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Impairment of HIV proviral reactivation by interfering with essential metabolic pathways in effector memory CD4+ T cells

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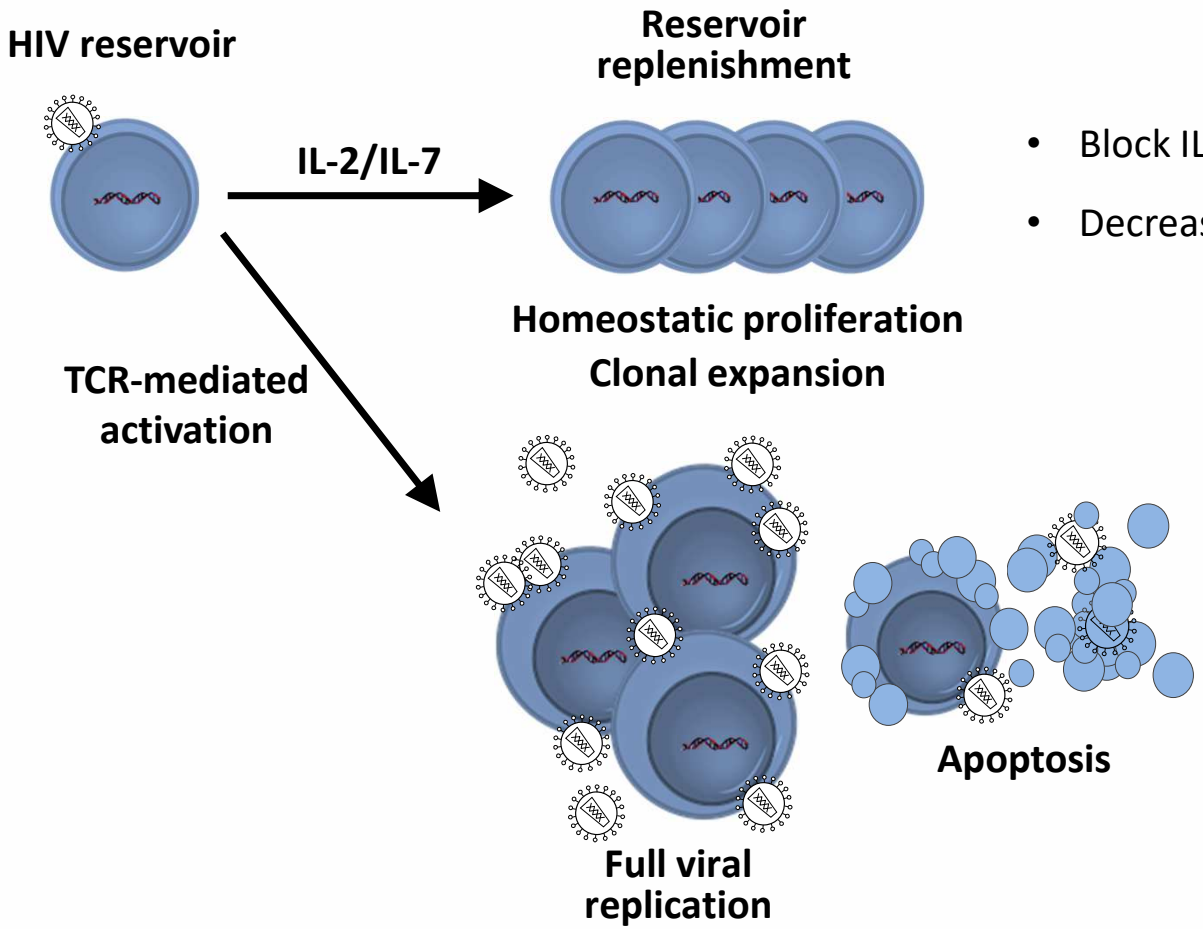
CONFLICTS OF INTEREST

I have no conflicts of interest to disclose

Potential ways to interfere with HIV reservoir

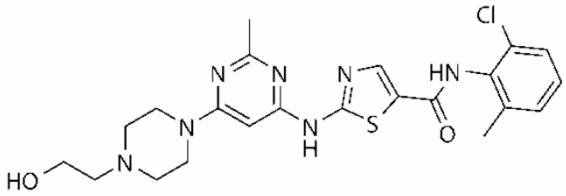


- Block IL-7-mediated homeostasis
- Decrease effector T cells (TEM, TEMRA)

- Interfere with TCR-mediated provirus reactivation
- Induce provirus reactivation and enhance cytotoxic activity against the reservoir

Tyrosine kinase inhibitors (TKIs) as adjuvants of ART

Dasatinib (BMS)



	Dasatinib
IC50	16.3 nM (8.26 ng/ml)
Cmax	41.52 ng/ml (50 mg once in healthy adults)
IC50/Cmax	0.2
Selectivity index	> 612

Estimated incidence of HIV-1 infection and CML 1:65,000

	Individuals HIV+ with CML on ART+dasatinib
Individuals, n	3
Male/female, n	3/0
Median age at HIV diagnosis (years)	33.0 (IQR 25.0 to 37.0)
Median age at CML diagnosis (years)	42.0 (IQR 37.0 to 58.0)
Median CD4/CD8 ratio	0.3 (IQR 0.11 to 1.6)
Median CD4 count (cells/milliliter)	786.0 (IQR 178 to 1014)
ART (n)	2 NRTI, 1 INI (2) 1 INI, 1 PI/c (1)
Time of treatment with dasatinib (years)	2.3 (IQR 2.3 to 5.3)

