Immunosuppressive drug withdrawal late after liver transplantation improves the lipid profile and reduces infections

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Abstract **Author Information** **Authors** **Article Outline** **Outline** **Article Metrics** **Metrics**

Background Treatment with immunosuppressive drugs (IS) after transplantation is accompanied by severe side effects. A limited number of studies have investigated the effect of IS withdrawal on IS-related comorbidities after liver transplantation (LTx) and the results are contradictory.

Patients and methods We determined in a retrospective case–control study the clinical effects of complete IS withdrawal in operationally tolerant (TOL) LTx recipients who discontinued IS 10.8 ± 5.1 years after LTx (n = 13) compared with a completely matched control (CTRL) group with a regular IS regimen (n = 22). TOL recipients have been IS and rejection free for 4.0 ± 2.8 years.

Results IS withdrawal in TOL recipients resulted in lower low-density lipoprotein levels (P = 0.027), whereas this was not observed in the CTRL group. Furthermore, persistent infections in individual recipients were resolved successfully by IS withdrawal. TOL recipients also had significantly fewer de novo infections after IS withdrawal (TOL pre vs. post withdrawal P = 0.0247) compared with recipients continued on IS during the same follow-up period (post withdrawal TOL vs. CTRL P = 0.044). Unfortunately, no improvement in kidney function, and lower rates of de novo occurrences of diabetes, hypertension, cardiovascular diseases, and malignancies were observed in the TOL group after IS withdrawal compared with the CTRL group during the same follow-up time period.

Conclusion IS withdrawal late after LTx reduces infection rates and low-density lipoprotein levels, but other IS-related side effects persist late after LTx. An accurate tolerance immune profile enabling identification of tolerant LTx recipients eligible for safe IS withdrawal earlier after transplantation is needed to prevent the development of

irreversible IS-related side effects.

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Introduction

Liver transplantation (LTx) is the only treatment for end-stage liver disease. To prevent allograft rejection after transplantation, the use of immunosuppressive drugs (IS) is indispensable. However, in a significant proportion of LTx recipients, long-term use of IS leads to severe side effects such as (persistent) infections, metabolic disorders (e.g. diabetes, dyslipidemia), renal dysfunction, cardiovascular disease, and malignancies [1–6]. Short-term post-transplant survival rates after LTx improved significantly over the last two decades because of improved surgical techniques and optimized IS regimens [7]. However, morbidity and mortality more than one year after LTx have showed little improvement and are still markedly higher compared with the general population [8]. As most causes of morbidity and mortality are related to IS therapy, most centers attempt to gradually reduce IS over time after LTx.

Occasionally, LTx recipients spontaneously develop operational tolerance to their graft, a state in which IS can be withdrawn completely without the occurrence of an acute rejection episode. This was first observed when individuals were withdrawn from IS for medical reasons or because of noncompliance [9]. Subsequently, a few clinical trials confirmed the possibility of achieving immunological tolerance to allogenic liver grafts in about 40% of selected adult [9–11] and 60% of selected pediatric LTx recipients withdrawn electively from IS [9].

Whether complete withdrawal of IS in LTx recipients could reduce IS-related side effects after LTx is still controversial. The number of studies that have assessed the long-term impact of IS withdrawal late after LTx on IS-related comorbidities is limited, and contradictory findings have been reported for both adult [11–15] as well as pediatric [9,16,17] recipients. Moreover, a study investigating the influence of IS withdrawal on IS-related side effects in adult LTx recipients compared with a completely matched control (CTRL) group on regular IS regimen has not been carried out as of yet. For this reason, the purpose of this retrospective case–control study was to determine the effect of complete IS withdrawal in tolerant (TOL) recipients late after LTx on liver function, kidney function, lipid metabolism, and occurrence of diabetes, hypertension, cardiovascular disease, malignancies, and infections compared with a completely matched CTRL group maintained on IS.

