TUPD203

Recovery of Functional Immunity over 48 Weeks with Darunavir-based Therapy in the GRACE Immunology Substudy

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Introduction

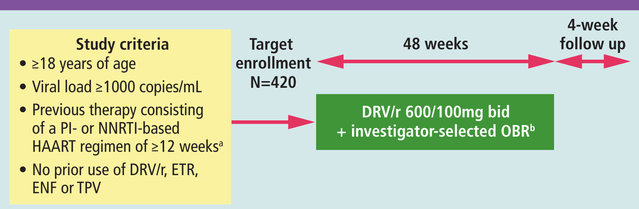
- During the course of HIV-1 infection, multifactorial T-lymphocyte (T-cell)-mediated mechanisms contribute to the progressive loss of host immune function, including:
  - Initial decreases in the number of CD4+ cells with concurrent increases in the CD8+ population[1]
  - Immune dysregulation and activation characterized by an increased expression of T-cell cellular activation markers, CD38 and human leukocyte antigen-DR (HLA-DR)[2–5]
    - Surface expression of CD38 on CD8+ cells is a powerful predictor of HIV progression[6,7]
  - Decreased proportion of CD4+ cells expressing interleukin (IL)-2 and interferon (IFN)-γ and an increased proportion expressing IL-4 and IL-10 due to a shift from the T helper (TH)1 to TH2 phenotype[8,9]
- In addition to reduced viral load, successful antiretroviral therapy (ART) results in improvements in CD4+ counts and decreases in immune activation
- The majority of studies of immune recovery with ART have been cross-sectional in design, conducted primarily in Caucasian males and have not assessed direct *in vitro* immune function
- The aim of this prospective substudy was to quantitatively and qualitatively evaluate the recovery of functional immunity (T-cell function) with a darunavir/ritonavir (DRV/r)-based regimen in a diverse treatment-experienced patient population from the GRACE (Gender, Race And Clinical Experience) study
  - GRACE is the largest ART trial to focus on women with HIV-1 in North America, and was designed to assess sex and race differences in efficacy, safety and tolerability of DRV/r plus an optimized background regimen over 48 weeks in treatment-experienced patients[10] [see poster MOPE042]

Methods

The GRACE study

- GRACE was a multicenter, open-label, single-arm, Phase IIIb study conducted at 65 sites across the United States, Puerto Rico and Canada, which enrolled treatment-experienced patients (viral load ≥1000 HIV-RNA copies/mL) aged ≥18 years with documented HIV-1 infection (**Figure 1**)

Figure 1. GRACE study design



[a]Patients were allowed to enter the study on treatment interruption of ≥4 weeks; [b]Investigator-selected NRTIs and NNRTIs were allowed; ENF, TPV or agents from novel classes were not allowed; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; HAART, highly-active antiretroviral therapy; DRV/r, darunavir/ritonavir; ETR, etravirine; ENF, enfuvirtide; TPV, tipranavir; bid, twice daily; OBR, optimized background regimen

GRACE immunology substudy

Patient population

- Patients at participating sites who were eligible for participation in GRACE could be enrolled into the single-arm prospective substudy
  - Virologic suppression in this analysis was defined as achieving HIV-RNA <50 copies/mL at Week 48

Study evaluations

- Immune function and immune phenotype were determined by flow cytometry at baseline and Weeks 12 and 48 in patients who were virologically suppressed and not suppressed
- Changes in immune phenotype were determined from subsets of CD4+ and CD8+ T cells, with immune activation defined as a change in CD38 and HLA-DR activation markers
  - Increased expression of HLA-DR and CD38 is a marker of immune activation
- Changes in immune function were assessed by:
  - Lymphocyte proliferation in response to candida and tetanus (recall antigens), phytohemagglutin (PHA) and pokeweed (mitogenic plant lectins), and anti-CD3/anti-CD28
  - Intracellular cytokine expression of IL-2, IFN-γ and tumor necrosis factor (TNF)-α in response to Staphylococcal enterotoxin B (SEB)
- The normal comparator group consisted of 34 healthy, HIV seronegative individuals; 50% were women and 74% Caucasian

