

SYNERGISTIC ACTIVITY OF ATAZANAVIR AND SAQUINAVIR ON VIRUSES WITH LOW SAQUINAVIR AND HIGH ATAZANAVIR RESISTANCE

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Introduction

The use of combinations of two protease inhibitors (PIs) boosted with low-dose ritonavir (*r*) is currently being investigated as an option for patients who are intolerant to nucleoside reverse transcriptase inhibitors or who harbour highly resistant virus.^{1,2} Saquinavir (SQV)/*r* has been shown to be potent and well tolerated, with a relatively benign lipid profile.³ Atazanavir (ATV) is a newly available PI possessing a unique resistance⁴ and favourable lipid profile.^{5,6} Co-administration of SQV/*r* and ATV has been suggested as a possible combination in treatment-experienced patients.² This is supported by the encouraging pharmacokinetic profile of SQV/ATV/*r*, which results in significantly increased exposure to SQV and ATV compared with each boosted single PI.^{2,7} We have previously reported a significant synergistic interaction between SQV and lopinavir (LPV) against HIV-1 with high resistance to LPV and lower resistance to SQV.⁸ We hypothesize that this phenomenon is due to enhancement of the intracellular activity of SQV by LPV via a pharmacological mechanism, an effect that can only be measured if the target virus retains sufficient susceptibility to SQV together with high resistance to the enhancing drug.

Objective

The objective of this study was to further evaluate this concept using the combination of SQV and ATV against HIV isolates presenting a range of resistance levels to both drugs.

Methods

Synergistic interactions between ATV and SQV were tested on a panel of PI-resistant recombinant viruses using a modified Phenoscript assay. Viral constructs (three obtained by site-directed mutagenesis of pNL4-3 and eleven reconstructed with Gag-Protease sequences from clinical samples) were

Figure 1. Plotting combination index as a function of inhibition

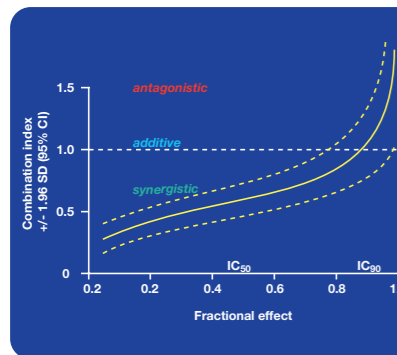


Table 1. IC₅₀ values and combination indices for site-directed mutants (SDMs)

