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Chimeric HLA antibody receptor T cell therapy for humoral transplant rejection

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ABSTRACT

Antibody-mediated rejection (ABMR) is a significant obstacle to achieving optimal long-term outcomes after solid organ transplantation. The presence of donor-specific antibodies (DSAs), particularly against human leucocyte antigen (HLA), increases the risk of allograft rejection and subsequent graft loss. No effective treatment for ABMR currently exists, warranting novel approaches to target the HLA-specific humoral alloimmune response. Cellular therapies may hold promise to this end. According to publicly available sources as of now, three independent laboratories have genetically engineered a chimeric HLA antibody receptor (CHAR) and transduced it into human T cells, based on the demonstrated efficacy of chimeric antigen receptor T cell therapies in malignancies. These CHAR-T cells are designed to exclusively eliminate B cells that produce donor-specific HLA antibodies, which form the cornerstone of ABMR. CHAR technology generates potent and functional human cytotoxic T cells to target alloreactive HLA-specific B cells, sparing B cells with other specificities. Thus CHAR technology may be used as a selective desensitization protocol and to treat ABMR after solid organ transplantation.

Keywords: antibody-mediated rejection, chimeric antigen receptor (CAR) therapy, donor-specific antibodies, HLA-specific B cells, long-lived plasma cells

INTRODUCTION

Kidney transplantation is the treatment of choice for patients with end-stage renal disease, as it has been shown to reduce morbidity and mortality and prolong patient survival compared with other renal replacement therapies [1]. However, antibody-mediated rejection (ABMR), both acute and chronic, is considered the main barrier limiting long-term outcomes [2, 3]. ABMR is characterized by the generation of donor-specific antibodies (DSAs), primarily against human leucocyte antigens (HLAs), deteriorating kidney function due to histological damage (Fig. 1). The presence of class I and II anti-HLA antibodies has been associated with graft loss [4].

CURRENT TREATMENT STRATEGIES FOR ANTIBODY-MEDIATED REJECTION IN SOLID ORGAN TRANSPLANTATION

The first approach to treat ABMR is the temporary elimination of DSAs through a combination of plasma exchange (PLEX) and intravenous immunoglobulin (IVIG) [5]. However, there is no strong evidence to support their use and there is no consensus on the frequency, mode and dose to be used [6, 7]. In contrast, the depletion of B cells using monoclonal antibodies against CD20 is widely practiced in the transplant community, despite limited evidence and few clinical trials investigating its utility and without demonstrating a clear benefit [7, 8]. Furthermore, mature plasma cells, which are the main cells responsible for producing antibodies, cannot be depleted by these monoclonal antibodies because they lack expression of CD20. Other approaches include the use of proteasome inhibitors (bortezomib, which exerts its effects mainly on plasma cells) [9] or complement inhibitors [10], whose efficacy is not clearly established. Novel agents targeting B cells have been tested, including monoclonal antibody against B cell activating factor (belimumab), targeting the interleukin-6 (IL-6)/IL-6 receptor (IL-6R) axis (clazakizumab), anti-CD38 [11] and CXCR4 antagonists.

Unfortunately, all these strategies lack specificity, as they focus on reducing all antibodies and B cells indiscriminately, regardless of their antigen specificity or function. Patients receiving B cell lymphocyte depletion agents have been shown to be particularly vulnerable to viral and bacterial infections and de novo malignant tumours [12]. In addition, it is known that B cells can have both effector and regulatory functions (Bregs). The depletion of Bregs may lead to acute cellular rejection, as these cells also have a regulatory function that can contribute to transplant tolerance [13, 14]. Therefore, the development of more effective and specific therapies could offer promising avenues for improving outcomes

