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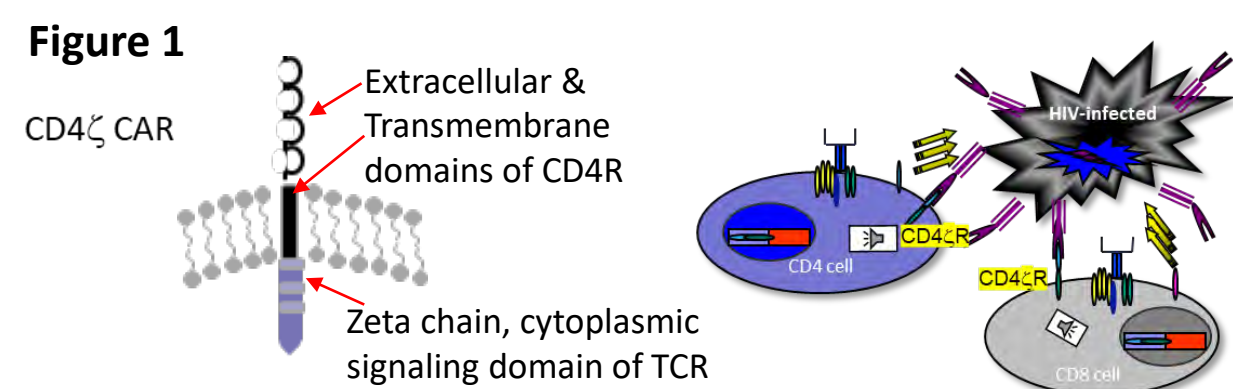
Poster# 00337

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## Background

- Despite effective antiretroviral therapy, the cellular HIV reservoir persists
- CD4ζ is a chimeric antigen receptor (CAR) T cell composed of the extracellular and transmembrane domains of CD4 linked to the cytoplasmic zeta signaling domain of the CD3 T cell receptor.



- Previously we have reported:
  - CD4ζ CAR-T cell infusions were well tolerated (Aronson 2008)
  - ζ transgene persists in PBMCs for 7 years (Scholler 2012)
  - Synergy of low dose IL-2 with ζ cells caused large but transient increases in CD4+ T cells (Aronson 2008)
- Other ζ CAR-T cell studies have reported:
  - ζ transgene persists in gut lymphatics at 1 year (Mitsuyasu 2000)
  - Slight decrease in HIV reservoir (Deeks 2002)

We report on the FDA-mandated 15 year follow up.

## Method

- Randomized controlled trial, laboratory-blinded
- Conducted at Walter Reed Army Medical Center, Washington D.C.
  - During 2001-2020
- CAR-T production at University of Pennsylvania:
  - Autologous T lymphocytes were cultured with anti-CD3/anti-CD28 coated magnetic beads.
  - ζ transgene transduction followed using a MMV γ retroviral vector.

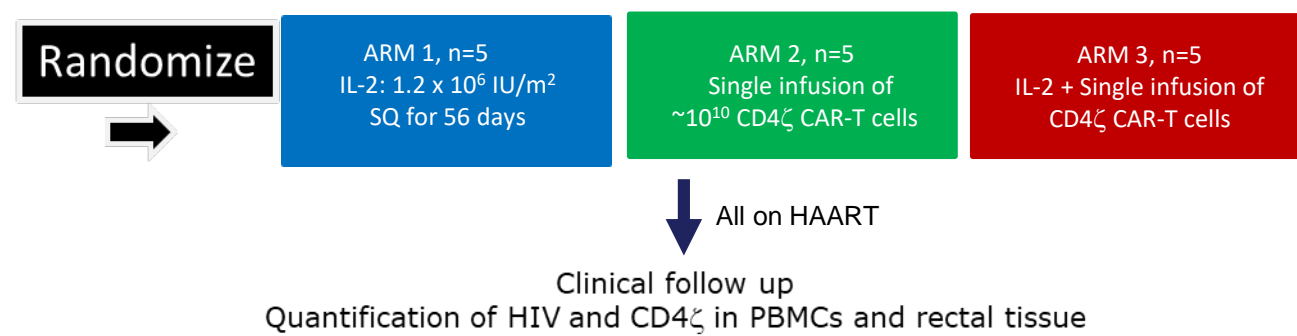
Table 1

Inclusion Criteria	Exclusion Criteria
Age >18 years	Prior CD4<200 for more than an 8 week period
Stable HAART	Inadequate organ function
HIV VL <50 copies for >8 weeks at enrollment	History of prior gene therapy
CD4 >200/mm <sup>3</sup>	Recent IL-2 treatment

- Data analysis includes descriptive statistics; mixed models and ANCOVA were used to assess the effects of treatment over time.

