

# Novel Tricyclic Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) with Improved Resistance Profiles

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## ABSTRACT

**Background:** NNRTI therapy has become a key component of HAART for the treatment of HIV infection. However, increasing resistance to currently marketed NNRTIs has been reported in clinical studies. The goal of this work was to identify agents that maintain potency against a range of clinically relevant mutated viruses while maintaining *in vivo* characteristics suitable for once-daily administration.

**Methods:** A series of 5,10-dihydrobenzo[*b*][1,8]naphthyridine N-oxides was synthesized and optimized for potency against WT virus and a panel of clinically relevant single and double mutant isolates of HIV-1. Serum protein binding and *in vivo* pharmacokinetic properties were assessed for compounds that met target potency criteria.

**Results:** Substituent-group optimization led to a series of compounds with potent antiviral activity against a panel of HIV-1 variants. The 7-fluoro-5-cyclopropylmethoxy-5-trifluoromethyl derivative with antiviral IC<sub>50</sub>s of 4.2 nM, 42 nM and 17 nM against wt, K103N/L100I and K103N/Y181C, respectively, an unbound fraction in human serum of 7.8%, and excellent animal pharmacokinetics was advanced into Phase I clinical trials with a target trough concentration of 410 nM. Development of this candidate was discontinued in response to adverse cardiovascular events in the clinic.

**Conclusions:** Medicinal optimization of a series of tricyclic NNRTIs led to the identification compounds with improved activity profiles against clinically relevant HIV mutant variants relative to currently marketed NNRTIs at clinically achievable concentrations.

## INTRODUCTION

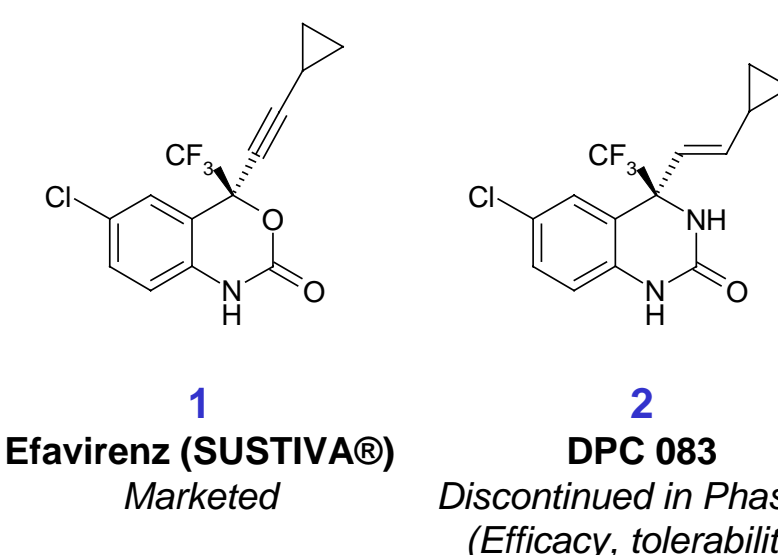
Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have become a key component of HAART therapy for the treatment of HIV infection. Combination therapy with other classes of anti-retroviral agents (nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs)) have yielded successful treatment regimens. Efavirenz (SUSTIVA®) has been approved by the FDA as an alternative first-line therapy to PI-containing regimens when used in combination with two NRTIs.

However, even with combination treatment virological failure occurs in ~20% of patients per year, concomitant with the appearance of resistant virus.

Some of the arising viral mutants, most notably K103N, have reduced susceptibility to the entire NNRTI class, precluding effective sequencing of NNRTI-containing therapies. The transmission rate of the K103N mutant virus to newly infected individuals is now estimated at 12.5%. Efforts have continued to identify new NNRTIs which maintain potency against a range of clinically relevant mutated viruses including variants containing the K103N mutation alone or in combination with other mutations while preserving ease of administration and maintenance of good overall tolerability.

The objective of this work was to build on our experiences with efavirenz (1) and related analogs such as DPC 083 (2) with the aim of identifying development candidates with virological profiles superior to existing agents and with *in vivo* characteristics suitable for once-daily dosing.

