

Immunosuppression minimization vs. complete drug withdrawal in liver transplantation

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Summary

Despite the increase in long-term survival, liver transplant recipients still exhibit higher morbidity and mortality than the general population. This is in part attributed to the lifelong administration of immunosuppression and its associated side effects. Several studies reported in the last decades have evaluated the impact of immunosuppression minimization in liver transplant recipients, but results have been inconsistent due to the heterogeneity of study designs and insufficient sample sizes. On the other hand, complete immunosuppression withdrawal has proven to be feasible in approximately 20% of carefully selected liver transplant recipients, especially in older patients and those with longer duration after transplantation. The long-term risks and clinical benefits of this strategy, however, also need to be clarified. As a consequence, and despite the general perception that a large proportion of liver recipients are over-immunosuppressed, it is currently not possible to derive evidence-based guidelines on how to manage long-term immunosuppression to improve clinical outcomes. Large clinical trials of drug minimization and/or withdrawal focused on clinically-relevant long-term outcomes are required. Development of personalized medicine tools and a deeper understanding of the pathogenesis of idiopathic inflammatory graft lesions will be pre-requisites to achieve these goals.

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Background

Long-term survival after solid organ transplantation has increased during the last decades [1] due to improvements in surgical technique, peri-operative care, and more efficient immuno-

suppressive (IS) drugs. However, transplant recipients still exhibit higher morbidity and mortality than the general population [2]. One of the main causes are co-morbidities negatively influenced by chronic IS drug usage [3–8]. The high prevalence of IS related toxicity and the fact that liver allograft rejection seldom impacts on clinical outcomes suggest that most liver recipients are likely to be over-immunosuppressed [9,10]. One of the most significant side effects of IS drugs is calcineurin inhibitor (CNI) nephrotoxicity, which contributes to the high rate of chronic renal failure observed in liver transplant recipients and is associated with the need to institute renal replacement therapies and with high mortality [11,12]. Minimization (or complete withdrawal) of immunosuppression, particular CNIs, may overcome these problems. The clinical opportunity is more tangible in the liver than in other transplantation settings due to the greater capacity of the liver allograft to cope with the cytolytic effects of alloimmune responses [13,14]. The potential benefits of IS minimization or withdrawal, however, still need to be balanced with the risks and inconveniences of prompting liver allograft rejection. This assessment has to take into account the fact that the individual recipient immunoreactivity evolves over time.

Over the past two decades, multiple studies on IS minimization have been reported in the liver transplantation literature. In parallel, a number of IS withdrawal trials have been performed. While the results of some of these studies have been promising, due to their heterogeneity and relatively small sample sizes, they have failed to provide truly generalizable information. As a consequence, we still lack evidence-based guidelines on how to reduce IS to improve clinical outcomes, and therefore, the long-term therapeutic management of liver transplant recipients remains an empirical practice. We review the benefits and limitations of the different strategies employed in liver transplantation to minimize or withdraw IS in an attempt to provide a framework to critically assess and/or design future studies in the field.

Immunosuppression minimization

In the absence of accurate tools to determine the optimal level of immunosuppression required by each individual patient, it is difficult to objectively define “immunosuppression minimization”. A commonly used definition is the administration of the lowest amount of immunosuppression compatible with a rejection-free state [15]. The IS levels required to prevent rejection, however, vary greatly, not only between different individuals, but also

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Abbreviations: IS, immunosuppression; CNI, calcineurin inhibitor; HCV, hepatitis C virus; RCT, randomized clinical trial; ATG, anti-thymocyte globulin; MMF, mofetil mycophenolate; mTOR, mammalian target of rapamycin; RISE, reprogramming the immune system for the establishment of tolerance; US, United States.



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Table 1. Strategies to minimize immunosuppression.

