Antiretroviral Drug Efflux Transporters and Metabolic Enzymes in Circulating Monocytes and Monocyte-Derived Macrophages of ART Treated People Living with HIV

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BACKGROUND

- Drug transporters and metabolic enzymes govern drug disposition and could render antiretroviral drug (ARV) intracellular concentrations suboptimal, thus facilitating HIV infection and HIV reservoir persistence in target cells, such as myeloid cells.[1]

- We investigated the expression of these transporters and metabolic enzymes in monocyte subsets and monocyte-derived macrophages (MDMs) of people living with HIV receiving viral suppressive antiretroviral therapy (HIV+ART) and HIV-uninfected individuals (HIV-). Plasma and intracellular ARV concentrations were quantified in HIV+ART cells.

HYPOTHESIS

Drug transporters and metabolic enzymes are expressed in monocytes and MDMs and could contribute to low drug penetration in these cell types that are known reservoirs of HIV-1.

HIV Reservoirs and Sanctuary Sites

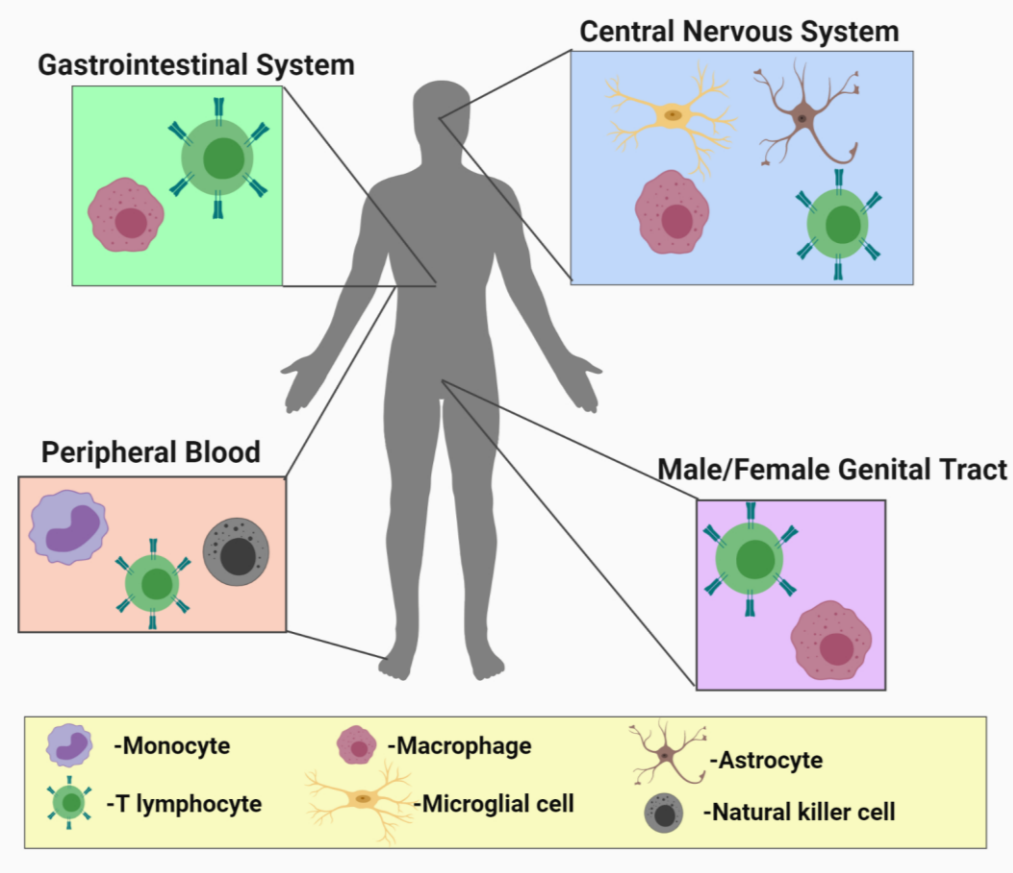

Figure 1. Illustration of several proposed cellular and anatomic HIV reservoirs. Adapted[1]

1. Whyte-Allman and Bendayan (2020). HIV-1 Sanctuary Sites—the Role of Membrane-Associated Drug Transporters and Drug Metabolic Enzymes. AAPS J, 22 (118).

