Primer on CRISPR and its Impact on Cancer Research and Treatment

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2024 International Ultmann Chicago Lymphoma Symposium

AT THE FOREFRONT
UChicago Medicine

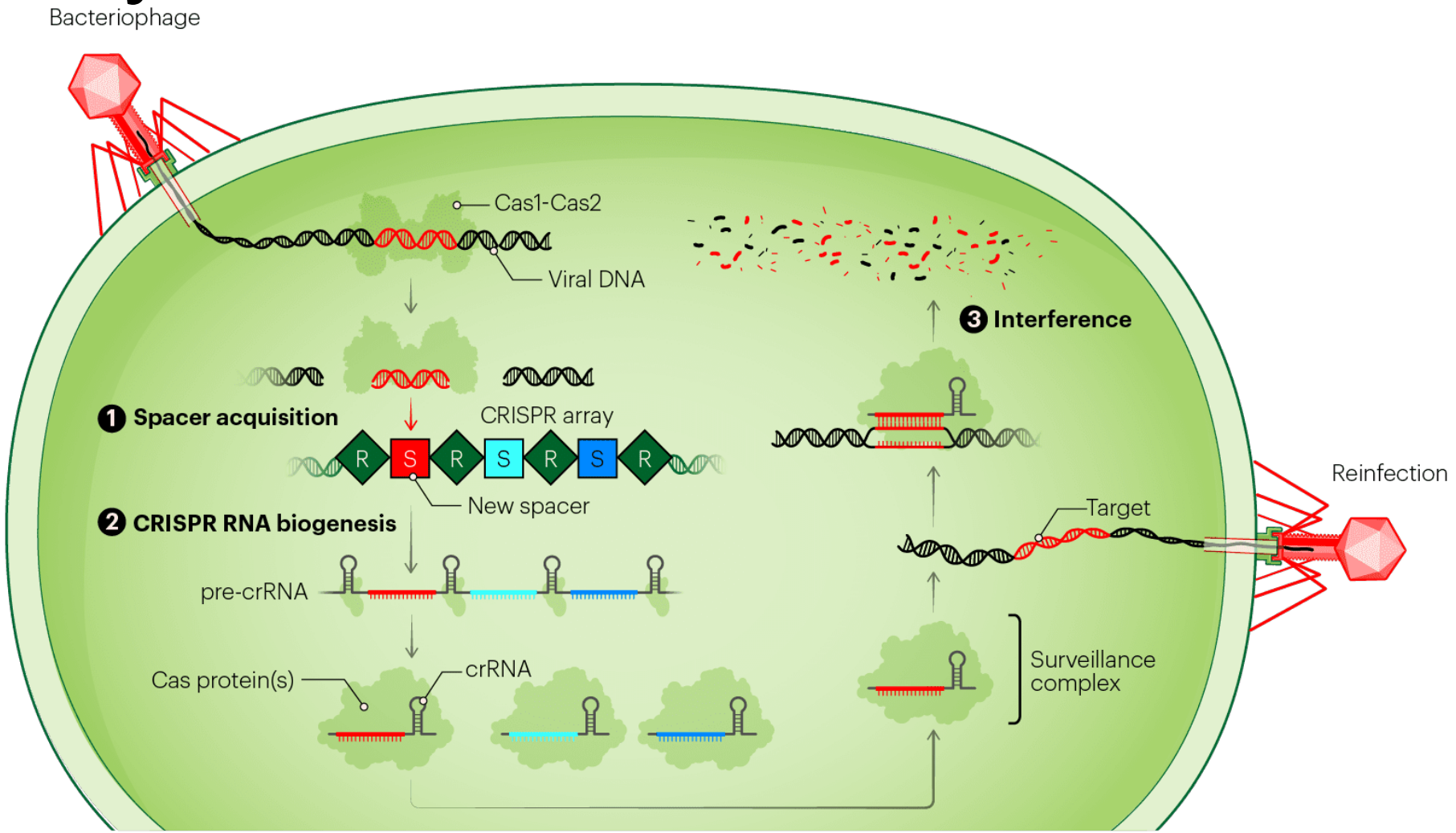
Disclosures

- None

The basics

CRISPR-Cas system in nature

A natural defense mechanism in bacteria against bacteriophages



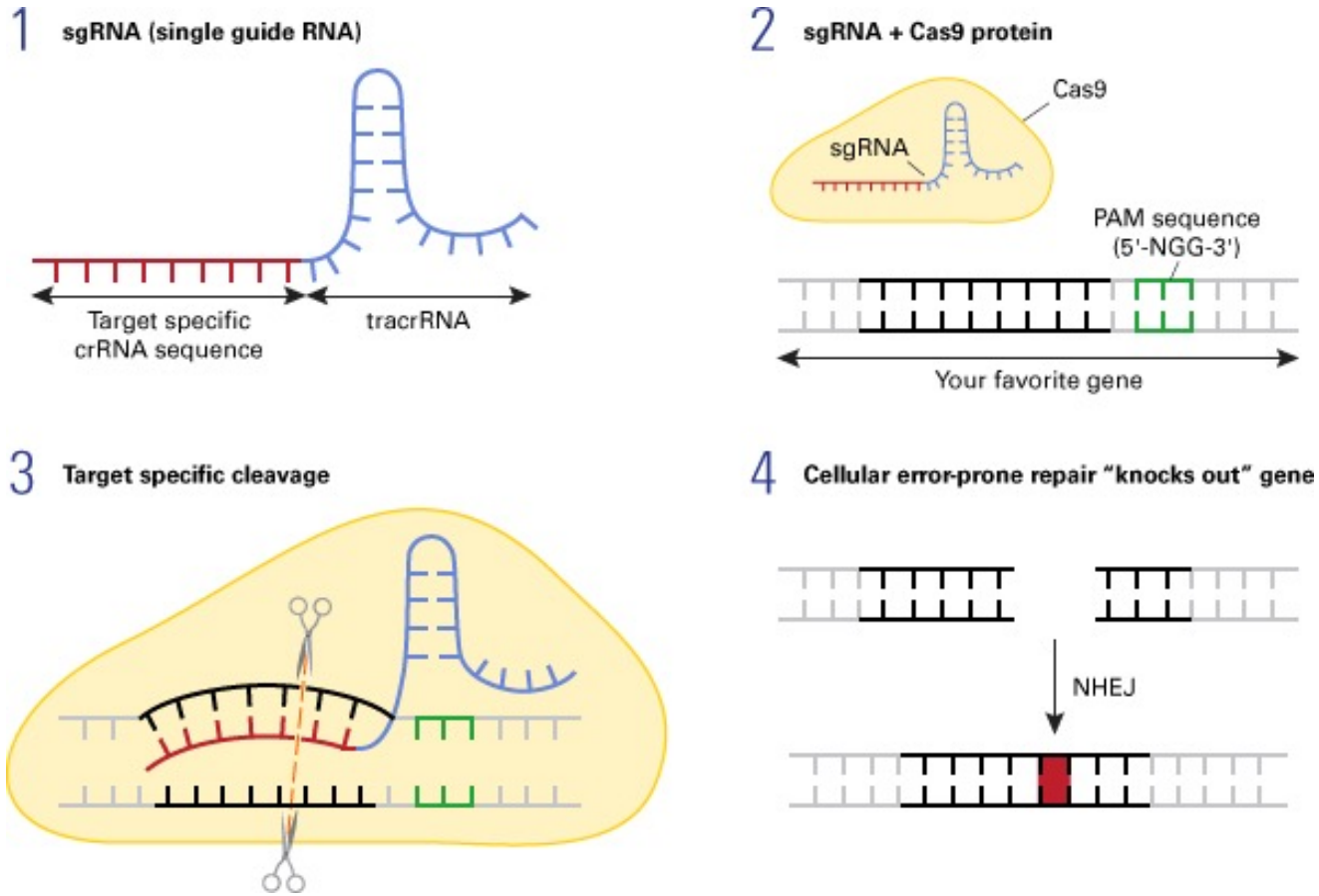
Innovative Genomics Institute | CRISPRpedia

How CRISPR works

