CDK INHIBITORS IMPACT HIV-1 SUSCEPTIBILITY BY MODULATING *IN VIVO* IMMUNOLOGIC RESPONSE

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BACKGROUND

Cyclin-dependent kinase inhibitors (CDKi) are currently used for the treatment of metastatic breast cancer (BC) with well-described effects on viral replication and immune cell responses *in vitro*. However, *in vivo* evidence from treated patients is limited. Here, using a longitudinal cohort of CDKi-treated BC patients, we determined treatment impact on HIV-1 susceptibility and immune response to define its potential as anti-HIV agents.

CLINICAL CHARACTERISTICS OF METASTATIC BREAST CANCER PATIENTS

Table 1. Baseline clinical characteristics of study participants

Variable	Whole cohort (n=40)	Infection and FACS analysis (n=13)
Age (y), mean	63.18	61.70
Type of therapy, n (%)		
Palbociclib	29 (72.5)	10 (76.92)
Ribociclib	7 (17.5)	3 (23.08)
Abemaciclib	4(10)	0
Line of therapy , n (%)		
First	21 (52.5)	9 (69.23)
Second	7 (17.5)	2 (15.39)
≥ Third	12 (30)	2 (15.4)
Site of metastasis, n (%)		
Visceral	54 (135)	10 (76.92)
Bone	34 (85)	11 (84.6)
Other	9 (22.5)	1 (7.69)

This study included 40 samples of metastatic breast cancer patients (n=40) treated with the CDKi palbociclib, ribociclib or abemaciclib. Plasma and PBMCs were longitudinally collected before and after treatment initiation. The study protocol was approved by the institutional review board of Hospital Germans Trias i Pujol. All participants provided written informed consent.

RESULTS

CDKi TREATMENT IMPACT ON T CELL SUBSETS AND IMMUNE CELL FUNCTION

A

Multicolor flow cytometry panel	
Immune cell subsets	**T-cell immune function**
B-cells	CTL4+
T-cell CD8+	CD25+
T-cell CD4+	PD1+
NKs	FOXP3+
Monocytes	CD19+

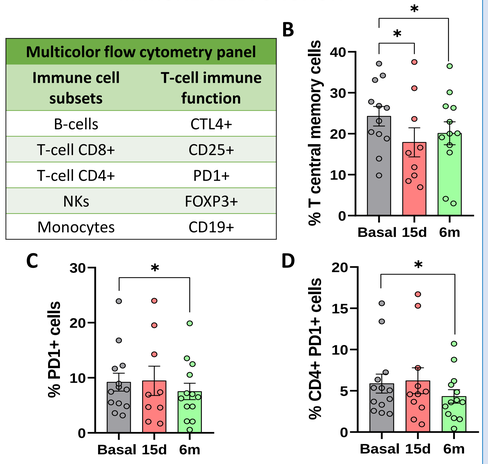


Fig. 1. Longitudinal characterization of immunophenotypic changes in PBMCs after CDKi treatment. (A) PBMCs subsets and T-cell immune function was evaluated by multicolor flow cytometry in BC patients treated with CDKi longitudinally collected before and after treatment initiation. No significant changes were observed after CDKi treatment, except for reduced proportion of CD4+ T-central memory cells 15 days and 6 months after treatment initiation (B) and decreased expression of the immune checkpoint PD1+ after 6 months (C, D). *p<0.05

