IMMUNOSUPPRESSION WITHDRAWAL IN LIVER GRAFT RECIPIENTS WITH RECURRENT HCV-INFECTION

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Hepatitis C Virus in Transplantation

- **HCV is a well adapted hepatotropic virus with elaborate immune evasion strategies and is infecting an immunopriveliged organ**

- **altogether this makes HCV a very elusive target for anti-viral therapy**

- **liver damage due to chronic HCV-infection is the leading cause for liver Tx**

- **HCV-infection universally recurs after Tx**

- **usually leads to accelerated progression of liver damage after recurrence**

The pathogenesis of recurrent HCV-infection is unknown

Several Hypotheses account for the accelerated pathogenesis of recurrent HCV-infection after liver transplantation:

•due to the presence of immunosuppressive drugs who blunt an active antiviral response

•bottleneck effect with a preferential expansion of HCV-quasispecies especially well fit for the proliferation in the hepatocytes of the newly grafted organ

•effect of the inflammatory situation due to ischemia-reperfusion injury immediately after Tx

IS withdrawal in recurrent HCV infection

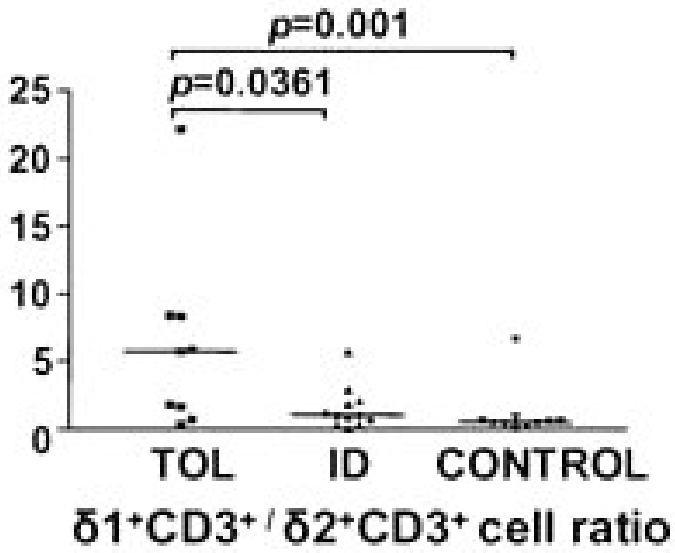
Due to these potential harmful effects of immunosuppression, HCV-infected liver recipients might be a population that particularly benefits from IS withdrawal strategies.

In previous studies it has been shown, that up to 20% of HCV infected liver recipients could be successfully weaned off IS and that withdrawal was ameliorating disease progression.

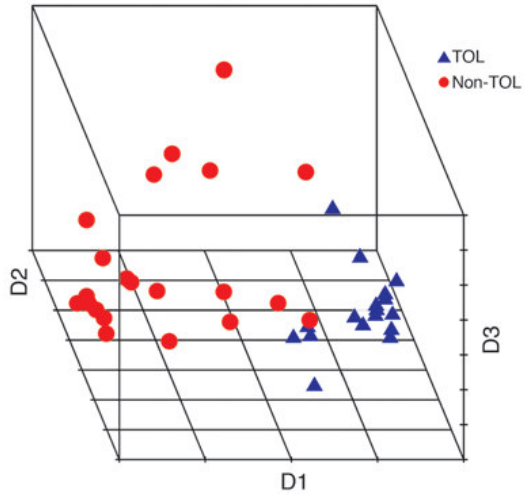
Tisone G., et al. Complete weaning off immunosuppression in HCV liver transplant recipients is feasible and favourably impacts on the progression of disease recurrence. *J. Hepatol.* (2006)

IS withdrawal in liver recipients

In previous case-controlled cross-sectional IS withdrawal studies we have characterized the immunologic parameters associated with tolerance in blood samples from liver recipients.