- Co-invented by Jennifer Doudna (UC Berkley) and Emmanuelle Charpentier (Umea University, Sweeden) circa 2012

- Gene editing technology that requires 2 components
  - A guide RNA (gRNA) that complements a desired target gene
  - Cas9 (CRISPR-associated protein 9) – endonuclease that catalyzes a double strand (ds) DNA break

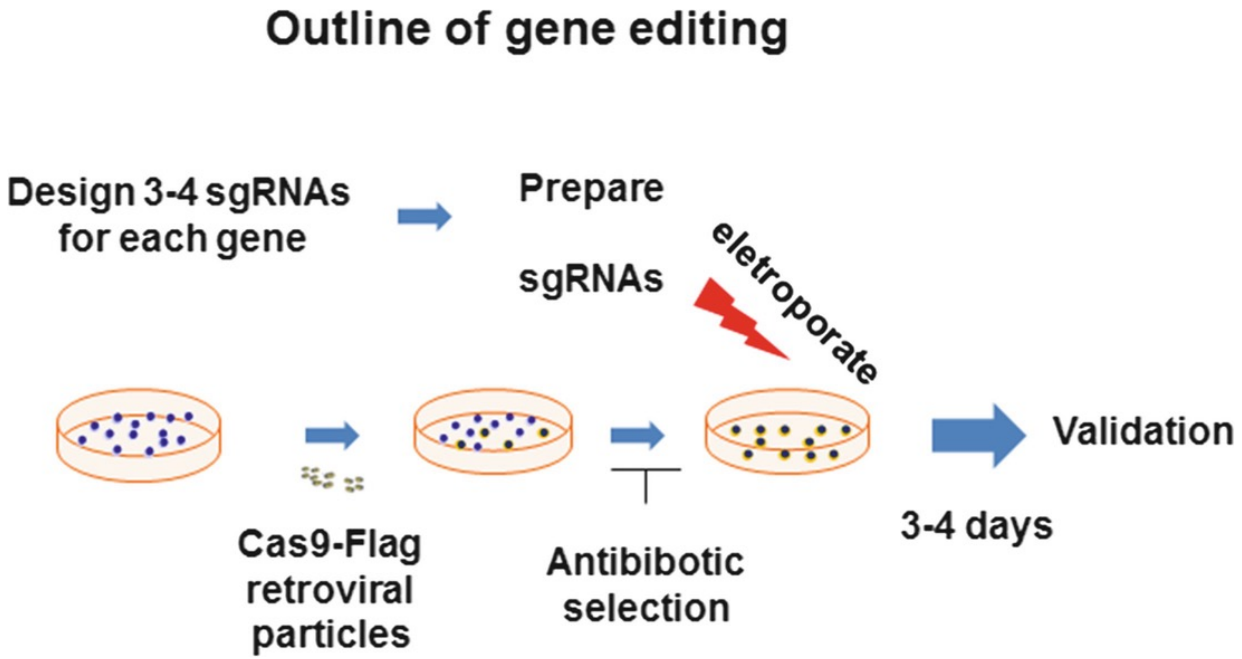
- Together, these components enable rapid and permanent genome modifications
  - Knock out/KO (make gene non-functional)
  - Knock in/KI (introduce a new gene)

