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TITLE: Association of ITPA polymorphisms rs6051702/rs1127354 instead of rs7270101/rs1127354 as predictor of ribavirin-associated anemia in chronic hepatitis C treated patients.

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SHORT TITLE: ITPA SNPs and anemia.

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List of Abbreviations: HCV, Hepatitis C virus; SVR, sustained virological response; PEG/IFN, pegylated interferon; RBV, ribavirin; Hb, haemoglobin; ITP, inosine triphosphate, ITPA, inosine triphosphatase; SNPs, single nucleotide polymorphisms; TDM, therapeutic drug monitoring; IQR, inter-quartile range; WT, wild type.

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ABSTRACT

Functional variants rs7270101 and rs1127354 of inosine triphosphatase (*ITPA*) were recently found to protect against ribavirin (RBV)-induced hemolytic anemia. However, no definitive data are yet available on the role of no functional rs6051702 polymorphism. Since a simultaneous evaluation of the three *ITPA* SNPs for hemolytic anemia has not yet been investigated, we aimed to understand the contribution of each SNPs and its potential clinical use to predict anemia in HCV treated patients.

A retrospective analysis included 379 HCV treated patients. The *ITPA* variants rs6051702, rs7270101 and rs1127354 were genotyped and tested for association with achieving anemia at week 4. We also investigated, using multivariate logistic regression, the impact of each single and paired associated polymorphisms on anemia onset.

All SNPs were associated with Hb decrease. The carrier of at least one variant allele in the functional *ITPA* SNPs was associated with a lower decrement of Hb, as compared to patients without a variant allele. In multivariate logistic regression analyses the carrier of a variant allele in the rs6051702/rs1127354 association (OR=0.11, $p=1.75x10^{-5}$) and Hb at baseline (OR=1.51, $p=1.21x10^{-4}$) were independently associated with protection against clinically significant anemia at week 4.

All *ITPA* polymorphisms considered were shown to be significantly associated with anemia onset. A multivariate regression model based on *ITPA* genetic polymorphisms was developed for predicting the risk of anemia. Considering the characterization of pre-therapy anemia predictors, rs6051702 SNP in association to rs1127354 is more informative in order to avoid this relevant adverse event.

Keywords: (Anemia; HCV; ITPA; Ribavirin; Single Nucleotide Polymorphism; Adverse Event).

1 INTRODUCTION.

Hepatitis C virus (HCV) is one of the major causes of liver cirrhosis and hepatocellular carcinoma (Barrera et al., 1995). Sustained viral response (SVR), defined as undetectable plasma HCV RNA 24 weeks after the end of treatment, is achieved in less than 50% of patients with genotype-1 infection using the dual regimen consisting of pegylated-interferon (PEG/IFN) and ribavirin (Hadziyannis et al., 2004).

Although two new antiviral drugs are being introduced into clinical use, the combination of pegylated-interferon-alfa (pegIFN-alfa) and ribavirin (RBV) still remain the backbone of anti-HCV therapy.

Anemia is a major untoward effect of anti-HCV chronic therapy. Both PEG/IFN and RBV play a role in haemoglobin (Hb) decrease, although most of the responsibility is by far attributable to the latter. Moreover, with the forthcoming introduction of the new HCV protease inhibitors, telaprevir and boceprevir, the incidence of anemia is likely to increase, as shown by the results of recent clinical trials (Hezode et al., 2009; Kwo et al.; Suzuki et al.).

Repeatedly, individual RBV dose and pharmacokinetic (Pk) exposure have been found to be consistent determinants of treatment-associated anemia, and recommendations on dose reduction have been released in order to mitigate the impact of RBV on the severity of anemia (Aguilar Marucco et al., 2008; D'Avolio et al., 2012a; Kubota et al.; Lindahl et al., 2004; Mac Nicholas and Norris; McHutchison et al., 2006).

Among the non-modifiable individual factors also contributing to anemia development in recipients of anti-HCV therapy, there are single nucleotide polymorphisms (SNPs) in the human DNA region coding for inosine triphosphatase (*ITPA*). These were identified recently as the most significant variables influencing the risk of anemia (Fellay et al., 2010; Suzuki et al., 2011;

Thompson et al., 2010a). In these studies, by using a genome-wide association approach, two functional variants of *ITPA*, including one coding and one intronic variant, were found to be associated with treatment-induced anemia in HCV-infected patients (Caviglia et al., 2012; D'Avolio et al., 2012a; Fellay et al.; Ochi et al.; Suzuki et al.), as defined by the magnitude of Hb reduction after 4 weeks of treatment. This genetically determined vulnerability was subsequently confirmed in several ethnically heterogeneous clinical cohorts. The association signal was accounted for by two functional variants in the *ITPA* gene on chromosome 20: a missense variant in exon 2 (rs1127354, P32T) and a splice-altering single nucleotide polymorphism in intron 2 (rs7270101). Both polymorphisms had previously been well characterized and validated as functional variants in studies of patients with ITPase deficiency, a benign inherited enzymopathy in which inosine triphosphate (ITP, the substrate for ITPase) accumulates in red blood cells (Shipkova et al., 2006; Sumi et al., 2002).

A recent study established that intraerythrocytic accumulation of ITP, as seen in carriers of such anemia-protecting mutants, provides an alternative source of nucleoside-triphosphates that eventually compensates for RBV-induced ATP reduction (Hitomi et al.).

The introduction of the *ITPA* rs1127354 and rs7270101 genetic polymorphisms substantially improves our ability to predict the individual risk of treatment-induced anemia(D'Avolio et al., 2012a), but it is not clear how the non-functional rs6051702 *ITPA* polymorphism should be considered in this scenario.

Similarly to rs12979860 and rs8099917 polymorphisms that are localized in the region upstream of the gene encoding for *IL28B*(Ge et al., 2009; Tanaka et al., 2009), rs6051702 polymorphism on chromosome 20 (20p13 region) (Fellay et al., 2010) is localized in a no coding region adjacent to *ITPA* gene (Fellay et al., 2010).

