



## Developing Resistance against HIV by Modifying the CCR5 receptor

Deepali Gangrade\*, Kalpita Nadkar

Vivekanand Education Society's College of Pharmacy, Chembur(E), Mumbai, India.

\*Corresponding author's E-mail: [gangrade.deepali@gmail.com](mailto:gangrade.deepali@gmail.com)

Accepted on: 26-08-2014; Finalized on: 31-10-2014.

### ABSTRACT

The data published by WHO for HIV rates is alarming. Today, people living with HIV are 34 million, newly infected are 2.5 million and total deaths are 1.7 million per year globally. Highly active antiretroviral therapy (HAART) for HIV infection results in restored immune function improved quality of life, the near normalization of expected lifespan, indefinite viral suppression and reduced viral transmission. However the drawback is that it does not eliminate viral reservoirs, and needs lifelong adherence to expensive regimens that have short-term and long-term toxic effects. Combination HAART basically comprises of reverse transcriptase inhibitors, integrase inhibitors, protease inhibitors. Despite virus control using HAART's, HIV-associated complications, including a higher than normal risk of cardiovascular disease, cancer, osteoporosis, and other end-organ diseases continue to persist. This increased risk might be due to the toxic effects of treatment or the consequences of persistent inflammation and immune dysfunction associated with HIV. Therefore novel treatment approaches that eliminate the HIV and thereby improve the immune system are needed. Inhibition or mutation of the CCR5 coreceptor using various techniques seems to be a promising approach to eradicate the virus.

**Keywords:** HIV, AIDS, CCR5 coreceptor, CD4 receptor, gene therapy, zinc finger nuclease.

### INTRODUCTION

The gp120 protein of the HIV has to bind the CD4 receptor as well as the coreceptor CCR5 or CXCR4. Inhibition using chemokine inhibitor drugs or mutation that is genetic modification that makes CCR5 resistant to HIV or allogeneic stem cell transplantation from an individual having a HIV resistant CCR5-Δ32 gene of these coreceptors can help to prevent or cure HIV/AIDS.

### Lifecycle of HIV

(1) HIV attaches the target cell. The surface glycoproteins (gp120 and gp41) of HIV must bind to a CD4 receptor and a chemokine receptor (CCR5 or CXCR4) on the host cell surface to penetrate the target cell (Fig.2). CCR5 is the major co-receptor for viral entry of HIV into macrophages. CXCR4 is the entry co-receptor for those HIV strains that have an affinity for T cells.

(2) The HIV releases its enzymes and RNA genome into the infected cell. (Fig. 2, 1)

(3) The viral RNA is converted to DNA, using the viral reverse transcriptase enzyme. (Fig. 1)

(4) The viral DNA (provirus) enters the nucleus of the host cell. The viral DNA (provirus) becomes integrated into the host cell's DNA using the viral integrase enzyme. (Fig. 1)

(5) The DNA of the infected cell now produces RNA as well as proteins that are required to assemble a new HIV virion. New viral RNA is used both as genomic RNA and as a template for the production of viral proteins. (Fig. 1)

(6) A new virus is assembled from RNA and short pieces of viral protein. The immature virus particle buds through the membrane of the host cell, enveloping itself in a fragment of the cell membrane of the host cell and it then pinches off from the infected cell (Fig. 1).

(7) A large number of viruses are liberated from an infected cell. The HIV replication cycle generates 1,010–1,012 virions every day.<sup>2</sup>

(8) The newly formed virus can infect other host cells only after it matures. HIV matures when a viral enzyme (HIV

