

Immunotherapeutic Potential of Inhibitory KIR/HLA Mismatched Allogeneic NK and gamma-delta T Cells as an HIV Cure Strategy

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Introduction

Background

- Despite successful antiretroviral therapies (ART), there is still a need for a cure for HIV.
 - Certain current HIV cure strategies that focus on cellular antiviral responses face challenges including limited antigen presentation, insufficient cytotoxic T cell responses, and immune exhaustion or tolerance.
 - Both natural killer (NK) and gamma/delta (gd)-T cells are innate immune cells known to be important in HIV responses.
 - Antigen recognition is achieved outside of MHC-restriction in these cells, which makes them a safe candidate for allogeneic cell therapies.
- In addition, certain persons with HIV (PWH) cannot tolerate ART, exhaust ART options, choose not to take, or do not have access to, therapy.

Rationale

- Therefore, we hypothesized that KIR/HLA-mismatched allogeneic NK and gd-T cells could be a potential adaptive cell therapy for HIV cure.

Methods

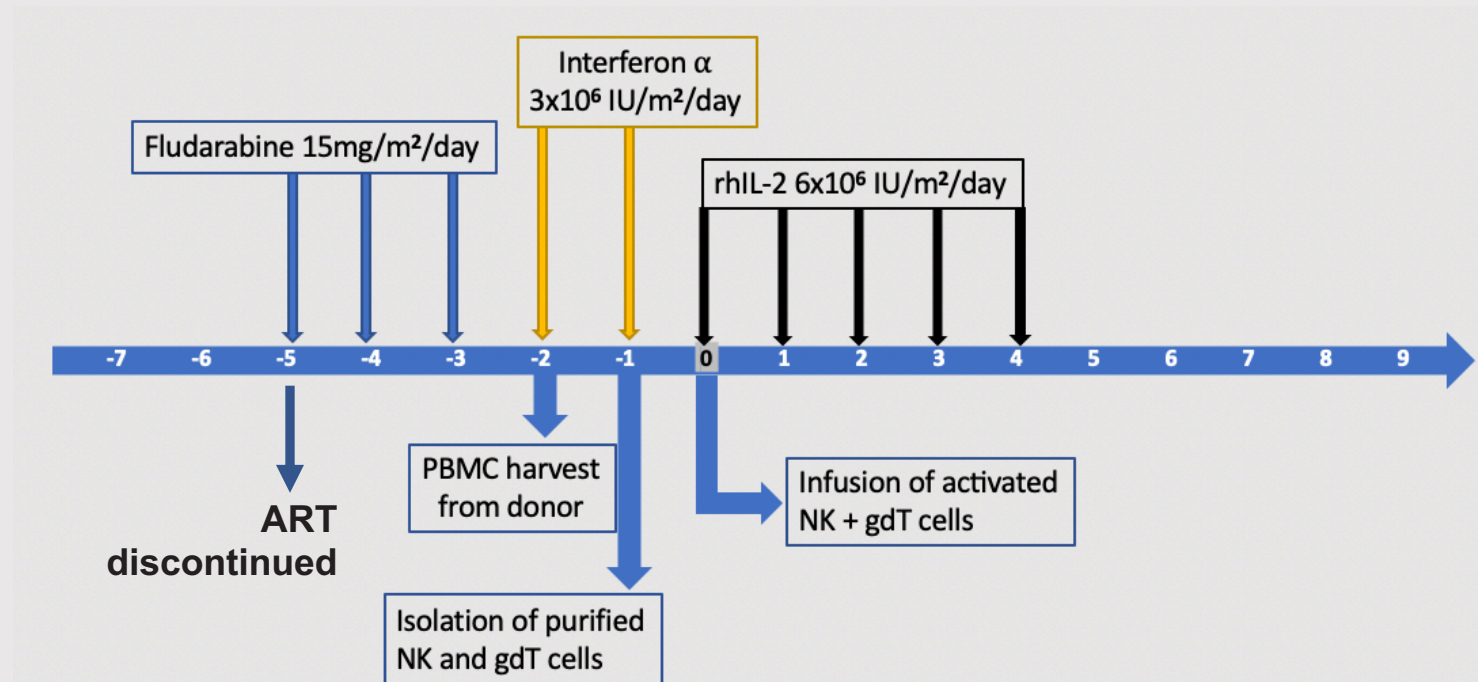
Pre-clinical

- PBMCs were isolated from two ART-controlled PWH and two seronegative KIR/HLA-mismatched individuals.
- gd-T cells were expanded using IL-2 and zoledronic acid.
