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Title:
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Date:
2019-01-01

Citation:
Beavis, P. A. & Darcy, P. K. (2019). CAR T cells take centre stage. Clinical and Translational Immunology, 8 (7), https://doi.org/10.1002/cti2.1068.

Persistent Link:
https://hdl.handle.net/11343/249655

Clinical & Translational Immunology

Australian and New Zealand Society for Immunology Inc.

Clinical & Translational Immunology 2019; e01068. doi: 10.1002/cti2.1068
www.wileyonlinelibrary.com/journal/cti

SPECIAL FEATURE EDITORIAL

CAR T cells take centre stage

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doi: 10.1002/cti2.1068

Over the last decade, we have witnessed unprecedented results for various forms of immunotherapy in patients with cancer, which has now become the fourth pillar of treatment behind conventional therapies involving surgery, radiotherapy and chemotherapy. The development of checkpoint inhibitors that block interaction of inhibitory receptors such as PD-1 and CTLA-4 with their respective ligands PD-L1/PD-L2 and CD80/CD86 to restore effector T-cell anti-tumor immunity has shown remarkable results across various cancers. This has led to FDA approval of anti-CTLA-4 in 2011 and anti-PD-1 in 2014.[1–3] The second form of immunotherapy that has shown remarkable results in patients with cancer is a specialised form of cellular immunotherapy known as chimeric antigen receptor (CAR) T-cell therapy. This approach involves the genetic modification of T cells with a CAR comprising an extracellular single-chain antibody (scFv) linked to transmembrane and cytoplasmic signalling domains such as the co-stimulatory CD28 domain and TCR-zeta chain. The scFv enables specific T-cell recognition of tumor antigen, and the signalling domains activate effector T-cell function against the tumor. Adoptive transfer of CAR T cells targeting the CD19 antigen on B-cell malignancies such as acute lymphoid leukaemia (ALL) has led up to 90% objective response rates in patients with many in long-term remission.[4–7]This striking efficacy resulted in recent FDA approval of two CAR T-cell products, Kymriah and Yescarta for the treatment of patients with $CD19^+$ ALL and non-Hodgkin lymphoma. However, despite these results, CAR T-cell responses have not met with the same success in the setting of solid cancer. There are a number of potential reasons underlying these moderate CAR T-cell responses, which include the immunosuppressive tumor microenvironment leading to reduced T-cell function, poor trafficking and infiltration of adoptively transferred T cells into the tumor site and the heterogenous expression of antigen on cancer cells. In this Special Feature of *Clinical & Translational Immunology*, we have invited several experts in the CAR T-cell field to discuss the latest developments that address these problems for potentially broadening the therapeutic effect by CAR T cells.

The reviews by Hartley and Abken[8] and Mardiana *et al.*[9] discuss the problem of tumor-induced immunosuppression and provide new avenues that can be explored to increase CAR T-cell anti-tumor responses. Harley and Abken focus on the problem of TGF-β immunosuppression resulting in resistance to immunotherapy. TGF-β is produced by both tumor cells and stromal cells including Tregs, myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages and neutrophils.[10] This can lead to increased accumulation of TGF-β within the tumor site that can inhibit T-cell function through binding to specific TGF-β receptors. One approach to overcome this problem is by using small molecule inhibitors. However, pharmacological TGF-β blockade may be associated with deleterious side effects due to the role of TGF-β in immune

homoeostasis. Therefore, intrinsic gene modification strategies of CAR T cells offer a way to address this problem. An example of this includes co-expression of a dominant-negative TGF-β type 2 receptor (dnTGF-βRII) or with a TGF-β CAR receptor that sequesters soluble TGF-β within the tumor site.[11,12] In the clinical setting, the expression of these receptors could be more tightly controlled by CAR T cells within the tumor tissue by using an NFAT-IL-2 promoter element, which would further alleviate safety concerns.

