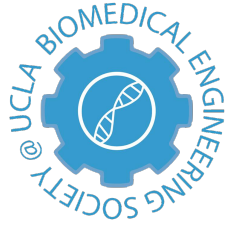
Journal Club 2: Efficient Gene Editing in T Cells

BMES Cell Team

Fall 2020



Outline

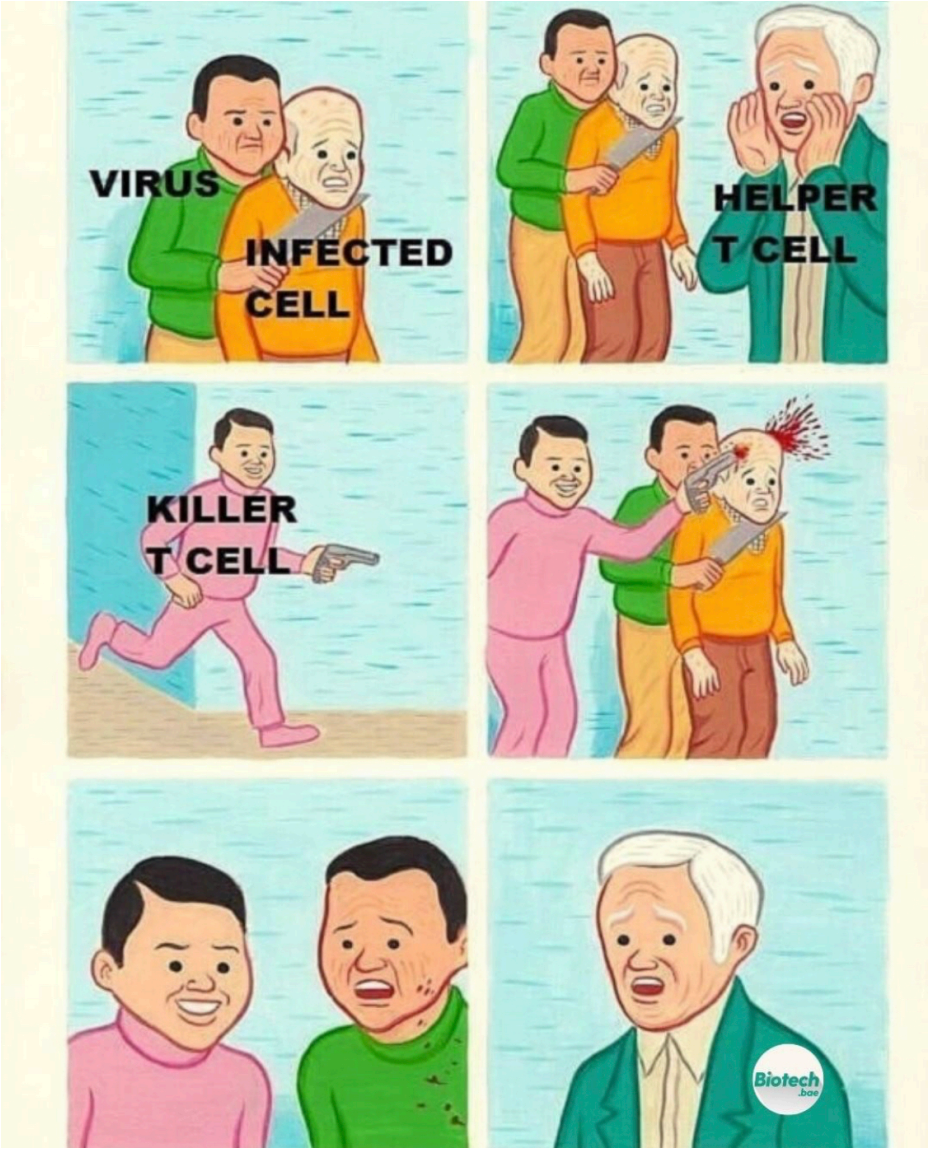
- Background information on T Cells
- Article Discussion
- Reminders and Announcements

Background Information on T Cells

- **Definition:** T Cells are part of the body's immune system. They are especially helpful in eliciting a response to fight infections.