## Bicyclic NNRTIs



## Criteria for Effective Antivirals

- Sufficient potency against clinically relevant viruses

AND

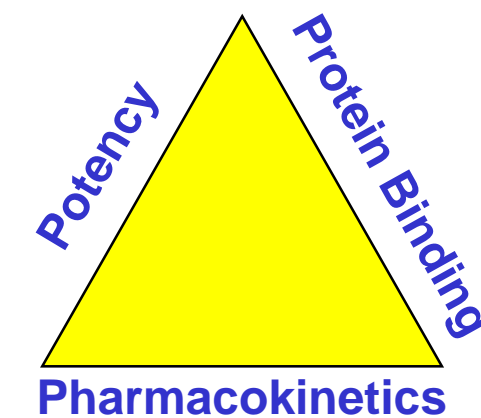
- Pharmacokinetics that produce sufficient total drug at trough

AND

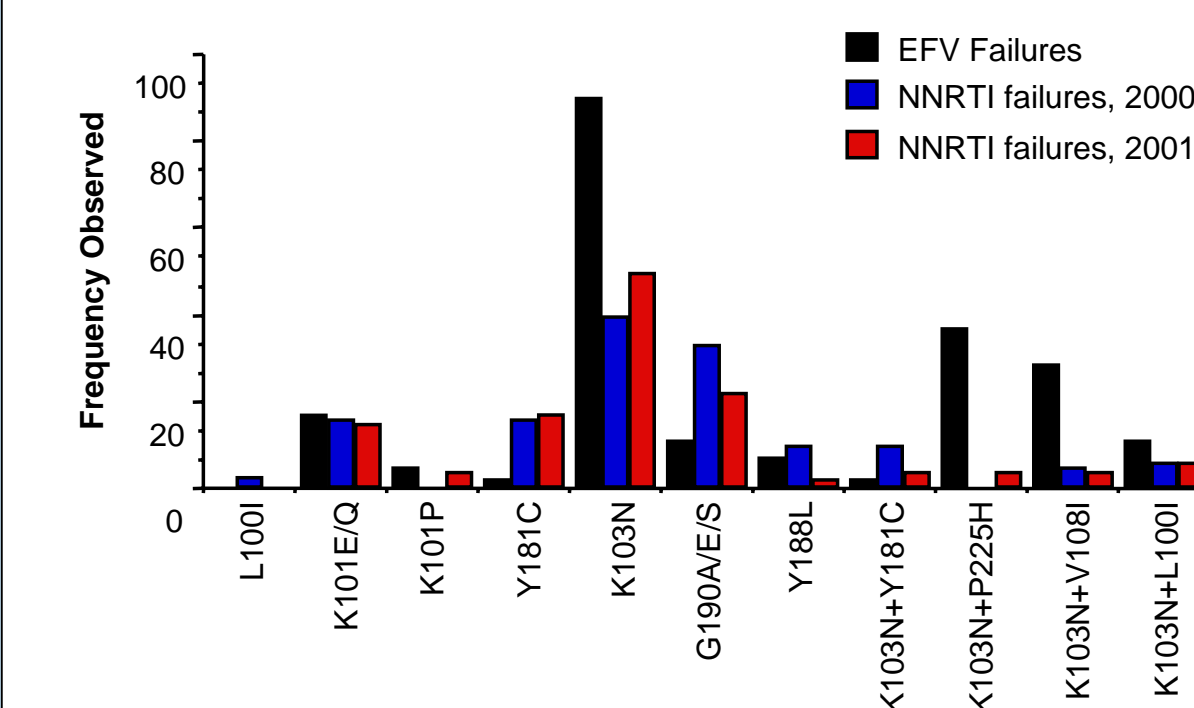
- Protein binding characteristics that result in sufficient free drug at trough

SO THAT

- Free drug at trough exceeds free drug concentration required for viral suppression



