Outcome and safety of a surveillance biopsy guided personalized immunosuppression program after liver transplantation

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Graft survival beyond year 1 has not changed after orthotopic liver transplantation (OLT) over the last decades. Likewise, OLT causes comorbidities such as infection, renal impairment and cancer. We evaluated our single-center real-world individualized immunosuppression program after OLT, based on 211 baseline surveillance biopsies (svLbx) without any procedural complications. Patients were classified as low, intermediate and high rejection risk based on graft injury in svLbx and anti-HLA donor-specific antibodies. While 32% of patients had minimal histological inflammation, 57% showed histological inflammation and 23% advanced fibrosis (F2), which was not predicted by lab parameters. IS was modified in 79% of patients after svLbx. After immunosuppression reduction in 69 patients, only 5 patients showed ALT elevations and three of these patients had a biopsy-proven acute rejection, two of them related to lethal comorbidities. The rate of liver enzyme elevation including rejection was not significantly increased compared to a svLbx control cohort prior to the initiation of our structured program. Immunosuppression reduction led to significantly better kidney function compared to this control cohort. In conclusion, a biopsy guided personalized immunosuppression protocol after OLT can identify patients requiring lower immunosuppression or patients with graft injury in which IS should not be further reduced.

KEYWORDS
clinical research/practice, immunosuppression/immune modulation, immunosuppressive regimens - maintenance, immunosuppressive regimens - minimization / withdrawal, kidney failure/injury, liver allograft function/dysfunction, liver transplantation/hepatology, rejection: subclinical

Abbreviations: AIH, autoimmune hepatitis; AILD, autoimmune liver disease; ALADIN, adult liver allograft dysfunction initiative; ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; ARFI, Acoustic Radiation Force Impulse; BanffMini, histological criteria justifying immunosuppression minimization; BMI, body mass index; bpclinTCMR, biopsy-proven clinical T cell-mediated rejection; Bx, biopsy; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CMV, cytomegalovirus; CN1, calcineurin inhibitor; CyA, cyclosporine A; DSA, donor-specific anti-HLA antibodies; eGFR, estimated glomerular filtration rate; FU, follow-up; GP, general practitioner; HCC, hepatocellular carcinoma; indLbx, indication liver biopsy; IS, immunosuppression; LAF score, liver allograft fibrosis score; Lbx, liver biopsy; LSM, liver stiffness measurements; MMF, mycophenolate mofetil; mTOR, mammalian Target of Rapamycin; OLT, orthotopic liver transplantation; pre-ALADIN, pre-ALADIN control cohort; PSC, primary sclerosing cholangitis; PTLD, posttransplant lymphoproliferative disease; RAI, rejection activity index; SOP, standard operating procedure; subTCMR, subclinical T cell-mediated rejection; svLbx, surveillance liver biopsy; TAC, tacrolimus; TCMR, acute T cell-mediated rejection.

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1 | INTRODUCTION

Side effects of immunosuppressive drugs, such as malignancy, infections and chronic kidney insufficiency still have a dramatic impact on patients after solid organ transplantation. Mortality associated with long-term administration of immunosuppression (IS) is twice as high as that of graft failure after OLT.1,2 These therapy-associated side effects have contributed to a stagnating long-term survival after OLT in the last four decades.3,4

Renal dysfunction in liver transplant recipients is considered to be mainly due to calcineurin inhibitor (CNI) induced toxicity and leads to higher costs and shorter long-term survival.2,5,6 In addition, significant graft fibrosis is one of the main reasons for retransplantation in long-term follow-up.7 Protocol biopsy programs have shown graft fibrosis (Ishak F ≥ 2) in 54% of non-HCV patients after 5 years, which were largely not detected by routine clinical follow-up.8 Graft biopsies are still the gold standard for diagnosing subclinical graft injury. However, few transplantation centers perform surveillance liver graft biopsies (svLbx) regularly, although Lbx are safe and previous studies have suggested an added value of svLbx beyond recurrent hepatitis C in order to reveal subclinical graft injury that is not reflected by liver function tests.9 Additionally, subclinical graft injuries including subclinical T cell-mediated rejection (subTCMR) are highly prevalent and are linked to a higher expression of TCMR associated transcripts, while high expression of such TCMR signatures is associated with fibrosis progression.10–13 Currently available non-invasive surveillance parameters are too insensitive to detect subclinical graft injury reliably.14

The clinical utility of svLbx for adjustment of IS was most obviously demonstrated by trials on intentional IS withdrawal.11,15–17 The knowledge gained from such trials led to a Banff consensus document summarizing histological criteria in which an IS minimization attempt appears reasonably safe (BanffMini).18 So far, only a few studies have evaluated the possibility of IS weaning in order to prevent long-term side effects of IS as well as graft failure due to acute or chronic rejection. Individualized IS programs, taking into account not only comorbidities such as kidney dysfunction, infections or malignancy, but also subclinical graft injury, have not yet been established as routine clinical care after OLT.

The aim of this study was to evaluate the safety and effectiveness of our clinical surveillance biopsy guided personalized immunosuppression initiative after liver transplantation, termed “Adult Liver Allograft Dysfunction INItiative” (ALADIN).

