CROI 2015
Seattle, WA
Feb 25th, 2015

541

BMS-986001: A Promising Candidate for HIV-2 Treatment

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Background

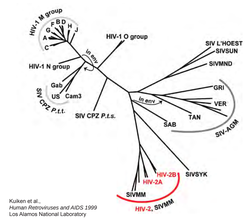
HIV-2 infection is a significant public health problem in West Africa and has been reported in other countries with economic ties to the region.

African countries reporting HIV-2 infections (red)



Schim van der Loeff, AIDS 13:569
Markowitz, Annals Internal Med 118:211

Phylogenetic relationship of HIV-2 to HIV-1 and SIV



Kuiken et al., *Human Retroviruses and AIDS 1999*
Los Alamos National Laboratory

HIV-2 is intrinsically resistance to many HIV-1–active antiretroviral drugs (ARV), including all NNRTI, several PI, and the fusion inhibitor T20.

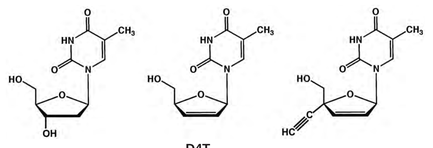
Antiretroviral therapy (ART) for HIV-2 infection in West Africa continues to rely heavily on PI-based regimens:

- before 2009: AZT/3TC/IDV; occasionallly 3 NRTI (ABC/3TC/AZT; Trizivir)
- 2009–present: AZT/3TC/LPV/r
- 2013: generic FTC and TDF available
- 2014: DRV/r and the integrase inhibitor raltegravir (RAL) available

Historically, clinical outcomes of ART for HIV-2 and HIV-1/HIV-2 dual infection have been poor, with high rates of immunovirologic failure. ~30% of individuals in our HIV-2 cohort in Senegal, West Africa harbor multi-class (NRTI+PI)-resistant strains.

Newer, HIV-2–active ARV are urgently needed.

BMS-986001 is a 4´-modified nucleoside analog inhibitor



Thymidine

D4T (stavudine)
2´-3´-didehydro-3´-deoxythymidine

BMS-986001
4´-ethynyl stavudine

A brief history of BMS-986001:

- Initially synthesized and evaluated at Yale (Hiragachi 2003)
- Phosphorylated by human TK-1 (Dutschman 2004)
- Active against HIV-1 in culture (Dutschman 2004)(Tanaka 2005)
- Selection for resistance → P119S+T165A+M184V (Nitanda 2005) (Yang 2009)
- 5´-TP incorporation by HIV-1 RT → chain termination (Yang 2008)(Sohl 2012)
- Active against most NRTI-resistant HIV-1 mutants (M184V or multiple TAMs confer resistance) (Nitanda 2005) (Li 2013)
- Phase IIb clinical trial (n=297): FTV (100, 200, 400 mg QD) or TDF (300 mg QD)+3TC+EFV. 89% suppressed (400 mg) @ wk 48 vs. 82% for TDF. Trial terminated due to higher rates of resistance (6–14%, vs. 1% for TDF) (ICAAC 2014).

The activity of BMS-986001 against HIV-2 has not been reported.

Aim

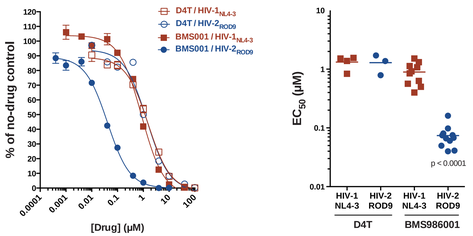
To evaluate the antiviral activity of BMS-986001 against HIV-2 in culture

Approach

- Single-cycle drug susceptibility assays using HeLa-CD4 indicator cells (MAGIC-5A).
- Multicycle assays measuring BMS-986001 sensitivity in an immortalized T cell line (CEMss).
- Resistance testing with site-directed and patient-derived RT mutants.