Virus (Protease mutations#)	Mean* IC ₅₀ , nM (FC relative to wild type)		Ratio (ATV/SQV)	Combination index (95% CI) for % inhibition of		
	ATV	SQV		50	75	90
WT 43XCS (None)	2.20	3.64	1:1	1.43 (0.60;2.17)	1.25 (0.76;1.69)	1.10 (0.58;1.58)
SDM B01 (L10I, G48V, L90M)	3.66 (1.7)	168 (46)	2:1	1.17 (0.38;1.93)	1.11 (0.57;1.63)	1.06 (0.58;1.52)
			4:1	1.15 (0.48;1.82)	1.07 (0.63;1.54)	1.00 (0.66;1.40)
			8:1	1.12 (0.45;1.78)	1.08 (0.57;1.58)	1.04 (0.60;1.47)
SDM B02 (M46I, I54V, A71V, V82A)	3.39 (1.5)	4.23 (1.2)	1:1	1.06 (0.48;1.63)	0.99 (0.49;1.49)	0.93 (0.60;1.26)
			2:1	1.13 (0.29;1.96)	1.08 (0.51;1.65)	1.04 (0.56;1.51)
			4:1	1.14 (0.58;1.70)	1.07 (0.58;1.56)	1.00 (0.59;1.41)
SDM B03 (I50L, A71V)	11.8 (5.3)	2.40 (0.66)	1:1	0.70 (0.51;0.90)	0.79 (0.59;0.99)	0.89 (0.72;1.07)
			2:1	0.60 (0.47;0.74)	0.70 (0.59;0.82)	0.82 (0.71;0.94)
			4:1	0.94 (0.79;1.08)	0.96 (0.83;1.09)	0.98 (0.86;1.09)
SDM B04 (36I, 54V, 63P, 71V, 82A, 88D)	54.7 (25)	58.8 (16)	1:1	1.22 (0.82;1.62)	1.19 (0.79;1.60)	1.16 (0.62;1.70)
			2:1	1.15 (0.71;1.58)	1.13 (0.71;1.54)	1.11 (0.61;1.60)
			4:1	1.27 (0.82;1.72)	1.15 (0.68;1.62)	1.05 (0.55;1.54)
SDM B05 (10R, 20I, 36I, 46I, 48V, 63P, 90M)	20.9 (9.5)	722 (200)	1:1	1.62 (1.13;2.12)	1.53 (1.13;1.93)	1.44 (1.14;1.74)
			2:1	1.72 (0.98;2.45)	1.54 (1.09;1.99)	1.38 (1.04;1.72)
			4:1	1.30 (0.56;2.04)	1.29 (0.75;1.83)	1.28 (0.89;1.67)
SDM B07 (10I, 20R, 32I, 36I, 46L, 63P, 71V, 82A, 90M)	45.5 (21)	45.0 (12)	1:1	1.09 (0.58;1.60)	0.96 (0.56;1.36)	0.84 (0.54;1.13)
			2:1	1.32 (0.63;2.02)	1.16 (0.72;1.60)	1.01 (0.62;1.40)
			4:1	0.98 (0.49;1.48)	0.92 (0.52;1.31)	0.86 (0.51;1.21)
SDM B08 (10I, 20R, 36I, 54V, 63P, 82A)	91.5 (42)	1200 (330)	1:1	1.12 (1.56;1.68)	1.05 (1.06;2.08)	1.04 (0.60;1.48)
			2:1	1.15 (0.61;1.68)	1.07 (0.60;1.54)	1.01 (0.49;1.53)
			4:1	1.16 (0.65;1.66)	1.12 (0.58;1.65)	1.08 (0.49;1.68)
SDM B09 (10I, 20R, 36I, 46I, 54V, 63P, 71V, 82A, 90M)	5.56 (2.5)	51.5 (14)	1:1	1.00 (0.41;1.59)	0.97 (0.55;1.40)	0.95 (0.36;1.54)
			2:1	2.24 (0.27;4.20)	1.88 (0.44;3.32)	1.59 (0.59;2.59)
			4:1	1.63 (-0.10;3.37)	1.45 (0.53;2.53)	1.29 (0.39;2.19)
SDM B10 (10I, 24I, 46IL, 77I, 82T, 90M)	8.66 (3.9)	13.9 (3.8)	1:1	1.85 (0.56;3.13)	1.85 (0.69;3.00)	1.85 (0.78;2.92)
			2:1	1.20 (-0.18;2.58)	1.23 (0.38;2.08)	1.25 (0.27;2.23)
			4:1	1.59 (0.69;2.48)	1.70 (0.92;2.48)	1.83 (0.74;2.92)
SDM B11 (10V, 36I, 63P, 71T, 88D)	13.5 (6.1)	19.6 (5.4)	1:1	1.13 (0.26;1.99)	1.10 (0.80;1.40)	1.26 (0.45;2.07)
			2:1	2.51 (0.93;4.09)	2.30 (0.94;3.65)	2.11 (0.80;3.42)
			4:1	1.92 (0.77;3.08)	1.70 (0.94;2.46)	1.51 (0.67;2.36)
CDV B01 (10F, 46L, 54V, 82A)	5.78 (2.6)	10.9 (3.0)	1:1	0.82 (0.55;1.09)	0.77 (0.54;0.99)	0.72 (0.50;0.93)
			2:1	0.89 (0.61;1.29)	0.69 (0.45;0.92)	0.73 (0.43;1.02)
			4:1	0.65 (0.36;0.94)	0.69 (0.45;0.92)	0.73 (0.43;1.02)
CDV B02 (30N, 63P, 71T, 77I, 88D)	6.40 (2.9)	13.8 (3.8)	1:1	0.84 (0.50;1.18)	0.68 (0.41;0.95)	0.68 (0.41;0.95)
			2:1	0.81 (0.39;1.22)	0.81 (0.45;1.17)	0.82 (0.39;1.24)
			4:1	1.04 (0.80;1.27)	1.13 (0.88;1.39)	1.24 (0.92;1.56)
CDV B03 (10F, 32I, 36I, 46I, 63P, 71T, 90M)	18.3 (8.3)	22.2 (6.1)	1:1	1.54 (0.83;2.26)	1.29 (0.73;1.85)	1.08 (0.57;1.59)
			2:1	0.74 (0.43;1.20)	0.82 (0.32;1.32)	0.82 (0.32;1.32)
			4:1	1.21 (0.51;1.91)	1.36 (0.69;2.02)	1.52 (0.12;2.93)
CDV B04 (36I, 54V, 63P, 71V, 82A, 88D)	54.7 (25)	58.8 (16)	1:1	1.23 (0.76;1.69)	1.19 (0.79;1.60)	1.16 (0.62;1.70)
			2:1	1.15 (0.71;1.58)	1.13 (0.71;1.54)	1.11 (0.61;1.60)
			4:1	1.27 (0.82;1.72)	1.15 (0.68;1.62)	1.05 (0.55;1.54)
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			4:1	1.30 (0.56;2.04)	1.29 (0.75;1.83)	1.28 (0.89;1.67)
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			2:1	1.32 (0.63;2.02)	1.16 (0.72;1.60)	1.01 (0.62;1.40)
			4:1	0.98 (0.49;1.48)	0.92 (0.52;1.31)	0.86 (0.51;1.21)
CDV B08 (10I, 20R, 36I, 54V, 63P, 82A)	91.5 (42)	1200 (330)	1:1	1.12 (1.56;1.68)	1.05 (1.06;2.08)	1.04 (0.60;1.48)
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			2:1	2.24 (0.27;4.20)	1.88 (0.44;3.32)	1.59 (0.59;2.59)
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			2:1	1.20 (-0.18;2.58)	1.23 (0.38;2.08)	1.25 (0.27;2.23)
			4:1	1.59 (0.69;2.48)	1.70 (0.92;2.48)	1.83 (0.74;2.92)
CDV B11 (10V, 36I, 63P, 71T, 88D)	13.5 (6.1)	19.6 (5.4)	1:1	1.13 (0.26;1.99)	1.10 (0.80;1.40)	1.26 (0.45;2.07)
			2:1	2.51 (0.93;4.09)	2.30 (0.94;3.65)	2.11 (0.80;3.42)
			4:1	1.92 (0.77;3.08)	1.70 (0.94;2.46)	1.51 (0.67;2.36)

