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Review

Review

Enhancing co-stimulation of CAR T cells to improve treatment outcomes in solid cancers

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Summary

Co-stimulation is a fundamental component of T cell biology and plays a key role in determining the quality of T cell proliferation, differentiation, and memory formation. T cell-based immunotherapies, such as chimeric antigen receptor (CAR) T cell immunotherapy, are no exception. Solid tumours have largely been refractory to CAR T cell therapy owing to an immunosuppressive microenvironment which limits CAR T cell persistence and effector function. In order to eradicate solid cancers, increasingly sophisticated strategies are being developed to deliver these vital co-stimulatory signals to CAR T cells, often specifically within the tumour microenvironment. These include designing novel co-stimulatory domains within the CAR or other synthetic receptors, arming CAR T cells with cytokines or using CAR T cells in combination with agonist antibodies. This review discusses the evolving role of co-stimulation in CAR T cell therapies and the strategies employed to target co-stimulatory pathways in CAR T cells, with a view to improve responses in solid tumours.

Keywords: chimeric antigen receptor, immunotherapy, T cell immunology, co-stimulation

Abbreviations:APC: Antigen presenting cell; CAR: Chimeric antigen receptor; CD: Cluster of differentiation; DAMP: Danger-associated molecular pattern; DC: Dendritic cell; FDA: American Food and Drug Administration; HSP: Heat shock protein; ICR: Inverted cytokine receptor; OV: Oncolytic virus; PAMP: Pathogen-associated molecular pattern; scFV: Single-chain fragment variable; Th_1: T helper type 1; TME: Tumour microenvironment; TLR: Toll-like receptor; TIL: Tumour-infiltrating lymphocyte; NK: Natural killer; VSV: Vesicular stomatitis virus.

Introduction

Immunotherapies are an increasingly prevalent therapeutic option for patients with cancer. Chimeric antigen receptor (CAR) T cell immunotherapy is a strategy to genetically engineer patient T cells with a synthetic receptor targeting a specific antigen [1]. The CARs are composed of an antigen binding single-chain fragment variable (scFV) extracellular domain, transmembrane domain, and the intracellular CD3ζ and co-stimulation signalling domains. CAR T cells are currently FDA approved for the treatment of certain B cell malignancies [2]. However the overall responses are disappointing in solid cancers [3]. This is due to several factors such as an immunosuppressive tumour microenvironment (TME), poor trafficking into the tumour and limited persistence of CAR T cells [4].

Optimal T cell activation results from cognate antigen recognition (signal 1), co-stimulation (signal 2), and cytokine support (signal 3). The precise timing and context of co-stimulation signals are understood to ultimately define the effectiveness of the T cell response [5]. Integrating this understanding with CAR T cell design will lead to more robust CAR T cell therapies for solid cancers. This review will summarize the role of co-stimulation in CAR T cell therapies with a focus on strategies to improve responses in solid cancers.

Importance of co-stimulation in CAR design

The first generation of CARs were developed more than 30 years ago. These CARs contained a single CD3ζ chain but did not include any co-stimulation intracellular domain, thus had limited anti-tumour function due to the lack of co-stimulation. In an early phase I study using the first-generation CAR against alpha-folate receptor (FR) in metastatic ovarian cancer, none of the treated patients developed any anti-tumour response, demonstrating the importance in incorporating co-stimulation in the CAR design [6]. The first studies exploring the use of co-stimulation in CAR T cells included a CD28 co-stimulation intracellular domain into the CAR receptor [7]. CD28 co-stimulation domain greatly enhanced CAR T cell function leading to early clinical responses to CAR T cell therapy, highlighting the importance of co-stimulation signalling [8, 9]. In an early trial, a patient with advanced follicular lymphoma was treated with a CD19-CAR that contained a CD28 co-stimulation domain. This patient's cancerous B cells were eliminated and absent for at least 39 weeks after CAR T cell transfusion. Inspired by the success, other co-stimulatory domains have been included in CARs and some trials have demonstrated great success [8, 9].

Until now only a limited number of co-stimulatory domains have been thoroughly investigated [10]. CD28 and 4-1BB (CD137) are the best characterized domains and the only two included in current FDA approved CAR T cell formulations (Table 1). These domains trigger distinct downstream signalling pathways resulting in either increased persistence or enhanced effector function of CAR T cells [11]. The selection of co-stimulatory domains within the CAR is believed to be key to overcoming barriers imposed by solid tumours. Screening approaches have demonstrated a wide range of novel candidate co-stimulatory domains which can be incorporated into CARs [12]. To this end, many groups are exploring additional domains such as OX40 (CD134), CD27, GITR (CD357), and ICOS (CD278) [13–17] (Fig. 1-1). CARs including one co-stimulatory domain are classified as second generation, while those including two co-stimulatory domains are classified as third generation. Third-generation CARs demonstrated superior anti-tumour responses and magnitude of *in vivo* expansion compared to second-generation CARs in some studies. Ramos *et al.* demonstrated that third-generation CAR T cells persisted longer and with superior *in vivo* expansion compared to second-generation CAR T cells in relapsed/refractory non-Hodgkin lymphoma patients [18]. However, other studies have demonstrated opposing results. For example, a study comparing the second-generation anti-PSCA-CD28 CAR with the third-generation anti-PSCA-CD28-4-1BB CAR indicated that the second-generation CAR was superior in their anti-tumour effect in a human pancreatic cancer xenograft model [19]. The superiority of third-generation CARs is therefore still debatable. Collectively, these studies demonstrated that co-stimulation within the CAR receptor is a key factor determining CAR T cell efficacy.

