Preclinical Evaluation of a Novel TALEN Targeting CCR5 Confirms Efficacy and Safety in Conferring Resistance to HIV-1 Infection

Marianna Romito, Alexandre Juillerat, Yik Lim Kok, Markus Hildenbeutel, Manuel Rhiel, Geoffroy Andrieux, Johannes Geiger, Carsten Rudolph, Claudio Mussolino, Aymeric Duclert, Karin J. Metzner, Philippe Duchateau, Toni Cathomen, and Tatjana I. Cornu*

Therapies to treat patients infected with human immunodeficiency virus (HIV) aim at preventing viral replication but fail to eliminate the virus. Although transplantation of allogeneic CCR5Δ32 homozygous stem cell grafts provided a cure for a few patients, this approach is not considered a general therapeutic strategy because of potential side effects. Conversely, gene editing to disrupt the C-C chemokine receptor type 5 (CCR5) locus, which encodes the major HIV coreceptor, has shown to confer resistance to CCR5-tropic HIV strains. Here, an engineered transcription activator-like effector nuclease (TALEN) that enables efficient CCR5 editing in hematopoietic cells is presented. After transferring TALEN-encoding mRNA into primary CD4+ T cells, up to 89% of CCR5 alleles are disrupted. Genotyping confirms the genetic stability of the CCR5-edited cells, and genome-wide off-target analyses established the absence of relevant mutagenic events. When challenging the edited T cells with CCR5-tropic HIV, protection in a dose-dependent manner is observed. Functional assessments reveal no significant differences between edited and control cells in terms of proliferation and their ability to secrete cytokines upon exogenous stimuli. In conclusion, a highly active and specific TALEN to disrupt CCR5 is successfully engineered, paving the way for its clinical application in hematopoietic stem cell grafts.

1. Introduction

As of today, there is no cure for infection with human immunodeficiency virus type 1 (HIV-1). The available antiretroviral therapies (ART) target different steps in the HIV replication cycle. Although ART manages to reduce the HIV burden below the detection limit as long as the medication is taken, it does not eradicate the virus. Inhibition of HIV entry has been considered a promising strategy that was explored in numerous ways. In particular, targeting C-C chemokine receptor type 5 (CCR5), the co-receptor of prevalent HIV-1 strains has been pursued using CCR5 inhibitors, or blocking antibodies.[1] But again, even when combined with other ARTs, they do not eradicate HIV.

On the other hand, the knowledge that an naturally occurring 32 nucleotide deletion (Δ32) mutation in the CCR5 locus leads to resistance to CCR5-tropic (R5-tropic) HIV-1 strains,[2,3] has spurred some encouraging
approaches. People with a homozygous Δ32 mutation are rare, clinically unremarkable, and conduct a normal life. In 2007, an HIV-positive patient, later referred to as the “Berlin Patient,” was transplanted with an allogeneic stem cell graft of an human leukocyte antigen (HLA)-matched donor homozygous for the CCR5Δ32 mutation after diagnosis of acute myeloid leukemia. After stopping ART, the patient was closely monitored for virus titers. As of today, that is, 13 years later, no viral RNA or proviral DNA has been detected, so that he was declared the first patient to be cured of HIV. Following the principle of the Berlin patient, two more patients, known as the London[9,10] and the Düsseldorf[11] patients, might be declared as cured. These observations underline the possibility to transfer HIV resistance from a donor to a patient. However, due to the potentially severe side effects of allogenic transplantations and the difficulty of finding matching homozygous Δ32 donors, this strategy is not considered a universal approach to treat patients affected by HIV.

