Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

CCR5Δ32 mutation and HIV infection: basis for curative HIV therapy

Kristina Allers and Thomas Schneider

The C–C chemokine receptor 5 (CCR5) is expressed on potential human immunodeficiency virus (HIV) target cells and serves as the predominant co-receptor for viral entry during initial transmission and through the early stages of infection. A homozygous Δ32 mutation in the *CCR5* gene prevents CCR5 cell surface expression and thus confers resistance to infection with CCR5-tropic HIV strains. Transplantation of hematopoietic stem cells from a CCR5Δ32/Δ32 donor was previously successful in eliminating HIV from the recipient's immune system, suggesting that targeted CCR5 disruption can lead to an HIV cure. Therefore, intense work is currently being carried out on CCR5 gene-editing tools to develop curative HIV therapy. Here, we review the natural function of CCR5, the progress made on CCR5 gene editing to date and discuss the current limitations.

Address
Department of Gastroenterology, Infectious Diseases, and Rheumatology, Campus Benjamin Franklin, Charité-Universitätsmedizin, Berlin, Germany

Corresponding author: Allers, Kristina (kristina.allers@charite.de)

Current Opinion in Virology 2015, **14**:24–29

This review comes from a themed issue on **Engineering for viral resistance**

Edited by **Albrecht von Brunn**

For a complete overview see the Issue and the Editorial

Available online 1st July 2015

http://dx.doi.org/10.1016/j.coviro.2015.06.007

Introduction

Current standard therapy for human immunodeficiency virus (HIV) infection requires the lifelong daily administration of a combination of antiretroviral drugs (combination antiretroviral therapy; cART). Although therapeutic control of viral replication allows the immune system to partially restore and delays disease progression, the cure of HIV infection remains unachievable with the use of the currently available drugs. Individuals who are naturally homozygous for the *CCR5* gene variant Δ32 are resistant to CCR5-tropic HIV infection because of the lack of cellular C–C chemokine receptor 5 (CCR5) surface expression [1]. Previously, we reported the cure of HIV infection in a patient who received hematopoietic stem cells from a donor with this homozygous Δ32 gene variant [2••]. After transplantation and discontinuation of cART, HIV became undetectable and $CD4^+$ T cell counts normalized, demonstrating effective protection from HIV replication [2••,3••]. Unfortunately, this outcome could not be repeated in a later study [4•]. The first case has nevertheless brought a lot of attention to the curative potential of treatment strategies targeting the *CCR5* gene in HIV-infected patients. Consequently, new technologies for gene editing have been developed over the last few years that aim to mimic natural CCR5 deficiency. In this review, we describe the physiological role of CCR5, the recent advances made in developing CCR5-modifying methods and discuss their application towards HIV therapy.

Natural immune functions of CCR5

The chemokine receptor CCR5 is a seven-transmembrane segment protein and can interact with several proinflammatory C–C motif chemokines that are typically released as part of innate or adaptive immune responses. Many of these chemokines are also capable of binding to other chemokine receptors, whereas chemokine (C–C motif) ligand 4 (CCL4) appears to be largely specific for CCR5 [5]. The most potent agonist of human CCR5 yet described is CCL3-like 1 (CCL3L1) [6]. CCR5 is naturally expressed on the surface of a wide range of leukocytes including memory/effector T cells, natural killer cells, B cells, monocytes, and antigen-presenting cells such as dendritic cells and macrophages. Interaction of surface CCR5 with agonist chemokines induces intracellular signaling pathways, which (i) mediate leukocyte migration along the chemokine gradient to the site of inflammation and (ii) enhance local inflammatory immune responses by stimulating the proliferation and effector molecule secretion of leukocytes. CCR5 is thus involved in the regulation of cell migration and local immune activation. For completeness, it should be noted that CCR5 is also expressed on non-hematopoietic cells including osteoclasts, fibroblasts, vascular endothelium, epithelium and vascular smooth muscle cells, liver cells, and neurons where it may have other physiological functions that are not directly related to immune response [7].

