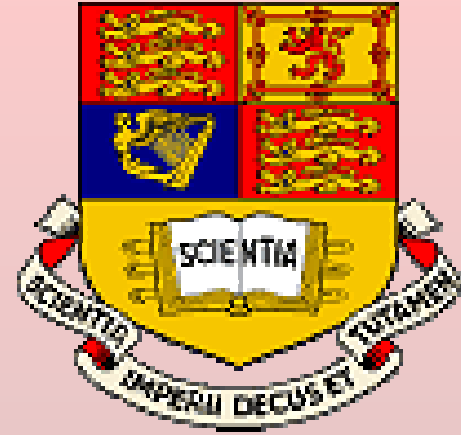


Imperial College of Science, Technology and Medicine



Limited thymic contribution to CD4 T cell restoration during supplementation of HAART with IL-2 and/or Remune.

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*This work was supported by The Luard Foundation and The Wellcome
Trust (Grant Numbers 058700 & 051541)*

Background

Treatment of HIV-1 infected individuals with highly active antiretroviral therapy (HAART) generally results in a dramatic decline in plasma and tissue HIV-1 RNA levels which is usually accompanied by obvious increases in blood CD4⁺ T cell numbers¹. Mechanisms proposed to account for the observed reconstitution of CD4⁺ T cells include proliferation of existing cells² and/or redistribution of sequestered cells from inflamed lymphoid tissues³. Increased generation of new T lymphocytes by the thymus (the primary site of T cell production) as a possible result of HAART induced decrease in viral mediated destruction of developing thymic T cells has been further proposed as a third potential mechanism⁴. The supplementation of HAART with the immunomodulatory agents interleukin-2 (IL-2) and Remune vaccine were found to be beneficial to CD4 reconstitution^{5,6}.

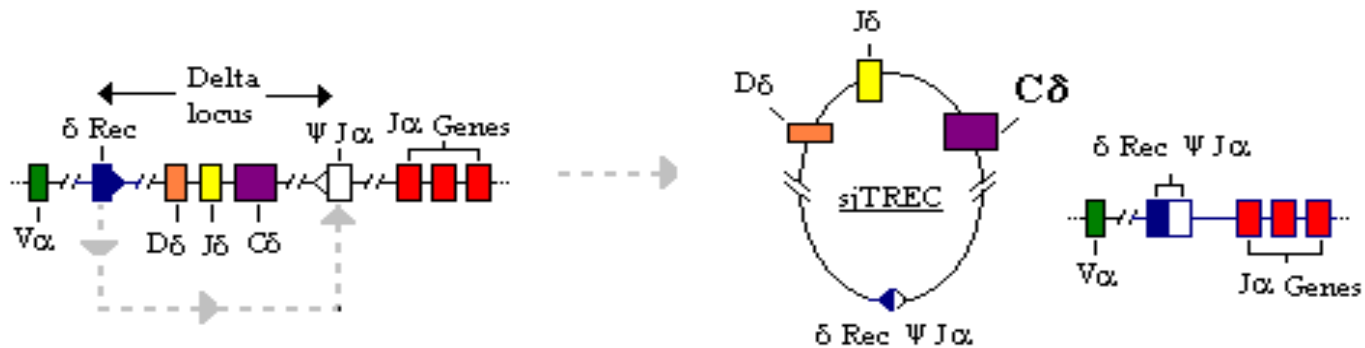
Aim

Through the use of the T cell receptor excision circle assay, measurement of thymic $\alpha\beta$ T cell generation by HIV-1 infected individuals receiving HAART or HAART and IL-2 and/or Remune was performed to determine the effects of such immunomodulatory agents on the contribution of the thymus to $CD4^+$ T cell restoration in chronically infected patients undergoing therapy.

Assessment of Thymic Output

Measurement of the number of new T cells that have recently emigrated from the thymus can be performed as an indirect method of quantifying thymic output⁷. The identification and measurement of recent thymic emigrant $\alpha\beta$ T cells can be achieved through the quantification of the extra chromosomal DNA by-products of the α T cell receptor (TCR) gene rearrangement process. These circular DNA molecules, known as signal joint TCR rearrangement excision circles (sjTRECs) are stable (remaining in the new T cells exiting the thymus), are not replicated (becoming diluted out during cell division) and are present in naïve $\alpha\beta$ T cells but are undetectable in memory T cells, $\gamma\delta$ T cells and B cells^{8,9}.