METHODS

- Total monocytes were isolated from PBMCs using magnetic beads, and differentiated into MDMs by six days culture in the presence of macrophage colony-stimulating factor.
- mRNA and protein expression of drug transporters and metabolic enzymes were analyzed by qPCR and flow cytometry, respectively.
- Plasma and intracellular (PBMCs, monocytes, MDM) ARV concentrations were quantified by LC-MS/MS analysis.

mRNA expression of drug transporters and metabolic enzymes in monocytes and MDMs

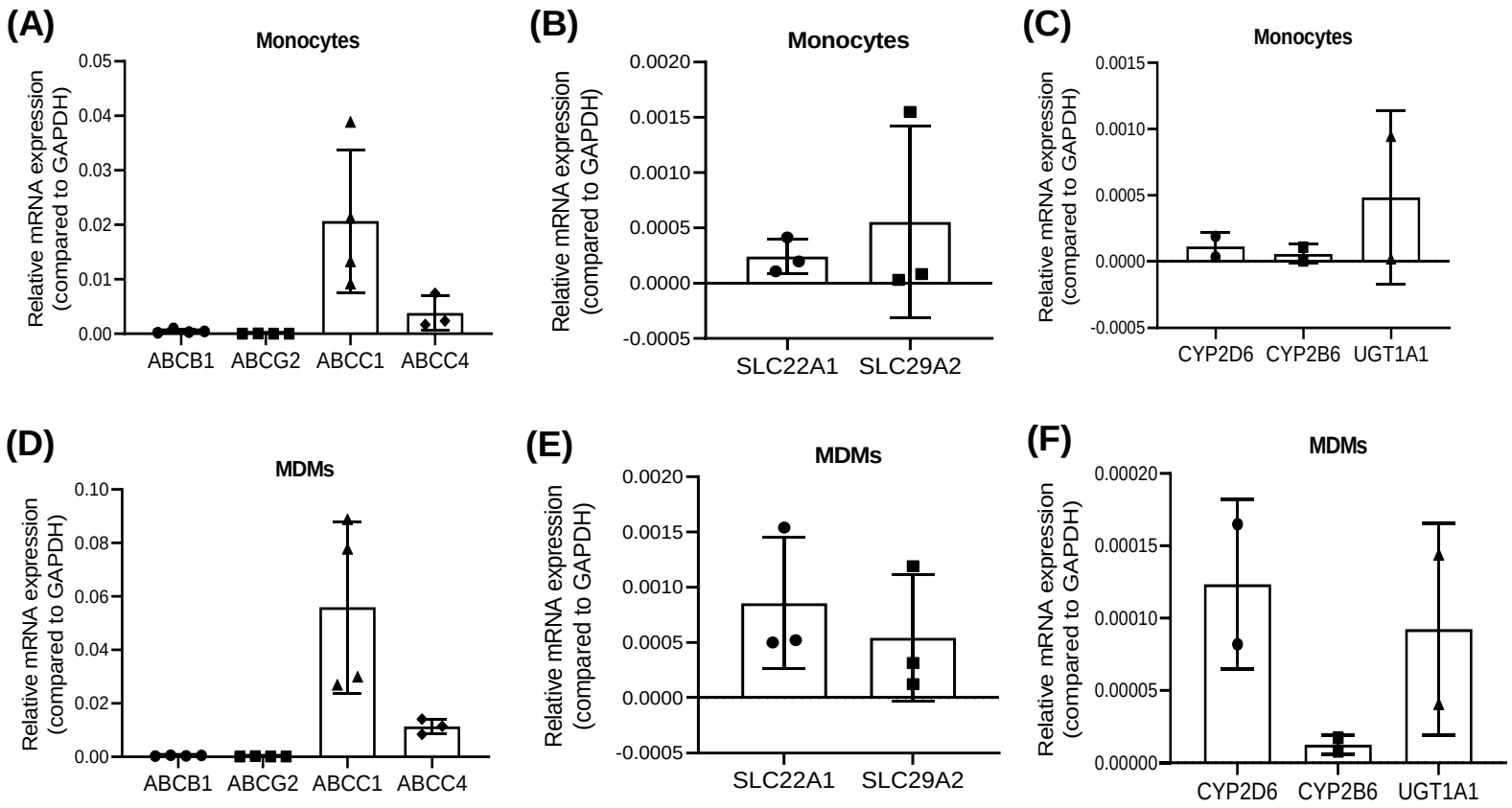

Figure 2. Relative mRNA expression of drug efflux/uptake transporters and metabolic enzymes in monocytes **(A-C)** and MDMs **(D-F)** isolated from HIV- donors. The results are expressed as mean relative mRNA expression ± SD normalized to the housekeeping gene GAPDH; n=2-4.