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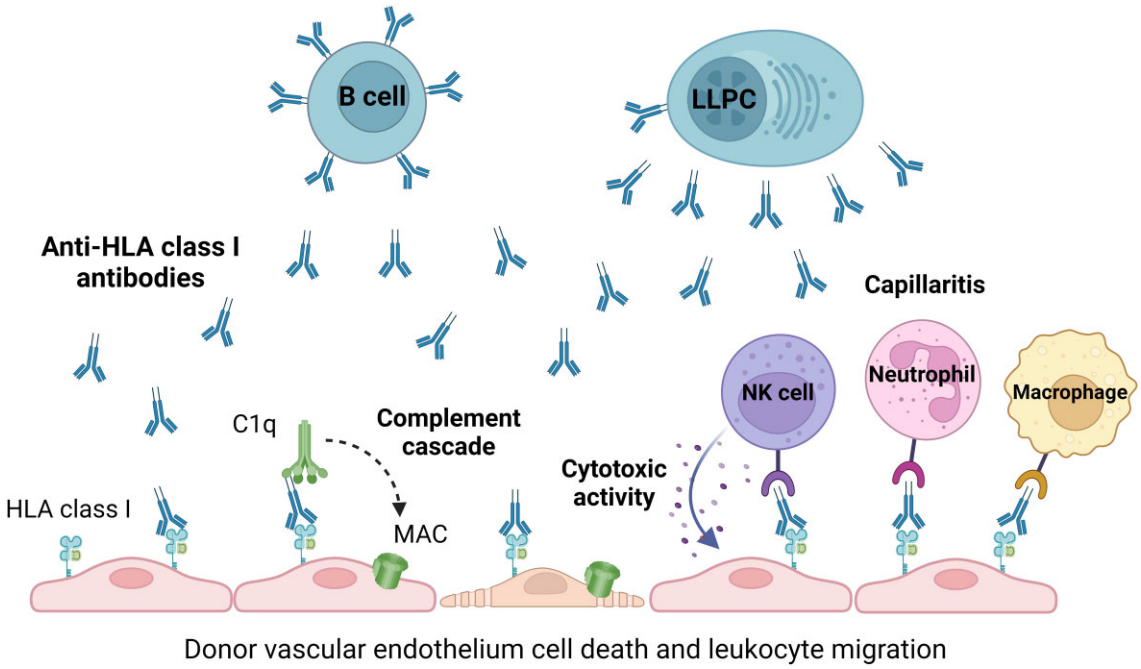

Figure 1: Mechanisms of ABMR. HLA-specific Bmems and LLPCs generate anti-HLA antibodies that bind to the HLA molecules on the kidney graft endothelium. The activation of the classical complement pathway occurs when the C1q binds to the Fc domain of the antibody in immune complexes. This leads to the establishment of the MAC on endothelial cells. In contrast, cells that possess Fc receptors, such as NK cells, neutrophils and macrophages, may be recruited and cause damage to the endothelium. Created with BioRender.

in ABMR management as well as increasing the chance of transplantation for highly sensitized patients through more potent and specific desensitization.

CHIMERIC ANTIGEN RECEPTORS (CARS) IN TRANSPLANTATION

CARs targeting CD19 and B cell maturation antigen (BCMA) have been shown to be effective therapies for B cell malignancies and multiple myeloma, respectively. Some groups are exploring the potential of CARs to eliminate memory B cells (Bmems) and long-lived plasma cells (LLPCs) in transplantation, including desensitization before transplantation and treatment of ABMR. The impact of CD19-CAR T cell therapy (CTL019 or CD19-CARTx) on pre-existing humoral immunity in patients with follicular lymphoma or refractory malignant B cell tumours was analysed. Two studies showed that anti-HLA antibodies are not reduced after CD19-CAR T cell therapy since it is ineffective against LLPCs ($CD19^-$), which continue to produce anti-HLA class I and class II antibodies [15, 16]. Furthermore, analysis of serum samples from patients in one clinical trial (NCT02030834) revealed the presence of anti-HLA antibodies in one patient prior to CTL019 treatment. These HLA antibodies remained elevated after CTL019 treatment, despite the absence of B cells [17]. These findings align with observations in animal models, where B cell depletion does not impact LLPCs that preferentially localize in bone marrow niches [18], suggesting relative efficacy in addressing pathogenic humoral immunity and, consequently, managing ABMR.

