Product of HIV co-receptor disrupted CD4+ T lymphocytes by using direct delivery of CRISPR/Cas9-Ribonucleoprotein

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Introduction

• Human immunodeficiency virus (HIV) infection causes a marked immune defect due to destruction of CD4+ T lymphocytes.

• HIV infects CD4+ T cells through binding of CD4 molecules and HIV co-receptors (i.e., chemokine receptors such as CCR5 and CXCR4).

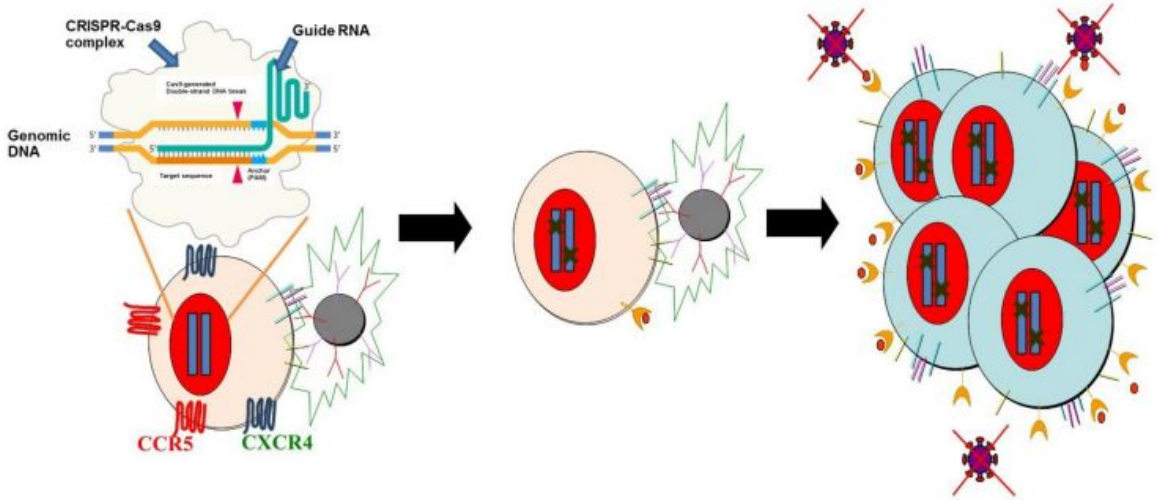
Anti-retroviral therapy (ART) is effective in lowering a circulating viral load to low or undetectable level and increasing a CD4+ T cell number.

• Although ART is the most effective treatment for HIV infection, it requires life long treatment .

• Sustained HIV remission after ceasing ART was achieved in patients who were treated with stem cell transplants from donors carrying CCR5O32 mutation that prevented expression of HIV co-receptor CCR5.

• An autologous transfusion of HIV co-receptor disrupted CD4+ T cells may provide an alternative treatment leading to repopulation of HIV resistant CD4+ T cells in HIV infected patients.

HIV co-receptor gene disruption by using CRISPR/Cas9 gene editing



- Single guide RNA (sgRNA) targets CCR5 and CXCR4 gene segments while Cas9 protein creates a double strand break. The occurrence of random base insertion and deletion during DNArepairing can induce frameshift mutations causing a functional knock out of the gene.

- Down-regulation of CCR5 and CXCR4 on the cell surface prevents HIV binding. Gene edited CD4+ T cells can be expanded by using anti-CD3/28 coated beads.

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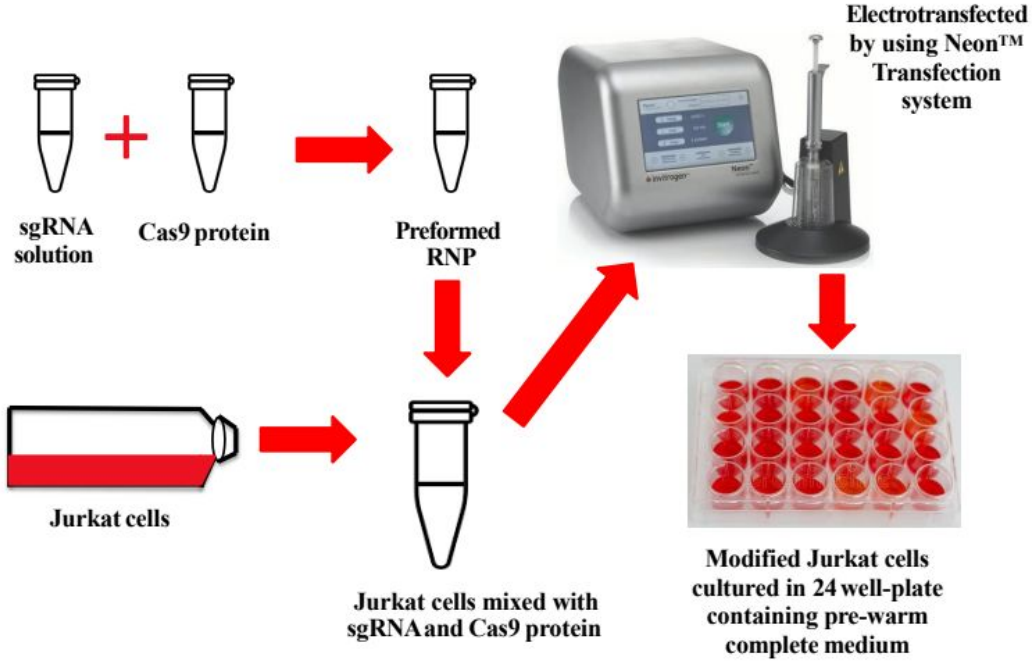
Our Objective