Co-stimulation delivered intrinsically within the CAR can be coupled with other methods of co-stimulation to overcome the key barriers imposed by solid cancers. Some novel designs include co-stimulatory domains from certain signalling pathways. CARs incorporating MyD88 domains along with intracellular domains of CD40 demonstrated improved efficacy. The incorporation of these 'MC' co-stimulatory domains resulted in increased long-lived central memory CAR T cells associated with improved clinical outcomes [20, 21]. Coupling co-stimulation and CAR engagement affords precise control over when and how co-stimulation is delivered. Other strategies may include transducing additional genes that code for cytokines, synthetic signalling domains and receptors into the CAR T cells. For example, a study included a JAK-STAT signalling domain into a CAR to resemble γ-chain cytokine signalling and

Table 1. FDA-approved CART therapies and their associated co-stimulatory domains. Data collected from Clinicaltrials.gov and fda.gov

Product	Company	Target	Disease	Co-stimulatory domain	Clinical Trial
KYMRIAH (tisagenlecleucel)	Novartis	CD19	Diffuse large B cell lymphoma (DLBCL), high grade B-cell lymphoma and DLBCL arising from follicular lymphoma.	4-1BB	NCT02445248
YESCARTA (axicabtagene ciloleucel)	Kite Pharma	CD19	DLBCL not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma.	CD28	NCT02348216
BREYANZI (lisocabtagene maraleucel)	Juno Therapeutics	CD19	DLBCL, high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B	4-1BB	NCT02631044 NCT03484702 NCT03744676 NCT03310619 NCT03483103 NCT03331198 NCT03743246 NCT03435796
ABECMA (Idecabtagene vicleucel)	Celgene Corporation	BCMA	Relapsed/refractory multiple myeloma	4-1BB	NCT03361748 NCT02215967 NCT02658929
TESCARTUS[a] (brexucabtagene autoleucel)	Kite Pharma	CD19	Relapsed/refractory mantle cell lymphoma	CD28	NCT02601313

[a]TESCARTUS employs the identical retroviral vector to YESCARTA however is manufactured using a distinct protocol which enriches for T cells.

resulted in increased CAR T cell proliferation *in vivo* in a model of oesophageal cancer [22]. Including domains such as this within the CAR circumvents potential cytokine release syndrome (CRS) associated with non-specific secretion of cytokine and avoids administration of toxic cytokines. Toll-like receptors (TLR) are known co-receptors in T cells, and CARs incorporating TLR domains are being developed [23]. TLR2 is expressed on memory T cell subsets and detects pathogen-associated molecular pattern (PAMPs) and endogenous danger-associated molecular patterns (DAMPs), such as heat shock protein (HSPs) and amyloids [24, 25]. Unlike commonly used domains, TLR2 signals through MyD88 to improve cytokine secretion and effector function in T cells [26, 27]. The incorporation of TLR2 domains improved the efficacy of MUC1-CAR T cell function in a solid tumour model [28].

Synthetic and combinatorial co-stimulatory receptors enhance CART cell function

CAR T cells can be transduced to express additional synthetic receptors, which act in *trans* or parallel with CAR receptors to provide co-stimulation to CAR T cells. These receptors often target molecules overexpressed by the TME. Switch receptors link a checkpoint extracellular domain to a co-stimulatory intracellular domain, for example, PD-1 (CD279) and CD28 [29] (Fig. 1-2). This PD-1-CD28 receptor delivers CD28 co-stimulation to CAR T cells when ligating PD-L1 (CD274), which is overexpressed by solid tumours. The ligation leads to enhanced cytokine secretion and restimulation of the switch CAR cells. In two models of mesothelin and several $PSCA^+$ solid tumours, the switch CAR anti-tumour effect is stronger than the non-switch CAR cells used in combination with pembrolizumab (anti-PD-1), indicating that signalling through CD28 of the switch receptor is driving this effect [30]. Inverted cytokine receptors (ICR) function similarly to switch receptors but leverage the abundance of immunosuppressive cytokines in the TME [31, 32]. ICRs couple an extracellular domain of an immunosuppressive cytokine receptor such as IL-4 with an intracellular signalling domain of a pro-survival cytokine receptor such as IL-7. These receptors deliver pro-survival cytokine signals (signal 3) in the presence of suppressive cytokines in the TME (Fig. 1-2). For

