Poster 391
Session P-F4

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Influenza Vaccination Increases HIV-1 Transcription During Antiretroviral Therapy

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Background

- The latent HIV-1 reservoir is widely recognized as the major barrier to eradication[1].
- Many curative strategies aim to reactivate latent virus, thereby exposing it to targeted therapy and facilitating clearance of the reservoir[2].
- Stimulators such as histone deacetylase inhibitors, disulfiram and IL-7 have thus far demonstrated only modest activity, often at the expense of considerable toxicity.
- In contrast, transient increases in viremia have been observed after administration of standard vaccines even during antiretroviral therapy (ART)[3,4].
- Clinically-approved vaccines present minimal side effects and long-term risks even in HIV-1 infected individuals.

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To study the effect of routine vaccination on HIV reservoir dynamics in peripheral blood mononuclear cells

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► **Clinical Trial Design:** A randomized clinical trial (NCT00329251) was conducted to study the effects of a vaccination schedule on viral rebound and immune function after structured treatment interruption. Participants were randomized to receive a vaccination schedule (n=13) or placebo (n=13). The vaccination schedule involved 7 clinically-approved vaccines given over the course of 12 months (Figure 1). Inclusion criteria for the trial were:

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► **DNA and RNA Extraction:** Cryopreserved peripheral blood mononuclear cells (PBMCs) from timepoints immediately pre- and 1 month post-vaccination were viably thawed. DNA and RNA were extracted using a Qiagen AllPrep DNA/RNA Mini Kit.

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Cell-associated RNA (caRNA): RNA was converted to cDNA with reverse transcriptase iScript (Biorad). ddPCR was performed with primer/probes *gag* HEX for unspliced mRNA (usRNA), *tat-rev* FAM for multispliced mRNA (msRNA), and *polyA* FAM for fully elongated and correctly processed HIV-1 mRNA. DNA and RNA values were adjusted for percentage of CD4 T cells as measured by flow cytometry.

► **Flow Cytometry:** Cell counting and immunophenotyping for T-cell markers (CD3, CD45, CD4, CD8) were performed by flow cytometry.

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Study Characteristics

Table 1: Baseline characteristics
Vaccinees and controls were not significantly different at baseline.

	Vaccinees (n=13)	Controls (n=13)
Age, Years, Median [IQR]	38 [29-41]	40 [38-52]
Males, n (%)	11 (85)	10 (77)
Risk Factor, n (%)		
Homosexual	9 (69)	5 (38)
Heterosexual	4 (31)	5 (38)
IVDU	0	3 (23)
Estimated Duration of HIV-1 Infection, Years, Median [IQR]	4.6 [2.1-7.9]	6.6 [3.3-11.0]
Time on ART, Years, Median [IQR]	1.4 [1.2-4.6]	4.5 [1.6-6.3]
ART		
NNRTI-based regimen, n (%)	3 (31)	7 (54)
PI-based regimen, n (%)	10 (69)	5 (38)
3-drug regimen, n (%)	0	1 (8)
Nadir CD4 T-cell Count, Cells/µl, Median [IQR]	414 [373-514]	411 [384-530]
Absolute CD4 T-cell Count at Month 0, Cells/µl, Median [IQR]	987 [767-1072]	898 [712-1073]
Plasma Viral Load at Month 0, log_{10}copies/ml, Median [IQR]	1.28 [1.28-1.28]	1.28 [1.28-1.4]

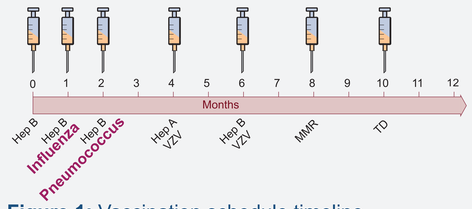


Figure 1: Vaccination schedule timeline
Hep A, B= Hepatitis A, B; VZV= Varicella; MMR= Measles-Mumps-Rubella; TD= Tetanus toxoid-Diphtheria toxoid.

	Vaccinees (n)	Controls (n)	Median fold-change (*gag*)	p-value
Influenza/ Hep B	12	11	2.4	**0.02**
Pneumococcus/ Hep B	8	1	7.0	**0.04**
VZV/ Hep A	10	6	1.6	0.06
VZV/ Hep B	8	4	0.5	0.38
MMR	12	9	1.1	0.97
TD	9	11	1.3	0.50

Table 2: Summary of individual vaccines
Samples available for each timepoint (n) and results for vaccine arm (median fold-change in *gag* transcripts after vaccination); p-value of Wilcoxon test.

