Clinical parameters and biomarkers predicting spontaneous operational tolerance after liver transplantation: a scoping review

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Abbreviations:

APC, antigen-presenting cell; CNI, calcineurin inhibitors; CENTRAL, Cochrane Central Register of Controlled Trials; DC, dendritic cells; DSA, donor specific antibodies; IS, immunosuppression; ISW, immunosuppression withdrawal; LFT, liver function tests; LT, liver transplantation; mTOR-I, mammalian target of rapamycin inhibitor; NINV, non-autoimmune/non-replicative viral; NK, natural killer; OT, operational tolerance; PBMC, peripheral blood mononuclear cells; SOT, solid organ transplantation; Treg, regulatory T cells

Abstract

Indefinite allograft acceptance after immunosuppression withdrawal (ISW), also known as operational tolerance (OT), can occur spontaneously after liver transplantation (LT) but reliable and reproducible prognosis of OT versus non-OT outcomes remains elusive. To prime this, systematic extraction of OT-predictive factors from the literature is crucial. We provide the first comprehensive identification and synthesis of clinical parameters and biomarkers predicting spontaneous OT in non-autoimmune/non-replicative viral LT recipients selected for ISW. We searched Embase, Medline, the Cochrane Central Register of Controlled Trials, clinicaltrials.gov, and the World Health Organization International Clinical Trials Registry Platform for articles, conference abstracts, and ongoing trials. We contacted principal investigators of stand-alone abstracts and ongoing trials for unpublished data and screened citations and references of eligible articles. 23 articles reporting on 11 completed ISW studies, 13 abstracts, and five trial registry entries were included.

Longer time between LT and ISW was the only clinical parameter that may increase the incidence of OT. Prognostic biomarkers conspicuously differed between pediatric and adult ISW candidates. These included allograft gene expression patterns and peripheral blood immune exhaustion markers for adults, and histological allograft scores for children. Our results will foster cross-validation efforts to facilitate safe and harmonized candidate selection for successful ISW.

1. Introduction

The introduction of potent pharmacological immunosuppression (IS) regimens in the late 1970s, in particular calcineurin inhibitors (CNI), kick-started the medical and surgical success story of solid-organ transplantation (SOT). While preventing acute and chronic rejection, these treatments are directly or indirectly responsible for various complications including recurrent or *de novo* malignancies, cardiovascular and metabolic diseases, chronic kidney disease and infections, which jeopardize patient and allograft survival.[1-3] Achieving drug-free allograft survival in SOT, known as operational tolerance (OT), would allow to overcome these adversities, while preserving a long-term allograft function.[4]

Among all solid-organ transplants, the liver exhibits unique and complex immunoregulatory properties, which render liver allografts less dependent on IS and less sensitive to immunological damage.[5] The putative underlying mechanisms of tolerance may include large antigen load, deficient antigen presentation by antigen-presenting cells (APC), neutralization of alloantibodies, deletion of effector lymphocyte clones, regulatory T cell (Treg) generation as well as long-term microchimerism.[6,7] Based on these particular features, clinical studies that examined IS minimization or complete IS withdrawal (ISW) following pediatric and adult liver transplantation (LT) have been initiated in the 1990s.[8] It is now estimated that 20-40% of carefully selected non-autoimmune/non-replicative viral (NINV) LT recipients can eventually achieve OT by stopping IS and yet maintaining a stable allograft function and histological integrity.[7] The majority of LT recipients would, however, still experience an acute cellular rejection episode or develop abnormal liver function tests (LFT) during the ISW process and require reinstitution of IS.[7,8] The timeline of the consecutive steps of an ISW study is illustrated in Figure 1.

The discovery of OT has promoted extensive research activity over the last two decades. One studied objective was to explore the factors associated with the development of OT to help refine the eligibility criteria for LT recipients to participate in ISW trials and increase the fraction of successful ISW.[9] Furthermore, researchers have started to address the question as to whether OT can be induced by immune manipulation prior to ISW.[4]

After two decades of OT research, we aim to systematically identify, review, and summarize the evidence on clinical parameters and biomarkers predicting spontaneous OT in prospective ISW LT recipients. The comprehensive mapping will likely foster inter-center cross-validation of OT-predictive factors with the final goal of concerted, safe, and successful ISW candidate selection after LT.

1.1. Study aim and objectives/questions

The objective of this scoping review was to map all published prognostic factors for spontaneous OT in NINV LT recipients who are being subjected to ISW. The obtained results may inform the subsequent conduct of a systematic review with a more targeted review question.

Specifically, the review questions were:

i) What are clinical parameters and biomarkers that predispose LT recipient ISW candidates to achieve spontaneous OT?

ii) What are the success rates of ISW and achievement of spontaneous OT in LT recipients?

iii) What are the rates of graft loss in LT recipients following ISW?

2. Methods

2.1. Protocol and registration

This scoping review was conducted along with the guidelines by the Joanna Briggs Institute[10] and reported according to the PRISMA-ScR statement.[11] A study protocol was prospectively peer-reviewed and published.[12]

2.2. Eligibility criteria

2.2.1.Population, Intervention, Outcomes

The primary eligibility criterion was the assessment of spontaneous OT, i.e. rejection-free liver allograft survival for at least one year following ISW. LT recipients of any age or stage were included, but recipients with underlying autoimmune diseases, replicative viral disease (i.e. patients with positive hepatitis B and/or C viremia detected by polymerase chain reaction) and/or multi-organ recipients were excluded. Studies reporting on mixed populations were included if NINV study population was 80% or greater. Studies that did not report the liver disease etiologies for LT in their population were also included. All pharmacological IS regimens, including combination treatments, being completely withdrawn were eligible. However, studies addressing dose reduction of IS including IS minimization, withdrawal of a subset of drugs from IS combination treatments (e.g. withdrawal of corticosteroids in patients on CNI maintenance treatment), or conversion between IS regimens [e.g. CNI to mammalian target of rapamycin inhibitor (mTOR-I) conversion vs. CNI continuation] were excluded.

We exclusively included studies that assessed an association of pre-ISW clinical parameters or pre-ISW biomarkers with OT development. Studies that addressed the effectiveness of induction or immunomodulation therapies on OT development were excluded. Owing to the risk of confounding by interrupted IS in the OT cohort, data on post-ISW biomarkers were excluded unless the same biomarkers had been measured in the same patients already before ISW.

2.2.2.Study types

We included prospective, retrospective, randomized, and non-randomized ISW studies irrespective of publication status, including case-control and cross-sectional designs. Conference abstracts and trial registry entries where the data was already published in a peer-reviewed article were excluded. Animal studies, case reports, case series (i.e. publications where patient histories of exclusively OT or non-OT LT recipients are

reported), reviews, letters, and editorials were excluded. No language or publication date restrictions were applied.

2.3. Information sources

An information specialist (CA-H) developed the search strategies, which were reviewed by a second information specialist (HE). Database-specific subject headings and text word synonyms for LT, ISW, OT, graft survival, or liver biopsy were used. We searched Embase via Elsevier, Medline via Ovid, and the Cochrane Central Register of Controlled Trials (CENTRAL) (last search July 17, 2019; Supplementary Information). We also searched the study registry clinicaltrials.gov as well as the World Health Organization's International Clinical Trials Registry Platform for ongoing studies (last search September 3, 2019; Appendix 1). All retrieved references were exported to EndNote X9 and deduplicated.

2.4. Selection of sources of evidence

One reviewer (CA-H) screened the references based on their titles and abstracts. All potentially relevant references were retrieved in full-text and independently assessed by two reviewers (CA-H, JV). Any disagreements over eligibility were resolved by consensus. Where necessary, a third review author (SH) made a final judgment.

To identify possible additional studies that escaped our database searches, we screened the references and citations in Scopus of all included articles (April 30, 2020). Furthermore, principal investigators of identified relevant ongoing studies and conference abstracts were contacted twice by email for the sharing of any unpublished data (personal communications, preprints, manuscripts in press).

2.5. Data charting process

Two reviewers (CA-H, JV) independently charted the data from each source of evidence using a jointly developed MS Excel 2016 charting form that was pilot-tested with four eligible full-text articles. The charting form was updated in an iterative process. Any disagreements over charted data items were solved by discussion.

2.6. Data items

Next to reported prognostic and non-prognostic factors (clinical parameters and biomarkers) for OT, which were the primary outcomes, we charted the incidence of OT and the number of graft losses in each trial as the secondary outcomes. All charted data items are listed in Table S1.

2.7. Synthesis of results

For the synthesis of prognostic clinical parameters and biomarkers, we used descriptive statistics to highlight sources of evidence that supported or invalidated each factor. For the calculation of the pooled incidence of OT from completed ISW studies, the reports with the highest number of study participants were chosen in case of multiple reports of the same study. In addition to tabular views, the results were narratively synthesized.

3. Results

3.1. Results of the search and study characteristics

Our bibliographic database searches returned 4,704 unique hits, which included 1,185 Embase- or CENTRAL-derived conference abstracts. Our clinical trial registry searches returned 87 unique hits and 20 additional potentially relevant articles were derived iteratively from registry entries or conference abstracts, or found by citation tracking of included articles, or by contact with study authors. Out of the 193 selected full-text records, 152 did not meet our inclusion criteria for the reasons indicated (Figure 2; Table S2). The remaining 23 articles[13-35] (Table S3), 13 conference abstracts[36-48] (Table S4), and five registry entries[49-53] (Table S5) were included (Figure 2). They were published between 2001 and 2020.

The 23 eligible articles reported on eleven completed ISW studies, as evidenced by cohort overlap (Table S3). Of the eleven completed ISW studies, six (three prospective and three retrospective studies) reported on pediatric and five (all prospective) on adult LT recipient cohorts (Tables S3, S6, S7). Three monocenter pediatric ISW studies were from Kyoto (Japan),[17,18,20,27] Tochigi (Japan),[22,33] and Taipei (Taiwan)[23] and three multicenter pediatric ISW studies[14,31,35] from North American liver transplantation units. Four monocenter adult ISW studies were from Chicago (US),[34] Murcia (Spain)[13,16,21,24,29] [two studies], and Pamplona (Spain)[26,28] [one report[32] integrated the clinical parameters from one Pamplona and three Murcia trials], and one multicenter adult ISW study[15,19,25,30] analyzed trial data from Barcelona (Spain), Rome (Italy), and Leuven (Belgium). Population characteristics of ISW study reports including living versus deceased donors and inclusion of patients with viral liver disease etiology or non-elective ISW are detailed in Table S3.

Of the 13 included conference abstracts, two[45,47] provided additional data derived from one of the completed ISW studies (WISP-R[14]), six[37-41,48] reported on work in progress derived from ongoing trials (see below), and five[36,42-44,46] added potentially new ISW data, which has not been published in an article yet (Table S4). The latter abstracts were from Pittsburgh (US),[42,44,46] Shanghai (China),[36] and London (UK).[43] 6/13 conference abstracts reported pediatric[36,42,44-47] and 7/13 adult[37-41,43,48] ISW research.

Four of five included trial registry entries related to the ongoing ISW trials ALLTOL (NCT02743793),[51] LIFT (NCT02498977),[53] LITMUS (NCT02541916),[52] and OPTIMAL (NCT02533180)[49] and one trial[50] was stopped due to poor recruitment (A. Sanchez-Fueyo, personal communication 20200709) (Table S5). ALLTOL and OPTIMAL are US multicenter ISW trials, LITMUS is a Canadian monocenter, and LIFT a European multicenter ISW trial, which all have completed their recruitment of adult LT recipients.

3.2. OT-predicting clinical parameters

Table 1 summarizes our results on clinical parameters. The most frequently examined prognostic clinical parameter (assessed in 10/11 completed studies) was the time between LT and ISW. Five studies[13,14,18-20,25-29,32] (two pediatric and three adult) found longer time to ISW to be OT-predictive, four studies[16,22,24,34,35] (two pediatric and two adult) found no effect (one with an OT-predictive trend), and one pediatric study[23] indicated an opposite, i.e. non-OT-predictive effect. All other potentially OT-predicting clinical parameters were reported in isolated ISW studies only, whereas for every suggested parameter several other ISW studies that assessed the same parameter found no prognostic value (Table 1, see also Tables S6 and S7 for further clinical parameters from pediatric and adult studies, respectively).

3.3. OT-predicting biomarkers

The quality of association of selected pre-ISW biomarkers with OT is summarized in Table 2. Full details of all reported pediatric and adult OT-predicting biomarkers are presented in Tables S6 and S7, respectively.

3.3.1. Pediatric results

Published evidence supporting non-invasive OT-predicting biomarkers in children is particularly scarce. Concerning serum biomarkers, one pediatric study found some post-LT but no pre-LT anti-HLA-antibodies to be associated with later OT,[22] but other studies could not endorse such association even when restricting their analysis to donor-specific antibodies (DSA).[14,35] Regarding pediatric OT-predicting peripheral blood mononuclear cell (PBMC) markers, none have been reported so far.

Additional non-invasive biomarkers that may predict pediatric OT came from conference abstracts (Table S4, not included in Table 2). Re-analysis of frozen pre-ISW PBMC from the WISP-R study[14] showed that half of the

OT samples were characterized by a two-fold increase in exhausted T cells (CD4+ PD-1+ cells), whereas the fraction of these cells in the remaining OT samples was indistinguishable from the one in non-OT samples.[45,47] The potential importance of PBMC exhaustion markers for pediatric OT-prediction is also supported by other conference abstracts.[36,44,46]

All invasive OT-predicting biomarkers in children were derived from histological analyses. Absence of portal inflammation (but not of lobular or perivenular inflammation) predicted OT in two multicenter studies.[14,35] Further pre-ISW OT-associated histological markers were fewer APC:lymphocyte pairs, lower C4d score, decreased load of leukocytes, and decreased load of infiltrating monocytes/macrophages.[14,35]

3.3.2.Adult results

Non-invasive OT-predicting biomarkers in adults are subdivided into immunoassays, PBMC subsets, PBMC gene expression patterns, and serum markers. Quite prominently among the former, *in vitro* stimulated proliferation was substantially lower for pre-ISW lymphocytes that were derived from prospective OT compared to non-OT patients.[26]

Two major studies suggested OT-predicting PBMC subset frequencies: a higher fraction of natural killer (NK) cells and a lower fraction of Vδ2-TCR γδT cells in the Barcelona/Rome/Leuven study[25] (the NK result was endorsed with borderline statistics by the Pamplona study[26] but not supported by two other studies;[24,34] and the γδT result was not endorsed by subsequent work[29]) and higher fractions of tolerogenic dendritic cells (DC) or of TEMRA+, and Eomes+ CD8+ T cells accompanied by a lower fraction of naïve CD8+ T cells in the Chicago study.[34] Of note, a higher fraction of Treg, a T cell subpopulation known to be crucial for achieving tolerance,[20,25,27,54-56] does not appear to have any pre-ISW prognostic value for OT.[24-26,29,34]

Regarding PBMC gene expression markers, a study from Murcia reported OT-predicting expression of *FEM1C*, *SENP6*, and the miRNAs *miR-95* and *miR-31*.[29] In addition, the Barcelona/Rome/Leuven study showed the two pre-ISW gene expression signatures *[NCR1, PDGFRB, PSMD14]* and *[SLAMF7, KLRF1, CLIC3, PSMD14, ALG8, CX3CR1]*,[25] the latter of which being related to NK cell expansion, to be moderately OT-predictive.