Alternatively, CCR5 gene editing in autologous cells represents a potent approach to provide patients with cells resistant to HIV. To this end, genome editing with programmable nucleases, in particular, zinc-finger nucleases (ZFN), transcription activation-like effector nucleases (TALENs), MegaTALs, and clustered regularly interspaced short palindromic repeat (CRISPR) associated protein (CRISPR-Cas) nucleases, have been used in several preclinical and clinical studies to disrupt the CCR5 gene in primary human CD4+ T cells or hematopoietic stem and progenitor cells (HSCs). In a clinical trial conducted by Tebas et al., 12 individuals living with HIV received a single dose of CCR5-edited autologous CD4+ T cells. Although the effect lasted only transiently, the blood level of HIV provirus decreased in most patients and the infusion of CCR5-modified autologous CD4+ T cells proved to be safe. To achieve long-lasting effects, however, CCR5 must be modified in HSCs. This ensures that HIV resistance will be conferred to all CD4+ immune cells, that is, also macrophages and dendritic cells. In reported clinical scale validation runs, 25–71% of CCR5 alleles were edited in HSCs but the used ZFN revealed high off-target (OT) activities, suggesting a relevant genotoxic potential in the stem cell compartment. HSCs were also edited with CRISPR-Cas nucleases for the treatment of an HIV-positive patient with acute lymphocytic leukemia. The CCR5 editing frequency in the graft was ≈18% and was further diluted by the concomitant transplantation of unedited HSCs. Not surprisingly, HIV rebound was observed during treatment interruption. Hence, while the concept of conferring HIV resistance by disrupting CCR5 has been known for over a decade, the employed designer nucleases revealed low to intermediated disruption efficiencies (usually 20–50% allelic knockout frequencies) and/or undesirable OT effects. We conjecture that for a CCR5 editing approach to be successful in the clinic, CCR5 editing in HSCs must be both highly efficient, >50% biallelic knockout, and highly specific, to provide the patient with a safe graft and a sufficient number of HIV-resistant immune cells.

In our study, we validated a novel TALEN for its ability to disrupt the CCR5 allele with both high activity and high specificity, using settings transferrable to clinical-grade manufacturing. To demonstrate resistance to infection with HIV-1, CCR5 editing was performed in primary CD4+ T cells. While TALENs were employed to disrupt CCR5 in human cells before,[16,23,24] here we show that an improved TALEN design in combination with novel RNA transfer technologies[25] enabled us to knockout CCR5 in CD4+ T cells with high activity and high specificity. The manufactured T cells were HIV resistant, genetically stable, and maintained their potency.

2. Results

2.1. CCR5 Knockout is Genetically Stable in CD4+ T Cells

The target locus in exon 3 of the CCR5 gene was chosen to be in the region coding for the N-terminus of the CCR5 protein (Figure 1A). The rationale was twofold: 1) out-of-frame insertion/deletion (indel) mutations would cause frameshift mutations that lead to early termination of translation, and 2) this sequence stretch codes for a conserved region of CCR5 allele with both high activity and high specificity, using settings transferrable to clinical-grade manufacturing. To demonstrate resistance to infection with HIV-1, CCR5 editing was performed in primary CD4+ T cells. While TALENs were employed to disrupt CCR5 in human cells before,[16,23,24] here we show that an improved TALEN design in combination with novel RNA transfer technologies[25] enabled us to knockout CCR5 in CD4+ T cells with high activity and high specificity. The manufactured T cells were HIV resistant, genetically stable, and maintained their potency.

and was further diluted by the concomitant transplantation of unedited HSCs. Not surprisingly, HIV rebound was observed during treatment interruption. Hence, while the concept of conferring HIV resistance by disrupting CCR5 has been known for over a decade, the employed designer nucleases revealed low to intermediated disruption efficiencies (usually 20–50% allelic knockout frequencies) and/or undesirable OT effects. We conjecture that for a CCR5 editing approach to be successful in the clinic, CCR5 editing in HSCs must be both highly efficient, >50% biallelic knockout, and highly specific, to provide the patient with a safe graft and a sufficient number of HIV-resistant immune cells.