Statistical analysis

- Wilcoxon rank-sum tests were used to assess immune parameter changes from baseline to Week 48

Results

- Patient demographics and baseline characteristics are shown in **Table 1**

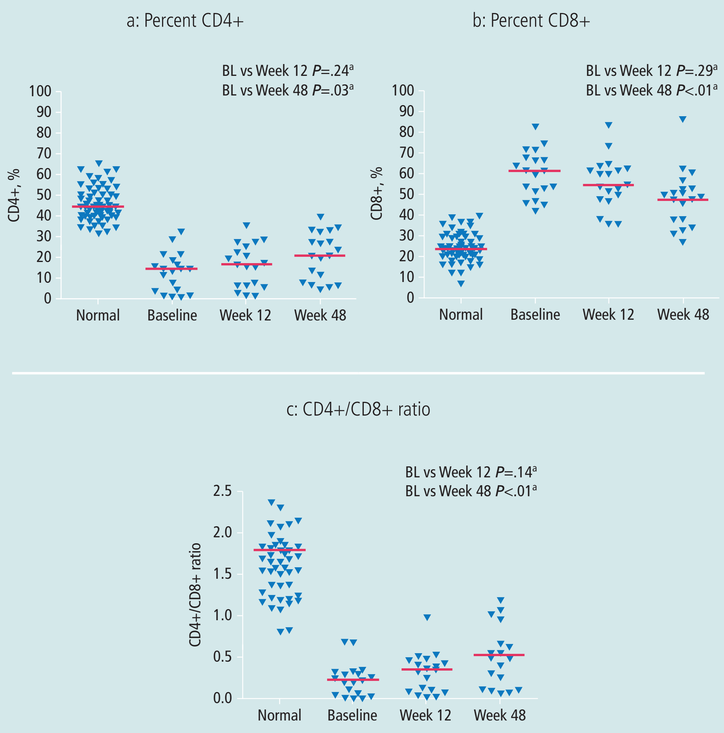
Table 1. Baseline characteristics and demographics

	All HIV+ patients (N=32)	Suppressed patients (n=19)
Sex, n (%)		
Men	19 (59.3)	13 (68.4)
Women	13 (40.6)	6 (31.6)
Race, n (%)		
Black	15 (46.9)	8 (42.1)
Hispanic	10 (31.3)	6 (31.6)
Caucasian	7 (21.9)	5 (26.3)
Mean (SD) viral load, log_{10} copies/mL	4.74 (0.84)	4.76 (0.82)
Mean (SD) CD4+ count, cells/mm³	183 (138)	202 (134)

SD, standard deviation

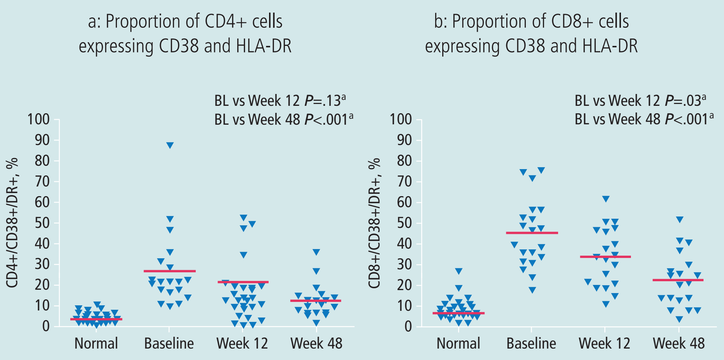
- Overall, mean CD4+ change (standard deviation[SD]) from baseline for the total patient population was 76 (86) cells/mm³ at Week 12 and 164 (149) cells/mm³ at Week 48
  - Mean CD4+ change (SD) from baseline for virologically suppressed patients was 82 (68) cells/mm³ at Week 12 and 195 (150) cells/mm³ at Week 48
- CD4+ and CD8+ counts increased and decreased, respectively, in virologically suppressed patients (**Figure 2**)
- Markers of T-cell activation decreased significantly in virologically suppressed patients (**Figure 3**)
- The ability of lymphocytes to respond to mitogens and recall antigens significantly improved in virologically suppressed patients (**Figure 4**)
  - Proliferation in response to anti-CD3/anti-CD28 and PHA was at, or near, normal levels by Week 12, and proliferation in response to pokeweed and candida was at normal levels by Week 48
  - Less pronounced improvements in lymphocyte proliferation were observed in unsuppressed patients (data not shown)

