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Disrupting B and T cell Collaboration in Autoimmune Disease: T cell engagers versus CAR T cell therapy?

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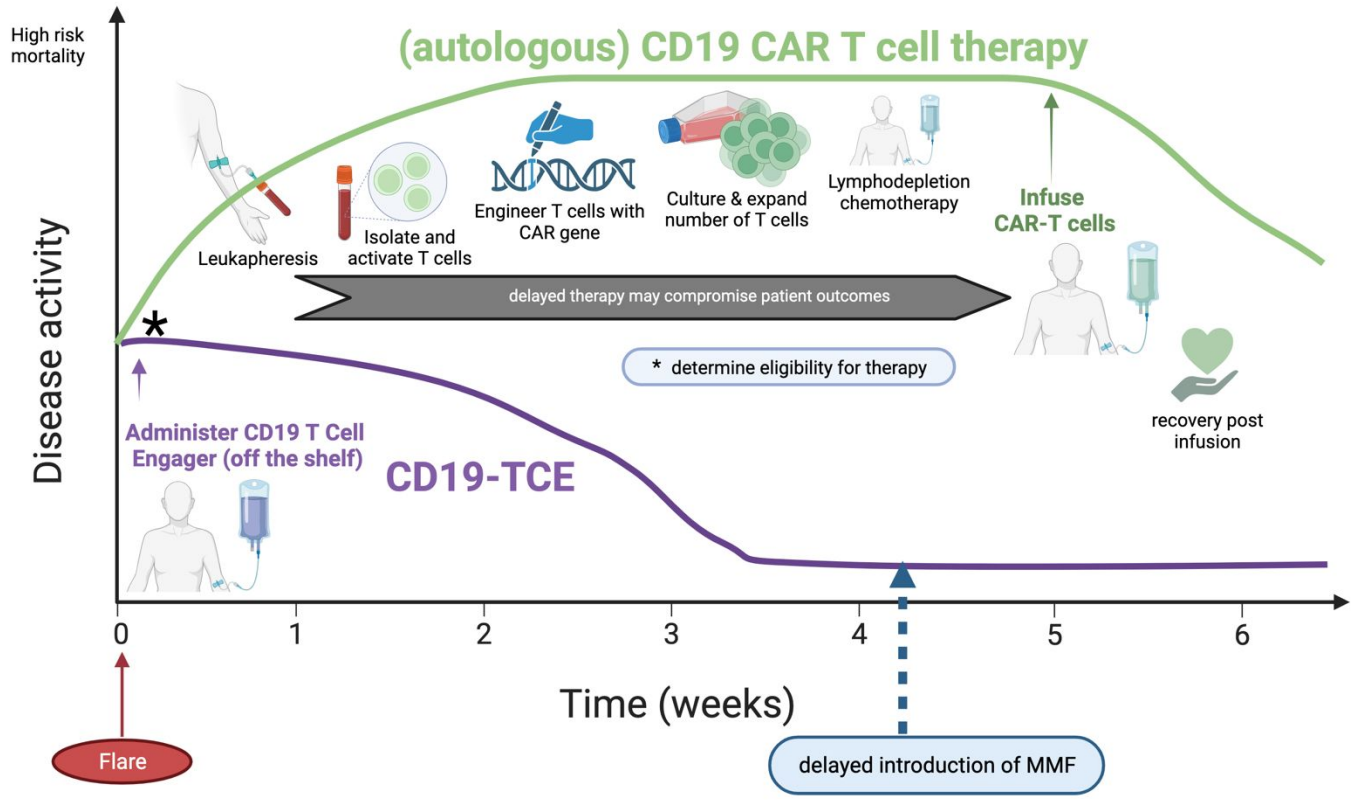
Key words
Systemic lupus erythematosus, rheumatoid arthritis, rituximab, CAR T-cell therapy, T cell engagers

Abstract

B and T cells collaborate to drive autoimmune disease (AID). Historically, B and T cell (B-T cell) co-interaction was targeted through different pathways such as alemtuzumab, abatacept, and dapirolizumab with variable impact on B cell depletion (BCD), whereas the majority of patients with AID including rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and organ transplantation benefit from targeted BCD with anti-CD20 monoclonal antibodies such as rituximab, ocrelizumab or ofatumumab. Refractory AID is a significant problem for patients with incomplete BCD with a greater frequency of IgD^{-} $CD27^{+}$ switched memory B cells, $CD19^{+}CD20^{-}$ B cells and plasma cells that are not directly targeted by anti-CD20 antibodies, whereas most lymphoid tissue plasma cells express CD19. Furthermore, B-T cell collaboration is predominant in lymphoid tissues and at sites of inflammation such as the joint and kidney, where BCD may be inefficient, due to limited access to key effector cells. In the treatment of cancer, chimeric antigen receptor (CAR) T cell therapy and T cell engagers (TCE) that recruit T cells to induce B cell cytotoxicity have delivered promising results for anti-CD19 CAR T cell therapies, the CD19 TCE blinatumomab and CD20 TCE such as mosunetuzumab, glofitamab or epcoritamab. Limited evidence suggests that anti-CD19 CAR T cell therapy may be effective in managing refractory AID whereas we await evaluation of TCE for use in non-oncological indications. Therefore, here, we discuss the potential mechanistic advantages of novel therapies that rely on T cells as effector cells to disrupt B-T cell collaboration toward overcoming rituximab-resistant AID.

Graphical Abstract



This graphical abstract demonstrates the distinct time courses of CD19 CAR T cell (green line) versus CD19-T cell engager (TCE) treatments (purple line) for a patient post flare of their autoimmune disease. Accessibility to CAR T cell therapy can be significantly limited to major centres of expertise. Significant disadvantages of CAR T therapy include: 1) high production costs; 2) labour intensive processes; 3) delay in establishing a sufficient effector T cell pool; and 4) tolerability of pre-requisite toxic lymphodepleting chemotherapy regimen which may restrict CAR T cell therapy to a smaller cohort of patients and potentially compromise patient outcomes. In contrast, CD19-TCE is readily available, off the shelf, and can be administered immediately post identification of a disease flare, thereby ameliorating the risks of disease progression associated with the lag time to receive CAR T cell therapy. The lack of pre-requisite chemotherapy associated with CD19-TCE is also positive as it reduces the associated risk of infections and cancers.

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Background

B-T cell collaboration in the pathogenesis of autoimmune disease

B and T cell (B-T cell) collaboration perpetuates chronic inflammation in a range of autoimmune diseases (AID) including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and multiple sclerosis (MS) (1, 2). This cellular collaboration may occur through contact -dependent or -independent pathways through cytokines and other immune stimuli. Within lymphoid aggregates and the germinal centre, B-T cell interactions involve an array of molecular pairings (3), summarised in Figure 1 and Table 1**.** These signals stimulate T cell secretion of cytokines and promote differentiation of naïve to memory B cells and plasma cells (PCs), Figure 1. Some of these pathways have been targeted, as discussed later, whereas others are the subject of novel therapeutic strategies.

In this context of an ongoing immune response, an appreciation of B cell biology is helpful. B cells originate from haematopoietic stem cells in the bone marrow and undergo differentiation in secondary lymphoid organs (4). Differential expression of various cell surface markers, including cluster of differentiation (CD) molecules and immunoglobulin isotypes help to define classical subpopulations including: naïve B cells (IgD+CD27-), unswitched memory B cells (IgD+CD27+), switched memory B cells (IgD-CD27+) and double negative memory B cells (IgD-CD27-) (4). Naïve B cells have not yet encountered antigen, whereas switched memory B cells are primed to respond to antigen and double negative memory B cells increase with ageing, autoimmunity and chronic infectious diseases (5). Until recently, the focus of B cell depletion therapy has been on rituximab, an anti-CD20 monoclonal antibody which is widely used in haematological malignancies and AID (discussed in more detail below). The first FDA approved targeted biologic therapy for SLE was Belimumab, a mAb directed at B-cell activating factor (BAFF, also known as BLyS) (6), however, real world data demonstrates variable success (7) (8). BAFF is a B cell survival and differentiation factor and is elevated in the serum of patients with SLE (9).

B-T cell interactions in the peripheral inflammatory sites of various AID including RA SLE, type I diabetes mellitus and coeliac disease exhibit a population of T cells which are termed T peripheral helper cells (1, 10, 11). Rao et al identified these cells, adjacent to B cells in lymphoid aggregates of the synovium in patients with RA as PD-1hiCXCR5^{-}CD4^{+} which lack Bcl6 but produce IL-21 and CXCL13, resulting in B cell differentiation into plasmablasts (PBs)(12). This perpetuates B-T cell networking in inflamed tissues, where ectopic lymphoid structures(13) are formed. Thus, B-T cell collaboration occurs in both lymphoid tissues and at sites of inflammation.

Disrupting the B-T cell networking in AID, historical perspectives

B-T cell collaboration is a dominant source of chronic inflammation in AID. Hence, disrupting this network is an appealing therapeutic strategy. Over the past four decades, B-T cell co-stimulation was targeted through

different pathways such as alemtuzumab (anti-CD52 monoclonal antibody, CAMPATH-1H), abatacept (cytotoxic T-lymphocyte antigen 4 immunoglobulin), and dapirolizumab (anti-CD40L) with variable impact on B cell depletion (BCD), Figure 2. In the 1980s, alemtuzumab was used to deplete CD52 expressing cells including B and T cells, providing the first insights into disrupting B-T cell networking. The 1990's trials of alemtuzumab in RA were terminated due to suboptimal therapeutic index probably owing to prolonged depletion of regulatory T cells(14), although it continues to be used to treat MS (albeit at lower doses). Abatacept inhibits the co-stimulatory CD28-CD80/86 pathway and is approved for RA(15) although the ALLURE trial of abatacept in lupus nephritis (LN) did not meet its primary end point(16). Attempts have been made to block other key co-stimulatory signalling pathways including the CD40-CD40L axis. Second generation agents have been developed including dapirolizumab-pegol which had favourable biomarker and safety response in SLE(17); phase III results are awaited (NCT04294667). Therefore, despite these advances, there remains a great unmet need for disrupting B-T cell collaboration in refractory patients with AID.

BCD with rituximab in RA and SLE; why is it suboptimal?

In the past three decades, BCD therapy with the CD20 monoclonal antibody rituximab, has revolutionised the treatment of severe or refractory AID and has been approved for use in RA(18), ANCA vasculitis(19), and pemphigus vulgaris (PV)(20) and is prescribed widely 'off-licence' in SLE(21) and in immune thrombocytopenic purpura (ITP) (22). Data from the Lupus Nephritis Assessment with Rituximab (LUNAR) study reported complete BCD with complete response, as defined in the study(23). However, there remains a significant proportion of patients, up to 30%, who have disease refractory to rituximab, particularly in the context of incomplete BCD (21) and/or repopulation with PB and switched memory B cells (IgD^-CD27^+, SwMBC)(24).

How do memory B cells and $CD19^+CD20^-$ PBs evade rituximab?

B cells can evade rituximab's effects either through intrinsic mechanisms (lacking CD20 expression and antigenic modulation) or extrinsic mechanisms such as restricted vascular access to effector cells as discussed previously(25). Upon activation naïve B cells solicit T cell co-stimulation in lymphoid tissues and at sites of inflammation such as the joint and the kidney to differentiate into memory B cells and antibody secreting cells including short-lived $CD19^+CD20^-$ PBs and long-lived $CD20^-$ PCs(12, 26). In RA, rituximab fails to completely deplete SwMBC and $CD19^+CD20^-$ PCs in lymphoid tissues(27), joints and bone marrow(28-30) contributing to poor response. In patients with ITP with poor response to rituximab, autoreactive splenic memory B cells down-regulate their BCR and up-regulate anti-apoptotic proteins and evade rituximab whilst retaining the capacity to reactivate and differentiate into autoantibody secreting $CD19^+CD20^-$ PBs(22). In muscle-specific kinase myasthenia gravis, autoreactive SwMBC evade rituximab and differentiate into autoantibody secreting $CD19^+CD20^-$ PBs contributing to relapse(31). Further, rituximab has no direct effect on $CD19^+CD20^-$ PBs and PCs, as they do not express CD20(32, 33). Thus, SwMBCs, $CD19^+CD20^-$PBs and $CD19^+CD20^-$PCs evade rituximab through distinct mechanisms, Figure 3.

Broadly, anti-CD20 mAbs can be grouped into type I and type II, where type I mAbs such as rituximab, are more efficient at clustering CD20 compared to type II anti-CD20 mAbs (34). This enables efficient complement activation and therefore enhanced complement-dependent cytotoxicity (CDC), however it also increases the propensity for internalisation of CD20:CD20 mAb complexes by B cells ((35)). In addition, incomplete BCD with rituximab may be related to its internalisation of rituximab(36). Type II anti-CD20 mAbs such as obinutuzumab may, at least in part, overcome this resistance mechanism(25). In a pivotal phase II study, obinutuzumab was shown to improve clinical response in LN(37) and phase III studies are ongoing. However, $CD19^{+}CD20^{-}$ PBs and $CD19^{+}CD20^{-}$ PCs are still not directly targeted. Furthermore, disease-associated macrophage phagocytic defects(38) and vascular access limitations may compromise the ability of anti-CD20 mAbs (and other B cell depleting mAbs, such as those directed to CD19) to evoke antibody dependent cellular phagocytosis (ADCP)(25, 39) as they rely on FcγR-bearing effector cells. In addition, NK cells are also scarce in tissues, limiting antibody dependent cellular cytotoxicity (ADCC). For example, we have previously reported that incomplete depletion and / or persistent infiltration of B cells in the kidneys was associated with active LN refractory to rituximab(40).

Through histological analysis of kidney(41) and skin(42) of patients with AID, and the synovium in patients with RA (12), we know that B cells interact with T cells in lymphoid tissues and at sites of inflammation, to differentiate into autoantibody secreting PBs and PCs. At these sites, limited access to rituximab's key effector cells, macrophages, and NK cells, may compromise depletion. Thus, antigen expression, modulation and access to effector cells influence the efficiency of rituximab-mediated BCD. Therefore, it is important to consider both alternative target antigens and therapies that recruit other effector cells to improve BCD.

Approaches to overcome rituximab resistance in AID

Is CD19 an ideal target?

CD19 regulates the threshold for B cell activation as a co-receptor of the BCR complex (43) with consequent implications for influencing autoimmunity(44). CD19 deficiency impairs humoral immunity, at least in part, due to an increased threshold for B cell activation(45) whereas overexpression is associated with AID such as SLE(26). When compared with $CD19^{-}CD20^{-}$ PCs, $CD19^{+}CD20^{-}$PCs accumulate more mutations and retain greater proliferative capacity, at least in vitro(32). These observations implicate a significant role for CD19 in B cell differentiation and activation.

When compared with CD20, B lineage cells express CD19 at an earlier stage in development and retain expression through all stages of differentiation into $CD19^{+}CD20^{-}$ PBs and some $CD19^{+}CD20^{-}$ PCs(26). $CD19^{hi}CD11c^{+}$ memory B cells in humans were shown to respond robustly to antigen challenge, in vitro(46). More recent evidence suggests that double negative (IgD-CD27-) DN B cells which express the

transcription factor T-box expressed in T cells (T-bet) encoded by *Tbx21*, termed DN-T-bet$^+$ B cells are expanded in ageing, are associated with higher mortality from COVID-19 infection and disease activity in SLE as well as disease pathogenesis in RA. Therefore they are of great interest in the field of B cell research (47).

Further, they demonstrate increased expression of CD19 which strengthens the argument to target CD19 in AID (Shah et al Manuscript in preparation). Considering the availability of newer therapies that target CD19 particularly in the field of oncology, we reappraise the concept of targeting CD19, put forward over a decade ago, to treat AID(26). In addition, evidence from oncology highlights that cancers refractory to monoclonal antibodies have been effectively treated with CD19-targeted chimeric antigen receptor (CAR) T cells, probably owing to the deeper depletion of B cells which provides promise for patients with AID resistant to current mAb therapy, highlighted by the published case series in SLE(48). These mechanistic considerations indicate that targeting CD19, particularly in AID, may overcome anti-CD20 mAb resistance.

How to target CD19 - T cell engagement as a mechanism of action?

Therapeutic options to target CD19$^+$ B cells and PCs include: 1) anti-CD19 mAbs; 2) CD19-targeted CAR T cells; and 3) CD19-directed T cell Engagers (TCE). The anti-CD19 mAb inebilizuzmab is approved for the treatment of neuromyelitis optica spectrum disorder(49) and showed initial promising results in a clinical trial in systemic sclerosis(50). BCD with inebilizumab was greater in transgenic mice blood and spleen as well as in an in vitro ADCC assay using human PBMCs when compared to rituximab(51). However, similar to rituximab, anti-CD19 mAbs are also disposed to internalisation(52) and would be limited by disease-associated macrophage phagocytic defects(38) and vascular access limitations. Therefore, CD19-directed CAR T cells and CD19 TCE may be of greater utility in AID and will be discussed in the following sections.

CAR T cell therapy

The introduction of CAR T cells to treat cancer has been instrumental in providing individualised, targeted treatment through genetically engineered T cells that express a CAR specific to a tumour associated antigen, such as CD19 in B cell(53)malignancies. Recognition of the target antigen bearing B cells activates CAR T cells to proliferate and selectively eliminate the target B cells. The basic structure of a CAR includes an extracellular surface domain for antigen recognition (typically derived from an antibody fragment), a transmembrane domain and an intracellular signalling domain which activates T cells (typically derived from CD3z chain). The evolution of CAR from first to fourth generation includes the addition of co-stimulatory domains (one in second generation and two in third generation CARs) as well as co-expression of additional transgenes for cytokine secretion (fourth generation) (54),

Figure 4.