HIV caRNA Increased after Influenza and Pneumococcus Vaccinations

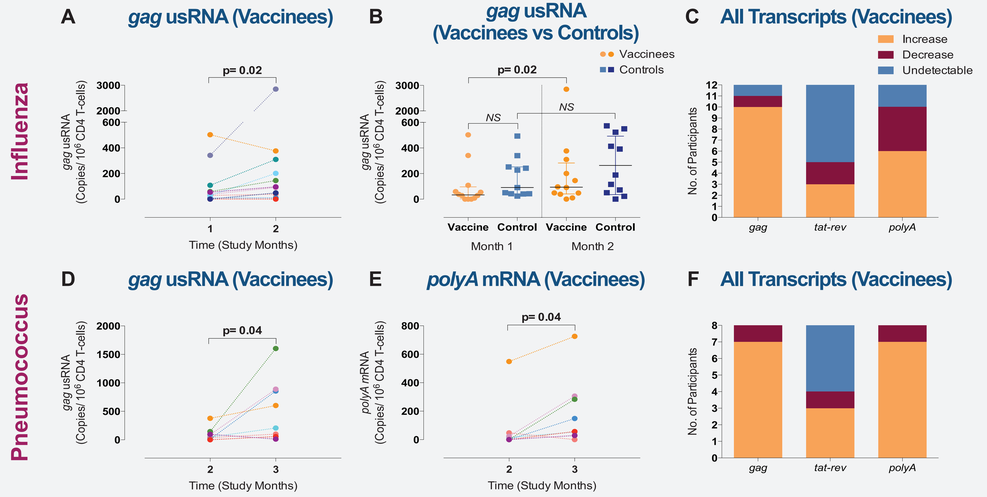


Figure 2: Absolute changes in HIV caRNA after Influenza and Pneumococcus vaccinations
Cell-associated HIV RNA (HIV caRNA) before and 1 month after Influenza (A-C) and Pneumococcus (D-F) vaccinations, respectively. Points represent single subjects with color-coding preserved throughout (A, D, E). p-values of Wilcoxon and Mann-Whitney tests for paired (A, B, D, E) and unpaired samples (B), respectively. Number of participants with increase, decrease or no change in measured transcripts (*gag, tat-rev, polyA*) after Influenza (C) and Pneumococcus (F) vaccinations.

	Influenza			Pneumococcus		
Transcript	*gag*	*tat-rev*	*polyA*	*gag*	*tat-rev*	*polyA*
Median	2.44	0	3.73	7.04	0	20.94
IQ Range	1.3 - 7.4	0 - 1.4	0 - 12.6	2.4 - 22.7	0 - 23.5	3.0 - 79.7

Table 3: Fold-changes in HIV caRNA after vaccination
Overall median fold-changes with interquartile ranges (IQ range) for *gag, tat-rev* and *polyA* transcripts after Influenza and Pneumococcus vaccinations (vaccinees).

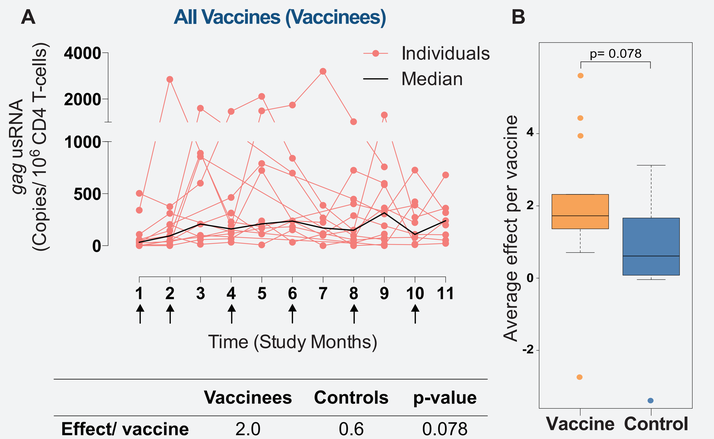
► There were no significant changes in HIV DNA or plasma HIV RNA after vaccination.

► Intra-host HIV RNA transcripts behaved differently:

- *gag* was detectable in 28 of 32 samples.
- *tat-rev* was least sensitive (undetectable in 11 samples).
- *polyA* was undetectable in 10 samples, but when detected showed the largest changes (**Table 3**).
- *gag* and *polyA* transcripts were significantly correlated for both Influenza (**p=0.003**) and Pneumococcus (**p=0.02**) vaccines.
- *tat-rev* did not correlate with either *gag* or *polyA*.

