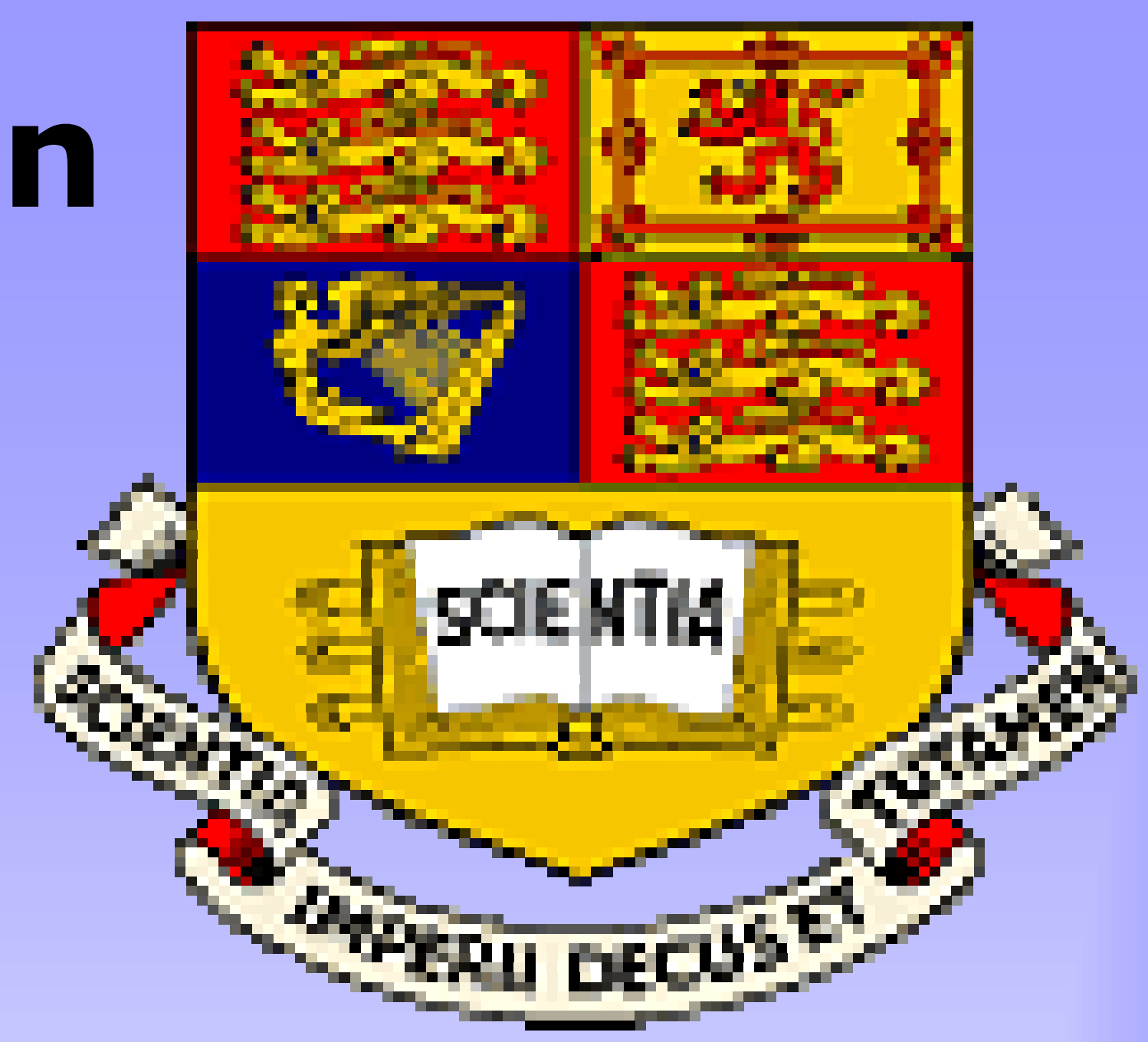


# Growth Hormone Enhances Thymocyte Development and Induces Differentiation into Functional Peripheral T Cells in HAART Treated HIV-1+ Patients

A. Pires<sup>1\*</sup>, J. Pido-Lopez<sup>1</sup>, G. Moyle<sup>2</sup>, B. Gazzard<sup>2</sup>, F. Gotch<sup>1</sup> and N. Imami<sup>1</sup>

Imperial Coll. of Sci. Technol. and Med<sup>1</sup>., and Dept. of GU Med<sup>2</sup>, Chelsea and Westminster Hosp., London, UK



Imperial College of Science Technology and Medicine.

