An Assessment of HIV Treatment Using CCR5 Targeted Cell Therapy and Preventing Viral Escape

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Abstract
In an HIV-positive person in 2008, allogeneic transplantation using homozygous CCR5-delta 32 (CCR5-d32) stem cells resulted in long-term viral control and likely HIV eradication. Since then, there has been a lot of interest in expanding the use of this strategy. There are two methods for doing this in cells. The first is to replicate the original discovery using a CCR5 negative cell source, such as hematopoietic stem cells (HSC). On the other hand, viral escape of CXCR4 quasi-species was seen in a recent example of a second allogeneic transplantation using homozygous stem cells expressing CCR5-d32. The second method involves using gene therapy to reduce CCR5 expression. There are now five procedures that show promise, three of which are undergoing clinical trials. Short hairpin RNA (shRNA), zinc finger nucleases (ZFN), transcription activator-like effectors nuclease (TALEN), clustered regularly interspaced palindromic repeats/CRISPR-associated protein 9 nuclease (CRISPR/Cas9), and a ribozyme are some of these methods. While many other gene therapy approaches are being investigated, in this review we focus on what we now know about the particular suppression of CCR5 and whether or not this allows for subsequent viral escape.

Keywords: HIV-1; CCR5; CCR5 delta-2; tropism; genetherapy; viral escape; chemokine receptor, etc,.

Introduction
HIV cell entrance is dependent on binding to the CD4 receptor and CCR5 or CXCR4, one of the two potential chemokine co-receptors. The capacity of a virus to attach to a particular co-receptor is known as tropism. Dual-tropic HIV strains can use either CCR5 or CXCR4, whereas strains that bind to CXCR4 are known as X4-tropic and those that bind to CCR5 as R5-tropic. CCR5 is the main receptor for HIV cell entrance out of the two potential co-receptors. It is significant to remember that X4-tropic strains appear later in the course of the disease, whereas R5-tropic strains are the most often transmitted and predominate during the early stages of infection **(Doms., 2000; Weiss., 2013; Connor *et al*., 1997; Scarlatti *et al*., 1997)**. The CCR5 gene has 32 base pairs deleted, which results in a nonfunctional gene product that is not produced at the cell surface. People who have a homozygous CCR5-d32 deletion do not express any CCR5 receptor, which provides them with a high level of protection against HIV-1 infection and no other apparent health risks **(Samson *et al*., 1996)**. The absence of a CCR5 co-receptor on the cell surface was initially identified as a defense mechanism against transmission in 1996. Consequently, a number of methods have been tried to leverage this HIV-1 "Achilles heel" to

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create novel CCR5-targeted treatment plans in addition to the widely used antiretroviral therapy (ART). Although there are other ways to prevent HIV infection, since CCR5 is the most common co-receptor, we will concentrate on tactics that include it in this article. There are several techniques to reduce or prevent the manufacture of CCR5, including as ribozymes, CRISPR/Cas9, TALEN, shRNA, small interfering RNAs (siRNA), and antisense RNA. Intrabodies and intrakines are two strategies to stop CCR5 from being expressed on the surface **(Nazari &Joshi., 2008; Schroers *et al*., 2002; Bai *et al*., 1998; Luis Abad *et al*., 2003)**.

Techniques for Down Regulating and Blocking CCR5 Synthesis

1. Zinc finger domains (ZFNs) are designed proteins that can attach to specific DNA areas and use double strand breaks to alter genes. Gene mutations can result from nonhomologous end joining or homologous recombination when donor DNA is inserted into the broken DNA region **(Li *et al*., 1992; Kim *et al*., 1996).** According to **Tebas *et al*., (2014),** ZFN was safely utilized to alter CCR5 on autologous CD4+ T cells that are used to treat HIV-positive patients. ZFN modifies CCR5 in CD4+ T cells in a trial that is now accepting volunteers in order to enhance engraftment by administering escalating doses of cyclophosphamide (NIH clinical trial NCT01543152).

2. More sophisticated methods of gene silencing have been developed recently. For instance, TALENs are less cytotoxic than ZFNs and may effectively target locations in the CCR5 locus **(Mussolino *et al*., 2011).** The TALENs binding domains, like ZFNs, employ the complex's already fused endonuclease portion to identify and break certain DNA. Adenoviruses are capable of carrying this. Instead of recognizing three nucleotides, TALENs only identify one **(Holkers *et al*., 2013).** TALEN has been used to knock down CCR5, according to Mock et al. (2015). It has been demonstrated that this method shields R5-tropic HIV from CCR5 T cells. It should be highlighted, nonetheless, that Mock et al. only reported one lengthy (12-day) HIV exposure that demonstrated insufficient suppression of HIV replication and that they only utilized temporary transfection techniques **(Mock *et al*., 2015).**

