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CHARACTERIZING NEW AND BOOSTED HIV-SPECIFIC T CELL RESPONSES ELICITED BY AN HIV THERAPEUTIC VACCINE

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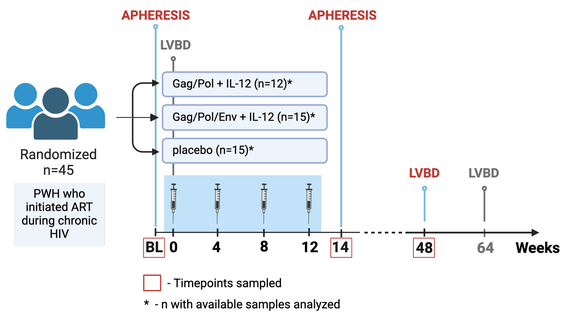
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BACKGROUND

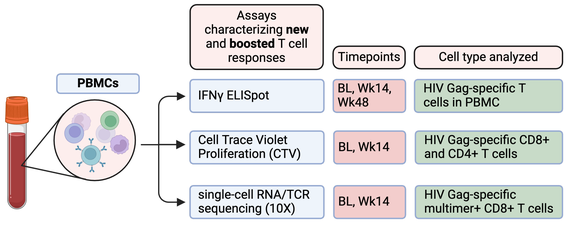
- Little is known about the capacity of HIV therapeutic vaccines to overcome pre-existing immunodominance hierarchies and elicit functional, long-lived new or boosted HIV-specific CD8+ T cell responses.
- We conducted a therapeutic vaccine study in PWH who were randomized to receive a DNA vaccine containing consensus Gag/Pol (**GP**) or Gag/Pol/Env (**GPE**) sequences (PENNVAX) plus IL-12 plasmid adjuvant via electroporation, or placebo (NCT03606213).
- HIV-specific T cell response magnitude was measured by IFNγ ELISpot using vaccine-matched peptide pools.
- The PENNVAX therapeutic vaccine was found to induce **new** and **boosted** HIV-specific T cell responses (Chew *et al.*, *CROI 2022*, poster #284).
- Here, we characterize the magnitude, durability, proliferative capacity, and TCR repertoire of these **new** and **boosted** HIV-specific T cell responses.

METHODS

Trial design:

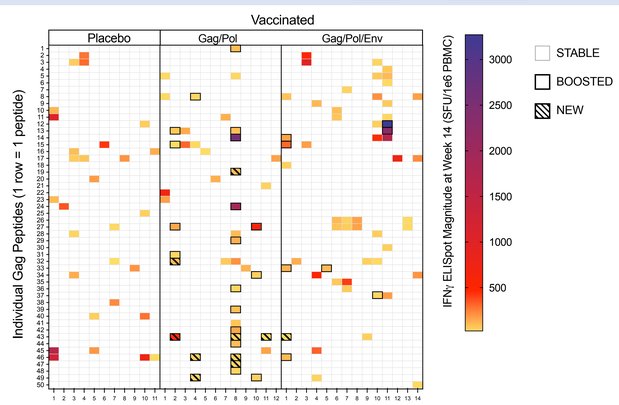


Assays:



RESULTS

Fig 1: THERAPEUTIC VACCINATION ELICITS NEW AND BOOSTED HIV GAG-SPECIFIC T CELL RESPONSES



Individual Gag peptide-specific T cell responses to vaccine-matched 15-mer peptides were measured via IFNγ ELISpot at baseline (BL) and 2 weeks after the last vaccine (Week [Wk] 14).

Responses were defined as **new** at Wk14 if they were absent at BL (even after 10-day *in vitro* peptide stimulation) with a fold change (FC)≥2, and **boosted** if present at BL and Wk14 with FC≥2.