As a simultaneous evaluation of the three *ITPA* SNPs for hemolytic anemia has not yet been investigated, we aimed to understand the contribution of each SNP and its potential clinical use to predict anemia in HCV treated patients at 1 month, date before which clinicians generally evaluate readjustments of ribavirin doses and the use of growth factors such as erythropoietin.

2 MATERIALS AND METHODS.

2.1 Patients

In this retrospective study, 379 patients with chronic HCV infection, treated in two university hospitals (Amedeo di Savoia and S. Giovanni Battista) of the city of Turin, Italy, between March 2005 and November 2012 were enrolled.

Patients were treated with PEG/IFN-α-2b (1.5 μg/kg s.c. once a week; sub dermal injection) or PEG/IFN-α-2a (180 μg once a week; sub dermal injection) plus RBV (600–1400 mg daily depending on bodyweight; orally). Sampling was performed after obtaining written informed consent in accordance with local ethics committee guidelines; patients were asked for an additional authorization for the genetic screening of their stored samples. Main inclusion criteria were: above 18 years old, no concomitant interacting drugs, self-reported adherence > 95%, no RBV and/or PEG/IFN dose modification up to week 4 of treatment and no treatment with growth factor before week 4. Patients with other forms of liver disease, active hepatitis A, hepatitis B infection, HIV infection, decompensated liver disease, hepatocellular carcinoma, severe depression or other psychiatric diseases, significant cardiac or renal disease, seizure disorders or pregnancy were excluded from this study. Data collected included: age, gender, weight, previous IFN therapy, concomitant drugs, baseline and week 2 and 4 biochemical parameters, such as white blood cells, Hb, alanine transaminase (ALT) level, serum HCV RNA level (logIU/mL).

2.2 Genotyping

Patients from whom DNA samples were available and who agreed to undergo genetic analyses were genotyped at polymorphic sites rs1127354, rs7270101 and rs6051702 on chromosome 20 using the ABI TaqMan allelic discrimination kit by real time PCR using standard methodology.

All primers, probes, and PCR conditions are available on request. The possible genotypes for each biallelic polymorphism are as follows: rs1127354: C/C, A/C, A/A (minor allele = A); rs7270101: A/A, A/C, C/C (minor allele = C); rs6051702: A/A, A/C, C/C (minor allele = C).

2.3 Definition of Clinical End Points

According to outcome definition in clinical trials for HCV chronic hepatitis treatment, Hb decline at week 4 on-therapy, was taken as the clinical endpoint. Patients were classified according to two clinical cut-off: (a) absolute Hb value lower than 10 g/dL, and (b) Hb reduction >3 g/dL at week 4.

The anemia grade was defined according to WHO (Groopman and Itri, 1999) as following: grade 0 (≥ 11g/dL), grade 1 (< 11-9.5g/dL), grade 2 (<9.5-8g/dL), grade 3 (<8-6.5g/dL), grade 4 (<6.5g/dL).

2.4 Statistical Analysis

For descriptive statistics, continuous variables were summarized as median (25th to 75th percentiles). Categorical variables were described as frequency and percentage. All data were assessed for normality using a Shapiro-Wilk test and categorical data were compared using a Mann Whitney or Kruskal-Wallis statistical test. To investigate continuous data, a Spearman Rank correlation was utilized. The association between individual *ITPA* SNP and the incidence of significant Hb decline was tested by a basic allelic test and calculated using the χ^2-test. Multivariate logistic regression analysis with stepwise forward selection was performed with *P*-values of less than 0.05 as the criteria for model inclusion. Statistical analyses were conducted by

using SPSS software package ver. 20.0 (Chicago, IL, USA). Linkage disequilibrium analysis was conducted considering r^2 and using Haploview 4.2.

3 RESULTS.

Twenty-nine out of 408 initial patients were excluded due to the lack of demographic and clinical information. Of the 379 patients included in the analysis, 231 patients (60.9%) were male, the median age was 46 years (IQR, 40–57 years) and the median Body Mass Index (BMI) was 24.3 kg/m^2 (IQR, 22.0–26.6 kg). 360 patients (94.9%) were Caucasian and 19 (5.1%) were African. 233 patients (61.5%) were treated with PEG/IFN-α-2a (180 µg/ once a week) and 146 (38.5%) were treated with PEG/IFN-α-2b (1.5 µg/kg s.c. once a week). The median dose of RBV was 13.88 mg/kg (12.70-15.33). The majority of patients were treated with 800 mg (n=108) or 1000 mg (n=201) of RBV; three patients received 600 mg, three received 400 mg, sixty 1200 mg and four 1400 mg. The baseline patients characteristics were summarized in Table 1.

The median Hb reduction was -2.3 g/dL (-3.4 to -1.3) at week 4. 129 patients (34%) had anemia (Hb reduction>3g/dL or Hb<10g/dL) after one month of therapy; moreover 64 (16.9%) and 10 (2.6%) patients developed WHO anemia grade 1 and 2, respectively, and none grade 3 or 4.

Baseline Hb was correlated with Hb reduction (rho=-0.350 $p=2.47x10^{-12}$).

The variant allele frequencies for rs1127354 C/A, rs7270101 A/C and rs6051702 A/C were 5.5%, 10.0%, and 14.5%, respectively; Specifically: 42 (11.1%) patients were CA and none AA for rs1127354 SNP; 64 (16.9%) patients were AC and 6 (1.6%) were CC for rs7270101 SNP; 84 (22.2%) patients were AC and 13 (3.4%) were CC for rs6051702 SNP. All SNPs were in Hardy-Weinberg equilibrium. The three SNPs in analysis result in low Linkage Disequilibrium (D'=0.65 for rs6051702/rs7270101 and D'=0.53 for rs6051702/rs1127354) in our population. No differences concerning demographic, racial, physical characteristics and biochemical parameters, (Hb, platelet count, alanine transaminase -ALT- level, serum HCV RNA level) were observed among genetically defined groups.