CAR therapies based on BCMA have been developed to specifically eliminate mature B lymphocytes and LLPCs [19]. BCMA-CAR therapy has been evaluated for the treatment of multiple myeloma (NCT02215967) [20] and relapsed/refractory neuromyelitis optica spectrum disorder (NCT04561557) [21], reporting a reduction in plasma cells within 2 months of treatment [20], and a decrease in specific antibodies alongside improvements in patient conditions and subsequent amelioration of other autoimmune comorbidities [21], respectively. A phase I clinical trial (NCT03549442), although characterized by a small cohort, showed that in the two subjects treated with CAR-BCMA monotherapy, there was no sustained reduction in alloantibodies.

Another potential target for therapeutic intervention is the BAFF receptor, primarily expressed on mature B cells and implicated in the promotion of autoreactive B cell responses [22].

In a recently published study, combined CART-19 and CART-BCMA therapy was used as an effective desensitization treatment in an experimental murine transplant model and in multiple myeloma patients with pre-existing anti-HLA antibodies [23]. The combination CART therapy will be evaluated in a recently planned clinical trial for the desensitization of highly sensitized patients on the kidney transplant waiting list (NCT06056102). However, the complete depletion of Bmems and LLPCs in kidney transplant recipients, which are already treated with immunosuppressive regimens, could compromise outcome due to infections, cardiovascular events or malignancies. For that reason, a more selective strategy should be addressed.

FROM CARS TO CHIMERIC AUTOANTIBODY RECEPTORS (CAARS)

The success of CAR T cell therapy in oncology sparked interest in its application for autoimmune diseases, leading to CAAR T cells. These cells have a modified CAR structure, with the extracellular domain targeting autoimmune disease antigens to selectively eliminate autoantibody-producing B cells. Initial

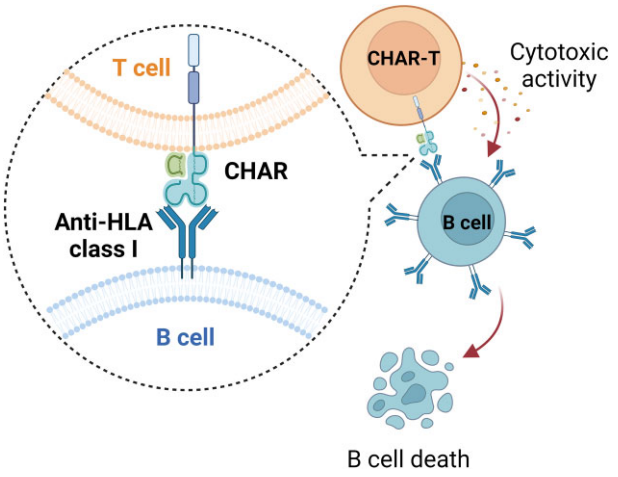

Figure 2: Mechanism of cytotoxic activation of CHAR T cells. The extracellular region of the CHAR construct contains the α1, α2 and α3 domains of a specific HLA class I molecule. Epitopes on this HLA class I molecule can be recognized by B cells, carrying BCRs specific for this epitope. Binding of the BCR activates CHAR T cells, which release cytokines and cytotoxic granules such as interferon-γ, granzyme B and perforin, specifically resulting in target cell death. Created with BioRender.

trials in a pemphigus vulgaris model demonstrated the ability of CAAR T cells to specifically target disease-causing B cells expressing desmoglein-3 [24, 25]. Currently, a phase 1 study is under way to determine the optimal dosing and infusion regimen for Dsg3-CAART (NCT04422912). Research extends to other autoimmune conditions like multiple sclerosis, myasthenia gravis and N-methyl-D-aspartate receptor (NMDAR) encephalitis, with promising developments in MBP-CAAR T cells [26], MuSK-CAART [27] and NMDAR-CAAR [28]. These therapies show potential in preclinical and phase 1 trials for treating autoimmune disorders.

