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How to cite

MOECKLI, Beat et al. Determining safe washout period for immune checkpoint inhibitors prior to liver transplantation : An international retrospective cohort study. In: Hepatology, 2025. doi: 10.1097/HEP.0000000000001289

This publication URL: https://archive-ouverte.unige.ch/unige:184160

Publication DOI: 10.1097/HEP.0000000000001289

Hepatology Publish Ahead of Print
DOI:10.1097/HEP.0000000000001289

OPEN

Determining safe washout period for immune checkpoint inhibitors prior to liver transplantation: An international retrospective cohort study

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Running head: IMMUNE CHECKPOINT INHIBITORS PRIOR TO LIVER TRANSPLANTATION

Keywords: Immune Checkpoint Inhibitor, Liver transplantation, rejection, hepatocellular carcinoma, recurrence

Electronic word count: 4532

Abbreviations: ICI, Immune Checkpoint Inhibitor; DBD, donor after brain death; HCC, hepatocellular carcinoma; LT, liver transplantation

Author Contributions: Conceptualization, BM, CHW, and CT; Methodology, BM, SEH, CT, SL, and CHW; Data curation, all authors; Writing—original draft preparation, BM, SEH, and CHW; Writing—review and editing, all authors; Supervision, CT and SL; Project administration, CT and BM; Funding acquisition, BM, CT, and SL.

Conflict of interest statement: Parissa Tabrizian consults for Boston Scientific and AstraZeneca. Manon Allaire consults for Roche and AstraZeneca. Constantine J. Karvellas consults for Grifols, Mallinckrodt, Baxter, and Morphocell. Bruno Sangro consults for, is on the speakers' bureau for, received grants from AstraZeneca. He consults for and is on the speakers' bureau for Roche, Sirtex Medical, and Eisai. He consults for or advises Bayer and Terumo. He consults for BMS, Boston Scientific, MSD, and Sanofi. He is on the speakers' bureau for Incyte. The remaining authors have no conflicts to report.

Financial support statement: The Swiss National Science Foundation (grant number 182471), The Fondation Francis & Marie-France Minkoff, and Prof. Dr. Max Cloëtta Foundation funded this research.

RG is funded by Swim Across America, Rally Foundation, StacheStrong, and Musella Foundation

Graphical Abstract

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Abstract

Background & Aims: Immune checkpoint inhibitors (ICI) are increasingly used in patients with advanced hepatocellular carcinoma (HCC) patients awaiting liver transplantation (LT). However, concerns about the risk of post-transplant rejection persist.

Methods: We conducted an international retrospective cohort study including 119 HCC patients who received ICIs prior to LT. We analyzed the incidence of allograft rejection, graft loss, and post-transplant recurrence with particular focus on the washout period between the last ICI dose and LT.

Results: In this study, 24 of the 119 (20.2%) patients experienced allograft rejection with a median time to rejection of 9 days (IQR 6-10) post-LT. A linear relationship was observed between shorter washout periods and higher rejection risk. Washout periods less than 30 days (OR 21.3, 95% CI: 5.93-103, $p<0.001$) and between 30 and 50 days (OR 9.48, CI 2.47-46.8, $p=0.002$) were significantly associated with higher rejection rates in the univariate analysis compared to the washout period above 50 days. Graft loss as a result of rejection occurred in 6 patients (25%) with rejection. No factors related to grafts were associated with rejection. A longer washout period was not associated with a lower recurrence-free survival post-transplantation at 36 months (71 vs. 67%, $p=0.71$).

Conclusions: Our findings suggest that a washout period longer than 50 days for ICIs before liver transplantation appears to be safe with respect to rejection risk. While these results may help guide clinical decision-making, future prospective studies are essential to establish definitive guidelines.

1. Introduction

The treatment landscape for hepatocellular carcinoma (HCC) is rapidly evolving. Curative treatments such as ablation, surgical resection or liver transplantation, offer the best survival and recurrence-free rates for HCC patients (1,2). However, even in countries with established HCC surveillance programs, only about 30% of patients can be potentially eligible for curative treatment (3). Until recently, advanced stages were treated with kinase inhibitors (e.g. sorafenib) as a systemic therapy.

Immune checkpoint inhibitors (ICI) represent a breakthrough in cancer immunotherapy (4,5). In HCC, antitumor activity was shown for CTLA4 inhibitors in 2013 (6) and PD-1 inhibitors in 2017(7). After mitigated results with ICI monotherapies, combinations have become the standard of care for advanced HCC. The combination of atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) (8) and the combination of tremelimumab (anti-CTLA-4) and durvalumab (anti-PD-L1) (9) resulted in increased overall survival (OS) compared to sorafenib. More recently, the combination of ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1)(10) improved OS compared to lenvatinib or sorafenib. Moreover, as it entered the clinical practice for advanced stages, we observed the capacity of ICI therapy to downstage patients to be considered for liver transplantation. Up to one third of patients present a durable long-term response (10,11). Ultimately, ICI therapy in combination with loco-regional therapies such as chemo- or radio-embolization have demonstrated their efficacy as bridging tools to curative treatments (12,13).

An increasing number of transplantation centers around the world now have patients on their waiting list under ICI therapy and this number is bound to increase as ICI therapy makes its way into international guidelines for the first-line treatment of advanced stage HCC (14). Allograft rejection occurs in 8-15% of patients after a liver transplantation and can lead to irreversible graft damage and even graft loss if not treated adequately (15–17). A short washout period may increase the risk of rejection and graft loss (18–20), whereas a longer washout period could unnecessarily delay a life-saving liver transplantation (21,22). A retrospective cohort study from China involving 83 patients reported higher rejection rates with washout periods shorter than 30 days (23). A recent individual patient data meta-analysis (91 patients) suggests a minimum washout period of 3 months (24). However the question of the optimal washout period remains unanswered to date.

This study aims to determine the impact of neoadjuvant ICI therapy on the rejection risk after liver transplantation through an international retrospective cohort study. Specifically, we aim to identify risk factors for rejection after liver transplantation, focusing on the determination of a safe washout period and graft-related factors. Our results will help to promote the safe use of ICI therapy in HCC patients potentially eligible for liver transplantation until high-quality prospective data will become available.

2. Materials and methods

2.1. Identification of study centers and search strategy

We conducted a structured search in Medline/PubMed and Embase (January 1, 2017, to November 25, 2024) (7,25) using a comprehensive strategy incorporating the concepts of liver transplantation, immune checkpoint inhibitors, and the pre-transplantation setting (Supplementary Appendix 1, http://links.lww.com/HEP/J741), and contacted authors of identified studies to request complete clinical details on patients receiving ICI prior to LT.

Additionally, we contacted individual expert centers in Asia, Europe and North America with significant liver transplantation activity to provide data on patients who received ICI before liver transplantation. The study selection strategy and a patient inclusion flowchart are detailed under Supplementary Figure 1, http://links.lww.com/HEP/J741.

2.2. Patient inclusion and data compilation

Patients were eligible for inclusion if they received at least one cycle of ICI such as PD1 inhibitor, PD-L1 inhibitor or CTLA-4 inhibitor before liver transplantation. No upper limit was set for the time between the discontinuation of ICI therapy and liver transplantation. A minimum follow-up of two weeks post-LT was required, as most rejection events typically occur within this period. Additionally, we ensured that patients included in multiple studies were counted only once in our cohort.

Data were extracted from the identified manuscripts on a per-patient level. Missing variables were completed by authors from the contributing centers wherever possible. Patients were excluded if data on the type of ICI, the duration between the last ICI dose and liver transplantation, or the occurrence of rejection were missing after the extraction of the data and contacting the corresponding author. Additionally, previously unpublished data for five patients were included in our cohort (Supplementary Fig. 1, http://links.lww.com/HEP/J741).

Of these, three patients were provided by UC San Diego Health, San Diego, CA, USA; one by Hôpital Universitaire Pitié-Salpêtrière, Paris, France; and one by Aster Hospitals, Bangalore, India. To increase data completeness, two follow-up emails were sent to all corresponding authors at two and four weeks after the initial contact.

2.3. Definition of rejection, rejection-free survival, recurrence-free survival and immunosuppression

We defined rejection as 1) acute cellular rejection proven by liver biopsy or 2) an increase in transaminase levels during the recovery after LT ≥2 times the upper limit in the absence of other causes for liver injury and responding to rejection treatment. According to the Banff criteria, the rejection activity index was reported if provided by the study center (26). A rejection activity index score of 3–5 was defined as mild rejection, 6–7 as moderate rejection, and 8–9 as severe rejection (27). To ensure clinically meaningful analysis, we used 100 days as a predefined time point for rejection-free survival, as our data show that most rejections occur within the first two weeks post-LT, with rejection becoming unlikely beyond 100 days. This is consistent with prior studies on post-transplant rejection in the setting of ICI use (23,24).