Dr. J. Geiger, Dr. C. Rudolph
ethris GmbH
Planegg 81249 Germany
Dr. A. Ducert, Dr. P. Duchateau
Cellectis S.A.
Paris 75013 France
Figure 1. Stable CCR5 knockout in CD4+ T cells. A) Schematic of the CCR5 locus with TALEN binding sites (boxes). B) Quantification of TALEN activity. T7E1 assays were performed in CD4+ T cells at days 7 or 21 post-electroporation. Where indicated, cells were transiently incubated at 32 °C for 24 h. Samples derived from a Δ32 heterozygous donor are highlighted in red (n = 3–7 donors; ±SD). C) CCR5 expression in CD4+ T cells. Cells were electroporated with only left TALEN arm (L–L) or both TALEN arms (L+R). Data is shown relative to untreated (UT) cells. Each dot represents a different donor (n = 2–7; ±SD; ns = not significant, nd = not determined, *p ≤ 0.05; ***p ≤ 0.001, paired t-test). D) Frequency of mutations. Targeted amplicon sequencing of the target site was performed after 7 and 21 days of culture (n = 3 donors; ±SD). Displayed are the frequencies of all indels with a frequency ≥1 in at least one of the three experiments. E) The sequence of induced mutations. Displayed are all sequences quantified in (D).
7 and 21, respectively (Figure 1D,E). The indel patterns and frequencies remained comparable between samples and over time. There was no significant enrichment or depletion of a specific indel type detected by a paired t-test during the culture period. This indicates that the clonal distribution of the edited cells between the two timepoints remained stable. In all three donors, the most frequent indel was a 19-nt deletion, followed by a 9-nt deletion, that combined represented about 20% of the total indel mutations. Of note, the four most frequent deletions
\[-19, -9, -13, \text{ and } -26\] are likely due to microhomologies, as detected by the Microhomology-Predictor tool.\(^{[29]}\)

2.2. CCR5-Edited CD4+ T Cells are Resistant to HIV-1 Infection

As a proof of concept, we challenged the edited cells at an early and late culture timepoint with GFP-expressing lentiviral vectors pseudotyped with either the gp160 glycoprotein of the HIV-1 R5-tropic strain BaL or VSV-G as control. The CCR5 edited CD4+ T cells showed \(\approx50\%\) reduced susceptibility to transduction with the HIV-gp160 pseudotyped vector when compared to control cells (Figure 2A). When challenging T cells from two \(\Delta32\) heterozygous donors, a decreased susceptibility—similar to cells from the other donors—was observed. As a CD4/CCR5-independent transduction control, we transduced the cells in parallel with VSV-G pseudotyped lentivectors. Under these conditions, we did not see any alteration in transduction efficiency.

Next, we tested the resistance of CCR5-edited CD4+ T cells to replication-competent HIV-1 by infecting them with either R5-tropic HIV-1\(_{\text{JRFL}}\) or CXCR4-tropic (X4-tropic) HIV-1\(_{\text{NL4-3}}\). HIV-1 p24 was quantified in the supernatant of infected cells as an measure of viral replication (Figure S1A,B, Supporting Information). In samples infected with HIV-1\(_{\text{JRFL}}\) (high or low multiplicity of infection [MOI]), a significant protective effect \((p = 8.8 \times 10^{-4} \text{ and } p = 5.7 \times 10^{-5})\) was observed when compared to infection of unedited cells. As expected, infections with X4-tropic HIV-1\(_{\text{NL4-3}}\) did not reveal significant differences (Figure S1B, Supporting Information). Furthermore, to investigate the impact of the editing rate on the viral replication, we spiked edited CD4+ T cells with unedited cells at ratios of 1:1 (50% edited cells) and 1:3 (25% edited cells). Low MOI infection with HIV-1JRFL, as determined by p24 in the supernatant, was significantly \((p = 2.72 \times 10^{-14})\) reduced in non-diluted edited cells (100%) when compared to unedited samples (Figure 2B). The protective effect was also observed in both the 50% and 25% mix \((p = 8.38 \times 10^{-11}, p = 1.13 \times 10^{-4})\), suggesting that a cell population with \(\approx20\%\) of edited CCR5 alleles was able to slow down virus replication. A significant protective effect \((p = 3.06 \times 10^{-2})\) was also observed when edited cells (100%) were infected with a high MOI (Figure 2C), albeit to a lower degree as compared to the low MOI infection. After dilution (50% mix), the protective effect was lost when cells were infected at high MOI \((p = 0.98)\). In the control settings with HIV-1\(_{\text{NL4-3}}\) infections, we did not see any impairment of virus replication in the edited cells (Figure 2D), as expected. We conclude...
that editing of CD4+ T cells with CCR5 targeting TALEN leads to protection against R5-tropic HIV-1 in an editing dose-dependent manner.

