

**Protocol for taking pharmacogenetic testing of tacrolimus into the clinical arena: patient-tailored anti-rejection therapy**

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**Aim of the study**

The primary aim was to verify in a well-powered prospective study the associations found in a smaller retrospective study that MDR-1 genotyping correlates with tacrolimus dose in individual patients and could therefore be used in future to predict tacrolimus starting dose.

**Study design**

This is a single centre, non-randomised, non-interventional, data-collection study. The study aimed to recruit 400 patients who have undergone kidney transplantation and are receiving oral tacrolimus as part of their clinical management. The study aimed to analyse single nucleotide polymorphisms of the cytochromes P450-3A4 and 3A5 and the MDR-1 genes in each recruited individual and to then assess the predictive value of MDR-1 and CYP3A4/5 genotyping in estimating tacrolimus dose concentration. Clinical dose records for recruited individuals would be accessed to correlate predicted dose range with actual dose at 3 months following transplantation.

**Patient participation**

This was an observational, non-interventional study. No visits outside normal follow-up appointments were required, no interventions or blood samples were taken for the purpose of the study. MDR-1 and CYP3A4/5 genotyping was performed for 138 individuals from stored serum in the tissue typing laboratory.

**Results**

Baseline characteristics for the 138 patients for whom genotyping was performed included mean age at recruitment 43.8 years, (+/- 12), range 18-73; gender male 78 (57%) and female 59 (43%); ethnicity white 120 (87%), black 5 (4%), and Asian 13 (9%).

**Table 1** Distribution of CYP3A and MDR allelic frequencies

Genotype	N	%	Predicted dose range
CYP3A	137		
AA	3	2	High
AG	16	12	Intermediate
GG	118	86	Low
MDR	136		
CC	44	32	High
TC	54	40	Intermediate
TT	38	28	Low
CYP3A + MDR	135		
AA CC	3	2	High
AG CC	6	4	High
AG TC	6	4	Intermediate
AG TT	4	3	Low
GG CC	34	25	High
GG TC	48	36	Intermediate
GG TT	34	25	Low

**Table 2** Tacrolimus pharmacokinetics after transplantation

	1 week	1 month	3 month	6 month	1 year
C0	11.6 (6.8)	10.2 (4.1)	9.2 (3)	8.6 (3.0)	8.3 (2.7)
Dose mg/day	9.4 (3.1)	8.2 (3.9)	6.8 (3.6)	6.1 (3.4)	6.8 (10.8)
Dose mg/kg	0.13 (0.04)	0.12 (0.06)	0.10 (0.05)	0.09 (0.05)	0.09 (0.08)
C0/mg/kg (ng/ml/mg/kg)	99.7 (6.9)	113 (74.8)	122.8 (71.7)	128 (91.4)	127 (66.5)
Serum creatinine	240.3 (211.8)	147.4 (60.5)	142.1 (55.6)	138.3 (43.4)	1.6 (48.17)

### **CYP3A4/5**

Individuals with the AA genotype had significantly higher dose requirements than other genotypes  $p=0.007$ , than AG  $p=\text{not significant}$ , GG  $p=0.004$ . AG genotype had higher dose requirements than GG  $p=0.0002$ . No significant differences in mean creatinine were seen between groups at 1 year. Trough levels at 1 week were  $<5\text{ng/ml}$  in all individuals with AA genotype, 31% with AG genotype and 6% with GG genotype. Trough levels were  $>15\text{ng/ml}$  in 26% GG, 8% AG and 0% AA.

**Table 3**

CYP3A	AA (3)			AG (16)			GG (118)		
	1 week	3 month	1 year	1 week	3 month	1 year	1 week	3 month	1 year
C0	3.5 (0.8)	8.3 (2.6)	9.6 (4.9)	7.6 (4.7)	7.3 (2.2)	8.2 (2.8)	12.4 (6.9)	9.5 (3.1)	8.3 (2.6)
Dose mg/day	17 (1.4)	15.7 (4.5)	14.3 (5.1)	10.3 (3.3)	11 (3.8)	9.7 (3.3)	9.1 (2.9)	5.9 (2.5)	6.1 (11.4)
Dose mg/kg	0.21 (0.03)	0.21 (0.02)	0.20 (0.05)	0.15 (0.05)	0.16 (0.06)	0.14 (0.05)	0.13 (0.04)	0.08 (0.04)	0.08 (0.08)
C0/mg/kg	16.6 (1.4)	38.9 (8.5)	46.3 (17.1)	50.9 (46.2)	49.3 (18.6)	65 (32.4)	108.3 (68.3)	135.1 (69.5)	137.6 (64.6)
Serum creatinine	142.5 (54.5)	147 (56.2)	144.3 (60.4)	301 (260.8)	144.6 (60.6)	148.8 (54.2)	234.7 (205.9)	141.6 (55.4)	138.1 (47.4)
Rejection	0			4 (25%)			23 (19%)		
ATG	0			1(25%)			3 (13)		
White	0			12 (74%)			107 (91%)		
Asian	1 (33%)			2 (13%)			10 (8%)		
Black	2 (67%)			2 (13%)			1 (1%)		

### **MDR-1**

Tacrolimus trough levels were low <5ng/ml at 1 week in 19% CC, 8% TC and 3% TT while levels were high >15ng/ml in 17% CC, 17% TC and 39% TT. Dose requirements at 3 months were not significantly different between the genotypes. No significant differences in serum creatinine or rejection rates at 1 year were seen between genotypes.

