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RDEA806, a Potent NNRTI with a High Genetic Barrier to Resistance

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Abstract

Background: RDEA806 is a novel NNRTI with potent in vitro activity against wild-type HIV (EC_{50}=3nM). NNRTIs are an important class of HIV drugs, but a single amino acid change, such as K103N, can confer resistance to the class. RDEA806 was designed with a flexible arrangement of pharmacophores that allow it to accommodate the amino acid changes associated with NNRTI resistance and may provide a more durable suppression of resistant viruses.
Methods: Fold-changes in susceptibility to NNRTIs were determined using VSV-g pseudotyped HIV-1 containing wild-type and NNRTI-resistant reverse transcriptase (RT) sequences. Selection of resistant virus was performed by serial passage in the presence of increasing concentrations of RDEA806 and efavirenz. RDEA806, efavirenz, and nevirapine were evaluated by ViroLogic (now Monogram) against a panel of 94 clinical isolates resistant to NNRTIs.
Results: Fold-changes (FCs) in EC_{50} against the most common NNRTI-resistant viruses found in patients compared to wild-type are significantly lower for RDEA806 (0.7 – 4-fold) than for efavirenz (16 – 1732-fold). RDEA806 also showed superior activity against a panel of 94 clinical isolates. It took 267 days of culturing in the presence of the EC_{50} concentration of RDEA806 before viral breakthrough was observed. In contrast, viral breakthrough was observed after 90 days for efavirenz. The antiviral profile of RDEA806 also compares favorably to the newer NNRTIs in development, TMC125 and TMC278; the FCs for the most common resistant strains for these agents were 0.2 – 2.0-fold and 0.2 – 12.0-fold, respectively.
Conclusion: (1) RDEA806 is a potent inhibitor of viral replication. (2) RDEA806 is superior to efavirenz against a panel of NNRTI-resistant viruses. (3) The longer time required for emergence of virus resistant to RDEA806 suggests that RDEA806 has a higher genetic barrier to resistance than efavirenz. (4) The antiviral activity of RDEA806 against resistant strains is comparable with the newer NNRTIs in development.

Introduction

Standard HIV therapies consist of combinations of nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs). Although they are generally effective and have resulted in reduced AIDS-related morbidity and mortality, none of them is curative. Treatment failures often occur when viruses that are resistant to one or more components of the regimen arise. Compared to the large numbers of drugs in the NRTI and PI classes, the NNRTI class has only two drugs (efavirenz and nevirapine) in extensive use. There are two important issues that impact the continued use of efavirenz or nevirapine and call for newer drugs in this class: (1) there is a low genetic barrier against resistance development and (2) cross-resistance among approved NNRTIs caused by single amino acid changes, such as the K103N mutation.

- RDEA806 is a novel NNRTI that resulted from optimization of a substituted triazole discovered from high throughput screening (De La Rosa et al (2006) and ICAAC Abstract #3285).
- RDEA806 is highly active against HIV-1 RT and inhibits the RT in an enzymatic assay with an IC_{50} of 2.3 nM.
- Profiling of RDEA806 against a panel of 22 mutant viruses arising from efavirenz treatment failures showed that it maintained its potency against most of these NNRTI resistant viruses.
- Resistance profiling against a larger panel of 94 NNRTI resistant clinical isolates showed that RDEA806 had better activity than either efavirenz or nevirapine against most of these viruses.