### 2.3. CCR5-Edited CD4+ T Cells Maintain Potency

Transfer of mRNA to T cells via electroporation can potentially affect cell viability and/or functionality. We addressed this point by evaluating viabilities 24 h post-treatment. CD4+ T cells electroporated with TALEN mRNA (both arms, or left arm only), GFP mRNA, or mock pulsed, showed a decrease in viabilities of ≈30% when compared to untreated samples (Figure 3A), implying that the electric pulse but not the RNA transfer had an impact on T cell viabilities. On the other hand, the transient temperature shift to 32 °C did not affect viability.

Next, we wanted to investigate if CCR5-edited cells retain their proliferative potential. To this end, the edited CD4+ T cells and
control samples were cultivated under proliferation activating conditions. The total number of cells at days 7, 14, and 21 were comparable for all tested conditions (Figure 3B,C). A time-series analysis confirmed that the samples, independent of cultivation temperature or treatment, did not show significant differences in proliferation. Hence, we conclude that editing CCR5 did not affect the expansion capabilities of CD4+ T cells.

To explore if editing CCR5 has an impact on CD4+ T cell function in terms of cytokine release upon stimulation, we stimulated the cells with either phorbol 12-myristate 13-acetate (PMA), ionomycin or CD3/CD28/CD2 to trigger the release of TNF-α, IFN-γ or IL-2, respectively. Supernatants of electroporated T cells were harvested 8–48 h after stimulation and analyzed by cytometric bead array. While we observed variations in the amounts of secreted cytokines among the four donors, the values did not significantly (two-sided t-test) differ when comparing the various treatment regimens (Figure 3D–F). Unstimulated cells did not release measurable amounts of cytokines. In sum, these results demonstrate that editing CCR5 did not impair the ability of the engineered CD4+ T cells to respond to the different stimuli.

### 2.4. Editing CCR5 in CD4+ T Cells with High Specificity

To assess the genotoxic potential of TALEN mediated editing of CCR5, we investigated the occurrence of OT activity by two different means: targeted amplicon sequencing of in silico predicted OTs or an unbiased OT detection approach. The top 20 potential OTs were predicted using the PROGNOS tool. Amplicons from edited samples were compared to amplicons of untreated samples (Figure 4A). Two of the top 20 predicted OTs revealed some low but significant OT activity over background (Table S2, Supporting Information). However, both OT01 (0.12% versus 0.10% background, p = 0.000211) and OT10 (0.08% versus 0.07% background, p = 0.004422) are situated in intronic regions. These data were complemented with an unbiased OT detection approach termed oligonucleotide capturing assay (OCA), which is based on GUIDE-seq. The highest score (OCA1) was obtained for the CCR5 target site (Figure 4B). While OT01 was also found by OCA (OCA03), OT10 did not match with any of the top 24 OCA hits (Table S3, Supporting Information). On the other hand, CCR2 did not come up as a potential OT in PROGNOS but a weak activity at CCR2 was picked up by OCA (OCA4). Importantly, all identified OCA sites had a considerably lower score than the CCR5 on-target site (OCA1). Together, these OT analyses demonstrate that the employed TALENs are highly specific designer nucleases with minimal OT activity.

### 3. Discussion

Approaches to disrupt CCR5 in human primary CD4+ T cells and HSCs have been previously described. These reports demonstrated the feasibility of abrogating HIV entry, albeit with some drawbacks. This includes low gene disruption efficiencies, high off-target effects or absence of specificity analyses, missing potency analyses, and/or lack of compatibility with good manufacturing practice (GMP).

In this study, we present efficient editing of the CCR5 locus in primary CD4+ T cells in a GMP-compatible manner. GMP compatibility is defined as a condition that uses GMP-compliant devices and research-grade reagents that are available as GMP-grade material as well. Under these specifications, we disrupted up to 90% of CCR5 alleles with no notable off-target activity, suggesting that the employed TALEN are highly specific. Disrupting the region that encodes the N-terminus of the CCR5 protein in CD4+ T cells gave rise to cells resistant to infection with R5-tropic HIV-1 but, as expected, not X4-tropic virus. Importantly, CCR5-edited CD4+ T cells did not show any differences to non-edited control cells in the applied proliferation and potency assays. This indicates that TALEN-mediated editing of CCR5 is efficacious and safe, opening a window for therapeutic applications in CD34+ HSCs.