Generation of Signal Joint TCR Rearrangement Excision Circles



Generation of sjTREC

1/ The TCR delta locus encompassed within the TCR alpha locus is first excised prior to alpha gene rearrangement.

2/ The delta Rec and Psi J alpha deleting elements are cleaved and brought towards each other.

3/ The cleaved portions of the delta Rec and Psi J alpha DNA segments are ligated to each other to form an extrachromosomal circular DNA by-product containing the deleted TCR delta locus - sjTREC.

Patients

Patients at Randomization

Age : 23-60 year (average = 38)

Therapy: 16 weeks pre-treated with 2 NA reverse transcriptase inhibitors + 1 or more protease inhibitors or 1 NNA reverse transcriptase (HAART)

Viral load : <50 RNA copies/ml plasma

CD4 count : >300 cells/ μ l blood

CD8 count : 530-1200 cells/ μ l blood (average = 920 cells/ μ l blood)

Randomization: **Group A**=HAART (n 7)

Group B =HAART + IL-2 (n 6)

Group C =HAART + IL-2 +Remune (n 6)

Group D =HAART +Remune (n 6)

Treatment Regimen: 5×10^6 IU BID IL-2 was administered subcutaneously for 5 days at 4 week intervals over a 12 week period. 100 μ g Remune in incomplete Freund's adjuvant was administered intramuscularly every 12 weeks for 36 weeks.

Method

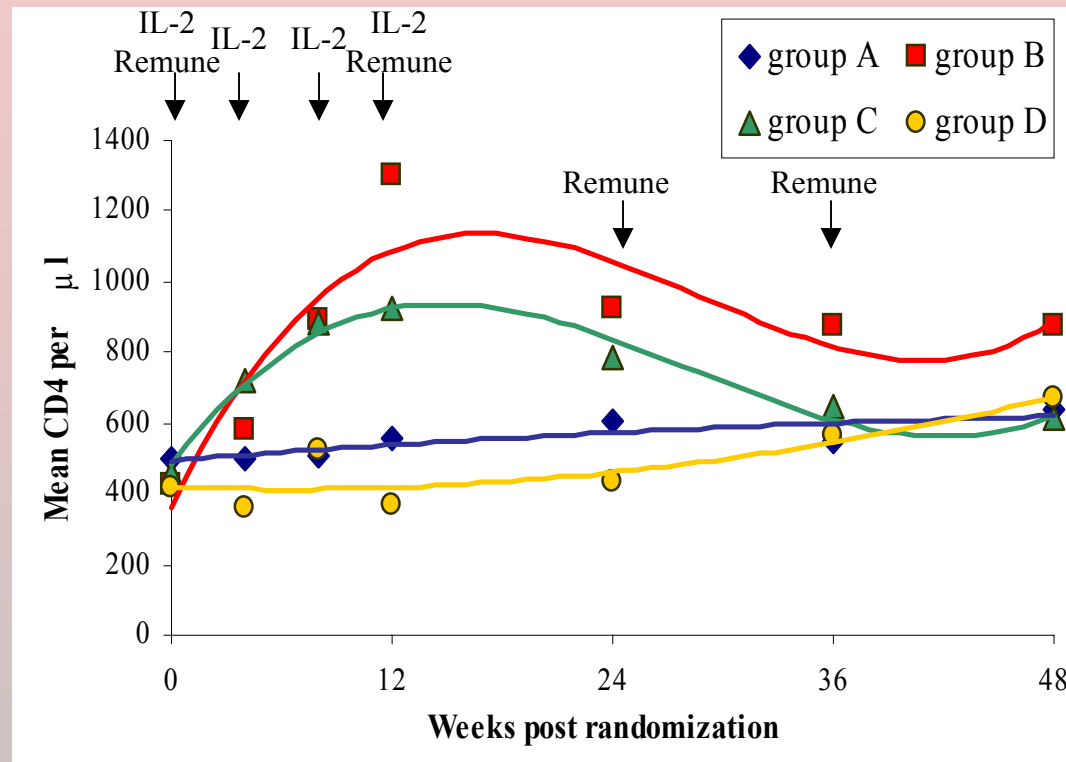
Quantification of sjTRECs

sjTREC levels in the DNA, purified from 5×10^6 peripheral blood mononuclear cell (PBMC) samples of patients obtained at weeks 0, 4, 8, 12, 24, 36 & 48 post randomization were measured via a PCR based technique involving the use of agarose gel electrophoresis, the Scion Image analysis system and an sjTREC standard curve (as previously described)⁸.