* Results shown are the means of three separate experiments; > 10-fold changes in IC₅₀ are indicated in bold. # Mutations associated with resistance to PIs according to the October/November IAS resistance tables. FC = fold-change. Antagonistic effects shown in red. Synergistic effects shown in green.

transfected into HeLa cells that were subsequently treated with various concentrations of PIs. The amount of infectious virus produced in the presence of the inhibitors was measured using P4 indicator cells. Inhibition by the combination of SQV and ATV was assessed at four fixed molar ATV/SQV ratios: 1:1, 2:1, 4:1 and 8:1. Assays were conducted in three replicate experiments with triplicate data points. Interactions were calculated using the multiple drug effect equation of Chou and Talalay based on the median effect principle, with CalcuSyn software (Biosoft, UK).⁹ This analysis yielded a combination index as a function of the inhibition rate (Figure 1). Confidence intervals (CIs) of combination indices were calculated by simulation of parameter estimates as suggested by Belen'kii and Schinazi.¹⁰ At a given inhibition rate, drugs were considered significantly synergistic when the upper limit of the 95% CI was < 1 and antagonistic when the lower limit of the 95% CI was > 1.

Results

- The three site-directed mutants carried two to four PI resistance mutations and exhibited low-level resistance to SQV and/or ATV (Table 1).
- The eleven viruses derived from clinical samples carried a median of six (range, four to nine) PI resistance mutations and exhibited higher levels of resistance to SQV and/or ATV (Table 2).
- One virus (CDV B06) had an IC₅₀ above the highest concentration of drug used in the assay and thus could not be assayed against drug combinations (data not shown).
- Our study showed no evidence of synergy between SQV and ATV with wild-type virus (Table 1 and Figure 2).
- Significant synergy was observed at all four combination ratios and all levels of inhibition for virus SDM B03 with combination indices ranging from 0.50 to 0.66 (Table 2 and Figure 2).
- This virus carried mutations I50L and A71V, resulting in low-level resistance to ATV (fold-change in IC₅₀ 5.35) and susceptibility to SQV (fold-change in IC₅₀ 0.66).
- For each inhibition rate at which combination indices were measured for this virus, synergy increased with increasing ATV/SQV ratio, highly suggestive of a mechanism that implies enhancement of SQV activity by ATV.
- Synergy was also observed at some combination ratios with two viruses susceptible to SQV (fold-change ≤ 3.0) (SDM B02 and CDV B01; Tables 1 and 2 and Figure 2).

