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## Original Research Article

# Role of CD5-negative CD8<sup>+</sup> T Cells in Adaptation to Antigenic Variation of Human Immunodeficiency Virus Type 1

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### Abstract

**Purpose:** To investigate the effect of 3-oxotirucalla-7, 24-dien-21-oic acid on CD8<sup>+</sup> T cell recovery in human immunodeficiency virus type 1 (HIV-1) disease.

**Methods:** The increase in the rates of CD8<sup>+</sup> T cells over 48 weeks following treatment with 3-oxotirucalla-7, 24-dien-21-oic acid was investigated. Plasma HIV-1 load was measured by Versant™ HIV-1 RNA 3.0 branched chain DNA assay while flow cytometry was used for blood CD4 cell counts. For the analysis of the data obtained, Stata version 9.0 was employed.

**Results:** 3-Oxotirucalla-7, 24-dien-21-oic acid treatment increased CD8<sup>+</sup> T cell count from a median of 89 % at baseline to 99 % at 48 weeks. The proportion of patients with CD8<sup>+</sup> T cell count < 90 % decreased from 50 % at baseline to 1 %. There was a similar rate of phase 1 CD8<sup>+</sup> T cell recovery and greater rates of phase 2 recovery in patients with baseline CD8<sup>+</sup> T counts < 50 cells/μL. Among those that achieved CD8<sup>+</sup> T cell count > 500 cells/μL at 48 weeks, 23 % had baseline CD8<sup>+</sup> T cell counts of < 50 cells/μL. However, the proportion of the patients that attained CD8<sup>+</sup> T count of 200 cells/μL at 48 weeks was lower than those with higher baseline CD4 cell counts.

**Conclusion:** 3-Oxotirucalla-7,24-dien-21-oic acid treatment induces greater tendency for CD8<sup>+</sup> T cell recovery in patients with baseline CD8<sup>+</sup> T cell counts < 50 cells/μL during 48 weeks of treatment. Therefore, 3-oxotirucalla-7,24-dien-21-oic acid is a promising agent for CD8<sup>+</sup> T cell count recovery in patients with HIV infection.

**Keywords:** CD8<sup>+</sup> T cells, HIV infection, Oleanolic acid, Lymphocyte cell, Cell recovery

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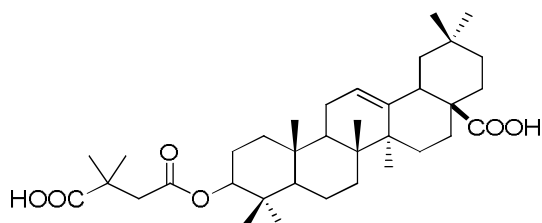
## INTRODUCTION

A number of medicinal plants reported to have anti-HIV properties were subjected to phytochemical investigation which resulted in the discovery of anti-HIV drug candidates [1-4]. This process leads to substantial progress in the isolation of different classes of molecules possessing anti-HIV properties. Among various classes of natural products, terpenes have also

been reported to possess anti-HIV activities. Betulinic acid, platanic acid and oleanolic acid abundant in the leaves of *Syzygium claviflorum*, exhibited anti-HIV activity in H9 lymphocyte cell [5-7]. To enhance the anti-HIV activity, oleanolic acid was subjected to structural modification for the development of more potent pharmacophore. Among the different analogs synthesized, 3-oxotirucalla-7,24-dien-21-oic acid (OTUA) (Figure 1) exhibited promising anti-HIV activity

compared to its parent molecule. OTUA showed inhibition of HIV protease with an  $IC_{50}$  value of 10 mg/mL [8].

The modulation of antigen-specific signalling on virtually all T cells in a ligand-independent manner is performed by group B cysteine-rich scavenger receptor CD5 [9-13]. The conjugation of T-cells with antigen-presenting cells is accompanied by co-localization of CD5 with T-cell receptors (TCRs). This leads to involvement of cognate MHC/peptide complexes within the immunological synapse and engagement of SHP phosphatases to the area through its cytoplasmic tyrosine inhibitory motif (ITIM) [11,14]. Therefore, there is suppression of TCR-derived activation signals by the antagonism of phosphorylation events caused by CD5 [15]. Stronger interaction between TCR and MHC/peptide complex is expected when CD5 concentration is higher. Thus CD5 expression on a T-cells TCR/MHC/peptide complex interaction is required for T cell activation [16]. In the present study, we investigated the effect of oleanolic acid ester, 3-oxotirucalla-7, 24-dien-21-oic acid on the  $CD8^+$  T cell count in HIV-infection.