Our data further demonstrate that low CCR5 editing frequency is not sufficient to abrogate HIV infection, putting forward that efficient disruption that mediates bi-allelic gene knockouts are paramount to see clinical effects. This is in line with a recently published case report confirming that a CCR5 knockout frequency of 18% in transplanted HSCs is not sufficient to achieve clinical benefit. Another crucial point for a clinical trial is to recruit patients exclusively positive for R5-tropic HIV variants since the absence of CCR5 would favor the propagation of X4-tropic viruses if present in the patient. The importance of proper examination was seen in the “Essen patient” who suffered from an X4 rebound after allogeneic transplantation of a Δ32 homozygous HSC graft. Performing a knockout of both coreceptor encoding loci, CCR5 and CXCR4, can be considered for CD4+ T cells but not for HSCs, as
CXC4 is essential, for example, for HSC homing and B cell development.\[^{39,40}\]

A side effect of gene editing approaches is genotoxicity as a consequence of OT effects. OT activity can induce mutagenesis but also provoke chromosomal rearrangements, both potentially inducing cellular transformation. Employing highly specific designer nucleases is therefore paramount to mitigate the risk of such effects. Several tools are available to identify OT events, including in silico predictions which were used in\[^{16,34}\] and known to miss important OTs. Another study\[^{31}\] performed OT analysis using whole-genome sequencing, which is unable to detect rare OT events. In our study, we carefully evaluated OTs using both in silico prediction as well as by employing the unbiased, cell-based oligonucleotide capturing assay (OCA). We detected two rare OT events occurring in introns of genes, thus minimizing the risks of adverse effects. For instance, OT1/OTA3 in CNOT10 was cleaved in 0.12% of cells. OCA identified three additional OTs, one of them in the CCR2 gene. The OCA scores of these OTs were similarly low as for OCA3, suggesting OT activities in the range of 0.1%. While editing in terminally differentiated cells, like CD4+ T cells, mitigates the risk of developing malignancies, this is not the case for genome editing in long-lived multipotent stem cells. Importantly, all functional assays we performed in this study demonstrated that the CCR5-edited cells behaved as unedited cells, suggesting that TALEN expression did not have any negative impact on these cells. All in all, we demonstrate that our novel designed TALEN mediates CCR5 disruption with high frequency and with low genotoxicity, so paving the way for clinical translation in CD34+ hematopoietic stem cells.

4. Experimental Section

CD4+ T Cells Cultivation and Editing: PBMCs were isolated from the leukocyte reduction system chambers obtained from the Blood Donation Center after informed donor consent. CD4+ T cells were isolated using CD4 MicroBeads (Miltenyi, Germany) and cryopreserved. After thawing, cells were cultivated in X-Vivo15 (Lonza, Switzerland) supplemented with 20 U mL\(^{-1}\) IL-2 (Miltenyi) and activated with T Cell Activation/Expansion Kit (Miltenyi). After 3 days, beads were removed and electroporation was performed by combining 5 \times 10^6 CD4+ T cells with 10 µg of each TALEN mRNA or 5 µg of GFP mRNA.\[^{41}\] Cells were electroporated in 100 µL electroporation buffer using P3 Primary Cell 4D-Nucleofector kit (Lonza) and program EO-115 or in 200 µL of BTXpress Cytoporation medium T (BTX, USA). The RNA pellets were resuspended in EB buffer (Qiagen) and stored at −80 °C. Flow Cytometry: GFP expression after lentiviral transduction and/or cell viability (7AAD, AppliChem, Germany) were assessed using BD Accuri C6 Flow Cytometer (BD Biosciences, USA). CCR5 expression was detected by labeling 1 \times 10^5 cells in 50 µL of PBS with 2 µL of APC-labeled mouse anti-human CD195 antibody (BD Biosciences) for 20 min at room temperature. Cells were analyzed on BD FACS Canto-II (BD Biosciences).