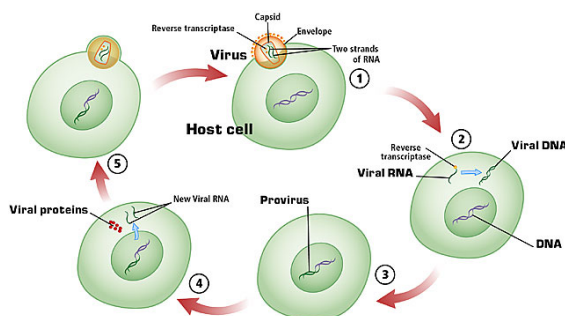


Figure 1: Lifecycle of HIV

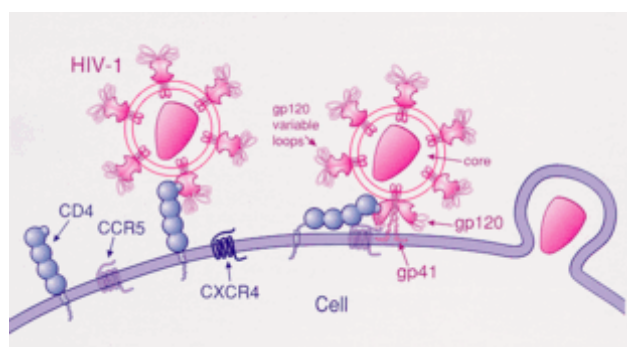


Figure 2: HIV interaction with receptors



protease) cleaves and releases structural proteins within the virus.<sup>1</sup>(Fig. 1)

### Importance of targeting the CCR5 receptor

The gp120 protein present on HIV binds to the CD4 receptor along with CCR5 or CXCR4 receptor. If the coreceptors are absent or mutated, the HIV will not be able to replicate. Thus, deletion or mutation of these coreceptors has become a target for both prevention as well as cure.

The CCR5 gene that encodes the CCR5 protein is located on the short (p) arm at position 21 on chromosome 3. A few individuals carry a mutation known as CCR5-Δ32 in the CCR5 gene, protecting them against certain strains of HIV. Some people have inherited the Δ 32 mutation resulting in the genetic deletion of a portion of the CCR5 gene. Homozygous carriers of this mutation are resistant to M-tropic strains of HIV-1 infection.<sup>3-8</sup>

This hypothesis was tested in an AIDS patient who had developed leukemia. He was treated with chemotherapy to suppress the cancer. A bone marrow transplant containing stem cells from a matched donor was then used to restore the immune system. However, the transplant was performed from a donor with 2 copies of CCR5-Δ32 mutation gene. Before the transplant, low levels of HIV X4, which does not use the CCR5 receptor, were also detected. After 600 days, the patient was healthy and had undetectable levels of HIV in the blood.<sup>9,4</sup> Following the transplant, HIVX4 type of HIV was not detected either, further baffling doctors.<sup>4</sup> However it was found that cells expressing the CCR5-Δ32 variant protein lack both the CCR5 and CXCR4 receptors on their surfaces. Thus it conferred resistance to a broad range of HIV could including HIV X4.<sup>10</sup> The patient is now pronounced cured of the HIV infection after a period of 6 years.

Enrollment of HIV-positive patients in a clinical trial was started in 2009 in which the patient's cells were genetically modified with a zinc finger nuclease to carry the CCR5-Δ32 trait and then reintroduced into the body as a potential HIV treatment. Results reported in 2014 were promising. This was done due to the fact that ARTs need lifelong adherence.<sup>11</sup>

### Gene therapy

Allogeneic transplantation with stem cells from very rare donors who are naturally resistant to HIV is not a feasible strategy; therefore, much interest is now focused on gene therapy to delete the virus from infected cells or to produce cells resistant to HIV infection. Three classes of DNA-editing enzymes are being studied for safety and efficacy to target HIV coreceptors and pro-viral sequences: zinc-finger nucleases, transcription activator-like effector nucleases, and homing endonucleases.<sup>12,13</sup> The targeted gene-therapy approach of blocking CCR5. CCR5-zinc-finger nuclease knockout T cells are in phase 1

clinical trial ex vivo investigation.<sup>14</sup> Phase 1 studies have shown that these approaches are safe and feasible.

HIV viral proteins are targeted by other gene treatments with either an anti-HIV ribozyme or antisense RNA oligonucleotide constructs. It was observed that they safely and feasibly reduce the viral load in phase 1 and phase 2a clinical trials.<sup>15</sup> Additionally, CD4 aptamer-CCR5-siRNA chimeras have proven to be safe and efficacious in the humanised mouse model.<sup>16</sup> Interventions that interfere with pre-integration steps in the viral-life cycle are promising, and are being assessed in phase 1 trials.<sup>17</sup> The safety and efficacy of gene delivery to specific cells and tissues, and access to these treatments, are major challenges for these approaches in the eradication of HIV infection.<sup>28</sup>

### Zinc finger nucleases

CCR5 is the major co receptor for the HIV. Using zinc finger nuclease, gene editing was done that is the infusion of autologous CD4 T cells in which the CCR5 gene was rendered permanently dysfunctional. A single dose of ZFN-modified autologous CD4 T cells. It was tested on patients who had chronic aviremic HIV infection while they were receiving highly active antiretroviral therapy. The primary outcome was safety as assessed by treatment-related adverse events. Secondary outcomes included measures of immune reconstitution and HIV resistance.