Once administered, CAR T cells can also expand and establish immune memory, thus providing long term surveillance of disease as described in malignancy(55). CAR T cell therapy has been approved for the treatment of B cell acute lymphoblastic leukaemia (ALL), lymphoma and multiple myeloma(53). Factors such as antigen overload are considered to contribute to undesirable effects including cytokine release syndrome (CRS) and neurotoxicity, leading to newer generation therapies with fewer toxicities being developed(56). Complete remission for at least three years, of various relapsed B cell malignancies was

demonstrated in 51% of patients treated with CAR T cell therapies, with few late onset side effects(57). This success led to CAR T cells being explored for treating refractory AID.

CAR T cell therapy in AID

The success of using CAR T cell therapy for the management of B cell malignancies inspired its research in a range of AID including SLE, myasthenia gravis and type 1 diabetes mellitus, as outlined in Table 1. In animal models of SLE, anti-CD19 CAR T cell treatment resulted in profound and sustained BCD with low circulating PCs and increased survival rates(58). This data provided the basis for the use of anti-CD19 CAR T cell therapy in the treatment of 5 patients with refractory multiorgan lupus which was well tolerated leading to serological and clinical remission at relatively short follow up(48). Probably owing to lower antigen load, the first cohort of patients with SLE treated with anti-CD19 CAR T cell therapy experienced only low grade CRS(59), of which tocilizumab (anti-IL-6 receptor mAb) was used (successfully) in only one patient owing to persistent fevers for 3 days(48). Thus, current preliminary evidence suggests that CD19 targeting CAR T cell therapy seems a safe and effective therapeutic strategy in AID such as SLE. Anti-CD19 CAR T cell therapy was associated with a reduction in autoantibodies and pro-inflammatory cytokines including IL-6 and TNF-α (60). Intriguingly, despite excellent clinical responses, the authors demonstrated an increase in serum BAFF levels.

With regards to other autoimmune diseases, single case studies of anti-CD19 CAR T cell therapy indicate a potential use of the approach also in anti-synthetase syndrome(61) and systemic sclerosis(62). To note, an important potential confounder when appraising the mechanisms of response to CAR T cell therapy is the use of lymphocyte depletion with fludarabine that may have contributed to response. Several studies exploring the safety, tolerability, and preliminary efficacy of anti-CD19 CAR T cell therapy in AID have been initiated (NCT05938725, NCT05869955, NCT03030976, NCT05798117, NCT05930314).

Limitations of CAR T cell therapy

Although the case examples of anti-CAR T cells in AID are promising, it is also important to understand the limitations. Two of the 5 patients treated with anti-CD19 CAR T cell therapy had persistence of clonotypic IgG in follow-up samples, demonstrating suboptimal depletion and/or rapid repopulation of memory B cells(48). Remarkably, despite lower antigen overload, three of five SLE patients treated with anti-CD19 CAR T cell therapy repopulated their B cells by day 50 after treatment(48) when compared with prolonged BCD achieved in B cell malignancies up to several years post infusion(53). Potential explanations for incomplete depletion and/or relatively early repopulation of B cells include: 1) complete depletion of target cells removing the sustained stimulus needed to maintain an optimal pool of CAR T cells, as CAR T cells had disappeared at week 4 after treatment; 2) higher proportion of senescent and/or exhausted SLE CAR T cells; and 3) potential inhibition of CAR T cell expansion due to the persistent effects of immunosuppression such as mycophenolate mofetil beyond cessation of therapy(63).

Implications of lymphodepletion in AID

Patients with AID, particularly SLE, are often lymphopenic owing to the underlying disease process and the effects of immunosuppression, which may impact the process of leukapheresis required to generate the CAR T cells. Nevertheless, patients with active SLE in the previously discussed case series (48) were successfully leukapheresed before CAR T cell therapy and concurrent treatment with steroids and immunosuppressive agents (64). The process of lymphodepletion itself increases the likelihood of infections and is an additional step preceding CAR T cell therapy, compared to 'off the shelf' TCE therapy.

Risks of hypogammaglobulinaemia

A major consideration with CAR T cell therapy is the risk of hypogammaglobulinaemia; this may be observed with TCE but likely to a lesser extent. In the treatment of cancer, approximately a third of patients develop hypogammaglobulinemia following CAR T cell infusion (65), owing to potent and persistent depletion of normal $CD19^{+}$ B cells. Very low IgG levels can arise from 9 weeks after treatment and continue beyond 4 years (65). This poses a risk of serious life threatening infections, necessitating intravenous immunoglobulin infusions as a prevention strategy, as per the majority of trials (66), however, this can be expensive and not readily accessible for all patients.

Importantly, B cell aplasia and hypogammaglobulinaemia result in suboptimal vaccine responses, which is also a significant concern especially in the current era of SARS-CoV-2 infection with only 29% of patients who receive CAR T cell therapy for lymphoma/myeloma mounting a clinically relevant antibody response to vaccination (67). Reassuringly, vaccine responses were stable following CAR T cell therapy in the SLE case series (48), likely related to the remaining pool of $CD19^{-}$ plasma cells which are able to secrete antibodies two years post treatment (68). These aspects also need to be accounted for during TCE trial design in AID.

Logistical limitations of CAR T cell therapy

Logistical limitations are also considerable. For example, in patients with rapidly progressing cancer or AID, the practical feasibility of CAR T cell therapy may be limited as there is typically a protracted vein-to-vein time of approximately 6-8 weeks, due to the time required for producing, transporting, and ensuring quality control of the personalised cell therapy, as illustrated in the graphical abstract. This process is typical for most CAR T cell therapies, although the novel YTB323 omits the ex-vivo expansion stage(NCT05798117).

Further disadvantages of CAR T cell therapy include the high cost involved with engineering and storage of CAR T cells and the specialist training required to administer treatment as detailed in Table 2. Therefore, readily available and effective novel treatments are required whilst awaiting CAR T cell therapy(69). One

approach to obviate the limiting factor of individual custom-made CAR T cells, is the generation of 'universal CAR T cells' as reviewed by Zhao et al (54). These can serve as 'off the shelf' therapies to treat a wide range of clinical indications as they are engineered to target multiple antigens. Further gene editing work is underway to ensure universal CAR T cells are not depleted by the recipient's immune system and are able to expand without causing harmful effects (70).

To this end, we consider alternative strategies, with the potential of TCE bispecific antibodies as a novel therapeutic option to disrupt B-T cell collaboration in AID. Table 2 outlines the major differences and similarities of using CAR T cell therapy and TCEs.

TCE: clinical trial experience and technical aspects

TCE represent a novel class of targeted therapeutics which recruit T cells(71). From a clinical perspective, in the late 1990's, the potential for bispecific antibodies as therapeutic interventions became clearer for cancers such as breast, leukaemia and lung(72), which led to a surge of interest in their use and FDA approval of catumaxomab for malignant ascites(73) and blinatumomab for refractory B-ALL(74) More recently, three CD20 T cell engagers, mosunetuzumab, glofitamab and epcoritamab have been approved for treatment of refractory/relapsed follicular lymphoma and refractory/relapsed diffuse large B cell lymphoma (75) . Technological advancements over time have enabled a range of modifications to enhance the flexibility and number of binding sites, half-life, production yield and potency of these therapeutics(76).

TCE technologies

TCEs can be broadly categorised into: 1) small, short half-life bispecific antibody fragments (single chain variable fragments) such as bispecific T cell engagers (BiTE®s) which require repeated administration (Figure 5A); and 2) larger IgG-based T cell bispecific antibodies (TCBs) with extended half-lives (Figure 5B and C). The development of TCBs has evolved from single chain variable fragments in the early 1990s(77), to the development of 'knobs into holes' (KiH) technology in the late 1990s(78) to the more advanced technologies including CrossMab to engineer bispecific antibodies(79, 80), Figure 5.

CD19-TCE

Blinatumomab, a BiTE® composed of two single chain antibodies targeting CD19 on B cells and CD3ε on T cells fused via a flexible linker (Figure 5A), is approved for B cell ALL(75). It is engineered to have a short half-life of 2 hours to enable tight control of serum levels in case of adverse events. Blinatumomab relies on the presence of $CD19^+$ target cells to activate T cells, with sensitive response from $CD8^+$ T cells to induce lysis of tumour cells as demonstrated in video-assisted microscopy studies(81). In vitro studies of human B lymphoma cells demonstrated a higher degree of tumour cell elimination with blinatumomab compared to rituximab(82). Interestingly, the combination of blinatumomab and rituximab was synergistically more

efficient, especially at low effector-to-target cell ratios and low Blinatumomab concentrations(82). This combined effect was found to be due to potent activation of pro-caspases 3 and 7 in target cells, which is instrumental in triggering granzyme-mediated apoptosis. The BiTE subtype is potent with regards to target cell killing. Regardless, the requirement for repeat dosing of Blinatumomab may limit its routine use in clinical practice.

CD20-TCE

Three CD20 TCE have been approved for refractory B cell lymphomas: mosunetuzumab, glofitamab and epcoritamab(75), Figure 5. Mosunetuzumab is an IgG based TCE with 1:1 binding to CD20 and CD3; it uses KiH technology and in vitro assembly to overcome incorrect light chain association (83). Epcoritamab is also IgG based, although employs the unique DuoBody® technology with point mutations in each Fc region (CH3 domain) to allow controlled Fab-arm exchange (84). Recent IgG-based TCEs have been developed for increased avidity. Glofitamab has two Fab regions which bind CD20, one Fab region which binds CD3 (so-called 2:1 format), and a longer half-life of 10 days, owing to its Fc region and interaction with FcRn(80). The Fc also includes the P329G LALA mutations (71), which abolish conventional effector functions and therefore it employs a different mechanism of action compared to rituximab. The 2:1 format (Figure 5C) enables greater potency with regards to B cell cytotoxicity compared to 1:1 antibodies, thought to be due to the close proximity of the CD20 binder and CD3 binder, resulting in a more stable T cell to target B cell synapse induced by the head-to-tail fusion design(85).

Effector mechanisms of TCEs: lessons learnt from treating malignant disease

Bispecific antibodies can redirect the effector function of various immune cells. T cells are promising as effector cells as they are abundant, able to expand rapidly, and have potent cytotoxic capacity. TCE are designed to by-pass the normal major histocompatibility complex–T cell receptor (MHC-TCR) interaction usually required between antigen presenting cells and T cells, and instead co-engage the CD3 molecules on the T cell and form an immunological synapse via the target antigen such as CD19 or CD20 on the surface of B cells that helps redirect co-stimulation to cytotoxicity(86, 87), Figure 6. This synapse is similar to that formed during cytotoxicity with CAR T cells. The CD20-TCE recruitment of T cells is evident in in vitro culture assays demonstrating that tumour lysis is dependent on T cell recruitment, activation, and expansion of $CD4^+$ and more profoundly $CD8^+$ subsets (71). Importantly, CD20-TCE depleted B cells in the spleen and lymph nodes, efficiently(71). These findings may be of relevance to AID where inefficient BCD in lymphoid tissues and inflammatory sites, as discussed earlier, contributes to refractory disease.

Employing T cells to disrupt the B-T collaboration: CAR T and TCE

As discussed above, in AID, B and T cells colocalise in lymphoid tissues and at inflammatory sites. Therefore, using CAR T cells or TCE that employ T cells as effector cells to deplete B cells may provide a

distinct advantage over rituximab-mediated BCD that relies on macrophages and/or NK cells as the dominant effector mechanism. The key differences and similarities between CAR T cell therapy and TCE therapy are described in Table 3.

Aside from requiring lymphodepletion, an important aspect to highlight is that the expansion of CARs in vivo cannot be controlled, demonstrated by the rapid rise in circulating CARs, reaching up to 59% by day nine post infusion(48).

In addition, the expansion and duration of CAR T cell action is not easily controlled, whereas a TCE can be given at a specific dose and the half-life of the molecule is expected to determine its duration of action. Overall, treatment with TCE may potentially overcome some of these limitations of CAR T therapy such as a lag time from decision to treatment to allow for engineering of CAR T cells, prior leukapheresis, and requirement for specialist centres with experience of cell-based immunotherapies.

Immunological/Biological pitfalls in recruiting T cells as effector cells

Despite the undoubted promise of CAR T cells and TCE, there remain potential hurdles. Both CAR T cells and TCE may evoke ‘bystander killing’ of antigen-negative cells directly in contact with antigen-positive cells(88). Whilst this local bystander effect is desirable in the treatment of solid tumours to prevent escape of antigen-negative cancer cells, the potential implications of this in AID are unknown.

More recently, there are an increasing number of reports of macrophage activation syndrome (MAS)/haemophagocytic lymphohistiocytosis (HLH) as a complication of CAR T cell therapy given for haematological malignancies, possibly as a distinct variant of CRS (89). MAS/HLH is a serious condition of hyperinflammation, fevers, cytopaenias, and can be life threatening. Patients with autoimmune disease such as SLE are already predisposed to developing secondary MAS/HLH (90), therefore initiation of CAR T cell therapy in this cohort needs careful consideration.

Another potential pitfall with recruiting T cells as effector cells is a possible reduction in T cell counts, which may increase the risk of infection, due to apoptosis noted with first generation CAR T cell treatments (91). Reassuringly, in studies with CD20-TCB, peripheral T cell counts decreased in the first 24 hours of drug administration before returning to baseline by 72 hours(71), considered to reflect an activation induced marginalisation. Therefore, the risk in the short term with these agents seems low but will need monitoring in the long-term.

Impact of the tissue microenvironment

An additional consideration is the tissue microenvironment, which is known to influence T cells cytotoxicity. AID related T cell subpopulations with features of anergy, exhaustion and senescence may compromise the efficiency of TCE(92). In addition, resistance to TCEs may arise from immune escape, through the expression of immune checkpoint molecules such as PD-1. In this context, combination treatment with checkpoint inhibitors, already explored in cancer immunotherapy may be limited by the potential activation of autoreactive T cells(93). Alternatively, next generation trispecific TCEs to additionally provide co-stimulation may be beneficial(94). As CD3 is a pan T cell marker, TCEs can recruit all T cell populations including naïve, regulatory T cells and exhausted T cells as effector cells. In AID, regulatory and exhausted T cells are associated with disease remission and improved prognosis(95). Mechanistic clinical studies will help us understand the clinical relevance of these potential limitations.

Clinical adverse effects of recruiting T cells as effector cells

The main adverse effect associated with both types of T cell therapy is CRS, which is the rapid systemic release of pro-inflammatory cytokines including IL-6, IL-10, TNF-α and IFN-γ, upon activation of the T cells(96). CRS manifests as fever, fatigue, vasodilation and can lead to multi-organ failure. Pre-treatment with corticosteroids such as dexamethasone may reduce the risk of CRS. Anti-IL-6 receptor antibody, tocilizumab, has been approved for use prior to CAR T cell therapy to attenuate CRS(97). In murine models, combination treatment with Janus Kinase (JAK) inhibitors or mammalian target of rapamycin (mTOR) inhibitor, restricted CD19-TCB related CRS whilst retaining their efficacy(98).

Immune effector cell-associated neurotoxicity syndrome (ICANS) is another dose-dependent unwanted side effect unique to patients receiving T cell engaging treatments, through adherence of T cells to cerebral microvascular endothelium and migration across the blood-brain barrier(99). In ALL, ICANS, characterised by headache, dizziness, tremor, confusion, and encephalopathy, was associated with high dose blinatumomab given in the first treatment cycle, probably owing to the higher tumour burden. As the target cell load is much lower in AID, the required dose of TCEs will be lower, consequently, the risk of CRS and ICANS should be lower than that reported for cancer immunotherapy.

What is the impact of immunosuppressive therapy on T cell cytotoxicity in the context of TCE and CAR T cells?

Other important considerations include AID-specific concurrent drug regimens. For example, transplant recipients and patients with AID and transplant recipients receive immunosuppressants to regulate immune response. In the context of T cell-based therapy, concurrent use of immunosuppressants may inhibit the effector function of the T cells, thereby, compromising the efficiency of CAR T cells and TCEs. For example, mycophenolate mofetil (MMF) can induce apoptosis in activated human T cells(100); and in a murine model, mycophenolic acid, the active form of MMF has shown dose-dependent reduction in the generation of cytotoxic T cells(101). Figure 6 illustrates the potential impact of immunosuppressants on T cell cytotoxicity in the context of TCE and CAR T cell therapies. Therefore withholding

immunosuppressants for a period of time to allow for T cell recovery to enhance performance may be considered in prospective trial design(102).

In a case series of renal transplant recipients requiring CAR T cell therapy for post-transplant lymphoproliferative disorders (PTLD), MMF was discontinued at the time of PTLD diagnosis (with DLBCL), and tacrolimus was stopped 2 weeks prior to leukapheresis for production of CAR T cells(103). Similarly, a report of CAR T cell infusion for anti-synthetase syndrome involved tapering azathioprine and steroids seven days before leukapheresis and starting MMF 35 days after CAR T cell infusion(104), which allowed for harvesting of fully functional T cells. This aligns with our proposition of correct sequencing of immunosuppressive treatments including the use of corticosteroids to allow full efficacy of TCE and/or CAR T therapies.

Developing personalised B cell targeting regimens

Where pathogenic B cell identity is well described, CAR T therapy can potentially enhance the prospects for personalised therapy. For example, desmoglein 3 targeting CAR T cells were engineered to selectively eliminate Dsg3 specific B cells, in vitro and in vivo in animal models(105) toward developing therapies for PV. Currently, a phase I study of BCMA CAR T therapy (NCT04561557) is ongoing for the treatment of neurological disorders including Aquaporin related neuromyelitis optica spectrum disorder (NMOSD). However, the identity of pathogenic B cells remains elusive for the majority of AID, where non-selective BCD therapy remains the current standard strategy.