- NK cells were isolated by negative selection via immunomagnetic separation with CliniMACS.
- HIV target cells were generated from CD4+ T cells from PWH by superinfecting them with HIV_{JR-CSF} *in vitro*.
- *In vitro* killing assays were run with 1:1, 1:10, 1:25, 1:50 effector/target cell ratios in co-cultures with allogeneic or autologous NK and gd-T cells and HIV-superinfected CD4+ T cells.
- Same killing experiments were done with latent CD4+ T cells that were unstimulated or treated with latency-reversal agent (LRA) vorinostat (VOR).
- HIV p24 antigen was measured in cells cultures by ELISA and with intracellular staining by FACS.

Methods

Clinical

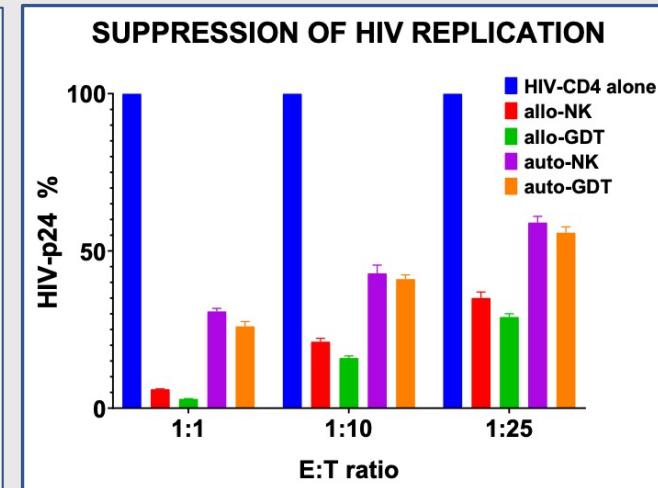
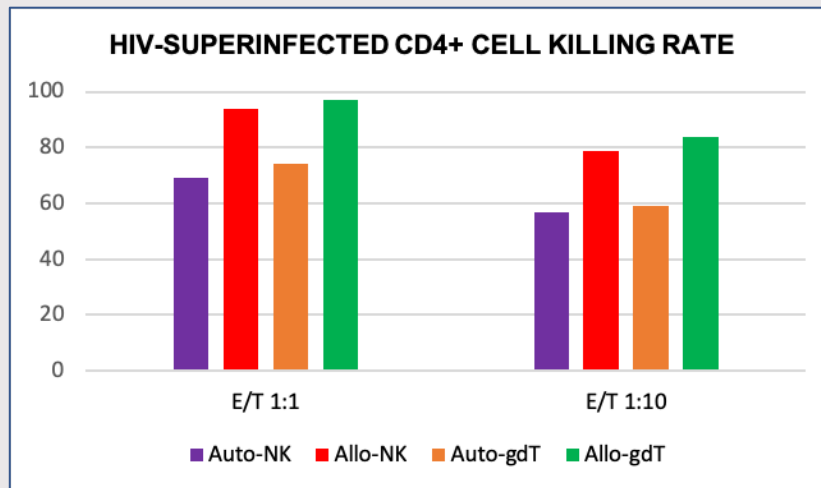
- An IRB-approved single-patient study was conducted on a virally unsuppressed person under combination (c)ART.
- **Patient:** 54yo, male
HIV seroconversion 1986
History of monotherapy and dual therapies
~2,000 (3.3 log₁₀) copies of HIV RNA under cART
~400 absolute CD4+ T cell count, ~22% CD4+ T cells
- Low dose lympho-suppressive pre-condition regimen for 3 days (day -5 to day -2)
- 25x10⁶/kg NK cells and 5x10⁶/kg gd-T cells on day 0 from a partially mismatched healthy donor
- Discontinued ART on day -5 and remained off for duration of study