CCR5 deficiency and natural HIV resistance

CCR5 is one of the major co-receptors for HIV entry into $CD4^+$ target cells. A natural occurring 32-base pair deletion in the CCR5 open reading frame (CCR5Δ32) introduces a premature stop codon and generates a shortened form of the protein that does not appear on the cell surface. The allelic frequency of the CCR5Δ32 deletion varies in populations from different ethnic groups. In

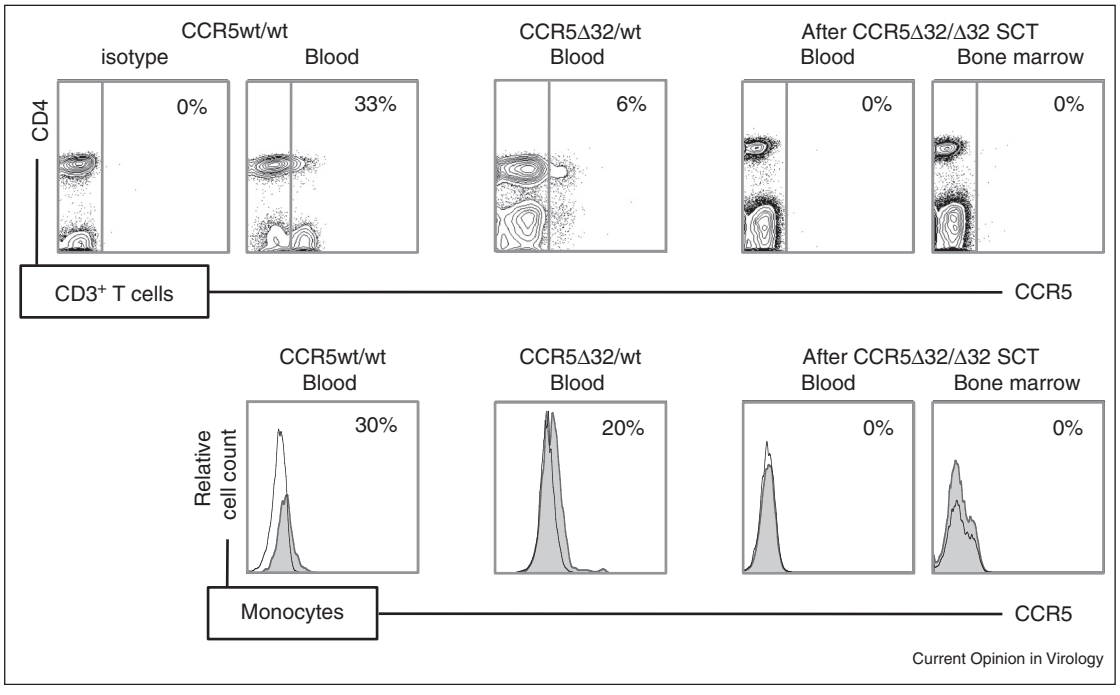
African and Asian people CCR5Δ32 is nearly non-existent, while in Caucasians, the frequency of the CCR5Δ32 allele is 10–20% and the prevalence of the homozygous mutation is 1–2% [8–10]. The homozygous genotype (CCR5Δ32/Δ32) leads to permanent absent cell surface expression of CCR5 and mediates resistance to HIV strains that use CCR5 for cell entry [11,12]. These observations have inspired the development of anti-HIV therapies that interrupt the interaction between the virus and CCR5.

Individuals with natural CCR5 deficiency are largely in healthy clinical conditions, except for impaired immune responses to some pathogens [13–16]. Absence of CCR5 surface expression may also exert a protective effect in inflammatory conditions including atherosclerosis and related cardiovascular disease, arthritis, and endotoxemia because of a defect in leukocyte and monocyte/macrophage trafficking [17–19]. In general, CCR5 seems to be dispensable for the proper function of the immune system, turning it into an excellent target for HIV therapy including cure approaches.

