Inhibition of HIV-1 Replication by a Novel Acylguanidine-based Molecule

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Background and Objective

Recent advances in HIV-1 antiretroviral therapy (ART) have substantially reduced morbidity and mortality, but the selection and transmission of drug-resistant strains necessitates ongoing discovery of new antiviral drugs. HIV-1 accessory proteins, including Vpu, enhance viral replication and in vivo pathogenesis and thus may be attractive targets for new classes of antiviral drugs.

Vpu promotes virion release by downregulating the host restriction factor BST-2/Tetherin. Vpu is also reported to be a viroporin (i.e. forming channels in cell membranes), but the role of this activity in HIV-1 replication remains unproven. A putative inhibitor of Vpu viroporin activity (BIT225) has been described; however, it displays high toxicity in T cell cultures.

We investigated the anti-HIV-1 activity of a novel acylguanidine compound, SM111, which has been shown to inhibit viroporin proteins encoded by Influenza, Dengue, and Hepatitis C viruses.

Materials and Methods

Drugs

i) SM111 (lead compound) is a novel acylguanidine-based small molecule
ii) BIT225 is an acylguanidine-based compound under investigation by Biotron, Ltd (Australia)
iii) Zidovudine (AZT) is a nucleoside reverse transcriptase inhibitor (NRTI)
iv) Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI)
v) Indinavir (IDV) is a protease inhibitor (PI)
vi) Raltegravir (RAL) is an integrase inhibitor (INI)

Viruses

Wild type NL43 and recombinant NL43 strains encoding patient-derived subtype B Pol sequences were used to assess antiviral activity of each drug. Details of 4 strains harbouring major NRTI, NNRTI, PI and INI resistance mutations are shown in Table 1.

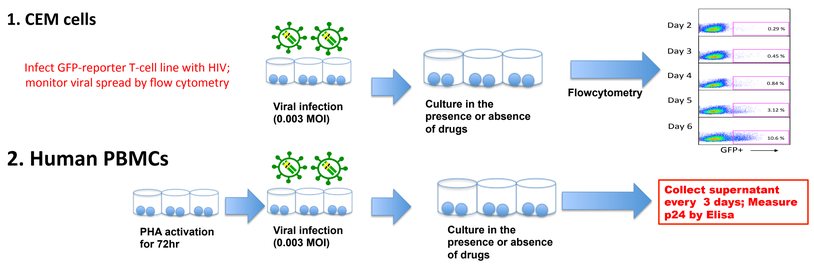
Table 1. Patient-derived pol sequences

HIV resistant strain	Drug resistance mutations
NRTI-RS (AZT)	D67N,T69N,K70R,T215Y
NNRTI-RS (EFV)	K103N, V108I,M230L
PI-RS (IDV)	M46L,I54V,I84V,L90M
INI-RS (RAL)	N155H

Cells

A GFP reporter CD4+ T cell line (CEM-GXR) and primary PBMCs were used in multi-cycle replication assays to propagate viruses in the presence or absence of SM111 (0-100μM) or other drugs (AZT, EFV, IDV, or RAL; 0-100nM). Infected CEM-GXR cells were quantified by flow cytometry as the % GFP+ events among viable cells in culture, while p24 Elisa was used to quantify viral growth in PBMCs. CEM-GXR cells were also transfected with WT Vpu or Vpu encoding SM111-selected mutations, and downregulation of CD4 or Tetherin (CD317) monitored by flow cytomery.

Assessment of viral replication



SM111 exhibited limited toxicity in cell lines and PBMCs

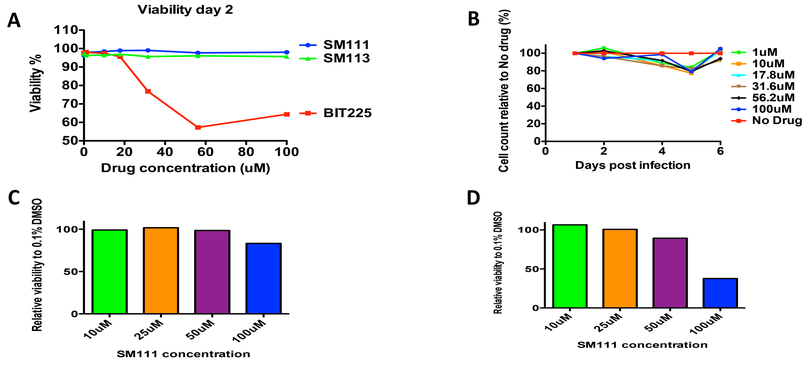


Figure 1. Effects of SM111 on cell viability and proliferation. A, CEM-GXR cells were incubated with various concentrations of SM111. Cell counts and viability were determined using ViaCount (Millipore). No toxicity was observed with up to 100uM SM111 or SM113 (an SM111 variant with no viroporin activity), while BIT225 was cytotoxic at >20uM. **B,** SM111 had minimal effects on cell proliferation in a 6 day assay. **C,** SM111 is non-toxic in HEK-293 cells up to 100uM. **D,** SM111 was non toxic in PBMCs up to 50uM; some toxicity was observed at 100uM.

