Published in final edited form as:

Nat Rev Clin Oncol. 2016 May ; 13(5): 273–290. doi:10.1038/nrclinonc.2016.25.

The future of cancer treatment: immunomodulation, CARs and combination immunotherapy

Danny N. Khalil[*], **Eric L. Smith**[*], **Renier J. Brentjens**, and **Jedd D. Wolchok**

Department of Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, New York 10065, USA

Abstract

In the past decade, advances in the use of monoclonal antibodies (mAbs) and adoptive cellular therapy to treat cancer by modulating the immune response have led to unprecedented responses in patients with advanced-stage tumours that would otherwise have been fatal. To date, three immune-checkpoint-blocking mAbs have been approved in the USA for the treatment of patients with several types of cancer, and more patients will benefit from immunomodulatory mAb therapy in the months and years ahead. Concurrently, the adoptive transfer of genetically modified lymphocytes to treat patients with haematological malignancies has yielded dramatic results, and we anticipate that this approach will rapidly become the standard of care for an increasing number of patients. In this Review, we highlight the latest advances in immunotherapy and discuss the role that it will have in the future of cancer treatment, including settings for which testing combination strategies and 'armoured' CAR T cells are recommended.

Immunotherapy is defined as the approach to treating cancer by generating or augmenting an immune response against it. This approach has been studied, mostly outside of mainstream cancer research, for over a century[1]. Nevertheless, cancer immunotherapy has only in the past decade been shown, in phase III clinical trials, to consistently improve the overall survival of patients with advanced-stage cancer[2–5], bringing unprecedented interest to this field. Despite the breakthroughs of the past decade, the successes to date do not fully capture the promise of immunotherapy.

Antitumour immunotherapy has broad potential and could be used to treat many different types of advanced-stage cancer owing to the durable and robust responses it elicits across a diverse spectrum of malignancies. Two types of immunotherapy have emerged as particularly effective over the past decade: immune-cell-targeted monoclonal antibody (mAb) therapy and adoptive cellular therapy (ACT). In this Review, we present current clinical progress in both modalities, discuss how each of them might be particularly

Correspondence to: J.D.W. wolchokj@mskcc.org.

*These authors contributed equally to this work.

Author contributions

All authors researched data for article, contributed to discussion of the content, wrote the manuscript and reviewed/edited the article before submission.

Competing interests statement

D.N.K. and E.L.S. and declare no competing interests. R.J.B. is a co-founder, stockholder, and consultant for Juno Therapeutics Inc. J.D.W. is a consultant for Bristol Myers Squibb, Genentech, Medimmune, Merck Pharmaceuticals and Polynoma Pharmaceuticals.

indicated for different types of cancer and we outline the potential therapeutic relevance of combination regimens.

Immune modulation with monoclonal antibodies

Immune modulation is based on the striking finding that stimulation of T-cell function with antibodies that block or activate regulatory receptors is sufficient to cause the regression of some tumours. Immunomodulatory mAbs target immune cells rather than cancer cells, and thus, are not necessarily specific to any cancer type. Indeed, the blockade of a single molecule, programmed cell-death protein 1 (PD-1), has resulted in antitumour activity and is now approved by the FDA to treat patients with mela-noma[2,3] and non-small-cell lung cancer (NSCLC)[6]. PD-1 is one of the receptors involved in immune-checkpoint signalling; in particular, in lymphocyte maintenance of self-tolerance. Checkpoint blockade is a method by which T-cell function is stimulated with mAbs that block their inhibitory receptors, whereas T-cell co-stimulation is the method that aims at activating T-cell function with mAbs that target their stimulatory receptors. Some tumour types, however, are more likely than others to respond to checkpoint blockade, which raises the possibility that T-cell-stimulatory mAbs can be applied to a broad spectrum of cancer types if they are administered in the proper therapeutic context.

The generation of immunological memory is another unique feature of immune modulation as an effective cancer therapy[7]. A persistent memory response would have a role in both preventing disease recurrence and in guarding against the evolution of therapy-resistant malignant cancer clones. The precise implications of immunological memory formation remain undefined, but evidence for extremely durable remissions has been shown in some patients with unresectable or metastatic melanoma treated with immunotherapy[8]. Furthermore, complete and rapid tumour regression has been observed among a subset of these patients[9,10], highlighting the fact that responses to immunotherapy are no less robust than those to cytotoxic chemotherapy and molecularly targeted therapy and can lead to tumour reduction and, in some cases, eradication.

The observation that mAbs targeting molecules on the T-cell surface are sufficient, in some patients, to mediate tumour regression is instructive. Therapeutic antitumour vaccination is based on the premise that an adaptive antitumour immune response can be elicited by presenting exogenous tumour antigens to the immune system. This strategy was at the forefront of cancer immunotherapy research in prior decades. Some vaccines were administered with so-called adjuvants, which, in the context of immunology, are agents designed to enhance the immune response to the antigen. One way to consider the current paradigm of cancer immunotherapy is a shift from administering an antigen to administering an adjuvant in the context of a pre-existing, but non therapeutic, vaccination event *in situ*, as will be described later.

The discovery that T-cell-stimulation alone (that is, without a co-administered vaccine to direct the immune response to a specific target) can have a therapeutic effect relies on a fundamental principle that surprised many in the field: it suggests that patients with cancer who derive benefit from T-cell-stimulatory therapy are immunologically primed, before

treatment, to mount an anticancer immune response. Correspondingly, successful immunotherapy in these patients merely needs to unmask this latent potential.

Numerous research groups have invested substantial resources into identifying patients who are most likely to benefit from T-cell stimulatory therapy. Such knowledge would not only spare some patients from unnecessary treatment with associated toxicities, but it would also expand the use of immunotherapy to treat new types of cancer. Among the initial candidates for predictive biomarkers were C-reactive protein (CRP) and the absolute lymphocyte count[11], because they correlated with improved outcomes. Subsequently, the measurement of circulating myeloid-derived suppressor cell (MDSC) levels before treatment emerged as a potential method to predict outcomes[12]. In 2015, elevated baseline levels of soluble CD25 were shown to correlate with poor survival outcomes[13]. In other studies[14–16], extensive whole-exome sequencing was performed on samples from patients with melanoma and from patients with NSCLC treated with checkpoint blockade agents with the purpose of identifying genomic properties that might predict a response to these immunotherapies. Genetic features, such as mutation burden, were identified but no consensus has been reached regarding the identity of specific genetic alterations encoding so-called ‘neoepitopes’ that would make malignant cells recognizable to T cells and offer good predictive value for a response to checkpoint blockade[16]. The discovery of such alterations and their validation in prospective clinical trials would be of immense importance as they could enable immunotherapy to be given selectively to patients who would benefit from it, merging immunotherapy with precision medicine in a manner that could benefit innumerable patients with cancer.

Immune-checkpoint blockade

In this section, we discuss the well-established role of cytotoxic T-lymphocyte protein 4 (CTLA-4) and PD-1in T-cell activation. We also highlight other promising inhibitory T-cell receptors for which mAbs are being developed.

CTLA-4—T cells are primed to acquire effector function at the immunological synapse with an antigen-presenting cell (APC). The initiating event, so-called signal 1, is the recognition by the T-cell receptor (TCR) of a cognate antigen peptide presented in the context of an MHC molecule on the surface of an APC (FIG. 1). This interaction, however, is insufficient to activate T-cell function. In fact, the T cell will go on to become anergic or apoptotic if no second signal is received[17]. In order to adequately prime T cells, a second signal is required, typically in the form of the T-cell receptor CD28 binding with either CD80 or CD86 on the APC. Once this occurs, the T-cell inhibitory CTLA-4 receptor is shuttled to the cell surface where it binds CD80 or CD86 with greater affinity than CD28 (REFS 18,19). Thus, CTLA-4 translocation to the T-cell surface peaks after TCR stimulation, and activation of this receptor limits T-cell stimulation by TCR/CD28 co-ligation, both by preventing signalling downstream of the TCR and by outcompeting CD28 for its ligands. Phenotypic evidence of the ability of CTLA-4 to dampen T-cell activation is well demonstrated in *Ctla4*-knockout mice[20,21]. These animals developed fatal autoimmune disorders caused by a marked expansion of the T-cell population and infiltration into multiple tissue types[20,21]. CTLA-4 thus has a central role in suppressing T-cell function, thereby restricting T cell-mediated

antitumour activity. Not surprisingly, CTLA-4 blockade has paved the way forward for modern cancer immunotherapy.

Regulatory T (T_{REG}) cells also use CTLA-4 to suppress antitumour immunity[22,23]. T_{REG} cells constitutively express CTLA-4, typically at levels higher than those of conventional T cells[24], and CTLA-4 is necessary for T_{REG} cells to exert maximal immune-suppressive function[23,25]. Aside from disrupting CTLA-4 ligation on T_{REG} cells, anti-CTLA-4 mAbs deplete intratumoural T_{REG} cells, at least in mouse models[26,27]. In 2015 it was observed that CTLA-4 blockade reduces the interaction time of conventional T cells with T_{REG} cells (REF. 22), thereby potentially freeing conventional T cells to be adequately primed by APCs.

Once it emerged that CTLA-4 constrains T-cell activity, the use of agents that block this receptor became an attractive candidate ‘adjuvant’ for therapeutic cancer vaccination[28]. This approach was used in preclinical studies to enhance the potency of the immune response generated by the administration of DNA vaccines[29] and of vaccines consisting of irradiated tumour cells transduced to express the granulocyte-macrophage colony-stimulating factor (GM-CSF)[30]. These results were followed by a phase III trial in which patients were randomly assigned to receive ipilimumab (anti-CTLA-4 human mAb) plus gp100 peptide vaccine, or each agent alone[4]. The overall survival of the patients in the ipilimumab group was improved compared with patients that received the peptide vaccine only, and addition of the peptide vaccine to ipilimumab did not confer any additional advantages. This result was a breakthrough for several reasons. Firstly, no treatment before ipilimumab had demonstrated any significant improvement of overall survival for patients with advanced-stage melanoma in the setting of a phase III clinical trial. Secondly, these results revealed for the first time that immune-checkpoint blockade can be sufficient to improve survival in patients with advanced-stage cancer. Finally, the survival plot in this study shows a plateau at approximately 2 years, after which the patients that survived (20%) went on to experience durable benefit for the remainder of the study[4]. This pattern was atypical of melanoma and, in fact, of other malignancies treated with conventional cancer therapy.