HIV cure by CCR5Δ32/Δ32 stem cell transplantation

Evidence for the curative potential of CCR5 disruption in HIV-infected persons comes from the success in eliminating HIV infection by allogeneic transplantation of naturally CCR5-deficient hematopoietic stem cells in a patient with long-known HIV infection and newly diagnosed acute myeloid leukemia that we have first reported about six years ago [2••,3••]. After depletion of the patient's CCR5Δ32/wild-type immune system, CCR5Δ32/Δ32 donor progenitor cells engrafted, expanded, and differentiated into mature lymphoid and myeloid cells that are resistant to HIV infection via CCR5 [2••] (Figure 1). The patient remained off cART following the transplantation and HIV in peripheral blood and certain tissues remained continuously undetectable. Today, this patient is regarded as cured of HIV infection and known as the 'Berlin patient'. Because of this remarkable success in clearing HIV from the immune system, permanent replacement of CCR5-expressing cells by CCR5-deficient cells is considered as the most promising approach to efficiently interrupt the interaction of HIV with its host cells. However, transplantation of naturally resistant donor cells for curative HIV therapy cannot find widespread application in clinical practice because allogeneic stem cell transplantations themselves are risky, with a 40–55% mortality rate [20–23], and are therefore only ethically acceptable in cancer patients without treatment alternatives. Also, the low prevalence of the CCR5Δ32/Δ32 gene variant in the general population limits the availability of naturally CCR5-deficient donor cells for stem cell

Figure 1



CCR5 surface expression on T cells and monocytes of individuals with the CCR5 wild-type genotype or heterozygosity for the CCR5Δ32 mutation and the Berlin patient after CCR5Δ32/Δ32 stem cell transplantation. T cells (upper part) and monocytes (lower part) in peripheral blood or bone marrow were analyzed for CCR5 surface expression. The frequency of CCR5-expressing cells is calculated by differences between the level of staining with a specific antibody (solid histogram) and the corresponding isotype control (open histogram).

transplantation. Alternative methods that mimic natural CCR5 deficiency and are broadly applicable to humans are therefore needed.

Artificial CCR5 deficiency

In theory, there are several possible ways to achieve artificial CCR5 deficiency: (i) extracellular blocking of CCR5 on HIV target cells, (ii) post-transcriptional down-regulation of *CCR5* gene expression by RNA interference-mediated gene silencing (knockdown), or (iii) permanent disruption of the *CCR5* gene (knockout). Blocking of cell surface CCR5 by exogenous drugs and gene silencing by RNA interference methods can reduce but not eliminate the CCR5 function as HIV coreceptor, and therefore can only serve as a supplement to the conventional anti-HIV therapy. By contrast, gene-editing methods change the genetic code and can provide a complete and irreversible elimination of gene function. In case of the *CCR5* gene, this would create a genetic resistance to CCR5-tropic HIV infection. Therefore, CCR5-targeted gene-editing methods have gained considerable attention in the field of HIV cure research.

Host genome editing

Novel technologies that enable site-specific changes in the genetic code include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic reapeats (CRISPR)-associated protein 9 (Cas9) systems. Currently the most widely used system in HIV-related human gene-editing studies is the ZFN system [24]. ZFNs are synthetic restriction enzymes composed of a target-specific zinc finger DNA-binding domain and an endonuclease domain that allow the sequence-selective cleavage of genomic target DNA [25,26]. After cleavage, cellular DNA repair pathways complete the precise edit. ZFNs can be used to disrupt the *CCR5* locus and allow the *de novo* generation of CCR5-deficient cells [27]. Thereby, biallelic *CCR5* gene disruption completely eliminates CCR5 surface expression, whereas monoallelic gene modification potentially reduces the molecule surface density. Most frequently used cell types for gene editing-based modification are $CD4^+$ T cells and $CD34^+$ hematopoietic stem cells. In theory, in the presence of CCR5-using viral strains, modified $CD4^+$ T cells provide a pool of HIV resistant cells with a survival advantage over unmodified cells and, consequently, expand within the recipient's immune system. Modified hematopoietic stem cells have the added advantage of continuously producing several distinct progeny cell types including HIV resistant monocytes/macrophages and dendritic cells. It has been demonstrated that application of CCR5-targeted ZFNs leads to 17–25% gene disruption in human stem cells and disrupts 50% of *CCR5* alleles in primary human $CD4^+$ T cells, whereby the frequency of biallelic gene disruption within the pool of modified cells varied between 33 and 40% [28•,29•,30•]. In immunodeficient mice, transplantation of total populations of human ZFN-treated cells led to the reduction of HIV levels [28•,30•]. Another study demonstrated safety and feasibility of ZFN-CCR5-modified autologous $CD4^+$ T cell infusions in HIV-infected patients [31••]. More recently, an optimized ZFN producing CCR5 modifications within the Δ32 region has been designed that confers high resistance against CCR5-tropic HIV infection with no significant off-target activity [32]. Collectively, these studies have established the artificial *CCR5* gene disruption by ZFNs systems followed by autologous cell transplantation as a reasonable and promising approach for the development of new strategies for HIV treatment. Consequently several trials are currently ongoing, as outlined below (Table 1). Compared to ZFN systems, TALEN and CRISPR technologies are still in earlier stages of development. However, first promising results for CCR5 modification using TALEN or CRISPR/Cas9 come from animal models and human cell studies [33–35] and indicate that these systems may become useful tools for the production of CCR5-deficient cells in the future. Interestingly, TALEN showed much lower cytotoxity and significantly lower off-target activity than ZFNs [33].

