Editorial

Synergizing genome editing and cancer immunotherapy

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Comment on: Eyquem J, Mansilla-Soto J, Giavridis T, *et al*. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature 2017;543:113-7.

Submitted May 18, 2017. Accepted for publication May 23, 2017.

doi: 10.21037/tcr.2017.06.11

View this article at: http://dx.doi.org/10.21037/tcr.2017.06.11

Cancer immunotherapy has recently become a powerful treatment option as several strategies harnessing the immune system to fight cancer achieved remarkable therapeutic benefits in clinical trials. Oncolytic viruses mediate selective tumor cell lysis (1), checkpoint inhibitors block receptors such as CTLA4 or PD1 on T cells and/or their ligands on tumor cells to reverse T cell suppression (2) and adoptive T cell therapy uses autologous tumor infiltrating lymphocytes (TIL) or genetically modified T cells to kill cancer cells. The latter has recently led to unprecedented efficacy, predominantly as a treatment for melanoma and leukemia. Similarly, gene editing—the precise modification of genes and genomic loci—holds enormous potential for clinical and research applications, primarily owing to the ease of use of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas9) system that has transformed gene editing into a widely-used technique (3). Research efforts in both fields are currently combined by exploiting gene editing to enhance efficacy and safety of adoptive T cell therapies. In a recent edition of Nature, Eyquem and colleagues showed the superior potency of gene edited T cells in eliminating tumor cells and promoting survival (4).

Adoptive T cell transfer aims at recognizing and eliminating tumor cells by the transplantation of tumor-reactive autologous or allogeneic T cells. T cell therapies have exhibited dramatic antitumor activity in numerous clinical trials. Autologous transfer is based on pre-existing *in vitro* expanded TIL that exhibit tumor reactivity. TIL therapy has been used primarily to treat melanoma as melanoma is able to induce strong antitumor T cell response in patients (5). Several clinical studies have shown tremendous success including durable complete responses beyond 3 years (6). The most impressive effects however were achieved using T cell receptor (TCR)- or chimeric antigen receptor (CAR)-engineered T cells. T cells can be genetically engineered to express tumor-specific TCR or artificial CAR that enable the cells to recognize selected antigens (7). CARs are composed of an extracellular single-chain variable fragment (scFv) derived from immunoglobulin variable domains that confers antigen-specificity. The scFv is fused to an intracellular domain composed of TCR signaling and co-stimulatory domains necessary for full T cell activation. While TCR recognize antigens presented by the major histocompatibility complex (MHC), CAR therapies are restricted to cell surface antigens. Compared to TIL therapy, adoptive transfer of engineered T cells has the potential to treat a broader spectrum of malignancies independently of preexisting tumor reactivity.

In a clinical phase I/II trial, adoptive transfer of TCR-engineered T cells mediated sustained antitumor effects in 80% of myeloma patients (8). CAR T cell therapies targeting CD19 or CD20 achieved clinical response rates of more than 90% in patients with B-cell malignancies such as chronic lymphocytic leukemia, non-Hodgkin's lymphoma and B cell acute lymphoblastic leukemia (B-ALL) (9). In addition, a number of CAR T cell clinical trials have been started targeting other hematological disorders and various solid tumors [reviewed in Fesnak *et al.* (10)].

The promising efficacy of T cell therapies has been accompanied by severe side effects in many studies. The potent immune responses and rapid clearance of large tumor burden by next-generation CAR T cells often induces cytokine release syndrome (CRS) and neurologic toxicities (11). In addition, due to their expression in healthy tissues, tumor associated antigens (TAA) can promote on-target/off-tumor toxicity of engineered T cells. In several clinical trials using TCR-engineered T cells, on-target toxicity and TCR cross-reactivity, led to severe adverse events including cardiogenic shock (12,13). Similarly, CAR T cell therapies have shown severe side-effects, including a lethal pulmonary adverse event and five deaths from cerebral edema/neurotoxicity (14). In addition to side effects, poor expansion and exhaustion of infused T cells is a major challenge of T cell therapies (15). T cell exhaustion could be a result of antigen-independent constitutive CAR T cell stimulation partly promoted by the receptor structure. Furthermore, many clinical studies reported high disease relapse rates that are predominantly based on poor persistence of the engineered cells or loss of the target antigen/epitope on tumor cells (9,16).

