

# Imperial College London Effects of Recombinant Growth Hormone on T-cell Phenotype and Function *In Vitro* and *In Vivo* During Treated HIV-1 Infection

POSTER # 721

Alison Cranage<sup>1</sup>, Graham Moyle<sup>2</sup>, Mark Bower<sup>2</sup>, Brian Gazzard<sup>2</sup>, Frances Gotch<sup>1</sup>, Nesrina Imami<sup>1</sup>

<sup>1</sup>Department of Immunology, Chelsea & Westminster Campus, Faculty of Medicine, Imperial College London, <sup>2</sup>Department of HIV/GU Medicine, Chelsea & Westminster Hospital, London, UK.

## ABSTRACT (# H-108)

**Background:** Recombinant human growth hormone (rhGH) has beneficial effects on the immune system. It can affect and improve thymic function, and also act locally on peripheral blood mononuclear cells (PBMC). In HIV-1 infection thymic function is impaired, resulting in reduced production of naive T cells, which are required to control infection. We studied the effects of rhGH *in vitro* and *in vivo* on T-cell function and phenotype, during treated HIV-1 infection. **Methods:** PBMC from healthy controls (HC) and HIV-1-infected individuals were cultured with rhGH for 72 hours, at two pharmacological concentrations. Cells were stained at baseline and after culture to determine CD4 and CD8 T-cell memory/differentiation status, activation and apoptosis markers. Lymphocyte proliferation was assessed using the conventional tritiated thymidine incorporation assay. Results were compared to those obtained from patients enrolled on a randomised, double blind, placebo controlled study to receive daily rhGH therapy, where we measured the proliferation of CD4 T cells, IFN $\gamma$ -production by CD8 T cells using ELISPOT assays, and assessed TREC and pro-viral DNA levels. The assays were carried out at baseline (before GH-therapy) and at 12 weeks (after GH-therapy) and at 24 and 48 week follow-ups. **Results:** We calculated the change from baseline in expression of several markers on CD4 and CD8 T cells cultured with rhGH. After culture, fewer CD4 cells expressed CD38 (marker of activation/disease progression) and HLA-DR (marker of activation), in HIV-1+ patients but not in HCs (p=0.008). There was a significantly larger decrease in CD8 T cells that expressed CD95 and CD57 (FasL and marker of senescence respectively) from baseline, when HIV-1+ patients were compared to HCs (p=0.015). *In vivo* daily administration of rhGH significantly increased HIV-1 specific CD4 proliferative responses and IFN $\gamma$  production by CD8 T-cells. These responses declined with less frequent dosing. There was a significant enhancement of T-cell maturation and proliferation, and an increase in naive CD4 T cells (all p values <0.05). TREC levels and pro-viral DNA remained stable. **Conclusions:** This data indicates that administration of rhGH has a beneficial effect on the thymus of HIV-1+ patients. Furthermore, the *in vitro* data indicates that beneficial effects of rhGH on T cells in the periphery may correlate with a reduction of activation and senescence markers.

## INTRODUCTION

Growth hormone (GH) secretion and subsequent insulin-like growth-factor-1 (IGF-1) release is essential for human growth and development. It has also been implicated to play a role in lymphocyte development and function. Studies in mice indicate that GH plays a role in the thymus, stimulating thymic growth as well as enhancing the function of thymic epithelial cells. In humans the mechanism by which GH affects thymic function is not clear. In HIV-1 infection thymic function is impaired, and it is therefore possible that administration of GH can complement HAART in restoring thymic function. In addition, GH can be produced and act locally on leukocytes in the periphery.

HIV-1-specific CD4+ helper (HTL) and CD8+ cytotoxic (CTL) T lymphocyte responses have been shown to inversely correlate with viral replication, disease progression and even protection from productive infection in HIV-1 infected and seronegative individuals. Immunomodulation with rhGH together with effective ART may boost the missing HIV-1-specific responses in chronic HIV-1 infection.

## AIMS AND OBJECTIVES

- Aim:** To assess the effects of recombinant GH (rGH) on T-cell function and phenotype *in vivo* and *in vitro*.
- Objectives:** To study the change in phenotype of T cells after *in vitro* culture with rGH  
 To study the effects of HIV-1 specific HTL and CTL function after daily administration of GH to HIV-1 patients on ART

