ORIGINAL ARTICLE

Early reduced liver graft survival in hepatitis C recipients identified by two combined genetic markers

Renato Romagnoli[1,*], Silvia Martini[2,*], Francesco Tandoi[1,*], Dominic Dell Olio[3], Paola Magistroni[3,4], Francesca E. Bertinetto[3,4], Ennia Dametto[3,4], Mario Rizzetto[2], Mauro Salizzoni[1,†] & Antonio Amoroso[3,4]

1 Liver Transplantation Center, General Surgery Unit 2U, AOU Città della Salute e della Scienza di Torino, University of Turin, Turin, Italy

2 Liver Transplantation Center, Gastrohepatology Unit, AOU Città della Salute e della Scienza di Torino, University of Turin, Turin, Italy

3 Regional Transplantation Center – Piedmont, AOU Città della Salute e della Scienza di Torino, University of Turin, Turin, Italy

4 Immunogenetics Laboratory, AOU Città della Salute e della Scienza di Torino, University of Turin, Turin, Italy

Correspondence

Professor Antonio Amoroso MD, Director, Department of Immunogenetics & Transplantation Biology, Regional Transplantation Center – Piedmont, AOU Città della Salute e della Scienza di Torino, Via Santena, 19-10126 Torino, Italy.
Tel.: +39 011 6334441;
fax: +39 011 6336529;
e-mail: antonio.amoroso@unito.it

*These authors contributed equally to this work.
†Director of Liver Transplant program.

SUMMARY

HLA and *IL-28B* genes were independently associated with severity of HCV-related liver disease. We investigated the effects of these combined genetic factors on post-transplant survival in HCV-infected recipients, aiming to provide new data to define the optimal timing of novel antiviral therapies in the transplant setting. HLA-A/B/DRB1 alleles and *IL-28B* rs12979860 (C > T) polymorphism frequencies were determined in 449 HCV viremic recipients and in their donors. Median follow-up was 10 years; study outcome was graft survival. HLA-DRB1*11 phenotype and *IL-28B* C/C genotype were significantly less frequent in recipients than donors (27.8% vs. 45.9% and 27.4% vs. 44.9%, respectively, $P < 0.00001$). Ten-year graft survival was better in patients with HLA-DRB1*11 ($P = 0.0183$) or *IL-28B* C/C ($P = 0.0436$). Conversely, concomitant absence of HLA-DRB1*11 and *IL-28B* C/C in 228 (50.8%) predicted worse survival ($P = 0.0006$), which was already evident at the first post-transplant year ($P = 0.0370$). In multivariable Cox analysis, absence of both markers ranked second as risk factor for survival (HR = 1.74), following donor age ≥ 70 years (HR = 1.77). In the current era of direct-acting antiviral agents, the negative effects of this common immunogenetic profile in HCV-infected recipients could be most effectively neutralized by peri-transplant treatment. This should be particularly relevant in countries where elderly donors represent an unavoidable resource.

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Key words
HCV recurrence, HLA-DRB1*11, *IL-28B* C/C, liver transplantation, post-transplant outcome

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Introduction

Hepatitis C virus (HCV) infects approximately 1.6% of the world's population [1], and HCV cirrhosis is the leading indication for liver transplantation (LT) in adults [2]. In recipients with viremia at LT, HCV recurrence is universal [3] and survival is reduced. Progression of liver disease is accelerated after LT, with chronic hepatitis developing in 50–90% of patients by 12 months and bridging fibrosis/cirrhosis in 20–54% by

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5 years after LT [4]. Recurrent HCV is the main cause of graft failure and patient death in HCV-infected recipients [5], and treatment achieving viral eradication before or after LT would improve patient and graft survival [6].

High viral load, genotype 1 virus, female gender, older donor age, treated cytomegalovirus (CMV) infection, and acute rejection were associated with an increased risk of severe recurrent hepatitis C [6].

Host immune responses may be crucial in determining the fate of the graft in the context of alloimmunity and immunosuppression. Several studies have correlated the expression of human leukocyte antigen (HLA) variants with spontaneous viral clearance or evolution of liver disease in the nontransplant setting, but only a few evaluated the association of HLA with severity of HCV disease after transplant [7,8]. Single nucleotide polymorphisms (SNP) upstream of the *interleukin-28B* (*IL-28B*) gene encoding interferon-lambda-3 (IFN-λ-3) were shown to influence the outcome of HCV infection, and the rs12979860 C/C genotype was found to be protective both in the immunocompetent [9,10] and in the immunosuppressed host [11–13]. However, no study addressed the impact of the combination of these two immunogenetic factors on the outcome of LT in HCV recipients.

Interferon and ribavirin were poorly tolerated in transplant candidates, while, even if initiated at an early stage of recurrent hepatitis C post-LT, they achieved sustained virological response (SVR) rates around 30% [6,14,15]. The addition of first-generation protease inhibitors improved SVR rates to 50–60% [16]; however, side effects and drug–drug interactions make this therapy cumbersome [17]. The recent advent of new direct-acting antiviral agents (DAAs) is revolutionizing anti-HCV therapies toward pan-genotypic, interferon-free regimens [18]. Results of compensated cirrhosis patients are similar to patients without cirrhosis [19], and the associations sofosbuvir–ledipasvir and sofosbuvir–daclatasvir are available also for child class B and C patients, although severity of liver disease influences response rates (around 80% compared to 95% for child A) [20,21]. Therefore, new management paradigms are emerging in the transplant field: to pursue viral eradication before LT (to improve or stabilize liver function in listed patients and to avoid graft infection) [22] or as early as possible after LT (preemptive therapy to prevent inflammation and fibrosis in the transplanted liver), rather than waiting for hepatitis C recurrence before starting treatment. However, unpredictable waiting time, risk of patient death and/or tumor progression on the list, occurrence of delayed graft function after LT or of severe kidney impairment both before and after transplant, and the new dilemmas about using HCV-positive donors are fueling the debate on the best timing for HCV therapy [23–27].

In this study on HCV patients managed in the interferon era, we investigated the effects of the recipient immunogenetic make-up on post-transplant graft survival, aiming to provide new data helpful to define the optimal timing to start novel antivirals in the transplant setting.

Materials and methods

Study population

This retrospective study is based on 1506 consecutive orthotopic LTs performed at the Turin Liver Transplant Center from January 1999 to December 2009. The time span was chosen to obtain long-term follow-up data before the introduction of new antivirals against the HCV. Excluded from the study were 86 pediatric, 11 living-related, 8 dominoes, and 125 retransplants as well as 27 primary transplants for which a recipient DNA sample was not available.