In keeping with the prognostic value of allograft gene expression related to iron metabolism (see below), increased ferritin levels and, somewhat less robustly, hepcidin-25 may represent OT-predictive adult serum biomarkers. This is based on Barcelona/Rome/Leuven data[25] and non-significant trends reported in a Murcia study.[29] Notably, although being associated with subclinical allograft injury and fibrosis,[57] pre-ISW anti-HLA antibodies (including DSA) appear not to have any prognostic value for OT.[19,34]

With regards to invasive pre-ISW biomarkers, robust and reproducible prediction of OT was achieved using gene expression analysis in liver biopsies of adult ISW candidates. Specifically, the Barcelona/Rome/Leuven study identified the iron metabolism-related gene expression signature *[CDHR2, MIF, PEBP1, SOCS1, TFRC]* (and four additional signatures each comprising more than five genes) as a predictor of OT with a sensitivity of 89%, a specificity of 86%, and an overall error rate of 13%.[25] These results were largely replicated in the Chicago study that specifically examined mTOR-I ISW.[34] OT-predicting histological markers were increased load of CD4+ cells[34] and higher iron deposition in hepatocytes.[25]

Further invasive biomarkers were extracted from conference abstracts (Table S4). Increased allograft expression of the miRNA *miR-193a-3p* was reported to denote adult patients that are likely to develop OT,[43] but this biomarker was later abandoned due to lack of robustness (A. Sanchez-Fueyo, personal communication 20200709). Finally, the prognostic value of allograft and PBMC positivity for an eight gene expression panel (six immunoregulatory genes decreased and two pro-inflammatory genes increased) that was derived from pre-clinical experiments[58] is currently under investigation.[39,40,48,52]

3.4. Incidence of OT and graft loss

The pooled incidence of OT in the 11 completed ISW studies was calculated with data from six articles on pediatric[14,20,23,31,33,35] and five articles on adult study populations.[13,16,19,26,34] The pediatric studies included 371 LT recipients, and a median of 36.5% (range 29 – 63) of recipients successfully completed ISW. The adult studies included 174 LT recipients, and a median of 42% (range 29 – 63) of recipients successfully completed ISW. It is important to stress that these numbers are neither a systematic result nor a meta-analysis, since all ISW studies that did not report any OT-predictive factors were excluded per protocol (see above and Table S2). Likewise, as

the study populations of all included ISW studies are highly selected (e.g. with no previous rejections or abnormal LFT), the incidence of OT in the general LT recipient population is expected to be substantially lower. In all included studies, only one graft in a pediatric recipient was lost,[22,33] confirming that – appropriate ISW eligibility provided – ISW failure is not per se associated with a significantly elevated risk of graft loss.[7]

4. Discussion

This review provides the first systematic overview of published prognostic factors to inform clinicians' and patients' decisions regarding whether or not to embark on ISW after LT. Since successful ISW has many benefits including cessation of toxicity of IS medication,[1-3] improved quality of life,[59] and cost-effectiveness[60] but ISW failure can be frustrating and associated with undesirable consequences (yet apparently not with graft loss), the clinical value of secured prognostic information on ISW outcomes is evident. To the best of our knowledge, the rigorous eligibility criteria that guided this review and led to the exclusion of all OT-associated biomarkers manifesting only during or after ISW were unique. Indeed, we could show that according to current evidence, frequently quoted OT biomarkers such as DC2:DC1 ratio,[61] HLA-G level,[54,62] or Vδ1:Vδ2 γδT cell subset ratio[63] cannot predict OT before ISW and guide ISW decisions (Table S2). Furthermore, the predictive capacities of most of the pre-ISW clinical parameters and of some biomarkers did not withstand inter-study cross-check, indicating that they were either subtle or based on chance findings.

The potentially OT-predictive value of longer time to ISW requires further investigation in children and adults through a systematic review with meta-analysis. It is possible that LT recipients that do not suffer from acute IS co-morbidity can benefit from delaying the initiation of ISW. Mechanistically, prolonged exposure to donor antigens and increased immune exhaustion can potentially explain the positive effects of delayed ISW start on withdrawal success.[7] Consistently, the A-WISH trial, which did not meet our eligibility criteria due to below-threshold proportion of NINV LT recipients (Table S2), examined early ISW (1 – 2 years posttransplant) and found a relatively low incidence of OT (10/77, 13%) and no prognostic value of time between LT and ISW.[64]

Bearing in mind that non-invasiveness increases the practical utility of a biomarker (and decreases the risk of complications), we synthesized the reports on non-invasive biomarkers separately. PBMC signatures that may reflect immune exhaustion such as high TEMRA+ and Eomes+ CD8+ T cells,[34] CD4+ PD-1+ cells,[45] or CD4+ CD57+ CD45RA- CCR7+ cells,[46] and low naïve CD8+ T cells[34] may be good candidate biomarkers to predict OT. Similarly, the promising results of an *in vitro* lymphocyte proliferation assay[26] warrant independent implementation in both pediatric and adult ISW studies and may be interpreted as a readout of immune exhaustion.

Analysis of invasive biomarkers revealed differences between children and adults. Accordingly, a transcriptional predisposition pattern for adult OT[25] did not show any prognostic value for pediatric OT.[35] Information on OT-predictive pediatric gene signatures, which likely exist, is still missing completely. Identified OT-predictive histological markers also conspicuously differed between pediatric and adult ISW candidates. Absence of portal inflammation was found to be OT-predictive in children,[14,35] whereas an adult study found no prognostic value for this parameter.[25] Furthermore, presence in liver tissue of fewer APC:lymphocyte pairs was OT-predictive in pediatric but non-prognostic in adult ISW candidates, whereas the opposite was true for an increased load of CD4+ cells.[34,35]

To best highlight the main review findings, we rated the overall value of different predictors based on our data and by expert opinion. Association of two invasive biomarkers with OT, namely no portal inflammation in children and allograft *[CDHR2, MIF, PEBP1, SOCS1, TFRC]* expression in adults, was rated strong, whereas association of several non-invasive adult biomarkers was deemed moderate only (Table 2). Similarly, the association of longer time between LT and ISW with OT was rated moderate (Table 1). Together, we hope that these preliminary overall judgements will help identify the next step towards reliable ISW prediction.

Given the fact that the best-documented and most promising biomarkers were all invasive (Table 2), our review strongly endorses the necessity of pre-ISW biopsies. The majority of included ISW trials did perform pre-ISW biopsies to assess liver histology (Table S3). Still, 8/23 publications did not report any pre-ISW or post-ISW liver histology findings. A liver biopsy is crucial for assessing subtle graft inflammation and fibrosis and patients' eligibility for ISW. According to the latest Banff update,[65] pre-ISW biopsy findings that focus on signs of subtle graft injury can be associated with ISW failure. In Figure 3, we have drafted a two-step selection algorithm for NINV LT recipients towards ISW that takes into account the inclusion/exclusion criteria of current ISW trials,[49,53] the Banff recommendations,[65] and our review findings (Table 2).

Our secondary outcomes – the ISW success rate and the number of graft losses – showed comparable results in children and adults (Tables S6 and S7). In highly selected ISW cohorts, the fraction of successful ISW is usually >30%. A general advantage of children resulting in presumable higher OT rates[8] could not be confirmed. At the

same time, graft loss very rarely occurred, indicating that IS reinstitution in response to ISW failure is mostly successful. We conclude that the risk of ISW in well-structured trials is relatively low, although there is a per se risk in the setting of every clinical trial that includes frequent and invasive monitoring. The current AASLD[66,67] and EASL[68] guidelines recommend a rigorous selection of candidates (see also Figure 3) and close monitoring. EASL and AASLD for children further suggest restricting ISW to trials.[66,68] Future guideline updates will give more guidance for clinicians to perform ISW.

5. Limitations

We acknowledge the following study limitations. (i) The present review exclusively included ISW studies that assessed and reported pre-ISW factors. While the clean presentation of OT-predictors is a strength, the exclusion of important ISW studies that assessed, e.g., biomarkers after OT achievement renders the mapping of ISW studies incomplete. (ii) Our results are irrelevant for ISW candidates with active viral hepatitis – one of our exclusion criteria – since allograft environment and ISW outcomes in these patients are evidently distinct.[25,69,70] (iii) Our finding that IS regimen appears not to impact OT outcome is likely limited by data availability. Indeed, ISW candidates on mTOR-I treatments display several tolerogenic features[71] and a relatively high OT success rate of >50% in a single-arm study.[34] (iv) Our eligibility criteria did not consider different criteria for patient inclusion into ISW studies, which led to some inter-study heterogeneity, e.g. in the times between LT and ISW (Tables S6 and S7) or the duration of follow-up (Table S3). (v) By limiting our study to spontaneous OT, the important research on OT-inducing treatments was not assessed.[4] (vi) We also included conference abstracts, which provide only limited data detail and are lacking the quality control of peer-review.

6. Conclusions

Our results highlight the need for validation and further development as well as new discovery of predictive biomarkers, since clinical parameters – with potential exception of time to ISW – appear to be of limited value to predict OT in LT recipients. The ideal biomarker would have to meet many criteria including being easy and rapid to assess, accurate, with high sensitivity and specificity, reproducible across centers, and affordable. While it is unlikely that a single biomarker will fulfill all these criteria, the results of the first prospective multicenter biomarker-guided ISW studies in adults[49,53] (Table S5) are eagerly awaited. Next to these ongoing validation efforts, all biomarkers demonstrating prognostic value in stand-alone studies (Table 2) will require further assessment and cross-cohort validation. Our comprehensive mapping of biomarkers, including those that are relevant in children, will help guide the design of such follow-up studies. Strong candidates for biomarkers to be scrutinized are allograft gene expression signatures, histopathological scores, and immune exhaustion markers.

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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

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Figure legends

Figure 1: *Timeline of immunosuppression withdrawal in liver transplant recipients.* Following LT (elapsed time variable), allograft recipients can be selected for ISW. Selection is guided by the likelihood of later ISW success (or OT), which can be assessed by liver function tests, biopsy findings, and biomarker assessment. LT recipients with unfavorable prognostic markers at baseline are deemed non-eligible. ISW takes 6-12 months until complete withdrawal (End of ISW) and OT is defined as rejection-free liver allograft survival for at least one year following ISW. Typically during these periods, a majority of patients experience allograft rejection and require reinstitution of IS (ISW failure). ISW success is documented with normal liver function tests and a liver biopsy that confirms the absence of rejection, and requires long-term follow-up.

Figure 2: *Study flow diagram for the selection of articles, conference abstracts, and trial registry entries.*

Figure 3: *Proposition for a two-step selection algorithm towards immunosuppression withdrawal in non-autoimmune/non-replicative viral liver transplant recipients.* Eligibility of LT recipients for ISW should be guided by patient history and liver biopsy. ISW candidates that fulfil the screening criteria (left text box) are subjected to a liver biopsy and detailed histologic analysis (right text box). For adults, also gene expression profile analysis of biopsies should be considered. Candidates that show favorable allograft histology can start ISW. The proposed algorithm is based on inclusion/exclusion criteria of current ISW trials,[49,53] the latest Banff update,[65] and the findings in this review (in italic letters). It should be noted that many details of OT-predictive allograft gene expression profiles (e.g. Which exact gene combinations? Which experimental platforms to quantify gene expression?) are still under active research.[52,53] AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

Supporting information statement

Additional supporting information may be found online in the Supporting Information section at the end of the article.