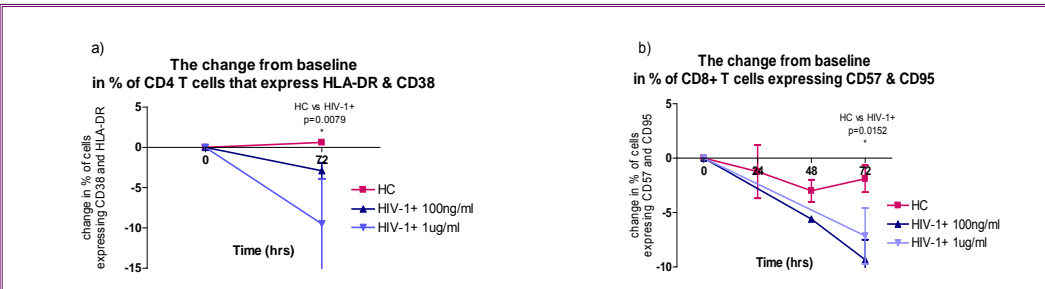
## METHODS - In Vivo Study

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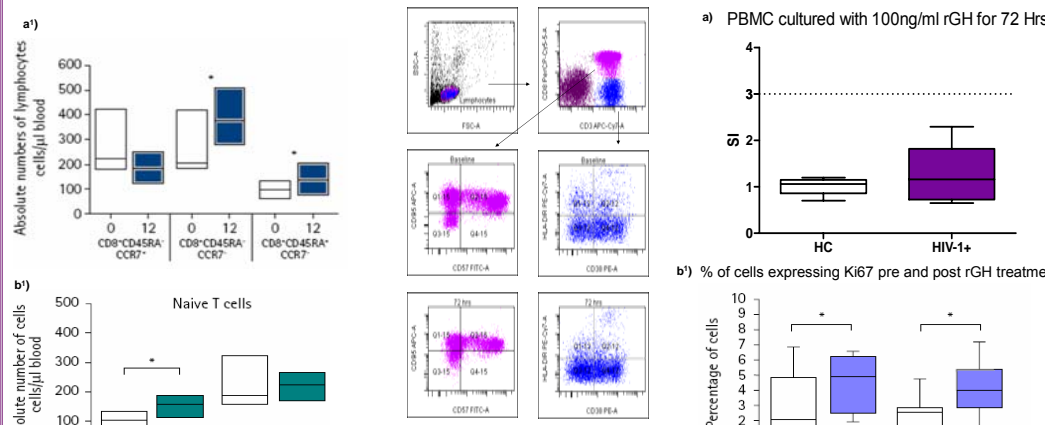
PBMC from 12 HIV-1 infected individuals with lipotrophy on long-term successful ART receiving rhGH (Serostim, Serono International, Geneva, Switzerland), were assessed for HTL proliferation and CTL IFN $\gamma$  production. Recombinant human growth hormone administration: Given at 4mg/day for 12 weeks to 12 HIV-1+ chronically infected individuals receiving successful ART. After 12 weeks alternate day dosing or twice weekly dosing, for a further 12 weeks. Weeks 24-48 patients received ART alone (no immunotherapy). Samples were collected at baseline and weeks 12, 24 and 48 of the study.

Lymphocyte Proliferation Reaction (LPR) was performed using the standard tritiated thymidine incorporation method. In the *in vitro* studies proliferation was measured in response to culture with rGH over 72 hrs. In the *in vivo* study PBMCs were taken at each time point and CD4 proliferation was measured in response to HIV-1 antigens (20mer peptides)

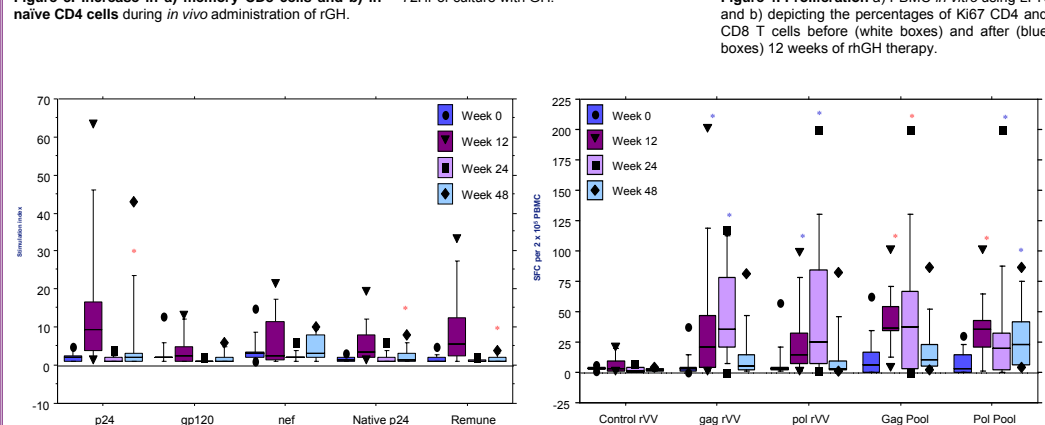
In addition PBMCs were phenotyped at each time point to assess the expression of CD3, CD4, CD8, CD45RA, CCR7 and Ki67.



