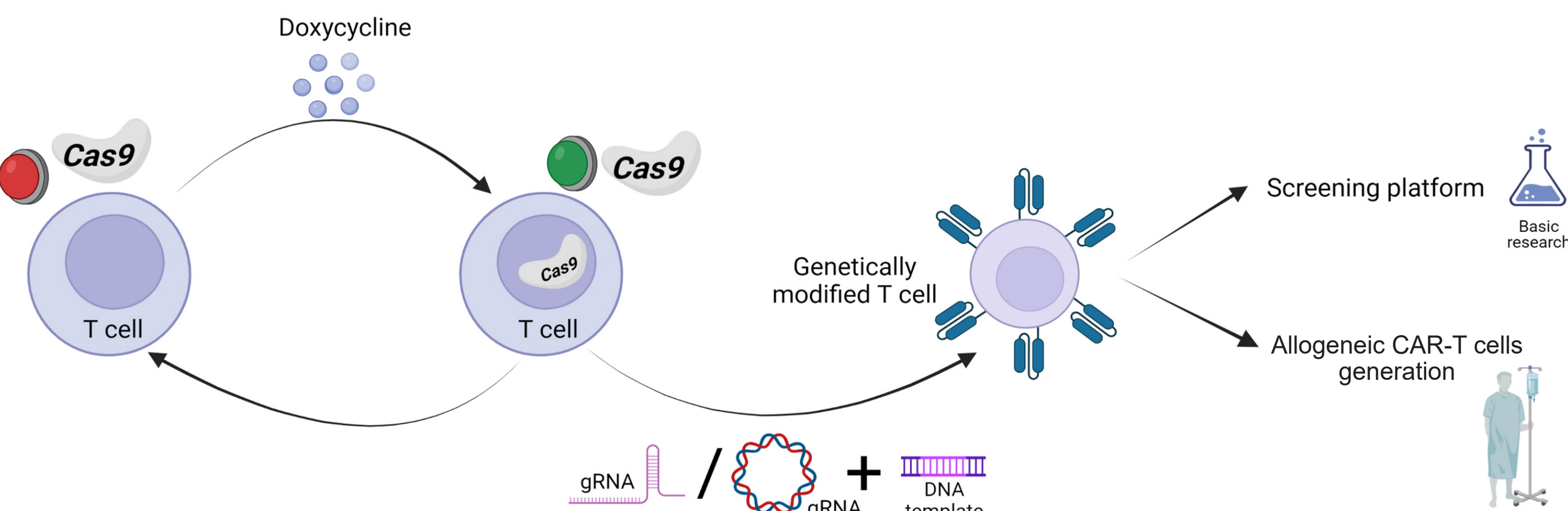


Inducible Cas9 T Cells: An Innovative Platform for Allogeneic CAR-T Cell Generation