2 | MATERIAL AND METHODS

2.1 | Study outline

This is a study on the safety and clinical effectiveness of our clinical biopsy guided personalized IS approach after OLT. With the publication of histological criteria justifying IS minimization (BanffMini: portal tract inflammation ≤ 1, interface hepatitis ≤ 1, central perivenulitis ≤ 1, lobular inflammation = 0, biliary inflammation = 0, endothelialitis = 0, portal microvasculitis = 0 and periportal fibrosis ≤ 3) in the Banff consensus 2016, a systematic ALADIN approach to personalized IS based on svLbx as routine clinical management after OLT with the primary aim of a sufficient control of biochemical and histological graft rejection to prevent graft fibrosis with the lowest possible IS levels was established.18 Since 2018, all patients who had undergone OLT were offered voluntary participation in the ALADIN svLbx program. Interdisciplinary conferences involving surgeons, hepatologists and standardized biopsy results graded for inflammation and fibrosis, were introduced to contemplate critically the IS regimen, guided by underlying diseases, comorbidities, adverse drug effects, liver function, presence of HLA-type donor-specific antibodies (DSA), and graft histopathology. For safety reasons, we applied a gradual decrease of IS, whenever justified, and no full weaning (Figure 1). In cases of persistence of at least moderate graft inflammation and/or at least moderate graft fibrosis beyond BanffMini, IS was usually increased incrementally and everolimus (EVR) was introduced because of antifibrotic effects shown in experimental studies.19–21

According to our local SOP, patients are on triple immunosuppressive therapy at discharge from the hospital after having undergone OLT, and steroids are stopped after 16 weeks (Figure 1). At several time points after OLT, starting around year one, each patient is offered a svLbx. According to the histopathological results and presence of DSA, patients were stratified into three groups: (A) low rejection risk: (liver enzyme levels of ≤ 2-fold upper limit of normal (ULN) and absence of relevant inflammation, according to BanffMini; (B) intermediate rejection risk: histological inflammation beyond BanffMini without evidence of DSA and without relevant fibrosis (Ishak F < 2); (C) high rejection risk: at least moderate inflammation activity with verified DSA and/or significant graft fibrosis (Ishak F ≥ 2). In addition to regarding these histopathological outcomes, comorbidities and adverse effects of immunosuppressive drugs are taken into account (Figure 1). mTOR inhibitors, which are started no earlier than 1 month after OLT, are preferred, for example, in patients with significant graft fibrosis, hepatocellular carcinoma before OLT, patients with moderate-to-severe kidney insufficiency or after CMV reactivation.22–24

This evaluation is covered by the ethical approval of our prospective biorepository and data base after OLT by the Ethics Committee of Hannover Medical School, Hannover/Germany (protocol numbers 933 for project Z2 of comprehensive research center 738 and 9754_BO_K_2021). Written informed consent was obtained from all subjects in advance.

2.2 | Subjects

Our study included all adult patients (≥18 years of age at time of enrollment) after OLT who underwent svLbx with the clinical ALADIN program and agreed to participate in this study (Table 1). A svLbx control cohort of 35 patients was selected from our prospective biorepository and data base. This comparator group included only patients with svLbx performed between 2010–2017, before the
implementation of ALADIN (pre-ALADIN). The pre-ALADIN cohort was selected to match as far as possible the study group (ALADIN follow-up cohort) used to assess the safety of svLbx-controlled immunosuppression alteration in terms of age, sex, body mass index (BMI) and number of immunosuppressants and, most of all, same kidney and liver graft function at baseline svLbx (Table 1). Due to high heterogeneity between ALADIN follow-up and pre-ALADIN cohorts the intended 1:1 ratio was not reached, but rather a 2:1 ratio.

### 2.3 | Immunosuppression scoring

For the allocation of an IS dosage score, we used a published semi-quantitative IS scoring system established by Vasudev et al., originally assigning one point to each of the following drug dosages: tacrolimus (TAC) 2 mg, MMF 500 mg, prednisolone 5 mg, cyclosporine 100 mg and azathioprine 100 mg. In addition, we assigned one score point to 1.5 mg of everolimus (EVR) as previously described by our group. Since ratios of doses and trough levels have a high inter-individual variability in CNI, we modified scoring points for trough level aims, assigning two points to a TAC aim of >5 ng/ml and a cyclosporine A (CyA) aim of >60 ng/ml, one point to a TAC aim of 3–5 ng/ml and a CyA aim of 30–60 ng/ml and finally 0.5 points to a TAC aim of <3 ng/ml and a CyA aim of <30 ng/ml.

### 2.4 | Statistical analysis

Statistical analysis was performed with SPSS version 25 software as described recently by our group. When testing for normal distribution, we used the Shapiro–Wilk test. Since the vast majority of variables were not normally distributed, non-parametric testing was applied in all comparisons. When analyzing for discrepancies and similarities between two independent groups, we used the Mann–Whitney U test. When analyzing changes over time in each of these groups, the paired Wilcoxon test was applied. Chi-square test and Fisher’s exact test, if Chi² was not possible, were used to compare contingency tables. A p-value of <.05 was considered significant.

Further methods are outlined in the supplemental material.

### 3 | RESULTS

#### 3.1 | Safety of liver biopsies and impact on post-transplant patient management

In total, 293 biopsies, including 82 indication Lbx and 211 surveillance Lbx (svLbx), performed to assess subclinical graft damage in order to individualize IS therapy, were performed on 287 patients between November 2018 and September 2020 without significant peri-interventional complications: no bleeding or drops in hemoglobin levels and no periprocedural infections.

