# Polyfunctional HIV-specific T cells in Post-Treatment Controllers

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To further understand the exceptional HIV-1 control observed in Post-Treatment Controllers (PTCs) from the Virological and Immunological Sustained CONtrol after Treatment Interruption study we investigated their HIV-specific T-cell responses. Polyfunctionality of HIV-specific CD4 and CD8 T cells and the ratios of HIV-specific CD4 T cells per infected cells were similar in post-treatment controllers, continuously early-treated patients and long-term non-progressors Overall early treatment appears to preserve robust HIV-specific $CD4^+$ T cells, which might contribute to the posttreatment control of HIV. 

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A model of HIV remission is represented by the Post-Treatment Controllers (PTCs) from the Virological and Immunological Sustained CONtrol after Treatment Interruption (VISCONTI) study, who control HIV over 7 years after interrupting combined antiretroviral therapy (cART) initiated shortly after primary HIV-1 infection (PHI) [1,2]. PTCs and Long-Term-Non-Progressors (LTNPs) display similar magnitude and distribution of their HIV reservoirs, whereas their clinical and genetic backgrounds differ. Indeed, PTCs are frequently HLA-B*35+, an allele previously associated with symptomatic PHI and rapid progression [3,4]. Contrarily, they are not enriched in the classical protective HLA-B*27 or B*57 alleles associated with spontaneous HIV control and robust anti-HIV $CD8^+$ T-cell responses [5–9]. Accordingly, low levels of HIV-specific $CD8^+$ T cells producing IFN-γ and limited inhibition of HIV production by $CD4^+$ T cells were previously reported in the VISCONTI PTCs [2]. The impact of HLA alleles was not examined in this study. To further understand whether T-cell-mediated immunity to HIV participated to the exceptional HIV-1 control observed in PTCs, we compared their HIV-specific T-cell responses to those of LTNPs and of Continuously Early-Treated patients (CETs).

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We included six HLA-B*35+ and six HLA-B*35− PTCs from the VISCONTI study [2], who had initiated cART within 10 weeks PHI. All 12 PTCs controlled HIV viremia for a median 3 years after 101-month (75−112) long cART interruption. They were compared with eight treatment-naive LTNPs (ANRS ALT-CO-15 cohort) [10] infected for at least 15 [11−13] years, and 10 CETs fully suppressed patients on cART initiated within 10 weeks PHI for a duration of 86 (38−150) months. Two CETs but no LTNPs carried HLA-B*35, whereas six LTNPs were either HLA-B*27 or B*57, in contrast to only one PTC and two CETs. Plasma viral loads were significantly lower in PTCs and CETs compared with LTNPs ($P = 0.005$). Levels of total cell-associated HIV-DNA measured in peripheral blood mononuclear cells (PBMCs) using the ANRS ultra-sensitive quantitative real-time PCR assay (Biocentric, Bandol, France) [2,9] did not significantly differ between the three groups despite a trend toward higher levels in HLA-B*35+ PTCs [127 (14−324)] compared with HLA-B*35- PTCs [25 (4−161)]. All patients' characteristics are described in Supplementary Table 1, http://links.lww.com/QAD/A949. Institutional review boards had approved all studies and patients signed informed consent.

The HIV-specific $CD4^+$ and $CD8^+$ T-cells intracellular cytokine-staining assay was performed [14] after stimulation with recombinant HIV-1 p24 (Protein-Sciences, Meriden, Connecticut, USA) or HIV-1 p24 15-mers synthetic peptide pools (Neosystem, Strasbourg, France), respectively. Staining was performed with anti-CD3-Pacific Blue, CD4-ECD (Beckman-Coulter Villepinte, France), CD8-APC-Cy7, IFN-γ-Alexa700, IL-2-APC, MIP-1β-FITC, TNFα-PECy7, and CD40L-PE (BD-Bioscience, San Jose, California, USA) monoclonal antibodies. At least one million cells were analyzed on Gallios Flow-Cytometer with Kaluza 1.2 Software (Beckmann-Coulter). The polyfunctionality index $(PI) = \sum i = 0nFüinq$ (with $q$ set conservatively to 1) was employed [15] and data were analyzed with the software SPICE (M. Roederer, Immuno Technology Section VRC/NIAID/ NIH, USA) and Funky Cells ToolBox (www.FunkyCells.com). All data were analyzed using the nonparametric Mann−Whitney $U$ test and Spearman's rank test and incorporated Bonferroni corrections for multiple comparisons. All values are medians and interquartile range.

