NanoART Facilitated HIV-1 Clearance by a Mixed-Lineage Kinase 3 Inhibitor

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Abstract

Background: Antiretroviral therapy (ART) has improved life quality and longevity for infected people. However, despite ART viral replication continues, albeit at low levels. Eradication of infection remains the major goal of HIV scientists and clinicians alike. Recently, a novel mixed-lineage kinase-3 (MLK3) inhibitor (URMC-099) with promising immune and neural modulatory activities was found. Surprisingly, the drug was found to potentiate the antiretroviral activities of our nanoformulated long-acting ART. **Results**: URMC-099 increased expression of the RAS-related GTP-binding proteins Rab 7, 8 and 14 in HIV-1 infected MDM. NanoATV antiretroviral activities were potentiated by URMC-099 with reductions in viral DNA and RNA, HIV-1gag and RT activity. URMC-099 increased ATV concentrations in Rab14 endosomal compartments. Highest levels of CD4+ T cells numbers were seen in infected humanized mice treated with URMC-099 and nanoATV with VL in plasma below the limit of assay detection in the mice (<500 copies/ml). Such URMC-099-ART synergy was not seen with native drug. Histopathological tests of spleen and lymph nodes showed decreased numbers of HIV-1p24 cells with both drugs as compared to nanoATV alone. Preservation of synaptic structures was also seen. iTRAQ and tandem mass spectrometry tests were confirmed by Western blot tests showing significant Rab7 and 8 changes in HIV-1 infected MDM. Conclusions: URMC-099 significantly potentiates nanoATV responses to restrict HIV-1 and protect CD4+ T lymphocytes. This appears to occur, in part, by sustaining ART levels in specific endosomal compartments of the cells. Such new effects of URMC-099 may represent a novel means for viral clearance.

Objective

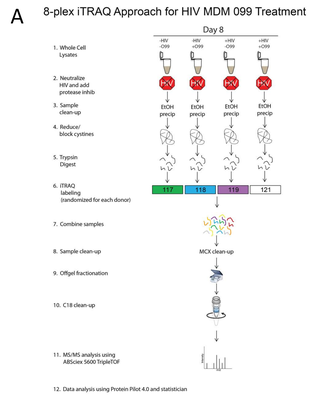
To evaluate the potentiation of URMC-099 effect on the antiretroviral actions of nanoATV to clear persistent HIV infection in MDM in vitro and in humanized mice.

Methods

Methodology: Isobaric tag for relative and absolute quantitation (iTRAQ) was performed in URMC-099 treated monocyte-derived macrophages (MDM) seeking potential antiretroviral actions. Nanoformulated atazanavir (nanoATV) with/out dose escalated URMC-099 was tested for antiretroviral activities in HIV-1_{ADA} exposed MDM. Viral DNA and RNA, Gag proteins and reverse transcriptase (RT) activities were assessed. Subcellular endosomal trafficking (Rab 5, 7, 8 and 14) of the ART nanoparticles were investigated by Western blot, confocal microscopy and HPLC. Humanized NOD/scid-IL-2R$^{\gamma c}$null (NSG) mice were infected with HIV-1_{ADA} followed by administration of URMC-099 daily and nanoformulated atazanavir and ritonavir (nanoART/r) for three weeks beginning at 10 weeks post-infection. Peripheral viral load (VL), human CD4+ and CD8+ T cells, and lymphoid and brain tissues were investigated for virus and immune profiling.

Results and Discussion

Protein profiling of URMC-099 effects in HIV-1 infection



B

Experimental Condition	Up regulated*	Down regulated*
099 +HIV-1 to Control	26	22
099 + HIV1 to HIV-1	152	4
HIV1 to Control	2	163
099 to Control	7	25

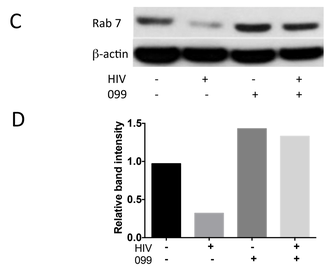


Figure 1. iTRAQ analysis of HIV-1-infected MDM cells. 100 ng/ml URMC-099 were treated with HIV-1-infected MDM cells for 8 days. The cells lysate were labeled by iTRAQ (A). URMC-099 induced protein level changes were sorted and shown in B. Rab 7 expression was evaluated by western blotting (C, D).