Analysis of T Cell Subsets' Numbers

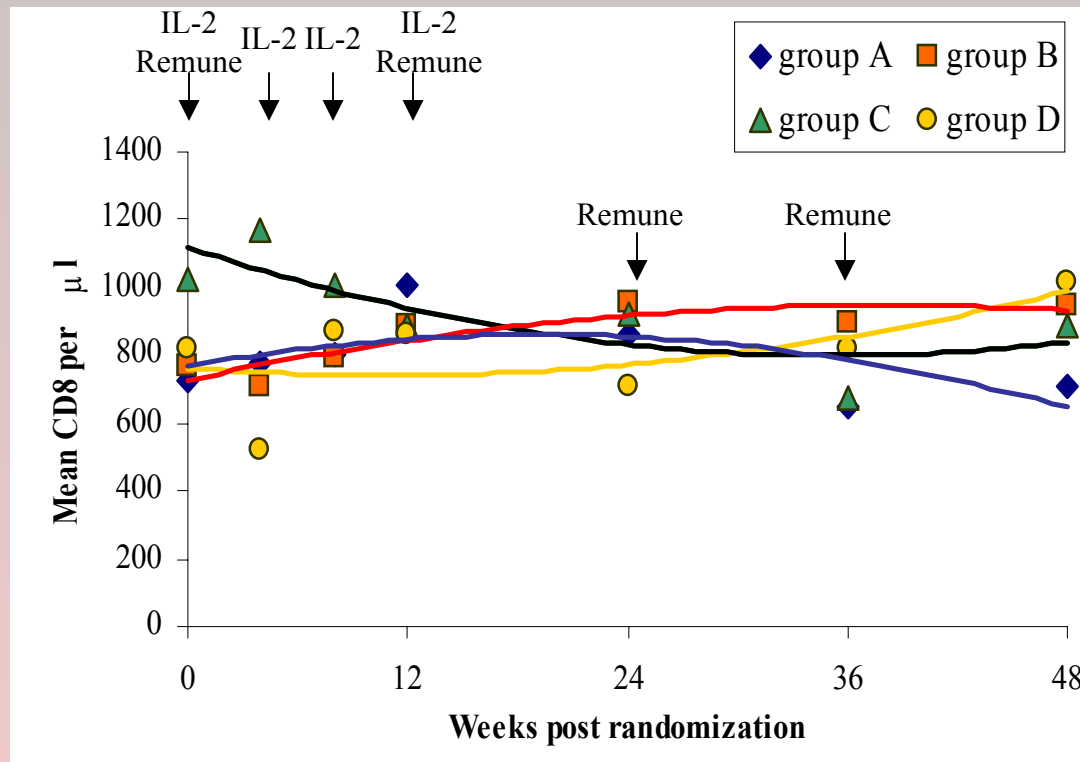
100 μ l of patients' peripheral blood were treated with fluorescence labeled anti-human monoclonal antibodies to T cell markers; CD3, CD4, and CD8 and analyzed by flow cytometry.