These clinical findings suggest that, once immune-checkpoint blockade successfully engages a patient’s immune system to control tumour growth, the immune response can be sustained even after the course of treatment has ended. This observation is consistent with the fact that such immunotherapeutic agents do not target the tumour itself, but rather they modify the patient’s immune system to control tumour progression, even after the exogenous agent has been withdrawn.

The introduction of ipilimumab in the clinic brought a new and different set of drug adverse effects now known as immune-related adverse events (irAE)[31]. These are defined as the mechanism-based toxicities that result from a ‘disinhibited’ immune response. Given the unique aetiology of these adverse events, limited overlap exists between irAEs and the toxicities associated with most other forms of cancer therapy in terms of type or severity of symptoms[31]. In principle, the immune stimulation caused by immune-checkpoint blockade can affect any organ system; however, some organ systems are particularly susceptible to the adverse effects of immune modulation by anti-CTLA-4 treatment (TABLE 1). Fortunately, even clinically moderate to severe irAEs can generally be controlled with medical

management without altering the antitumour effects of the therapy. Whereas most irAEs are typically reversible, endocrinopathies such as hypophysitis and thyroiditis frequently require chronic hormone replacement. Discontinuation of ipilimumab should be considered if corticosteroids cannot be tapered below 10 mg daily, which is the case for a minority of patients. Clinicians, however, should keep in mind the unique response kinetics of ipilimumab and other checkpoint-blocking agents. Some patients who ultimately derive benefit from ipilimumab will often experience an initial phase of tumour growth on commencing therapy[32], a phenomenon that has not been observed to the same extent with cytotoxic chemotherapy or molecularly targeted anticancer therapy, which are directed against the tumour and not the patient's immune system. An appropriate use of checkpoint blocking agents (particularly anti-CTLA-4 monotherapy), thus, requires careful clinical judgment to avoid discontinuing the therapy too early in the treatment course. Immune-related response criteria (irRC) have been developed to provide guidance for avoiding premature discontinuation of therapy under specific circumstances[33]. For example, whereas the development of a small new lesion during therapy would be designated as progression of disease by standard RECIST criteria[34] and imply treatment failure; however, such a designation by irRC criteria would also take into account the overall disease burden[33].

Ipilimumab was approved by the FDA for the treatment of metastatic melanoma in 2011. This agent's antitumour properties, however, are not limited to melanoma[35] nor even to cancers that are historically thought to be immune responsive, such as renal-cell carcinoma (RCC)[36]. Nevertheless, in our opinion, the future role of CTLA-4 blockade in cancer therapy will be primarily in the context of combination regimens. CTLA-4 blockade might enable local antitumour therapy to trigger a systemic response; for example, there is anecdotal evidence of widespread tumour regression after localized radiation therapy in the setting of systemic ipilimumab treatment[37]. Furthermore, preclinical evidence exists showing that intratumoural viral therapy[38] and cryo-ablation[39] can trigger a systemic response in the setting of CTLA-4 blockade.

PD-1 axis—PD-1 is a second inhibitory receptor expressed on T cells. The PD-1 ligands, PD-L1 and PD-L2, are expressed on the surface of APCs and malignant cells, particularly in response to local inflammatory cytokines, such as IFNγ. Similarly to CTLA-4, PD-1 ligation inhibits signalling downstream of the TCR[40,41] (FIG. 1). PD-L1 can also ligate to CD80 expressed on T cells as a second mechanism of T-cell suppression[42,43]. Autoimmune processes developed in *pd1* knockout mice include arthritis, nephritis, and myocarditis[44,45]. PD-1 ligands present within tumours can function as potent mediators of T-cell suppression and intratumoural PD-L1 expression is associated with a poor prognosis in some tumour types, including lung, ovarian or colon cancer, among others[46].

PD-1 and PD-L1 blockade are currently among the most promising endeavours in clinical oncology. Two anti-PD-1 mAbs, pembrolizumab and nivolumab, were approved by the FDA in 2014 after the publication of robust data showing that up to 40% of patients with advanced-stage melanoma, including those who previously had no response to ipilimumab, experienced objective responses when treated with these agents, compared with approximately 12% for ipilimumab monotherapy[3]. In 2015, the combination of ipilimumab and nivolumab was approved by the FDA for the treatment of patients with advanced-stage

melanoma based on phase III data showing improved response rates and progression-free survival rates compared with either agent alone[47]. Nivolumab was also approved in 2015 for the treatment of squamous-cell lung cancer that is refractory to platinum-based therapy based on the results of a phase III study showing a 3.2-month improvement in overall survival, and a 17% improvement in 2-year survival for patients with advanced-stage squamous-cell NSCLC receiving nivolumab compared with those receiving docetaxel for disease[48]. The indication for the use of nivolumab was then expanded to patients with other types of advanced-stage NSCLC[49], as was pembrolizumab for patients with PD-L1-positive NSCLC[50]. Thus, antibodies that target the PD-1 axis have been approved for the treatment of patients with melanoma[3,51] and NSCLC[48], and ongoing efforts are seeking to expand the indication for the treatment of RCC[51,52], bladder cancer[53], ovarian cancer[52], Hodgkin lymphoma[54], and a growing list of other malignancies.

Intratumoural PD-L1 can suppress T-cell activity through interactions with both PD-1 and CD80 on T cells[42,43], and for this reason some investigators have predicted that anti-PD-1 and anti-PD-L1 therapies might have distinct antitumour effects and adverse-effect profiles[55]. PD-1 blockade would leave the CD80–PD-L1 interaction intact, whereas PD-L1 blockade would leave the PD-L2–PD-1 interaction intact. In spite of these mechanistic differences, distinct clinical features of each approach have not yet become apparent.

Some similarity is shared between the inflammatory toxicities associated with the blockade of the PD-1 axis and CTLA-4; high-grade toxicities, however, are much less common with PD-1 pathway blockade[47], with the exception of pneumonitis, which is a particular concern for patients receiving anti-PD-1 or anti-PD-L1 mAbs[6,51]. Earlier in the development of anti-PD-1 therapy, 3% of the treated patients developed pulmonary toxicity, which was fatal for approximately one third of them[6,51]. The vigilance for pulmonary toxicities has increased as a result of these observations, and their management has improved[47] (TABLE 1). The development of life-threatening pneumonitis can be avoided in the vast majority of patients by early intervention with corticosteroids and withholding anti-PD-1 treatment when appropriate; clinical experiences reflect an evolved approach to pneumonitis management, with no deaths from this toxicity reported in two phase III trials of anti-PD-1 therapy in 2015 (REFS 47,48).

Anti-CTLA-4/anti-PD-1 combinations—We anticipate that the greatest antitumour effect of blocking CTLA-4 as well as the PD-1 axis will come in the form of combination therapy. Dual anti-CTLA-4 and anti-PD-1 therapy has already shown significant promise[9,10,47]. For example, this approach has demonstrated an unprecedented 58% response rate and an 11.5% complete response rate in patients with advanced-stage melanoma in a global phase III trial[47]. Combinations with other forms of immune modulation and agents historically thought not to function through immune modulation are being actively investigated for cancer therapy.

Lymphocyte-activation gene 3 (LAG-3)—LAG-3 is expressed on activated conventional T cells, T_{REG} cells, B cells and plasmacytoid dendritic cells[56]. Upon binding MHC class II molecules on APCs, LAG-3 transmits an inhibitory signal in conventional T cells[57], whereas this signalling event enhances the suppressive function of T_{REG} cells (REFS

58,59) (FIG. 1). Co-expression of LAG-3 and PD-1 is a marker of exhausted T cells (dysfunctional T cells classically associated with chronic infection) and, therefore, the blockade of both receptors confers additive therapeutic activity in preclinical models of chronic infection and cancer[60–62]. Interestingly, a soluble form of LAG-3 has been detected in the serum of patients with breast cancer and its presence correlates with a more-favourable prognosis[63]. A soluble LAG-3-Ig fusion protein, designed to promote dendritic-cell (DC) maturation through MHC II binding, has been tested in patients with RCC in a phase I clinical trial, resulting in disease stabilization and enhanced $CD8^+$ T-cell activation[64]. Finally, a blocking mAb targeting LAG-3 is currently being tested in the clinic (NCT01968109)[65].

T-cell membrane protein 3 (TIM-3)—TIM-3 (also known as hepatitis A virus cellular receptor 2; HAVCR2) is another exhaustion-associated inhibitory receptor that serves to blunt T-cell-effector function[66] and induce apoptosis of T cells[66]. To date, four natural ligands of TIM-3 have been identified: galectin-9 (REF. 67), HMGB1 (REF. 68), phosphatidyl serine[69] and CEACAM-1 (REF. 70) (FIG. 1). TIM-3 blockade has demonstrated antitumour activity in mouse models of colon adenocarcinoma, melanoma, and sarcoma, particularly when combined with PD-L1 blockade[71,72]. Furthermore, anti-TIM-3 treatment has been shown to enhance the proliferation and cytokine production of $CD8^+$ T cells derived from patients with melanoma[73].