Efficient and safe adoptive transfer of engineered T cells relies on the stable expression of transferred TCR or CAR and on the *in vivo* proliferation, survival and reactivity of T cells. T cells with a central memory or memory stem phenotype are capable of self-renewal, long term survival and show elevated proliferative capacity (17). Preselection of certain T cell phenotypes could lead to enhanced antitumor reactivity and better defined T cell products (18). At present, receptor genes are predominantly transferred using integrating gammaretroviral or lentiviral vectors. Both vectors integrate semi-randomly into the genome of target cells preferentially near or within transcribed genes (19,20). Semi-random integration leads to variegated expression levels and can result in transcriptional silencing (4,21). In addition, usage of these vectors entails the risk of vector-mediated insertional T cell transformation. However, no adverse events related to insertional mutagenesis have been observed to date in T cell therapies (22).

The simultaneous expression of endogenous and transferred TCR can result in the formation of mixed-dimers of TCR α and β chains. Such mispaired TCR have been shown to trigger autoimmunity in a mouse model and neoreactivity and autoimmunity in human T cells (23,24). To reduce the risk of graft-versus-host disease (GvHD), expression of endogenous TCR can be knocked-out by designer nucleases such as CRISPR/Cas9 or transcription activator-like effector nucleases (TALEN) (25-29). TCR knockout can similarly be used in healthy donor T cells to provide universal off-the-shelf CAR T cell therapies (25,30). The lack of TCR expression prevents infused allogeneic T cells from recognizing recipient alloantigens thereby abolishing the risk of GvHD. Additionally, knockout of self-antigens such as the MHC complex can protect engineered T cell products from clearance by the host immune system (30). Universal CAR T cell therapies would be highly valuable to treat lymphopenic patients, to save manufacturing costs, time and resources as well as to reduce the heterogeneity of treatment effects (31). Recently two patients with B-ALL were successfully treated by universal CAR T cells targeting CD19 (UCART19). The allogeneic CAR19 expressing T cells were infused following TALEN-mediated disruption of the endogenous TCR α chain as well as CD52 (32). Another promising gene editing approach is the knockout of PD1 on engineered T cells to block T cell suppression in the microenvironments of solid tumors (31). The combination of PD1 blockade by immune checkpoint inhibitors with CAR T cell therapy has demonstrated increased antitumor reactivity in preclinical studies (33).

The presence of a donor DNA template exhibiting homology to a designer nuclease target site enables targeted integration of genes. Researchers from the Sadelain lab have recently developed a sophisticated strategy to knockout the endogenous TCR and simultaneously target the integration of a CAR coding sequence to the *TRAC* locus in mice. Compared to conventional CAR T cells engineered with integrating viral vectors, endogenous regulation promoted uniform CAR expression and resulted in delayed T cell exhaustion, prolonged median T cell survival *in vivo* and superior therapeutic effects (4). In addition to enhancing the antitumor potency of engineered CAR T cells, this approach increases the safety of the therapy by avoiding use of integrating viral vectors and by preventing endogenous TCR expression.

The strategy of using designer nucleases to target CAR or TCR genes into the *TRAC* locus solves several of the safety and efficiency concerns associated with engineered T cells. A remaining challenge associated with gene editing, in particular for the translation into clinical applications, is designer nuclease off-target activity (34). Double strand breaks introduced at genomic loci other than the nuclease target site could result in severe side-effects such as oncogenesis. Thus, assessing the specificity of designer nucleases with genome-wide experimental approaches that have been developed to detect *bona fide* off-target sites is of

major importance (35,36).

Administration of engineered T cells has shown dramatic antitumor responses in various clinical trials, especially in patients with B cell tumors. Combing conventional T cell engineering with one of the most exciting recent technological developments—gene editing—opens up a whole new world of manufacturing possibilities and T cell engineering strategies. Off-the shelf gene edited T cells generated from single donors can serve as cell therapy products for numerous recipients. Transcriptional regulation of transferred TCR or CAR by targeted integration into the *TRAC* locus promoted superior performance of engineered T cells in tumor eradication. As first reports of adoptive transfer of gene edited T cells are very promising, further investigation in patients is eagerly anticipated to confirm these first clinical and preclinical results.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Chen Qian (Center for Inflammation & Epigenetics, Houston Methodist Hospital Research Institute, Houston, TX, USA).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2017.06.11). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Gabriel R, Knipping F, von Kalle C. Synergizing genome editing and cancer immunotherapy. Transl Cancer Res 2017;6(Suppl 6):S969-S972. doi: 10.21037/tcr.2017.06.11