## Method

Figure 2: Study Plan NCT 01013415

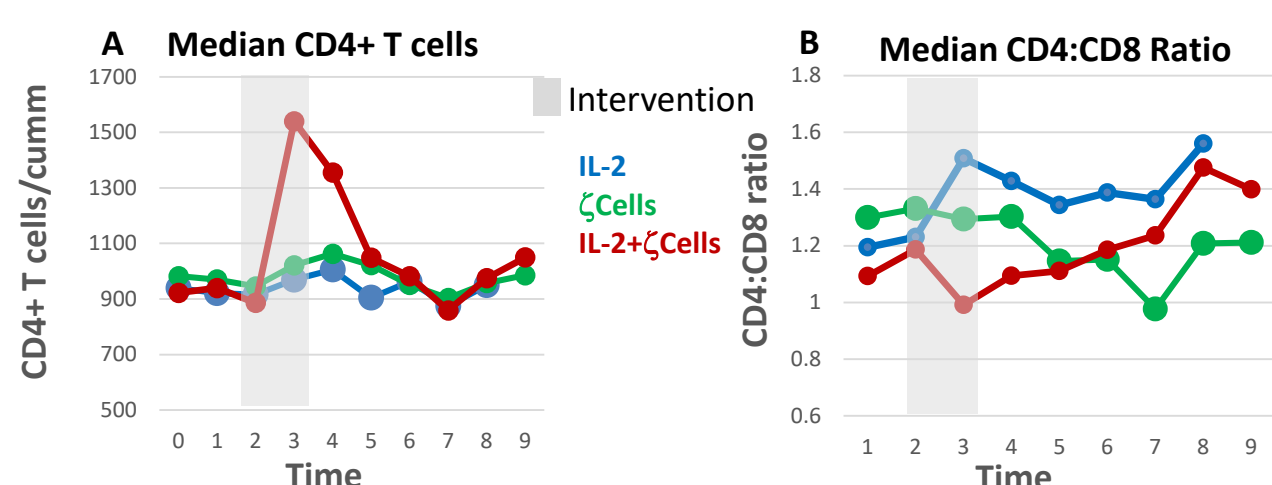


- CD4ζ and HIV-1 gag gene were quantified in PBMCs and rectal tissue using real time quantitative PCR
- RNAscope with HIV-1 clade B probe was performed on FFPE rectal tissue at 15 years follow up
- A highly sensitive nested PCR assay was used to determine total and integrated HIV DNA in PBMCs

## Results

Table 2: Demographics	IL-2 N=4	ζCells N=5	IL-2 + ζCells N=4
Sex: Male N (%)	4 (100)	5 (100)	2 (50)
Race: White:Black:Hispanic:Asian N (%)	4W (100)	2W (40) 3B (60)	2H (50) 1B (25) 1A (25)
Median years follow up	15.9 (14.4-17)	15.6 (13.9-16.1)	15.6 (13.8-16.1)
Median Age at LTF	54.2 (47.7-60)	53.8 (49.5-56.1)	58.6 (39.3-69.5)
Median Years Sero+ at LTF	24.8 (19.4-29)	21.6 (16.4-27.9)	24.4 (17.5-30.6)
Median viable ζcells infused (x 10 <sup>9</sup> )	NA	5.1 (3.8-6.5)	7.9 (5.0-8.2)
Median transduction efficiency (%)	NA	21.8 (6.9-24.7)	9.4 (4.3-18.1)
Median VL (mRNA copies/ml plasma) pre	BD (<50)	BD (BD-292)	BD
Median VL (mRNA copies/ml plasma) LTF	BD (<20)	BD	BD