HIV-1 Infection: HIV-1 provirus encoding plasmids were obtained from the NIH AIDS Research and Reference Reagent Program.\[^{45,46}\] Virus stocks HIV-1\(^{\text{R5}}\) (R5-tropic) and HIV-1\(^{\text{X4}}\) (X4-tropic) were generated and titrated as previously described.\[^{47}\] For infection, 2 \times 10^6 CD4+ T cells were activated as above and infected with either HIV-1\(^{\text{R5}}\) or HIV-1\(^{\text{X4}}\) at MOIs of 0.01 and 0.001. At indicated timepoints, 50 µL of cell culture supernatant was harvested and used to determine p24 concentration by ELISA.\[^{48}\]

Cytokine Release: 200 µL of supernatants were harvested from 1 \times 10^5 cells per sample at indicated timepoints. Cytokine concentrations were determined using the Cytometric Bead Array for IFN-γ, IL-2, or TNF-α (BD Biosciences). Cells were stimulated with CD3/CD28/CD2 beads (Miltenyi), 1 µg mL\(^{-1}\) ionomycin (Merck-Millipore, Germany), and 10 ng mL\(^{-1}\) PMA (Sigma-Aldrich).

Off-Target Analyses: Potential OTs were predicted with PROGNOS (http://www.advancedsciencenews.com) using the TALEN v2.0 algorithm.\[^{31}\] Five mismatches per half-sites were allowed. Loci were PCR amplified using primers listed in Table S1, Supporting Information. Libraries were prepared with NEBNext Ultra II DNA Library Prep Kit (NEB), quantified with ddPCR Library Quantification Kit for Illumina Technologies (Mountain, Germany), sequenced on Illumina MiSeq platform using MiSeq Reagent Kit v2, 500-cycles (Illumina, USA), and data analyzed with CRISPResseq\[^{49}\] p values were adjusted with the Benjamini & Hochberg\[^{50}\] method. For OCA, 1 \times 10^6 PBMCs (ALLCELLS, USA) mL in X-Vivo15 medium (Lonza), supplemented with 5% human AB serum (Gemini, USA) and 20 µg mL\(^{-1}\) of IL-2 (Miltenyi), were activated using human T activator CD3/CD28 (ThermoFisher). 4 days later, 5 \times 10^6 T cells in cytometry medium T were electroporated with 20 µg of TALEN mRNA (10 µg per subunit) and 10 µg of pre-anneled oligodeoxynucleotides (dsODN, 100 µm) using AgilePulse MAX system (Hamburg, Germany) and a 0.4 cm cuvette. Genomic DNA was extracted 3 days later, randomly sheared to 300 bp fragments by sonication (Covaris LE220 plus), fragments end-repaired-A-tailed (NEBNext Ultra End Repair/dA-Tailing Module), and NGS Y-adapters (TruSeq Annealed Adapter) added. Two rounds of anchored PCR using dsODN-specific and adapter-specific primers were performed. Adapter-specific (PS_1) and dsODN-specific primers were used in the first PCR. Adapter-specific (PS_2) primers, dsODN-specific primers P7, and primers adding the barcode and P7 sequence to the ends of the PCR product were used in the second PCR. PCR products were pooled and sequenced using Illumina NextSeq (2 \times 350 bp). The resulting sequences reads were mapped to the human genome to identify potential OT sites.

Statistical Analyses: A time series analysis was performed to compare the samples in both the HIV-1 challenge and growth curves.
experiments. The goodness of the fit between the full and the reduced models was tested by Anova where p-value indicates the probability that the two models were the same. p-values are indicated with * ≤ 0.05; ** ≤ 0.001; *** ≤ 0.0001. For all other analyses, the Student’s t-test was applied.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors thank Boris Fehse and the NIH AIDS Research and Reference Reagent Program (Division of AIDS, NIAID) for providing plasmids and PM1 cells, Kay Ole Chmielewski for help with OT analysis, Ilona Skatulla and Petra Scheliga-Marschner for technical support, the Lighthouse Core Facility and the Blood Donation Center (both Medical Center—University of Freiburg) for flow cytometry support and for providing PBMCs. This work was supported by a grant of the German Federal Ministry of Education and Research (BMBF-01EO1303 to T.I.C. and T.C.) and funded research cooperation with Cellectis (ZVS20170614 to T.I.C. and T.C.).

