

Impaired responses to immunization in HIV-infection despite CD4+ T-cell restoration after suppressive antiretroviral therapy

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Abstract

BACKGROUND: Responses to immunization are indices of general immune competence requiring integrity of both afferent and efferent limbs of the immune system. This study was designed to examine the responses to immunization with recall and neoantigens in persons with "normal" CD4+ T-cell counts after successful antiretroviral therapy.

METHODS: 29 HIV-infected patients with pre-treatment CD4+ T-cell nadirs below(A)and above(B) 250 cells/μL and recent CD4+ T-cells > 450/μL and HIV-RNA (VL)< 400 copies/mL for > 12 months and 9 HIV- seronegative controls(C) were immunized at study entry and 4 weeks later with tetanus toxoid (TT), diphtheria toxoid (DT) and keyhole limpet hemocyanin (KLH).Lymphocyte proliferation (LP) and delayed type hypersensitivity (DTH) responses were analysed. Lymphocyte subpopulations were analysed by three color flow cytometry.

RESULTS: CD4+ T-cell nadirs averaged 40/μL in group A and 405/μL in Group B. VL was <400 copies/mL for a median of 41 (A) and 30 (B) months. The median current CD4+ T-cell counts were 744 (A) and 724 (B). Absolute naive and memory CD4+, CD4+ CD28+, CD8+ and CD8+ HLA DR38+ cell numbers were comparable among the patient groups. Following immunization, lymphoproliferation in response to TT, DT and KLH and DTH in response to KLH were comparable in the patient groups, although responses were lower in patients compared to controls (p<0.05). The median LP stimulation index (SI) in response to TT was 45, 48 and 174, in response to DT 2, 6 and 34 and in response to KLH 38, 38 and 223 in groups A, B and C respectively. Percentages of DTH responders to KLH increased from 0 to 46 in A, from 0 to 64 in B and from 0 to 100 in C.

CONCLUSION: Despite suppression of HIV replication and restoration of CD4+ T-cells to "normal" levels, responses to immunization remained impaired in HIV-1 infected patients. This persistent immune dysfunction has implications for the design of vaccine strategies in HIV-1 infection

Introduction

Responses to immunization are indices of general immune competence requiring integrity of both afferent and efferent limbs of the immune system.

Optimal timing of treatment initiation may depend on the magnitude of immunologic restoration in early versus advanced HIV-1 infection.

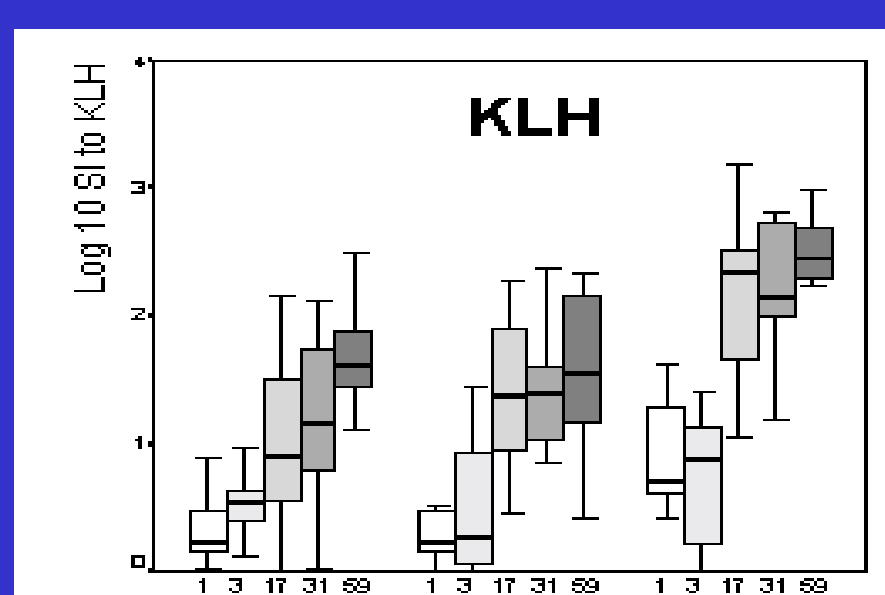
This study was designed to examine the relationship between the CD4+ T-lymphocyte nadir (CD4+ nadir), and the magnitude of immune restoration after successful suppression of HIV-1 replication on HAART.