T-cell immunoreceptor with Ig and ITIM domains (TIGIT)—TIGIT is expressed on $CD4^+$ T cells, in which it marks T_{REG} cells; on $CD8^+$ T cells, in which it is a marker of exhausted cytotoxic cells; and on other immune cells[74,75]. TIGIT blockade in animal models mediates antitumour activity in combination with anti-TIM-3 or anti-PD-L1 mAbs. By contrast, a clear autoimmune phenotype has not been described for *Tigit*-deficient mice[74,76], which suggests that TIGIT blockade might have a potential role in future immunotherapy regimens without adding significant toxicity.

T-cell co-stimulation

In part as a response to the potent antitumour activity observed when inhibitory T-cell receptors are blocked, substantial interest has been generated towards the activation of co-stimulatory T-cell receptors to control cancer. Upon engagement of the TCR by peptides presented on MHC by APCs, co-stimulatory receptors on T-cells receive a crucial second signal from cell-surface proteins on APCs that enable T-cell activation[77] (FIG. 1). The encouraging results of several preclinical studies prompted clinical trials to investigate several agonist mAbs targeting co-stimulatory molecules. These co-stimulatory receptors are members of the tumour necrosis factor receptor (TNFR) family, a group of non-enzymatic cell-surface proteins that mediate proliferation, activation and differentiation responses in T cells.

T-cell antigen 4-1BB homologue (4-1BB)—Therapeutic mAbs targeting the co-stimulatory molecule 4-1BB (also known as CD137 or TNFR superfamily member 9) are among the most advanced co-stimulatory agonists currently in clinical development. 4-1BB is expressed on T cells, natural killer (NK) cells and monocytes[78]. Stimulation of T cells by

4-1BBL, its cognate ligand expressed on dendritic cells, results in proliferation and upregulation of the antiapoptotic proteins Bcl-2-like protein 1 (Bcl-xL)[79], Bcl-2-related protein A1 (Bfl-1)[79,80] and CASP8 and FADD-like apoptosis regulator (c-FLIP)[80], which protect T cells from activation-induced cell death[79–82]. Data from preclinical studies have shown the antitumour activity of anti-4-1BB mAb therapy alone and in combination with CTLA-4 blockade[83], CD40 activation[84], cellular vaccines[83,85] or radiation therapy[86]. Urelumab, a fully human anti-4-1BB agonistic mAb, has demonstrated antitumour activity in patients with melanoma[87]. A subsequent clinical study, however, was suspended owing to the development of severe hepatotoxicity[88]. Further clinical testing of urelumab at reduced doses in combination with several regimens is ongoing[89]. Given the role of 4-1BB in augmenting NK-cell activity, therapies that combine anti-4-1BB mAbs with mAbs targeting tumour cells (such as rituximab or cetuximab) are being developed to increase the ability of tumour-targeting mAbs to mediate antibody-dependent cell-mediated cytotoxicity via NK cells[90,91].

Glucocorticoid-induced TNFR-related protein (GITR)—GITR (also known as TNFR superfamily member 18) is another clinically relevant co-stimulatory receptor[92]. GITR is upregulated when conventional T cells are activated, and is constitutively expressed by T_{REG} cells (REF. 92). GITR activation augments effector T-cell proliferation, cytokine production and resistance to T_{REG}-cell-mediated suppression[93–95]. Treatment with anti-GITR agonist mAbs has been shown to mediate tumour rejection and the generation of immunological memory in syngeneic mouse models of fibrosarcoma[96], colorectal carcinoma[97] and melanoma[98]. GITR ligation is also able to disrupt T_{REG}-cell lineage stability and impart T-effector function[99]. Treatments using anti-GITR approaches are currently being evaluated in early phase clinical trials across a broad spectrum of malignancies[100,101].

CD40—CD40 is a member of the TNFR family, but it is predominantly expressed on dendritic cells, macrophages, monocytes and B cells, and also in malignant melanoma, lymphoma, leukaemia, and carcinoma cells[102,103]. $CD4^+$ T cells express CD40L, the ligand for CD40, which enables APCs to activate T cells. Upon CD40 ligation, dendritic cells upregulate MHC class II and secrete proinflammatory cytokines, such as IL-12 (REF. 103) (FIG. 1). On B cells, CD40 is important for immunoglobulin class switching[104]. Therapeutic mAbs that target CD40 on tumour cells have been developed[105]; however, CD40 agonist mAbs that target nonmalignant immune cells to elicit an antitumour response are of potentially even broader applicability. The macrophage-mediated robust antitumour activity that agonist CD40 mAbs show when combined with chemotherapy for the treatment of patients with pancreatic cancer provides a striking example of their efficacy[106].

OX40—OX40 (also known as tumour necrosis factor ligand superfamily member 4) is present on the surface of T cells, NK cells and neutrophils, whereas its ligand, OX40L, is expressed on a number of different immune cells, including APCs[107]. OX40 engagement on T cells promotes proliferation, survival, and the secretion of cytokines associated with both type 1 and type 2 T helper cell responses[108,109]. OX40 ligation also blunts the suppressive effects and promotes activation-induced cell death of T_{REG} cells (REFS 110,111). In preclinical models, the ligation of OX40 exerts antitumour activity mediated by $CD4^+$ and

$CD8^+$ T cells, and confers immunological memory manifested as resistance to tumour rechallenge[112]. Results of a phase I trial[113] demonstrated that an agonist mAb targeting OX40 given as monotherapy has antitumour activity in patients with melanoma or RCC. A number of follow-up clinical trials using OX40 agonists in combination regimens are currently underway[114–117].

Checkpoint blockade plus co-stimulation

The concept of augmenting T-cell activity with co-stimulatory mAbs and concurrently liberating activated T cells to lyse malignant cells by blocking PD-1 or PD-L1 is an appealing antitumour approach. Several ongoing clinical trials involving patients across a broad range of solid organ and haematological malignancies are investigating this possibility; with most of them employing agents that target the PD-1 axis as the means of checkpoint blockade[118]. In the near future, we will have more data to evaluate the toxicity–benefit ratio of such immunotherapeutic combinations; we anticipate that this knowledge will ultimately lead to the design of new therapies for a wide range of advanced-stage cancers.

CAR-T-cell therapy

The goal of adoptive T-cell therapy is to generate a robust immune-mediated antitumour response through the *ex vivo* manipulation of T cells. This aim can be accomplished through the selection and expansion of tumour-infiltrating lymphocytes (TILs), or through gene transfer of a synthetic TCR (sTCR) or a chimeric antigen receptor (CAR) into T cells. We will focus on CAR T-cell therapy, which differs from TIL or sTCR-based therapies in that it uses a single-chain variable fragment (scFv) derived from the variable heavy and variable light chains of an antibody to target an extracellular antigen independent of the peptide–HLA complex[119,120]. In its simplest form, a CAR is encoded by a single gene consisting of an scFv, a transmembrane region, and the CD3ζ chain (the signalling domain of the TCR complex)[119]. This molecule, a so-called first-generation CAR, provides only activation signal 1 to T cells, and has been shown to lead to T-cell anergy upon repeated antigen stimulation[120,121]. Second-generation CARs contain an additional co-stimulatory domain that provides activation signal 2 upon the scFv engaging the target antigen[121]. The most frequently used co-stimulatory molecules, to date, have been the signalling domains of CD28 (REFS 121,122) or 4-1BB[123,124], although others[125,126] have also been studied. Almost all clinical trials performed to date, and all the trials discussed herein, have used second-generation CARs. In third-generation CARs, two co-stimulatory domains are added to the above design, although direct comparisons with second-generation CARs have not yet been conducted in the clinical setting. Finally, ‘armoured’ CAR T cells have been evaluated in preclinical experiments; the first clinical trials using armoured CAR T cells are now enrolling patients (NCT02498912)[127]. An armoured CAR vector includes a second gene, encoding a protein that either provides the resulting T cell with a survival or cytotoxicity advantage, or modulates the tumour microenvironment. Examples of such proteins include the proinflammatory cytokine IL-12 (REF. 128), or the immunostimulatory molecules 4-1BBL[129] or CD40L[130].

CD19-targeted CAR-T-cell therapy

Initial clinical trials using CAR T cells have all focused on targeting CD19, which is an ideal antigen because it is ubiquitously expressed on a broad range of differentiated B cells (from pro-B cells to memory B cells), but it is not expressed on haematopoietic stem cells or any other essential cell types, limiting potential 'on target-off tumour' toxicity. For this reason, CD19-targeted CAR T cells have been used to treat diseases from B-cell acute lymphoblastic leukaemia (B-ALL) to more-differentiated non-Hodgkin lymphomas. We and others have found that the expected normal B-cell aplasia is well tolerated and can be managed with monthly administration of intravenous immunoglobulin[122,124,131].

B-ALL—To date, the single greatest success of ACT has been achieved with CD19-targeted CAR T cells for the treatment of relapsed and/or refractory paediatric and adult B-ALL[122,124,131]. The efficacy of CD19-targeted CAR T-cell therapy can be put in perspective when considering the results of a large US–UK cooperative pre-CAR-T-cell therapy era group study that treated adults with ALL after first relapse (n = 609): the median survival was 24 weeks, and the 5-year overall survival was 7%[132]. Complete response rates from investigations conducted at several institutions testing CAR T-cell therapy have been reported in the range of ~70–90% in heavily pretreated patients[122,124,131]. Similar response rates have been reported in studies across institutions; however, understanding the key similarities and differences in CAR design, gene transfer technology, and the effects of different trial designs on patient outcomes are key for the field to advance.

Different scFv, co-stimulatory domains, and gene transfer methods have been employed by researchers from several institutions involved in several trials with CAR-T-cell therapy[133] (TABLE 2). Most of these trials included patients with relapsed or refractory B-ALL who received salvage chemotherapy, and regardless of the response, were administered CD19-targeted second-generation CAR T cells, most often after lymphodepleting preconditioning chemotherapy. Each study used one of two different anti-CD19 scFvs, different gene-transfer methods and infused either bulk or selected CAR-T-cell populations.