Frequencies of HIV-specific $CD4^+$ T cells producing at least one function, mainly IFN-γ and MIP-1β, or displaying CD40L did not differ between PTCs [0.35% (0.09- 0.67)], LTNPs [0.16% (0.10−0.32)], or CETs [0.52% (0.17−1.64)] (Fig. 1a). Interestingly, anti-HIV $CD4^+$ T cells were even more frequent in CETs than in LTNPs [$P = 0.013$ (NS after Bonferroni correction)], confirming that early cART preserves HIV-specific $CD4^+$ T cells [11−13,16,17]. In addition, 28.1 and 30.3% HIV-specific $CD4^+$ T cells from PTCs and CETs, respectively, mediated at least 2 functions, not different from 49% observed in LTNPs, with a similar polyfunctionality index between PTCs, LTNPs, and CETs (30, 35, and 31, respectively) (Fig. 1b).

The highest HIV-specific $CD8^+$ T-cell frequencies producing at least one function were observed in LTNPs [1.24% (1.14−3.72)] compared with whole PTC group [0.29% (0.17−0.62) $P = 0.006$ (NS after Bonferroni correction)], but not different from CETs [0.46% (0.24−1.72)] (Fig. 1c). Furthermore PTCs $CD8^+$ T cells producing IFN-γ and/or MIP-1β were five to 10-fold fewer [0.04% (0.01−0.15); 0.083% (0.04−0.13), respectively] than in LTNPs [0.57% (0.45−1.81) $P = 0.015$ (NS after Bonferroni correction); 0.66% (0.43−1.84) $P = 0.001$ (significant after Bonferroni correction), respectively] because of lower levels in HLA-B*35+ [0.006% (0.003−0.061); 0.08% (0.008−0.1)] than in B*35− PTCs [0.131% (0.022−0.382); 0.106% (0.048−0.321)]. CETs had also fewer cells producing MIP-1β [0.19% (0.04−0.54)] than LTNPs [$P = 0.012$ (NS after Bonferroni correction)]. Altogether, we cannot exclude the LTNPs' higher viremia might stimulate higher $CD8^+$ responses compared with PTCs or CETs.

In contrast, polyfunctional HIV-specific $CD8^+$ T cells producing at least two functions and polyfunctionality indices tended to be similar in PTCs, LTNPs, and CETs (44.6, 57.5, 47.1% and 33, 32, and 30, respectively) independently of HLA-B*35 (Fig. 1d). Therefore, despite an HLA-B*35 effect on MIP-1β production, early treatment also appears to preserve functionality of HIV-specific $CD8^+$ T cells. The importance of robust polyfunctional HIV-specific $CD4^+$ and $CD8^+$ T cells is supported by our recent correlation between polyfunctionality and in-vitro cytotoxic capacity [18,19], as well as by recent demonstration that T-cell polyfunctionality assessed either using our polyfunctional index or the newly described COMPASS index, predicts protection against HIV acquisition [20].

Finally, as one infected cell harbors only one HIV-DNA copy, we calculated the 'in-vivo immune effector/target cell ratios' (E/T), by dividing the HIV-specific T cell by the HIV-infected cell numbers per million PBMCs (Fig. 1e and f) [9,21]. The CD4 E/T ratios [65 (12−172)] did not differ between PTCs and LTNPs [62 (19−155)] or CETs [179 (105−1372)], whereas the CETs ratio was significantly higher than in LTNPs ($P = 0.043$; Fig. 1g). The CD8 E/T ratios were higher in LTNPs [417 (105−1980)] compared with PTCs [84 (16−305)], though significant only in HLA-B*35+ [35 (12−91), $P = 0.005$] and not in non-HLA-B*35 PTCs [240 (67−781)], whereas the CETs CD8 E/T ratio was intermediate [221 (82−1622)].

