PA-101 **FUNCTIONAL AND PHENOTYPIC CHARACTERISATION
OF REGULATORY T (TREG) CELLS IN ANTIRETROVIRAL
NAÏVE HIV-1 INFECTED PEOPLE**

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Background Regulatory T cells (Tregs) function in dampening excessive immune activation in steady state. However during HIV-1 infection there is sustained immune activation and it is not known how Tregs function in this context. To optimise immunotherapeutic strategies based on Tregs for HIV-1 infected people we assessed the phenotypic and functional properties of these cells from antiretroviral naïve HIV-1 infected adults in Cameroon.

Methods Tregs were purified by magnetic sorting from PBMCs obtained from adults aged 21 to 65 years using microbeads according to the manufacturer's protocol (Miltenyi Biotec). The phenotypic properties of the purified Tregs were then determined by multiparametric flow cytometry. Tregs functions were assessed by measuring inflammatory cytokine formation by monocytes following co-culture with autologous Tregs in the presence of either polyICLC or CLO97. Samples were acquired on BD Fortessa X5 cytometer using BDFACS Diva Software and data analysed with FlowJo version 9.8.5. Graph Pad Prism 5 was used for statistical analysis.

Results Tregs were defined as CD4+CD25+CD127LoFoxP3+ cells. However, the strong correlation between Foxp3 with the combination of CD25+CD127Lo ($r=0.965$; $p<0.001$, Pearson's correlation) allowed us to use these surface markers as previously reported for tracking Tregs in subsequent experiments. With respect to surface expression there was a significant elevation of HLA-DR /CD38 in Tregs from HIV-1-infected people when compared to HIV-participants. When purified Tregs were co-cultured with autologous monocytes in the presence polyICLC (a TLR 3 agonist) and CLO97 (TLR7/8 agonist) they escalated the intracellular formation of both TNF- α and IL-6 by monocytes. The escalation was significantly higher in co-cultures of cells from antiretroviral naïve HIV-1-infected people relative to seronegative participants.

Conclusions Dysregulation in Tregs function can exacerbate inflammatory cytokine formation.