- There are two types of T Cells
  - **Helper T cells** communicate with other cells of the immune system by releasing cytokines
  - **Cytotoxic T cells** kill infected cells; they are activated by cytokines secreted by helper T cells

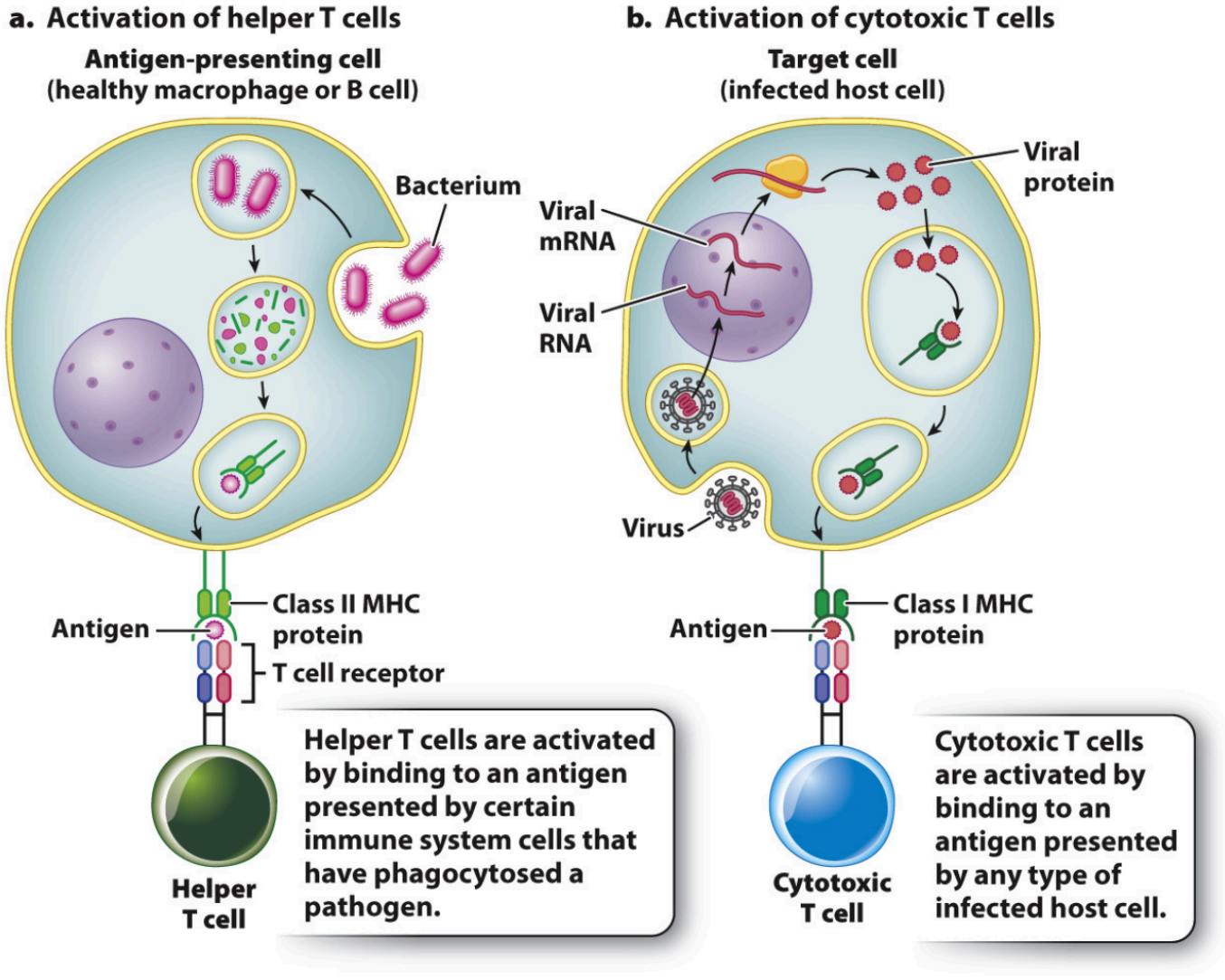
Background Information on T Cells



Background Information on T Cells

- There are different two different **glycoproteins** on T cells
    - **Helper T cells** contain **CD4**
    - **Cytotoxic T cells** contain **CD8**, which is a more powerful glycoprotein
- T cells originate from stem cells in bone marrow
- A **mature** T cell will contain T cell receptors (TCR) on its membrane
    - Once a specific receptor binds to a target antigen, it will trigger the T cell to divide into clones
    - This results in a pool of T Cells that are specific for a particular antigen

Background Information on T Cells



Source: *Biology - How Life Works* (2e)