Rejection-free survival was defined as the time from liver transplantation to the occurrence of acute rejection, with patients who did not experience rejection censored at their last follow-up. Death without rejection was treated as a censoring event.

Graft loss probability was analyzed using the Kaplan-Meier estimator, with graft loss defined as graft failure due to acute rejection. Patients who lost their grafts for reasons unrelated to rejection and were re-transplanted, were not censored. Death was treated as a censoring event. Due to the low frequency of deaths in the post-operative period in our cohort, we did not consider death as a competing risk.

Recurrence-free survival was defined as the length of time from liver transplantation to the date of the first loco-regional or systemic recurrence. Recurrence-free survival was analyzed using a competing risk model, with death without recurrence treated as a competing event. This approach provides an accurate estimation of recurrence rates and aligns with the standard definition of recurrence-free survival used in the literature. The cumulative incidence function was used to estimate the probability of recurrence over time, while the Fine-Gray model assessed the association of covariates with recurrence risk (28).

The contributing centers managed the immunosuppression based on per center protocols.

2.4. Statistical analysis

Descriptive statistics summarized group data as median (interquartile range) with standardized mean difference. Groups were compared by nonparametric Wilcoxon Signed Rank test, parametric unpaired t-test, one-way or two-way repeated-measures analysis of variance (ANOVA) with post hoc Tukey's multiple comparison test or Kruskal-Wallis test with post hoc nonparametric unpaired Wilcoxon, or Pearson's Chi-squared test.

All clinically relevant variables from our dataset were included in the univariate logistic regression. Continuous variables were categorized based on clinically relevant thresholds from prior literature where available (e.g., age ≥60 years, cold ischemia time ≥10 hours) (29–31). When no established cutoffs existed but categorization was appropriate for data interpretability, we used data-driven approaches, such as median-based categorization (e.g., for ICI duration, perioperative transfusions). When categorization would lead to a loss of statistical power or oversimplification of associations, we retained variables in their continuous form to preserve the full range of data. For the primary analysis, the washout period was grouped into categories to simplify interpretation and avoid overfitting, given the limited sample size and number of rejections. To ensure robustness, we also performed a sensitivity analysis treating the washout period as a continuous variable.

Regarding the multivariable analysis, we assessed each variable's association with rejection in univariate analysis, and variables with a p-value <0.2 were pre-selected for multivariable analysis. The stepwise selection of variables for multivariable analysis was performed using both forward and backward approaches, based on the Akaike Information Criterion (AIC), to identify the best set of predictors associated with rejection. Final model fit was evaluated using AIC and residual deviance (32).

Time-to-event outcomes were visualized with Kaplan-Meier survival curves and analyzed using a log-rank test when no significant competing risks were present. For outcomes involving competing risks, such as recurrence-free survival, we applied a competing risks analysis using the Fine and Gray model. (28)

All statistical analyses and graphical representations were performed using the R statistical programming environment (R version 4.4.0 (2024-04-24) / RStudio Version 2024.04.0+735)

(33,34). The tbl_regression function of the gtsummary package was used for the logistic regression analysis (35). Survival analysis and Kaplan-Meier plots were performed with the survminer package. Competing risk cumulative incidence was analysed with the ggsurvfit package (36). For all analyses, a two-tailed probability of type I error of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the overall population

After excluding three patients due to incomplete data on the ICI type and the length of the washout period, a total of 119 patients from 29 study centers were included in the analysis. Among these, 12 centers contributed data for more than one patient. Missing data were addressed by contacting all study centers, with 55% of centers providing additional information (Supp. Fig 2). Asian centers provided 61% of patients, North American 34% and European 5%. Details and references of the included studies are provided in Supplementary Table 1, http://links.lww.com/HEP/J741. Out of the 119 patients studied, 24 experienced graft rejection acutely following LT with a median time to rejection of 9 days (IQR 6-10) post-LT. A confirmation biopsy in the case of rejection was less frequently carried out in the Asian centers compared to North American centers (62.5 vs. 87.5%), but this difference was not statistically significant ($p = 0.427$) (Figure 1A). In patients biopsied, the stratification according to rejection severity reflected by the Rejection Activity Index was similar among patients from Asian and North American centers (p=0.762) (Figure 1B).

Data related to the population characteristics are presented in Table 1. When comparing the non-rejection to the rejection groups, we observed a different median age, respectively 58 years (IQR: 53-63) and 55 years (IQR: 48-62) ($p = 0.2$). Sex distribution was similar showing 15% females in the non-rejection group, versus 13% females in rejection group (p>0.9). The underlying liver diseases were comparable between groups, with hepatitis B virus being the most common (59% in the non-rejection versus 55% in the rejection group), followed by hepatitis C virus (16% non-rejection, 23% rejection). The type of immune checkpoint inhibitor (ICI) used was not significantly different between groups (p=0.3). Specifically, atezolizumab was administered to 23% of the non-rejection group and 8.3% of the rejection group, nivolumab to 34% and 25%, and pembrolizumab to 19% and 38%, respectively. The

types of ICIs were classified as PD-L1 (25% non-rejection, 13% rejection) and PD1 (75% non-rejection, 88% rejection) ($p = 0.2$). The majority of patients (74%) received a single-agent ICI, while 26% received combination regimens, with atezolizumab-bevacizumab being the most common (18%). Rejection occurred in 23.9% of patients treated with single-agent ICIs and in 9.7% of those treated with combination regimens. While rejection rates appeared lower in the combination group, this difference was not statistically significant (Fisher's exact test, $p = 0.12$). Combinations such as PD-1/CTLA-4 inhibitors, PD-1/PD-1 inhibitors, and PD-1/PD-L1 inhibitors, were used in smaller proportions. Rejection rates did not significantly differ between the different combination regimens ($p = 0.5$) (Table 1). Of note, the median number of ICI cycles was similar between groups (5 vs. 5, $p = 0.4$), and complete responses to ICI were similarly observed in both groups (34% of non-rejection and 50% of rejection cases, $p = 0.3$). We did not observe any significant differences in induction immunosuppression regiments ($p = 0.5$) or in the maintenance immunosuppression regiments ($p = 0.6$). Recurrence patterns were predominantly intrahepatic across the cohort, accounting for 86% of all recurrences. Two patients (9.5%) experienced both intra- and extrahepatic recurrence, while one patient (4.8%) had exclusively extrahepatic recurrence. No statistically significant differences in recurrence sites were observed between the rejection and no-rejection groups ($p > 0.9$).

Tumor stage pre-ICI differed between groups, with a higher proportion of BCLC stage A tumors in the rejection group ($p = 0.015$). Of the 15 BCLC A patients, 27% had a single tumor and 73% had up to three nodules. For these BCLC A patients, ICI was administered within clinical trials (60%), after HCC recurrence post-resection (27%), or based on decisions by community oncologists (13%). Excluding the 28 BCLC-C patients (23.5%) from the analysis did not alter key findings.

3.2. Rejection post liver transplantation

In our international cohort, 24 (20.2%) patients experienced rejection at a median of 9 days after liver transplantation. Out of the 24 patients diagnosed with rejection, 17 rejections (70.8%) were confirmed by liver biopsy (Figure 1). Six patients suffered a graft loss due to their acute rejection event (25.0%) (Supplementary Table 2, http://links.lww.com/HEP/J741). Four of the patients with graft loss died and two were retransplanted. All patients presenting with rejection were treated with an increase in the immunosuppression therapy.

3.3. The interaction between washout period and rejection

To identify the optimal washout duration, we plotted the proportion of patients experiencing rejection against washout period increments of 10 days (Figure 3A). A linear relationship was observed between the length of the washout period and rejection risk, with the risk decreasing below 10% at 50 days. This level of baseline rejection aligns with the allograft rejection rate previously reported in the general liver transplantation population not exposed to ICI therapy (15,17).

