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Jean-Baptiste Woillard[1,2], Nicolas Picard[1,2], Antoine Thierry[3,4], Guy Touchard[3,4], Pierre Marquet[1,2] on behalf of the DOMINOS study group

[1]INSERM UMR-S850, Université de Limoges, 2 rue du Dr Marcland, 87025 Limoges, France

[2]Service de Pharmacologie et Toxicologie, CHU de Limoges; 2 avenue Martin Luther King 87042 Limoges, France

[3]INSERM U1082, Hôpital La Milétrie, 2 rue de La Milétrie 86021 Poitiers Cedex, France

[4]Service de Néphrologie et Transplantation Rénale, Hôpital La Milétrie, 2 rue de La Milétrie 86021 Poitiers Cedex, France

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Running head: SNPs and clinical effects under EC-MPS

Address for Correspondence:

Prof. Pierre Marquet,

INSERM UMR-S850

2 rue du Dr Marcland,

87025 Limoges, France

Tel: +33555435895; fax: +33555435936

e-mail: pierre.marquet@unilim.fr

Abstract

Objectives: Different associations between Single Nucleotide Polymorphisms (SNPs) in cellular target, metabolism enzymes or transport proteins, and biopsy proven acute rejection (BPAR) or adverse events have been reported in transplant patients receiving mycophenolate mofetil (MMF). This work aimed at studying them all in patients on enteric-coated mycophenolate sodium (EC-MPS). **Methods:** The study included 189 renal transplant patients from the DOMINOS trial. Fifteen SNPs in *IMPDH2*, *IMPDH1*, *ABCC2*, *SLCO1B3*, *UGT1A8, UGT1A9, UGT2B7, CYP2C8, HUS1* and *IL12A* were genotyped in all patients. Associations between SNPs and the first event of BPAR or diarrhea were investigated using multivariate logistic regressions. Associations between SNPs and leucopenia or anemia at 9 different visits between days 0 and 190 post-transplantation, were studied using time dependent Cox proportional hazards regression models. **Results:** Multivariate analyses showed that the *CYP2C8* rs11572076 wild-type genotype was significantly associated with a lower risk of leucopenia (GG *vs.* GA: HR [95%CI]=0.14[0.03,0.59]; p=0.00783). Higher EC-MPS doses and the *UGT2B7* c.-840 G>A variant allele were associated with an increased risk of anemia (EC-MPS per unit dose increase: 1.004[1.003-1.005], p<0.0001; *UGT2B7* GA *vs.* AA: 1.65 [1.12-2.43], p=0.01043; GG *vs.* AA: 1.88 [1.23-2.88], p=0.00343). However, no significant association was found between any of the SNPs studied and diarrhea or BPAR. **Conclusions:** Two pharmacogenetic associations previously reported with MMF were found in a population of 189 renal transplant patients treated with EC-MPS.

Keywords: pharmacogenetics, mycophenolate sodium, BPAR, adverse effects

Introduction

The enteric-coated formulation of mycophenolate sodium (EC-MPS) has been developed with the aim of improving the gastro-intestinal tolerability of mycophenolic acid (MPA). Contrary to mycophenolate mofetil (MMF) for which many studies on potential associations between pharmacogenetics and pharmacokinetic or pharmacodynamic characteristics have been performed, no such study had been reported for EC-MPS so far.

Due to its particular formulation, the absorption phase of EC-MPS is different from that of MMF. Indeed, for EC-MPS the delayed absorption of MPA results in delayed entero-hepatic recirculation and subsequently higher and more variable MPA trough levels compared with MMF [1]. However, there are no differences regarding their inhibitory effect on inosine mono-phosphate dehydrogenases (IMPDH) I and II, two enzymes involved in the *de novo* purine synthesis [2]. MPA is metabolized by glucuronidation in a major, inactive metabolite, mycophenolate-phenyl-glucuronide (MPAG). This reaction is catalyzed by UDP-glucuronosyl–transferases (UGT), particularly UGT1A9 in the kidney and the liver and UGT1A8 in intestinal cells [3, 4]. UGT2B7 produces a minor metabolite, mycophenolate-acyl-glucuronide (AcMPAG) [5], which is highly reactive [6] and might be implicated in adverse events, although not in MPA activity [7] . MPAG is mainly eliminated by the kidney [8], probably involving the MRP2 drug transporter [9]. However, it is also subject to biliary excretion by the active, vectorial transport system OATP and MRP2 [10, 11]. Part of MPAG then undergoes bacterial deglucuronidation in the intestine back to MPA, which is then reabsorbed [8] giving rise to MPA entero-hepatic cycling.