Several large clinical trials have used CD19-targeted CAR-modified T cells to treat B-ALL at various research centres in the USA, including the Memorial Sloan Kettering Cancer Center (MSKCC)[122,134,135], the University of Pennsylvania (UPenn)[124,136,137], the US National Cancer Institute (NCI)[131,138], the Fred Hutchinson Cancer Center[139] and the MD Anderson Cancer Center[140] (TABLE 3). Differences in design between these trials included patient populations, conditioning therapies, tumour burden, and CAR-T-cell dose, among other variables. Additionally, the end points defined for each study and the methods for reporting clinical efficacy and safety varied between different institutions[122,124,131,134–140].

In the trials conducted at MSKCC[122,134,135], UPenn[124,141,142], NCI[131,138], and Fred Hutchinson[139], investigators reported similar remarkably high overall response rates, complete remission rates, minimal residual disease (MRD) negativity (when reported) and comparable toxicity. This is in contrast with the design of the trial carried out at MD Anderson[140], in which, uniquely, lymphodepleting conditioning chemotherapy was omitted and an electroporation method of gene transfer that involved co-culture on artificial antigen presenting cells (aAPCs) was employed. Conditioning chemotherapy is likely to deplete

immune-suppressive regulatory cells, a process that might be essential to the effectiveness of CAR-T-cell therapy. Similarly, an electroporation process that includes *ex vivo* culture on aAPCs can potentially exhaust T cells before they are infused.

CAR T-cell persistence is likely an important factor in determining the efficacy of the antitumoural response, although the optimal time of survival of CAR T cells required to eradicate disease in patients is not known, and likely highly variable between tumour types and individual patients. Most clinical trials conducted to date have not routinely detected, as might have been expected, the occurrence of lifelong memory against the target antigen; this is evidenced by only transient B-cell aplasias observed in the majority of patients treated[122,131,134,135,138]. Investigators conducting the UPenn study uniquely reported persistence of B-cell aplasia of >26 months in the patient with the longest ongoing response (range 1–26 months)[124,136]. Data from few patients with follow-up durations of over 1 year have been reported to date; however, it is estimated that B-cell aplasia at 6 months was 73% in this trial[124,136]. This persistence could be caused by several factors that were unique to the UPenn study: the young paediatric population treated (median age 11), the use of fludarabine-based conditioning chemotherapy in the majority of patients (see further discussion below), or the use of 4-1BB as opposed to the CD28 co-stimulatory domain. Paediatric B-ALL is very different compared with B-ALL in adult patients: as adults have a far greater rate of relapse and associated mortality in response to standard of care cancer therapies than children with B-ALL[143]. Moreover, differences between paediatric and adult patients' thymic function[144] and T-cell-subset populations and immunosenescence[145] could potentially explain why CAR T cells might have superior persistence in paediatric patients. In addition, 4-1BB is considered a 'late' co-stimulatory signal[146] and thus might have a role in increased persistence. Whether any of these factors, alone or in combination with each other and/or conditioning chemotherapy, contribute to increased persistence must be validated by further preclinical and clinical studies.

Despite the differences between the clinical trials discussed earlier[122,124,131,134–140], the efficacy and safety outcomes were remarkably similar. Therefore, drawing generalizable conclusions about optimal trial and vector design from the comparison of these studies is difficult. Important lessons on the influence of the conditioning regimen on CAR T-cell persistence and efficacy, however, can be garnered from the results of the trial conducted at Fred Hutchinson, in which changes to the lymphodepleting conditioning regimen were made as the trial progressed[139]. Preclinical studies had shown that lymphodepleting conditioning chemotherapy is necessary before CAR-T-cell infusion to obtain maximal antitumour efficacy[128], an observation also noted in the first cohort of patients with CLL treated with this therapy[147,148]. Inferior response rates were noted in trials that did not include conditioning chemotherapy, such as those carried out at MD Anderson[140] and Baylor[149] (TABLE 3).

In the clinical trial carried out at Fred Hutchinson, the addition of fludarabine to the use of cyclophosphamide-based conditioning regimens was investigated in patients with B-ALL (as well non-Hodgkin lymphoma (NHL)). These trials offered a unique opportunity to compare the effects of changes in conditioning regimens in patient cohorts that had otherwise been treated identically[139]. In this trial, 13 patients with B-ALL received the same dose of CAR T

cells, but eight were given fludarabine and the other five were given conditioning regimens lacking fludarabine[139]. In patients that received fludarabine, CAR-T-cell numbers were found to peak earlier and expand to numbers >100-fold greater than those of patients treated with conditioning regimens lacking fludarabine. At day 28 post-CAR-T-cell infusion, this difference was even more apparent because, in patients who received conditioning without fludarabine, CAR T cells became minimally detectable[139]. This increased cell expansion and persistence correlated with enhanced clinical responses and toxicity, indicating its functional relevance. Similarly, in a trial that included 19 patients with NHL, nine of them received fludarabine conditioning. In these patients, an identical trend of increased peak CAR-T-cell expansion, and persistence at 28 days was observed. The overall response rate of patients with NHL that received a fludarabine-containing regimen was 83%, compared with 50% of patients treated with conditioning regimens lacking fludarabine. Interestingly, no $CD19^+$ relapses and only one $CD19^-$ relapse was observed[139].

Another open-ended question is whether CART-cell therapy should serve as a 'bridge' to an allogeneic haematopietic stem cell transplantion (alloHSCT), or whether alloHSCT could be avoided. The standard of care for adults with relapsed B-ALL who develop a second complete response is alloHSCT[150,151]. For patients who would not be eligible for an alloHSCT owing to their disease burden, CAR T-cell therapy helps to reach a second or third complete response, making them newly eligible for transplant. A group of MSKCC investigators have reported on the largest series of adult patients with relapsed B-ALL treated to date ($n = 38$), with a strategy that encouraged patients to undergo an alloHSCT whenever possible[135]. However, two-thirds of patients achieving a complete response to CAR-T-cell therapy did not proceed to alloHSCT because of the lack of an available donor, a personal preference, or, most commonly, because of medical co-morbidities that excluded alloHSCT. The overall survival of the patients with >6 months of follow-up who achieved a complete response and proceeded to alloHSCT was 70%[135]. This is similar to the overall survival of patients who were ineligible or declined alloHSCT (62%; $P = 0.5$). Additionally, a subset of patients who were followed expectantly without alloHSCT have had long-term disease-free survival of >12 months post-CAR-T-cell therapy[135]. This study is the largest of its kind performed to address this question, but these values were certainly underpowered and the patients in the study were not randomly allocated, thus precluding any firm conclusions on post-CAR-T-cell alloHSCT. These results[135], however, suggest that CART-cell therapy might replace alloHSCT for patients with B-ALL in the future.

CLL—The initial trials investigating CAR T-cell therapy and, therefore, the first responses to this therapy were reported in patients with chronic lymphocytic leukaemia (CLL)[147,152]. The responses in patients treated with CAR T cells for CLL have been more modest than those observed in patients with B-ALL; although exceptional responses have been reported initially among the first two out of three patients achieving a complete response[152,153]. In an updated analysis by investigators from UPenn, five (inclusive of the initial two) out of 23 evaluable patients achieved a complete response[141]. In a study carried out at MSKCC, in which nine patients were treated, the first three patients did not receive conditioning therapy, and the following six patients received cyclophosphamide or bendamustine conditioning[147,148]. None of the patients who did not receive conditioning therapy had a

response, and only one of the six patients who received it achieved a complete response[147,148].

There are several potential causes for this relative paucity of responses seen in CLL when compared with B-ALL. One reason is that CAR T cells generated from patients with CLL might have inherent effector T-cell dysfunction[154,155]. Furthermore, it remains to be confirmed whether CAR T cells migrate towards, and penetrate into lymph nodes as efficiently as they do bone marrow. Additionally, immune suppression via T-cell checkpoint inhibitory receptors[156], cell types associated with immunosuppression, such as T_{REG} cells (REF.157) and MDSCs[158] or supportive cell types, such as CLL-nurse cells[159,160], and inhibitory cytokine production[161] might influence CAR-T-cell efficacy in patients with CLL to varying degrees. CLL cells might exist in a tumour microenvironment that is suppressive to CAR-T-cell function (FIG. 2). Additional modifications in the design of CAR-T-cell therapies are needed to further optimize their efficacy for the treatment of CLL.

NHL

Several groups have reported their initial experience treating patients with relapsed and/or refractory NHL (mostly of aggressive histology subtypes) using the same CARs as those used to treat B-cell leukaemias[139,162–165]. Investigators from the NCI carried out a trial that included nine patients with aggressive NHL; either diffuse-large B-cell lymphoma (DLBCL) or primary mediastinal B-cell lymphoma (PMBCL)[162]. Using cyclophosphamide-conditioning chemotherapy before administration of CAR T cells, four out of seven evaluable patients with aggressive NHL achieved a complete response, which included three patients who had a persistent complete response 9–22 months after treatment. The T-cell counts peaked in the peripheral blood 7–17 days after infusion and persisted for a period between 2 weeks and 2 months[162].

Similar to the NCI trial, investigators from UPenn also reported on their CAR-T-cell trials for aggressive NHL with a cohort of 13 patients with DLBCL that had been heavily pretreated[163]. Different non-myeloablative preconditioning chemotherapy regimens were used in this trial. Five patients achieved a complete response, in all cases ongoing at the time of reporting between 6 months to >1 year[163].

At the same time as UPenn investigators reported their results from treating patients with aggressive NHL with CAR-T-cell therapy, researchers at MSKCC reported on 10 evaluable patients with relapsed, aggressive-histology NHL who were chemosensitive to salvage therapy, but with tumour presence, as confirmed by post-treatment PET scans[164]. Unlike at the NCI or UPenn, the protocol investigated administering CAR T cells in the setting of high-dose chemotherapy (BEAM regimen; carmustine, etoposide, cytarabine, melphalan) followed by autologous stem-cell rescue (ASCR). CAR T cells were administered on days +2 and +3 post-ASCR. Six out of 10 evaluable patients achieved a complete response, with four of them remaining free from disease progression after 13–21 months of follow-up[164].