Methods

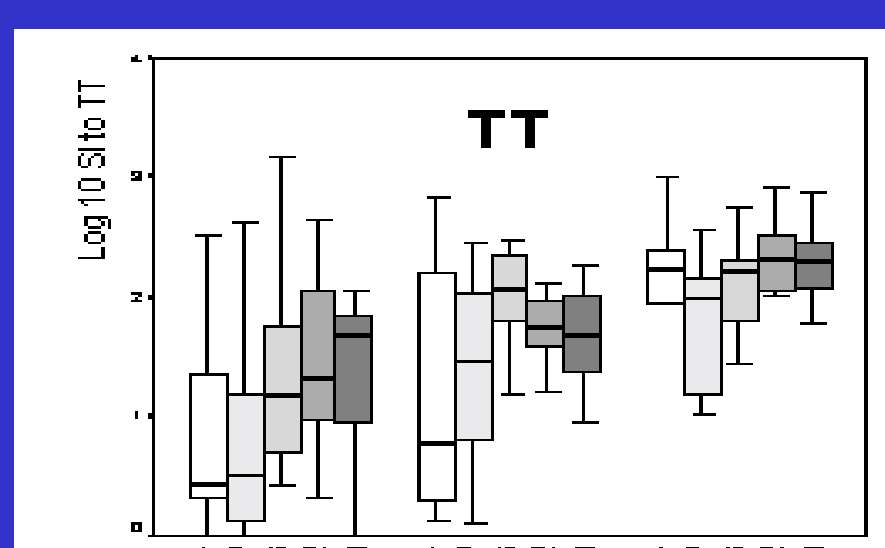
- 29 HIV-1 infected patients with recent CD4+ T-cell counts above 450/μL and plasma HIV-RNA of less than 400 copies/mL during the previous 12 months on HAART were enrolled. Nine age matched non-HIV-1 infected controls were included in the study.
- Individuals were immunized at study entry and 4 weeks later with tetanus toxoid (TT), diphtheria toxoid (DT) and keyhole limpet hemocyanin (KLH).
- Lymphoproliferation to antigens of TT, DT and KLH as well as *Candida albicans* (CA), *Cytomegalovirus* (CMV), HIVp24, *Mycobacterium avium-intracellulare complex* (MAC), Streptokinase (SK) and the pokeweed mitogen (PWM) was assayed on days 1, 3, 17, 31 and 59. Results are expressed as stimulation index (SI), defined as the ratio of counts per minute with antigen to the counts per minute without antigen. A SI > 3 was considered a response.
- Delayed type hypersensitivity (DTH) responses were analysed at baseline and after two months. An induration > 5mm was considered a response.
- Actual T-lymphocyte subsets were enumerated by flow cytometry using murine monoclonal antibodies against CD4 CD8, CD28, CD45RA, CD45RO, CD62L CD95 and HLA DR CD38 at baseline.
- Patients were stratified according to the lowest pre-treatment CD4+ T-lymphocyte count (CD4+ nadir) into two groups with greater or less than 250 cells/μL.
- Comparisons between groups were performed by post hoc multiple comparison ANOVA Bonferroni analysis.