Figure 2. Immune phenotype in virologically suppressed patients



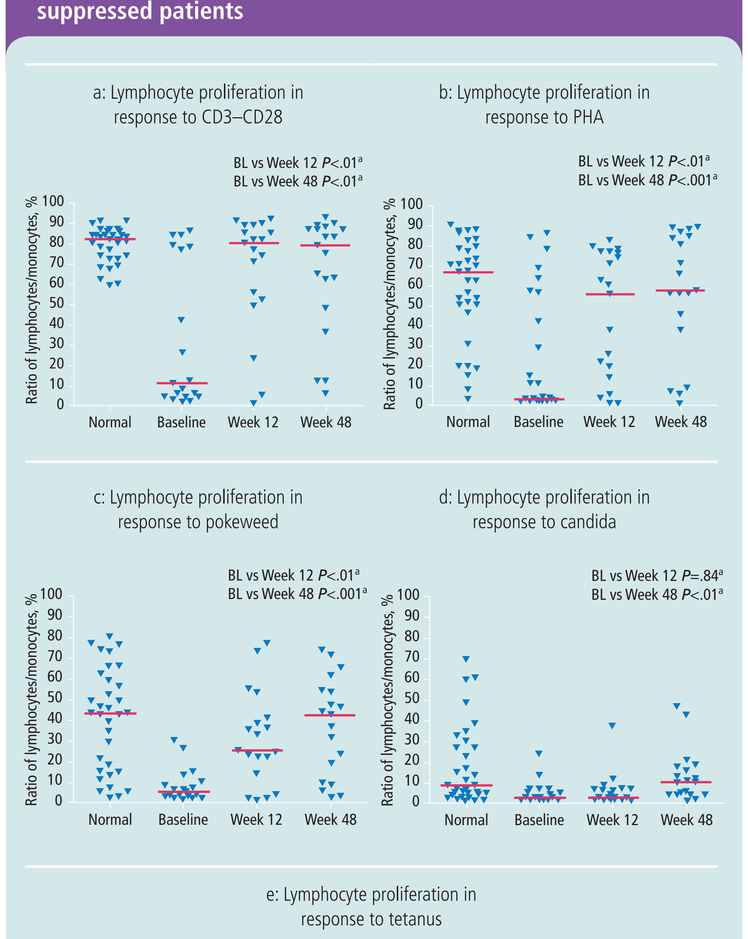
[a]Wilcoxon rank-sum test; Red line represents median; BL, baseline

Figure 3. Markers of T-cell activation in virologically suppressed patients



[a]Wilcoxon rank-sum test; Red line represents median; HLA-DR, human leukocyte antigen-DR; BL, baseline