3. Bacteria have an efficient defensive mechanism called the CRISPR/Cas9 system to resist harmful substances. By generating site-specific double strand breaks, it functions as an intracellular defense mechanism against plasmids or viral DNA. It was decided to use the CRISPR/Cas9 system as a molecular tool to disrupt individual human genes. Indeed, tests on human cells have been effective. 18% of the CCR5 genes were affected there by Kim et al., a percentage that would be necessary for a successful clinical application (**Cho *et al*., 2013).**

4. Small synthesized RNA fragments known as siRNAs are used to direct an endonuclease to cleave a specific location in mRNA. siRNAs are fragile (21-23 mers short), produced exogenously, and quickly degraded. To get to the specific RNA of interest, they must be taken in large doses. Numerous research have employed siRNAs to target CCR5, however the results have included off-target effects and insufficient suppression of HIV-1 **(Martinez *et al*., 2002); Qin *et al*., 2003)**. Viral escape mutants have been shown to render the usage of siRNAs—which are target specific—less than optimal for therapeutic applications **(Boden *et al*., 2003; Das *et al*., 2004).**

5. The more stable secondary structure (hairpin loop) of shRNAs sets them apart from siRNAs. Because of its structure, researchers may achieve their goal with a very minimal amount of it. Moreover, shRNAs can be produced via a gene cassette in the target cell's nucleus. ShRNAs may be effectively expressed using lentiviral vectors. Indeed, it has been demonstrated lately that they suppress HIV in both animal models and human cells **(Burke *et al*., 2015;**

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Wolstein *et al*., 2014; Shimizu *et al*., 2009). NIH clinical trial NCT01734850 is an ongoing study that uses a lentiviral vector to produce shRNA against CCR5 in conjunction with C46.

6. Single-stranded complementary RNAs known as antisense RNAs have the ability to block translation at the mRNA level**. Li et al., (2006)** demonstrate that a recombinant adenovirus encoding antisense CCR5 RNA downregulates CCR5. The vector is only momentarily expressed, according to the authors, and repeated dosage would be necessary if it were employed as a therapy since the host's immune system would eliminate it. Consequently, this method falls short of becoming the perfect gene therapy.

7. Small catalytic RNA molecules called ribozymes can be designed to target particular RNA sequences and function similarly to protein enzymes **(Sarver *et al*., 1990; Rossi., 2007; Macpherson *et al*., 2005; Sun *et al*., 1995).** The long-term stability, viability, and safety of employing ribozymes targeted to tat and tat-vpr HIV components have been positively demonstrated by three clinical trials **(Macpherson *et al*., 2005; Amado *et al*., 2004; Mitsuyasu *et al*., 2009).** The transduction efficiency, however, might have been higher. Furthermore, none of the experimental subjects had myeloablation. Since then, technology related to gene transfer has advanced. Myeloablation is now being studied as a possible HIV treatment in combination with gene therapy. **Di Giusto *et al*., (2010)** describe a combinatorial strategy for genetically modifying autologous peripheral blood derived CD34+ HSC from AIDS patients using Tat/Rev shRNA, Tat activation-response region (TAR) decoy, and CCR5 ribozyme. Results from this ongoing clinical investigation (NIH clinical trial NCT00569985) showed that CCR5 ribozyme stability was maintained for up to 24 months and that transduction procedures needed to be improved.

Methods for Inhibit CCR5 Cell Surface Expression

I. Intrakines are intracellular chemokines that can target endoplasmic reticulum-synthesised CCR5 by obstructing its transit to the cell surface **(Schroers *et al*., 2002; Bai *et al*., 1998); Luis Abad *et al*., 2003).** Published in 1997, this was likely one of the earliest attempts to prevent the usage of chemokine co-receptors to produce HIV-resistant cells. The group employed intrakines to target CCR5 **(Yang *et al*., 1997).** However, it was noted that the primary issue with this strategy was insufficient CCR5 inhibition.

