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Blockade of PD-L1 Does Not Reverse HIV Latency in CD4+ T Cells *Ex Vivo*

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ABSTRACT

Background: Blockade of the PD-1/PD-L1 pathways using monoclonal antibodies (mAb) to PD-1 has been reported to activate HIV expression from latently infected CD4 T cells ex vivo (1). To further evaluate this approach, we tested the ability of the human anti-PD-L1 mAb BMS-936559 to activate virion production from mononuclear cells obtained from patients on suppressive ART.
Methodology: PBMC, total (t) CD4, and resting (r) CD4 cells were purified by negative selection of large-volume blood draws from HIV-infected donors. Freshly isolated PBMCs were cryopreserved and immunophenotyped for PD-1/PD-L1 expression. The remaining cells were incubated (1 million cells/well in triplicate) for 1 week with 20, 5, or 1.25μg/mL anti-PD-L1 mAb, with 20μg/mL isotype control [Zymogen DT-1D12g-4P (hIgG4)], or with anti-CD3/CD28-coated microbeads plus either anti-PD-L1 mAb or isotype control. On Day 8, cells were assessed for viability and supernatants tested for HIV RNA using the Roche Taqman v.2.0. A virologic response was defined as a >3-fold increase in HIV RNA over isotype control. Donors whose cells responded initially to anti-PD-L1 mAb were redrawn and tested again.
Results: PBMC, tCD4, and rCD4 cells were purified from ten long-term (mean 8 years) suppressed donors. Cell viability was not reduced by treatment with anti-PD-L1 mAb. 9 of 10 donors responded to anti-CD3/CD28 in all cell types (mean fold-increases of 742, 1353, and 272 for PBMC, tCD4 and rCD4, respectively). Anti-PD-L1 mAb did not enhance responses to anti-CD3/CD28. PBMC from 2 of 10 donors (donor 3: 61-fold; donor 4: 7-fold) showed an initial response to anti-PD-L1 mAB that was not reproduced upon repeat blood draw. tCD4 cells from 2 of 10 donors (donor 2: 583-fold; donor 6: 84-fold) initially responded to anti-PD-L1. However, cells from donor 2 did not respond after a repeat draw. Cells from donor 6 responded on the first repeat draw but not the second. rCD4 from 0 of 10 donors responded to anti-PD-L1. PD-1/PD-L1 expression on CD4 and CD8T cells was evident on the day of cell isolation in all donors, but expression levels did not differ between responders, non-responders, and a healthy control.
Conclusions: Despite detectable PD-1/PD-L1 expression, increased HIV production from PBMC, total CD4 T cells or resting CD4 T cells after treatment with anti-PD-L1 antibody was infrequent and not reproduced longitudinally. Alternate strategies will be needed to activate proviral expression from latently infected CD4 T cells.

BACKGROUND

- Reservoirs of latently infected CD4 cells that persist despite anti-retroviral therapy (ART) present a major barrier to curing HIV infection (2,3).
- Effective methods are required to reactivate latently infected cells so they can be targeted for elimination.
- Blockade of the PD-1 pathway using monoclonal antibodies (mAB) against PD-1 has been reported to activate HIV virus expression from latently infected CD4 T cells *ex vivo* (1).
- Here, we investigated the ability of a mAB against PD-L1 (PD-1 ligand) to induce virus production from PBMC, tCD4 and rCD4 cells in an *ex vivo* assay using cells from HIV-1-infected donors on long-term ART.

METHODS

Study Population:
- Experiments performed using 10 HIV-1-infected donors, ART-suppressed for an average of 8 years, with plasma HIV-RNA ≤ 15.8 cps/mL by iSCA.

Cell Purification
- PBMC were isolated from large-volume (180mL) blood draws using ficoll separation.
- Total (t) and resting (r) CD4+ T-cells were purified from PBMC using Stemcell Technologies negative selection kits.

Immunophenotyping
- Freshly isolated PBMCs were cryopreserved and immunophenotyped for PD-1/PD-L1 expression.

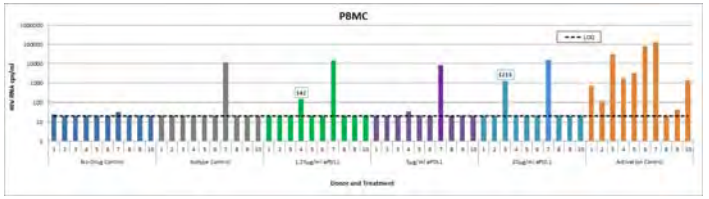
Assay Design
- 1 million cells/well (PBMC, tCD4 or rCD4) were incubated in triplicate for 1 week with 20, 5, or 1.25μg/mL anti-PD-L1 mAB, with 20 μg/ml isotype control, or with anti-CD3/CD28-coated microbeads plus either anti-PD-L1 antibody or isotype control.
- On Day 8, cellular viability was assessed using Promega CellTiter Fluor kit. Plasma HIV-1 RNA was measured by Roche TM (quantification limit 20 cps/mL).
- Donors whose cells responded initially to anti-PD-L1 mAB were redrawn and tested again.