Discussion

We previously demonstrated that, in our assay system, LPV appeared to enhance the antiviral activity of SQV, but this phenomenon was only measurable when LPV had lost most of its antiviral activity and when sufficient susceptibility to SQV was retained. The results presented here are consistent with these observations in that synergy between SQV and ATV was only observed with the viruses that were most susceptible to SQV. It would be expected that SQV and ATV would act synergistically on viruses with high levels of resistance to ATV and low levels of resistance to SQV. None of the clinically derived viruses included in this study, which were obtained

from ATV-naïve patients and lacked the I50L mutation, had such a resistance profile.

This is unlike our previous study on LPV/SQV synergy, where, using the same panel of viruses, resistance to LPV was found to be often markedly higher than resistance to SQV. In these instances, strong synergistic interaction between LPV and SQV was seen.

One possible mechanism for this synergy is that the presence of ATV or LPV in some way results in an increase in intracellular levels of SQV, for example by saturation of intracellular proteins or inhibition of drug transporter molecules by ATV or LPV. In support of this hypothesis, *in vitro* studies have shown that SQV has a particularly long intracellular half-life compared with other PIs.¹¹ Although the mechanism for this long half-life has not been defined, it is possible that it could involve binding of SQV to an intracellular protein. Furthermore, increased intracellular levels of SQV have been reported when ATV/SQV/*r* is administered.¹²

Consistent with our results, a modest increase in the intracellular SQV concentration caused by this mechanism would be expected to be insufficient to result in a measurable difference from SQV-resistant virus and would therefore be masked by the antiviral effect of LPV or ATV. However, if the virus were resistant to LPV or ATV but retained susceptibility to SQV then the majority of the actual antiviral effect seen would be due to SQV, and thus even relatively small increases in intracellular concentration could significantly increase virus inhibition.

Conclusions

- Significant synergistic interaction between ATV and SQV can be observed in viruses resistant to ATV but susceptible to SQV.
- Further studies are needed in order to define the mechanism(s) for this observed synergy.

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Figure 2. Combination index as a function of inhibition