References

1. Chak E, Saab S. Risk factors and incidence of de novo malignancy in liver transplant recipients: a systematic review. *Liver International* 2010;30(9):1247-1258.
2. Ojo AO, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW et al. Chronic renal failure after transplantation of a nonrenal organ. *The New England journal of medicine* 2003;349(10):931-940.
3. Memaran N, Borchert-Mörlins B, Schmidt BMW, Sugianto RI, Wilke H, Blöte R et al. High Burden of Subclinical Cardiovascular Target Organ Damage After Pediatric Liver Transplantation. *Liver Transplantation* 2019;25(5):752-762.
4. Mathew JM, Leventhal JR. Clinical transplant tolerance: Coming of age. *Human Immunology* 2018;79(5):255-257.
5. Heymann F, Tacke F. Immunology in the liver--from homeostasis to disease. *Nature reviews Gastroenterology & hepatology* 2016;13(2):88-110.
6. Demetris AJ, Bellamy CO, Gandhi CR, Prost S, Nakanuma Y, Stolz DB. Functional Immune Anatomy of the Liver-As an Allograft. *American Journal of Transplantation* 2016;16(6):1653-1680.
7. Thomson AW, Vionnet J, Sanchez-Fueyo A. Understanding, predicting and achieving liver transplant tolerance: from bench to bedside. *Nature reviews Gastroenterology & hepatology* 2020.
8. Levitsky J, Feng S. Tolerance in clinical liver transplantation. *Human Immunology* 2018;79(5):283-287.
9. Vionnet J, Sánchez-Fueyo A. Biomarkers of immune tolerance in liver transplantation. *Human Immunology* 2018;79(5):388-394.
10. Peters MDJ, Godfrey C, McInerney P, Munn Z, Tricco AC, Khalil H. Chapter 11: Scoping Reviews (2020 version). JBI Manual for Evidence Synthesis 2020; Available from: https://wiki.joannabriggs.org/display/MANUAL/Chapter+11%3A+Scoping+reviews
11. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Annals of internal medicine* 2018;169(7):467-473.
12. Appenzeller-Herzog C, Hartleif S, Vionnet J. Clinical parameters and biomarkers predicting spontaneous operational tolerance after liver transplantation: a scoping review protocol. *F1000Res* 2019;8:2059.
13. Baroja-Mazo A, Revilla-Nuin B, de Bejar Á, Martínez-Alarcón L, Herrero JI, El-Tayeb A et al. Extracellular adenosine reversibly inhibits the activation of human regulatory T cells and negatively influences the achievement of the operational tolerance in liver transplantation. *American Journal of Transplantation* 2019;19(1):48-61.
14. Feng S, Ekong UD, Lobritto SJ, Demetris AJ, Roberts JP, Rosenthal P et al. Complete immunosuppression withdrawal and subsequent allograft function among pediatric recipients of parental living donor liver transplants. *JAMA - Journal of the American Medical Association* 2012;307(3):283-293.
15. Millán O, Benitez C, Guillén D, López A, Rimola A, Sánchez-Fueyo A et al. Biomarkers of immunoregulatory status in stable liver transplant recipients undergoing weaning of immunosuppressive therapy. *Clinical Immunology* 2010;137(3):337-346.
16. Pons JA, Ramírez P, Revilla-Nuin B, Pascual D, Baroja-Mazo A, Robles R et al. Immunosuppression withdrawal improves long-term metabolic parameters, cardiovascular risk factors and renal function in liver transplant patients. *Clinical Transplantation* 2009;23(3):329-336.
17. Takatsuki M, Uemoto S, Inomata Y, Egawa H, Kiuchi T, Fujita S et al. Weaning of immunosuppression in living donor liver transplant recipients. *Transplantation* 2001;72(3):449-454.
18. Nafady-Hego H, Li Y, Ohe H, Elgendy H, Zhao X, Sakaguchi S et al. Utility of CD127 combined with FOXP3 for identification of operational tolerance after liver transplantation. *Transplant Immunology* 2016;36:1-8.
19. Benítez C, Londoño MC, Miquel R, Manzia TM, Abraldes JG, Lozano JJ et al. Prospective multicenter clinical trial of immunosuppressive drug withdrawal in stable adult liver transplant recipients. *Hepatology* 2013;58(5):1824-1835.
20. Ohe H, Waki K, Yoshitomi M, Morimoto T, Nafady-Hego H, Satoda N et al. Factors affecting operational tolerance after pediatric living-donor liver transplantation: Impact of early post-transplant events and HLA match. *Transplant International* 2012;25(1):97-106.

21. Pons JA, Yélamos J, Ramírez P, Oliver-Bonet M, Sánchez A, Rodríguez-Gago M et al. Endothelial cell chimerism does not influence allograft tolerance in liver transplant patients after withdrawal of immunosuppression. *Transplantation* 2003;75(7):1045-1047.
22. Waki K, Sugawara Y, Mizuta K, Taniguchi M, Ozawa M, Hirata M et al. Predicting operational tolerance in pediatric living-donor liver transplantation by absence of HLA antibodies. *Transplantation* 2013;95(1):177-183.
23. Lin NC, Wang HK, Yeh YC, Liu CP, Loong CC, Tsai HL et al. Minimization or withdrawal of immunosuppressants in pediatric liver transplant recipients. *Journal of Pediatric Surgery* 2015;50(12):2128-2133.
24. Pons JA, Revilla-Nuin B, Baroja-Mazo A, Ramírez P, Martínez-Alarcón L, Sánchez-Bueno F et al. FoxP3 in peripheral blood is associated with operational tolerance in liver transplant patients during immunosuppression withdrawal. *Transplantation* 2008;86(10):1370-1378.
25. Bohne F, Martínez-Llordella M, Lozano JJ, Miquel R, Benítez C, Londoño MC et al. Intra-graft expression of genes involved in iron homeostasis predicts the development of operational tolerance in human liver transplantation. *Journal of Clinical Investigation* 2012;122(1):368-382.
26. De La Garza RG, Sarobe P, Merino J, Lasarte JJ, D'Avola D, Belsue V et al. Trial of complete weaning from immunosuppression for liver transplant recipients: Factors predictive of tolerance. *Liver Transplantation* 2013;19(9):937-944.
27. Nafady-Hego H, Li Y, Ohe H, Zhao X, Satoda N, Sakaguchi S et al. The generation of donor-specific CD4+CD25 ++CD45RA+ naive regulatory T cells in operationally tolerant patients after pediatric living-donor liver transplantation. *Transplantation* 2010;90(12):1547-1555.
28. García de la Garza R, Sarobe P, Merino J, Lasarte JJ, D'Avola D, Belsue V et al. Immune monitoring of immunosuppression withdrawal of liver transplant recipients. *Transplant Immunology* 2015;33(2):110-116.
29. Perez-Sanz F, Revilla-Nuin B, Marinez-Alarcon L, Herrero JI, Ramirez P, Pons JA et al. Tolerance Biomarkers in Liver Transplantation: Independent External Validation of the Predictive Strength of SENP6 and FEM1C Gene Expression. *Transplantation* 2019;30:30.
30. Bonaccorsi-Riani E, Pennycuick A, Londono MC, Lozano JJ, Benitez C, Sawitzki B et al. Molecular Characterization of Acute Cellular Rejection Occurring During Intentional Immunosuppression Withdrawal in Liver Transplantation. *American Journal of Transplantation* 2016;16(2):484-496.
31. Talisetti A, Hurwitz M, Sarwal M, Berquist W, Castillo R, Bass D et al. Analysis of clinical variables associated with tolerance in pediatric liver transplant recipients. *Pediatric Transplantation* 2010;14(8):976-979.
32. Revilla-Nuin B, de Bejar Á, Martínez-Alarcón L, Herrero JI, Martínez-Cáceres CM, Ramírez P et al. Differential profile of activated regulatory T cell subsets and microRNAs in tolerant liver transplant recipients. *Liver Transplantation* 2017;23(7):933-945.
33. Waki K, Sugawara Y, Mizuta K, Fujita H, Kadowaki T, Kokudo N. Living-donor liver transplantation at the University of Tokyo, 1996-2011: the impact of HLA matching and a positive crossmatch on long-term survival and tolerance. *Clinical transplants* 2011:223-235.
34. Levitsky J, Burrell BE, Kanaparthi S, Turka LA, Kurian S, Sanchez-Fueyo A et al. Immunosuppression Withdrawal in Liver Transplant Recipients on Sirolimus. *Hepatology* 2019.
35. Feng S, Bucuvalas JC, Mazariegos GV, Magee JC, Sanchez-Fueyo A, Spain KM et al. Efficacy and Safety of Immunosuppression Withdrawal in Pediatric Liver Transplant Recipients: Moving Towards Personalized Management. *Hepatology* 2020.
36. Gu G, Lu T, Xu N, Xia Q. Liver transplant tolerance induced by severe or consistent infection revealed the association with T cell exhaustion. *Transplantation* 2018;102(5):112.
37. Sanchez-Fueyo A, Douiri A, Elstad M, Kodela E, Wall J, Riani EB et al. Prevalence of subclinical histological lesions and tolerance biomarkers in long-term adult liver transplant recipients considered for immunosuppression withdrawal. *Journal of Hepatology* 2018;68:S27.
38. Chandran S, Abecassis M, Levitsky J, Feng S, Humar A, Emond J et al. Subclinical histologic findings are observed in 25% of stable adult liver transplant recipients (ALTRs) screened for immunosuppression withdrawal (ISW): OPTIMAL (NCT02533180). *American Journal of Transplantation* 2017;17:778-779.
39. Chruscinski A, Rojas-Luengas V, Macarthur M, Issachar A, Luo J, Yowanto H et al. Results of litmus (NCT 02541916): The liver immune tolerance biomarker utilization study. *Transplant International* 2017;30:108.

40. Chruscinski A, Rojas-Luengas V, Issacher A, Luo J, Yowanto H, Selzner N et al. Results of litmus (nct 02541916): The liver immune tolerance bio marker utilization study. *American Journal of Transplantation* 2018;18:477-478.
41. Vionnet J, Mxquel R, Douiri A, Elstad M, Kodela E, QuagHa A et al. Identification of adult liver transplant recipients eligible to participate in an immunosuppression withdrawal trial employing non-invasive assessments of allograft status. *American Journal of Transplantation* 2019;19:1165.
42. Ramaswami B, Osamu Y, Ippolito R, Metes D, Zeevi A, Thomson A et al. Operationally tolerant pediatric liver transplant recipients exhibit features of immune activation and exhaustion. *Transplantation* 2014;98:231.
43. Danger R, Lozano J, Bonaccorsi-Riani E, Kodela E, Mas-Malavila R, Londono MC et al. Hepatocyte-related miR-193a-3p is over-expressed in allograft biopsy from liver tolerant recipients. *American Journal of Transplantation* 2015;15.
44. Ramaswami B, Yoshida O, Ippolito R, Metes D, Zeevi A, Thomson A et al. Pediatric liver transplant recipients with operational tolerance exhibit features of immune activation and exhaustion. *Journal of Immunology* 2014;192(1).
45. Burrell B, Feng S, Bridges N, Ekong U, Kanaparthi S, Kirk A et al. Tolerant pediatric liver transplant (LT) recipients (TOL) from WISP-R (NCT00320606) split into two phenotypically distinct groups by PD1 expression on CD4+ T cells at baseline, prior to immunosuppression withdrawal (ISW). *American Journal of Transplantation* 2017;17:275-276.
46. Ramaswami B, Yoshida O, Ippolito R, Marrari M, Bentlejewski C, Metes D et al. Pediatric liver transplant recipients with operational tolerance exhibit features of immune activation and exhaustion. *Transplantation* 2015;99(7):115-116.
47. Feng S, Tchao N, Ekong U, Stempora L, Kirk A, Turka L et al. High percentage of PD-1+ CD4+ T cells in a subset of tolerant pediatric liver recipients. *Transplantation* 2014;98:872.
48. Chruscinski A, Rojas-Luengas V, Macarthur M, Issacher A, Luo J, Yowanto H et al. Results of LITMUS (NCT 02541916): The liver immune tolerance bio marker utilization study. *Transplantation* 2018;102(5):307-308.
49. NCT02533180. Evaluation of Donor Specific Immune Senescence and Exhaustion as Biomarkers of Tolerance Post Liver Transplantation. 2015; Available from: https://clinicaltrials.gov/show/NCT02533180
50. NCT01445236. Pilot Study of Immunosuppression Drug Weaning in Liver Recipients Exhibiting Biomarkers of High Likelihood of Tolerance. 2011; Available from: http://clinicaltrials.gov/show/NCT01445236
51. NCT02743793. A Cohort Study of Operationally Tolerant Allograft Recipients. 2016; Available from: https://ClinicalTrials.gov/show/NCT02743793
52. NCT02541916. Liver Immune Tolerance Marker Utilization Study. 2015; Available from: https://ClinicalTrials.gov/show/NCT02541916
53. NCT02498977. Liver Immunosuppression Free Trial. 2015; Available from: https://ClinicalTrials.gov/show/NCT02498977
54. Castellaneta A, Mazariegos GV, Nayyar N, Zeevi A, Thomson AW. HLA-G level on monocytoid dendritic cells correlates with regulatory T-cell Foxp3 expression in liver transplant tolerance. *Transplantation* 2011;91(10):1132-1140.
55. Tokita D, Mazariegos GV, Zahorchak AF, Chien N, Abe M, Raimondi G et al. High PD-L1/CD86 ratio on plasmacytoid dendritic cells correlates with elevated T-regulatory cells in liver transplant tolerance. *Transplantation* 2008;85(3):369-377.
56. Li Y, Zhao X, Cheng D, Haga H, Tsuruyama T, Wood K et al. The presence of Foxp3 expressing T cells within grafts of tolerant human liver transplant recipients. *Transplantation* 2008;86(12):1837-1843.
57. Höfer A, Jonigk D, Hartleben B, Verboom M, Hallensleben M, Manns MP et al. Non-invasive screening for subclinical liver graft injury in adults via donor-specific anti-HLA antibodies. In. *Scientific reports*. 2020: 14242.
58. Xie L, Ichimaru N, Morita M, Chen J, Zhu P, Wang J et al. Identification of a novel biomarker gene set with sensitivity and specificity for distinguishing between allograft rejection and tolerance. *Liver Transplantation* 2012;18(4):444-454.