Bermejo et al., Biochemical Pharmacology 156 (2018) 248–264

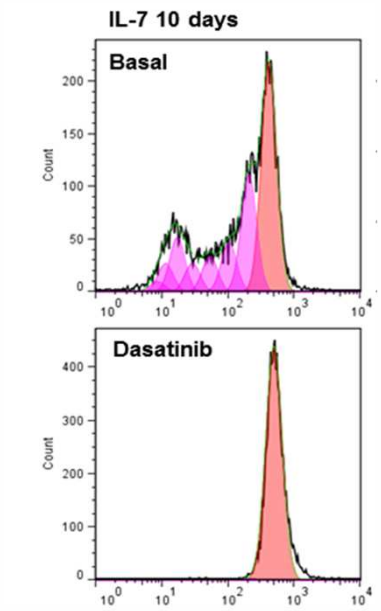
Dasatinib reduces T cell proliferation mediated by IL-7

CD4+ T cells from healthy donors

10 days

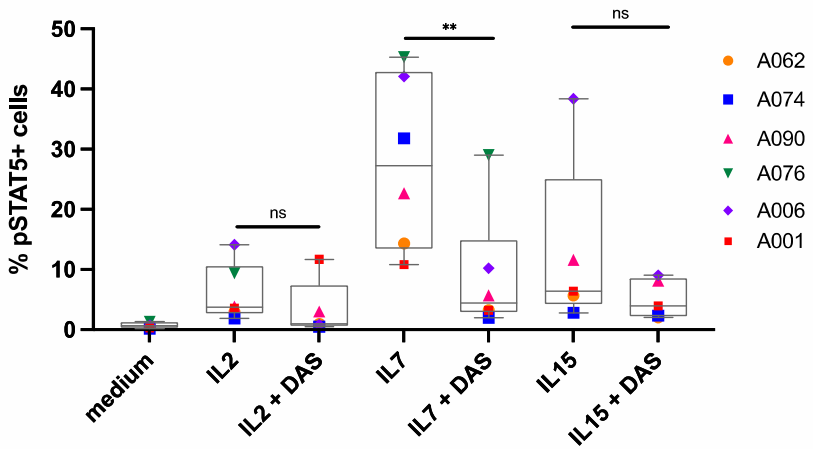
Cell proliferation by flow cytometry

+ Dasatinib + IL-7 **+ CFSE**



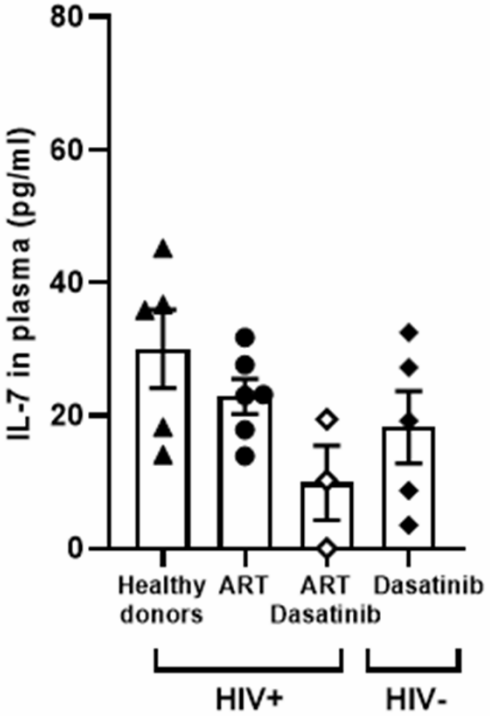
Bermejo et al., Biochem Pharmacol. 2018 Oct;156:248-264

Dasatinib restricts IL-7 induced proliferation through STAT5 phosphorylation inhibition



Innis et al., Biochem Pharmacol. 2021 Oct 26:114816

IL-7 plasma levels are reduced in individuals HIV+ on ART+dasatinib



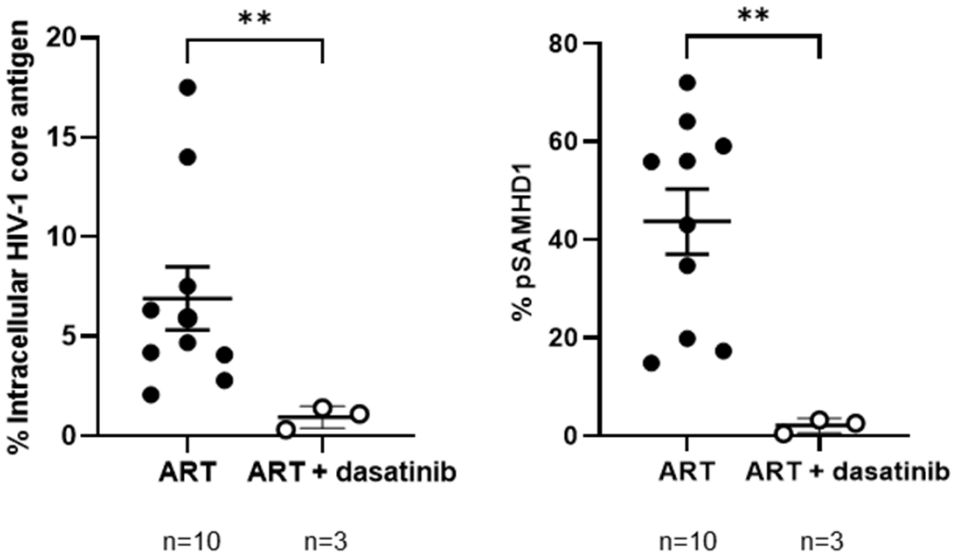
Vigón et al., Biochemical Pharmacology 192 (2021) 114666

BACKGROUND

HIV reservoir of individuals on ART and dasatinib show impaired proviral reactivation