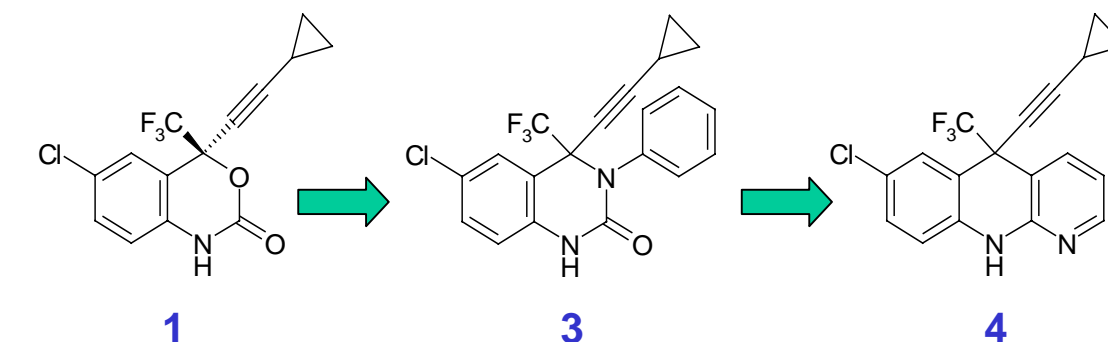
## Clinically Relevant Mutations for NNRTIs



## Mutations Cluster Around the Binding Site

**Observation:** Most mutations in the NNRTI pocket involve changes to smaller amino acid residues; **increasing pocket size**

**Hypothesis:** Larger NNRTIs might improve potency against mutant viruses by more effectively filling the mutant enzyme pocket



## Azaacridine Ring System

Fuse the phenyl ring to the core  
Use a heteroatom as the hydrogen bond acceptor moiety

## Improved Resistance Profile

ID	WT	K103N	K103N/L100I
1	2	51 (25x)	6600 (3300x)
3	19	90 (5x)	460 (24x)
5	8.7	4.5 (0.3x)	194 (15x)

(fold loss)

- Azaacridine exhibited flatter resistance profile
- No loss to the K103N virus

## Effect of Size of 5-Alkoxy Moiety

R	IC <sub>50</sub> (nM)		
	WT	K103N	K103N/L100I
-CH <sub>3</sub>	3.2	16	1700
-CH <sub>2</sub> CH <sub>3</sub>	5.2	3.4	300
-CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	7.9	2.9	230
-CH <sub>2</sub> cycPr	12	4.5	190
-CH <sub>2</sub> cycBu	24	7.0	77
-CH <sub>2</sub> Ph	6.7	9.5	710

## Larger side chain:

- Decreases potency against wild type virus
- Increases potency against mutant viruses

## Azaacridine N-Oxides

X	R	WT IC <sub>50</sub> (nM)	K103N'	Y188L'	K103N/L100I'
Cl	N-propyl	8.6	0.67x	5.4x	
Cl	cyclopropylmethyl	10	0.68x	4.2x	17x
F	cyclopropylmethyl	8.8	0.66x	5.5x	24x
F	cyclobutylmethyl	4.6	4.9 x	33 x	

\*Fold Loss

- N-oxide: Maintains wild type activity of original Azaacridines
  - Improved potency against mutant viruses
  - Improved aqueous solubility
  - Improved PB free fraction
- Cyclobutylmethoxy moiety now too big for mutant virus

## Azaacridine SAR Summary

Resistance: n-Bu, -OR, -CH<sub>2</sub>OR > -CH<sub>2</sub>NHR > -CH<sub>2</sub>NR<sub>2</sub> > -NHR  
PB: Reverse order of resistance

Resistance: -CF<sub>3</sub> > -CH<sub>2</sub>CF<sub>3</sub> > -CF<sub>3</sub>H  
PB: Reverse order of resistance