Background Information on T Cells

Summary Table

	Helper T Cells	Cytotoxic T Cells
Functions	Activates other immune cells by secreting cytokines	Kills infected cells
Surface Molecule	CD4	CD8
MHC Protein	Class II	Class I

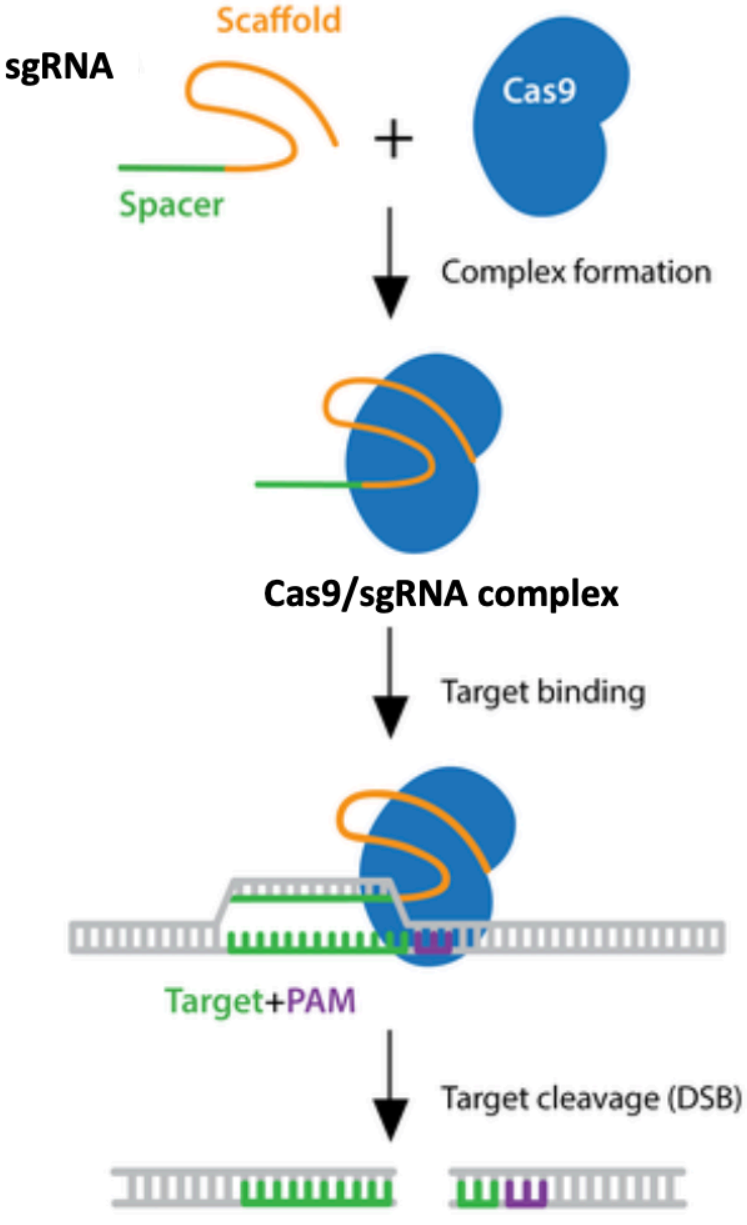
Article Discussion

- There are several genetic editing techniques mentioned in the article, but the most prominent is CRISPR/Cas9