TARGETED CELL THERAPY SPECIFICALLY AGAINST ABMR IN SOLID ORGAN TRANSPLANTATION

In the field of transplantation, three laboratories independently recently engineered chimeric receptors to recognize HLA-specific B cells and generate a cytotoxic response to eliminate them, following the CAAR-Tc concept (Fig. 2). The constructs were named chimeric HLA antibody receptor T cells (CHAR-Tc) [29, 30] or T cells overcoming rejection by antibodies (CORA-Ts) [31] (Fig. 3). For nomenclature consistency, the three constructs will now be called CHAR. The CHAR construct is characterized by presenting the sequence of the HLA molecule of interest in its extracellular domain. To date, works have been published expressing the class I HLA molecule, specifically HLA-A*02: 01 (A2-CHAR) [29–31] or HLA-A*03: 01 (A3-CHAR) [30], including the extracellular region composed by the α1, α2 and α3 domains. This structure is completed with the β2-microglobulin (B2M) molecule, provided by the T cell endogenously, as is the case with the sequence of the class I HLA molecule. Gille *et al.* [30] confirmed this feature by transducing the same HLA-A*02 CHAR T cell construct into B2M knock-out cells, observing the absence of CHAR expression.

Following the extracellular region there is a transmembrane domain, which is responsible for anchoring the CHAR to the T cell membrane. Most of these domains derive from natural proteins, such as CD8 [29] and CD28 [30], which have been used in CAR therapy. Several studies in CAR T cells for the treatment of oncological diseases suggest that the CAR transmembrane domain influences its expression level and stability, with CD8 and CD28 showing superiority compared with CD3ζ [32, 33].

The intracellular signalling domains are composed by one major signalling domain derived from CD3ζ and by one co-stimulatory domain. The co-stimulation domain is perhaps the most important structure of CAR therapy, as it regulates metabolism and influences T cell survival and effector function. Two domains have been used thus far in CHAR T cells, 4-1BB [29, 31] and CD28 [30], both domains are also used in US Food and Drug Administration–approved CAR therapies. These CAR T cells show a high response rate in oncohaematological pathologies; however, they present some differences. The CD28 domain promotes the growth of memory effector T cells with a gene signature consistent with enhanced glycolysis [34]. Additionally, a persistence of up to 3 months after activation is observed [35]. On the other hand, the 4-1BB domain promotes the differentiation of $CD8^+$ central memory cells by increasing mitochondrial biogenesis and oxidative metabolism. Furthermore, it has been described that these cells can persist for several years after infusion [34, 36, 37]. Thus the question arises as to which strategy would be ideal for desensitization or the treatment of ABMR in transplantation. In our opinion, the CD28 domain should be used for desensitization protocols because we required the alloimmune reset before the transplantation, whereas the 4-1BB domain should be more useful for ABMR therapy since the alloimmune stimulus persists in the patient and long-term control of memory B cells may be required. Moreover, in certain types of cancer, T cells with third-generation CAR molecules, which combine multiple costimulatory signalling domains, have been employed with favourable safety profiles and enhanced persistence and proliferation. However, no augmented efficacy has been observed compared with T cells with a second-generation CAR (with one costimulatory signalling domain, like CHAR constructs). A recent study on CD19 CAR T cells for the treatment of systemic lupus erythematosus demonstrated a profound depletion of B cells [38]. Nevertheless, the vaccination antibodies indicated that the humoral immune system is not fully reset, as the LLPCs are not affected by CD19 CAR T cells.