59. Feng S, Demetris AJ, Spain KM, Kanaparthi S, Burrell BE, Ekong UD et al. Five-year histological and serological follow-up of operationally tolerant pediatric liver transplant recipients enrolled in WISP-R. *Hepatology* 2017;65(2):647-660.
60. Maria Manzia T, Angelico R, Toti L, Angelico C, Quaranta C, Parente A et al. Longterm Survival and Cost-Effectiveness of Immunosuppression Withdrawal After Liver Transplantation. *Liver Transplantation* 2018;24(9):1199-1208.
61. Mazariegos GV, Zahorchak AF, Reyes J, Chapman H, Zeevi A, Thomson AW. Dendritic cell subset ratio in tolerant, weaning and non-tolerant liver recipients is not affected by extent of immunosuppression. *American Journal of Transplantation* 2005;5(2):314-322.
62. Zarkhin V, Talisetti A, Li L, Wozniak LJ, McDiarmid SV, Cox K et al. Expression of soluble HLA-G identifies favorable outcomes in liver transplant recipients. *Transplantation* 2010;90(9):1000-1005.
63. Zhao X, Li Y, Ohe H, Nafady-Hego H, Uemoto S, Bishop GA et al. Intragraft Vδ1 γδ T cells with a unique T-Cell receptor are closely associated with pediatric semiallogeneic liver transplant tolerance. *Transplantation* 2013;95(1):192-202.
64. Shaked A, DesMarais MR, Kopetskie H, Feng S, Punch JD, Levitsky J et al. Outcomes of immunosuppression minimization and withdrawal early after liver transplantation. *American Journal of Transplantation* 2019;19(5):1397-1409.
65. Demetris AJ, Bellamy C, Hubscher SG, O'Leary J, Randhawa PS, Feng S et al. 2016 Comprehensive Update of the Banff Working Group on Liver Allograft Pathology: Introduction of Antibody-Mediated Rejection. *American Journal of Transplantation* 2016;16(10):2816-2835.
66. Kelly DA, Bucuvalas JC, Alonso EM, Karpen SJ, Allen U, Green M et al. Long-term medical management of the pediatric patient after liver transplantation: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Liver Transplantation* 2013;19(8):798-825.
67. Lucey MR, Terrault N, Ojo L, Hay JE, Neuberger J, Blumberg E et al. Long-term management of the successful adult liver transplant: 2012 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Liver Transplantation* 2013;19(1):3-26.
68. European Association for the Study of the Liver. Electronic address eee. EASL Clinical Practice Guidelines: Liver transplantation. *Journal of Hepatology* 2016;64(2):433-485.
69. Bohne F, Londoño MC, Benítez C, Miquel R, Martínez-Llordella M, Russo C et al. HCV-induced immune responses influence the development of operational tolerance after liver transplantation in humans. *Science Translational Medicine* 2014;6(242).
70. Orlando G, Manzia T, Baiocchi L, Sanchez-Fueyo A, Angelico M, Tisone G. The Tor Vergata weaning off immunosuppression protocol in stable HCV liver transplant patients: The updated follow up at 78 months. *Transplant Immunology* 2008;20(1-2):43-47.
71. Levitsky J, Mathew JM, Abecassis M, Tambur A, Leventhal J, Chandrasekaran D et al. Systemic immunoregulatory and proteogenomic effects of tacrolimus to sirolimus conversion in liver transplant recipients. *Hepatology* 2013;57(1):239-248.

Table 1: Association of pre-immunosuppression withdrawal clinical parameters with OT

Clinical Parameter	Studies Endorsing Association	Evidence[#] OT	Evidence[#] non-OT	Studies with Discordant Findings (*Trend for Association*, Opposite Conclusion)	Association[§]
Pediatric Studies					
Longer Time Between LT and ISW	"Kyoto"[18]	4725 ± 1102 d	4135 ± 1099 d	"Taipei"*[23], "Tochigi"[22], "iWITH"[35]	**+/-**
	"WISP-R"[14]	100.6 (71.8-123.5) mo	73.0 (57.6-74.9) mo		
		mean ± SD			
		median (IQR)			
Age at LT	"Stanford"[31]	1.4 (0.3 - 4.9) yr	3.4 (0.3 - 16) yr	"Tochigi"[33], "WISP-R"[14], "Kyoto"[20], "Taipei"[23], "iWITH"[35]	-
		mean (range)			
Female Sex	"Tochigi"[33]	89%	52%	"Kyoto"[18], "Stanford"[31], "WISP-R"[14], "Taipei"[23], "iWITH"[35]	-
Liver Disease Etiology	"Taipei"[23]	3:2	1:9	"Kyoto"[20], "Stanford"[31], "Tochigi"[22], "WISP-R"[14]	-
		Metabolic:Parenchymal			
History of Rejection	"Kyoto"[20]	83%	60%	"Tochigi"[22], "WISP-R"[14], "iWITH"[35]	-
		Patients with absence of early rejection			
HLA Mismatch	"Kyoto"[20]	75%, 94%	89%, 89%	"Tochigi"[22], "WISP-R"[14], "Taipei"[23], "iWITH"[35]	-
		Patients (HLA-A, HLA-B)			
IS Regimen	"Kyoto"[20]	17 ± 12 ng/ml	11 ± 5 ng/ml	"WISP-R"[14]	-
		TAC trough levels 1 wk post-LT, mean ± SD			
Additional clinical parameters with no current evidence of prognostic value are reported in Table S5.					
Adult Studies					
Longer Time Between LT and ISW	"Barcelona/Rome/Leuven"[25]	131 ± 47 mo	83 ± 40 mo	*"Chicago"*[34]	**+/-**
	"Murcia 1-3 & Pamplona"[32]	10.8/12 (4-18) yr	6.1/5 (4-14) yr		
		mean ± SD			
		mean/median (range)			
Age at LT	"Barcelona/Rome/Leuven"[19,25]	50 ± 10 yr	45 ± 11 yr	"Murcia 1"[16], "Murcia 3"[13], "Chicago"[34]	-
		mean ± SD			
Male Sex	"Barcelona/Rome/Leuven"[19]	83%	58%	"Murcia 1"[16], "Pamplona"[26], "Murcia 3"[13], "Chicago"[34]	-
Liver Disease Etiology				"Barcelona/Rome/Leuven"[19], "Pamplona"[26], "Murcia 3"[13]	-
History of Rejection				"Murcia 1"[16], "Barcelona/Rome/Leuven"[19]	-
HLA Mismatch				"Barcelona/Rome/Leuven"[19]	
IS Regimen	"Barcelona/Rome/Leuven"[19]	56%	79%	"Murcia 1"[16], "Pamplona"[28], "Murcia 3"[13]	-
		Patients with CNI			
Additional clinical parameters with no current evidence of prognostic value are reported in Table S6.					

[#] From report with largest sample size, * For numerical evidence, see Table S4, [§] Expert opinion: +, strong; +/-, moderate; -, weak

CNI, calcineurin inhibitor; d, days; HLA, human leukocyte antigen; IQR, interquartile range; IS, immunosuppression; ISW, immunosuppression withdrawal; LT, liver transplantation; ml, milliliters; mo, months; ng, nanograms; SD, standard deviation; wk, weeks; yr, years

Table 2: Association of pre-immunosuppression withdrawal biomarkers with OT

Biomarker	Studies Endorsing Association	Evidence		Studies with Discordant Findings (*Trend for Association*)	Association[§]
		OT	non-OT		
Pediatric Studies					
Non-Invasive Biomarkers					
Lower Anti-HLA Antibodies (incl. DSA)	"Tochigi"[22]	p < 0.03 Post-LT class I, HLA-B, HLA-C, class II, HLA-DQ, HLA-DR		"WISP-R"[14], "iWITH"[35]	-
Additional non-invasive biomarkers with no current evidence of prognostic value are reported in Table S5.					
Invasive Biomarkers					
No Portal Inflammation	"WISP-R"[14] "iWITH"[35]	91.7 (61.5-99.8)% Patients (95CI) 0.36 (0.14-0.90) Mild vs No Portal Inflammation, OR (95CI)	42.9 (9.9-81.6)%		+
Fewer APC:Lymphocyte Pairs	"iWITH"[35]	0.82 (0.74-0.92) OR (95CI)			+/-
Lower C4d Score	"WISP-R"[14]	6.1 (5.1-9.3) median (IQR)	12.5 (9.3-16.8)		+/-
Lower Load of Leukocytes	"iWITH"[35]	0.97 (0.95-0.99) OR (95CI)			-
Lower Load of Infiltrating Monocytes/Macrophages	"iWITH"[35]	0.91 (0.85-0.97) OR (95CI)			-
***[CDHR2, MIF, PEBP1, SOCS1, TFRC]* Expression in Allograft**				"iWITH"[35]	-
Adult Studies					
Non-Invasive Biomarkers					
Lower Lymphocyte Proliferation	"Pamplona"[26,28]	7.5 (2.1-23) *In vitro* Stimulated Lymphocyte Proliferation Index, median (IQR)	41.7 (19-65)		+/-
NK Cells Higher in PBMC	"Barcelona/Rome/Leuven"[25] "Pamplona"[26,28]	NR 14.0 (9.9-20.7)% median (IQR), p = 0.07	8.3 (6.0-13.7)%	*"Murcia 1"*[24], "Chicago"[34]	+/-
Vδ2-TCR γδT Cells Higher in PBMC	"Barcelona/Rome/Leuven"[25]	p = 0.03		*"Murcia 1"*[24], "Murcia 3"[29]	+/-
"Tolerogenic DC" Higher in PBMC	"Chicago"[34]	p < 0.01			+/-
Naive CD8+ Cells Lower in PBMC	"Chicago"[34]	p < 0.01			+/-
TEMRA CD8+ Cells Higher in PBMC	"Chicago"[34]	p < 0.05			+/-
Eomes+ CD8+ Cells Higher in PBMC	"Chicago"[34]	p < 0.05			+/-
Higher *FEM1C* Expression in PBMC	"Murcia 3"[29]	AUC 0.967			+/-
Higher *SENP6* Expression in PBMC	"Murcia 3"[29]	AUC 0.933			+/-
Higher *miR95* Expression in PBMC	"Murcia 3"[29]	AUC 0.867			+/-
Higher *miR31* Expression in PBMC	"Murcia 3"[29]	AUC 0.967			+/-

Higher Serum Ferritin	"Barcelona/Rome/Leuven"[25]	185.5 (26-864) ng/ml vs 73.5 (3-304) ng/ml mean (range), p < 0.01	*"Murcia 3"*[29]	**+/-**
Lower Cytokine Secretion	"Barcelona/Rome/Leuven"[15]	p < 0.05 *In vitro* IFNγ secretion	"Pamplona"[28]	**-**
Treg Cells Higher in PBMC			"Murcia 1"[24], "Barcelona/Rome/Leuven"[25], "Pamplona"[26,28], "Murcia 3"[29], "Chicago"[34]	**-**
Higher *FoxP3* Expression in PBMC	"Barcelona/Rome/Leuven"[30]	NR	"Murcia 1"[24]	**-**
***[NCR1, PDGFRB, PSMD14]* Expression in PBMC**	"Barcelona/Rome/Leuven"[25]	AUC 0.76		**-**
***[SLAMF7, KLRF1, CLIC3, PSMD14, ALG8, CX3CR1]* Expression in PBMC**	"Barcelona/Rome/Leuven"[25]	AUC 0.71		**-**
Lower Anti-HLA Antibodies (incl. DSA)			"Barcelona/Rome/Leuven"[19], "Chicago"[34]	**-**
Higher Serum Hepcidin-25	"Barcelona/Rome/Leuven"[25]	p < 0.05	*"Murcia 3"*[29]	**-**
Additional non-invasive biomarkers with no current evidence of prognostic value are reported in Table S6.				
Invasive Biomarkers				
***[CDHR2, MIF, PEBP1, SOCS1, TFRC]* Expression in Allograft**	"Barcelona/Rome/Leuven"[25]	49 (7-343) [89+80% SN, 86+100% SP, 80+100% PPV, 92+85% NPV, 13+9.5% ER, AUC 0.83]		**+**
	"Chicago"[34]	OR (95CI) 88% SN, 83% SP, 88% PPV, 83% NPV		
Higher Load of CD4+ Cells	"Chicago"[34]	178 (168-205) vs 85 (69-158) median (IQR)		**+/-**
Higher Hepatocytic Iron	"Barcelona/Rome/Leuven"[25]	p < 0.01		**+/-**
No Portal Inflammation			"Barcelona/Rome/Leuven"[25]	**-**

§ Expert opinion: +, strong; +/-, moderate; -, weak

APC, antigen-presenting cell; AUC, area under the curve; DC, dendritic cell; DSA, donor-specific antibodies; ER, overall error rate; HLA, human leukocyte antigen; IFNγ, interferon gamma; IQR, interquartile range; ISW, immunosuppression withdrawal; LT, liver transplantation; ml, milliliters; ng, nanograms; NK, natural killer; NPV, negative predictive value; NR, not reported; OR, odds ratio; PBMC, peripheral blood mononuclear cells; PPV, positive predictive value; SN, sensitivity; SP, specificity; Treg, regulatory T-cell; 95CI, 95% confidence interval