Methods

Construction and testing of NNRTI resistant viruses. A panel of 22 clinically prevalent NNRTI-resistant mutation patterns was constructed into the HIV-1 molecular clone pNL4-3.Luc.R-E- by using QuikChange® II XL Site-Directed Mutagenesis. To generate infectious luciferase-expressing virus stocks, a plasmid encoding VSV-G protein was co-transfected with pNL4-3.Luc.R-E-derived plasmids (0.4:2 w/w ratio, respectively) into 293T cells. Supernatants containing virus were collected and aliquots frozen 64 hours post-transfection. Antiviral assays were carried out in 96-well (100 µl) flat-bottom tissue culture plates with 10 µl of 1:4 serially diluted compounds. Five µl of virus was mixed with 85 µl of HeLa-JC53 cells (15,000/well). All cultures were maintained at 37°C and 5% CO_2 for 48 h. Following incubation, an equal volume of Bright-Glo™ reagent (Promega) was added to each well and luminescence was read on an LJL Analyst. The EC_{50} values were determined by non-linear regression using XLfit4 or Prism 4 (Graph Pad).
Selection and determination of RDEA806-resistant mutations. SupT1 cells (2 x 10^6 in 1 ml of RPMI-1640 containing 10% FBS) were exposed to wt NL4-3 virus (MOI=0.05) for 3 hours. The culture was subsequently maintained in 1 ml of growth medium containing 6 nM RDEA806. Every 3 – 4 days, 100 µl of culture was passed to 900 µl of medium containing fresh drug and 9 x 10^5 SupT1 cells. Virus replication was monitored microscopically by observing the formation of syncytia. At each virus breakthrough (massive syncytia formation), the concentration of inhibitor was doubled. Culture media and cell pellets from each breakthrough point were collected. Cellular DNA was purified with Wizard® Genomic DNA Isolation Kit (Promega, Madison, WI). The RT coding region of proviruses were amplified using Platinum® Taq DNA Polymerase (Invitrogen, Carlsbad, CA). The amplified fragments were cloned into TOPO® TA Cloning Vector (Invitrogen, Carlsbad, CA). The RT coding sequences of breakthrough viruses at 6, 12,24, 48, 96, 192, 384, 768, and 1500 nM were determined by sequencing 10 – 12 individual PCR clones from each point.

Results

RDEA806 is Broadly Active Against Efavirenz-resistant Viruses

Virus	Prevalence[a]	Fold-Changes vs. Wild-type (EC_{50})	
		RDEA806 (3.0 nM)[b]	Efavirenz (0.37 nM)[b]
K103N	88.5	0.76	16
K103N-P225H	32.7	3.4	212
K103N-V108I	28.8	0.67	42
K103N-K101Q	16.3	3.8	60
K103N-L100I	10.6	0.53	1732
G190S	10.6	1.1	63
K103N-V108I-P225H	9.6	1.0	190
Y188L	6.7	6.3	130
K101E	4.8	5.4	4.3
K103N-F227L	4.8	1.7	11
V106I-Y188L	4.8	76	742
G190A	3.8	2.9	3.0
K103N-G190A	3.8	7.8	135
K103N-K101Q-P225H	3.8	0.99	250
K103N-V108I-A98G	3.8	2.1	180
K103N-Y188L	3.8	32	Max[c]
A98G	2.9	3.6	3.2
K103N-Y181C	2.9	3.0	23
V106I	1.9	2.0	1.1
Y181C	1.9	3.2	1.6
Y181C-G190A	<1	126	8.7
Y188C	<1	0.94	2.2

[a] Prevalence of mutations from patients failing efavirenz therapy from Bacheler et al. 2000.
[b] $EC_{50\ wt}$
[c] The EC_{50} was above the highest concentration tested, 2,500 nM.

Comparison of RDEA806, TMC125, and TMC278 Against the Most Prevalent NNRTI – Resistant Viruses

Virus	Fold-Change vs Wild-type		
	RDEA806	TMC125*	TMC278*
Wt	1.0	1.0	1.0
K103N	0.76	0.6	1.1
K103N-P225H	3.4	1.5	1.4
K103N-V108I	0.67	0.3	0.7
K103N-K101Q	3.8	1.3	1.4
K103N-L100I	0.53	1.9	10.8
G190S	1.1	0.3	0.2
K103N-V108I-P225H	1	0.8	0.8

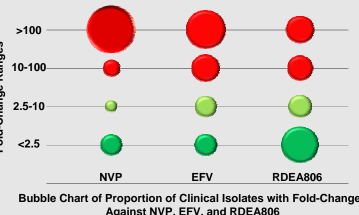
*TMC Compounds were synthesized and tested at Ardea Biosciences

RDEA806 is More Active Against a Panel of Clinical Isolates Containing NNRTI Mutations than Nevirapine (NVP) and Efavirenz (EFV).