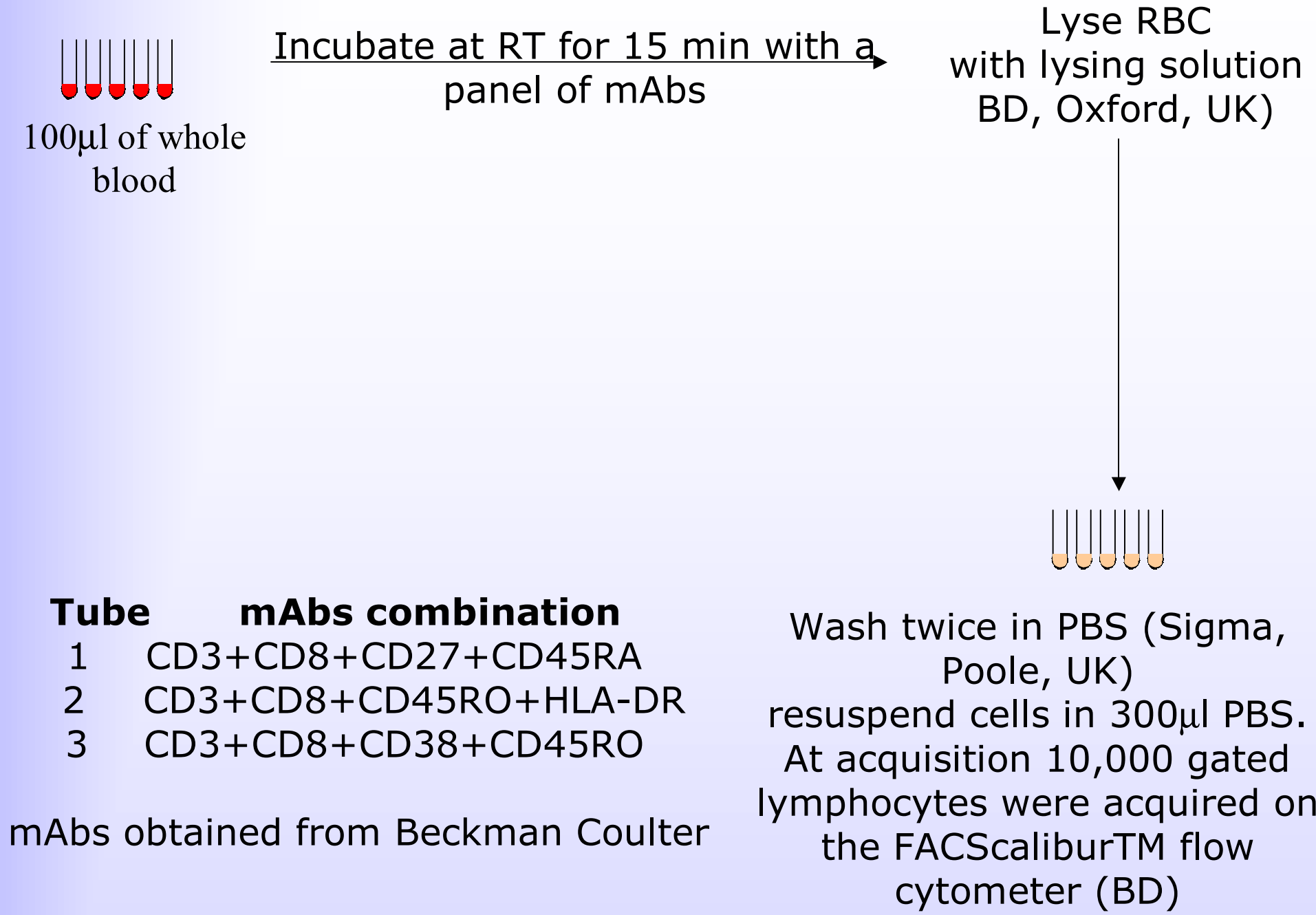
Abstract # B7e  
Poster Board # 513M

Administration of recombinant human growth hormone (rhGH) with HAART in HIV-1 infection may increase thymic activity, resulting in the production of naive T cells, and drive differentiation into functional peripheral memory/effector cells. Twelve chronic HIV-1 infected individuals mean age 43.4 ± 7.4 years on HAART received rhGH. We assessed the effects on the immune system at baseline, 12 weeks post receiving 4mg/day of hrGH and 24 weeks after randomisation into 3 groups (receiving placebo or, alternate day dosing or twice weekly dosing of rhGH). Our data reveals that rhGH appears to have a direct effect on thymic function, promotes thymocyte development resulting in an increase in naive T cells, and induces differentiation into functional memory/effector T cells in a dose dependant manner.