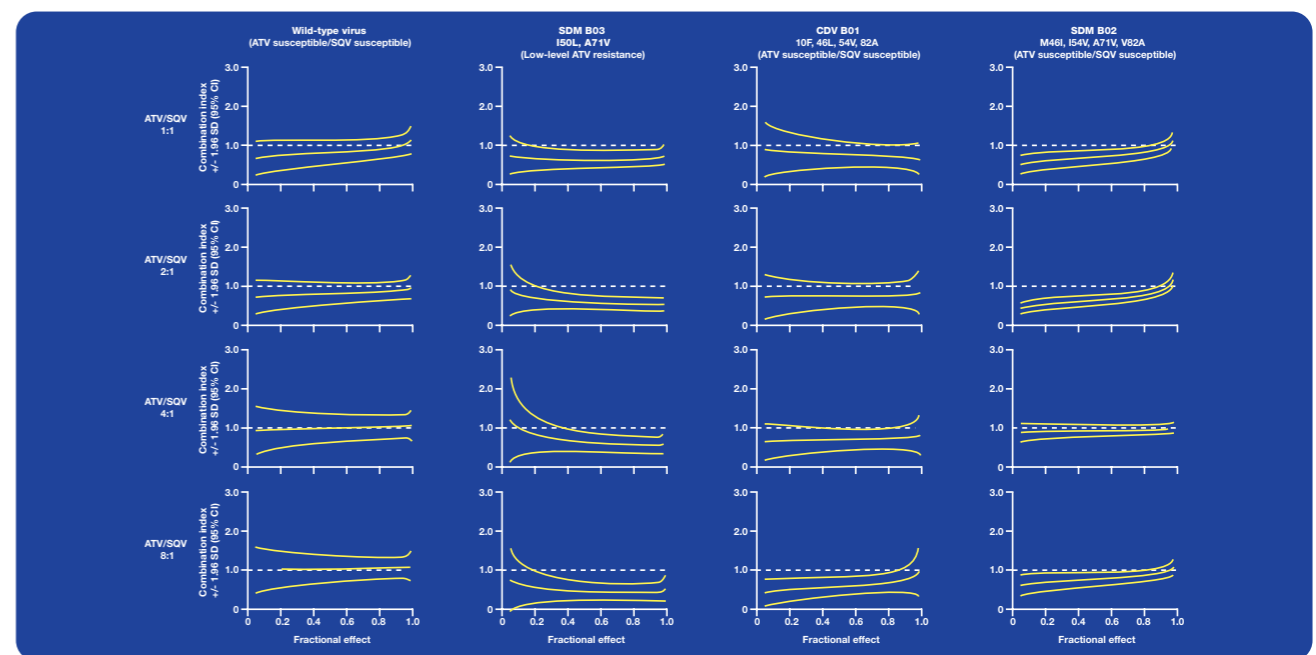


Table 2. IC₅₀ values and combination indices for clinically derived viruses (CDVs)

Virus (Protease mutations#)	Mean* IC ₅₀ , nM (FC relative to wild type)		Ratio (ATV/SQV)	Combination index (95% CI) for % inhibition of		
	ATV	SQV		50	75	90
CDV B01 (10F, 46L, 54V, 82A)	5.78 (2.6)	10.9 (3.0)	1:1	0.82 (0.55;1.09)	0.77 (0.54;0.99)	0.72 (0.50;0.93)
			2:1	0.89 (0.61;1.29)	0.69 (0.45;0.92)	0.73 (0.43;1.02)
			4:1	0.65 (0.36;0.94)	0.69 (0.45;0.92)	0.73 (0.43;1.02)
			8:1	0.66 (0.40;0.92)	0.71 (0.50;0.96)	0.77 (0.50;1.13)
CDV B02 (30N, 63P, 71T, 77I, 88D)	6.40 (2.9)	13.8 (3.8)	1:1	0.84 (0.50;1.18)	0.68 (0.41;0.95)	0.68 (0.41;0.95)
			2:1	0.81 (0.39;1.22)	0.81 (0.45;1.17)	0.82 (0.39;1.24)
			4:1	1.04 (0.80;1.27)	1.13 (0.88;1.39)	1.24 (0.92;1.56)
			8:1	0.96 (0.61;1.31)	1.14 (0.85;1.42)	1.34 (0.88;1.80)
CDV B03 (10F, 32I, 36I, 46I, 63P, 71T, 90M)	18.3 (8.3)	22.2 (6.1)	1:1	1.54 (0.83;2.26)	1.29 (0.73;1.85)	1.08 (0.57;1.59)
			2:1	0.74 (0.43;1.20)	0.82 (0.32;1.32)	0.82 (0.32;1.32)
			4:1	1.21 (0.51;1.91)	1.36 (0.69;2.02)	1.52 (0.12;2.93)
			8:1	1.23 (0.76;1.69)	1.19 (0.79;1.60)	1.16 (0.62;1.70)
CDV B04 (36I, 54V, 63P, 71V, 82A, 88D)	54.7 (25)	58.8 (16)	1:1	1.71 (1.11;2.30)	1.57 (1.06;2.08)	1.44 (0.87;2.01)
			2:1	1.15 (0.71;1.58)	1.13 (0.71;1.54)	1.11 (0.61;1.60)
			4:1	1.27 (0.82;1.72)	1.15 (0.68;1.62)	1.05 (0.55;1.54)
			8:1	1.22 (0.82;1.62)	1.10 (0.80;1.40)	0.99 (0.61;1.36)
CDV B05 (10R, 20I, 36I, 46I, 48V, 63P, 90M)	20.9 (9.5)	722 (200)	1:1	1.62 (1.13;2.12)	1.53 (1.13;1.93)	1.44 (1.14;1.74)
			2:1	1.72 (0.98;2.45)	1.54 (1.09;1.99)	1.38 (1.04;1.72

**12th Conference on
Retroviruses and
Opportunistic Infections
Boston, MA, USA
February 22–25, 2005**

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