Using this 50 day threshold and findings from previous studies (23,24) we divided our cohort into four washout periods: 0-30 days, 30-50 days, 50 to 90 days and more than 90 days (Figure 3B). Patients with a washout period of 0 to 30 days presented the highest rejection risk with 50.0% of patients experiencing rejection. However, even patients with a washout period between 30 and 50 days rejected their grafts in 26.0% of cases. Only patients with a washout period above 50 days presented with rejection rate comparable to the rate of rejections in the general liver transplant population(16). Length of ICI therapy prior to LT did not seem to influence the occurrence of rejection (Figure 4).

3.4. Variables associated with rejection

Variables associated with rejection in the univariate logistic regression are shown in Table 2. Univariate analysis did not demonstrate a significant association between age over 60 years and rejection risk (odds ratio (OR) 0.58, 95% confidence interval (CI) 0.19-1.56, p=0.3). Compared to nivolumab (n=40), patients treated with pembrolizumab (n=27) showed a higher risk of rejection in the univariate analysis (OR 2.67, CI 0.83–9.14, p=0.10), though this difference was not statistically significant. The length of the washout period was significantly associated with rejection in the univariate analysis. Patients with a washout period below 30 days experienced the highest risk of rejection (OR 21.3, 95% CI 5.93–103, p < 0.001), while those with a washout period of 30–50 days still had a markedly increased risk of rejection (OR 9.48, 95% CI 2.47–46.8, p = 0.002). A sensitivity analysis treating the washout period as a continuous variable confirmed its significant association with rejection (OR 0.97, 95% CI 0.95–0.99, p=0.001).

After univariate analysis, variables with a p-value <0.2 were considered for multivariable logistic regression. Stepwise logistic regression with both forward and backward AIC based

selection identified the Washout Periods as the only independent predictor of rejection (Table 2).

3.5. Influence of graft-related factors on rejection risk

In this study we specifically looked at the influence of graft-related factors on the risk of rejection (Figure 2). Most patients in our cohort received an organ from a donor after brain death, with a minority of patients having received an organ from a living donor (Figure 2A). We did not observe a statistical differences between groups in any of the studied factors (Figure 2B-F).

3.6. Impact of the washout period on rejection-free and graft survival

To study the rejection-free survival, we divided the cohort into patients with an extended washout period above 50 days (n=66) and a short washout period below 50 days (n=53). The rejection-free survival probability at 100 days post liver transplantation for the patients in the extended washout group was 95% (95% CI: 90-100%) versus 60% in the short washout group (95% CI: 48-74%, $p<0.0001$) (Figure 5A). In some cases, acute rejection can result in graft loss, considerably more relevant for patient's survival outcome. To address this particular complication of acute rejection, we calculated the impact of a short washout period below 50 days on graft loss using the Kaplan-Meier estimator (Figure 5B). Patients with a shorter washout period below 50 days experienced a higher graft loss risk at 100 days (88 vs. 98%, $p=0.029$). The characteristics of the patients having experienced a graft loss are detailed in the Supplementary Table 2, http://links.lww.com/HEP/J741.

3.7. Impact of the washout period on recurrence

The increased risk of rejection with a short washout period needs to be balanced with the potentially worse oncological outcomes if the washout period increases. To study the impact of a long washout period, we looked at post-transplant recurrence of HCC. The median post-transplant follow-up was 18 (IQR: 11-27) months. Twenty-four patients presented with recurrence during their follow-up period (21.8%). Patients with a long washout period did not present a higher risk for recurrence (Figure 6), and their recurrence free survival at 36 months was 71% (95% CI: 58-87%) compared to 66% in the short washout group (95% CI: 50-89%, $p=0.71$).

4. Discussion

The emergence of ICI therapy promises new treatment possibilities for both downstaging and bridging HCC patients to liver transplantation. In the future a greater number of patients will be exposed to ICI therapy before liver transplantation. Early reports have raised concerns about the safety of ICI therapy (19,20) and there is an urgent need to determine how these therapies can be used safely before liver transplantation. Previous studies have addressed this question with conflicting clinical recommendations (23,24). Our study represents currently the largest international observational studies on this topic, adding important insights to the current literature (24). We found that a washout period of more than 50 days appears safe and is not associated with an increased risk of allograft rejection post liver transplantation.

Current guidelines from major hepatology organizations, including the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases, recommend ICIs as a first-line treatment option for advanced (HCC) (37,38). However, these guidelines lack specific recommendations for their use in liver transplantation candidates, primarily due to concerns about graft rejection risk and limited availability of data (39). Numerous prospective trials are currently evaluating ICI therapy before liver transplantation both as bridging or downstaging treatment (39) (NCT05879328, NCT04425226, NCT05027425). In these trials, the washout period is not always clearly defined; when specified, it ranges from >30 to >90 days, reflecting the lack of consensus and the wide variability in clinical practice (40,41).

Our study builds on two previously published studies including each more than 80 patients. In a retrospective multicenter cohort study from China by Guo et al. (83 patients), the authors found an overall rejection rate of 27.7%, similar to the 20.2% observed in our cohort (23). The authors concluded that a washout period below 30 days should be avoided due to higher rejection risk. Our findings further suggest that a longer washout period may be necessary to minimize the risk of acute rejection and graft loss due to rejection. Indeed 26.1% of patients with a washout between 30 and 50 days experienced rejection (Figure 3) and two thirds of rejection cases resulting in graft loss occurred in patients with a washout period longer than 30 days.

Additionally, a recently published individual patient meta-analysis by Rezaee-Zavareh et al. proposed a 90-day washout period as safe in patients with ICI exposure (24). Less than half

of our cohort overlaps with this meta-analysis. Despite this, our analysis demonstrates that extending the washout period beyond 50 days provides only marginal additional benefit in reducing rejection rates (Supplementary Figure 2, http://links.lww.com/HEP/J741). Already a washout period of 50 days or longer may reduce rejection risk to levels comparable to those in patients without prior ICI exposure. This finding is critical, as an excessively long washout period could unnecessarily extend waiting times for liver transplantation and discourage the use of ICI therapy in potential transplantation candidates.

Graft-related factors such as ischemia time, younger age and autoimmune liver disease have been reported as risk factors for acute rejection (16,42). Another focus of this study was to investigate the importance of graft-related factors on the risk of rejection in the context of pre-transplant ICI use. We found no significant differences in graft-related factors between patients presenting with rejection and without (Figure 2). A significant transfusion requirement during transplantation might lead to faster serum clearance of the ICI and packed red blood cells might exert intrinsic immunomodulatory properties (43,44). We observed a higher number of transfusions in patients without rejection, though this difference was not statistically significant (Figure 2C). Even though transfusion requirements were not associated with rejection risk in the logistic regression analysis, this variable may still be an important confounding factor and should be considered in future randomized trials.

Nine different immune checkpoint inhibitors all targeting the programmed death 1 (PD1) programmed death ligand 1 (PD-L1) interaction were used in our cohort. It has been previously suggested that alloimmune reactions are more likely with PD-L1 blockade (45–47). We did not observe such a trend in our cohort. However, some anti PD-1 agents such as pembrolizumab were associated with a higher risk of rejection in the univariate analysis but not in the multivariable analysis. Given the low number of patients, no conclusions can be drawn at this stage, future studies will show if some ICIs are associated with a higher alloimmune response.

Although our study offers important insights into the feasibility and safety of the use of ICIs before liver transplantation, several limitations need to be acknowledged. First, this study is retrospective, and patient management protocols differed among the study centers, this included the management of immunosuppression in the perioperative period. One third of allograft rejections were diagnosed based on clinical criteria without histological confirmation, potentially leading to an overestimation of the rejection incidence, however an

analysis including only patients with biopsy proven rejection showed similar results. Given the retrospective nature of our study, detailed immune status data, such as PD-1 and PD-L1 expression and the immunological status of the recipients were not collected, limiting our understanding of the immune mechanisms underlying post-transplant rejection. Such data should be collected in future randomized trials. Another limitation of this study is the lack of standardized radiological follow-up protocols among the contributing centers. Most centers did not report specific details about their imaging schedules after liver transplantation, and those that did showed variability in the frequency and modality of imaging. This heterogeneity could influence the calculation of progression-free survival. The inherent publication bias in retrospective studies like ours should also be considered, as the selective reporting of patients with rejection may influence the perceived safety and efficacy of pre-transplant ICI therapy. Finally, we did not look at the oncologic progression during the washout period, as longer washout period might lead to a higher number of dropouts before liver transplantation which could represent an intrinsic bias for the cohort with an extended washout regarding recurrence. The risk of recurrence with a longer washout should be balanced with the risk of rejection.