As described earlier, investigators at the Fred Hutchinson conducted a trial that included 19 patients with NHL of different grades[139]. These patients' T cells were separated into $CD4^+$ and $CD8^+$, and independently transduced in parallel with a 4-1BB containing CAR. $CD8^+$

cells were further selected to have a potentially favourable central memory immunophenotype (T_{cm}). The final infusion product was formulated to consist of 1:1 $CD4^+$:$CD8^+$ T_{cm} cells. The conditioning regimen included included various combinations involving cyclophosphamide and/or fludarabine. The overall response rate in all patients with NHL who received treatment was 63%. Similarly as observed in the trial for B-ALL[139], these researchers oberved that T-cell persistence and the complete response rate were significantly improved in the group of patients that received a conditioning treatment containing fludarabine, as previously described.

Finally, investigators at Baylor reported on a trial that included five patients with NHL[165]. The design of this trial was unique because it involved infusing two CAR-T-cell products simultaneously: one containing a CD28-bearing CAR and the other consisting of an equal dose of T cells containing a first-generation CAR with a CD3ζ signalling domain but without an additional co-stimulatory domain. Furthermore, in contrast with other groups, it did not include lymphodepleting preconditioning chemotherapy. The efficacy of this design was limited, and no sustained remissions were observed in the patients who received treatment. The investigators determined that second-generation CAR T cells persisted longer (up to 9 months as detected in peripheral blood using quantitative PCR) than first-generation CAR T cells[165].

Toxicity—The adoption of CAR-T-cell therapy, has been followed by the emergence of a novel set of adverse effects including cytokine-release-syndrome (CRS), macrophage activation syndrome (MAS; or haemophagocytic lymphohistiocytosis, HLH), and neurological toxicities. CRS is a constellation of symptoms derived from the cytokines released by activated T cells and/or activated macrophages[122,138,166]. A concomitant MAS is evidenced by the presence of elevated levels of ferritin and, in some cases, hypofibrinogenaemia[166] in addition to elevated levels of cytokines, such as IL-6 and IL-10. MAS can occur owing to a positive feedback loop that affects signalling pathways activated by the cytokines released by activated CAR T cells[138,166]. These potential cytokine mediated toxicities range from mild, with isolated fever, to severe (sCRS), with symptoms that include hypotension and respiratory distress requiring the intervention of an intensive care unit[122,138]. CRP is a parameter that can be measured in routine clinical laboratory tests and serves as a surrogate marker of CRS in patients with B-ALL. A monitored rise in CRP levels in serum is indicative of a high likelihood of impending sCRS[122]. sCRS and MAS are managed with the administration of antibodies targeting the IL-6 pathway, and with lymphodepleting doses of corticosteroids[122,138]. Neurological toxicity seems to be distinct from CRS and can occur together with, or independent from, sCRS. The incidence of both toxicities (neurological and sCRS) seems to correlate with disease burden, tumour histology, CAR-T-cell dose, and conditioning chemotherapy. The timing of CRS-mediated toxicity correlates with T-cell expansion in blood because the onset occurs around the time of peak T-cell expansion and usually resolves as T cells contract[122,138].

Major differences between CD19-targeted CART-cell trials exist, including differences in how sCRS is defined. In reports from the four major clinical trials for B-ALL mentioned above[131,135,137,139], however, authors report similar rates of grade 3/4 CRS or sCRS, ranging from 23–29%. Treatment-related mortality from CRS and neurotoxicity is low. In

adults treated for B-ALL, treatment-related mortality is reported as 8% (three out of 38 patients), 4% (one out of 24 patients), and 25% (three out of 12 patients) by MSKCC[135], Fred Hutchinson[139], and UPenn[167] investigators, respectively. Treatment of paediatric B-ALL and of other indications (such as CLL and NHL) in adults have even lower mortality rates, with UPenn investigators reporting no mortalities out of 85 non-adult B-ALL patients treated for all other indications[167] and NCI investigators reporting no mortalities in 21 paediatric or young-adult patients treated for B-ALL[131]. Given the heavy pretreatment history and poor prognosis of the patients included in these trials, the toxicity data need to be considered in the proper context when weighing this type of intervention in a risk–benefit discussion with patients. The modulation of CAR-T-cell infusion doses (for example, administering lower doses to patients with larger tumour burdens), and the selection of patients with lower tumour burdens might be promising approaches to minimize the occurrence of CRS and related toxicities in the future.

Additional targets in haematologic cancer

CD19 is an excellent target for CAR-T-cell therapy because it is expressed across a broad range of B-cell differentiation stages, and is ubiquitously expressed in many patients with a range of B-cell malignancies. Additional potential CAR targets for patients with haematological malignancies, however, have been identified and their use has been validated, with substantial preclinical data available; some of these potential targets have already been applied in the clinic. A selected list of these targets include, for the treatment of B-cell malignancies: CD22 (REF. 168), ROR1 (inactive tyrosine-protein kinase transmembrane receptor ROR1)[169,170], CD30 (REF. 171) and Ig kappa (κ) light chain[149]; for the treatment of multiple myeloma: B-cell maturation antigen (BCMA)[172], SLAMF7 (CS1)[173], CD38 (REFS 174,175) and CD138 (REF. 176); and for the treatment of AML: CD33 (REF. 177) and CD123 (REFS 178–180). The discussion of these and other targets is beyond the scope of this Review, but they have been discussed in detail elsewhere[181].

CAR T cells for solid tumours

The demonstration of clinical efficacy in trials using CAR-T-cell therapy are, at present, limited to haematological malignancies, but this modality is beginning to be explored clinically in the treatment of solid tumours. Solid tumours present three unique challenges not seen in B-ALL. Firstly, when compared with B-ALL, their microenvironment can be considerably more immunosuppressive (FIG. 2). Secondly, antigen selection is, in general, more difficult because the antigen heterogeneity across the same malignancy is generally higher in solid tumours[182,183]. Thirdly, ‘on-target, off-tumour’ toxicity is more problematic because potential target antigens in solid tumours are more likely to be expressed in other essential organs. New targets for solid tumours that are beginning to enter clinical studies include mesothelin for the treatment of mesothelioma[184–186], pancreatic[142,186] and ovarian cancer[186]; disialoganglioside GD2 (REFS 187,188) and EGFRvIII[189] for CNS malignancies; and mucin-16 (REFS 190,191) for the treatment of ovarian cancer. The results of the initial clinical trials for these and other targets are awaited. A more detailed discussion of CAR-T-cell therapy for solid tumours can be found elsewhere[181].

Potential escape mechanisms

Different physiological mechanisms can prevent durable CAR-T-cell-mediated antitumour responses. These mechanisms include target tumour-antigen escape, lack of CAR-T-cell persistence, and lack of CAR T-cell function. Antigen escape occurs in the setting of an initial response to CAR T cells, in which the target extracellular tumour-associated antigen is down regulated or when a minor tumour subclone that lacks antigen expression outgrows the other clones[192]. In either situation, malignant cells become undetectable to CAR T cells. Antigen escape was reported as a major cause of relapse in the UPenn trial for paediatric B-ALL, in which 10 out of 15 relapses involved this mechanism and resulted in the expansion of CD19-negative malignant cell populations[137]. Antigen escape could be addressed by targeting multiple antigens. Lack of CAR T-cell persistence has been previously discussed in this Review. The ideal length of CAR T-cell persistence is unknown; however, it seems that some minimum degree of persistence (weeks to months) is required for optimal CAR-T-cell efficacy and complete tumour eradication. Conditioning chemotherapy, T-cell immunophenotype, the patients' age and health status, the CAR-T-cell host and CAR vector design are among the factors that appear to influence persistence. Finally, lack of function can be further divided into two main causes. The first is an inability of CAR T cells to access the site of disease owing to a lack of homing signals and/or the presence of exclusion signals in the site of disease. The second is the suppression of CAR-T cell-mediated cytotoxicity at the site of disease by signals from the microenvironment. The lack of persistence or function can be addressed by administering CAR T cells in combination with immunomodulatory antibodies or by administering armoured CAR T cells.

Armoured CAR T cells

In an immunosuppressive tumour microenvironment, CAR T cells are likely to suffer the same loss of cytotoxic functionality as endogenous T cells (FIG. 1A). This phenomenon has been demonstrated by *in vivo* experiments, in which the injection of CAR T cells into mice bearing large, established tumours led to the upregulation at the protein level of the T-cell inhibitory enzymes diacylglycerol kinase and SHP-1, the cell surface expression of the inhibitory receptors PD-1, LAG-3, and TIM-3, and the inability to clear the tumour[193]. One strategy to overcome the effects of an immunosuppressive microenvironment is through the further modification of CAR T cells to additionally express immune-modulatory proteins, including ligands and cytokines (FIG. 1B). Examples of three classes of armoured CAR T cells that are currently in preclinical development are described below.

The first example is the inclusion of a second chimeric gene in the CAR vector, in which the PD-1 extracellular receptor domain is fused to the CD28 intracellular signalling domain. This design was tested in the setting of a synthetic TCR (sTCR), but could equally apply to CAR T cells[194,195]. T cells that included both the PD-1/CD28 fusion gene and the sTCR outperformed T cells that included the sTCR alone in *in vitro* studies of cytokine secretion and proliferation[194,195]. In murine xenograft models, and in a syngeneic model of human melanoma, the PD-1/CD28 chimera expressing targeted T cells demonstrated increased clearance rechallenge[194,195].