The two functional *ITPA* SNPs responsible for inosine triphosphatase deficiency (rs1127354 and rs7270101) and the co-segregate SNP (rs6051702) were associated with the magnitude of hemoglobin decrease after one month of therapy. Patients with rs1127354CC (n=337) had the greater hemoglobin decrement (-2.4 g/dL, IQR -3.5 to -1.5), compared to patients with CA/AA genotypes (-0.9 g/dL, IQR -1.6 to -0.2, $p=1.28x10^{-11}$) (Figure 1B). Similarly patients with genotype rs7270101AA (n=309) had a greater hemoglobin decrement (-2.4 g/dL, IQR -3.6 to -1.3), compared to patients with AC/CC (-1.7 g/dL, IQR -2.5 to -0.9, p=0.001) (Figure 1C) and patients with rs6051702AA (n=282) had a greater hemoglobin decrement (-2.5 g/dL, IQR -3.6 to -1.6) compared to patients with AC/CC (-1.4 g/dL, IQR -2.3 to -0.7, $p=2.35x10^{-10}$) (Figure 1A).

As shown in Table 2, the factors related to anemia after 1 month of therapy are the baseline hemoglobin and *ITPA* genetic polymorphisms.

The associations in pair of mutation carriers of the three polymorphisms were evaluated on their ability to predict anemia with a logistic regression analysis. As shown in Table 3 only the carrier of variant allele in the association in pair of rs6051702 AC/CC and rs1127354 CA/AA is able to predict anemia ($p<0.001$) in multivariate logistic analysis.

Many factors previously linked to anemia were also included in multivariate logistic regression analysis and only the rs6051702/rs1127354 genetic association has been considered on the basis of the results in table 3. In the evaluation of the factors in the logistic regression analysis, hemoglobin at baseline (OR=1.51, $p=1.21x10^{-4}$) and the carrier of at least one variant allele of SNPs association (OR=0.11, $p=1.75x10^{-5}$) in *ITPA* gene (Table 4) were independently associated with achievement of significant anemia.

Finally, the positive and negative likelihood ratios (LR+ and LR-) between the presence of at least one protective variant allele of rs6051702/rs1127354 SNPs and absence of anemia showed to be 1.44 and 0.30 respectively, with 88.4% of sensibility and 38.8% of specificity.

4 DISCUSSION.

Recently several genetic variants associated with substantial effects on both efficacy and toxicity of PEG/IFN and ribavirin therapy have been identified. SNPs in the *IL28B* gene were found to be strongly associated with response to therapy of chronic genotype 1 HCV infection (D'Avolio et al., 2011; D'Avolio et al., 2012b; Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Thompson et al.) and SNPs in the *ITPA* gene were identified as predictors of RBV treatment-associated anemia in the European-American and Japanese populations (Caviglia et al., 2012; D'Avolio et al., 2012a; Fellay et al., 2010; Kurosaki et al., 2013; Naggie et al., 2012; Ochi et al.; Rau et al., 2012; Sakamoto et al., 2010; Suzuki et al., 2011; Thompson et al., 2010a). The anemia experienced as a consequence of PEG/IFN and RBV combination therapy is primarily caused by a RBV-induced hemolysis and secondarily by interferon-induced bone marrow toxicity. Ribavirin toxicity can be explained by the accumulation of RBV phosphate metabolites in erythrocytes, oxidative damage and consequent cell lysis (De Franceschi et al., 2000). The impact of anemia on the outcome of anti-HCV infection therapy is substantial, since RBV dose-reduction or early treatment interruption due to anemia often leads to suboptimal drug intake and a higher chance of treatment failure (Fried et al., 2002). The possibility of identifying those patients who are more likely to undergo significant Hb loss while on treatment might be helpful in order to implement those measures which might limit the impact of anemia on treatment outcome.

In this study the newly identified genetic polymorphisms of *ITPA* confirmed their value as predictors of treatment-induced anemia, as established by HB reduction after 4 weeks of PEG/IFN-RBV treatment. Furthermore, we observed that baseline Hb was correlated with Hb reduction and, strikingly, RBV dose was not significantly correlated to anemia by a statistical

point of view, but shows a borderline statistical significance (p=0.056) respect to other paper (Sulkowski et al., 2010), confirming, however, that anemia is even a pharmacodynamic marker of RBV exposure.

However having a higher baseline HB concentration is a biologically benign condition, which testifies to a higher functional reserve as compared to the other patients. For pure numerical reasons such condition is more vulnerable to a HB loss, since HB loss is here measured in terms of absolute values rather than percentage HB loss from baseline.

Beside *ITPA* polymorphisms, a series of individual features such as RBV concentration, age, platelet count, haemoglobin concentration and haptoglobin phenotype were found to have some degree of association with the risk of anemia (Caviglia et al., 2012; D'Avolio et al., 2012a; Lindahl et al., 2004; Nomura et al., 2004; Rendon et al., 2005; van Vlerken et al., 2011; Van Vlierbergh et al., 2001). Among these, the only modifiable feature is RBV pharmacokinetic exposure, which shows a remarkable variability among HCV-infected patients (Tsubota et al., 2003). In fact, early assessment of RBV concentration is suggested by some studies (Caviglia et al., 2012; D'Avolio et al., 2012a; Loustaud-Ratti et al., 2008; Morello et al., 2008; Rendon et al., 2005) in order to adjust individual RBV dose.