Within the remaining 1249 adult primary LT recipients, 738 were anti-HCV antibody negative and 511 anti-HCV antibody positive; the latter underwent HCV RNA plasma level and genotype determination as part of their pretransplant work-up. The donors were similarly studied for HCV in blood collected during the brain death observation period.

Twenty-one anti-HCV-positive but HCV RNA-negative recipients and 41 anti-HCV-positive patients who received the graft from an anti-HCV-positive donor were not considered.

Ultimately, 449 HCV viremic recipients of grafts from HCV-negative brain-dead heart-beating donors represent the study population (Fig. 1).

The recipient and donor features of 449 HCV and 738 non-HCV transplants are shown in Table S1.

Informed consent was signed by all patients upon entering the waiting list. Due to the retrospective design, no specific approval was sought from the local Institutional Review Board; by Italian law, Regional Transplantation Centers are custodians of donor/recipient biomedical data also for research purposes. All study procedures complied with the ethical standards of the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008.

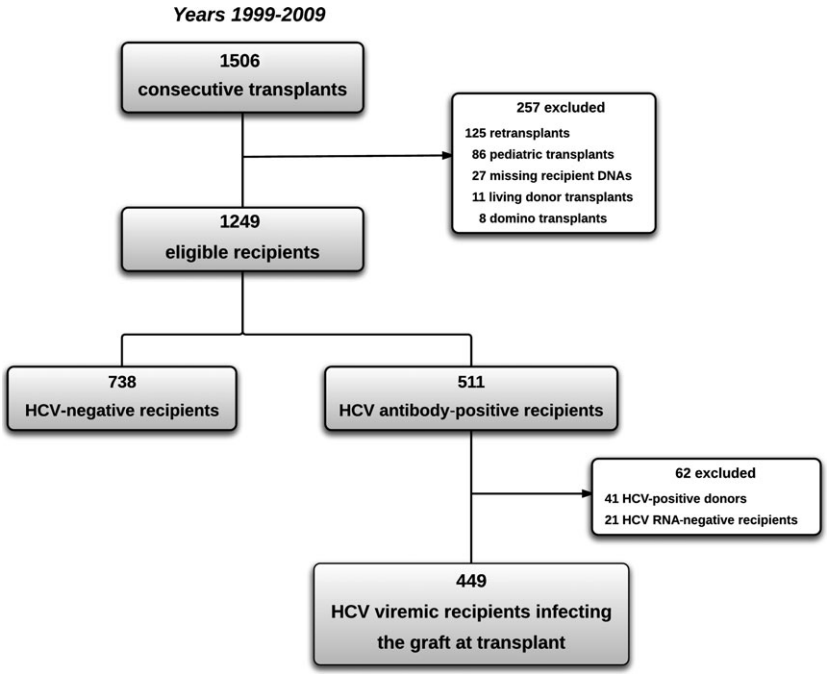

Figure 1 Identification of patients included in the study. HCV, hepatitis C virus.

HCV testing

Antibodies to HCV were detected in recipient and donor sera by the Architect assay, Abbott Laboratories, Abbott Park, IL, USA.

Between January 1999 and April 2007, HCV RNA detection and quantitation were performed with a qualitative and quantitative assay. Qualitative detection was obtained by COBAS Amplicor® HCV system (Roche Molecular Systems Inc, Branchburg, NJ, USA) with a detection limit of 50 IU/mL. Quantitative detection was performed by signal amplification Branched-DNA test (Versant® HCV version 3.0, Bayer Diagnostic Corporation, Tarrytown, NY, USA) with a dynamic range of quantitation from 615 to 7.7×10^6 IU/mL. After April 2007, plasma HCV RNA was detected and quantified by the automated high-sensitivity system COBAS AmpliPrep®/COBAS TaqMan® HCV version 1 (Roche Molecular Systems Inc). The dynamic range of quantitation went from 43 to 6.9×10^7 IU/mL. After October 2012, the version 2 of the AmpliPrep®/COBAS TaqMan® HCV test was introduced, with a dynamic range of quantitation from 15 to 1×10^8 IU/mL.

HCV genotypes were determined with a reverse hybridization line probe assay (INNO-LIPA, Innogenetics, Ghent, Belgium) after nested-polymerase chain reaction amplification of the 5′NC viral region.

HLA typing

Recipient and donor peripheral blood was prospectively collected in ethylenediaminetetraacetic acid, and genomic DNA was banked after extraction by "salting out" method or automatically (Macherey-Nagel GmbH, Düren, Germany). HLA loci A and B were typed by serology, while HLA-DRB1 was typed by serology until 2006 and then by molecular low resolution with sequence-specific primer technology. A sample of recipients and donors was also typed at high resolution by sequence-specific primer technology to detect HLA-DRB1*11 subtypes.

IL-28B SNP typing

We investigated the *IL-28B* rs12979860 (C > T) polymorphism using the biorepository of recipient and donor DNAs of our Immunogenetics Laboratory. The Custom TaqMan® Allelic Discrimination Kit (Life Technologies Applied Biosystems®, Thermo Fisher Scientific, Waltham, MA, USA) was used for the allelic discrimination of the selected SNP. Upstream and downstream primers (forward: GCCTGTCGTGTACTGAACCA; reverse: GCG CGGAGTGCAATTCAAC) and two TaqMan® probes (5′-VIC-TGGTTCGCGCCTTC-3′ and 5′-FAM-CTGGTT CACGCCTTC-3′) were used, one probe for each allele in a two-allele system. The TaqMan® method was carried

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out with Applied Biosystems® 7500 Real-Time PCR Systems (Thermo Fisher Scientific).

Clinical protocol

Immunosuppression was based on calcineurin inhibitors (mainly cyclosporine), antimetabolites, and steroids (tapered to suspension in 6 months). Moderate or severe acute rejection episodes were treated with high-dose methylprednisolone boluses on three consecutive days; monoclonal anti-CD3 antibodies were used in steroid-resistant rejections.

Follow-up liver biopsies were performed as dictated by clinical needs. Recurrent hepatitis C was graded and staged by Ishak score [28].

Therapy against recurrent HCV infection was based on ribavirin and interferon-α or peginterferon-α given for 12 months or according to tolerance. No patient was treated while on the waiting list. Criteria for HCV treatment after LT were absence of contraindications (mainly neutropenia, anemia, thrombocytopenia, renal failure, biliary complication, psychiatric disorders), a minimum 3-month interval from LT, and mild-to-moderate fibrosis (score ≥ 2/6 Ishak) or evidence of fibrosing cholestatic hepatitis.

The variables assessed in the study population are shown in Table 3.

Study outcome

Graft survival was the sole study outcome; patient survival would have been biased by factors influencing the decision whether to retransplant or not a patient with a failing graft in the setting of recurrent HCV disease.