Result 1: Patients' Mean CD4⁺T Cell Numbers During the Course of Therapy



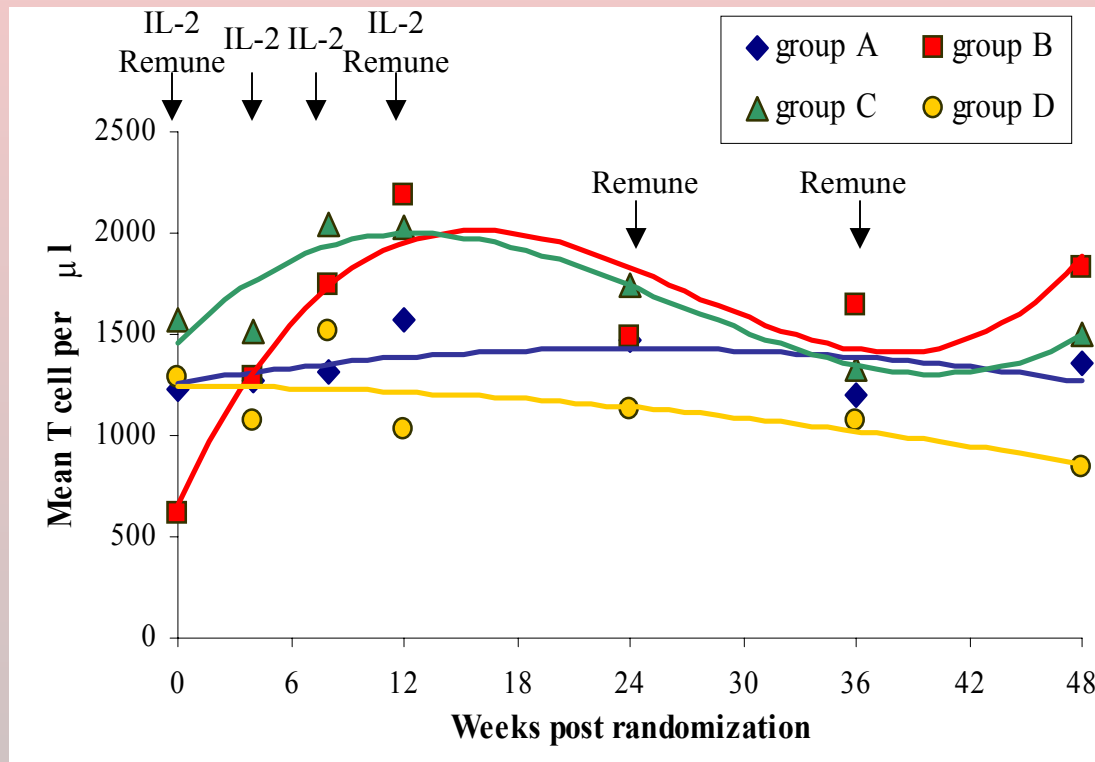
In groups B & C mean CD4⁺ T cell numbers display a biphasic pattern, primarily increasing during the first 12 weeks of therapy and then decreasing thereafter. In groups A & D mean CD4⁺ T cell numbers remain relatively unchanged throughout the 48 week period of therapy. This data suggests a positive effect of IL-2 on CD4⁺ T cell restoration. 7