Another strategy involves CAR T cells that are genetically modified to constitutively express stimulatory ligands. T cells co-expressing CD40L[130] or 4-1BBL[129] with a second-generation CAR have been shown to increase survival of mice with difficult-to-treat systemic lymphoma xenograft. CD40L mediates its effect on $CD40^+$ tumours through T cells directly, by enhancing their immunogenicity, and via stimulation of dendritic cells[130]. 4-1BBL expression has been shown to bind T-cell co-stimulatory receptors and stimulate, not only the transduced cells themselves, but also through *trans*-co-stimulation of adjacent T cells[129].

A third example is the inclusion of a gene construct that leads to the secretion of a pro-inflammatory cytokine. IL-2 (REF. 196), IL-15 (REF. 197) and IL-12 (REF. 128) have all been studied in this context. The first trial using armoured CAR T cells secreting IL-12 has recently opened and is testing a CAR targeting mucin-16 in patients with ovarian cancer (NCT02498912)[191,127]. Systemic administration of IL-12 was shown to be toxic in early phase clinical trials[198], but local administration delivered to the site of the tumour can be achieved through secretion by CAR-targeted T cells. In this capacity, T-cells act as 'micropharmacies' and, owing to the low IL-12 levels achieved through localized secretion, systemic toxicities might be avoided. IL-12 secretion benefits CAR T cells through pleotropic effects. In preclinical studies, IL-12 secretion has been shown to obviate the need for preconditioning chemotherapy[128], enhance CAR T-cell persistence[199], provide resistance to T_{REG} cell (REF. 128) and MDSC[200,202] inhibition and result in enhanced antitumour efficacy[128,190,199–202].

Given that ovarian cancer, similarly to many other solid tumours, has a high TIL burden, which is indicative of a strongly immunosuppressive microenvironment, we are investigating the efficacy of mucin-16-targeted IL-12-secreting CAR T cells in a phase I trial for patients with relapsed ovarian cancer[191].

mAbs, CAR T cells, or combined therapy?

Durable responses are seen using immune checkpoint blockade for the treatment of patients with metastatic melanoma or NSCLC, and CAR T-cell therapy has produced dramatic responses in B-ALL. An appreciation of why each therapy has been so effective for these malignancies, and less so in others (FIG. 2), might lead to more rational design in clinical trials to investigate immunotherapy for other tumour types.

It is understood that, as tumour cells evolve, they are eliminated by immune surveillance, particularly by T cells that respond to tumour neoantigen-derived peptides presented by MHCs[203–208]. Tumour types such as melanoma and NSCLC, which harbour a high frequency of somatic mutations[209], leading to increased presentation of neoantigens, are more likely to escape immune surveillance through co-evolution in an immunosuppressive microenvironment. This same immunosuppressive microenvironment, which thwarts endogenous TILs, might also prevent CAR T cells from generating a robust antitumour response through the same suppressive mechanisms[193,210] (FIG. 1).

Thus, we hypothesize that, for tumours with high neoantigen-presenting capacity in an immunosuppressive microenvironment, immune-modulating mAbs, such as those that confer checkpoint blockade, will likely be necessary for the generation of immune-mediated antitumour responses. Additionally, as evidenced by the high rates of durable responses observed in patients with melanoma[1–3,9,47] and NSCLC[48,211] treated with PD-1 targeting mAbs alone, and especially in patients with melanoma treated with PD-1/CTLA-4 dual targeting[10,47], immune-modulating mAbs are frequently sufficient to induce such a response in this setting. Evidence for this hypothesis is supported by data from studies in which a subset of lung adenocarcinomas with higher levels of somatic mutations had increased levels of inflammation- related gene expression and immune-checkpoint effector molecules, including PD-L1 (REF. 212). Furthermore, even within a given malignancy, the prevalence of neo-antigens can be predictive of the response to checkpoint blockade with PD-1-targeted therapy in patients with NSCLC[14], and with CTLA-4-targeted therapy in those with melanoma[15,16].

We further hypothesize that tumours with low neoantigen-presenting capacity, such as those that have a reduced number of potentially immunogenic somatic mutations (for example, B-ALL[209]) or, otherwise, do not present neoantigens through downregulated antigen processing, presentation or HLA expression might be overlooked by endogenous T cells. These tumour types might not have had the pressure to co-evolve in an immunosuppressive microenvironment. In this situation, antigen presentation and TIL burden will likely be low, and immune-modulating mAbs alone would be less likely to generate a robust antitumour response. CAR T cells, however, are not inhibited by these barriers, and, as demonstrated with CD19-targeted CAR-T-cell therapy for B-ALL, can induce rapid complete responses in up to 90% of patients in this tumour type, which has a low somatic mutation rate[209].

For tumours between both extremes of the neoantigen spectrum, immunotherapies involving either CAR T cells or immune-modulating mAbs alone have not shown the dramatic results seen with melanoma and lung cancer on the one hand, and B-ALL on the other. The limited available preclinical data supports the use of combination cellular and mAb therapy in syngeneic models of sarcoma and breast cancer, in which the combination of PD-1 blockade with murine CAR T cells showed a significantly enhanced antitumour effect compared with either intervention alone[210]. We predict that, in patients with tumour types of an intermediate neoantigen-presentation capacity, the maximal immune-mediated antitumour responses might be best achieved in patients in which both the microenvironment can be modified and HLA-independent targeted effectors added, such as with combination immune modulating mAbs plus CAR-T-cell therapy or, potentially, armoured CAR-T-cell therapy. However, a risk of toxicities being exacerbated does exist when these approaches are used. We eagerly await the results of the first trial investigating the combination of CTLA-4 blockade with CAR T cells (NCT00586391)[213], and the first trial using an armoured CAR vector (NCT02498912)[127].

This neoantigen burden-based response hypothesis is not predicted to apply to other types of ACT. Unlike CAR-T-cell therapy, bulk TIL-based therapy requires tumour antigen peptides to be presented on MHCs. Thus, TIL therapy would more likely be successful in a tumour type with high neoantigen burden, as has been demonstrated with the successful use of TIL

therapy in treatment of melanoma[214–216]. This requirement would be overcome, however, if T cells targeting a specific neoantigen epitope in a particular patient could be identified and their numbers expanded *ex vivo*[217]. In such patients, the efficacy of neoantigen-directed TILs or sTCR-based therapies would remain subject to downregulation of HLA, defects in antigen processing and/or presentation machinery, or an immunosuppressive microenvironment, all of which can be found more frequently in tumour types with a high neoantigen burden (FIG. 2). Overall, this might partially explain the uniquely robust success among ACT, at least to date, of CAR-T-cell monotherapy in B-ALL.

Conclusions

The remarkable clinical results observed in trials investigating immunotherapy since 2010 have generated a large amount of interest in this therapeutic modality. Clinical trials using checkpoint blockade inhibitors to treat patients with metastatic mela-noma[1–3,9,47] and NSCLC[48,211], and trials using CAR T cells to treat relapsed or refractory B-ALL[122,124,131] have demonstrated that treating cancer ‘indirectly’ by acting on the immune system can yield durable disease control in patients with malignancies previously thought to be uniformly fatal (BOX 1). We have focused on mAbs and CAR T-cell therapy, but a number of other modalities of immunotherapy also offer great hope. These include immunomodulatory small molecules[218], oncolytic viruses[38], vaccines[219], and tumour-targeting mAbs[220], as well as attempts to overcome T-cell exclusion[221], and to exploit the immunomodulatory potential of chemotherapy and radiation therapy. The efficacy of these methods can be complementary to that of the technologies discussed here. Conceptualizing which tumour types are most likely to respond to different immunotherapies by categorizing those tumours according to their neoantigen presentation ability and their microenvironment will help investigators choose the appropriate combinations of immunotherapy for each particular cancer; and with the ongoing advances in precision medicine, to facilitate personalized therapeutic selection for each patient.

Box 1

Anticipated challenges

Immunomodulatory antibodies

- Identify optimal combinations of immunomodulatory monoclonal antibodies with each other or with other forms of cancer therapy for individual cancer types. Such combinations are key to expanding the role of immunotherapy
- Minimize impact of ‘on-target, off-tumour’ adverse effects. Immunomodulatory antibodies are not inherently cancer-specific, and therefore inflammation in benign tissues can occur. With new combinations emerging, investigators must remain wary of new or more-severe adverse effects
- Define ideal treatment duration. Unlike many cancer treatments, the activity of immunotherapy can persist after the drugs have been cleared

CAR T cells

- The challenge for relapsed/refractory B-ALL is to routinely translate deep remissions into cures. Fine-tuning conditioning chemotherapy regimens and targeting multiple antigens simultaneously to avoid antigen escape might address this issue
- The challenge for CLL and solid tumours is to achieve response rates similar to those for B-ALL. Obstacles are microenvironment-mediated immunosuppression, lack of persistence, lack of appropriate homing and/or access to the site of disease and, in some cases, lack of tumour-specific ubiquitously expressed target antigens. Modulation of the microenvironment with armoured CAR T cells or with co-administration of immunomodulatory antibodies might minimize these hurdles
- To mitigate cytokine-release syndrome and related toxicities: modifying cell-dose and frequency of administration, including administering lower doses of CAR T cells initially to patients with bulky disease followed by larger subsequent doses and treating patients in earlier stages of disease. Additionally, with the improvement of biomarkers of CRS, early intervention for this adverse event might be possible

Combinations

- Identify the safest/most efficacious combinations of immunomodulatory antibodies and/or adoptive cellular therapeutics. Rational preclinical evidence-based approaches need to be taken, but optimization in patients will be time-consuming
- In addition to selecting the appropriate combinations of agents, unique challenges to be addressed in phase I trials include selecting initial doses, determining sequencing and timing of agents, estimating maximum tolerated dose, identifying agents with limited single agent activity/toxicity, but with considerable potential for synergy

B-ALL, B-cell acute lymphoblastic leukaemia; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukaemia; CRS, cytokine-release syndrome.

