Chemical Facilitated Endosomal Storage of Long-Acting Antiretroviral Nanoparticles

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Abstract

Background: Suboptimal adherence to antiretroviral therapy (ART) for HIV-1 begets viral mutations and drug resistance. This might be reduced or eliminated by maintenance of plasma and tissue drug levels above an effective dose for prolonged time periods after single dose administration. To achieve this, we sought pharmacologic approaches that would augment ART depots in intracellular organelles free from pathways operative for drug elimination.

Methods: A mixed lineage kinase-3 inhibitor URMC-099, developed in our laboratories, was investigated for its abilities to affect ART storage and antiviral activities. Humanized HIV-1 infected NOD/SCID/IL2R γ c$^{-/-}$ (NSG) mice and monocyte-derived macrophages (MDM) were treated with or without nanoformulated ritonavir (RTV)-boosted atazanavir (nanoATV/r), co-administered with URMC-099. 22-weeks old humanized-NSG mice were infected with HIV-1_{ADA}, followed by administration of URMC-099 daily and nanoformulated ATV and RTV (nanoART) weekly for three weeks starting 10 weeks post-infection, when the mice were at the peak of their viral load (VL). Peripheral VL, ratio of CD4$^+$ and CD8$^+$ T-lymphocytes, drug level in plasma and organs and pathological changes in lymphoid and brain tissues were investigated.

Results: Co-administration of URMC-099 and nanoATV/r reduced viral load (VL) below detectable levels in plasma and reduced HIV-1p24+ lymphocyte numbers in spleen. We found very small amounts of residual HIV-1 found in infected humanized mice significantly below what was seen by nanoATV/r alone treatment. This effect on VL was associated with elevated nanoATV/r depots in macrophages and paralleled URMC-099 induction of ATV and RTV in spleen and liver. Proteomic and Western blot validation assays demonstrated that the antiretroviral effect was associated with accumulation of HIV-1 and nanoATV/r as well as reduced progeny virions in Rab 7- and 11-labeled recycling and late endosomes. URMC-099 induced dose-dependent reductions in HIV-1p24 and reverse transcriptase activity within Rab 5-, 7- and 11-labeled endosomes in infected human monocyte-derived macrophages but only when administered with nanoformulated drugs.

Conclusions: Combination URMC-099 and nanoATV/r treatments offers a unique means to increase antiretroviral efficacy by enhancing accumulation of drug particle depots at endosomal sites of progeny virion maturation.

Results

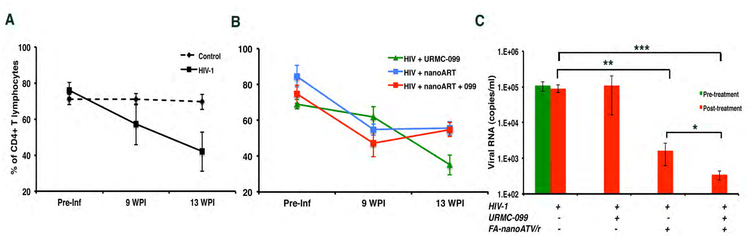

Fig. 1. URMC-099 potentiates FA-nanoATV/r antiretroviral activities in humanized CD34+ NSG mice. (A) Circulating human CD4+ T-cells in blood of untreated and HIV-1 infected animals (B) CD4+ T cells in FA-nanoATV/r with/without URMC-099 mice before and after treatment and (C) Plasma VL at the endpoint of the study.