Survival data were collected up to June 30, 2014, because starting from July 2014 new direct anti-HCV agents became available in our center. Any surviving patient with the original graft was censored, while graft losses and their causes were recorded at the time of patient death or retransplantation.

Survival results obtained in the 738 non-HCV recipients were compared with the HCV-infected study population.

Statistical analysis

Allelic, phenotypic, and genotypic frequencies were assessed by direct counting. HLA-A,-B,-DRB1 phenotypic frequencies and all other categorical variables were compared by $n \times m$ cells chi-square test and Bonferroni post hoc correction for multiple comparisons. A P value < 0.05 was otherwise considered statistically significant.

Results for ordinal variables are expressed as mean ± standard deviation or as median and interquartile range, as appropriate. A two-tailed t-test was adopted to compare differences between normally distributed variables (normality assessed by Kolmogorov–Smirnov test). Differences between not normally distributed variables were assessed by the Mann–Whitney U-test.

To evaluate the effect of individual categorical variables on graft survival, Kaplan–Meier analysis was employed and survival curves were compared using the log-rank test. Then, a Cox regression model was used to analyze the one-year graft survival with artificial censoring of all graft losses after 1 year; short-term hazard ratios (HR), 95% confidence intervals, and P values were thus calculated.

To obtain a parsimonious set of pretransplant predictors of graft survival, recipient and donor variables were selected based on the background knowledge (6), irrespective of their performance at univariate analysis. They were fitted simultaneously into a multivariable Cox proportional hazards regression model and a backward selection method was used. In the backward elimination, the results of the Wald test for individual variables were examined and the least significant parameter that did not meet the level for staying in the model (set at P-value = 0.157, corresponding to selection by Akaike's information criterion) was removed.

Finally, we checked for interactions by including variables in the model as well as their cross-product and by testing the statistical significance of the cross-product term.

Data were analyzed by StatSoft STATISTICA software package version 8.0 (Tulsa, OK, USA) and IBM SPSS version 21 (New York, NY, USA).

Results

Comparison of immunogenetic markers

HLA frequencies

Table 1 shows the statistically significant differences in HLA phenotypic frequencies between the 449 HCV-infected recipients and their HCV-negative donors, the complete pattern of HLA frequencies being depicted in Table S2.

Comparing recipients with donors, HLA-B*13 and HLA-B*16 were significantly less frequent in donors, while HLA-DRB1*11 was significantly less frequent in recipients. In the latter, the frequencies of the other HLA-DRB1 antigens, especially HLA-DRB1*7, were proportionately increased.

Table 1. Significant differences in HLA phenotypic frequencies between recipients and donors in HCV liver transplants.

HLA alleles	HCV-positive recipients (n = 449)	HCV-negative donors (n = 449)	P value
HLA-DRB1*11	125 (27.8%)	206 (45.9%)	**<0.00001**
HLA-DRB1*7	163 (36.3%)	112 (24.9%)	**0.0002**
HLA-B*13	58 (13.2%)*	31 (6.9%)	**0.0018**
HLA-B*16	52 (11.8%)*	21 (4.7%)	**0.0001**

In bold significant P values after Bonferroni correction (locus DRB1 <0.0042; locus B <0.0019).

*8 missing data.

HCV, hepatitis C virus; HLA, human leukocyte antigen.

The relative frequencies of the most common HLA-DRB1*11 subtypes (DRB1*11:01, *11:02, *11:03, *11:04) were examined in a sample of HLA-DRB1*11-positive recipients and donors. No differences were found, and they were in line with those of the Italian population (Table S3).

IL-28B SNP frequencies

As shown in Table 2, C allele and C/C genotype were significantly less frequent in HCV-infected recipients than in their HCV-negative donors, and allelic/genotypic frequencies showed Hardy–Weinberg equilibrium in the donors, but not in the recipients (P = 0.0039).

The frequency of C/C genotype was significantly higher in donors aged 70 years or more (Table S4), and C/C donors were older than non-C/C ones (median age: 63 vs. 58 years, P = 0.0286).

Graft survival after LT

The median follow-up for surviving grafts was 10 years (range 4.5 to 15.5).

The 10-year graft survival rate was significantly lower in the 449 HCV than in the 738 non-HCV recipients (P < 0.00001). The two survival curves started to diverge few months after LT and the gap widened progressively over time (Fig. S1).

Within the HCV patients, the HLA-DRB1*11-positive recipients had a graft survival significantly longer than the HLA-DRB1*11-negative ones, with a difference of 17% at 10 years (P = 0.0183) (Fig. 2a). The recipient status for HLA-DRB1*7, HLA-B*13, and HLA-B*16 (Fig. S2) as well as the full donor/recipient mismatch at the DRB1 locus (Fig. S3) did not affect the outcome.

The graft survival of HCV recipients was influenced also by the *IL-28B* genotype of both the recipient and the donor, results being better in C/C recipients (P = 0.0436) (Fig. 2b) and with non-C/C donors (P = 0.0257) (Fig. S4). Looking at donor/recipient match for *IL-28B*, the outcome was significantly worse in non-C/C recipients transplanted with a C/C donor (P = 0.0240) (Fig. S5).

While the contemporary presence of HLA-DRB1*11 phenotype and *IL-28B* C/C genotype in HCV recipients did not improve survival (Fig. S6), the absence of both variants predicted significantly worse LT results (P = 0.0006) (Fig. 3a). The subdivision of HCV recipients in two groups according to the combination of these markers (HLA-DRB1*11-negative and *IL-28B*

Table 2. Allelic and genotypic frequencies of *IL-28B* rs12979860 in HCV liver transplants.

	HCV-positive recipients (n = 449)	HCV-negative donors (n = 443)*	P value
IL-28B alleles			
C	498 (55.5%)	591 (66.7%)	**<0.00001**
T	400 (44.5%)	295 (33.3%)	
IL-28B genotypes			
C/C	123 (27.4%)	199 (44.9%)	**<0.00001**
C/T	252 (56.1%)	193 (43.6%)	**0.0002**
T/T	74 (16.5%)	51 (11.5%)	**0.0326**

In bold significant P values.

*Six missing data.

HCV, hepatitis C virus; *IL-28B*, *interleukin-28B*.