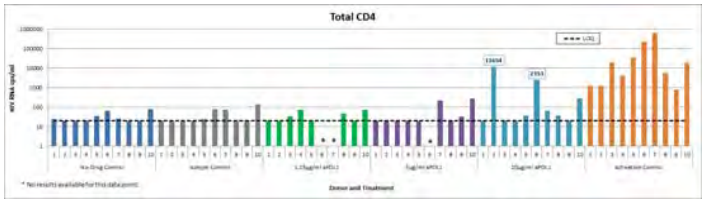
RESULTS

Table 1: Donor Clinical Characteristics

Donor	Draw Date	Plasma HIV RNA (cps/mL) by ISCA	Age	Gender	Race	CD4 Count	Antiretroviral Regimen when Blood was Donated	Duration of HIV RNA Suppression (< 50 cps/mL)
1	05.13.2013	1.0	55	Female	African American	1373	Atripla	12 yrs, 7 mos
2	06.10.2013	< 0.6	40	Male	Caucasian	699	Combivir, Raltegravir	6 yrs, 6 mos
3	06.12.2013	1.9	51	Female	African American	792	Truvada, Raltegravir	2 yrs, 6 mos
4	02.24.2014	1.0	60	Female	African American	784	Atripla	> 8 years
5	04.23.2014	15.8	59	Male	African American	1023	Sustiva, Epivir, Ziagen	17 years, 5 months
6	11.19.2013	2.0	58	Male	Caucasian	656	Truvada, Retrovir, Etravirine, Raltegravir	9 years, 2 months
7	12.03.2013	0.6	51	Male	African American	472	Norvir, Raltegravir, Ziagen, Prezista, Truvada	6 years, 6 months
8	03.31.2014	< 0.7	60	Male	African American	898	Complera	16 years, 8 months
9	04.04.2014	2.0	42	Male	African American	605	Reyataz, Truvada, Norvir	1 year, 5 months
10	01.22.2014	10.0	42	Male	African American	524	Complera	1 year, 10 months
	AVG:	3.6	51.8		AVG:	782.6	AVG:	8 years, 2 months

Figure 1: HIV RNA (cps/mL) by Roche Taqman in Day 8 Cell Culture Supernatants (Experiment 1)





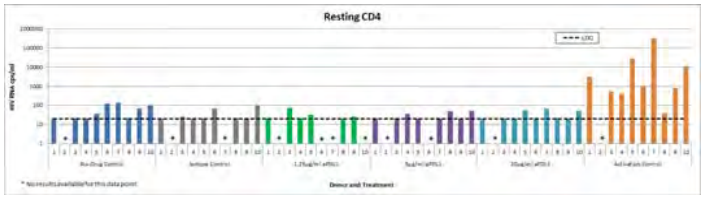
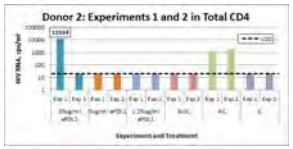
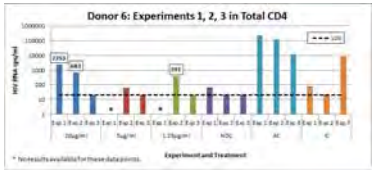
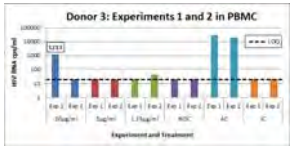


Figure 2: Donors whose cells responded initially to anti-PD-L1 mAb treatment were redrawn and tested again (Experiments 2 and 3). mAB-induced viral expression not reproducible longitudinally.







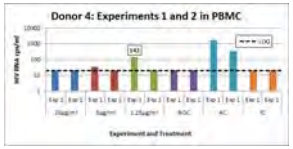


Table 2: Immunophenotype Data: Both PD-1 and PD-L1 expression on Day 0 cells. No difference betweeen "responders" and "non-responders."

Day 0 Cells: % PD-1 Expression

Donor	Potential Responder?	Resting CD4 T Cells	CD8 T Cells	Monocytes
HIV Neg	NA	22.1	42.3	Not detected
6 (Exp 1)	Yes	36.4	32.1	0.2
6 (Exp 2)	Yes	41.3	36.4	0.32
2	Yes	19.2	22.7	Not detected
5	No	27.5	14.8	1.32
10	No	41.9	24.7	0.19

Day 0 Cells: PD-L1 Expression as Fold-Change in MFI Relative to Isotype

Donor	Potential Responder?	Resting CD4 T Cells	CD8 T Cells	Monocytes
HIV Neg	NA	2.82	2.77	1.71
6 (Exp 1)	Yes	3.52	3.19	1.41
6 (Exp 2)	Yes	3.89	3.46	1.58
2	Yes	2.92	2.93	1.55
5	No	1.52	2.93	1.29
10	No	3.24	3.02	1.36

RESULTS

- Day 8 viability was not affected by treatment with anti-PD-L1 mAB or isotype control (data not shown).
- 9 of 10 donors responded to anti-CD3/CD28 in all cell types **(Figures 1-3)**.
- PBMC from 2 of 10 donors showed an initial response to anti-PD-L1 mAB that was not reproduced upon a repeat blood draw **(Figure 2)**.
- Total CD4 from 2 of 10 donors showed an initial response to anti-PD-L1 mAB treatment. One of these donors (Donor 6) responded again in a second but not a third repeat blood draw. The second donor (Donor 2) did not respond following a repeat blood draw **(Figure 2)**.
- Resting cells from 0 of 10 donors responded to anti-PD-L1 mAB **(Figure 1)**.
- PD-1/PD-L1 expression on CD4 and CD8 T cells was evident on the day of isolation in all donors, but expression levels did not differ between responders, non-responders, and a healthy control (HIV Neg) **(Table 2)**.

CONCLUSIONS

- Despite detectable PD-1/PD-L1 expression, increased HIV production from PBMC, total CD4 T cells, or resting CD4 T cells after treatment with anti-PD-L1 antibody was infrequent and not reproduced longitudinally.
- Alternate strategies will be needed to activate proviral expression from latently infected CD4 T cells.
- Additional experiments with anti-PD-1 mAB are in progress.

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