Martinez-Llordella M. , Am. J. Transplant (2007)



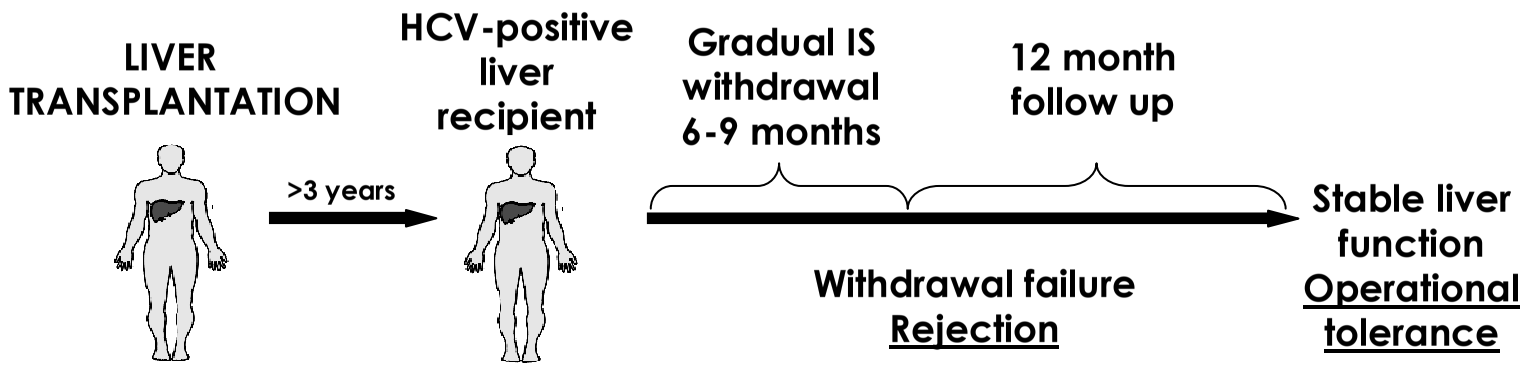
gene	*fold change*	
KLRF1	1.879	(p<0.05)
SLAMF7	1.414	(p<0.05)

Martinez-Llordella M. , J. Clin. Invest. (2007)

Rationale

On the basis of this previous work we hypothesize, that the assessment of these phenotypic and transcriptional biomarkers could be useful to identify HCV-infected liver recipients with a high likelyhood of developing tolerance.

Prospective study of immunosuppression withdrawal in HCV infected recipients



Inclusion criteria:

i) Vδ1/Vδ2 T-cell ratio above the predefined threshold of 2.33 or increased expression of SLAMF7/KLRF1

ii) no indication for peg IFNγ/ribavirin during the weaning procedure

iii) stable liver function tests for at least 6 months

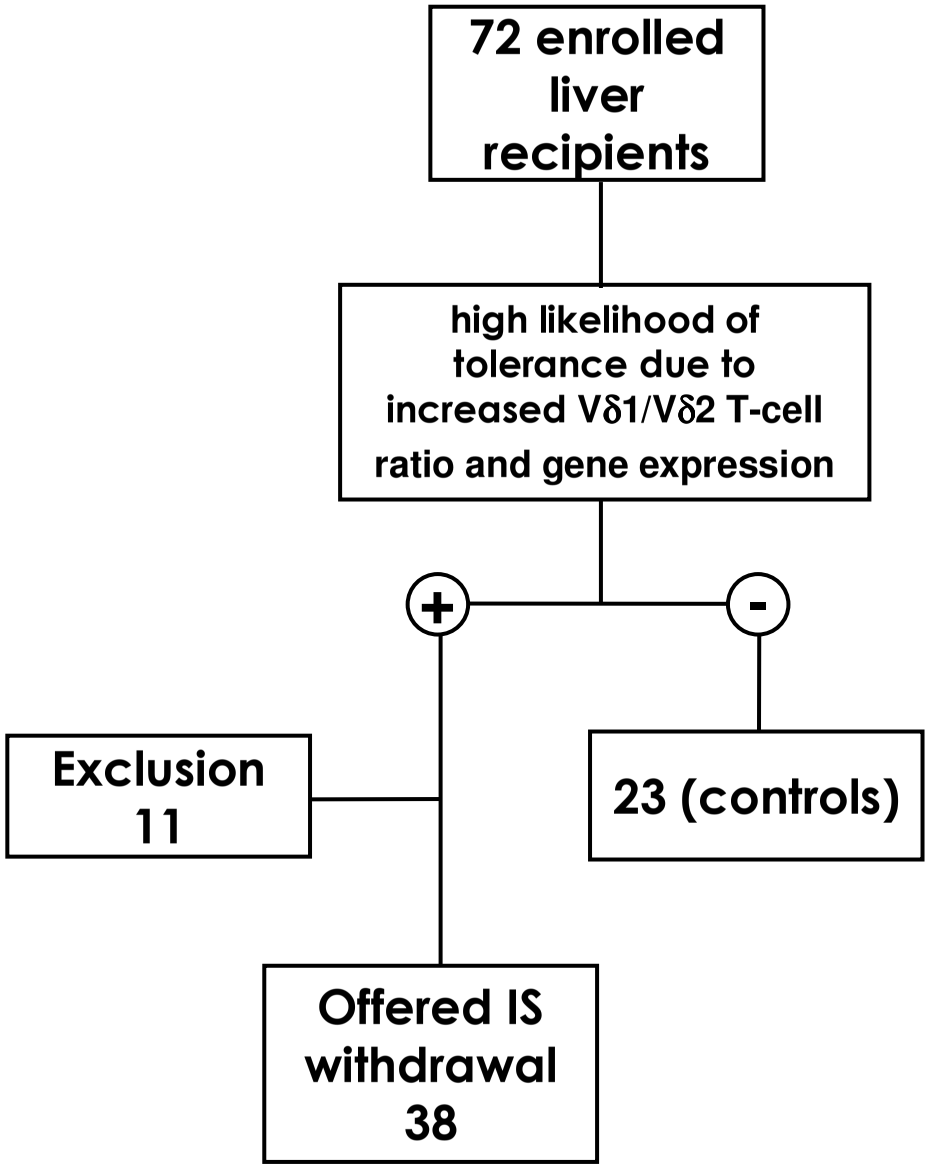
iv) no evidence of autoimmune liver disease