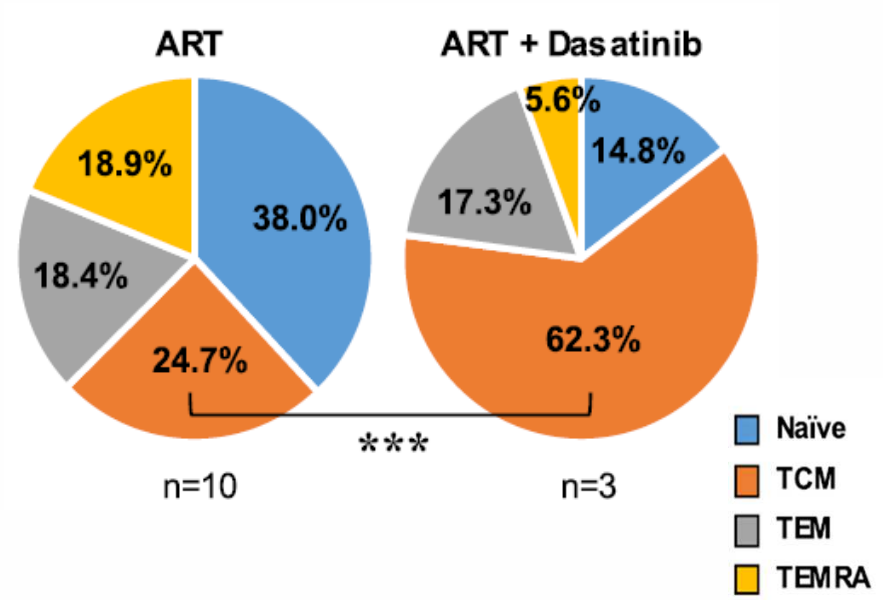
CD4+ T cells isolated from HIV+ individuals on ART + dasatinib

CD3/CD28/IL-2 → 7 days → **Intracellular measurement of p24 (kc57)**



Vigón et al., Biochemical Pharmacology 192 (2021) 114666

Dasatinib reduces the level of CD4 TEM and TEMRA in HIV+ individuals on ART + dasatinib

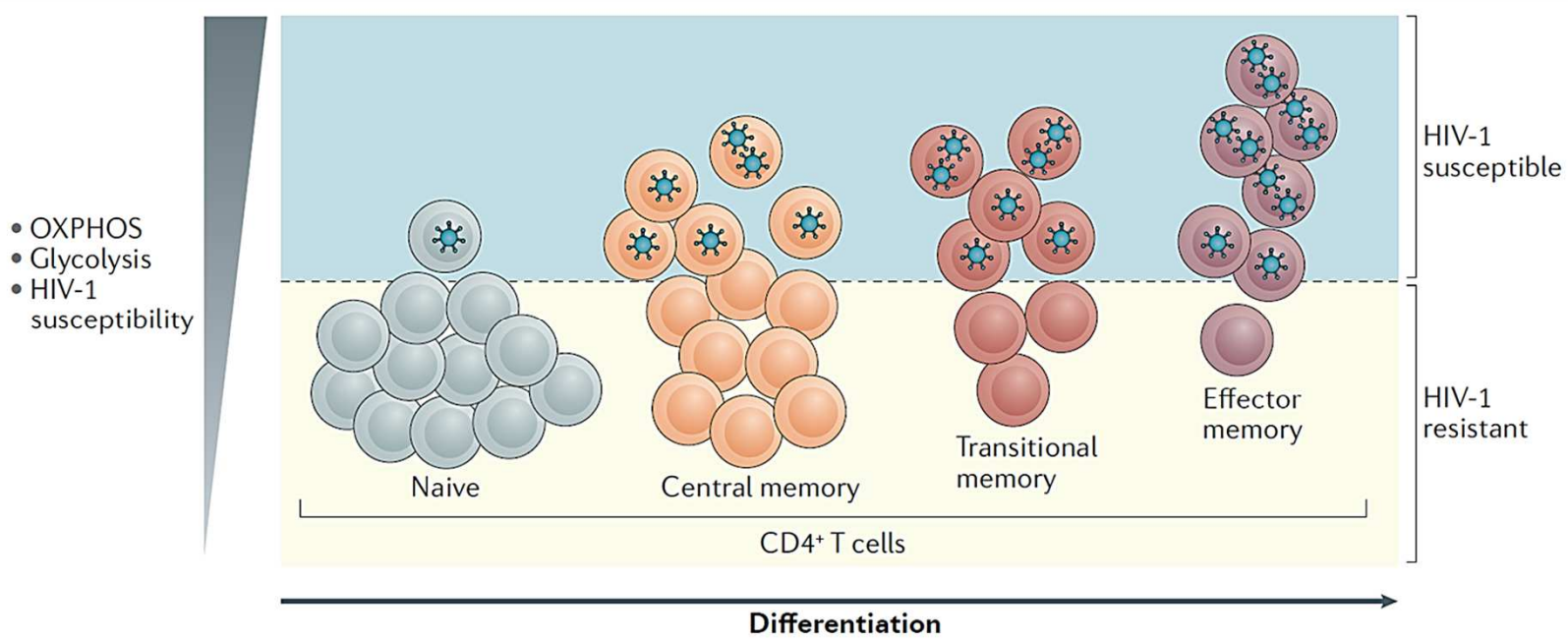


Vigón et al., Biochemical Pharmacology 192 (2021) 114666

BACKGROUND

HIV-1 selectively infects CD4+ T cells with enhanced glycolysis and OXPHOS

- The susceptibility of CD4+ T cell subsets to HIV-1 matches their metabolic activity
- Inhibition of glycolysis impairs HIV-1 replication

