Product name: **INVIRASE**
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SCIENTIFIC DISCUSSION

Detailed description of Invirase antiviral activity *in vitro*

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Antiviral activity *in vitro*:

Saquinavir demonstrates antiviral activity against both laboratory strains and clinical isolates of HIV-1 with typical EC_{50} and EC_{90} values in the range 1–10 nM and 5–50 nM, respectively, using acutely infected T cell lines or primary human lymphocytes/monocytes. In vitro antiviral activity was observed against a panel of HIV-1 group M non-clade B isolates (A, AE, C, D, F, G and H) and HIV-2 with EC_{50} values ranging from 0.3-2.5 nM. In the presence of 50% human serum or alpha-1 acid glycoprotein (1 mg/ml) the antiviral activity of saquinavir decreases by an average factor of 25-fold and 14-fold respectively.

Table 1 Activity against Laboratory and Clinical Isolate Wild-Type Viruses

Parameters	Median EC_{50} (nM)	Range (nM)	Median EC_{90} (nM)	Range (nM)
Laboratory Virus Data [a]				
GB8 (n=12)	2.7	1.1-7.0	14	3.9-28 (n=11)
RF (n=2)	4.0	2-6	0.9	0.9
MN (n=1)	4.0	4	22	22
NIT (n=2)	15	1.5-28	3.1	3.1 (n=1)
HXB2 (n=1)	1.7	1.7	8.9	8.9
BaL (n=2)	11.0	1.4-20	102	4.6-200
ROD (n=1)	4.0	4	n/a[e]	n/a
IIIB (n=2)	9.0	1.7-14	3.0	0.3-6.4
Serum Shift Data				
50% Human Serum (n=3) [b]	250	180-680 (0% HS: 9-21)	n/a	n/a
Clinical Isolate Data				
Subtype A (n=5) [c]	0.9	0.9-1.3	n/a	n/a
Subtype AE (n=5) [c]	1.3	1.2-1.4	n/a	n/a
Subtype B (n=5) [c]	1.4	1.1-1.7	n/a	n/a
Subtype C (n=5) [c]	1.5	1.0-1.6	n/a	n/a
Subtype D (n=5) [c]	1.4	1.0-1.9	n/a	n/a
Subtype F (n=5) [c]	1.7	1.3-2.2	n/a	n/a
Subtype G (n=5) [c]	1.3	1.1-2.3	n/a	n/a
Subtype H (n=2) [c]	2.0	1.6-2.5	n/a	n/a
HIV-2 (n=6) [d]	1.2	0.3- 2.4	n/a	n/a

a. Laboratory strains of HIV-1 using a multi-cycle assays in acutely infected T cell lines or primary human lymphocytes/monocytes and p24, MTT, RT, or syncytia readout performed in five different laboratories (Roche Report No. W-142331)

b. Phenotypic Assay: Serum shift in EC_{50} observed with laboratory viruses IIIB, NL4-3, and HXB2 in multicycle infection of MT4 cells in the presence to 50% human serum and 10% fetal bovine serum (Molla 1998)

c. Phenotypic Assay: Single cycle assay with protease cloned into NL4-3 expression vector (Heilek-Synder 2004)

d. Phenotypic Assay: Multi-cycle assay with clinical isolates infecting PBMCs (Heilek-Synder 2004)

e. n/a – data not available

***In vitro* resistance:**

In vitro selection of resistance from wild type HIV-1 virus:

The most commonly reported mutations observed to develop during in vitro passage of HIV-1 wild type virus in the presence of increasing concentrations of saquinavir are G48V and L90M. Recombinant virus harbouring the G48V or L90M mutations respectively, exhibited 7.9 and 3.3-fold reduced susceptibility to saquinavir. Additional protease mutations observed to develop less frequently were M36I, I54V, K57R, and L63V.

***In vivo* resistance:**

Treatment naïve patients: Four studies have investigated ritonavir boosted saquinavir regimens in ART naïve patients (saquinavir/ritonavir 1600 mg/100 mg once daily n=349; 1000 mg/100 mg twice daily n=92). Baseline and on-therapy resistance analyses were available for 26 patients experiencing virological rebound, and not harbouring resistance mutations at baseline (n=1) or developing signature protease mutations associated with other PIs (n=1). Virus from two patients developed protease mutations (M36I and M46i/m respectively) not typically associated with saquinavir resistance. No saquinavir-associated protease mutations were observed to develop following virological failure.

Treatment experienced patients: Baseline and on-therapy genotype was available for 22 previously PI-experienced patients experiencing virological failure after receiving a ritonavir boosted saquinavir regimen (MaxCmin1 & 2 studies; 1000/100mg twice daily, n=171). Virus from eight (8/22; 36%) patients developed additional protease mutations following virological failure. The relative incidence of each mutation was: I84V (n=4, 18%); F53L, A71V or G73S (n=2, 9%); L10V, M46I, I54V, V82A or L90M (n=1, 4.5%).