Reference	Minimization strategy	N	Study design	Rejection	Impact on co-morbidities
Margarit <i>et al.</i> , [26]	Steroid avoidance	60	Randomized TAC vs. TAC + steroids	Acute rejection 39 vs. 32%; $p = \text{n.s.}$	No differences in survival rate and infections
Samonakis <i>et al.</i> , [24]	Steroid avoidance	56	Randomized TAC vs. TAC + steroids + AZA	Acute rejection 70 vs. 86%; $p = \text{n.s.}$	No differences in renal function, metabolic complications and survival rate
Lerut <i>et al.</i> , [25]	Steroid avoidance	156	Randomized TAC vs. TAC + steroids	Acute rejection 20 vs. 23%; $p = \text{n.s.}$ Steroid resistant 13 vs. 3%; $p = 0.04$	No differences in renal function, metabolic complications and PTLT
Herrero <i>et al.</i> , [27]	MMF	11	Progressive CNI reduction (6 patients free of CNI)	Acute rejection 2 episodes	Improvement in renal function in patients free of CNI
Schlitt <i>et al.</i> , [28]	MMF	28	MMF replacement vs. CNI	Acute rejection 3 vs. 0 episodes	Significant improvement in renal function in MMF patients. No differences in lipid profile and blood pressure
Orlando <i>et al.</i> , [30]	MMF	42	Conversion to MMF	Acute rejection 9 patients	Renal function improved in 89% of the patients. Cholesterol and triglycerides decreased in 76% of the patients. Blood pressure improved 80% of the patients
Abdelmalek <i>et al.</i> , [36]	Sirolimus	607	Randomized (2:1) Conversion to sirolimus vs. CNI	Acute rejection 11 vs. 6%; $p = 0.02$	No differences in renal function or patients and graft survival
De Simone <i>et al.</i> , [40]	Everolimus	719	Randomized 1 month after LT TAC + everolimus vs. everolimus vs. TAC	Acute rejection 4 vs. 11%; $p = \text{n.s.}$ Everolimus monotherapy was early terminated due to high rate of acute rejection (19%)	Improvement in renal function
Fischer <i>et al.</i> , [39]	Everolimus	203	Randomized 1 month after LT everolimus vs. TAC	Acute rejection 15 vs. 18%	No differences in renal function, infection or metabolic complications

TAC, tacrolimus; AZA, azathioprine; PTLT, post-transplant lymphoproliferative disorder; CNI, calcineurin inhibitor; LT, liver transplant; n.s., not significant.

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within the same individuals over time. Thus, to comply with this definition, recipients would need to decrease IS doses until rejection occurs. A more useful definition of minimization is the attainment of a state in which immunosuppressive drugs are decreased down to levels that do not cause clinically significant side effects and yet prevent rejection. In liver transplantation, this has been attempted by reducing CNI doses following induction with T-cell depleting agents, by avoiding steroids and administering CNIs on monotherapy, or by withdrawing CNIs at later time points (Table 1). In addition, a variety of trials designed to prevent specific side effects (e.g., diabetes, renal failure) by combining various IS drugs at reduced doses have also been performed, but these do not fall within the scope of what would be considered drug minimization. An important consideration when assessing these studies is the fact that the standard recommended CNI dosing targets for liver transplantation were originally set within clinical trials that replicated the doses employed in kidney transplantation, where overall immunosuppression requirements are higher than in liver transplantation. The optimal ranges for CNI levels in liver transplantation have not been studied in depth and are still unclear.

CNI minimization and induction with T-cell depleting antibodies early after LT

The use of T-cell depleting therapies in combination with drastically reduced CNI doses has been proposed as a strategy potentially capable of maximizing the tolerogenic properties of liver allografts [16] by achieving a “prope” tolerant state in which normal graft function is maintained with minimal (spaced-dose) immunosuppression [16,17]. Starzl *et al.* [16] first reported the results of this strategy in liver transplant recipients in 2003. In this study, a single pre-transplant dose of thymoglobulin was administered followed by monotherapy with tacrolimus at doses that were gradually decreased, starting 4 months after transplantation. The trial was successful in that 11 out of the 14 survivors, 13–17 months after transplantation, were receiving spaced-dose tacrolimus administration (every other day) with very low blood levels. The real applicability of the strategy was however difficult to ascertain, since a very large number of protocol violations occurred. Furthermore, the rejection rate and response to therapy were not clearly described. The same group performed a subsequent cohort study in which they compared 76 liver patients treated with alemtuzumab and tacrolimus monotherapy followed by gradual tapering of tacrolimus doses with a cohort of 84 patients who received tacrolimus plus steroids at conventional doses. At 14 to 22 months after transplantation, 62% of surviving alemtuzumab-treated recipients were on spaced dosed tacrolimus, and this was achieved without an increased rate of rejection. In this study, hepatitis C virus (HCV+) infection was associated with very low patient and graft 1-year survival, regardless of the treatment arm (70% and 71% in alemtuzumab-treated patients and 65% and 54% in patients under conventional treatment) [18]. By contrast, De Ruvo *et al.* [19] retrospectively compared 22 HCV+ liver transplant recipients treated with thymoglobulin and tacrolimus monotherapy to 30 HCV+ patients under standard immunosuppression (tacrolimus plus steroids). Patients treated with thymoglobulin achieved lower tacrolimus trough levels and this was not associated with increased mortality, rejection episodes, or severity of HCV recurrence [19].

