IMPACT OF IMMUNE CHECKPOINT INHIBITORS IN VACCINE-INDUCED ANTI-HIV RESPONSES

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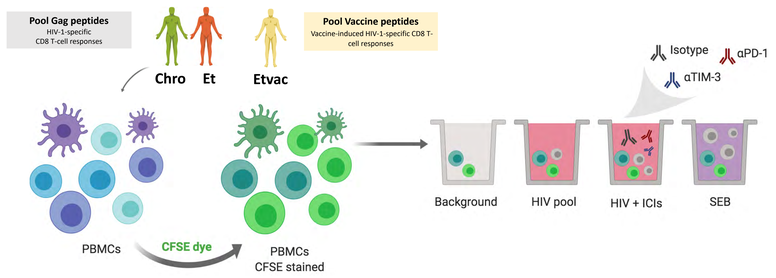
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BACKGROUND

To attain the control or elimination of HIV-1 infection it is critical to delineate immune interventions capable of boosting or reinvigorating HIV-1-specific CD8+ T-cell responses. Immune interventions, including therapeutic vaccines or immune checkpoint inhibitors (ICIs), have been postulated to achieve this goal. However, the potency of combining both immune interventions has not yet been tested.

AIM: To assess ex vivo the impact of ICIs on vaccine-induced HIV-1 CD8+ T cell responses in samples from a vaccine trial conducted in early-treated HIV-1 infected individuals.

METHODS



We selected PBMCs of individuals from the BCN01 (NCT01712425) trial receiving early treatment and a ChAdV63.HIVconsv/MVA.HIVconsv prime-boost regimen (Etvac; n=12), from early treated not vaccinated individuals (Et; n=13) and chronically treated individuals (Chro; n=11). Functional assays; PBMCs were CFSE-stained and stimulated with an HIV-1 peptide pool in the presence of anti-PD-1, anti-TIM-3, anti-PD-1+TIM-3 or isotype antibodies. After seven days, we quantified the frequency of CFSE-, IFNγ+ and HLA-DR+/CD38+ HIV-1-specific CD8+ T cells by polychromatic flow cytometry. Also, 17 human cytokines in the culture supernatants were measured by multiplex assay.

STUDY GROUPS

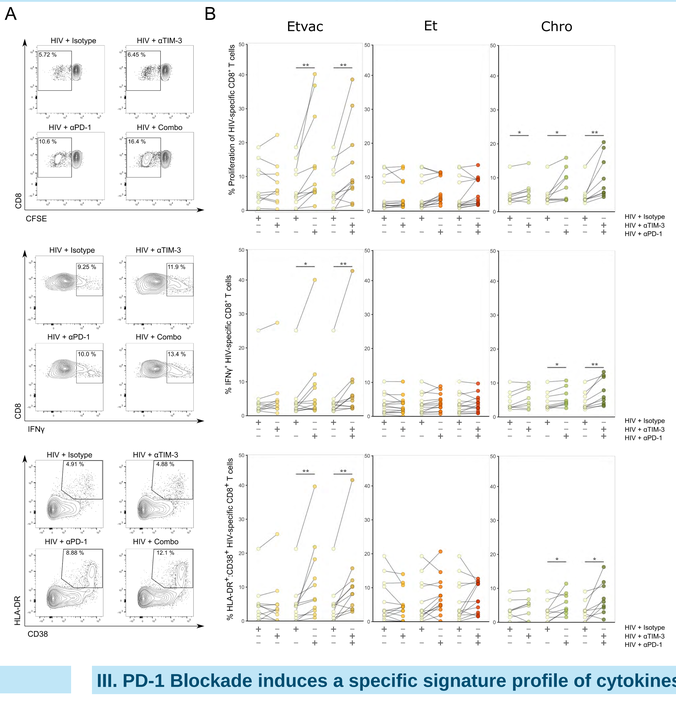
	Early treated HIV-infected individuals vaccinated (Etvac, n= 12)	Early treated HIV-infected individuals (Et, n=13)	Chronic HIV-infected individuals (Chro, n=11)
Male sex - *no (%)*	11 (91,7%)	13 (100%)	9 (75%)
Female sex - *no. (%)*	1 (8.3%)	0 (0%)	2 (25%)
Age - *mean years (range)**	40 (30 - 49)	33 (22 - 62)	46 (38 - 60)
CD4 count - *mean no. cells/µl (range)**	821 (283 - 1302)	871 (273 - 1322)	839 (542 - 1267)
Viral load *	Undetectable	Undetectable	Undetectable
Years since HIV diagnosis - *mean (range)**	0.9 (0.7 - 1.0)	0.9 (0.6 - 1.0)	13.0 (6.3 - 19.0)
Time undetectable - mean years (range)*	> 0.5	> 0.5	6.9 (2.1 - 16.0)
Time since HIV-1 diagnosis to cART - *mean month (range)*	0	0	0.9 (0 - 4.2)
Time since HIV-1 infection to diagnosis/cART - *median days (range)*	89 (28 - 164)	68 (28 - 98)	NA