In conclusion, our study highlights that the increased risk of allograft rejection was associated with a short washout period (<50 days) between ICI administration and liver transplantation in HCC patients. We found that a washout period greater than 50 days appears safe regarding rejection, while not increasing the risk of HCC recurrence post-transplant. These results underscore the importance of optimizing the timing of liver transplantation following ICI therapy to ensure better post-transplant outcomes. Further prospective studies are essential to validate these findings and establish definitive guidelines for the integration of ICIs into the pre-transplant management of HCC patients.

5. Acknowledgments

We thank Myriam Benichou from the Geneva University Library for her expert assistance with the literature search on this topic. We express gratitude to Ilaria Di Meglio for her valuable assistance in English language editing of the manuscript. We would also like to express our gratitude to Dr. François Cauchy for his critical review of the manuscript. His

pertinent feedback on both the methodology and content was invaluable and greatly enhanced the quality of this work.

6. Data availability statement

Data will be made available to other researchers upon any reasonable request and can be obtained through the corresponding authors.

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7.1. Table 1 – Baseline characteristics of patients

Characteristic	N	Overall N = 119[1]	No Rejection N = 95[1]	Rejection N = 24[1]	Difference[2]	95 % CI[23]	p-value[4]
Age	111	57 (50, 63)	58 (53, 63)	55 (48, 63)	0.29	-0.18, 0.76	0.2
Sex	118				0.05	-0.41, 0.50	>0.9
F		17 (14%)	14 (15%)	3 (13%)			
M		101 (86%)	81 (85%)	20 (87%)			
Region	119				0.40	-0.05, 0.85	0.4
Asia		72 (61%)	56 (59%)	16 (67%)			
North America		40 (34%)	32 (34%)	8 (33%)			
Europe		7 (5.9%)	7 (7.4%)	0 (0%)			
Immune Check Point Inhibitor	119				0.69	0.24, 1.1	0.4
nivolumab		38 (32%)	32 (34%)	6 (25%)			
pembrolizumab		27 (23%)	18 (19%)	9 (38%)			
atezolizumab		24 (20%)	22 (23%)	2 (8.3%)			
camrelizumab		16 (13%)	13 (14%)	3 (13%)			

Characteristic	N	Overall N = 119[1]	No Rejection N = 95[1]	Rejection N = 24[1]	Difference[2]	95% CI[2,3]	p-value[4]
sintilimab		9 (7.6%)	6 (6.3%)	3 (13%)			
toripalimab		2 (1.7%)	1 (1.1%)	1 (4.2%)			
cadonilimab		1 (0.8%)	1 (1.1%)	0 (0%)			
durvalumab		1 (0.8%)	1 (1.1%)	0 (0%)			
penpulimab		1 (0.8%)	1 (1.1%)	0 (0%)			
ICI Type	119				0.33	-0.12, 0.78	0.3
PD-L1		27 (23%)	24 (25%)	3 (13%)			
PD1		92 (77%)	71 (75%)	21 (88%)			
ICI Indication	99				0.43	-0.07, 0.92	0.3
bridging		43 (43%)	33 (42%)	10 (50%)			
downstaging		51 (52%)	43 (54%)	8 (40%)			
non-transplant-related		4 (4.0%)	2 (2.5%)	2 (10%)			
other		1 (1.0%)	1 (1.3%)	0 (0%)			
ICI Combination Treatment	119				0.43	-0.02, 0.88	0.2

Characteristic	N	Overall N = 119[1]	No Rejection N = 95[1]	Rejection N = 24[1]	Difference[2]	95% CI[2,3]	p-value[4]
Single Agent		88 (74%)	67 (71%)	21 (88%)			
Combination Treatment		31 (26%)	28 (29%)	3 (13%)			
ICI Combination	119				0.52	0.07, 0.97	0.4
Single Agent		88 (74%)	67 (71%)	21 (88%)			
Atezo/Bev		22 (18%)	20 (21%)	2 (8.3%)			
PD1/CTLA-4		4 (3.4%)	4 (4.2%)	0 (0%)			
PD1/PD1		4 (3.4%)	3 (3.2%)	1 (4.2%)			
PD1/PD-L1		1 (0.8%)	1 (1.1%)	0 (0%)			
Underlying Liver Disease	104				0.65	0.17, 1.1	0.4
HBV		60 (58%)	48 (59%)	12 (55%)			
HCV		18 (17%)	13 (16%)	5 (23%)			
Unknown		10 (9.6%)	7 (8.5%)	3 (14%)			
MASH		8 (7.7%)	8 (9.8%)	0 (0%)			
ALD		5 (4.8%)	4 (4.9%)	1 (4.5%)			
CPS		1 (1.0%)	1 (1.2%)	0 (0%)			
Hemochromatosis		1 (1.0%)	1 (1.2%)	0 (0%)			

Characteristic	N	Overall N = 119[1]	No Rejection N = 95[1]	Rejection N = 24[1]	Difference[2]	95% CI[2,3]	p-value[4]
PSC		1 (1.0%)	0 (0%)	1 (4.5%)			
Cycles ICI	107	5 (3, 9)	5 (3, 9)	5 (3, 10)	-0.29	-0.77, 0.19	0.3
Response ICI	76				0.44	-0.11, 0.98	0.5
CR		29 (38%)	20 (34%)	9 (53%)			
PR		32 (42%)	26 (44%)	6 (35%)			
SD		14 (18%)	12 (20%)	2 (12%)			
PD		1 (1.3%)	1 (1.7%)	0 (0%)			
Washout Period [d]	119	56 (30, 122)	67 (39, 138)	25 (15, 38)	0.76	0.30, 1.2	**<0.001**
Graft Type	72				0.68	0.12, 1.2	0.2
DBD		53 (74%)	38 (69%)	15 (88%)			
DCD		10 (14%)	10 (18%)	0 (0%)			
LDLT		9 (13%)	7 (13%)	2 (12%)			
Donor Age	35	42 (31, 56)	39 (31, 54)	53 (44, 60)	-0.31	-1.3, 0.64	0.6
Cold Ischemia Time [min]	54	343 (189, 444)	347 (195, 444)	289 (189, 367)	0.20	-0.52, 0.91	0.6

Characteristic	N	Overall N = 119[1]	No Rejection N = 95[1]	Rejection N = 24[1]	Difference[2]	95% CI[2,3]	p-value[4]
Warm Ischemia Time [min]	37	30 (26, 36)	30 (25, 37)	28 (26, 35)	0.32	-0.47, 1.1	0.4
Steatosis [%]	23	5 (0, 10)	3 (0, 13)	5 (0, 10)	0.40	-0.81, 1.6	0.4
Perioperative Transfusions [U PRBC]	49	2 (0, 4)	2 (0, 5)	1 (0, 2)	0.55	-0.31, 1.4	0.084
POD1 ALT [IU/L]	51	580 (343, 956)	602 (334, 945)	476 (385, 1,000)	-0.22	-0.89, 0.45	0.6
Induction Immunosuppression	116				0.61	0.16, 1.1	0.5
MTP		44 (38%)	34 (37%)	10 (42%)			
anti-ILR2/MTP		32 (28%)	24 (26%)	8 (33%)			
anti-ILR2		20 (17%)	15 (16%)	5 (21%)			
No		11 (9.5%)	11 (12%)	0 (0%)			
rATG/MTP		6 (5.2%)	5 (5.4%)	1 (4.2%)			
rATG		3 (2.6%)	3 (3.3%)	0 (0%)			
Maintenance Immunosuppression	116				0.64	0.18, 1.1	0.6
Tacro/MMF		52 (45%)	38 (41%)	14 (61%)			
Prednisone/Tacro/MMF		36 (31%)	31 (33%)	5 (22%)			

Characteristic	N	Overall N = 119[1]	No Rejection N = 95[1]	Rejection N = 24[1]	Difference[2]	95% CI[2,3]	p-value[4]
Tacro/MMF/Sirolimus		12 (10%)	10 (11%)	2 (8.7%)			
Tacro/MMF/Everolimus		6 (5.2%)	6 (6.5%)	0 (0%)			
Tacro		3 (2.6%)	2 (2.2%)	1 (4.3%)			
Tacro/MMF/Sirolimus / Prednisone		3 (2.6%)	2 (2.2%)	1 (4.3%)			
Tacro/Sirolimus		3 (2.6%)	3 (3.2%)	0 (0%)			
Prednisone/CsA/MMF		1 (0.9%)	1 (1.1%)	0 (0%)			
Recurrence	110	24 (22%)	19 (21%)	5 (24%)	-0.06	-0.53, 0.42	>0.9
Recurrence Site	21				0.59	-1.4, 2.6	>0.9
both		2 (9.5%)	2 (10%)	0 (0%)			
extrahepatic		1 (4.8%)	1 (5.0%)	0 (0%)			
intrahepatic		18 (86%)	17 (85%)	1 (100%)			
Death	92	9 (9.8%)	5 (6.9%)	4 (20%)	-0.39	-0.89, 0.11	0.2
Cause of Death	9				3.7	1.6, 5.9	0.2
MOF due to Rejection		3 (33%)	0 (0%)	3 (75%)			
Tumor Progression		2 (22%)	1 (20%)	1 (25%)			