SM111 inhibits in vitro HIV-1 replication

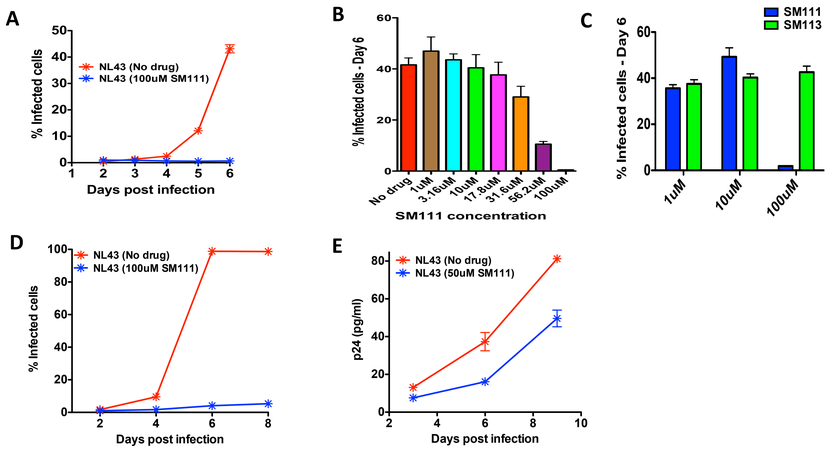


Figure 2. Anti-HIV activity of SM111. A, CEM-GXR cells were infected at MOI of 0.3% and SM111 (various doses) was added at 24hrs. Negative (no drug) wells were used as controls. Viral growth was monitored at days 2-6 by flow cytometry. **B**, HIV-1 replication was inhibited by SM111 in a dose-dependent manner. Results are displayed as % infected cells at day 6. **C**, HIV-1 replication is not affected by SM113, an acylguanidine analog that has no anti-viroporin activity. Results are displayed as % infected cells at day 6. **D,** Inhibition of HIV replication in SupT1 cells (cell line lacks BST2/CD317-Tetherin) in a multi cycle 8 day assay. **E,** Inhibition of HIV replication in human primary PBMCs, by 50uM SM111 in a multi cycle 9 day assay.

SM111 inhibits drug resistant HIV-1 strains

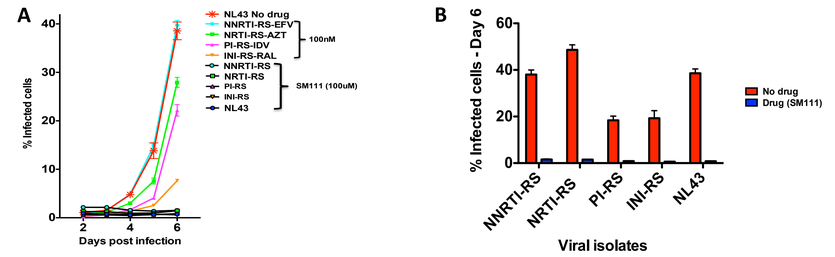


Figure 3: SM111 is inhibits replication of NRTI, NNRTI, PI and INI resistant HIV-1 strains. A, Replication kinetics of recombinant NL43 strains encoding resistance mutations to current ARVs in the presence of the respective ARV or SM111. **B**, Spread of drug resistant HIV-1 strains and wild type NL43 control in the absence or presence of SM111. Results are displayed as % infected cells at day 6.

SM111 selects mutations in the transmembrane domain of Vpu

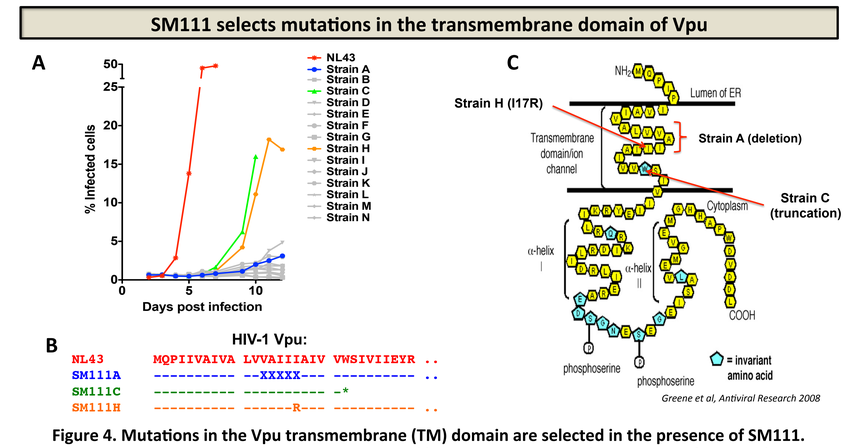


Figure 4. Mutations in the Vpu transmembrane (TM) domain are selected in the presence of SM111. A, SM111 resistant HIV-1 variants were generated by passaging NL43 in the presence of 100uM drug. **B** and **C**, Unique Vpu mutations were observed in three outgrowth strains, resulting in either a 5 AA deletion (strain A), a truncation (strain C) or a substitution I17R (strain H) of the TM sequence.