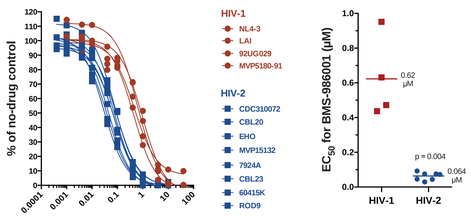
Results

$HIV\text{-}2_{ROD9}$ is more sensitive to BMS-986001 than $HIV\text{-}1_{NL4\text{-}3}$ in a single cycle of infection



All data are from single-cycle assays with MAGIC-5A indicator cells (Smith et al. PLoS One 7:e45372). *(left)* Results of a single representative experiment for D4T and BMS-986001. Y-axis represents the relative titer (%) of HIV compared to solvent-only controls. Error bars represent standard deviations from two independent MAGIC-5A cultures. *(right)* Results of multiple experiments with ROD9 and NL4-3. Y axis indicates 50% effective concentration as determined by sigmoid regression of dose-response data (GraphPad Prism v 6.0f). P value for the comparison between ROD9/D4T and ROD9/BMS-986001, calculated by ANOVA of Log10-transformed EC50 values with Tukey's post-test.

Other HIV-2 isolates are more sensitive to BMS-986001 than HIV-1



All data are from single-cycle assays with MAGIC-5A indicator cells. *(left)* Results of a single experiment with BMS-986001. All strains shown in the graph were assayed on the same date. Error bars are omitted for clarity. *(right)* EC50 values calculated from the data shown in the left panel. Bars and values indicate the mean EC50s for all HIV-1 and HIV-2 isolates. P value was obtained via two-tailed Mann-Whitney test.

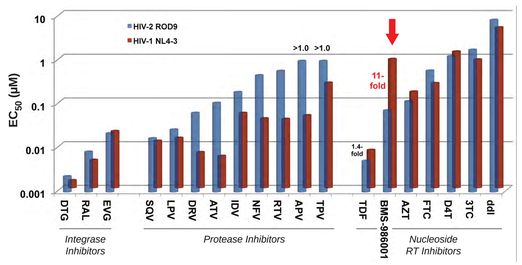
Results

Compared to HIV-1, EC_{50} values for BMS-986001 are ~10-fold lower for Group A and B HIV-2

HIV Type	Strain	Group/ Subtype	EC_{50} (μM) [a]	n= [b]
HIV-1	NL4-3[c]	M/B	0.91 ± 0.43	7
	LAI	M/B	0.61 ± 0.34	3
	92UG029	M/A	0.45 ± 0.11	3
	MVP5180-91	O	0.47 ± 0.09	3
	mean ± SD for HIV-1		0.61 ± 0.21	
HIV-2	ROD9[c]	A	0.081 ± 0.041	7
	7924A	A	0.069 ± 0.009	3
	MVP15132	A	0.064 ± 0.019	3
	60415K	A	0.078 ± 0.009	3
	CBL20	A	0.081 ± 0.010	3
	CBL23	A	0.075 ± 0.005	3
	CDC310072	A	0.030 ± 0.012	3
	EHO	B	0.042 ± 0.009	3
	mean ± SD for HIV-2		0.065 ± 0.019	

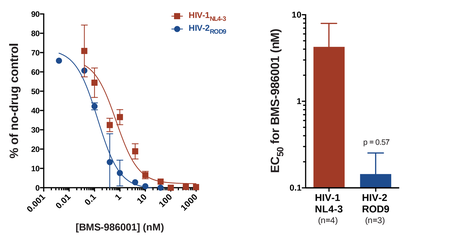
[a]50% effective concentration (mean ± standard deviation) determined via single-cycle focus reduction assay.
[b]Number of assay runs performed for each strain.
[c]Virus generated by transfection of wild-type pNL4-3 or pROD9 in 293T/17 cells.

Relative to other NRTI, BMS-986001 is a potent inhibitor of HIV-2 replication



All measurements were made using MAGIC-5A cells and our established single-cycle assay protocol. Bars indicate the mean of at least three independent dose-response experiments. Drug abbreviations: DTG, dolutegravir; RAL, raltegravir; EVG, elvitegravir; SQV, saquinavir; LPV, lopinavir; DRV, darunavir; ATV, atazanavir; IDV, indinavir; NFV, nelfinavir; RTV, ritonavir; APV, amprenavir; TPV, tipranavir; TDF, tenofovir disoproxil fumarate, AZT, zidovudine; FTC, emtricitabine; D4T, stavudine; 3TC, lamivudine; ddI, didanosine. Figure includes previously-published data from our laboratory (Smith et al. Retrovirology 12:10, Raugi et al. Antimicrob Agents Chemother 57:2751, Smith et al. J Infect Dis 199:1323, Smith et al. Retrovirology 7:70).