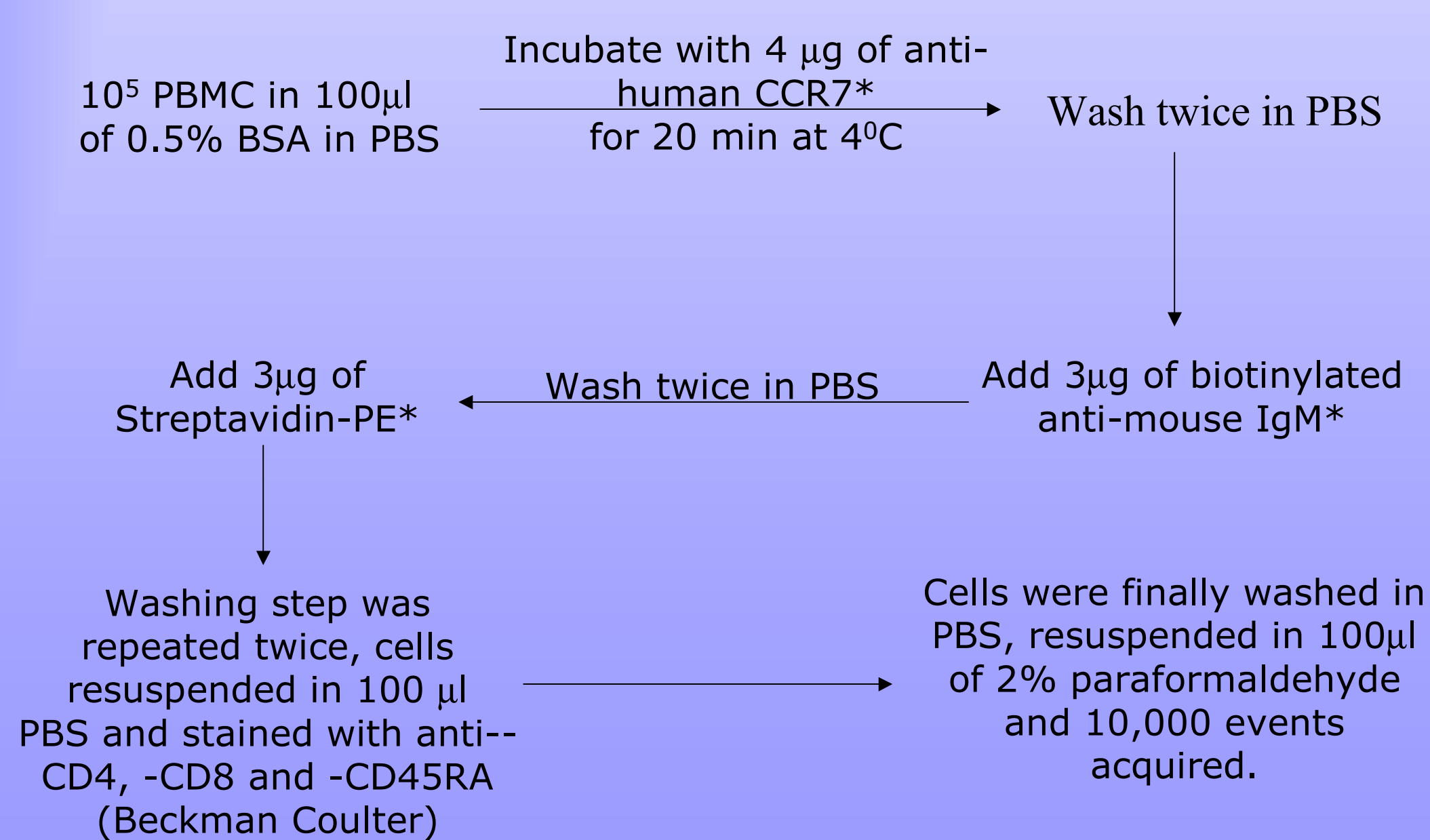
## Patient cohort

Blood was collected from 12 HIV-1+ individuals with lipoatrophy receiving successful HAART (9 on NNRTI and 3 on PI). Mean age was 43.4 ± 7.4 years, viral load was undetectable in 83% of patients, and absolute mean CD4<sup>+</sup> T cell counts were 478.4 ± 192.7 cells/μl blood. Samples were collected at baseline, 12 weeks post administration of 4mg/day of rhGH (Serostim, Serono International, Geneva, Switzerland), and a further 12 weeks post randomisation into three groups: a) receiving placebo, b) alternate-day dosing, c) twice-per-week dosing of rhGH. The patients' informed consent and Ethics Committee approval were obtained for the studies described.