BCRP, MRP1 and P-gp Protein Expression in HIV+ART and HIV- Monocytes

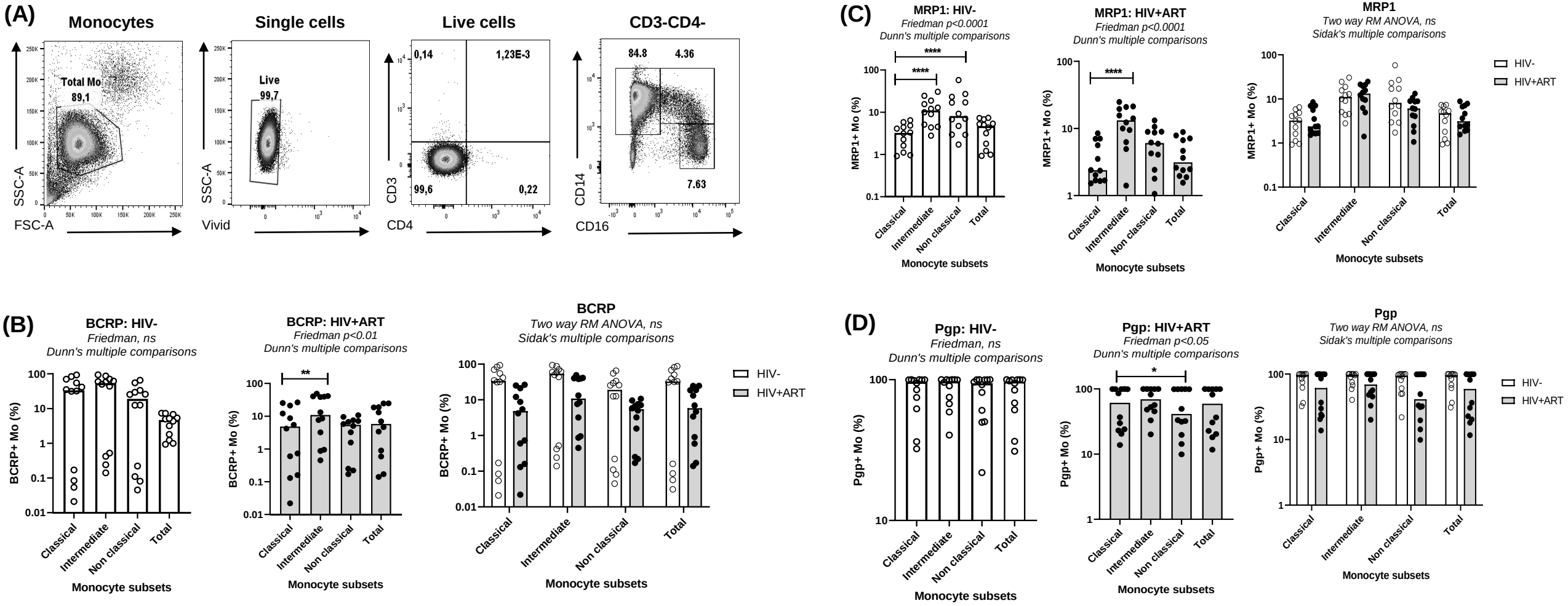

Figure 3. (A) Flow cytometry gating strategy for monocytes isolation from PBMCs of a representative donor. **(B)** BCRP, **(C)** MRP1 and **(D)** Pgp expression in monocyte subsets were compared in classical (CD14++CD16−), intermediate (CD14++CD16+), and nonclassical (CD14+CD16++) monocytes isolated from HIV- and HIV+ART donors. Data is expressed as median percentage of monocyte subsets expressing each transporter.

Friedman or two-way RM ANOVA tests using Dunn's or Sidak's multiple comparisons tests, respectively. *, p<0.05. **, p<0.01. ***, p<0.001. n = 12 donors per group;