**Table 4**

MDR	CC (44)			TC (55)			TT (38)		
	1 week	3 month	1 year	1 week	3 month	1 year	1 week	3 month	1 year
C0	11.0 (5.8)	8.7 (3.7)	7.8 (2.7)	10.7 (5.9)	9.2 (2.6)	8.4 (2.8)	13.4 (8.5)	9.6 (2.8)	8.7 (2.5)
Dose mg/day	10.3 (3.5)	7.4 (4.8)	6.4 (4.2)	8.3 (2.2)	6.7 (2.5)	8.5 (17. 3)	9.4 (3.2)	6.2 (3.4)	5.3 (3.3)
Dose mg/kg	0.14 (0.04 )	0.10 (0.06)	0.09 (0.0 6)	0.12 (0.05)	0.10 (0.05)	0.10 (0.1 2)	0.14 (0.04)	0.09 (0.04)	0.08 (0.04)
C0/mg/kg	88.8 (54)	111.5 (70.4)	115. 6 (60. 3)	110.4 (81.8)	120.6 (70.2)	131. 9 (74. 9)	104.9 (68.3)	137.3 (74.7)	131.9 (64.1)
Serum creatinine	235. 2 (165. 4)	152.1 (62.3)	136. 7 (40. 4)	287.9 (266.6 )	136.2( 2.8)	142. 4 (50. 7)	188 (176.4 )	138.9 (48.2)	138.3 (55)
Rejection	5 (11% )			9 (16%)			5 (13%)		
ATG	0			3 (33%)			2 (40%)		
White	36 (82% )			50			33		
Asian	3 (7%)			5			5		
Black	5 (11% )			0			0		

**Ethnicity:**

At 3 months individuals of Black ethnicity had increased dose requirements mg/kg than other ethnicities  $p=0.03$ , Asians  $p=0.03$  and whites  $p=0.03$ . No significant difference was found in dose requirement between Asians and whites. Blacks also had lower dose adjusted trough concentrations than others  $p=0.007$ , Asians  $p=0.03$  and whites  $p=0.007$ . Black ethnicity trended toward lower trough levels at 1 week  $p=0.08$ , mean difference  $-7.7$  ng/ml  $(-17.4-1.95)$  with 4/5 black individuals with  $C_0 < 5$  ng/ml. No significant difference in creatinine at 1 year was seen between ethnic groups or in rejection rates.

**Table 5**

Ethnicity	White (120)			Black (5)			Asian (13)		
	1 week	3 month	1 year	1 week	3 month	1 year	1 week	3 month	1 year
$C_0$	12.1 (7)	9.4 (3)	8.3 (2.7)	4.9 (3.1)	5.7 (3)	8.4 (4.4)	10.9 (4.6)	8.9 (2.7)	7.6 (1.6)
Dose mg/day	9.6 (2.8)	6.8 (3.1)	7.7 (13.8)	11.4 (16.5)	10.8 (7.2)	12 (6.2)	6.3 (2.1)	4.8 (3)	3.9 (3.1)
Dose mg/kg	0.14 (0.04)	0.1 (0.05)	0.09 (0.10)	0.15 (0.07)	0.14 (0.08)	0.15 (0.07)	0.10 (0.02)	0.08 (0.05)	0.07 (0.07)
$C_0$ /mg/kg	99.6 (69.5)	123.3 (64.3)	125.9 (55)	44.7 (43.3)	48.5 (20.1)	59.7 (35.9)	133.1 (59.2)	159.7 (112.5)	158.9 (130)
Serum creatinine	246.2 (220.6)	141.7 (55.4)	138.8 (44.5)	215.2 (118.2)	126.5 (46.2)	126 (50.3)	197.9 (171.7)	141.7 (55.4)	151.4 (72)
Rejection	26 (22%)			0			1 (8%)		
ATG	6 (23%)			0			0		
AA	0			2			1		
AG	12			2			2		
GG	107			1			10		
CC	36			5			3		
TC	49			0			5		
TT	33			0			5		

**Conclusions:**

Individuals with black ethnicity and AA genotype have significantly higher dose requirements than those of other ethnicity and CYP3A4/5 genotypes. Although only approaching levels of significance in our study those of black ethnicity, AA and CC genotype are at risk of subtherapeutic tacrolimus trough concentrations in the early post-transplant period while those with MDR-1 TT genotype and CYP3A4/5 GG have greater proportions with potentially toxic early trough concentrations.