In routine practice of managing AID, rituximab induction therapy incorporates two doses of 1 gram, given two weeks apart. Retreatment with the same or lower dose of rituximab, is usually at six months or longer for optimal management of disease activity(15). Current evidence highlights that response can be improved with better depletion with a lower frequency of memory B cells and PB in RA and SLE (25). As discussed previously, presumably due to more efficient BCD, obinutuzumab treatment seems to be effective in LN(37). To this end, targeting CD19 and disrupting the B-T cell networking in AID, with CD19/CD3 TCEs or CAR T cells would be expected to provide mechanistic advantages. For example, targeting CD19, expressed on memory B cells, $CD19^{+}CD20^{-}$PBs and $CD19^{+}CD20^{-}$PCs should help deplete these ‘rituximab-resistant cells’ whereas the use of TCEs would help direct T cells from B cell ‘co-stimulation to cytotoxicity’ to disrupt B -T networking. Key lessons from previous SLE rituximab trials include 1) patient selection with regards to disease manifestations, severity of disease activity, serological parameters, and previous treatment are important to consider so as not to exclude the most active patients, 2) defining standard concomitant therapy in the comparator and placebo arms as variable usage of glucocorticoid and immunosuppressants such as MMF can impact outcomes, 3) defining endpoints in particular the steroid sparing effect, 4) selecting the right disease activity index and 5) defining follow up duration and side effects. These serve as a reminder of the importance of optimal trial design to evaluate the ‘real’ potential of TCE(23, 106).

Optimising co-therapies with immunosuppressants, and sequential therapy with rituximab

Co-therapy with immunosuppressants and/or rituximab therapy may influence the efficacy and safety of TCEs. As demonstrated in Figure 6, patients with AID are often being treated with immunosuppression such as MMF and corticosteroids. Therefore, considering discontinuation of MMF for three(48) to six weeks may optimise the effector function of T cells to disrupt the B-T cell network in AID. Thereafter, a delayed introduction of MMF may be considered as needed for optimal control of disease activity.

Sequential therapy with rituximab, which is already competitively priced as a biosimilar, followed by CD19-TCE will enable targeting of B-T cell networks in ectopic lymphoid tissue within peripherally inflamed tissues in AID, Figure 3. A potential limitation of this sequence is that rituximab therapy may result in lower expression of CD19(22), probably through internalisation as shown in vitro(36), thus, compromising the efficiency of CD19-TCE or CD19-CAR T therapy. Therefore, treatment with CD19-TCE first followed by rituximab, as needed, could be considered as an alternative strategy for those with poor depletion with CD19-TCE alone. In this context, it would be important to have strategies to detect B cells using novel antibodies that bind an alternative epitope to the therapeutic mAbs, less challenging for CD19 as it is a bigger antigen than CD20.

Conclusions

CD19 CAR T cell or CD19-TCE therapy to convert B and T cell co-stimulation into conflict and disrupt their networking could prove to be a paradigm shift in treating AID. TCE, designed and developed through advanced antibody engineering methods, offer a mechanistically sound, logistically convenient, and favourable alternative therapeutic strategy in the management of refractory AID. To this end, mechanistic studies of TCE in AID, particularly during early phase clinical trials, are of critical importance to optimise the use of TCE in combination with standard-of-care therapy as an alternative strategy to deplete B lineage cells to improve outcomes for people with refractory AID.

Figure 1

Figure 1

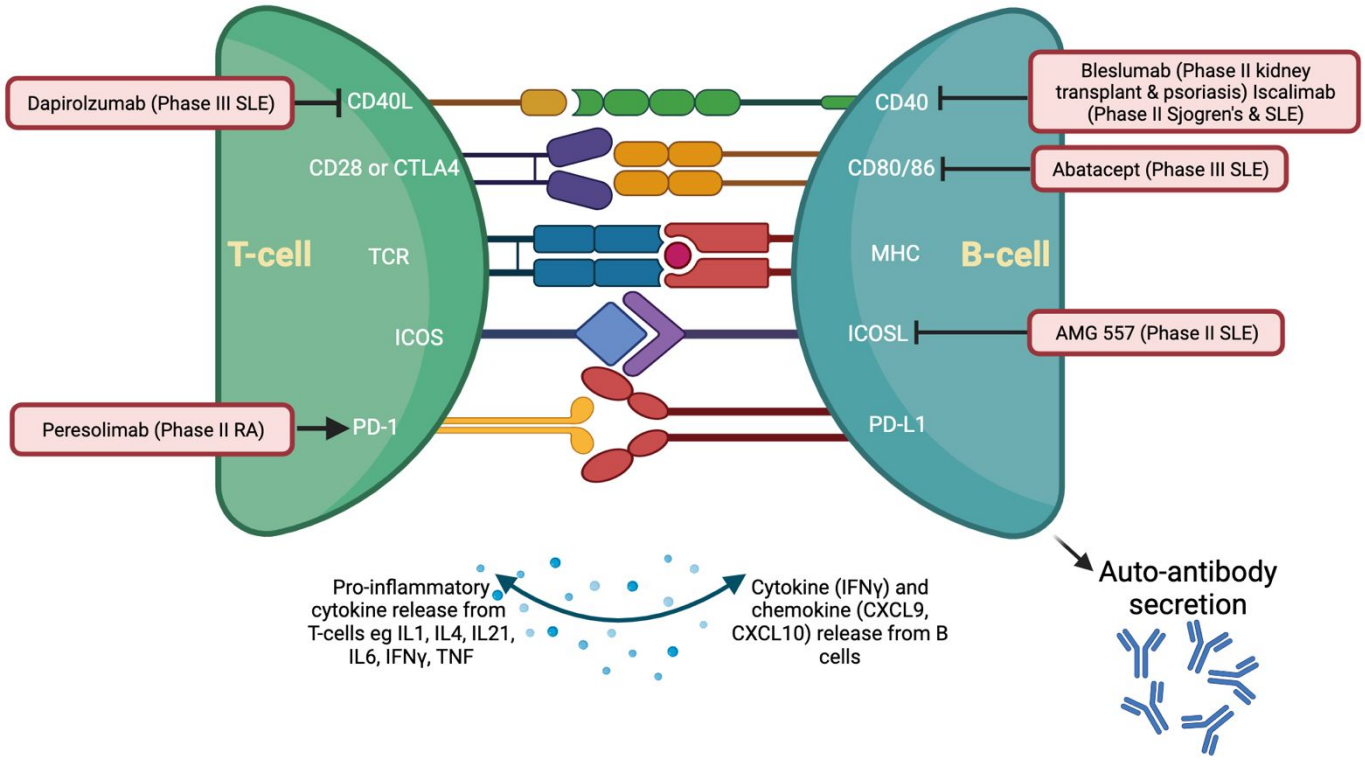

Figure 1. Pathways of B-T cell co-stimulation and trials of therapeutic agents. Molecular pairings are explained in Table 1. Drugs which target co-stimulation are outlined here. Dapirolizumab is an anti-CD40L mAb, currently in phase III study in SLE (NCT04294667). Bleslumab is an IgG4 mAb that targets CD40 which underwent phase II trial in plaque psoriasis with no clinical improvement compared to placebo (107), and demonstrated non-inferiority compared with standard of care for acute rejection in renal transplant recipients (108). Iscalimab is another anti-CD40 mAb which is undergoing phase II trial in SLE and Sjogren's Syndrome (NCT03656562, NCT04541589). Abatacept inhibits CD80/86 to prevent engagement with CD28 and is approved for use in RA but failed to meet the primary end point in the lupus nephritis phase III trial. AMG 557, anti-ICOSL antibody, underwent phase II trial in SLE and a newer therapy inhibiting ICOSL and BAFF is undergoing phase II trial (NCT04058028). PD-1 agonist, Peresolimab demonstrated modest improvement in disease activity in a phase II trial for patients with RA.
Image created using Biorender.com

Figure 2

Figure 2

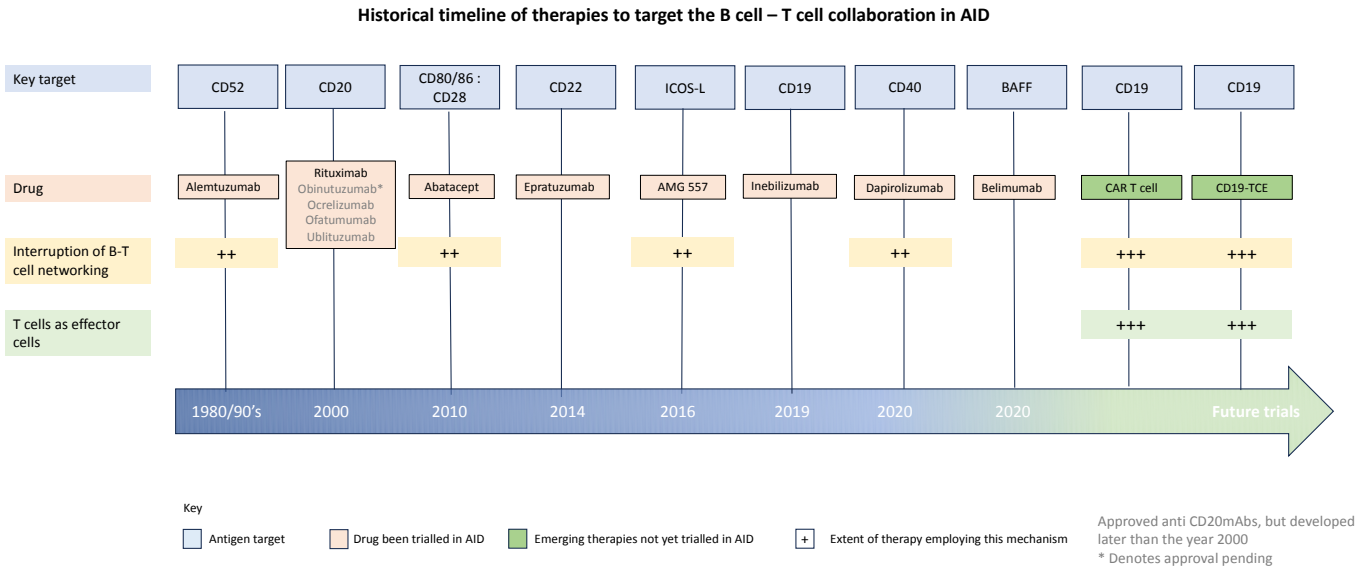

Figure 2. Historical timeline of therapies that target B-T cell collaboration in autoimmune disease. These agents were designed either to deplete B cells and/or disrupt the B-T cell collaboration. Text in blue boxes denote the target antigen, peach shaded boxes are drugs which have undergone clinical trial, drugs in dark green boxes are yet to undergo clinical trial in AID. Yellow bars represent therapies which interrupt B-T cell networking and light green bars represent treatments which employ T cells as effector cells. Text in grey represents other approved anti-CD20 mAbs, * *denotes pending approval.*

Figure 3

Figure 3

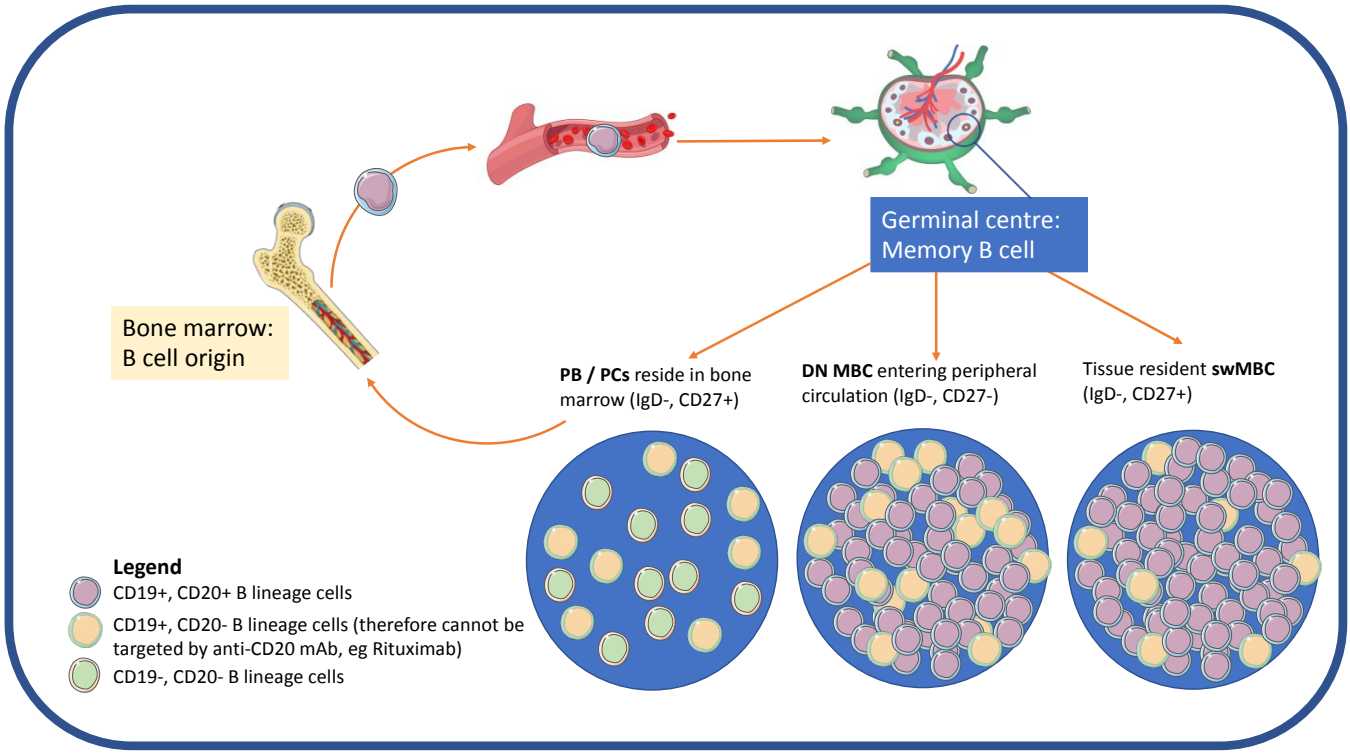

Figure 3. Life cycle of B lineage cells. B cells originate in the bone marrow and migrate through peripheral circulation into lymphoid tissues such as lymph nodes and the spleen. Naïve B cells mature into memory B cells which then differentiate into switched memory B cells, SwMBC (IgD-,CD27+), or double negative memory B cells (DN MBC; IgD-, CD27-) entering the peripheral circulation or plasma blasts (PBs) and plasma cells (PCs) a majority of which reside in the bone marrow, tissues, and inflammatory sites. Proportions of $CD19^{+}CD20^{+}$ vs $CD19^{+}CD20^{-}$ B cells are demonstrated pictorially within each subpopulation. Anti-CD20 monoclonal antibodies such as rituximab may not completely deplete $CD19^{+}CD20^{+}$ B cells in tissue and do not target $CD19^{+}CD20^{-}$ B cells, therefore, alternative strategies of depletion including CD19 targeting approaches may help to overcome rituximab resistance in autoimmunity.

Figure 4

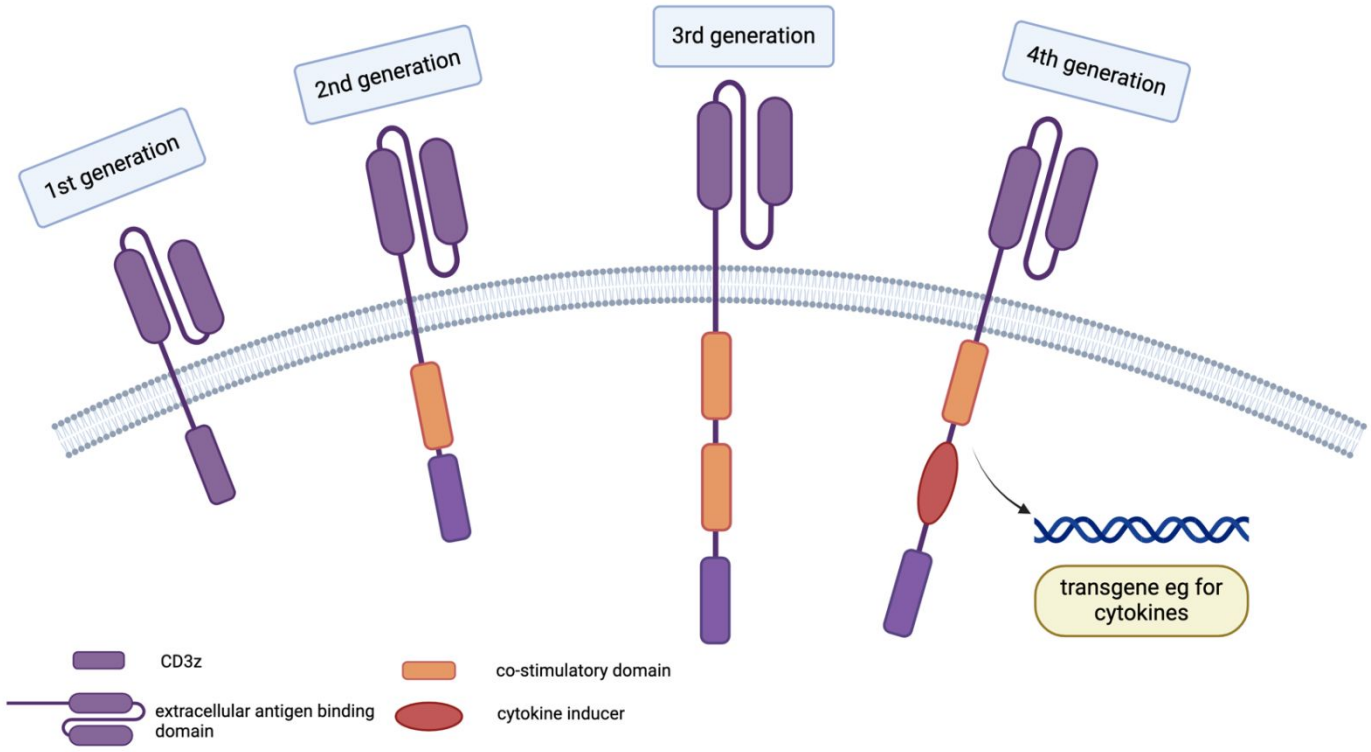

Figure 4. Evolution of CARs across the generations

All CARs have a single chain variable region of a mAb. A) first generation CARs contain an intracellular signalling domain of CD3 zeta chain alone; B) second generation includes a single co-stimulatory domain (CD28 or 4-1BB). C) third generation CARs combine two of the above co-stimulatory domains. D) fourth generation CARs are diversified in that they can express cytokines.
Image created using Biorender.com