Clinical trials

We searched http://clinicaltrials.gov/for clinical trials of the application of CCR5-targeted gene editing in HIV-infected patients. A summary is outlined in Table 1. Target cells for ZFN-mediated *CCR5* gene disruption used in all of these trials are autologous $CD4^+$ T cells. The study designs focus on various conditions, which could affect the persistence of modified cells, including those aiming at increasing the engraftment of reinfused cells through the administration of low non-myeloablative doses of cyclophosphamide.

A recently published Phase I study describes the feasibility and safety of CCR5-modified autologous $CD4^+$ T cell infusions [31••]. In this case series study, twelve aviremic HIV-infected patients on cART received a single-dose infusion of *ex vivo* expanded, autologous $CD4^+$ T cells that had been modified at the *CCR5* gene by ZFNs. One serious adverse event occurred in a single patient who developed fever, chills and joint/back pain within one day after infusion. All study patients showed a significant increase in the peripheral $CD4^+$ T cell count at one week post-infusion and cells carrying a modification in one or both alleles of the *CCR5* gene constituted, on average, around 14% of circulating $CD4^+$ T cells. Six of the patients underwent treatment interruption from week 4 to 16 post infusion. There was a rapid viral rebound in all six patients and treatment interruption had to be terminated in two of these patients due to high viral loads. In patients who completed the treatment interruption period, the viral load decreased continuously from the peak level during the absence of cART. Interestingly, one patient had a relatively low and late peak level of viremia

Table 1

Clinical trials of CCR5 gene editing-based cell therapy for the treatment of HIV-infected persons.

Intervention	Phase	Recruited subjects (*n*)	Outcome measures	Status (ClinicalTrials.gov identifier)	Institution/ Company
Single dose of ZNF-modified autologous $CD4^+$ T cells	I	Patients on cART with or without treatment failure (12)	PR: Safety, Side-effect profile SRY: Effect on viral load and T cells	Published [31••] (NCT00842634)	University of Pennsylvania, Albert Einstein College of Medicine, Sangamo Biosciences
Escalating doses of ZNF-modified autologous $CD4^+$ T cells	I	Patients on cART with or without heterozygosity for CCR5Δ32 mutation (19)	PR: Safety SRY: Long-term persistence and activity of modified cells	Completed (NCT01044654)	Sangamo Biosciences
Single dose of ZFN-modified autologous $CD4^+$ T cells	I/II	Untreated viremic patients (21)	PR: Safety and tolerability SRY: Persistence of modified cells, Effect on HIV and $CD4^+$ T cells	Completed (NCT01252641)	Sangamo Biosciences
Single dose of ZFN-modified autologous $CD4^+$ T cells with and without cyclophosphamide conditioning/pretreatment	I	Aviremic patients on cART with or without CCR5Δ32 mutation (15)	PR: Safety	Recruiting (NCT02388594)	University of Pennsylvania, National Institute of Allergy and Infectious Diseases
Escalating doses of cyclophosphamide administered before single dose infusion of ZFN-modified autologous $CD4^+$ T cells	I/II	Aviremic patients on cART (26)	PR: Safety SRY: Engraftment of modified cells, Effect on HIV and $CD4^+$ T cells	Recruiting (NCT01543152)	Sangamo Biosciences

PR: primary outcome; SRY: secondary outcome.

and then controlled HIV to an undetectable level until week 16. This patient was subsequently found to be heterozygous for the CCR5Δ32 mutation. It is therefore likely that the rate of biallelic knockout of the *CCR5* gene and consequent complete disruption of CCR5 surface expression in the reinfused pool of $CD4^+$ T cells was higher in comparison with the remaining patients. Effective viral control thus probably depends on the degree of biallelic disruption of the *CCR5* gene, highlighting the need for strategies that ensure highly efficient *CCR5* gene knockout on a single cell level. However, in all twelve patients total $CD4^+$ T cells and numbers of gene-modified progressively declined during the treatment interruption of 12 weeks. Gene-modified cells remained detectable during the long-term follow up and represented $<2\%$ of $CD4^+$ T cells in the peripheral blood after the longest observation period of 42 months.