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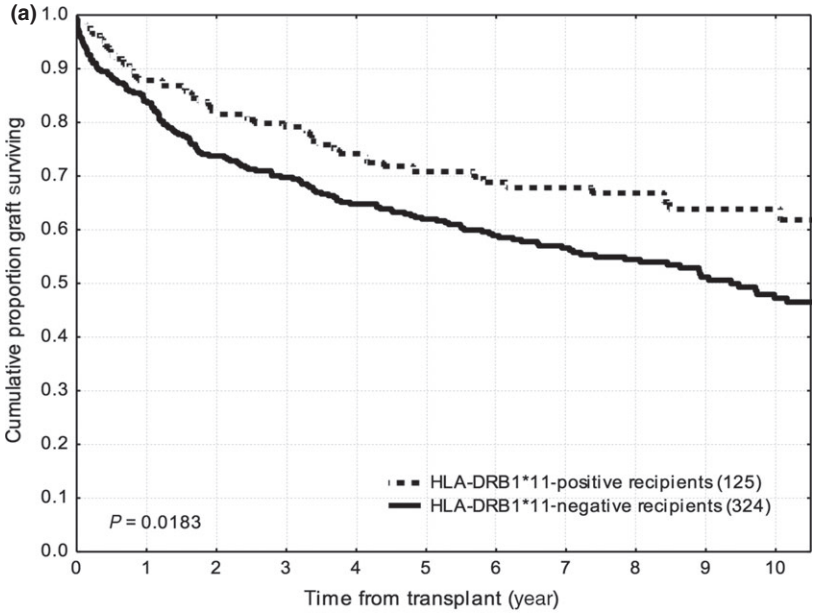
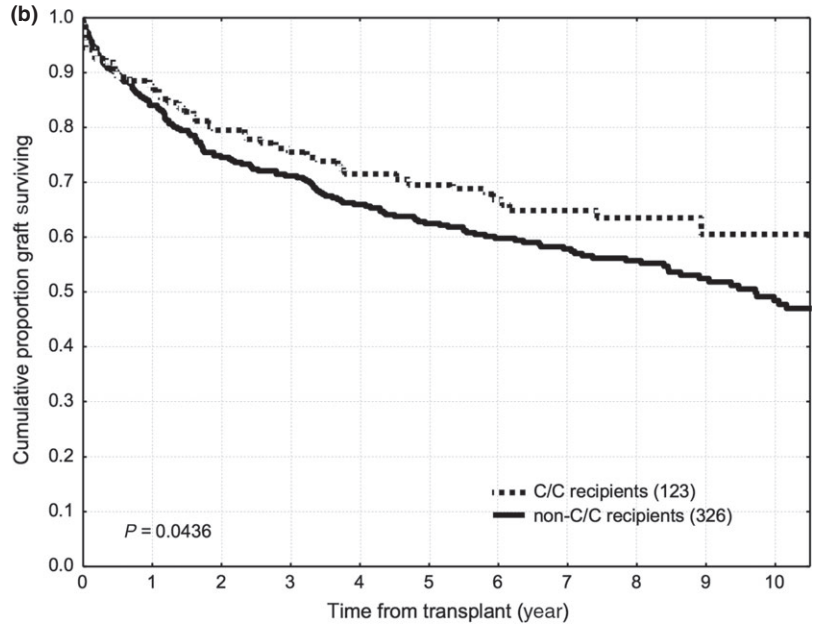
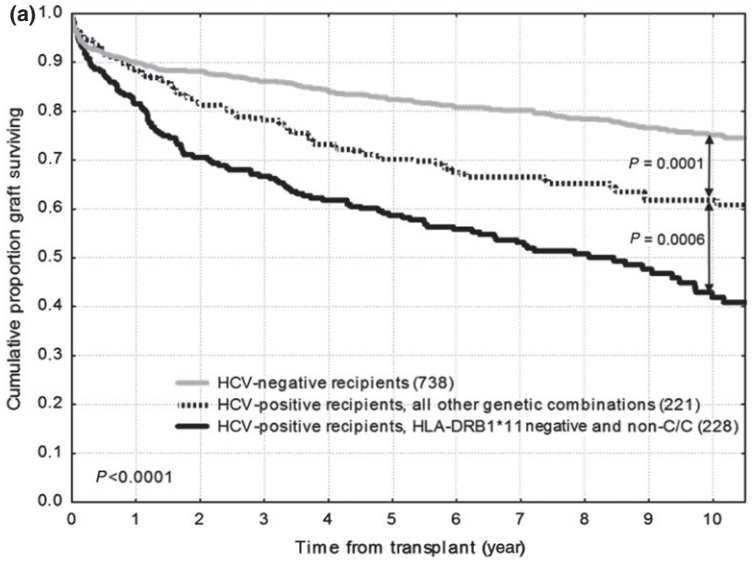


HCV-positive recipients	1 year	2 years	3 years	5 years	10 years
HLA-DRB1*11 positive	0.88	0.82	0.79	0.71	0.64
(No. at risk)	(*n* = 110)	(*n* = 102)	(*n* = 99)	(*n* = 82)	(*n* = 43)
HLA-DRB1*11 negative	0.84	0.74	0.70	0.62	0.47
(No. at risk)	(*n* = 272)	(*n* = 239)	(*n* = 226)	(*n* = 191)	(*n* = 68)

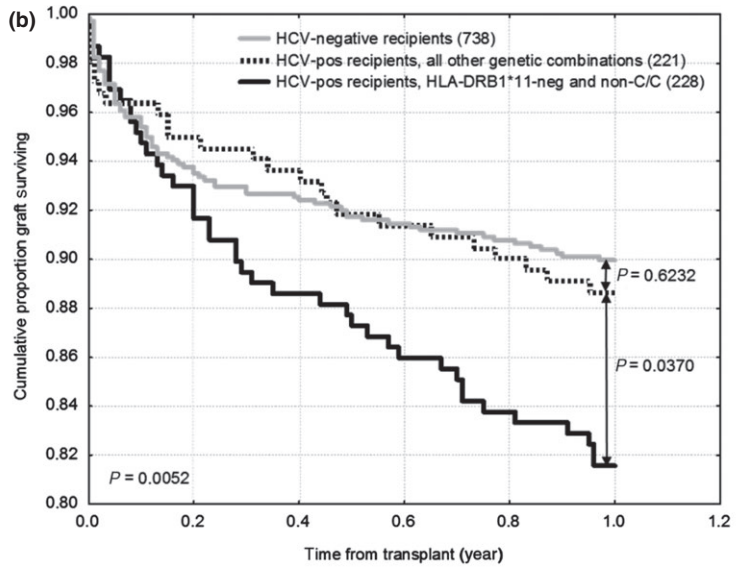


HCV-positive recipients	1 year	2 years	3 years	5 years	10 years
IL28B C/C	0.88	0.80	0.76	0.70	0.61
(No. at risk)	(*n* = 108)	(*n* = 98)	(*n* = 93)	(*n* = 83)	(*n* = 41)
IL28B non-C/C	0.84	0.75	0.71	0.63	0.48
(No. at risk)	(*n* = 274)	(*n* = 243)	(*n* = 232)	(*n* = 194)	(*n* = 68)

Figure 2 Kaplan–Meier curves for liver graft survival in HCV-infected recipients. (a) Stratification by HLA-DRB1*11 phenotype (positive vs. negative). (b) Stratification by *IL-28B* rs12979860 genotype (C/C vs. non-C/C). HCV, hepatitis C virus; HLA, human leukocyte antigen; *IL-28B*, *interleukin-28B*.