Following the identification of *ITPA* polymorphism as the strongest predictor of anemia in PEG/IFN-RBV recipients, the question arises as to how to integrate this newly identified genetic marker in order to predict and manage the individual risk of anemia. The three SNPs analyzed in this study, two functional and one no-functional *ITPA* polymorphisms, retain their independent predictive values and display limited overlap in terms of predictive value (Table 2 and Figure 1).

As shown in Table 3, the pairwise associations of the three studied polymorphisms showed that the combination rs6051702/rs1127354 is the most significant in predicting anemia at 1 month after initiation of therapy.

These data are partially unexpected because between the two polymorphisms considered in the combination only the rs1127354 SNP is functional, while rs6051702 SNP is in the non-coding region of the *ITPA* gene. This finding is similar to what has been found for the two non-coding polymorphisms rs1297860 and rs8099917 *IL28B* gene identified as the most powerful genetic factors influencing IFN therapy response.

The polymorphism rs7072101, which is functional and often it is studied in association with the rs1127354 (Caviglia et al., 2012; D'Avolio et al., 2012a; Fellay et al., 2010; Naggie et al., 2012; Rau et al., 2012; Thompson et al., 2010a; Thompson et al., 2010c) seems to have minor clinical significance, compared to the other.

Therefore, considering the importance of pre-therapy anemia prognostic factors rs6051702 SNP should be considered in place of rs7270101 in association to rs1127354. According to our LR-results, for a patient carrier of at least one mutation for rs6051702/ rs1127354 SNPs, the probability to not develop anemia is nearly 3 times higher respect to the probability to develop anemia.

The clinical use of *ITPA* genetic polymorphisms substantially improves our ability to predict the individual risk of treatment-induced anemia, particularly including the non-coding polymorphism rs6051702. The main implication of our results suggests that the carriage of *ITPA* variants is associated with a rather limited median Hb loss on-therapy, independently of RBV concentration (D'Avolio et al., 2012a).

Otherwise, it has been demonstrated that anemia is related to the intra-erythrocytic and plasma concentrations of RBV (Caviglia et al., 2012; D'Avolio et al., 2012a; D'Avolio et al., 2013; D'Avolio et al., 2012c; De Franceschi et al., 2000; De Nicolò et al., 2013; Kubota et al., 2010; Loustaud-Ratti et al., 2008; Loustaud-Ratti et al., 2011; Morello et al., 2008; Rendon et al., 2005), moreover RBV plasma and intra-erythrocytic concentrations are correlated with the RBV doses taken by patients. Otherwise, RBV concentration is not only dependent on the administered dose, but also on body weight, absorption (nucleoside transporters and food effect) and excretion. Then the integration of the early therapeutic drug monitoring of RBV (after 1-4 weeks of therapy) (D'Avolio et al., 2012a; De Nicolò et al., 2013; Loustaud-Ratti et al., 2008; Loustaud-Ratti et al., 2011) and RBV dose selection by clinicians on the basis of ITPA genetic profile could improve the management of anemia.

Genetic testing is widespread and as short turnaround time compared to therapeutic drug monitoring (TDM) for ribavirin. The characterization of the most significant *ITPA* SNPs pairs at the baseline may to able the estimation of the individual risk of treatment-induced anemia. Ribavirin TDM might maintain a role in the management of the prevailing proportion of wild type *ITPA* genotype patients, whose RBV concentration may be fruitfully modified by dose adjustment. Since RBV pharmacokinetic exposure is also associated with the rate of SVR (Aguilar Marucco et al., 2008; Breilh et al., 2009; D'Avolio et al., 2011; Maynard et al., 2008; Pedersen et al.; van Vlerken et al.), TDM might also apply to carriers of *ITPA* variants with suboptimal RBV concentration, in whom RBV dose may be increased with a smaller risk of anemia.

5 CONCLUSION

In conclusion, this retrospective study confirms the high impact of *ITPA* SNPs on hemolytic anemia and suggest that rs6051702/rs1127354, and not rs7270101/rs1127354 combination, is the best genetic predictor able to predict anemia after 1 month of therapy. Although more studies are needed to clarify and confirm the relationship between anemia, *ITPA* polymorphism and RBV pharmacokinetics, these results show that estimating the risk of anemia by genotyping these two SNPs is more accurate than genotyping for the two functional SNPs. Moreover, these data might suggest the development of an individual screening algorithm, based on *ITPA* genetic profiles, able to reduce the impact of anemia on the rate of response to anti-HCV treatment, maybe limiting the need of TDM RBV plasma concentrations in this setting.

6 REFERENCES

Aguilar Marucco, D., Gonzalez de Requena, D., Bonora, S., Tettoni, C., Bonasso, M., De Blasi, T., D'Avolio, A., Sciandra, M., Siccardi, M., Baietto, L., Trentini, L., Sinicco, A., Cariti, G., Di Perri, G., 2008. The use of trough ribavirin concentration to predict sustained virological response and haematological toxicity in HIV/HCV-co-infected patients treated with ribavirin and pegylated interferon. J Antimicrob Chemother 61, 919-924.

Barrera, J.M., Bruguera, M., Ercilla, M.G., Gil, C., Celis, R., Gil, M.P., del Valle Onorato, M., Rodes, J., Ordinas, A., 1995. Persistent hepatitis C viremia after acute self-limiting posttransfusion hepatitis C. Hepatology 21, 639-644.

Breilh, D., Foucher, J., Castera, L., Trimoulet, P., Djabarouti, S., Merrouche, W., Couzigou, P., Saux, M.C., de Ledinghen, V., 2009. Impact of ribavirin plasma level on sustained virological response in patients treated with pegylated interferon and ribavirin for chronic hepatitis C. Aliment Pharmacol Ther 30, 487-494.

Caviglia, G., D'Avolio, A., Ciancio, A., De Nicolò, A., Boglione, L., Abate, M., Rosso, C., Smedile, A., Cariti, G., Cusato, J., 2012. F-33 Early ribavirin plasma concentration and ITPA polymorphisms as determinants of anemia after one month of anti-HCV therapy. Digestive and Liver Disease 44, S42.