**Figure 1:** Structure of 3-oxotirucalla-7, 24-dien-21-oic acid

## EXPERIMENTAL

### Study population

The patients accessing 3-oxotirucalla-7, 24-dien-21-oic acid treatment were studied at the Yunnan's Care Centre, Anning City, Southwest China's Yunnan Province. The study was approved by the Ethics Committee of the Yunnan's Care Centre (approval ref no. 158/2014/YCC) and followed the guidelines of World Health Organisation (WHO) 2002 recommendations [17]. The patients were treated free of charge. 3- Oxotirucalla-7, 24-dien-21-oic acid treatment was given to the patients with  $CD8^+$  T cell counts < 200 cells/ $\mu$ L daily without interruption.

### Materials and data collection

Versant™ HIV-1 RNA 3.0 branched chain DNA assay (Bayer HealthCare, Leverkusen,

Germany), was used to measure plasma HIV-1 load at baseline and after every 4 months. Flow cytometry using FACSCount™ (Becton Dickinson Inc., Franklin Lakes, NJ, USA), was used for blood  $CD8^+$  T cell counts. Patients' records were maintained and transferred weekly to a computer database. At the end of the programme, the data were analysed. The study of these patients was approved by the Research Ethics Committee of the Yunnan's Care Centre. A written consent was obtained from all the patients enrolled, in order to ensure that they were aware of the type of study.

### Data analysis

For the analysis of the data obtained, we used Stata version 9.0 (College Station, Texas, USA). The absolute responses in  $CD8^+$  T cell counts were calculated during the intervals (from baseline to 24 weeks of 3-oxotirucalla-7, 24-dien-21-oic acid treatment, and 24 to 48 weeks). Increase in  $CD8^+$  T-cell count (cells/ $\mu$ L/month) during each interval was also calculated. The effects of 3-oxotirucalla-7,24-dien-21-oic acid treatment on  $CD8^+$  T-cell responses were observed in terms of  $CD8^+$  T cell count below 90 %; achievement of  $CD8^+$  T-cell count of 90-95 % and  $CD8^+$  T-cell count above 95 % at 48 weeks. Wilcoxon rank-sum and sign-rank tests for comparison of medians and  $\chi^2$  tests for proportions were used in bivariate analyses.