II. When intrabodies were used, CCR5 was more completely inhibited than when intrakines were used. An intrabody is an intracellular single chain variable fragment antibody (scFv) that has the ability to attach to a target protein and perhaps cause malfunction. In order to prevent HIV infection in gene-modified cells, **Steinberger *et al*., (2000)** created a CCR5-specific intrabody that could suppress CCR5 surface expression. Since the late 1990s, patients with HIV infection and cancer have been evaluated for allogeneic stem cell transplantation using CCR5-d32/d32 cells. Having correctly matched human leukocyte antigens (HLA) is essential when receiving donor cells. If not, the host's immune system is more likely to reject you. Over the past 20 years, there has been no rise in the scarce supply of HLA-matched unrelated donors. The CCR5 null allele is present in just ~1% of Caucasians. Because of this, the strategy is almost impossible. In 2001, the cord blood bank StemCyte (Covina, CA, USA) began testing all of its units that were kept for CCR5-deletion in order to provide transplantation hospitals with a source of CCR5-negative stem cells. This was done in order to get around this restriction. However, after identification of many hundreds of CCR5-d32/d32 units, there was still little chance of obtaining an HLA-matched transplant with a sufficient cell count (>2.5 × 107 total

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nucleated cells). More specifically, only about 27 percent of patients who were Caucasian were matched adequately **(Petz *et al*., 2013).** This becomes extremely rare when combined with homozygous CCR5-d32/d32 of 1%.

The first successful allogeneic transplantation with a perfect HLA match from a donor homozygous for the CCR5-d32 deletion provided validity to the idea of CCR5-depleted HIV cell therapy. This patient, known as the "Berlin patient," has not seen a viral rebound in seven years since ART was discontinued at the time of transplantation. Furthermore, even the most sensitive methods have not been able to find any replication-competent viral material, suggesting that the patient has had their HIV-1 infection sterilized **(Hutter *et al*., 2009; Yukl *et al*., 2013).**

A few years later, a second patient (referred to as the "Essen patient") underwent care akin to that of the "Berlin patient." In this instance, it was discovered that the engraftment of the Essen patient resulted in a resurgence of the viral load due to the utilization of different chemokine receptors by the HIV quasi-species **(Kordelas *et al*., 2014).** The possibility of using gene therapy approaches for CCR5 down-regulation to a broader patient population has been called into doubt by this incidence in a number of ways. This review looks at the issue of non-CCR5 tropic viruses regaining their ability to replicate after CCR5 down-regulation and discusses potential preventative measures. HIV can evade immune responses through a variety of mechanisms, including sequestration, latent reservoirs, switching to X4-tropism, epitope mutation or deletion, and exhaustion of cytotoxic T lymphocytes.

Distinctions between the "Essen Patient" and the "Berlin"

The "Essen patient's" results were released in 2014, seven years following the "Berlin patient's" complete recovery. There was a palpable sense of disappointment among the scientific community and the general population. Because both patients got HLA-matched unrelated stem cells from a homozygous donor who carried the CCR5-d32 gene, both instances were strikingly comparable. On the other hand, upon transplantation with an X4 strain, the virus recovered in the "Essen patient" instance. Examining the specifics of the clinical course and the circumstances of both individuals reveals some significant discrepancies.

Table 1. Differences between the "Berlin" patient and the "Essen" patient receiving a CCR5-delta32 homozygous allogeneic stem cell transplantation.

	Berlin patient	Essen patient
Age, sex	40 years, male	27 years, male
Malignancy	Acute myeloid leukemia	Anaplastic large T-cell lymphoma
Time between infection and ART	7 years	3 years
Time between infection and Tx	12 years	5 years
Tx regimen	Intermediate intensity	Myeloablative +12 Gy TBI
Immunosuppression	ATG, CSA, MTX, MNF	ATG, CSA, MTX
GVHD	Max. grade 1 (skin)	Max, grade 1-2 (skin)
Engarftment	Day +11	Day +39
ART discontinuatio	On day of Tx	7 days before Tx

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V3 sequence	CIRPNNNTRK**G**IHIGPGR**A**FYTTG EIIGDIRQAHC	CTRPNNNTRK**G**I**PL**GPG**KV**FY**A**T EII**R**DIR**K**A**Y**C
>3 months prior Tx		
X4 prediction		
3 months prior Tx	Capable	Intermediate
Immediate prior Tx	Nd	Capable

The "Essen patient" had a far less favorable clinical history of HIV infection. It didn't take long for a cancer to be discovered after the first diagnosis and the start of ART. The "Essen patient" quickly became afflicted with AIDS after acquiring a T-cell lymphoma. In contrast, the "Berlin patient" never experienced an opportunistic infection and before developing leukemia, they consistently maintained a high enough CD4 T-cell count.

A thorough examination of the tropism-predicting V3 region of the virus demonstrated a significant change in the "Essen patient" that was consistent with the clinical history. A few more mutations gave rise to a larger chance of the "Essen patient" changing the tropism from R5 to X4 in comparison to the consensus sequence.

One week before to transplantation, ART was discontinued in the "Essen patient," which was another significant distinction. This seems to have allowed the virus enough time to multiply so much that it changed to utilize different co-receptors. The "Essen patient's" extremely late engraftment—a stable hematopoiesis is typically attained two to three weeks following transplantation—may indicate that the mutant virus proliferated quickly, having cytopathic consequences on the growing hematopoiesis as a result. This could have had a part in the delayed engraftment. Uncontrolled viral replication and the "Essen patient" case's late engraftment of CCR5-negative cells for over six weeks both seem to have had a role in the shift in tropism.

