Metformin Therapy With Immune Checkpoint Inhibitors Impacts anti-HIV T cell Responses

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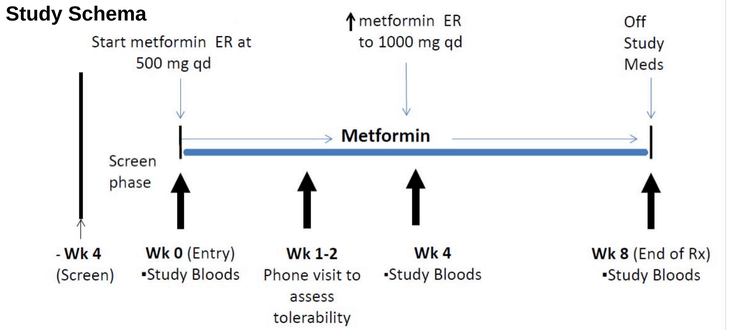
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Introduction

- Despite life saving combination antiretroviral therapy (cART), chronic HIV infection is associated with T cell exhaustion defined by the overexpression of negative immune checkpoint receptors
- The efficacy of immune checkpoint blockade in reversing T cell dysfunction and improving cancer survival is variable among treated individuals
- Metformin is an FDA-approved, widely used oral hypoglycemic agent for diabetes with short and long term documented record of safety and has previously unrecognized therapeutic effects against age-related conditions
- Metformin in combination with immune checkpoint blockade yielded favorable clinical outcomes in oncology (MZ. Afzal et.al. 2018)
- Based on reports of Metformin's pleiotropic effects, we utilized banked blood specimens from a previously conducted open-label, 8-week adjunctive metformin clinical trial and assessed the *ex vivo* anti-HIV CD8 T cell responses to immune checkpoint blockades before and after adjunctive metformin therapy in cART suppressed HIV infected adults

Study Schema



An open-label, single-arm, 8-week pilot study of seven euglycemic HIV-infected individuals on ART, stable for >1 year with plasma HIV RNA <50 copies/ml. 500mg once daily of metformin extended release was administered at entry, increasing to 1000mg once daily at week 4 until study completion at week 8.

Table 1. Study Participant Characteristics

Characteristics	Metformin (*n=7*)
Age, Years	61 (50, 65)
Gender, Male	7 (100)
Ethnicity, Caucasian	5 (83)
Fasting Glucose, mg/ml	107 (99, 112)
Current CD4 Counts, cell/µl	739 (659, 1083)
Plasma HIV RNA, copies/ml	Undetectable (<20)

Median (IQR) presented for continuous variables, n (%) presented for categorical variables

Methods

Immunophenotyping: Frozen PBMCs were rapidly thawed, washed, then stained with a Live/Dead cell discrimination (AARD) marker followed by fluorochrome conjugated monoclonal antibodies (mAbs) to identify lymphocyte or monocyte populations (CD3, CD4, CD8, CCR7, CD28, CD45RA, TIGIT, PD-1, TIM-3, CD14, CD16, HLA-DR, CD11b, CD11c, CD7). Cells were washed twice and fixed with 1% PFA before acquiring on a custom BD Bioscience four laser LSR Fortessa flow cytometer in our lab. All compensation and gating analysis was performed in FlowJo (Treestar).

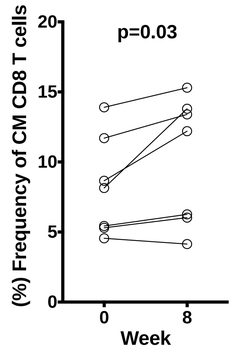
Functional Characterization: Six day *ex vivo* HIV gag peptide pool stimulation in the presence of monoclonal blocking antibody targeting PD-L1 or TIGIT was performed on cryopreserved PBMCs. Day 0 set up with stimulation conditions, day 2 expansion with IL-2, day 5 re-stimulation with original conditions + BFA/MON, day 6 assay takedown. HIV-specific cytokine and degranulation responses were assessed by intracellular cytokine staining and acquired previously mentioned.