Result 2: Patients' Mean CD8⁺T Cell Numbers During the Course of Therapy



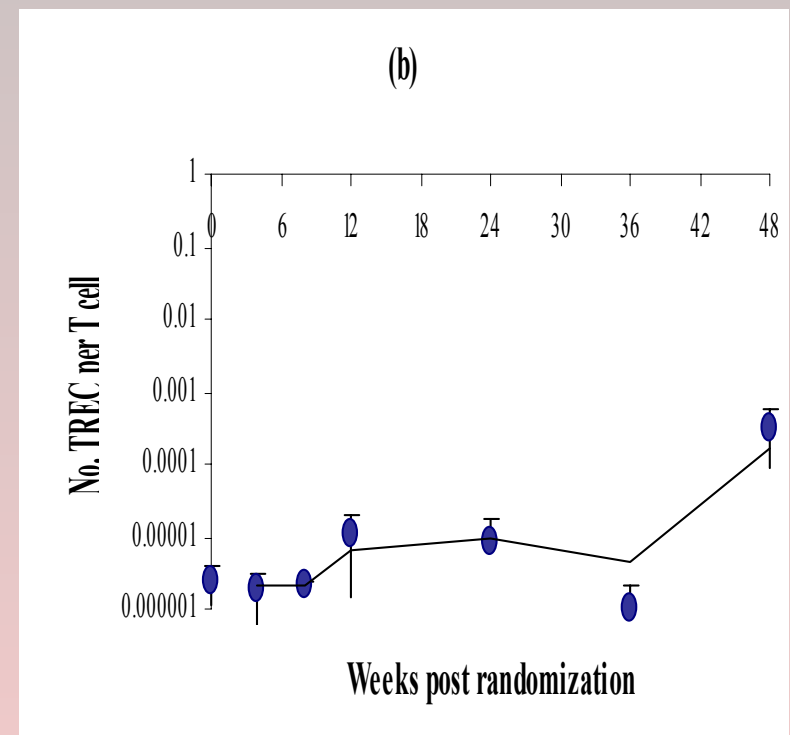
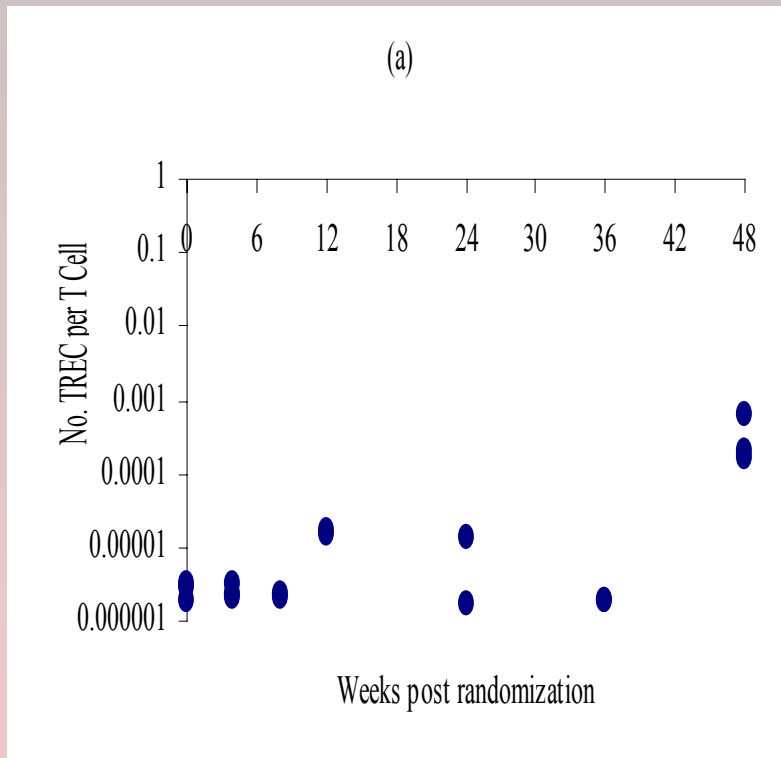
Mean CD8⁺ T cell numbers remain relatively unchanged throughout the course of therapy for all patient groups. With group C patients displaying a gradual but insignificant decrease commencing at randomization point to week 36.