Finally, a year after the initial transplant, the "Berlin patient" experienced a recurrence of his leukemia and underwent a second transplant from the same donor. It is possible that the double transplantation has increased the procedure's ability to purge HIV reservoirs. But in the interim between these treatments, the "Berlin patient" was HIV free without antiretroviral medication, suggesting that eradication could have occurred prior to the second transplant.

The most important thing we can take up from the "Essen patient" is the need to keep up ART throughout the conditioning regimen in order to achieve 100% chimerism and steady engraftment. We believe that concerns regarding the possibility of graft failure due to the cytotoxic effects of antiretrovirals are exaggerated **(Hutter *et al.*, 2011).** However, there are several notable and occasionally challenging drug-drug interactions between antiretrovirals and drugs used during the transplant surgery (such as cyclosporine A). The fact that antiretroviral drugs are only taken orally presents another unresolved issue and might be problematic for individuals who have severe mucositis during their aplasia. To make informed clinical judgments, guidelines and recommendations for the safe use of antiretroviral treatment (ART) during chemotherapy and allogeneic transplantation are required **(Flepisi *et al.*, 2014).**

HIV Tropism and CCR5 Suppression

The primary receptor for HIV cell entrance is CCR5. HIV-1, however, has the potential to partially alter its tropism during infection (to CXCR4, for example). This flip can happen even while the viral load is controlled and is linked to low CD4+ T cell count, AIDS, high viral load,

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and ART pre-treatment. In order to prevent viral escape, it is crucial to comprehend the prerequisites of the tropism change for CCR5 targeted therapeutic approaches. Our observations are mostly based on three cases in which tropism change and CCR5 were examined.

- **Entry Inhibitor of HIV**

A competitive CCR5 inhibitor called maraviroc was given clinical approval in 2007. Maraviroc is an example of the novel HIV medication class (entry inhibitors), which shown increased efficacy in individuals on antiretroviral therapy (ART) **(Fatkenheuer *et al*., 2008).** Ongoing entry inhibitor usage has, however, been linked to the reemergence of X4-tropic viruses, which indicates viral failure. Numerous research were conducted on this phenomena, and the majority of them discovered the existence of even very tiny populations of HIV strains other than CCR5 before maraviroc commencement. According to evolutionary study, X4-tropic viruses originate from pre-existing populations rather than evolving de novo as a result of enhanced selection pressure **(Westby *et al*., 2006; Archer *et al*., 2009).**Consequently, if the R5 viral suppression is not fully achieved and/or the HIV reservoir size has not been reduced to an unacceptable degree, X4-variants of the virus may reappear.

- **CCR5 Gene Therapy of HIV Disease**

The Sangamo trial is the most advanced HIV gene therapy trial in terms of patient recruitment.
The ZFN against CCR5 was used in this experiment to manipulate the peripheral autologous T-cells. After receiving cell infusion, some individuals experienced a break in their therapy. Since they all quickly recovered, it was clear that the modified cells lacked defense against viral reproduction. CCR5 negative cells were used at a comparatively low dose. Remarkably, one patient who tested positive for both CCR5 and d32 deletions spontaneously acquired the ability to regulate viral replication in the absence of antiretroviral therapy. Prior to enrollment, every candidate for these studies only possessed R5-tropic strains of HIV. However, information about potential changes in HIV tropism in the patient who managed viral replication and after zinc-finger administration is lacking **(Tebas *et al*., 2014).**

- **Problem unsolved: alternative chemokines**

Generally speaking, X4-tropic viruses infect HIV-positive individuals with the natural CCR5-d32/d32 mutation. It is generally known, meanwhile, that HIV may occasionally utilise other chemokine receptors. Analysis of tropism from HIV-infected CCR5-d32 homozygotes, where cases of infection with non-R5-tropic viruses have been reported, is particularly intriguing in this context **(Gray *et al*., 2006; Henrich *et al*., 2015).** HIV rebound's "back door" may pose a serious threat to entry-targeted treatment plans.

The reservoir's size and probability of rebound

When antiretroviral medicine is stopped in patients, the rebound usually happens in a few weeks. It's interesting to note that even after taking antiretrovirals for a long period, patients still have a reasonably quick comeback (minimization of the reservoir is presumed) **(Davey *et al*., 1999).** Moreover, there is typically a detectable proviral reservoir in individuals with spontaneous inhibition of viral replication (elite controllers). Additionally, elite controllers may have an unplanned, sporadic, and spontaneous outbreak of HIV replication **(Cortes *et al*., 2015).**
Most recently, reports of patients with non-detectable viral reservoirs were published and deserve closer attention.

