Zaprinast Reprogrammes Resting CD4+ T Cell Metabolism and Induces HIV Latency Reversal ex vivo.

Valentin de Masson d'Autume[1], Valentin LeDouce[1], Sophie O'Reilly[1], Nathan François[1], Matthew Angieladis[1], Alejandro Abner Garcia Leon[1], Tara McGinty[2], Jose Carlos Valle Casuso[3], Alan Macken[2], Olivier Rohr[5], Carine Van Lint[6], Asier Sáez-Cirión[4], Aoife Cotter[2], Eoin R. Feeney[3], Mallon W. G. Paddy[1,2,3], Virginie Gautier[1]

[1]UCD Centre for Experimental Pathogen Host Research, School of Medicine, University College Dublin, Ireland; [2]Mater Misericordiae University Hospital, Dublin, Ireland;[3] St Vincent University Hospital, Dublin, Ireland; [4]Institut Pasteur, Unité Réservoirs viraux et contrôle immunitaire, Paris, France; [5]University of Strasbourg, EA7292, DHPI, Institute of Parasitology and Tropical Pathology, Strasbourg, France; [6]Université Libre de Bruxelles, Service of Molecular Virology, Institute for Molecular Biology and Medicine, Gosselies, Belgium

577

BACKGROUND

The silent HIV long-lived cellular reservoir (mainly CD4+ memory T cells) but replication-competent constitute the main obstacle to HIV cure. Current HIV latency reversal strategies aim to force viral replication in the presence of ART so reactivated reservoirs can be eliminated by boosting immune approaches.

Accumulating evidence supports that the establishment and persistence of HIV latent reservoirs despite suppressive ART are intricately linked to the quiescent metabolism of CD4+ memory T cells. Especially glycolysis and oxidative phosphorylation (OXPHOS), which produce the ATP, nucleotides and amino acids required.

Pyruvate, a glycolysis product, can either be transformed into lactate in the cytoplasm or enter mitochondria through the Mitochondrial Pyruvate Carrier (MPC) to fuel TCA and support OXPHOS. By controlling the fate of pyruvate with Mitochondrial Pyruvate Carrier inhibitor (MPCi), we aimed to reprogram resting CD4+ T cells toward a more glycolytic metabolism, which could support HIV reversal latency.

OBJECTIVES

- Primary screening of metabolic drug of interest for promoting HIV latency reversal.
- Examine the capacity of Zaprinast to revert HIV from latency on circulating HIV reservoirs from ART-suppressed individuals.
- Evaluate the impact of Zaprinast on primary CD4+ T cell metabolism.

METHODS

HIV latency reversal was performed on CD4+ T cells infected *in vitro*. Total HIV gene expression was measured by targeting 3' end of viral transcripts.

HIV latency reversal was performed *ex vivo* on CD4+ T cells or CD8-depleted PBMC from ART-suppressed individuals. HIV gene expression and viral production were measured by RT-qPCR targeting the HIV *tat-rev* msRNA or HIV *gag* RNA, respectively.

Metabolic analysis was performed on CD4+ T cells isolated by negative selection (Stemcell). Mitochondrial respiration and ATP production were assessed using the Mitostress test and ATP real-time kits (Seahorse, Agilent). Aerobic glycolytic activity was measured using pH Xtra assay (Agilent) and glucose uptake with 2-NBDG. Flow cytometry was used to evaluate mitochondrial mass (MitoView Green, Generon), membrane potential (MitoTracker Red CMXRos, Invitrogen), ROS production (MitoSOX Red, Thermofisher), viability (Viobility 405/452, Miltenyi) and activation (CD69 and CD25 antibodies, Biolegend).