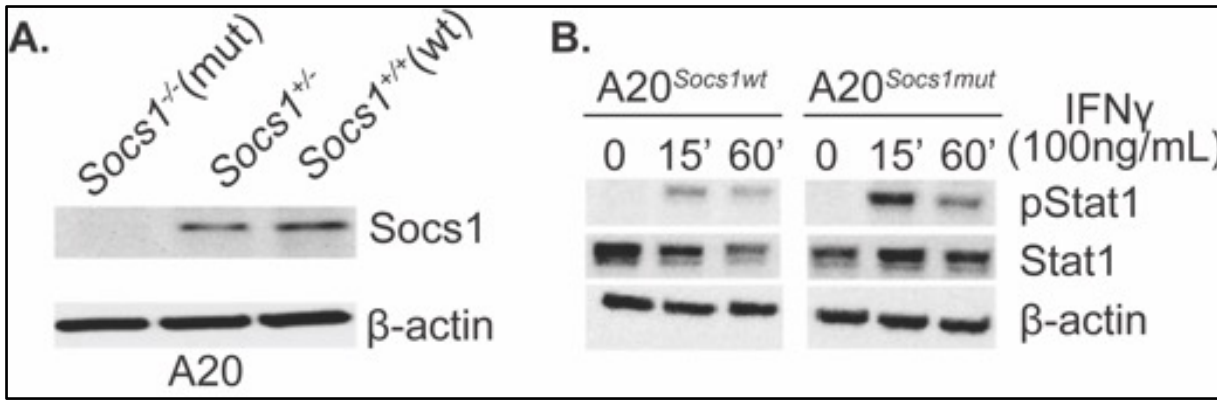


Takara Bio

CRISPR in the lab

Studying individual genes





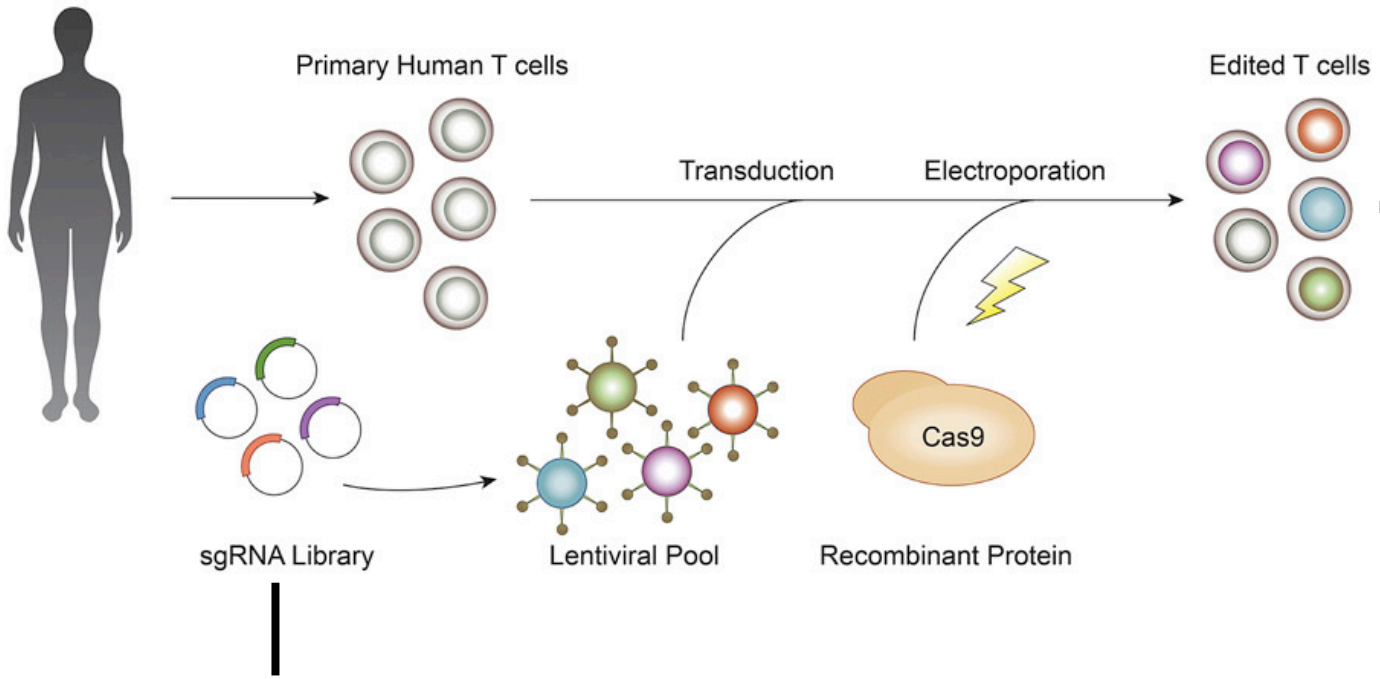
Modeling cancer at the genetic level

- Knock out tumor suppressors
- Knock in oncogenic mutations

Bai et al. *Methods Mol Biol.* 2020.

Tumuluru and Godfrey et al. *bioRxiv.* 2024.