## RESULTS

### Effect of HIV- infection on level of $CD8^+$ T cells circulating with expression of CD5

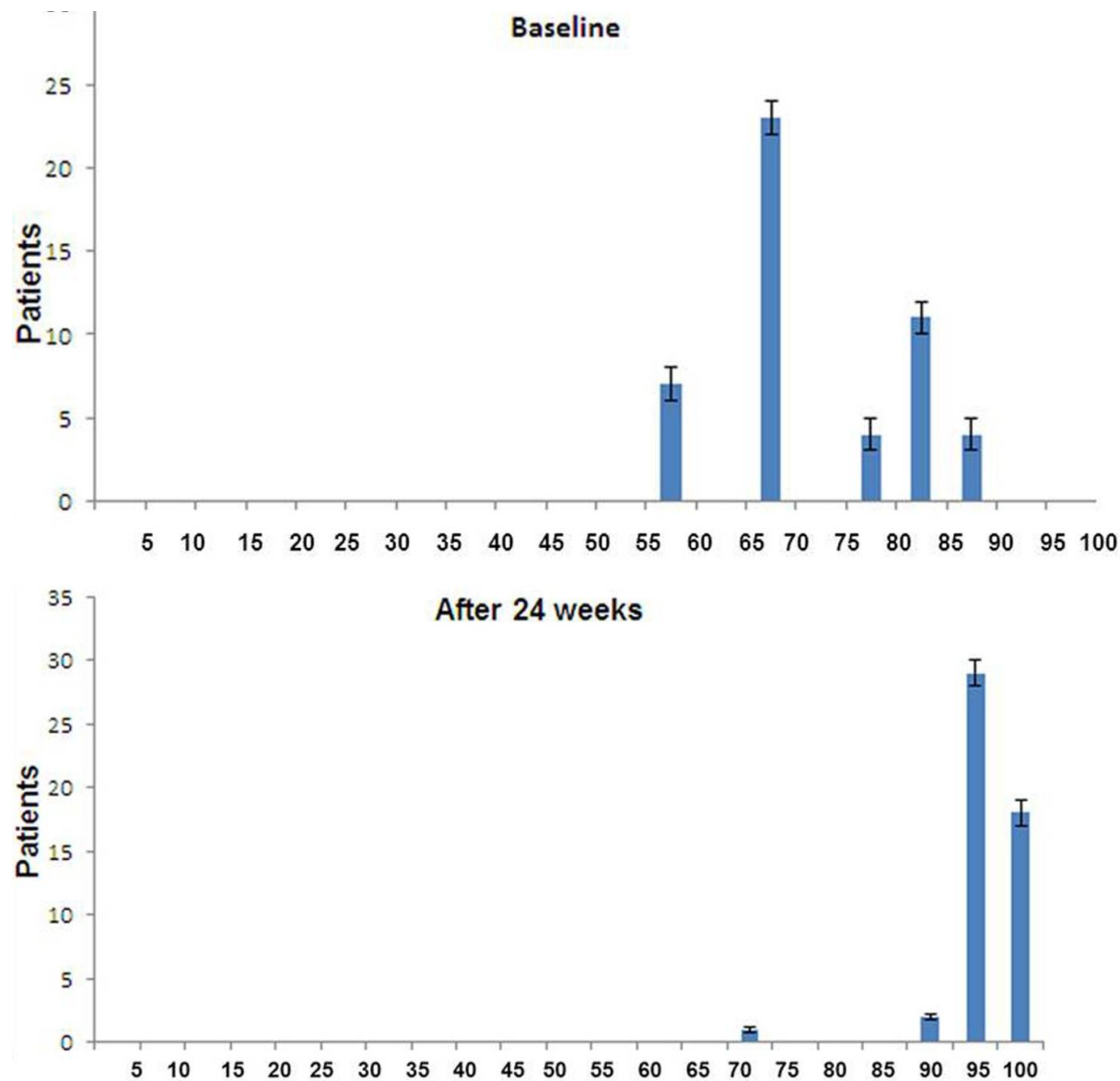
The results revealed that in HIV-positive patients, the proportion of  $CD8^+$  T-cells circulating with CD5 expression was significantly lower than those in the normal controls. The median level of  $CD8^+$  T-cells in the HIV-positive patients was 89.5 with an interquartile range of 91.2-82.4 versus 99.0 with interquartile range 98.7-99.8 ( $p < 0.0001$ , Mann Whitney test) in normal controls. Thus, HIV replication effectively suppressed  $CD8^+$  T cells which lead to decrease of CD5 expression.

### Effect of 3- oxotirucalla-7,24-dien-21-oic acid on virological and $CD8^+$ T cells response

3- oxotirucalla-7, 24-dien-21-oic acid treatment led to a significant decrease in viral level at each follow-up time point. The viral load was decreased to < 350 copies per mL at each of the follow-up time-points (Table 1). We also observed a remarkable change in the level of

CD8<sup>+</sup> T-cells in the blood of patients treated with 3-oxotirucalla-7, 24-dien-21-oic acid for 48 weeks (Figure 2). Treatment for 48 weeks resulted percentage of HIV-positive patients having median level of CD8<sup>+</sup> T-cells 89.5 was reduced to 1 % only (Table 1).

While comparing the increase in CD8<sup>+</sup> T cell count during two phases, we observed that rate of increase was faster during first phase of 24 weeks compared to second phase of 24 weeks.