v) absence of acute/chronic rejection episodes in the previous 12 months

vi) absence of fibrosis stage III or IV in baseline liver biopsy

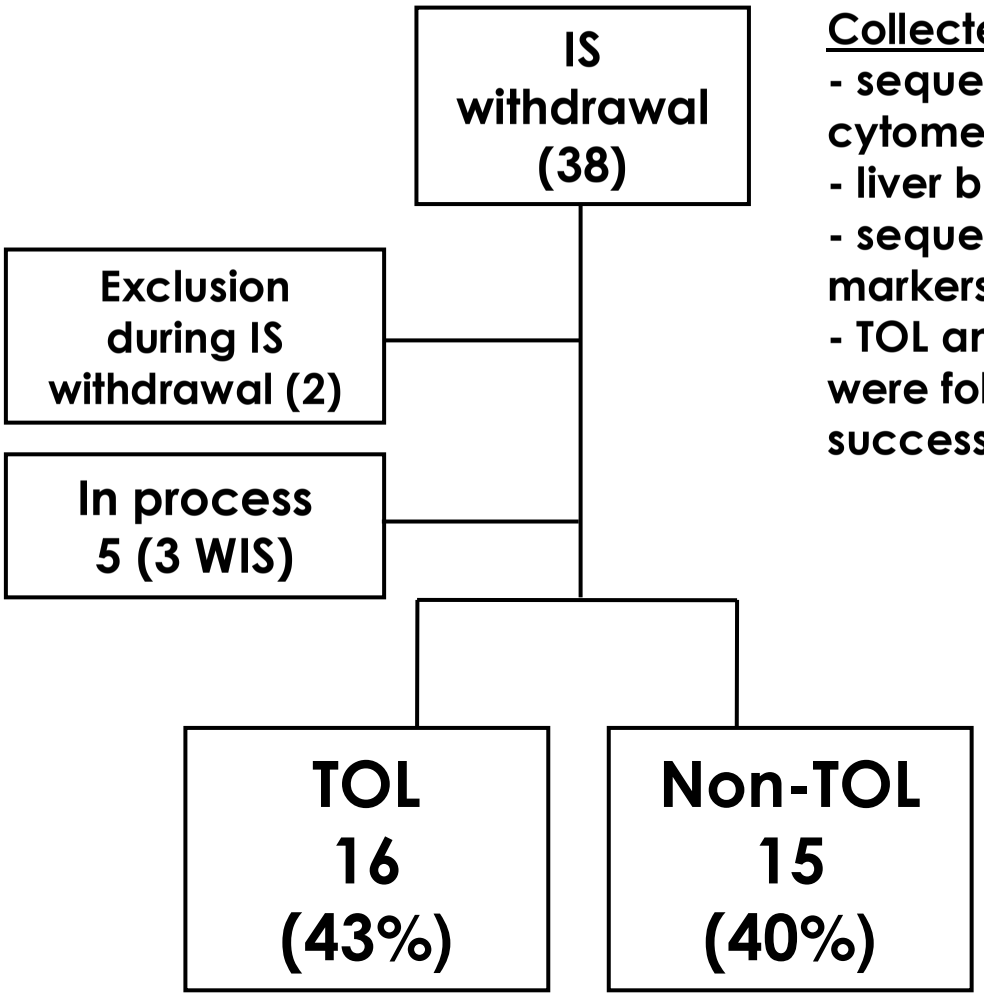
In case of conformity, gradual IS withdrawal was offered.