Whether this resulted in clinical benefits (e.g., improved renal function or decreased metabolic co-morbidities) was, however, not reported. Benitez *et al.* performed a randomized controlled trial (RCT) in which recipients were allocated to either conventional immunosuppression (tacrolimus plus steroids) or to induction with ATG-Fresenius followed by tacrolimus monotherapy. In the experimental group, tacrolimus doses were gradually decreased starting 3 months after transplantation to achieve spaced dose administration or single day doses with <5 ng/ml trough levels. Patients treated with the experimental regimen received lower tacrolimus and steroid doses, but this was associated with an increased rate of early acute rejection episodes (66% vs. 31% in the control group). Furthermore, at 3 months after transplantation, only a minority of ATG-treated recipients met the criteria for partial tacrolimus weaning, and in those in whom weaning was attempted, the development of late acute rejection episodes precluded the attainment of the primary end point. Once more, obvious clinical benefits in terms of decreased immunosuppression-related adverse events were not seen [20].

Altogether, it is clear from these studies that the use of T-cell depleting therapies allows for an overall reduction in the doses of conventional immunosuppressive drugs. Anecdotally, this treatment can facilitate the complete discontinuation of immunosuppression [21]. However, high rejection rates may occur when immunosuppressive drugs are rapidly reduced. Furthermore, whether aggressive T-cell depletion should be included under the scope of what is considered IS minimization is unclear, in particular following alemtuzumab treatment, whose immunosuppressive effects can last for years. Whether the strategy results in clinical benefits has not been formally demonstrated yet. These studies need to be assessed in the light of recent reports indicating that CNI trough levels lower than those traditionally recommended (i.e., tacrolimus 10–15 ng/ml during the first month after transplantation) can be achieved within conventional immunosuppressive regimens, resulting in improved clinical outcomes without increasing rejection [10,22].

Steroid minimization or avoidance

Steroid-free immunosuppressive regimens and early steroid withdrawal protocols have been explored in multiple RCTs, 21 of which were included in a recent meta-analysis [23]. In 18 out of the 21 studies included in the meta-analysis, no attempt at immunosuppression minimization was made in that steroids were replaced by other immunosuppressive agents (daclizumab, thymoglobulin, mycophenolate mofetil [MMF], or basiliximab). The 3 remaining studies [24–26], by contrast, directly compared standard immunosuppression (tacrolimus plus steroids \pm azathioprine) with tacrolimus monotherapy. Overall, steroid-free cohorts exhibited decreased development of *de novo* diabetes mellitus, lower cholesterol levels, and lower incidence of cytomegalovirus infection. These benefits were not observed in the 3 trials that specifically analysed clinical outcomes following tacrolimus monotherapy. Margarit *et al.* [26] randomized 60 patients to receive tacrolimus monotherapy or tacrolimus plus steroids. Survival rate, acute rejection incidence, infections, and side effects were comparable between the 2 groups of treatment. A separate analysis of HCV+ patients showed a significantly lower Ishak fibrosis score in the tacrolimus monotherapy group. Samonakis *et al.* [24] randomized 56 HCV+ patients to tacrolimus monotherapy or triple therapy

(tacrolimus, steroids and azathioprine). There were no differences between the 2 groups in rejection, renal dysfunction, metabolic complications, retransplantation, and survival rates. Lerut *et al.* [25] prospectively randomized 156 patients to tacrolimus monotherapy of tacrolimus plus a short steroid course. There were no differences between the 2 groups in the overall incidence of acute rejection, albeit steroid-resistant rejection was higher in recipients treated with tacrolimus monotherapy (12.8% vs. 3.8%, $p = 0.04$). Again, no differences in metabolic complications, infections, and 1-year survival were observed. Taken together, these results suggest that clinical outcomes following tacrolimus monotherapy are fully comparable to those obtained with more “conventional” immunosuppressive regimens. Despite this, the use of tacrolimus as monotherapy has not been further explored and remains marginal within the current state of immunosuppressive therapy. This is likely due to the current emphasis on preventing renal dysfunction by reducing CNIs rather than steroids [9].