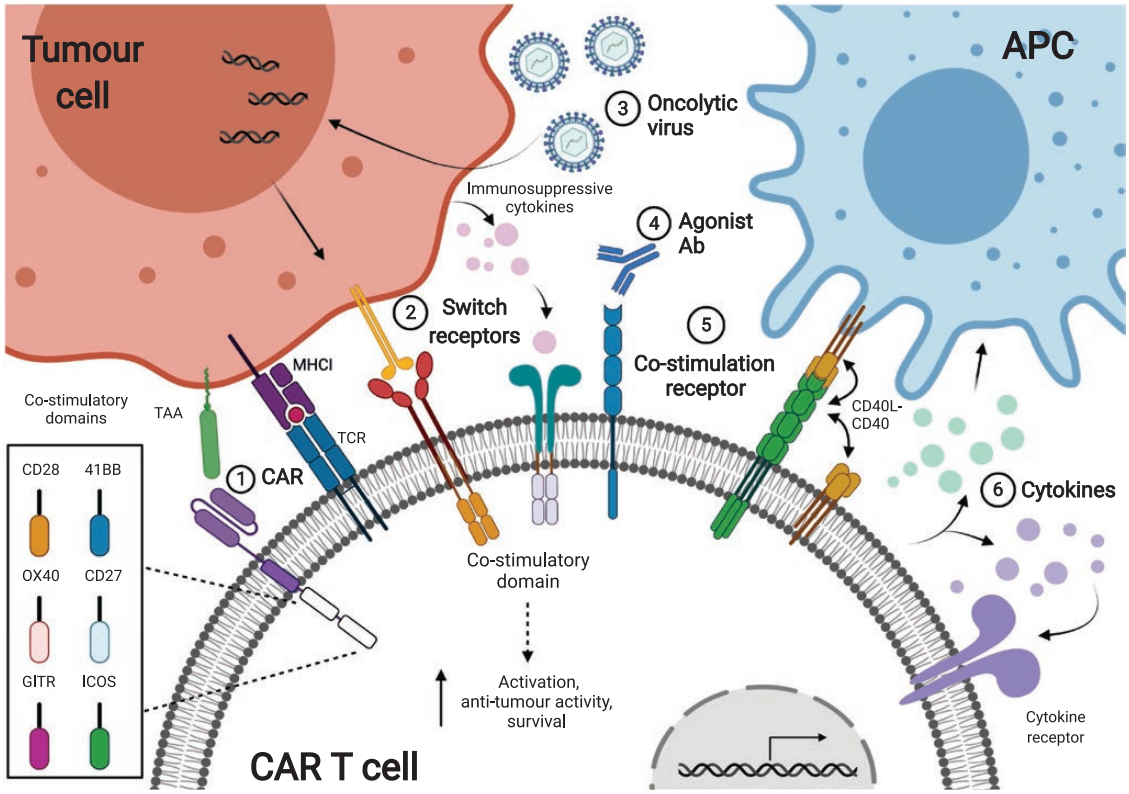

Figure 1. Strategies enhancing co-stimulation of CAR T cells. 1-1: Co-stimulation and synthetic signalling domains can be integrated directly within the CAR receptor. These domains provide co-stimulatory signals when the CAR is activated. 1-2: Switch receptors (e.g. PDL1-CD28) and inverted cytokine receptors (e.g. GM-CSF-IL-18) transduce a co-stimulation signal when ligating immunosuppressive cytokines such as PDL1 or GM-CSF. 1-3: Oncolytic viruses target tumor cells and remodel the TME with immunostimulatory molecules such as OX40. 1-4: CAR T cell secreting agonist antibodies against CD40 or 4-1BB activate CAR T or endogenous immune cells. 1-5: CD40 (represented by the yellow molecule) can act in *cis* and *trans* when expressed on CAR T cells. 1-6: CAR T cells secreting cytokines such as IL-18 or IL-12 license APCs or act on T cells to drive an antitumor response.

example, CAR T cells expressing a GM-CSF-IL-18 ICR were able to mediate tumour regression in HER2 and EphA2 solid tumour models. In this design, the ICR contained an extracellular domain of the GM-CSF receptor and the signalling domains of the IL-18 receptor (GM18). GM18 can be activated in the tumour by endogenous GM-CSF of the TME, leading to enhanced CAR T cell survival and tumour cell clearance [33]. GM-CSF has also been targeted with an IL-2-based ICR [34]. These additional co-stimulatory triggers may synergize with CAR signalling by including distinct domains, effectively augmenting CAR signalling localized within tumour tissues. Additional synthetic co-stimulatory receptors also flip key interactions within the TME to deliver additional pro-survival signals to CAR T cells.

CD40 is a receptor expressed on antigen presenting cells (APCs) and is central to developing tumour-specific T cell responses [35]. When expressed on T cells, CD40 is able to act in *cis* and *trans* by binding to CD40-L expressed on T cells, ultimately enhancing the survival of CAR T cells in tumours [36] (Fig. 1-*5*). Solid tumours evade the immune system through a number of mechanisms including a large degree of antigen heterogeneity, as well as their immunosuppressive microenvironment. Enhancing co-stimulation of both CAR T and endogenous T cells may boost endogenous immune responses to recognise neoantigens and reduce tumour immune escape. $CD40L^{+}$ CAR T cells are shown to be superior in their anti-tumour effect and provide a rational to incorporate CD40-CD40-L signal in CAR T design [36].

Cytokine co-stimulation is a crucial component of a CAR T cell response

Cytokines are secreted proteins with a range of effects on all sets of immune cells. In the context of CAR T cells, these proteins constitute the 'signal 3' checkpoint for activation. γ-chain cytokines such as IL-2 and