Figure 5

Figure 5

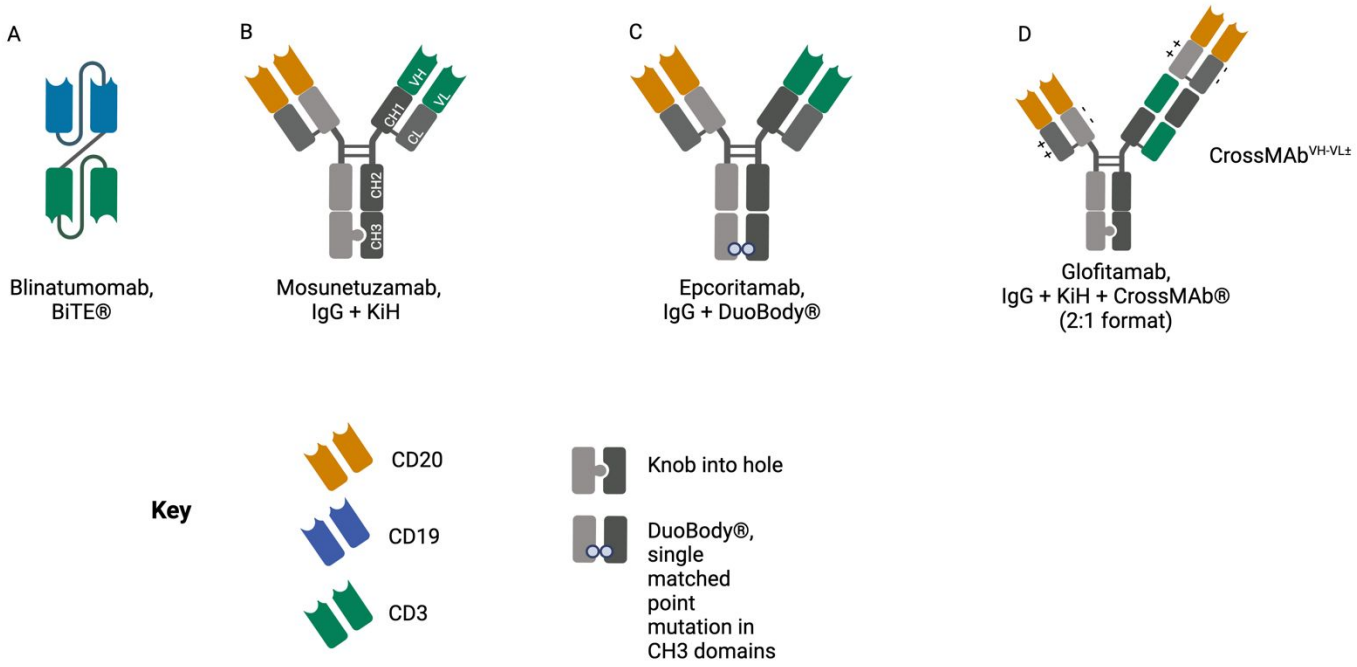

Figure 5: Selected TCE formats in a schematic representation used for T cell redirecting therapies. A) Blinatumomab, tandem scFv (single chain variable fragment) (BiTE) format. B) Mosunetuzumab, IgG based-TCE with monovalent binding using a native antibody structure with 1 Fab arm to bind CD20 (target antigen) and 1 Fab arm to bind CD3 on T cells, combined with the KiH technology as demonstrated in the CH3 domain to achieve heavy chain heterodimerisation. C) Epcoritamab, IgG based TCE with point mutations in each Fc region (CH3 domain) to allow controlled Fab-arm exchange, termed DuoBody®. D) Glofitamab, bivalent binding to increase the avidity of TCE binding to the target antigen, CD20, with additional KiH and CrossMab^{VH-VL} with charge interactions using variable regions.
Image created using Biorender.com

Figure 6

Figure 6

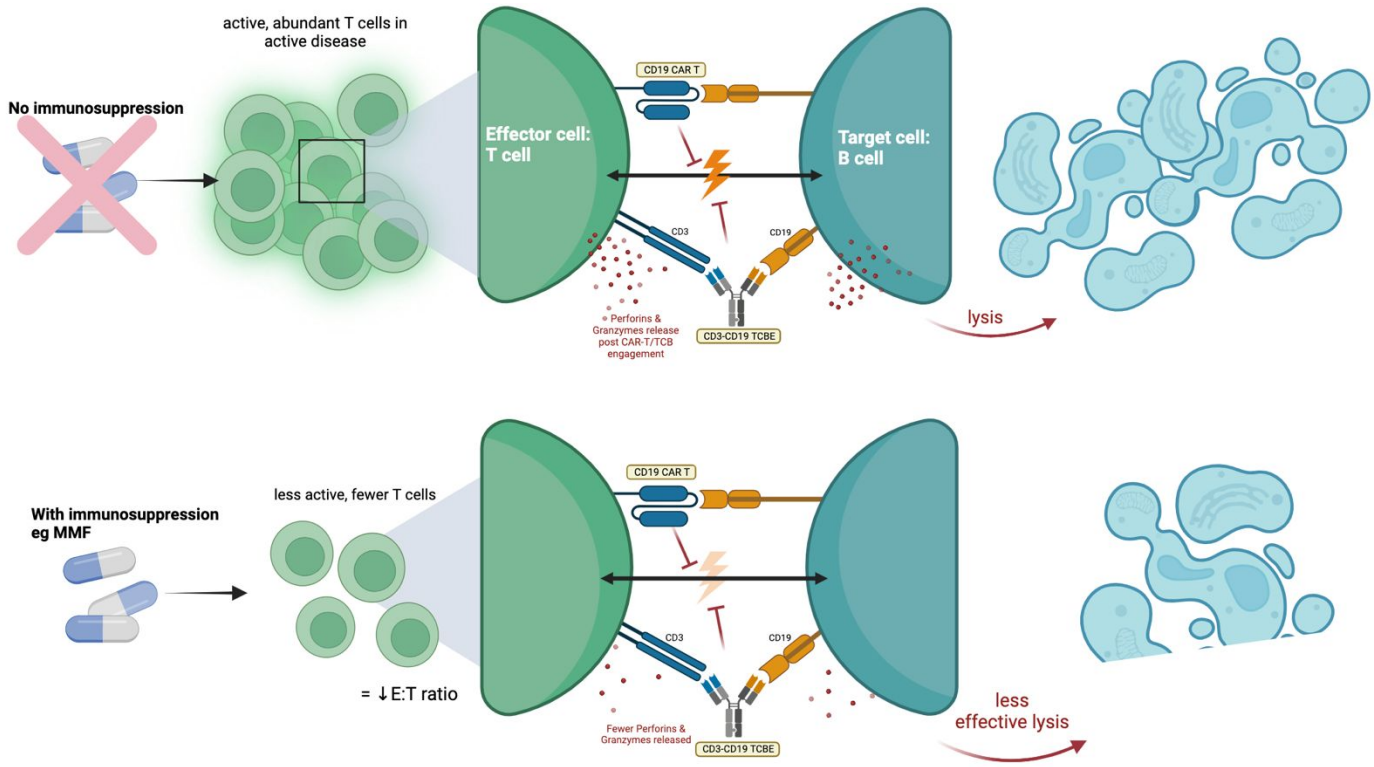

Figure 6. The potential effect of immunosuppressive treatments on T cell effector function. Mycophenolate mofetil (MMF) as per the bottom panel, results in fewer T cells to serve as effector cells for therapies such as CD19 TCE and CD19 CAR T cells. MMF can directly reduce the number of T cells and impair their activation and reduce their cytotoxicity against target B cells with lower release of perforin and granzyme molecules.
Image created using Biorender.com

Table 1

Table 1 Overview of CD (cluster of differentiation) antigens and other molecules involved in B and T cell collaboration along with their their function /utility

Marker (+/- Ligand/Receptor)	**Meaning / function / application**
CD3 (TCR)	T cell activation signalling and regulation of TCR expression.
CD4 (MHC II)	T helper cell
CD8 (MHC I)	Cytotoxic T cell
CD19 (co-receptor for BCR)	Pan B cell marker. Regulates B cell development, activation and differentiation.
CD20	B cell activation and proliferation. Also present on a minority of T cells.
CD27 (CD70)	Marker of B and T cell memory
CD28 (CD80/86)	Co-stimulation between B and T cells.
CD40 (CD40L)	Co-stimulation between B and T cells.
BAFF-R (BAFF) or BLyS	B-cell activating factor, enhances B cell survival
PD-1 (PD-L1 and PD-L2)	Programmed Cell Death, Down regulates the immune response
CXCL-10 (CXCR3)	Recruitment of monocytes, T cells, NK cells
CXCL-13 (CXCR5)	B cell chemoattractant
CCR2 (CCL-2 also known as MCP-1)	Trafficking of monocytes to inflammatory sites
ICOS-ICOSL	ICOS part of the CD28 superfamily, provides co-stimulatory signal to activated T cells upon binding to ICOS-L
IL21-IL21R	Promotes proliferation and function of T and B cells, an enhance cytotoxicity of $CD8^+$ T cells and NK cells
TCR-MHCII	MHC displays peptides to the TCR, TCR can discriminate foreign from self-peptides

CXCL, CXC chemokine ligand; CCR, C-C Motif Chemokine Receptor; ICOS, MCP, Monocyte chemoattractant protein; MHC, major histocompatibility complex; TCR, T cell receptor

Table 2

Table 2 Evidence for the use of CAR T cell therapies in non-malignant settings.

Specialty	Indication	Study phase/type	Outcome	Ref
Neurology	Multiple sclerosis (murine model = experimental autoimmune encephalomyelitis)	Murine model	Depleted B cells in peripheral blood & CNS Improved clinical scores of EAE	(109)
	Myasthenia Gravis (using anti-B cell maturation antigen CAR T cells)	Phase 1b/2a (human)	Safe, well tolerated, clinical improvement Phase IIb ongoing (NCT04146051)	(110)
Transplant medicine	Post transplant lymphoproliferative disorder (PTLD) post renal transplant	Case series (n=3) (human)	Demonstrated safety & feasibility (with regards to stopping immunosuppression) however only 1 of 3 patients maintained in remission at 3 months follow-up	(103)
	Case series of 3 patients with refractory PTLD post solid organ transplants (cardiac transplant, kidney transplant, pancreas transplant)	Case series (n=3) (human)	Poor outcomes, multiple complications including CRS, immune effector cell-associated neurotoxicity syndrome (ICANS), acute kidney injury, lack of response to CAR T cell therapy, mortality.	(111)
	Refractory PTLD post heart and kidney transplant	Case report (human)	6 months post CAR T cell infusion, clinically well and normal ejection fraction on echocardiography	(112)
Rheumatology	Systemic lupus erythematosus	Case series (n=5) (human)	Deep depletion of B cells, clinical improvement, normalisation of anti-ds-DNA antibodies and all achieved remission after 3 months. 3 patients repopulated B cells less than 50 days post CAR T cell therapy (although mainly naïve B cells)	(48)
	Systemic sclerosis (diffuse cutaneous)	Case report (human)	Extensive fibrosis (skin, heart, lung) – all showing improvement post treatment) Well tolerated, mild CRS (Grade 1), no signs of ICANS.	(62)
	Anti-synthetase syndrome (myositis and interstitial lung disease)	Case report (n=2) (human)	Treated with CD19-targeting CAR T cells. Excellent outcome with biochemical, serological, and radiological resolution of myositis and improvement in pulmonary function tests / CT chest.	(104) (61)

Dermatology	Pemphigus Vulgaris – target antigen Desmoglein 3	Preclinical study, ex-vivo (human)	Depletion of Dsg3 cells and antibodies in human pemphigus vulgaris model	(113)
Endocrinology	Type I Diabetes Mellitus – target antigen Insulin	Murine model	Delayed onset of diabetes but no long-term protection	(114)

Table 3

Table 3 Mechanistic differences and similarities between CAR T and TCE: Experience in Oncology

	CAR T cell therapy	**TCE**
Side effect profile	Variable between CAR T regimens. In some oncological indications, about 80% suffer CRS, longer lasting and at a higher grade Neurotoxicity: Immune effector cell-associated neurotoxicity syndrome (ICANS) occurs in approx 13-21% of patients, lasting 4-5 times longer than with TCE.	Variable between different TCE and indications. Approx. 50% suffer CRS, earlier onset but shorter duration. Obinutuzumab (anti-CD20mAb) pre-treatment limits CRS Neurologic side effects eg headache but less severe than ICANS, much less frequent than CAR T cells.
Efficacy	Higher rates of complete response in haematological malignancies	Dose dependent response, but can be up to 30% less effective than CAR T cell therapy
Pre-conditioning	Leukodepleting so higher rates of infection and risk of rejection in transplant patients.	No preconditioning, but pre-medication with dexamethasone to reduce cytokine production and with obinutuzumab for glofitamab
Hypogammaglobulinaemia	Persistence of engineered T cells in vivo resulting in sustained B cell asplasia and hypogammaglobulinaemia, may require IVIg	TCB can deplete normal B cells and plasma precursor cells leading to a higher risk of hypogammaglobulinaemia, but therapeutic regimen could be personalised according to clinical need
Effector cell type	Engineered T cells Less differentiated T cells (naïve and memory) show better efficacy than effector T cells	Endogenous T cells Antigen-experienced T cells mediate TCE induced cell death, whereas naïve T cells are not activated
Logistical differences/similarities		
Cost	+++ (~£300,000 in the UK) (115)	++ (~£56,000 per cycle UK) (116)
Production	Personalised therapy requiring individual engineering of patient's T cells – labour intensive, time consuming (resulting in disease progression), higher risk of production error. Also requires the patient to have sufficient peripheral T cell counts for successful isolation of T cells from leukapheresis.	'Off the shelf' medication, so technically less delay to administration than CAR T cell therapy. Can be manufactured in large quantities. Can be used independent of peripheral lymphocyte counts
Administration	Single intravenous administration, however, from decision to treat to administering therapy can be 6-8 weeks when disease may progress.	Shorter half-life so may need repeat dosing. Quick to administer so can treat patient promptly and halt progression of disease.

	Specialist training of staff required to administer CAR T cell therapy and monitor for complications during infusion	No additional specialist training required, similar administration to routine mAbs used such as rituximab.
Approval for use	ALL, large B cell lymphoma, mantle cell lymphoma, multiple myeloma (FDA approval)	Blinatumomab (CD3-CD19) for ALL, epcoritamab-bysp and glofitamab (CD3-CD20) for DLBCL (FDA approval), mosunetuzumab (CD3-CD20) for follicular lymphoma
Repeat treatment	Complicated due to maintenance of T cell pool, patient factors (risk of infection).	More convenient and standardised

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Highlights (5)

- B-T cell collaboration in lymphoid tissue and at peripheral sites sustains inflammation in AID
- Some memory B cells and plasmablasts evade anti-CD20 mAb treatment, are not efficiently deleted and contribute to poor disease control
- CAR T cell therapy & TCEs can redirect B-T cell collaboration towards conflict by employing T cells as effector cells to deplete B cells
- CD19-CAR T and CD19-TCEs offer an alternative novel therapeutic strategy to deplete $CD19^{+}CD20^{-}$ plasmablasts and plasma cells to overcome rituximab resistance
- TCEs may provide practical advantages as off the-shelf-reagents to CAR T cell strategy in AID

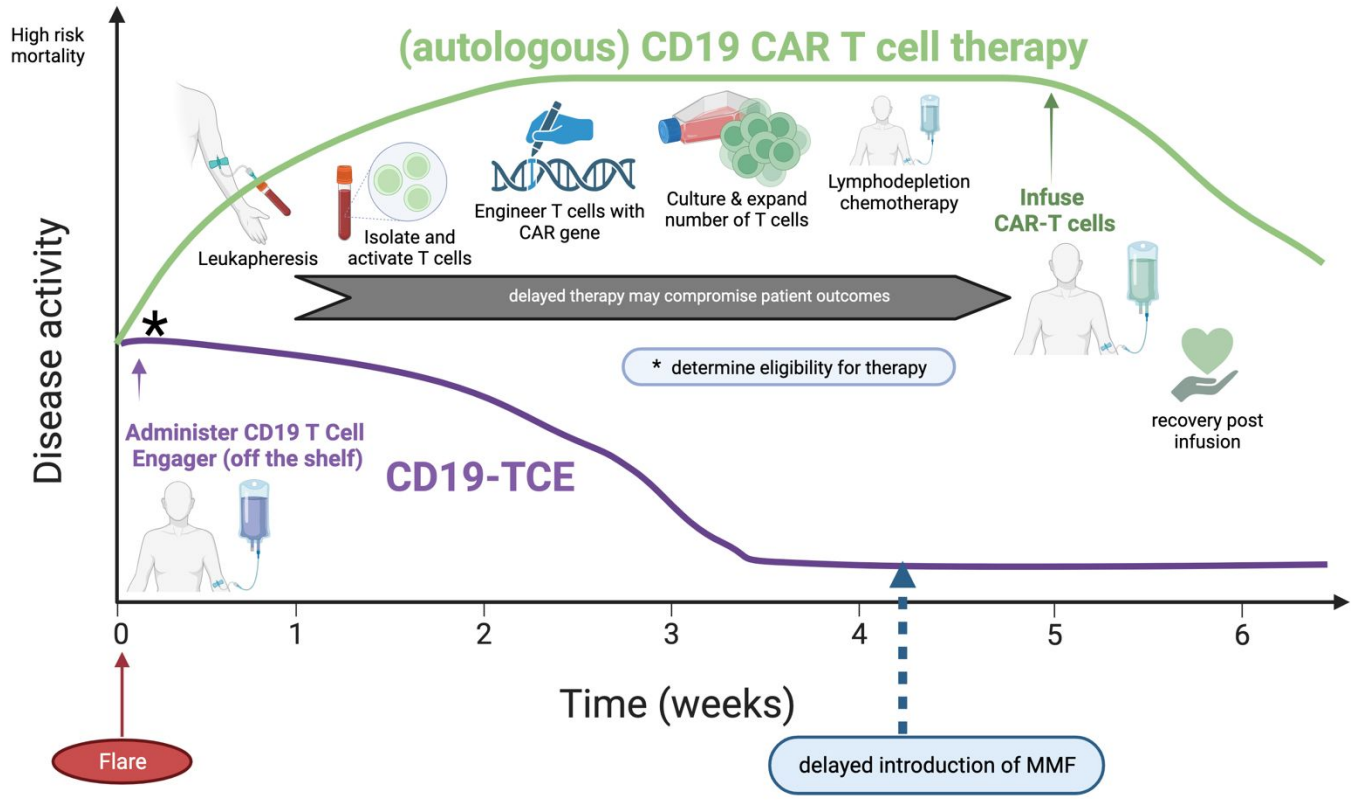
Disrupting B and T cell Collaboration in Autoimmune Disease: T cell engagers versus CAR T cell therapy?