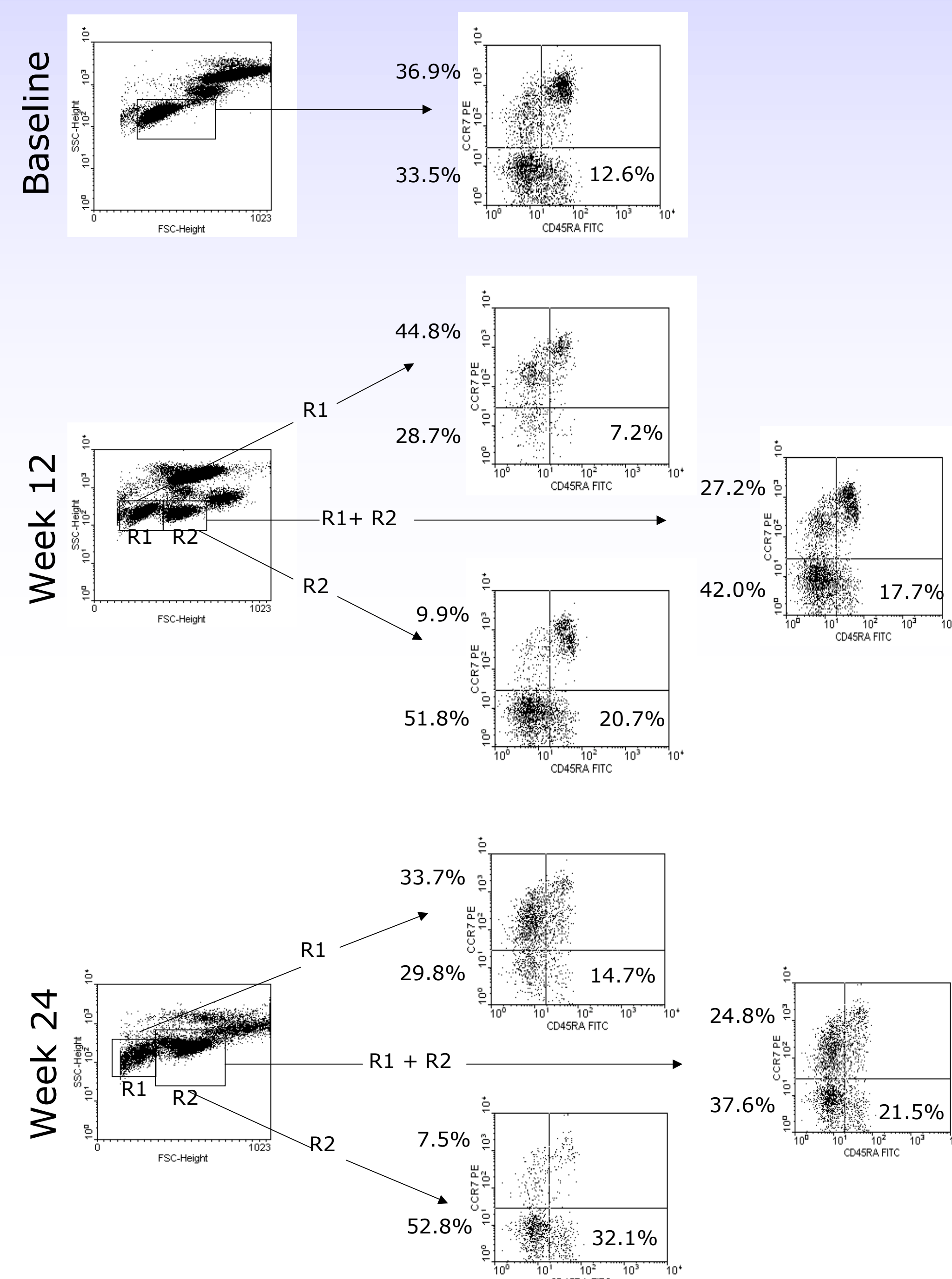
## Flow cytometry



## CCR7 staining



## Effects of rhGH on maturation/differentiation of CD8<sup>+</sup> T cells



• Before initiation of rhGH therapy, the terminally differentiated CD8<sup>+</sup>CD45RA<sup>+</sup>CCR7<sup>-</sup> subsets comprised of a mean 12.6 ± 2.3%