D'Avolio, A., Ciancio, A., Siccardi, M., Baietto, L., Simiele, M., Cariti, G., Calcagno, A., Smedile, A., Cusato, J., Bonora, S., Rizzetto, M., Di Perri, G., 2011. Ribavirin pharmacokinetics and interleukin 28B plus cytochrome P450 27B1 single-nucleotide polymorphisms as predictors of response to pegylated interferon/ribavirin treatment in patients infected with hepatitis C virus genotype 1/4. Hepatology 54, 2279.

D'Avolio, A., Ciancio, A., Siccardi, M., Smedile, A., Baietto, L., Simiele, M., Marucco, D.A., Cariti, G., Calcagno, A., de Requena, D.G., Sciandra, M., Cusato, J., Troshina, G., Bonora, S., Rizzetto, M., Di Perri, G., 2012a. Inosine triphosphatase polymorphisms and ribavirin pharmacokinetics as determinants of ribavirin-associate anemia in patients receiving standard anti-HCV treatment. Ther Drug Monit 34, 165-170.

D'Avolio, A., Ciancio, A., Siccardi, M., Smedile, A., Simiele, M., Cusato, J., Baietto, L., Marucco, D.A., Cariti, G., Calcagno, A., de Requena, D.G., Sciandra, M., Troshina, G., Caviglia, G.P., Bonora, S., Rizzetto, M., Di Perri, G., 2012b. Negative predictive value of IL28B, SLC28A2 and CYP27B1 SNPs and low RBV plasma exposure for therapeutic response to PEG/IFN-RBV treatment. Ther Drug Monit 34, 722-728.

D'Avolio, A., Cusato, J., Calcagno, A., Di Perri, G., 2013. Estimating ribavirin plasma exposure: Genetics or therapeutic drug monitoring? J Hepatol.

D'Avolio, A., De Nicolo, A., Simiele, M., Turini, S., Agnesod, D., Boglione, L., Cusato, J., Baietto, L., Cariti, G., Calcagno, A., Sciandra, M., Di Perri, G., Bonora, S., 2012c. Development and validation of a useful HPLC-UV method for quantification of total and phosphorylated-ribavirin in blood and erythrocytes of HCV+ patients. J Pharm Biomed Anal 66, 376-380.

De Franceschi, L., Fattovich, G., Turrini, F., Ayi, K., Brugnara, C., Manzato, F., Noventa, F., Stanzial, A.M., Solero, P., Corrocher, R., 2000. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. Hepatology 31, 997-1004.

De Nicolò, A., Cusato, J., Boglione, L., Ciancio, A., Smedile, A., Agnesod, D., Cariti, G., Rizzetto, M., Di Perri, G., D'Avolio, A., 2013. P_21 Early ribavirin plasma concentrations as

predictor of anemia onset after one month of anti-HCV therapy in ITPA stratified population. Reviews in antiviral therapy & infectious diseases 3, 51.

Fellay, J., Thompson, A.J., Ge, D., Gumbs, C.E., Urban, T.J., Shianna, K.V., Little, L.D., Qiu, P., Bertelsen, A.H., Watson, M., Warner, A., Muir, A.J., Brass, C., Albrecht, J., Sulkowski, M., McHutchison, J.G., Goldstein, D.B., 2010. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. Nature 464, 405-408.

Fried, M.W., Shiffman, M.L., Reddy, K.R., Smith, C., Marinos, G., Goncales, F.L., Jr., Haussinger, D., Diago, M., Carosi, G., Dhumeaux, D., Craxi, A., Lin, A., Hoffman, J., Yu, J., 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 347, 975-982.

Ge, D., Fellay, J., Thompson, A.J., Simon, J.S., Shianna, K.V., Urban, T.J., Heinzen, E.L., Qiu, P., Bertelsen, A.H., Muir, A.J., Sulkowski, M., McHutchison, J.G., Goldstein, D.B., 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461, 399-401.

Groopman, J.E., Itri, L.M., 1999. Chemotherapy-induced anemia in adults: incidence and treatment. J Natl Cancer Inst 91, 1616-1634.

Hadziyannis, S.J., Sette, H., Jr., Morgan, T.R., Balan, V., Diago, M., Marcellin, P., Ramadori, G., Bodenheimer, H., Jr., Bernstein, D., Rizzetto, M., Zeuzem, S., Pockros, P.J., Lin, A., Ackrill, A.M., 2004. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 140, 346-355.

Hezode, C., Forestier, N., Dusheiko, G., Ferenci, P., Pol, S., Goeser, T., Bronowicki, J.P., Bourliere, M., Gharakhanian, S., Bengtsson, L., McNair, L., George, S., Kieffer, T., Kwong, A.,

Kauffman, R.S., Alam, J., Pawlotsky, J.M., Zeuzem, S., 2009. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 360, 1839-1850.

Hitomi, Y., Cirulli, E.T., Fellay, J., McHutchison, J.G., Thompson, A.J., Gumbs, C.E., Shianna, K.V., Urban, T.J., Goldstein, D.B., 2011. Inosine Triphosphate Protects Against Ribavirin-Induced Adenosine Triphosphate Loss by Restoring Adenylosuccinate Synthase Function. Gastroenterology.

Kubota, R., Komiyama, T., Kumagai, N., Kimijima, M., Mitsuki, K., Uetake, J., Kaneko, F., Tsunematsu, S., Tsuchimoto, K., 2010. Optimal erythrocyte ribavirin level to reduce the risk of anemia and obtain an early virological response in patients with chronic hepatitis C caused by genotype 1b infection. Hepat Res Treat 2010, 495928.