Study design



Demographics:	
Age	61.3
Time since Tx	7.1 (years)
Sex	79% male
Baseline IS:	
CNI	88%
Antimetabolite	4%
Rapamycin	8%

Immunosuppression withdrawal in HCV infected recipients



Collected Samples:

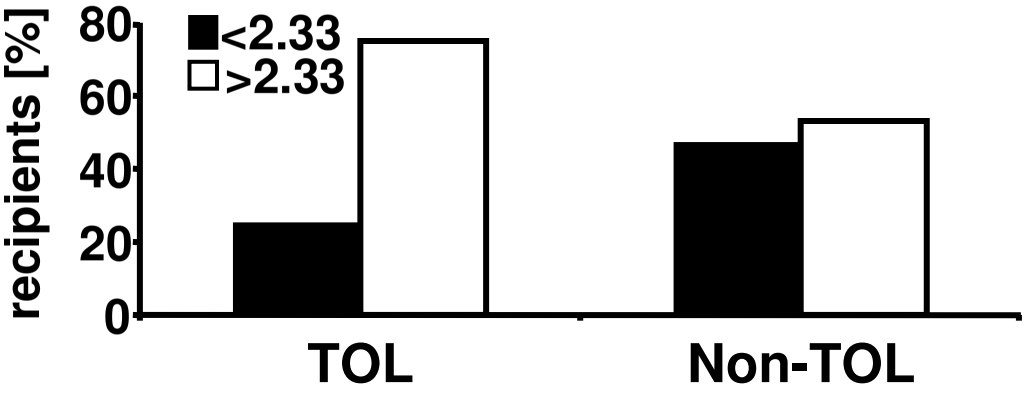
- sequential peripheral blood (Elsipot/Flow cytometry/gene expression)
- liver biopsies (gene expression)
- sequential serum samples (protein markers)
- TOL and Non-TOL patients were followed-up for 12 months following successful IS withdrawal or rejection.

Phenotypical biomarkers facilitat the selection of recipients with high likelihood of tolerance

Vδ1/Vδ2 T-cell ratio (threshold 2.33)

		clinical phenotype: TOL	clinical phenotype: Non-TOL
prediction	TOL (>2.33)	12	8
	Non-TOL (<2.33)	4	7
	Totals	16	15

Sensitivity = 75%
Specificity = 46%



Assessment of gene expression didn´t result in discriminative power

Characterization of virus-specific T-cell responses

HCV-specific IFNγ-Elispot analysis:

- 7-33 synthetic peptides per pool (excluded: E1, NS1, NS2)
-13- to 18-mers with 11-12 amino acid overlaps (8 µg/ml final concentration)