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Background

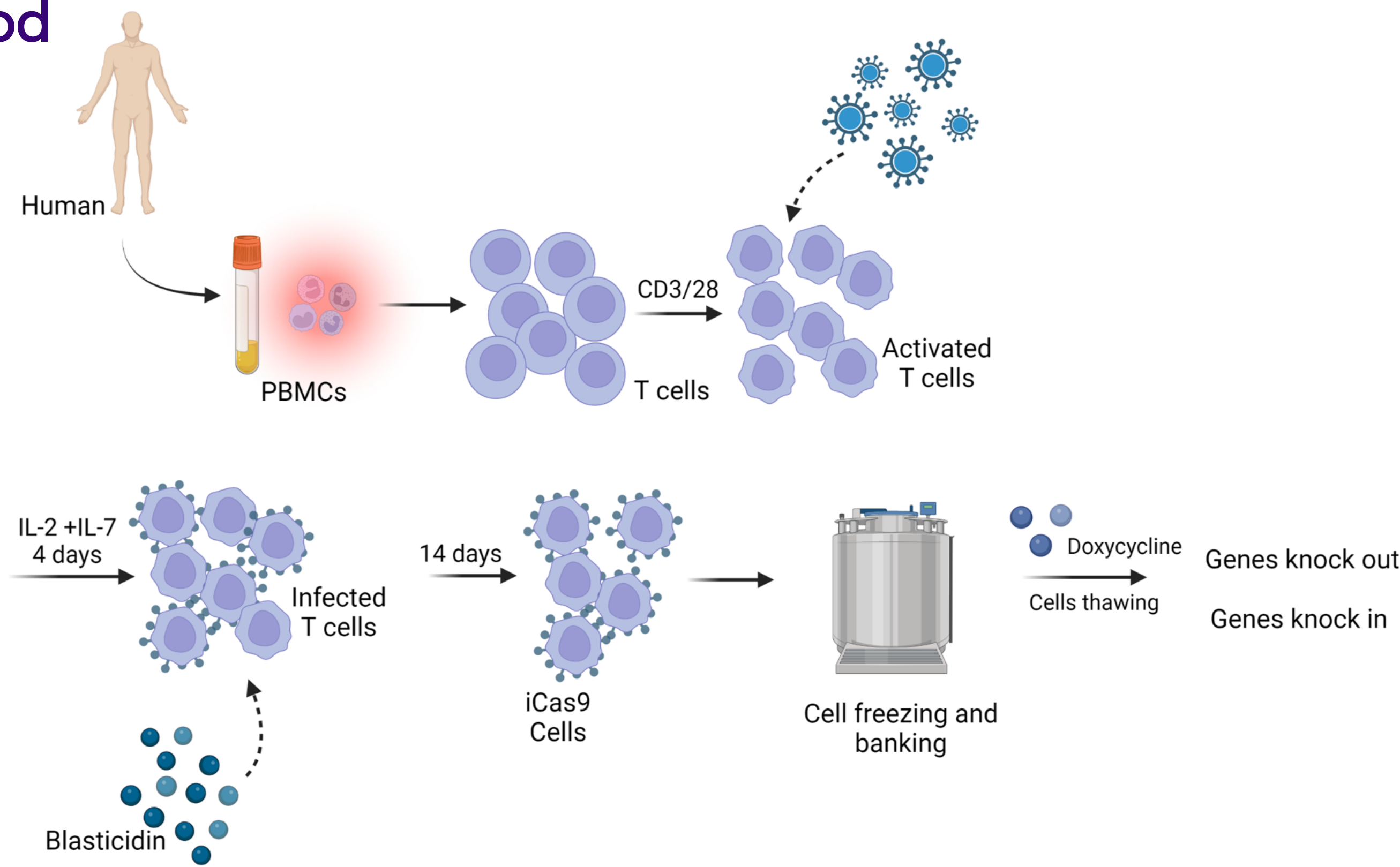
- CAR-T cells show great promise in treating many types of cancers
- Genetically engineered allogeneic T