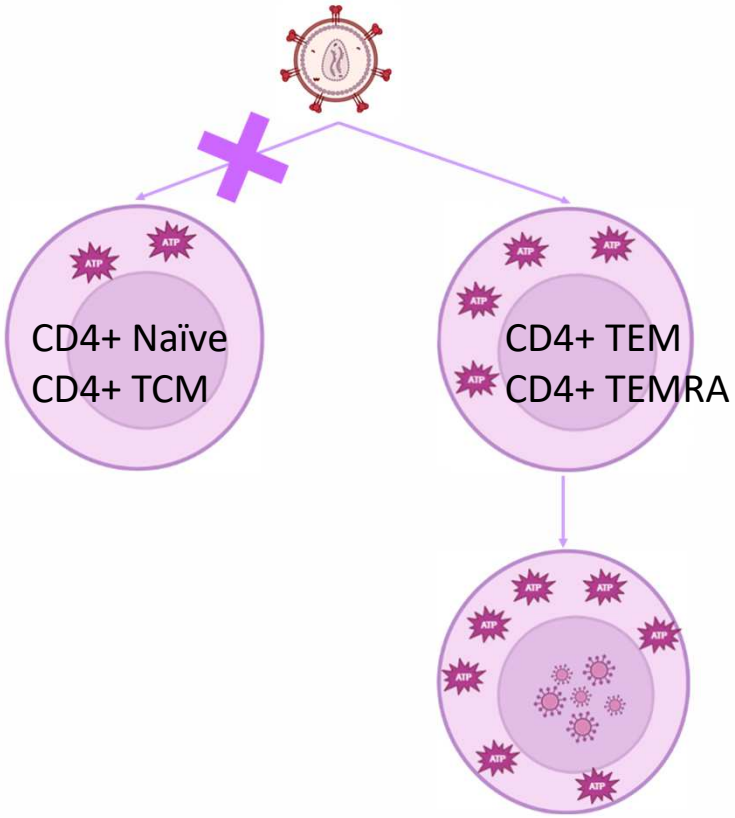


Sáez-Cirión & Sereti. Nat Rev Immunol 21, 5–19 (2021).

BACKGROUND

CD4 TEM and TEMRA show the highest metabolic activity

- CD4 TEM and TEMRA are responsible for the reservoir replenishment and have the highest metabolic activity in comparison with naïve and TCM
- ART does not reverse metabolic reprogramming
- Metabolic reprogramming is the main driver of initial immune activation that ends up leading to exhaustion of the immune system
- Metabolic reprogramming is a new target for drug development against HIV



Chronic inflammation
Comorbidities

OBJECTIVES

To determine the effect of dasatinib on the metabolic activity of PBMCs

To evaluate if dasatinib may induce metabolic reprogramming of CD4 TEM and TEMRA cells, reducing their glycolytic activity to interfere with the replenishment of HIV reservoir

RESULTS

Dasatinib modified the phosphorylation of >130 proteins involved in metabolic pathways

Analysis of phosphoproteome by liquid chromatography followed by tandem mass spectrometry on Orbitrap

