Docket #: S22-235

A multiplexed RNA regulation platform for primary immune cell engineering

To overcome current gene editing safety, efficacy, and scope limitations, Stanford researchers in the Mackall Lab and Stanley Qi Lab developed MEGA (Multiplexed Effector Guide Arrays), a versatile and multifunctional platform for programmable and scalable regulation of the T cell transcriptome using RNA-guided, RNA-targeting activity of CRISPR/Cas13d, and successfully enhanced the anti-tumor activity of CAR T cells (see figure 1). MEGA uses molecular scissors to cut RNA, not DNA, to activate reversible changes to gene expression in T cells with expected lower genotoxicity and chromosomal rearrangements compared to CRISPR/Cas9. MEGA is quantitative, tunable, and regulatable without binary changes to genome. It acts quickly and downstream of chromatin remodeling. Researchers demonstrated they could make 10 edits at once to human T cells, compared to 3 with Cas9. MEGA is a safer, more effective, more versatile, and reliable addition to the synthetic immunology toolkit, with many applications in cancer immunotherapy and research.

Figure

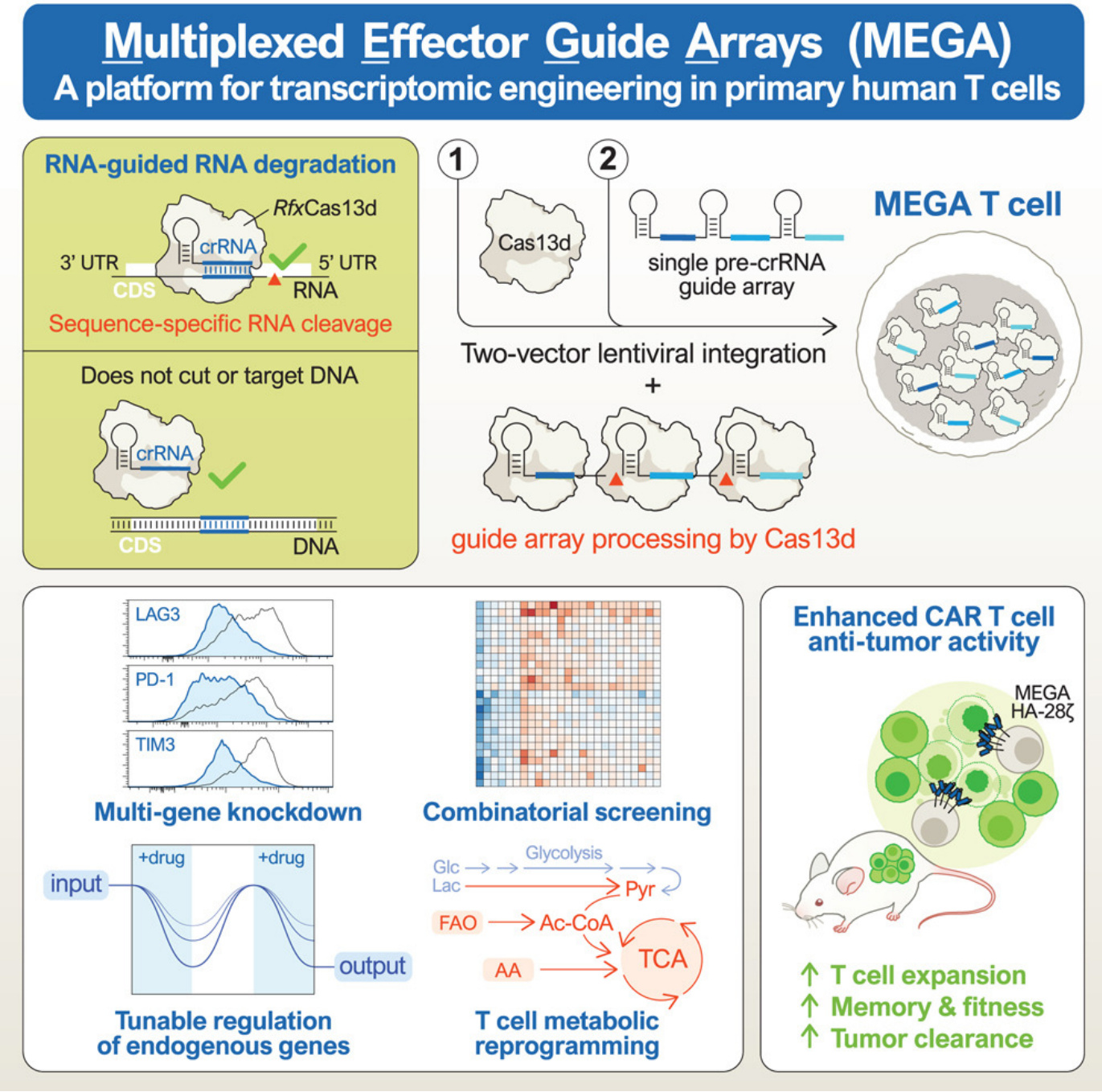

Figure Description: Multiplexed Effector Guide Arrays (MEGA) for transcriptomic engineering in T cells. (*Image courtesy the Mackall and Qi Labs*)

Stage of Development

Research - In Vivo: Stanford researchers continue *in vivo* work to verify the safety and efficacy of the technology, and to enhance anti-tumor activity of CAR T cells.

Applications

- Cancer immunotherapy treatments and research
- Combinatorial CRISPR screening

Advantages

- **Safer, more effective, more versatile, and reliable** than CRISPR/Cas9:
  - Expected **lower genotoxicity** and chromosomal rearrangements compared to Cas9 induced double-strand breaks.
  - **High editing efficiency** and **low off-target effects**.
  - **Reversible** knockouts or base editing.
  - Quantitative, tunable, and regulatable without binary changes to genome.
  - Acts quickly and downstream of chromatin remodeling, so **chromatin remodeling is unaffected**.
  - Compact CRISPR/Cas13d effector **is easily expressed in T cells**.
  - **Robust multiplexed knockdown** in single cells due to the guide array processing ability of Cas13d.
  - **MEGA can repress 10 genes simultaneously** (compared to 3 with Cas9).
  - **Flexible and unconstrained targeting** of arbitrary RNA sequences as Cas13d does not require PAM sequences.
  - Sophisticated guide design tools exist for Cas13d gene knockdown.

Publications

- Tieu, V., Sotillo, E., Bjelajac, J.R., Klysz, D., Mackall, C.L., Qi, L.S. (2024). A versatile CRISPR-Cas13d platform for multiplexed transcriptomic regulation and metabolic engineering in primary human T cells. *Cell*.
- A new RNA editing tool could enhance cancer treatment. (FEBRUARY 21, 2024). *Stanford News*.

Patents

- Published Application: WO2024044672

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