Key words
Systemic lupus erythematosus, rheumatoid arthritis, rituximab, CAR T-cell therapy, T cell engagers

Abstract

B and T cells collaborate to drive autoimmune disease (AID). Historically, B and T cell (B-T cell) co-interaction was targeted through different pathways such as alemtuzumab, abatacept, and dapirolizumab with variable impact on B cell depletion (BCD), whereas the majority of patients with AID including rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and organ transplantation benefit from targeted BCD with anti-CD20 monoclonal antibodies such as rituximab, ocrelizumab or ofatumumab. Refractory AID is a significant problem for patients with incomplete BCD with a greater frequency of IgD^{-} $CD27^{+}$ switched memory B cells, $CD19^{+}CD20^{-}$ B cells and plasma cells that are not directly targeted by anti-CD20 antibodies, whereas most lymphoid tissue plasma cells express CD19. Furthermore, B-T cell collaboration is predominant in lymphoid tissues and at sites of inflammation such as the joint and kidney, where BCD may be inefficient, due to limited access to key effector cells. In the treatment of cancer, chimeric antigen receptor (CAR) T cell therapy and T cell engagers (TCE) that recruit T cells to induce B cell cytotoxicity have delivered promising results for anti-CD19 CAR T cell therapies, the CD19 TCE blinatumomab and CD20 TCE such as mosunetuzumab, glofitamab or epcoritamab. Limited evidence suggests that anti-CD19 CAR T cell therapy may be effective in managing refractory AID whereas we await evaluation of TCE for use in non-oncological indications. Therefore, here, we discuss the potential mechanistic advantages of novel therapies that rely on T cells as effector cells to disrupt B-T cell collaboration toward overcoming rituximab-resistant AID.

Graphical Abstract



This graphical abstract demonstrates the distinct time courses of CD19 CAR T cell ~~therapy~~ (green line) versus CD19-T cell engager (TCE) treatments (purple line) for a patient post flare of their autoimmune disease. Accessibility to CAR T cell therapy can be significantly limited to major centres of expertise. Significant disadvantages of CAR T therapy include~~such as~~: 1) high production costs; 2) labour intensive processes; 3) ~~a~~ delay in establishing a sufficient effector T cell pool; and 4) tolerability of pre-requisite toxic lymphodepleting chemotherapy regimen which may restrict CAR T cell therapy to a smaller cohort of patients and potentially compromise patient outcomes. In contrast, ~~On the contrary,~~ CD19-TCE is readily available, off the shelf, and can be administered immediately post identification of a disease flare, thereby ameliorating the risks of disease progression associated with the lag time to receive CAR T cell therapy. The lack of pre-requisite ~~preceding~~ chemotherapy associated with CD19-TCE is ~~equally favourable~~ also positive as it reduces the associated risk of infections and cancers.

Image created in Biorender.com

Background

B-T cell collaboration in the pathogenesis of autoimmune disease

B and T cell (B-T cell) collaboration perpetuates chronic inflammation in a range of autoimmune diseases (AID) including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and multiple sclerosis (MS) (1, 2). This cellular collaboration may occur through contact -dependent or -independent pathways through cytokines and other immune stimuli. Within lymphoid aggregates and the germinal centre, B-T cell interactions involve an array of molecular pairings ~~including TCR-MHCII, CD28-CD80/86, CD40-CD40L, ICOS-ICOSL, SLAMF-SAP, PD-1-PD-L1, IL-21-IL-21R~~(3), summarised in Figure 1 and ~~explained in~~ Table 1**.** These signals stimulate T cell secretion of cytokines ~~IL-4 and IL-21~~ and promote differentiation of naïve to memory B cells and plasma cells (PCs), Figure 1~~1~~. Some of these pathways have been targeted, as discussed later, whereas others are the subject of novel therapeutic strategies ~~are being explored~~.

In this context of an ongoing immune response, an appreciation of B cell biology is helpful~~of relevance~~. B cells originate from haematopoietic stem cells in the bone marrow and undergo differentiation in secondary lymphoid organs (4). Differential expression of various cell surface markers, including cluster of differentiation (CD) molecules and immunoglobulin isotypes help to define classical subpopulations including: naïve B cells (IgD+CD27-), unswitched memory B cells (IgD+CD27+), switched memory B cells (IgD-CD27+) and double negative memory B cells (IgD-CD27-) (4). Naïve B cells have not yet encountered antigen, whereas switched memory B cells are primed to respond to antigen and double negative memory B cells increase with ageing, autoimmunity and chronic infectious diseases (5). Until recently, the focus of B cell depletion therapy has been on rituximab, an anti-CD20 monoclonal antibody which is widely used in haematological malignancies and ~~in~~ AID (discussed in more detail below). The first FDA approved targeted biologic therapy for SLE was Belimumab, a mAb directed at ~~BAFF,~~ B-cell activating factor (BAFF, also known as BLyS) (6), however, real world data demonstrates variable success (7) (8). BAFF is a B cell survival and differentiation factor and is ~~found to be~~ elevated in the serum of patients with SLE (9).

B-T cell interactions in the peripheral inflammatory sites of various AID including RA SLE, type I diabetes mellitus and coeliac disease exhibit a population of T cells which are termed T peripheral helper cells (1, 10, 11). Rao et al identified these cells, adjacent to B cells in lymphoid aggregates of the synovium in patients with RA as PD-1hiCXCR5^{-}CD4^{+} which lack Bcl6 but produce IL-21 and CXCL13, resulting in B cell differentiation into plasmablasts (PBs)(12). This perpetuates B-T cell networking in inflamed tissues, where ectopic lymphoid structures(13) are formed. Thus, B-T cell collaboration occurs in both lymphoid tissues and at sites of inflammation.

Disrupting the B-T cell networking in AID, historical perspectives

B-T cell collaboration is a dominant source of chronic inflammation in AID. Hence, disrupting this network is an appealing therapeutic strategy. Over the past four decades, B-T cell co-stimulation was targeted through different pathways such as alemtuzumab (anti-CD52 monoclonal antibody, CAMPATH-1H), abatacept (cytotoxic T-lymphocyte antigen 4 immunoglobulin), and dapirolizumab (anti-CD40L) with variable impact on B cell depletion (BCD), Figure 222. In the 1980s, alemtuzumab was used to deplete CD52 expressing cells including B and T cells, providing the first insights into disrupting B-T cell networking. The 1990's trials of alemtuzumab in RA were terminated due to suboptimal therapeutic index probably owing to prolonged depletion of regulatory T cells(14), although it continues to be used to treat MS (albeit at lower doses). Abatacept inhibits the co-stimulatory CD28-CD80/86 pathway and is approved for RA(15) although the ALLURE trial of abatacept in lupus nephritis (LN) did not meet its primary end point(16). Attempts have been made to block other key co-stimulatory signalling pathways including the CD40-CD40L axis. Second generation agents have been developed including dapirolizumab-pegol which had favourable biomarker and safety response in SLE(17); phase III results are awaited (NCT04294667). Therefore, despite these advances, there remains a great unmet need for disrupting B-T cell collaboration in refractory patients with AID.

BCD with rituximab in RA and SLE; why is it suboptimal?

In the past three decades, BCD therapy with the CD20 monoclonal antibody rituximab, has revolutionised the treatment of severe or refractory AID and has been approved for use in RA(18), ANCA vasculitis(19), and pemphigus vulgaris (PV)(20) and is prescribed widely 'off-licence' in SLE(21) and in immune thrombocytopenic purpura (ITP) (22). Data from the Lupus Nephritis Assessment with Rituximab (LUNAR) study reported complete BCD with complete response, as defined in the study(23). However, there remains a significant proportion of patients, up to 30%, who have disease refractory to rituximab, particularly in the context of incomplete BCD (21) and/or repopulation with PB and switched memory B cells ($IgD^{-}CD27^{+}$, SwMBC)(24).

How do memory B cells and $CD19^{+}CD20^{-}$ PBs evade rituximab?

B cells can evade rituximab's effects either through intrinsic mechanisms (lacking CD20 expression and antigenic modulation) or extrinsic mechanisms such as restricted vascular access to effector cells as discussed previously(25). Upon activation naïve B cells solicit T cell co-stimulation in lymphoid tissues and at sites of inflammation such as the joint and the kidney to differentiate into memory B cells and antibody secreting cells including short-lived $CD19^{+}CD20^{-}$ PBs and long-lived $CD20^{-}$ PCs(12, 26). In RA, rituximab fails to completely deplete SwMBC and $CD19^{+}CD20^{-}$ PCs in lymphoid tissues(27), joints and bone marrow(28-30) contributing to poor response. In patients with ITP with poor response to rituximab, autoreactive splenic memory B cells down-regulate their BCR and up-regulate anti-apoptotic proteins and

evade rituximab whilst retaining the capacity to reactivate and differentiate into autoantibody secreting $CD19^+CD20^-$ PBs(22). In muscle-specific kinase myasthenia gravis, autoreactive SwMBC evade rituximab and differentiate into autoantibody secreting $CD19^+CD20^-$ PBs contributing to relapse(31). Further, rituximab has no direct effect on $CD19^+CD20^-$ PBs and PCs, as they do not express CD20(32, 33). Thus, SwMBCs, $CD19^+CD20^-$PBs and $CD19^+CD20^-$PCs evade rituximab through distinct mechanisms, Figure 333.

Broadly, anti-CD20 mAbs can be ~~divided~~ grouped into type I and type II, where type I mAbs such as rituximab, are more efficient at clustering CD20 ~~into detergent insoluble lipid rafts~~ compared to type II anti-CD20 mAbs (34). This enables efficient complement activation and therefore enhanced complement-dependent cytotoxicity (CDC), however it also increases the propensity for internalisation of CD20:CD20 mAb complexes by B cells ((35)).

In addition, incomplete BCD with rituximab may be related to its internalisation of rituximab(36). Type II anti-CD20 mAbs such as obinutuzumab may, at least in part, overcome this resistance mechanism(25). In a pivotal phase II study, obinutuzumab was shown to improve clinical response in LN(37) and phase III studies are ongoing. However, $CD19^+CD20^-$ PBs and $CD19^+CD20^-$ PCs are still not directly targeted. Furthermore, disease-associated macrophage phagocytic defects(38) and vascular access limitations may compromise the ability of anti-CD20 mAbs (and other B cell depleting mAbs, such as those directed to CD19) to evoke antibody dependent cellular phagocytosis (ADCP)(25, 39) as they rely on FcγR-bearing effector cells. In addition, NK cells are also scarce in tissues, limiting antibody dependent cellular cytotoxicity (ADCC). For example, we have previously reported that incomplete depletion and / or persistent infiltration of B cells in the kidneys was associated with active LN refractory to rituximab(40).

Through histological analysis of kidney(41) and skin(42) of patients with AID, and the synovium in patients with RA (12), we know that B cells interact with T cells in lymphoid tissues and at sites of inflammation, to differentiate into autoantibody secreting PBs and PCs. At these sites, limited access to rituximab's key effector cells, macrophages, and NK cells, may compromise depletion. Thus, antigen expression, modulation and access to effector cells influence the efficiency of rituximab-mediated BCD. Therefore, it is important to consider both alternative target antigens and therapies that recruit other effector cells to improve BCD.

Approaches to overcome rituximab resistance in AID

Is CD19 an ideal target?

CD19 regulates the threshold for B cell activation as a co-receptor of the BCR complex (43) with consequent implications for influencing autoimmunity(44). CD19 deficiency impairs humoral immunity, at least in part, due to an increased threshold for B cell activation(45) whereas overexpression is associated

with AID such as SLE(26). When compared with $CD19^{-}CD20^{-}$ PCs, $CD19^{+}CD20^{-}$PCs accumulate more mutations and retain greater proliferative capacity, at least in vitro(32). These observations implicate a significant role for CD19 in B cell differentiation and activation.

When compared with CD20, B lineage cells express CD19 at an earlier stage in development and retain expression through all stages of differentiation into $CD19^{+}CD20^{-}$ PBs and some $CD19^{+}CD20^{-}$ PCs(26). $CD19^{hi}CD11c^{+}$ memory B cells in humans were shown to respond robustly to antigen challenge, in vitro(46). More recent evidence suggests that double negative (IgD-CD27-) DN B cells which express the transcription factor ~~T-bet (~~T-box expressed in T cells (T-bet) encoded by *Tbx21 ~~gene~~*, termed DN-T-bet^{+} B cells are expanded in ageing, are associated with higher mortality from COVID-19 infection and disease activity in SLE as well as disease pathogenesis in RA.~~,~~ T~~t~~herefore they are of great interest in the field of B cell research ~~in autoimmunity~~ (47).

Further, ~~double negative (IgD-CD27-) T-bet^{+} B cells, which are implicated in SLE pathogenesis and flare~~they~~,~~ demonstrate increased expression of CD19 which strengthens the argument to target CD19 in AID (Shah et al Manuscript in preparation). Considering the availability of newer therapies that target CD19 particularly in the field of oncology, we reappraise the concept of targeting CD19, put forward over a decade ago, to treat AID(26). In addition, evidence from oncology highlights that cancers refractory to monoclonal antibodies have been effectively treated with CD19-targeted chimeric antigen receptor (CAR) T cells, probably owing to the deeper depletion of B cells which provides promise for patients with AID resistant to current mAb therapy, highlighted by the published case series in SLE(48). These mechanistic considerations indicate that targeting CD19, particularly in AID, may overcome anti-CD20 mAb resistance.

How to target CD19 - T cell engagement as a mechanism of action?

Therapeutic options to target $CD19^{+}$ B cells and PCs include: 1) anti-CD19 mAbs; 2) CD19-targeted CAR T cells; and 3) CD19-directed T cell Engagers (TCE). The anti-CD19 mAb inebilizuzmab is approved for the treatment of neuromyelitis optica spectrum disorder(49) and showed initial promising results in a clinical trial in systemic sclerosis(50). BCD with inebilizumab was greater in transgenic mice blood and spleen as well as in an in vitro ADCC assay using human PBMCs when compared to rituximab(51). However, similar to rituximab, anti-CD19 mAbs are also disposed to internalisation(52) and would be limited by disease-associated macrophage phagocytic defects(38) and vascular access limitations. Therefore, CD19-directed CAR T cells and CD19 TCE may be of greater utility in AID and will be discussed in the following sections.

CAR T cell therapy

The introduction of CAR T cells to treat cancer has been instrumental in providing individualised, targeted treatment through genetically engineered T cells that express a CAR specific to a tumour associated antigen, such as CD19 in B cell(53)malignancies. Recognition of the target antigen bearing B cells activates CAR T cells to proliferate and selectively eliminate the target B cells. The basic structure of a CAR includes an extracellular surface domain for antigen recognition (typically derived from an antibody

fragment), a transmembrane domain and an intracellular signalling domain which activates T cells (typically derived from CD3z chain). The evolution of CAR ~~structures is depicted in Figure 6, however in brief the modifications~~ from first to fourth generation ~~CARs~~ includes the addition of co-stimulatory domains (one in second generation and two in third generation CARs) as well as co-expression of additional transgenes for cytokine secretion (fourth generation) (54), **Figure 4.**

Once administered, CAR T cells can also expand and establish immune memory, thus providing long term surveillance of disease as described in malignancy(55). CAR T cell therapy has been approved for the treatment of B cell acute lymphoblastic leukaemia (ALL), lymphoma and multiple myeloma(53). Factors such as antigen overload are considered to contribute to undesirable effects including cytokine release syndrome (CRS) and neurotoxicity~~pathy~~, leading to newer generation therapies with fewer toxicities being developed(56). Complete remission for at least three years, of various relapsed B cell malignancies was demonstrated in 51% of patients treated with CAR T cell therapies, with few late onset side effects(57). This success led to CAR T cells being explored for treating refractory AID.