Patients and methods

Study design

The study cohort included in this retrospective single-center study consisted of all operationally TOL adult LTx recipients who visited the outpatient LTx clinic of Erasmus MC between 2014 and 2017 (n = 13). Operational tolerance was defined as complete withdrawal of IS for medical reasons or noncompliance for at least 1 year without the occurrence of an acute rejection episode. TOL recipients had been completely withdrawn of IS between 2008 and 2017. To avoid risks associated with liver biopsies after transplantation, biopsies were not taken during or after complete IS withdrawal in stable LTx recipients. However, in four TOL recipients, a biopsy was taken on indication of elevated liver enzymes by their attending physician in the time period after complete IS withdrawal (on average 3.1 ± 2.2 years). All biopsies were evaluated by a pathologist and in all cases, rejection was excluded using the BANFF criteria, and an alternative diagnosis was given. Acute rejection was defined as at least a two-fold increase in serum bilirubin and liver enzymes aspartate aminotransferase (AST) and/or alanine transaminase (ALT). A CTRL group of LTx recipients on a regular IS regimen (n = 22) was matched to the TOL group for sex, age, time after LTx, cytomegalovirus (CMV) serostatus, and primary disease (Table 1). For each TOL LTx recipient, one or two (when available) CTRL LTx recipient(s) was matched. No other inclusion or exclusion criteria were used. All recipients, both TOL and CTRL, were seen at regular intervals at the outpatient LTx clinic of Erasmus MC. All clinical and laboratory information was retrieved from electronic patient records. Follow-up of recipients ended in December 2017. All recipients provided written informed consent to participate in the study. This study was approved by the medical ethics committee of Erasmus MC (MEC 2014–232) and was carried out in accordance with the 1975 Declaration of Helsinki.

Table 1

Laboratory assessments

The following parameters were analyzed for both groups: total bilirubin, AST, ALT, alkaline phosphatase (AP), and γ-glutamyltransferase for liver function, creatinine, and estimated glomerular filtration rate (derived from MDRD formula with four variables [18]) for kidney function, glycated hemoglobin (HbA1c) for the glycemic index, and low-density lipoproteins (LDL), high-density lipoproteins (HDL), cholesterol, and triglycerides for lipid metabolism. Blood levels of the above-mentioned parameters (except for HbA1c levels) were evaluated 1 year before (−1), immediately before (0), and 2 and 4 years after (2, 4) complete IS withdrawal for the TOL group and at matching

time-points after LTx for the CTRL group. HbA1c levels were included 2 years before (−2), immediately before (0), and 2 years after (2) complete withdrawal for the TOL group and at matching time-points after LTx for the CTRL group as information was not available for other time-points.

Side effects

The occurrence of events possibly related to IS was analyzed during the complete post-LTx period and before and after complete IS withdrawal in TOL recipients or matching time-points after LTx in CTRL recipients. De novo development of malignancies and cardiovascular diseases was scored when diagnosed. De novo development of diabetes and hypertension was scored when disease-specific medication had been administered. Infections were noted when a PCR confirmed viral infection or a positive culture confirmed a bacterial, parasitic, or fungal infection, and when appropriate treatment had been administered. The total number of infections was divided by the total number of years in the time period analyzed for each individual. The recipients who received a LTx because of hepatitis B virus (HBV) have been HBV-DNA negative after LTx. Recipients remained HBV-DNA negative either without antiviral therapy or after immediate antiviral therapy with anti-HBV hyper-immunoglobulins combined with either lamivudine, entecavir, or tenofovir. For the recipients who underwent transplantation for HCV-induced cirrhosis, recipients either showed spontaneous clearance of the virus after LTx, or were cured after direct antiviral therapy in 2015.

Statistical analysis

Statistical analyses were carried out using IMB SPSS statistics version 24 (SPSS Inc., Chicago, Illinois, USA). The Wilcoxon signed-rank test was used to compare values of liver function, kidney function, and metabolic parameters and infections for either the TOL or the CTRL group longitudinally. The Mann–Whitney rank-sum test was used to compare time-points between the two groups for these parameters. For sex, CMV status, primary disease, diabetes, hypertension, cardiovascular disease, and malignancies, two-sided Fisher's exact test was used to compare both groups, whereas McNemar's test was used to compare occurrence within the TOL or the CTRL group before and after withdrawal. Data are presented as mean ± SD, median (range), or percentage.