Figure 3A-B Peripheral T-lymphocyte counts

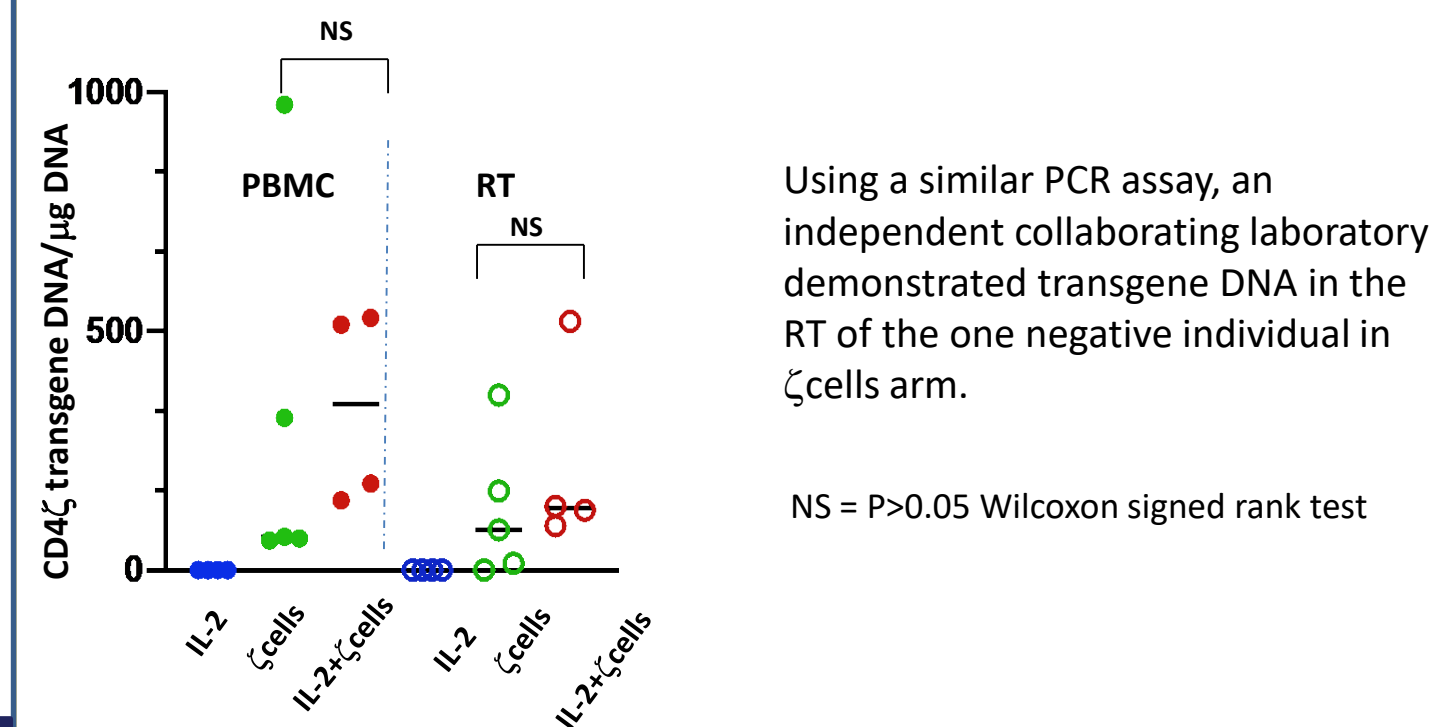


- A: IL-2+ζcells recipients experience a brief increase in CD4+ T cells during intervention
- B: The CD4:CD8 ratio increases over the course of the study in IL-2 and IL-2+ζcell recipients

Timepoint 0 is a single pre-intervention data point. The remaining are the medians of data from 5 aggregated visits

## Results

Figure 4: CD4ζ transgene was detected in PBMCs and RT at LTF



Using a similar PCR assay, an independent collaborating laboratory demonstrated transgene DNA in the RT of the one negative individual in ζcells arm.

