positive clinical outcomes, a decrease in HbA1c, increase in c-peptide, and gradual reduction in insulin requirement until the patient was insulin independent.[155] However, credible concerns remain regarding the classification of the patient's disease, ethics surrounding this experimental procedure, the true characterization of the transplanted cells, and their contribution to glycemic control.[156] In another patient with 11-year history of type 1 diabetes mellitus and 2 liver transplants, autologous iPSC-derived islets were injected underneath the abdominal anterior rectus muscle sheath.[157] The patient did not experience any episodes of hypoglycemia after the transplant and achieved insulin independence. Unfortunately, since both patients were solid organ transplant recipients, they remained on immunosuppression despite the autologous nature of their islet grafts. This completely masks the immunological response to the autologous tissue, thus missing an opportunity to answer an important question in the field.[156] Allogeneic cell replacement therapeutics in regenerative medicine also possess a multitude of advantages over autologous approaches. However, cell replacement therapy also competes with medical management or allogeneic organ transplantation, which can either be pancreas or islet transplantation. The appeal of immune-evasive allogeneic cell replacement therapy is the restoration of glycemic control with disease mitigation superior to medical management without the need for immunosuppression. Pancreatic islet cells have negligible proliferation

potential, very long lifespans, and the success of restoring endocrine function depends on their long-term survival. For cell replacement therapy, complete immune evasion that provides full protection is critically required because even a slowed rejection process eventually leads to graft failure and recurrence of the disease. Also, diabetic patients are not immunocompromised by their disease or medication, and neither toxic preconditioning nor long-term immunosuppression seems acceptable. The bar for immune evasion in immunocompetent patients with benign diseases is undoubtedly high.

Unprotected allogeneic islet cell therapeutics

Early clinical trials testing the feasibility of using pluripotent-derived pancreatic islet cells to alleviate diabetes are ongoing. Twelve type 1 diabetes mellitus patients with impaired hypoglycemic awareness and severe hypoglycemic events (SHEs) received an infusion of unprotected allogeneic embryonic-stem-cell-derived islet cells into the hepatic portal vein. All patients required chronic immunosuppressive therapy to protect the islet cells from immune rejection. An interim analysis reported that 11 of 12 patients had a reduction or elimination of exogenous insulin use at their last visit, and all patients had elimination of SHEs from day 90 onward.[158] This study demonstrated that stem-cell-derived cell therapy can contribute to glycemic control in patients with type 1 diabetes mellitus.

Encapsulated allogeneic islet cell therapeutics

Encapsulation devices have been proposed to shield cells mechanically from the host immune system as an alternative to gene editing. Microencapsulation had first been tested with human donor islets. Alginate microencapsulated islets implanted intraperitoneally in non-immunosuppressed patients with type 1 diabetes mellitus (T1D) demonstrated c-peptide secretion for 1–2.5 years, but the amount of insulin released was too small to alter the blood glucose levels in a meaningful way.[159,160] Macroencapsulated islets within the bioartificial pancreas βAir, a cell chamber connected to a refillable oxygen tank, survived for 3–6 months, but insulin release was too low to improve glucose control.[161] The first evidence of meal-regulated insulin secretion by stem-cell-derived pancreatic endoderm cells implanted in a non-immunoprotective macroencapsulation device has recently been reported.[162] Patients in this study were still required to be on an immunosuppressive regimen. The downside of all encapsulation concepts is their restriction for proper oxygenation, and beta cells are uniquely sensitive to hypoxia.[163] Despite comprising only 1%–2% of the total pancreatic mass, islets physiologically receive 10%–20% of the pancreatic blood flow.[164] They have a dense vascular network with directed blood flow from the core of the islet outward[165] and connecting every beta cell with a capillary.[166] Improved encapsulation technologies are currently being developed.