Results

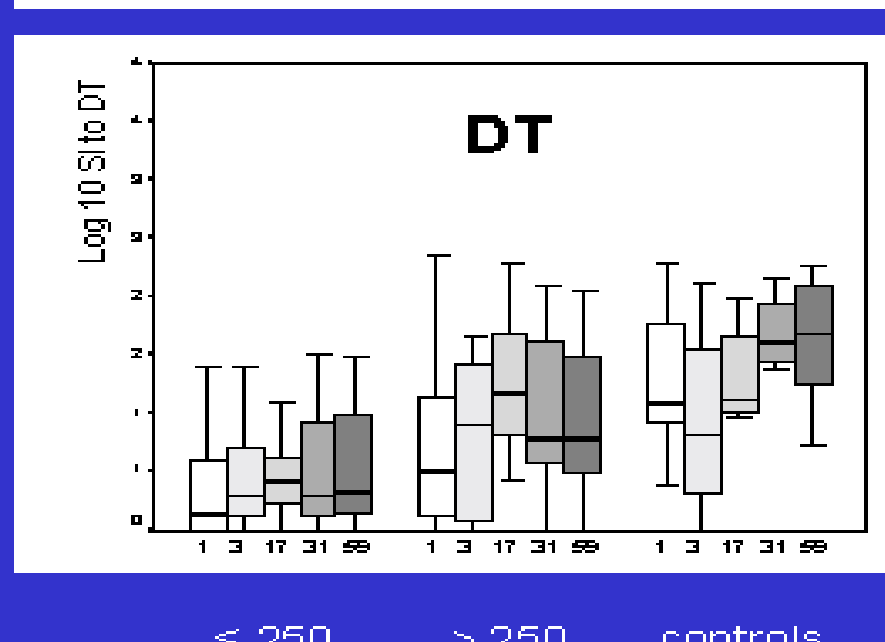
Figure 1: Lymphoproliferation in response to immunization with KLH, TT and DT. LP is comparable between the groups of patients at any time after immunization.



Median SI to KLH is greater in controls after immunization (p<0.005)



Median SI to TT is greater in controls after immunization (p<0.05)



Median SI to DT is greater in controls after immunization (p<0.05)