The review by Mardiana *et al.*[9] discusses various strategies to overcome the immunosuppressive tumor microenvironment and for increasing the specificity and safety of CAR T cell therapy. One approach discussed involves the use of immunostimulatory antibodies in combination with CAR T therapy to increase anti-tumor responses. A good example includes the use of the checkpoint inhibitor anti-PD-1 that has been shown to enhance CAR T-cell therapy in mouse tumor models and is now showing some promise in early Phase I clinical trials.[13,14] There are a vast array of different reagents available targeting both inhibitory and activating checkpoint pathways that have shown promise for increasing CAR T-cell responses in preclinical mouse models that are under clinical development. Another elegant approach for modulating the tumor microenvironment involves the use of 'armoured' CAR T cells that deliver immune stimulatory molecules. This has been shown for CD40L, IL-12 and IL-18 that has resulted in profound effects on modulating both host immune cells and adoptively transferred CAR T cells within the tumor microenvironment resulting in increased therapeutic responses in preclinical models.[15–17] Other important areas discussed in this review were potential strategies for enhancing CAR T-cell infiltration into tumors through either genetic modification with specific chemokine receptors such as CXCR2[18] or alternatively using certain drugs to modify the chemokine profile of tumors. Finally, the potential use of allogeneic CAR T cells as an 'off-the-shelf product' was discussed for potentially broadening the applicability of CAR T-cell therapy to diverse patient populations. The advent of gene-editing technologies such as CRISPR/Cas9 has been a significant development for potentially using CAR T cells in an allogeneic setting as it has enabled inactivation of the TCR on the CAR T cells for reducing potential graft vs host disease (GvHD) following transfer. Alternatively, the use of effector cells other than T cells such as NK cells that do not induce GvHD may provide another therapeutic option.

The review by Weinkove *et al.*[19] summarises the functional effects of different co-stimulatory domains used alone or in combination within the CAR design and the potential impact of these co-stimulatory domains on the safety of this approach. There are a number of co-stimulatory domains that have been incorporated into CARs and tested preclinically that include CD28, 4-1BB, OX-40 and CD27 that have been shown to enhance CAR T-cell function. In the clinical setting, with the incorporation of either CD28 or 4-1BB into the CAR design, which have been the predominant co-stimulatory signalling domains employed, and the optimal use of these signalling domains may be highly dependent on the clinical indication. For example, in the setting of aggressive B-NHL, CD28 containing CARs resulted in higher response rates than 4-1BB containing CARs despite the loss of persistence of the CAR T cells.[20] However, in B-ALL the opposite was the case where the loss of CAR T-cell persistence was associated with disease relapse.[21] The use of third-generation CARs containing multiple co-stimulatory domains was discussed with results in the early clinical trials demonstrating greater proliferative potential of the CAR T cells and increased clinical benefit. However, there was also toxicity including cytokine release syndrome and neurotoxicity reported in some patients in this trial,[22] and therefore, further refinement and characterisation of third-generation CARs is required. Finally, the authors discuss several studies exploring the positioning of co-stimulatory domains within the CAR and the influence this has on CAR T-cell function.

The final review by Brown *et al.*[23] discusses the clinical use of CAR T-cell therapy for the treatment of glioblastoma, which presents as one of the most challenging cancers to effectively treat given the poor response to conventional treatments and the poor immunogenic nature of these cancers. The review describes three separate CAR T-cell trials for glioblastoma that differ in the antigen targeted, the phenotype of the CAR T cells transferred and route of administration. The results of these clinical studies highlight the need to adopt new approaches for enhancing overall CAR T-cell responses in patients with glioblastoma and other solid cancers. The authors discuss potential combination therapies using drugs to block immunosuppressive inhibitory factors such

as PD-1/PDL1 or CD73/CD39 to overcome the hypoxic tumor microenvironment. The phenotype of the CAR T-cell product is an important factor where the transfer of CAR T cells retaining a greater memory phenotype or containing a higher number of $CD4^+$ T-cells present may result in stronger anti-tumor responses. The route of administration of CAR T cells either within the tumor site or by intravenous delivery and strategies to potentially enhance endogenous T-cell immunity are other important considerations that may enhance overall CAR T-cell anti-tumor responses. All of these factors require further validation in both preclinical models and early-phase clinical trials.

In summary, this Special Feature of *Clinical & Translational Immunology* presents a comprehensive view of the tremendous promise of CAR T-cell therapy and discusses current and future developments in the field that may broaden the effectiveness of this approach in both blood and solid cancers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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