Results

Patient characteristics

The study group of TOL LTx recipients included all recipients who visited the Erasmus MC LTx outpatient clinic between 2014 and 2017 and who had not received any IS regimen for at least one year without indication of graft rejection (n = 13) (Supplementary Fig. 1, Supplemental digital content 1, http://links.lww.com/EJGH/A410). The CTRL group (n = 22) included LTx recipients on a regular IS regimen matched with the TOL group for sex, age, follow-up time after LTx, CMV serostatus, and primary liver disease (all $P > 0.05$). Demographic characteristics of the TOL and the CTRL group of LTx recipients are shown in Table 1. Two-third of the patients in both the TOL and the CTRL group were male, which is representative of the total Erasmus MC LTx cohort. The BMI of the TOL and CTRL recipients at LTx did not differ. Age at LTx was 38.8 ± 17.5 years for TOL and 36.0 ± 17.9 years for CTRL and time from LTx to the end of follow-up was 14.9 ± 3.7 years for TOL and 14.5 ± 5.6 years for CTRL. Most individuals had undergone transplantation because of cholestatic or virus-related liver disease. TOL recipients were withdrawn completely from IS 10.8 ± 5.1 years after LTx because of noncompliance, persistent infection, or renal dysfunction and have been IS and rejection free for 4.0 ± 2.8 years.

Liver function

In the first 2 years after complete IS withdrawal, slight to moderate, but transient, elevations of hepatocellular enzymes AST and ALT were observed in two TOL LTx recipients, but these elevations were because of other medical complications and not rejection activity (Supplementary Fig. 2, Supplemental digital content 1, http://links.lww.com/EJGH/A410). As shortly before complete IS withdrawal the IS trough levels were low in the

TOL group (Supplementary Fig. 1, Supplemental digital content 1, http://links.lww.com/EJGH/A410), 1 year before complete IS withdrawal was included in the analysis of all clinical parameters. Overall, liver function parameters bilirubin, AST, and ALT improved in TOL recipients 4 years after complete IS withdrawal compared with 1 year before complete withdrawal (Fig. 1a–c), whereas this was not observed in the CTRL group. AP levels increased in the TOL group 4 years after complete IS withdrawal compared with 1 year before, but in the CTRL group an increase in AP levels was also observed when comparing time-point four versus time-point zero (Fig. 1d). Moreover, AP levels did not differ between both groups 4 years after IS withdrawal. γ-Glutamyltransferase levels were increased significantly in the CTRL group, whereas only an increasing trend in the TOL group was observed 4 years after complete withdrawal compared with before withdrawal (Fig. 1e). In conclusion, on the basis of bilirubin, AST, and ALT parameters, no deterioration in liver graft function was observed in TOL after complete IS withdrawal.

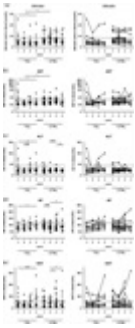

Fig. 1

Immunosuppressive drug-related side effects

Kidney function, measured as estimated glomerular filtration rate, as well as creatinine levels, did not improve in TOL after complete IS withdrawal (Fig. 2, Supplementary Fig. 3, Supplemental digital content 1, http://links.lww.com/EJGH/A410). However, deterioration in renal function was mitigated in one individual and a stable renal function was induced in another individual after complete IS withdrawal (Table 2).

Recipient	Primary reason for withdrawal	Secondary reason for withdrawal	Result after withdrawal
TOL 1	Norovirus infection	Renal dysfunction	Infection resolved
TOL 2	EBV related lymphoma	NA	Resolved
TOL 3	HHV-8 related Kaposi sarcomas	NA	Mitigated
TOL 4	Active EBV infection	NA	Resolved
TOL 5	Microsporidia infection	NA	Resolved
TOL 6	Renal dysfunction	NA	Stable
TOL 7	Renal dysfunction	NA	Mitigated

EBV, Epstein-Barr virus; HHV-8, human herpesvirus-8; NA, not applicable; TOL, tolerant.

