Establishment of HIV Resistance by Genome Editing

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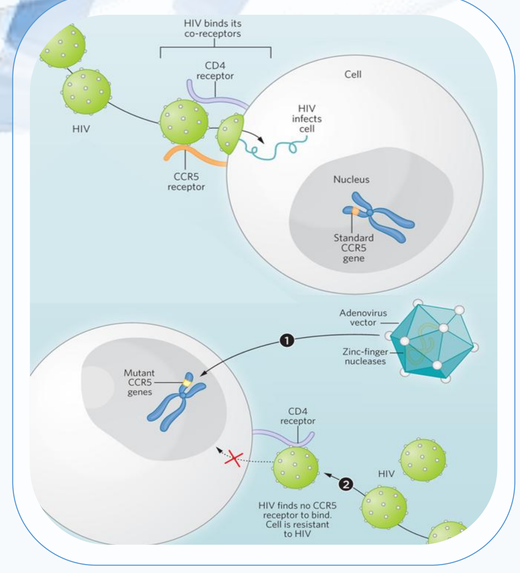


Introduction

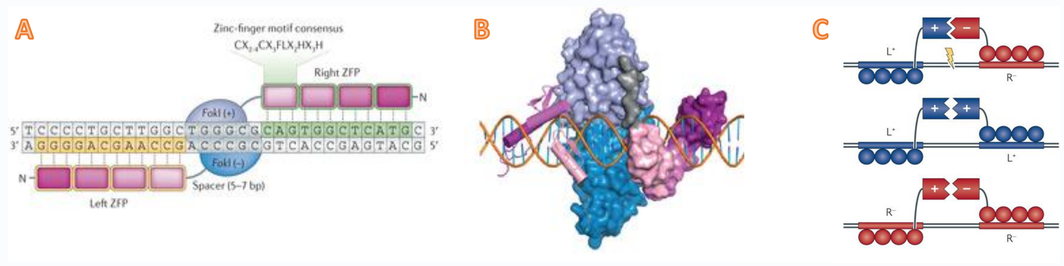
The human immunodeficiency virus, known as HIV, is the lentivirus responsible of the acquired immune deficiency syndrome, AIDS, the main cause of death by infective agent around the world. HIV infects T-lymphocytes and macrophages by the recognition of the cellular receptor CD4 and a chemokine coreceptor CCR5 or CXCR4, being CCR5 the most common.

Interestingly, individuals with homozygous 32bp deletion in the CCR5 gene are highly resistant to HIV-1. New therapies try to mimic the natural mutation and have proved that the establishment of a stable HIV resistance is possible thanks to the genome editing by zinc finger nucleases.

Image. Foundations of the genome editing therapy to establish VIH-resistance. (1)

Zinc-finger Nucleases

Targeted genome editing is an approach that introduces stable genetic changes at preselected loci. Zinc-finger nucleases (ZFN) are chimeric proteins that function as a pair and consist in zinc finger peptides that bind to DNA specific sequences, fussed to the enzyme *Fok*I. The double-stranded breaks are dominantly repaired by error-prone non-homologous end joining (NHEJ), introducing mutations that produce truncated or non-functional proteins.

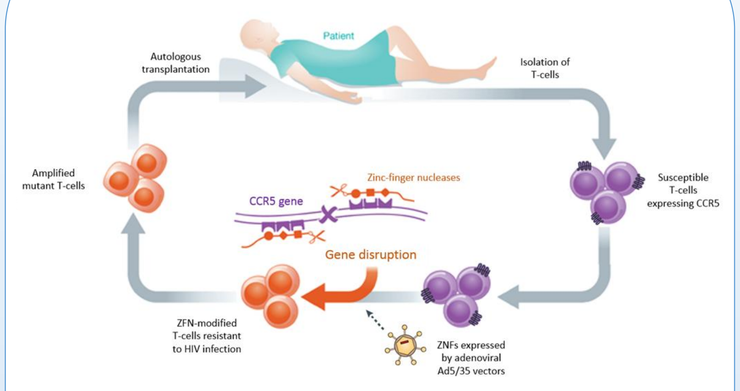


Structure of Zinc-finger nucleases. A. Schematic representation of a ZFN pair. B. A computer model structure of a ZFN pair bound to DNA.(2) C. Variants of the *Fok*I cleavage domain that function as obligate heterodimers. (3)

Ex vivo Genome Editing

The *ex vivo* cell therapy is based on the ability to isolate cells from a patient, expand them *ex vivo* before and after genetic modification, and administer them to the patient to stablish a stable graft of the infused cells and their progeny.