Characteristic	N	Overall N = 119[1]	No Rejection N = 95[1]	Rejection N = 24[1]	Difference[2]	95 % CI[2,3]	p-value[4]
Cardiac Death		1 (11%)	1 (20%)	0 (0%)			
Graft vs. Host Disease		1 (11%)	1 (20%)	0 (0%)			
Post-Operative Hemorrhage		1 (11%)	1 (20%)	0 (0%)			
Septic shock		1 (11%)	1 (20%)	0 (0%)			
Follow Up [months]	112	18 (11, 27)	20 (11, 27)	16 (7, 24)	0.15	-0.31, 0.61	0.6
Number of Tumors	72	2 (1, 4)	2 (1, 4)	3 (2, 4)	0.16	-0.57, 0.90	0.5
Size of largest tumor [cm]	95	4.4 (3.0, 7.5)	4.6 (3.3, 7.5)	4.2 (2.5, 7.3)	0.21	-0.29, 0.70	0.3
AFP pre-LT [(µg/L]	104	12 (4, 136)	10 (4, 124)	28 (5, 149)	-0.10	-0.60, 0.39	0.7
Tumor stage pre ICI (BCLC)	99				0.65	0.14, 1.2	0.054
A		28 (28%)	19 (24%)	9 (47%)			
B		43 (43%)	39 (49%)	4 (21%)			
C		28 (28%)	22 (28%)	6 (32%)			

[1]Median (Q1, Q3); n (%) [2]Standardized Mean Difference [3]CI = Confidence Interval
[4]Welch Two Sample t-test; Pearson's Chi-squared test

Characteristic	N	Overall N = 119[1]	No Rejection N = 95[1]	Rejection N = 24[1]	Difference[2]	95% CI[2,3]	p-value[4]

Abbreviations: AFP, Alpha-Fetoprotein; ALD, Alcoholic Liver Disease; ALT, Alanine Aminotransferase; Atezo/Bev, Atezolizumab/Bevacizumab; BCLC, Barcelona Clinic Liver Cancer; CPS, Congenital Portosystemic Schunt; CR, Complete Response; CsA, Cyclosporine A; DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; ICI, Immune Checkpoint Inhibitor; ILR2, Interleukin-2 Receptor; LDLT, Living Donor Liver Transplantation; MMF, Metabolic dysfunction-associated steatohepatitis; Mycophenolate mofetil; MOF, Multiple Organ Failure; MTP, Methylprednisolone; NR, No Response; PD, Progressive Disease; PD1, Programmed Cell Death Protein 1; PD-L1, Programmed Cell Death Protein Ligand 1; POD, Postoperative Day; PR, Partial Response; PRBC Packed Red Blood Cell; PSC, Primary sclerosing cholangitis; rATG, rabbit Anti-Thymocyte Globulin; SD, Stable Disease.

7.2. Table 2 - Factors associated with acute allograft rejection in logistic regression

	Univariate analysis				Multivariable analysis		
Characteristic	**N**	**OR**[1]	**95% CI**[1]	**p-value**	**OR**[1]	**95% CI**[1]	**p-value**
Age	111						
0-60		—	—				
60+		0.58	0.19, 1.56	0.3			
Sex	118						
F		—	—				
M		1.15	0.34, 5.34	0.8			
Region	119						
Asia		—	—				
North America		0.88	0.32, 2.22	0.8			
Europe		0.00		>0.9			
Immune Check Point Inhibitor	119						
nivolumab		—	—				
pembrolizumab		2.67	0.83, 9.14	0.10	3.81	0.88, 19.8	0.087
atezolizumab		0.48	0.07, 2.33	0.4	1.36	0.15, 10.4	0.8
camrelizumab		1.23	0.23, 5.45	0.8	1.88	0.26, 13.6	0.5
sintilimab		2.67	0.47, 13.6	0.2	1.74	0.25, 11.7	0.6
ICI Type	119						
PD-L1		—	—				

PD1		2.37	0.73, 10.6	0.2
ICI Indication	99			
bridging		—	—	
downstaging		0.61	0.21, 1.72	0.4
non-transplant-related		3.30	0.36, 30.5	0.3
other		0.00		>0.9
ICI Combination Treatment	119			
Single Agent		—	—	
Combination Treatment		0.34	0.08, 1.09	0.10
ICI Combination	119			
Single Agent		—	—	
Atezo/Bev		0.32	0.05, 1.22	0.14
PD1/CTLA-4		0.00		>0.9
PD1/PD1		1.06	0.05, 8.82	>0.9
PD1/PD-L1		0.00		>0.9
Underlying Liver Disease	104			
HBV		—	—	
HCV		1.54	0.43, 5.02	0.5
Unknown		1.71	0.33, 7.24	0.5
MASH		0.00		>0.9
ALD		1.00	0.05, 7.57	>0.9
Cycles ICI	107			
0-8		—	—	
8+		1.69	0.60, 4.55	0.3

Response ICI	76						
CR		—	—				
PR		0.51	0.15, 1.66	0.3			
SD		0.37	0.05, 1.75	0.2			
PD		0.00		>0.9			
Washout Period [d]	119						
I >50 days		—	—				
II 30-50 days		9.48	2.47, 46.8	**0.002**	10.8	2.3, 78.6	**0.006**
III 0-30 days		21.3	5.93, 103	**<0.001**	34.0	7.17, 267	**<0.001**
Graft Type	72						
DBD		—	—				
DCD		0.00		>0.9			
LDLT		0.72	0.10, 3.42	0.7			
Donor Age	35	1.01	0.96, 1.06	0.6			
Cold Ischemia Time [min]	54						
0-10h		—	—				
10+h		0.00		>0.9			
Warm Ischemia Time [min]	37	0.97	0.89, 1.05	0.5			
Steatosis [%]	23	0.96	0.77, 1.08	0.6			
Perioperative Perfusions [U PRBC]	49						
0-4 PRBC		—	—				
4+ PRBC		0.58	0.03, 4.16	0.6			
POD1 ALT [IU/L]	51	1.00	1.00, 1.00	0.4			

Induction Immunosuppression	119			
MTP		—	—	
No MTP		0.52	0.16, 1.43	0.2
Maintenance Immunosuppression	119			
No Prednisone		—	—	
Prednisone		0.60	0.20, 1.58	0.3
Tumor stage pre ICI (BCLC)	99			
A		—	—	
B		0.22	0.05, 0.75	0.021
C		0.58	0.17, 1.89	0.4

[1]OR = Odds Ratio, CI = Confidence Interval

Abbreviations: AFP, Alpha-Fetoprotein; ALD, Alcoholic Liver Disease; ALT, Alanine Aminotransferase; Atezo/Bev, Atezolizumab/Bevacizumab; BCLC, Barcelona Clinic Liver Cancer; CPS, Congenital Portosystemic shunt; CR, Complete Response; CsA, Cyclosporine A; DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; ICI, Immune Checkpoint Inhibitor; ILR2, Interleukin-2 Receptor; LDLT, Living Donor Liver Transplantation; MASH, Metabolic dysfunction-associated steatohepatitis; MMF, Mycophenolate mofetil; MOF, Multiple Organ Failure; MTP, Methylprednisolone; NR, No Response; PD, Progressive Disease; PD1, Programmed Cell Death Protein 1; PD-L1, Programmed Cell Death Protein Ligand 1; POD, Postoperative Day; PR, Partial Response; PRBC Packed Red Blood Cell; PSC, Primary sclerosing cholangitis; rATG, rabbit Anti-Thymocyte Globulin; SD, Stable Disease.