CHAR T CELL THERAPY SPECIFICITY

According to the HLA antigen specificity, each CHAR confers the capacity to drive a cytotoxic response against HLA-specific B cells with corresponding specificity. This concept is reflected in studies by Betriu *et al.* [29], where T cells transduced with HLA-A2-CHAR were exposed to B cell hybridomas expressing an HLA-A2-specific BCR. While complete elimination of target cells at an effector:target ratio of 1:2 was observed, B cell hybridomas that did not express HLA-A2-specific BCR were not targeted, as corroborated in both *in vitro* and *in vivo* experiments. Similarly, Gille *et al.* [30] demonstrate cytotoxic activity of CHAR T cells towards cells expressing specific BCRs, and further confirmed this by analysing hybridoma IgG production through ELISpot assays. Importantly, specificity was corroborated by using both HLA-A2 and HLA-A3 CHARs and hybridoma target cells specific for either HLA-A2 or HLA-A3 [30]. Dragon *et al.* [31] used mouse hybridoma cells specific for HLA-A2 or HLA-B35 and again showed high specificity of lysis by flow cytometry and lactate dehydrogenase release. Additionally, an increase in cytokine release was observed in all three studies when CHAR T cells were exposed to the appropriate target cells [29–31].

Undoubtedly, the specificity of this therapy could make a difference with current CAR T cell therapies by preserving B lymphocytes that do not produce antibodies against donor HLA,