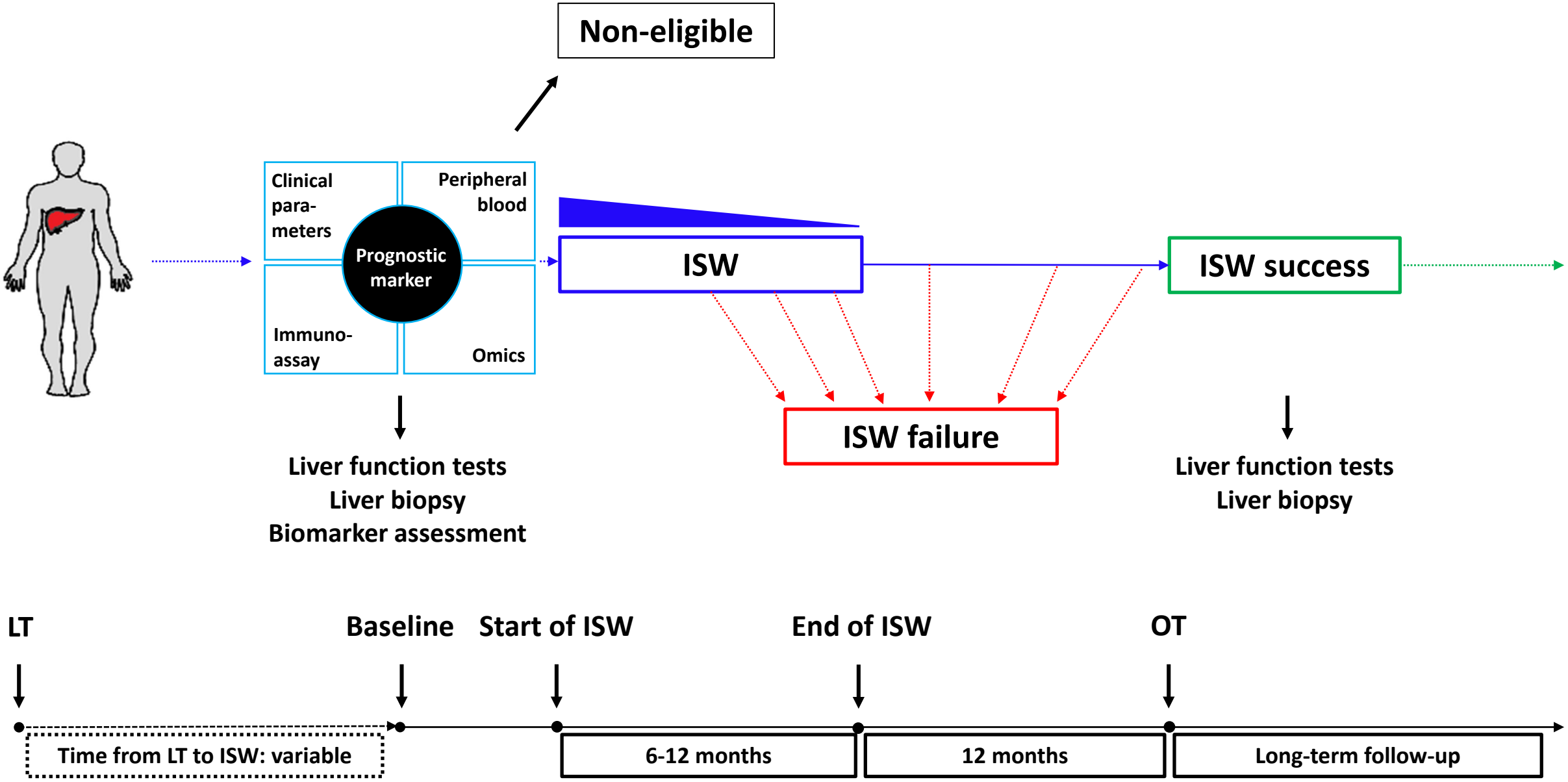
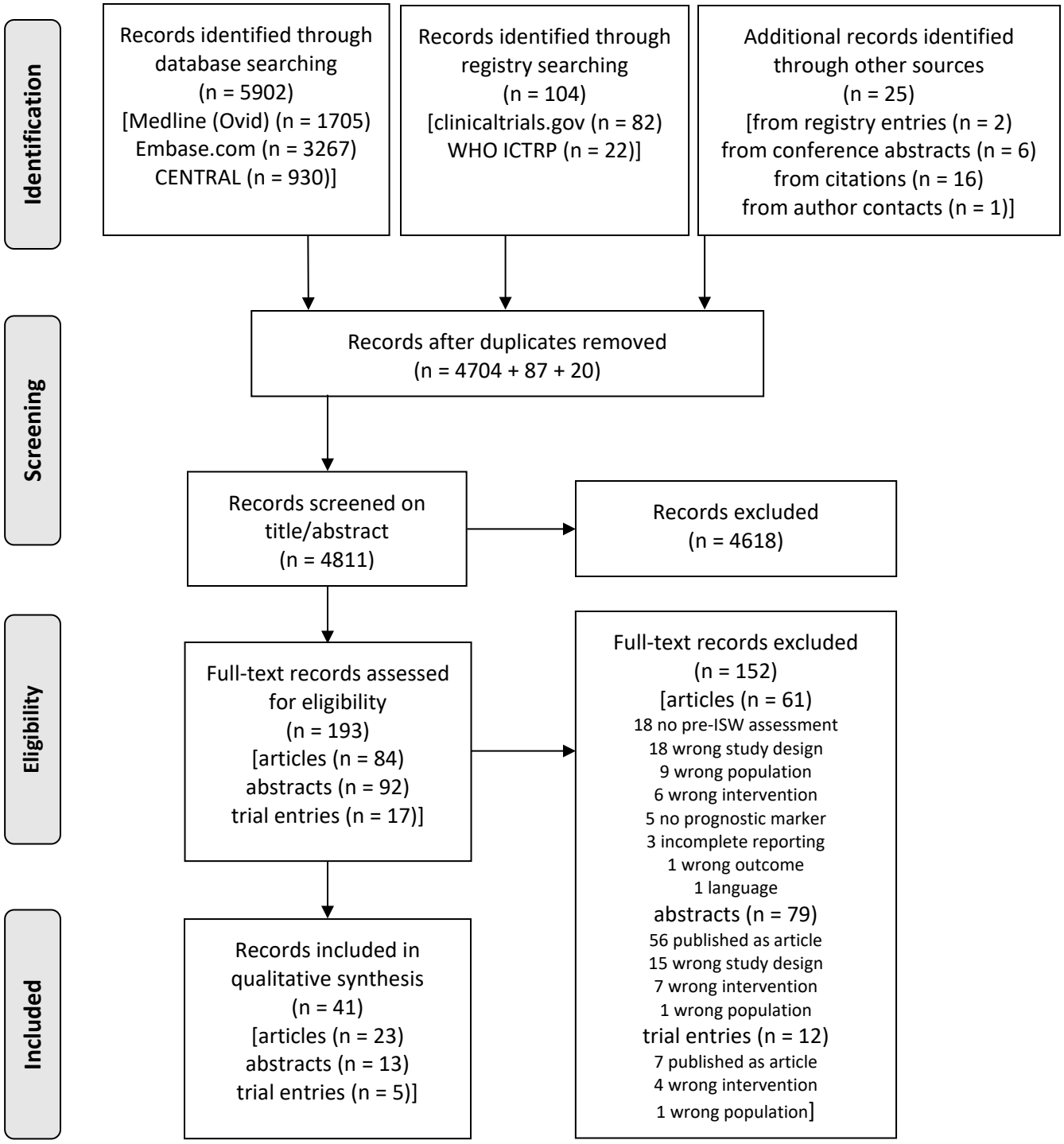

Non-eligible
Clinical para-meters
Peripheral blood
Prognostic marker
Immuno-assay
Omics
ISW
ISW success
ISW failure
Liver function tests
Liver biopsy
Biomarker assessment
Liver function tests
Liver biopsy
LT
Baseline
Start of ISW
End of ISW
OT
Time from LT to ISW: variable
6-12 months
12 months
Long-term follow-up

Figure 1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Records identified through registry searching (n = 104) [clinicaltrials.gov (n = 82) WHO ICTRP (n = 22)]
Additional records identified through other sources (n = 25) [from registry entries (n = 2) from conference abstracts (n = 6) from citations (n = 16) from author contacts (n = 1)]
Screening
Records after duplicates removed (n = 4704 + 87 + 20)
Records screened on title/abstract (n = 4811)
Records excluded (n = 4618)
Eligibility
Full-text records assessed for eligibility (n = 193) [articles (n = 84) abstracts (n = 92) trial entries (n = 17)]
Full-text records excluded (n = 152) [articles (n = 61)
18 no pre-ISW assessment
18 wrong study design
9 wrong population
6 wrong intervention
5 no prognostic marker
3 incomplete reporting
1 wrong outcome
1 language
abstracts (n = 79)
56 published as article
15 wrong study design
7 wrong intervention
1 wrong population
trial entries (n = 12)
7 published as article
4 wrong intervention
1 wrong population]
Included
Records included in qualitative synthesis (n = 41) [articles (n = 23) abstracts (n = 13) trial entries (n = 5)]

Figure 2

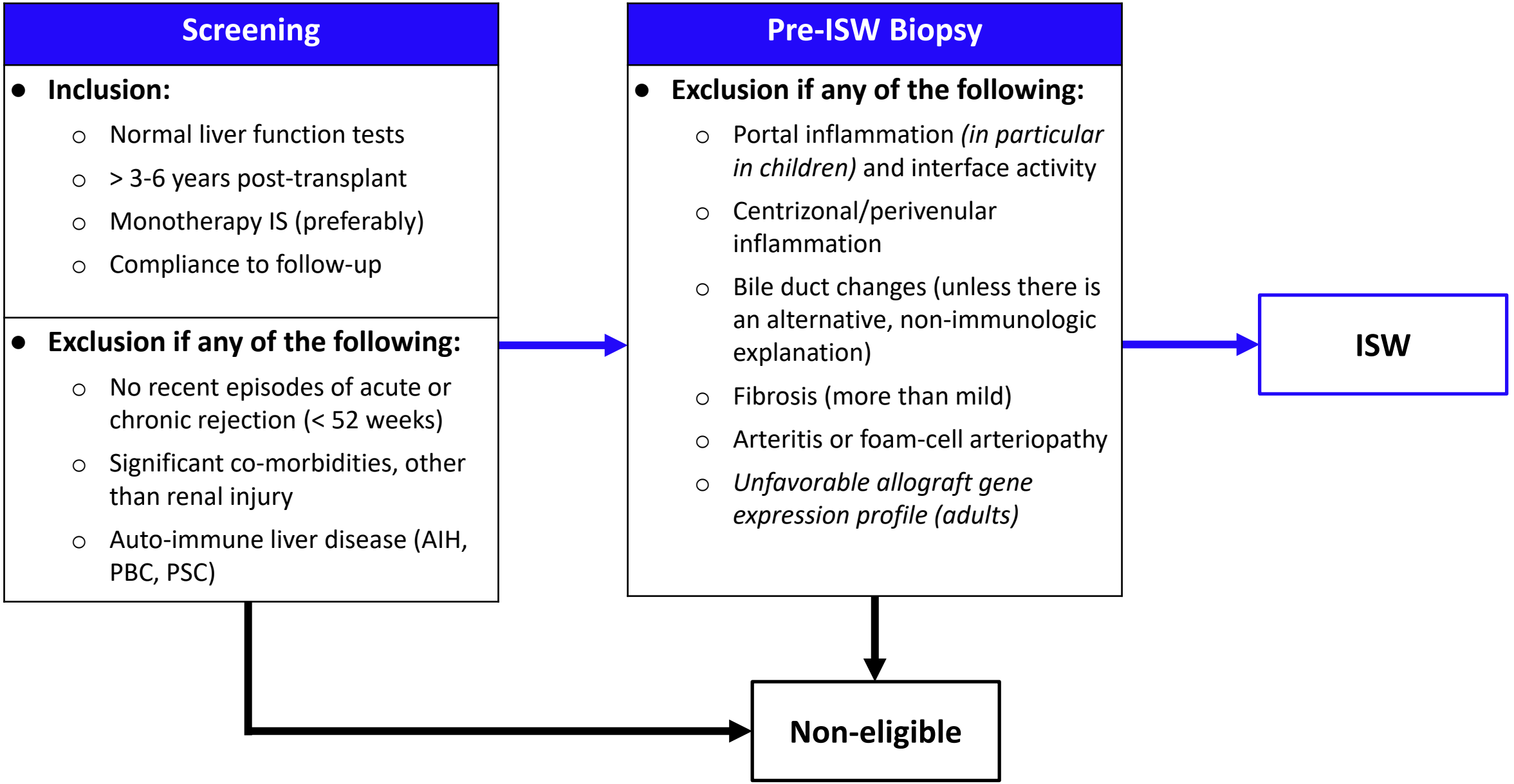
Screening
Inclusion:
Normal liver function tests
> 3-6 years post-transplant
Monotherapy IS (preferably)
Compliance to follow-up
Exclusion if any of the following:
No recent episodes of acute or chronic rejection (< 52 weeks)
Significant co-morbidities, other than renal injury
Auto-immune liver disease (AIH, PBC, PSC)
Pre-ISW Biopsy
Exclusion if any of the following:
Portal inflammation (in particular in children) and interface activity
Centrizonal/perivenular inflammation
Bile duct changes (unless there is an alternative, non-immunologic explanation)
Fibrosis (more than mild)
Arteritis or foam-cell arteriopathy
Unfavorable allograft gene expression profile (adults)
ISW
Non-eligible

Figure 3

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('liver transplantation'/exp OR (OLT OR LTx):ab,ti OR (('liver'/de OR 'liver lobe'/exp OR 'liver disease'/exp OR 'obstructive bile duct disease'/exp OR 'bile duct atresia'/de OR (liver OR hepatic OR hepato* OR hepatis OR hepatitis OR intrahepatic OR extrahepatic OR cirrhosis OR cirrhotic OR 'periportal fibrosis' OR jaundice OR icterus OR bilirubinaemia OR cholestasis OR cholestatic OR ((bile OR biliary OR choledoch*) NEAR/3 (obstruction OR stasis OR occlusion OR stenosis OR stricture OR obliteration OR atresia OR agenesi*))):ab,ti) AND ('transplantation'/de OR 'organ transplantation'/de OR 'allotransplantation'/de OR 'orthotopic transplantation'/de OR 'recipient'/exp OR (transplant* OR Tx OR allotransplant* OR graft* OR allograft* OR recipient*):ab,ti)))

AND

((('immunosuppressive agent'/exp OR 'calcineurin inhibitor'/exp OR 'mammalian target of rapamycin inhibitor'/de OR 'immunosuppressive treatment'/de) AND ('treatment withdrawal'/exp OR 'weaning'/de)) OR ((((immunosuppress* OR immuno-suppress* OR immune-suppress* OR immunodepress* OR immuno-depress* OR immune-depress* OR anti-rejection OR antirejection OR 'immune system-suppressing' OR 'transplantation reaction inhibition' OR anti-metaboli* OR antimetaboli* OR azathioprine OR belatacept OR cyclophosphamide OR daclizumab OR 'mycophenolate mofetil' OR MMF OR 'mycophenolic acid' OR cellcept OR 'calcineurin inhibitor*' OR 'protein phosphatase 2B inhibitor*' OR cyclosporin* OR ciclosporin* OR neoral OR sandim* OR tacrolimus OR advagraf OR prograf* OR fk506 OR fk-506 OR 'mammalian target of rapamycin inhibitor*' OR 'mammalian target of rapamycin kinase inhibitor*' OR 'mechanistic target of rapamycin inhibitor*' OR 'mechanistic target of rapamycin kinase inhibitor*' OR 'mTOR inhibitor*' OR 'mTOR kinase inhibitor*' OR everolimus OR rad001* OR rad-001* OR rapamune OR rapamycin OR sirolimus) NEAR/4 (withdraw* OR taper* OR wean* OR minimization OR minimisation OR minimizing OR minimising OR sparing OR eliminat* OR reduction OR reducing OR lower* OR cessation OR discontinu* OR interrupt* OR abstinence OR avoid* OR stop* OR downgrad* OR diminish* OR free*)) OR is-withdraw* OR is-taper* OR is-wean* OR is-minimization OR is-minimisation OR is-minimizing OR is-minimising OR is-sparing OR is-eliminat* OR is-reduction OR is-reducing OR is-lower* OR is-cessation OR is-discontinu* OR is-interrupt* OR is-abstinence OR is-avoid* OR is-stop* OR is-downgrad* OR is-diminish* OR is-free):ab,ti))

