

Resistance to RDEA806 Requires Multiple Mutations Which Have Limited Cross-Resistance to Other NNRTIs

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Abstract

BACKGROUND: RDEA806 is a novel non-nucleoside reverse transcriptase inhibitor (NNRTI) with potent *in vitro* activity against wild-type and NNRTI-resistant HIV-1. Phase 2a clinical trial data demonstrated robust antiviral activity in naive patients. RDEA806 suppressed viral breakthrough in an *in vitro* resistance selection study for 9 months, indicating a high genetic barrier to resistance. The genotypic and phenotypic analyses of the mutant viruses selected by RDEA806 in this resistance selection study will be presented.

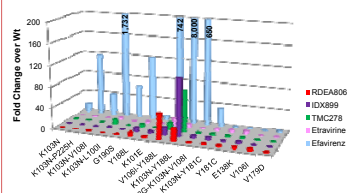
METHODS: Selection of HIV-1 virus resistant to RDEA806 was performed in infected SupT1 cells. The mutations identified in the RT region at the time of virus breakthrough for each drug combination were engineered into a wild-type vector. Activity was measured using the VSV-G protein pseudotyped NL4-3.Luc.R-E- virus containing the mutation(s).

RESULTS: RDEA806 concentration was increased to 1500 nM in the virus culture over the course of 13 months. The first consensus mutation selected, K104E, was identified after > 300 days in culture and showed essentially no loss of susceptibility to RDEA806. Other NNRTIs tested also retained maximal activity against this mutant virus. RDEA806 showed a minor loss of activity to the next virus selected, K104E-E138K-T240I, while efavirenz retained full activity. Virus with the ultimate combination contained 5 RT mutations, K104E-E138K-T240I-V179D-F227L, and had very low replication capacity. While RDEA806 lost potency against this virus, other NNRTIs showed only minor to moderate cross resistance.

CONCLUSIONS: Prolonged suppression of viral breakthrough in this resistance selection study suggests that RDEA806 has a high genetic barrier to resistance. Phenotypic analysis of selected mutations indicates that multiple mutations are necessary for loss of susceptibility to RDEA806. Minor cross-resistance to some of the mutation patterns is observed by some of the NNRTIs tested.

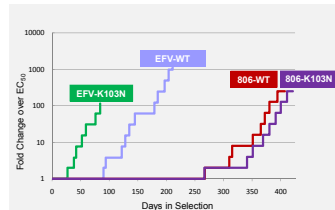
Results

Figure 1. RDEA806 Activity Against Prevalent NNTRI-resistant Viruses



- RDEA806 has broad spectrum activity against NNTRI-resistant viruses
- RDEA806 has excellent activity (FC <1) against the K103N mutation, the most commonly transmitted resistant virus in antiretroviral naive patients (up to 11.7%)¹
- The resistance profile of RDEA806 is superior to that of EFV and similar to those of other NNRTIs

Figure 2. Time Course of Viral Breakthrough



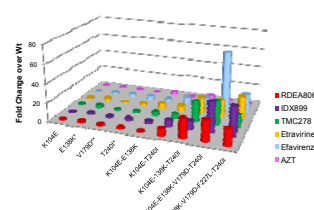
- RDEA806 provides more durable suppression of WT and K103N virus than EFV
- WT HIV-1 required 267 days of culturing in RDEA806 at the EC₅₀ level to break through, compared with 90 days in EFV
- RDEA806 required multiple nucleotide changes for high level resistance

Figure 3. Mutants Selected by RDEA806 in Wild-type Virus

Consensus Genotype			Total Days									
			K104E		K104E E138K T240I		K104E E138K T240I V179D		K104E E138K T240I V179D F227L		K104E E138K T240I V179D F227L	
Total Days			310	315	351	365	372	380	394	407		
Fold EC ₅₀			2	4	8	16	32	64	128	250		
RT aa #	Wt	Mut	# of clones									
75	V	L										
104	K	E										
138	E	K										
179	V	D										
227	F	L										
240	T	I										
			11	10	10	10	10	10	10	10	10	10