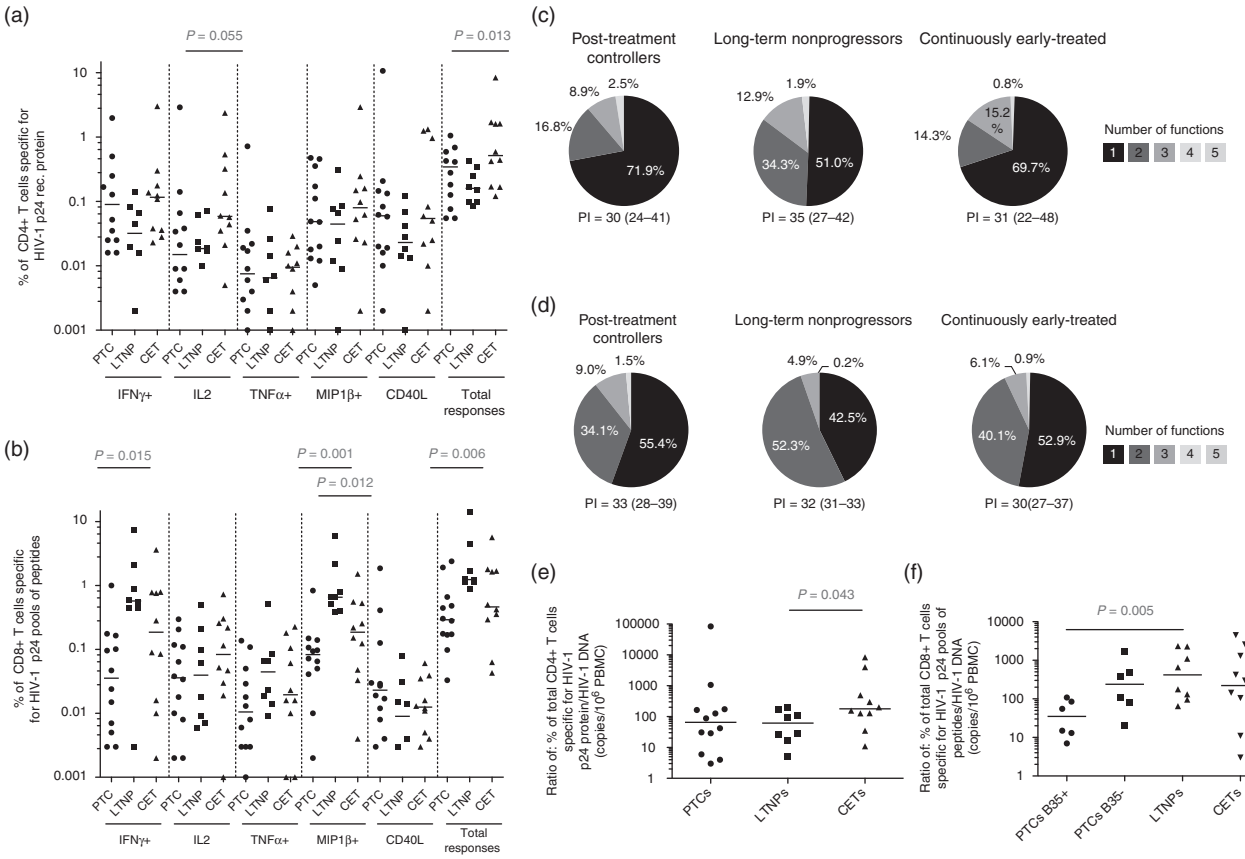


**Fig. 1. T-cell responses in the three groups of patients were assessed after peripheral blood mononuclear cell (PBMC) stimulation with HIV-1 p24 recombinant protein for $CD4^+$ T-cell responses and with HIV-1 p24 peptide pools for $CD8^+$ T-cell response.** PBMCs were then stained simultaneously for CD40L, IL-2, IFN-γ, MIP-1β, and TNFα and analyzed by flow cytometry. Frequencies of HIV-specific T cells expressing CD40L, producing IL-2, IFNγ, MIP-1β, and TNFα, are presented for $CD4^+$ T-cells (a) or $CD8^+$ T-cells (c) black line representing median values in Post-Treatment Controllers (PTCs), Continuously Early-Treated patients (CETs), and Long-Term-Non-Progressors (LTNPs). The background value from unstimulated peripheral blood mononuclear cells was subtracted. The pie charts depict the polyfunctional profile (1–5 functions) of HIV-specific $CD4^+$ T-cell (b) or HIV-specific $CD8^+$ T-cells. (d) Polyfunctional index is indicated [median (interquartile range)]. Ratio between (e) the total of $CD4^+$ T cells specific for HIV-1 p24 protein (f) the total of $CD8^+$ T cells specific for HIV-1 p24 pools of peptides and HIV-1 DNA reservoir measured in PBMC are presented for PTCs, CETs, and LTNPs and for HLA*B35+ and HLA*B35− PTC. The *P* value was determined by the Mann–Whitney test and we used Bonferroni correction for multiple comparisons. CETs, continuously early treated; LTNPs, long-term nonprogressors; PTCs, posttreatment controllers.

Of note, in the small samples studied here, no correlation was observed between the magnitudes and polyfunctionality of HIV-specific $CD4^+$ and $CD8^+$ T cells. Whatever the group and after a Bonferroni correction for multiple comparisons, many *P* values would no longer be statistically significant.

Thus, our results, obtained in this small number of samples, strongly suggest the robust polyfunctional anti-HIV $CD4^+$ T-cell responses preserved by prolonged early cART may have allowed high CD4 E/T ratios in PTCs similarly to LTNPs, and therefore might contribute to virus control, even in HLA-B*35+ individuals. In contrast, $CD8^+$ T-cell control might not be as contributive because of the HLA-B*35 effect, whereas other mechanisms, such as natural killer cells, might also participate in control of viral reservoirs and in establishment of remission [22] in the VISCONTI model of functional HIV cure.

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A.S.C., C.R., B.A. Performed the experiments: A.S., C.B.S., B.D., V.A.F., I.T. Analyzed the data: A.S., M.L. Wrote the paper: A.S. Contributed to reviewing the manuscript. A.S., L.H., V.A.F., M.L., A.S.C., C.R., B.A.

## Conflicts of interest

M.L. is inventor of the polyfunctionality index (patent number: WO2013127904) and proprietary owner of the Funky Cells ToolBox software (www.FunkyCells.com).

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