Minimization of CNIs at late time points after transplantation

Employment of MMF to reduce CNI exposure

Given the association between chronic renal dysfunction and decreased recipient survival, most attempts at drug minimization conducted at late time points after transplantation have focused on the reduction or substitution of CNIs. The first attempts to minimize CNIs in long-term surviving liver recipients employed MMF to reduce CNI exposure. Herrero *et al.* [27] first described the effect of administering MMF to 11 recipients with impaired kidney function as a means to reduce CsA dosing. Patients who were free from CsA experienced a reduction in serum creatinine and increased creatinine clearance. Comparable results were reported by Schlitt *et al.* [28]. Patients who were switched from CNIs to MMF improved renal function, arterial blood pressure, and uric acid as compared with control patients on CNI. Three patients in the group on monotherapy with MMF developed acute rejection. Pageaux *et al.* [29] employed the same strategy within an RCT that recruited 56 liver recipients with renal dysfunction. A switch to low-dose CNI (>50% dose reduction) plus MMF was associated with an improvement in renal function at 12 months without an increase in the incidence of acute rejection [29]. Orlando *et al.* converted 42 long-term recipients on CNI maintenance IS (mostly cyclosporine A) with renal dysfunction, hypertension or hyperlipidemia to MMF monotherapy. This resulted in improvements in renal function, cholesterol and triglyceride levels and/or blood pressure levels in approximately 80% of the cases [30]. By contrast, in a retrospective study, involving 1075 patients in which chronic renal dysfunction was identified as a risk factor for decreased survival within the first year of transplantation, CNI reduction and addition of MMF in patients with advanced renal dysfunction did not improve creatinine levels [31].

Employment of mTOR inhibitors to reduce CNI exposure

A number of single-centre studies employing mTOR (mammalian target of rapamycin) inhibitors as replacement for CNIs to improve renal function have been performed. Results ranged from no benefit [32–34] to mild increases in glomerular filtration rate [33–35]. Abdelmalek *et al.* [36] recently published the results of the first multicentre prospective RCT evaluating safety and efficacy of sirolimus conversion in liver recipients 6 months after liver transplantation. No significant differences in renal function between patients

switched to sirolimus and those maintained on CNIs were observed. Furthermore, sirolimus conversion was associated with increased rejection and treatment discontinuation. The latter was probably related to the fact that the sirolimus doses employed in the study were significantly higher than those commonly used in clinical practice [37]. Conversion to everolimus has also been analyzed in a few prospective randomized studies. De Simone *et al.* randomized 145 patients to continue CNI exposure ($n = 72$) or CNI reduction or discontinuation ($n = 73$). At 6 months, 80% of the patients on everolimus stopped CNIs. Everolimus conversion was safe but there were no significant improvement in renal function in patients without CNIs [38]. Fischer *et al.* published a prospective randomized study to evaluate the efficacy and safety of conversion to everolimus in renal function. Four weeks after LT, patients were randomized to continue CNI or to convert to everolimus. There were no differences between the groups of patients in terms of acute rejection or mortality. However, at 1 year of conversion, the authors were not able to find a significant improvement in renal function in patients converted to everolimus [39]. More recently, De Simone reported the results of a prospective trial in which *de novo* liver transplant recipients were randomized to tacrolimus elimination after everolimus introduction, everolimus plus reduced dose tacrolimus, and standard-dose tacrolimus. The everolimus plus low-dose tacrolimus group presented better renal function as compared with standard-dose tacrolimus. However, the tacrolimus elimination group was prematurely stopped due to high rate of acute rejection [40]. Conversion to mTOR inhibitors could have an additional beneficial effect by favouring the expansion of immunoregulatory T cells [41–43], which in experimental animal models has been linked to transplantation tolerance. Thus, Levitsky *et al.* [42] described an increase in the number of regulatory T cells following conversion from tacrolimus to sirolimus. Whether these changes would favour successful discontinuation of immunosuppression in human liver recipients is however currently unknown.