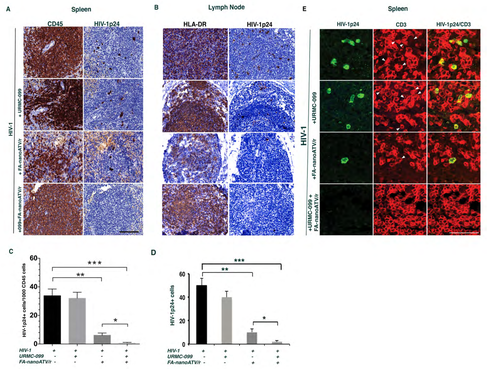

Fig. 2. Histopathological evaluation of Spleen and Lymph nodes in HIV-1 infected mice. Rare HIV-1p24+ cells found in FA-nanoATV/r mice, which were eliminated by addition of URMC-099 as shown in spleen (A) and lymph nodes (B). (C-D) quantitations of A-B. (E) immuno double staining of spleen sections with CD3 and HIV-1p24. URMC-099 co-treatment with FA-nanoATV/r reduced residual lymphocyte infection.

		HIV-1		HIV+nanoATV+099	
	Protein	Ztest	Regulation	Z test	Regulation
Early endosomes	Rab5	4.22	↑	--	↔
	TAP	2.39	↑	2.11	↑
	TfR	3.91	↑	--	↔
	vATPase	4.48	↑	--	↔
	Stx13	--	↔	--	↔
	EEA1	--	↔	-3.27	↓
	VPS34	--	↔	--	↔
	CALR	--	↔	--	↔
	Calnexin	--	↔	--	↔
Late endosomes	Rab7	2.12	↑	2.89	↑
	vATPase	4.48	↑	--	↔
	Dynein	4.49	↑	5.27	↑
	TUBB	--	↔	-3.38	↓
	TUBA	--	↔	--	↔
	RILP	--	↔	--	↔
	Stx7	--	↔	--	↔
Lysosomes	LAMP	--	↔	--	↔
	vATPase	4.48	↑	--	↔
	TAP	2.39	↑	2.11	↑
	M6PR	2.00	↑	--	↔
	Cathepsin	-2.52	↓	3.17	↑

↓ Downregulated ↔ Unchanged ↑ Upregulated

Table 1. Proteomic analysis showing changes in endolysosomal proteins in HIV-1 or HIV-1+nanoATV +URMC-099 treated MDM.

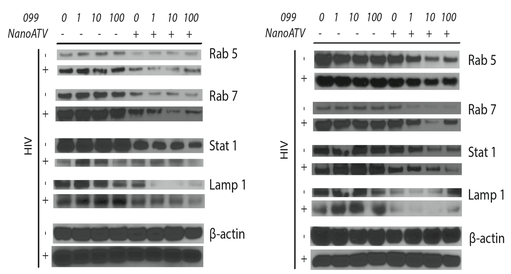

Fig. 3. Western blot analysis showing expression of Rab5, 7, STAT1 and LAMP1 in URMC-099/nanoATV treated MDM. HIV-1 infection was performed on day-0 and 5 after drug treatment.

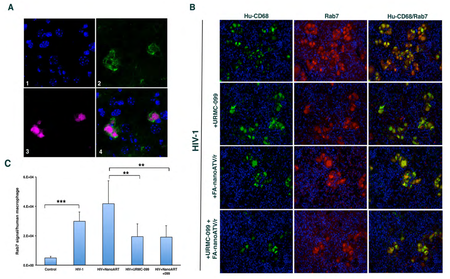

Fig. 4. Nanoparticle uptake and Characterization of human Rab7 expression in human macrophages (A) IF staining of mouse macrophages (F4/80-green) and CF-633 labeled nanoATV (pink) in liver. (B) double staining of spleens with human macrophages (CD68-green) and human Rab7 (red), (C) quantitation of panel B.

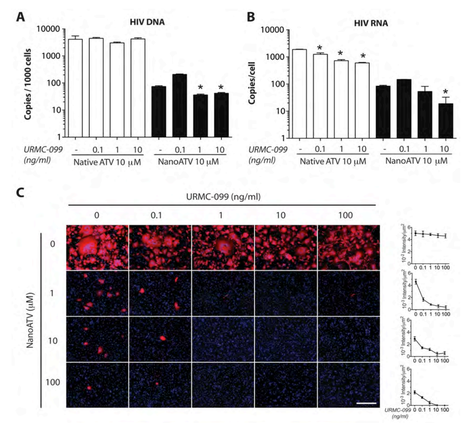

Fig. 5. URMC-099 Potentiates nanoATV antiretroviral activities for HIV-1 infected MDM. (A) HIV-1 LTR DNA was measured by Alu-gagPCR (B) HIV-1 RNA was measured by gag amplificatio and (C) Immunocytochemical staining of HIV-1p24 expression.