	1 year	2 years	3 years	5 years	10 years
HCV-negative recipients	0.90	0.88	0.86	0.82	0.75
(No. at risk)	(*n* = 664)	(*n* = 651)	(*n* = 635)	(*n* = 554)	(*n* = 243)
HCV-positive recipients					
all other genetic combinations	0.89	0.81	0.78	0.71	0.62
(No. at risk)	(*n* = 196)	(*n* = 180)	(*n* = 173)	(*n* = 145)	(*n* = 73)
HLA-DRB1*11 negative and *IL28B* non-C/C	0.82	0.71	0.67	0.59	0.42
(No. at risk)	(*n* = 186)	(*n* = 161)	(*n* = 152)	(*n* = 130)	(*n* = 41)



	1 month	2 months	3 months	6 months	1 year
HCV-negative recipients	0.96	0.94	0.93	0.92	0.90
(No. at risk)	(*n* = 707)	(*n* = 694)	(*n* = 686)	(*n* = 677)	(*n* = 664)
HCV-positive recipients					
all other genetic combinations	0.96	0.95	0.95	0.92	0.89
(No. at risk)	(*n* = 213)	(*n* = 210)	(*n* = 209)	(*n* = 203)	(*n* = 196)
HLA-DRB1*11 negative and *IL28B* non-C/C	0.96	0.93	0.91	0.88	0.82
(No. at risk)	(*n* = 218)	(*n* = 212)	(*n* = 207)	(*n* = 200)	(*n* = 186)

Figure 3 Kaplan–Meier curves for liver graft survival in HCV-negative recipients compared with HCV-positive ones divided into two groups according to HLA-DRB1*11 phenotype and *IL-28B* genotype. (a) At 10 years post-transplant. (b) At 1 year post-transplant. HCV, hepatitis C virus; HLA, human leukocyte antigen; *IL-28B*, *interleukin-28B*.

non-C/C, $n = 228$, 50.8% vs. all other genetic combinations, $n = 221$, 49.2%) demonstrated markedly different outcomes. Compared with the HCV recipients exhibiting all other genetic combinations, the patients lacking both these markers experienced a significant survival disadvantage as early as the first post-transplant year ($P = 0.0370$), the one-year risk of graft loss being increased by 68% (HR = 1.68 [95% confidence interval: 1.026–2.761], $P = 0.0390$). In contrast, the one-year survival rate of HCV recipients with all other genetic combinations was equivalent to that of non-HCV ones (89% vs. 90%, $P = 0.6232$) (Fig. 3b).

In the two groups of HCV recipients, graft survival at 1 and 10 years remained significantly different also when SVR patients were censored at the beginning of interferon-based therapy (Fig. S7, Panel A and B).

Table 3 summarizes demographics and LT details of HCV patients divided according to their immunogenetic markers. While they did not differ for gender, Model for End-stage Liver Disease (MELD) score, donor age and donor *IL-28B* genotype, the recipients lacking both HLA-DRB1*11 and *IL-28B* C/C showed: (i) significantly higher HCV RNA levels before and 3 months after LT; (ii) higher prevalences of genotype 1 virus, HLA-DRB1 donor/recipient full mismatch, *IL-28B* C/C donor to non-C/C recipient, positive cross-match, treated acute rejection, and treated CMV infection; (iii) lower SVR rate to interferon therapy.

Comparing causes of graft loss recorded by attending physicians at the time of the event, severe recurrent hepatitis C was more frequent (31.6% vs. 20.3%, $P < 0.0001$) and twofold increased in both the short (<1 year: 6.1% vs. 2.7%) and long term (>5 years: 12.3% vs. 6.3%) in HLA-DRB1*11-negative and *IL-28B* non-C/C recipients, while the rates of graft loss due to recurrent hepatocellular carcinoma (HCC) or not primarily attributed to HCV recurrence were not significantly different between the two groups (Table S5).

Lastly, the relevance of donor features was analyzed in the HCV recipients lacking both HLA-DRB1*11 and *IL-28B* C/C. Regarding donor immunogenetic profile, receiving a graft from an HLA-DRB1*11-positive donor did not exert an appreciable protective effect, while a trend toward a worse graft survival was detected with *IL-28B* C/C donors (Table S6). It is worth noting that the C/C genotype was significantly more frequent in donors aged 70 years or more compared with younger than 70 (34/61, 55.7% vs. 63/164, 38.4%; $P = 0.0197$). Focusing on donor age, survival rates markedly decreased with donors aged 70 years or more (the upper quartile in our study), being 71% at 1 year, 44% at 5 years, and 29% at 10 years (Fig. 4a). With older donors, the survival disadvantage was already evident from the very first months after transplant, adding up to a difference of 15% at 1 year compared with recipients of a donor younger than 70 ($P = 0.0095$), the one-year risk of graft loss being increased by more than 120% (HR = 2.23 [95% confidence interval: 1.210–4.111], $P = 0.0100$) (Fig. 4b).

Multivariable analysis

Table 4 summarizes the results of a backward selection multivariable analysis (Cox model) performed on a set of covariables with potential impact on graft survival (*donor* age, body mass index, anticore HBV antibodies status, *IL-28B* genotype; *recipient* age, gender, MELD score, HCV genotype, HCV RNA level, combined HLA-DRB1*11/*IL-28B* profile; *cross-match*). The independent pretransplant variables significantly affecting the outcome of LT in HCV recipients were as follows: donor age $\geq$ 70 years (HR = 1.77), recipient lacking both HLA-DRB1*11 phenotype and *IL-28B* C/C genotype (HR = 1.74), MELD score $\geq$ 25 (HR = 1.74), HCV RNA level before LT $\geq 1 \times 10^6$ IU/mL (the median viremia level in the study population) (HR = 1.45), and donor with *IL-28B* C/C genotype (HR = 1.45).

Checking interaction, no synergy was found between HLA-DRB1*11 phenotype and *IL-28B* genotype ($P = 0.886$; HR = 1.05; 95% confidence interval: 0.522–2.121).

Discussion

In this study on primary transplants performed for HCV cirrhosis in the interferon era, we found that the combination of HLA-DRB1 and *IL-28B* recipient markers identifies a large population of patients at increased risk of liver graft loss from the early post-transplant period.