Studying many genes at once: CRISPR screens



Genome-wide library that covers 19,000 genes

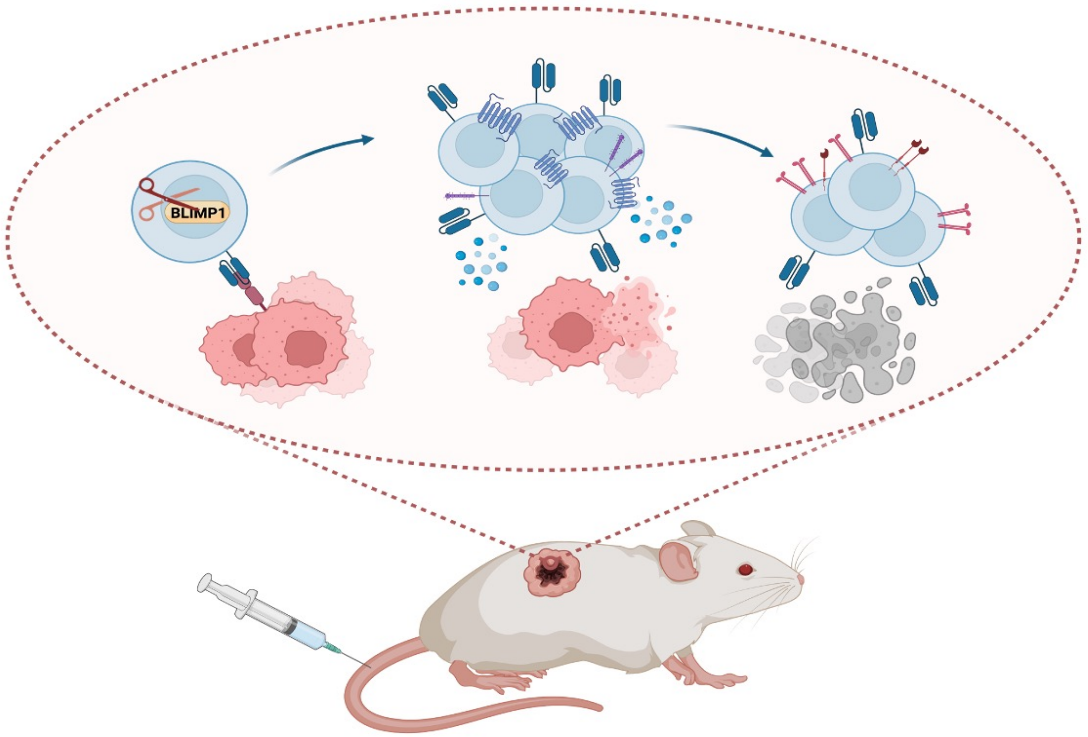
Unbiased analysis of T cell function

- *What genes contribute to T cell proliferation?*
- *In vivo persistence?*
- *Cytokine production?*
- *Tumor cell killing?*

….

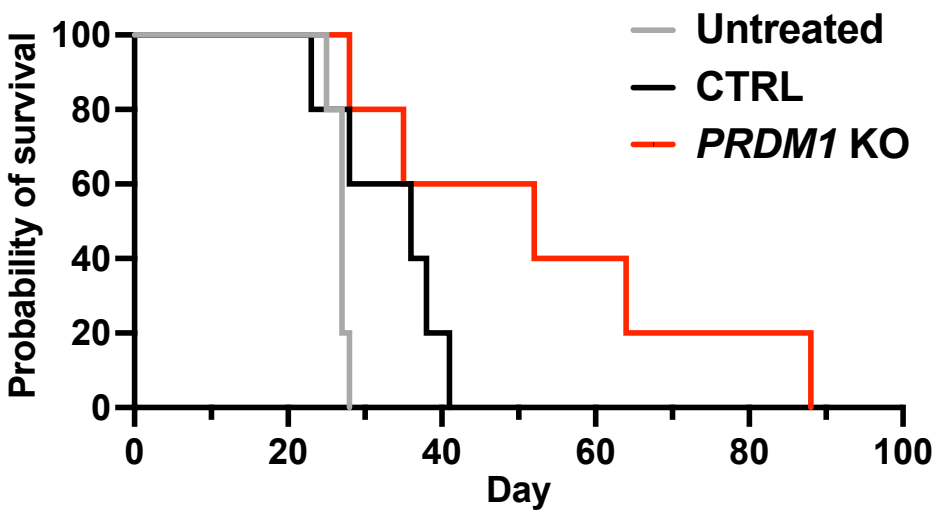
Shifrut et al. *Cell.* 2018.

CRISPR and CAR T cell engineering



Hypothesis

Disrupting *PRDM1* in CAR T cells will enhance *in vivo* persistence and antitumor activity of CAR T cells.

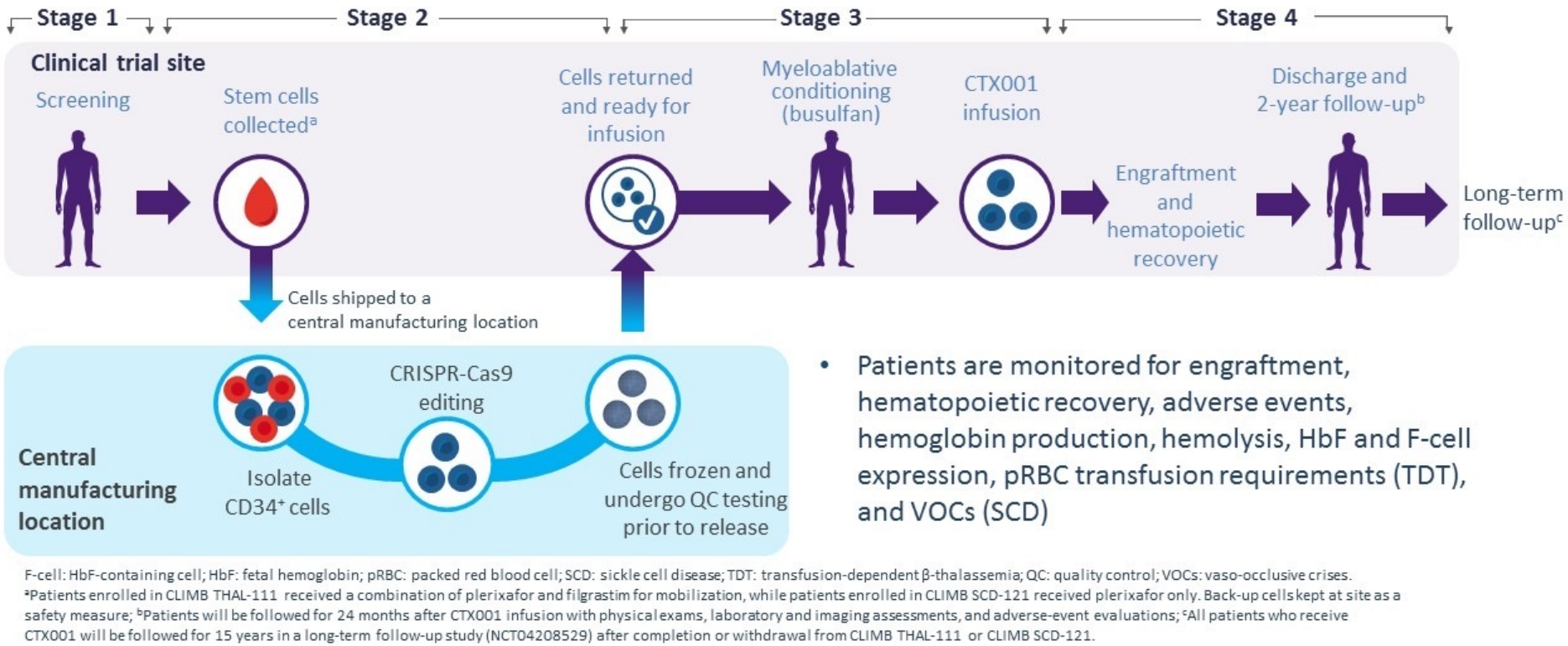


Sidney Wang from the Kline Lab (https://klinelab.uchicago.edu/)