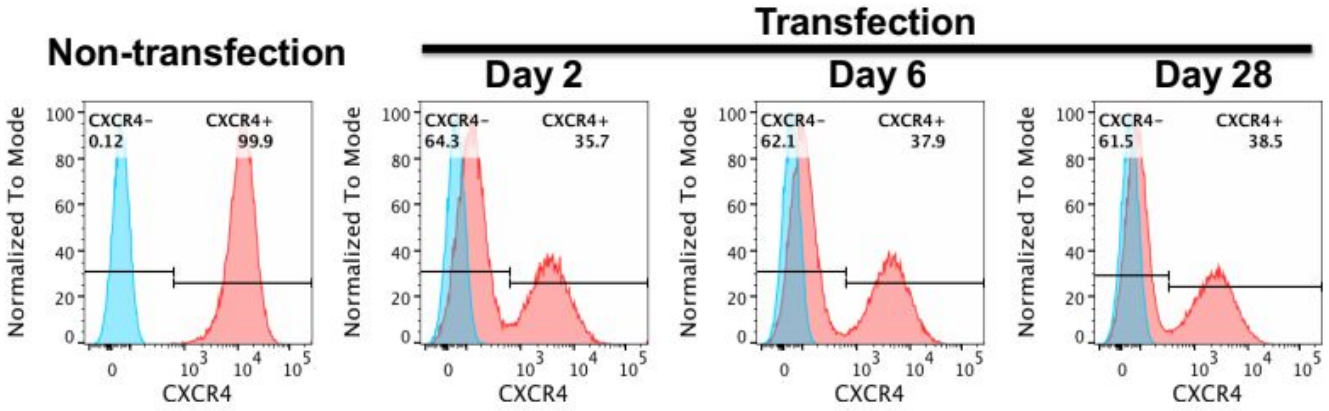
- CRISPR/Cas9 gene editing was used to create HIV co-receptor disrupted CD4+ Tcells.

- Direct delivery of Cas9 ribonucleoprotein (Cas9 RNP) complexes for CCR5 and CXCR4 gene disruption was evaluated.

Cas9 RNP transfection



Flow cytometric analyses of CXCR4 expression in Cas9 RNP transfected Jurkat cells



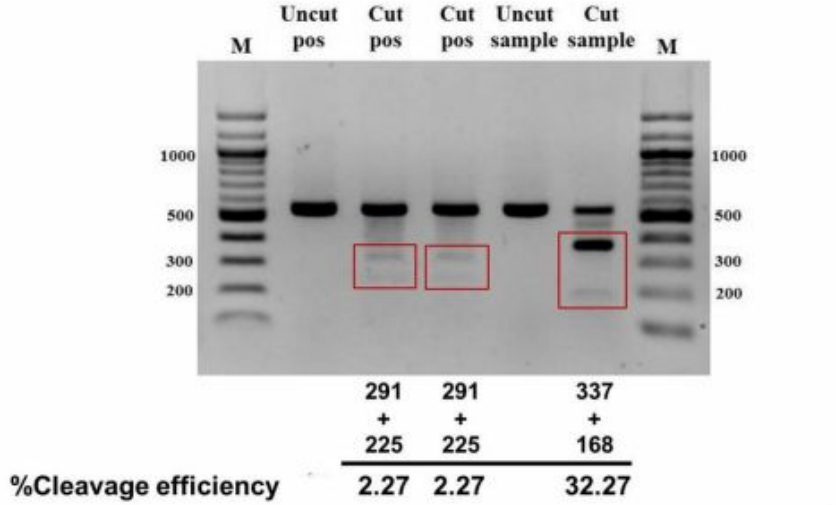
• Comparison between non-transfected cells (Non-transfection) and transfected cells (on days 2, 6 and 28 after transfection) is demonstrated in overlaid histograms. Blue color indicates the isotype control whereas red color indicates CXCR4 staining. Most of non-transfected cells expressed CXCR4 molecules(99.9%).

• In contrast, percentages of transfected Jurkat cells expressing CXCR4 were similarly reduced to an average value of 37.4 ± 1.5% (mean ± SD) throughout the observation period.