Altogether the overall clinical benefits of CNI minimization at late time points after transplantation are still unclear. While in some circumstances a renal-sparing effect is observed, there is almost no data on whether late CNI minimization influences other clinical end points. Furthermore, the potential impact of IS minimization protocols on long-term subclinical histological graft damage (e.g., idiopathic chronic hepatitis and/or progressive fibrosis) also remains to be properly investigated [44]. This is relevant considering that most protocol biopsy studies have revealed substantial histological abnormalities in long-term surviving liver recipients with unremarkable liver function tests [44–47]. The likelihood of improving renal function and other co-morbidities is probably higher if CNIs are minimized early after transplantation (i.e., within 2 years of transplantation). The risk of rejection early after transplantation is, however, higher than at late time points.

Immunosuppression withdrawal

The notion that immunosuppressive drugs can be completely discontinued from selected liver recipients has been known for more than two decades [48]. These patients, who maintain normal graft function in the absence of histological signs of progressive graft damage and do not exhibit manifestations of immunocompromise, are conventionally referred to as operationally tolerant [49]. Following the original report from Starzl *et al.* in 1993 describing the cases of 6 non-compliant patients who discontinued immunosuppression and yet maintained normal liver function for 5–

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Table 2. Spontaneous operational tolerance in liver transplantation: clinical experience with elective IS weaning.

Reference	n	Time since transplantation (yr)	IS withdrawal (%)	Acute rejection (%)	Chronic rejection (%)	Graft loss (%)
Mazariegos <i>et al.</i> , [50]	95	8.4 (1.7-25)	19	26	0	0
Devlin <i>et al.</i> , [51] Girlanda <i>et al.</i> , [52]	18	6.5 (5-10)	16.7	28	5.6	5.6
Takatsuki <i>et al.</i> , [53]	26	>2	23.8	12	0	0
Eason <i>et al.</i> , [54]	18	>6 mo	5.6	61	0	0
Tryphonopoulos <i>et al.</i> , [55]	104	4	19	67	1.9	0.96
Tissone <i>et al.</i> , [56]	34	5.3	23.4	76.4	0	0
Assy <i>et al.</i> , [57]	26	4.6	8	58	0	0
Pons <i>et al.</i> , [58]	21	5.2 (24-127)	38	22	0	0
Feng <i>et al.</i> , [64]	20	7.6 (4.4-12.7)	60	35	0	0
Benitez <i>et al.</i> , [66]	102	8.5 (3-19)	40.2	59.8	0	0

13 years [48], several reports describing retrospective and/or single-centre experiences with immunosuppression withdrawal were published (Table 2) [14,50–59]. On the basis of these studies, a 20% prevalence of operational tolerance in liver transplantation was proposed [60], although this estimate did not take into consideration the heterogeneity of the study designs and of the criteria employed to select and enrol patients. The incidence of acute rejection episodes within these studies was very high. These episodes, however, were in most cases mild, and often resolved by return to baseline IS without administration of steroid boluses. Reassuringly, only 2 cases of graft loss due to chronic rejection among patients involved in these IS weaning protocols were reported [52,55]. The long-term histological outcome of drug-free liver recipients was, however, difficult to assess due to small sample sizes and/or lack of sequential protocol biopsies. In most studies, patients off immunosuppression exhibited no obvious progressive liver histological damage. Yoshitomi *et al.* [61], however, reported slightly increased fibrosis progression in operationally tolerant recipients as compared with patients under maintenance immunosuppression, although the lack of pre-weaning liver biopsies and marked differences in post-transplant follow-up between cases and controls reduced the robustness of the study. The potential clinical benefit of drug withdrawal was also only partially explored. Thus, after a mean follow-up of 6.5 years, Orlando *et al.* reported that a small group of 7 tolerant recipients exhibited decreased HCV-RNA levels, reduced infection rate, and less medication requirement to treat co-morbidities than the 22 patients who remained under maintenance IS [62]. No differences were, however, noted in terms of fibrosis stage, fibrosis progression rate, and necro-inflammatory activity. In a parallel study by Pons *et al.* with a similarly prolonged drug-free follow-up (range 43–132 months), a group of 8 tolerant liver recipients exhibited significant improvements in creatinine, glucose, and uric acid serum levels as compared with the 12 recipients in whom drug withdrawal failed [63]. Overall, these studies demonstrated the feasibility of discontinuing IS from stable liver recipients, but small sample sizes and/or lack of homogeneous well-standardized algorithms for patient screening, drug withdrawal, and patient follow-up somehow reduced the generation of truly generalizable information. The recent reports of the first two prospective multi-centre and independently monitored clinical trials of IS withdrawal in paediatric and adult liver recipients, respectively [64–67], have addressed some of the limitations of previous studies. The paediatric multi-centre study [64], sponsored by the Immune Tolerance Network in the US, included 20 carefully selected recipients of

