

The HIV-1 RT Mutant Q151L Shows Decreased Replication Capacity, Selective High-Level Resistance to GS-9148 and Hypersusceptibility to Tenofovir and Zidovudine

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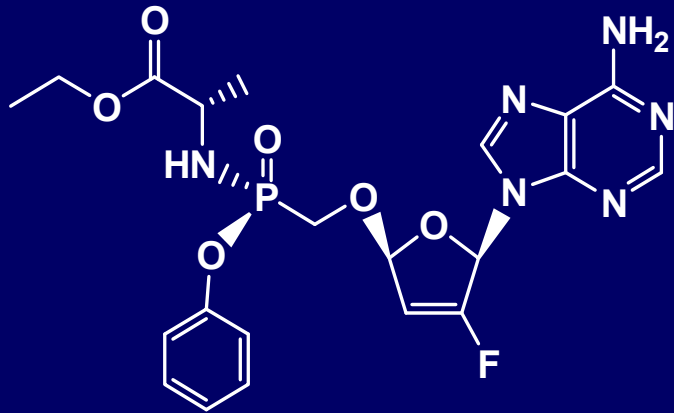
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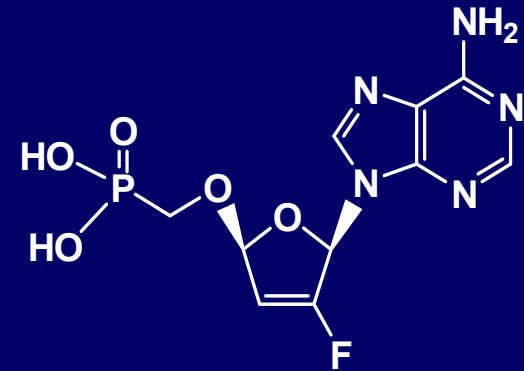
Abstract No. 18

GS-9148 is an HIV N(t)RTI



GS-9131 Prodrug

$EC_{50} = 0.018 \mu\text{M}$
(PhenoSense Assay)



GS-9148

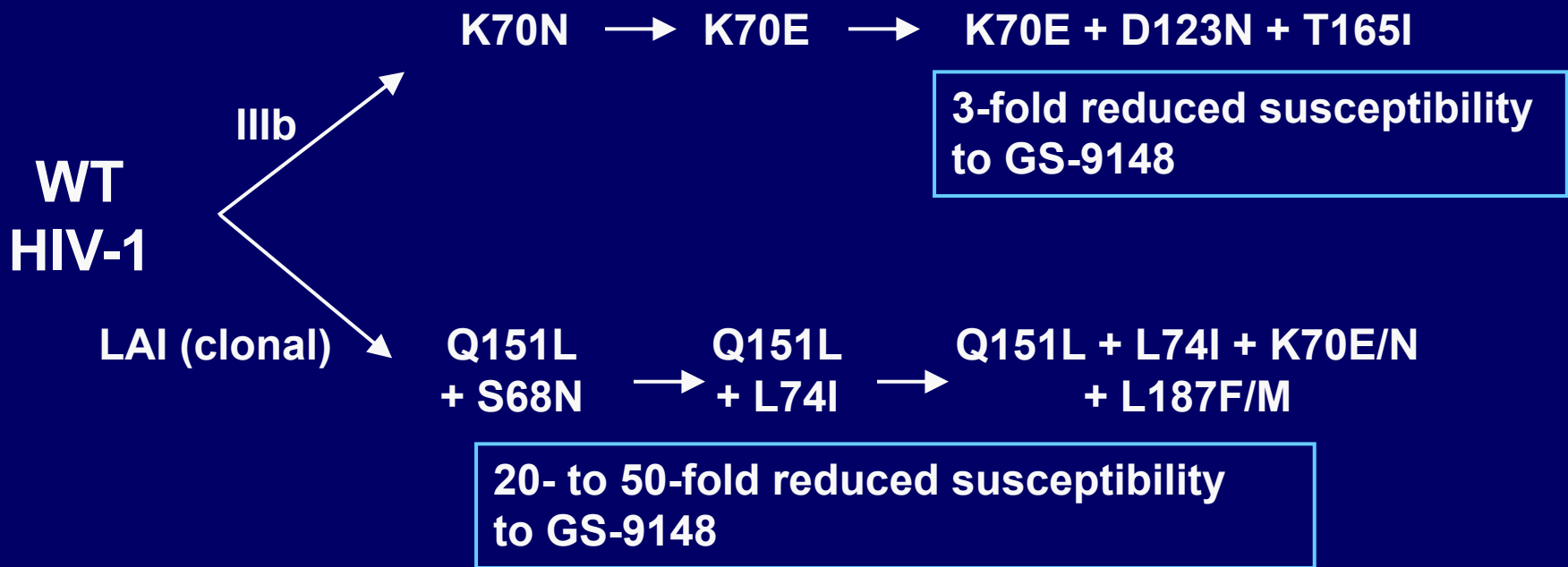
$EC_{50} = 3.39 \mu\text{M}$
(PhenoSense Assay)