Statistical Analysis: Non-parametric Wilcoxon Rank Sum test was used to compare entry and 8 week changes in CD8 T cell phenotype and function. Spearman's rho was used for correlations. A p-value <0.05 was regarded as statistically significant for all analyses and all results were conducted using SAS v9.4

Results

Figure 1. Metformin therapy increased the proportion of Central Memory CD8 T cells

A)

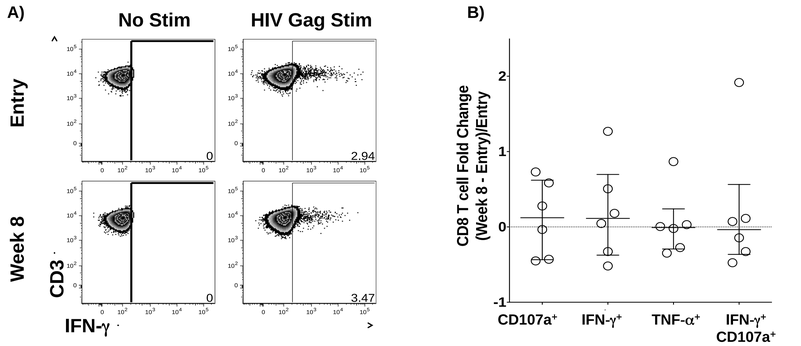


B)

CD8 T cell Subset	Week 0	Week 8	p value
Naïve (CD45RA+CCR7+CD28+)	15.9 (4.58, 20.8)	14.9 (4.87, 17)	0.15
Central Memory (CD45RA-CCR7+CD28+)	8.14 (5.3, 11.7)	12.2 (6.03, 13.8)	0.03
Transitional Memory (CD45RA_CCR7-CD28+)	38.3 (31.3, 49)	44.1 (29.6, 48.7)	0.46
Effector Memory (CD45RA-CCR7-CD28-)	61.7 (51, 68.7)	55.9 (51.3, 70.4)	0.46
Terminally Differentiated (CD45RA+CCR7-CD28-)	41.4 (27.2, 48.1)	37.4 (27.1, 43.8)	0.6

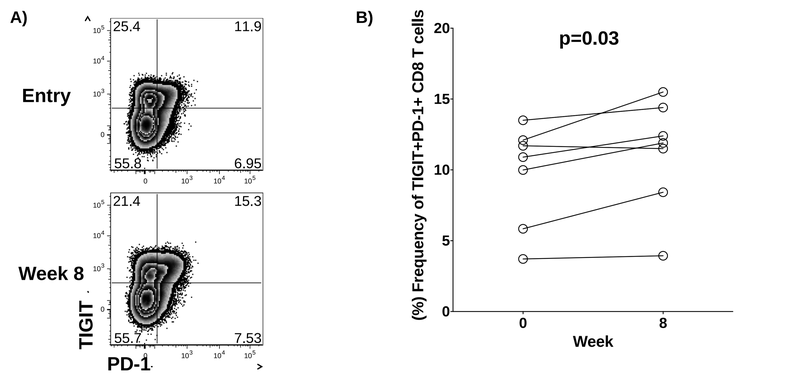
A) Graphs show changes in the the the frequency of Central Memory CD8 T cell populations B) Table shows median (IQR) frequencies of CD8 T cell subsets over the 8-week course of adjunctive metformin therapy (*n = 7*)

Figure 2. Metformin alone did not improve anti-HIV CD8 T cell responses *ex vivo*