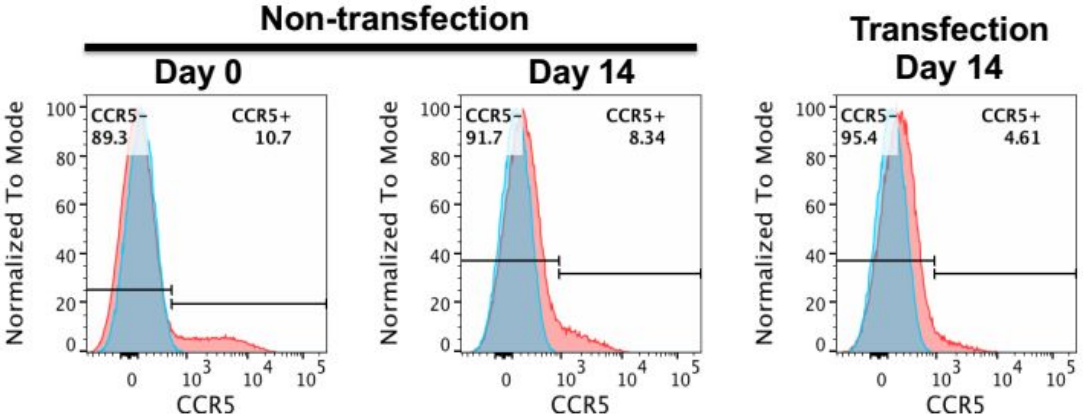
Genomic cleavage analysis of CXCR4

- A representative result compared between a positive control sample (pos) and transfected Jurkat cells (sample) with T7 endonuclease I (T7E1) digestion (Cut) and non-digestive control (Uncut). Red boxes and numbers at the bottom indicate the predicted cut sizes.

- The analysis was used to determine a specific site in genome where gene editing occurs. A mismatch DNA duplex was created from denaturation and hybridization of the wild-type and mutant sequences which were able to be digested by T7E1. The result shows 2 predicted cleaved bands at 337 and 168 base pairs with a percentage of cleavage efficiency at 32.27%.



Flow cytometric analysis of CCR5 expressionin Cas9 RNP transfected primary CD4+ T cells



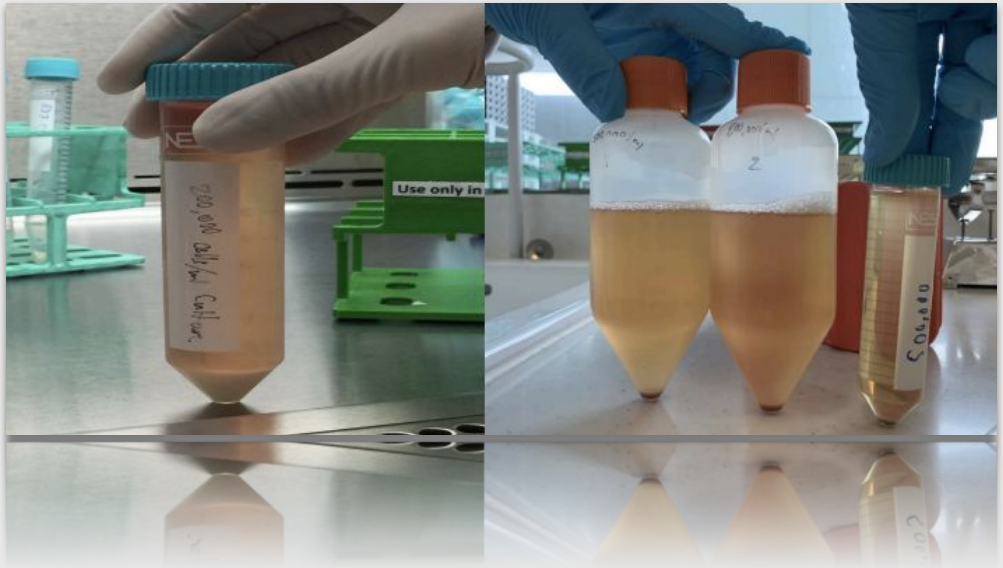
- Comparison between non-transfected cells (Non-transfection) and transfected cells (on day 14 after transfection) is demonstrated in overlaid histograms. Blue color indicates the isotype control whereas red color indicates CCR5 staining.

- Approximately 10% of non-transfected cells expressed CCR5 whereas transfected cells reduced CCR5 expression by half after 14 days of cell expansion with anti-CD3/28 coated beads.

Conclusion

- This study demonstrated a disruption of CCR5 and CXCR4 genes by direct delivery of preformed Cas9 ribonucleoprotein complex using electroporation.
- Disruption of CCR5 gene expression was successfully achieved in human primary CD4+ T lymphocytes.
- More importantly, these gene edited cells can be in vitro expanded suggesting a capability for a large scale production to use in autologous transfusion.

Breakthrough Cell Culture Protocol for CD4 T Cell Expansion.



With new protocol, we can get 4000-fold expansion.
In this experiment, starting from 1 million CD4:

- Old Method : 384,000,000 Viable Cells
- New Method : 3,936,000,000 Viable Cells

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