Many association studies have been performed between SNPs in genes coding MPA metabolism (UGTs), transport (MRP2 and OATPs) or target proteins (IMPDH) on the one hand, and biopsy-proven acute rejection (BPAR) or the most frequent adverse effects of MPA

(diarrhea, anemia and leucopenia) on the other hand. However, these studies were all conducted with MMF as the administered drug and lead to contradictories results [12-14]. Moreover, none considered all these different genes simultaneously.

Recently, Jacobson et al observed in 978 renal transplants that single-nucleotide polymorphisms in interleukin IL 12A (IL12A; rs568408 G>A), cytochrome P450 (CYP) 2C8 (CYP2C8; rs11572076 G>A), and checkpoint homolog protein (HUS1; rs1056663 C>T) were associated with the occurrence of mycophenolic acid (MPA)-related anemia but not leucopenia [15]. Similarly, Bouamar et al observed in the fixed-dose group of the FDCC study (n=178) that the CYP2C8 (rs11572076 G>A) variant allele was significantly associated with an increased risk of anemia and leucopenia [16].

The present work aimed at studying the effect of SNPs in MPA target, metabolism and transport proteins on BPAR, diarrhea, anemia and leucopenia in adult kidney transplant recipients on EC-MPS.

Methods

Patients and data

Patients (n=189) of the DOMINOS study [17] were enrolled in this sub-study after they had signed a specific written informed consent for the pharmacogenetic analyses involved. DOMINOS was a multicentre, randomized, parallel group, open-label 6-month trial conducted in 14 transplant centers in France between April 2007 and March 2009. The study was undertaken in accordance with the Declaration of Helsinki and the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, ethically approved by the Comité de Protection des Personnes (Poitiers) and authorized by the French Drug Agency (AFSSAPS). Briefly, in this study adult renal transplant patients treated with cyclosporine (CsA) and EC-MPS received 2160 mg/day MPS from the day of transplantation to week 6, and then the dose

was reduced to 1440 mg/day. Cyclosporine concentration was adjusted based on C2 blood levels. Patients were randomized into two arms: one with steroid avoidance (patients only received 250 mg intravenous methylprednisolone on day 0 and 1) and a control group where patients received corticosteroids until the third month posttransplantation, after which the physician had the possibility to stop. At month 6, 38.1% and 86.6% of the patient actually received corticosteroids in the steroid avoidance and the control groups, respectively. Study visits were performed on days 1, 3, 5 and 7, weeks 2 and 4 and months 3 and 6 posttransplantation and laboratory test results (leukocyte counts, hemoglobin levels), CsA C2 levels, steroid and EC-MPS doses were collected. A kidney biopsy was performed in case of suspected rejection, and a blinded central review of all biopsy samples was performed at the end of the study. In the present study, we considered as BPAR biopsies with evidence of cellular acute rejection or with borderline lesions. In order to exclude the variability in the definition of diarrhea, only the diarrhea episodes leading to EC-MPS dose reduction or prolonged hospitalization were considered. Mycophenolate-related anemia was defined as the use of mycophenolic acid for at least 14 days prior to a hemoglobin level <10g/dL and/or initiation of erythropoietin therapy within 30 days of the onset of anemia. Attributable leucopenia was defined as the use of mycophenolate at least for 14 days prior to a leucocyte count <3 G/L and/or initiation of granulocyte colony stimulating factor or granulocyte-macrophage colony stimulating factor therapy within 30 days of the onset of leucopenia.