This approach exploits *ex vivo* expanded T-cells or HSCs with genetic engineering to disrupt CCR5 gene, increasing their resistance and immune activity against HIV. The main advantage is that the patient is not expose to the immunogenic vector.



Proposed concept of designer nuclease-mediated correction of patient-specific T-cells for autologous transplantation. (4)

Different approaches

Pre-clinical studies

- ✓ CCR5-deficient CD4 T Lymphocytes
- ✓ Validation in Hematopoietic Stem Cells
- ✓ CCR5/CXCR4 ZFNs Combined Strategy

Clinical trials

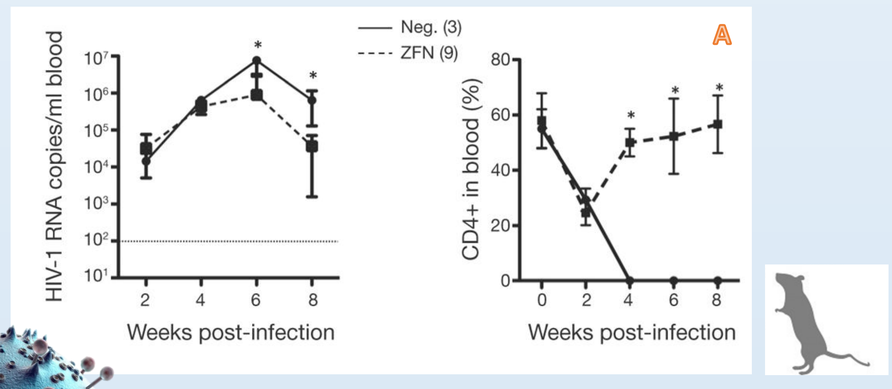
- ✓ Phase I/II study of safety and efficacy
- ✓ Dose escalation phase I/II in patients with inadequate response to antiretroviral therapy
- ✓ Phase I/II study with cyclophosphamide pre-treatment
- ✓ Phase II clinical trial

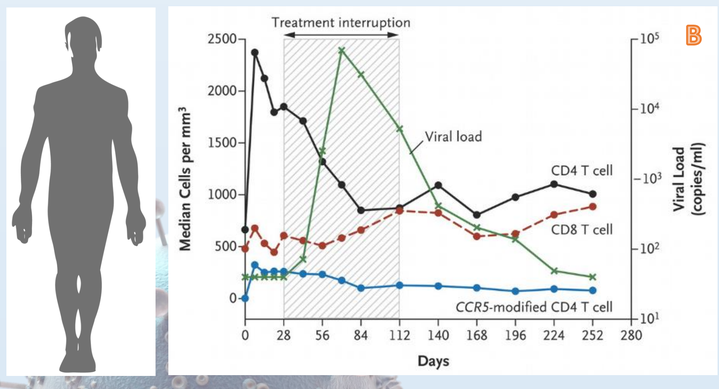
Pre-clinical & Clinical Results

Pre-clinical studies showed that in the presence of the selective pressure of a R5 HIV infection, ZFN modification conferred significant survival advantage and real protection to the treated T cells in humanized mice. Moreover, CCR5-negative HSCs retained full functionally and provided long-term resistance in all hematopoietic linage, even in critical compartments. Additionally, the combined disruption of both coreceptors gave protection in front of all tropism strains.

Clinical trials make evident the therapeutic potential of the strategy. The infusion was generally safe and most of the patients showed increasing CD4 T cells counts, significant persistence of modified cells and their accumulation in the mucosa. Furthermore, the viral load importantly decreased even during the HAART interruption.

The results suggest that viral eradication would probably require increased input and sustained generation of T cells carrying biallelic CCR5 disruption. Other improvements include the depletion of endogenous T cells before the infusion and the use of RNA electroporation to administrate the ZFNs into the lymphocytes.





Changes in viremia and T-cells counts. A. In mice into which untreated (Neg.) or ZFN-treated CD34⁺ HSPCs were transplanted. (5) B. In human HIV patients during treatment interruption. (6)

Conclusion

The huge amount of open fronts the HIV fight has, make evident the enormous effort that the scientific community is doing to eradicate the pandemic infection and get us closer to find a definitive cure. Regarding genome editing, future studies will be directed towards increasing the engraftment, demonstrating that it is possible to generate a stable population of HIV-resistant cells that can fight the virus and opportunistic infections thereby mimicking the characteristics of individuals that carry the natural mutation. In the future, multiple diseases may benefit from gene-editing strategies coupled to hematopoietic cell transplantation and immunotherapy approaches. Overall, these studies contribute to the birth of a new era in cell and gene therapy and bring us closer to the goal of precise site-specific gene editing and personalized medicine.

References

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