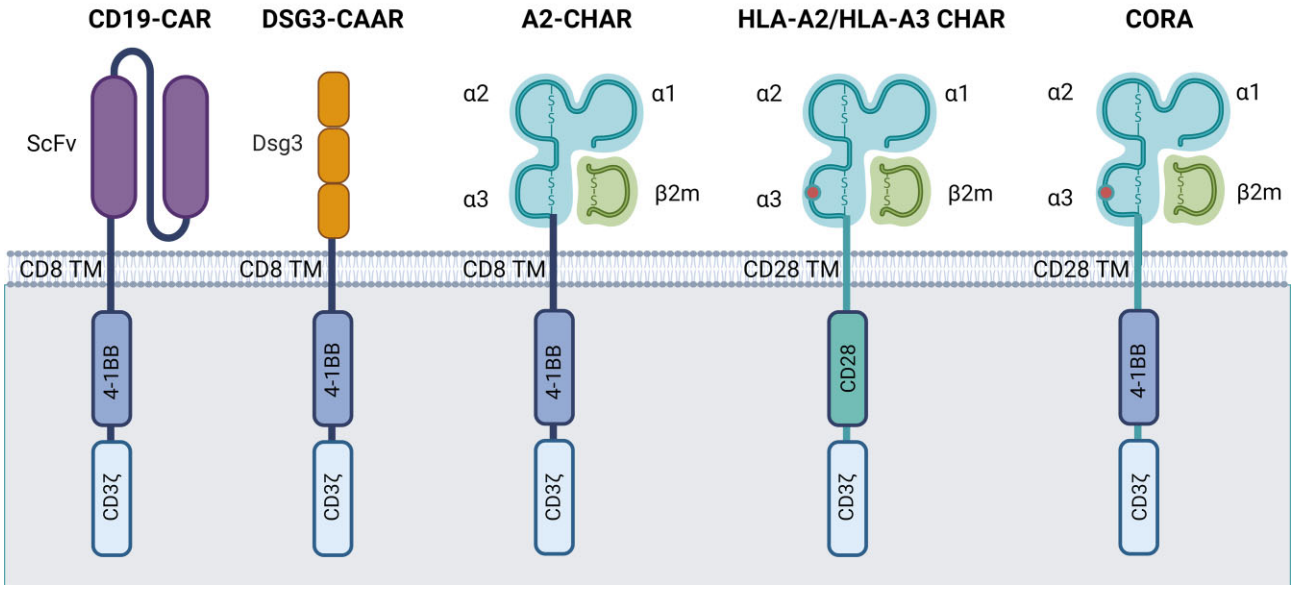

Figure 3: Schematic structure of the different chimeric receptors: chimeric antigen CD19 receptor (CD19-CAR), chimeric autoantibody desmoglein 3 receptor (DSG3-CAAR), chimeric HLA antibody receptor (CHAR) and T cells overcoming rejection by antibodies (CORA-Ts). All chimeric receptors share the transmembrane and intracellular structure; CD8 hinge and transmembrane (or CD28), a co-stimulatory molecule (4-1BB or CD28) and a signalling domain (CD3ζ). They differ in the extracellular domains, as CD19-CAR has the single-chain variable fragment (ScFv) originating from a monoclonal antibody to recognize CD19 molecules. In DSG3-CAAR, desmoglein 3 (Dsg3) is expressed instead of the ScFv domain. CHAR and CORA constructs possess the specific domains of the HLA-A*02: 01 allele or HLA-A*03: 01, that will form a functional complex with the endogenous β2M. Red dot in the α3 domain represents a mutation (D227K/T228A) in the HLA-A2 molecule that abolishes CD8 binding and reduces T cell alloresponse against the CHAR T cell therapy. Created with BioRender.

but against viral pathogens, allowing for the development of a therapy with a significant reduction in side effects and paving the way towards personalized therapy.

STIMULATION AND EXPANSION OF PRIMARY HUMAN T CELLS

A crucial aspect in CAR therapy is the selection of the initial cell population for CAR T therapy manufacturing, as this significantly impacts both the production process and the efficacy of the final product [39]. CAR T therapy is recognized for its ability to induce cytotoxicity through two distinct pathways: direct killing, achieved via antigen–antibody recognition, and indirect killing, facilitated through the release of cytokines or activation of other cells [40, 41]. In several comparative studies it was observed that CAR T cells transduced in CD8$^+$ cells exhibit a mechanism of direct contact, unlike those transduced in CD4$^+$ cells, which more frequently employ the indirect pathway [40–42]. It has been described that CAR T therapy in CD8$^+$ cells show superiority in potency compared with CD4$^+$ cells, as a higher percentage of tumour lysis has been observed with the former [42]. However, it has been demonstrated that the persistence of CAR-T therapy depends on the number of CD4$^+$ cells and central memory cells (CD45RO$^+$ CD62L$^+$) in the infused product [43]. The combination of both subgroups (CD8$^+$ and CD4$^+$) enhances the adoptive transfer of T cells and supports the development of memory functions in CD8$^+$ cells, making this combination potentially beneficial in therapy [44, 45]. As discussed above, it remains to be seen whether long-term persistence of CHAR T cells is desirable in a non-malignant setting. In addition, the production of vast quantities of inflammatory cytokines upon target recognition by transduced CD4$^+$ T cells could potentially result in off-target effects.

In the available studies on CHAR therapy, Betriu *et al.* [29] described the transduction of CD3$^+$ lymphocytes with a variable proportion of CD4$^+$ and CD8$^+$ cells depending on the patient or cell donor, like in oncohaematology CAR therapy. However, Gille *et al.* [30] and Dragon *et al.* [31] transduced exclusively CD8$^+$ cells, in order to infuse only cytotoxic cells and prevent potential off-target effects.

Given that all previously analysed studies have been conducted in an oncological context, it would be interesting to evaluate the effect of different therapies in an animal model reflecting the immunosuppressive environment and determine which strategy is most beneficial. Establishing the most suitable cellular phenotype for transduction or adjusting the percentage of different populations (CD4$^+$ and CD8$^+$) should also be considered. Additionally, the patient's T cell profile should be taken into account, as it differs significantly from that of a person without prolonged immunosuppression.

POTENTIAL HURDLES FOR CHAR T CELL THERAPY

In the following section we describe potential interferences that may reduce the effectiveness of CHAR T cell therapy.