CAR T cell therapy in AID

The success of using CAR T cell therapy for the management of B cell malignancies inspired its research in a range of AID including SLE, myasthenia gravis and type 1 diabetes mellitus, as outlined in Table 1~~Table 1~~. In animal models of SLE, anti-CD19 CAR T cell treatment resulted in profound and sustained BCD with low circulating PCs and increased survival rates(58). This data provided the basis for the use of anti-CD19 CAR T cell therapy in the treatment of 5 patients with refractory multiorgan lupus which was well tolerated leading to serological and clinical remission at relatively short follow up(48). Probably owing to lower antigen load, the first ~~seven~~ cohort of patients with SLE treated with anti-CD19 CAR T cell therapy experienced only low grade CRS(59), of which tocilizumab (anti-IL-6 receptor mAb) was used (successfully) in only one patient owing to persistent fevers for 3 days(48). Thus, current preliminary evidence suggests that CD19 targeting CAR T cell therapy seems a safe and effective therapeutic strategy in AID such as SLE. Anti-CD19 CAR T cell therapy was associated with a reduction in autoantibodies and pro-inflammatory cytokines including IL-6 and TNF-α (60). Intriguingly, despite excellent clinical responses, the authors demonstrated an increase in serum BAFF levels.

~~Additionally,~~With regards to other autoimmune diseases, single case studies of anti-CD19 CAR T cell therapy indicate a potential use of the approach also in anti-synthetase syndrome(61) and systemic sclerosis(62). To note, an important potential confounder when appraising the mechanisms of response to CAR T cell therapy is the use of lymphocyte depletion with fludarabine that may have contributed to response. Several studies exploring the safety, tolerability, and preliminary efficacy of anti-CD19 CAR T cell therapy in AID have been initiated (NCT05938725, NCT05869955, NCT03030976, NCT05798117, NCT05930314).

Limitations of CAR T cell therapy

Although the case examples of anti-CAR T cells in AID are promising, it is also important to understand the limitations. Two of the 5 patients treated with anti-CD19 CAR T cell therapy had persistence of clonotypic IgG in follow-up samples, demonstrating suboptimal depletion and/or rapid repopulation of memory B cells(48). Remarkably, despite lower antigen overload, three of five SLE patients treated with anti-CD19 CAR T cell therapy repopulated their B cells by day 50 after treatment(48) when compared with prolonged BCD achieved in B cell malignancies up to several years post infusion(53). Potential explanations for incomplete depletion and/or relatively early repopulation of B cells include: 1) complete depletion of target cells removing the sustained stimulus needed to maintain an optimal pool of CAR T cells, as CAR T cells had disappeared at week 4 after treatment; 2) higher proportion of senescent and/or exhausted SLE CAR T cells; and 3) potential inhibition of CAR T cell expansion due to the persistent effects of immunosuppression such as mycophenolate mofetil beyond cessation of therapy(63).

Implications of lymphodepletion in AID

Patients with AID, particularly SLE, are often lymphopenic owing to the underlying disease process and the effects of immunosuppression, which may impact the process of leukapheresis required to generate the CAR T cells. Nevertheless, patients with active SLE in the previously discussed case series (48) were successfully leukapheresed before CAR T cell therapy and concurrent treatment with steroids and immunosuppressive agents (64). The process of lymphodepletion itself increases the likelihood of infections and is an additional step preceding ~~leading up to receiving~~ CAR T cell therapy, compared to 'off the shelf' TCE therapy.

Risks of hypogammaglobulinaemia

A major consideration with CAR T cell therapy is the risk of hypogammaglobulinaemia; this may be observed with TCE but likely to a lesser extent. In the treatment of cancer, approximately a third of patients develop hypogammaglobulinemia following CAR T cell infusion (65), owing to potent and persistent depletion of normal $CD19^{+}$ B cells. Very low IgG levels can arise from 9 weeks after treatment and continue beyond 4 years (65). This poses a risk of serious life threatening infections, necessitating intravenous immunoglobulin infusions as a prevention strategy, as per the majority of trials (66), however, this can be expensive and not readily accessible for all patients.

Importantly, B cell aplasia and hypogammaglobulinaemia result in suboptimal vaccine responses, which is also a significant concern especially in the current era of SARS-CoV-2~~COVID-19~~ infection with only 29% of patients who receive CAR T cell therapy for lymphoma/myeloma mounting a clinically relevant antibody response to ~~COVID-19~~ vaccination (67). Reassuringly, vaccine responses were stable following CAR T cell therapy in the SLE case series (48), likely related to the remaining pool of $CD19^{-}$ plasma cells which are able to secrete antibodies two years post treatment (68). ~~2 years post CD19-targeted CAR T cell therapy, 2~~

years post CD19-targeted CAR T-cell therapyThese aspects also need to be accounted for during TCE trial design in AID.

Logistical limitations of CAR T cell therapy

Here we consider the potential risks of sustained BCD. The process of lymphodepletion itself increases the likelihood of infections and is an additional step leading up to receiving CAR T cell therapy, compared to 'off the shelf' TCE therapy. A major consideration with CAR T cell therapy is the risk of hypogammaglobulinaemia; this may be observed with TCE but likely to a lesser extent. In the treatment of cancer, approximately a third of patients develop hypogammaglobulinemia following CAR T cell infusion(54), owing to potent and persistent depletion of normal $CD19^{+}$ B cells. Very low IgG levels can arise from 9 weeks after treatment and continue beyond 4 years(54). This poses a risk of serious life threatening infections, necessitating intravenous immunoglobulin infusions as a prevention strategy, as per the majority of trials(55), however, this can be expensive and not readily accessible for all patients.

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Logistical limitations are also considerable. For example, in patients with rapidly progressing cancer or AID, the practical feasibility of CAR T cell therapy may be limited as there is typically a protracted vein-to-vein time of approximately 6-8 weeks, due to the time required for producing, transporting, and ensuring quality control of the personalised cell therapy, as illustrated in the graphical abstract. This process is typical for most CAR T cell therapies, although the novel YTB323 omits the ex-vivo expansion stage(NCT05798117). (57)

Further disadvantages of CAR T cell therapy include the high cost involved with engineering and storage of CAR T cells and the specialist training required to administer treatment as detailed in Table 2. Therefore, readily available and effective novel treatments are required whilst awaiting CAR T cell therapy(69). One approach to obviate the limiting factor of individual custom-made CAR T cells, is the generation of 'universal CAR T cells' as reviewed thoroughly by Zhao et al (54). These can serve as 'off the shelf' therapies to treat a wide range of clinical indications as they are engineered to target multiple antigens.

Further gene editing work is underway to ensure universal CAR T cells are not depleted by the recipient's immune system ~~(NK cells)~~ and are able to expand without causing harmful effects (70).

To this end, we consider alternative strategies, with the potential of TCE bispecific antibodies as a novel therapeutic option to disrupt B-T cell collaboration in AID. Table 2 outlines the major differences and similarities of using CAR T cell therapy and TCEs.

TCE: clinical trial experience and technical aspects

TCE represent a novel class of targeted therapeutics which recruit T cells(71). From a clinical perspective, in the late 1990's, the potential for bispecific antibodies as therapeutic interventions became clearer for cancers such as breast, leukaemia and lung(72), which led to a surge of interest in their use and FDA approval of catumaxomab for malignant ascites(73) and blinatumomab for refractory B-ALL(74) More recently, three CD20 T cell engagers, mosunetuzumab, glofitamab and epcoritamab have been approved for treatment of refractory/relapsed follicular lymphoma and refractory/relapsed diffuse large B cell lymphoma (75) . Technological advancements over time have enabled a range of modifications to enhance the flexibility and number of binding sites, half-life, production yield and potency of these therapeutics(76).

TCE technologies

TCEs can be broadly categorised into: 1) small, short half-life bispecific antibody fragments (single chain variable fragments) such as bispecific T cell engagers (BiTE®s) which require repeated administration (Figure 544A); and 2) larger IgG-based T cell bispecific antibodies (TCBs) with extended half-lives (Figure 544B and C). The development of TCBs has evolved from single chain variable fragments in the early 1990s(77), to the development of 'knobs into holes' (KiH) technology in the late 1990s(78) to the more advanced technologies including CrossMab to engineer bispecific antibodies(79, 80), Figure 544.

CD19-TCE

Blinatumomab, a BiTE® composed of two single chain antibodies targeting CD19 on B cells and CD3ε on T cells fused via a flexible linker (Figure 544A), is approved for B cell ALL(75). It is engineered to have a short half-life of 2 hours to enable tight control of serum levels in case of adverse events. Blinatumomab relies on the presence of $CD19^+$ target cells to activate T cells, with sensitive response from $CD8^+$ T cells to induce lysis of tumour cells as demonstrated in video-assisted microscopy studies(81). In vitro studies of human B lymphoma cells demonstrated a higher degree of tumour cell elimination with blinatumomab compared to rituximab(82). Interestingly, the combination of blinatumomab and rituximab was synergistically more efficient, especially at low effector-to-target cell ratios and low Blinatumomab concentrations(82). This combined effect was found to be due to potent activation of pro-caspases 3 and 7 in target cells, which is instrumental in triggering granzyme-mediated apoptosis. The BiTE subtype is potent

with regards to target cell killing. Regardless, the requirement for repeat dosing of Blinatumomab may limit its routine use in clinical practice.

CD20-TCE

Three CD20 TCE have been approved for refractory B cell lymphomas: mosunetuzumab, glofitamab and epcoritamab(75), Figure 544. Mosunetuzumab is an IgG based TCE with 1:1 binding to CD20 and CD3; it uses KiH technology and in vitro assembly to overcome incorrect light chain association (83). Epcoritamab is also IgG based, although employs the unique DuoBody® technology with point mutations in each Fc region (CH3 domain) to allow controlled Fab-arm exchange (84). Recent IgG-based TCEs have been developed for increased avidity. Glofitamab has two Fab regions which bind CD20, one Fab region which binds CD3 (so-called 2:1 format), and a longer half-life of 10 days, owing to its Fc region and interaction with FcRn(80). The Fc also includes the P329G LALA mutations (71), which abolish conventional effector functions and therefore it employs a different mechanism of action compared to rituximab. The 2:1 format (Figure 544C) enables greater potency with regards to B cell cytotoxicity compared to 1:1 antibodies, thought to be due to the close proximity of the CD20 binder and CD3 binder, resulting in a more stable T cell to target B cell synapse induced by the head-to-tail fusion design(85). ~~About 24 hours following administration of the CD20-TCE in stem cell humanized NSG mice, peripheral blood CD4⁺ and CD8⁺ T cell activation results in secretion of cytokines including interferon-γ (IFNγ), tumour necrosis factor α (TNFα), monocyte chemoattractant protein-1 (MCP-1), Macrophage Inflammatory Protein-1β (MIP-1β), interleukin (IL)-2, IL-5, IL-6, IL-8 and IL-10 which return to baseline by 72 hours(59). This spike in secretion of cytokines was not seen after a second administration of CD20-TCE at day 10 due to almost complete BCD in the peripheral blood following the first dose.~~

Effector mechanisms of TCEs: lessons learnt from treating malignant disease

Bispecific antibodies can redirect the effector function of various immune cells. T cells are promising as effector cells as they are abundant, able to expand rapidly, and have potent cytotoxic capacity. TCE are designed to by-pass the normal major histocompatibility complex–T cell receptor (MHC-TCR) interaction usually required between antigen presenting cells and T cells, and instead co-engage the CD3 molecules on the T cell and form an immunological synapse via the target antigen such as CD19 or CD20 on the surface of B cells that helps redirect co-stimulation to cytotoxicity(86, 87), Figure 655. This synapse is similar to that formed during cytotoxicity with CAR T cells.

The CD20-TCE recruitment of T cells is evident in in vitro culture assays demonstrating that tumour lysis is dependent on T cell recruitment, activation, and expansion of $CD4^+$ and more profoundly $CD8^+$ subsets. ~~Following CD20-TCE administration in humanized NSG mice, T cell activation, based on upregulation of CD25, PD-1, Granzyme B, and other markers of TCR complex cross-linking, was detectable on CD4⁺ and CD8⁺ T cells~~(71). Importantly, CD20-TCE depleted B cells in the spleen and lymph nodes, efficiently(71).

These findings may be of relevance to AID where inefficient BCD in lymphoid tissues and inflammatory sites, as discussed earlier, contributes to refractory disease.

Employing T cells to disrupt the B-T collaboration: CAR T and TCE

As discussed above, in AID, B and T cells colocalise in lymphoid tissues and at inflammatory sites. Therefore, using CAR T cells or TCE that employ T cells as effector cells to deplete B cells may provide a distinct advantage over rituximab-mediated BCD that relies on macrophages and/or NK cells as the dominant effector mechanism. The key differences and similarities between CAR T cell therapy and TCE therapy are described in Table 3~~Table 2~~.

~~Patients with AID, particularly SLE, are often lymphopenic owing to the underlying disease process and the effects of immunosuppression, which may impact the process of leukapheresis required to generate the CAR T cells. Nevertheless, patients with active SLE in the previously discussed case series(43) were successfully leukapheresed before CAR T cell therapy and concurrent treatment with steroids and immunosuppressive agents(77).~~

Aside from requiring lymphodepletion, an important aspect to highlight is that the expansion of CARs in vivo cannot be controlled, demonstrated by the rapid rise in circulating CARs, reaching up to 59% by day nine post infusion(48).

In addition, the expansion and duration of CAR T cell action is not easily controlled, whereas~~,~~ a TCE can be given at a specific dose and the half-life of the molecule is expected to determine its duration of action. Overall, treatment with TCE may potentially overcome some of these limitations of CAR T therapy such as a lag time from decision to treatment to allow for engineering of CAR T cells, prior leukapheresis, and requirement for specialist centres with experience of cell-based immunotherapies.

Immunological/Biological pitfalls in recruiting T cells as effector cells

Despite the undoubted promise of CAR T cells and TCE, there remain potential hurdles. Both CAR T cells and TCE may evoke ‘bystander killing’ of antigen-negative cells directly in contact with antigen-positive cells(88). Whilst this local bystander effect is desirable in the treatment of solid tumours to prevent escape of antigen-negative cancer cells, the potential implications of this in AID are unknown.

More recently, there are an increasing number of reports of macrophage activation syndrome (MAS)/haemophagocytic lymphohistiocytosis (HLH) as a complication of CAR T cell therapy given for haematological malignancies, possibly as a distinct variant of CRS (89). MAS/HLH is a serious condition of

hyperinflammation, fevers, cytopaenias, and can be life threatening. Patients with autoimmune disease such as SLE are already predisposed to developing secondary MAS/HLH (90), therefore initiation of CAR T cell therapy in this cohort needs careful consideration.

Another potential pitfall with recruiting T cells as effector cells is a possible reduction in T cell counts, which may increase the risk of infection, due to apoptosis noted with first generation CAR T cell treatments (91). Reassuringly, in studies with CD20-TCB, peripheral T cell counts decreased in the first 24 hours of drug administration before returning to baseline by 72 hours(71), considered to reflect an activation induced marginalisation. Therefore, the risk in the short term with these agents seems low but will need monitoring in the long-term.

Impact of the tissue microenvironment

An additional consideration is the tissue microenvironment, which is known to influence T cells cytotoxicity. AID related T cell subpopulations with features of anergy, exhaustion and senescence may compromise the efficiency of TCE(92). In addition, resistance to TCEs may arise from immune escape, through the expression of immune checkpoint molecules such as PD-1. In this context, combination treatment with checkpoint inhibitors, already explored in cancer immunotherapy may be limited by the potential activation of autoreactive T cells(93). Alternatively, next generation trispecific TCEs to additionally provide co-stimulation may be beneficial(94). As CD3 is a pan T cell marker, TCEs can recruit all T cell populations including naïve, regulatory T cells and exhausted T cells as effector cells. In AID, regulatory and exhausted T cells are associated with disease remission and improved prognosis(95). Mechanistic clinical studies will help us understand the clinical relevance of these potential limitations.

Clinical adverse effects of recruiting T cells as effector cells

The main adverse effect associated with both types of T cell therapy is CRS, which is the rapid systemic release of pro-inflammatory cytokines including IL-6, IL-10, TNF-α and IFN-γ, upon activation of the T cells(96). CRS manifests as fever, fatigue, vasodilation and can lead to multi-organ failure. Pre-treatment with corticosteroids such as dexamethasone may reduce the risk of CRS. Anti-IL-6 receptor antibody, tocilizumab, has been approved for use prior to CAR T cell therapy to attenuate CRS(97). In murine models, combination treatment with Janus Kinase (JAK) inhibitors or mammalian target of rapamycin (mTOR) inhibitor, restricted CD19-TCB related CRS whilst retaining their efficacy(98).

Immune effector cell-associated neurotoxicity syndrome (ICANS) is another dose-dependent unwanted side effect unique to patients receiving T cell engaging treatments, through adherence of T cells to cerebral microvascular endothelium and migration across the blood-brain barrier(99). In ALL, ICANS, characterised by headache, dizziness, tremor, confusion, and encephalopathy, was associated with high dose blinatumomab given in the first treatment cycle, probably owing to the higher tumour burden. As the target

cell load is much lower in AID, the required dose of TCEs will be lower, consequently, the risk of CRS and ICANS should be lower than that reported for cancer immunotherapy.

What is the impact of immunosuppressive therapy on T cell cytotoxicity in the context of TCE and CAR T cells?

Other important considerations include AID-specific concurrent drug regimens. For example, transplant recipients and patients with AID and transplant recipients receive immunosuppressants to regulate immune response. In the context of T cell-based therapy, concurrent use of immunosuppressants may inhibit the effector function of the T cells, thereby, compromising the efficiency of CAR T cells and TCEs. For example, mycophenolate mofetil (MMF) can induce apoptosis in activated human T cells(100); and in a murine model, mycophenolic acid, the active form of MMF has shown dose-dependent reduction in the generation of cytotoxic T cells ~~cytotoxicity by mycophenolic acid, the active form of MMF~~(101). Figure 6~~5~~5 illustrates the potential impact of immunosuppressants on T cell cytotoxicity in the context of TCE and CAR T cell therapies. Therefore withholding immunosuppressants for a period of time to allow for T cell recovery to enhance performance may be considered in prospective trial design(102).