Conclusions

Some promising progress has been made in gene-editing technology since proof-of-principle for CCR5-deficient stem cell therapy in HIV infection was first published in 2009 [3••]. The efficiency of biallelic CCR5 gene disruption seems to need further improvement in order to achieve an HIV-resistant cell pool with high effectiveness. The critical threshold for the number of reinfused CCR5-deficient cells required for effective viral control is unknown at this point but, if efficient enough, CCR5-directed manipulation of the host immune system may indeed have potential as curative HIV therapy. However, while disabling replication of CCR5 using viral strains, this manipulated host immune system would still be susceptible for C–X–C chemokine receptor 4 (CXCR4)-using HIV variants, which usually emerge during later stages of HIV infection, are associated with high viral loads, and can replicate in the absence of CCR5 expression. Consequently, therapeutic application of CCR5-disrupting gene-editing methods will be limited to HIV-infected persons not harboring CXCR4-using viral strains. Manipulation of the *CXCR4* gene locus in humans is generally problematic as it can have serious consequences as a result of its indispensable immunological functions [36,37]. Also, in the setting of reinfused *ex vivo* CCR5-modified $CD4^+$ T cells, other cell types such as monocytes, macrophages, and dendritic cells remain potent target populations of CCR5-using HIV and facilitate ongoing viral replication, which in turn enables the evolution and outgrowth of CXCR4-using viral variants. One approach to create a lifelong source of both lymphoid as well as myeloid HIV resistant immune cells would be to infuse CCR5-disrupted autologous hematopoietic stem cells. However,

efficient engraftment of stem cells requires the preconditioning with agents known to induce a broad range of complications including toxic injuries, severe cytopenias, and to increase the risk for the development of malignancies.

Finally, elimination of the latent viral reservoir remains to be addressed. Long-lived cells latently infected with replication-competent HIV will continue to produce virions even after engraftment of genetically modified cells. Therefore, residual non-modified $CD4^+$ cells would still be significant sources of viral growth that may eventually lead to the emergence of CXCR4-using HIV variants and consequent therapeutic failure. In that context, comparing the outcomes of the recently published case of an HIV-infected patient who experienced a rapid viral rebound after allogeneic CCR5Δ32/Δ32 stem cell transplantation [4•] and the Berlin patient case [2••] is interesting. Based on the dramatic difference in the outcomes, it can be speculated that the viral reservoir differed in size, distribution and/or quality between the two patients and, as a result, was more efficiently eliminated in the Berlin patient. Homozygosity for the CCR5Δ32 mutation that was present in the HIV-infected Berlin patient before the transplantation could have been beneficial in this regard because monoallelic CCR5 expression in HIV target cells may have a protective effect against the formation and/or stability of viral reservoirs [38,39]. This is also suggested by the two cases of HIV-infected patients in Boston who were heterozygous for the CCR5Δ32 mutation before they received CCR5 wild-type stem cells and converted to full donor chimerism [40]. Although HIV typically rebounds from persistent viral reservoirs within days of cART interruption after stem cell transplantation [41,42], HIV remained undetectable for three and 8 months after cART discontinuation in the two Boston patients suggesting that the CCR5Δ32/wt immune system may harbor reduced reservoirs of replication-competent HIV [43]. Fortunately, our knowledge about the biology of the viral reservoir continually improves and the development of therapeutic strategies aimed at the elimination of the latent HIV reservoir is progressing [44,45].

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft (grant SCHN616/6-2).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E *et al.*: **Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study**. *Science* 1996, **273**:1856-1862.