parental living donor liver transplants in whom IS was prospectively withdrawn over approximately 36 weeks. Drug withdrawal was successful in 12 recipients, who maintained normal graft function after at least 1 year following complete IS discontinuation, and in whom liver biopsies obtained more than 2 years after complete IS withdrawal showed no significant change compared with baseline biopsies. The most significant clinical factor associated with successful IS withdrawal was an increased time interval between transplantation and initiation of IS weaning (100.6 months in operationally tolerant vs. 73 months in those who failed to discontinue IS; $p = 0.03$). No patient developed irreversible graft damage. However, the beneficial effect of drug withdrawal could not be adequately assessed. The adult multi-centre study supported by the European Union Riset Consortium enrolled 102 adult recipients in whom IS were gradually discontinued over 6 to 9 months [65]. Forty-two patients were successfully weaned, maintained stable graft function for at least 12 months after drug withdrawal, and exhibited no signs of rejection in protocol liver biopsies obtained 12 months following withdrawal. The successful discontinuation of IS was associated with longer duration after transplantation, more advanced age of the recipients at the time of transplant, and male gender. The effect of time after transplantation was surprisingly strong, in that a striking 79% of recipients enrolled in the study more than 10.6 years after transplant could be successfully wean from IS, while this occurred in <15% of those transplanted for less than 6 years [67]. No significant beneficial effect of IS withdrawal on renal function, hypertension, diabetes, and hyperlipidemia was however noted.

Preliminary data from an on-going US randomized adult multi-centre trial in which IS withdrawal was initiated within the second year after transplantation, further supports the notion that time after transplantation is a critical parameter associated with successful drug withdrawal. At the time these results were reported in 2011, 67 adult recipients had been enrolled in the study, 53 of whom were randomized to IS withdrawal and 14 to maintain conventional IS. In contrast to the two studies described above, IS withdrawal was successful in only 2 out of the 18 patients in whom IS withdrawal was attempted [68].

Immunosuppression withdrawal in hepatitis C positive patients

The course of hepatitis C recurrence is influenced, among other factors, by the strength of IS. Several factors support this

statement. Fibrosing cholestatic hepatitis, the most severe presentation of hepatitis C recurrence, has only been described in immunosuppressed patients. Besides, it is well known that the rate of fibrosis progression is higher in immunosuppressed patients and in HIV-co-infected patients [69]. Steroid boluses to treat rejection are clearly associated with higher viral load, earlier and more severe HC recurrence, and higher liver-related mortality [70]. Beyond this, however, the role of specific IS agents is difficult to assess, with most of the published studies being retrospective and lacking long follow-ups and/or protocol biopsies [69,71,72]. Overall, the global immunosuppressive status appears to be more important than the effects of individual drugs. For instance, Berenguer *et al.* [73] showed that when over-IS was avoided (steroid boluses and triple or quadruple therapies), the rate of severe HC recurrence decreased from 54% to 33%.

On the basis of these evidence, IS withdrawal could benefit hepatitis C recipients by delaying the progression of viral-induced liver damage. IS withdrawal is indeed feasible in hepatitis C positive patients [51,52,54,55]. The impact of drug withdrawal on hepatitis C disease progression, however, has only been formally investigated by the Tor Vergata group in Rome [56]. The authors evaluated 34 hepatitis C positive liver transplant recipients with more than 1 year after transplantation and normal liver function. Twenty-four percent of the patients achieved an IS-free state. After 3 years of follow-up, operational tolerant recipients showed a significant reduction in fibrosis progression (fibrosis progression rate of -0.16 ± 0.19 vs. $+0.16 \pm 0.31$ in patients who developed rejection and needed to be maintained on IS; $p = 0.0098$). After 10 years of follow-up, however, fibrosis progression was comparable between the 2 groups and tolerant patients showed only a trend towards slower progression rate (0.26 ± 0.16 vs. 0.48 ± 0.16 ; $p = 0.06$) and slightly lower Ishak fibrosis score (1.5 ± 0.9 vs. 2.8 ± 1.5 ; $p = 0.07$) [74]. There were no significant differences between the 2 groups regarding renal function, diabetes, cardiovascular complications, and lipid profile.

