

Genotypic Resistance and Phenotypic Cross-Resistance Profile *in vitro* for a Novel NNRTI: IDX899

DD Richman¹, J Jakubik², C Chapron², L Hubbard², L Gray², M Seifer² and DN Standing²
¹University of California San Diego and VA San Diego Healthcare System, CA, USA; ²Idenix Pharmaceuticals, Inc., Cambridge, MA, USA

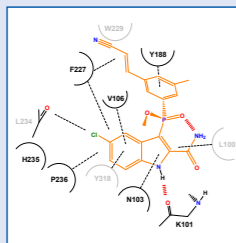
#729

Email: drichman@ucsd.edu
 Tel.: (858) 552-8585 (ext 7439); Fax: (858) 552-7445

Background

- Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are valuable components of antiretroviral combination therapy for the treatment of human immunodeficiency virus type 1 (HIV-1)-infection.
- In treatment-naïve patients, the most widely used NNRTIs are limited by a low genetic barrier leading to rapid emergence of drug-resistant mutants.
- IDX899 (see Figure 1) is a novel NNRTI drug candidate for the treatment of HIV-1 infection with potent and selective *in vitro* antiviral characteristics.⁴
- Our data suggest that this compound may offer an enhanced resistance threshold with favorable pharmacokinetic and safety profiles, suitable for once-daily oral dosing in treatment-naïve and NNRTI-experienced HIV-1-infected individuals.⁵
- IDX899 is currently in a proof of concept study in HIV-1 infected patients.

Figure 1. Schematic structure of HIV-1 K103N/Y181C RT with IDX899



Methods

Compounds

- IDX899, etravirine (TMC125), rilpivirine (TMC278) and efavirenz (EFV) were synthesized by Idenix Pharmaceuticals, Cambridge, MA.

Antiviral efficacy

- Activity profiles for IDX899, TMC125, TMC278 and EFV were generated using *in vitro* selected viral pools and site-directed mutants in the BH10 backbone.

Identification of IDX899^R (resistant) mutants

- In vitro* selection of drug-resistant variants was performed in HIV-1 BH10 WT infected MT-2 cells grown in escalating drug concentrations. Virus replication was monitored by syncytium formation indicative of viral breakthrough. Since pilot experiments showed that doubling of IDX899 concentrations after a single syncytial event led to loss of HIV-1 infection, drug concentrations were only doubled following two passages showing massive syncytium formation to ensure mutant survival. Emerging viruses were subjected to genotypic analysis via RT-PCR and population sequencing to identify the selected resistance mutations.

Phenotypic cross-resistance testing

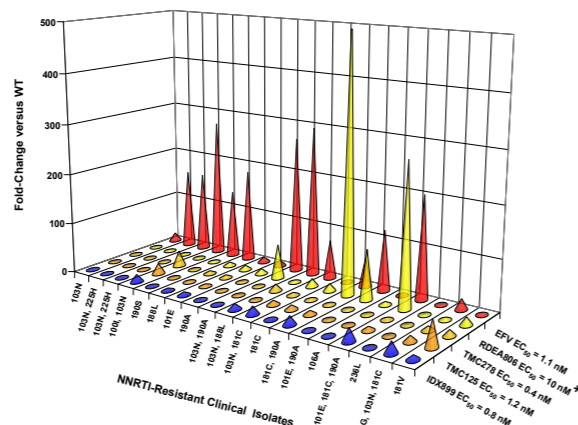
- Performed using *in vitro* selection supernatant normalized to 75 ng of capsid antigen (p24) per million MT-4 cells.
- Contribution of each mutation to resistance was determined with site-directed HIV-1 (BH10 strain) NNRTI mutants.

Results

Activity of IDX899 against a panel of NNRTI^R clinical isolates

- IDX899 had potent antiviral activity against NNRTI^R clinical isolates (n=19) in the PhenoSense™ assay (Figure 2).
- IDX899, TMC125 or TMC278 remained active against the bulk of the NNRTI^R mutant panel implying a higher barrier to resistance than EFV.
- Based on data presented by Ardea Biosciences² and summarized in Figure 2, IDX899 retained antiviral activity against more NNRTI^R mutants than RDEA806.

Figure 2: Activity of IDX899 against NNRTI^R clinical isolates (Monogram Panel). Resistance profiling was performed by Monogram Biosciences using the PhenoSense™ HIV assay.



*Ardea Biosciences Abstract #1662, 47th Annual ICAAC, Sept 2007

In vitro selection of IDX899^R virus pools

- Resistance mutations selected with IDX899 differed from those selected with EFV.³
- A series of *in vitro* selection studies revealed two pathways to IDX899 resistance (Table 1):
 - Pathway 1 via E138K
 - Pathway 2 via V90I

Antiviral efficacy of IDX899 and EFV against IDX899^R virus pools

Virus pools selected by IDX899 were tested against each drug. Results are shown in Table 1. The key findings can be summarized as follows:

- 26 to 30 passages and at least 3 mutations were typically required to produce virus pools with high level resistance [>100 -fold] to IDX899.
- Virus pools containing V90I, S134I, Y181C and M230L mutations at passage 29 (Experiment 2) or E138K, Y181I and M230L mutations at passage 31 (Experiment 1) showed 343- and >1136 -fold resistance to IDX899, respectively, and were also resistant to EFV.
- Virus pools tend to show higher levels of resistance than the equivalent site-directed mutant viruses, presumably due to contributions from minor mutant species.