Only the 211 svLbx (Table 1) were included in the biopsy guided personalized immunosuppression program. The majority of svLbx (68%) exhibited abnormal histology and only a minority showed absence of, or only mild inflammatory activity within the histological criteria justifying IS minimization (BanffMini; approximately 32%) according to the 2016 Banff consensus (Table 2).

All 211 svLbx were discussed in the monthly interdisciplinary conferences guided by a standardized protocol (Figure 1).
TABLE 1  Baseline characteristics of patients included in the study

|                                | Total ALADIN surveillance biopsy group, n = 211 | ALADIN follow-up (FU) group, n = 112 | Pre-ALADIN cohort (pre-ALADIN), n = 35 | p-values ALADIN FU vs. pre-ALADIN |
|--------------------------------|-------------------------------------------------|---------------------------------------|----------------------------------------|----------------------------------|
| Age at biopsy (years)          | 53 (18–72)                                      | 54 (18–71)                            | 51 (22–66)                             | .176                             |
| Male gender                    | 129 (61.1)                                      | 72 (64.3)                             | 16 (45.7)                              |                                  |
| BMI (kg/m²)                    | 25.3 (17.4–50.4)                                | 25.07 (18.1–37.6)                     | 24.84 (17.0–39.4)                      | .539                             |
| **Underlying disease**         |                                                |                                       |                                        |                                  |
| Chronic viral hepatitis        | 43 (20.4)                                       | 27 (24.1)                             | 7 (20.0)                               |                                  |
| Autoimmune liver disease       | 59 (28.0)                                       | 29 (25.9)                             | 12 (34.3)                              |                                  |
| Cryptogenic                    | 30 (14.2)                                       | 18 (16.1)                             | 4 (11.4)                               |                                  |
| Alcoholic liver disease        | 22 (10.4)                                       | 14 (12.5)                             | 3 (8.6)                                |                                  |
| Other                          | 57 (27.0)                                       | 31 (27.7)                             | 13 (37.1)                              |                                  |
| **Reason for OLT**             |                                                |                                       |                                        |                                  |
| Cirrhosis                      | 58 (27.5)                                       | 36 (32.1)                             | 6 (17.1)                               |                                  |
| Autoimmune liver disease       | 50 (23.7)                                       | 24 (21.4)                             | 7 (20.0)                               |                                  |
| Hepatocellular carcinoma       | 27 (12.8)                                       | 17 (15.2)                             | 6 (17.1)                               |                                  |
| Acute liver failure            | 29 (13.7)                                       | 11 (9.8)                              | 4 (11.4)                               |                                  |
| Other                          | 47 (22.3)                                       | 27 (24.1)                             | 13 (37.1)                              |                                  |
| Age at OLT                     | 45 (0–67)                                       | 47.5 (2–67)                           | 45 (17–60)                             | .802                             |
| Time from OLT to surveillance biopsy (months) | 88 (9–452)                                      | 78.5 (10–406)                        | 48 (12–164)                            | .005                             |
| **Immunosuppression**          |                                                |                                       |                                        |                                  |
| Tacrolimus                     | 150 (71.1)                                      | 84 (75.0)                             | 21 (60.0)                              |                                  |
| MMF                            | 158 (74.9)                                      | 83 (74.1)                             | 28 (80.0)                              |                                  |
| Prednisolone                   | 63 (29.9)                                       | 30 (26.8)                             | 18 (51.4)                              |                                  |
| Cyclosporine                   | 52 (24.6)                                       | 23 (20.5)                             | 14 (40.0)                              |                                  |
| mTOR                           | 31 (14.7)                                       | 19 (17.0)                             | 0                                      |                                  |
| Azathioprine                   | 5 (2.4)                                         | 3 (2.7)                               | 1 (2.9)                                |                                  |
| Monotherapy                    | 13 (6.2)                                        | 7 (6.3)                               | 1 (2.9)                                |                                  |
| Dual therapy                   | 145 (68.7)                                      | 77 (68.8)                             | 21 (60.0)                              |                                  |
| Triple therapy                 | 52 (24.6)                                       | 27 (24.1)                             | 13 (37.1)                              |                                  |
| **IS aim score (pts)**         | 3.0 (0–7.0)                                     | 3.0 (0–7.0)                           | 4.0 (2.0–6.5)                          | <.001                            |
| Tacrolimus trough levels (ng/ml) | 5.2 (1.5–10.9)                               | 5.3 (2.3–10.9)                       | 5.8 (2.2–8.7)                          | .414                             |
| Cyclosporine trough levels (ng/ml) | 55 (18–177)                                    | 52 (18–125)                          | 72.5 (26–99)                           | .009                             |
| MMF dose (mg/d)                | 1000 (500–2000)                                 | 1000 (500–2000)                      | 1000 (500–2000)                        | .279                             |
| Prednisolone dose (mg/d)       | 5 (1–10)                                        | 5 (1–10)                             | 5 (2.5–7.5)                            | .154                             |
| mTOR trough levels (ng/ml)     | 4.5 (2.7–10.5)                                  | 4.6 (2.7–10.5)                       | 0                                      | N/A                              |
| **AST (U/L)**                  | 25 (8–60)                                       | 25 (8–60)                            | 24 (15–61)                             | .476                             |
| **ALT (U/L)**                  | 21 (6–72)                                       | 20 (7–72)                            | 16 (8–64)                              | .043                             |
| **AP (U/L)**                   | 91 (32–565)                                     | 90 (45–565)                          | 77 (28–270)                            | .029                             |
| **GGT (U/L)**                  | 31 (7–927)                                      | 30 (7–749)                           | 23 (7–758)                             | .304                             |
| **Bilirubin (µmol/L)**         | 10 (3–55)                                       | 10 (3–55)                            | 9 (4–20)                               | .543                             |
| eGFR (ml/min×1.73)             | 67 (5–129)                                      | 61.5 (5–127)                         | 63.2 (27–101)                          | .413                             |
| Cholesterol (mg/dl)            | 170 (89–317)                                    | 170 (112–317)                       | 178 (104–282)                          | .246                             |
| Triglycerides (mg/dl)          | 107 (39–539) (n = 210)                          | 110.5 (43–539)                      | 106 (56–368)                           | .868                             |
| HbA1c (%)                      | 5.6 (3.8–10.1) (n = 67)                         | 5.7 (3.8–9.6) (n = 38)               | 5.6 (4.7–7.4) (n = 6)                  | .644                             |
| **Liver stiffness measurements**|                                               |                                       |                                        |                                  |
| ARFI (m/s)                     | 1.26 (0.67–4.07) (n = 85)                       | 1.26 (0.81–3.95) (n = 44)            | N/A                                    | N/A                              |
| Transient Elastography (kPa)   | 6.1 (3.4–26.9) (n = 89)                         | 5.9 (3.4–25.9) (n = 43)              | N/A                                    | N/A                              |