Both innate and adaptive immunity play a role in HCV infection, leading to virus elimination in 20–30% of the patients during the acute phase [29,30]. Presentation of viral peptides to CD4 T cells occurs preferentially in the context of specific HLA class-II antigens, and several studies demonstrated a protective role for HLA-DRB1*11 in HCV infection [7,31]. HLA-DRB1*11 is less prevalent among HCV patients undergoing LT compared with healthy controls, and a fully mismatched donor/recipient pair at the DRB1 locus is associated with an increased severity of recurrent hepatitis C [8]. On the innate arm of the immune response, the *IL-28B*

Table 3. Demographic and clinical characteristics of HCV liver transplant recipients divided into two groups according to their immunogenetic markers.

	HLA-DRB1*11-negative and *IL-28B* non-C/C (n = 228)	All other genetic combinations (n = 221)	P value
Recipient features			
Age (years)	56 [48–60]	55 [51–59]	0.9061
Male	184 (80.7%)	179 (81%)	0.9370
MELD at LT	15 [12–20]	15 [12–18]	0.4422
HCC	96 (42.1%)	102 (46.2%)	0.3877
HCV RNA before LT (log_{10} IU/mL)	6.14 [5.61–6.49]	5.92 [5.30–6.43]	**0.0324**
Viral genotype 1/2/3/4†	167 (75.6%)/21/15/18	137 (66.8%)/36/28/4	**0.0002**
PRA Max 0–9/10–50/51–80/>80%‡	198/6/13/3	203/1/9/1	0.1525
Waiting list time (months)	2.6 [1.5–4.5]	3.0 [1.3–5.2]	0.3275
Donor features			
Age (years)	61 [49–71]	58 [47–69]	0.4154
Male	126 (55.3%)	131 (59.3%)	0.3902
HBV-core Ab-positive donor	37 (16.2%)	36 (16.3%)	0.9859
Body mass index	25 [23–27]	25 [23–28]	0.9840
Macrovesicular steatosis <15%/≥15%§	167/22	154/25	0.5040
Graft quality* optimal/suboptimal§	83/106	84/95	0.5619
HLA-DRB1*11-positive phenotype	103 (45.2%)	103 (46.6%)	0.7610
IL-28B genotype C/C vs. non-C/C¶	97 (43.1%)/128	102 (46.8%)/116	0.4366
Split liver graft	7 (3.1%)	7 (3.2%)	0.6145
Cold ischemic time (minutes)	500 ± 133	496 ± 142	0.7444
Donor/recipient match			
D-MELD	899 [619–1166]	845 [598–1152]	0.4052
IL-28B C/C D to non-C/C R yes/no¶	97 (43.1%)/128	49 (22.5%)/169	**<0.0001**
HLA-A, HLA-B, HLA-DRB1 match 0/1/2/3/4/5/6‖	69/80/58/13/3/0/0	47/86/53/24/7/0/0	0.0518
HLA-A match 0/1/2††	141/76/8	123/89/5	0.2466
HLA-B match 0/1/2‡‡	170/49/4	161/52/5	0.8235
HLA-DRB1 match 0/1/2	135 (59.2%)/88/5	107 (48.4%)/95/19	**0.0031**
Positive cross-match	45 (19.7%)	17 (7.7%)	**0.0009**
Post-transplant features			
Main IS regimen CsA/Tac/other	210/16/2	201/16/4	0.3835
Treated acute rejection	71 (31.1%)	47 (21.3%)	**0.0175**
Treated CMV infection	36 (15.8%)	19 (8.6%)	**0.0201**
HCV RNA at 3 months (log_{10} IU/mL)	7.20 [6.62–7.60]	6.91 [6.38–7.47]	**0.0062**
Antiviral treatment after LT	117 (51.3%)	106 (48%)	0.4776
SVR at 24 weeks	25 (21.4%)	36 (34%)	**0.0351**

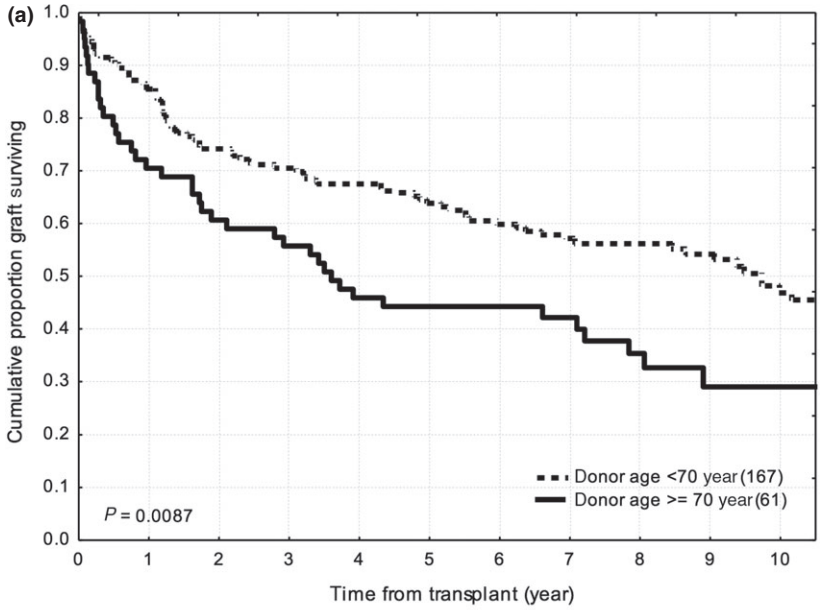
Numerical variables are expressed as median [Q1–Q3] or mean ± standard deviation according to their nonparametric or parametric distribution. Categorical variables are expressed as numbers (prevalence, %).

In bold significant P values.

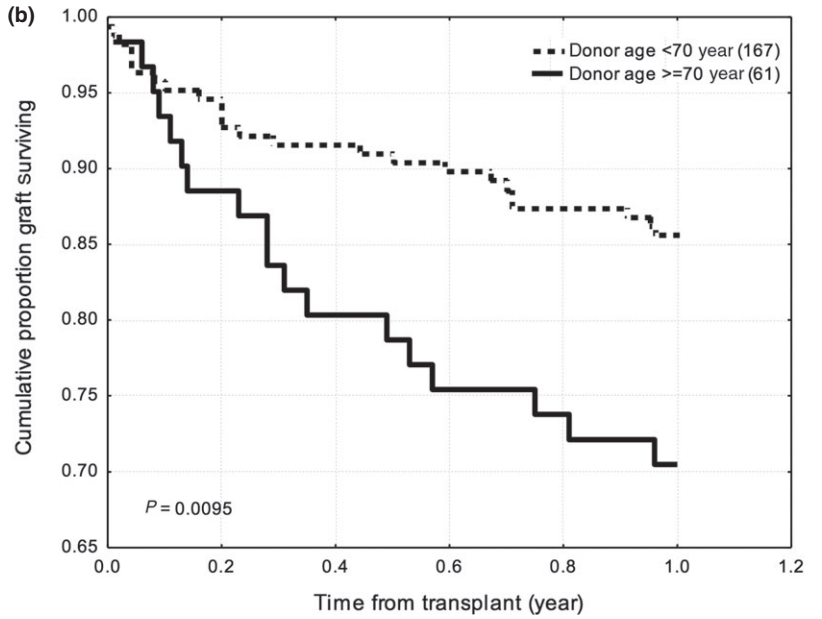
*Graft quality: suboptimal if graft from donor ≥65 years and/or with macrovesicular steatosis ≥15%, according to Salizzoni M *et al. Transpl Int* 2003.