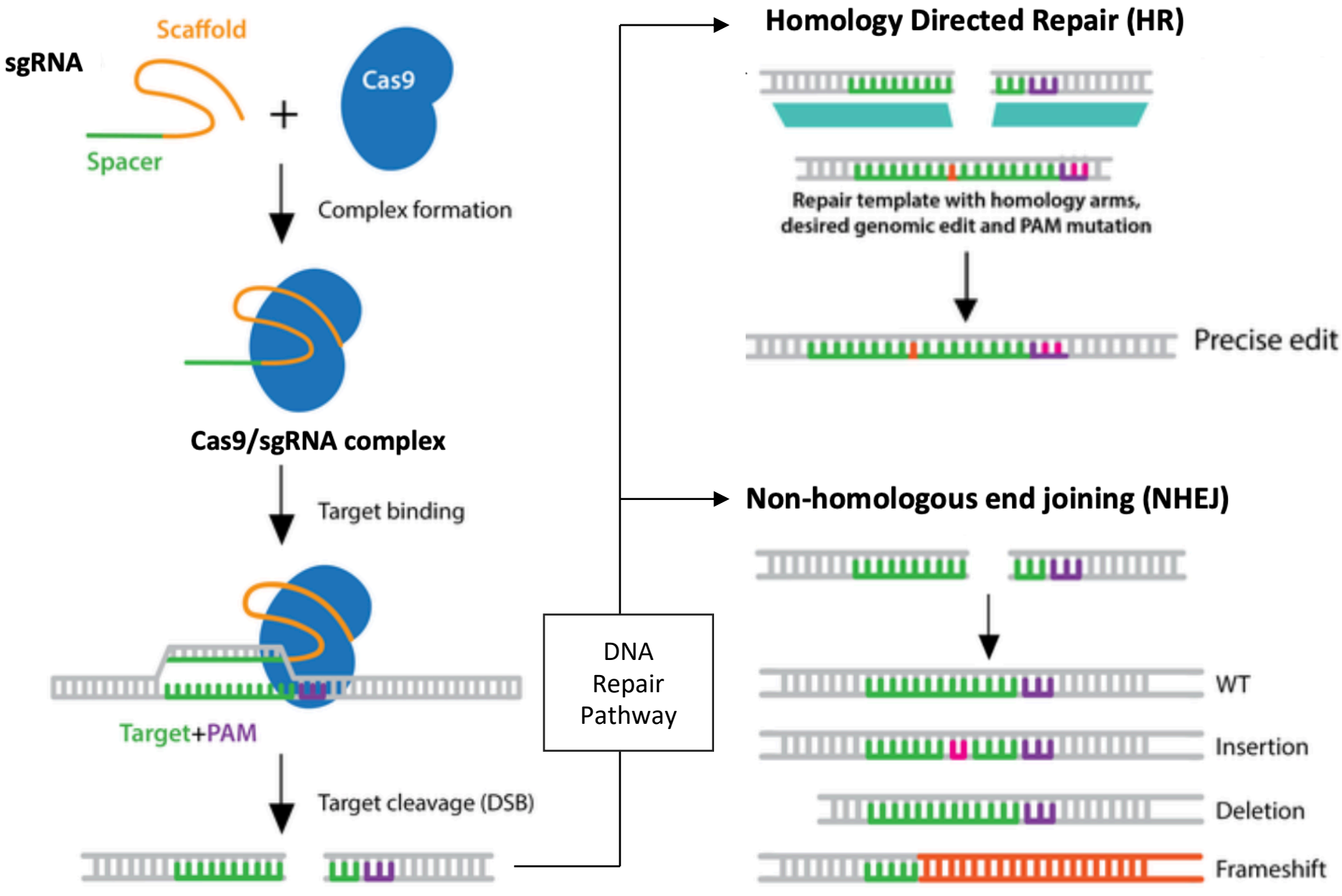
Emmanuelle Charpentier
Max Planck Institute

Jennifer Doudna
UC Berkeley

How does CRISPR/Cas9 work in the lab?



How does CRISPR/Cas9 work in the lab?



How does CRISPR/Cas9 work in the lab?

As shown, the two repair mechanisms for CRISPR are HR and NHEJ:

- **Homology Repair**
    - Used for CRISPR experiments that require extreme precision
- **Non-Homologous End Joining**
    - Typically introduces mutations within genetic material
    - Also known as “sloppy repair”