Kurosaki, M., Tanaka, Y., Nishida, N., Sakamoto, N., Enomoto, N., Matsuura, K., Asahina, Y., Nakagawa, M., Watanabe, M., Sakamoto, M., Maekawa, S., Tokunaga, K., Mizokami, M., Izumi, N., 2013. Model incorporating the ITPA genotype identifies patients at high risk of anemia and treatment failure with pegylated-interferon plus ribavirin therapy for chronic hepatitis C. J Med Virol 85, 449-458.

Kwo, P.Y., Lawitz, E.J., McCone, J., Schiff, E.R., Vierling, J.M., Pound, D., Davis, M.N., Galati, J.S., Gordon, S.C., Ravendhran, N., Rossaro, L., Anderson, F.H., Jacobson, I.M., Rubin, R., Koury, K., Pedicone, L.D., Brass, C.A., Chaudhri, E., Albrecht, J.K., 2010. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. Lancet 376, 705-716.

Lindahl, K., Schvarcz, R., Bruchfeld, A., Stahle, L., 2004. Evidence that plasma concentration rather than dose per kilogram body weight predicts ribavirin-induced anaemia. J Viral Hepat 11, 84-87.

Loustaud-Ratti, V., Alain, S., Rousseau, A., Hubert, I.F., Sauvage, F.L., Marquet, P., Denis, F., Lunel, F., Cales, P., Lefebvre, A., Fauchais, A.L., Liozon, E., Vidal, E., 2008. Ribavirin exposure after the first dose is predictive of sustained virological response in chronic hepatitis C. Hepatology 47, 1453-1461.

Loustaud-Ratti, V., Carrier, P., Rousseau, A., Maynard, M., Babany, G., Alain, S., Trepo, C., De Ledinghen, V., Bourliere, M., Pol, S., Di Martino, V., Zarski, J.P., Pinta, A., Sautereau, D., Marquet, P., 2011. Pharmacological exposure to ribavirin: a key player in the complex network of factors implicated in virological response and anaemia in hepatitis C treatment. Dig Liver Dis 43, 850-855.

Mac Nicholas, R., Norris, S., 2010. Review article: optimizing SVR and management of the haematological side effects of peginterferon/ribavirin antiviral therapy for HCV - the role of epoetin, G-CSF and novel agents. Aliment Pharmacol Ther 31, 929-937.

Maynard, M., Pradat, P., Gagnieu, M.C., Souvignet, C., Trepo, C., 2008. Prediction of sustained virological response by ribavirin plasma concentration at week 4 of therapy in hepatitis C virus genotype 1 patients. Antivir Ther 13, 607-611.

McHutchison, J.G., Manns, M.P., Longo, D.L., 2006. Definition and management of anemia in patients infected with hepatitis C virus. Liver Int 26, 389-398.

Morello, J., Rodriguez-Novoa, S., Jimenez-Nacher, I., Soriano, V., 2008. Usefulness of monitoring ribavirin plasma concentrations to improve treatment response in patients with chronic hepatitis C. J Antimicrob Chemother 62, 1174-1180.

Naggie, S., Rallon, N.I., Benito, J.M., Morello, J., Rodriguez-Novoa, S., Clark, P.J., Thompson, A.J., Shianna, K.V., Vispo, E., McHutchison, J.G., Goldstein, D.B., Soriano, V., 2012. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia in HIV/HCV-coinfected patients with all HCV genotypes. J Infect Dis 205, 376-383.

Nomura, H., Tanimoto, H., Kajiwara, E., Shimono, J., Maruyama, T., Yamashita, N., Nagano, M., Higashi, M., Mukai, T., Matsui, Y., Hayashi, J., Kashiwagi, S., Ishibashi, H., 2004. Factors contributing to ribavirin-induced anemia. J Gastroenterol Hepatol 19, 1312-1317.

Ochi, H., Maekawa, T., Abe, H., Hayashida, Y., Nakano, R., Kubo, M., Tsunoda, T., Hayes, C.N., Kumada, H., Nakamura, Y., Chayama, K., 2010. ITPA polymorphism affects ribavirin-induced anemia and outcomes of therapy--a genome-wide study of Japanese HCV virus patients. Gastroenterology 139, 1190-1197.

Pedersen, C., Alsio, A., Lagging, M., Langeland, N., Farkkila, M., Buhl, M.R., Morch, K., Westin, J., Sangfelt, P., Norkrans, G., Christensen, P.B., 2011. Ribavirin plasma concentration is a predictor of sustained virological response in patients treated for chronic hepatitis C virus genotype 2/3 infection. J Viral Hepat 18, 245-251.

Rau, M., Stickel, F., Russmann, S., Manser, C., Becker, P., Weisskopf, M., Schmitt, J., Dill, M.T., Dufour, J.F., Moradpour, D., Semela, D., Mullhaupt, B., Geier, A., 2012. Impact of genetic SLC28 transporter and ITPA variants on ribavirin serum level, hemoglobin drop and therapeutic response in patients with HCV infection. J Hepatol.

Rendon, A.L., Nunez, M., Romero, M., Barreiro, P., Martin-Carbonero, L., Garcia-Samaniego, J., Jimenez-Nacher, I., Gonzalez-Lahoz, J., Soriano, V., 2005. Early monitoring of ribavirin plasma concentrations may predict anemia and early virologic response in HIV/hepatitis C virus-coinfected patients. J Acquir Immune Defic Syndr 39, 401-405.

Sakamoto, N., Tanaka, Y., Nakagawa, M., Yatsuhashi, H., Nishiguchi, S., Enomoto, N., Azuma, S., Nishimura-Sakurai, Y., Kakinuma, S., Nishida, N., Tokunaga, K., Honda, M., Ito, K., Mizokami, M., Watanabe, M., 2010. ITPA gene variant protects against anemia induced by pegylated interferon-alpha and ribavirin therapy for Japanese patients with chronic hepatitis C. Hepatol Res 40, 1063-1071.

