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How to make a better T cell: *in vivo* CRISPR screens have some answers

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Understanding what regulates CD8$^+$ T cell responses is key to effectively harnessing these cells in human disease. In this issue of *Cell*, Huang et al. and Chen et al. use *in vivo* CRISPR screens to discover novel regulators of CD8$^+$ T cell immunity to engineer a more efficacious response against cancer and infections.

Together with immune checkpoint blockade therapy, adoptive cell transfer has changed the treatment of cancer. Although the former aims at interfering with a steadily increasing number of "molecular breaks," the best-known being PD-1 and CTLA-4, that restrain CD8$^+$ T cell responses and drive exhaustion, adoptive cell therapy expands and reinforces patient-derived CD8$^+$ T cells to recognize and clear cancer cells or pathogens. In recent years, CRISPR-Cas9 technology has received considerable attention for its unprecedented potential for specific and efficient gene editing (Doudna and Charpentier, 2014). In this issue of *Cell*, Huang et al. and Chen et al. use CRISPR-Cas9-based screens to investigate the molecular mechanisms that underlie CD8$^+$ T cell activation, effector function, and memory formation *in vivo* (Huang et al., 2021; Chen et al., 2021). Upon targeting two distinct selections of genes putatively involved in the development of CD8$^+$ T cell responses, the authors identify novel metabolic and transcriptional regulators of CD8$^+$ T cell-mediated immunity, which can be targeted to enhance the efficacy of adoptive cell therapies against infection and cancer (Figure 1).

Engagement of different metabolic pathways underlies cell fate and function of CD8$^+$ T cells. Although effector T cells (T_{EFF}) commit to anabolic metabolism, coupling nutrient uptake with energy production and the synthesis of biomass to support their activation and proliferation, memory T cells (T_M) engage a catabolic metabolic phenotype to sustain cellular fitness and their long-term survival (Geltink et al., 2018). Huang et al. screened over 3,000 metabolism-associated genes to identify modulators of CD8$^+$ T cell responses and used the ratio between T_{EFF} and T_M as an indicator of the quality of the response. The authors found that loss of the amino acid transporters encoded by the genes *Slc7a1* and *Slc38a2* dampened mTORC1 signaling and in turn promoted T_M formation, in line with previous findings (Araki et al., 2009). Interestingly, they also showed that deletion of *Pofut1*, which encodes an enzyme that couples the sugar fucose to proteins (i.e., fucosylation), concomitantly promoted expansion of the T_{EFF} pool and efficient T_M formation. *Pofut1*-deficient CD8$^+$ T cells were retained in a less differentiated state, were more metabolically active, and showed enhanced proliferation, underlying the expansion of the T_{EFF} pool. Finally, the authors elegantly linked the availability of fucose, *Pofut1* fucosyltransferase function, and Notch signaling to the commitment of CD8$^+$ T cells to different fates. Although it remains unclear how fucose and Notch modulate the molecular events behind T cell function, the study of Huang et al. highlights novel targets to enhance the magnitude and quality of CD8$^+$ T cell responses.

Taking a similar approach, Chen et al. performed an *in vivo* CRISPR screen targeting 120 transcription factors selected for their known relevance in CD8$^+$ T cell biology and on the assumption that their function shapes the transcriptional landscape of CD8$^+$ T cell responses. The authors screened for negative regulators of antigen-specific T_{EFF} responses at 8- and 15-days post infection, using models of both acute and chronic viral infection. Comparing hits found across all conditions led to the identification of the ETS family transcription factor *Fli1* as a new negative regulator of T_{EFF} responses. Deletion of *Fli1* enhanced T_{EFF} development and function without compromising T_M formation, whereas its enforced expression restrained T_{EFF} differentiation. Analysis of the chromatin landscape using ATAC-sequencing revealed binding of *Fli1* to *cis*-regulatory elements of T_{EFF}-driving genes. Loss of *Fli1* increased chromatin accessibility at ETS:RUNX motifs, allowing RUNX3-mediated transcription of T cell effector genes. Functionally, loss of *Fli1* yielded T_{EFF} with increased cytotoxic potential, ultimately conferring better protection against infection with lymphocytic choriomeningitis virus (LCMV), influenza virus, and *Listeria monocytogenes*, as well as against multiple tumors. Importantly, increased effector differentiation did not enhance T cell exhaustion, which is a major factor limiting T_{EFF} responses against chronic infections and tumors (McLane et al., 2019). The work of Chen et al. leverages *in vivo* CRISPR screening to identify novel safeguards of T_{EFF} differentiation and describes the transcription factor *Fli1* as a new checkpoint of CD8$^+$ T cell development.