Figure 2: Frequency of pre- and post-immunization LP responses

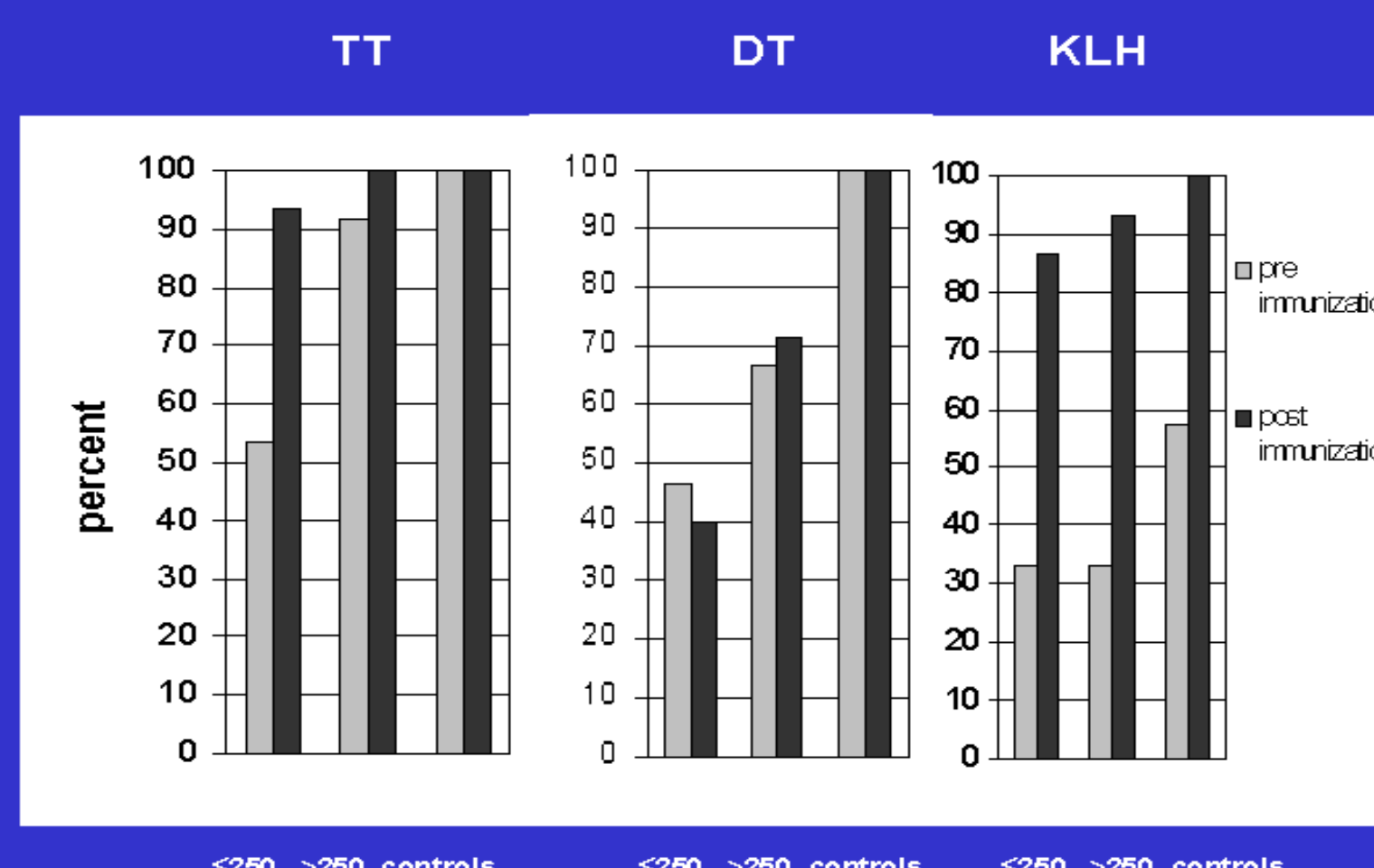