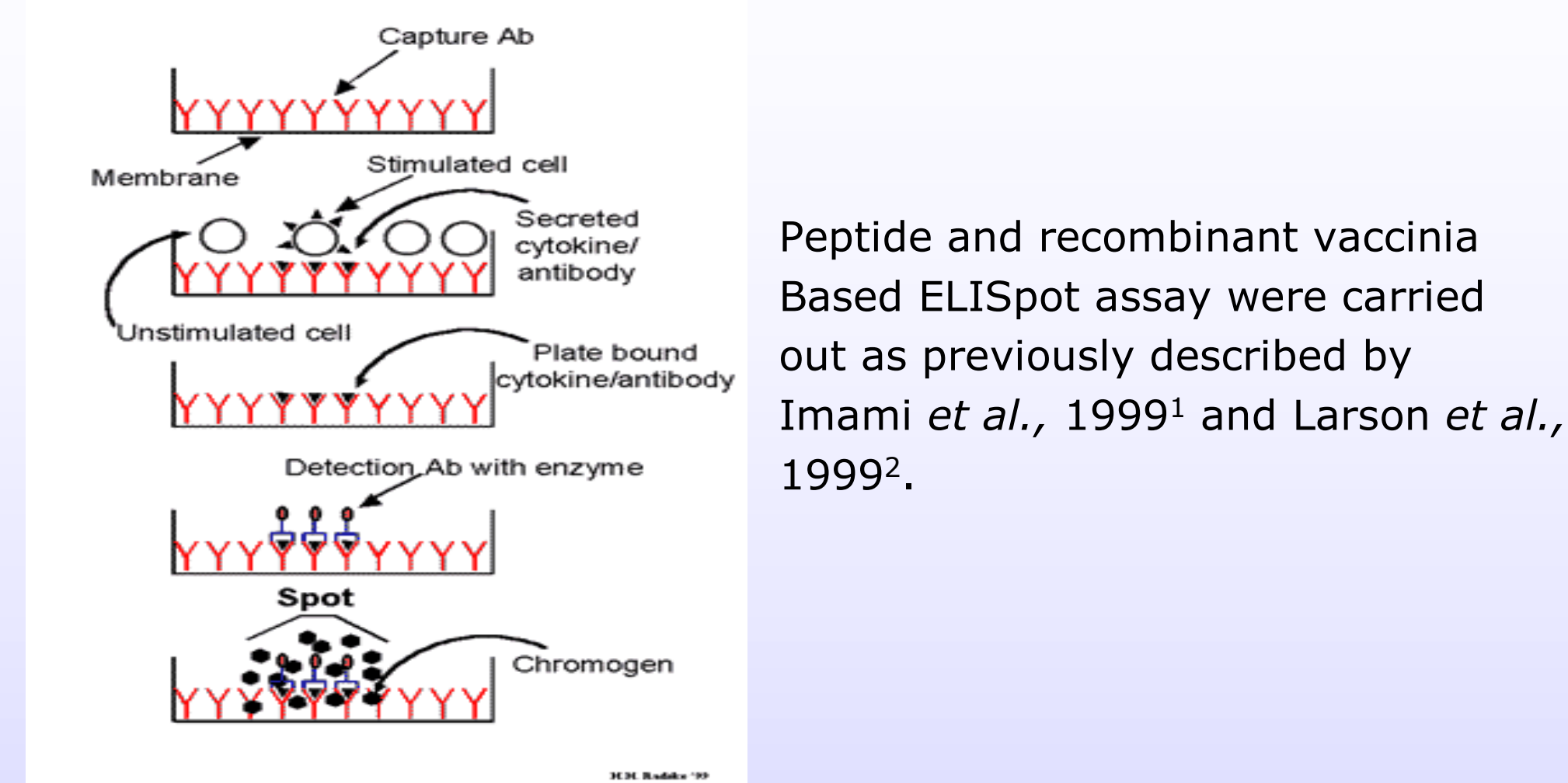
• After 12 weeks of therapy, proliferating CD8<sup>+</sup>CD45RA<sup>-</sup>CCR7<sup>+</sup> lymphocytes were mainly found in the smaller subset (R1), mean 44.8±5.5% compared to 9.9 ± 1.2% in the larger T cell subset (R2)

• The larger activated lymphocytes (R2) consisted mainly of CD8<sup>+</sup>CD3<sup>+</sup> T lymphocytes expressing a memory/effector phenotype of which a mean 20.7±2.6% were fully differentiated CD8<sup>+</sup>CD45RA<sup>+</sup>CCR7<sup>+</sup> effector cells and 51.8±5.4% were pre-terminally differentiated CD8<sup>+</sup>CD45RA<sup>-</sup>CCR7<sup>-</sup> T cells

• By week 24, there were no significant changes in proliferating, pre- and terminally differentiated CD8<sup>+</sup> T cells, however, the trend observed was an increase in the latter.