SNP selection and identification of genotypes

Genomic DNA was extracted from EDTA-treated blood using the QIAamp DNA Blood Mini kit (QIAGEN S.A., Courtaboeuf, France). For all patients, we genotyped the following SNPs: *IMPDH2* (IVS7+10 T>C, rs11706052); *IMPDH1* (rs2278923 and rs2278924); *ABCC2* (c.-24 C>T, rs717620); *ABCC2* (c.1249 G>A, rs2273697); *ABCC2* (c.3972 C>T, rs3740066); *ABCC2* (c.4544 G>A, rs8187710); *SLCO1B3* (c.334 T>G, rs4149117); *UGT1A8* (c.518 C>G,

rs1042597); *UGT1A9* (c.-2152 C>T rs17868320 and c.-275 T>A rs6714486); *UGT2B7* (c.-840 G>A, rs7438135); *CYP2C8* (G>A rs11572076); *HUS1* (G>A rs1056663); *IL12A* (G>A rs568408). Genotyping was performed using the TaqMan Real-Time Polymerase Chain Reaction discrimination assay on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Courtaboeuf, France) or a Rotorgene-Q (Qiagen, Courtaboeuf, France) following the manufacturer's protocol. Assays were ordered from Life-Technologies as functionally tested assays (references C__1842928_10, C__15966663_10, C__15966664_10, C___2814642_10, C__25639181_40, C__11742072_10, C__34418857_10, C__27843087_10, C__26058102_10, C__31658115_10, C__1825446_20, C__2423981_10, C__11214910_20) or custom TaqMan SNP genotyping assays (Assays ID: AHBKDMW (rs2273697) and AHI12PD (rs8187710).

Statistical analysis

The statistical analyses were performed using R software version 2.13.1 (R foundation for statistical computing, http://www.r-project.org). Deviations from Hardy–Weinberg equilibrium were assessed using the Fisher exact test with the package 'SNPassoc'.

SNPs were recoded following dominant, recessive or co-dominant genetic models, as a function of their frequency and of literature results.

In order to study the impact of the time-dependent CsA exposure on time-independent phenotypes (BPAR and diarrhea), the global exposure of each individual to CsA was computed as the area under the curve of all the available C2 levels in the period of follow-up (AUC_{C2} = equivalent to (mean C2)×follow-up duration). The EC-MPS cumulative dose ($CD_{EC\text{-}MPS}$ = (mean EC-MPS dose)×follow-up duration) was calculated in the same way. In a first step, the association between BPAR, diarrhea and each SNP, exposure index or demographic variable was studied by univariate analysis using logistic regression. The potential associations of SNPs, EC-MPS daily dose at each visit, patient randomization group

(with *vs.* without steroids) and demographic data (age and sex) with time-dependent phenotypes, such as leucopenia and anemia were investigated by univariate analysis using time-dependent Cox proportional hazards regression models.

For multivariate analyses, all the variables leading to $p<0.1$ at this step were then included together in an intermediate model. The final model was selected by a backward stepwise selection process based on the Bayesian Information criterion (BIC).

Results

Clinical data

The characteristics of the 189 patients included in this pharmacogenetic sub-study are presented in Table 1. A total of 46 (27%) patients were diagnosed with BPAR, out of 168 patients with such data available. Eight patients had 2 BPAR events, which led to a total of 54 BPAR episodes. The 21 patients with no biopsy results were completely at random. A total of 15 (8.6%) patients out of the 189 patients had at least one diarrhea episode. Among them, 2 patients had 2 diarrhea events, which led to a total of 17 diarrhea episodes. Twenty three episodes of leucopenia were found in 19 patients, among the 1398 data available over 9 visits. A hundred seventy five episodes of anemia were found in 98 patients, among the 1279 data available over 9 visits. In the present substudy, 9 patients (9.6%) in the steroid avoidance group were actually given corticosteroids in the course of the study, based on clinician decision. All the SNPs respected the Hardy Weinberg equilibrium.

BPAR and pharmacogenetic associations

No significant association was found between SNP, age, sex, randomization group, $CD_{EC\text{-}MPS}$, AUC_{C2} and BPAR in univariate or multivariate analysis (Table 2).

Diarrhea and pharmacogenetic associations

Univariate analysis showed that no genetic covariate was significantly associated with diarrhea (Table 3), but suggested that diarrhea episodes were more frequent in female patients. This factor was kept in the final model, with increased risk of diarrhea in females (F *vs.* M: OR (95%CI) = 3.11 (1.05-9.15); p=0.040)

Leucopenia and pharmacogenetic associations

Univariate analysis of leucopenia (Table 4) showed that *IMPDH2* rs11706052, *IMPDH1* rs2278923, *ABCC2* rs3740066 and *CYP2C8* rs11572076 had a p value < 0.1. However, only the *CYP2C8* rs11572076 was retained in the final, multivariate model, with a lower risk of leucopenia in carriers of the wild-type genotype (GG *vs.* GA: HR [95%CI] = 0.14 [0.03-0.59]; p=0.00783).