- K104E was the first mutation to emerge, followed gradually by E138K, T240I, V179D and F227L

Figure 4. NNRTI Activity Against RDEA806-selected Mutant Viruses: WT HIV-1



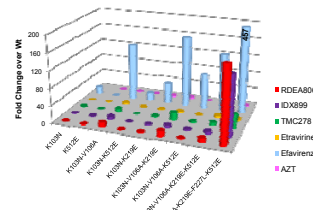
- * ≥ 1 one prior mutation required
** ≥ 3 prior mutations required
- RDEA806 requires at least 3 mutations for > 10x loss in activity
- Other NNRTIs are active against most of the RDEA806-selected mutant viruses
- Viruses with ≥ 3 mutations have very low replication capacity

Figure 5. Mutants Selected by RDEA806 in K103N Virus

Consensus Genotype		K103N		K103N K512E		K103N V106A		K103N V106A K219E		K103N V106A K219E F227L	
Total Days		267	341	351	369	380	391	400	411	421	
Fold EC ₅₀		1	2	4	8	16	32	64	128	250	
RT aa #	Wt/Mut	# of Clones									
103	K/N										
106	V/A										
219	K/E										
227	F/L										
512	K/E										
		21	10	10	10	10	10	10	12	10	10

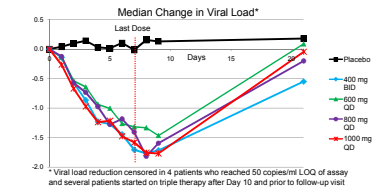
- Starting with K103N, selection of predominant mutations was slow; V106A was followed by K219E, K512E and F227L

Figure 6. NNRTI Activity Against RDEA806-selected Mutant Viruses: K103N HIV-1



- RDEA806 requires at least 4 mutations for significant loss in activity
- Other NNRTIs remain active against the RDEA806-selected mutant viruses, with TMC278 and efavirenz effective against the most resistant virus
- Viruses with ≥ 3 mutations have very low replication capacity

Figure 7. RDEA806 Demonstrated Excellent Viral Load Suppression with No Genotypic Changes in a Phase 2a Monotherapy Trial



- in 36 patients treated with RDEA806, 144 samples were analyzed for RT genotype in the VircoSTYPE HIV-1 assay.
- No genotypic changes characteristic of known NNRTI resistance or of the mutations identified in the *in vitro* resistance study were observed in 36 patients at the end of treatment or two weeks after the last dose.

Conclusions

- In this long-term selection study, RDEA806 provided more durable suppression against WT and K103N viruses than efavirenz.
- The pathways to RDEA806 resistance were different for WT and K103N viruses.
- Other than efavirenz activity on the K103N-containing viruses, most of the viruses selected remained susceptible to other NNRTIs.
- The viruses engineered with the mutation patterns that showed high levels of resistance replicated very poorly.
- The prolonged suppression of viral breakthrough and the requirement for multiple amino acid changes for high level resistance suggest that RDEA806 has a high genetic barrier to resistance.
- Consistent with this *in vitro* study, no genotypic or phenotypic changes occurred during RDEA806-201, a Phase 2a monotherapy study in treatment-naïve HIV-infected individuals (ICAAC Oral Presentation #4707)

Reference
1. Smith D, Momi N, Pessano R, Little S, et al. Clinical utility of HIV-1 genotyping among antiretroviral naive individuals with unknown duration of infection. *Clin Infect Dis* 2007;44:456-8

Disclosures
W. Xu, Z. Zhang, D. Bellows, R. Hamatake are former employees of Ardea Biosciences. B. Groschel, R. Straney, J-L. Girardet, B. Quart and A. Raney are current employees of Ardea Biosciences.