Engineering Human Hematopoietic Stem Cells to Encode the HIV Broadly Neutralizing Antibody VRC01

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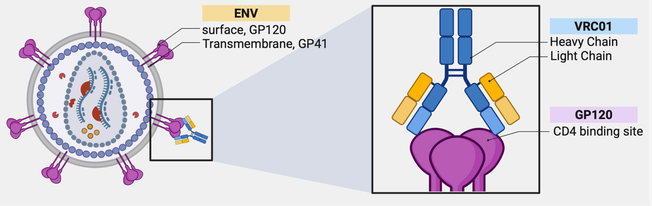
Background - *What is VRC01?*

- **HIV (Human Immunodeficiency Virus)** is known for its high mutation rate, evading the binding of typical antibodies produced by the adaptive immune system.[1]
- **Broadly neutralizing antibodies (bnAbs)** are specialized antibodies that target and neutralize viruses like HIV by binding to conserved regions of the viral surface.[2] Specifically, the bnAb **VRC01** has been shown to reduce viral load when administered intravenously.[2]

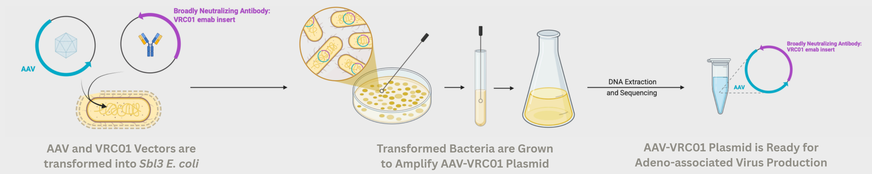
HIV Virion



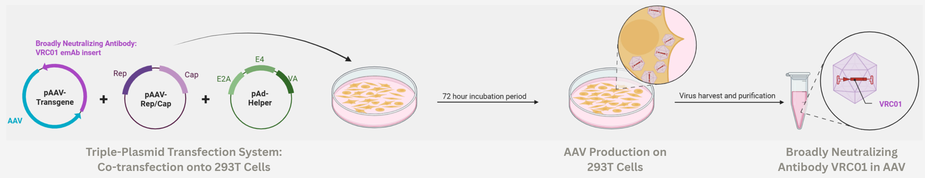
- Intravenous administration of VRC01 has demonstrated high safety, tolerability, and peak serum concentrations[2], but due to its short half-life, doses must be administered regularly over the lifetime of patients.[2]
- **VRC01-edited Hematopoietic Stem Cells (HSCs)** have the potential to negate this issue by integrating this key bnAb into autologous cells. These cells can later mature into VRC01-presenting B cells or other hematopoietic cells.[3]
- Gene editing of VRC01 into human Hematopoietic Stem Cells using **CRISPR-Cas9 technology** and an **Adeno-Associated Virus (AAV)** delivery system may mitigate this limitation.

Methods - *from antibody DNA to edited stem cells*

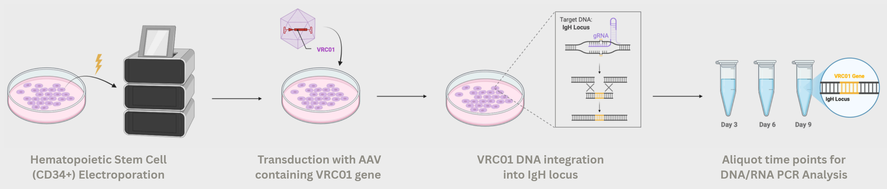
1 Cloning AAV-VRC01 Plasmids



2 Adeno-Associated Virus Production



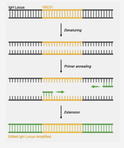
3 VRC01 Integration into Hematopoietic Stem Cells



4 PCR Analysis of VRC01 Insertion

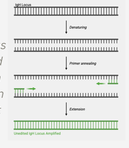
VRC01 in-out PCR
Edited Target:

With one primer targeting the VRC01 sequence and another the IgH locus, amplification of edited HSCs in the correct locus occurs.



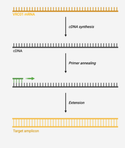
IgH Locus
Edited vs Unedited Target:

Amplification of the IgH locus reveals the number of edited and unedited loci, which can then be analyzed for insetion through sequence alignment



VRC01 mRNA PCR
Edited Target Expression:

Levels of VRC01 mRNA in the edited HSCs reflect the amount of active VRC01 expression, confirming VRC01 insertion and functional transcription.