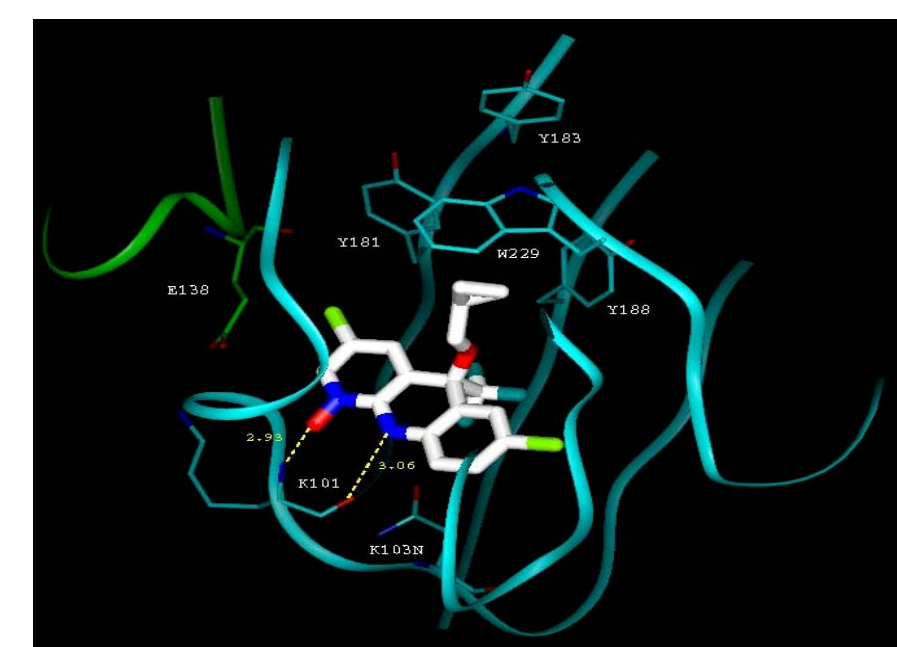
Resistance: -Cl > -H  
PB: Reverse order of resistance  
PK: -Cl > -H

Resistance: -Cl > -F, -CN  
PB: -CN > -F > -Cl  
PK: Reverse order of resistance

Resistance: N-oxide > pyridine  
PB: N-oxide >> pyridine  
PK: N-oxide >> pyridine

- Best groups for resistance are lipophilic - except N-oxide
- PB Free fraction correlates with polarity
- Need to balance activity and polarity for optimal compound**

## Azaacridine N-Oxide Bound to HIV-1 RT



## Establishing Target Trough Levels

- NNRTIs of the efavirenz, DPC 083 and azaacridine classes bind to serum proteins

- Bind to the serum present in tissue culture medium (typically 47% to 80%)

- The effect of this protein binding is to effectively inflate the observed IC<sub>50</sub> values

- Bind to protein in human serum

- The effect of this protein binding is to effectively decrease the drug available to enter cells

- DEFINE PLASMA IC<sub>90</sub>: The concentration of total drug in the plasma required for 90% inhibition of a particular virus. Determined by:

$$\text{Potency} \times \text{Free fraction in culture media} = \text{Free fraction in human plasma}$$

## N-Oxides: Improved Pharmacokinetics

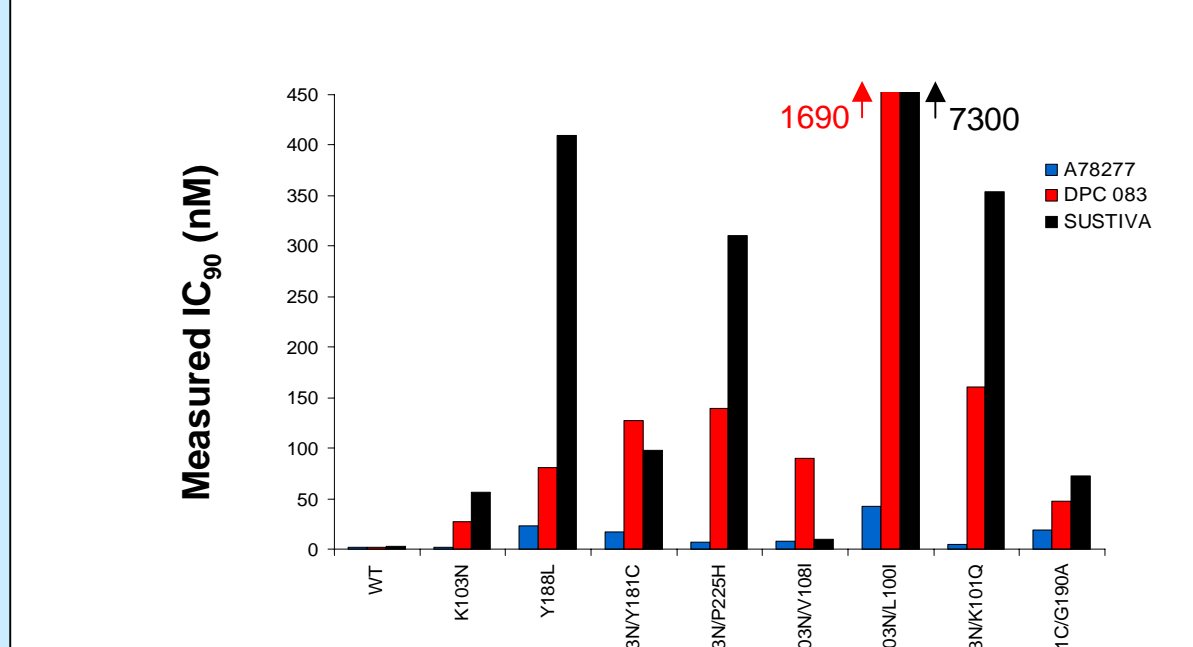
Structure	HPLC log P	Solubility pH 7.4 (µg/mL)	% free human plasma	WT IC <sub>50</sub> (nM)	K103N/L100I IC <sub>50</sub> (nM)	Plasma IC <sub>90</sub> K103N/L100I (nM)	C24h Chimp 10 mg/kg (nM)
	4.9	<-0.1	<-0.2	7	73	10,000	BQL
	4.2	13	3	3	51	1000	60
	3.8	47	7.8	4.2	42	410	750