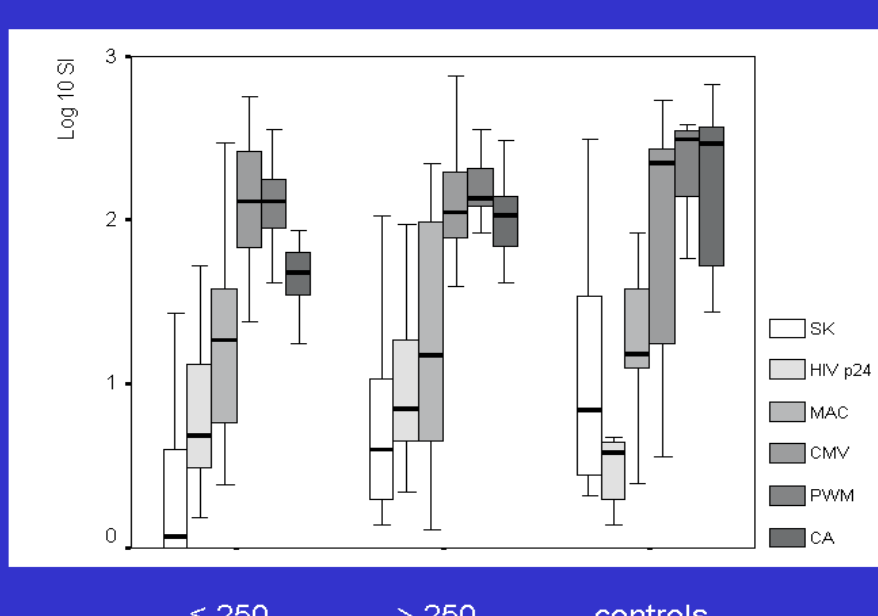
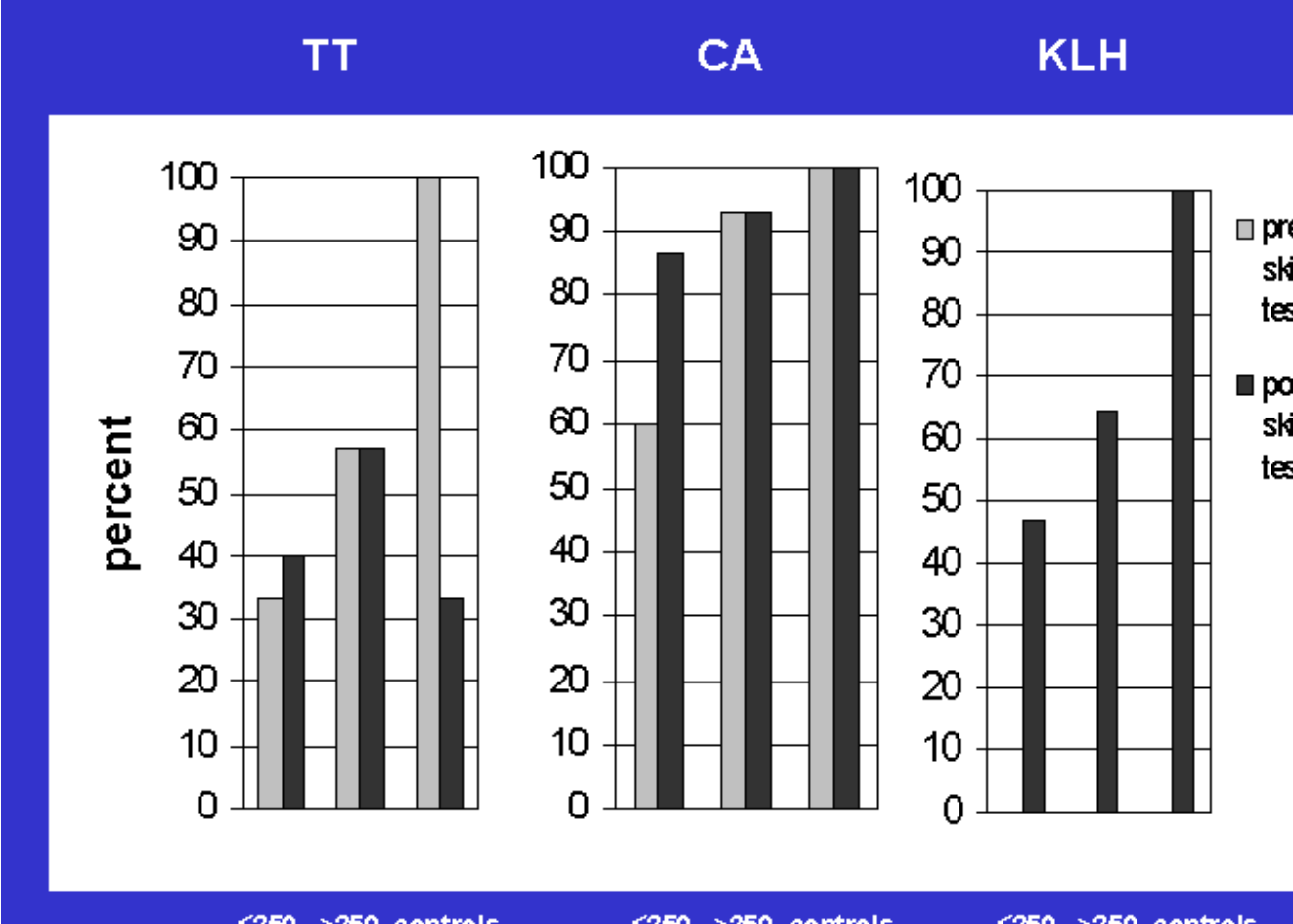