†23, ‡15, §81, ¶6, ‖9, ††7, ‡‡8 missing data.

CMV, cytomegalovirus; CsA, cyclosporine; D, donor; D-MELD, donor age × recipient MELD; HBV, hepatitis B virus; HBV-core Ab, anticore HBV antibodies; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HLA, human leukocyte antigen; *IL-28B*, *interleukin-28B*; IS, immunosuppression; LT, liver transplantation; MELD, Model for End-stage Liver Disease; PRA, panel-reactive antibodies; R, recipient; SVR, sustained virological response; Tac, Tacrolimus.



HLA-DRB1*11-negative and *IL28B* non-C/C HCV-positive recipients	1 year	2 years	3 years	5 years	10 years
Donor age < 70 years	0.86	0.74	0.71	0.64	0.47
(No. at risk)	(*n* = 143)	(*n* = 124)	(*n* = 118)	(*n* = 106)	(*n* = 37)
Donor age ≥ 70 years	0.71	0.61	0.56	0.44	0.29
(No. at risk)	(*n* = 43)	(*n* = 37)	(*n* = 34)	(*n* = 27)	(*n* = 8)



HLA-DRB1*11-negative and *IL28B* non-C/C HCV-positive recipients	1 month	2 months	3 months	6 months	1 year
Donor age < 70 years	0.96	0.95	0.93	0.91	0.86
(No. at risk)	(*n* = 160)	(*n* = 158)	(*n* = 155)	(*n* = 152)	(*n* = 143)
Donor age ≥ 70 years	0.95	0.89	0.87	0.79	0.71
(No. at risk)	(*n* = 58)	(*n* = 54)	(*n* = 53)	(*n* = 48)	(*n* = 43)

Figure 4 Kaplan–Meier curves for liver graft survival in HCV-infected recipients lacking both HLA-DRB1*11 phenotype and *IL-28B* C/C genotype stratified by the 70-year donor age cutoff. (a) At 10 years post-transplant. (b) At 1 year post-transplant. HCV, hepatitis C virus; HLA, human leukocyte antigen; *IL-28B*, *interleukin-28B*.

Table 4. Multivariable analysis output using Cox model in HCV liver transplant recipients.

Covariable		Exp(β)	95% CI lower	95% CI upper	*P* value
Recipient HLA-DRB1*11 phenotype and *IL-28B* genotype	Baseline value: all other genetic combinations				
	At risk: HLA-DRB1*11-negative and *IL-28B* non-C/C	**1.74**	1.29	2.35	**<0.0001**
HCV RNA before LT (IU/mL)	Baseline value: $<1 \times 10^6$				
	At risk: $\geq 1 \times 10^6$	**1.45**	1.07	1.96	**0.017**
MELD at LT	Baseline value: <15				**0.025**
	At risk: 15–24	0.95	0.69	1.31	0.756
	At risk: ≥25	**1.74**	1.10	2.78	**0.019**
Donor *IL-28B* genotype	Baseline value: non-C/C				
	At risk: C/C	**1.45**	1.08	1.96	**0.014**
Donor age (years)	Baseline value: <48				**0.004**
	At risk: 48–58	0.86	0.53	1.38	0.517
	At risk: 59–69	1.34	0.85	2.09	0.208
	At risk: ≥70	**1.77**	1.14	2.74	**0.011**
Recipient age (years)	Baseline value: <49				0.132
	At risk: 49–54	0.73	0.46	1.17	0.191
	At risk: 55–59	1.23	0.81	1.87	0.335
	At risk: ≥60	0.98	0.63	1.52	0.938
Recipient gender	Baseline value: male				
	At risk: female	1.36	0.96	1.93	0.082
Donor HBV-core Ab positive	Baseline value: negative				
	At risk: positive	1.41	0.98	2.04	0.067

Multivariable analysis was performed on 411 cases with fully available data.

Model $\chi^2 = 65.576$, $P < 0.00001$.

In bold significant *P* values and relevant hazard ratios.

HBV, hepatitis B virus; HBV-core Ab, anticore HBV antibodies; HCV, hepatitis C virus; HLA, human leukocyte antigen; *IL-28B*, *interleukin-28B*; LT, liver transplantation; MELD, Model for End-stage Liver Disease.

gene product IFN-λ-3 is induced by viral infections and is upregulated in hepatocytes and peripheral blood mononuclear cells of individuals with HCV infection [32]. IFN-λ-3 enhances cellular immunity, and it may be the main inducer of infection suppressor genes following HCV infection [33]. *IL-28B* SNP rs12979860 affects expression of IFN-λ-3, with higher levels of IFN-λ-3 and a higher rate of spontaneous clearance of HCV in patients presenting a C/C genotype [10]. HCV-infected LT recipients with an *IL-28B* genotype different from C/C (i.e., C/T or T/T) have been shown to suffer from earlier HCV recurrence and more severe fibrosis progression; on the contrary, the *IL-28B* C/C genotype in the donor was associated with an adverse outcome in the recipient. Finally, an *IL-28B* C/C genotype both in the recipient and in the donor was found to favor the response to interferon-based therapies [11–13].

In our study, we confirmed that in HCV recipients, HLA-DRB1*11 is uncommon, and the frequency of other HLA-DRB1 alleles is increased; similarly, the frequencies of *IL-28B* C allele and C/C genotype are reduced.

Even if in HCV transplants a full mismatch at the HLA-DRB1 locus was shown to be associated with severity of hepatitis C recurrence [8], we failed to detect an impact of this donor/recipient combination on graft survival. Conversely, we were able to extend from fibrosis progression to graft survival the findings of Duarte-Rojo [12] about the detrimental effect of the *IL-28B* mismatch represented by C/C donor to non-C/C recipient.