IL-15 have essential non-redundant roles in supporting the survival and differentiation of T cells, as well as CAR T cells [37]. *Ex vivo* production of CAR T cells using these γ-chain cytokines drives CAR T cell differentiation to effective subtypes for solid cancers, and these cytokines have also been used as direct therapies *in vivo* [38]. Cytokines such as IL-2 and IL-12 have been used to activate and expand tumour-infiltrating lymphocytes (TILs) in solid cancers resulting in some curative responses, but are associated with toxicity [39]. Therefore, 'armoured CAR' T cells have been developed to secrete such cytokines specifically within the TME to reduce toxicity as well as recruit endogenous T cells to overcome tumour heterogeneity [40] (Fig. 1-6). CAR T cells transduced to secrete IL-12 increased macrophage and innate cell-mediated clearance of TAA-negative cells, leading to enhanced control of tumours [41]. However, excessive cytokine co-stimulation with IL-12 has been documented to drive CART cell exhaustion [42]. IL-1 family cytokines are a group of proinflammatory cytokines including IL-1, IL-18, and IL-36γ [43]. These cytokines are generally proinflammatory and can act on both T cells and dendritic cells (DCs) to drive a Th_1 type response and increase IFNγ secretion by T cells [43]. IL-18, best known for inducing antigen-independent bystander T cell activation, can act synergistically with IL-12 to inhibit solid cancer progression [44, 45]. CAR T cells expressing IL-18 were able to mediate effective responses in a model of colon cancer while also activating endogenous TILs [46]. Similarly, CAR T cells expressing IL-36γ also mediated tumour regression but with different kinetics to previously tested IL-1 family cytokines, demonstrating non-redundant signalling within this cytokine family [47]. Chemotactic cytokines, or chemokines, can also be used to enhance trafficking of CAR T cells to solid tumours. CAR T cells secreting IL-7 and CCL19 provide both pro-survival signals to CAR T cells in the tumour as well as recruit and license intertumoral APCs in a model of lung cancer [48]. This resulted in increased immune cell infiltration and memory formation as cured mice were resistant to tumour re-challenge, and these results have now been extended to human xenograft models. Manipulation of the cytokine milieu by direct CAR T cell secretion has demonstrated effects directly on the function of CAR T cells and endogenous cells, remodelling the TME to a more permissive immune environment. Understanding the role of cytokines in sustaining, improving or hampering intra-tumoral immune response will facilitate their optimal incorporation into CAR T cell therapy regimes.

Antibody-based approaches utilizing co-stimulation in CAR T cell therapies

Checkpoint blockade therapies are an indirect method of modulating T cell co-stimulation by utilising antibodies to inhibit negative regulators of co-stimulatory molecules. These therapies have demonstrated to enhance CAR T cell efficacy and have been reviewed elsewhere [49, 50]. Antibodies directly targeting co-stimulatory molecules can also boost the immune response to cancer. CD40 antibodies are approved therapeutics for cancer and have both T cell intrinsic and pleiotropic effects [51]. When used in combination with IL-15, CD40 agonists were able to increase CD8 T cell and NK cell infiltration into pancreatic cancers, leading to establishment of immune memory response [52]. In a novel approach, CAR T cells were engineered to secrete CD40 agonist antibodies. Compared with traditional CAR T cells, these anti-CD40 secreting CAR T cells demonstrated elevated cytotoxic effect on cancer cells and increased proportion of central memory phenotype [53]. 4-1BB agonist antibodies have also been investigated in the context of solid cancers and were able to increase the cytokine secretion of CAR T cells as well as remodelling of endogenous T cells in a model of breast cancer [54] (Fig. 1-4). However, these agonist antibodies have not progressed beyond clinical trials due to systemic toxicity and requirement of FcγRIII to facilitate hyper clustering of 4-1BB [55].

Co-stimulatory bispecific antibodies have been developed which combine two antibody or ligand specificities [56]. This strategy allows for agonist antibodies being targeted to the TME by coupling with an antibody specific for a TAA [57, 58]. For example, a bispecific composed of 4-1BBL (CD137L) and fibroblast activator protein was able to provide co-stimulation to T cells [59]. Similarly, coupling antibodies to collagen factors in tumour-associated vasculature has been used to deliver checkpoint antibodies, IL-2 or chemokine factors to the TME, leading to APC recruitment [60, 61]. A CD27-PD-L1 bispecific was able to simultaneously deliver co-stimulation and checkpoint blockade, leading to increased T cell function [62]. These bispecific antibodies have great potential to be used together with CAR T cells to boost CAR T cell anti-tumour effect. For example, bispecific engager antibodies targeting CD40 and the c-Myc tag expressed within CAR was able to eliminate tumours in mouse models of breast cancer [63]. The eradication of tumour was due to enhanced co-stimulation of CAR T cells by APCs mediated by this bispecific antibody. Currently CD27, CD28, CD40, and 4-1BB co-stimulation have been tested in the form of a bispecific engagers.

Antibody-based therapies offer precise dose control and targeting to the TME to limit toxicity. Additionally, antibody therapies offer a high degree of flexibility for combination with many CAR T formats already in use and have pleiotropic effects to enhance both CAR T cell and endogenous immune responses.

Non-antibody-based approaches utilizing co-stimulation in CAR T cell therapies

Nanotechnology and biotechnology are increasingly utilized in health and medicine. In the context of CAR T cell therapies, these fields offer alternative methods of delivering co-stimulation to antibody-based methods. Nanoparticle vaccines have been demonstrated to engage the host APCs to activate T cells and can be used in cancer immunotherapy [64]. For example, a nanoparticle targeting CLEC-9A was able to effectively deliver antigen to host cross presenting DCs promoting the activation of CAR-TCR dual-specific cells [65]. Additionally, a nanoparticle RNA vaccine enabled claudin-presentation by APCs to claudin-specific CAR T cells, and enhanced CAR T cell trafficking to tumour tissues, leading to eradication of disease [66]. A similar technology utilised APC targeting 'amph ligands' to direct CAR T cell interactions with endogenous DCs. This platform utilises the CAR-specific ligand attached to a DC targeting phospholipid polymer, resulting in CAR T cell and DCs interactions [67]. The co-stimulatory signals delivered by DCs to CAR T cells leads to increased proliferation and tumour control [68].