Results

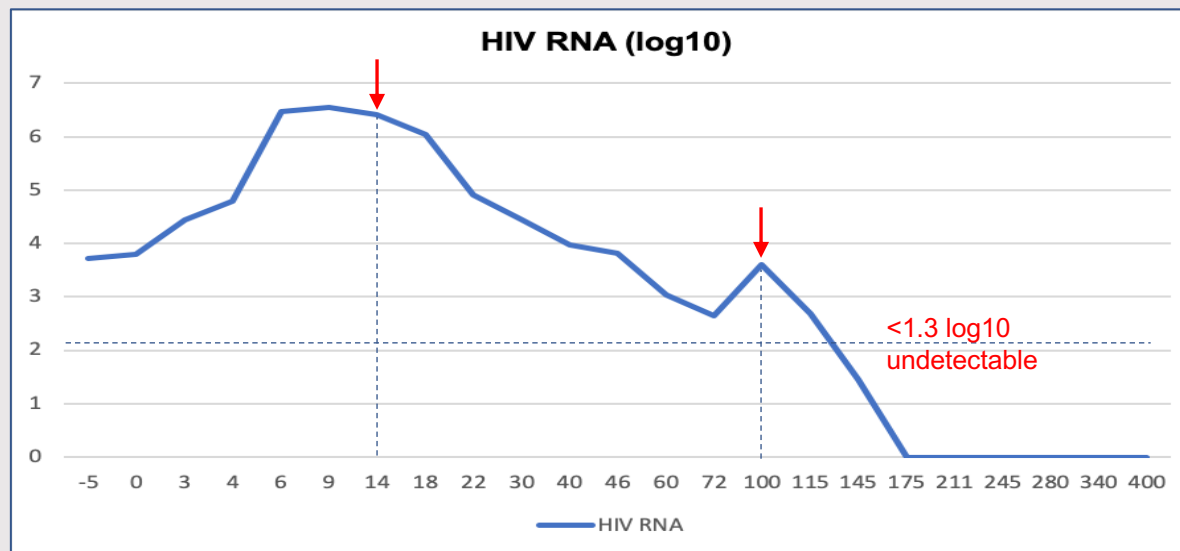
In Vitro

- Allogeneic NK and gd-T cells inhibited HIV replication in superinfected CD4+ cells substantially at 1:1 (94% and 97%) and 1:10 ratio (79% and 84%) compared to autologous cells (69% and 74% at 1:1, 57% and 59% at 1:10)
- Similar results were obtained with VOR-treated latent target cells.
- Unstimulated latent cells co-cultured with either allogeneic cell at 1:1 ratio demonstrated no p24 production after VOR treatment.



Clinical

- There were no grade >2 adverse events or treatment-related toxicity observed in the single-patient study.
- Off ART throughout, after initial increase in plasma HIV RNA, decline began at day 14 and reached pre-intervention levels at day 100. The viral load then rapidly decline to <20 or 20 copies/ml for 225 days



Conclusion

- KIR/HLA mismatched NK and gd-T cells demonstrated superior ability to target HIV-infected CD4+ T cell and suppress HIV viral replication *in vitro* compared to autologous cells.
- KIR/HLA mismatched NK and gd-T cells successfully suppressed HIV replication in LRA-stimulated latent CD4+ T cells *in vitro*, potentially suggesting the mismatched cells' ability to recognize latently infected cells.
- KIR/HLA mismatched NK and gd-T cell therapy led to an initial increase in plasma HIV followed by persistent virologic control (<20 or 20 copies/ml for 10 months) of HIV in a subject who previously had uncontrolled viremia under ART, without observed toxicity or severe adverse event.
- Studies to investigate the patient's immunological profiling and anti-HIV immunity are planned and underway.
- Allogeneic NK and gd-T cells present important potential as an HIV immunotherapy and warrant further investigation potentially to achieve functional cure.
- An investigator-initiated IND is being prepared to submit to the FDA.

Acknowledgements



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