



The Role of Genetic Polymorphisms of the MDR1 (ABCB1) Gene in the MaxCmin1 Study

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On behalf of the MaxCmin 1 trial group



INTRODUCTION

- The MaxCmin1 study assessed rate of virological failure at 48 weeks in adult HIV-1 infected patients assigned indinavir / ritonavir (IDV/RTV; 800/100 mg bd) or saquinavir / ritonavir (SQV/RTV; 1000/100 mg bd). Greater treatment-limiting adverse events were observed in the IDV/RTV arm. However, RTV-boosted SQV and IDV had comparable antiviral effects.
- P-glycoprotein (Pgp) is a drug efflux pump coded for by the MDR1 gene. The C to T transition at position 3435 is the most extensively studied MDR1 single nucleotide polymorphism (SNP). The T allele at this position has been related to better immune recovery (Fellay *et al*, 2002) and a trend toward virological failure on HAART has been reported in CC homozygotes (Brumme *et al*, 2003). Furthermore, a non-significant association between this SNP and infectivity was reported (Ifergan *et al*, 2002). However, these observations are currently subject to substantial debate.
- Pgp expression was unrelated to the C3435T genotype in placenta (Tanabe *et al*, 2001) and CD34+ hematopoietic stem cells (Calado *et al*, 2002). In placenta, Pgp expression was correlated with the G2677T SNP, which results in an Ala→Ser change at position 893 of the protein. G2677T has been shown to be in linkage disequilibrium with C3435T with the most common haplotypes being 3435C/2677G and 3435T/2677T.
- The aim of this study was to investigate the impact of C3435T, G2677T and the resultant haplotypes on the MaxCmin1 endpoints.

METHODS

- Study design:** Phase IV, open-label, randomised (1:1, stratified by region & HIV-1 RNA \leq 400 cps/ml). Clinical indication for a ritonavir-boosted PI treatment was:
 - Protease inhibitor (PI)-naïve (all) or
 - PI-experienced, = 400 cps/ml (all) or
 - PI-experienced, < 400 cps/ml (adherence problems and/or toxicity to PI).
 All patients were followed at weeks 4, 12, 24, 36, and 48 and received >2 NRTI(s)/NNRTI(s) (decided prior to randomisation).
- Pharmacokinetic analyses were previously conducted in a separate substudy (Justesen *et al*, 9th EACS, Warsaw, Abstract F2/5)

- Genotyping:** Of 306 patients who initiated treatment, 230 available patients were genotyped for either C3435T, G2677T or both. Genomic DNA was extracted from cell suspensions and both C3435T and G2677T SNPs were genotyped by taqman allelic discrimination. Haplotype assignment was carried out as shown in table 1.

Table 1: MDR1 haplotype assignments and corresponding patient numbers.

3435	2677	Haplotype assignment	Patient numbers
CC	GG	1	46
CC	GT	2	7
CC	TT	3	1
CT	GG	4	24
CT	GT	5	71
CT	TT	6	0
TT	GG	7	7
TT	GT	8	22
TT	TT	9	32

- Statistical analysis:** Statistical analysis: Statistical analyses were performed using STATA software (version 7). Kruskal Wallis non-parametric tests were used to compare the CD4 counts at baseline. Cox proportional hazard models were constructed in order to compare time to virological failure according to genotype and haplotype in addition to determining whether clinical progression or time to an adverse event were dependent on these SNPs. Finally, linear regression models were developed to examine the change in CD4 up to week 48, the change in viral load up to week 48 and also to predict the trough concentrations of SQV, IDV and RTV according to the MDR1 genetics.

RESULTS

- No significant differences in the time to virological failure were found between different genetic assignments (Figure 1).
- A significant difference was observed in baseline CD4 count between G2677T polymorphic status (Table 2). Furthermore, TT homozygotes had a significantly lower increase in CD4 count up to week 48 (Table 3).
- No significant differences were observed for change in viral load from baseline to week 48 between MDR1 SNPs and haplotype. PK were not correlated to either C3435T, G2677T (data not shown) or MDR1 haplotype (Table 5).

- MDR1 genetics were found not to influence clinical progression or time to an adverse event (data not shown).

Figure 1: Kaplan Meier plots for virological failure (maxCmin primary endpoint) according to treatment arm (A), G2677T (B), C3435T (C) and haplotype (D).

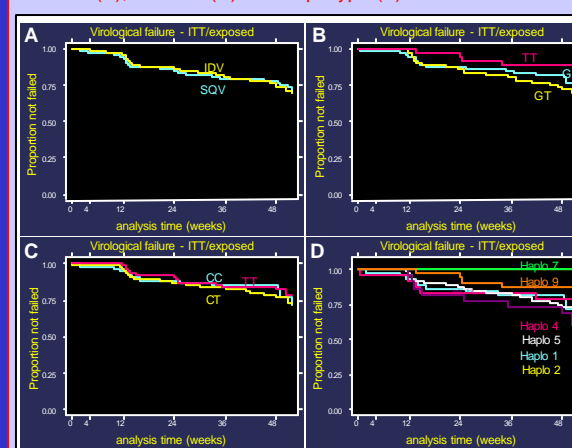


Table 2: Baseline CD4 counts according to genotype and haplotype (cells/ml).