2. •• Allers K, Hutter G, Hofmann J, Loddenkemper C, Rieger K, Thiel E, Schneider T: **Evidence for the cure of HIV infection by CCR5Delta32/Delta32 stem cell transplantation**. *Blood* 2011, **117**:2791-2799.
This is the follow-up of the first case study of a HIV-infected patient who received an allogenic transplantation with CCR5Δ32/Δ32 stem cells. After transplantation and discontinuation of cART, HIV remained undetectable and $CD4^+$ T cells normalized. This patient is considered cured of HIV infection and known as the 'Berlin patient'.

3. •• Hutter G, Nowak D, Mossner M, Ganepola S, Mussig A, Allers K, Schneider T, Hofmann J, Kucherer C, Blau O *et al.*: **Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation**. *N Engl J Med* 2009, **360**:692-698.
This is the first case study of a HIV-infected patient who received an allogenic transplantation with CCR5Δ32/Δ32 stem cells. After transplantation and discontinuation of cART, HIV was not detectable.

4. • Kordelas L, Verheyen J, Beelen DW, Horn PA, Heinold A, Kaiser R, Trenschel R, Schadendorf D, Dittmer U, Esser S: **Shift of HIV tropism in stem-cell transplantation with CCR5 Delta32 mutation**. *N Engl J Med* 2014, **371**:880-882.
This is the second case study of a HIV-infected patient who received an allogenic transplantation with CCR5Δ32/Δ32 stem cells. By contrast to the ‚Berlin patient' case, the authors describe here a rapid viral rebound after transplantation with a shift from dominantly CCR5-tropic HIV toward CXCR4-using HIV strains. This case shows that viral escape mechanisms can prevent the control of HIV after CCR5-targeted treatments.

5. Wells TN, Power CA, Shaw JP, Proudfoot AE: **Chemokine blockers — therapeutics in the making?** *Trends Pharmacol Sci* 2006, **27**:41-47.

6. Nibbs RJ, Yang J, Landau NR, Mao JH, Graham GJ: **LD78beta, a non-allelic variant of human MIP-1alpha (LD78alpha), has enhanced receptor interactions and potent HIV suppressive activity**. *J Biol Chem* 1999, **274**:17478-17483.

7. Rottman JB, Ganley KP, Williams K, Wu L, Mackay CR, Ringler DJ: **Cellular localization of the chemokine receptor CCR5. Correlation to cellular targets of HIV-1 infection**. *Am J Pathol* 1997, **151**:1341-1351.

8. Martinson JJ, Chapman NH, Rees DC, Liu YT, Clegg JB: **Global distribution of the CCR5 gene 32-basepair deletion**. *Nat Genet* 1997, **16**:100-103.

9. Novembre J, Galvani AP, Slatkin M: **The geographic spread of the CCR5 Delta32 HIV-resistance allele**. *PLoS Biol* 2005, **3**:e339.

10. Su B, Sun G, Lu D, Xiao J, Hu F, Chakraborty R, Deka R, Jin L: **Distribution of three HIV-1 resistance-conferring polymorphisms (SDF1-3'A CCR2-641, and CCR5-delta32) in global populations**. *Eur J Hum Genet* 2000, **8**:975-979.

11. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA, Landau NR: **Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection**. *Cell* 1996, **86**:367-377.

12. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapoumeroulie C, Cognaux J, Forceille C *et al.*: **Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene**. *Nature* 1996, **382**:722-725.

13. Kindberg E, Mickiene A, Ax C, Akerlind B, Vene S, Lindquist L, Lundkvist A, Svensson L: **A deletion in the chemokine receptor 5 (CCR5) gene is associated with tickborne encephalitis**. *J Infect Dis* 2008, **197**:266-269.

14. Lim JK, Murphy PM: **Chemokine control of West Nile virus infection**. *Exp Cell Res* 2011, **317**:569-574.

15. Telenti A: **Safety concerns about CCR5 as an antiviral target**. *Curr Opin HIV AIDS* 2009, **4**:131-135.

16. Glass WG, McDermott DH, Lim JK, Lekhong S, Yu SF, Frank WA, Pape J, Cheshier RC, Murphy PM: **CCR5 deficiency increases risk of symptomatic West Nile virus infection**. *J Exp Med* 2006, **203**:35-40.

17. Jones KL, Maguire JJ, Davenport AP: **Chemokine receptor CCR5: from AIDS to atherosclerosis**. *Br J Pharmacol* 2011, **162**:1453-1469.