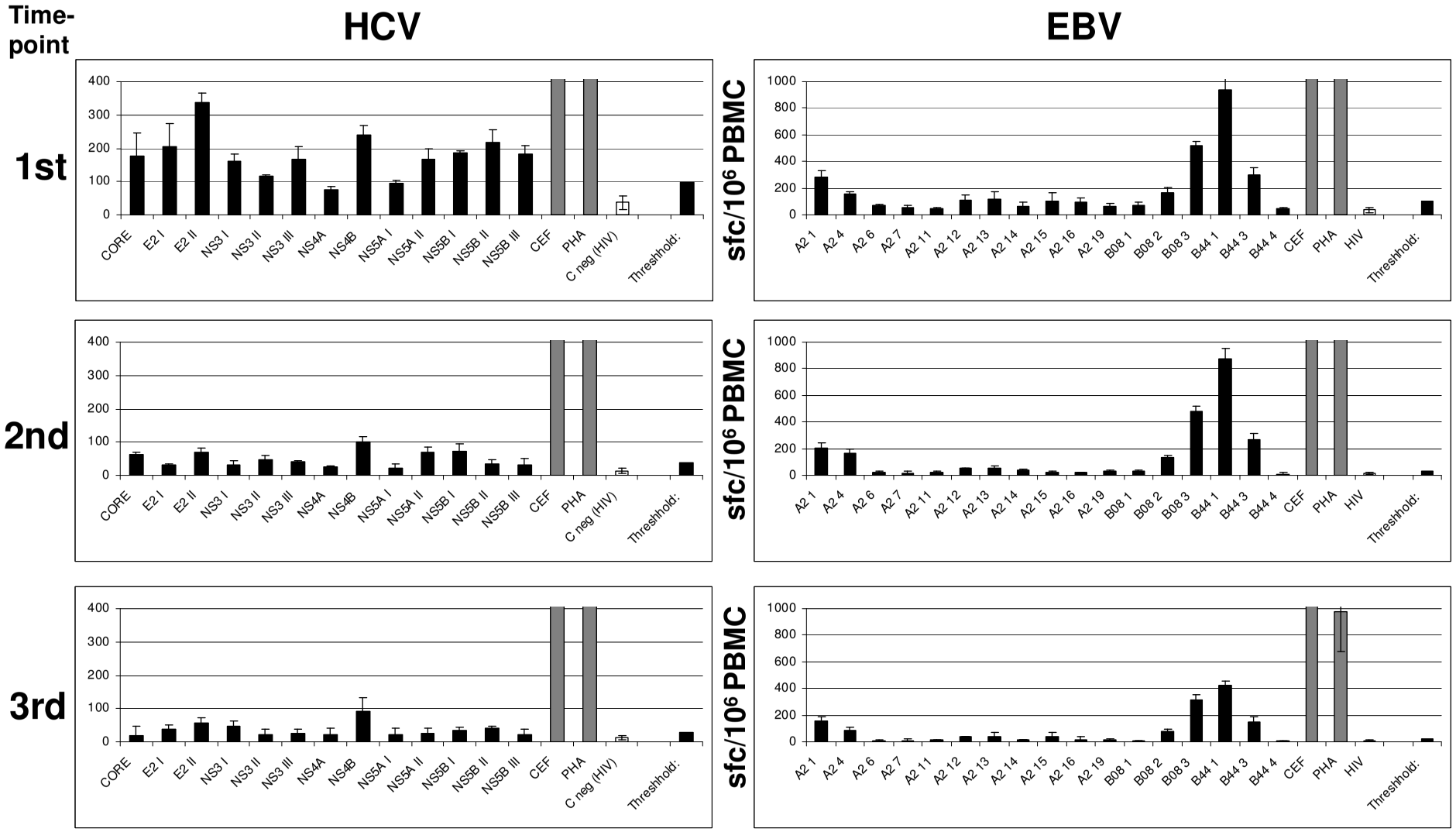
EBV-specific IFNγ-Elispot analysis:

-set of defined HLA-restricted (Class I/CTL) EBV-epitopes (8-15 mers;12 µg/ml final concentration)

Controls:

-HIV GAG peptide pool as negative control
-Antigen specific positive control (CEF: CMV, EBV, Influenza)
-Mitogenic positive control (PHA)
-Threshold: negative control plus three STDVs

Sequential characterization of virus-specific T-cell responses in HCV-positive graft recipients



Conclusions

- We confirmed that IS withdrawal can be accomplished in transplant recipients with active recurrence of HCV-infection.

- The use of immunophenotypic markers on PBMCs can be useful to identify HCV-infected recipients with a high likelihood of being tolerant.

- The potential benefit of IS withdrawal on the progression of HCV-disease remains to be demonstrated.

- We are currently completing the sequential Elispot analyses, flow cytometry and gene expression in peripheral blood and liver tissue

- We expect that the results of these studies will yield a refined set of biomarkers to accurately predict the outcome of IS withdrawal in future studies of HCV-infected recipients

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