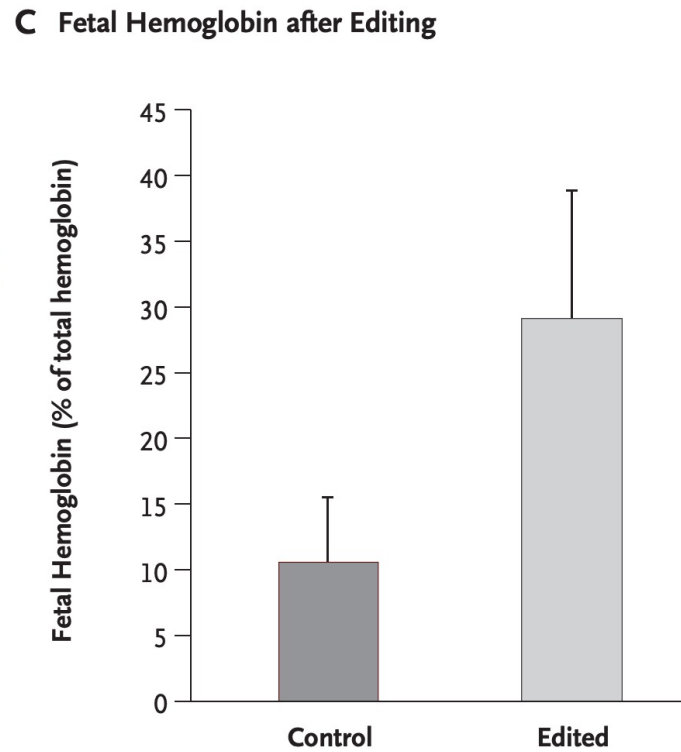
CRISPR in the clinic

CRISPR in benign hematology

Editing BCL11A to restore fetal Hb for patients with sickle cell disease or β-thalassemia



F-cell: HbF-containing cell; HbF: fetal hemoglobin; pRBC: packed red blood cell; SCD: sickle cell disease; TDT: transfusion-dependent β-thalassemia; QC: quality control; VOCs: vaso-occlusive crises.
[a]Patients enrolled in CLIMB THAL-111 received a combination of plerixafor and filgrastim for mobilization, while patients enrolled in CLIMB SCD-121 received plerixafor only. Back-up cells kept at site as a safety measure; [b]Patients will be followed for 24 months after CTX001 infusion with physical exams, laboratory and imaging assessments, and adverse-event evaluations; [c]All patients who receive CTX001 will be followed for 15 years in a long-term follow-up study (NCT04208529) after completion or withdrawal from CLIMB THAL-111 or CLIMB SCD-121.



CRISPR Therapeutics.
Frangoul et al. *NEJM*. 2020.