Target level exceeded

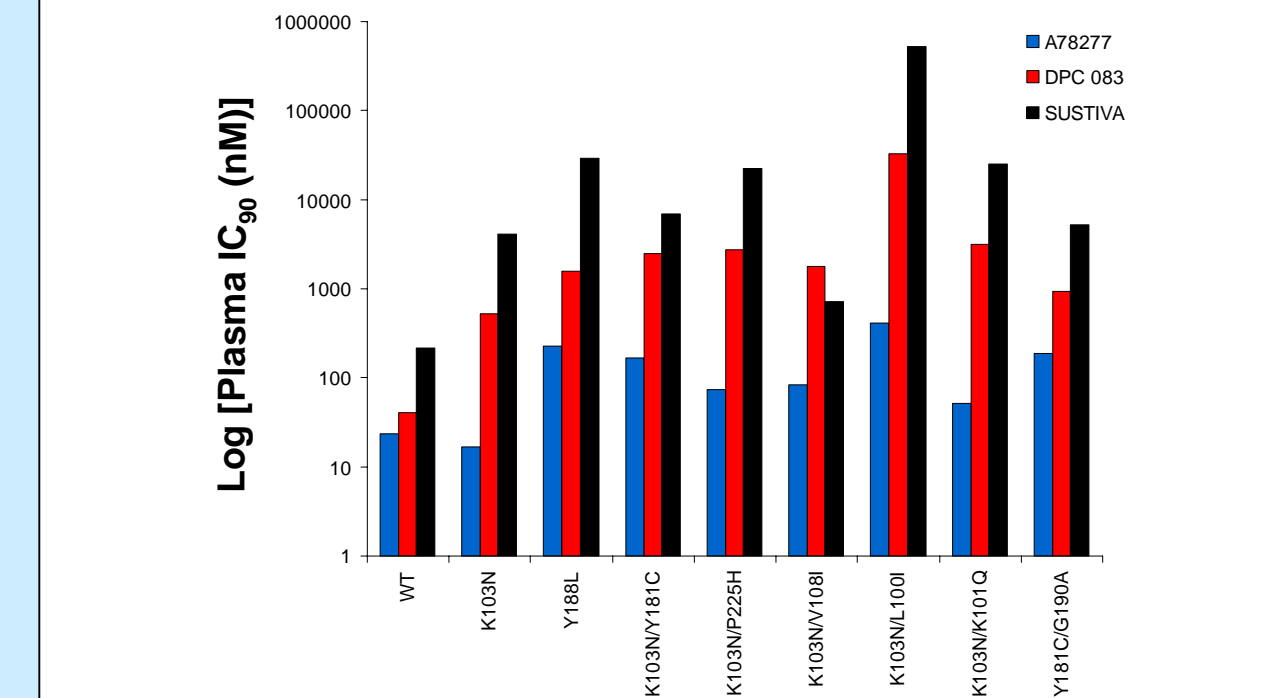
## Virological Profile of DPC-A78277

Virus	IC <sub>50</sub> (nM)	Fold Increase over wt
NL4-3 or HXB2	2.4	
RF	3.6	
IIIB	2.5	
L100I	2.7	0.8x
K103N	1.7	0.7x
Y188L	23	9.6x
K103N/Y181C	17	6.8x
K103N/P225H	7.5	3.1x
K103N/V108I	8.5	1.5x
K103N/L100I	42	17x
K103N/K101Q	5.3	2.2x
Y181C/A98G	7.9	3.3x
Y181C/L190A	19	7.9x
K103N/Y181C/G190A (trip)	150	62.5x
K101P	8480	3500x
HIV-2	2820	

