15-12-2017

CAR-T cell therapies in Glioblastoma

Executive summary

The immune system surveys the body throughout a person's life to detect and eliminate tumour cells as well as infections such as influenza. In cancer patients, tumour cells have escaped these body defences and grow unchecked. Scientists and clinicians now have the tools to create new defences, in the form of CAR T-cells, which are manufactured from the patient's own white blood cells using genetic engineering. This article describes the results of preliminary clinical trials using specially designed CAR-T-cells in glioblastoma patients. The results provide a starting point from which potentially curative therapies can be developed.

Background

The recent application of immunotherapies to control or even cure some cancers has been hailed as a 'game changer' in the field of cancer therapy and has prompted an explosion of related activity in academia and industry. It is perhaps not too fanciful to predict the eventual elimination of at least some cancers as chronic diseases[1]. Several different strategies are being employed, including vaccination with tumour antigens[2], checkpoint inhibition with antibodies to cell surface molecules expressed on tumours[3] and adoptive immunotherapy with patient-derived cytotoxic T lymphocytes[4]. While each approach has potential utility in treating glioblastomas (which will be highlighted in future *Window on Glioblastoma* articles), here we review some of the first clinical studies in this disease using adoptive immunotherapy with CAR T-cells.

CAR T-cells

Chimeric antigen receptor-modified (CAR) T-cells are generated from the patient's white blood cells using lentiviral transfection to introduce specific genes that allow recognition of defined tumour antigens. Pioneering work by Carl June and others have shown that CAR T-cells directed against the CD19 antigen present in acute lymphoblastic leukaemia (ALL) can kill the target B cells causing the disease, and lead to disease remission[5]. Specifically, an anti-CD19 single-chain Fv domain is grafted to intracellular T-cell signalling (CD3-zeta) domains of the T cell receptor and a co-stimulatory signal provided by the CD137 (4-1BB) domain. This results in the redirection of cytotoxic T lymphocytes to cells expressing this antigen. The success of CAR T-cell therapy in treating refractory ALL has been such that the engineered cells are being marketed as tisagenlecleucel by Novartis and have been approved for clinical use by the FDA[6].

CAR T-cells and glioblastoma

The success of CAR T-cell therapy against ALL is due in part to there being a well-defined antigen (CD19) which is uniformly expressed on the target cells. This does not always apply to other cancers, including glioblastoma, where the targets may not be as immunogenic or present in the majority of tumour cells. Nevertheless, some early attempts are being made to treat GBM patients with a variety of CAR constructs, as reviewed recently by Migliorini et al [7]. CAR T-cells have potential in treating GBM as they can be generated outside the immunosuppressive microenvironment of brain tumours before being introduced to the site of disease to target the chosen antigens.
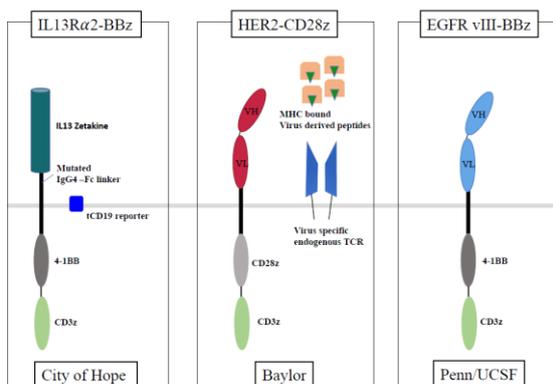
To quote the authors: "Thus, translational research investments combining the preclinical optimization of CAR constructs with innovative engineering strategies to circumvent immunosuppression are imperative in addition to evaluating the standard variables related to routes of administration, persistence and cell trafficking to CNS and dose-schedules. These questions have been addressed in three Phase I trials recently reported by investigators from the City of Hope[8], Baylor College of Medicine[9], and the University of Pennsylvania/UCSF[10]."

A schematic diagram of the three CAR T-cell constructs used in these studies is shown in the figure below (taken from [7]).

Here, we summarize the key elements of these trials in turn.

Target: interleukin-13 receptor alpha 2 (IL13Rα2)

Target rationale

The interleukin-13 receptor α2 isoform was chosen as a GBM target molecule based on earlier observations about its expression on most tumour cells, but not normal brain cells, and its lack of responsiveness to the related anti-inflammatory cytokine interleukin-4[12].

CAR T-cell construct

CAR T-cells were engineered from a so-called IL-13 zetakine, in which an IL-13 binding domain was fused to signalling and co-stimulation domains as with the CD19 construct (along with a mutated Fc domain to minimise Fc interactions). The IL-13 domain was itself modified as a mutein to enhance biding to the IL-13 α2 receptor. The CAR T cells therefore bind to the tumour cells bearing the IL-13 receptor which are then killed in a cytotoxic response[8].

Study design

IL13BBζ-CAR T-cells were administered to a single patient whose GBM had recurred six months after completing the standard therapy of tumour resection, radiation therapy, and temozolomide treatment. Clinical outcomes were reported from a period of 298 days after enrolment.

Results

As quoted from Migliorini et al[7]: "Clinical benefit was significant as assessed by several objective measures including 7.5 months duration of response, ability to discontinue corticosteroids, and complete response by RANO criteria with magnetic resonance imaging (MRI) and Fluoro-deoxy-glucose positron emission tomography (FDG-PET)".