During treatment interruption and the resultant viremia, the decline in circulating CCR5-modified cells was significantly less than the decline in unmodified cells. HIV RNA became undetectable in one of four patients who could be evaluated. The blood level of HIV DNA decreased in most patients.

Conclusions CCR5-modified autologous CD4 T-cell infusions are safe within the limits of this study.<sup>19</sup>

### Silencing genes

CCR5 is one of the two coreceptors that are utilized by the HIV to enter the cell. CCR5Δ32 a deletion variant of the CCR5 gene, has been found in the Caucasian population and codes for a nonfunctional protein. Homozygous carriers of CCR5Δ32 are resistant to infection with the R5tropic virus, and heterozygosity for the variant correlates with a slower disease progression in HIV infected patients, while no pathology associated with the CCR5 mutation has been observed in its carriers. The findings provide a basis for developing drugs to inhibit CCR5, in particular using gene therapy.

The efficacy and safety of CCR5 gene silencing has been demonstrated upon bone marrow transplantation from a CCR5Δ32/Δ32 donor to a HIV infected patient. The patient did not display any clinical or virological evidence of HIV infection for more than 3.5 years after the transplantation had been performed and highly active antiretroviral therapy terminated. This first case of a documented cure of the disease has given new impetus



to developing methods of HIV treatment based on the silencing of the CCR5 gene. Various technologies have been utilized over the past years to inhibit the CCR5 expression at various steps from the gene to the protein. In particular, the technologies include RNA interference, mRNA cleavage with ribozymes, CCR5 neutralization with intracellular antibodies, and gene inactivation via deletions introduced using sitespecific nucleases.<sup>23</sup>

Short interfering RNAs (siRNAs) provide one of the most efficient tools for gene silencing. Stable siRNA synthesis in the cell can be achieved via integration into the chromosome and subsequent expression of short hairpin RNAs (shRNAs) whose processing yields siRNAs. Expression of shRNAs directed to CCR5 was shown to substantially decrease the CCR5 amount on the cell surface and to confer HIV resistance on cells. Gene silencing requires high level shRNA expression, which can be achieved with RNA polymerase III promoters. However, an excess of shRNAs or their precursors is toxic for the cell. The cytotoxicity possibly occurs because protein complexes involved in RNA interference are saturated and because shRNAs compete with endogenous miRNAs, distorting the regulation of genes. A decrease in shRNA expression level, for instance, by using a weak promoter alleviates the toxicity, but the efficiency of inhibition also decreases in this case.<sup>20</sup>

## CONCLUSION

In addition to ART there are a lot of techniques by which the prognosis can be significantly altered by newer approaches.

Allogeneic transplantation with stem cells from very rare donors who are naturally resistant to HIV is one way to eradicate the virus from the human body over a period of time. But humans having the CCR5-Δ32 genetic mutation are very rare and therefore are not always a feasible strategy.

By using zinc finger nuclease, our strategy is to repopulate the immune system with CCR5-deficient central memory T lymphocytes by infusion of SB-728-T.

In the concept of silencing genes, miRNA based genetic constructs can be used to silence human CCR5. The indicator cell lines constructed provide a convenient adequate model for rapidly assessing the CCR5 expression level. Exploring ways to eradicate the HIV by inhibiting the CCR5 receptor seems to be a promising way.