Acknowledgments

The authors would like to thank their funders. D.N.K. receives support through the American Association for Cancer Research Amgen fellowship in Clinical/Translational Cancer Research and the American Philosophical Society Daland Fellowship in Clinical Investigation. E.L.S. receives support from the Conquer Cancer Foundation of ASCO, Lymphoma Research Foundation, MSKCC Technology Development Fund, and the Multiple Myeloma Research Foundation. R.B.J. receives support from the Annual Terry Fox Run for Cancer Research (New York, NY) organized by the Canada Club of New York, Carson Family Charitable Trust, Emerald Foundation, the Experimental Therapeutics Center of Memorial Sloan Kettering Cancer Center (Innovations in the structures, functions and targets of monoclonal antibody-based drugs for cancer), Kate's Team, National Institutes of Health Grants (R01CA138738-05, PO1CA059350, PO1CA190174-01), and the William Lawrence and Blanche Hughes Foundation. J.D.W. receives funding support from Bristol-Myers Squibb, Emerald Foundation, Genentech, the Ludwig Center for Cancer Immunotherapy, Medimmune, Merck Pharmaceuticals, Polynoma Pharmaceuticals and Swim Across America.

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Key points

- Cancer immunotherapies have the potential to generate robust antitumour responses; this can be achieved through several methods, such as modulatory antibodies or adoptive cellular therapy
- Since 2010, clinical trials using different immunotherapeutic approaches to treat patients with several tumour types have yielded unprecedented results
- In contrast with therapies that act on the tumour itself, immunotherapy-dependent antitumour responses can be sustained after the treatment has finished
- The optimal efficacy of immunotherapy will likely be achieved with designs that include combinations of different immunotherapeutic approaches, or immunotherapy combined with other cancer treatments

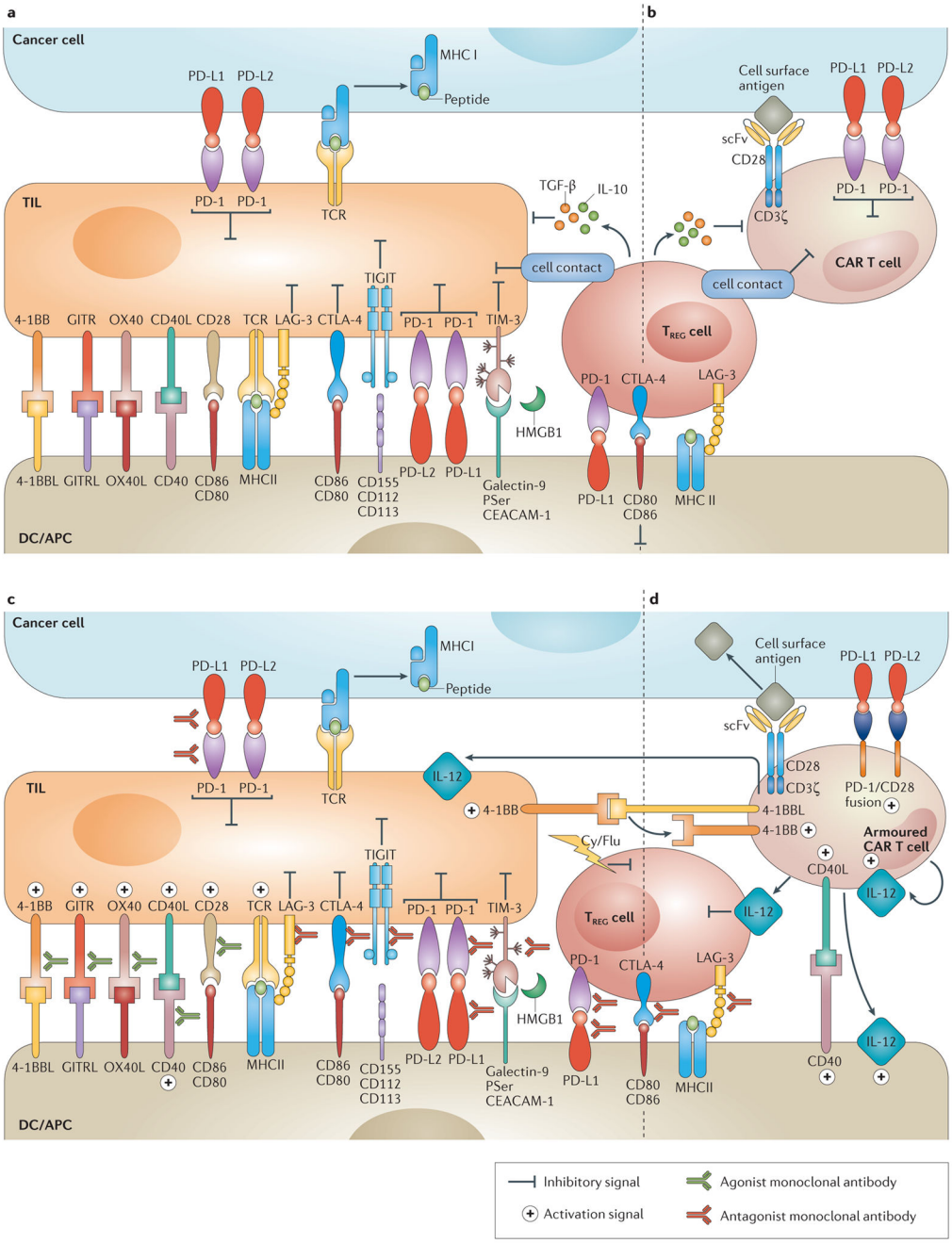

Figure 1. Immunomodulatory monoclonal antibodies and armoured chimeric antigen receptor (CAR) T cells overcome immune suppression

a | Overview of the immune inhibitory molecules that compromise endogenous T–cell antitumour activity. T cells are susceptible to immune inhibitory factors associated within the microenvironment that prevent their full antitumour activity. Such factors include cell surface proteins (such as programmed cell death 1 ligands 1 and 2 (PD-L1 and PD-L2)) and cytokines (such as TGF-β and IL-10). Regulatory T (T_{REG}) cells are representative of inhibitory cellular components of the tumour microenvironment (TME), which also include myeloid-derived suppressor cells, tumour-associated macrophages and other cell types non-

depicted. **b** | Similarly to endogenous T cells, CAR T cells are susceptible to immune inhibitory factors present in the TME. **c** | Immunomodulatory monoclonal antibodies can be used to overcome local immunosuppression by either activating stimulatory receptors (such as TNFRSF9 (4-1BB) or OX40), or blocking suppressive receptors (for example, programmed cell-death 1 (PD-1) or cytotoxic T-lymphocyte antigen 4 (CTLA-4)). **d** | Armoured CAR T cells are engineered to express proteins that overcome immunosuppression associated with the TME (such as CD40L, IL-12 or TNFSF9 (4-1BBL)). Ag, antigen; APC, antigen-presenting cell; CD40L, CD40 ligand; Cy, cyclophosphamide; DC, dendritic cell; Flu, fludarabine; GITR, glucocorticoid-induced TNFR family related protein; GITRL, GITR ligand; HMGB1, high mobility group 1 protein; LAG-3, lymphocyte activation gene-3; PSer, phosphatidyl serine; scFv, single-chain variable fragment; TCR, T-cell receptor; TIGIT, T-cell immunoreceptor with immunoglobulin and ITIM domains; TIL, tumour -nfiltrating lymphocyte; TIM-3, T cell immunoglobulin and mucin domain 3.