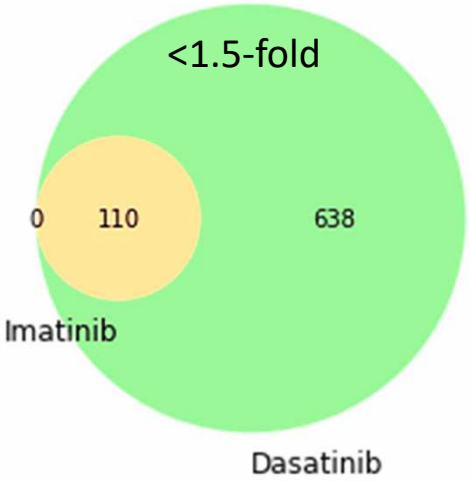
PBMCs from healthy donors

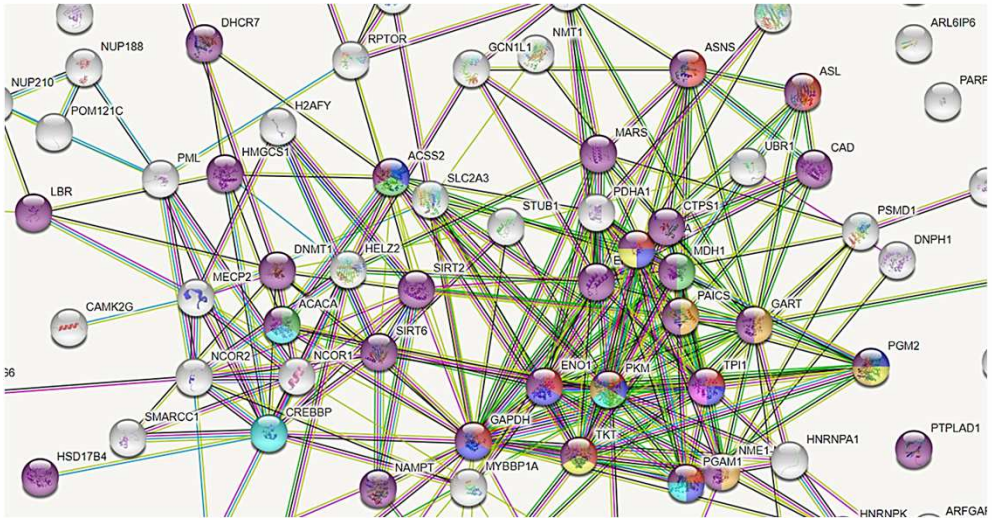
48h → **Phosphoproteome**

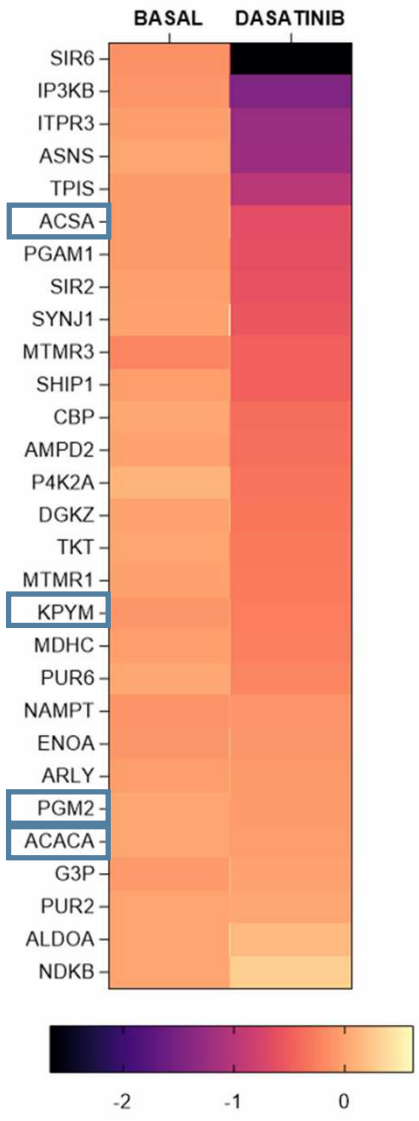
1. PHA/IL-2
2. Dasatinib 75 nM + PHA/IL-2

Deregulated proteins

- Glycolysis / glyconeogenesis
- Pyruvate metabolism
- Pentose phosphate pathway
- Inositol phosphate metabolism
- Glucagon signaling pathway







RESULTS

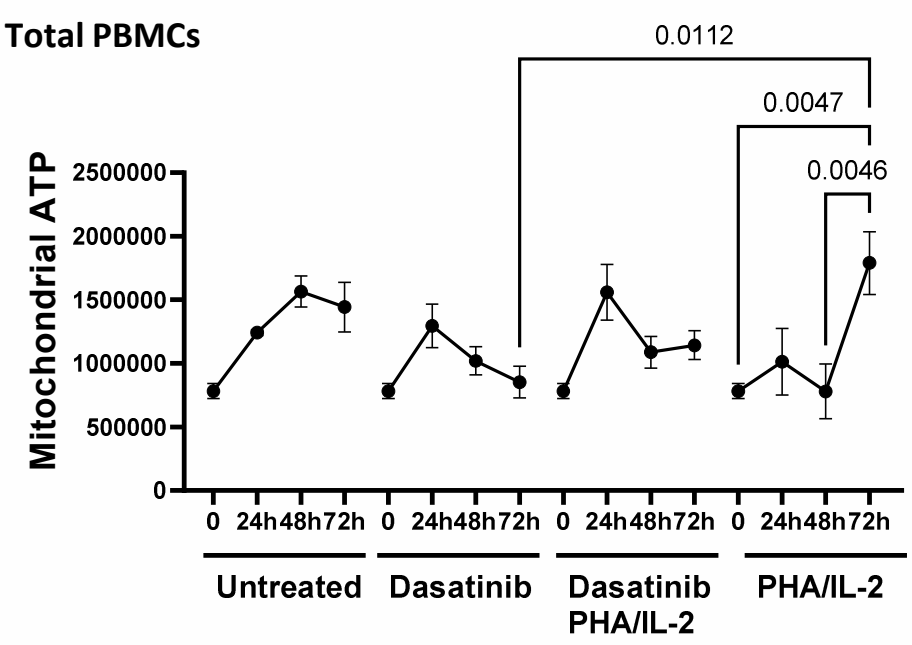
Dasatinib interferes with the metabolic activity of PBMCs