18. Zhao Q: **Dual targeting of CCR2 and CCR5: therapeutic potential for immunologic and cardiovascular diseases**. *J Leukoc Biol* 2010, **88**:41-55.

19. Prahalad S: **Negative association between the chemokine receptor CCR5-Delta32 polymorphism and rheumatoid arthritis: a meta-analysis**. *Genes Immun* 2006, **7**:264-268.

20. Pavletic ZS, Arrowsmith ER, Bierman PJ, Goodman SA, Vose JM, Tarantolo SR, Stein RS, Bociek G, Greer JP, Wu CD *et al.*: **Outcome of allogeneic stem cell transplantation for B cell chronic lymphocytic leukemia**. *Bone Marrow Transplant* 2000, **25**:717-722.

21. Sobecks RM, Leis JF, Gale RP, Ahn KW, Zhu X, Sabloff M, de Lima M, Brown JR, Inamoto Y, Hale GA *et al.*: **Outcomes of human leukocyte antigen-matched sibling donor hematopoietic cell transplantation in chronic lymphocytic leukemia: myeloablative versus reduced-intensity conditioning regimens**. *Biol Blood Marrow Transplant* 2014, **20**:1390-1398.

22. Doney KC, Chauncey T, Appelbaum FR: **Allogeneic related donor hematopoietic stem cell transplantation for treatment of chronic lymphocytic leukemia**. *Bone Marrow Transplant* 2002, **29**:817-823.

23. Toze CL, Shepherd JD, Connors JM, Voss NJ, Gascoyne RD, Hogge DE, Klingemann HG, Nantel SH, Nevill TJ, Phillips GL *et al.*: **Allogeneic bone marrow transplantation for low-grade lymphoma and chronic lymphocytic leukemia**. *Bone Marrow Transplant* 2000, **25**:605-612.

24. Owens B: **Zinc-finger nucleases make the cut in HIV**. *Nat Rev Drug Discov* 2014, **13**:321-322.

25. Urnov FD, Miller JC, Lee YL, Beausejour CM, Rock JM, Augustus S, Jamieson AC, Porteus MH, Gregory PD, Holmes MC: **Highly efficient endogenous human gene correction using designed zinc-finger nucleases**. *Nature* 2005, **435**:646-651.

26. Isalan M: **Zinc-finger nucleases: how to play two good hands**. *Nat Methods* 2012, **9**:32-34.

27. Yao Y, Nashun B, Zhou T, Qin L, Qin L, Zhao S, Xu J, Esteban MA, Chen X: **Generation of CD34+ cells from CCR5-disrupted human embryonic and induced pluripotent stem cells**. *Hum Gene Ther* 2012, **23**:238-242.

28. • Perez EE, Wang J, Miller JC, Jouvenot Y, Kim KA, Liu O, Wang N, Lee G, Bartsevich VV, Lee YL *et al.*: **Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases**. *Nat Biotechnol* 2008, **26**:808-816.
Using engineered ZFNs, the authors demonstrate *CCR5* gene disruption in primary human $CD4^+$ T cells at a mean frequency of 50% of total alles within the cell population. HIV-infected mice engrafted with these ZFN-modified human $CD4^+$ T cells had lower viremia and higher peripheral $CD4^+$ T cell counts than control mice.

29. • Li L, Krymskaya L, Wang J, Henley J, Rao A, Cao LF, Tran CA, Torres-Coronado M, Gardner A, Gonzalez N *et al.*: **Genomic editing of the HIV-1 coreceptor CCR5 in adult hematopoietic stem and progenitor cells using zinc finger nucleases**. *Mol Ther* 2013, **21**:1259-1269.
Using engineered ZFNs, the authors demonstrate *CCR5* gene disruption in human stem/progenitor cells at a mean frequency of 25% of total alles within the cell population. The authors demonstrate that the rate of *CCR5* gene disruption can be enhanced by treatment of the cells with protein kinase C activators.

30. • Holt N, Wang J, Kim K, Friedman G, Wang X, Taupin V, Crooks GM, Kohn DB, Gregory PD, Holmes MC *et al.*: **Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo**. *Nat Biotechnol* 2010, **28**:839-847.
Using engineered ZFNs, the authors demonstrate *CCR5* gene disruption in human stem/progenitor cells at a mean frequency of 17% of total alles within the cell population. Tranplantation of immunodeficient mice with these ZFN-modified human stem cells led to multilineage immune reconstitution and, after challenge with CCR5 tropic HIV, to the reduction of HIV levels.