Although genetically deleting these new checkpoints proved effective in modulating CD8$^+$ T cell immune responses in mice, the potential for translation of these findings into clinical applications and their suitability as therapeutic targets remains to be addressed. As previously mentioned, adoptive cell transfer is

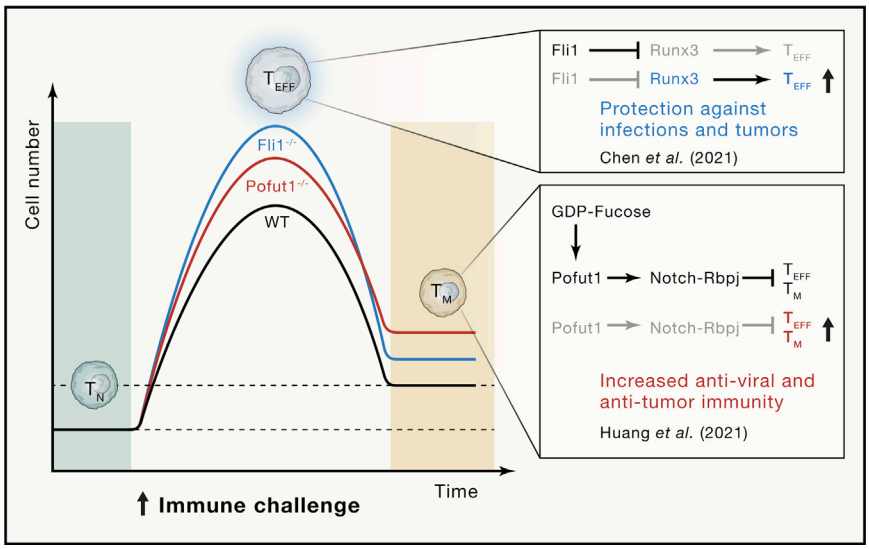

Figure 1. Kinetics of a T cell response: *Fli1* and *Pofut1* as negative regulators of CD8$^+$ T cell differentiation

Upon antigen encounter, naive CD8$^+$ T (T_N) cells proliferate extensively to form the pool of T_{EFF}. T_{EFF} produce cytokines (such as interferon-γ and tumor necrosis factor) as well as cytotoxic proteins (such as granzymes) to kill infected and cancer cells. To keep the response in check and to prevent immunopathology, T_{EFF} largely undergo apoptosis upon clearance of infection or cancer. A subset of cells will survive the contraction phase and persist as T_M, conferring long-lasting protection against re-encounter of the same threat. The works of Chen et al. (2021) and Huang et al. (2021) highlight the transcription factor *Fli1* and the fucosyltransferase *Pofut1* as important regulators of the magnitude and quality of CD8$^+$ T cell responses.

employed in the treatment of cancer and could hold promise for treating chronic infections where T cell exhaustion is also an issue. However, whether using CAR T cells or *ex vivo*-expanded patient-derived T cells, adoptive cell transfer is only efficacious in a subset of cancer patients and faces many scientific, regulatory, and economic obstacles. In addition, the majority of currently available immunotherapies aim to augment the endogenous anti-tumor immune response and are not readily compatible with gene editing. Future work will be needed to reconcile some of the conflicting findings regarding the role of fucosylation in CD8$^+$ T cell development (Yao et al., 2011), activation (Liang et al., 2018), and function (Alatrash et al., 2019; Okada et al., 2017), as well as to address the feasibility of targeting T cell fucose metabolism *in vivo* (Schneider et al., 2018). Similarly, the therapeutic potential of targeting *Fli1* in other RUNX3-dependent settings remains to be explored, such as improving the generation and accumulation of resident T_M cells in peripheral tissues to augment vaccination strategies (Milner et al., 2017). Ultimately, all approaches aiming to generate improved genetically modified effector CD8$^+$ T cells will need to balance enhanced anti-tumor activity with the potential for adverse immune-mediated effects and the development of autoimmunity. Approaches targeting transcription factors also need to weigh the potential risk for malignant transformation due to the unrestricted activity of potentially oncogenic drivers. Although much work still needs to be done, understanding the fundamental biology of T cells is essential for the rational choice of targets for clinical development and synergistic combination treatments. Upcoming studies will hopefully shed light on these exciting key questions in this rapidly evolving field.

DECLARATION OF INTERESTS

E.L.P. is an SAB member of Immunomet, a Founder and Advisor of Rheos Medicines, and an Advisory Board member of *Cell*. A family member of E.L.P. is a Founder and Advisor of Rheos Medicines.

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