Shipkova, M., Lorenz, K., Oellerich, M., Wieland, E., von Ahsen, N., 2006. Measurement of erythrocyte inosine triphosphate pyrophosphohydrolase (ITPA) activity by HPLC and correlation of ITPA genotype-phenotype in a Caucasian population. Clin Chem 52, 240-247.

Sulkowski, M.S., Shiffman, M.L., Afdhal, N.H., Reddy, K.R., McCone, J., Lee, W.M., Herrine, S.K., Harrison, S.A., Poordad, F.F., Koury, K., Deng, W., Noviello, S., Pedicone, L.D., Brass, C.A., Albrecht, J.K., McHutchison, J.G., 2010. Hepatitis C virus treatment-related anemia is associated with higher sustained virologic response rate. Gastroenterology 139, 1602-1611, 1611 e1601.

Sumi, S., Marinaki, A.M., Arenas, M., Fairbanks, L., Shobowale-Bakre, M., Rees, D.C., Thein, S.L., Ansari, A., Sanderson, J., De Abreu, R.A., Simmonds, H.A., Duley, J.A., 2002. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. Hum Genet 111, 360-367.

Suppiah, V., Moldovan, M., Ahlenstiel, G., Berg, T., Weltman, M., Abate, M.L., Bassendine, M., Spengler, U., Dore, G.J., Powell, E., Riordan, S., Sheridan, D., Smedile, A., Fragomeli, V., Muller, T., Bahlo, M., Stewart, G.J., Booth, D.R., George, J., 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41, 1100-1104.

Suzuki, F., Suzuki, Y., Akuta, N., Sezaki, H., Hirakawa, M., Kawamura, Y., Hosaka, T., Kobayashi, M., Saito, S., Arase, Y., Ikeda, K., Chayama, K., Kamatani, N., Nakamura, Y., Miyakawa, Y., Kumada, H., 2011. Influence of ITPA polymorphisms on decreases of

hemoglobin during treatment with pegylated interferon, ribavirin, and telaprevir. Hepatology 53, 415-421.

Tanaka, Y., Nishida, N., Sugiyama, M., Kurosaki, M., Matsuura, K., Sakamoto, N., Nakagawa, M., Korenaga, M., Hino, K., Hige, S., Ito, Y., Mita, E., Tanaka, E., Mochida, S., Murawaki, Y., Honda, M., Sakai, A., Hiasa, Y., Nishiguchi, S., Koike, A., Sakaida, I., Imamura, M., Ito, K., Yano, K., Masaki, N., Sugauchi, F., Izumi, N., Tokunaga, K., Mizokami, M., 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41, 1105-1109.

Thompson, A.J., Fellay, J., Patel, K., Tillmann, H.L., Naggie, S., Ge, D., Urban, T.J., Shianna, K.V., Muir, A.J., Fried, M.W., Afdhal, N.H., Goldstein, D.B., McHutchison, J.G., 2010a. Variants in the ITPA Gene Protect Against Ribavirin-Induced Hemolytic Anemia and Decrease the Need for Ribavirin Dose Reduction. Gastroenterology.

Thompson, A.J., Muir, A.J., Sulkowski, M.S., Ge, D., Fellay, J., Shianna, K.V., Urban, T., Afdhal, N.H., Jacobson, I.M., Esteban, R., Poordad, F., Lawitz, E.J., McCone, J., Shiffman, M.L., Galler, G.W., Lee, W.M., Reindollar, R., King, J.W., Kwo, P.Y., Ghalib, R.H., Freilich, B., Nyberg, L.M., Zeuzem, S., Poynard, T., Vock, D.M., Pieper, K.S., Patel, K., Tillmann, H.L., Noviello, S., Koury, K., Pedicone, L.D., Brass, C.A., Albrecht, J.K., Goldstein, D.B., McHutchison, J.G., 2010b. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 139, 120-129 e118.

Thompson, A.J., Santoro, R., Piazzolla, V., Clark, P.J., Naggie, S., Tillmann, H.L., Patel, K., Muir, A.J., Shianna, K.V., Mottola, L., Petruzzellis, D., Romano, M., Sogari, F., Facciorusso, D., Goldstein, D.B., McHutchison, J.G., Mangia, A., 2010c. Inosine triphosphatase genetic variants

are protective against anemia during antiviral therapy for HCV2/3 but do not decrease dose reductions of RBV or increase SVR. Hepatology 53, 389-395.

Tsubota, A., Hirose, Y., Izumi, N., Kumada, H., 2003. Pharmacokinetics of ribavirin in combined interferon-alpha 2b and ribavirin therapy for chronic hepatitis C virus infection. Br J Clin Pharmacol 55, 360-367.

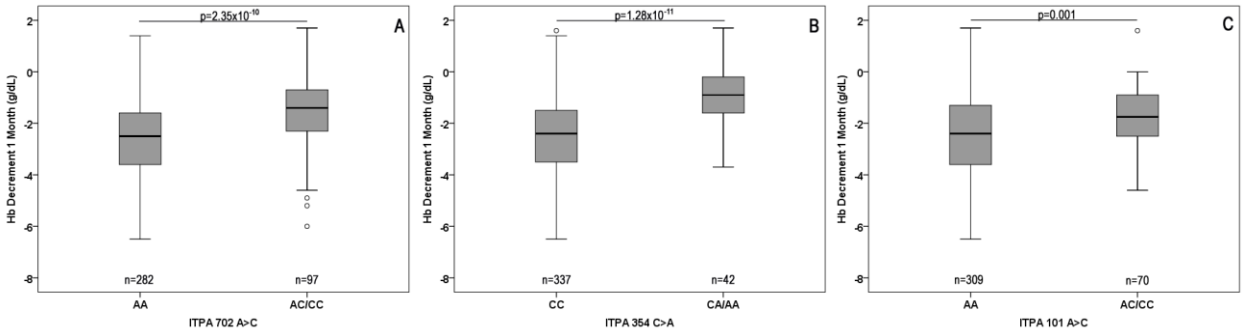
van Vlerken, L.G., van Oijen, M.G., van Erpecum, K.J., 2011. Ribavirin concentration is a more important predictor of sustained viral response than anemia in hepatitis C patients. Gastroenterology 140, 1693-1694.