1. Allogeneic stem cell transplantation (CCR5 wild type graft) was performed on two HIV+ patients. Antiretroviral therapy was administered to both 2.5 and 4.3 years following transplantation. In terms of anti-HIV antibodies, both showed a steady sero-deconversion,

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suggesting that no substantial replication had taken place over this period. If not, detectable levels of anti-HIV antibodies would have remained. Additionally, peripheral blood outgrowth testing and tissue samples came out negative. Allogeneic transplantation has been suggested to have eliminated the viral reservoir by latently infected cells turning over and (assumed) graft T-cell cytotoxicity against the reservoirs (graft against HIV impact). Nevertheless, after three and seven weeks, respectively, both individuals recovered with HIV. It's interesting to note that both individuals had a very tiny reservoir but had not completely eliminated the virus, suggesting that latently infected cells "hid" in undetectable niches. This is supported by the abnormally extended time interval between stopping treatment and rebound **(Henrich *et al*., 2013).**

2. After receiving antiviral therapy within 30 hours of birth, a perinatally infected kid was weaned off of the medicine after 18 months. Remarkably, after stopping antiretroviral therapy, the youngster was shown to have no HIV replication. Very minute amounts of viral material have occasionally been recovered, but no virus capable of reproduction has been identified. There was no immunological response in the patient in terms of the generation of anti-HIV antibodies. It was believed that the immune system could manage these few infected cells and that early ART commencement reduced the reservoir. However, the child's HIV replication was discovered to be active around 27.6 months after ART was stopped **(Luzuriaga *et al*., 2015).**

By achieving viral control and reducing the amount of the latent reservoir, a functional cure was presumed for all three patients. Regretfully, all three individuals had HIV recurrence after remarkably extended intervals, suggesting that viral resurgence might originate from minuscule cell origins that may be inaccessible to existing detection methods. The development and optimization of assays for assessing the latent reservoir is still ongoing. While more recent, more sensitive RT-PCR assays require only seven days, outgrowth experiments utilizing ELISA take fourteen days. Although the reservoir has been quantified, the source of latently infected cells is still unclear, and the precision of these measurements is questionable.

Methods for minimize the viral reservoir

As previously mentioned, the size of the viral reservoir may influence the likelihood and timing of viral replication following the cessation of antiretroviral therapy. Enhancing the efficacy of CCR5-driven medicines is one strategy that will be crucial in minimizing the reservoir.

1. The use of chemotherapy

During the course of treating cancer, autologous and allogeneic stem cell transplants are frequently used with chemotherapy, either alone or in conjunction with radiation therapy. Chemotherapy has a prolonged, but often transient, myelosuppressive impact. Fludarabine is one example of a chemotherapy drug that has longer-lasting and more cell line-specific side effects. There is no long-term impact on the size of the viral reservoir, according to experiences with HIV+ patients undergoing autologous stem cell transplantation and high dosage chemotherapy **(Zaia *et al*., 2013).**

2. Using HIV cytopathic effects

Due to HIV's cytopathic impact on cells, externalization and replication of the virus may result in the death of the corresponding cell. In an unchecked infection, freshly infected target cells make up for the loss of the infected cell pool. With the introduction of antiretroviral therapy (ART), there was a glimmer of optimism because latently infected cells underwent a notable turnover,

 https://doi.org/10.5281/zenodo.13818835

suggesting that continued ART might eventually eradicate HIV. When it became clear that eradication was not possible during a normal life span and that the half-life of the cell turnover is significantly longer than predicted (due to resting and non-replicating cell sources), strategies to increase the turnover rate were put forth. Various drugs were tried under the names "shock and kill" or "kick and kill." Using these drugs, latently infected cells were able to be "kick/shocked" into multiplying their virus, which was then eliminated (or "killed"). To stop cells from being infected again, antiretrovirals were given concurrently, which included entrance inhibitors. Testing has been done on extra-terminal protein inhibitors, protein kinase C activators, and histone deacetylase inhibitors for this strategy **(Archin *et al*., 2014).** Histone deacetylase inhibitor vorinostat, however, showed promise in early clinical trials for "kick[ing]" the virus out of the reservoir, but no discernible impact on the reservoir's size was seen **(Elliott *et al*., 2014).**