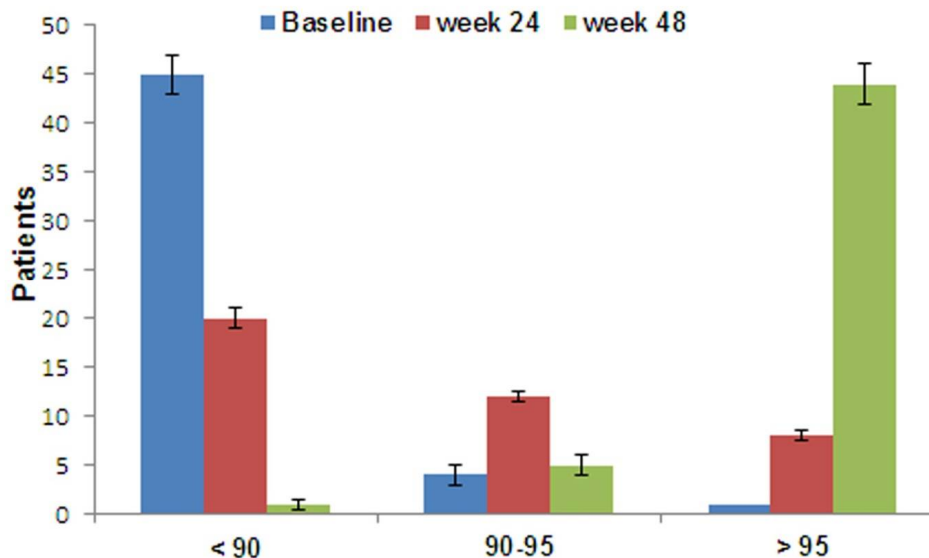


**Figure 2:** The frequency distribution of absolute blood CD8<sup>+</sup> T cell counts (a) at baseline and (b) after 48 weeks of 3-oxotirucalla-7, 24-dien-21-oic acid treatment

**Table 1:** Changes in blood CD8<sup>+</sup> T cell counts and plasma viral load of patients (n = 50) during 3-oxotirucalla-7, 24-dien-21-oic acid treatment

Variable	Baseline	Week 24	Week 48
<b>Virological response</b>	72480	< 50	< 50
Average VL			
Patients with VL ≥ 350 (No: and %)	50 (100)	8 (4)	2 (2.8)
Patients with VL ≥ 50 (No: and %)	50 (99)	4 (20)	1 (3)
<b>CD8<sup>+</sup> T Cell count response</b>			
Median CD8 <sup>+</sup> T cell count (cells/μL)	89.5 (91.2-82.4)	96 (98.3-89.4)	99.4 (99.7-90.6)

VL = viral load (copies/μL)



**Figure 3:** Graph showing the median CD8<sup>+</sup> T cell counts at baseline and during 3-oxotirucalla-7, 24-dien-21-oic acid treatment stratified by baseline CD8<sup>+</sup> T cell count

#### Effect of 3- oxotirucalla-7, 24-dien-21-oic acid with respect to baseline CD8<sup>+</sup> T cell count

Increase in CD8<sup>+</sup> T-cell count during the phase 1 (0-24 weeks) after 3- oxotirucalla-7,24-dien-21-oic acid treatment was almost same irrespective of the base line cell count (Figure 3). However, phase 1 CD8<sup>+</sup> T-cell recovery was found to be dependent on baseline plasma viral load. The phase 1 CD8<sup>+</sup> T-cell slope was 13.6 cells/ $\mu$ L/month in patients with baseline viral load < 5 log<sub>10</sub> copies/ $\mu$ L. On the other hand, the slope was 24. 4 cells/ $\mu$ L/month among those with baseline viral load of > 5 log<sub>10</sub> copies/  $\mu$ L.

During phase 2 (24-48 weeks), after 3-oxotirucalla-7, 24-dien-21-oic acid treatment, the increase in CD8<sup>+</sup> T cell count was greater among the patients with a baseline CD8<sup>+</sup> T-cell count < 90 cells/ $\mu$ L compared to those with higher baseline counts. The age of patients was also an important factor that influences the CD8<sup>+</sup> T-cell count during phase 2. The rate of increase was also greater among younger compared to older patients. Therefore, both the low baseline CD8<sup>+</sup> T-cell count and the younger age were the only baseline characteristics that were significantly associated with higher rates of phase 2 CD8<sup>+</sup> T cell recovery.

#### Baseline CD4 CD8<sup>+</sup> T cell count and risk of immunological non-response

After 48 weeks of 3- oxotirucalla-7, 24-dien-21-oic acid treatment, the blood CD8<sup>+</sup> T-cell count increased by < 50 cells/ $\mu$ L among 20 (9.7 %) patients; of these, the viral load was suppressed

< 50 copies/mL among 4 % patients, representing a treatment discordance rate of 7 %. Contrary to our initial hypothesis, low baseline CD8<sup>+</sup> T-cell counts were not associated with increased risk of immunological non-response.