- **GS-9148 maintains potent activity against a majority of clinically observed resistance mutations including M184V, K65R, L74V, and ≥ 4 TAMs**
- **GS-9148 has low-level cross-resistance to Q151M + complex**

Objectives

- **To further characterize the resistance that develops to GS-9148 *in vitro***
- **To evaluate the cross-resistance of the selected mutations to other N(t)RTIs**
- **To characterize the developing resistance mutations for their impact on viral replication capacity**

Two Pathways of GS-9148 Resistance *in vitro*



- Q151L is a possible intermediate to Q151M, but is infrequent in patients
- Q151M was not observed during prolonged selection with GS-9148

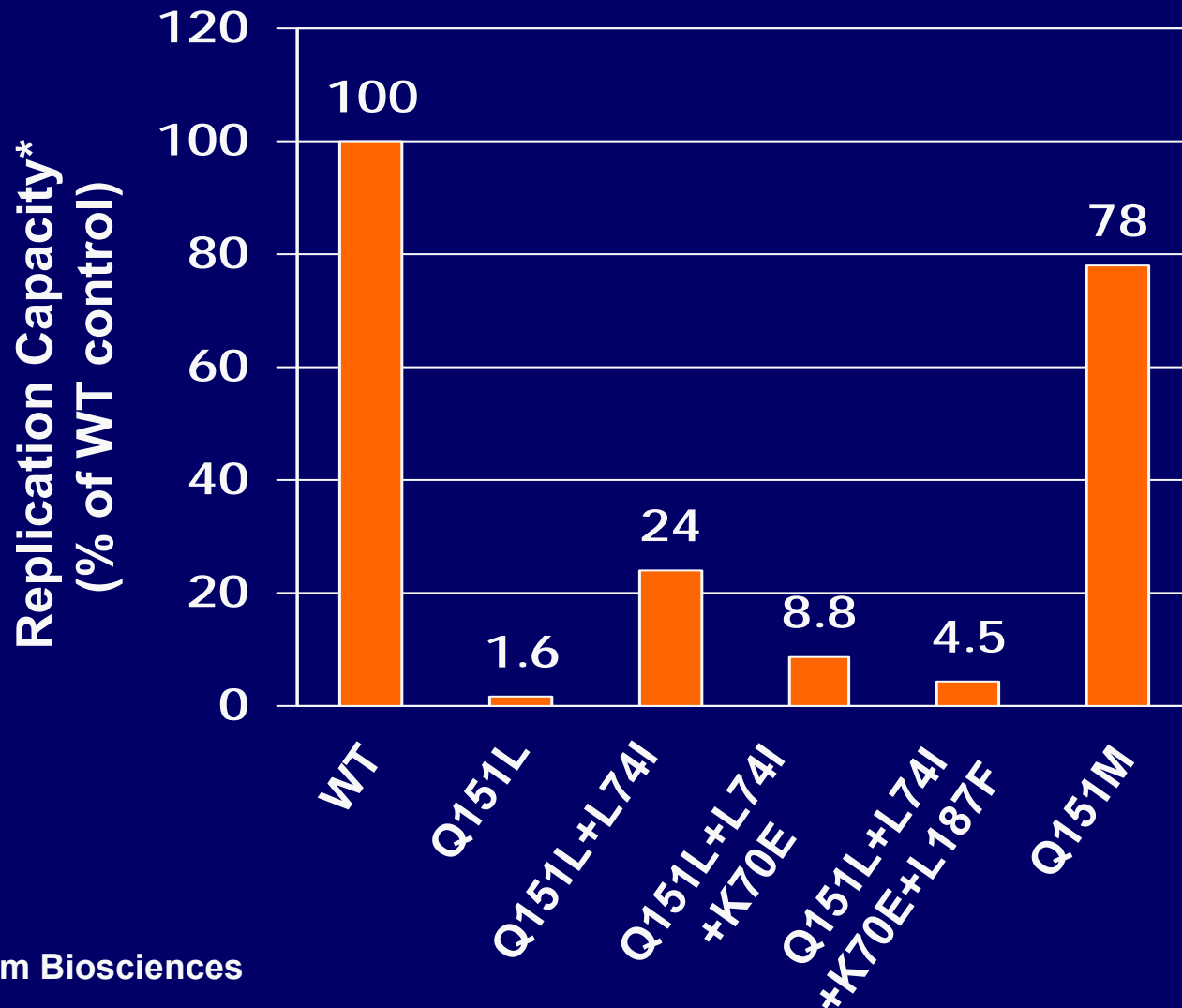
Susceptibility Profile of Q151L Mutants (PhenoSense Assay)