**Note:** Data are provided as n (%) or median (range).

*a*Calculated by CKD-EPI-formula.
Immunosuppression was altered following svLbx in 166 (79%) cases. In 47 (22%) cases further diagnostic measures, most of all metabolic counseling for liver steatosis, were advised (Figure 2A).

### 3.2 Longitudinal safety and effectiveness of a svLbx guided personalized immunosuppression approach

To evaluate the effectiveness and safety of the ALADIN approach, patients with at least one outpatient visit to our center after svLbx were longitudinally assessed. Follow-up data were available at a median of 13 months (range: 8–22) from 112 (53%) patients (ALADIN follow-up cohort [ALADIN FU]). With regard to histological findings, the ALADIN FU cohort was representative of the total svLbx cohort (Table 2). Strength of overall immunosuppression was compared using a published semi-quantitative immunosuppression score.13 In the ALADIN FU group, IS strength at FU was reduced in 69 (62%); median relative decrease 33%; range: 9%–67% and increased in 11 (10%); median relative increase: 25%; range: 6%–300%. In 32 (29%) cases, the immunosuppression score did not change, although in 13 (41%) of these patients, IS was slightly modified (Table 3; Figure 2A, Figure S1). IS regimen at baseline and FU are outlined in Figure 3 and Figure S2. In short, at FU triple IS was reduced by one third (16%), mono IS was more than doubled (15%) and rate of double IS remained stable (68%) compared to pre-Lbx time-point.

To describe the success of the IS adjustment, we stratified patients into the following categories: (1) increased liver enzymes after IS adjustment; (2) suggested IS adjustment was not initiated or not continued for other reasons, for example compliance issues, drug intolerance, or comorbidities; (3) successful IS adjustment without evidence of graft dysfunction (Figure 2A, Figures S3 and S4). At follow-up, 80% of patients were still on the intended IS regimen suggested by the interdisciplinary conference.

The outcomes of the ALADIN FU patients with IS reduction are outlined in Figure 2A. After IS reduction, 5 (7%) patients showed liver enzyme elevation without evidence of drug toxicity or infections. Liver enzyme elevation improved after IS was re-increased without a further Lbx in 2/5 patients. Biopsy-proven clinTCMR (bp-clinTCMR) was seen in one patient at 6 months after IS reduction coming from a baseline svLbx that fulfilled BanffMini criteria (Figure S4). After high-dosage steroids and re-initiation of baseline IS, liver values normalized and are still <1.5× ULN 1 year later. The remaining two patients had bp-clinTCMR in the course of severe and finally lethal systemic diseases (pneumocystis pneumonia and recurrent PTLD) (Figure 2A, Figure S4). In both cases IS had been reduced for clinical reasons, although both patients were showing inflammation at baseline beyond BanffMini.

Besides this, liver enzymes and liver function remained stable in those ALADIN FU patients with IS reduction (Table 3). However, there was a small, non-significant trend to an ALT increase within the normal range after IS reduction. This trend reached significance in those patients with later FU (15–22 months compared to 8–14 months) (Figure 4A). Apart from a marginal difference in baseline ALT levels, there was no difference in the baseline parameters between patients with or without an increasing ALT after IS reduction (Table S1).

The outcome at FU after IS reduction according to the degree of subclinical graft injury within (n = 23) or beyond BanffMini criteria (n = 46) is outlined in Figure 4B,C and Figure S4. The outcome of ALADIN FU patients in whom IS strength increased showed no liver enzyme elevation and no bp-clinTCMR, while in the patient cohort that maintained their IS score, 2 (6%) showed an increase in liver values (Figure 2). There was no overall change in the ALT levels between svLbx and FU in those patients with increased or maintained IS strength (Table 3).