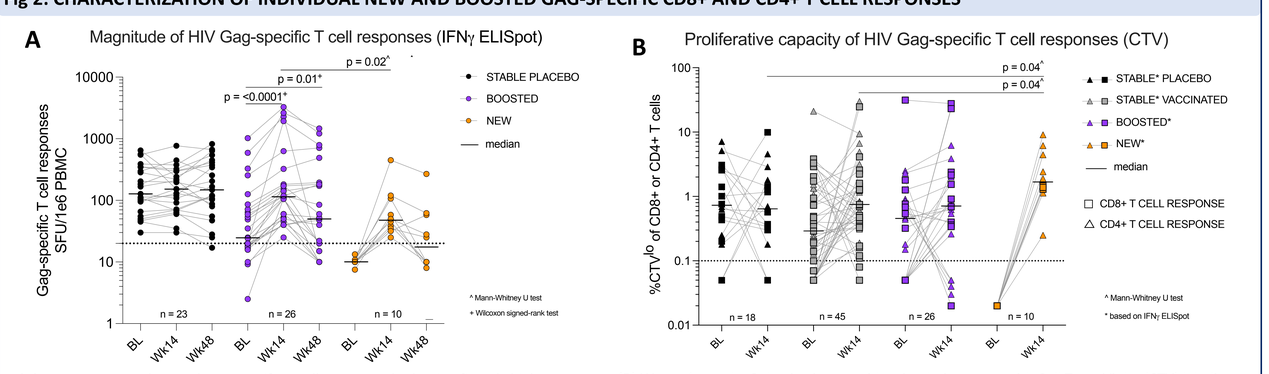
Only peptides and participants with ≥1 response are shown

Fig 4: BOOSTED RESPONSES RECRUIT NEW T CELL CLONES AND EXPAND PRE-EXISTING CLONES AFTER VACCINATION



Paired 10X single-cell RNA/TCR sequencing was performed on sorted multimer+ CD8+ T cells from participants. Flow cytometry plots show the HIV-specific multimer+ CD8+ T cell responses at baseline and Week 14. Bar plots display the frequency of clonotypes within each sorted multimer+ response.

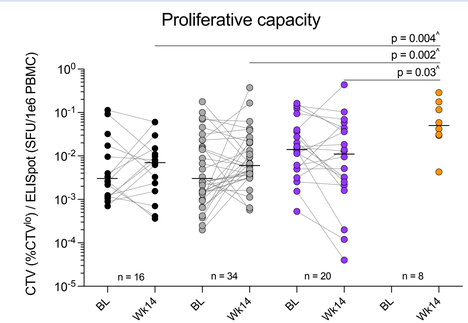
Fig 2: CHARACTERIZATION OF INDIVIDUAL NEW AND BOOSTED GAG-SPECIFIC CD8+ AND CD4+ T CELL RESPONSES

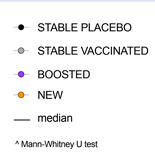


(A) By IFNγ ELISpot, boosted Gag-specific T cell responses had a significantly higher magnitude at Wk14 vs new responses. At Wk48, 11 of the boosted responses remained ≥2 fold above BL magnitude, and 5 of the new responses remained detectable.

(B) We performed a 6-day *in vitro* peptide stimulation assay with Cell Trace Violet (CTV) labeling to quantify the proliferative capacity of the responses (%CTV^{lo} of total CD8+ or CD4+ T cells after 6-day stim). Both CD8+ and CD4+ T cell responses were identified in all response categories.

Fig 3: NEW RESPONSES EXHIBIT HIGHER PROLIFERATIVE CAPACITY COMPARED TO BOOSTED AND STABLE RESPONSES





For each individual Gag-specific T cell response, proliferative capacity, relative to T cell response magnitude, was determined by taking the ratio of proliferated (CTV^{lo}) T cells to the magnitude as measured by IFNγ ELISpot. Based on this ratio, new responses at Wk14 had a significantly higher proliferative capacity compared to boosted or stable responses.

Experimental design for the Cell Trace Violet (CTV) proliferation assay

CONCLUSIONS

- HIV DNA therapeutic vaccination both boosted pre-existing T cell responses and also elicited responses against new epitopes.
- HIV DNA therapeutic vaccination elicited both CD8+ and CD4+ new and boosted T cell responses, with ~half of the vaccine-elicited responses persisting at Wk48.
- While boosted responses had the highest IFNγ ELISpot magnitude at Wk14, new responses demonstrated higher proliferative capacity at Wk14, relative to their magnitude.
- Eliciting new T cell responses may be required in order for HIV therapeutic vaccines to enhance T cell function.
- **Future Directions:** Investigate further whether therapeutic vaccination can overcome pre-existing immunodominance and recruit new T cell clones to the HIV-specific T cell response.

ADDITIONAL INFORMATION

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