Key Points

- Transplant recipients exhibit higher morbidity and mortality as compared to the general population. Co-morbidities related to the chronic use of immunosuppression are one of the main causes
- Immunosuppression minimization is desirable in liver transplant recipients
- No minimization strategy (T-cell depleting agents, steroid avoidance, or CNIs reduction) has been proved to be superior and prospective randomized trials are needed
- Immunosuppression drug withdrawal is feasible in liver transplant recipients. However, a potential clinical benefit of completely withdrawing immunosuppression has not been clearly demonstrated and the long-term histological stability of the operational tolerance phenotype is still unclear
- Currently, there is no clear justification to discontinue IS outside of carefully monitored clinical trials

Conclusions

While there is a general perception that a large proportion of liver recipients are over-immunosuppressed, how to minimize immunosuppression in order to improve clinical outcomes remains unclear. Recent reports have shown that lower tacrolimus trough concentrations early after transplantation are associated with improved renal function [22], and in some cases with longer graft survival [10]. Many of these studies, however, achieved reduced CNI levels by employing “cocktail” multi-drug IS regimens. These regimens cannot be considered IS minimization *per se*, albeit this cannot be confirmed in the absence of reliable tools to individually quantify the overall degree of immunosuppression. On the other hand, what have been traditionally considered *bona fide* IS minimization strategies (i.e., the reduction or avoidance of CNI and/or steroids in the absence of additional IS drugs), have yielded inconsistent results [75–77]. Some studies showed moderate improvement in renal function and/or metabolic complications, but others reported no significant clinical benefits. This is not entirely surprising considering that several of these studies were poorly designed and inadequately powered. Impact on hard clinical outcomes (i.e., survival) has not been intentionally investigated. There is a clear need therefore for large clinical trials of IS minimization conducted at relatively early time points after transplantation and focused on clinically-relevant long-term outcomes. Long-term effects on liver histology would need to be investigated in parallel to unambiguously determine if IS minimization is associated with idiopathic inflammatory and fibrotic lesions.

The recent multi-centre IS withdrawal trials have unambiguously established that drug weaning can be conducted according to standardized protocols with good short-term results. Furthermore, they have demonstrated that time is the most important parameter influencing the development of operational tolerance. This finding will certainly shape future trials of drug withdrawal. A potential clinical benefit of completely withdrawing immunosuppression, however, has not been clearly demonstrated. This is not unexpected, considering the small sample sizes of most studies and their still limited patient follow-up. On the other hand, benefits might not be apparent unless drug withdrawal is achieved much earlier after transplantation, before long-term exposure to immunosuppression-related toxicities has occurred. In order to achieve this, however, effective tolerance-promoting strategies such as those recently employed in kidney transplantation [78] would need to be employed, which is currently difficult to envisage in their current form given their inherent toxicity and the poor clinical state of most liver recipients at the time of transplantation. An additional issue to clarify is the long-term histological stability of the operational tolerance phenotype. Given the lack of obvious clinical benefits, uncertainties regarding long-term histological outcomes, and the high rate of rejection associated with IS withdrawal in unmanipulated liver recipients even when conducted very late after transplantation, no clear justification currently exists to discontinue IS outside of carefully monitored clinical trials. Indeed, the mechanistic insight provided by these studies has been so far their most valuable contribution. If validated in future trials, this information might open the door to novel therapeutic approaches. Furthermore, confirmation that operational tolerance can be predicted employing a combination of clinical parameters and molecular biomarkers

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would modify the equipoise in favour of discontinuing IS in previously identified operationally tolerant recipients. The availability of accurate biomarkers of tolerance would also facilitate the implementation of novel immunotherapeutic strategies such as the use of immunoregulatory cell therapy.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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