31. •• Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, Spratt SK, Surosky RT, Giedlin MA, Nichol G *et al.*: **Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV**. *N Engl J Med* 2014, **370**:901-910.
In this phase I study, twelve patients received a single-dose infusion of *ex vivo* expanded ZFN-CCR5-modified autologous $CD4^+$ T cells. This is the first study demonstrating safety of CCR5-modified autologous $CD4^+$ T cell infusions in HIV-infected patients.

32. Badia R, Riveira-Munoz E, Clotet B, Este JA, Ballana E: **Gene editing using a zinc-finger nuclease mimicking the CCR5Delta32 mutation induces resistance to CCR5-using HIV-1**. *J Antimicrob Chemother* 2014, **69**:1755-1759.

33. Mussolino C, Morbitzer R, Lutge F, Dannemann N, Lahaye T, Cathomen T: **A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity**. *Nucleic Acids Res* 2011, **39**:9283-9293.

34. Cho SW, Kim S, Kim JM, Kim JS: **Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease**. *Nat Biotechnol* 2013, **31**:230-232.

35. Ye L, Wang J, Beyer AI, Teque F, Cradick TJ, Qi Z, Chang JC, Bao G, Muench MO, Yu J *et al.*: **Seamless modification of wild-type induced pluripotent stem cells to the natural CCR5Delta32 mutation confers resistance to HIV infection**. *Proc Natl Acad Sci U S A* 2014, **111**:9591-9596.

36. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR: **Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development**. *Nature* 1998, **393**:595-599.

37. Lapidot T: **Mechanism of human stem cell migration and repopulation of NOD/SCID and B2mnull NOD/SCID mice. The role of SDF-1/CXCR4 interactions**. *Ann N Y Acad Sci* 2001, **938**:83-95.

38. van Rij RP, Portegies P, Hallaby T, Lange JM, Visser J, de Roda Husman AM, van 't Wout AB, Schuitemaker H: **Reduced prevalence of the CCR5 delta32 heterozygous genotype in human immunodeficiency virus-infected individuals with AIDS dementia complex**. *J Infect Dis* 1999, **180**:854-857.

39. Wang C, Abdel-Mohsen M, Strain MC, Lada SM, Yukl S, Cockerham LR, Pilcher CD, Hecht FM, Sinclair E, Liegler T *et al.*: **Decreased HIV type 1 transcription in CCR5-Delta32 heterozygotes during suppressive antiretroviral therapy**. *J Infect Dis* 2014, **210**:1838-1843.

40. Henrich TJ, Hu Z, Li JZ, Sciaranghella G, Busch MP, Keating SM, Gallien S, Lin NH, Giguel FF, Lavoie L *et al.*: **Long-term reduction in peripheral blood HIV type 1 reservoirs following reduced-intensity conditioning allogeneic stem cell transplantation**. *J Infect Dis* 2013, **207**:1694-1702.

41. Avettand-Fenoel V, Mahlaoui N, Chaix ML, Milliancourt C, Burgard M, Cavazzana-Calvo M, Rouzioux C, Blanche S: **Failure of bone marrow transplantation to eradicate HIV reservoir despite efficient HAART**. *AIDS* 2007, **21**:776-777.

42. Mavigner M, Watkins B, Lawson B, Lee ST, Chahroudi A, Kean L, Silvestri G: **Persistence of virus reservoirs in ART-treated SHIV-infected rhesus macaques after autologous hematopoietic stem cell transplant**. *PLoS Pathog* 2014, **10**:e1004406.

43. Henrich TJ, Hanhauser E, Marty FM, Sirignano MN, Keating S, Lee TH, Robles YP, Davis BT, Li JZ, Heisey A *et al.*: **Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases**. *Ann Intern Med* 2014, **161**:319-327.

44. Barouch DH, Deeks SG: **Immunologic strategies for HIV-1 remission and eradication**. *Science* 2014, **345**:169-174.

45. Passaes CP, Saez-Cirion A: **HIV cure research: advances and prospects**. *Virology* 2014, **454-455**:340-352.