AND

('transplantation tolerance'/de OR 'immunological tolerance'/de OR 'immunoregulation'/de OR 'immunoreactivity'/de OR 'graft survival'/de OR 'liver biopsy'/de OR (tolerogen* OR 'tolerant patient*' OR 'tolerant state' OR 'state of tolerance' OR 'sustained weaning' OR ((transplant* OR posttransplant* OR operational* OR immune OR immunologic* OR alloimmune OR allograft* OR graft* OR alloantigen* OR antigen* OR chimerism OR donor-specific OR peripheral) NEAR/3 (tolerance OR tolerant OR tolerated OR tolerating OR acceptance OR protect* OR quiescen* OR unresponsive* OR nonresponsive* OR un-responsive* OR non-responsive*)) OR immunoregulat* OR immunosurveill* OR immunoreactiv* OR immunoactiv* OR ((immune OR immunologic*) NEXT (regulat* OR surveill* OR reactiv* OR activ*)) OR ((graft OR allograft OR transplant* OR liver OR hepatic) NEAR/3 (survival OR health OR function OR 'resistance to rejection')) OR ((inhibit* OR decrease OR abolish OR suppress* OR reduc* OR ameliorat* OR improve* OR absent OR avoid* OR prevent*) NEAR/3 (graft OR allograft OR transplant* OR liver OR hepatic) NEAR/3 (injury OR complication* OR dysfunction OR inflammation OR fibrosis OR infiltration)) OR ((inhibit* OR decrease OR abolish OR suppress* OR reduc* OR ameliorat* OR improve* OR absent OR avoid* OR prevent*) NEAR/3 (rejection OR 'immune response*' OR 'alloimmune response*' OR 'T-cell response*' OR 'B-

cell response*' OR 'antibody response*' OR 'humoral response*')) OR ((liver OR hepatic) NEAR/3 (biopsy OR biopsies OR puncture*))):ab,ti)

NOT

(('animal'/de OR 'animal experiment'/exp OR 'nonhuman'/de) NOT ('human'/exp OR 'human experiment'/de))

NOTE: The subject heading "graft rejection" (and respective free text terms) was omitted, because its inclusion resulted in a non-manageable increase of hits.

Medline (Ovid)

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(liver transplantation/ OR (OLT OR LTx).ab,ti. OR ((liver/ OR exp liver diseases/ OR exp cholestasis/ OR biliary atresia/ OR (liver OR hepatic OR hepato* OR hepatis OR hepatitis OR intrahepatic OR extrahepatic OR cirrhosis OR cirrhotic OR periportal fibrosis OR jaundice OR icterus OR bilirubinaemia OR cholestasis OR cholestatic OR ((bile OR biliary OR choledoch*) ADJ3 (obstruction OR stasis OR occlusion OR stenosis OR stricture OR obliteration OR atresia OR agenesi*))).ab,ti.) AND (transplantation/ OR organ transplantation/ OR transplant recipients/ OR (transplant* OR Tx OR allotransplant* OR graft* OR allograft* OR recipient*).ab,ti.)))

AND

(((exp immunosuppressive agents/ OR calcineurin inhibitors/ OR exp sirolimus/ OR tacrolimus/ OR cyclosporine/ OR azathioprine/ OR cyclophosphamide/ OR daclizumab/ OR mycophenolic acid/ OR immunosuppression/) AND (ad.fs OR substance withdrawal syndrome/ OR withholding treatment/)) OR (((immunosuppress* OR immuno-suppress* OR immune-suppress* OR immunodepress* OR immuno-depress* OR immune-depress* OR anti-rejection OR antirejection OR immune system-suppressing OR transplantation reaction inhibition OR anti-metaboli* OR antimetaboli* OR azathioprine OR belatacept OR cyclophosphamide OR daclizumab OR mycophenolate mofetil OR MMF OR mycophenolic acid OR cellcept OR calcineurin inhibitor* OR protein phosphatase 2B inhibitor* OR cyclosporin* OR ciclosporin* OR neoral OR sandim* OR tacrolimus OR advagraf OR prograf* OR fk506 OR fk-506 OR mammalian target of rapamycin inhibitor* OR mammalian target of rapamycin kinase inhibitor* OR mechanistic target of rapamycin inhibitor* OR mechanistic target of rapamycin kinase inhibitor* OR mTOR inhibitor* OR mTOR kinase inhibitor* OR everolimus OR rad001* OR rad-001* OR rapamune OR rapamycin OR sirolimus) ADJ4 (withdraw* OR taper* OR wean* OR minimization OR minimisation OR minimizing OR minimising OR sparing OR eliminat* OR reduction OR reducing OR lower* OR cessation OR discontinu* OR interrupt* OR abstinence OR avoid* OR stop* OR downgrad* OR diminish* OR free*)).ab,ti.))

AND

(transplantation tolerance/ OR immune tolerance/ OR graft survival/ OR (tolerogen* OR tolerant patient* OR tolerant state OR state of tolerance OR sustained weaning OR ((transplant* OR posttransplant* OR operational* OR immune OR immunologic* OR alloimmune OR allograft* OR graft* OR alloantigen* OR antigen* OR chimerism OR donor-specific OR peripheral) ADJ3 (tolerance OR tolerant OR tolerated OR tolerating OR acceptance OR protect* OR quiescen* OR unresponsive* OR nonresponsive* OR un-responsive* OR non-responsive*)) OR immunoregulat* OR immunosurveill* OR immunoreactiv* OR immunoactiv* OR ((immune OR immunologic*) ADJ

(regulat* OR surveill* OR reactiv* OR activ*)) OR ((graft OR allograft OR transplant* OR liver OR hepatic) ADJ3 (survival OR health OR function OR resistance to rejection)) OR ((inhibit* OR decrease OR abolish OR suppress* OR reduc* OR ameliorat* OR improve* OR absent OR avoid* OR prevent*) ADJ3 (graft OR allograft OR transplant* OR liver OR hepatic) ADJ3 (injury OR complication* OR dysfunction OR inflammation OR fibrosis OR infiltration)) OR ((inhibit* OR decrease OR abolish OR suppress* OR reduc* OR ameliorat* OR improve* OR absent OR avoid* OR prevent*) ADJ3 (rejection OR immune response* OR alloimmune response* OR T-cell response* OR B-cell response* OR antibody response* OR humoral response*)) OR ((liver OR hepatic) ADJ3 (biopsy OR biopsies OR puncture*))).ab,ti.)

NOT

(exp animals/ NOT humans/)

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((OLT OR LTx OR ((liver OR hepatic OR hepato* OR hepatis OR hepatitis OR intrahepatic OR extrahepatic OR cirrhosis OR cirrhotic OR 'periportal fibrosis' OR jaundice OR icterus OR bilirubinaemia OR cholestasis OR cholestatic OR ((bile OR biliary OR choledoch*) NEAR/3 (obstruction OR stasis OR occlusion OR stenosis OR stricture OR obliteration OR atresia OR agenesi*))) AND (transplant* OR Tx OR allotransplant* OR graft* OR allograft* OR recipient*))):ab,ti) AND ((((immunosuppress* OR immuno-suppress* OR immune-suppress* OR immunodepress* OR immuno-depress* OR immune-depress* OR anti-rejection OR antirejection OR 'immune system-suppressing' OR 'transplantation reaction inhibition' OR anti-metaboli* OR antimetaboli* OR azathioprine OR belatacept OR cyclophosphamide OR daclizumab OR 'mycophenolate mofetil' OR MMF OR 'mycophenolic acid' OR cellcept OR 'calcineurin inhibitor*' OR 'protein phosphatase 2B inhibitor*' OR cyclosporin* OR ciclosporin* OR neoral OR sandim* OR tacrolimus OR advagraf OR prograf* OR fk506 OR fk-506 OR 'mammalian target of rapamycin inhibitor*' OR 'mammalian target of rapamycin kinase inhibitor*' OR 'mechanistic target of rapamycin inhibitor*' OR 'mechanistic target of rapamycin kinase inhibitor*' OR 'mTOR inhibitor*' OR 'mTOR kinase inhibitor*' OR everolimus OR rad001* OR rad-001* OR rapamune OR rapamycin OR sirolimus) NEAR/4 (withdraw* OR taper* OR wean* OR minimization OR minimisation OR minimizing OR minimising OR sparing OR eliminat* OR reduction OR reducing OR lower* OR cessation OR discontinu* OR interrupt* OR abstinence OR avoid* OR stop* OR downgrad* OR diminish* OR free*)) OR (is NEXT withdraw*) OR (is NEXT taper*) OR (is NEXT wean*) OR (is NEXT minimization) OR (is NEXT minimisation) OR (is NEXT minimizing) OR (is NEXT minimising) OR (is NEXT sparing) OR (is NEXT eliminat*) OR (is NEXT reduction) OR (is NEXT reducing) OR (is NEXT lower*) OR (is NEXT cessation) OR (is NEXT discontinu*) OR (is NEXT interrupt*) OR (is NEXT abstinence) OR (is NEXT avoid*) OR (is NEXT stop*) OR (is NEXT downgrad*) OR (is NEXT diminish*) OR (is NEXT free)):ab,ti) AND ((tolerogen* OR 'tolerant patient*' OR 'tolerant state' OR 'state of tolerance' OR 'sustained weaning' OR ((transplant* OR posttransplant* OR operational* OR immune OR immunologic* OR alloimmune OR allograft* OR graft* OR alloantigen* OR antigen* OR chimerism OR donor-specific OR peripheral) NEAR/3 (tolerance OR tolerant OR tolerated OR tolerating OR acceptance OR protect* OR quiescen* OR unresponsive* OR nonresponsive* OR un-responsive* OR non-responsive*)) OR immunoregulat* OR immunosurveill* OR immunoreactiv* OR immunoactiv* OR ((immune OR immunologic*) NEXT (regulat* OR surveill* OR reactiv* OR activ*)) OR ((graft OR allograft OR transplant* OR liver OR

hepatic) NEAR/3 (survival OR health OR function OR 'resistance to rejection')) OR ((inhibit* OR decrease OR abolish OR suppress* OR reduc* OR ameliorat* OR improve* OR absent OR avoid* OR prevent*) NEAR/3 (graft OR allograft OR transplant* OR liver OR hepatic) NEAR/3 (injury OR complication* OR dysfunction OR inflammation OR fibrosis OR infiltration)) OR ((inhibit* OR decrease OR abolish OR suppress* OR reduc* OR ameliorat* OR improve* OR absent OR avoid* OR prevent*) NEAR/3 (rejection OR 'immune response*' OR 'alloimmune response*' OR 'T-cell response*' OR 'B-cell response*' OR 'antibody response*' OR 'humoral response*')) OR ((liver OR hepatic) NEAR/3 (biopsy OR biopsies OR puncture*))):ab,ti)

ClinicalTrials.gov

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tolerance AND liver transplantation *(in OTHER TERMS)*

WHO ICTRP

(20190903; 22 hits)

tolerance *(in TITLE)*

AND

liver transplantation *(in CONDITION)*

Table S1: Data Items

No.	Description
1	Article characteristics
1a	First author
1b	Year of publication
1c	Bibliographic details
2	Country/ies of study
3	Trial ID
4	Study design
4a	Monocenter/multicenter study
4b	Prospective/retrospective
4c	IS maintenance or ISW control group yes/no
4d	IS drug(s)
4e	Pre-ISW biopsy
4f	ISW schedule
4g	Method(s) for assessing OT
4h	Duration of follow-up
5	Study population
5a	Paediatric/adult/mixed
5b	DDLT/LDLT/mixed
5c	Recipient age at LT
5d	Liver disease aetiology
5e	Viral status during ISW
5f	Time from LT to ISW
5g	Reasons for ISW elective/non-elective
6	Clinical parameters predicting OT
6a	Numerical evidence for positive associations
7	Biomarkers predicting OT
7a	Numerical evidence for positive associations
8	Clinical parameters not predicting OT
9	Biomarkers not predicting OT
10	Total number of patients that are included in the prognostic analyses
11	Incidence of OT
12	Number of graft losses

Table S2: Characteristics of excluded studies

[ordered by articles, abstracts, and registry entries and by first-author names]

Study (Article)	Reason for exclusion
Aini 2014[1]	Wrong Study Design
Aini 2012[2]	Wrong Study Design
Assy 2007[3]	Incomplete Reporting (unclear how 11 non-rejection patients relate to 2 OT patients)
Bourdeaux 2013[4]	Wrong Intervention (IS minimization)
Castellaneta 2011[5]	No pre-ISW Assessment
Craciun 2007[6]	Wrong Intervention (immunomodulation with stem cells)
Devlin 1998[7]	Wrong Population (> 20% viral/autoimmune)
Duizendstra 2019[8]	Wrong Study Design (no non-OT group)
Feng 2017[9]	Wrong Study Design (no non-OT group)
Geng 2017[10]	Incomplete Reporting (non-OT group incompletely described)
Geng 2018[11]	Wrong Study Design (no ISW study)
Girlanda 2005[12]	Wrong Population (> 20% viral/autoimmune)
Girnita 2010[13]	No pre-ISW Assessment
Haarer 2016[14]	Wrong Study Design (no ISW study)
Hsu 2007[15]	Wrong Study Design (no ISW study)
Inomata 1999[16]	LANGUAGE (cases likely correspond to the 26 elective cases in included Takatsuki report)
Jhun 2018[17]	No Prognostic Marker Reported
Jucaud 2019[18]	Wrong Population (> 20% viral/autoimmune)
Kasahara 2002[19]	Wrong Study Design (no ISW study)
Kawasaki 2007[20]	No pre-ISW Assessment
Koshiba 2007[21]	No pre-ISW Assessment
Koshiba 2015[22]	Wrong Study Design (book chapter)
Lau 2016[23]	No pre-ISW Assessment
Lee 2009[24]	Wrong Study Design (no non-OT group)
Levine 2017[25]	Wrong Study Design (editorial)
Li 2012[26]	No pre-ISW Assessment
Li 2008[27]	No pre-ISW Assessment
Li 2004[28]	No pre-ISW Assessment
Manez 1994[29]	Wrong Intervention (temporary ISW)
Manzia 2018[30]	No Prognostic Marker Reported
Martínez-Llordella 2008[31]	No pre-ISW Assessment
Martinez-Llordella 2007[32]	No pre-ISW Assessment
Mazariegos 1997[33]	Wrong Population (> 20% viral/autoimmune)
Mazariegos 1997[34]	Wrong Population (> 20% viral/autoimmune)
Mazariegos 2007[35]	No pre-ISW Assessment
Mazariegos 2005[36]	No pre-ISW Assessment
Mazariegos 2003[37]	No pre-ISW Assessment
Miura 2016[38]	Wrong Study Design (no non-OT group)