Table 1. *In vitro* phenotypic cross-resistance profile of IDX899 and EFV against IDX899^R virus pools (fold change from WT)

	IDX899 ^R			
	Experiment 1		Experiment 3	
Pathway 1 (Passage)	E138K (P5)	E138K, Y181I (P13)	E138K, Y181I, M230L (P31)	E138K, G190E, L214F (P32)
Fold change to IDX899	3.1	25.9	>1136.4	20.2
Fold change to EFV	3.7	1.4	132.5	3.5
	Experiment 2			
	Pathway 2 (Passage)	V90I, Y181C (P17)	V90I, S134I, Y181C (P23)	V90I, S134I, Y181C, M230L (P29)
Fold change to IDX899	8.5	4.7	343.0	172.5
Fold change to EFV	37.6	11.7	306.4	238.9

Antiviral efficacy of IDX899 and EFV against EFV^R virus pools

- Mutant virus pools selected by EFV were tested against EFV and IDX899 (Table 2 and Figure 3).
- For EFV, one to four mutation(s) generated virus pools highly resistant to EFV, ranging from 124- to >1136 -fold (Table 2).
- All EFV-resistant virus pools selected through passage 28 and carrying up to 4 mutations remained susceptible to IDX899 (<12 -fold resistance).

Table 2. *In vitro* phenotypic cross-resistance profile of IDX899 and EFV against EFV^R virus pools (fold change from WT)

Mutations (Passage)	EFV ^R					
	Experiment 1			Experiment 2		
V179D (P14)	L100I, V179D (P20)	V35L, L100I, V179D (P26)	V179D, G190A (P13)	L100I, V179D, G190A (P19)	R83K, L100I, K103R, V179D (P28)	
Fold change to IDX899	2.8	1.2	5.1	6.4	9.0	11.8
Fold change to EFV	124.0	588.2	360.2	>694.4	>1136.4	>1136.4

Figure 3: Fold change in susceptibility to IDX899 and EFV against EFV^R mutant virus pools.

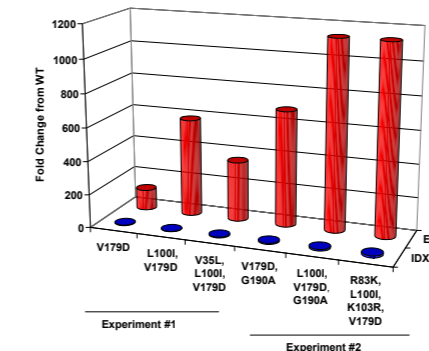
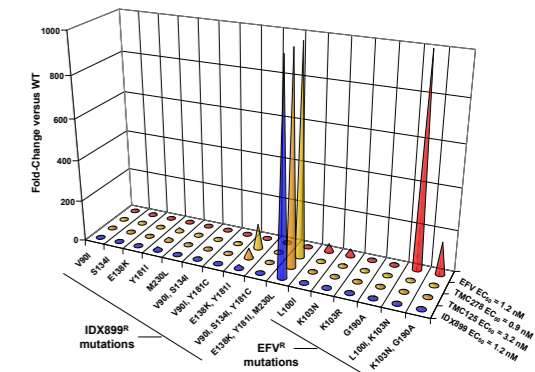


Figure 4: Cross-resistance profiles of site-directed NNRTI^R HIV-1 mutants.



Conclusions & Discussion

- Compared to EFV, emergence of IDX899-resistant HIV-1 isolates *in vitro* was slower and required more mutations suggesting a higher barrier to resistance for IDX899.
- Two pathways to IDX899 resistance were identified in a panel of *in vitro* selection studies, one starting with the mutation E138K and the other with V90I.
- In vitro* cross-resistance data imply that IDX899 has a different resistance profile than EFV and remains active against EFV^R virus pools containing up to 4 mutations.
- Conversely, our studies suggest that IDX899-selected HIV-1 resistant virus pools bearing up to 3 mutations can remain susceptible to EFV. The significance of these *in vitro* findings will need to be established in clinical studies.

Acknowledgments

We thank Monogram Biosciences for phenotypic analysis and Dr Valérie Philippon for assistance with presentation preparation.

Disclosures

J Jakubik, C Chapron, L Hubbard, M Seifer and D Standing are current employees and L Gray is a former employee of Idenix Pharmaceuticals Inc.

References

- Brilliant J, Klumpp K, Swallow S, et al (2004). *Antiviral Therapy*; 9:S20.
- Hamatake R, Zhang Z, Xu W, et al (2007). 47th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Chicago, USA.
- Jakubik J, Seifer M, Gray L, et al (2007). *Antiviral Therapy* 2007;12:S32.
- Richman DD, Dousson CB, Storer R, et al (2007). 14th Conference on Retroviruses and Opportunistic Infections (CROI), Los Angeles, USA.
- Mayers D, Hard M, Dampousse D, et al (2008). 15th Conference on Retroviruses and Opportunistic Infections (CROI), Boston, USA.