CAR T-cell therapy: opportunities and challenges

A letter in response to: Milena Kalaitidou, Gray Kueberuwa, Antje Schütt & David Edward Gilham. CAR T-cell therapy: toxicity and the relevance of preclinical models. *Immunotherapy* 7(5), 487–497 (2015).

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In the May 2015 issue of *Immunotherapy*, Kalaitidou *et al.* describe adoptive chimeric antigen receptor (CAR) engineered T-cell therapy from another angle: the issue of toxicity and novel strategies to overcome these toxicities [1].

From first through to fourth generation, CAR T-cell technology has developed rapidly. A patient's own T cells can be modified with a CAR, which not only targets the antigen that is expressed on the surface of the tumor cells specifically, but plays an independent role to the major histocompatibility complex. The results of some clinical trials have reversed conventional cognition for tumor treatment, at least the therapeutics for acute lymphoblastic leukemia [2–4]. A number of clinical reports suggested that CD19-CARs were notable for remission induced in patients with refractory and heavily pretreated B cell malignancies, and thereby they begin to appear to be the most successful design [5].

With the promising results in acute lymphoblastic leukemia, the complete remission rates increased from 70 to 90% [2–4]. Various CARs were designed for lymphomas and solid tumors [6,7], which were followed by a variety of side effects such as hypotension, respiratory distress, transient neurotoxicity, renal insufficiency and serious life-threatening

events. The subsequent clinical trial results indicated that once the transgene products return to the body, CAR modified T cells can selectively bind to the targeted antigens expressed on the tumor as well as the normal tissue, which also express these antigens at a relatively lower level. This also contributes to the adverse events after infusion. Adverse events of CAR T cells infusion can be summarized as 'on-target on-tumor, on-target off-tumor (Figure 1) and off-target off-tumor' [1]. On-target on-tumor reactions, including cytokine release syndrome and tumor lysis syndrome. They can be controlled with symptomatic, supportive strategy, glucocorticoid and even the anti-IL-6 receptor antibody Actemra (Tocilizumab) according to the grading of adverse reactions [8]. On-target off-tumor and off-target off-tumor events did not seem as easy to deal with. Persistent eradication of the healthy B-cell population induced by CD19-CAR could be managed by intravenous immune globulin [9]. However, the counter measure for targeted toxicities caused by other designed CARs remain limited. Even more, we cannot predict what events will occur after receiving the new CARs that we designed as the perfect pattern. Once the unpredictable events occur, we may activate the inhibitory signal pathway of CAR T cell (-iCAR) [10],

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Figure 1. Toxicities and barriers of the CAR-T therapy. Single chain variable fragments also bind the antigen expressed by normal cells that may trigger the on-target on-tumor and on-target off-tumor toxicities. Tumor microenvironment presents itself as physical barriers and immune barriers to prevent T cells from directly contacting with targeted cells.

CAR: chimeric antigen receptor; Treg: Regulation T cell; MDSC: Myeloid-derived suppressor cell.

removing the toxic side effects at the expense of the therapeutic effect. Hence, if we do not get a handle on its toxicity, even this very potent therapy could self-destruct. Novel tactics, not only for the encouraging results, but the controllable toxicities, remain to be one of the urgent but unmet needs today.

Aside from these toxicities, CAR T techniques were highly controversial and questioned whether they could bring a similar surprise in application of lymphoma and solid tumors. First, the tumor microenvironment, interdependent with lymphoma or solid tumors, presents as physical barriers to prevent T cells from direct contact with targeted cells [11] (Figure 1). Second, the peritumoral areas are surrounded by a tumor immune friendly environment which is composed by regulation T cells (Tregs), myeloid-derived suppressor cells (MDSCs) and M2 macrophage, all of which are working hard for weakening the function of T cells and promoting its exhaustion [12–14] (Figure 1). Third, the lack of homing receptors, CAR T cells are transformed into passing T cells rather than penetrating into the

tumor [15]. Finally, repetitive CAR targeting may facilitate selective emergence of the CAR-negative oncocty clone and promote immune escape. Emerging data indicate that CD19 proteins may be lost or truncated with therapeutic CD19-CAR T cells, therefore, some cases underwent a relapse or progression with the CD19 negative clone [3,4]. Those mentioned above constitute powerful challenges to immunotherapy targeting lymphoma and solid tumors.

CAR T technology, endowing the specific T cells ability to recognize and kill tumor cells directly, has brought the dawn of conquering malignant tumors, however, current issues to be solved are as follows. First, there is no uniform consensus on the dose of transfusion cells. Small dose infusions may not obtain the ideal curative effect, but induce tumor antigen deletion, then develop into immune escape; large dose infusions and serious tumor load will increase the cytokine release syndrome and tumor lysis syndrome. Whether we can seize the relationship between CAR T-cell infusion dosage and influence factors, including tumor load, effi-

cacy and side effects, seems particularly critical. Second, the reasonable targets need to be set, which is possible to maximize the destruction of the tumor and minimize the toxicities. Third, the preconditioning scheme before infusion is needed. Chemotherapy induced lymphocyte depletion may reduce the anti-CAR immune response and induce bone marrow cytokine production that restores the immune cell populations and favors the activation of antitumor responses [15]. From another point of view, these cytokines can aggravate the cytokine release syndrome. Tregs and MDSCs are also increased after preprocessing, which is an unexpected by product result. Finally, due to the existence of tumor microenvironment, not only the physical barriers but the immune barriers for CAR-T therapy, combined with other targeting drugs or designed novel CARs have become a new trend [16–18].

In conclusion, CAR T technology not only brings us spectacular opportunities, but also severe challenges. Precise targeting, accurate infusion dose, ideal efficacy and slight side effects will be considered as a starting point for the novel designed CARs, and a new era for tumor immunotherapy will emerge to overcome these hurdles mentioned above.

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