3. Agents Toxic to Viral Reservoir

It has been demonstrated that a number of substances are cytotoxic to latently infected cells. Amanofin is a gold complex that has shown promise in the treatment of rheumatoid arthritis. It works by inhibiting redox (reduction/oxidation) enzymes, which are vital to many cellular processes, especially those that involve maintaining intracellular levels of reactive oxygen species (ROS). Maintaining the equilibrium of reactive oxygen species (ROS) is essential for the survival of all cell types, including parasites, cancer cells, and memory T cells that carry proviral HIV DNA. Through the down-modulation of CD27 in non-activated central and transitional memory T-cells (TCM and TTM, respectively), as well as a putative caspase pathway, auranofin exhibits pro-apoptotic effects. A hallmark of memory T cells with persistent phenotypes that carry proviruses is CD27 **(Chirullo *et al*., 2013).**

The Vaccine and Gene Therapy Institute in Florida, USA, reported the start of an early clinical study using auranofin in HIV-positive individuals; however, the experiment was canceled before any patients could be enrolled (NIH clinical trial NCT02176135).

4. Gene Therapy

Gene therapy may be helpful in reducing the amount of the reservoir by focusing on latently infected cells in addition to being a viable strategy for addressing the HIV entry mechanism. A novel strategy for eliminating contaminated cells gained notice in 2007. Using a very selective tre-recombinase was the method utilized here. An enzyme that could cleave DNA sections with a sequence comparable to the long terminal repeats (LTR) seen in HIV-1 insertion sites was the source of this enzyme. The provirus can be entirely eliminated by inserting the tre-recombinase into the infected cell **(Sarkar *et al*., 2007).**

The most current method to be disclosed uses Cas9/guide RNA (gRNA). In this case, testing of only a portion of the integrated provirus was successful. Effectively blocking the HIV-1 LTR U3 region, Cas9/gRNA transfection inhibited HIV gene expression and latently infected cells' ability to replicate **(Hu *et al*., 2014).**

Strategies to Overcome Viral Rebound

1. Additional HIV Entry Inhibition

Given the contradictory findings on CCR5 down-regulation (as previously mentioned), dual entry inhibition may be justified. Using an agent with an extra impact on CCR5 down-regulation is one option. The von Laer laboratory produced the HIV-fusion inhibitor C46 (M87o), which has demonstrated potent anti-HIV action in non-human primates and tissue culture systems **(Hildinger *et al*., 2001; Schambach *et al*., 2006; Younan *et al*., 2013).** Constructed from the second heptad repetition of the HIV-1 envelope glycoprotein gp41, C46 is a 46-amino acid

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protein that functions to prevent the fusion of the viral and cellular membranes after virus entrance.The fusion-inhibitory peptide C36 (T-20/enfurvitide), the first HIV-fusion inhibitor authorized for clinical use, is represented by the 36 C-terminal amino acids of C46. C46 is produced as a fusion protein that has a membrane-spanning domain followed by a C-terminal linker and an N-terminal signal peptide that travels to the cell surface via the endoplasmic reticulum. This inhibitor has demonstrated safety both in vitro and in a clinical study **(Van Lunzen *et al*., 2007).** It has been demonstrated that a lentiviral vector containing both a shRNA targeting CCR5 and a fusion inhibitor for C46 (LVsh5/C46) works in concert to limit HIV replication in T cell lines, peripheral blood mononuclear cells, and an in vivo humanized animal model. Molt 4/CCR5 cells were transduced in LVsh5/C46 experiments, and the cells were then challenged with HIV Bal (R5-tropic). Triplicate experiment results indicated that gene marking increased over time (10 to >75%) and that, for up to nine weeks, there was no sign of escape mutants based on PCR examination of the HR1, HR2, and V3 loop areas **(Ledger *et al*., 2015).** Another combination treatment that is presently undergoing clinical trials employs a triple combination of anti-CCR5 ribozyme, nucleolar-localizing transactivation response (TAR) decoy, and shRNA targeting the HIV-1 tat and rev mRNAs (NIH clinical study NCT00569985) **(DiGuisto *et al*., 2010; Li *et al*., 2005; Anderson *et al*., 2007).** HIV replication requires the regulatory proteins Tat and rev; however, shRNAs that target these proteins have demonstrated anti-HIV-1 efficacy in human cells in a mouse model that is humanized. For transcription, Tat needs to attach to TAR. Thus, it has been demonstrated that using the TAR decoy, which may simulate tat binding to TAR, can counteract its action **(Michienzi *et al*., 2002).**This triple combination treatment targets both HIV and cellular components through several ways in addition to CCR5 knockdown via a ribozyme. It is envisaged that multiple combination treatment will limit any potential for viral escape by increasing the capacity to block HIV on several levels.