Immune-evasive islet cell therapeutics

Partial immune evasion of pancreatic islets by depleting HLA class I[93] or class I and II[126] without protection from innate immune cells has led to dismal results in preclinical models. Conflicting results have been reported for the efficacy of using transgenic PD-L1 overexpression to protect beta cells from allorejection. While PD-L1-overexpressing HLA-replete human ESC-derived islets were rejected within 10 days in B6 recipients,[93] PD-L1 overexpressing iPSC-derived islets engrafted and ameliorated diabetes in both B6 and humanized mice.[167] The secretion of IL-2 mutein, TGF-β, and IL-10 as vehicles for localized immunosuppression has been described to enable the survival of human ESC-derived islets in diabetic NOD mice and reverse diabetes.[93] Human iPSC-derived pancreatic progenitor cells depleted of HLA class I and the potentially NK-cell-activating ligands B7-H3 and CD155 showed prolonged survival in NSG mice supplemented with human NK cells.[75] Human islets derived from HIP iPSCs were transplanted into immunocompetent, allogeneic, diabetic humanized mice and showed resistance against allorejection. Survival, engraftment, and alleviation of diabetes could be achieved, and no immune response against HIP islets was observed.[126] To study autoimmune killing, islets were generated from iPSCs of a T1D patient and transplanted into autologous humanized mice reconstituted with immune cells from the same T1D patient.[168] In this autoimmune model, autologous iPSC-derived islets got rejected. HIP engineering, however, made the islets resistant against autoimmunity and showed that HIP islets are protected from allorejection and autoimmunity. Gene editing of primary human islets has been challenging, but a stepwise protocol including multiple rounds of islet dispersion, engineering, and re-clustering has recently been developed.[168] HIP-edited primary human donor islets were endocrinologically more competent than iPSC derivatives and were able to survive, engraft, and restore blood glucose control in fully allogeneic, diabetic, immunocompetent humanized mice.[168] Again, no adaptive, innate, or humoral response against the HIP cells was observed. In preparation for clinical trials, primary rhesus monkey islets were HIP-edited and transplanted into an allogeneic, diabetic cynomolgus monkey.[169] The HIP islets were able to control blood glucose levels and restore c-peptide without any supportive medication for over half a year. No immune response against the HIP islets could be detected during the close follow-up. In addition, the elevated CD47 levels of HIP islets could overcome the susceptibility of islets to macrophage phagocytosis in inflammatory environments[170] or myeloid cell clearance as during instant blood-mediated inflammatory reaction (IBMIR) when transplanted intraportally.[171] Most recently, Sana Biotechnology and Uppsala University Hospital announced via press release the successful transplantation of HIP-engineered allogeneic primary islet cells into a patient with T1D without the use of any immunosuppression.[172] Results of the study at 4 weeks after cell transplantation demonstrate the survival and function of pancreatic beta cells as measured by the presence of circulating c-peptide. C-peptide levels also increased with a mixed meal tolerance test, consistent with insulin secretion in response to a meal. MRI scanning also demonstrated a sustained signal at the site of transplanted cells over time, which is consistent with graft survival. The study identified no safety issues, and the HIP-modified islet cells fully evaded immune responses for 1 month, the most critical period of allograft rejection. More follow-up data will be presented in the future about long-term survival, engraftment, and endocrinological function.

CONCLUSIONS

The development of allogeneic cell therapy products is incredibly complex given the shear infinite number of variables that

could affect the efficacy of the engineered cells. Allorejection is a major hurdle to their success, and much research has been devoted to overcoming the immune barrier and making truly immune-evasive universal cells. Given the recent progress in this arena, there is justified optimism that allogeneic products are on their way to clinical success.

DECLARATION OF INTERESTS

T.D. is a consultant to Sana Biotechnology and owns stock. T.D. is a consultant to Shinobi Therapeutics and owns stock. S.S. is an employee of Sana Biotechnology and owns stock. T.D. and S.S. have patent applications covering immune evasion topics.

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