Viruses can alter the TME to enhance CAR T cell infiltration, activation, and anti-tumour effects. Oncolytic viruses (OV) naturally infect malignant cells and are therefore good theoretical candidates for synergy with CAR T cell therapy. OV can remodel the TME, as well as cause tumour cell death and release of neoantigens [69, 70]. Some studies armed OVs with molecules such as cytokines or co-stimulatory ligands, which are expressed by tumours after OV infection. The expression of these immune modulatory molecules subsequently drives CAR T cell activation (Fig. 1-3). OV-mediated expression of a bispecific engager worked synergistically with CAR T cell activity in two tumour models [71]. In a tumour model of B16 melanoma, modified OV expressing IL-21 enhanced the survival of mice compared to a panel of co-stimulatory molecules including CD86 and 4-1BB [72]. Other therapies utilising OVs to express molecules such as OX40, IL-2, and CD40 have also been studied [73, 74]. OV therapies can be further refined to enhance tropism for tumour cells through the inclusion of tumour-specific promoters such as survivin or hTERT, or modification of OV capsid proteins [75]. For example, a chimeric OV created from vesicular stomatitis virus (VSV) and Newcastle disease virus generated potent anti-tumour effect with greatly reduced hepatotoxicity and neurotoxicity compared to wild-type VSV OV. [69].

Platforms for delivering co-stimulation specifically to the TME or specific subsets of APC within the immune system can be used to drive CAR T cell proliferation and persistence *in vivo*. These methods offer several advantages over antibody-based methods, including delivering flexible payloads or antigens. Therefore, these technologies should be developed further to deliver specific co-stimulatory payloads for each tumour type.

Conclusions and future directions

The understanding of the role of co-stimulation for the design of immunotherapies including CAR T cell therapies has expanded rapidly. Co-stimulatory pathways are demonstrating potential to overcome barriers specifically associated with the TME such as impeded cell trafficking, persistence and exhaustion. The identification and thorough characterisation of novel co-stimulatory pathways and their potential role in improving CAR T cell persistence and avoiding exhaustion in the solid tumour TME is one of the most pressing areas to develop for CAR T cell research. To date, the majority of CARs have incorporated CD28 or 4-1BB domains, but T cells are known to utilize a multitude of co-stimulatory signals to develop a potent immune response. Novel platforms such as OV, nano-emulsion vaccines and combination therapies with antibody therapeutics offer bespoke strategies for delivering such broad co-stimulatory signals to allow CAR T cells to overcome these barriers. Solid tumours continue to be a major human and economic toll in our society. Understanding and refining the use of co-stimulation in CAR T cell design is critical for the future application of CAR T cell therapy enabling all of us to live longer, healthier lives.

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Author contributions

Conception and design: A.J.H. and C.S. Write, review, and revision of the manuscript: A.J.H., Bv.S., X.D., C.S., and M.K.

Conflict of interest

None declared.

Ethical approval information

Not applicable.

Data availability

Not applicable.