Focusing on the recipient side, we found a significantly longer survival for HCV patients carrying either HLA-DRB1*11 phenotype or *IL-28B* C/C genotype, independently from the donor's genetic features. More importantly, we identified in half of our HCV recipients an immunogenetic profile (i.e., concurrent absence of both HLA-DRB1*11 and *IL-28B* C/C) which predicted a much worse outcome starting from the first months post-transplant. These "at-risk" subjects did not differ from their "protected" counterparts for other characteristics which are known to affect LT results such as recipient age, gender, liver disease severity, hepatocellular carcinoma; donor age, *IL-28B* genotype, hepatitis B-core antibody positivity; graft macrovesicular steatosis, cold ischemic time; donor age times recipient's MELD (D-MELD) [6,12,34]. On the other hand, they were more often infected with genotype 1 virus, viremia was significantly higher before and 3 months after LT, and SVR with interferon-based therapies was less frequent, in accordance with the knowledge that the *IL-28B* C/C genotype is a robust predictor of response to interferon [9] and that non-C/C recipients show higher viral loads at hepatitis C recurrence after LT [12]. These data indicate that "at-risk" subjects are less resistant to the HCV both before and after LT.

The immunological risk in these disadvantaged patients was further compounded by the higher prevalence of HLA-DRB1 donor/recipient full mismatch due to the marked phenotypic prevalence of the HLA-DRB1*11 in the general population [8,35], the same selection bias possibly explaining also the higher prevalence of cross-match positivity. In the "at-risk" recipients, alloimmunity may have increased the occurrence of acute rejection requiring treatment, with consequent weakening of immune defenses and increase in both CMV reactivations and HCV viremic levels 3 months after LT. It is a reasonable inference that this chain of events may favor a more severe course of HCV recurrence, with rapid and progressive injury leading to a higher rate of graft loss starting early after LT.

Although its retrospective nature may be a limit of this study, a high number of patients were consecutively recruited at a single center. They were referred from all parts of Italy; therefore, they are representative of an European, mainly Caucasian, population. Secondly, in our center, protocol biopsies are not envisaged, and liver elasticity studies were not available in the initial follow-up years; thus, no data are available on the role of HLA-DRB1*11 and *IL-28B* on fibrosis progression [36]. To offset this disadvantage, graft survival from the time of transplant was selected as a more robust outcome than disease evolution.

The recent advent of new highly effective and safe antivirals is providing the mean to eradicate the HCV also in the transplant setting [37]. In the current transition phase in which many HCV candidates are still viremic when accessing to LT waiting lists, pretransplant DAA-based treatment has been advocated as the most likely to reap most benefits [23]. Other authors plead, instead, for a more selective use of DAAs in wait-listed patients [25] and suggest to delay HCV therapy after LT in most recipients until their clinical course has stabilized [38] or recurrent hepatitis C has been histologically recognized, because solid data on preemptive therapy are still lacking (Belli LS, Oral Presentation at the Specialty Update Symposium ELITA—Advances and challenges of liver transplantation for HCV liver cirrhosis—ESOT Congress, Brussels, Belgium, September 13, 2015). Unfortunately, immediately

after transplant, distinction between acute rejection and early recurrent hepatitis C [39] as well as between biliary obstruction and fibrosing cholestatic hepatitis C [40] remains a challenging histological and clinical problem. Furthermore, HCV-infected transplant patients presenting early organ preservation injury, which is more marked in elderly grafts, show the poorest survival outcomes [41].

In this complex scenario, while waiting for universal hepatitis C eradication before listing, the pre-LT typing of HLA-DRB1 and *IL-28B* helps identifying HCV viremic recipients who are at an increased risk of graft loss starting from the first months after LT. Therefore, our study findings provide a new useful, low-cost decisional element for the timing of DAA-based therapy in the transplant setting and suggest that immunogenetically "at-risk" patients are high priority candidates for pre-transplant or immediately post-transplant preemptive treatment [42,43]. This indication should be all the more relevant in high MELD candidates and in countries where elderly donors represent an unavoidable resource [44,45]. Conversely, immunogenetically "low-risk" patients with viremia at LT could undergo antiviral therapy beyond 3 months following LT, when their clinical course has stabilized and early mortality risk has been overcome.

Authorship

AA, RR, SM, and FT: contributed substantially to the study conception and design, data interpretation, and drafting and revision of the manuscript. FEB and ED: participated in the acquisition of data, and in the drafting of the manuscript. PM: carried out the statistical analyses. MS and MR: participated in its critical revision. DD: participated in the analysis and interpretation of data, in the drafting of the manuscript, and its critical revision for important intellectual content. All authors participated in final approval of the version to be published. All authors agreed to be accountable for all aspects of their work in ensuring that questions related to the accuracy or integrity of any part of the paper are appropriately investigated and resolved.

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Conflicts of interest

All authors of this manuscript declare no conflicts of interest to disclose as described by *Transplant International*. MR is an Advisory Board member for Janssen, Merck, Abbvie, and BMS. The study was already presented in Full Oral Session at ESOT 2015 Congress, Brussels, and in Transplant Plenary Session at AASLD 2015 Congress, San Francisco.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Recipient and donor demographic and clinical features in HCV and non-HCV transplants.

Table S2. Phenotypic frequencies of HLA-A/B/DRB1 in HCV liver transplants.

Table S3. Relative frequencies of the most frequent HLA-DRB1*11 subtypes in a sample of HLA-DRB1*11-positive transplant recipients, liver donors and Italian controls.

Table S4. Distribution of *IL28B* rs12979860 genotypes (C/C vs. non-C/C) in HCV-negative donors stratified according to age quartiles.

Table S5. Causes of graft loss in the two groups of HCV liver transplant recipients.

Table S6. Liver graft survival rates in HCV recipients lacking both HLA-DRB1*11 and *IL28B* C/C stratified by donor HLA-DRB1*11 phenotype and donor *IL28B* genotype.

Figure S1. Ten-year liver graft survival according to HCV infection at transplant (1187 consecutive adult primary transplants from HCV-negative donors, years 1999–2009).

Figure S2. Kaplan-Meier curves for graft survival in HCV recipients according to: A) HLA-DRB1*7, B) HLA-B*13 and C) HLA-B*16 phenotype (positive vs. negative).

Figure S3. Kaplan-Meier curves for liver graft survival according to HLA-DRB1 donor/recipient match in HCV transplants.

Figure S4. Kaplan-Meier curves for liver graft survival in HCV recipients according to donor *IL28B* genotype.

Figure S5. Kaplan-Meier curves for liver graft survival according to *IL28B* genotype donor/recipient match in HCV transplants.

Figure S6. Effect on liver graft survival of the concurrent presence of HLA-DRB1*11 phenotype and *IL28B* C/C genotype in HCV recipients.

Figure S7. Kaplan-Meier curves for graft survival at 1 year (Panel A) and 10 years (Panel B) in HCV recipients stratified according to HLA-DRB1*11 phenotype and *IL28B* genotype. Patients who attained SVR were censored at the beginning of interferon-based therapy.

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