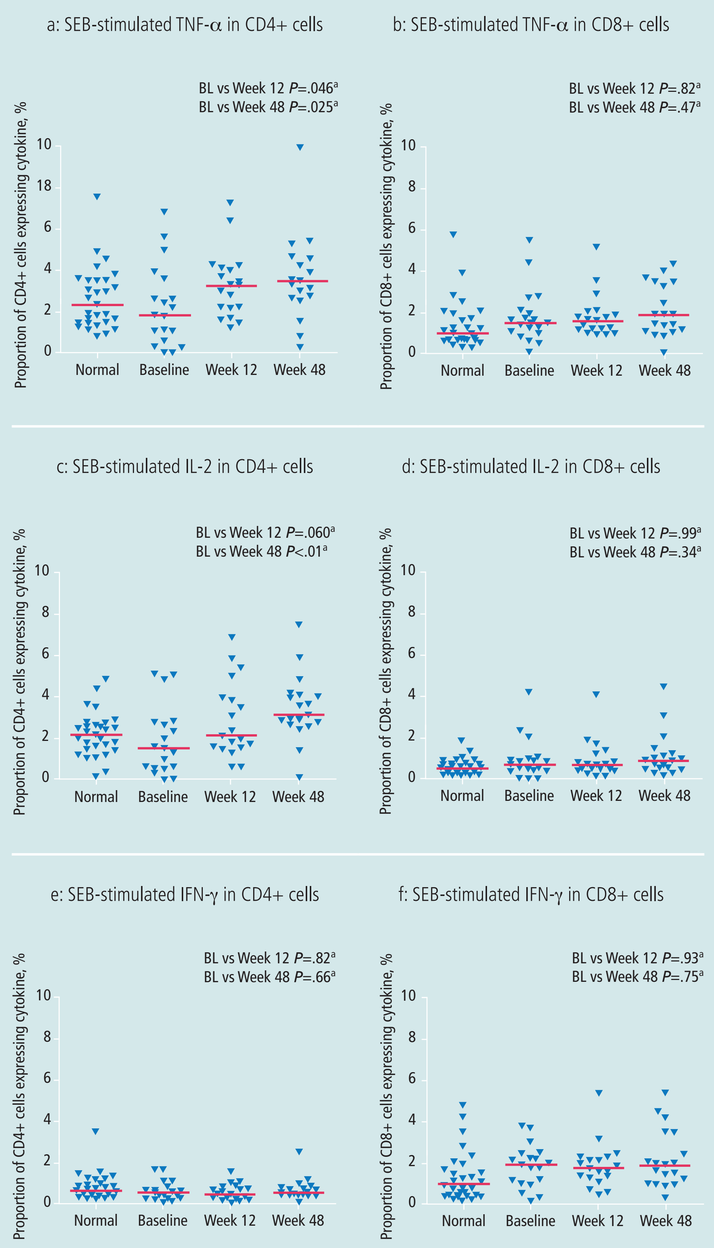
Figure 4. Lymphocyte proliferation in virologically suppressed patients



[a]Wilcoxon rank-sum test; Red line represents median; PHA, phytohemagglutin; BL, baseline

- TNF-α and IL-2 expression significantly increased in stimulated CD4+ cells (**Figure 5**)
  - Less pronounced improvements in cytokine expression were observed in unsuppressed patients (data not shown)

Figure 5. Cytokine expression in virologically suppressed patients



[a]Wilcoxon rank-sum test; Red line represents median; SEB, Staphylococcal enterotoxin B; TNF, tumor necrosis factor; BL, baseline; IL, interleukin; IFN, interferon

Conclusions

- Few, if any, studies have prospectively assessed changes in *in vitro* immune function in response to ARV therapy
  - This substudy from GRACE evaluated T-cell function in a racially diverse population comprised of more than 30% women
- DRV/r (600/100mg twice daily)-based ART was associated with progressive immune recovery over 48 weeks in virologically suppressed patients
  - Both immune phenotype and function of CD4+ and CD8+ cells were significantly improved as evidenced by positive changes in the capacity to proliferate and the expression of intracellular cytokines by CD4+ cells
- Functional recovery, as assessed by proliferative response and intracellular cytokine expression was also seen in unsuppressed patients, although to a lesser degree than in suppressed patients
- Results from this substudy validate that virologic suppression with highly-active ART not only leads to increased CD4+ cell counts, but also improves *in vitro* immune function

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Acknowledgments

The authors would like to thank the study sites, the patients and their families, and the principle investigators for their participation in the trial. The authors would like to acknowledge Gilead for supplying tenofovir, emtricitabine and emtricitabine/tenofovir. The authors would also like to thank internal study support staff, as well as Samantha Taylor, PhD, Medicus International, for her editorial assistance. Funding for editorial support was provided by Tibotec Therapeutics.

Presented at the 5th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention, Cape Town, South Africa, July 19–22, 2009

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