DSA were found only in a minority of patients in the ALADIN FU cohort (8/61 [7%]; HLA B 1/8; HLA DR 3/8; HLA DQ 6/8) in median 97 days before the svLbx, while S1 had no or non-valid (meaning positive anti-HLA antibodies but with incomplete donor typing) DSA testing. It was difficult to characterize new DSA findings as de novo (2/8) because, in most cases, these were the first DSA quantifications after OLT (6/8). C4d staining was not performed because of a low rate of positivity in our center.12 The histological findings of the baseline svLbx, the IS adjustments and the outcome

### Table 2: Histological findings in liver graft biopsies (Lbx)

|                          | Surveillance Lbx, n = 211 | ALADIN follow-up, n = 112 | p-values total svLbx vs. ALADIN FU |
|--------------------------|----------------------------|----------------------------|-----------------------------------|
| subTCMR                  | 40 (19)                   | 26 (23)                   | .387                              |
| TCMR                     | 0                          | 0                         | 1.0                               |
| BanffMini                | 67 (32)                   | 34 (30)                   | .900                              |
| Graft hepatitis          | 7 (3)                     | 5 (5)                     | .758                              |
| Recurrence of pre-tx disease | 5 (2); suspected: 2 (1) | 4 (4); suspected: 1 (1) | .188                              |
| NASH/MAFLD               | 11 (5)                    | 10 (9)                    | .237                              |
| Fatty degeneration       | 38 (18)                   | 23 (21)                   | .654                              |
| Ishak Fibrosis ≥2        | 48 (23)                   | 23 (21)                   | .675                              |
| Re-cirrhosis Ishak F ≥ 5 | 8 (4)                     | 6 (5)                     | .570                              |

Note: Data are provided as n (%).
FIGURE 2  Flow chart outlining availability and selection of patients. (A) The main selection criteria, grouping, and outcomes are outlined. Further details on changes in immunosuppression and outcomes in different patient groups can be found in supplemental Figures 1, 3, and 4.  (B) Selection criteria, grouping and outcome are shown for the pre-ALADIN FU control cohort.
Regarding the DSA findings are outlined in Figure S3. So far, we have no longitudinal assessment of DSA within the medium-term follow-up of the ALADIN FU. In short, DSA were associated with more graft injury and fewer DSA patients were eligible for IS reduction.

So far, we have no follow-up svLbx for the patients of the first two ALADIN years. Due to the corona pandemic, even follow-up elastographies were limited to 44 patients (25/44 had baseline elastographies). Within the normal range of interindividual variation of follow-up elastographies, there was no significant trend to an increase or decrease in liver stiffness (Figure S5). In the patient group with IS reduction, 16 underwent follow-up transient elastography and 12 had follow-up ARFI examinations, both showing no overall elevation (median TE: 6.4 kPa; median ARFI 1.23 m/s).