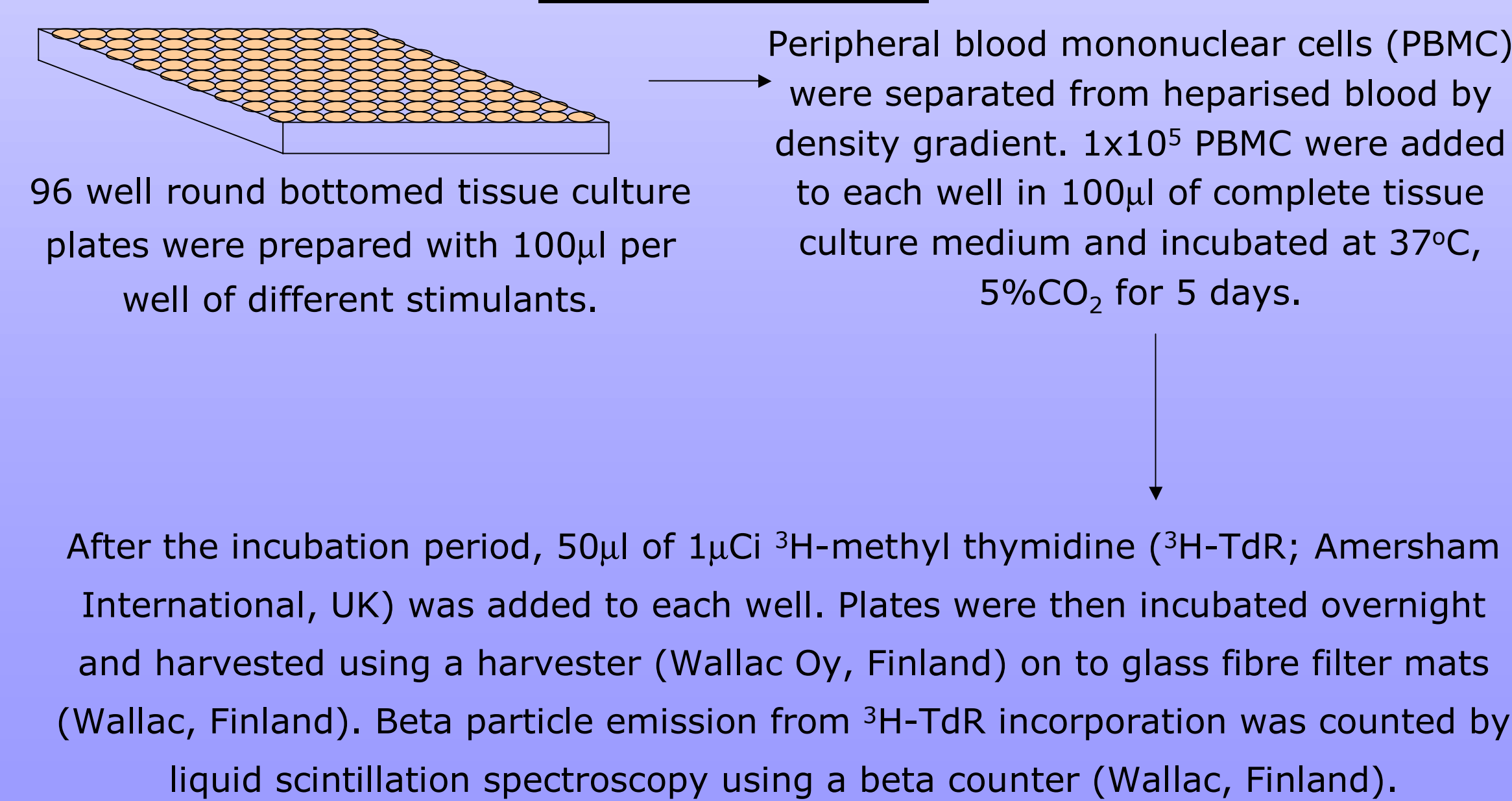
• By contrast, CD45RA<sup>-</sup>CCR7<sup>+</sup> and CD45RA<sup>-</sup>CCR7<sup>-</sup> CD8<sup>+</sup> T cells decreased between weeks 12 and 24, suggesting that lower rhGH dosing affects the differentiation lineage of CD8<sup>+</sup> T cells either directly or indirectly through CD4<sup>+</sup> T cell help.

