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CCR5-edited gene therapies for HIV cure: Closing the door to viral entry

KEVIN G. HAWORTH[1], CHRISTOPHER W. PETERSON[1,2] & HANS-PETER KIEM[1,2,3]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA, [2]Department of Medicine, University of Washington, Seattle, Washington, USA, and [3]Department of Pathology, University of Washington, Seattle, Washington, USA

Abstract

Human immunodeficiency virus (HIV) was first reported and characterized more than three decades ago. Once thought of as a death sentence, HIV infection has become a chronically manageable disease. However, it is estimated that a staggering 0.8% of the world's population is infected with HIV, with more than 1 million deaths reported in 2015 alone. Despite the development of effective anti-retroviral drugs, a permanent cure has only been documented in one patient to date. In 2007, an HIV-positive patient received a bone marrow transplant to treat his leukemia from an individual who was homozygous for a mutation in the CCR5 gene. This mutation, known as CCR5Δ32, prevents HIV replication by inhibiting the early stage of viral entry into cells, resulting in resistance to infection from the majority of HIV isolates. More than 10 years after his last dose of anti-retroviral therapy, the transplant recipient remains free of replication-competent virus. Multiple groups are now attempting to replicate this success through the use of other CCR5-negative donor cell sources. Additionally, developments in the use of lentiviral vectors and targeted nucleases have opened the doors of precision medicine and enabled new treatment methodologies to combat HIV infection through targeted ablation or down-regulation of CCR5 expression. Here, we review historical cases of CCR5-edited cell-based therapies, current clinical trials and future benefits and challenges associated with this technology.

Key Words: *CCR5 receptor, genetic therapy, HIV*

Introduction

Human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), has become one of the most costly and deadly epidemics in human history. As of 2015, more than 70 million people worldwide have been infected by HIV, and 35 million people have died due to the disease (Joint United Nations Programme on HIV/AIDS [UNAIDS]; www.unaids.org). These numbers are astounding considering the virus was first characterized in humans only 34 years ago, in 1983 [1,2]. Despite significant efforts during the past three decades, HIV infection remains a chronic disease. From the discovery of the first approved anti-retroviral drug, azidothymidine (AZT), to the current regimens of highly active anti-retroviral therapy (ART), it has become possible for infected individuals to maintain a relatively normal life expectancy from what was once considered a death sentence. These ART regimens, while effective, require strict adherence because viral rebound occurs rapidly after treatment interruption [3–5]. Despite advances in treatment, several limitations remain, most significantly being the cost and availability of ART. As of 2010, the estimated lifetime treatment cost of an individual living in the United States with HIV was $379,000 (Center for Disease Control and Prevention; www.cdc.gov). This problem is compounded by the fact that the vast majority of HIV cases worldwide occur in impoverished countries where patients lack reliable access to ART and/or financial resources to afford these medications. The ultimate goal of HIV cure research is the development of a permanent, portable and relatively inexpensive treatment, capable of reaching and benefiting all individuals worldwide infected with HIV. While the world still waits for such a breakthrough, a single clinical case has both inspired the scientific field and provided hope to the community that an HIV cure is achievable.

In 2009, the "Berlin Patient" was reported as the first and, to date, the only known patient to be cured of HIV [6,7]. This patient was diagnosed with HIV in 1995 and subsequently initiated anti-retroviral therapy. After developing acute myeloid leukemia (AML) in

Correspondence: **Hans-Peter Kiem**, MD, PhD, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave North Mail Stop D1-100, PO Box 19024, Seattle, WA 98109-1024, USA. E-mail: hkiem@fredhutch.org

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2007, he received two allogeneic hematopoietic cell transplants (HCTs) from an individual who carried a specific mutation in the CCR5 gene, which is required as a co-receptor for most strains of HIV to efficiently bind and enter a target cell [8]. Individuals who carry a naturally occurring mutation of this gene, CCR5 *Delta*32 (Δ32), have been shown to be resistant to HIV infection [9]. Likewise, following numerous quantitative assays performed by multiple laboratories, no live virus has been detected in the Berlin Patient during the past decade, and he now is widely considered to be the only individual "cured" of his HIV infection [10]. This revelation has spurred a new approach to the treatment of HIV, which revolves around genetically engineering an immune system that can resist HIV infection. In this review, we will cover the various attempts to replicate the Berlin Patient's success and discuss the different approaches researchers are taking to move one step closer to a permanent cure.