Criteria	Assignment	Median (IQR)	Patients (N)	P
Baseline CD4	N/A	277 (137-450)	300	
G2677T	GG	293 (130-450)	107	0.02
	GT	279 (160-416)	78	
	TT	387 (250-665)	34	
C3435T	CC	300 (160-440)	51	0.74
	CT	296 (143-515)	98	
	TT	290 (182-516)	61	
Haplotype	1	276 (160-425)	42	0.15
	2	432 (300-450)	7	
	3	-	1	
	4	301 (117-373)	23	
	5	299 (142-580)	67	
	6	-	0	
	7	280 (210-580)	7	
	8	260 (106-359)	21	
	9	377 (250-656)	32	

Table 3: Changes in CD4 count between baseline and week 48 according to genotype and haplotype (cells/ml). All multivariable analyses are adjusted for the effect of treatment arm.

Criteria	Assignment	Single variable analysis		Multivariable analysis	
		CD4 count change	P	CD4 count change	P
G2677T	GG	0	0.05	0	0.04
	GT	3.5 (-42.9 - 50.0)		3.9 (-42.8 - 50.5)	
	TT	-69.5 (-132.4 - -6.5)		-69.9 (-133.1 - -6.7)	
C3435T	CC	0	0.72	0	0.73
	CT	2.3 (-46.4 - 51.0)		3.9 (-44.8 - 52.7)	
	TT	-15.8 (-68.7 - 37.0)		-13.7 (-66.6 - 39.2)	
Haplotype	1	0	0.57	0	0.53
	2	-1.0 (-109.5 - 107.5)		-1.5 (-107.2 - 110.1)	
	3	-		-	
	4	27.4 (-45.5 - 100.3)		33.4 (-40.4 - 107.3)	
	5	-18.4 (-72.8 - 35.9)		-18.4 (-72.7 - 36.0)	
	6	-		-	
	7	5.1 (-105.4 - 113.6)		7.0 (-101.0 - 116.2)	
	8	-5.2 (-78.0 - 67.7)		-4.1 (-77.0 - 68.8)	
	9	-19.2 (-113.0 - 14.7)		-15.9 (-110.1 - 18.3)	

Table 4: Changes in HIV-RNA between baseline and week 48 according to genotype and haplotype (log₁₀ cps/ml). All multivariable analyses are adjusted for the effect of treatment arm and the HIV-RNA level at baseline (\leq 400 cps/ml).

Criteria	Assignment	Single variable analysis		Multivariable analysis	
		Log HIV-RNA change	P	Log HIV-RNA change	P
G2677T	GG	0	0.26	0	0.70
	GT	0.13 (-0.36 - 0.62)		0.15 (-0.20 - 0.49)	
	TT	0.54 (-0.12 - 1.20)		0.07 (-0.40 - 0.53)	
C3435T	CC	0	0.66	0	0.49
	CT	0.25 (-0.30 - 0.80)		0.22 (-0.15 - 0.60)	
	TT	0.21 (-0.39 - 0.80)		0.11 (-0.30 - 0.52)	
Haplotype	1	0	0.22	0	0.62
	2	1.47 (0.19 - 2.74)		0.70 (-0.20 - 1.60)	
	3	-		-	
	4	0.34 (-0.49 - 1.18)		0.23 (-0.37 - 0.82)	
	5	0.52 (-0.10 - 1.15)		0.38 (-0.06 - 0.82)	
	6	-		-	
	7	0.49 (-0.79 - 1.76)		0.36 (-0.53 - 1.26)	
	8	0.08 (-0.76 - 0.91)		0.17 (-0.42 - 0.76)	
	9	0.71 (-0.02 - 1.45)		0.22 (-0.31 - 0.74)	

Table 5: Regression models to show the effect of the SNP type on PK values at week 4. Ritonavir PK are pooled and no differences were observed between treatment arms (p=0.57).

Haplotype	Saquinavir		Indinavir		Ritonavir	
	Mean PK (ng/ml) (95% CI)	P	Mean PK (ng/ml) (95% CI)	P	Mean PK (ng/ml) (95% CI)	P
1	1166.1 (706.6-1952.6)	0.41	1472.0 (719.7-3011.0)	0.58	546.5 (386.2-765.5)	0.83
2	890.4 (275.3-1741.2)		1369.5 (379.1-4523.0)		594.2 (321.3-1099.6)	
3	-		-		-	
4	334.4 (241.1-1138.3)		1419.0 (740.0-2376.7)		532.7 (376.1-796.8)	
5	892.8 (486.8-1313.7)		1176.0 (737.6-1909.5)		486.9 (359.2-634.5)	
6	-		-		-	
7	230.8 (46.8-1130.4)		351.8 (129.9-2517.3)		267.4 (112.0-608.1)	
8	733.1 (417.9-1237.3)		1627.8 (877.6-3914.5)		479.4 (317.8-711.1)	
9	882.7 (493.8-1636.1)		1661.2 (843.5-3279.3)		526.1 (357.4-799.1)	

CONCLUSIONS

- MDR1 genetics did not impact on viral load in the MaxCmin1 trial.
- However, a marked difference was observed in baseline CD4 count between G2677T polymorphism with higher baseline counts (and consequently less rise at 48 weeks) in TT homozygotes.
- These results indicate that the effects of these polymorphisms may have been exerted prior to initiation of this trial.
- MDR1 polymorphisms could be important in influencing the outcome of HIV treatment, but this is best evaluated in a study of treatment naive patients commencing therapy.

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