### Table 3: IS score and blood values at biopsy and follow-up

|                      | IS score not altered, n = 32 | IS increased, n = 11 | IS reduced, n = 69 | pre-ALADIN svLbx cohort n = 35 | p-value IS reduced vs. pre-ALADIN |
|----------------------|-------------------------------|----------------------|-------------------|-------------------------------|----------------------------------|
| IS score Lbx         | 3.0 (1.0–7.0)                 | 2.5 (0–5.0)          | 3.5 (1.5–6.0)     | 4.0 (2.0–6.5)                 | .001                             |
| IS score FU          | 3.0 (1.0–7.0)                 | 3.5 (1.5–6.0)**      | 2.0 (0.5–5.0)**   | 4.0 (2.0–6.5)*                | <.001                            |
| Fold change IS (%)   | 0 (0)                         | 25 (6–300)           | −33 (−9 to −67)   | 0 (−50 to 30)                 | <.001                            |
| AST Bx (U/L)         | 26 (16–50)                    | 29 (22–56)           | 25 (8–60)         | 24 (15–61)                    | .912                             |
| AST FU (U/L)         | 24 (14–69)                    | 26 (14–44)           | 26 (12–250)       | 24 (12–70)                    | .618                             |
| ΔAST (U/L)           | −1 (−21 to 35)                | −8 (−14 to 13)       | 1 (−34 to 220)    | −1 (−29 to 40)                | .434                             |
| ALT Bx (U/L)         | 23 (8–64)                     | 34 (14–61)           | 18 (7–72)         | 16 (8–64)                     | .194                             |
| ALT FU (U/L)         | 22 (8–73)                     | 31 (11–73)           | 22 (8–274)*       | 20 (8–48)*                    | .639                             |
| ΔALT (U/L)           | 0 (−31 to 33)                 | −2 (−30 to 31)       | 1 (−47 to 242)    | 2 (−26 to 25)                 | .788                             |
| AP Bx (U/L)          | 98 (47–351)                   | 91 (67–530)          | 85 (45–565)       | 77 (28–270)                   | .092                             |
| AP FU (U/L)          | 92 (40–347)                   | 120 (69–296)         | 93 (40–682)       | 84 (36–273)*                  | .394                             |
| ΔAP (U/L)            | 0 (−106 to 256)               | −17 (−234 to 42)     | 3 (−185 to 626)   | 7 (−36 to 131)                | .169                             |
| GGT Bx (U/L)         | 31 (8–410)                    | 90 (13–504)          | 28 (7–749)        | 23 (7–758)                    | .620                             |
| GGT FU (U/L)         | 32 (10–760)                   | 101 (11–339)         | 30 (8–1052)       | 30 (8–640)                    | .726                             |
| ΔGGT (U/L)           | 2 (−227 to 432)               | 6 (−177 to 231)      | 1 (−133 to 639)   | 2 (−201 to 437)               | .259                             |
| Bilirubin Bx (µmol/L)| 10 (3–55)                     | 8 (3–26)             | 11 (3–45)         | 9 (4–20)                      | .422                             |
| Bilirubin FU (µmol/L)| 8 (3–57)                      | 7 (3–19)             | 9 (3–117)         | 9 (3–26)                      | .135                             |
| ΔBilirubin (µmol/L)  | 0 (−6 to 16)                  | −1 (−22 to 2)        | 0 (−12 to 72)     | −1 (−9 to 16)                 | .140                             |
| eGFR² Bx (ml/min×1.73)| 63 (9–107)                    | 57 (35–119)          | 61 (5–127)        | 63 (27–101)                   | .288                             |
| eGFR² FU (ml/min×1.73)| 64 (8–111)                    | 51 (33–104)          | 62 (6–127)        | 63 (19–88)**                  | .131                             |
| ΔeGFR (ml/min×1.73)  | −2 (−26 to 16)                | −3 (−20 to 14)       | 0 (−41 to 55)     | −2 (−27 to 9)                 | .047                             |
| Fold Change eGFR (%) | −5 (−50 to 21)                | −6 (−18 to 32)       | 0 (−57 to 122)    | −7 (−31 to 21)                | .045                             |
| Cholesterol Bx (mg/dl)| 168 (124–240)                 | 186 (135–247)        | 166 (112–317)     | 178 (104–282)                 | .107                             |
| Cholesterol FU (mg/dl)| 184 (120–278)*                | 213 (155–259)        | 162 (108–352)     | 178 (97–313)                  | .017                             |
| ΔCholesterol (mg/dl) | 8 (−24 to 81)                 | 4 (−31 to 66)        | 1.5 (−143 to 209) | 4 (−46 to 50)                 | .317                             |
| Triglycerides Bx (mg/dl)| 108 (52–238)                 | 128 (84–500)         | 108 (43–539)      | 106 (56–368)                  | .715                             |
| Triglycerides FU (mg/dl)| 112 (47–299)                | 113 (88–261)         | 109 (27–381)*     | 116 (47–292)                  | .873                             |
| ΔTriglycerides (mg/dl)| 2.5 (−99 to 107)              | 2 (−256 to 69)       | 8.5 (−403 to 154) | 10 (−168 to 125)              | .810                             |
| HbA1c Bx (%)         | 6.2 (4.9–7.8)                 | N/A                  | 6.2 (5.0–9.6)     | N/A                           | N/A                              |
| HbA1c FU (%)         | 7.0 (5.0–9.2)                 | N/A                  | 6.5 (4.4–9.2)     | N/A                           | N/A                              |
| ΔHbA1c (%)           | −0.1 (−1.3–2.1)               | N/A                  | 0.1 (−2.9–1.1)    | N/A                           | N/A                              |

Note: Data are provided as n (%) or median (range). Baseline values at biopsy (Bx) and at follow-up (FU) were compared by a paired Mann–Whitney-U test within each group. Comparing patients with IS reduction to pre-ALADIN, significant p-values (<.05) are printed in bold. Nonsignificant p-values comparing time of follow-up to time of biopsy are not indicated (**p-value < .001; *p-value < .01; *p-value < .05; #p-value .05–.1).
3.3 Preserved kidney function after introduction of biopsy guided personalized immunosuppression reduction