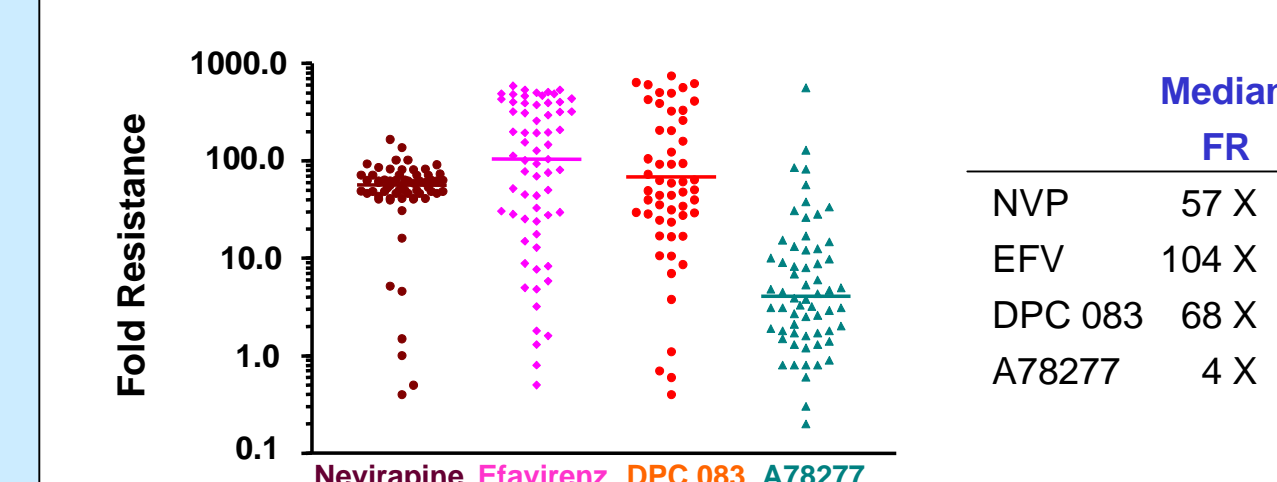
## Relative Potency of DPC-A78277, DPC 083 and SUSTIVA®