Engineered T cell therapies

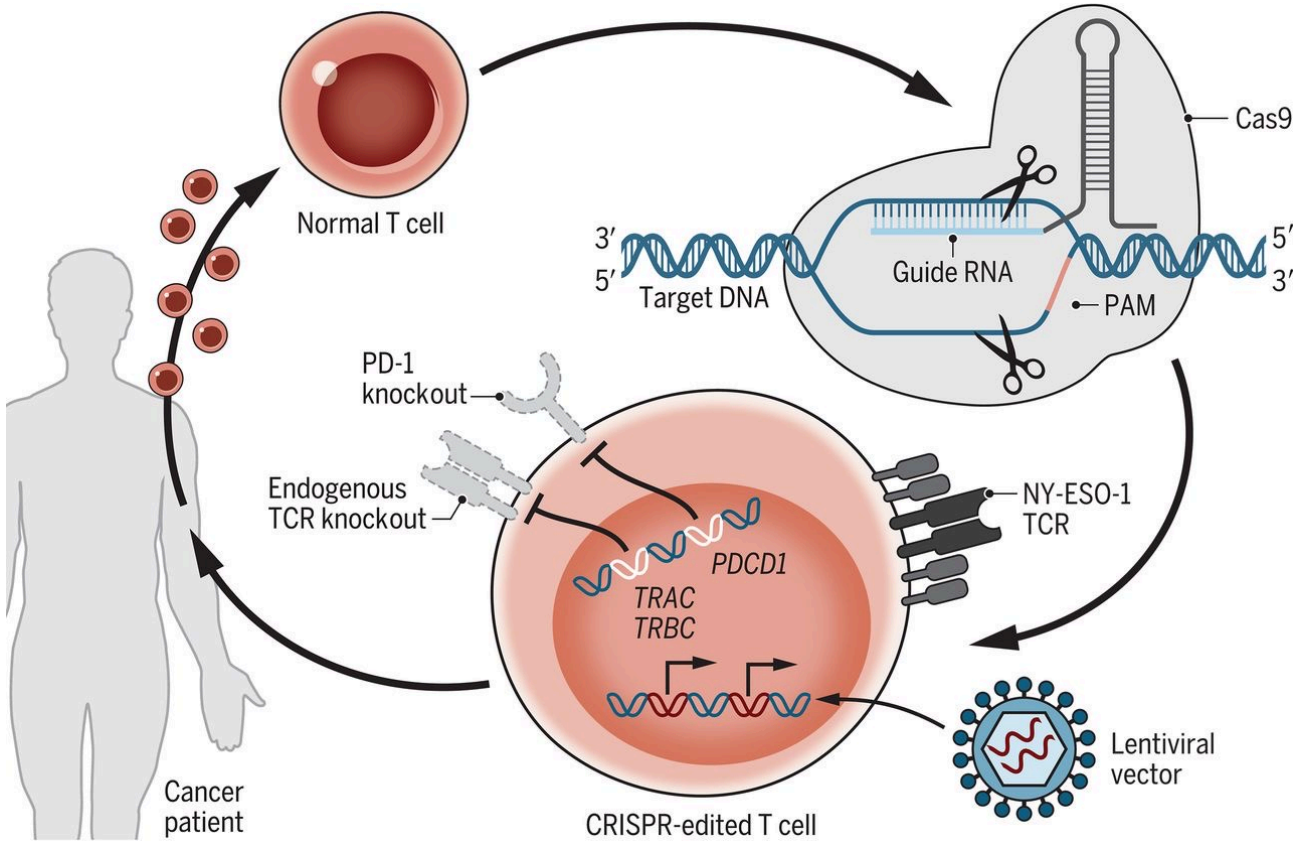
CRISPR-engineered TCR T cells

- Phase 1 study, enrolled 3 patients

- Introduce T cell receptor against NY-ESO-1
- KO endogenous TCR and PD-1

- T cells persisted in the blood > 200 days
- T cells trafficked to the tumor

- No reported CRS
- Stable disease for 2 patients

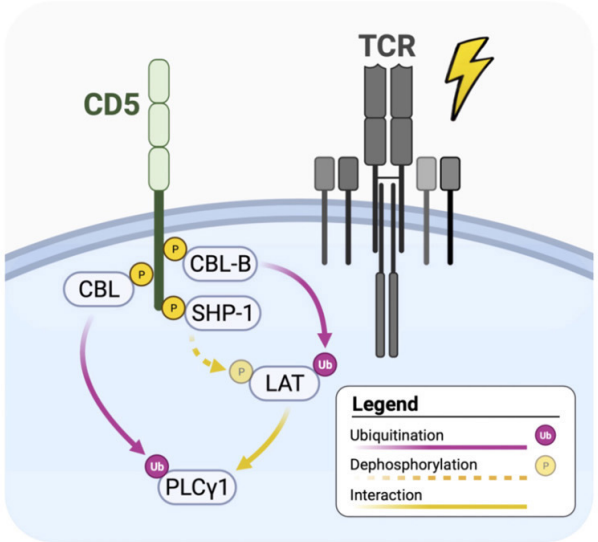


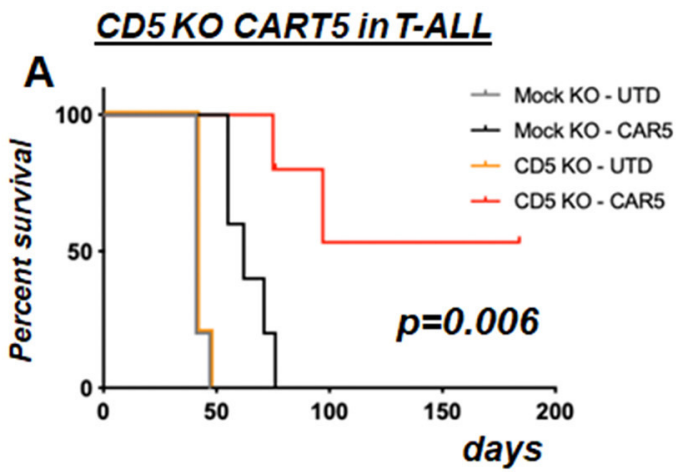
Stadtmauer and Fraietta et al. *Science*. 2020.

Engineered T cell therapies

CRISPR-engineered CAR T cells

- CD5 KO CAR T
  - Improved T cell function and reduced fratricide
- CD7 KO CAR T
  - Phase 1 study for T-ALL
  - KO CD7, CD52, and the TCR
  - 3 patients treated, 2 had d28 molecular remission





CD5 KO CAR T cells improve survival in a T cell leukemia mouse model

Adapted from Chun et al. ASH 2020 and Snook et al. ASH 2023.
Chiesa et al. *NEJM.* 2024.