cells have the potential to provide universal CAR-T cells to treat large number of patients
- CRISPR/Cas9 is used for generating allogeneic T cells



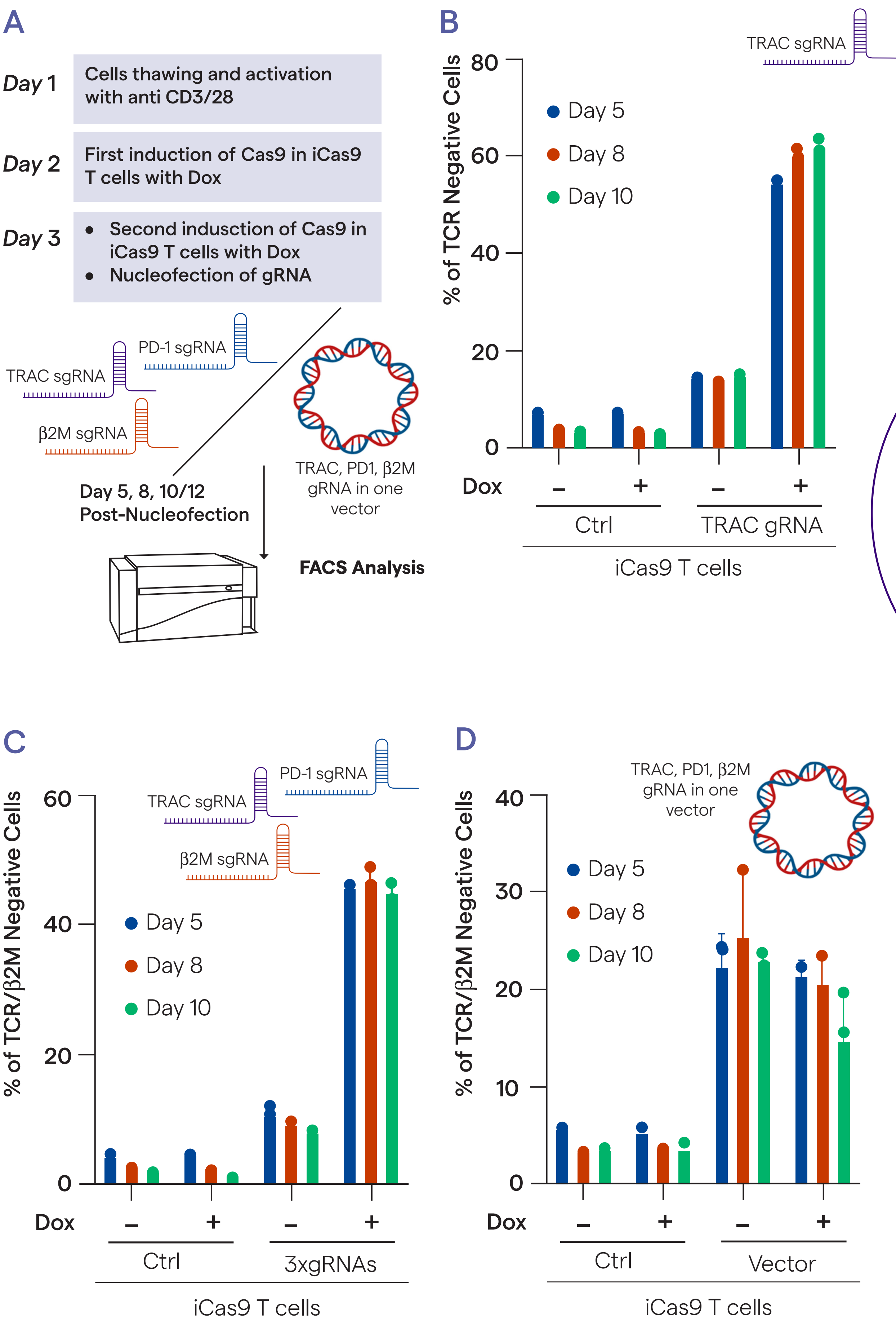
Why is it Necessary?