Result 3: Patients' Mean CD3⁺ T Cell Numbers During the Course of Therapy



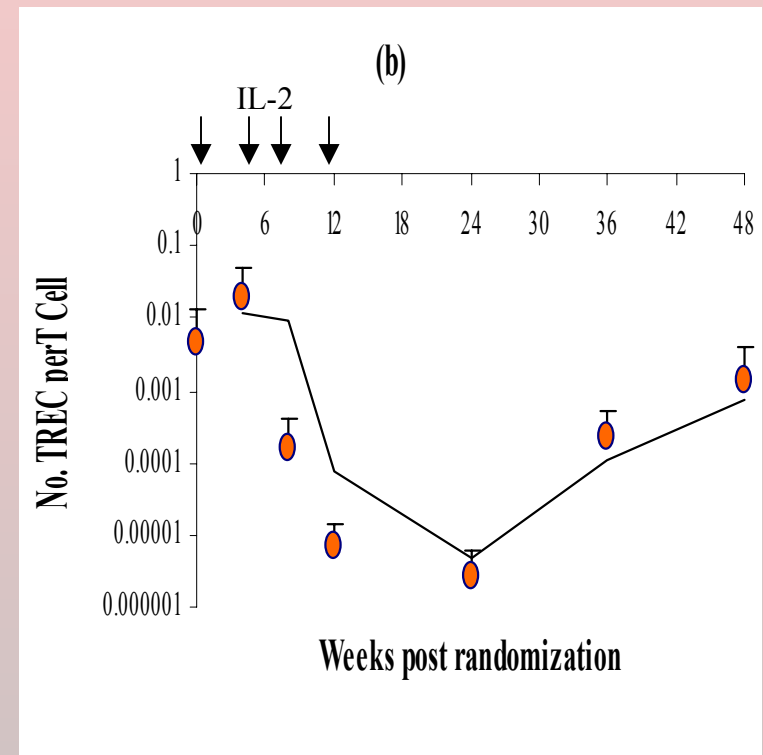
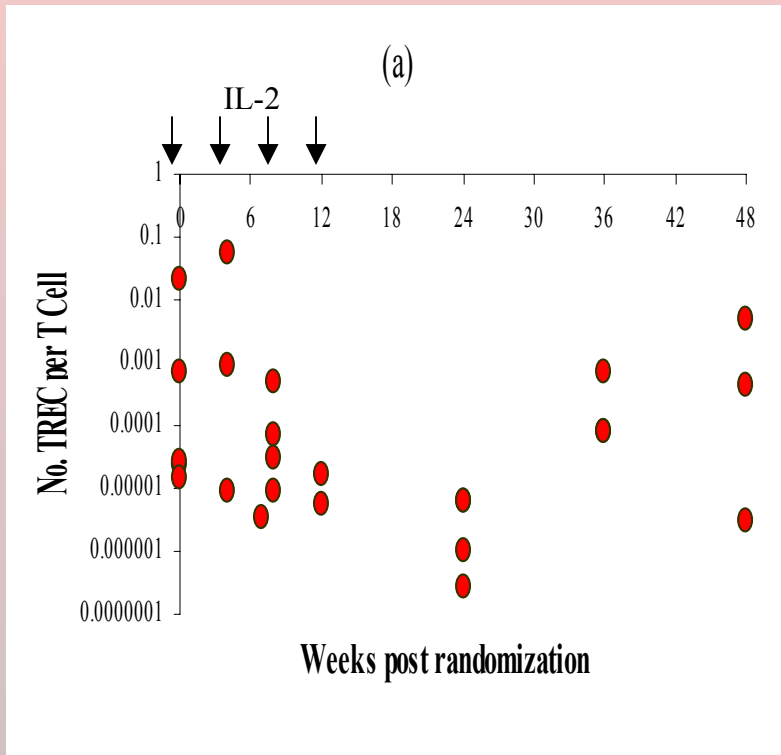
Similar to the observation for CD4⁺ T cell numbers, group B & C's mean CD3⁺ T cell numbers primarily increase between week 0 to 12 of therapy and then slowly declines thereafter and later show signs of recovery at week 36. In groups A & D mean CD3⁺ T cell numbers remain relatively unchanged throughout the 48 weeks of immunotherapy.

Result 4: Group A (HAART Alone) Patients' Individual (a) and Mean (b) sjTREC/T Cell Levels During the Course of Therapy ($n=7$)