Anemia and pharmacogenetic associations

Univariate analysis of anemia (Table 5) showed that EC-MPS dose, *ABCC2* -24 C>T, *UGT1A9* -275T>A and *UGT2B7* -840 G>A had a p value < 0.1. Multivariate analyze only retained EC-MPS dose (per unit dose increase: HR [95%CI]: 1.004 [1.0035-1.0047], p<0.0001) and *UGT2B7* c.-840 G>A variant allele (GA *vs.* AA: 1.65 [1.12-2.43], p=0.01043; GG *vs.* AA: 1.88 [1.23-2.88], p=0.00343) as significantly associated with an increased risk of anemia.

Discussion

This study investigating potential associations between polymorphisms in target, metabolism or transport proteins of mycophenolate sodium and drug-related clinical events in kidney transplant recipients. The result showed that there was no association between any SNP or EC-MPS dose on BPAR, there was a significant influence of: female gender on the risk of

diarrhea; *CYP2C8* rs11572076 G>A on leucopenia and *UGT2B7* c.-840 G>A variant allele and EC-MPS dose on anemia.

In the present study, patient exposure to the immunosuppressants was taken into account as the time-weighted mean value (AUC of (EC-MPS dose or cyclosporine C2) per time unit). This approach is not common and has not been, to our knowledge, applied and validated previously. Usually, to take into account the level of immunosuppression, authors test the effect of CsA C2 or EC-MPS dose at each protocol visit. However, it increases the number of tests and thus the alpha risk, and it considers each period independently, so that the influence of slight underexposure or overexposure for long periods of time will not be detected. As a consequence, little is known about the relationships between immunosuppressant exposure (in terms of Cmax, Cmin, AUC, cumulated exposure, nadir values, etc.) over time and clinical phenotypes. Another advantage of estimating time-weighted mean exposure is that it is compatible with missing data and is easier than modelling multiple time occurrences, which requires describing the relationships between observations at different times. A limitation may be that such an exposure index obviously dampens local variations. However, in the context of a pharmacogenetic study, our goal was not to investigate the direct associations between immunosuppression highs or lows and clinical phenotypes, but rather to take into account overall exposure as a covariate, if pertinent. We are working on different approaches to such estimation of global exposure and its association with clinical outcomes, one of which has been submitted for publication elsewhere.

In the present study, no association was found between CsA C2 and BPAR. As already mentioned above, a limitation may be that such an exposure index obviously averages local variations. It seems that initial studies showed a benefit of C2 monitoring; however, recent studies questioned this benefit [18].

In the present ancillary study, we considered BPAR and borderline lesions together, which partly explains the high percentage of BPAR obtained. When we consider only acute rejection, there were 37 episodes (=22%), but this is still quite high and we have no explanation for this. We cannot exclude a selection bias by which clinicians would have encouraged more patients with rejection to participate in this pharmacogenetic substudy. Such a selection bias, if any, would increase the statistical power of our tests but would not bias the gene-outcome association found. .

Stern et al reported that female Sprague-Dawley rats treated chronically with oral MPA had more GI toxicity than male rats and that intestinal microsomes from the upper jejunum of the female rats had 3-fold lower MPA glucuronidation rates than males [19]. The authors made the hypothesis that the greater susceptibility of female rats to diarrhea could thus result from reduced protection of enterocytes by *in situ* glucuronidation. This is consistent with the present finding of a significant association between female gender and increased risk of diarrhea. A possible limitation to this finding is that different attitudes of females and males with respect to diarrhea declaration to the physician could have led to a false association between diarrhea and sex. However, by choosing a conservative definition of diarrhea (requiring EC-MPS dose reduction or prolonging hospitalization), we hope we minimized this reporting bias, if any. Such an effect of sex on diarrhea was not found in the princeps study and in the clinical trials with EC-MPS [1, 17, 20], maybe because it was not investigated, or maybe because it is a spurious result here. It would be of interest to analyze in more detail the results of previous trials to confirm or turn down this apparent association. It must also be admitted that it could be chance finding in our study, owing to an imbalance between the different risk factors of diarrhea between males and females. In any case, we do not think that this finding could lead to clinical recommendations.