2. CXCR4 Blockage

The late 1980s saw the synthesis of plerixafor, a CXCR4 inhibitor, apart from the discovery of maraviroc as a CCR5 inhibitor. Later, plerixafor emerged as a promising HIV medication. It was notable that it might prevent the hematopoietic stem cell homing pathway, despite the generally unsatisfactory effect on HIV. Plerixafor is now used as a secure and effective mobilizer of stem cells during the extraction of autologous or allogeneic stem cells **(Herbert *et al*., 2014).**

It has not yet been tried to utilize plerixafor in conjunction with gene therapy. In order to prevent the emergence of additional quasi-species of HIV, plerixafor or other CXCR4 inhibitors may be helpful in enriching CCR5-manipulated cells (Figure 1). The existing theory of gene therapy suggests that a very small percentage of cells are transduced and, as a result, develop resistance. In this case, the cytopathic impact of HIV would put other "unprotected" cells at a disadvantage and decrease their population. The protective agent-containing cells would so grow enriched and may eventually constitute a sizable cell population.In this case, viral replication would act as a selective agent allowing protected population of cells to expand before any untoward effects of HIV replication manifest themselves.

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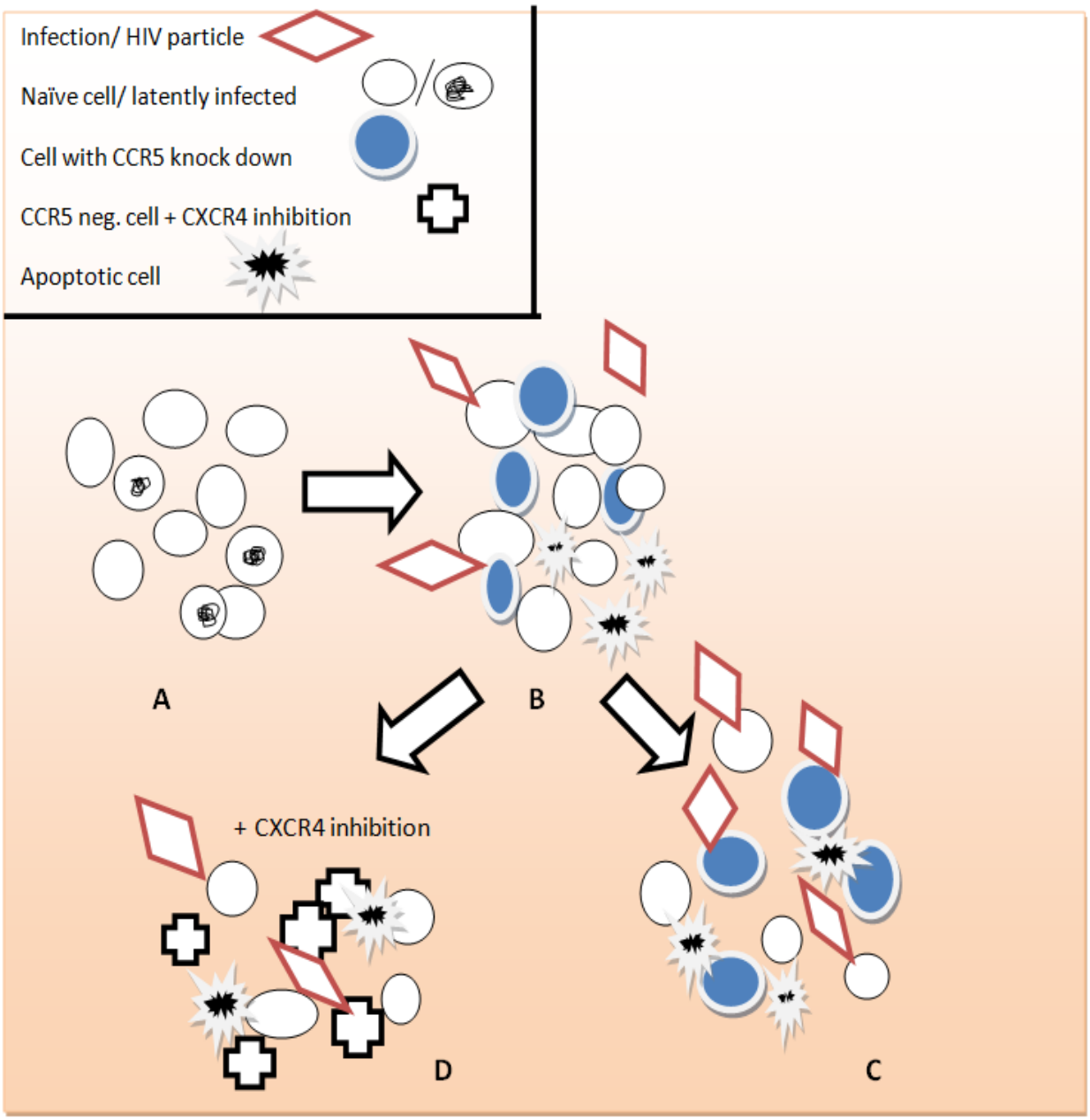