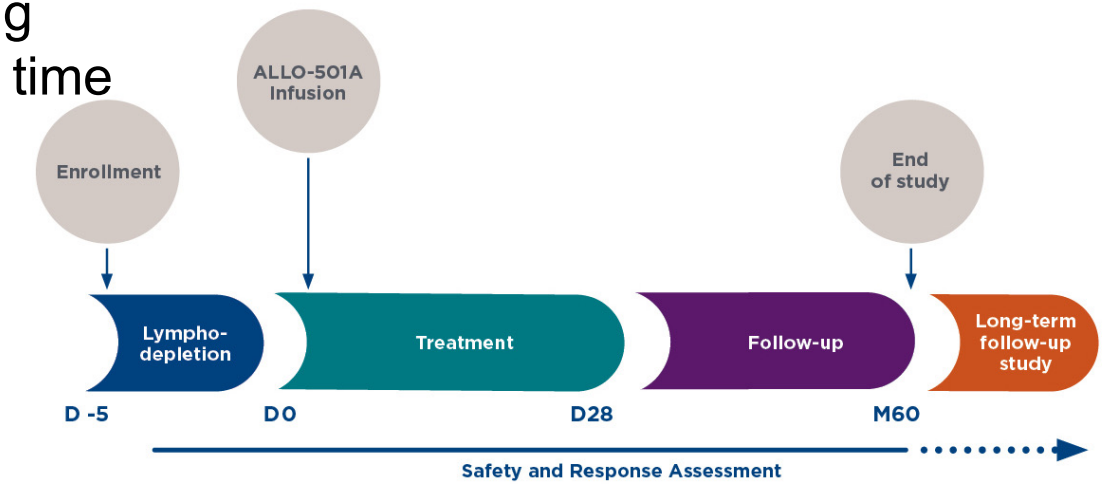
Allogeneic CAR T cells

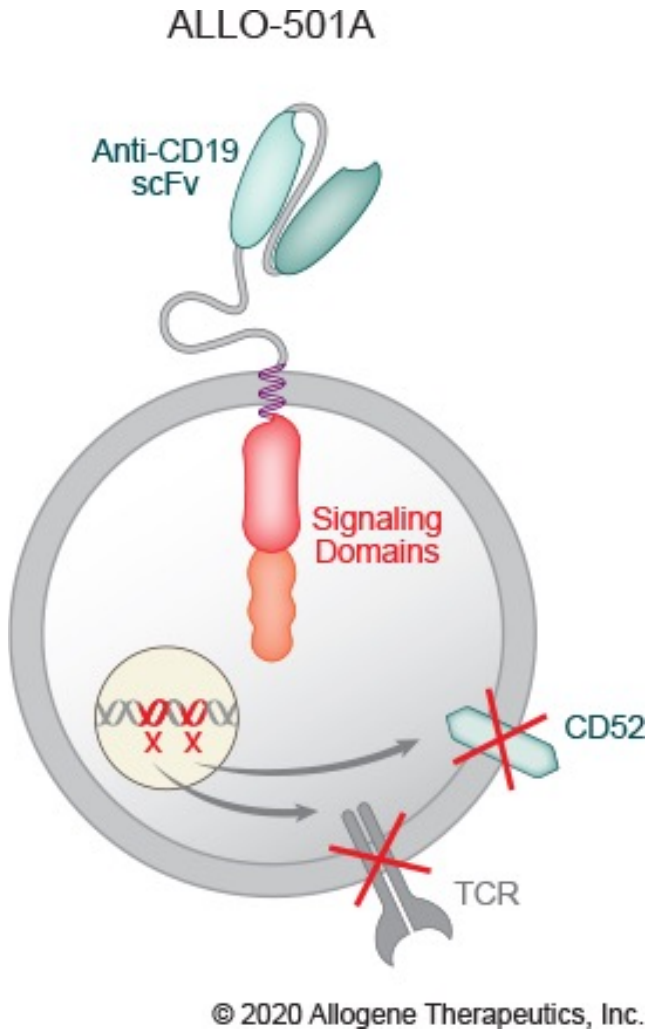
Phase 1 study in LBCL

- CAR-T-naïve population
- 66.7% ORR 58.3% CR
- mDOR 23.1 months
- No reported GvHD

Why is off-the-shelf better?

- Manufacturing
- Brain-to-vein time
- T cell fitness
- Toxicity





Locke et al. ASCO 2023.
Allogene Therapeutics.

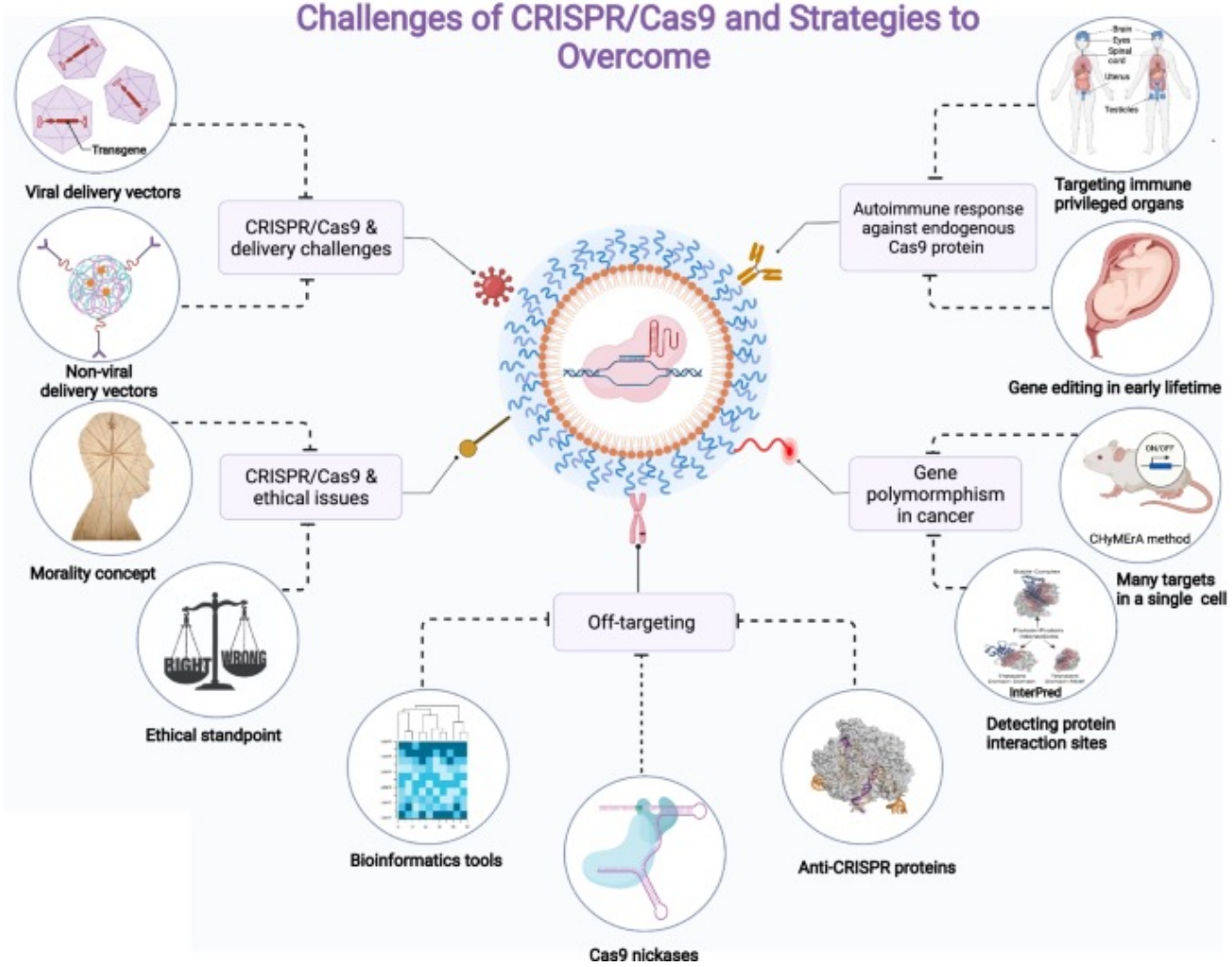
Where we are going

The future of CRISPR-Cas technology

Better DNA edits
- Multiple edits
- More complex edits (point mutations, indels,…)
- Reduced off-target effects