The *CYP2C8* rs11572076 variant allele has been previously associated with an increased risk of leucopenia in the fixed dose arm of the FDCC study [16]. This may be partly influenced by a decreased of MPA metabolism associated to the *CYP2C8* variant allele and to a higher exposure to MPA, which is well known to favor leucopenia [21]. However, it is rather unlikely that this SNP could explain on its own such an increased in MPA exposure given the minor involvement of this enzyme in MPA metabolism [22]. It is of note that there was no difference in EC-MPS dose between CYP2C8 genotype groups (data not shown), as confirmed by the non-significant statistical interaction between the CYP2C8 SNP and EC-MPS dose.

In contrast to the results reported by Jacobson et al. [15], no effect of HUS1 on anemia was observed. The major difference between their study and the present one was the galenic form of mycophenolate (EC-MPS *vs.* MMF), but it may not be sufficient to explain this discrepancy. Another possible explanation could be an insufficient statistical power in the present study to detect such an effect on anemia. However, another group did not observe any impact of this polymorphism on anemia despite a larger group of renal transplant patients [16].

An increasing risk of anemia was found with increasing doses of EC-MPS. These results are in conformity with one of the phase 3 MMF studies, which found that MMF dosing was related to anemia [23]. In this study, we also found an increased risk of anemia in carriers of the *UGT2B7* c.-840 G>A variant allele. A previous study performed in our team found a higher AcMPAG area under the curve in patient carriers of this *UGT2B7* c.-840 G>A variant allele [24]. Moreover, higher AcMPAG AUCs were reported to be significantly associated with an increased risk of anemia in thoracic transplantation [25]. A possible explanation of this relationship between the *UGT2B7* c.-840 G>A variant allele and anemia could thus be the

increased production of AcMPAG, with high reactive properties [26] leading to a chronic inflammation and anemia.

A general limitation of this study is that MPA exposure was not measured. Indeed, the EC-MPS formulation is associated with a higher intra- and inter-patient variability in MPA exposure than MMF at equivalent doses [1, 27]. Thus, EC-MPS dose cannot be considered the best marker of MPA exposure.

In conclusion, in a population of 189 renal transplant patients treated with EC-MPS, two pharmacogenetic associations previously reported with MMF were found. The clinical implementation of pharmacogenetic markers would be of particular interest for this particular formulation, which has a hardly predictable pharmacokinetics. However, it is premature to recommend such genotyping in routine practice because the exact mechanisms behind these findings are still unclear. These results have to be confirmed by independent studies.

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Conflict of interest

Jean-Baptiste Woillard, Nicolas Picard, Antoine Thierry have no conflict of interest.

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Table 1- Demographic, treatment and genetic characteristics of the 189 patients included in the DOMINOS pharmacogenetic substudy (continuous data are expressed as mean±sd).

Variable	Value
Age (years)	51 ± 11
Cyclosporine AUC_{C2}* (mg*month/L)	1156 ± 275
$CD_{EC\text{-}MPS}$** (mg/month)	1568 ± 195
Randomization group (number without steroids/with steroids)	94/95
BPAR yes/no	42/126
$Diarrhea yes/no	15/174
Anemia yes/no	175/1279
Leucopenia yes/no	23/1398
Male/Female	124/65
IMPDH2 IVS7+10 T>C (rs11706052)	
TT	151 (80%)
TC	36 (19%)
CC	2 (1%)
IMPDH1 C>T (rs2278923)	
CC	52 (28%)
CT	97 (51%)
TT	40 (21%)
IMPDH1 C>T (rs2278924)	
CC	85 (45%)
CT	84 (44%)
TT	20 (11%)
ABCC2 -24 C>T (rs717620)	
CC	124 (66%)
CT	65 (34%)
ABCC2 1249 G>A (rs2273697)	
GG	120 (64%)
GA	57 (30%)
AA	12 (6%)
ABCC2 3972 C>T (rs3740066)	
CC	71 (38%)
CT	87 (46%)
TT	31 (16%)
ABCC2 4544 G>A (rs8187710)	
GG	161 (85%)
GA	26 (14%)
AA	2 (1%)
SLCO1B3 334 T>G (rs4149117)	
TT	5 (3%)
TG	45 (24%)
GG	139 (73%)
UGT1A8 518 C>G (rs1042597)	
CC	111 (59%)