* at sampling point

WE PROPOSE THE COMBINED USE OF IMMUNE CHECKPOINT INHIBITORS AND THERAPEUTIC VACCINES TO BOOST VACCINE-INDUCED ANTI-HIV CD8+ RESPONSES

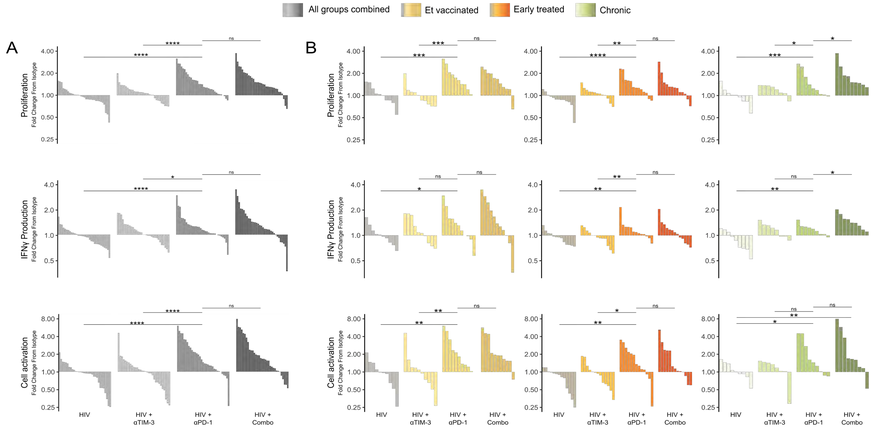
RESULTS

I. PD-1 Blockade boosted Etvac and Chro HIV-specific CD8+ T cell responses



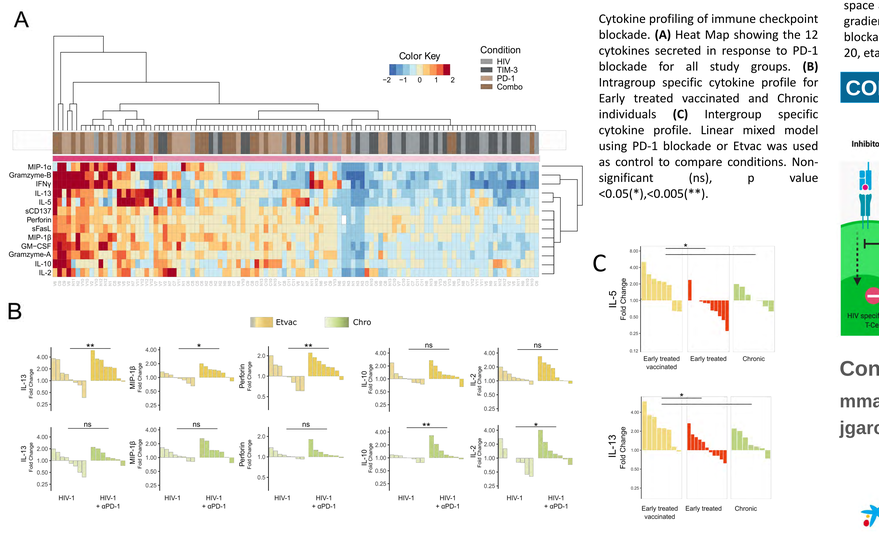
Response to Immune Checkpoint Blockade in Early treated vaccinated (Etvac), Early treated (Et) and Chronic (Chro) individuals. **(A)** Representative flow cytometry plots showing the frequency of proliferation, by total loss of CFSE dye, the production of IFN-gamma, and the frequency of cell activation ($CD38^+$:$HLA\text{-}DR^+$) of HIV-specific $CD8^+$ T-cells in the presence of Isotype control, αTIM-3, αPD-1 or αTIM-3 + αPD-1 antibodies. **(B)** Pairwise comparison of the proliferation, the IFNγ production, and the frequency of cell activation in HIV-specific CD8+ T-cells in responses to immune checkpoint blockade in Etvac, Et and Chro individuals. For each study group and from left to right, pairwise analysis compare HIV-1 pool peptide plus isotype control condition versus HIV-1 pool peptide plus αTIM-3, αPD-1 or αTIM-3 + αPD-1 (Combo) blockade antibodies. Wilcoxon matched-pairs signed-rank test was used to compare isotype conditions versus immune checkpoint blockade conditions. Non-significant (not shown), p value <0.05(*), p value<0.005(**).