Figure 5A-C: HIV RNA and HIV DNA were detected in PBMCs and RT

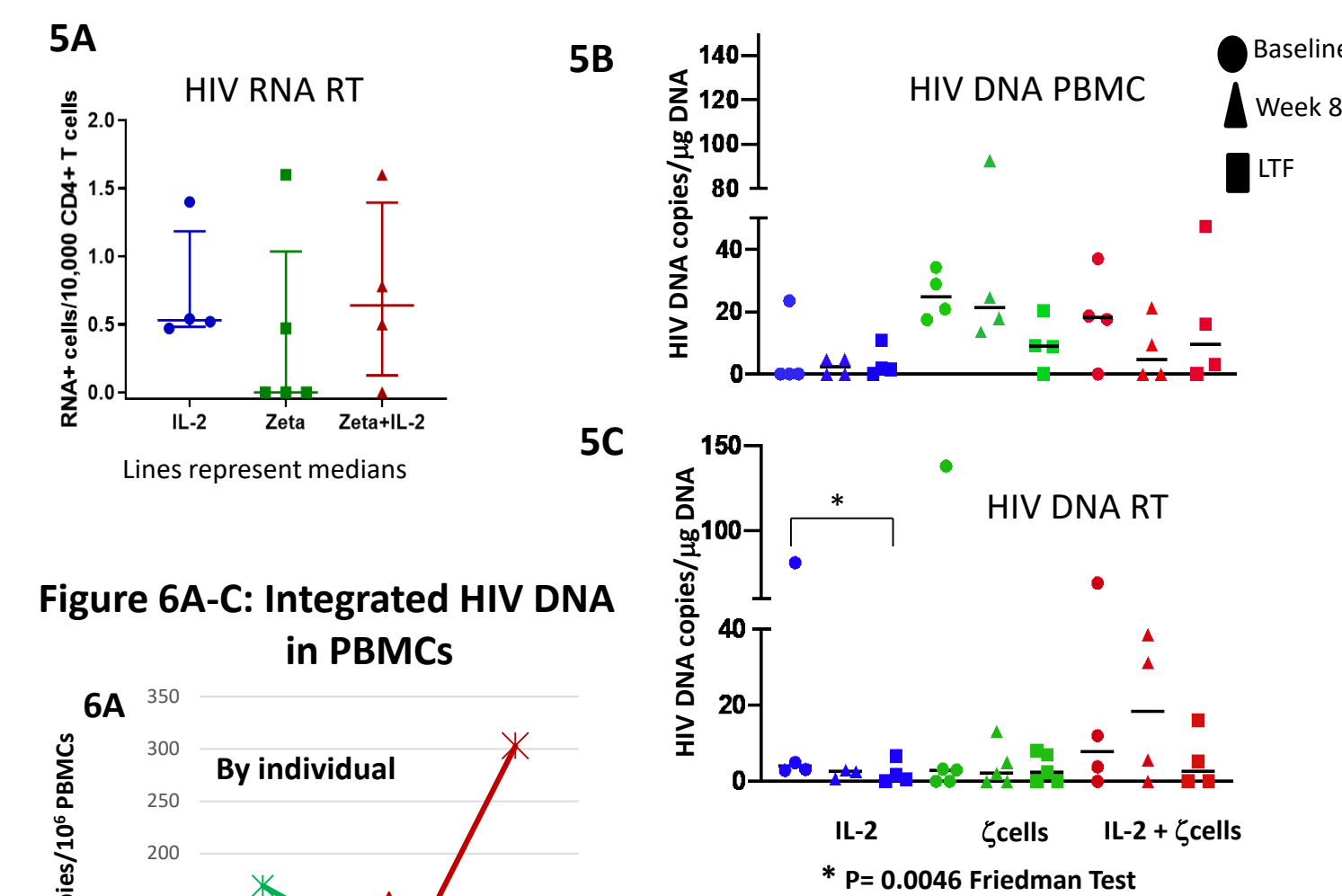
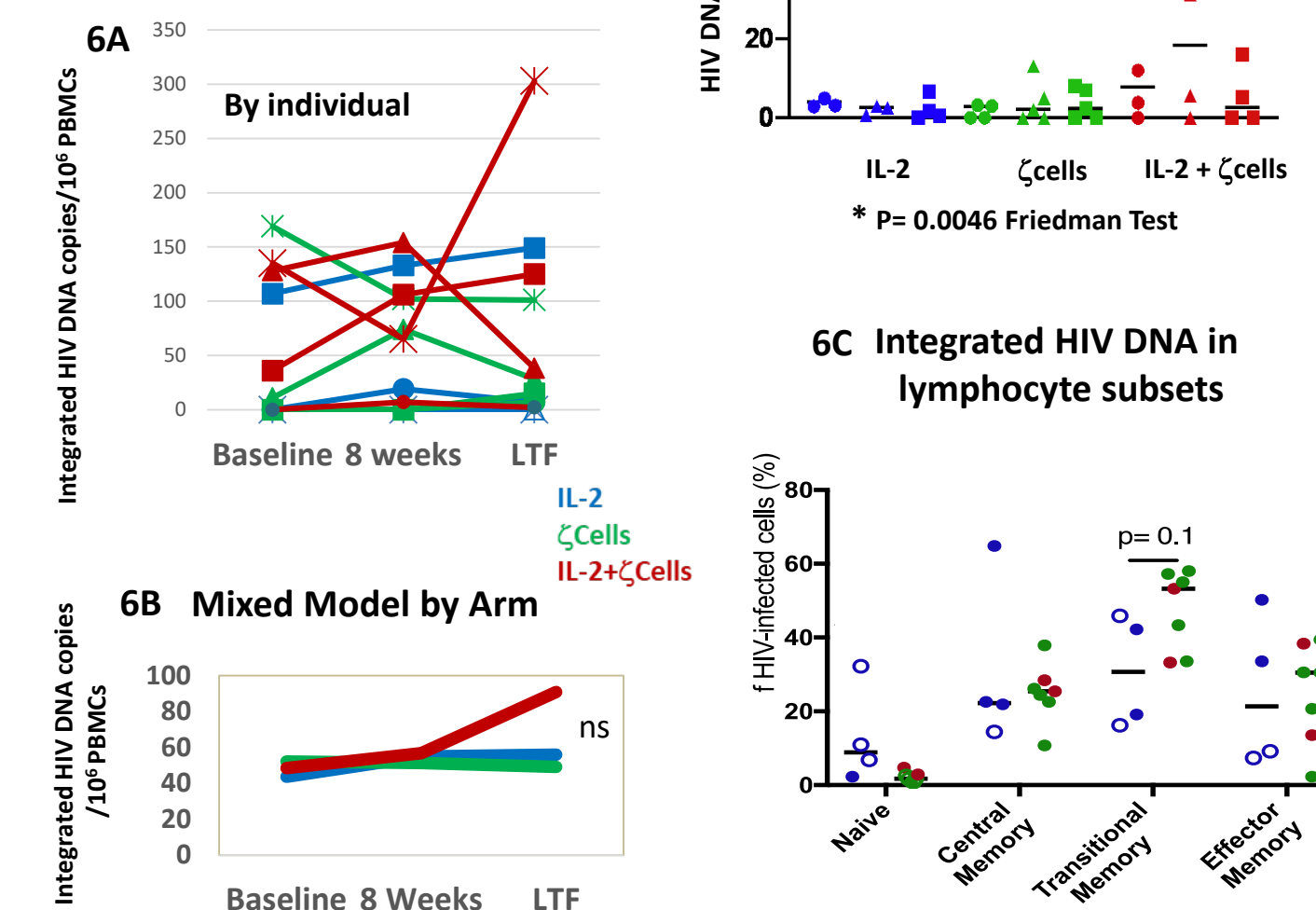


Figure 6A-C: Integrated HIV DNA in PBMCs



## Conclusions

- Persistence of the CD4ζ transgene was measured in all 13 subjects' PBMC and RT at 15 years.
- Low levels of HIV RNA and DNA were detected in PBMC and/or RT at the long term follow up in all arms.
- In recipients of ζ CAR-T cells, there is a trend towards transitional memory cells harboring more integrated HIV DNA compared to the IL-2 arm.
- Findings are limited by the small heterogenous study population with intra subject variability, low transduction efficiency, low cell dose, methodologic limits of detection of the HIV reservoir, and the absence of a HAART-only comparison arm.

## References

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