The use of autologous CAR-T cells requires individual manufacturing for each patient which is challenging in terms of quality, mass production and logistics. Allogeneic CAR-T cells have the potential to address these challenges

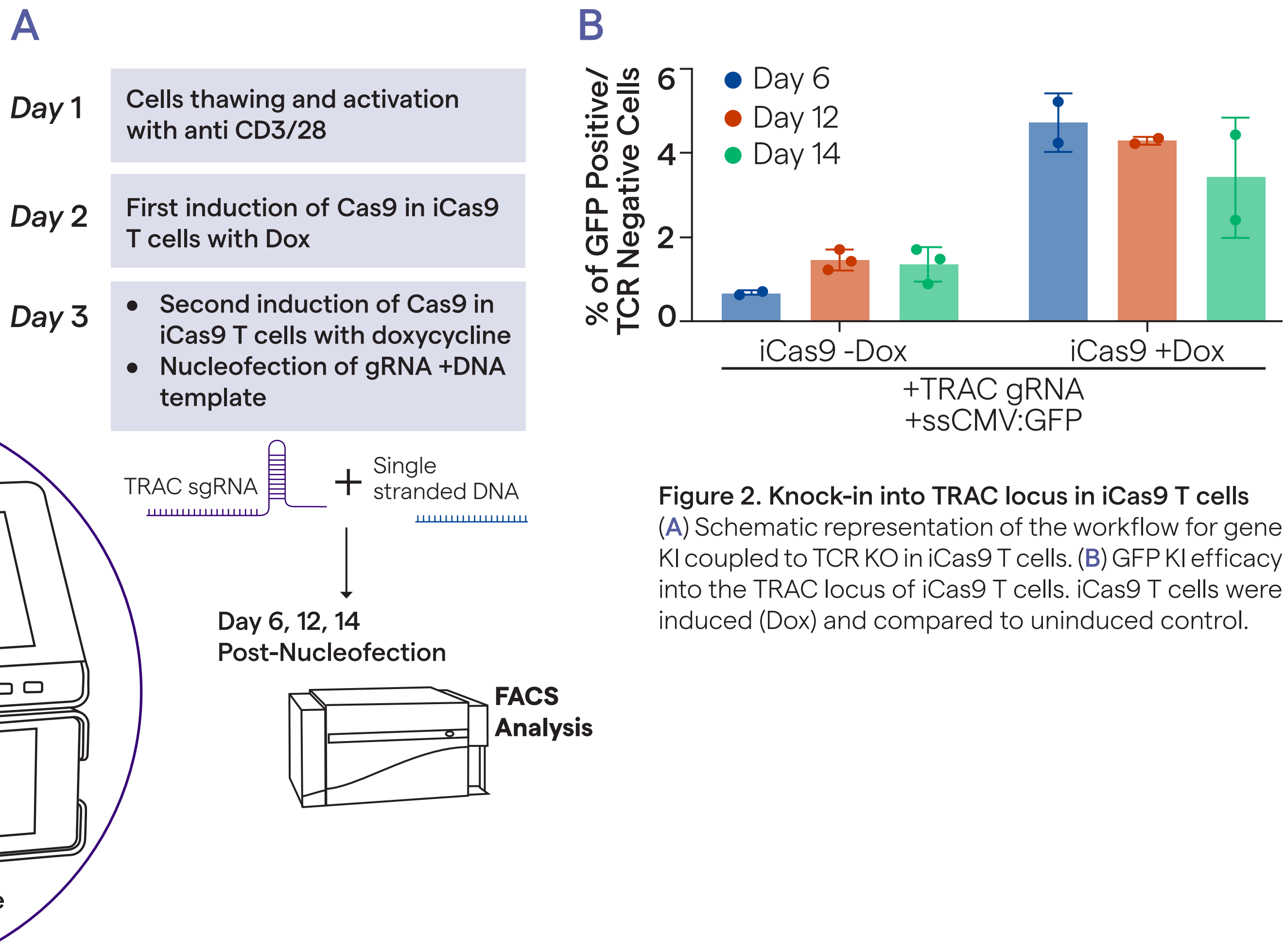
Method



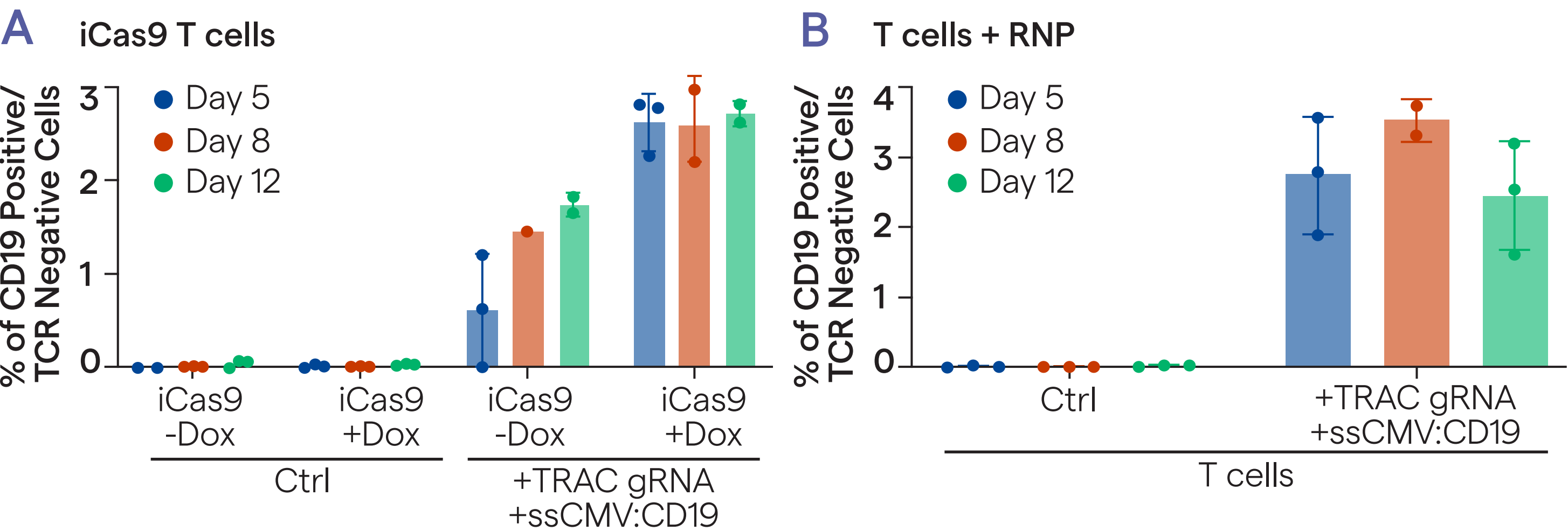
Knock-out (KO) Using iCas9 T Cells



Knock-in (KI) Using iCas9 T Cells



Generation of CAR-T Cells Using iCas9 T Cells vs Cas9 RNP



Conclusions

- Successful generation of inducible Cas9 expressing T cells
- Generation of inducible Cas9 expressing T cells master cell bank
- The platform allows for single/multiple knock-outs and knock-ins using iCas9 T cells transfected with single/multiple gRNAs and/or plasmid encoding multiple gRNAs
- Transfection of single stranded DNA template with promoter yield the highest knock-in efficiency in iCas9 T cells
- iCas9 T cells and Cas9-RNP reach comparable KO and KI efficiencies

References:

- Waldman, A.D. et al. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol. 2020; 20: 651-668.
- Gaj T, et al. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013; 31(7):397-405

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Take Home Message

The iCas9 T cells platform is an innovative approach for the generation of allogeneic CAR-T cells that allows the KO of genes involved in a range of immunogenic responses at high efficiency and more importantly for KI of relevant CARs.