Ohe 2014[39]	Wrong Study Design (classified according to fibrosis grade, not OT)
Oike 2002[40]	Incomplete Reporting
Perito 2015[41]	Wrong Outcome (post-transplant metabolic syndrome parameters)
Picascia 2012[42]	No Prognostic Marker Reported
Ramos 1995[43]	Wrong Population (> 20% viral/autoimmune)
Reding 2005[44]	Wrong Intervention (no ISW)
Reyes 1993[45]	Wrong Study Design (no non-OT group)
Savage 2020[46]	No Prognostic Marker Reported
Scheenstra 2006[47]	Wrong Intervention (no complete ISW)
Shaked 2019[48]	Wrong Population (> 20% viral/autoimmune)
Shaked 2017[49]	Wrong Study Design (focus on rejection)
Shin 2013[50]	Wrong Study Design (no non-OT group)
Takatsuki 2001[51]	No pre-ISW Assessment
Taubert 2016[52]	No Prognostic Marker Reported
Tokita 2008[53]	No pre-ISW Assessment
Tryphonopoulos 2010[54]	Wrong Population (> 20% viral/autoimmune)
Varela-Fascinetto 1997[55]	Wrong Intervention (IS minimization)
Wong 1998[56]	Wrong Population (> 20% viral/autoimmune)
Wozniak 2015[57]	No pre-ISW Assessment
Yoshida 1998[58]	Wrong Study Design (comment)
Yoshitomi 2009[59]	Wrong Study Design (no non-OT group)
Zarkhin 2010[60]	No pre-ISW Assessment
Zhao 2013[61]	No pre-ISW Assessment

Study (Abstract)	**Reason for exclusion**
Benitez 2009[62]	Published as Article
Benitez 2010[63]	Published as Article
Benitez 2010[64]	Published as Article
Bohne 2011[65]	Published as Article
Bohne 2010[66]	Published as Article
Bourdeaux 2010[67]	Published as Article
Castellaneta 2010[68]	Published as Article
Castellaneta 2010[69]	Published as Article
Castellaneta 2010[70]	Published as Article
Celik 2016[71]	Wrong Study Design
Danger 2014[72]	Published as Article
Danger 2014[73]	Published as Article
De La Garza 2013[74]	Published as Article
Demetris 2010[75]	Published as Article
Ekong 2009[76]	Wrong Intervention
Feng 2016[77]	Published as Article
Feng 2016[78]	Published as Article
Feng 2014[79]	Published as Article
Feng 2009[80]	Published as Article
Feng 2011[81]	Published as Article

Feng 2011[82]	Published as Article
Fens 2011[83]	Published as Article
García De La Garza 2013[84]	Published as Article
Girnita 2010[85]	Published as Article
Herrero 2012[86]	Published as Article
Jucaud 2018[87]	Published as Article
Jucaud 2018[88]	Published as Article
Kawagishi 2014[89]	Wrong Study Design
Kim 2015[90]	Published as Article
Kim 2017[91]	Wrong Study Design
Kim 2017[92]	Wrong Study Design
Laue 2019[93]	Wrong Study Design
Levitsky 2017[94]	Published as Article
Levitsky 2016[95]	Published as Article
Li 2010[96]	Published as Article
Li 2010[97]	Published as Article
Li 2011[98]	Published as Article
Manzia 2014[99]	Published as Article
Manzia 2013[100]	Wrong Study Design
Manzia 2018[101]	Published as Article
Manzia 2012[102]	Wrong Population
Manzia 2015[103]	Published as Article
Manzia 2015[104]	Published as Article
Manzia 2010[105]	Published as Article
Manzia 2010[106]	Published as Article
Manzia 2010[107]	Published as Article
Nafady Hego 2010[108]	Published as Article
Nafady Hego 2010[109]	Published as Article
Nafady-Hego 2011[110]	Published as Article
Nafady-Hego 2012[111]	Published as Article
Ohe 2010[112]	Wrong Intervention
Ohe 2010[113]	Wrong Intervention
Reding 2009[114]	Published as Article
Scapa 2018[115]	Wrong Study Design
Schulz-Juergensen 2010[116]	Wrong Study Design
Shaked 2014[117]	Published as Article
Shaked 2016[118]	Published as Article
Shaked 2012[119]	Published as Article
Shaked 2012[120]	Published as Article
Shaked 2011[121]	Published as Article
Soyama 2012[122]	Wrong Intervention
Takatsuki 2011[123]	Wrong Intervention
Takatsuki 2017[124]	Wrong Intervention
Takatsuki 2013[125]	Wrong Intervention
Taubert 2014[126]	Published as Article
Taubert 2015[127]	Published as Article

Teisseyre 2012[128]	Wrong Study Design
Teisseyre 2014[129]	Wrong Study Design
Toti 2013[130]	Wrong Study Design
Tryphonopoulos 2010[131]	Published as Article
Tryphonopoulos 2010[132]	Published as Article
Tryphonopoulos 2012[133]	Wrong Study Design
Uchida 2012[134]	Published as Article
Wozniak 2010[135]	Published as Article
Yoshitoshi 2012[136]	Wrong Study Design
Yoshizawa 2018[137]	Wrong Study Design
Yoshizawa 2017[138]	Wrong Study Design
Zarkhin 2010[139]	Published as Article
Zhao 2012[140]	Published as Article
Study (Trial Entries)	**Reason for exclusion**
NCT00135694	Published as Article
NCT00320606	Published as Article
ISRCTN16781831	Wrong Intervention
NCT00647283	Published as Article
NCT01034345	Wrong Intervention
NCT01198314	Published as Article
NCT01444079	Wrong Intervention
NCT01678937	Wrong Intervention
ISRCTN42322435	Wrong Population
NCT01638559	Published as Article
NCT02062944	Published as Article
ISRCTN15775356	Published as Article

References

1. Aini W, Miyagawa-Hayashino A, Ozeki M, et al. Accelerated telomere reduction and hepatocyte senescence in tolerated human liver allografts. *Transplant Immunology* 2014; **31**(2): 55-9.
2. Aini W, Miyagawa-Hayashino A, Tsuruyama T, et al. Telomere shortening and karyotypic alterations in hepatocytes in long-term transplanted human liver allografts. *Transplant International* 2012; **25**(9): 956-66.
3. Assy N, Adams PC, Myers P, et al. Randomized controlled trial of total immunosuppression withdrawal in liver transplant recipients: Role of ursodeoxycholic acid. *Transplantation* 2007; **83**(12): 1571-6.
4. Bourdeaux C, Pire A, Janssen M, et al. Prope tolerance after pediatric liver transplantation. *Pediatric Transplantation* 2013; **17**(1): 59-64.
5. Castellaneta A, Mazariegos GV, Nayyar N, Zeevi A, Thomson AW. HLA-G level on monocytoid dendritic cells correlates with regulatory T-cell Foxp3 expression in liver transplant tolerance. *Transplantation* 2011; **91**(10): 1132-40.
6. Craciun L, Stordeur P, Troisi R, et al. A Rapid Test of Alloreactivity Based on Interleukin-2 mRNA Expression Might Identify Liver Transplant Recipients With Donor-Specific Hyporesponsiveness. *Transplantation Proceedings* 2007; **39**(8): 2665-7.
7. Devlin J, Doherty D, Wong T, Donaldson P, Portmann B, Williams R. Defining the outcome of immunosuppression withdrawal after liver transplantation. *Hepatology* 1998; **27**(4): 926-33.
8. Duizendstra AA, de Knegt RJ, Betjes MGH, et al. Immunosuppressive drug withdrawal late after liver transplantation improves the lipid profile and reduces infections. *European journal of gastroenterology & hepatology* 2019.
9. Feng S, Demetris AJ, Spain KM, et al. Five-year histological and serological follow-up of operationally tolerant pediatric liver transplant recipients enrolled in WISP-R. *Hepatology* 2017; **65**(2): 647-60.
10. Geng L, Huang JJ, Lin BY, Chen TC, Shen T, Zheng SS. Characteristics of recipients with complete immunosuppressant withdrawal after adult liver transplantation. *Hepatobiliary and Pancreatic Diseases International* 2017; **16**(4): 437-9.
11. Geng L, Liu J, Huang J, et al. A high frequency of CD8+CD28–T-suppressor cells contributes to maintaining stable graft function and reducing immunosuppressant dosage after liver transplantation. *International Journal of Medical Sciences* 2018; **15**(9): 892-9.
12. Girlanda R, Rela M, Williams R, O'Grady JG, Heaton ND. Long-term outcome of immunosuppression withdrawal after liver transplantation. *Transplantation Proceedings* 2005; **37**(4): 1708-9.
13. Girnita A, Mazariegos GV, Castellaneta A, et al. Liver transplant recipients weaned off immunosuppression lack circulating donor-specific antibodies. *Human Immunology* 2010; **71**(3): 274-6.
14. Haarer J, Riquelme P, Hoffmann P, et al. Early Enrichment and Restitution of the Peripheral Blood Treg Pool Is Associated With Rejection-Free Stable Immunosuppression After Liver Transplantation. *Transplantation* 2016; **100**(7): e39-40.
15. Hsu LW, Goto S, Nakano T, et al. Immunosuppressive activity of serum taken from a liver transplant recipient after withdrawal of immunosuppressants. *Transplant Immunology* 2007; **17**(2): 137-46.
16. Inomata Y, Takatsuki M, Uemoto S, Tanaka K. Weaning of immunosuppression in living-related liver transplantation. *Nihon Rinshō Men'eki Gakkai kaishi = Japanese journal of clinical immunology* 1999; **22**(6): 431-5.
17. Jhun J, Lee SH, Lee SK, et al. Serial monitoring of immune markers being represented regulatory T cell/T helper 17 cell ratio: Indicating tolerance for tapering immunosuppression after liver transplantation. *Frontiers in Immunology* 2018; **9**(MAR).
18. Jucaud V, Shaked A, DesMarais M, et al. Prevalence and Impact of De Novo Donor-Specific Antibodies During a Multicenter Immunosuppression Withdrawal Trial in Adult Liver Transplant Recipients. *Hepatology* 2019; **69**(3): 1273-86.

19. Kasahara M, Kiuchi T, Uryuhara K, et al. Role of HLA compatibility in pediatric living-related liver transplantation. *Transplantation* 2002; **74**(8): 1175-80.
20. Kawasaki M, Iwasaki M, Koshiba T, et al. Gene expression profile analysis of the peripheral blood mononuclear cells from tolerant living-donor liver transplant recipients. *Int Surg* 2007; **92**(5): 276-86.
21. Koshiba T, Li Y, Takemura M, et al. Clinical, immunological, and pathological aspects of operational tolerance after pediatric living-donor liver transplantation. *Transplant Immunology* 2007; **17**(2): 94-7.
22. Koshiba T, Li Y, Zhao X, Ohe H, Nafady-Hego H, Bishop GA. Implication of VôiyôT Cells in Operational Tolerance after Human Liver Transplantation. Advances in Medicine and Biology: Nova Science Publishers, Inc.; 2015: 113-27.
23. Lau AH, Vitalone MJ, Haas K, et al. Mass cytometry reveals a distinct immunoprofile of operational tolerance in pediatric liver transplantation. *Pediatric Transplantation* 2016; **20**(8): 1072-80.
24. Lee JH, Lee SK, Lee HJ, et al. Withdrawal of immunosuppression in pediatric liver transplant recipients in Korea. *Yonsei Medical Journal* 2009; **50**(6): 784-8.
25. Levine MH, Akimova T, Murken DR, Hancock WW. Regulatory T cell signatures in liver transplant recipients successfully weaned from immunosuppression: Getting from here to there. *Liver Transplantation* 2017; **23**(7): 875-7.
26. Li L, Wozniak LJ, Rodder S, et al. A common peripheral blood gene set for diagnosis of operational tolerance in pediatric and adult liver transplantation. *American Journal of Transplantation* 2012; **12**(5): 1218-28.
27. Li Y, Zhao X, Cheng D, et al. The presence of Foxp3 expressing T cells within grafts of tolerant human liver transplant recipients. *Transplantation* 2008; **86**(12): 1837-43.
28. Li Y, Koshiba T, Yoshizawa A, et al. Analyses of peripheral blood mononuclear cells in operational tolerance after pediatric living donor liver transplantation. *American Journal of Transplantation* 2004; **4**(12): 2118-25.
29. Manez R, Kusne S, Linden P, et al. Temporary withdrawal of immunosuppression for life-threatening infections after liver transplantation. *Transplantation* 1994; **57**(1): 149-51.
30. Maria Manzia T, Angelico R, Toti L, et al. Longterm Survival and Cost-Effectiveness of Immunosuppression Withdrawal After Liver Transplantation. *Liver Transplantation* 2018; **24**(9): 1199-208.
31. Martínez-Llordella M, Lozano JJ, Puig-Pey I, et al. Using transcriptional profiling to develop a diagnostic test of operational tolerance in liver transplant recipients. *Journal of Clinical Investigation* 2008; **118**(8): 2845-57.
32. Martinez-Llordella M, Puig-Pey I, Orlando G, et al. Multiparameter immune profiling of operational tolerance in liver transplantation. *American Journal of Transplantation* 2007; **7**(2): 309-19.
33. Mazariegos GV, Reyes J, Marino I, Flynn B, Fung JJ, Starzl IE. Risks and benefits of weaning immunosuppression in liver transplant recipients: Long-term follow-up. *Transplantation Proceedings* 1997; **29**(1-2): 1174-7.
34. Mazariegos GV, Reyes J, Marino IR, et al. Weaning of immunosuppression in liver transplant recipients. *Transplantation* 1997; **63**(2): 243-9.
35. Mazariegos GV, Sindhi R, Thomson AW, Marcos A. Clinical tolerance following liver transplantation: Long term results and future prospects. *Transplant Immunology* 2007; **17**(2): 114-9.
36. Mazariegos GV, Zahorchak AF, Reyes J, Chapman H, Zeevi A, Thomson AW. Dendritic cell subset ratio in tolerant, weaning and non-tolerant liver recipients is not affected by extent of immunosuppression. *American Journal of Transplantation* 2005; **5**(2): 314-22.
37. Mazariegos GV, Zahorchak AF, Reyes J, et al. Dendritic cell subset ratio in peripheral blood correlates with successful withdrawal of immunosuppression in liver transplant patients. *American Journal of Transplantation* 2003; **3**(6): 689-96.
38. Miura K, Kobayashi T, Zhang Z, et al. Study of Immune Tolerance Cases in Adult Living Donor Liver Transplantation. *Transplantation Proceedings* 2016; **48**(4): 1119-22.