Resistance profiling was performed by Monogram Biosciences using the PhenoSense™ HIV Assay.

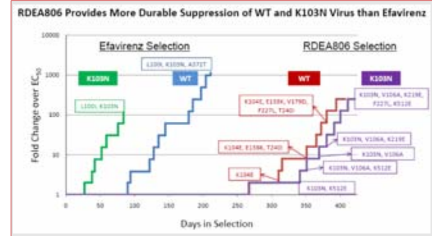
Mutations	Fold-Changes vs wild- type (EC_{50})		
	NVP (225 nM)	EFV (2.8 nM)	RDEA806 (10 nM)
None (wild- type)	2.3	1.4	1.1
103N	51.0	14.0	0.5
103N, 225H	>206	162.0	1.8
103N, 225H	93.0	89.0	1.1
103N, 108I	136.0	172.0	1.6
101Q, 103N	>206	133.0	4.1
100I, 103N	42.0	>253	0.7
190S	>201	97.0	2.3
103N, 108I, 225H	>201	>247	2.9
188L	>206	86.0	8.0
101E	42.0	16.0	9.3
106I, 188L	>201	232.0	34.0
190A	>206	18.0	11.0
103N, 190A	>206	>253	68.0
101Q, 103N, 225H	>229	>264	6.7
98G, 103N, 108I	>201	148.0	1.4
103N, 188L	>206	>253	3.7
98G	5.9	2.9	1.6
103N, 181C	>206	212.0	16.0
106I	1.1	0.7	0.8
181C	>206	6.7	15.0
181C, 190A	>206	14.0	539.0
101Q	2.8	2.5	2.4
179D	4.2	5.7	3.5
108I	2.3	1.7	1.4
103N, 181C, 190A	>206	241.0	393.0
101E, 190A	>206	>253	99.0
103N, 238T	108.0	31.0	0.6
98G, 103N	>206	220.0	2.5
101P, 103N	>206	>253	1.6
179E	1.9	4.1	2.2
103N, 108I, 181C	>206	72.0	10.0
106A	>206	5.7	6.7
108I, 181C	>206	5.3	38.0
101E, 181C, 190A	>206	>253	280.0
179A	0.4	0.5	0.6
106A, F227L	>206	>253	>563
98G, 181C	>206	17.0	21.0
101Q, 181C, 190A	>206	43.0	408.0
101E, 190S	>201	>247	11.0
190E	>201	>247	>500
103N, 106A	>201	26.0	4.5
179G	0.1	0.2	0.2
227L	2.10	0.80	0.66
103R, 179D, 188L	>201	>247	261.0
101E, 181C	>201	118.0	280.0
103N, 106M	>201	>247	6.9
103N, 106I	122.0	28.0	1.3

