Road to HIV cure; from Berlin to London and beyond

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Around 37 million people are living with HIV worldwide, with a million deaths due to HIV in 2017. While only ~60% of the infected population are receiving antiretroviral therapy (ART), by taking a combination of drugs suppressing different stage of the HIV lifecycle to lower the viral burden. While the treatment is very effective it does not eliminate HIV from the patient's body and non-AIDS comorbidities (cardiovascular diseases and cancers) and unrelenting rate of new infections (around 2 million infections per year) have become a major concern and, thus new approaches are needed that no longer continuously suppress HIV but actually cure people.

The C-C Chemokine receptor type 5 (CCR5) is a protein on the surface of white blood cells and acts as a receptor for chemokines (signaling proteins). The CCR5 plays an important role in HIV replication, as the virus can use this protein as a co-receptor to enter the target cell (e.g. CD4+ helper T cells).[1] Upon entering the cell, the virus embeds itself in the host chromosomal DNA and uses the cell machinery to induce progeny viral replication and subsequent viral spreading. While a CCR5 antagonist is available none of the current recommended initial antiretroviral (ARV) regimens include the CCR5 antagonist (i.e. Maraviroc). The CCR5 receptor antagonist exerts its function by blocking the interaction between HIV and CCR5 on immune cells and inducing HIV suppression.[2] The alteration of the CCR5 coreceptor on immune cells could be an exciting approach to break the HIV lifecycle and potentially induce a HIV cure. Recently, the deletion of CCR5 expression on immune cells has shown great promise towards HIV cure, but has been celebrated or scrutinized in the science community depending on its approach.

The first belief that CCR5 deletion could induce a HIV cure was shown a decade ago in the HIV-infected 'Berlin patient'. For the treatment of his acute myeloid leukemia he first underwent chemotherapy and whole-body radiation to get rid of the native immune and progenitor stem cell populations, and then received hematopoietic stem cell transplantations (HSCT) (so as to reintroduce the immune system) from a donor expressing a mutant non-functional version of CCR5 (CCR5Δ32/Δ32) that is not expressed on the cell surface.[3] Approximately 1% of people carry this specific homozygous CCR5 deletion and the lack of CCR5 expression renders the HIV unable to infect cells.[4] Though cured of HIV, the patient had to endure extremely risky and potentially lethal clinical procedures including total body irradiation followed by two rounds of allogeneic-HSCTs. A new report by Gupta et al. used a similar approach of allogeneic stem cell transplantation to treat for Hodgkin's lymphoma using a donor carrying CCR5Δ32/Δ32, but now utilized a less toxic treatment regime.[5] This patient (termed London patient) only experienced mild donor immune reactivity targeting his own tissues (graft versus host disease) and antiretroviral therapy was interrupted 16 months following transplantation. HIV-1 remission has been maintained for a further 19 months. Another patient in Düsseldorf who also received a CCR5Δ32/Δ32 allo-HSCT has not shown viral rebound in the 3 months following cART interruption.[6] These cases demonstrate that HIV remission can be obtained without additional treatment and with a less toxic treatment regime than the 'Berlin patient'. While more patients have received a CCR5Δ32/Δ32 or CCR5 wild-type allo-HSCT, the virus unfortunately rebounded after transplantation. While a delay in viral rebound is noticed in the CCR5 wild-type allo-HSCT [7,8], the viral rebound in the CCR5Δ32/Δ32

allo-HSCT was due to a shift from a dominantly CCR5-tropic HIV-1 to a CXCR4-tropic HIV-1 that does not need CCR5 to enter the cell.[9] While CCR5-deficient stem cell transplantation seems to confer a robust and prolonged HIV remission in the case of CCR5-tropic HIV, this treatment still has serious and potentially lethal side-effects and for now seems to be reserved for patients who have other life threatening diseases.

While the exact mechanism of the HIV cure via CCR5Δ32/Δ32allo-HSCT is still under investigation, it is largely believed that the CCR5 mutation was a key factor. This initiated the utilization of gene editing technologies to manually reconstruct the CCR5Δ32 mutation (reviewed in [10]). Gene editing in hematopoietic stem cells or peripheral blood mononuclear cells (PBMCs) would then result in cells that are naturally resistant to HIV-1 infection, with the goal of providing the same curative benefits without the challenges of allogeneic transplantation. Several *in vitro* and murine *in vivo* models have shown that gene editing of HIV coreceptors lowers the number of cells HIV-1 can infect.[10,11] In a clinical trial of 12 patients who received infusion of CCR5 gene edited CD4+ T cells, a slow viral rebound was noticed after stopping ART and the treatment was well tolerated.[12] One patient cleared the virus completely and another two experienced 10-fold drops in circulating virus levels. The patient who cleared the virus was already heterozygous for the CCR5Δ32 deletion, which could potentially be the reason for the better response exhibited by the patient. For now, gene editing shows great promise as a complimentary therapeutic approach in conjunction with existing ART regimes as gene editing is currently not 100% effective and will therefore not target all latent viral reservoirs (mostly memory CD4+ T cells). Further pursuit of this technology could optimize the efficacy and minimize off-target effects and play a more prominent role in a functional HIV cure (immune system-mediated durable suppression of viral replication without completely eliminating HIV) or even a sterilizing cure by eliminating all latent HIV reservoirs. For instance, prior depletion of the patient's native CD4+ T cells might increase the expansion of the transferred gene edited HIV-resistant cells and might augment therapeutic response.

In the end of 2018 gene editing of CCR5 was taken to another level and the scientific world was shocked by the first reported CCR5 gene-edited babies, reported on YouTube. The Chinese researcher He Jiankui conducted the controversial experiment to genetically alter the CCR5 expression, via gene editing technology, in embryos of twins to create resistance to HIV. The father of the twins was HIV positive and this procedure was conducted to protect the twins from a similar fate, according to the researcher. While a worldwide scientific consensus against human germline genome editing was ignored, the benefits from this procedure are also likely a lot smaller than initially thought. The mutations created in CCR5 were not similar to the naturally occurring CCR5Δ32 mutation and have never before been seen in humans, making it unsure if this mutation actually provides protection. One of the twins had only one CCR5 allele edited resulting in serious undercutting of the potential benefits of the gene editing. Furthermore, the twins are still very susceptible to non-CCR5 tropic HIV, such as CXCR4 tropic HIV and HIV-2 and are therefore not protected from HIV infection as intended and suggested. In addition, the gene editing could have also made unintended edits in the genome which could theoretically lead to health problems.

Overall, recent successful allo-HSCTs involving a donor lacking the co-receptor CCR5 has resulted in the reinvigorated believe of the possibility of a HIV Cure. Allo-HSCT is however only reserved for certain conditions (malignancies) and not as sole treatment for HIV due to its toxic treatment regime. Adoptive transfer of CCR5 gene edited stem cells or PBMCs is a far less toxic approach that shows great promise in suppressing HIV. Gene editing of humans is however not an option as the current technology is not sufficiently safe or effective and outweigh the potential benefits from such a treatment,

besides numerous ethical concerns. While current advances show great promise, it is still a long road ahead towards a broadly applicable CCR5-mediated HIV cure.

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