In a case series of renal transplant recipients requiring CAR T cell therapy for post-transplant lymphoproliferative disorders (PTLD), MMF was discontinued at the time of PTLD diagnosis (with DLBCL), and tacrolimus was stopped 2 weeks prior to leukapheresis for production of CAR T cells(103). Similarly, a report of CAR T cell infusion for anti-synthetase syndrome involved tapering azathioprine and steroids seven days before leukapheresis and starting MMF 35 days after CAR T cell infusion(104), which allowed for harvesting of fully functional T cells. This aligns with our proposition of correct sequencing of immunosuppressive treatments including the use of corticosteroids to allow full efficacy of TCE and/or CAR T therapies.

Developing personalised B cell targeting regimens

Where pathogenic B cell identity is well described, CAR T therapy can potentially enhance the prospects for personalised therapy. For example, desmoglein 3 targeting CAR T cells were engineered to selectively eliminate Dsg3 specific B cells, in vitro and in vivo in animal models(105) toward developing therapies for PV. Currently, a phase I study of BCMA CAR T therapy (NCT04561557) is ongoing for the treatment of neurological disorders including Aquaporin related neuromyelitis optica spectrum disorder (NMOSD). However, the identity of pathogenic B cells remains elusive for the majority of AID, where non-selective BCD therapy remains the current standard strategy.

In routine practice of managing AID, rituximab induction therapy incorporates two doses of 1 gram, given two weeks apart. Retreatment with the same or lower dose of rituximab, is usually at six months or longer for optimal management of disease activity(15). Current evidence highlights that response can be improved

with better depletion with a lower frequency of memory B cells and PB in RA and SLE (25). As discussed previously, presumably due to more efficient BCD, obinutuzumab treatment seems to be effective in LN(37). To this end, targeting CD19 and disrupting the B-T cell networking in AID, with CD19/CD3 TCEs or CAR T cells would be expected to provide mechanistic advantages. For example, targeting CD19, expressed on memory B cells, $CD19^{+}CD20^{-}$PBs and $CD19^{+}CD20^{-}$PCs should help deplete these 'rituximab-resistant cells' whereas the use of TCEs would help direct T cells from B cell 'co-stimulation to cytotoxicity' to disrupt B -T networking. Key lessons from previous SLE rituximab trials include 1) patient selection with regards to disease manifestations, severity of disease activity, serological parameters, and previous treatment are important to consider so as not to exclude the most active patients, 2) defining standard concomitant therapy in the comparator and placebo arms as variable usage of glucocorticoid and immunosuppressants such as MMF can impact outcomes, 3) defining endpoints in particular the steroid sparing effect, 4) selecting the right disease activity index and 5) defining follow up duration and side effects. These serve as a reminder of the importance of optimal trial design to evaluate the 'real' potential of TCE(23, 106).

Optimising co-therapies with immunosuppressants, and sequential therapy with rituximab

Co-therapy with immunosuppressants and/or rituximab therapy may influence the efficacy and safety of TCEs. As demonstrated in Figure 6~~5~~5, patients with AID are often being treated with immunosuppression such as MMF and corticosteroids. Therefore, considering discontinuation of MMF for three(48) to six weeks may optimise the effector function of T cells to disrupt the B-T cell network in AID. Thereafter, a delayed introduction of MMF may be considered as needed for optimal control of disease activity.

Sequential therapy with rituximab, which is already competitively priced as a biosimilar, followed by CD19-TCE will enable targeting of B-T cell networks in ectopic lymphoid tissue within peripherally inflamed tissues in AID, Figure 3~~3~~3. A potential limitation of this sequence is that rituximab therapy may result in lower expression of CD19(22), probably through internalisation as shown in vitro(36), thus, compromising the efficiency of CD19-TCE or CD19-CAR T therapy. Therefore, treatment with CD19-TCE first followed by rituximab, as needed, could be considered as an alternative strategy for those with poor depletion with CD19-TCE alone. In this context, it would be important to have strategies to detect B cells using novel antibodies that bind an alternative epitope to the therapeutic mAbs, less challenging for CD19 as it is a bigger antigen than CD20.

Conclusions

CD19 CAR T cell or CD19-TCE therapy to convert B and T cell co-stimulation into conflict and disrupt their networking could prove to be a paradigm shift in treating AID. TCE, designed and developed through advanced antibody engineering methods, offer a mechanistically sound, logistically convenient, and favourable alternative therapeutic strategy in the management of refractory AID. To this end, mechanistic studies of TCE in AID, particularly during early phase clinical trials, are of critical importance to optimise the

use of TCE in combination with standard-of-care therapy as an alternative strategy to deplete B lineage cells to improve outcomes for people with refractory AID.

~~Figures~~

Figure 1

Figure 1~~1~~

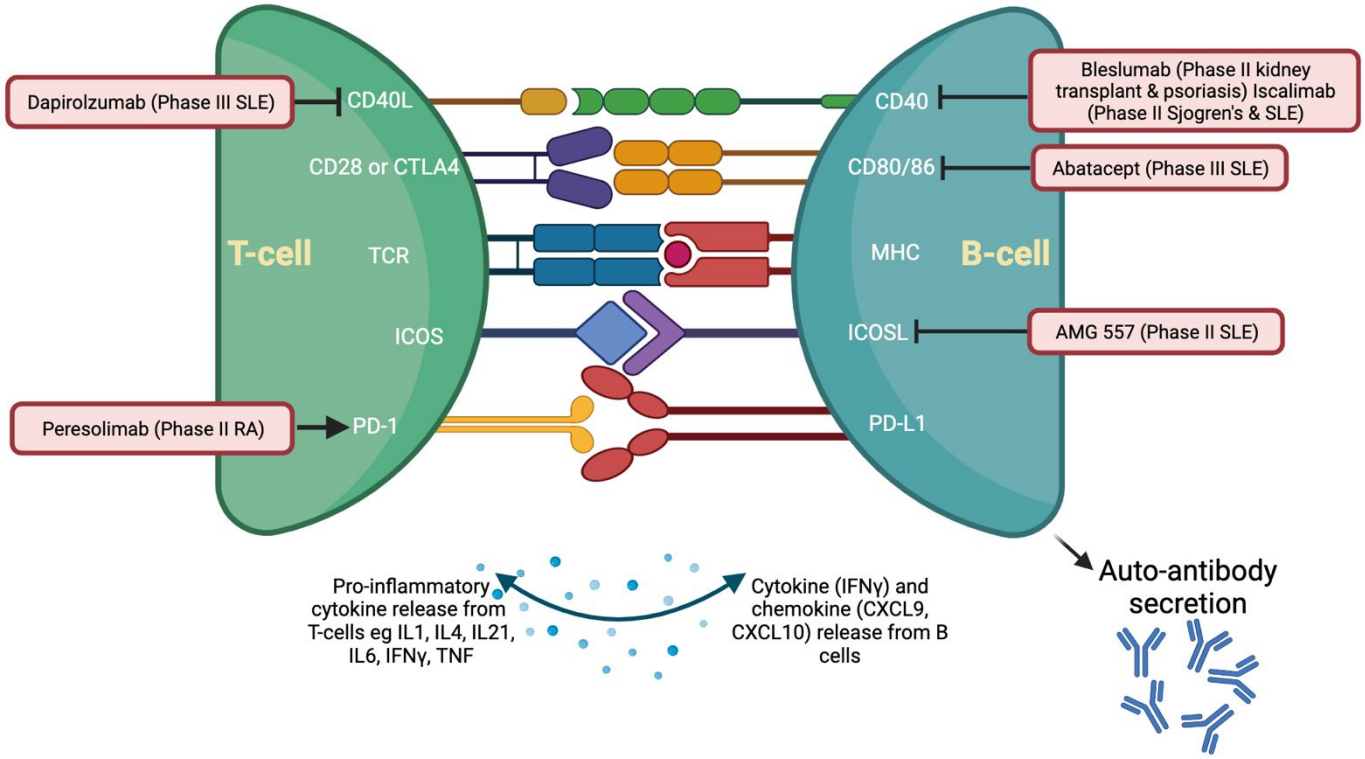

Figure 111. Pathways of B-T cell co-stimulation and trials of therapeutic agents. Molecular pairings are explained in Table 1. Drugs which target co-stimulation are outlined here. Dapirolizumab is an anti-CD40L mAb, currently in phase III study in SLE (NCT04294667). Bleslumab is an IgG4 mAb that targets CD40 which underwent phase II trial in plaque psoriasis with no clinical improvement compared to placebo (107), and demonstrated non-inferiority compared with standard of care for acute rejection in renal transplant recipients (108). Iscalimab is another anti-CD40 mAb which is undergoing phase II trial in SLE and Sjogren's Syndrome (NCT03656562, NCT04541589). Abatacept inhibits CD80/86 to prevent engagement with CD28 and is approved for use in RA but failed to meet the primary end point in the lupus nephritis phase III trial. AMG 557, anti-ICOSL antibody, underwent phase II trial in SLE and a newer therapy inhibiting ICOSL and BAFF is undergoing phase II trial (NCT04058028). PD-1 agonist, Peresolimab demonstrated modest improvement in disease activity in a phase II trial for patients with RA.
Image created in Biorender.com

Figure 2

Figure 222

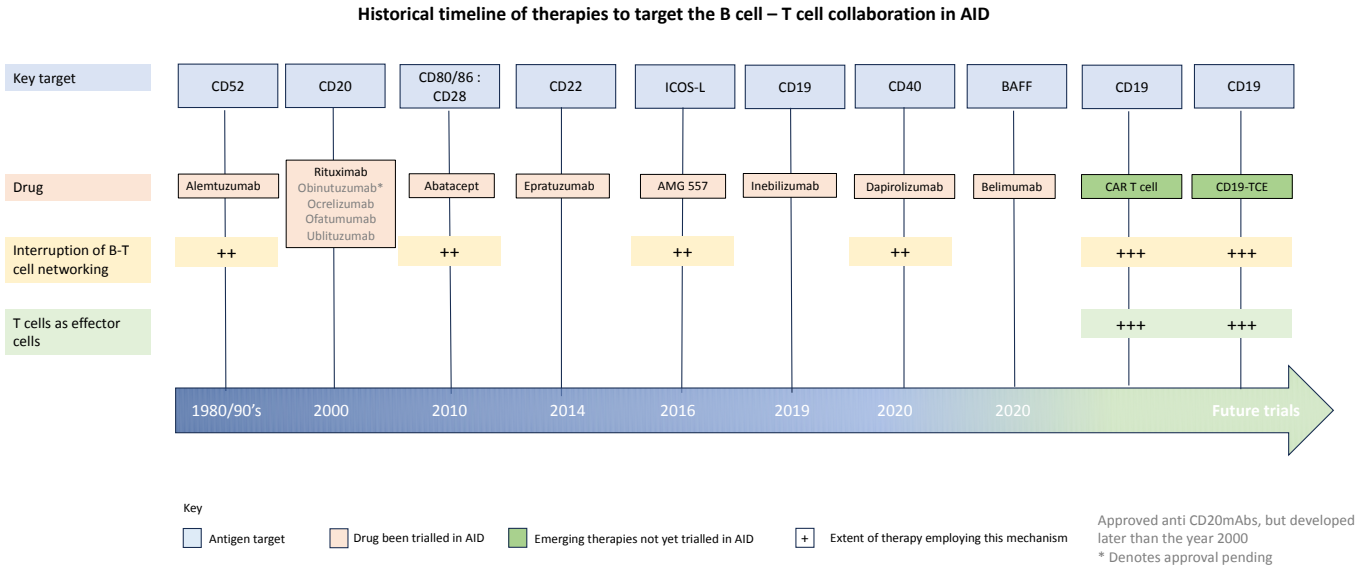

Figure 222. Historical timeline of therapies that target B-T cell collaboration in autoimmune disease. These agents were designed either to deplete B cells and/or disrupt the B-T cell collaboration. Text in blue boxes denote the target antigen, peach shaded boxes are drugs which have undergone clinical trial, drugs in dark green boxes are yet to undergo clinical trial in AID. Yellow bars represent therapies which interrupt B-T cell networking and light green bars represent treatments which employ T cells as effector cells. Text in grey represents other approved anti-CD20 mAbs, ** denotes pending approval.*

Figure 3

Figure 333

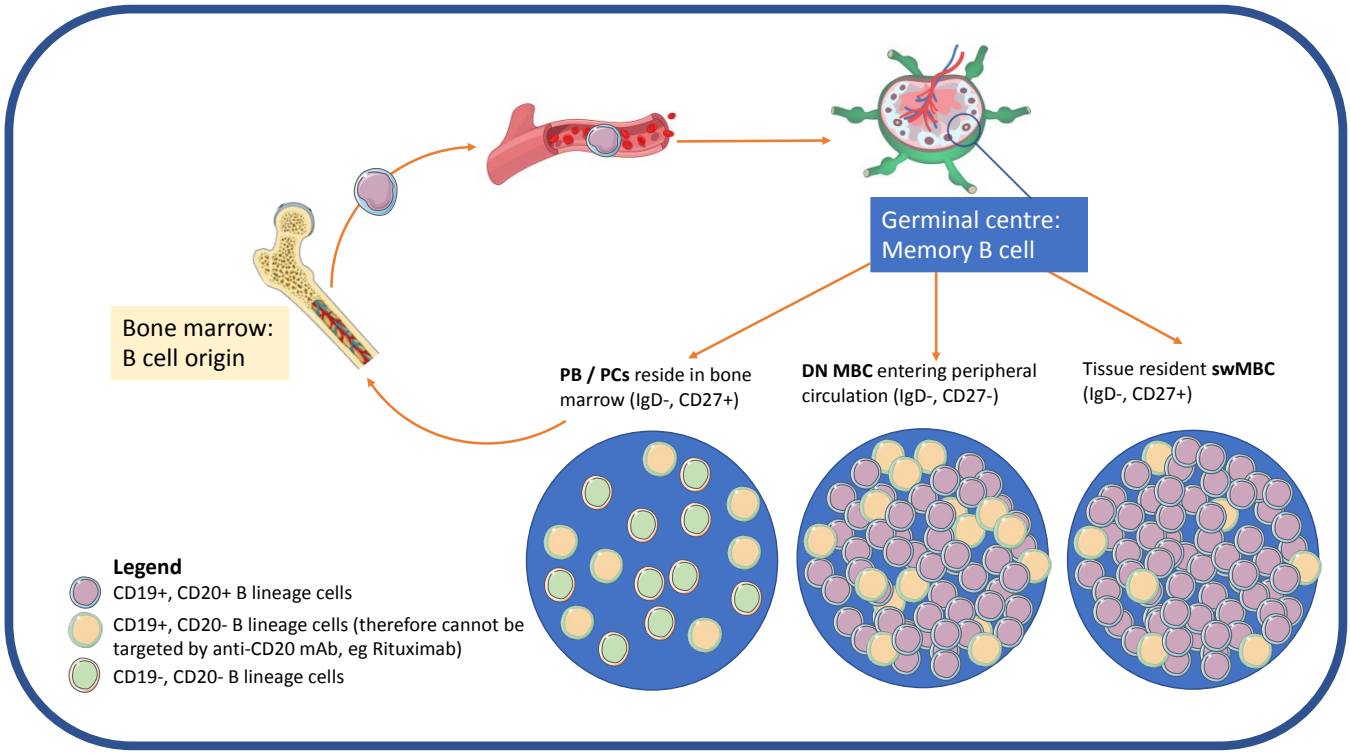

Figure 333. Life cycle of B lineage cells. B cells originate in the bone marrow and migrate through peripheral circulation into lymphoid tissues such as lymph nodes and the spleen. Naïve B cells mature into memory B cells which then differentiate into switched memory B cells, SwMBC (IgD-,CD27+), or double negative memory B cells (DN MBC; IgD-, CD27-) entering the peripheral circulation or plasma blasts (PBs) and plasma cells (PCs) a majority of which reside in the bone marrow, tissues, and inflammatory sites. Proportions of $CD19^{+}CD20^{+}$ vs $CD19^{+}CD20^{-}$ B cells are demonstrated pictorially within each subpopulation. Anti-CD20 monoclonal antibodies such as rituximab may not completely deplete $CD19^{+}CD20^{+}$ B cells in tissue and do not target $CD19^{+}CD20^{-}$ B cells, therefore, alternative strategies of depletion including CD19 targeting approaches may help to overcome rituximab resistance in autoimmunity.