II. Lack of additive effect of PD-1 and TIM-3 blockade in Etvac



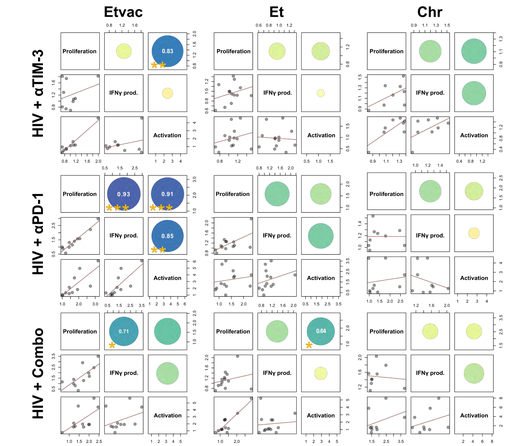
Waterfall plots illustrating the magnitude of HIV-specific CD8+ T-cell Proliferation (top), IFNγ production (middle), and activation (bottom) in Fold Change from Isotype control. From left to right, HIV-1, HIV + αTIM3, HIV + αPD-1, and HIV + Combo conditions **(A)** Fold change for all groups combined is represented for a grey bar. **(B)** Fold change for each individual is represented for a colored bar according to the study group. Linear mixed model using PD-1 blockade as control was used to compare conditions. Non-significant (ns), p value<0.05(*), p value<0.005(**), p value<0.0005(***), p value<0.00005(****).

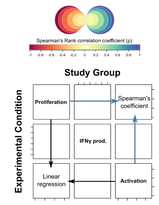
III. PD-1 Blockade induces a specific signature profile of cytokines in Etvac



Cytokine profiling of immune checkpoint blockade. **(A)** Heat Map showing the 12 cytokines secreted in response to PD-1 blockade for all study groups. **(B)** Intragroup specific cytokine profile for Early treated vaccinated and Chronic individuals **(C)** Intergroup specific cytokine profile. Linear mixed model using PD-1 blockade or Etvac was used as control to compare conditions. Non-significant (ns), p value <0.05(*),<0.005(**).

IV. Correlation of functional markers are exclusive of Etvac after PD-1 blockade



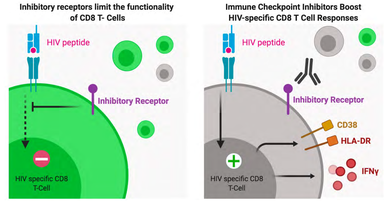


Correlograms between functional markers in responses to Immune Checkpoint Blockade. Each individual correlogram shows the correlated parameters; Proliferation, IFNγ production, and cell activation of HIV-1-specific CD8 T cells in Fold change from the Isotype control. Correlation with a p-value greater than 0.05 shows the Spearman rank correlation coefficient in white. The color and size of circles indicate the strength and direction of correlation. P value<0.05(*), <0.005(**), <0.0005(***).

V. Single-cell HIV-specific CD8+ T cell responses in Etvac after PD-1 blockade

Multidimensionality-reduction of single-cell cytofluorimetric data in Etvac cells in the PD-1 blockade condition. **(A)** Cells represented in a tSNE space are colored based on the intensity of either the CFSE dye or the fluorochrome-conjugated antibodies; IFNγ, CD38 and HLA-DR. The color gradients display the relative loss of CFSE dye, production of IFNγ and the expression of CD38 and HLA-DR. **(B)** Clustering analisis Etvac in PD-1 blockade condition. tSNE clustering was performed with the following parameters: Downsampling – 1500 cells, Iterations – 1000, Perplexity – 20, eta/learning rate – 200, Theta – 0.05.

CONCLUSIONS



ICIs boost the magnitude of vaccine-induced HIV-1-specific CD8+ T-cell responses in Etvac. These responses are define by a concomitant proliferation, activation and IFNg secretion at the single-cell level. Also, response to ICIs was linked to personalized cytokine signature profile in Etvac.

We propose a novel therapeutic use of ICIs in combination with therapeutic vaccines to boost vaccine-induced immune responses.

Contac us!

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