Results

1 Cloning Flow Diagram

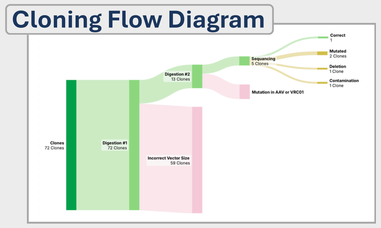


Figure 1. AAV-VRC01 plasmid cloning workflow.
Individual bacterial clones (n = 72) were screened to identify the correct AAV-VRC01 construct for AAV production using gel electrophoresis and DNA sequencing. Of the 72 clones analyzed, 70 contained sequence variations relative to the intended AAV-VRC01 construct.

2

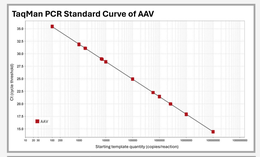


Figure 2. Standard curve used to quantify AAV Production
Serial standards were analyzed to generate a calibration curve (R^2=1.00) for estimating the concentration of the AAV Produced.

Table 1. Quantification of the AAV containing VRC01 preparation.
AAV estimation is calculated from the average of two dilution factors estimated from the standard curve

AAV Dilution	Concentration(AAV Copies/ml)
1:20	5.71E+11
1:100	6.38E+11
Average	6.05E+11

3 Pilot Experiment

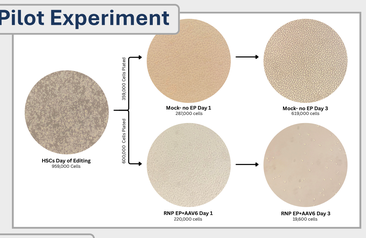


Figure 3. Pilot experiment of human CD34+ hematopoietic stem cells following gene-editing treatment.
Microscopy images showing mock-treated and experimental cultures before editing (day 0) and on days 1 and 3 post-editing. Live cells were counted at each time point to assess post-treatment recovery and growth.

Experiment 1

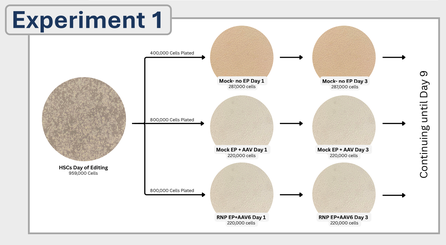
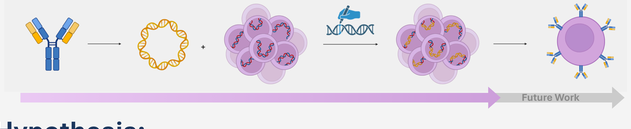


Figure 4. Human CD34+ hematopoietic stem cells following gene-editing treatment.
Microscopy images of 3 million total cells, comparing mock-treated and experimental cultures before editing (day 0) and on days 1 and 3 post-editing. Cells will continue to be evaluated until day 9 post-editing.

Objective

Determine the efficacy of inserting the bnAb VRC01 into primary human HSCs with CRISPR-Cas9 gene editing and an AAV delivery system



Hypothesis:

CRISPR-Cas9 editing with an AAV can successfully integrate the VRC01 broadly neutralizing antibody gene into the IgH locus of human CD34+ hematopoietic stem cells, enabling subsequent VRC01 expression following B cell differentiation.

Conclusions

- AAV-VRC01 plasmid was successfully developed and amplified in *Sbl3 E. coli* and verified via sequencing
- AAV carrying the VRC01 gene was produced and quantified at approximately 6.05e11 vg/mL.
- Primary human CD34+ HSCs remained viable until day 3 post-editing during preliminary editing experiments.
- Targeted integration and VRC01 expression have not yet been confirmed. PCR and downstream expression assays are in progress.

Future Directions

- PCR results in the near future will reflect the integration of VRC01
- **Stem Cell Maturation:** Edited stem cell lineages should be evaluated to identify which hematopoietic populations express VRC01.
- **In vivo editing:** Virus-like particles could enable targeted delivery of gene-editing tools directly to stem cells in vivo.

Acknowledgments

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