PALBOCICLIB, RIBOCICLIB AND ABEMACICLIB IMPAIRED HIV-1 INFECTION *IN VITRO* AND *EX VIVO*

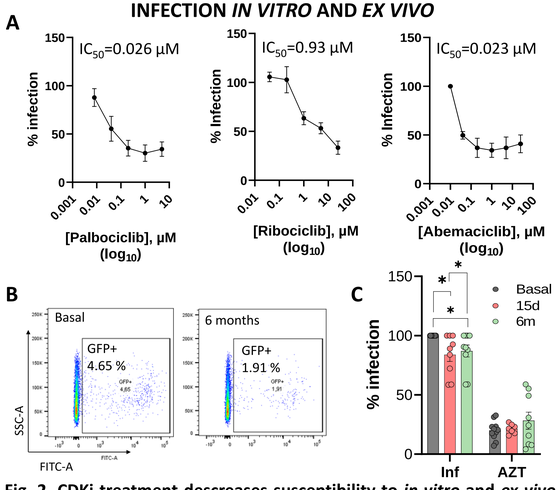


Fig. 2. CDKi treatment descreases susceptibility to *in vitro* and *ex vivo* HIV infection. (A) Percentage of HIV-1 infection (% GFP) in primary monocyte-derived macrophages from healthy donors *in vitro* treated with different concentrations of palbociclib, ribociclib or abemaciclib. (B) Flow cytometry plots of HIV-1 infection in stimulated PBMCs from CDKi treated BC pattents *ex vivo* infected with a GFP-expressing HIV-1. Infection was measured 48h after infection. A representative sample is shown. (C) *Ex vivo* susceptibility to HIV infection was impaired 15 days and 6 months after treatment initiation. AZT (retro-transcriptase inhibitor) was used as control. *p<0.05

TREATMENT WITH CDKi MODULATES PLASMA INFLAMMATION MARKERS *IN VIVO*

A

MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel - Premixed 29 Plex©			
EGF	IL-2	IL-12 (p40)	MIP-1β
G-CSF	IL-3	IL-12 (p70)	TNF-α
GM-CSF	IL-4	IL-13	TNF-β
IFN-α2	IL-5	IL-15	VEGF
IL-1α	IL-6	IL-17A	Eotaxin
IFN-γ	IL-7	IP10	-
IL-1β	IL-8	MCP-1	-
IL-1ra	IL-10	MIP-1α	-

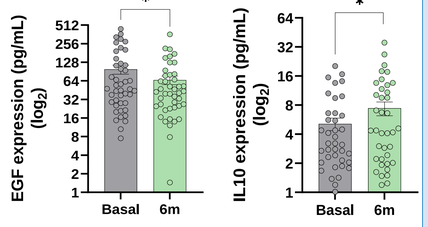


Fig. 3. Evaluation of plasma inflammation markers expression. (A) Plasma samples from CDKi treated patients were evaluated with HCYTMAG-60K-PX29 Milliplex©. (B) No significant changes in plasma cytokine expression was observed, except for significantly less EGF and increased IL-10. *p<0.05

CD4+ T-CENTRAL MEMORY CELLS POSITIVELY CORRELATE WITH HIV-1 INFECTION

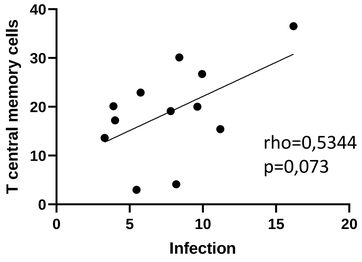


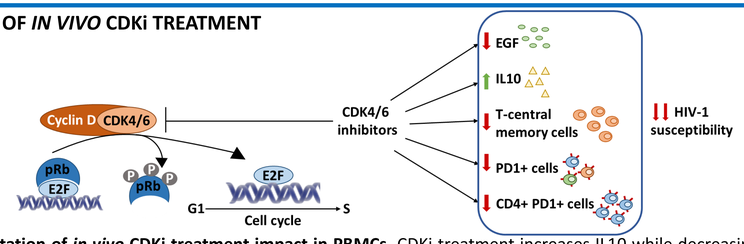
Fig. 4. Correlation between T central memory cells and infection. Percentage of CD4+ T-central memory cells positively correlated with HIV-1 infection, demonstrating the direct impact of *in vivo* CDKi treatment on CD4+ T-cell populations and HIV-1 susceptibility.

CONCLUSIONS

- *In vivo* treatment with CDKi decreases PD1+ and T-central memory cells (the main source of virus reservoir), suggesting improved immune cell function in the absence of significant changes in T cell activation.
- All CDKi inhibited *in vitro* HIV-1 infection in MDMs from healthy controls. Susceptibility to *ex vivo* HIV infection was impaired in CDKi-treated compared to untreated samples.
- Plasma cytokine expression showed a modulation of antiviral immune response 6 months after treatment initiation, presenting significantly less EGF and increased IL-10.

CDKi may represent a putative therapeutic strategy against HIV.

IMPACT OF *IN VIVO* CDKi TREATMENT



Representation of *in vivo* CDKi treatment impact in PBMCs. CDKi treatment increases IL10 while decreasing EGF, T-central memory cells subset and the immunecheckpoint PD1+ expression in cells, leading to decreased *ex vivo* HIV-1 susceptibility

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