To assess safety and effectiveness of a personalized IS approach, we selected a control group of patients who had received a svLbx for research purposes with similar FU outpatient visits, but without a personalized IS adjustment, because svLbx were taken before the introduction of the systematic ALADIN approach. This pre-ALADIN FU cohort (pre-ALADIN) was matched to the ALADIN FU cohort (Table 1). However, some overall trends before initiation of ALADIN, for example declining use of CyA and increasing use of EVR, could not be balanced completely without reducing the sample number too far for statistical analysis (Table 3). Four patients of the pre-ALADIN FU cohort exhibited a liver enzyme elevation (12%) including one bp-clinTCMR at 38 months post-svLbx and three patients without a further biopsy (median 19.5 months; range: 8–42 months post-Lbx). Bp-clinTCMR and liver enzyme elevations as a combined end point was not significantly more prevalent in ALADIN FU with reduced IS (5/69 during 81 patient years of follow-up) compared to pre-ALADIN (4/35 patients; $p = .481$). Beyond that, the pre-ALADIN cohort exhibited a stable liver function with a non-significant trend towards an ALT increase within the normal range until the post-svLbx FU, although IS strength was kept unchanged (Table 3, Figure 2B). Although both cohorts, ALADIN FU with reduced IS and pre-ALADIN, started from similar baseline kidney function, the reduced IS within ALADIN was associated with a stable kidney function, while the pre-ALADIN exhibited a significant longitudinal decline in absolute ($p = .047$) and percental kidney function ($p = .045$) leading to a percental difference of 7% between ALADIN FU with reduced IS and pre-ALADIN (Figure 5A). Here the preservation of kidney function was associated with CNI reduction (Figure 5B). Similar non-significant trends to a decrease in GFR were seen for ALADIN FU cohorts with maintained IS strength and increased IS, with kidney function declining by 5% and 6% until FU compared to ALADIN FU with reduced IS (Table 3). When the total ALADIN FU cohort was analyzed, irrespective of the change of IS, kidney function was better preserved or increased in those with reduced CNI levels (Figure 5C). Baseline parameters were not significantly different between patients with improving renal function compared to those with declining eGFR (Tables S2 and S3). When focusing on metabolic parameters, IS reduction was associated with a marginal decrease in triglycerides, but not in cholesterol. Compared to the pre-ALADIN cohort, IS reduction was associated with a significantly lower total cholesterol at FU. Unfortunately, cholesterol subclassification into LDL and HDL was not systematically available in older samples. Furthermore, statin prescription increased over time (7% at svLbx and 17% at FU; pre-ALADIN: 6% at Lbx and 9% at FU), due to declining LDL targets suggested by current guidelines. 26 IS associated comorbidities (infections, malignancies, metabolic complications) were recorded from the patients’ records at the outpatient visits (Table 3). The ALADIN FU cohorts and the pre-ALADIN cohort showed no difference in incidence rates of these comorbidities during the FU after svLbx.

4 DISCUSSION

In the past, svLbx programs have been critically discussed, in the light of biopsy risks and frequent abnormal histology, with ambiguous consequences for IS management. 27 IS withdrawal studies and our center’s 12 years of experience did not exhibit relevant complications from screening and svLbx. 9,15–17,28 Multiple prospective IS weaning trials demonstrated that spontaneous operational tolerance could be reached, even when svLbx before IS withdrawal showed minimal inflammation. On the other hand, many of the patients in weaning trials experienced a rejection despite initial absence of relevant histological abnormalities. 15,17 Nonetheless, the attempts of IS minimization, even when they failed, were safe for patients in these IS withdrawal trials. These experiences led to the release of consensus recommendations on which histological findings justify an IS reduction attempt. 18 The histological findings in this study, with abnormalities in the vast majority of svLbx, are well in line with previous studies. 10,11,14,29 Immunosuppression regimens
FIGURE 4  Alanine aminotransferase at biopsy and follow-up. (A) ALT values at Lbx and at early (8–14 months post-Lbx) and late FU (15–22 months post-Lbx) in the ALADIN IS reduction group (n = 67) are depicted. Two patients were excluded from this graph because ALT elevation occurred in the course of chemotherapy due to PTLD and during a severe systemic infection at time of FU. For paired comparison between Lbx and respective FU timepoint, the paired Wilcoxon test was used. For comparison of ALT values between early and late FU we used the Mann-Whitney-U test. (B) ALT values at Lbx and FU in patients who fulfilled BanffMini criteria (n = 34). The Wilcoxon test was used to compare the ALT levels at the different timepoints. (C) ALT values at Lbx and FU in patients who did not fulfill BanffMini criteria (n = 43). The Wilcoxon test was used to compare the ALT levels at the different timepoints.

![Graph A](image1)

**A**

\( p = 0.009 \)

\( p = 0.768 \)

\( p = 0.214 \)

![Graph B](image2)

**B**

\( p = 0.036 \)

![Graph C](image3)

**C**

\( p = 0.397 \)
FIGURE 5  Longitudinal changes in renal function after individualized immunosuppression adjustment. (A) Percentage changes in eGFR from Lbx to FU in ALADIN FU group with IS reduction (n = 69), pre-ALADIN FU control cohort (pre-ALADIN; n = 35), and others in ALADIN FU (IS increased/IS modified with similar score/IS not altered; n = 43). Right: Mean Percentage changes in ALADIN FU with reduced IS, pre-ALADIN, and others in ALADIN FU. (B) Percentage changes in eGFR in ALADIN FU with CNI reduction (n = 24) comparing to ALADIN FU with other IS reductions (n = 45). Right: Mean Percentage changes in eGFR in ALADIN FU with CNI reduction and ALADIN FU with other IS reductions. All group comparisons were performed with Mann-Whitney-U test. (C) Percentage changes in eGFR from Lbx to FU in ALADIN FU (n = 112), comparing CNI reduction (n = 28) to all others (n = 84). Right: Mean Percentage changes in eGFR in ALADIN FU with CNI reduction and all others in ALADIN FU group.
without svLbx would have missed relevant graft injury including subTCMR in ~20%, and subclinical re-fibrosis or even re-cirrhosis in ~25% of svLbx (Table 2), and inherited the risk of progressive graft injury by inappropriate lowering of IS per protocol.

In contrast to the current study, most of the published svLbx programs did not modify IS according to a structured protocol. IS was modified more often in our structured ALADIN program (79%) in comparison to previous studies without structured assessment (30%–41% IS adjustments after Lbx).10,21

In view of patient safety, we applied a step-down approach, with small to moderate steps of IS reduction (Figure S1), even when patients would have fulfilled the criteria for ongoing reweaning trials, such as the LIFT trial (NCT02498977). This was also necessary because we included a larger number of AILD patients with a higher rejection risk, who were usually excluded from IS withdrawal trials. Similarly, AILD represented only a minority in many trials on mTOR inhibitors, with or without combination with tacrolimus, to study ways of reducing CNI dosages.32–37 Our study showed that at least moderate IS reduction is feasible in the majority of patients who were within or slightly beyond BanffMini (Figure S4) and that a moderate IS reduction attempt was safe in terms of graft loss or steroid resistant rejection.