HIV-1 Genotype	GS-9148	GS-9131	TFV	ddI	ABC	FTC	3TC	AZT	d4T
Q151L	12	17	< 0.1	1.2	0.6	1.1	0.5	0.1	0.7
Q151L + L74I	7.7	8.5	0.1	1.1	1.0	1.8	0.9	0.1	0.8
Q151L + L74I + K70E	32	50	0.1	1.4	1.5	3.9	0.8	0.1	1.3
Q151L + L74I + K70E + L187F	36	49	0.1	1.3	1.1	3.4	1.1	0.1	1.1

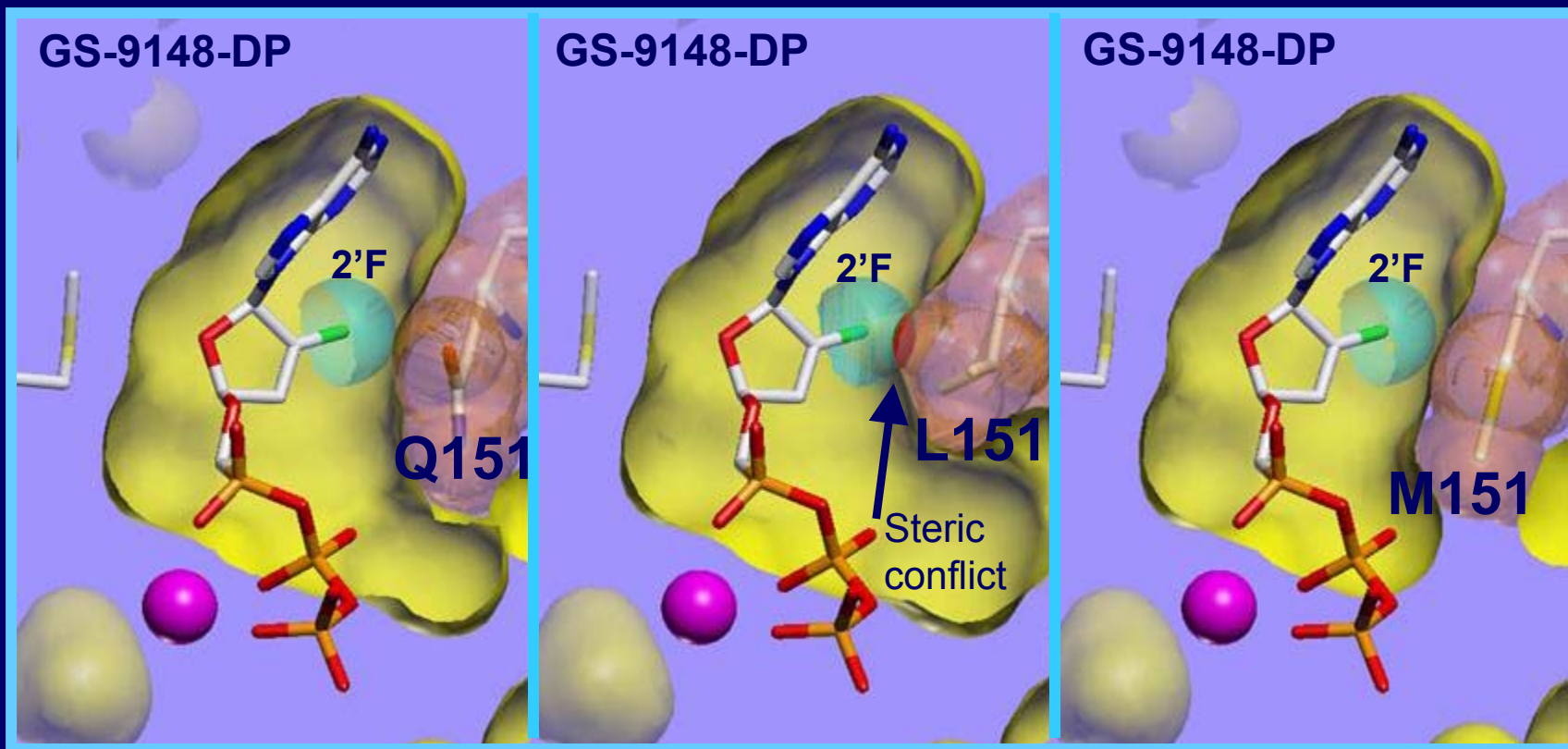
a. Fold change in EC₅₀ vs. NL4-3.