Figure Analysis

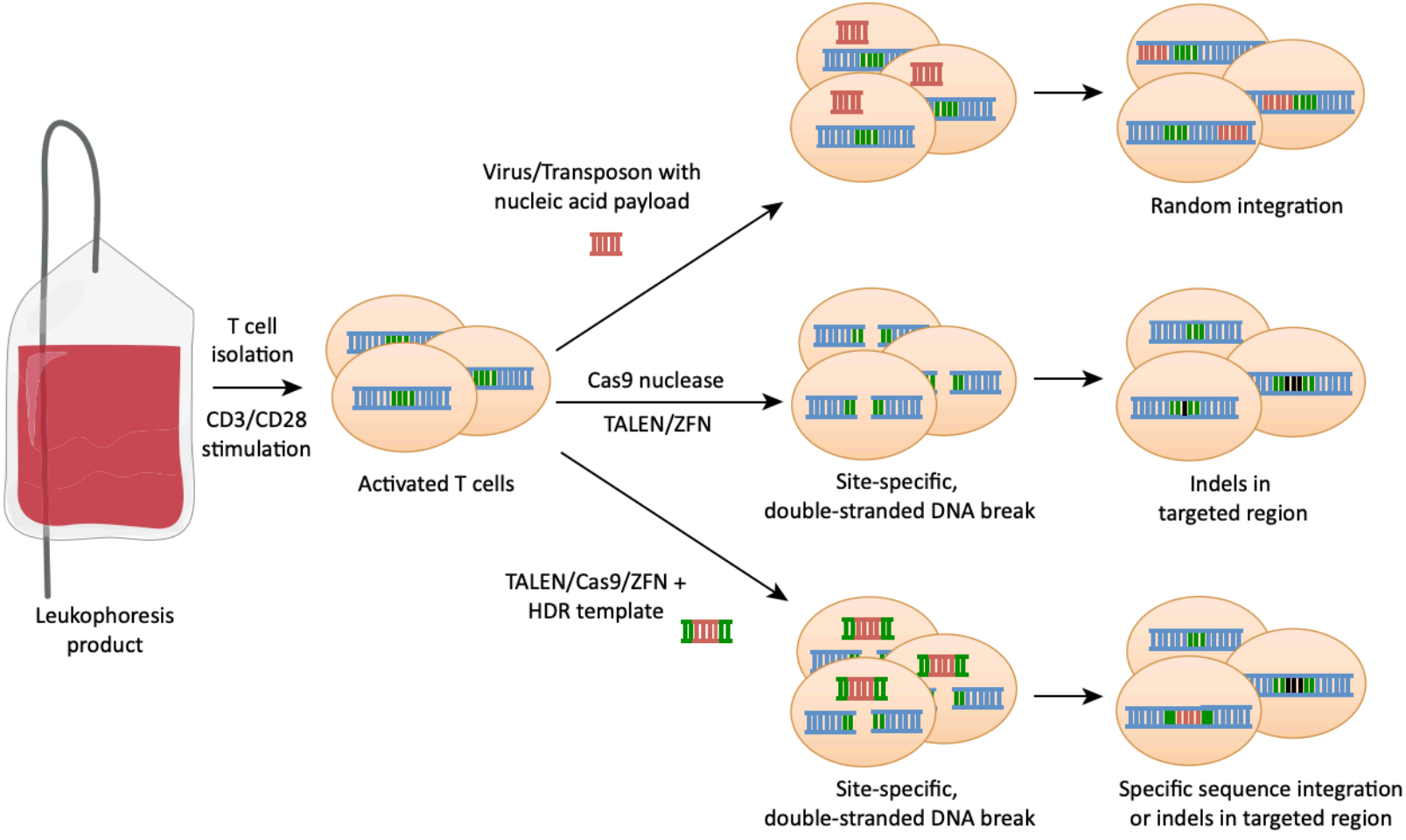


Figure 1. Primary Human T Cells Can be Genetically Modified to Introduce Exogenous Sequences or Disrupt Endogenous Genes. T cells isolated from donor blood are activated through CD3/CD28 stimulation. Activated T cells can be transduced with lenti- or retroviruses or electroporated with Sleeping Beauty transposon system components to achieve non-site-specific integration of transgenic constructs. Alternatively, activated T cells can be electroporated with DNA, mRNA, or proteins encoding Cas9 nuclease, transcription effector nuclease (TALEN), or zinc-finger nuclease (ZFN) to achieve site-specific gene disruption. Finally, Cas9, TALEN, and ZFN can be co-delivered with homology-directed repair (HDR) templates to site-specifically integrate transgenic constructs. None of the editing methods achieves 100% efficiency, thus some cells will remain unedited. In the case of genome editing with HDR templates, a fraction of the cells will integrate the desired exogenous sequences while others will experience insertions/deletions (indels) in the targeted region without integration of the desired sequence.

Article Discussion

- Define the following terms or acronyms:
    - CAR
    - NHEJ
    - HR (or HDR)
    - sgRNA
    - Transposon
    - Exogeneous DNA
    - CRISPR
    - Cas9
    - Indels
- What is the main idea of the article?
- What did you find interesting about the article?

Article Discussion

- What other applications of CRISPR/Cas9 could you think of?
- Would you use HR or NHEJ for the following experiments and why?
    - Engineering CAR T cells so that they can be more efficient in fighting cancer
    - You want to disable the effectiveness of a bacteriophage by modifying its genes
    - Developing an mRNA-based vaccine to simulate the immune system
    - Introducing a gene to enhance the growth rate of plants (GMOs)