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Editorial

From CARs to TRUCKs and Beyond: Safely en Route to Adoptive T-cell Therapy for Cancer

This year's American Society of Hematology (ASH) Annual Meeting (December 2016, San Diego, USA) had an emphasis on leukemia and adoptive therapy, as did previous ASH meetings. But this year, ASH also had a dedicated session on "Adoptive Immunotherapy: CAR-T Toxicity and Clinical Trials", and experience with chimeric antigen receptor (CAR) T-cell therapy in various clinical trials to date was presented in hundreds of talks and posters throughout the conference.

There are numerous exciting developments in this relatively young field of cancer immunotherapy. Following the success of immune checkpoint inhibitors–a topic touched upon in the November 2016 *EBioMedicine* editorial–all eyes are now on CAR T cells, which are being tested in more than 100 ongoing clinical trials in various cancers. CAR T cells are genetically engineered *in vitro* so that their chimeric receptors contain an extracellular antigen-recognition domain of a monoclonal antibody and an intracellular T-cell activation domain (such as CD3ζ or FcRγ). Newer-generation CAR T cells also incorporate intracellular costimulatory domains (such as CD28, ICOS, OX-40 and 4-1BB). As such, CAR T cells can recognize antigens and become activated independently of MHC-I restriction, thus bypassing two principal mechanisms that tumors use to evade the immune system (MHC-I downregulation and proteasomal antigen processing).

Among several antigen candidates, CD19 is arguably an ideal model antigen to demonstrate the efficacy of CAR T-cell therapy in hematological malignancies. CD19 is expressed exclusively on B lymphocytes and their progenitors, and anti-CD19 CAR T cells have been tested in various clinical trials in acute lymphoblastic leukemia (ALL), chronic lymphoblastic leukemia, multiple myeloma and lymphoma–in fact nearly half of all clinical trials using CAR T cells involve CD19 as a target. Given that these patients had advanced stage, lethal diseases that were refractory to standard treatments at trial enrollment, it is impressive that CAR T-cell infusion can induce remission in many patients, some of whom have not had disease recurrence since the therapy. Because CD19 is also expressed on healthy B cells, there is a potential risk of B-cell aplasia following the therapy, but this can be mitigated by IgG replacement therapy. In practice, most of these patients already had chemoradiotherapy-induced lymphocytopenia so the on-target/off-tumor effect of CAR T cells on healthy B cells is virtually absent.

A serious safety concern with CAR T-cell therapy is cytokine release syndrome (CRS), whereby activation of CAR T cells can lead to production of several proinflammatory cytokines such as IL-6, TNFα and IFNγ, which can manifest as high fever, hypotension, tissue edema, hypoxia and organ failure. Studies have shown that CRS can be controlled using cytokine-blocking agents such as tocilizumab or corticosteroids. Neurotoxicity has also been reported in a few cases following CAR T-cell infusion, including delirium, dysphasia, akinetic mutism and seizures, but the mechanisms of these symptoms remain elusive. In November 2016, two ALL patients died of cerebral edema in a clinical trial testing the investigational anti-CD19 CAR T cell JCAR015 (NCT02535364), adding to the previous three deaths in July. The trial has been halted, and whether these deaths are related to CRS or have other etiologies remains unknown. Now more than ever, extra vigilance is needed for patient safety monitoring in other ongoing CAR T-cell therapy clinical trials.

Many innovative approaches to improve CAR T-cell safety have been investigated. A favorite and clinically tested method is the "suicide switch" whereby inducible suicide genes, such as caspase 9, caspase 8 and herpes simplex thymidine kinase, are incorporated into CAR T cells to eliminate those cells if treatment-related toxicity occurs. An alternative strategy is to use mRNA-transfected T cells that only transiently express CARs. In another approach, T cells that co-express both activating CAR and inhibiting CAR (iCAR) can function as logic gates whereby the activating signal is turned off by iCAR if the T cells encounter healthy cells, thus improving tumor specificity in a preclinical model.

Beyond CD19 and hematological malignancies, CAR T cells encounter a unique set of challenges when it comes to treating solid tumors. The on-target/off-tumor toxicity becomes more evident due to the lack of tumor-specific antigens and the intrinsic heterogeneity of solid tumors sharing many self-antigens with healthy tissues. The tumor immunosuppressive microenvironment also presents a major obstacle for CAR T cells to infiltrate and persist, necessitating higher infusion doses. All these factors can result in autoimmunity and graft-versus-host disease following treatment, which can be potentially lethal. More than 20 tumor-associated antigens have been tested preclinically in various solid tumors, but only some have made it into clinical trials, including CEA for adenocarcinoma, EGFRvIII for glioblastoma, GD2 for neuroblastoma, Her2 for Her2 + solid tumors, PSMA for prostate cancer, to highlight a few. Responses have been very modest compared to what has been observed in blood cancers, and complete remission is rare.

In order to combat the tumor immunosuppressive microenvironment, CAR T cells redirected for universal cytokine killing (TRUCKs) are equipped with an inducible cytokine expression cassette, such as IL-12. Upon antigen engagement, these armored CAR T cells secrete IL-12 in a locally restricted manner, and recruit both primary adaptive and innate immune cells, such as cytotoxic T cells and NK cells, to the tumor sites. TRUCKs have been shown preclinically to enhance antitumor activity and modify tumor microenvironment, but clinical experience with TRUCKs is currently limited.

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The use of CAR T-cell therapy to date remains within clinical trial settings. There is still much to be learnt about the behavior of these cells and their interaction with other cells and tumors in the human body. This is what drives both basic and clinical sciences–to design better CAR T cells, to anticipate desirable responses, to counteract unwanted adverse outcomes, and to simplify and accelerate manufacturing processes. Hopefully we will see the first CAR T-cell products being approved for clinical use in the coming years.

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