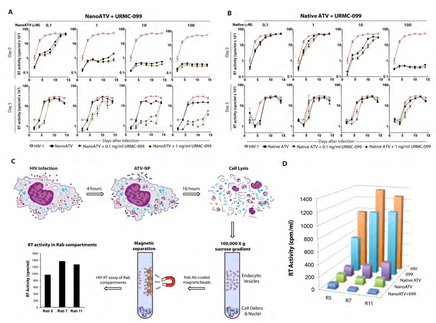

Fig. 6. URMC-099 facilitates nanoATV antiretroviral activities in HIV-1 infected human MDM. (A-B) RT activity in MDM treated with nano/native ATV and URMC-099 and infected on day-0 and 5. (C-D) isolation and measurement of RT activity in Rab5, 7 and 11 endsomoal compartments.

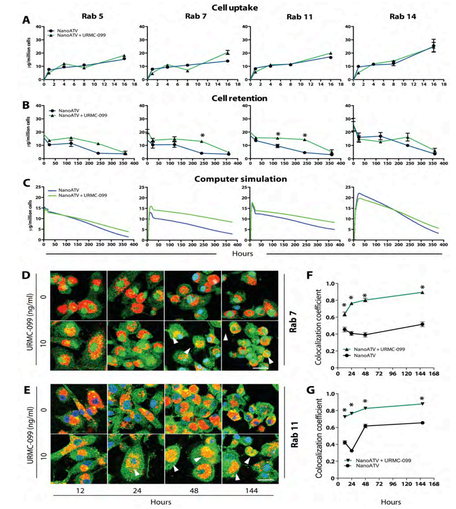

Fig. 7. URMC-099 facilitates retention of nanoATV in endosomal compartments. Uptake (A) and retention (B) of nanoATV in individual endosomal copartments of MDM over time. (C) mathematical simulation. (D-E) potentiation of nanoATV accumulation in Rab7 and 11 compartments by URMC-099. (F-G) quantitation of Rab7 and 11. CF568-labeled nanoATV (red) and Alexa Fluor-488 labeled endosomal compartments (green).

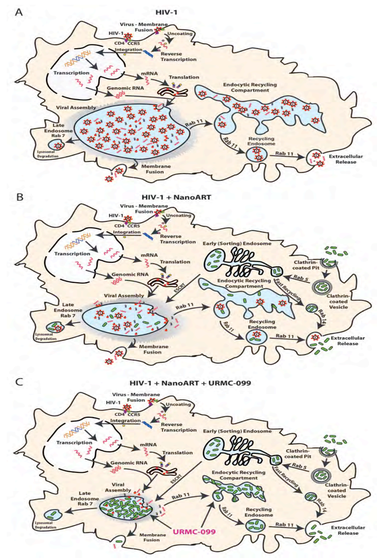

Fig. 8. Schematic diagrams reveal URMC-099 affects nanoART endosomal trafficking and HIV-1 progeny virion production. (A) HIV-1 budding, assembly and maturation in macrophage Rab7 and 11 endosomal compartments. (B) nanoATV taregts the same for storage and inhibits viral maturation at the site of assembly. (C) URMC-099 boosts nanoATV antiviral activity through increased nanoATV accumulation in macrophage Rab7 and 11 endosomal compartments.

Conclusions

- **FA-nanoATV/r + URMC-099 targets lymph nodes**
- **Both drugs are synergistic in attenuating HIV-1 infection in humanized mice.**
- **Rab5, 7 and 11 expressions are linked to pro-inflammatory cytokines and inhibited by URMC-099.**

Acknowledgements

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