Antiretroviral activity

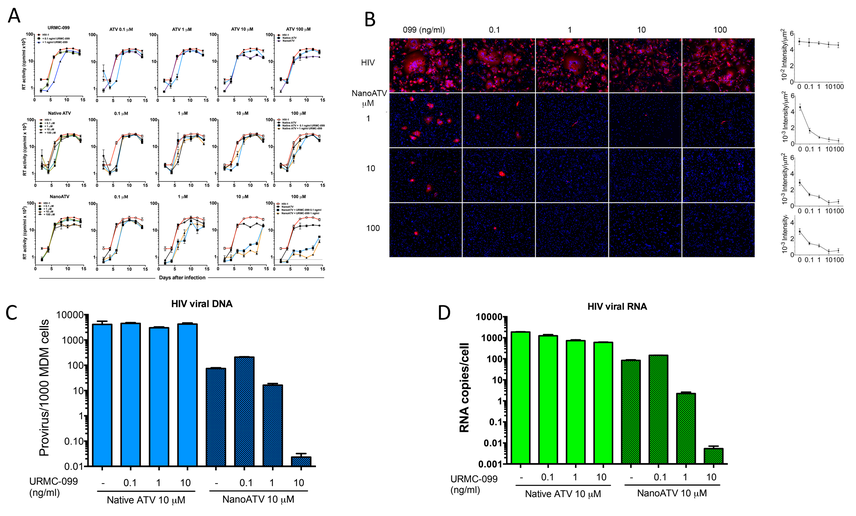


Figure 3. URMC-099 improved nanoATV effects on antiretroviral activity. Assays of RT activity in supernatant fluids of HIV-1 exposed MDM treated with free and nanoATV at 0.1, 1, 10 and 100 μM with increasing doses of URMC-099 (A).Immunocytochemical staining of HIV-1 infected MDM after 14 days infection are shown in B. HIV-1 LTR DNA was amplified by real time PCR to measure integrated HIV-1 (C). HIV-1 RNA expression was measured by HIV-1 gag amplification by real-time PCR.

Figure 5. HIV-1 infected cells in spleen and lymph nodes following nanoATV/r and URMC-099 treatments. Immunohistology for human cells (HLA-DR) and HIV-1 infected cells (HIV-1 p24) was performed in parafiin embedded sections. A. Spleen and B. lymph node sections show comparativly similar amount of human immune cell (HLA-DR) reconstitution in all groups of mice and numbers of p24 stained cells are reduced following nanoATV/r treatment and potentiated by URMC-099.

URMC-099 modulates macrophage subcellular trafficking

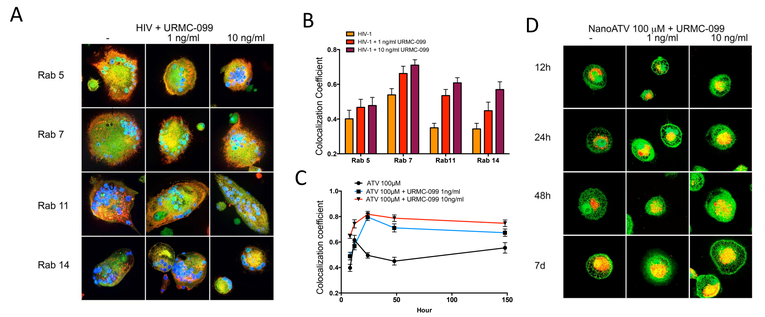
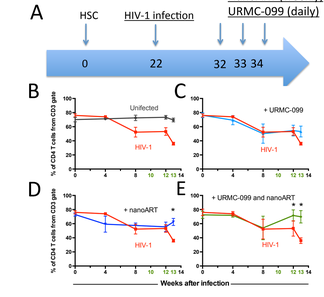


Figure 2. URMC-099 increased HIV-1 gag protein accumulating in macropahge endosome and potentiate nanoATV drug level in Rab 14 positive subcellular compartments. After 14 days HIV-1 infection and URMC-099 co-treatment, macrophages were double stained by endosomal marker Rab and HIV p24 protein (A). The dynamic nanoATV subcellar trafficking in Rab 14 endsome was shown in D. All the confocal images were quantified (B,C).

CD4+ T cells monitoring and viral load testing



F

Groups	Mice ID	Viral load (RNA copies/ml) Before*	After*	% decrement*	Mean ± SD
HIV-1	1612	187000	74500	60.1	
	1613	432000	232000	46.2	59.9 ± 18.1
	1614	830000	128000	85.5	
	1615	103000	53500	48.1	
HIV-1 + URMC-099	1597	770000	470000	38.9	
	1599	870000	417000	47.9	58.8 ± 27.1
	1602	20200	2070	89.7	
HIV-1 + nanoART	1594	16000	340	97.8	
	1596	8750	2470	71.7	79.8 ± 23.2
	1619	3010000	22100	99.2	
	1628	61200	30200	50.6	
HIV-1 + URMC-099 + nanoART	1572	3870000	0#	100	
	1573	65000	0#	100	99.0 ± 1.67
	1591	3150000	93200	97.1	

* Before ART treatment, after ART treatment.

Viral load <500 copies/ml (undetectable level)

Figure 4. Immune and antiretroviral activities of nanoATV/r and URMC-099 in humanized mice. Schematic diagram of the experimental procedure including generation of humnized mice, infection with HIV-1 and drug treatments (A). Graphs represent the percentage of human CD4+ T cells (from CD3 gate) in peripheral blood collected at different time points as analyzed by flow cytometry (B, C, D,E). Viral load in HIV-1-infected humanized mice was show in F.

HIV infection in spleen and lymph node