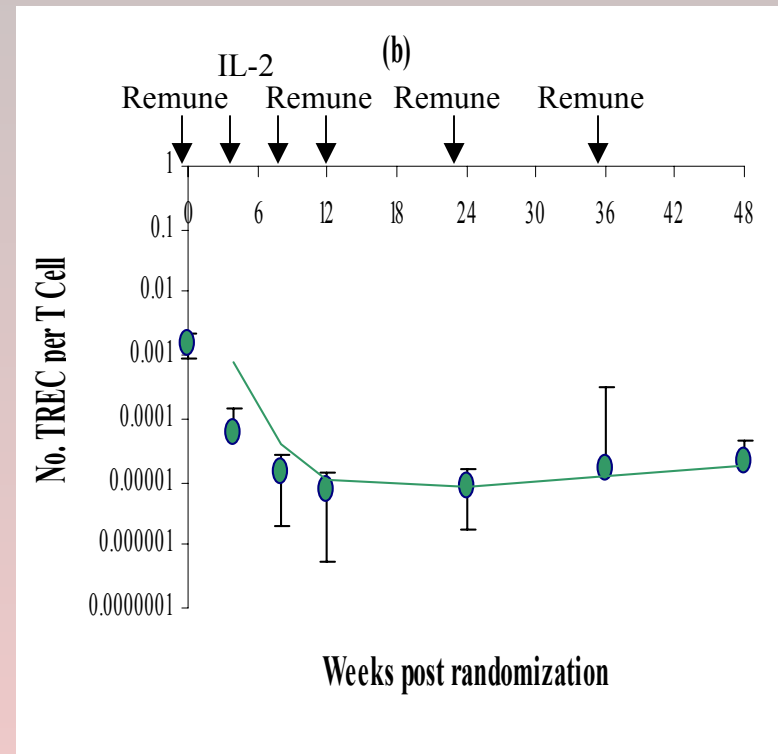
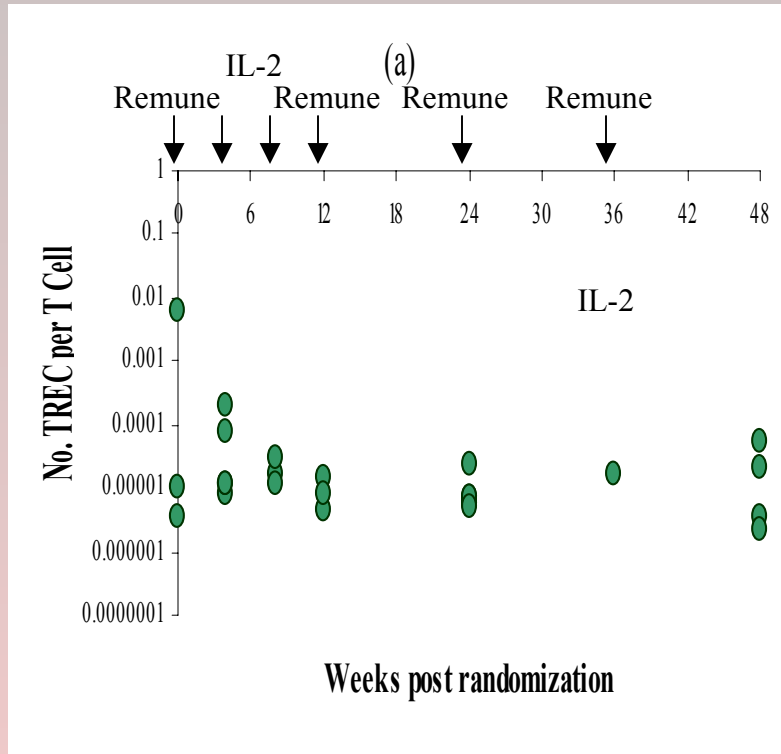
sjTREC levels of patients receiving HAART alone remained relatively constant for the first 36 weeks after randomization, then dramatically rise thereafter. The number of sjTREC at week 48 was found to be significantly higher compared to the value at randomization ($P < 0.05$).

Result 5: Group B (HAART + IL-2) Patients' Individual (a) and Mean (b) sjTREC/T Cell Levels During the Course of Therapy (n=6)



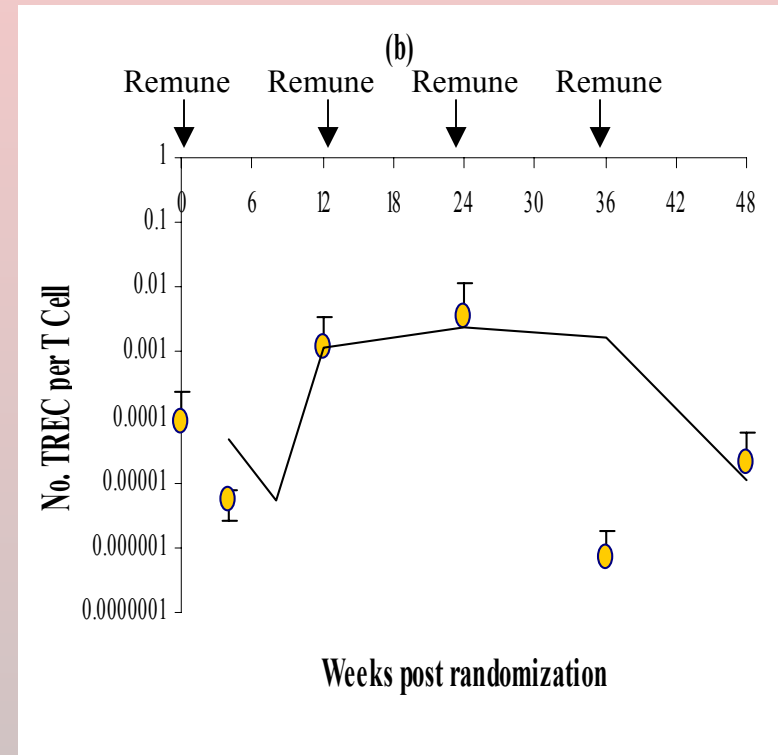
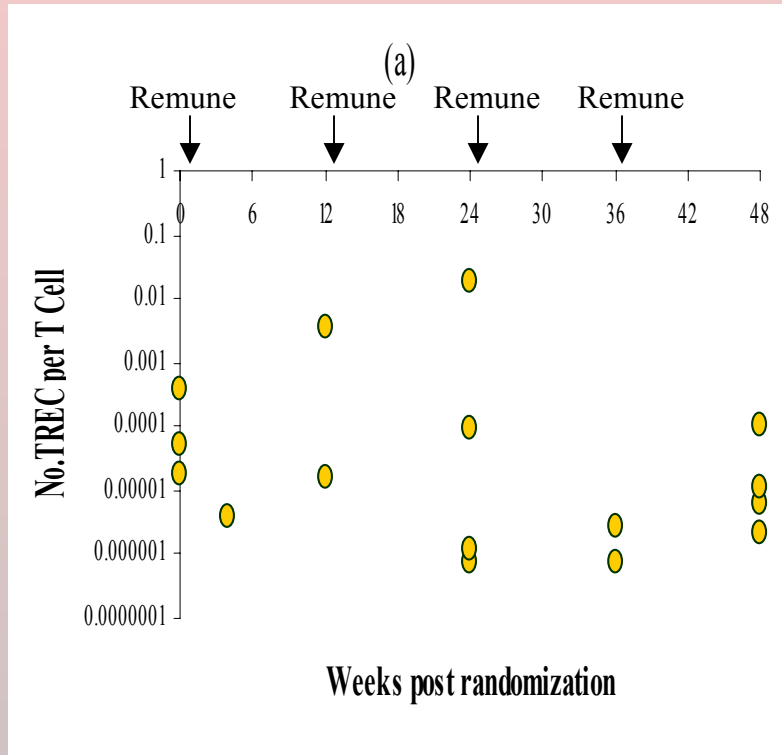
Overall sjTREC levels for patients receiving HAART+IL-2 initially declined at an average rate of approximately 13%/week for a period of 24 weeks after randomization, so that by week 12 sjTREC levels are significantly lower than the value at randomization ($P < 0.05$). From week 24 sjTREC levels begin to increase progressively at a rate of approximately 9%/week.