## ELISA SPOT ASSAY\*

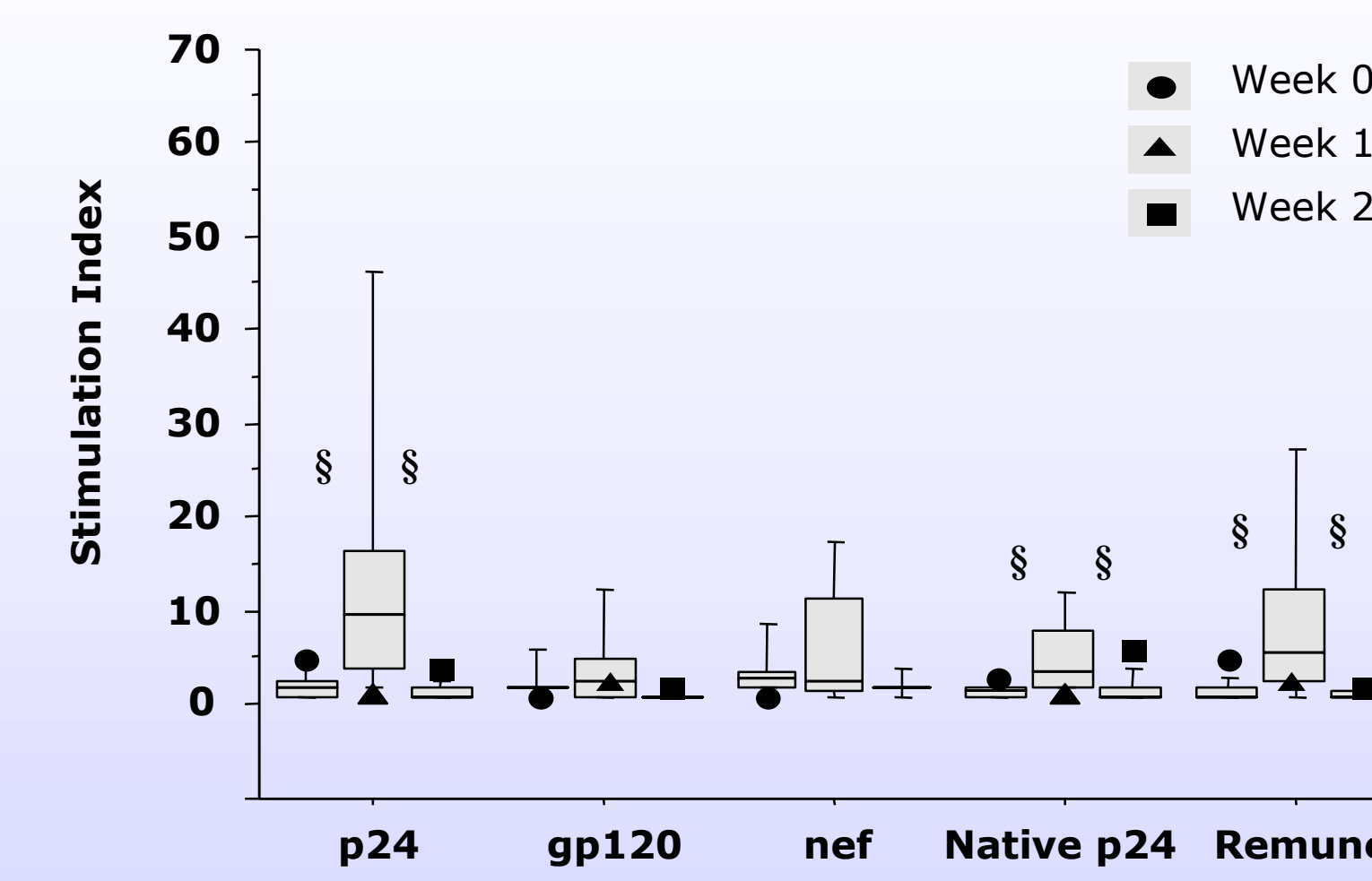


<sup>1</sup> Imami *et al.*, 1999 *Clin Exp. Immunol.* 118:78-86  
<sup>2</sup> Larsson *et al.*, 1999 *AIDS* 13:767-77