References

1. Kershaw MH, Teng MW, Smyth MJ *et al*. Supernatural T cells: genetic modification of T cells for cancer therapy. *Nat Rev Immunol* 2005;**5**(12):928–40. http://doi.org/10.1038/nri1729
2. Calmes-Miller J. FDA approves second CAR T-cell therapy. *Cancer Discov* 2018;**8**(1):5–6. http://doi.org/10.1158/2159-8290.CD-NB2017-155
3. Chan JD, Harrison AJ, Darcy PK *et al*. Chimeric antigen receptor T cell therapies for thoracic cancers-challenges and opportunities. *J Thorac Dis* 2020;**12**(8):4510–5. http://doi.org/10.21037/jtd.2020.03.34
4. Tantalo DG, Oliver AJ, von Scheidt B *et al*. Understanding T cell phenotype for the design of effective chimeric antigen receptor T cell therapies. *J Immunother Cancer* 2021;**9**. http://doi.org/10.1136/jitc-2021-002555
5. Azuma M. Co-signal molecules in T-cell activation: historical overview and perspective. *Adv Exp Med Biol* 2019;**1189**:3–23. http://doi.org/10.1007/978-981-32-9717-3_1
6. Kershaw MH, Westwood JA, Parker LL *et al*. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res* 2006;**12**(20 Pt 1):6106–15. http://doi.org/10.1158/1078-0432.CCR-06-1183
7. Maher J, Brentjens RJ, Gunset G *et al*. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta /CD28 receptor. *Nat Biotechnol* 2002;**20**(1):70–5. http://doi.org/10.1038/nbt0102-70
8. Kochenderfer JN, Wilson WH, Janik JE *et al*. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood* 2010;**116**(20):4099–102. http://doi.org/10.1182/blood-2010-04-281931
9. Grupp SA, Kalos M, Barrett D *et al*. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013;**368**(16):1509–18. http://doi.org/10.1056/NEJMoa1215134
10. Weinkove R, George P, Dasyam N *et al*. Selecting costimulatory domains for chimeric antigen receptors: functional and clinical considerations. *Clin Transl Immunology* 2019;**8**(5):e1049. http://doi.org/10.1002/cti2.1049
11. Cheng Z, Wei R, Ma Q *et al*. In vivo expansion and antitumor activity of coinfused CD28- and 4-1BB-engineered CAR-T cells in patients with B cell leukemia. *Mol Ther* 2018;**26**(4):976–85. http://doi.org/10.1016/j.ymthe.2018.01.022
12. Duong CP, Westwood JA, Yong CS *et al*. Engineering T cell function using chimeric antigen receptors identified using a DNA library approach. *PLoS One* 2013;**8**(5):e63037. http://doi.org/10.1371/journal.pone.0063037
13. Guedan S, Posey AD, Shaw Jr C *et al*. Enhancing CAR T cell persistence through ICOS and 4-1BB costimulation. *JCI Insight* 2018;**3**:1. http://doi.org/10.1172/jci.insight.96976
14. Hombach AA, Abken H. Of chimeric antigen receptors and antibodies: OX40 and 41BB costimulation sharpen up T cell-based immunotherapy of cancer. *Immunotherapy* 2013;**5**(7):677–81. http://doi.org/10.2217/imt.13.54
15. Song DG, Powell DJ. Pro-survival signaling via CD27 costimulation drives effective CAR T-cell therapy. *Oncoimmunology* 2012;**1**(4):547–9. http://doi.org/10.4161/onci.19458
16. Song DG, Ye Q, Poussin M *et al*. CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo. *Blood* 2012;**119**(3):696–706. http://doi.org/10.1182/blood-2011-03-344275
17. Golubovskaya VM, Berahovich R, Xu Q *et al*. GITR domain inside CAR co-stimulates activity of CAR-T cells against cancer. *Front Biosci (Landmark Ed)* 2018;**23**:2245–54.
18. Ramos CA, Rouce R, Robertson CS *et al*. In vivo fate and activity of second- versus third-generation CD19-specific CAR-T cells in B cell Non-Hodgkin's Lymphomas. *Mol Ther* 2018;**26**(12):2727–37. http://doi.org/10.1016/j.ymthe.2018.09.009
19. Abate-Daga D, Lagisetty KH, Tran E *et al*. A novel chimeric antigen receptor against prostate stem cell antigen mediates tumor destruction in a humanized mouse model of pancreatic cancer. *Hum Gene Ther* 2014;**25**(12):1003–12. http://doi.org/10.1089/hum.2013.209
20. Collinson-Pautz MR, Chang WC, Lu A *et al*. Constitutively active MyD88/CD40 costimulation enhances expansion and efficacy of chimeric antigen receptor T cells targeting hematological malignancies. *Leukemia* 2019;**33**(9):2195–207. http://doi.org/10.1038/s41375-019-0417-9
21. Prinzing B, Schreiner P, Bell M *et al*. MyD88/CD40 signaling retains CAR T cells in a less differentiated state. *JCI Insight*, 2020;**5**:21. http://doi.org/10.1172/jci.insight.136093
22. Kagoya Y, Tanaka S, Guo T *et al*. A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects. *Nat Med* 2018;**24**(3):352–9. http://doi.org/10.1038/nm.4478
23. Komai-Koma M, Jones L, Ogg GS *et al*. TLR2 is expressed on activated T cells as a costimulatory receptor. *Proc Natl Acad Sci USA* 2004;**101**(9):3029–34. http://doi.org/10.1073/pnas.0400171101
24. Cottalorda A, Mercier BC, Mbitikon-Kobo FM *et al*. TLR2 engagement on memory CD8(+) T cells improves their cytokine-mediated proliferation and IFN-gamma secretion