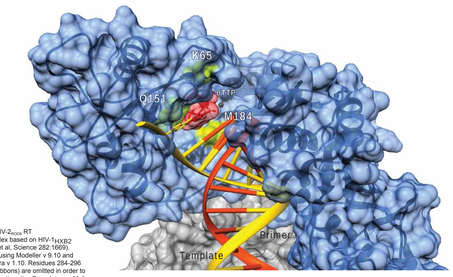
$HIV\text{-}2_{ROD9}$ is highly sensitive to BMS-986001 in spreading infections of CEMss cells



Supernatants from drug-treated CEMss cultures were collected four days after infection and transferred to MAGIC-5A indicator cells for quantification of infectious virus. *(left)* Y-axis represents the relative titer (%) of HIV in the CEMss supernatants compared to solvent-only controls. *(right)* Results of multiple experiments. For three assays in which the two viruses were tested in parallel, 50% effective concentrations for NL4-3 were 4-, 10- and 220-fold higher than those of ROD9. In both panels, error bars indicate standard deviations.

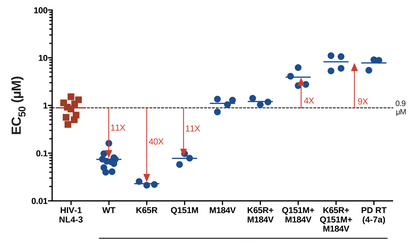
Results

Canonical NRTI resistance mutations in HIV-2 map to the polymerase active site of RT



Homology model of the HIV-2_{ROD} RT polymerase ternary complex based on HIV-1_{HXB2} RT model 1RTD (Huang et al, Science 282:1669). Structure was generated using Modeller v 9.10 and rendered in UCSF Chimera v 1.10. Residues 284-296 of the p66 domain (blue ribbons) are omitted in order to visualize the polymerase active site. Grey dots are Mg^{2+}.

BMS-986001 retains partial activity against NRTI-resistant HIV-2 mutatants



All data were obtained with the MAGIC-5A single-cycle assay. Red arrows and values are fold resistance (*up*) or hypersusceptibility (*down*) relative to wild-type HIV-1 NL4-3 (mean EC50 shown with dotted line). PD RT (4-7a) is a full length molecular clone of HIV-2 in which the RT-encoding region (codons 12-297) of pROD9 was replaced with a sequence amplified from an ARV-treated individual. The resultant clone harbors RT replacements K64R, K65R, N69S, V111I, Q151M and M184V, as well as other polymorphisms in RT.

Conclusions

- BMS-986001 is a more potent inhibitor of $HIV\text{-}2_{ROD9}$ than D4T. Relative to HIV-1, HIV-2 isolates from ART-naïve individuals are more sensitive to BMS-986001 in culture.
- BMS-986001 retains partial activity against HIV-2 variants that are resistant to other chain-terminating nucleoside analogs.
- To our knowledge, this is the first report of an antiviral compound that exhibits more potent activity against HIV-2 than HIV-1.

Acknowlegments

These studies were supported by grants to GSG from the National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID; 2R01-AI060466), the UW Center For AIDS Research (CFAR, an NIH-funded program; P30 AI027757), and the UW Royalty Research Fund (A92723). We thank the NIH AIDS Reagent Program for providing wild-type HIV-1 and HIV-2 strains. Additional UW-Dakar HIV-2 Study Group members are as follows: Macoumba Toure, Selly Ba, Ndeye Mery Dia Badiane, Louise Fortes, Cheikh T. Ndour, Jacques Ndour, Fatou Niasse, Fatima Sall, Fatou Traore, Khadim Faye, Sophie Chabils, Marie Pierre Sy, Bintou Diaw, Mbaye Ndoye, Amadou Bale Diop, Marianne Fadam Dionne (Clinique des Maladies Infectieuses Ibrahima DIOP Mar, Centre Hospitalier Universitaire de Fann, Universite´ Cheikh Anta Diop de Dakar, Dakar, Senegal); Alassane Niang, ElHadji Ibrahima Sall, Ousseynou Cisse, Jean Philippe Diatta, Raphael Bakhoum, Juliette Gomis, (Région Médicale de Ziguinchor, Ziguinchor, Casamance, Senegal), Stephen Hawes, Donna Kenney, Joshua Stern, Qinghua Feng, John Lin, Steve Cherne, Nancy Kviat, Brad Church, Matthew Coyne, Alexandra Hernandez, Alexandra Montano, Kara Parker, Kate Parker, Lindsey Blankenship, Mariah Oakes, Julia Olson, Sally Leong, Vincent Wu and Noelle Benzekri (University of Washington, Seattle, Washington).