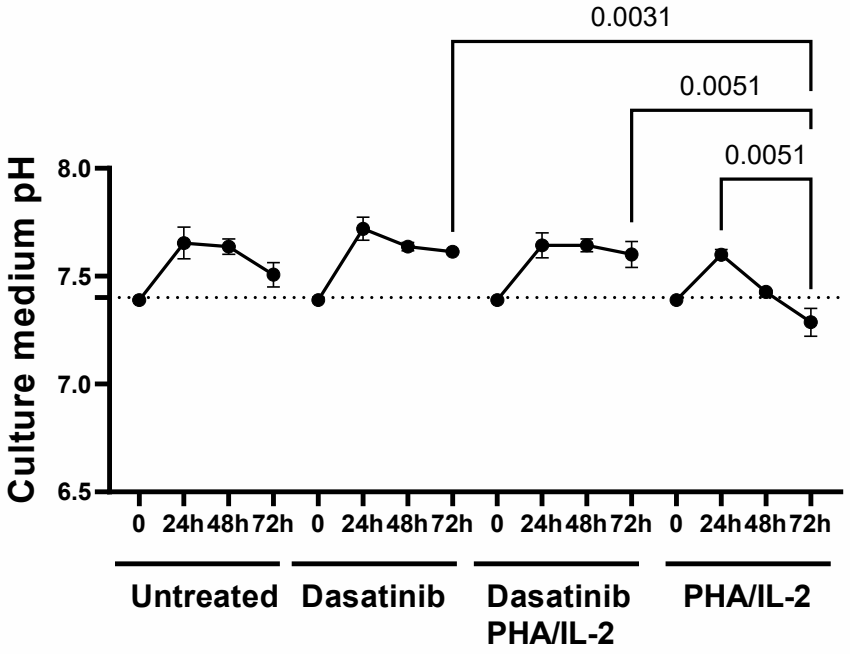
PBMCs from healthy donors

1. Basal
2. Dasatinib 75 nM
3. PHA/IL-2
4. Dasatinib 75 nM + PHA/IL-2

24h 48h 72h

- **Mitochondrial ATP**
- **Culture medium pH**





RESULTS

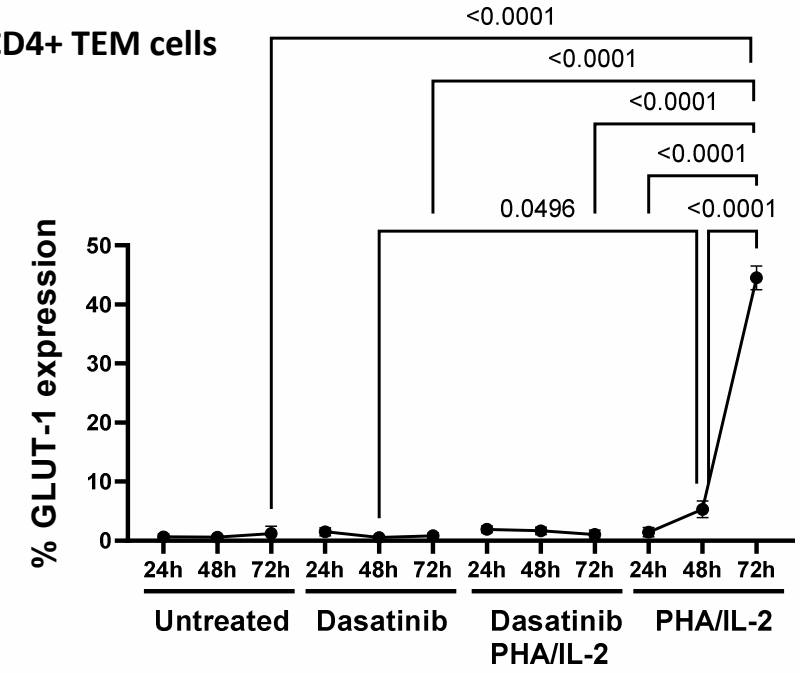
Dasatinib interferes with the expression of the glucose transporter GLUT-1 in CD4 TEM and TEMRA cells

PBMCs from healthy donors

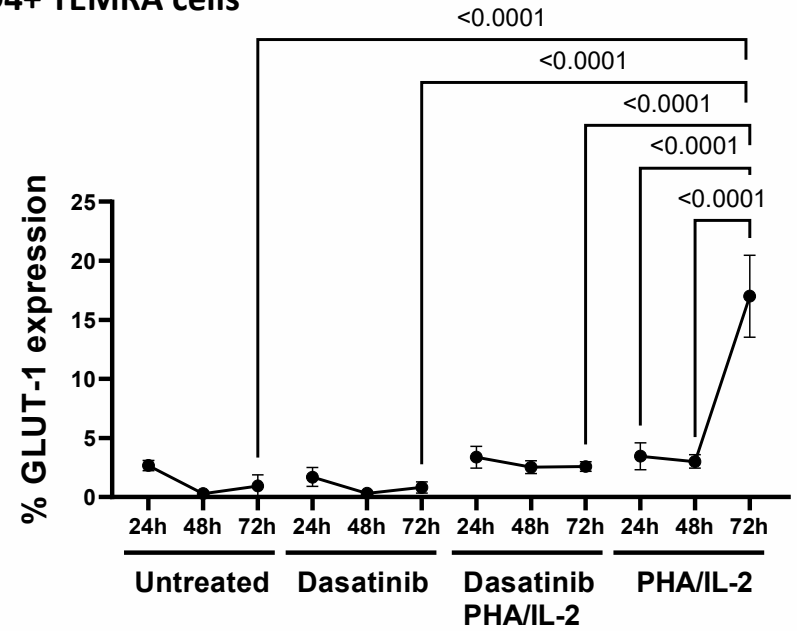
1. Basal
2. Dasatinib 75 nM
3. PHA/IL-2
4. Dasatinib 75 nM + PHA/IL-2

24h 48h 72h → **GLUT-1 expression on the cell surface**

CD4+ TEM cells



CD4+ TEMRA cells



RESULTS

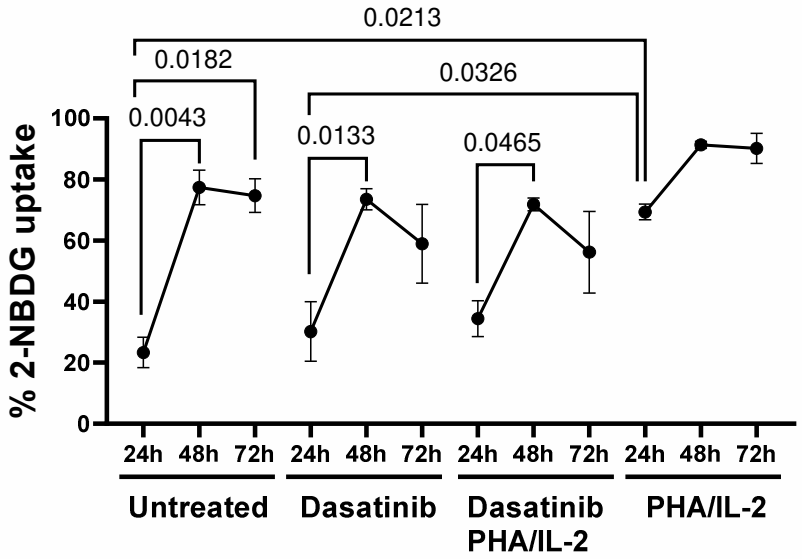
Dasatinib interferes with the glucose uptake in CD4 TEM and TEMRA cells