7.3. Figure 1 – Regional differences in biopsy

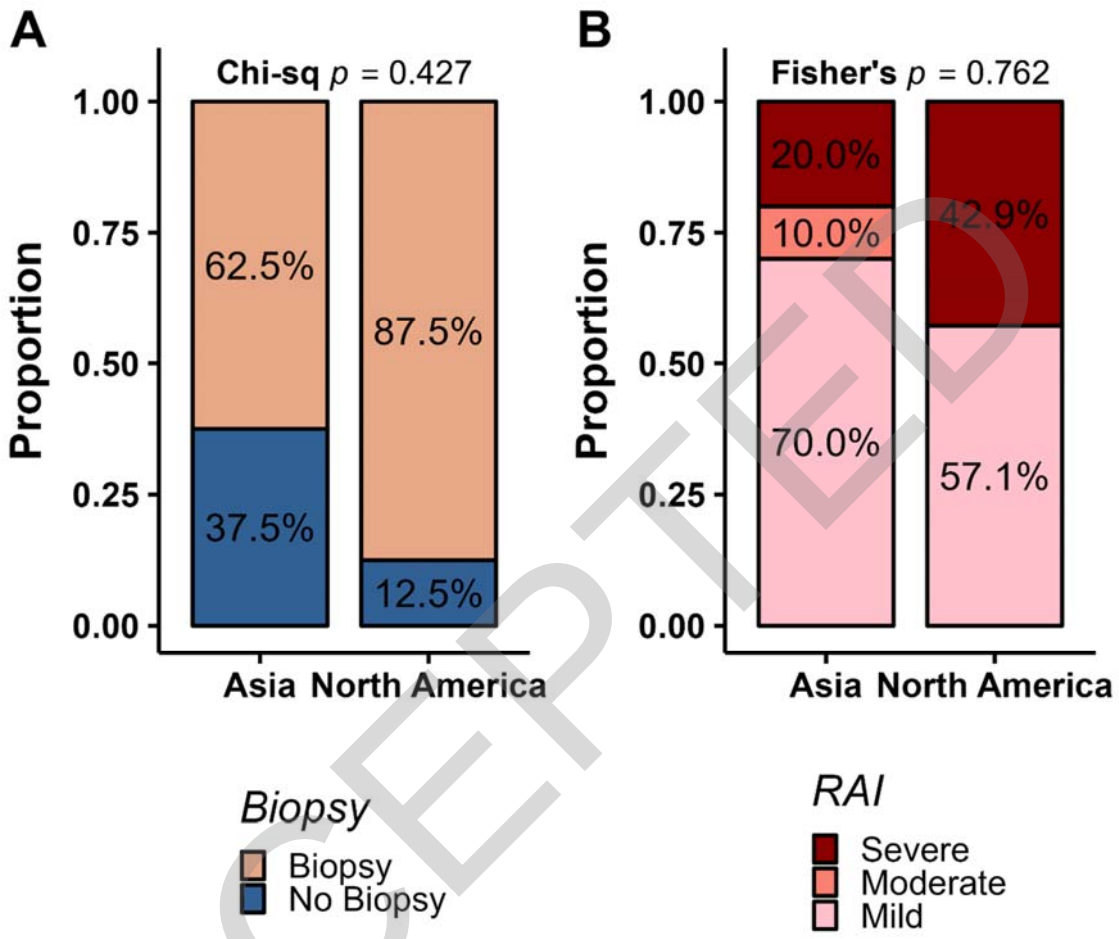

Fig. 1. Regional differences in liver biopsy for rejection (**A**) Proportion of patients presenting with rejection, that were biopsied according to the region. (**B**) Proportion of rejection severity on the biopsy according to the Rejection Activity Index (RAI). Statistical analysis: Panels A: Statistical analysis was performed by Chi square test, Panel B: Statistical analysis was performed by Fisher's exact test. Data represented as proportion of patients, level of significance of p=0.05

7.4. Figure 2 – Graft related factors

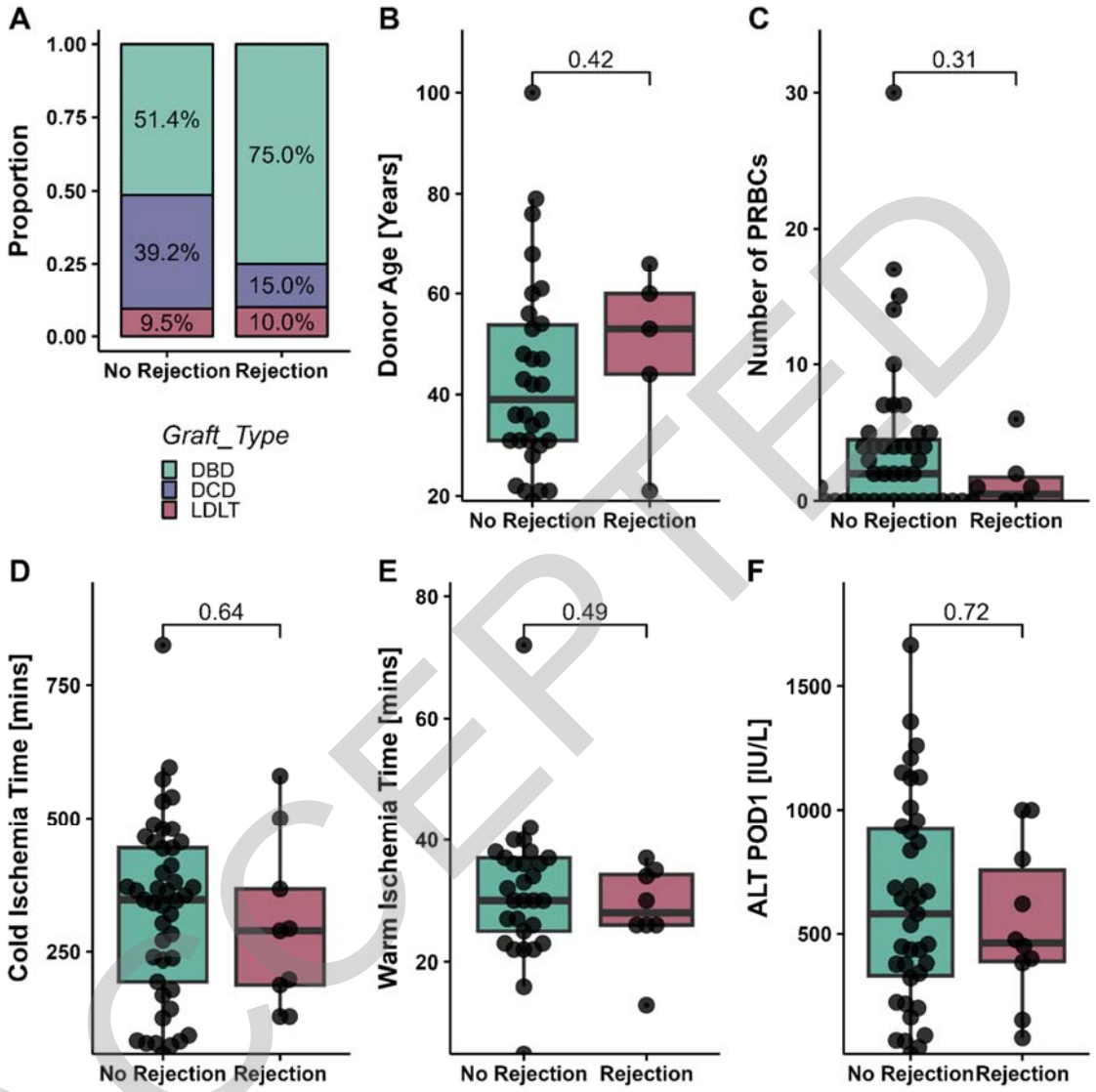

Fig. 2. Differences in graft related factors between patients who presented with rejection and those without (**A**) Graft Type, (**B**) age of the donor, (**C**) number of transfusions with packed Red Blood Cells during the liver transplantation. (**D**) Cold ischemia time and (**E**) warm ischemia time of the graft in minutes. (**C**) Opening alanine transaminase on post-operative day 1. Panels B-E: Data presented as median ± IQR, one dot represents one patient, level of significance of p=0.05. Statistical analysis was performed by Wilcoxon–Mann–Whitney test. Abbreviations: DCD, Donation after Circulatory Death; DBD, Donation after Brain Death; LDLT, Living Donor Liver Transplantation; POD, Postoperative Day; ALT, Alanine Aminotransferase; PRBC, Packed Red Blood Cells