The investigators made several new and important observations. Again, to quote directly from the paper, "First, the route of delivery appeared decisive, with complete regression of multiple intracranial and spinal tumors after intraventricular rather than intracavitary administration. Cells and effect appears confined to the CNS with no detection of CAR+ T-cells or elevated cytokines in the peripheral blood. Second, glioma with heterogeneous target expression exhibited marked response, with eventual relapse consistent with tumor editing and growth of antigen loss variants. The use of T-cells enriched for those with a 'central memory' phenotype (Tcm) gave impressive pre-clinical data[13]; however, in the context of glioblastoma treatment, more evidence is required before any efficacy claims in favour of Tcm over bulk T cells can be postulated."

Target: HER2

Target rationale

The epidermal growth factor receptor variant HER2 is expressed on GBM cells and can stimulate class I HLA restricted cytotoxic T-cells responses which could be exploited in immunotherapy strategies[11].

CAR T-cell construct

The CAR T cells produced by Ahmed et al[9] used a HER2 recognition element engineered from the ectodomain of an anti-HER2 antibody and fused with a CD28 endodomain (for co-stimulation), and CD3zeta signalling domain. The T cells used for engineering the constructs were primed with virus antigens to facilitate the cytotoxic response after recognition of the HER2 on glioma cells.

Study design

Sixteen evaluable patients were treated with intravenous doses of CAR T-cells infusions. Clinical outcomes were measured after six weeks using brain MRI.

Results

According to Ahmed et al[9]: "For the entire study cohort, median overall survival was 11.1 months from the first T-cell infusion and 24.5 months from diagnosis. Three patients with stable disease are alive without any evidence of progression during 24 to 29 months of follow-up."

Target: EGFRvIII

Target rationale

EGF receptor variant III (EGFR vIII) results from an in-frame deletion of exons 2 to 7 and the generation of a novel glycine residue at the junction of exons 1 and 8 in the extracellular domain of the EGFR creating a tumour-specific, immunogenic epitope[10]. The EGFRvIII, expressed in about 30% of newly diagnosed GBM cases, is thought to be a negative prognostic indicator. For review of the different approaches used to target this variant in GBM, see [14].

CAR T-cell construct

The O'Rourke study[10] under review here, employed a CAR T-cell construct based on humanised single-chain variable fragments (scFvs) derived from monoclonal antibodies to the vIII epitope[15]. As with other constructs, these antigen-binding regions were fused with costimulatory (4-1BB) and signalling (CD3 zeta) domains.

Study design

CAR T-cells were administered to ten patients in a single dose by intravenous infusion followed by brain MRI and, in some cases, histopathological analysis of brain tissue.

Results

CAR-T cell expansion peaked 3-10 days post infusion in all subjects and were undetectable in peripheral blood samples after day 30 post-infusion. Biopsies from seven patients showed detectable CAR T cells in tumour accompanied by a marked infiltration of non-CAR infiltrating T cells, albeit at various time points. Two patients with the highest levels of immune-mediated changes as reflected by CD8+ T cell and CAR T-cell infiltration remain alive (one patient had stable disease that persisted for >18 months and remains alive with no additional treatment). Evidence of target (EGFRvIII) tumor cell elimination was found based on diminished expression of EGFRvIII positive cells within the resection specimen.

Overall conclusions

The results of these three trials, while not showing the dramatic clinical responses seen with leukaemias, do at least give some reason for cautious optimism, some of which are listed below:

1) Due to the absence of a cytokine release syndrome, no systemic or neurotoxicity was seen. This is particularly important in the context of the brain, where inflammation can be fatal. A balance must be struck between proving an anti-inflammatory environment (steroids, anti-IL-6 antibodies etc) and one where an immune response to the tumour can occur.

2) The intraventricular route of administration appears highly promising, being used with the only patient who showed a complete radiographic response. The IVT route enables trafficking of CAR-T-cells to multiple tumour sites in the CNS, as well as circulating tumour cells.

3) The time from initiation of T-cell manufacturing to CAR-T cell infusion (the 'vein to vein' interval), currently takes ~4 weeks, too long for many patients with recurrent GBM. One opportunity for clinical investigation would be to include CAR T-cell therapy immediately after completion of 6 weeks of standard chemoradiotherapy.

4) Elimination of target antigen positive GBM cells (tumour editing) was documented in the IL13Rα2 and the EGFRvIII-targeted patients, and supports the idea that a tumour-antigen specific immune response is occurring *in vivo*.

5) Infiltration of T cells other than the engineered CAR constructs was observed in one study, which begs the question as to whether they are also contributing to an anti-tumour response. Migliorini et al [7] speculate that checkpoint inhibitors targeting the PD1-PDL1 axis could be used in combination with cell therapy to enhance the latter's efficacy.

6) Antigen escape and tumour heterogeneity are two major issues, so future studies targeting multiple antigens should evaluate bi- and tri-specific CAR-T cells[16]. Additional antigens for this purpose include CD133, and EphA2[16,17].

To conclude, these three studies have provided valuable insights that will guide the design of future studies. Tumour editing is clearly a desirable consequence of effective therapeutic intervention and thus, selection of multiple targets is an essential first step in creating combinatorial therapy with curative potential. Each target chosen (IL13Rα2, HER2, and EGFRvIII) has been preliminarily validated and each will warrant further study.

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