Mutations	Fold-Changes vs wild- type (EC_{50})		
	NVP (225 nM)	EFV (2.8 nM)	RDEA806 (10 nM)
None (wild-type)	2.3	1.4	1.1
103S, 190A	>201	>247	>500
181I	>201	1.4	5.1
179T	0.5	0.8	1.0
101E, 181C, 190S	>201	>247	>500
101Q, 103R	1.3	1.2	0.5
103R, 179D	1.3	0.9	0.3
101P, 190A	>201	>247	99.0
101R, 103N	189.0	37.0	1.0
101Q, 190S	>201	>247	1.4
98G, 190A	>201	64.0	9.8
106A, 190A	>201	181.0	374.0
101Q, 181C, 190S	>201	>247	320.0
101E, 103N	145.0	52.0	0.9
101Q, 181C	>201	4.4	17.0
236L	0.5	0.2	0.8
101H, 190A	>201	77.0	28.0
98G, 188L	>201	>247	131.0
98G, 103N, 181C	>201	172.0	4.7
101E, 108I, 181C, 190A	>201	>247	>500
103N, 108I, 238T	>201	230.0	1.8
98G, 100I, 103N	>201	>247	1.0
100I, 103N, 108I	>229	>264	1.4
103N, 188H	>229	256.0	2.5
188H	178.0	8.7	1.2
98G, 101E, 181C, 190A	>229	71.0	393.0
103Q	1.0	1.7	1.2
101H	12.0	4.1	2.2
103N, 108I, 181C, 190A	>229	>264	>549
179D, 188L	>229	>264	262.0
103N, 108I, 190A	>229	>264	23.0
108I, 181C, 190A	>229	40.0	>549
98G, 103N, 181C, 190A	>229	>264	494.0
103N, 190S	>229	>264	16.0
106A, 181C	>229	8.2	109.0
98G, 108I, 181C	>229	47.0	26.0
238N	1.9	0.8	0.6
238T	10.0	3.0	1.9
103N, 238N	>229	>264	1.6
103S	39.0	8.6	1.9
230L	46.0	16.0	8.1
101R, 103N, 181C, 190A	>229	>264	>549
101E, 103N, 190A	>229	>264	69.0
101N, 103N	32.0	26.0	0.6
181V	>229	4.5	16.0
106L, 188L	>229	>264	80.0
179D, 181C	>229	216.0	189.0
100F	2.4	1.6	1.5



Bubble Chart of Proportion of Clinical Isolates with Fold-Changes Against NVP, EFV, and RDEA806

RDEA806 Provides More Durable Suppression of WT and K103N Virus than Efavirenz



Time Course of Viral Breakthrough and Determination of Viruses Resistant to RDEA806. SupT1 cells were infected with wild-type or K103N HIV-1 in the presence of the EC_{50} concentration of RDEA806 or EFV. The concentrations were doubled when viral breakthrough occurred. RT mutations present in at least 40% of cloned sequences are indicated at the observed times for breakthrough.
Selection of viruses resistant to RDEA806 required 267 days of culturing in media containing RDEA806 at the EC_{50} level for viral breakthrough. Under the same conditions, viral breakthrough occurred in 90 days with efavirenz. The longer time for the emergence of resistant viruses and the requirement for multiple nucleotide changes for high level resistance suggests that RDEA806 has a high genetic barrier to resistance.

Conclusions

- RDEA806 is a novel NNRTI that is highly active against wild-type and many NNRTI-resistant HIV-1 viruses
- RDEA806 is more active than efavirenz against a panel of NNRTI-resistant viruses found in patients failing efavirenz therapy
- RDEA806 is more active than nevirapine and efavirenz against a panel of clinical isolates containing the most prevalent NNRTI-resistant mutation patterns
- RDEA806 has fold changes against the most common NNRTI-resistant viruses comparable to or better than TMC125 and TMC278
- The prolonged time for suppression of viral breakthrough suggests that RDEA806 has a high genetic barrier to resistance
- Low potential for drug-drug interactions (ICAAC Abstract #3390) and good safety and PK in humans (ICAAC Abstract #1609) make RDEA806 an attractive candidate for further development

References

1. De La Rosa M., H.-W. Kim, E. Gunic, C. Jenket, U. Boyle, Y.-H. Koh, I. Korboukh, M. Allan, W. Zhang, H. Chen, W. Xu, S. Nilar, N. Yao, R. Hamatake, S. Lang, Z. Hong, Z. Zhang, and J.L. Girardet. 2006 *Bioorg. Med. Chem. Lett.* 16:4444-4449
2. Bacheler LT, ED Anton, P Kudish et al. 2000 *Antimicrob. Agents Chemother.* 44:2475-2484