Study Participants Characteristics	Cell-associated HIV RNA N = 21[1]	HIV Viral load N = 17[1]
Age (Years)	39.6 (35.2, 41.7)	41.4 (37.5, 46.3)
Sex at birth		
F	3 / 21 (14%)	3 / 17 (18%)
M	18 / 21 (86%)	14 / 17 (82%)
CD4+ T cell count	625.5 (499.5, 765.5)	670.5 (468.0, 838.0)
Missing	1	1
CD4/CD8 ratio	1.1 (0.8, 1.6)	0.7 (0.6, 1.2)
Missing	1	1
Integrated Pro Viral Load	69.9 (27.6, 174.3)	45.0 (4.0, 230.0)
Missing	8	0
cART Started in	2019 (2018, 2021)	2,015.0 (2,013.0, 2,016.0)
Missing	4	1
ART Regiment		
Atripla	0 / 17 (0%)	6 / 16 (38%)
Biktarvy	4 / 17 (24%)	0 / 16 (0%)
Delistrigo(Doravirine, Lamivudine, Tenofovir)	1 / 17 (5.9%)	0 / 16 (0%)
Dolutegravir/ Relipivirine	1 / 17 (5.9%)	0 / 16 (0%)
Dovato(Dolutegravir/ lamivudine)	1 / 17 (5.9%)	0 / 16 (0%)
Elvitegravir, Cobicistat, Emitrictabine, TAF	1 / 17 (5.9%)	0 / 16 (0%)
Eviplera	2 / 17 (12%)	0 / 16 (0%)
Genvoya	0 / 17 (0%)	4 / 16 (25%)
Raltegravir_Darunavir_Ritonavir	0 / 17 (0%)	1 / 16 (6.3%)
Rezolsta_Dolutegravir	0 / 17 (0%)	1 / 16 (6.3%)
Striblid	1 / 17 (5.9%)	1 / 16 (6.3%)
TDF/FTC/ EFV	1 / 17 (5.9%)	0 / 16 (0%)
Triumeq	2 / 17 (12%)	2 / 16 (13%)
Truvada_Dolutegravir	3 / 17 (18%)	1 / 16 (6.3%)
Missing	4	1
NADIR	574.5 (359.0, 609.0)	127.5 (49.5, 482.0)
Missing	5	1
Year of Nadir	2020 (2019, 2022)	2011 (2008, 2014)
Missing	5	1

[1] Median (Q1, Q3); n / N (%)

Table 1: Study participant characteristics

Mitochondrial Pyruvate Carrier inhibitors are a **potent new class** of **Latency Reversing Agents** (**LRAs**) capable of inducing **viral particle release** from **circulating viral reservoirs ex vivo** by **shaping CD4+ T** cell **metabolic profile**.

RESULTS

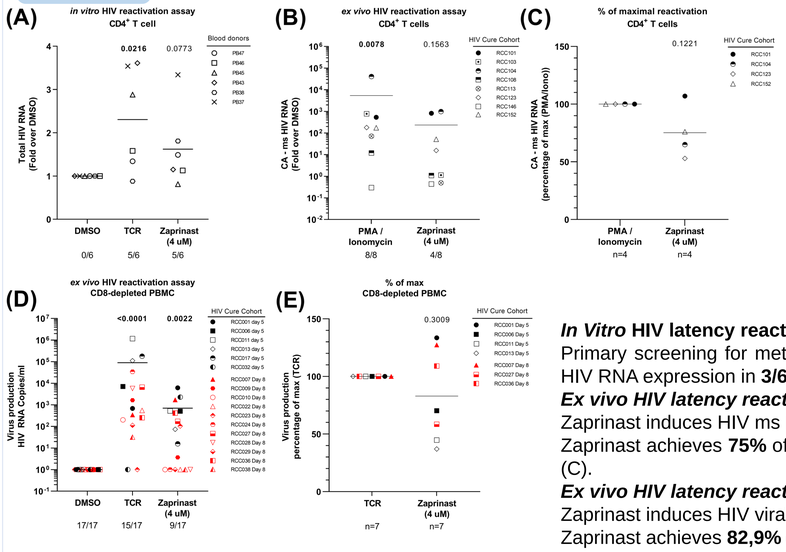


Figure 1: Zaprinast induces HIV latency reversal *in vitro* and *ex vivo*.