## Proliferation Assays



## HIV-1-specific CD4<sup>+</sup> T cell responses

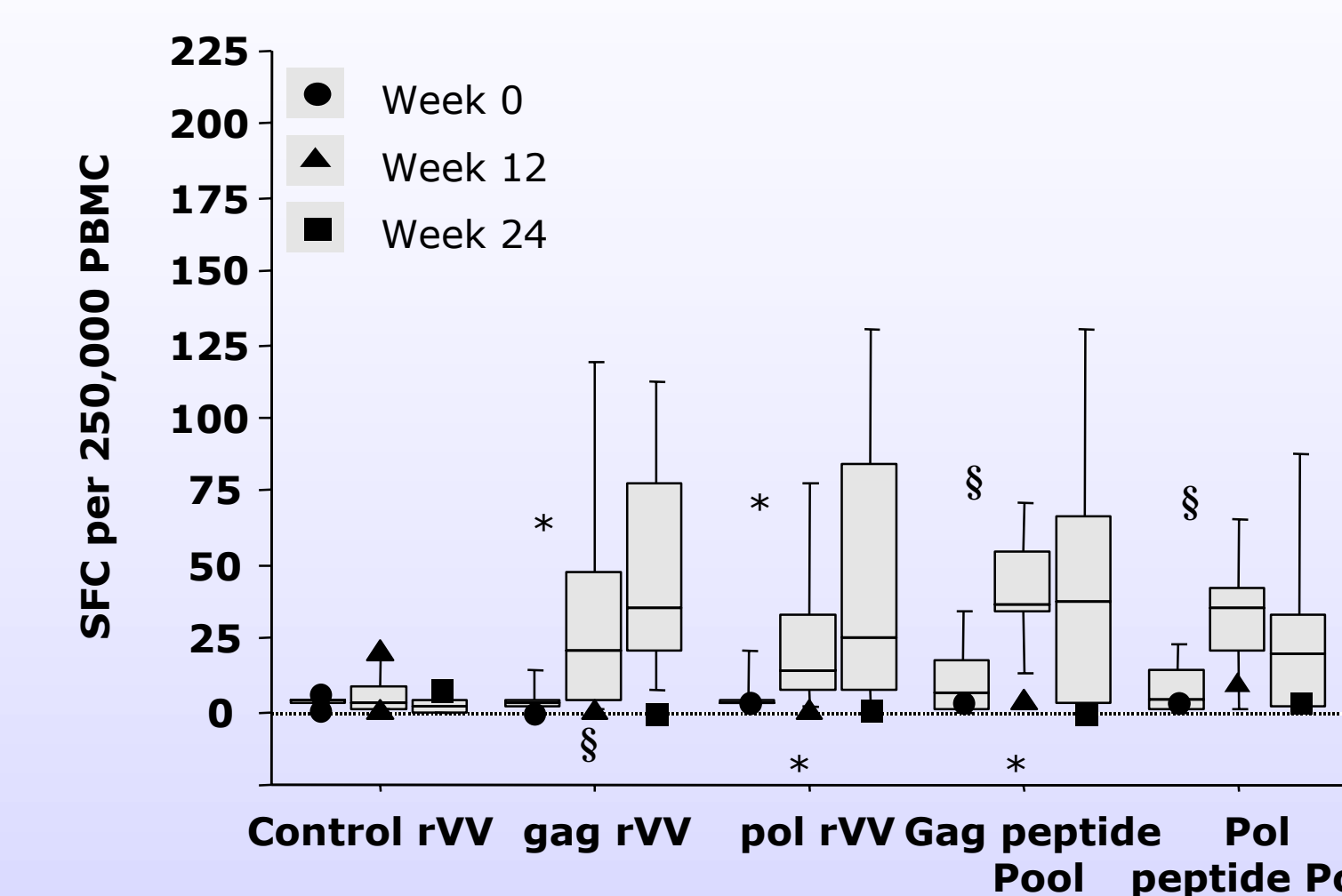


• Daily administration of rhGH, induced a significant (p<0.005) increase in both gag-p24 and whole HIV-1 (Remune) antigen specific CD4<sup>+</sup> T cell responses over a 12-week period in 9 of the 12 patients.

• Improved CD4<sup>+</sup> T cell responses at week 12 were lost by week 24 in all patients including those who received alternate- day or twice-per-week dosing of rhGH.

§ indicates significant change (p<0.005).

## HIV-1-specific CD8<sup>+</sup> T cell responses



• There was a significant increase in gag- and pol- specific CD8<sup>+</sup> T cell responses over a 12-week period, evaluated by ELISpot using both rVV (p<0.05) and peptide pools of gag or pol proteins (p<0.005).

• These virus-specific CD8<sup>+</sup> T cell responses were maintained even at week 24 regardless of which arm of randomisation the patients were in.

\* denotes a p value <0.05; §denotes a p value <0.005. \* or § on the x-axis depicts p value between baseline and week 24.

• In the smaller lymphocyte subset, there was a significant increase in mean naive CD45RA<sup>+</sup>CD27<sup>+</sup> circulating CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes from 24.9±5.4% to 37.8±4.5% and from 23.6±3.7% to 32±4.3% respectively (p<0.01 for both).

• In the activated T cell subset an increase in naive compartment of CD4<sup>+</sup> lymphocytes was observed from week 12 to week24 (p<0.05).

• Analysis of both subsets together revealed a decrease in memory cells in the CD4<sup>+</sup> T cell compartment (p<0.01).

• TREC levels detected in 8 of 12 patients at baseline (range undetectable - 1.2x10<sup>6</sup> copies/4x10<sup>6</sup> cells) decreased in 7 of 8 patients by week 12 (range undetectable - 6.6x10<sup>5</sup> copies/4x10<sup>6</sup> cells) despite the significant increase in naive T cells, and reached undetectable levels in all patients by week 24.

## Conclusions

• Daily administration of 4mg rhGH to HIV-1 infected patients on HAART over a 12-week period, results in the increase of the activated subset of lymphocytes, which was observed in 11/12 patients.

• We suggest that rhGH exerts strong effects on the maturation pathway of CD8<sup>+</sup> T cells resulting in the apparent increase of pre- and terminally differentiated CD8<sup>+</sup> T lymphocytes.

• The smaller subset of lymphocytes contains the majority of naive and resting memory cells whereas the larger subset comprises of activated effector/memory lymphocytes.

• We suggest that the increase in circulating naive lymphocytes was rapidly followed by maturation of these cells into proliferating memory cells, which rapidly differentiated into CD45RA<sup>+</sup>CCR7<sup>-</sup> and CD45RA<sup>-</sup>CCR7<sup>+</sup> effector cells, thus resulting in dilution of TREC numbers.

• Responses to other viral pathogens or PHA did not present any significant changes (data not shown), implying that original defective responses are mainly directed at HIV-1 antigens and newly formed cells may preferentially differentiate into functional HIV-1-specific lymphocytes.

• The reappearance of HIV-1-specific HTL and CTL responses, is also suggestive that rhGH exerts a role in purging HIV-1 from reservoirs which may suffice for priming of newly formed T cells.

• Some of the effects exerted on the immune system by rhGH disappeared with less frequent dosing suggesting that the compound is beneficial in a dose dependant manner.

• The instigation of rhGH as an adjuvant therapy for treated HIV-1 infection proves to have beneficial effects on thymic output, T cell differentiation/maturation and function, and may play an important role as a future therapeutic immunomodulation.