Soluble donor-specific HLA antibodies

CHAR T cells can be readily activated by circulating HLA antibodies. Gille *et al.* [30] identified intracellular activation markers in T lymphocytes transduced with A2-CHAR and exposed to microspheres coated with specific αHLA-A2 antibodies; however, no such signals were observed when exposed to non-specific αHLA-A3 antibodies. Furthermore, cytokine release is observed,

confirming the activation of CHAR T cells and their cytotoxic capacity even without direct contact between those cells and target HLA-specific B lymphocytes. This unintended effect of the therapy should be avoided [29, 30]. Similarly, Ellebrecht *et al.* [24] investigated how the presence of anti-Dsg3 antibodies in serum affects the ability of Dsg3-CAAR T cells to eliminate target cells. It was found that soluble anti-Dsg3 antibodies decrease the cytotoxicity of CAAR T cells against some target cells but increase cytotoxicity against others. Moreover, it is mentioned that the presence of soluble anti-Dsg3 antibodies may enhance the efficacy and persistence of CAAR T cells due to co-stimulatory signals that improve their activity, although this interaction is complex and depends on several factors. However, in an *in vivo* scenario, exposure to specific antibodies could potentially increase cytokine secretion and trigger cytokine release syndrome (CRS) [46].

In addition to causing ill-directed CHAR activation, circulating HLA-specific antibodies could also result in complement-dependent cytotoxicity and antibody-dependent cytotoxicity of CHAR T cells. Since HLA-specific antibodies are often of the IgG1 and IgG3 subclasses, it is to be expected that potent antibody-mediated effector mechanisms will affect CHAR T cell efficacy. To avoid both CRS and lysis of CHAR T cells through antibody-mediated mechanisms, plasma exchange prior to therapy administration has been proposed as a possible solution.

Allo-HLA-reactive T cell reactivity against CHAR T cells

One challenge that the CHAR T cell therapy may encounter post-infusion is recognition by cytotoxic T lymphocytes capable of targeting the extracellular domain of the HLA molecule and killing the infused cells. To address the likelihood of this scenario, Gille *et al.* [30] investigated the effect of allo-HLA-reactive T cells by transducing Jurkat triple parameter reporter (TPR) cells with an HLA-A2 alloreactive T cell receptor (TCR) specific for the endogenous peptide USP11 presented in HLA-A*02: 01. Results demonstrated that the Jurkat TPR cells transduced with the HLA-A2 alloreactive USP11 TCR become activated after ligation with the HLA-A2-CHAR T cells. In contrast, CHAR T cells did not become activated upon ligation with cells expressing the allo-HLA-reactive TCR. Since previous studies demonstrated that the mutation (D227K/T228A) in the extracellular α3 domain of the HLA-A2 molecule abolished CD8 binding and reduced T cell activation [47], CHAR constructs were genetically modified with this mutation [30, 31]. Subsequent co-incubation of the allo-HLA-reactive T cell clone with cells expressing the mutated CHAR showed a reduction, but not full abolishment, in allo-HLA reactivity [30]. Although CHAR T cells carrying the same mutation fully abolished the response of $CD8^+$ T cells from HLA-$A2^+$ individuals, this autologous system may lack sensitivity [31].

Immunosuppression

CHAR T cell therapy, besides being utilized as a desensitization strategy, aims to serve as a treatment for ABMR, acting within an environment of immunosuppressive drugs that diminishes lymphocyte proliferation. Studies have been conducted to assess the efficacy of CHAR therapy in the presence of immunosuppressants. No impact on cytotoxicity was observed when exposing CHAR T cells to monotherapy [tacrolimus, mycophenolate, prednisone, mammalian target of rapamycin (mTOR) inhibitors]; however, a reduction in cytotoxicity was noted with exposure to triple therapy (tacrolimus, prednisone, mycophenolate or mTOR inhibitor). Additionally, CHAR therapy in the presence of immunosuppressants showed reduced release of interferon-γ, IL-2, tumour necrosis factor-α and IL-10, with the exception of granzyme B [29]. These findings suggest a reduction in therapy efficacy within the context of post-kidney transplantation immunosuppressive drugs.