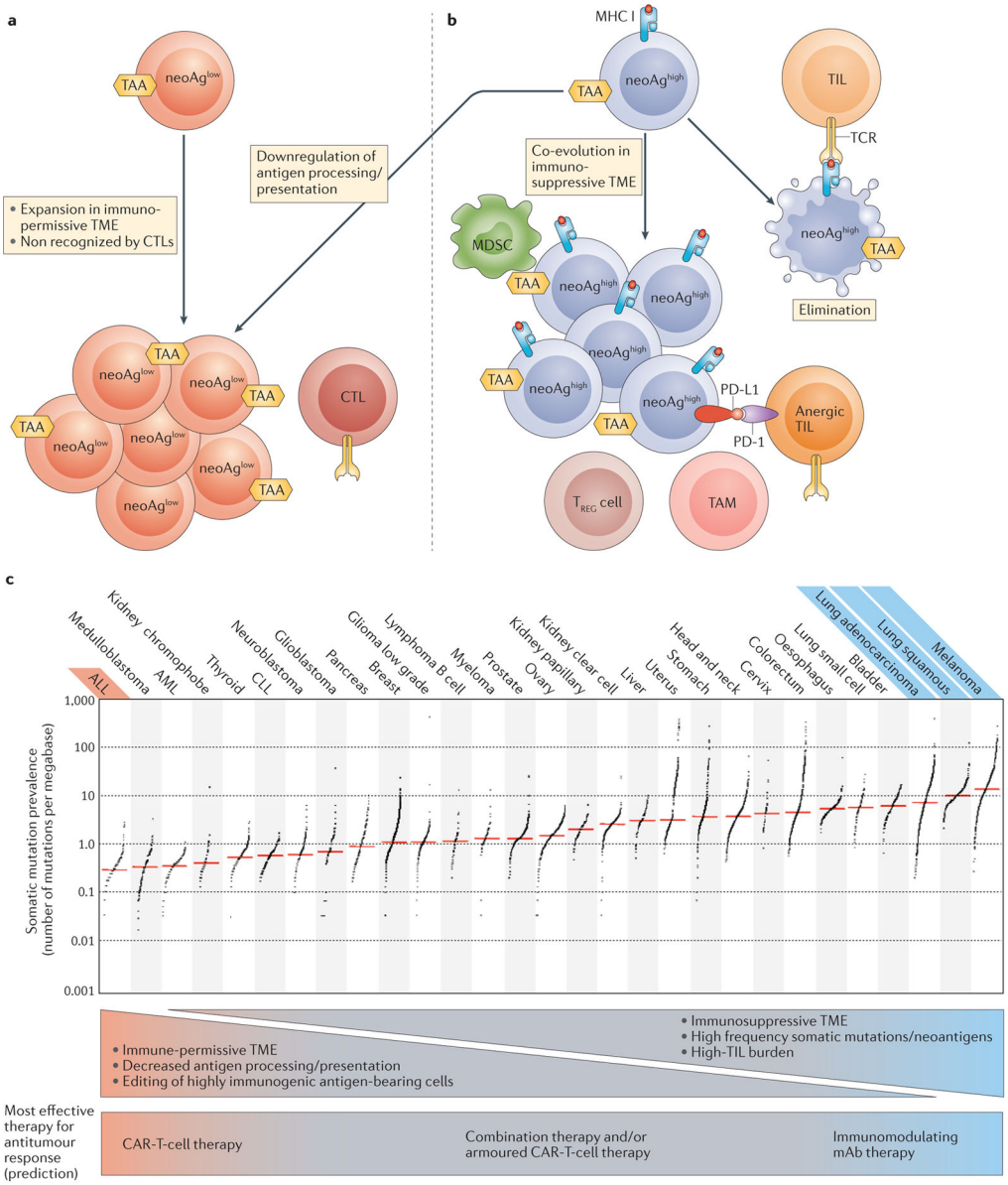

Figure 2. Neoantigen presentation in the tumour microenvironment

a | Tumour cells can avoid elimination by immunoediting because of low neoantigen presentation (neoAg^{low}). This occurs as a result of a low level of somatic mutations, or because tumour cells might downregulate antigen processing or presentation. NeoAg^{low} tumours might escape detection by endogenous CTLs early in tumorigenesis, and therefore would expand without the pressure to co-evolve within an immunosuppressive microenvironment. This is the ideal setting for CAR T cells to direct a robust antitumour response. **b** | Tumours that present a high neoantigen burden (neoAg^{high}) can be eliminated during the immunoediting process (far right). Alternatively, neoAg^{high} tumour cells might co-evolve within an immunosuppressive tumour microenvironment (TME) that mediates avoidance of T-cell surveillance. This setting is the ideal situation for immune-modulating therapies with monoclonal antibodies to direct a robust antitumour response. **c** | Tumour

types can be ranked by their relative presence of somatic mutations[209], and this classification can be used to indirectly estimate neoantigen burden. Tumours can be stratified on the basis of somatic mutation prevalence; tumour types with the most robust clinical responses to CAR T-cell therapy (red) or immune modulating mAbs (blue) are stratified by somatic mutation prevalence. ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukaemia; CTL, cytotoxic T cell; mAb, monoclonal antibody; MDSC, myeloid-derived suppressor cell; neoAg, neoantigen; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; TAA, tumour-associated antigen; TAM, tumour-associated macrophage; TCR, T-cell receptor; TIL, tumour infiltrating lymphocyte; T_{REG} cell, regulatory T cell. Image reproduced from Alexandrov, L. B. *et al.* Signatures of mutational processes in human cancer. *Nature* **500**, 415–421 (2013),

Table 1

Adverse events associated with immune-checkpoint blockade

Immune-mediated adverse event	Manifestations	Management
Enterocolitis	Diarrhoea, abdominal pain, mucus or blood in stool	Antidiarrhoeals followed by systemic corticosteroids if persistent; infliximab if refractory
Pneumonitis	Dyspnoea, cough	Systemic corticosteroids
Hepatitis	ALT/AST, bilirubin elevation	Systemic corticosteroids; mycophenolate mofetil if refractory
Dermatitis	Pruritic/macular/papular rash, Stevens–Johnson syndrome (rare), toxic epidermal necrolysis (rare)	Topical betamethasone or oral antihistamines; systemic corticosteroids if refractory
Neuropathy	Sensory/motor neuropathy, Guillain–Barre syndrome (rare), myasthenia gravis (rare)	Systemic corticosteroids
Endocrinopathy	Hypothyroidism, hyperthyroidism, hypopituitarism, adrenal insufficiency, hypogonadism, Cushing's syndrome (rare)	Systemic corticosteroids, appropriate hormone replacement (potentially long-term)
Other irAEs	Arthritis, nephritis, meningitis, pericardidits, uveitis, iritis, anaemia, neutropenia	Organ-system specific

Severe immune-mediated adverse events require permanent discontinuation of therapy and initiation of high-dose systemic corticosteroids. Therapy should be withheld for moderate immune-mediated adverse events or symptomatic endocrinopathy. Non-immune aetiology should be ruled out when possible, and manufacturer recommendations should be reviewed for the latest guidance and dosing information. ALT, alanine aminotransferase; AST, aspartate aminotransferase; irAEs, immune-related adverse events.

Table 2

CD19-targeted CAR T-cell design by institution[120]

Study	Anti-CD19 scFv clone	Spacer regions	Signalling domains	Genetic modification method	T-cell population
Baylor[165]	FMC63	Human IgG1 (CH2 and CH3 domains)	CD28 and CD3ζ or CD3ζ alone	Retroviral transduction	Bulk
Fred Hutchinson[139]	FMC63	Modified human IgG4	4-1BB co-stimulatory CD3ζ activation	Lentiviral transduction	1:1 $CD4^+$: $CD8^+$ T_{cm}
MD Anderson[140]	FMC63	Modified human IgG4	CD28 co-stimulatory CD3ζ activation	Sleeping beauty transposon electroporation	Bulk
MSKCC[122,134,135,147,164]	SJ25C1	No hinge	CD28 co-stimulatory CD3ζ activation	Retroviral transduction	Bulk
NCI[131,138,162]	FMC63	No hinge	CD28 co-stimulatory CD3ζ activation	Retroviral transduction	Bulk
UPenn/CHOP[124,141,142,152,153,163]	FMC63	CD8a hinge	4-1BB co-stimulatory CD3ζ activation	Lentiviral transduction	Bulk

Baylor, Baylor College of Medicine; CAR, chimeric antigen receptor; CHOP, Children's Hospital of Philadelphia; Fred Hutchinson, Fred Hutchinson Cancer Center; MD Anderson, MD Anderson Cancer Center; MMSKC, Memorial Sloan Kettering Cancer Center; NCI, National Cancer Institute; scFv, single-chain variable fragment; T_{cm}, central memory T cells; UPenn, University of Pennsylvania.

Table 3

Clinical outcomes of CD19-targeted CAR-T-cell therapy for patients with B-ALL

Study/CAR design	Conditioning/CAR-T-cell dose	Patient population	Clinical responses	CAR-T-cell persistence
Fred Hutchinson[139] FMC63-BBζ	• Cy 60 mg/kg + Flu 25 mg/m^2 daily × 3–5 (46%) or other Cy-based conditioning (54%) • 0.2–20 × 10^6 CAR T cells/kg	• *n* = 24 adults with R/R disease • Age range: 22–71 years	• 91% CR • 87% MRD (by PCR) • 29% sCRS	• Persistence in responding patients >1 month • Improved with Flu conditioning
MD Anderson[140] FMC63-28ζ	• No conditioning • 1–500 × 10^6 CAR T cells/m^2	• *n* = 10 • Post-alloHSCT uniquely, no active disease	• 5 month DFS: 30% • No GVHD or sCRS	Detectable up to 3 months
MSKCC[122,134,135] SJ25-28ζ	• Cy 1.5–3 g/m^2 × 1 dose • 1–3 × 10^6 CAR T cells/kg (fractionated over 2 days)	• Adults with R/R disease • Median age: 45 years (22–74 years) • *n* = 38 evaluable for safety (*n* = 28 evaluable for response)	• 87% CR • 81% MRD-negative (by deep sequencing) • Median time to CR: 23 days • 6-month DFS: 50% • 6-month OS: 59% • 14% CD19-negative relapses • 23% sCRS	• Peak day: 7–14 • Persistence 1–3 months
NCI[131,138] FMC63-28ζ	• Cy 900 mg/m^2 × 1 dose + Flu 25 mg/m^2 × 3 doses • 1–3 × 10^6 CAR T cells/kg	• Paediatric/young adults with R/R disease • Median age: 13 years (1–30 years) • *n* = 21 (*n* = 20 with B-ALL) • Uniquely reported intention-to-treat-analysis	• 67% CR • 10-month OS: 52% • Two CD19-negative relapses • 29% sCRS	Detectable up to day 68 (by qPCR)
UPenn/CHOP[124,141,142] FMC63-BBζ	• Variable conditioning:>50% received Flu 30 mg/m^2 daily ×4 doses + Cy 500	• Paediatric patients with R/R disease	• 94% CR • 82% MRD-negative (by flow cytometry)	Range detectable:1 month–2 years (by qPCR)

Study/CAR design	Conditioning/CAR-T-cell dose	Patient population	Clinical responses	CAR-T-cell persistence
	mg/m^2 daily × 2 doses • 0.76–20.6 × 10^6 CAR T cells/kg	• Median age: 11 years • *n* = 48 evaluable	• 6-month DFS: 76% • 6-month OS: 78% • 67% CD19-negative relapses • 29% sCRS	

alloHSCT, allogeneic haematopoietic stem cell transplantation; B-ALL, B-cell acute lymphoblastic leukaemia; CAR, chimeric antigen receptor; CHOP, Children's Hospital of Philadelphia; CR, complete response; Cy, cyclophosphamide; DFS, disease free survival; Flu, fludarabine; Fred Hutchinson, Fred Hutchinson Cancer Center; GVHD, graft-versus-host disease; MD Anderson, MD Anderson Cancer Center; MRD, minimal residual disease; MSKCC, Memorial Sloan Kettering Cancer Center; NCI, National Cancer Institute; OS, overall survival; PCR, polymerase chain reaction; qPCR, quantitative PCR; R/R; relapsed and/or refractory; sCRS, severe cytokine-release syndrome; UPenn, University of Pennsylvania.