Among patients with baseline CD8<sup>+</sup> T-cell counts of < 50, 50–99, 100–149 and 150 cells/ $\mu$ L, the proportions of patients who were immunological non-responders were 5, 4, 11 and 19 %, respectively. Furthermore, in multivariate analyses, an increment of < 50 cells/ $\mu$ L was independently associated with higher baseline CD8<sup>+</sup> T-cell count as well as older age, lower baseline viral load, and a viral load > 400 copies/mL at any follow-up time-point (Table 2).

## DISCUSSION

The current study represents the first report to demonstrate the effects of 3-oxotirucalla-7, 24-dien-21-oic acid (OTUA) treatment on CD8<sup>+</sup> T-cell count among patients suffering from HIV infection. The results revealed increased rates of CD8<sup>+</sup> T-cell count recovery in the second phase (24 – 48 weeks) in patients having CD8<sup>+</sup> T-cell concentration during baseline < 50 cells/ $\mu$ L. On the other hand, the rates of phase 1 (0 – 24 weeks) recovery were similar, irrespective of baseline CD8<sup>+</sup> T-cell counts. 3-oxotirucalla-7, 24-dien-21-oic acid treatment led to increased response to CD8<sup>+</sup> T-cell recovery in patients with low baseline counts. The increased response to enhance count of CD8<sup>+</sup> T-cells by patients having baseline CD8<sup>+</sup> T cells more than 50 cells/ $\mu$ L, could not reach to the value of 200 CD8<sup>+</sup> T/ $\mu$ L at 48 weeks.

**Table 2:** Change in blood CD8<sup>+</sup> T cell count during anolignan A treatment using logistic regression models

Variable		Immunological non-response risk			CD8 <sup>+</sup> T cell count of ≥ 200 cells/μL risk		
		OR	(95% CI)	P value	OR	(95% CI)	P-value
Age (years)	< 35		1.0		1.0		
	35–40	2.95	(0.96, 8.86)	0.043	1.56	(0.48, 3.10)	0.38
	> 40	4.12	(0.98, 14.23)	0.029	4.23	(1.79, 8.34)	0.002
Baseline CD4 count	> 150	1.0			1.0		
	100–149	0.54	(0.17, 2.43)	0.26	3.12	(0.65, 7.76)	0.06
	50–99	0.12	(0.02, 0.93)	0.009	6.54	(3.32, 17.45)	<0.002
WHO clinical stage	< 50	0.12	(0.02, 0.93)	0.012	14.21	(4.43, 33.14)	<0.002
	1 & 2	1.0			1.0		
	3	0.96	(0.19, 4.36)	0.53	0.89	(0.34, 4.56)	0.43
Baseline viral load	4	3.76	(0.20, 8.67)	0.11	2.93	(0.21, 4.87)	0.97
	< 5 log <sub>10</sub>	1.0			1.0		
Follow-up viral load	> 400 any visit	12	(0.11, 0.87)	0.004	0.23	(0.18, 0.24)	0.05
	< 400 all visits	1.0			1.0		
	> 400 any visit	5.45	(2.03, 18.21)	0.005	5.34	(0.98, 8.54)	0.009

3-Oxotirucalla-7, 24-dien-21-oic acid treatment resulted in decrease of percentage of patients with a CD8<sup>+</sup> T-cell count < 100 cells/μL from 61 % at baseline to 5 % at after 48 weeks. It has been reported that the patients with the lowest baseline CD8<sup>+</sup> T-cell counts have higher death risks [26]. During phase 2, the sustenance of suppression of viral replication also has a key role in the CD8<sup>+</sup> T-cell recovery [27,28]. In our study, the proportion of the patients having more than 50 D8<sup>+</sup> T-cells/μL was only 6 % following 48 week treatment with 3-oxotirucalla-7, 24-dien-21-oic acid and 4 % were immunologically non-responsive even after a marked reduction in viral level. Moreover, 23 % of 'super-responders' had baseline CD8<sup>+</sup> T-cell counts < 50 cells/μL, indicating that a very low baseline CD8<sup>+</sup> T-cell count does not preclude an excellent CD4 cell count response to 3-oxotirucalla-7, 24-dien-21-oic acid treatment.

## CONCLUSION

The study revealed that patients with CD4 counts in the lower range have a significant tendency for immune recovery. 3-Oxotirucalla-7, 24-dien-21-oic acid treatment suppresses the viral level and enhances the probability of immune system recovery. Therefore, 3-oxotirucalla-7, 24-dien-21-oic acid can be an effective candidate for CD4 cell recovery.

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