Figure 4

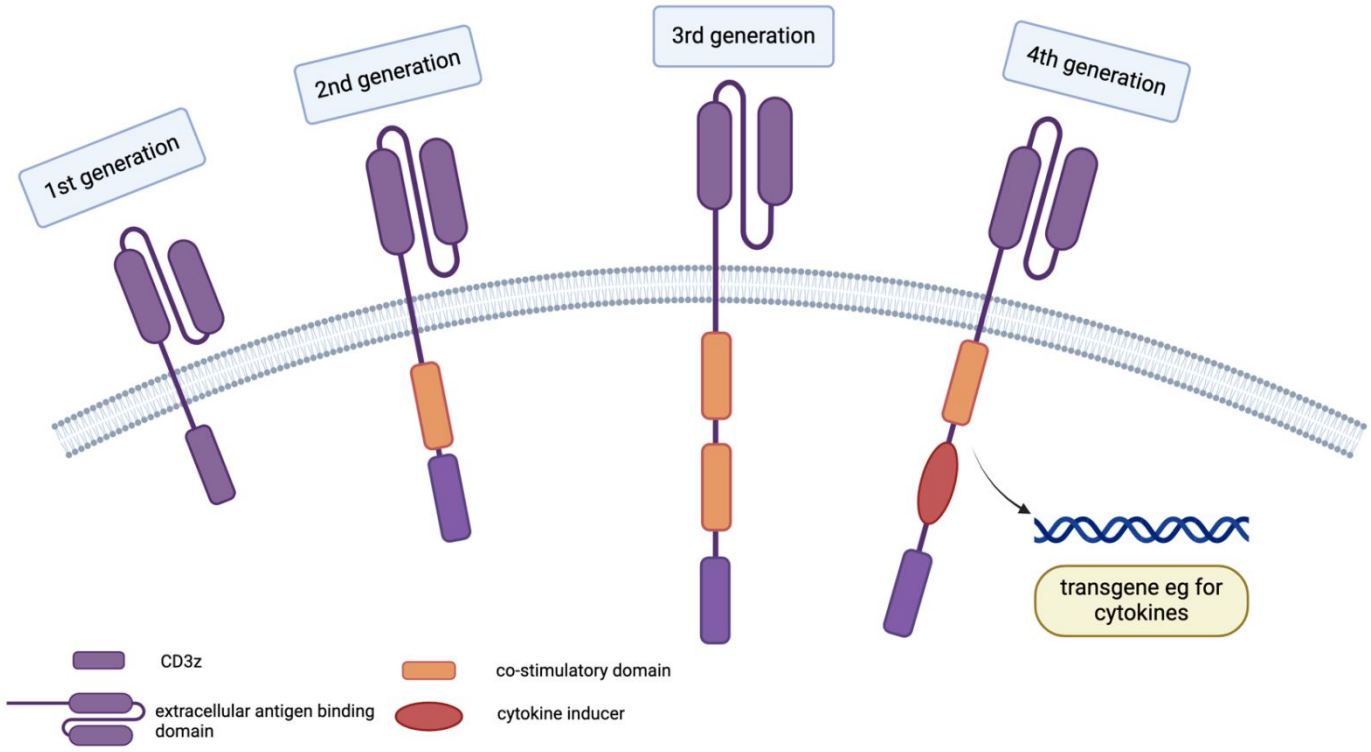

Figure 4 Evolution of CARs across the generations

All CARs have a single chain variable region of a mAb. A) first generation CARs contain an intracellular signalling domain of CD3 zeta chain alone; B) second generation includes a single co-stimulatory domain (CD28 or 4-1BB). C) third generation CARs combine two of the above co-stimulatory domains. D) fourth generation CARs are diversified in that they can express cytokines.
Image created in Biorender.com

Figure 5

Figure 544

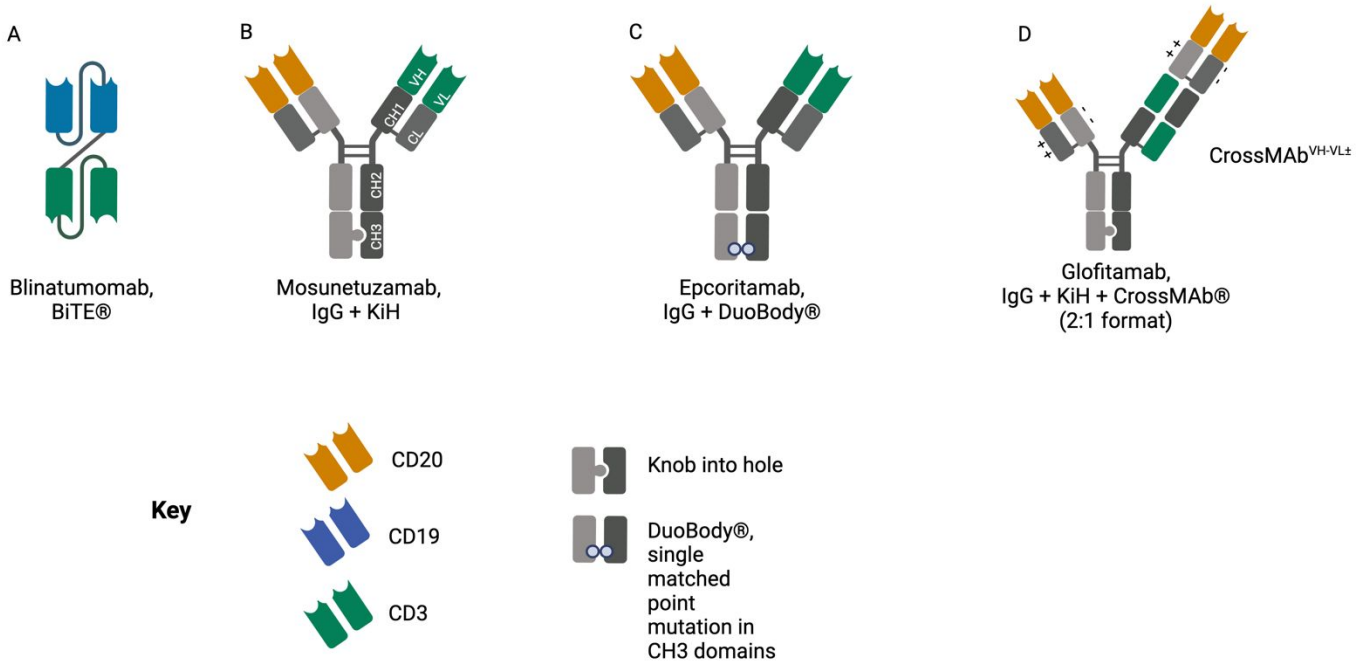

Figure 544: Selected TCE formats in a schematic representation used for T cell redirecting therapies. A) Blinatumomab, tandem scFv (single chain variable fragment) (BiTE) format. B) Mosunetuzumab, IgG based-TCE with monovalent binding using a native antibody structure with 1 Fab arm to bind CD20 (target antigen) and 1 Fab arm to bind CD3 on T cells, combined with the KiH technology as demonstrated in the CH3 domain to achieve heavy chain heterodimerisation. C) Epcoritamab, IgG based TCE with point mutations in each Fc region (CH3 domain) to allow controlled Fab-arm exchange, termed DuoBody®. D) Glofitamab, bivalent binding to increase the avidity of TCE binding to the target antigen, CD20, with additional KiH and CrossMab^{VH-VL} with charge interactions using variable regions.
Image created in Biorender.com

Figure 65

Figure 655

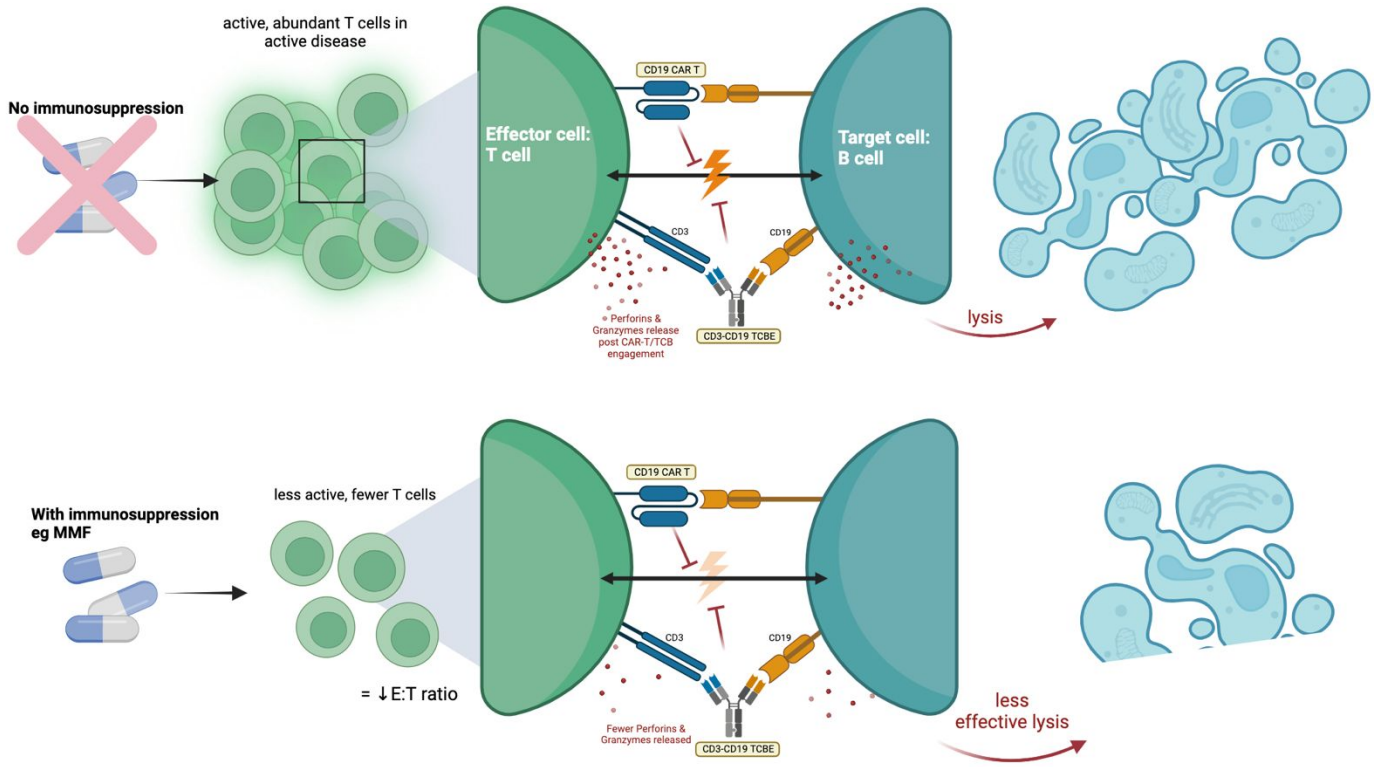

Figure 655. The potential effect of immunosuppressive treatments on T cell effector function. Mycophenolate mofetil (MMF) as per the bottom panel, results in fewer T cells to serve as effector cells for therapies such as CD19 TCE and CD19 CAR T cells. MMF can directly reduce the number of T cells and impair their activation and reduce their cytotoxicity against target B cells with lower release of perforin and granzyme molecules.

~~Figure 6 ? needed – would make this figure 4 if keeping~~

~~Figure 6 Evolution of CARs across the generations~~

~~All CARs have a single chain variable region of a mAb. A) first generation CARs contain an intracellular signalling domain of CD3 zeta chain alone; B) second generation includes a single co-stimulatory domain (CD28 or 4-1BB). C) third generation CARs combine two of the above co-stimulatory domains. D) fourth generation CARs are diversified in that they can express cytokines.~~

Table 1~~Tables~~

Table 1 Overview of CD (cluster of differentiation) antigens and~~,~~ other molecules involved in B and T cell collaboration ~~ar markers including co-stimulation and~~along with their their function /utility

Marker (+/- Ligand/Receptor)	**Meaning / function / application**
CD3 (TCR)	T cell activation signalling and regulation of TCR expression.
CD4 (MHC II)	T helper cell
CD8 (MHC I)	Cytotoxic T cell
CD19 (co-receptor for BCR)	Pan B cell marker. Regulates B cell development, activation and differentiation.
CD20	B cell activation and proliferation. Also present on a minority of T cells.
CD27 (CD70)	Marker of B and T cell memory
CD28 (CD80/86)	Co-stimulation between B and T cells.
CD40 (CD40L)	Co-stimulation between B and T cells.
BAFF-R (BAFF) or BLyS	B-cell activating factor, enhances B cell survival
PD-1 (PD-L1 and PD-L2)	Programmed Cell Death, Down regulates the immune response
CXCL-10 (CXCR3)	Recruitment of monocytes, T cells, NK cells
CXCL-13 (CXCR5)	B cell chemoattractant
CCR2 (CCL-2 also known as MCP-1)	Trafficking of monocytes to inflammatory sites
ICOS-ICOSL	ICOS part of the CD28 superfamily, provides co-stimulatory signal to activated T cells upon binding to ICOS-L
IL21-IL21R	Promotes proliferation and function of T and B cells, an enhance cytotoxicity of $CD8^+$ T cells and NK cells
TCR-MHCII	MHC displays peptides to the TCR, TCR can discriminate foreign from self-peptides

CXCL, CXC chemokine ligand; CCR, C-C Motif Chemokine Receptor; ICOS, MCP, Monocyte chemoattractant protein; MHC, major histocompatibility complex; TCR, T cell receptor

Table 2

~~Evidence for the use of CAR T cell therapies in non-malignant settings.~~

Table 2 Evidence for the use of CAR T cell therapies in non-malignant settings.

Specialty	Indication	Study phase/type	Outcome	Ref
Neurology	Multiple sclerosis (murine model = experimental autoimmune encephalomyelitis)	Murine model	Depleted B cells in peripheral blood & CNS Improved clinical scores of EAE	(109)
	Myasthenia Gravis (using anti-B cell maturation antigen CAR T cells)	Phase 1b/2a (human)	Safe, well tolerated, clinical improvement Phase IIb ongoing (NCT04146051)	(110)
Transplant medicine	Post transplant lymphoproliferative disorder (PTLD) post renal transplant	Case series (n=3) (human)	Demonstrated safety & feasibility (with regards to stopping immunosuppression) however only 1 of 3 patients maintained in remission at 3 months follow-up	(103)
	Case series of 3 patients with refractory PTLD post solid organ transplants (cardiac transplant, kidney transplant, pancreas transplant)	Case series (n=3) (human)	Poor outcomes, multiple complications including CRS, immune effector cell-associated neurotoxicity syndrome (ICANS), acute kidney injury, lack of response to CAR T cell therapy, mortality.	(111)
	Refractory PTLD post heart and kidney transplant	Case report (human)	6 months post CAR T cell infusion, clinically well and normal ejection fraction on echocardiography	(112)
Rheumatology	Systemic lupus erythematosus	Case series (n=5) (human)	Deep depletion of B cells, clinical improvement, normalisation of anti-ds-DNA antibodies and all achieved remission after 3 months. 3 patients repopulated B cells less than 50 days post CAR T cell therapy (although mainly naïve B cells)	(48)
	Systemic sclerosis (diffuse cutaneous)	Case report (human)	Extensive fibrosis (skin, heart, lung) – all showing improvement post treatment) Well tolerated, mild CRS (Grade 1), no signs of ICANS.	(62)
	Anti-synthetase syndrome (myositis and interstitial lung disease)	Case report (n=2) (human)	Treated with CD19-targeting CAR T cells. Excellent outcome with biochemical, serological, and radiological resolution of myositis and improvement in pulmonary function tests / CT chest.	(104) (61)

~~Gastroenterology~~	~~Colitis – target antigen CEA~~	~~Murine model~~	~~Migration of CEA-CAR T regs in colon mucosa; Improvement in colitis & survival~~	~~(99)~~
Dermatology	Pemphigus Vulgaris – target antigen Desmoglein 3	Preclinical study, ex-vivo (human)	Depletion of Dsg3 cells and antibodies in human pemphigus vulgaris model	(113)
Endocrinology	Type I Diabetes Mellitus – target antigen Insulin	Murine model	Delayed onset of diabetes but no long-term protection	(114)

Table 32

~~Table 2 – Mechanistic differences and similarities between CAR T and TCE: Experience in Oncology~~

Table 3 Mechanistic differences and similarities between CAR T and TCE: Experience in Oncology

	CAR T cell therapy	TCE
Side effect profile	Variable between CAR T regimens. In some oncological indications, about 80% suffer CRS, longer lasting and at a higher grade Neurotoxicity: Immune effector cell-associated neurotoxicity syndrome (ICANS) occurs in approx 13-21% of patients, lasting 4-5 times longer than with TCE.	Variable between different TCE and indications. Approx. 50% suffer CRS, earlier onset but shorter duration. Obinutuzumab (anti-CD20mAb) pre-treatment limits CRS Neurologic side effects eg headache but less severe than ICANS, much less frequent than CAR T cells.
Efficacy	Higher rates of complete response in haematological malignancies	Dose dependent response, but can be up to 30% less effective than CAR T cell therapy
Pre-conditioning	Leukodepleting so higher rates of infection and risk of rejection in transplant patients.	No preconditioning, but pre-medication with dexamethasone to reduce cytokine production and with obinutuzumab for glofitamab
Hypogammaglobulinaemia	Persistence of engineered T cells in vivo resulting in sustained B cell asplasia and hypogammaglobulinaemia, may require IVIg	TCB can deplete normal B cells and plasma precursor cells leading to a higher risk of hypogammaglobulinaemia, but therapeutic regimen could be personalised according to clinical need
Effector cell type	Engineered T cells Less differentiated T cells (naïve and memory) show better efficacy than effector T cells	Endogenous T cells Antigen-experienced T cells mediate TCE induced cell death, whereas naïve T cells are not activated
Logistical differences/similarities		
Cost	+++ (~£300,000 in the UK) (115)	++ (~£56,000 per cycle UK) (116)
Production	Personalised therapy requiring individual engineering of patient's T cells – labour intensive, time consuming (resulting in disease progression), higher risk of production error. Also requires the patient to have sufficient peripheral T cell counts for successful isolation of T cells from leukapheresis.	'Off the shelf' medication, so technically less delay to administration than CAR T cell therapy. Can be manufactured in large quantities. Can be used independent of peripheral lymphocyte counts
Administration	Single intravenous administration, however, from decision to treat to administering therapy can be 6-8	Shorter half-life so may need repeat dosing. Quick to administer so can treat patient

	weeks when disease may progress. Specialist training of staff required to administer CAR T cell therapy and monitor for complications during infusion	promptly and halt progression of disease. No additional specialist training required, similar administration to routine mAbs used such as rituximab.
Approval for use	ALL, large B cell lymphoma, mantle cell lymphoma, multiple myeloma (FDA approval)	Blinatumomab (CD3-CD19) for ALL, epcoritamab-bysp and glofitamab (CD3-CD20) for DLBCL (FDA approval), mosunetuzumab (CD3-CD20) for follicular lymphoma
Repeat treatment	Complicated due to maintenance of T cell pool, patient factors (risk of infection).	More convenient and standardised

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