Table 2

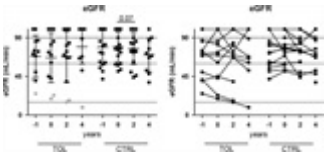

Fig. 2

In TOL recipients, a significant decrease in LDL levels was observed 4 years after complete withdrawal, whereas this was not observed in the CTRL group (Fig. 3a). Before IS withdrawal, cholesterol/HDL ratios were significantly higher in TOL recipients, whereas HDL/LDL ratios were significantly lower in TOL individuals compared with CTRL recipients (Fig. 3b and c). However, these differences between groups disappeared after complete IS withdrawal. For both groups, no significant changes in HDL, cholesterol, or triglyceride levels were observed (Supplementary Fig. 4A–C, Supplemental digital content 1, http://links.lww.com/EJGH/A410). HbA1c levels did not improve over time after complete IS withdrawal (Fig. 3d). Unfortunately, no reduction was observed in de novo cardiovascular disease or malignancies, nor was a significant reduction in de novo occurrence of diabetes and

hypertension found in the TOL group after IS withdrawal compared with CTRL recipients during the same post-LTx period (Table 3). Interestingly, all TOL recipients who developed diabetes developed this within 2 years after LTx, whereas none developed diabetes after complete IS withdrawal.

Complications	TOL	CTRL	TOL vs. CTRL (P)
Diabetes			
Total (%)	23.1	13.6	0.65
Before withdrawal (%)	23.1	9.1	0.34
After withdrawal (%)	0.0	4.5	1.00
pre/post (P)	0.50	1.00	
Hypertension			
Total (%)	46.2	27.3	0.29
Before withdrawal (%)	38.5	13.6	0.12
After withdrawal (%)	7.7	13.6	1.00
pre/post (P)	0.06	1.00	
Cardiovascular*			
Total (%)	23.1	13.6	0.65
Before withdrawal (%)	0	4.5	1.00
After withdrawal (%)	23.1	9.1	0.34
pre/post (P)	0.50	1.00	
Malignancy†			
Total (%)	30.8	18.2	0.43
Before withdrawal (%)	15.4	13.6	1.00
After withdrawal (%)	15.4	4.5	0.54
pre/post (P)	1.00	0.63	

De novo development of diabetes and hypertension was scored when dis- ease-specific medication had been administered. De novo development of malignancies and cardiovascular diseases was scored when diagnosed. Percentages of recipients with events during the total follow-up period after liver transplantation and before or after immunosuppressive drug withdrawal time-points are indicated. Statistical analyses were carried out using the two-sided Fisher's exact test or McNemar's test.
CTRL, control; TOL, tolerant.
*Includes myocardial infarction, aneurysm, and atrioventricular block.
†Includes lung carcinoma, colon carcinoma with liver metastasis, renal cell carcinoma, yolk sac tumor, testicular seminoma, Kaposi sarcoma, and lymphoma.

Table 3

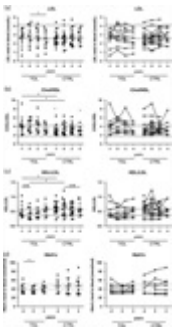

Fig. 3

In the TOL group, the total number of infections per year was significantly reduced after complete IS withdrawal compared with before (Fig. 4a), whereas such a decrease was not observed in the CTRL group. Furthermore, total numbers of infections after complete IS withdrawal in the TOL group were significantly lower than those in the CTRL group in the same follow-up time period. Moreover, in every TOL LTx recipient withdrawn from IS because of a persistent infection, the infection was resolved completely after IS withdrawal (Table 2). The types of infections present in TOL and CTRL before and after IS withdrawal are presented in Fig. 4b. When the total numbers of infections per year were split into bacterial and viral infections, a decreasing trend in the infection rate was observed for both types of infections in the TOL group, whereas only the rate of viral infections decreased in the CTRL group (Fig. 4c).

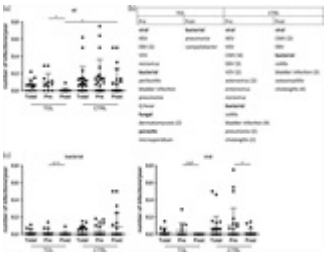

Fig. 4

Discussion

In this study, we retrospectively compared a group of TOL LTx recipients completely withdrawn from IS for on average 4years with a completely matched CTRL group on a regular IS regimen. A significant decrease in the total number of infections and LDL levels was observed in the TOL group after complete IS withdrawal. Furthermore, total numbers of infections after complete IS withdrawal in the TOL group were significantly lower than those in the

CTRL group in the same time period. Moreover, complete IS withdrawal led to successful resolution of all persistent infections in individual recipients. Thus, even late withdrawal (on average 11 years after LTx) of IS may offer benefits for LTx recipients.