in the absence of Ag. *Eur J Immunol* 2009;**39**(10):2673–81. http://doi.org/10.1002/eji.200939627
25. Erridge C. Endogenous ligands of TLR2 and TLR4: agonists or assistants? *J Leukoc Biol* 2010;**87**(6):989–99. http://doi.org/10.1189/jlb.1209775
26. Rohrs JA, Siegler EL, Wang P *et al*. ERK activation in CAR T cells is amplified by CD28-mediated increase in CD3ζ phosphorylation. *Iscience* 2020;**23**(4):101023. http://doi.org/10.1016/j.isci.2020.101023
27. Sun C, Shou P, Du H *et al*. THEMIS-SHP1 recruitment by 4-1BB Tunes LCK-mediated priming of chimeric antigen receptor-redirected T cells. *Cancer Cell* 2020;**37**(2):216–25.e6. http://doi.org/10.1016/j.ccell.2019.12.014
28. Lai Y, Weng J, Wei X *et al*. Toll-like receptor 2 costimulation potentiates the antitumor efficacy of CAR T Cells. *Leukemia* 2018;**32**(3):801–8. http://doi.org/10.1038/leu.2017.249
29. Ankri C, Shamalov K, Horovitz-Fried M *et al*. Human T cells engineered to express a programmed death 1/28 costimulatory retargeting molecule display enhanced antitumor activity. *J Immunol* 2013;**191**(8):4121–9. http://doi.org/10.4049/jimmunol.1203085
30. Liu X, Ranganathan R, Jiang S *et al*. A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T Cells in advanced solid Tumors. *Cancer Res* 2016;**76**(6):1578–90. http://doi.org/10.1158/0008-5472.CAN-15-2524
31. Bajgain P, Tawinwung S, D'Elia L *et al*. CAR T cell therapy for breast cancer: harnessing the tumor milieu to drive T cell activation. *J Immunother Cancer* 2018;**6**(1):34. http://doi.org/10.1186/s40425-018-0347-5
32. Weimin S, Abula A, Qianghong D *et al*. Chimeric cytokine receptor enhancing PSMA-CAR-T cell-mediated prostate cancer regression. *Cancer Biol Ther* 2020;**21**(6):570–80. http://doi.org/10.1080/15384047.2020.1739952
33. Lange S, Sand LGL, Bell M *et al*. A chimeric GM-CSF/IL18 receptor to sustain CAR T-cell function. *Cancer Discov* 2021;**11**:1661–71. http://doi.org/10.1158/2159-8290.CD-20-0896
34. Evans LS, Witte PR, Feldhaus AL *et al*. Expression of chimeric granulocyte-macrophage colony-stimulating factor/interleukin 2 receptors in human cytotoxic T lymphocyte clones results in granulocyte-macrophage colony-stimulating factor-dependent growth. *Hum Gene Ther* 1999;**10**(12):1941–51. http://doi.org/10.1089/10430349950017301
35. Elgueta R, Benson MJ, de Vries VC *et al*. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev* 2009;**229**(1):152–72. http://doi.org/10.1111/j.1600-065X.2009.00782.x
36. Kuhn NF, Purdon TJ, van Leeuwen DG *et al*. CD40 Ligand-modified chimeric antigen receptor T Cells enhance antitumor function by eliciting an endogenous antitumor response. *Cancer Cell* 2019;**35**(3):473–88.e6. http://doi.org/10.1016/j.ccell.2019.02.006
37. Leonard WJ, Lin JX, O'Shea JJ. The gammac family of cytokines: Basic biology to therapeutic ramifications. *Immunity* 2019;**50**(4):832–50. http://doi.org/10.1016/j.immuni.2019.03.028
38. Sabatino M, Hu J, Sommariva M *et al*. Generation of clinical-grade CD19-specific CAR-modified CD8+ memory stem cells for the treatment of human B-cell malignancies. *Blood* 2016;**128**(4):519–28. http://doi.org/10.1182/blood-2015-11-683847
39. Lacy MQ, Jacobus S, Blood EA *et al*. Phase II study of interleukin-12 for treatment of plateau phase multiple myeloma (E1A96): a trial of the Eastern Cooperative Oncology Group. *Leuk Res* 2009;**33**(11):1485–9. http://doi.org/10.1016/j.leukres.2009.01.020
40. Chmielewski M, Hombach AA, Abken H. Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. *Immunol Rev* 2014;**257**(1):83–90. http://doi.org/10.1111/imr.12125
41. Chmielewski M, Kopecky C, Hombach AA *et al*. IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression. *Cancer Res* 2011;**71**(17):5697–706. http://doi.org/10.1158/0008-5472.CAN-11-0103
42. Wijewarnasuriya D, Bebernitz C, Lopez AV *et al*. Excessive costimulation leads to dysfunction of adoptively transferred T cells. *Cancer Immunol Res* 2020;**8**(6):732–42. http://doi.org/10.1158/2326-6066.CIR-19-0908
43. Muñoz-Wolf N, Lavelle EC. A guide to IL-1 family cytokines in adjuvanticity. *Febs J* 2018;**285**(13):2377–401. http://doi.org/10.1111/febs.14467
44. Kim TS, Shin EC. The activation of bystander CD8+ T cells and their roles in viral infection. *Exp Mol Med* 2019;**51**(12):1–9. http://doi.org/10.1038/s12276-019-0316-1
45. Coughlin CM, Salhany KE, Wysocka M *et al*. Interleukin-12 and interleukin-18 synergistically induce murine tumor regression which involves inhibition of angiogenesis. *J Clin Invest* 1998;**101**(6):1441–52. http://doi.org/10.1172/JCI1555
46. Chmielewski M, Abken H. CAR T Cells releasing IL-18 convert to T-Bethigh FoxO1low effectors that exhibit augmented activity against advanced solid Tumors. *Cell Rep* 2017;**21**(11):3205–19. http://doi.org/10.1016/j.celrep.2017.11.063
47. Li X, Daniyan AF, Lopez AV *et al*. Cytokine IL-36γ improves CAR T-cell functionality and induces endogenous antitumor response. *Leukemia* 2021;**35**(2):506–21. http://doi.org/10.1038/s41375-020-0874-1
48. Adachi K, Kano Y, Nagai T *et al*. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat Biotechnol* 2018;**36**(4):346–51. http://doi.org/10.1038/nbt.4086
49. Rafiq S, Yeku OO, Jackson HJ *et al*. Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. *Nat Biotechnol* 2018;**36**(9):847–56. http://doi.org/10.1038/nbt.4195
50. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science* 2018;**359**(6382):1350–5. http://doi.org/10.1126/science.aar4060