Figure 4: Lymphoproliferation in response to antigens of Streptokinase (SK) HIVp24, *Mycobacterium avium-intracellulare complex* (MAC), *Cytomegalovirus* (CMV), *Candida albicans* (CA) and the pokeweed mitogen (PWM).



LP responses to prevalent antigens are restored in HIV-1 infected patients regardless of the CD4+ T-cell nadir

Figure 3: Frequency of pre- and post-immunization DTH skin test responses



Skin test responses were more frequently present in controls as compared to patients with CD4+ T-cell nadirs below 250 cell/μL in response to recall antigens (TT, CA) pre immunization (p<0.05) and to a neoantigen (KLH) post immunization (p<0.05).

Conclusions

Despite quantitative restoration of CD4+ T-lymphocytes and normal functional immune responses to prevalent antigens after longstanding suppression of viral replication on HAART, phenotypic reconstitution and functional immune responses to immunization with recall- and neoantigens are incomplete in HIV-1 infected patients.

Functional responses to immunization are weaker and may be less durable in patients with CD4+ T-cell nadirs below 250 cell/μL when compared to controls.

Incomplete responses to immunizations should be expected in chronically HIV-1 infected patients, especially when the initiation of therapy had been delayed.

Persistent immune dysfunction despite CD4+ T-cell restoration and longstanding suppression of viral replication has implications for the design of vaccine strategies in HIV-1 infection

Acknowledgment

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Table 1: Characteristics of patient groups with CD4+ T-cell nadirs below and above 250 cells/μL

| | CD4+ T-lymphocyte nadir <250 cells/μL | CD4+ T-lymphocyte nadir >250 cells/μL | p |
|---|---------------------------------------|---------------------------------------|--------|
| number | 15 | 14 | |
| age (years) | 41.0 (39-48) | 40.5 (38-49) | ns |
| duration of therapy (months) | 44 (41-52) | 34.8 (29-40) | <0.005 |
| duration of HIV-RNA < 400 copies/mL (months) | 41 (35-45) | 30.0 (22-36) | ns |
| pre-treatment HIV-RNA (log ₁₀ copies/mL) | 4.7 (3.6-5.5) | 4.2 (3.6-4.6) | ns |
| duration of known HIV-infection (years) | 9 (5-13) | 6 (4-11) | ns |
| history of AIDS defining illness | 9 | 0 | <0.001 |
| CD4+ T-lymphocyte increase (cells/month) | 13 (11-16) | 11 (6-20) | ns |
| CD4+ T-lymphocyte nadir (cells/μL) | 40 (20-180) | 405 (318-506) | <0.001 |
| CD4+ T-lymphocytes (cells/μL) | 744 (497-849) | 725 (565-853) | ns |

ns denotes non-significant

Table 2: Lymphocyte phenotypes after longstanding suppression of viral replication in patients with CD4+ T-cell nadirs below and above 250 cells/μL and controls

| | CD4+ T-cell nadir <250 cells/μL (a) | CD4+ T-cell nadir >250 cells/μL (b) | a-b p | controls (c) | a-c p | b-c p |
|---------------------------------|-------------------------------------|-------------------------------------|-------|------------------|--------|-------|
| number of patients | 15 | 14 | | 9 | | |
| absolute lymphocytes (cells/μL) | 2330 (1749-2930) | 1955 (1733-2185) | ns | 1880 (1720-2165) | ns | ns |
| CD4+ T-lymphocytes (cells/μL) | 744 (497-849) | 725 (565-853) | ns | 810 (704-1166) | ns | ns |
| CD4+ CD45 RA+CD62L+ (cells/μL) | 256 (132-330) | 289 (228-400) | ns | 457 (345-706) | <0.05 | <0.05 |
| CD4+ CD45 RA+CD62L+ (%) | 43 (30-53) | 44 (30-55.5) | ns | 55 (46-64) | <0.05 | ns |
| CD4+ CD45 RA+RO+ (cells/μL) | 428 (259-524) | 339 (285-494) | ns | 366 (308-457) | ns | ns |
| CD4+ CD45 RA+RO+ (%) | 58(45-61) | 56 (42-60.5) | ns | 43 (35-54) | <0.05 | ns |
| CD4+ CD28+ (cells/μL) | 567 (407-725) | 691 (497-729) | ns | 810 (683-1079) | <0.05 | ns |
| CD4+ CD28+ (%) | 94(75-99) | 93.0 (85.5-97) | ns | 99. (94-99) | ns | ns |
| CD4+ HLADR+ CD38+ (cells/μL) | 22 (17-36) | 20.7 (14-35) | ns | 24.3 (15-36) | ns | ns |
| CD4+ HLADR+ CD38+ (%) | 3 (3-5) | 3 (2-5) | ns | 3 (2-4) | ns | ns |
| CD8+ T-lymphocytes (cells/μL) | 1114 (674-1335) | 893 (647-1109) | ns | 493 (387-721) | <0.005 | <0.05 |
| CD8+ HLADR+ CD38+ (cells/μL) | 93 (55-186) | 99 (57-245) | ns | 21 (15-40) | <0.05 | <0.05 |
| CD8+ HLADR+ CD38+ (%) | 9 (5-17) | 12 (4-40) | ns | 6 (2-7) | <0.05 | <0.05 |

ns denotes non-significant