Here, we observed a significantly lower de novo infection rate and persistent infections were all resolved after complete IS withdrawal in the TOL group, which resulted in significantly fewer infections compared with the CTRL group in the same follow-up period. This finding is supported by two follow-up studies of the Tor Vergata clinical trial [13,14], in which eight adult stable HCV-positive LTx recipients were withdrawn successfully from IS, whereas 26 needed to restart IS and were considered non-tolerant. After 6.5 and 10 years of IS-free follow-up, significantly fewer recurrent infections were found in the tolerant group compared with the non-tolerant group. However, only recurrent infections were analyzed in their study and not de novo infections. In contrast, Benitez *et al.* [11] reported that there was no significant difference in the numbers of infections requiring in-hospital admission and treatment between the tolerant and non-tolerant recipients 3 years after the initiation of their IS withdrawal study. One explanation for the contradictory results could be the difference between the definitions of infections used in these and our studies: infections requiring in-hospital admission versus recurrent infections versus de novo infections. Another reason could be the difference in matching between the two groups in the study of Benitez and colleagues, in which the control group included non-tolerant LTx recipients that needed to restart IS. This control group differed in age at withdrawal, time after LTx, and sex compared with the tolerant group, whereas these variables were matched completely in our study.

The LDL levels of the TOL group decreased significantly after complete IS withdrawal, but no significant changes were found in total cholesterol, triglyceride levels, and HDL levels. In accordance with our data, Benitez and colleagues observed no differences between the tolerant and the non-tolerant group in hypercholesterolemia and hypertriglyceridemia. Furthermore, our data were confirmed by the Tor Vergata withdrawal study, in which cholesterol and triglyceride levels also did not improve in the tolerant group after complete IS withdrawal. However, none of the hitherto published IS withdrawal studies investigated LDL levels. Thus, we are the first to report that LDL levels do improve after IS withdrawal, leading to a more favorable lipid profile after IS withdrawal.

Nevertheless, de novo occurrence of cardiovascular disease was not reduced after IS withdrawal in the TOL group compared with the CTRL group. In addition, we found no reduction in de novo occurrence of diabetes, hypertension, and malignancies after IS withdrawal in the TOL group compared with the CTRL group during the same follow-up time period. Similarly, Benitez and colleagues did not observe significant differences between TOL and non-TOL LTx recipients in the occurrence of all four mentioned parameters after IS withdrawal. In contrast, the 6.5 and 10-year follow-up of the Tor Vergata IS withdrawal study showed a significantly lower incidence of new-onset cardiovascular diseases and diabetes in tolerant compared with non-tolerant recipients. These discrepancies may be because of earlier IS withdrawal after LTx in the Tor Vergata study (5.3 years) and therefore shorter IS toxicity versus the later IS withdrawal after LTx in our study and Benitez and colleagues (about 11 years). Another reason could be the type of recipients included in the studies. In the Tor Vergata study, only LTx recipients with HCV as the primary disease and HCV RNA serum positivity after LTx were included. Recipients with deteriorated liver function, cirrhosis, or other hepatic or non-hepatic diseases after LTx were excluded. In our study and in the study carried out by Benitez and colleagues, recipients who had comorbidities because of IS were included and Benitez and colleagues also included recipients who had a higher risk of developing a neoplasm. Tryphonopoulos *et al.* [12], did not observe significant differences in de novo neoplasms between the tolerant and the rejecter group after IS withdrawal, which is similar to our results and those of Benitez and colleagues. These data may suggest that pre malignant cell changes had already occurred in the immunosuppressed state before IS withdrawal, which evolved into malignancies after withdrawal.

Finally, the size of the TOL cohort in our study may be too small and the follow-up duration of our study is probably too short to observe a significant decrease in malignancies and cardiovascular disease after IS withdrawal. Also, the higher incidence of cardiovascular disease after withdrawal compared with before in the TOL group in our study is probably related to the higher incidence of diabetes and hypertension before withdrawal among these recipients. Overall, this possibly indicates that recipients with a pre-existing disease are more prone to developing comorbidities and consequently the positive effects of IS withdrawal can be less evident than it actually is in our

study. A combination of a long time period between LTx and IS withdrawal and a short follow-up time in the study by Benitez and colleagues, Tryphonopoulos and colleagues, and our own study could obscure the positive effects of IS withdrawal on de novo occurrence of cardiovascular diseases and malignancies.