Total HIV-1 RNA expression in primary CD4+ T cells latently infected with HIV-1 *in vitro* after 24-hour treatment with DMSO (control), 4 µM Zaprinast or anti-CD3/CD28 antibodies (TCR). Statistical analysis: Wilcoxon test (A). Cell-Associated (CA) HIV-1 multiply spliced (ms) RNA expression in CD4+ T cells from ART-suppressed individuals after 72 hours of treatment with DMSO, 4 µM Zaprinast or 20nM PMA and 280 nM Ionomycin (PMA/Ionomycin). Statistical analysis: Wilcoxon test (B). CA HIV-1 msRNA expression as a percentage of maximal HIV reactivation (PMA/Ionomycin). Statistical: paired t-test (C). HIV-1 viral loads in CD8-depleted PBMCs from ART-suppressed individuals after 5 or 8 days of DMSO (control), anti-CD3/CD28 antibodies (TCR) or 4µM Zaprinast. Statistical analysis was performed using paired t-test (D). Viral loads expressed as a percentage of TCR stimulation. Statistical analysis: paired t-test (E).

***In Vitro* HIV latency reactivation assay (Planelles model):**
Primary screening for metabolic drugs identified Zaprinast to induce Total HIV RNA expression in **3/6** samples (**50%**) (A).

***Ex vivo HIV latency reactivation assay (CD4+ T cells)*:**
Zaprinast induces HIV ms RNA expression in **4/8** samples (**50%**) (B).
Zaprinast achieves **75%** of maximal HIV reactivation level (PMA-Ionomycin) (C).

***Ex vivo HIV latency reactivation assay (CD8-depleted PBMC)*:**
Zaprinast induces HIV viral particles production in **9/17** samples (**64%**) (D).
Zaprinast achieves **82,9%** of maximal HIV reactivation level (E).

Figure 2: Zaprinast reprogrammes resting CD4+ T cell metabolism towards a more active state.

Immediate effects of increasing Zaprinast concentrations on Maximal Respiratory Capacity and Spare Respiratory Capacity calculated from Oxygen Consumption Rate (OCR) measurements using Mitostress test (Agilent). Data shown from one representative experiment performed in triplicate. Statistical analysis: paired t-tests (A). Maximal Respiratory Capacity, Spare Respiratory Capacity and Basal Respiration of primary CD4+ T cells calculated from OCR measurements using Mitostress test (Agilent) after immediate or 18-hour treatment with DMSO or 4 µM Zaprinast. Statistical analysis: paired t-tests (C). Glucose uptake in primary CD4+ T cells after 18-hour treatment with 4 µM Zaprinast, measured using glucose analogue 2-NBDG (Invitrogen). Statistical analysis: paired t-tests (D). Extracellular Acidification Rate (ECAR) of primary CD4+ T cells following immediate or 18 hours treatment with DMSO or 4 µM Zaprinast treatment, measured using pH Xtra assay (Agilent) with a microplate reader (Molecular Device ID3) over a 2-hour kinetic. Statistical analysis: paired t-tests (E). MitoATP and GlycoATP production in primary CD4+ T cells following immediate or 18 hours treatment with 4 µM Zaprinast, measured using ATP real-time assay (Seahorse Analyser, Agilent). Statistical analysis: paired t-tests (F).

Zaprinast modulates mitochondrial respiration in CD4+T cells: a biphasic effect: Zaprinast immediately and dose-dependently reduced mitochondrial respiration (**MRC 36%**, **SRC 56%** at **4 µM**) (A)(B). An **18-hour** exposure led to moderate increases in **basal respiration** (**16%**), **MRC** (**28%**), and **SRC** (**28%**). Even greater after the removal of Zaprinast, with **basal respiration** increasing by **41%**, **MRC** by **83%**, and **SRC by 89%** (C). This increase could be the result of T cell metabolic plasticity, where cells compensate for reduced mitochondrial Pyruvate entry by using alternative carbon sources like glutamate or fatty acid (FA) to maintain TCA cycle function, as observed in previous studies

Zaprinast upregulates glycolysis and glucose uptake: Zaprinast inhibition of pyruvate uptake induces upregulates **Glucose** uptake (**2,04-fold**) (D), to support an immediate (**1.55-fold**), sustained (**1.41-fold**) and reversible upregulation of **ECAR** due to Lactate secretion(E).

Zaprinast modulates ATP production: Zaprinast inhibition of pyruvate uptake results in an immediate reduction of **mitoATP** production (**0.8-fold**), followed by an upregulation of **mitoATP** (**1.41-fold**) and **glycoATP** (1.32-fold) production after 18 hours (F).