HIV requires the expression of a co-receptor for target cell infection

HIV entry requires interaction of the viral particle with two surface receptors found on the target cell. The primary receptor for viral entry is the surface marker CD4 [11]. This receptor is an essential molecule in the immune system, and is responsible for eliciting adaptive immunity against invading pathogens. Once HIV successfully binds to CD4, it then requires a secondary co-receptor to complete the infection process (Figure 1) [12]. There are two known co-receptors for the virus: the dominant co-receptor for most HIV isolates is CCR5 ("R5-tropic viruses"), whereas the secondary alternative co-receptor is CXCR4 ("X4-tropic viruses"). Primary infection is thought to initiate most often with R5-tropic viruses [13]. The switch from R5 to X4 tropism over time is associated with specific mutations in the V3 loop of the HIV envelope glycoprotein and more rapid disease progression, which in turn may be caused by a decrease in the lack of $CD4^{+}CCR5^{+}$ target cells [14–16]. After the discovery of CCR5 as the predominant co-receptor for HIV infection, researchers demonstrated that by binding small beta-chemokine molecules to surface-expressed CCR5, cells were rendered resistant to infection with R5-tropic viruses. This discovery led to an entire class of ART drugs called entry inhibitors, such as the small molecule Maraviroc, which prevent HIV infection of cells at the initial stages of viral infection [17].

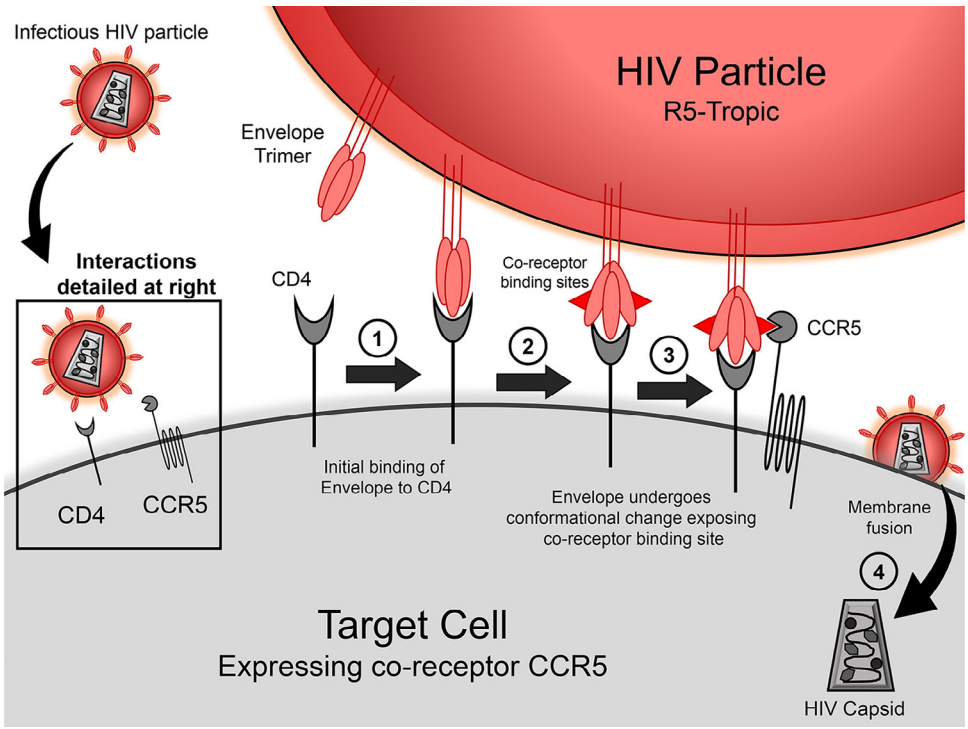

Figure 1. This illustration shows the interactions between HIV particle and cell surface receptors during virus entry. Schematic overview for an R5-tropic virus using the co-receptor CCR5. (1) HIV envelope glycoprotein trimer first interacts with surface-expressed CD4 protein. (2) After binding CD4, the envelope trimer undergoes a conformational change, exposing the secondary co-receptor binding site. (3) Surface-expressed CCR5 co-receptor molecules bind envelope. After engaging with both primary and secondary receptors, additional conformation changes occur in the envelope timer, exposing the fusion peptide complex, which is inserted into the CD4+ cell plasma membrane ("virus fusion"). (4) HIV capsids, containing the viral RNA and associated viral proteins, translocate to the nucleus and integrate the viral genome into cellular chromosomal DNA.