Results

Multiple Vaccines have Cumulative Effect



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Effect/ vaccine	2.0	0.6	0.078
p-value	**0.004**	0.19	

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gag usRNA transcripts in vaccinees (A) over study period; solid black line represents median cohort values, black arrows denote vaccination timepoints. Comparison of vaccinated versus control subjects (B, table inset) using average effect per vaccine (or placebo) calculated by multiple regression of logdomain *gag* usRNA levels onto predictor variables representing vaccine boost and temporal decay; regression coefficient with interquartile range represented in (B), p-values compare between groups (B) or single groups to null hypothesis (table).

Conclusions

► Influenza and Pneumococcus vaccinations were associated with significant increases in HIV caRNA during suppressive ART.

► Although no changes in HIV caRNA were seen with individual Hep A, VZV, MMR or TD vaccines, sequential administration led to overall significant effects of vaccination.

► Levels of HIV caRNA vary amongst unspliced, multi-spliced and overall transcripts (as measured by polyA), possibly reflecting different assay sensitivities.

► Plasma viral loads, total HIV DNA and 2-LTR circle copies did not change significantly after vaccination, reflecting successful suppression of *de novo* infection by ART.

► Routine vaccination is unlikely to present a cure for HIV-1 infection. Nevertheless, our findings suggest that multiple sequential immune stimulatory "hits" may act syngeristically to reactivate latent HIV and may be important in future curative strategies.

References

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Acknowledgements

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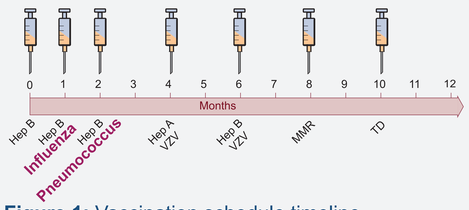

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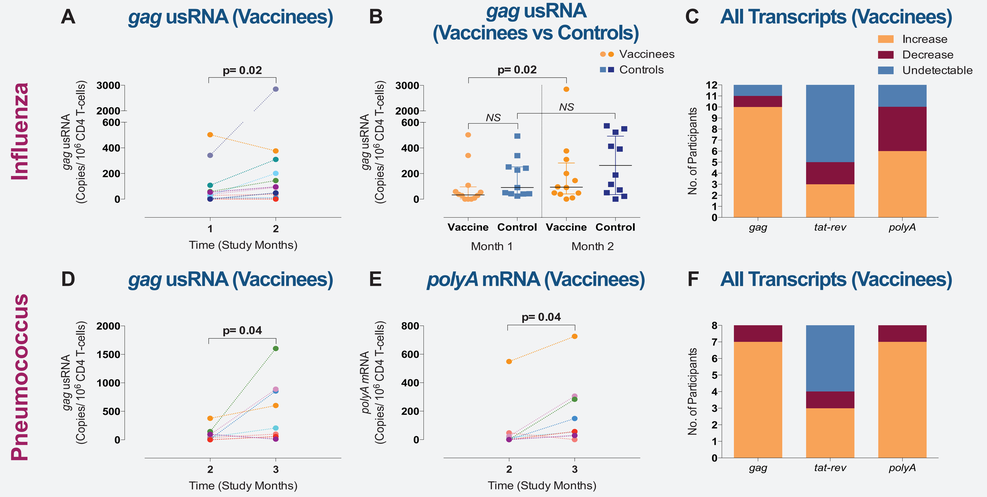

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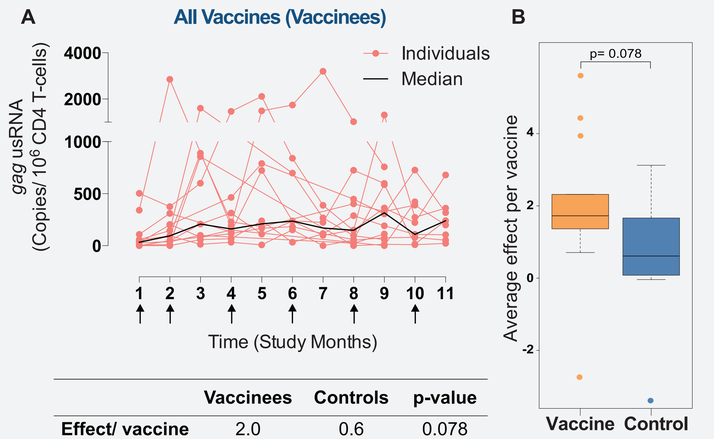

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