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

A.J., A.D., and P.D. are employees of Cellectis. J.C. and C.R. are employees of Ethris. T.I.C. and T.C. have sponsored research collaborations with Cellectis and Miltenyi Biotec. K.J.M. received travel grants and honoraria from Gilead Sciences, Roche Diagnostics, GlaxoSmithKline, Merck Sharp & Dohme, Bristol-Myers Squibb, ViiV, and Abbott. The University of Zurich received advisory board honoraria from Gilead Sciences and research grants from Gilead Science, Roche, and Merck Sharp & Dohme for studies in which K.J.M. serves as principal investigator.

Author Contribution

M.R. contributed to the formal analysis, investigation, methodology, validation, visualization, and writing the original draft. A.J. contributed to the formal analysis and methodology, Y.L.K. formal analysis, investigation, and methodology. M.H. contributed to the formal analysis, methodology, software, and validation. M.R. contributed to the formal analysis, visualization, and writing the original draft. C.A. contributed to the formal analysis and software. J.C., C.R., and C.M. provided the resources. A.D. contributed to the formal analysis and investigation. K.M. contributed to the conceptualization, formal analysis, funding acquisition, methodology, supervision, and writing the original draft. P.D. contributed to the conceptualization, funding acquisition, and resources. T.C. contributed to the conceptualization, funding acquisition, supervision, and writing the original draft. T.I.C. led the conceptualization, methodology, supervision, and visualization and contributed to the formal analysis, funding acquisition, and writing the original draft.

Data Availability Statement

The data that support the findings of this study are mainly available in the supplementary material of this article. Additional data are available upon request.

Keywords

C-C chemokine receptor type 5 editing, CCR5 knockout, human immunodeficiency virus type 1, HIV cure, transcription activator-like effector nuclease
Zhang, J. Xu, C. Li, H. Wu, H. Deng, H. Chen, *N. Engl. J. Med.* 2019, 381, 1240.

[21] T. I. Corru, C. Mussolino, T. Cathomen, *Nat. Med.* 2017, 23, 415.

[22] C. H. June, *N. Engl. J. Med.* 2019, 381, 1281.

[23] C. Mussolino, J. Alzubi, E. J. Fine, R. Morbitzer, T. J. Cradick, T. Lahaye, G. Bao, T. Cathomen, *Nucleic Acids. Res.* 2014, 42, 6762.

[24] C. Mussolino, R. Morbitzer, F. Lutge, N. Dannemann, T. Lahaye, T. Cathomen, *Nucleic Acids. Res.* 2011, 39, 9283.

[25] A. S. Gautron, A. Juillerat, V. Guyot, J. M. Filhol, E. Desesse, A. Duclert, P. Duchateau, L. Poirot, *Mol. Ther.–Nucleic Acids* 2017, 9, 312.

[26] E. G. Cormier, D. N. Tran, L. Ykhayeva, W. C. Olson, T. Dragic, *J. Virol.* 2001, 75, 5541.

[27] T. Dragic, A. Trkola, S. W. Lin, K. A. Nagashima, F. Kajumo, L. Zhao, W. C. Olson, L. Wu, C. R. Mackay, G. P. Allaway, T. P. Sakmar, J. P. Moore, P. J. Maddon, *J. Virol.* 1998, 72, 279.

[28] H. Meijerink, A. R. Indrati, R. van Crevel, H. Koenen, A. J. van der Ven, *BMC Infect. Dis.* 2014, 14, 683.

[29] S. Bae, J. Kweon, H. S. Kim, J. Kweon, *BMC Infect. Dis.* 2014, 14, 683.

[30] J. C. Mar, J. Quackenbush, *PLoS Comput. Biol.* 2009, 5, e1000626.

[31] E. J. Fine, T. J. Cradick, C. L. Zhao, Y. Lin, G. Bao, *Nucleic Acids. Res.* 2014, 42, e42.