Van Vlierbergh, H., Delanghe, J.R., De Vos, M., Leroux-Roel, G., 2001. Factors influencing ribavirin-induced hemolysis. J Hepatol 34, 911-916.

Figure Legend

Figure 1. The possession of at least one variant allele in the ITPA rs6051702 (ITPA 702 A>C) (n=97), rs1127354 (ITPA 354 C>A) (n=42) and rs7270101 (ITPA 354 C>A) (n=70) SNPs were associated with a smaller decrement of Hb (g/dL) at week 4 ($p=2.35x10^{-10}$) [A], ($p=1.28x10^{-11}$) [B] and (p=0.001) [C], respectively.

Median values (horizontal line), IQR (bars), patient values (black square), highest and lowest value (whiskers) are shown.



Tables.

Table 1. Baseline characteristics of 379 patients.

Characteristics	
Male sex n,%	231, 60.9
Age median (IQR)	46.0 (40.5-57.0)
BMI median (IQR)	23.4 (22.0-26.6)
Caucasian Ethnicity n,%	360, 94.9
HCV genotypes n, %	
1	187, 49.3
2	81, 21.4
3	85, 22.4
4	26, 6.9
ALT baseline median (IQR)	82.5 (48.7-144.0)
HCV-RNA baseline median (IQR)	1176280 (234290-2827480)
PEG-IFN n, %	
α 2a	233, 61.5
α 2b	146, 38.5
Ribavirin dose mg/day n,%	
400	3, 0.8
600	3, 0.8
800	108, 28.5
1000	201, 53.0
1200	60, 15.8
1400	4, 1.1
Anemia WHO grade n,%	
Grade 0 (≥ 11g/dL)	371, 97.9%
Grade 1 (< 11-9.5g/dL)	8, 2.1%
Grade 2 (< 9.5-8g/dL)	-, 0%
Grade 3 (< 8-6.5g/dL)	-, 0%
Grade 4 (< 6.5g/dL)	-, 0%

Table 2. Factors associated with anemia (absolute Hb value lower than 10 g/dL, and Hb reduction >3 g/dL) at week 4.

Factors	Anemia at week 4		P value
	Yes	No	
Hb baseline (g/dL)	**15.4 (14.3 to 16.1)**	**14.7 (13.5 to 15.7)**	**<0.001**
RBV dose (mg/kg)	14.2 (13.3 to 15.5)	13.7 (12.5 to 15.0)	0.056
Weight (kg)	70 (62 to 80)	70 (62 to 77)	0.807
Age (years)	48 (42 to 61)	46 (39 to 55)	0.101
Peg Interferon 2a	76 (58.9%)	157 (62.8%)	0.463
Peg Interferon 2b	53 (41.1%)	93 (37.2%)	
Gender (female)	**47 (36.4%)**	**101 (40.4%)**	**0.039**
Gender (male)	**82 (63.6%)**	**149 (59.6%)**	
rs6051702 AA (Wild-type)	**116 (89.9%)**	**166 (66.4%)**	**<0.001**
rs6051702 AC/CC (Carrier of variant allele)	**13 (10.1%)**	**84 (33.6%)**	
rs1127354 CC (Wild-type)	**127 (98.4%)**	**210 (84.0%)**	**<0.001**
rs1127354 CA/AA (Carrier of variant allele)	**2 (1.6%)**	**40 (16.0%)**	
rs7270101 AA (Wild-type)	**115 (89.1%)**	**194 (77.6%)**	**0.006**
rs7270101 AC/CC (Carrier of variant allele)	**14 (10.9%)**	**56 (22.4%)**	

Table 3. Genetic associations of polymorphisms in pairs as predictors of anemia (absolute Hb value lower than 10 g/dL, and Hb reduction >3 g/dL at week 4) in univariate and multivariate logistic regression analysis.

Factors	Univariate		Multivariate	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
rs6051702 AC/CC + rs1127354 CA/AA (Carrier of variant allele)	**0.20 (0.11 to 0.38)**	**<0.001**	**0.20 (0.11 to 0.37)**	**<0.001**
rs6051702 AC/CC + rs7270101 AC/CC (Carrier of variant allele)	**0.27 (0.16 to 0.48)**	**<0.001**	1.22 (0.40 to 3.73)	0.724
rs1127354 CA/AA + rs7270101 AC/CC (Carrier of variant allele)	**0.23 (0.13 to 0.42)**	**<0.001**	0.51 (0.22 to 1.15)	0.106

Table 4. Predictors of anemia (absolute Hb value lower than 10 g/dL, and Hb reduction >3 g/dL at week 4) in univariate and multivariate logistic regression analysis.

Factors	Univariate		Multivariate	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Gender (male)	1.18 (0.76 to 1.83)	0.454		
Weight (kg)	1.00 (0.98 to 1.01)	0.912		
Hb baseline (g/dL)	**1.27 (1.10 to 1.47)**	**0.001**	**1.52 (1.22 to 1.88)**	**$1.21x10^{-4}$**
Age (years)	1.01 (0.99 to 1.04)	0.087	n.s.	
RBV dose (mg/kg)	1.09 (0.99 to 1.20)	0.080	n.s.	
Peg Interferon 2a	0.592 (0.28 to 1.04)	0.110	n.s.	
rs6051702 AC/CC + rs1127354 CA/AA (Carrier of variant allele)	**0.20 (0.11 to 0.38)**	**<0.001**	**0.11 (0.04 to 0.31)**	**$1.75x10^{-5}$**