- Q151L shows specific, high-level resistance to GS-9148 and GS-9131
- Q151L is hypersusceptible to TFV and AZT

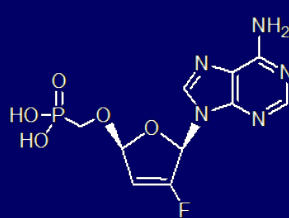
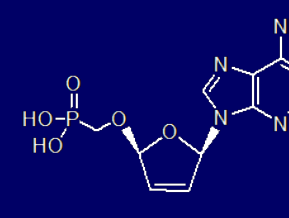
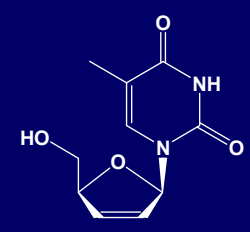
Q151L Shows a Severe Replication Defect



Molecular Models Show the 2'Fluoro of GS-9148-DP Has Steric Conflict With Q151L



Reduced Susceptibility to GS-9148 by Q151L Mutant HIV Maps to the 2'Fluoro Moiety

	Fold Change in EC ₅₀ vs. wild-type ¹		
	GS-9148	d4AP	d4T
Structures			
Q151L	15.2**	2.2*	1.4
Q151L+L74I	24.6**	1.5	1.1**
Q151L+L74I+K70E	>40.5**	2.2*	1.2
Q151L+L74I+K70E+L187F	>39.2**	3.1**	2.9**
Q151M	2.7**	5.6**	17.6*

1. In-house susceptibility assay in MT-2 cells.
2. p-value from Student's t-test: p≤0.01 (**); p≤0.05 (*).

Conclusions

- HIV with a Q151L mutation in RT was selected after >100 days of GS-9148 treatment from clonal LAI
 - Q151L showed ~ 20-fold reduced susceptibility to GS-9148 and GS-9131
 - Q151L was hypersusceptible to TFV and AZT
 - Q151L reverted to wild-type with TFV or AZT selection pressure and evolved to Q151M in the absence of drug
 - Q151M shows lower resistance to GS-9148 than Q151L, potentially explaining its lack of appearance during prolonged *in vitro* selection
- Q151L showed strongly decreased replication capacity *in vitro*
 - Replication capacity increased with the addition of other mutations
- From structural studies, the unique 2' fluoro moiety of GS-9148 appears to be directly hindered by Q151L resulting in high-level resistance to GS-9148

Acknowledgements

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