Several strategies have been postulated and tested to avoid the malfunction in transferred T cells caused by ongoing immunosuppressive therapies. Amini *et al.* [48] demonstrated that tacrolimus resistance can be conferred to adoptive antiviral T cells in transplant recipients by knocking out the gene encoding the adaptor protein FK506-binding protein 12 (FKBP12) using CRISPR/Cas9 gene editing. FKBP12 is required for the immunosuppressive function of tacrolimus. Similarly, Dragon *et al.* [31] used this technique to protect their CHAR T cells against tacrolimus treatment. Another strategy could involve utilizing a different cellular population, such as natural killer (NK) cells, which may be less affected by immunosuppressives and are already used in CAR-NK approaches [49, 50].

Lack of efficacy in LLPCS

Betriu *et al.* [29] showed that A2-CHAR-Tc can eliminate B cells that produce anti-HLA-A*02 antibodies, regardless of whether they are of the IgG or IgM class, as long as these Bmem cells express IgG or IgM on their cell surface. It is well established that LLPCs are no longer dependent upon antigen binding for antibody production. However, while it is known that BCR expression is reduced in IgG-producing LLPCs, it has been described that IgM-producing LLPCs express a functional membrane BCR associated with the Igα/Igβ heterodimer [51, 52]. This might suggest that CHAR T cell therapy can effectively eliminate IgM-producing LLPCs, while IgG-producing LLPCs may be more resistant. Accordingly, for desensitization protocols or ABMR treatments, it seems reasonable to propose that the CHAR T cells could be used as a selective therapy to deplete alloreactive B cells long term without affecting other B cell responses when combined with a plasma cell depletion therapy such as BCMA-CAR or anti-CD38.

NEXT STEPS AND FUTURE STRATEGIES

Currently, CHAR T cell therapy has been generated to target HLA class I–specific B lymphocytes. However, it is important to note that the presence of *de novo* anti-HLA class II antibodies is associated with a higher incidence of rejection and worse outcomes in solid organ transplantation [53]. Therefore, it is necessary to address the development of CHAR T cell technology using HLA class II molecules. Supposedly with a limited number of HLA entities a large range of sensitized patients could be covered for the purpose of increasing the chances of a transplant by HLA-specific desensitization. However, the treatment of ABMR may require a greater number of CHAR constructs to enable personalized treatment based on the DSAs presented by the recipient. In addition, it should be noted that CHAR T cell therapy alone may not effectively eliminate IgG-LLPCs, therefore the development of a selective therapy for donor-specific antibody-producing LLPCs should be addressed.

A therapy with the potential to revolutionize the treatment of ABMR has been described and could serve as a valuable strategy to desensitize patients with high immunologic risk and limited chances of finding a compatible graft. However, several questions remain unanswered, one of which is the potential immunogenicity that the therapy could cause by exposing the antigen to which the patient is sensitized. It is essential to keep in mind that patients are constantly exposed to graft cells expressing

HLA on their surfaces. Furthermore, it is documented that during rejection episodes, the release of soluble HLA molecules [54], as well as exosomes [55] (with HLA molecules on the membrane) is observed. Given this existing exposure, it is plausible that CHAR T cell therapy does not induce additional changes. These considerations could not necessarily be made in the case of a desensitization. Nevertheless, further *in vitro* and *in vivo* studies are necessary to elucidate these questions and to ensure the safe implementation of this therapy in clinical practice.

A multidisciplinary approach including immunology, haematology, nephrology and advanced therapies is required for this project. The goal is to develop multicentre studies at both the national and international levels to create an effective therapy with fewer side effects, ultimately improving the quality of life of patients.

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AUTHORS' CONTRIBUTIONS

J.R. and F.D. contributed substantially to the conception and design of the work. C.A., A.G.-B., M.N. and S.B. drafted the initial version of the manuscript. All authors revised the content, offered critical insights and endorsed the finalized version prior to submission.

DATA AVAILABILITY STATEMENT

All the data are available in the article.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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