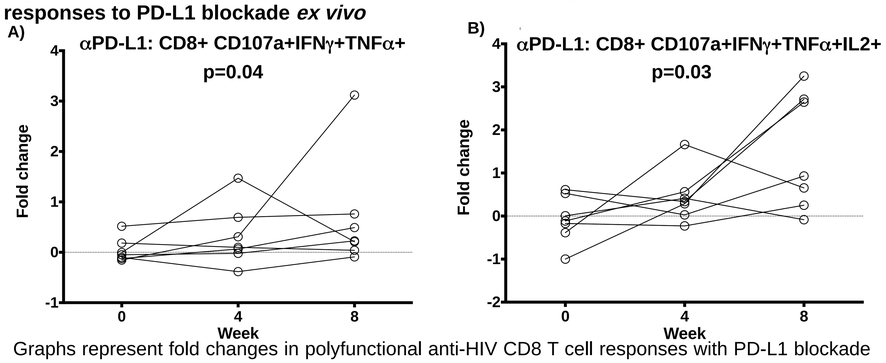
A) Representative flow cytometry plots showing IFN-γ producing HIV-specific CD8 T cell responses at entry and week 8 of adjunctive metformin therapy. B) Graph shows the fold change in anti-HIV CD8 T cell effector capacities from entry to week 8. (*n = 7*)

Figure 3. Increased expression of TIGIT+PD-1+ CD8 T cells over the 8-week course of adjunctive metformin



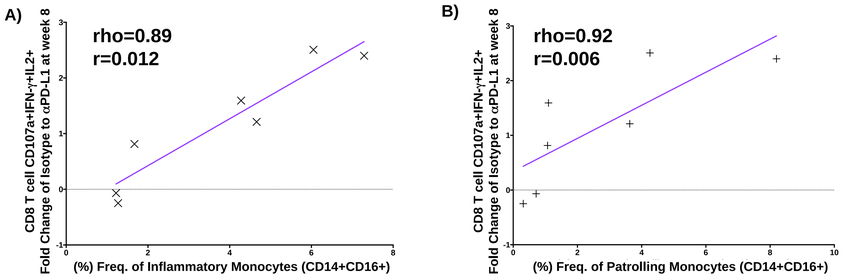
A) Representative flow cytometry plots showing the frequency of TIGIT and PD-1 expressing CD8 T cells. B) Graph shows changes in TIGIT+PD-1+ CD8 T cells over the 8-week course of metformin. (*n = 7)*

Figure 4. Metformin therapy increased the rejuvenated polyfunctional anti-HIV CD8 T cell responses to PD-L1 blockade *ex vivo*



Graphs represent fold changes in polyfunctional anti-HIV CD8 T cell responses with PD-L1 blockade over the course of 8 weeks of metformin therapy (*n = 7*)

Figure 5. Baseline monocyte population frequencies correlate with the anti-HIV functional improvements at week 8 of adjunctive metformin therapy with PD-L1 blockade



Graphs represent the association between A) inflammatory monocytes and B) patrolling monocytes with week 8 fold changes in polyfunctional anti-HIV CD8 T cell responses with PD-L1 blockade (*n = 7*)

Summary

- **The 8-week course of adjunctive metformin increased the central memory population of CD8 T cells**
- **Among 7 virally suppressed euglycemic HIV infected adults, a 8-week course of adjunctive metformin did not improve anti-HIV CD8 T cell function *ex vivo***
- **Observed significant increase of TIGIT+PD-1+ CD8 T cells with metformin therapy**
- **Metformin increased the *ex vivo* immunotherapeutic reinvigoration of polyfunctional anti-HIV CD8 T cells to PD-L1 blockade over the 8-week adjunctive therapy**
- **Baseline subset monocyte frequencies associated with the magnitude of anti-PD-L1 polyfunctional anti-HIV CD8 T cell restoration *ex vivo***

Conclusion

Metformin in combination with immune checkpoint blockade may improve polyfunctional HIV-specific CD8 T cell effector responses. The presence of certain monocyte subsets correlated with favorable restored CD8 T cell responses, suggesting that monocytes may play an important role in immune checkpoint efficacy. Metformin may have additional value as an adjunctive therapy in "Shock and Kill" HIV remission studies.

Further study of metformin in a randomized placebo controlled study is warranted

Acknowledgements

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