CG	62 (33%)
GG	16 (8%)
UGT1A9 -2152 C>T (rs17868320)	
CC	177 (94%)
CT	12 (6%)
UGT1A9 -275 T>A (rs6714486)	
TT	170 (90%)
TA	18 (9%)
AA	1 (1%)
UGT 2B7 -840 G>A (rs7438135)	
GG	45 (24%)
GA	86 (45%)
AA	58 (31%)
CYP2C8 G>A (rs11572076)	
GG	187 (99%)
GA	2 (1%)
HUS1 (rs1056663)	
GG	58 (31%)
GA	91 (48%)
AA	40 (21%)
IL12A (rs568408)	
GG	154 (82%)
GA	33 (17%)
AA	2 (1%)

* (mean C2)×follow-up duration ; **(mean EC-MPS dose)×the follow-up period duration; $ diarrhea episodes leading to EC-MPS dose reduction or prolonged hospitalization; for BPAR and diarrhea, the first episode was taken into account, for anemia and leucopenia, the total number of events among the whole protocol visits were taken into account.

Table 2- Results of univariate logistic regressions of potential factors favoring the first biopsy-proven acute rejection (BPAR)

Covariate	Category	OR (95%CI)	p
$CD_{EC\text{-}MPS}$* (mg*month)	Per unit increase	0.999 (0.997,1.001)	0.708
Cyclosporine AUC_{C2}** (mg*month/L)	Per unit increase	1.000 (0.999,1.001)	0.594
Age	Per year increase	0.99 (0.96,1.02)	0.542
Sex	M *vs.* F	1.22 (0.61,2.47)	0.571
Randomization group	with *vs.* without steroids	0.98 (0.5,1.93)	0.951
IMPDH2 IVS7+10 T>C (rs11706052)	TT vs TC/CC	1.35 (0.56,3.24)	0.501
IMPDH1 C>T (rs2278923)	CT *vs.* CC TT *vs.* CC	0.94 (0.43,2.06) 0.87 (0.33,2.30)	0.883 0.772
IMPDH1 C>T (rs2278924)	CT *vs.* CC TT *vs.* CC	0.83 (0.4,1.7) 0.94 (0.3,2.93)	0.606 0.910
ABCC2 -24 C>T (rs717620)	CT *vs.* CC	0.95 (0.46,1.92)	0.877
ABCC2 1249 G>A (rs2273697)	GG *vs.* GA/AA	1.65 (0.79,3.44)	0.185
ABCC2 3972 C>T (rs3740066)	CT *vs.* CC TT *vs.* CC	1.36 (0.63,2.97) 2.31 (0.88,6.05)	0.434 0.088
ABCC2 4544 G>A (rs8187710)	GG *vs.* GA/AA	1.39 (0.52,3.69)	0.513
SLCO1B3 334 T>G (rs4149117)	TG/TT *vs.* GG	0.75 (0.34,1.64)	0.472
UGT1A8 518 C>G (rs1042597)	CG/GG *vs.* CC	1.04 (0.52,2.05)	0.920
UGT1A9 -2152 C>T (rs17868320)	CC *vs.* CT	1.36 (0.39,4.74)	0.632
UGT1A9 -275 T>A	TT *vs.* TA/AA	0.55 (0.20,1.52)	0.252
UGT2B7 -840 G>A (rs7438135)	GA *vs.* AA GG *vs.* AA	1.54 (0.7,3.41) 0.89 (0.34,2.33)	0.282 0.808
CYP2C8 G>A rs11572076	GG *vs.* GA	0.37 (0.02,6.07)	0.488
HUS1 G>A rs1056663	GA *vs.* AA GG *vs.* AA	1.15 (0.46,2.83) 1.35 (0.52,3.49)	0.767 0.542
IL12A G>A rs568408	GG *vs.* GA/AA	0.66 (0.29,1.51)	0.326

*(mean EC-MPS dose)×the follow-up period duration ; ** (mean C2)×follow-up duration