## Relative Plasma Potencies of DPC-A78277, DPC 083 and SUSTIVA®

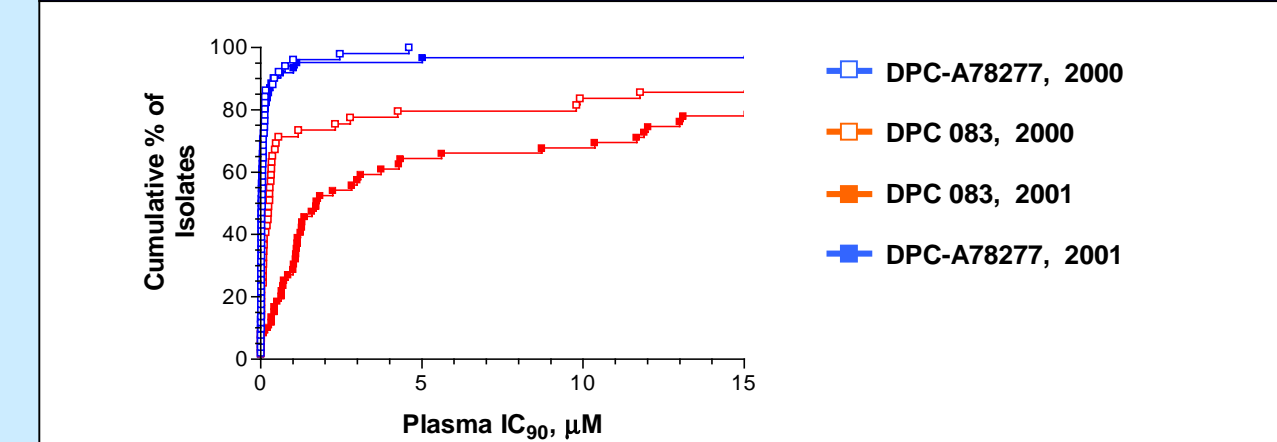


## Activity Against Resistant Clinical Isolates



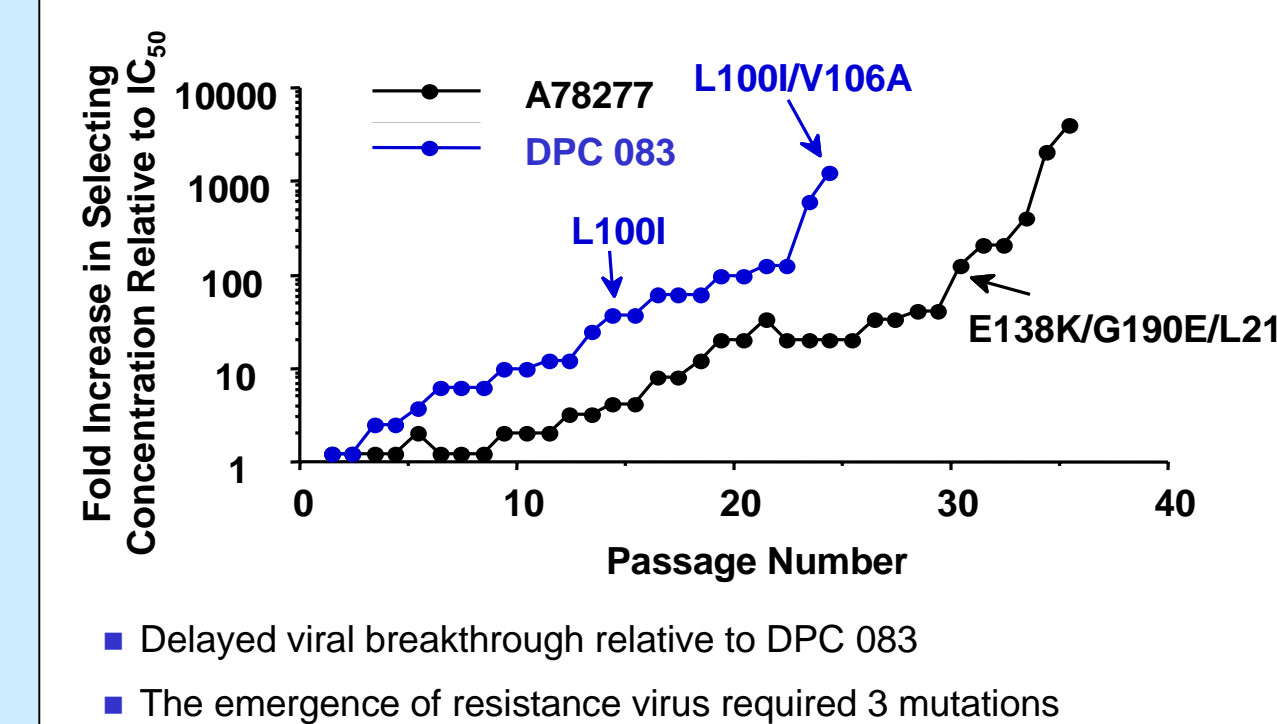
Activity versus a panel of 64 isolates derived from patients failing a NNRTI therapy in 2001