## REFERENCES

- Moss JA, HIV-AIDS review, Radiology technology, 2013, 268-270.
- Soriano V, Perelson AS, Zoulim F, Why are there different dynamics in the selection of drug resistance in HIV and hepatitis B and C viruses?, Journal of antimicrobial chemotherapy, 2008, 1-4.
- DeSilva E, Stumpf MP, HIV and the CCR5-Delta32 resistance allele, FEMS Microbiology Letters 241 (1), 2004, 1-12.
- Hütter G, Nowak D, Mossner M, Ganepola S, Müssig A, Allers K, Schneider T, Hofmann J, Kücherer C, Blau O, Blau IW, Hofmann WK, Thiel E, Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation, New England Journal of Medicine, 360(7), 2009, 692–8.
- Allers K, Hütter G, Hofmann J, Lodenkemper C, Rieger K, Thiel E, Schneider T, Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation, Blood, 117 (10), 2011, 2791–9.
- Zhen A, Kitchen S, infection, Viruses, 6(1), 2014, 1–12.
- Kay MA, Walker BD, Engineering cellular resistance to HIV, New England Journal of Medicine, 370(10), 2014, 968–9.
- Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, Spratt SK, Surosky RT, Giedlin MA, Nichol G, Holmes MC, Gregory PD, Ando DG, Kalos M, Collman RG, Binder-Scholl G, Plesa G, Hwang WT, Levine BL, June CH, Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV, New England Journal of Medicine, 370 (10), 2014, 901–10.
- Schoofs M, A Doctor, a Mutation and a Potential Cure for AIDS, The Wall Street Journal, 2010, 12-15.
- Agrawal L, Lu X, Qingwen J, VanHorn-Ali Z, Nicolescu IV, McDermott DH, Murphy PM, Alkhatib G, Role for CCR5Delta32 protein in resistance to R5, R5X4, and X4 human immunodeficiency virus type 1 in primary CD4+ cells, Journal of Virology, 78(5), 2004, 2277–87.
- Katlama C, Deeks SG, Autran B, Picado JM, Lunzen JV, Rouzioux C, Miller M, Vella S, Schmitz JE, Ahlers JR, Richman DD, Sekaly R, Barriers to a cure for HIV: new ways to target and eradicate HIV-1 reservoirs, The Lancet, 381, 2013, 2109–17
- Mussolino C, Morbitzer R, Lütge F, Dannemann N, Lahaye T, Cathomen T, A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity, Nucleic Acids Research, 39, 2011, 9283–93.
- Schiffer JT, Aubert M, Weber ND, Mintzer E, Stone D, Jerome KR, Targeted DNA mutagenesis for the cure of chronic viral infections, Journal of Virology, 86, 2012, 8920–36.
- Tebas P, Disruption of CCR5 in zinc finger nuclease-treated CD4 T cells: phase I trials. 18th conference on retroviruses and opportunistic infections; Boston, MA, USA; Feb 27–March 2, 2011. 165.
- Mitsuyasu RT, Merigan TC, Carr A, et al, Phase 2 gene therapy trial of an anti-HIV ribozyme in



- autologous CD34+ cells, *Nature Medicine*, 15(3), 2009, 285–92.
16. Wheeler LA, Trifonova R, Vrbanac V, et al, Inhibition of HIV transmission in human cervicovaginal explants and humanized mice using CD4 aptamer-siRNA chimeras, *Journal of Clinical Investigation*, 2011, 2401–12.
  17. Van Lunzen J, Glaunsinger T, Stahmer I, et al. Transfer of autologous gene-modified T Cells in HIV-infected patients with advanced immunodeficiency and drug-resistant virus. *Molecular Therapy*, 15, 2017, 1024–33.
  18. Laer D, Baum C, Schambach A, Gene therapeutic approaches for immune modulation in AIDS, *Anti Inflammatory and Anti Allergy Agents Medicinal Chemistry*, 6, 2007, 121–40.
  19. Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, Spratt SK, Surosky RT, Giedlin MA, Nichol G, Holmes MC, Gregory PD, Ando DG, Kalos M, Collman RG, Scholl GB, Plesa G, Hwang WT, Levine BT, and June CH, Gene Editing of CCR5 in Autologous CD4 T Cells of Persons Infected with HIV, *The New England Journal of Medicine*, 370(10), 2014, 901-910.
  20. Glazkova DV, Vetchinova AS, Bogoslovskaya BV, Zhogina YA, Markelov ML, and Shipulina GA, Downregulation of Human CCR5 Gene Expression with Artificial microRNAs, *Molecular biology*, 47(3), 2013, 475-85.

**Source of Support:** Nil, **Conflict of Interest:** None.