Table 3- Results of univariate logistic regressions of potential factors favoring the first diarrhea episode leading to EC-MPS dose reduction or prolonged hospitalization

Covariate	Category	OR (95%CI)	p
CD_{EC-MPS}* (mg*month)	Per unit increase	1.00 (0.997,1.003)	0.979
Cyclosporine AUC_{C2}** (mg*month/L)	Per unit increase	1.0009(0.999,1.003)	0.351
Age	Per year increase	1.01 (0.96,1.06)	0.604
Sex	F vs M	3.11(1.05,9.15)	0.040
Randomization group	with *vs.* without steroids	2.09 (0.69,6.38)	0.193
IMPDH2 IVS7+10 T>C (rs11706052)	TT *vs.* TC/CC	1.00 (0.27,3.74)	1
IMPDH1 C>T (rs2278923)	TC *vs.* CC	0.40 (0.10,1.58)	0.192
	TT *vs.* CC	1.61 (0.45,5.71)	0.460
IMPDH1 C>T (rs2278924)	TC *vs.* CC	0.42 (0.12,1.43)	0.166
	TT *vs.* CC	0.89 (0.18,4.46)	0.886
ABCC2 -24 C>T (rs717620)	CT *vs.* CC	0.46 (0.12,1.68)	0.237
ABCC2 1249 G>A (rs2273697)	GG *vs.* GA/AA	1.67 (0.51,5.44)	0.399
ABCC2 3972 C>T	CT *vs.* CC	0.82 (0.23,2.94)	0.757
(rs3740066)	TT *vs.* CC	2.58 (0.69,9.64)	0.160
ABCC2 4544 G>A (rs8187710)	GG *vs.* GA/AA	2.67 (0.34,21.1)	0.353
SLCO1B3 334 T>G (rs4149117)	TT/TG *vs.* GG	1.02 (0.31,3.36)	0.974
UGT1A8 518 C>G (rs1042597)	CG/GG *vs.* CC	1.28 (0.44,3.69)	0.646
UGT1A9 -2152 C>T (rs17868320)	CT *vs.* CC	1.06 (0.13,8.86)	0.954
UGT1A9 -275 T>A	TT *vs.* AA/TA	1.61 (0.20,12.93)	0.657
UGT2B7 -840 G>A	GA *vs.* AA	3.23 (0.67,15.53)	0.143
(rs7438135)	GG *vs.* AA	2.73 (0.48,15.63)	0.259
CYP2C8 G>A rs11572076	GG *vs.* GA	Inf (0,Inf)	0.993
HUS1 G>A rs1056663	GA *vs.* AA	0.74 (0.2,2.69)	0.649
	GG *vs.* AA	0.67 (0.16,2.84)	0.583
IL12A G>A rs568408	GG *vs.* GA/AA	3.38 (0.43,26.57)	0.248

*(mean EC-MPS dose)×the follow-up period duration ; ** (mean C2)×follow-up duration

Table 4- Univariate analysis of factors influencing leucopenia using time dependent Cox proportional hazards regression models. The total number of events among the whole protocol visits were studied.