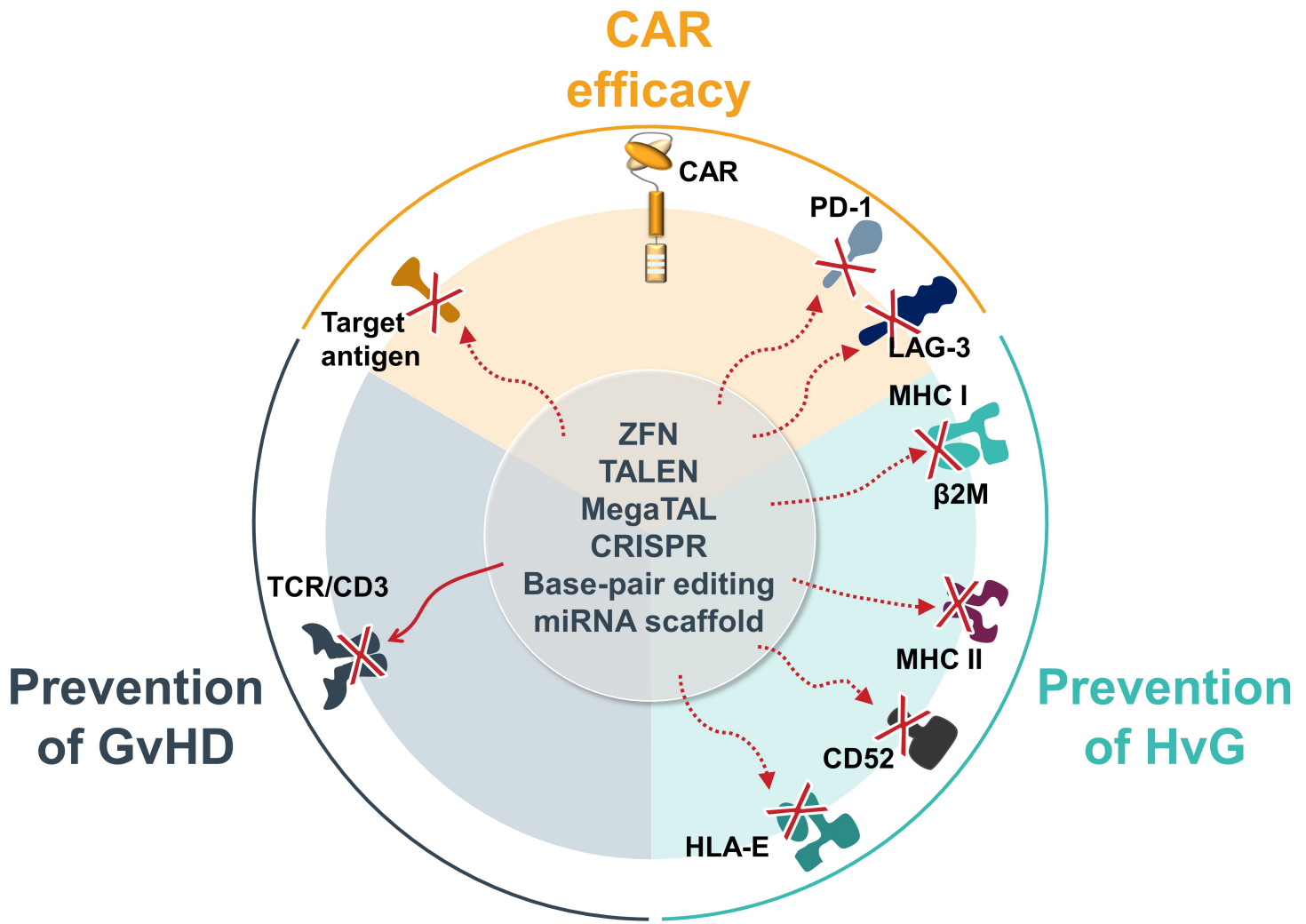
- CRISPR-Cas9 alternatives
  - Prime editing (nickase)

- Modifying RNA instead of DNA



Rasul et al. *Molecular Cancer*. 2022.

Supercharged CAR T cells



Trials to watch

NCT	Trial	Product	Gene editing
NCT04416984	Safety and Efficacy of ALLO-501A Anti-CD19 Allogeneic CAR T Cells in Adults With Relapsed/Refractory Large B Cell Lymphoma, Chronic Lymphocytic Leukemia and Small Lymphocytic Lymphoma (ALPHA2) (ALPHA2)	ALLO-501A	CD52 KO *TRAC* KO
NCT04637763	CRISPR-Edited Allogeneic Anti-CD19 CAR-T Cell Therapy for Relapsed/Refractory B Cell Non-Hodgkin Lymphoma (ANTLER)	CB-010	PD-1 KO *TRAC KO*
NCT05643742	A Safety and Efficacy Study Evaluating CTX112 in Subjects With Relapsed or Refractory B-Cell Malignancies	CTX112	Regnase-1 KO TGFBR2 KO B2M KO *TRAC* KO CD70 KO
NCT04443907	Study of Safety and Efficacy of Genome-edited Hematopoietic Stem and Progenitor Cells in Sickle Cell Disease (SCD)	OTQ923	*HBG1* *HBG2*

Conclusions and outstanding questions

CRISPR-Cas technology and gene editing

- CRISPR-Cas9 is a powerful tool for understanding cancer genetics
- CRISPR screens enable unbiased genome-wide discovery of relevant genes
- *Are new gene editing approaches more efficient with fewer off-target effects?*
- *Can CRISPR-Cas9 alternatives be done at scale?*

Gene editing in the clinic

- Allogeneic CAR T cells are a promising alternative to autologous CAR T cells
- Gene editing can be used to improve T cell therapies
  - *Can we engineer CAR T cells that work for more patients?*
  - *Even patients with solid tumors?*
- *Do CAR T cells for T cell lymphoma need to be edited?*
  - CD5-directed CAR T cells in Snook et al. ASH 2023. and LaQuisa et al. *Blood*. 2024
- <u>Long-term follow-up of gene-edited products in humans is needed</u>
  - Monitoring for clonal hematopoiesis, MDS, or AML after gene therapy
  - T cell lymphomas after CAR T cell therapy

Frangoul et al. *NEJM*. 2020; Sharma et al. *NEJM*. 2024.
Ghilardi et al. *Nature Medicine.* 2024; Elsallab et al. *Blood*. 2024; Harrison et al. ASH 2023.