PBMCs from healthy donors

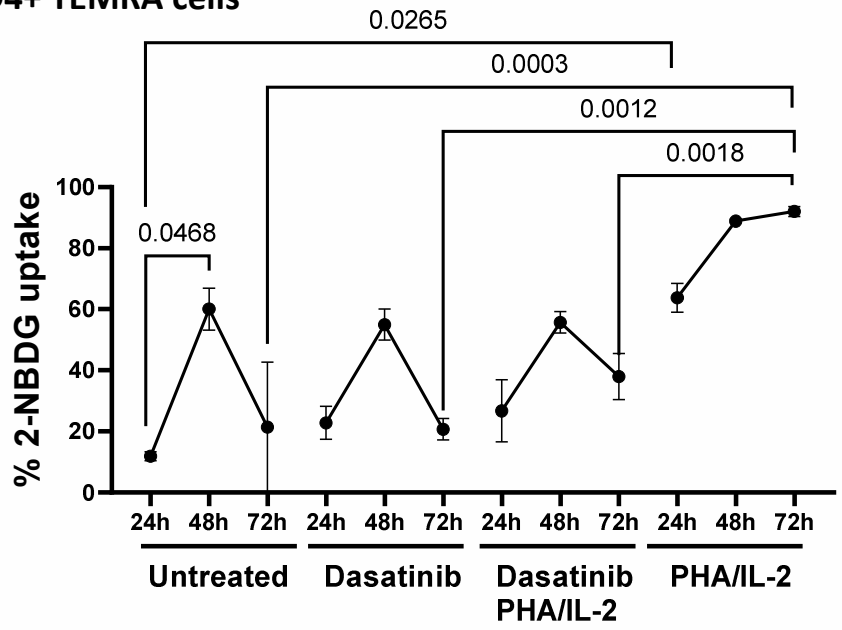
1. Basal
2. Dasatinib 75 nM
3. PHA/IL-2
4. Dasatinib 75 nM + PHA/IL-2

24h 48h 72h → **Uptake of fluorescent glucose analog 2-NBDG**

CD4+ TEM cells



CD4+ TEMRA cells



RESULTS

Dasatinib does not modify GLUT-1 expression in CD8 but it reduces the glucose uptake

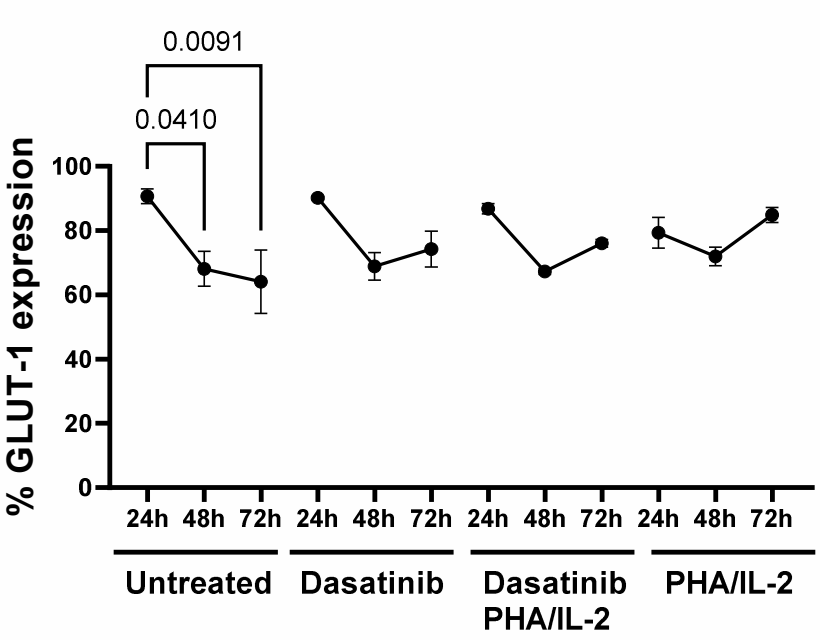
PBMCs from healthy donors

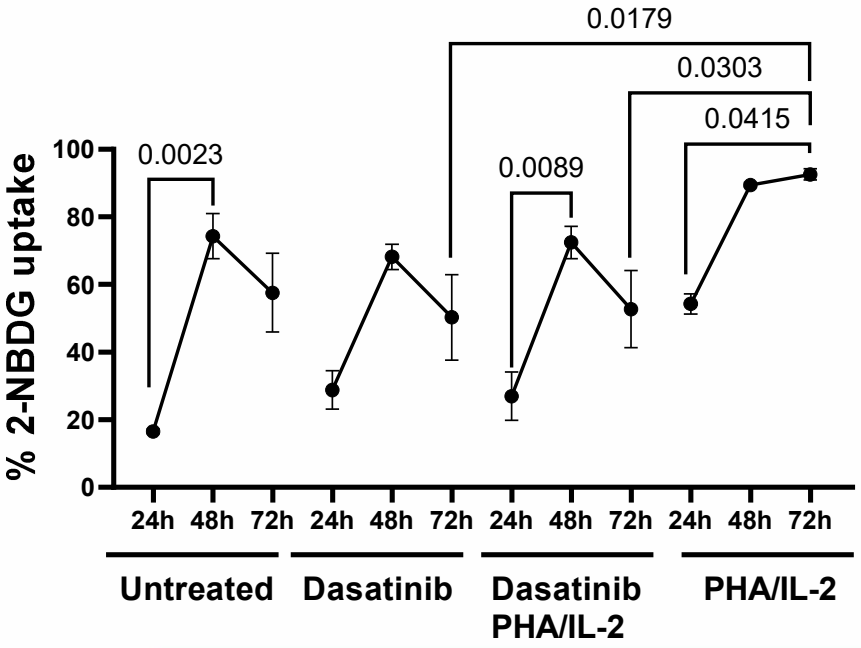
1. Basal
2. Dasatinib 75 nM
3. PHA/IL-2
4. Dasatinib 75 nM + PHA/IL-2

24h 48h 72h

- **Uptake of fluorescent glucose analog 2-NBDG**
- **GLUT-1 expression on the cell surface**