The stepwise IS reduction approach (Figure S1) was not associated with a significantly increased frequency of rejection (biopsy-proven or not) compared to pre-ALADIN svLbx without structured IS modification. In this context, patients with biopsy proven TCMR patterns in the course of severe systemic and lethal disease, like pneumocystis pneumonia and recurrent PTLD, were not excluded from the safety analysis. This stepwise and moderate IS reduction, between 20%–40% in over 50% of the patients, seems to confirm the unchanged rejection rate as seen in the IS withdrawal studies.15,17 Despite the long cumulative follow-up period of 81 patient years after IS reduction, the results have to be interpreted with caution because of the short follow-up, potentially missing smoldering graft injury with progressive fibrosis after years.

CNI nephrotoxicity has proven to be a major issue in the long-term care of transplant recipients, and especially after OLT.2,5,6 The current study was able to show that particularly IS regimes with continued moderate to high CNI were associated with progressive deterioration of renal function. So far, we are not aware of comparable results of a significantly preserved kidney function after IS reduction in other svLbx studies. GFR preservation was not only associated with a switch to mTOR-inhibitors, but also with the significant reduction of CNI levels. In contrast to CNI sparing studies that point to a benefit for kidney function mostly early after OLT, CNI reduction in the current study took place comparatively late after OLT (median: 79 months), and kidney function was still significantly preserved.32,37–39

The majority of controlled studies showing preserved kidney function as a result of reduced CNI levels started in the first weeks after transplantation.32–37 Furthermore, IS withdrawal studies, which mostly started late after OLT, were unable to demonstrate a benefit for renal function.15,17 In contrast to a large register study from France on OLT patients converted to EVR with subsequent CNI reduction, we found no influence of gender or time after OLT on preserved kidney function (Figures S2 and S3).22 Based on the median short-term decline slope of kidney function in those patients without CNI reduction, we estimated the need for dialysis (eGFR <15 ml/min/1.73 m²) after 26 years (data not shown).

As regards other adverse events of chronic immunosuppression, such as malignancy and severe infections, our data was not sufficient to make secure statements. Longer follow-up periods are necessary to evaluate whether personalized IS, and explicitly not only complete IS weaning, has an impact on incidence of malignancies and long-term survival. Cardiovascular diseases, associated with dyslipidemia and diabetes, and the effect of our metabolic counselling and increasing statin prescription also remain to be evaluated in the long-term follow-ups. Likewise, IS withdrawal studies also had difficulty showing benefits for these IS associated comorbidities.15–17

Obvious limitations of the current study are that it is a single center evaluation of a clinical practice but not of a controlled study. In addition, changes in clinical routines (regular and longitudinal assessment of DSA and LSM) need time, and the resulting lack of data limits the scope of our real-world study. The COVID-19 pandemic with its subsequent lock down measures, including reduced capacity for svLbx, and the voluntary social distancing of the OLT patients, including refusal of svLbx during the pandemic, limited the follow-up surveillance due to a reduction in visits to our center. The comparison to a not completely matched pre-ALADIN is another source of bias, which could not be overcome, due to limited patient numbers. The COVID-19 pandemic led to a delay in outpatient follow-up visits and we cannot rule out that this influenced the detection rate of infections and routine tumor surveillance after OLT. In addition, a longer follow-up including LSM and follow-up svLbx is needed in order to exclude progression of subclinical graft injury by IS reduction. Although none of the classical immunosuppression studies, for example, those on mTOR inhibitors, included such an assessment of subclinical graft injury via svLbx beyond bp-clinTCMR in their study protocols, a longitudinal paired svLbx is state of the art in intentional IS withdrawal studies.15–17

The classical pharmaceutical studies established the cornerstones of IS management in the first year after OLT, but without the assessment of subclinical graft injury by biopsy, while IS withdrawal studies demonstrated that operational tolerance is feasible and safe in highly selected patients with low graft injury late after OLT. The current study suggests how IS could be adjusted stepwise and personalized on the basis of subclinical graft injury beyond year 1 and for all patients after OLT irrespective of the underlying diseases.

In summary, the combination of biopsy, DSAs and clinical parameters could identify a large group of patients in which an IS reduction was safe and resulted in preserved kidney function without increased rejection risk. In addition, we identified an intermediate risk group with subTCMR but normal LFTs in which IS reduction should not be attempted. Finally, we identified re-fibrosis (≥F2) in 26% of patients. Future clinical trials should attempt to change the outcomes for these high-risk patients.
With this encouraging retrospective single center data, the safety and effectiveness of both interventional ALADIN approaches, IS reduction in patients with low subclinical graft injury and IS modification/increase in those with advanced graft injury, will have to be confirmed in prospective controlled interventional trials.

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DISCLOSURE
The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

DATA AVAILABILITY STATEMENT
The data that support the plots within this paper and other findings of this study are available from the corresponding author upon reasonable request.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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