[32] S. Q. Tsai, Z. Zheng, N. T. Nguyen, M. Liebers, V. V. Topkar, V. Thapar, N. Wyvekens, C. Khayer, A. J. Iafrate, L. P. Le, M. J. Aryee, J. K. Joung, *Nat. Biotechnol.* 2015, 33, 187.

[33] Q. Xiao, S. Chen, Q. Wang, Z. Liu, S. Liu, H. Deng, W. Hou, D. Wu, Y. Xiong, J. Li, D. Guo, *Retrovirology* 2019, 16, 683.

[34] B. Shi, J. Li, X. Shi, W. Jia, Y. Wen, X. Hu, F. Zhuang, J. Xi, L. Zhang, J. Acquir. Immune Defic. Syndr. 2017, 74, 229.

[35] L. Kordelas, J. Verheyen, D. W. Beelen, P. A. Horn, A. Heinold, R. Kaiser, R. Trenschel, D. Schadendorf, U. Dittmer, S. Esser, H. I. V. A. G. Essen, *N. Engl. J. Med.* 2014, 371, 880.

[36] C. A. Didigu, C. B. Wilen, J. Wang, J. Duong, A. J. Secreto, G. A. Danet-Desnoyers, J. L. Riley, P. D. Gregory, C. H. June, M. C. Holmes, R. W. Doms, *Blood* 2014, 123, 61.

[37] Z. Liu, S. Chen, X. Jin, Q. Wang, K. Yang, C. Li, Q. Xiao, P. Hou, S. Liu, S. Wu, W. Hou, Y. Xiong, C. Kong, X. Zhao, L. Wu, C. Li, G. Sun, D. Guo, *Cell Biosci.* 2017, 7, 47.

[38] R. Mohle, F. Bautz, S. Rafi, M. A. Moore, W. Brugger, L. Kanz, *BLOOD* 1998, 97, 4523.

[39] Q. Ma, D. Jones, P. R. Borghesani, R. A. Segal, T. Nagasawa, T. Kishimoto, R. T. Bronson, *Proc. Natl. Acad. Sci. USA* 1998, 95, 9448.

[40] M. S. Kormann, G. Hasenpusch, M. K. Aneja, G. Nica, A. W. Flemmer, S. Herber-Jonat, M. Huppmann, L. E. Mays, M. Illenyi, A. Schams, M. Grieser, I. Bittmann, R. Handgretinger, D. Hartl, J. Rosenhecker, C. Rudolph, *Nat. Biotechnol.* 2011, 29, 154.

[41] A. K. Dreyer, D. Hoffmann, N. Lachmann, M. Ackermann, D. Steinemann, B. Timm, U. Siler, J. Reichenbach, M. Grez, T. Moritz, A. Schambach, T. Cathomen, *Biomaterials* 2015, 69, 191.

[42] T. I. Corru, T. Cathomen, *Mol. Ther.* 2007, 15, 2107.

[43] P. Lusso, F. Cocchi, C. Balotta, P. D. Markham, A. Louie, P. Farci, R. Pal, R. C. Gallo, M. S. Reitz, *J. Virol.* 1995, 69, 3712.

[44] Y. Koyanagi, S. Miles, R. T. Mitsuysasu, J. E. Merrill, H. V. Vinters, I. S. Chen, *Science* 1987, 236, 819.

[45] A. Adachi, H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, M. A. Martin, *J. Virol.* 1986, 59, 284.

[46] Y. L. Kok, V. Vongrad, M. Shilaih, F. Di Giallonardo, H. Kuster, R. Kouyos, H. F. Gunthard, K. J. Metzner, *Sci. Rep.* 2016, 6, 24157.

[47] J. P. Moore, J. A. McKeating, R. A. Weiss, Q. J. Sattentau, *Science* 1990, 250, 1139.

[48] K. Clement, H. Rees, M. C. Canver, J. M. Gehrke, R. Farouni, J. Y. Hsu, M. A. Cole, D. R. Liu, J. K. Joung, D. E. Bauer, L. Pinello, *Nat. Biotechnol.* 2019, 37, 224.

[49] Y. Benjamini, Y. Hochberg, *J. R. Stat. Soc., Ser. B* 1995, 57, 289.