BCRP, MRP1 and P-gp Protein Expression in HIV+ART and HIV- MDM

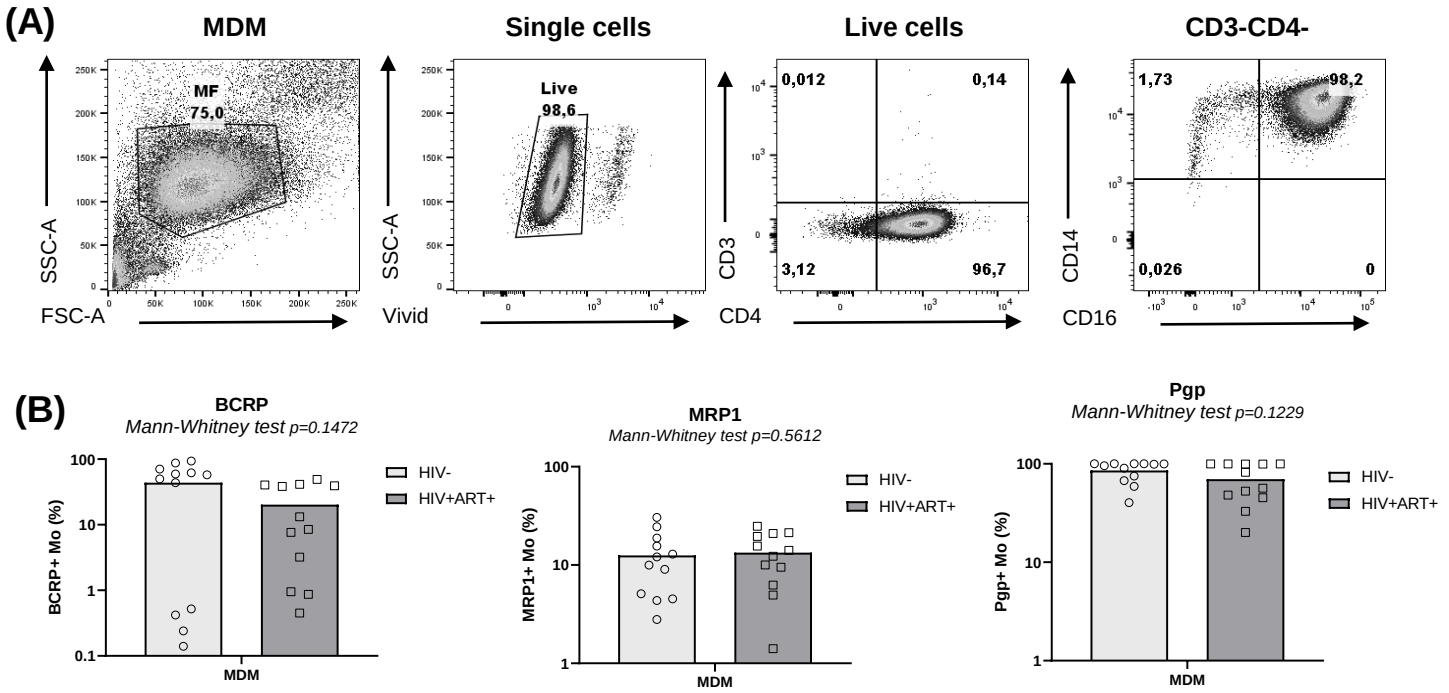

Figure 3. (A) Representative flow cytometry gating strategy for MDMs. **(B)** BCRP, MRP1 and Pgp expression in MDMs isolated from HIV- and HIV+ART donors. Data is expressed as median percentage of cells expressing each transporter. Comparisons between groups were performed using the the Mann-Whitney Test; n=12/group.

CONCLUSION

Herein, we demonstrated the highest frequencies of ARV efflux transporters on intermediate monocytes, a subset expanded during HIV infection, expressing the highest levels of the HIV coreceptor CCR5. These transporters could potentially limit ARV intracellular concentrations and contribute to persistent HIV infection of these cells. These novel findings prompt future investigations on HIV reservoir persistence in tissue-resident myeloid cells in relationship with specific ART regimens.

ARV Quantification in Plasma, PBMCs, and Monocytes of HIV+ART Donors

Patient ID	Drug	Plasma Concentration (ng/ml)[a]	Intracellular Concentration (fmol/10^6 cells)[b]	
		Plasma	PBMC	Monocyte
HIV+ART#3	Tenofovir-DF	167	8.5	22.8
	Emtricitabine	916.9	457.7	1,111
HIV+ART#4	Tenofovir-DF	73.13	26.87	83.45
	Emtricitabine	120.6	1,256.5	2,927.5
	Nelfinavir	NQ	NQ	NQ
HIV+ART#6	Tenofovir-DF	68.85	24.655	49.085
	Emtricitabine	163.4	1,919.5	3,472.5
	Cobicistat	172.1	8.955	12.635
	Elvitegravir	745.4	4.734	BLQ
HIV+ART#7	Tenofovir-DF	57.32	27.975	49.81
	Emtricitabine	73.46	2,787	3,588
	Efavirenz	923.1	BLQ	BLQ
HIV+ART#8	Tenofovir-DF	41.94	30.88	60.1
	Rilpivirine	18.4	BLQ	BLQ
	Dolutegravir	1310	BLQ	BLQ
HIV+ART#10	Tenofovir-DF	33.53	28.67	BLQ
	Emtricitabine	36.34	787	BLQ
	Rilpivirine	8.54	BLQ	BLQ
HIV+ART#11	Abacavir	1,563	25.11	31.89
	Lamivudine	1,169	4,487	8,315
	Dolutegravir	3,640	BLQ	BLQ

Table 1. ARV Concentrations in Plasma, PBMCs and Monocytes of several HIV+ART Donors. BLQ – Below limit of quantification; NQ- not quantified; Tenofovir-DF- Tenofovir Disoproxil Fumarate; Tenofovir-AF- Tenofovir alafenamide. [a]NRTIs were measured as parent compounds in plasma and [b]phosphorylated metabolites in PBMCs and monocytes.