7.5. Figure 3 – Proportion of post-transplant rejections according to washout period

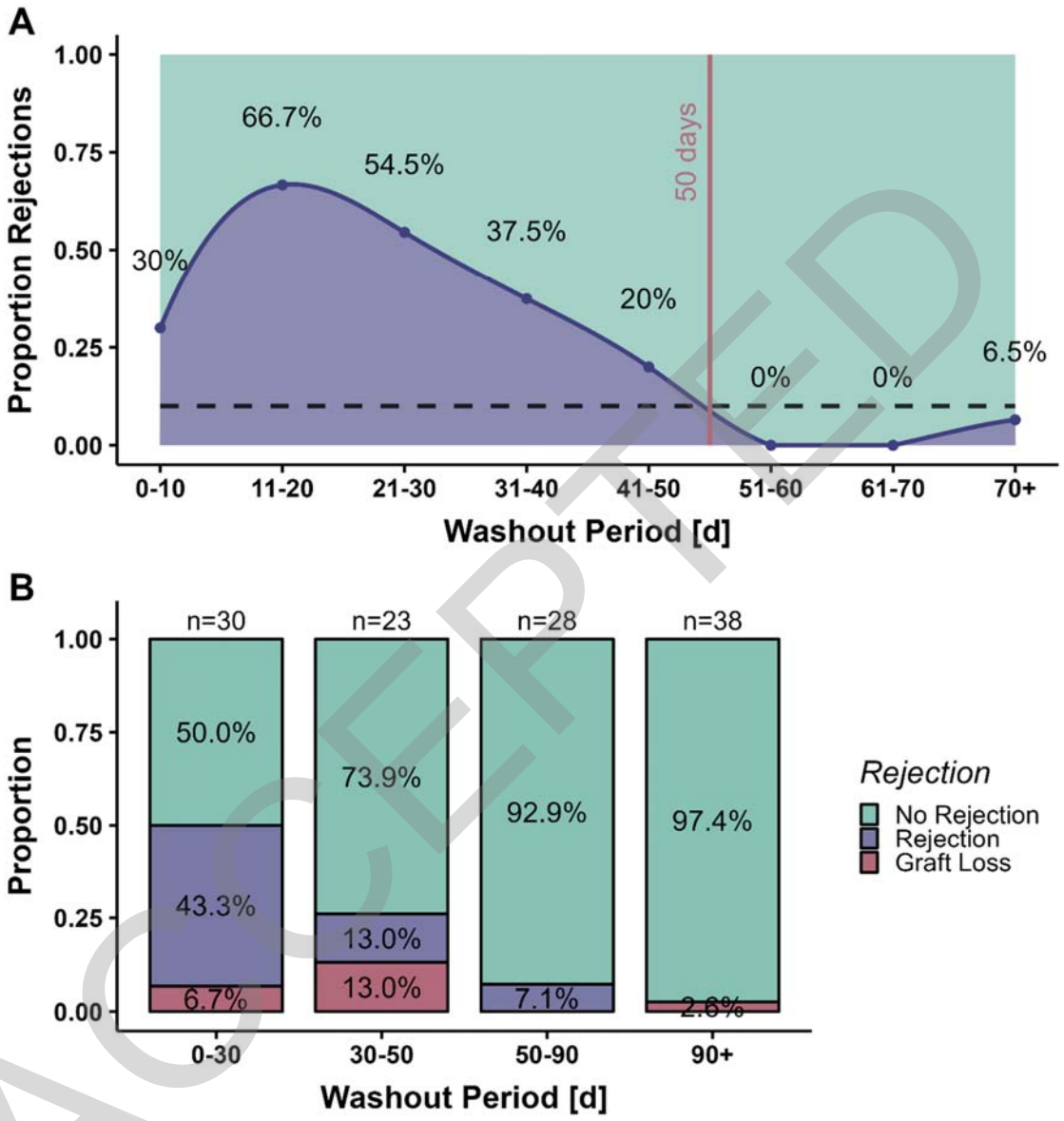

Fig. 3. Proportion of post-transplant rejections according to washout period (**A**) Proportion of patients presenting with rejection per 10-day increments. The black dashed line represents the baseline rejection rate of 10%. The vertical line represents the washout period of 50 days. (**B**) Histogram of the washout period with the corresponding proportion of rejections. The number of patients is displayed on each column.

7.6. Figure 4 – Relation of washout and ICI therapy to post-transplant rejection and graft loss

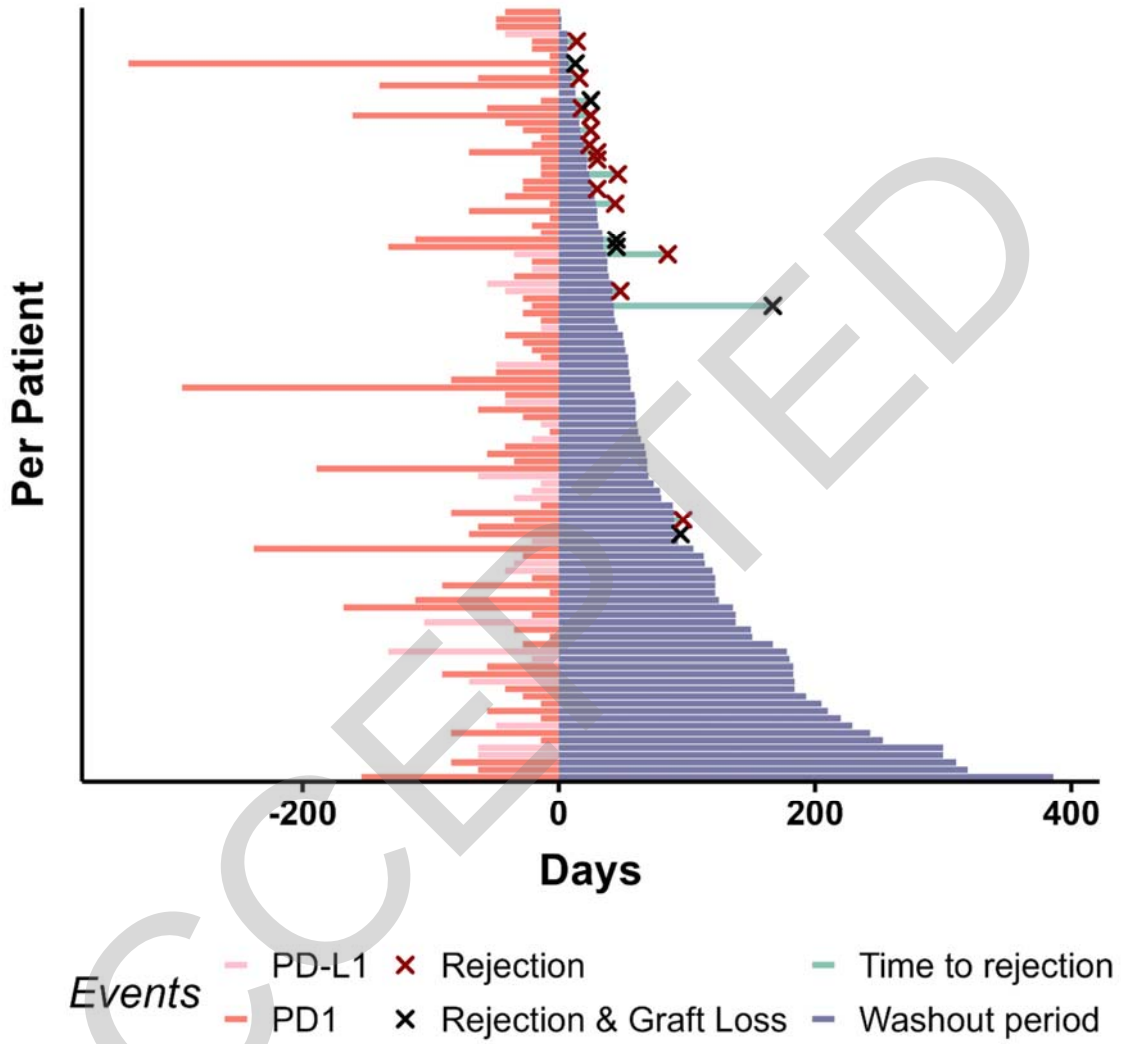

Fig. 4. Relation of length of washout period and ICI therapy to post-transplant rejection and graft loss Each line represents one patient. Negative values on the x-axis visualizes periods prior to liver transplantation, positive values periods after liver transplantation. Patients with a red cross experienced rejection, patients with a black cross suffered rejection leading to graft loss. Abbreviations: PD1, Programmed Cell Death Protein 1, PD-L1, Programmed Cell Death Protein Ligand 1.