Result 6: Group C (HAART + IL-2 + Remune) Patients' Individual (a) and Mean (b) sjTREC/T Cell Levels During the Course of Therapy (n=6)



Overall sjTREC levels of group C patients initially declined at an average rate of approximately 13%/week for up to 12 weeks post randomization so that by week 12 the sjTREC level is significantly lower than the value at randomization ($P < 0.05$). sjTRECs then increased slowly from week 12 at a rate of 1%/week for the remainder of the therapy.

Result 7: Group D (HAART + Remune) Patients' Individual (a) and Mean (b) sjTREC/T Cell Levels During the Course of Therapy (n=6)



Overall sjTREC levels of patients receiving HAART+Remune remained relatively unchanged throughout the course of therapy. With sjTREC levels showing no significant change/difference from the value at the start of randomization ($P > 0.05$).

Conclusions

1/ The immediate increases in CD4 T cell numbers observed in patients supplemented with IL-2 (Group B and C) indicate a positive effect of IL-2 immunomodulation on the T cell reconstitution of HIV-1⁺ patients during HAART.

2/ The lack of CD4⁺ T cell increase in patients given Remune in conjunction with HAART (Group D) suggest no notable effect of Remune administration on the reconstitution CD4⁺ T cells during treatment with HAART.

3/ The absence of any notable increases in sjTREC levels in patients receiving HAART alone during the first 36 weeks post randomization indicate a poorly functioning thymus in HIV-1 chronically infected individuals, whilst the significant increases thereafter suggests an ability of the thymus to recover its function during the later stages of therapy.

4/ The progressive increases in CD4⁺ T cell numbers of patients receiving IL-2 supplementation during the first 12 weeks post randomization is accompanied by a gradual decrease in sjTREC levels indicating little or no thymic contribution to the observed IL-2 induced T cell restoration.

5/ The inability of sjTRECs to be replicated during mitosis and their subsequent dilution during cell division strongly suggests cellular proliferation as the mechanism involved in the observed IL-2 mediated CD4⁺ T cell regeneration.

6/ The late increase in sjTREC levels from week 36 after IL-2 administration ceases (Group B) further indicates a capacity of the thymus to recover its functionality and contribute to the T cell reconstitution of HIV-1⁺ patients during the later stages of therapy.

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