Similar to Benitez and colleagues, we did not observe an improvement in GFR after IS withdrawal in TOL LTx recipients. In contrast, Pons *et al.* [15] found that the GFR increased significantly after IS withdrawal, whereas in non-tolerant recipients, the GFR decreased significantly. One explanation for these discrepancies could be the time between LTx and IS withdrawal and induced toxicity. IS withdrawal was performed on average of 3.4 years after LTx in the study by Pons and colleagues, whereas in our study and that of Benitez and colleagues, recipients were withdrawn on average 11 years after LTx. Another explanation could be that in the study carried out by Pons and colleagues, none were withdrawn from IS because of renal dysfunction, whereas in our study and that of Benitez and colleagues, some recipients were withdrawn because of these comorbidities. Both the tolerant groups are thus more biased with respect to pre-existing renal dysfunction as a cause of IS withdrawal, and it is therefore possible that we did not observe an improvement in GFR after IS withdrawal. In the study carried out by Geng *et al.* [19], it appeared that lower trough levels of tacrolimus late after LTx did not have a significant beneficial effect over higher trough levels late after LTx with respect to renal dysfunction in LTx recipients. In several studies, renal function improved in LTx recipients when the CNI IS regimen was converted into a CNI-low or a CNI-free IS regimen early after LTx, which seems to indicate that renal function could improve with complete IS withdrawal in tolerant LTx recipients at a earlier time point after LTx [20,21]. Therefore, IS minimization and complete withdrawal should occur as soon as possible after LTx to limit the IS-related nephrotoxicity in tolerant LTx recipients as it may then be still largely reversible.

The strength of our study is that we compared a TOL group with a completely matched CTRL group, and can therefore eliminate potential confounders. Multiple significant differences between important factors, such as age, sex, primary disease, and time after LTx, were present in all other studies that investigated the clinical effects of IS withdrawal by comparing tolerant adult LTx recipients with a non-tolerant or a rejecter group. However, our study also has some limitations. We carried out a retrospective case–control study with a small cohort of TOL LTx recipients. Because of the retrospective nature of our study, we could not address the safety of complete IS withdrawal in TOL LTx recipients. There is probably a population bias in our TOL group, as about half of the TOL LTx recipients were withdrawn from IS for medical reasons, and recipients with a pre-existing disease could be more prone to comorbidities. Furthermore, the time period between LTx and IS withdrawal in the TOL group is extensive and may result in persistent IS-related morbidities. However, this is a limitation of most other follow-up studies, except for two [11,15]. Despite these limitations, we do find positive effects of IS withdrawal in our TOL group. Compared with other studies, it does suggest that the benefits of IS withdrawal could be more extensive when IS withdrawal is performed earlier.

Unfortunately, time after LTx is a strong predictor of tolerance after LTx, that is, the longer the time after LTx, the higher the chance of being tolerant towards the liver graft [11]. In the study by Benitez and colleagues, no more than 13% of selected stable LTx recipients could be withdrawn completely from IS less than six years after LTx. Similarly, a recently published study [22] reported that complete IS withdrawal in the 2nd year after transplantation was possible in only 13% of selected adult LTx recipients. Therefore, to withdraw LTx recipients safely from IS earlier after LTx, tolerant recipients need to be identified carefully from a larger group of LTx recipients with regular IS regimen. Consequently, an accurate tolerance identification profile enabling identification of LTx recipients eligible for safe IS withdrawal early after transplantation is needed. Prospective IS withdrawal studies excluded LTx recipients who were expected to have an enhanced risk of graft rejection, for example, recipients with a pre-existing (hepatic) autoimmune disease, recipients with a rejection episode during a preceding period of up to 12 months, and recipients with elevated serum bilirubin, AST, and/or ALT levels. Nevertheless, the majority of recipients included in these studies experienced rejection activity during IS withdrawal early after LTx [11,22]. Therefore, clinical parameters are not sufficient to select tolerant LTx recipients early after transplantation. Different studies have already examined possible markers to identify these tolerant LTx recipients [23–29]. However, an accurate immune profile that could be validated in independent studies has not been determined as of yet. When these tolerant LTx recipients can be recognized earlier and withdrawn from IS, more IS-related side effects could be reversed or avoided.

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Conflicts of interest

There are no conflicts of interest.

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