Total CD8+ T cells





RESULTS

Dasatinib does not modify GLUT-1 expression in NKs but it reduces the glucose uptake

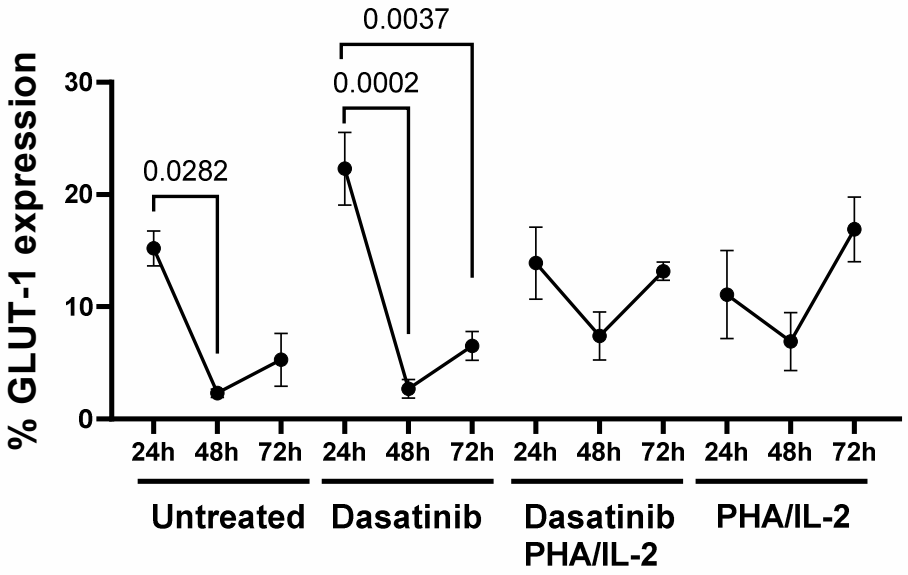
PBMCs from healthy donors

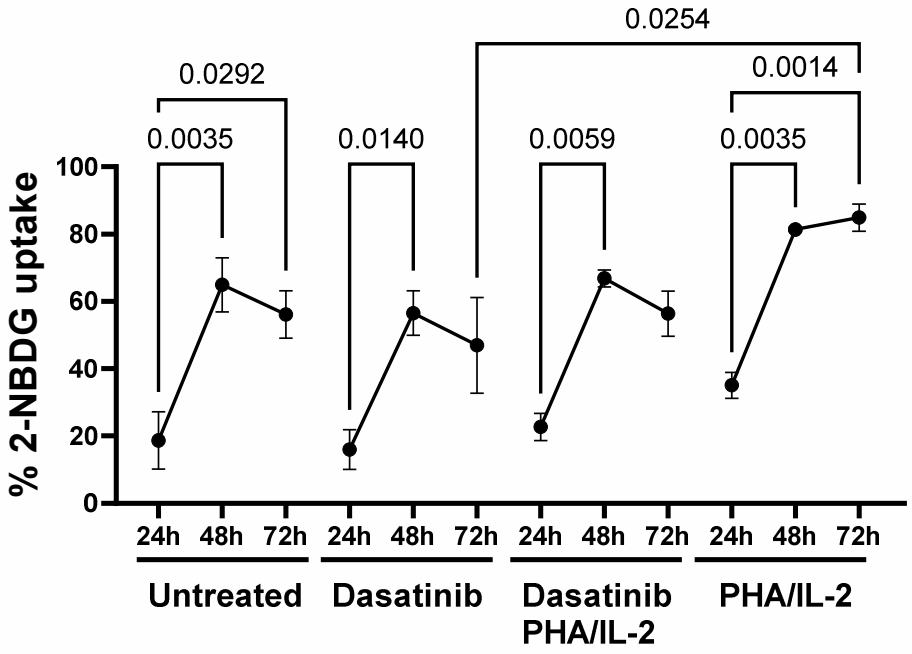
1. Basal
2. Dasatinib 75 nM
3. PHA/IL-2
4. Dasatinib 75 nM + PHA/IL-2

24h 48h 72h

- **Uptake of fluorescent glucose analog 2-NBDG**
- **GLUT-1 expression on the cell surface**

NK cells





CONCLUSIONS

- Treatment with dasatinib and ART is safe for individuals with HIV and CML
- Individuals HIV+ on ART and dasatinib show low levels of IL-7 in plasma and low reservoir size that is resistant to reactivation
- Dasatinib interferes with several metabolic pathways induced by activating stimuli in PBMCs, including glycolysis
- Dasatinib relegates viable CD4 cells to a resting state in which glucose uptake was impaired in both TEM and TEMRA subpopulations, acting as a metabolic immunomodulator
- Although glucose metabolism was partially affected in CD8 and NK, PLWH on treatment with ART and dasatinib do not present higher susceptibility to opportunistic infections or proviral reactivation
- Dasatinib may be used as a latency-promoting agent by metabolic reprogramming of CD4 and used along with ART may contribute to silence the viral reservoir as part of block & lock strategy

Key questions

May dasatinib act as a latency-promoting agent (LPA) and contribute to promote control of the viral reservoir in PLWH?
LPAs should act mainly on CD4 effector cells that may reactivate the latent provirus without affecting the antiviral activity of cytotoxic cells.

Key findings

Treatment with dasatinib induces metabolic reprogramming of CD4 TEM and TEMRA, interfering with essential metabolic pathways such as glycolysis. Both CD4 subpopulations are reduced in peripheral blood of PLWH on treatment with ART and dasatinib.

Next steps

The potential of dasatinib to induce metabolic reprogramming of CD4 effector cells will be analyzed in PBMCs from PLWH, as well as the effect on cytotoxic cells with capacity to control and eliminate the reservoir.

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