## Maintenance of Activity Against Increasingly Resistant Viral Isolates



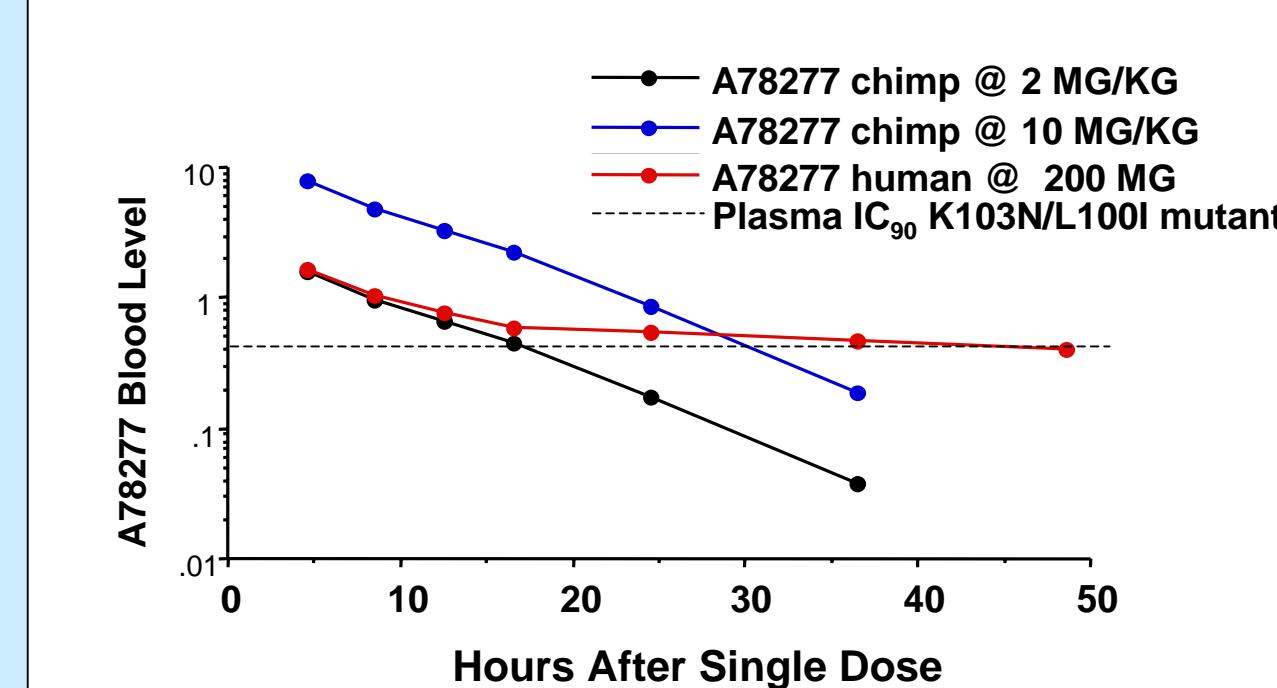
Measured IC<sub>50</sub> values were converted to Plasma IC<sub>90</sub> values based on protein binding to human serum and tissue culture medium. The cumulative percentage of recombinant isolates with a given Plasma IC<sub>90</sub> was plotted. At the target level ~ 90% of isolates from the 2000 Panel and > 85% of the isolates from the 2001 panel should be suppressed by DPC-A78277.

## In Vitro Selection with DPC-A78277



- Delayed viral breakthrough relative to DPC 083
- The emergence of resistance virus required 3 mutations

## Human Pharmacokinetics of DPC-A78277



- Chimp provides an adequate approximation of human PK
  - DPC-A78277 had a longer t<sub>1/2</sub> in humans (37 h cf. 5.7 h in chimp)
- Phase I data in healthy male volunteers suggest a 200 mg dose of DPC-A78277 QD would be sufficient to supply trough levels in excess of the K103N/L100I double mutant IC<sub>90</sub> value

## SUMMARY

- Increasing the size of the NNRTI scaffold provided a series of compounds with improved resistance profiles
- Balancing protein binding, pharmacokinetics and resistance led to the identification of DPC-A78277 as first clinical candidate in this series
- DPC-A78277 exhibited a flat virological profile against the clinically relevant single and double mutant viruses of HIV-1 with significant improvements in activity over DPC 083 and SUSTIVA®
- In vitro* selection experiments identified a triple mutant with A78277 resistance after 30 passages (E138K/G190E/L214F) indicating a potential increased genetic barrier over DPC 083
- Human PK studies indicated that DPC-A78277 had a long half in humans (37 h) and that the 200 mg dose would provide adequate trough levels to cover the plasma IC<sub>90</sub> of the K103N/L100I mutant
- DPC-A78277 was dropped from clinical development due to cardiovascular effects observed in Phase I

## ACKNOWLEDGEMENTS

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