[Figure 1. Dual entry inhibition's selective advantage. (A) Reinfused autologous cells with CCR5 downregulation into a patient receiving continuing antiretroviral therapy (ART); (B) stopping ART and allowing HIV to proliferate and infect naïve cells (CCR5+ cells). Theoretically, HIV's cytopathic impact will lead to an enrichment of CCR5-negative cells; (C) infected cells' death reduces CCR5 as a possible target for cell entrance. HIV may use different chemokine receptors, such as CXCR4, to enter CCR5 negative cells by increasing the selective pressure; (D) Dual entrance inhibition (CCR5 negative and CXCR4 inhibited cells) may stop HIV from entering the cells and consequently infection.]

Burixafor (TG-0054), a newly developed CXCR4 inhibitor, provides an alternative to plerixafor. Burixafor is more efficient and less hazardous than Plerixafor. Burixafor may potentially contribute to a CCR5-targeted therapy strategy**(Hsu *et al.*, 2015).** Recently, ZFNs were employed to disrupt both CCR5 and CXCR4 in an in vitro experiment**(Yang *et al.*, 1997).** In a model of cell lines, resistance to both R5 and X4 strains was exclusively demonstrated by the

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double-manipulated group, suggesting a highly selective advantage against the cytopathic impact of HIV infection. However, because CXCR4 appears to be important for immune responses and is also critical for human health, knocking it down in vivo carries some danger. This strategy's viability requires more investigation **(Didigu *et al*., 2014).**

3. Chemokine Receptor Down-regulation

The amount of chemokine receptors present on the surface of cells may influence how susceptible those cells are to HIV**(Reynes *et al*., 2001); (Lin *et al*., 2002).** Conversely, reversal down-regulation may also be a safeguard. It is well known that chemokine receptor expression is strictly regulated. The transcriptional attenuation of CCR5 mediated by prostaglandin E2 (PGE2) is one instance of such control**(Mahic *et al*., 2006)**. It has been shown that during differentiation in the peripheral, inducible regulatory T cells (iTregs) express cyclooxygenase-2 (COX-2) and generate large levels of PGE2. iTregs grow in peripheral lymphoid tissues, in contrast to normally occurring CD4+CD25+Foxp3+ Tregs (nTregs), which arise in the thymus and do not produce COX-2.Through the bystander effect, the COX-2-mediated PGE2 generation of iTregs can significantly affect the epigenetic down-regulation of CCR5 surface expression on both CD4+ T cells and myeloid cells. It is noteworthy that research has demonstrated the crucial role that PGE2 and its more stable derivative dimethyl PGE2 (dmPGE2) play in controlling HSC homeostasis**(Cutler *et al*., 2013).** The "effective dose" of HSCs in allogeneic transplantation may be increased by short ex vivo manipulation with dmPGE2, according to recent evidence from clinical studies. This raises the prospect that the same therapy may potentially epigenetically down-regulate CCR5 expression. Similar outcomes for the down-regulation of CXCR-4 mediated by PGE2 have also been hypothesized.

Conclusions

A new era in HIV therapy has begun today. The primary barrier to HIV prevention is the absence of an effective vaccine; given the effectiveness of antiretroviral therapy (ART) in reducing infection and prolonging the life span of HIV-positive individuals, perhaps it is time to think about HIV eradication. The "Berlin patient" case created a window of opportunity for cell-based treatment. Nonetheless, the area of cell-based gene therapy has some restrictions. Clinical trials are now being conducted on gene treatment techniques.

These days, there are many new instances of HIV patients that may be successfully controlled by the virus. However, these claims could sway the conversation away from HIV treatment, creating erroneous expectations and rash judgments. Ultimately, each novel advancement remains only a component of the whole picture rather than a conclusive answer to the optimization of HIV treatment.

As far as we now know, inhibiting the chemokine receptor to low levels can prevent HIV from re-emerging following CCR5 targeted treatment, imitating the practically complete protection against transmission observed in persons who are homozygous for CCR5-d32. Rebound of HIV quasi-species utilizing alternative chemokine receptors may be prevented by inhibiting viral replication during this phase and by employing CCR5-independent entry inhibitors.

Future prospects include the development of combination medicines to improve efficacy, advances in gene-editing technologies for improved accuracy and safety, and attempts to make

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these therapies more accessible and scalable. Ethical considerations in genetic modification, as well as practical ones such as cost and healthcare infrastructure, are critical to ensuring that these medicines may be widely applied, possibly altering HIV management and eradication throughout the world.

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