51. Vonderheide RH. CD40 agonist antibodies in cancer immunotherapy. *Annu Rev Med* 2020;**71**:47–58. http://doi.org/10.1146/annurev-med-062518-045435
52. Van Audenaerde JR, Marcq E, von Scheidt B *et al*. Novel combination immunotherapy for pancreatic cancer: potent anti-tumor effects with CD40 agonist and interleukin-15 treatment. *Clin Transl Immunology* 2020;**9**(8):e1165. http://doi.org/10.1002/cti2.1165
53. Zhang Y, Wang P, Wang T *et al*. Chimeric antigen receptor T cells engineered to secrete CD40 agonist antibodies enhance antitumor efficacy. *J Transl Med* 2021;**19**(1):82. http://doi.org/10.1186/s12967-021-02750-4
54. Mardiana S, John LB, Henderson MA *et al*. A multifunctional role for adjuvant Anti-4-1BB Therapy in augmenting antitumor response by Chimeric Antigen Receptor T Cells. *Cancer Res* 2017;**77**(6):1296–309. http://doi.org/10.1158/0008-5472.CAN-16-1831
55. Li F, Ravetch JV. Antitumor activities of agonistic anti-TNFR antibodies require differential FcγRIIB coengagement in vivo. *Proc Natl Acad Sci USA* 2013;**110**(48):19501–6. http://doi.org/10.1073/pnas.1319502110
56. Baeuerle PA, Reinhardt C. Bispecific T-cell engaging antibodies for cancer therapy. *Cancer Res* 2009;**69**(12):4941–4. http://doi.org/10.1158/0008-5472.CAN-09-0547
57. A novel antibody-4-1BBL fusion protein for targeted costimulation in cancer immunotherapy. 2008;**31**(8):714–22. http://doi.org/10.1097/CJI.0b013e31818353e9
58. Aigner M, Janke M, Lulei M *et al*. An effective tumor vaccine optimized for costimulation via bispecific and trispecific fusion proteins. *Int J Oncol* 2008;**32**(4):777–89.
59. Claus C, Ferrara C, Xu W *et al*. Tumor-targeted 4-1BB agonists for combination with T cell bispecific antibodies as off-the-shelf therapy. *Sci Transl Med* 2019;**11**:496. http://doi.org/10.1126/scitranslmed.aav5989
60. Ishihara J, Ishihara A, Sasaki K *et al*. Targeted antibody and cytokine cancer immunotherapies through collagen affinity. *Sci Transl Med* 2019;**11**:487. http://doi.org/10.1126/scitranslmed.aau3259
61. Williford JM, Ishihara J, Ishihara A *et al*. Recruitment of CD103+ dendritic cells via tumor-targeted chemokine delivery enhances efficacy of checkpoint inhibitor immunotherapy. *Sci Adv* 2019;**5**(12):eaay1357. http://doi.org/10.1126/sciadv.aay1357
62. Vitale LA, He LZ, Thomas LJ *et al*. Development of CDX-527: a bispecific antibody combining PD-1 blockade and CD27 costimulation for cancer immunotherapy. *Cancer Immunol Immunother* 2020;**69**(10):2125–37. http://doi.org/10.1007/s00262-020-02610-y
63. von Scheidt B, Wang M, Oliver AJ *et al*. Enterotoxins can support CAR T cells against solid tumors. *Proc Natl Acad Sci USA* 2019;**116**(50):25229–35. http://doi.org/10.1073/pnas.1904618116
64. Kranz LM, Diken M, Haas H *et al*. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature* 2016;**534**(7607):396–401. http://doi.org/10.1038/nature18300
65. Chan JD, von Scheidt B, Zeng B *et al*. Enhancing chimeric antigen receptor T-cell immunotherapy against cancer using a nanoemulsion-based vaccine targeting cross-presenting dendritic cells. *Clin Transl Immunology* 2020;**9**(7):e1157. http://doi.org/10.1002/cti2.1157
66. Reinhard K, Rengstl B, Oehm P *et al*. An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. *Science* 2020;**367**(6476):446–53. http://doi.org/10.1126/science.aay5967
67. Liu H, Moynihan KD, Zheng Y *et al*. Structure-based programming of lymph-node targeting in molecular vaccines. *Nature* 2014;**507**(7493):519–22. http://doi.org/10.1038/nature12978
68. Ma L, Dichwalkar T, Chang JYH *et al*. Enhanced CAR-T cell activity against solid tumors by vaccine boosting through the chimeric receptor. *Science* 2019;**365**(6449):162–8. http://doi.org/10.1126/science.aav8692
69. Abdullahi S, Jakel M, Behrend SJ *et al*. A Novel Chimeric Oncolytic Virus vector for improved safety and efficacy as a platform for the treatment of Hepatocellular Carcinoma. *J Virol* 2018;**92**:23. http://doi.org/10.1128/JVI.01386-18
70. Zheng M, Huang J, Tong A *et al*. Oncolytic viruses for cancer therapy: barriers and recent advances. *Mol Ther Oncolytics* 2019;**15**:234–47. http://doi.org/10.1016/j.omto.2019.10.007
71. Wing A, Fajardo CA, Posey AD Jr *et al*. Improving CART-Cell therapy of solid tumors with Oncolytic Virus-driven production of a bispecific T-cell Engager. *Cancer Immunol Res* 2018;**6**(5):605–16. http://doi.org/10.1158/2326-6066.CIR-17-0314
72. Chen T, Ding X, Liao Q *et al*. IL-21 arming potentiates the anti-tumor activity of an oncolytic vaccinia virus in monotherapy and combination therapy. *J Immunother Cancer* 2021;**9**(1). http://doi.org/10.1136/jitc-2020-001647
73. Andarini S, Kikuchi T, Nukiwa M *et al*. Adenovirus vector-mediated in vivo gene transfer of OX40 ligand to tumor cells enhances antitumor immunity of tumor-bearing hosts. *Cancer Res* 2004;**64**(9):3281–7. http://doi.org/10.1158/0008-5472.can-03-3911
74. Feder-Mengus C, Schultz-Thater E, Oertli D *et al*. Nonreplicating recombinant vaccinia virus expressing CD40 ligand enhances APC capacity to stimulate specific CD4+ and CD8+ T cell responses. *Hum Gene Ther* 2005;**16**(3):348–60. http://doi.org/10.1089/hum.2005.16.348
75. Ulasov IV, Zhu ZB, Tyler MA *et al*. Survivin-driven and fiber-modified oncolytic adenovirus exhibits potent antitumor activity in established intracranial glioma. *Hum Gene Ther* 2007;**18**(7):589–602. http://doi.org/10.1089/hum.2007.002