*Covariate	Category	HR (95%CI)	p value
EC_MPS dose	Per unit increase	0.999 (0.998,1.000)	0.2220
Age	Per year increase	1.03 (0.99,1.07)	0.1400
Sex	F vs M	1.66 (0.73,3.76)	0.2270
Randomization group	with *vs.* without steroids	0.61 (0.27,1.38)	0.2380
IMPDH2 IVS7+10 T>C (rs11706052)	TT *vs.* TC/CC	6.14 (0.83,45.58)	0.0761
IMPDH1 C>T (rs2278923)	CT *vs.* CC	4.84 (1.12,20.97)	0.0351
	TT *vs.* CC	3.35 (0.65,17.33)	0.1489
IMPDH1 C>T (rs2278924)	CT *vs.* CC	1.42 (0.60,3.34)	0.4200
	TT *vs.* CC	0.98 (0.21,4.56)	0.9770
ABCC2 -24 C>T (rs717620)	CT *vs.* CC	0.83 (0.35,2.02)	0.6890
ABCC2 1249 G>A (rs2273697)	GG *vs.* GA/AA	0.89 (0.39,2.03)	0.7880
ABCC2 3972 C>T (rs3740066)	CT *vs.* CC	0.42 (0.14,1.23)	0.1147
	TT *vs.* CC	2.45 (0.99,6.07)	0.0533
ABCC2 4544 G>A (rs8187710)	GG *vs.* GA/AA	4.82 (0.65,35.81)	0.1240
SLCO1B3 334 T>G (rs4149117)	TG/TT *vs.* GG	1.60 (0.70,3.65)	0.2620
UGT1A8 518 C>G (rs1042597)	CG/GG *vs.* CC	1.64 (0.73,3.67)	0.2270
UGT1A9 -2152 C>T (rs17868320)	CT *vs.* CC	0 (0,Inf)	1.0000
UGT1A9 -275 T>A	TT *vs.* TA/AA	2.38 (0.32,17.67)	0.3970
UGT 2B7 -840 G>A (rs7438135)	GA *vs.* AA	0.57 (0.22,1.46)	0.2430
	GG *vs.* AA	0.69 (0.24,1.99)	0.4910
CYP2C8 G>A rs11572076	GG *vs.* GA	0.14 (0.03,0.59)	0.0078
HUS1 G>A rs1056663	GA *vs.* AA	0.70 (0.23,2.08)	0.5180
	GG *vs.* AA	1.07 (0.36,3.21)	0.9000
IL12A G>A rs568408	GG *vs.* GA/AA	0.55 (0.22,1.41)	0.2140

*cyclosporine C2 was not evaluated due to a high number of missing values (487/1398), totally at random

Table 5- Univariate analysis of factors influencing anemia using time dependent Cox proportional hazards regression models. The total number of events among the whole protocol visits were studied.

Covariate	Category	HR (95%CI)	p value
EC_MPS dose	Per unit increase	1.004 (1.003,1.005)	<0.0001
Age	Per year increase	1.003(0.989, 1.016)	0.690
Sex	F vs M	0.95 (0.69,1.31)	0.768
Randomization group	with *vs.* without steroids	0.83 (0.61,1.11)	0.207
IMPDH2 IVS7+10 T>C (rs11706052)	TT *vs.* TC/CC	1.11 (0.77,1.62)	0.572
IMPDH1 C>T (rs2278923)	CT *vs.* CC TT *vs.* CC	0.95 (0.67,1.34) 1.09 (0.72,1.63)	0.769 0.682
IMPDH1 C>T (rs2278924)	CT *vs.* CC TT *vs.* CC	0.99 (0.72,1.36) 1.12 (0.70,1.80)	0.958 0.630
ABCC2 -24 C>T (rs717620)	CT *vs.* CC	1.42 (1.06,1.92)	0.0202
ABCC2 1249 G>A (rs2273697)	GG *vs.* GA/AA	1.14 (0.83,1.56)	0.4170
ABCC2 3972 C>T (rs3740066)	CT *vs.* CC TT *vs.* CC	1.35 (0.97,1.89) 1.43 (0.91,2.25)	0.0772 0.1235
ABCC2 4544 G>A (rs8187710)	GG *vs.* GA/AA	0.77 (0.53,1.12)	0.1790
SLCO1B3 334 T>G (rs4149117)	TG/TT *vs.* GG	1.14 (0.82,1.58)	0.447
UGT1A8 518 C>G (rs1042597)	CG/GG *vs.* CC	1.02 (0.75,1.37)	0.914
UGT1A9 -2152 C>T (rs17868320)	CT *vs.* CC	1.54 (0.85,2.76)	0.150
UGT1A9 -275 T>A	TT *vs.* TA/AA	0.65 (0.43,0.99)	0.0473
UGT 2B7 -840 G>A (rs7438135)	GA *vs.* AA GG *vs.* AA	1.51 (1.03,2.22) 1.63 (1.07,2.50)	0.036 0.023
CYP2C8 G>A rs11572076	GG *vs.* GA	1.88 (0.26,13.43)	0.529
HUS1 G>A rs1056663	GA *vs.* AA GG *vs.* AA	0.76 (0.52,1.12) 0.89 (0.59,1.33)	0.162 0.567
IL12A G>A rs568408	GG *vs.* GA/AA	0.98 (0.66,1.43)	0.905

*cyclosporine C2 was not evaluated due to a high number of missing values (514/1279), totally at random