SM111-selected mutations impair Vpu-mediated Tetherin and CD4 down regulation, and confer decreased susceptibility to SM111

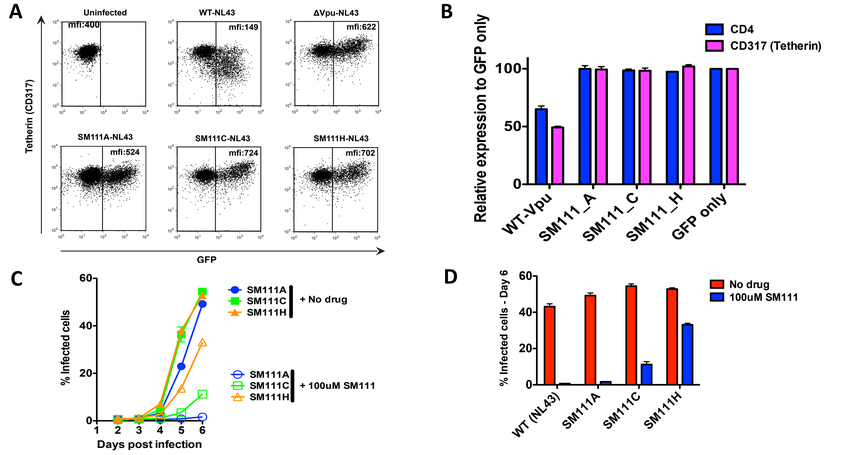


Figure 5. Effects of Vpu mutations on Tetherin (CD317)/CD4 downregulation activity, viral spread and SM111 resistance. A, Dot plots showing Tetherin cell surface expression at day 6 post-infection. **B,** % Tetherin and CD4 expression on CEM T cells transfected with wild-type Vpu or Vpu mutants demonstrates impaired down regulation activity by all SM111-selected mutants. **C,** Vpu mutant viruses displayed enhanced viral spread in the presence of SM111. **D,** Mutant strains , showed a range of susceptibility to SM111, with the 5AA deletion (SM111A) remaining most sensitive, truncation (SM111C) moderately sensitive, and I17R substitution (SM111H) least sensitive to the drugs antiviral activity.

HIV-1Δvpu displays partial resistance to SM111 that differs by cell type

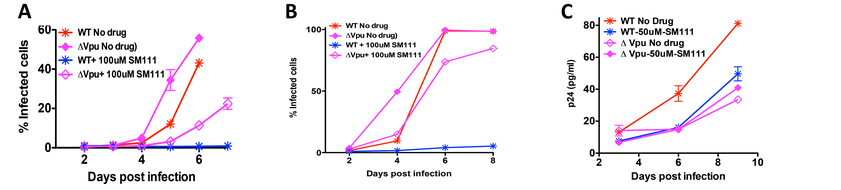


Figure 6. NL4-3 Δvpu exhibits susceptibility to SM111 that is dependent in part on cell type. A, NL4-3 Δvpu displayed minimal resistance to SM111 in CEM-GXR cells but was more resistant to drug in SupT1 cells as shown in **(B)**. **C,** NL4-3 Δvpu replicated poorly in PBMC and displayed minimal resistance to SM111.

SM111 inhibits HIV-1 viral egress

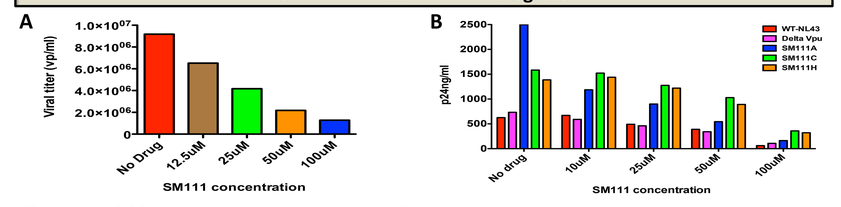


Figure 7. Inhibition of HIV-1 release by SM111. A, Titers of NL4-3 virions from the supernatants of HEK293 cells harvested at 48hrs post-transfection with pNL4-3 displayed an SM111 dose-dependent decrease. Viral titer was measured using CEM-GXR cells. **B,** Supernatant p24 concentrations of WT and mutant HIV-1 particles released from HEK293 cells 48hrs post transfection under different concentrations of SM111.

Conclusions

- SM111 is a novel acylguanidine-based small molecule with broad antiviral activity
- SM111 displays anti-HIV-1 activity with minimal cytotoxicity in T cell lines and PBMCs
- SM111 inhibits the replication of HIV-1 strains resistant to NRTI, NNRTI, PI and INI drugs, indicating a distinct mechanism of action
- SM111 selects for mutations located in the HIV-1 Vpu TM domain that impair Tetherin and CD4 down regulation activity
- Mutant HIV-1 strains and strains lacking Vpu remain at least partially sensitive to SM111, indicating that additional host and/or viral target(s) may be required for drug activity
- SM111 represents a promising lead molecule for future ART studies