RESULTS

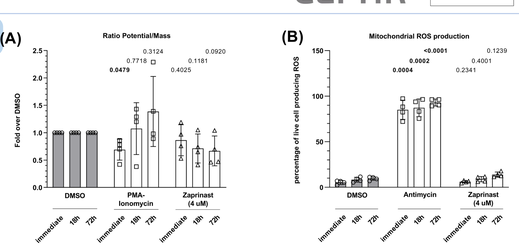


Figure 3: Zaprinast treatment moderately modulates mitochondrial membrane potential without inducing ROS production.

Normalised mitochondrial membrane potential relative to mitochondrial mass and DMSO treatment for each time point (n=4). Statistical analysis: paired t-tests (A). Mitochondrial Reactive Oxygen Species (ROS) production in CD4+ T cells measured immediately, 18-hours and 72-hours after treatment with DMSO or 4 µM Zaprinast (n=4). Statistical analysis: paired t-tests (B). Mean Fluorescence Intensity (MFI) for all measurements was determined using flow cytometry with Cytoflex S (Beckman Coulter).

CONCLUSIONS

Zaprinast treatment blocks pyruvate entry in the mitochondria and redirects pyruvate towards lactate formation and secretion. After 18 hours of treatment, Zaprinast induces an increase in overall metabolic activity, including enhanced glycolysis, elevated mitochondrial respiration, and increased ATP production, supported by increased glucose uptake. This metabolic reprogramming ultimately leads to HIV-1 latency reversal evidenced by the production of HIV-1 RNA and viral particles.

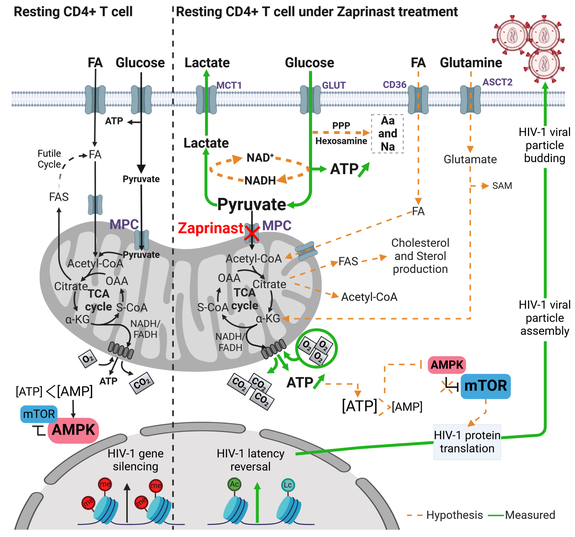


Figure 4: Zaprinast-induced metabolic reprogramming of resting CD4+ T cells promotes HIV latency reversal.

Graphical representation of the metabolic changes in resting CD4+ T cells without treatment (left) and after Zaprinast treatment (right). In the basal state, resting CD4+ T cells primarily rely on oxidative phosphorylation (OXPHOS) for ATP production, with naïve cells utilising glycolysis to fuel the tricarboxylic acid (TCA) cycle and memory cells employing both glycolysis and fatty acid (FA) oxydation. Green arrows indicate the observed effects of Zaprinast , while dashed orange arrows represent potential effects based on the literature review. Abbreviation: FA (Fatty acid), FAS (Fatty Acid Synthesis), TCA (Tricarboxylic Acid Cycle), OAA (Oxaloacetate), S-CoA (Succinyl-CoA), α-KG (α-Ketoglutaric acid), Aa (Amino Acid), Na (Nucleic Acid), me (methyl group), Ac (acetyl group), Lc (Lactyl group), SAM (S-Adenosyl methionine), PPP (Pentose Phosphate Pathway).

ADDITIONAL INFORMATION

Statistical analysis was performed on GraphPad. P-values <0,05 are in bold.

We thank Health Research Board for the support of this study (No 691119 — EU4HIVCURE — H2020-MSCA-RISE-2015), all participants of HIV CURE MMUH Cohort (IE) for the PLWH samples, and blood donors from Irish Blood Transfusion Services (St James Hospital).

Contact:
Associate Professor Virginie Gautier: virginiegautier@ucd.ie