7.7. Figure 5 – Survival analysis for rejection and graft loss

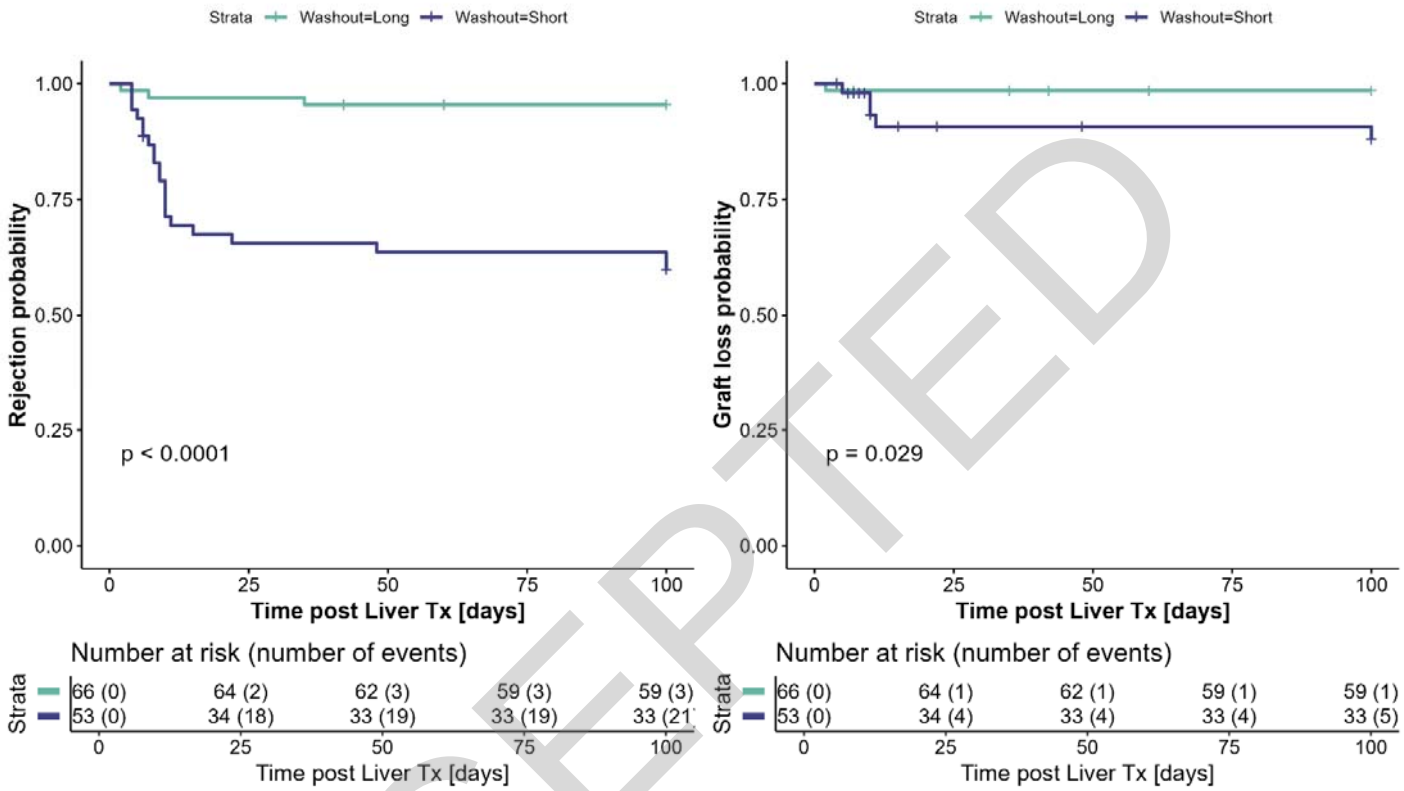

Fig. 5. Survival analysis for rejection and graft loss (**A**) Kaplan-Meier survival curve of rejection post liver transplantation according to washout-period. (**B**) Kaplan-Meier survival curve of graft loss post liver transplantation according to washout-period. The cohort is divided into patients with a long washout period above 50 days (Washout=Long, n=66) and patients with a short washout period below 50 days (Washout=Short, n=53). Statistical analysis panels A and B: Level of significance of p=0.05. Statistical analysis was performed by log-rank test.

7.8. Figure 6 – Survival analysis for recurrence

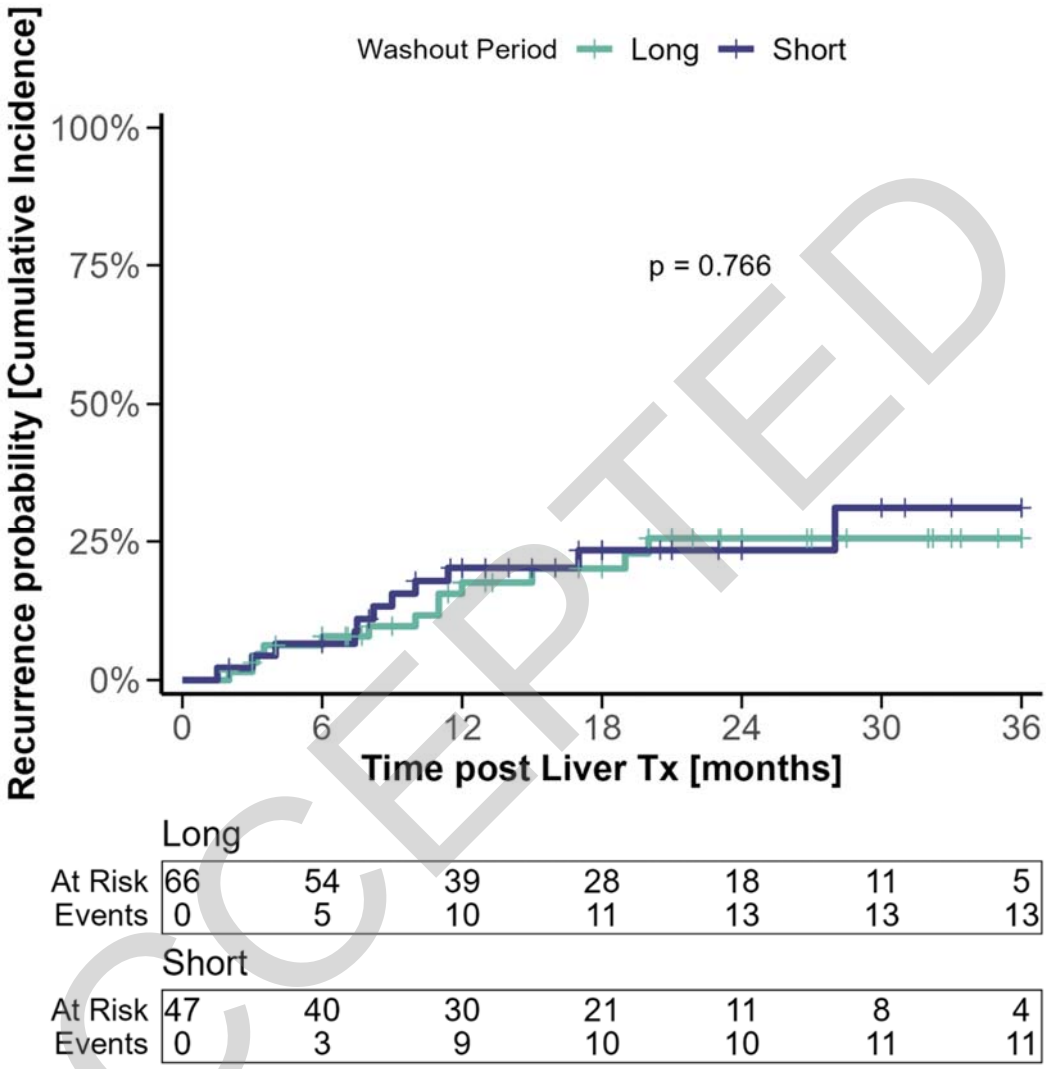

Fig. 6. Competing risk analysis for recurrence post transplantation according to washout period Cumulative incidence curves of recurrence post liver transplantation according to washout-period. The total cohort is divided into patients with a long washout period above 50 days (Washout=Long, n=66) and patients with a short washout period below 50 days (Washout=Short, n=47). Statistical analysis: Level of significance of *p*=0.05. Statistical analysis was performed by Fine-Gray model.