**Figure 1. *In vivo* treatment of PBMCs with rGH.** Phenotypic changes from baseline in expression of a) HLA-DR and CD38 on CD4 cells and b) CD57 and CD95 on CD8 cells cultured with 100ng rGH; healthy controls (HC) and HIV-1+ and 1ug/ml rGH; HIV-1+



**Figure 3. Increase in a) memory CD8 cells and b) in naive CD4 cells during *in vivo* administration of rGH.**



**Figure 5. Immunotherapy with rhGH in the presence of effective ART induced a significant (p<0.005) increase in HIV-1-specific CD4 T-cell responses, measured by LPR (stimulated with 20mer peptides).**

## RESULTS AND DISCUSSION

As shown in figure 1, culturing T cells with rGH induced changes in phenotype. A smaller % of CD4+ T cells from HIV-1 infected individuals expressed HLA-DR and CD38 after 72 of culture compared to baseline. This change was significantly different between HIV-1+ individuals and HC. There was also a decrease in the % of CD8 cells expressing CD57 and CD95 from baseline. Again the change was significantly different in HIV-1+ individuals compared to HC. These results indicate that rGH can directly affect the phenotype and therefore possibly the function of T cells in the periphery. The observation that HIV-1+ infected patients show a greater change compared to HC could be indicative of differential levels of expression at baseline, or that cells from HIV-1 infected individuals are more prone to apoptosis. Figure 2 shows a representative example of the staining before (baseline) and after cells were cultured with rGH for 72hrs. The change in % of cells in each quadrant from baseline to 72 hours was calculated.

In figure 3a, the absolute numbers of naive (CD45RA+CD27+) CD4 and CD8 T cells before (white box) and after (blue box) 12 weeks of rGH therapy are shown. There was a significant increase in naive CD4 cells over this time. Figure 3b shows the median absolute numbers of CD8 T cells expressing each phenotype (given on the x-axis) describing the maturation pathway of CD8 T cells (TCM, CD8+CD45RA-CCR7+; terminally differentiated TEM, CD8+CD45RA-CCR7-; terminally differentiated TEMRA, CD8+CD45RA+CCR7-) at baseline (white) and week 12 (blue). Data depicted as median values with interquartile ranges. \*P<0.05. There was an increase in CD8 cells with a TEM phenotype.

Figure 4 indicates that rGH does not cause proliferation of T-cells *in vitro* directly. This is shown by an LPR measuring proliferation of PBMCs cultured in 100ng/ml rGH for 72 hrs. *In vivo*, using Ki67 staining (which binds to cells in G2 phase of the cell cycle), as shown in figure 5b, rGH administration did induce an increase of proliferating CD4 and CD8 T cells. Although TREC levels remained unchanged, the Ki67 data indicates that rGH is having a indirect effect on T cell function – possibly affecting the thymus.

Figure 5 shows the CD4 HTL responses in patients on rGH therapy. At baseline there was a lack of proliferative CD4 virus-specific responses to any HIV-1 antigen in 11 of 12 individuals. Daily rhGH increased both gag-specific and whole HIV-1 antigen (Remune)-specific CD4 T-cell responses over a 12-week period in 9 of 12 patients with chronic infection on ART (Fig 2). These effects were not maintained with less frequent dosing.

Figure 6 shows the induction of HIV-1 specific CD8 T-cell responses with administration of rGH and effective ART. Immunotherapy with rGH in the presence of ART induced a significant increase in HIV-1-specific CD8 T-cell responses evaluated with rVV constructs (p<0.05) and peptide pools (p<0.005) of gag or pol proteins in IFN $\gamma$  ELISPOT assays.

## CONCLUSIONS

- Strong HIV-1-specific CD4 and CD8 T-cell responses were amplified by daily administration of rhGH over the 12 week period in HIV-1+ individuals on successful ART. Randomisation (into placebo, alternate day dosing or twice weekly dosing) at week 12, showed that HIV-1-specific CD8 T-cell responses were maintained at week 24 regardless of randomisation, despite the disappearance of HIV-1-specific CD4 T-cell responses. At week 48, on ART alone (no immunotherapy) for the final 24 weeks, HIV-1-specific CD8 T-cell responses declined (except for anti-pol). HIV-1-specific CD4 T-cell responses remained undetectable. Disappearance of CD4 responses suggests that cells may require stronger or continuous signals from GH to provide ongoing help. CD8 responses may be maintained for a limited period without CD4 T cell help.
- *In vitro* data indicate that rGH can have an effect on T cells in the periphery, specifically on phenotype. This effect may be beneficial in that activation markers associated with disease progression and also markers of senescence may be reduced.
- We provide novel data inferring that concomitant administration of rhGH with effective ART may reverse defects exerted on the immune system by HIV-1.
- We show novel effects of *in vitro* rGH on T-cell phenotype.

## ACKNOWLEDGEMENTS REFERENCES

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