39. Ohe H, Uchida Y, Yoshizawa A, et al. Association of anti-human leukocyte antigen and anti-angiotensin II type 1 receptor antibodies with liver allograft fibrosis after immunosuppression withdrawal. *Transplantation* 2014; **98**(10): 1105-11.
40. Oike F, Yokoi A, Nishimura E, et al. Complete withdrawal of immunosuppression in living donor liver transplantation. *Transplantation Proceedings* 2002; **34**(5): 1521.
41. Perito ER, Mohammad S, Rosenthal P, et al. Posttransplant metabolic syndrome in the Withdrawal of Immunosuppression in Pediatric Liver Transplant Recipients (WISP-R) pilot trial. *American Journal of Transplantation* 2015; **15**(3): 779-85.
42. Picascia A, Grimaldi V, Napoli C. HLA match in operational tolerance after pediatric living-donor liver transplantation. *Transplant International* 2012; **25**(10): e106-e7.
43. Ramos HC, Reyes J, Abu-Elmagd K, et al. Weaning of immunosuppression in long-term liver transplant recipients. *Transplantation* 1995; **59**(2): 212-7.
44. Reding R, Gras J, Bourdeaux C, et al. Stepwise minimization of the immunosuppressive therapy in pediatric liver transplantation: A conceptual approach towards operational tolerance. *Acta Gastro-Enterologica Belgica* 2005; **68**(3): 320-2.
45. Reyes J, Zeevi A, Ramos H, et al. Frequent achievement of a drug-free state after orthotopic liver transplantation. *Transplantation Proceedings* 1993; **25**(6): 3315-9.
46. Savage TM, Shonts BA, Lau S, et al. Deletion of donor-reactive T cell clones after human liver transplant. *American Journal of Transplantation* 2020; **20**(2): 538-45.
47. Scheenstra R, Torringa MLJ, Waalkens HJ, et al. Cyclosporine a withdrawal during follow-up after pediatric liver transplantation. *Liver Transplantation* 2006; **12**(2): 240-6.
48. Shaked A, DesMarais MR, Kopetskie H, et al. Outcomes of immunosuppression minimization and withdrawal early after liver transplantation. *American Journal of Transplantation* 2019; **19**(5): 1397-409.
49. Shaked A, Chang BL, Barnes MR, et al. An ectopically expressed serum miRNA signature is prognostic, diagnostic, and biologically related to liver allograft rejection. *Hepatology* 2017; **65**(1): 269-80.
50. Shin M, Song S, Moon HH, et al. Characteristics of recipients who achieved spontaneous operational tolerance in adult liver transplantation. *Transplantation Proceedings* 2013; **45**(8): 3024-7.
51. Takatsuki M, Uemoto S, Inomata Y, et al. Analysis of alloreactivity and intragraft cytokine profiles in living donor liver transplant recipients with graft acceptance. *Transplant Immunology* 2001; **8**(4): 279-86.
52. Taubert R, Danger R, Londono MC, et al. Hepatic Infiltrates in Operational Tolerant Patients After Liver Transplantation Show Enrichment of Regulatory T Cells Before Proinflammatory Genes Are Downregulated. *Am J Transplant* 2016; **16**(4): 1285-93.
53. Tokita D, Mazariegos GV, Zahorchak AF, et al. High PD-L1/CD86 ratio on plasmacytoid dendritic cells correlates with elevated T-regulatory cells in liver transplant tolerance. *Transplantation* 2008; **85**(3): 369-77.
54. Tryphonopoulos P, Ruiz P, Weppler D, et al. Long-term follow-up of 23 operational tolerant liver transplant recipients. *Transplantation* 2010; **90**(12): 1556-61.
55. Varela-Fascinetto G, Treacy SJ, Vacanti JP. Approaching operational tolerance in long-term pediatric liver transplant recipients receiving minimal immunosuppression. *Transplantation Proceedings* 1997; **29**(1-2): 449-51.
56. Wong T, Nouri-Aria KT, Devlin J, Portmann B, Williams R. Tolerance and latent cellular rejection in long-term liver transplant recipients. *Hepatology* 1998; **28**(2): 443-9.
57. Wozniak LJ, Hickey MJ, Venick RS, et al. Donor-specific HLA Antibodies Are Associated with Late Allograft Dysfunction after Pediatric Liver Transplantation. *Transplantation* 2015; **99**(7): 1416-22.
58. Yoshida EM, Chung SW. Outcomes after complete immunosuppression withdrawal in liver transplant recipients: Rejection-free graft tolerance or late acute rejection? *Canadian Journal of Gastroenterology* 1998; **12**(6): 387-9.

59. Yoshitomi M, Koshiba T, Haga H, et al. Requirement of protocol biopsy before and after complete cessation of immunosuppression after liver transplantation. *Transplantation* 2009; **87**(4): 606-14.
60. Zarkhin V, Talisetti A, Li L, et al. Expression of soluble HLA-G identifies favorable outcomes in liver transplant recipients. *Transplantation* 2010; **90**(9): 1000-5.
61. Zhao X, Li Y, Ohe H, et al. Intragraft Vδ1 γδ T cells with a unique T-Cell receptor are closely associated with pediatric semiallogeneic liver transplant tolerance. *Transplantation* 2013; **95**(1): 192-202.
62. Benitez C, Lozano JJ, Martinez-Llordella M, et al. Use of transcriptional biomarkers to identify liver transplant (Table 1). Thus the transcriptome in renal allograft biopsies predicts the risk of recipients who can successfully discontinue immunosuppressive graft loss beyond the predictive value of time post transplant, renal function, and therapy. *American Journal of Transplantation* 2009; **9**: 252.
63. Benitez CE, Lozano JJ, Martínez-Llordella M, et al. Use of transcriptional biomarkers to identify liver transplant recipients who can successfully discontinue immunosuppressive therapy. *American Journal of Transplantation* 2010; **10**: 2-3.
64. Benitez CE, Lozano JJ, Martinez-Llordella M, et al. Use of transcriptional biomarkers to identify liver transplant recipients who can successfully discontinue immunosuppressive therapy. *American Journal of Transplantation* 2010; **10**: 191-2.
65. Bohne F, Martinez LM, Lozano JJ, et al. Transcriptional profiling of liver grafts in spontaneous operational tolerance. *American Journal of Transplantation* 2011; **11**: 390.
66. Bohne F, Martinez-Llordella M, Lozano JJ, et al. Transcriptional profiling of liver grafts in spontaneous operational tolerance. *Transplantation* 2010; **90**: 13.
67. Bourdeaux C, Pire A, Janssens M, et al. Liver transplantation during infancy provides similar overall results and might enhance prope/operational tolerance in the long term. *Liver Transplantation* 2010; **16**: S116.
68. Castellaneta A, Mazariegos G, DeVera ME, et al. Elevated hla-g and ilt4 expression on circulating dendritic cell subsets in pediatric liver transplant recipients successfully weaned off immunosuppression. *Transplantation* 2010; **90**: 1077.
69. Castellaneta A, Mazariegos GV, DeVera M, et al. Elevated HLA-G and ILT4 expression on circulating dendritic cell subsets in pediatric liver transplant recipients successfully weaned off immunosuppression. *American Journal of Transplantation* 2010; **10**: 1.
70. Castellaneta A, Mazariegos GV, Girnita A, et al. Liver transplant recipients successfully weaned off immunosuppression lack donor-specific anti-HLA antibodies. *American Journal of Transplantation* 2010; **10**: 481.
71. Celik N, Ganoza A, Vyas F, et al. Long-term outcomes in pediatric live-donor liver transplantation. *Transplantation* 2016; **100**(7): S531.
72. Danger R, Taubert R, Londono M, et al. Immune-regulatory pathways are transiently activated during the spontaneous establishment of operational tolerance following liver transplantation. *Transplantation* 2014; **98**: 25.
73. Danger R, Taubert R, Londoño MC, et al. Immune-regulatory pathways are transiently activated during the spontaneous establishment of operational tolerance following liver transplantation. *Liver Transplantation* 2014; **20**: S138.
74. De La Garza RG, Merino J, D'Avola D, et al. Evolution of lymphocyte subpopulations after immunosuppression withdrawal in tolerant liver transplant recipients. *Hepatology* 2013; **58**(4): 1037A.
75. Demetris AJ, Ruppert K, Ekong U, Lobritto SJ, Tchao NK, Feng S. Prospective evaluation of histopathologic biopsy features that predict successful weaning and comparison to protocol follow-up biopsies 2 years after complete immunosuppression withdrawal in living-related pediatric liver allograft recipients. *Hepatology* 2010; **52**: 322A-3A.
76. Ekong UD, Lokar J, Cymerman I, Alonso EM. Successful immunosuppression minimization in pediatric LT. *American Journal of Transplantation* 2009; **9**: 254.

77. Feng S, Bucuvalas J, Demetris A, et al. Primary outcome of iWITH: A multi-center clinical trial of complete immunosuppression withdrawal (ISW) in stable pediatric liver transplant (LT) recipients. *American Journal of Transplantation* 2016; **16**: 269.
78. Feng S, Bucuvalas J, Demetris AJ, et al. Primary outcome of iWITH: A 12 center North American clinical trial of immunosuppression withdrawal in stable pediatric liver transplant recipients. *Transplantation* 2016; **100**(7): S348-S9.
79. Feng S, Demetris AJ, Isse K, et al. Serum and tissue DSA subclass, stellate and endothelial phenotype monitoring in itn029st tolerant pediatric liver transplant recipients over 5+ years of follow-up. *Liver Transplantation* 2014; **20**: S117.
80. Feng S, Ekong U, Lobritto S, et al. ITN029ST: Immunosuppression withdrawal in pediatric recipients of parental living donor liver transplants: Preliminary results of a pilot study. *American Journal of Transplantation* 2009; **9**: 244-5.
81. Feng S, Ekong U, Lobritto S, et al. Clinical and histological predictors of operational tolerance in pediatric liver transplant recipients. *American Journal of Transplantation* 2011; **11**: 76.
82. Feng S, Ekong U, Lobrttto S, et al. Clinical and histological predictors of operational tolerance in pediatric liver transplant recipients. *Liver Transplantation* 2011; **17**: S107.
83. Fens S, Punch J, Reyes J, et al. Evolution of donor specific alloantibodies (DSA) with immunosuppression (IS) withdrawal among adult liver transplant recipients in 1TNQ3QST. *Liver Transplantation* 2011; **17**: S85.
84. García De La Garza R, Sarobe P, Merino J, et al. Factors predictive of tolerance after liver transplantation. *Journal of Hepatology* 2013; **58**: S231.
85. Girnita A, Mazariegos GV, Castellaneta A, et al. Liver transplant recipients successfully weaned off immunosuppression lack donor-specific anti-HLA antibodies. *American Journal of Transplantation* 2010; **10**: 25-6.
86. Herrero JI, Sarobe P, Merino J, et al. Factors predictive of tolerance after liver transplantation. *Liver Transplantation* 2012; **18**: S187.
87. Jucaud V, Shaked A, DesMarais M, Sayre P, Everly M. Development of post-transplant DSA following immunosuppression minimization/withdrawal in liver transplant recipients. *American Journal of Transplantation* 2018; **18**: 472-3.
88. Jucaud V, Shaked A, DesMarais M, Sayre P, Everly MJ. Development of post-transplant DSA in adult liver transplant patients from the ITN a-wish trial. *Transplantation* 2018; **102**(7): S203.
89. Kawagishi N, Tokodai K, Takeda I, Miyagi S, Satoh K, Ohuchi N. Long-term results on the immunosuppression withdrawal in pediatric recipients of parental living donor liver transplants. *Transplantation* 2014; **98**: 713.
90. Kim HY, Choi JY, Jhun JY, Cho ML, Kim DG. Prospective immunosuppression withdrawal under immunological monitoring among adult liver transplant recipients. *Transplantation* 2015; **99**(7): 275.
91. Kim IG, Hu XG, Wang HJ, Kim BW, Hong SY. Minimization of immunosuppression after liver transplantation from the perspectives of 'de novo malignancy' and 'nephrotoxicity'. *Transplant International* 2017; **30**: 479.
92. Kim IG, Hu XG, Wang HJ, Kim BW, Hong SY. Minimization of immunosuppression after liver transplantation from the perspectives of 'de novo malignancy' and 'nephrotoxicity'. *Journal of Hepato-Biliary-Pancreatic Sciences* 2017; **24**: A128.
93. Laue T, Goldschmidt I, Pfister E, et al. Biomarkers for Immune function after liver transplantation in children and adolescents with standard calcineurin inhibitor-based versus reduced minimal immunosuppression. *Journal of Pediatric Gastroenterology and Nutrition* 2019; **68**: 947-8.
94. Levitsky J, Kurian SM, Sanchez-Fueyo A, Mathew JM, Heinrichs J, Burrell BE. Blood and graft biomarkers predictive of operational tolerance in liver transplant recipients on sirolimus. *Hepatology* 2017; **66**: 3A-4A.
95. Levitsky J, Mathew JM, Miller J, Heinrichs J, Huang X, Abecassis MM. Immunosuppression withdrawal in liver transplant recipients on sirolimus. *Hepatology* 2016; **64**(1): 1A.
96. Li L, Talisetti A, Hsieh S, et al. A peripheral blood 12 gene-set for diagnosis of pediatric liver allograft tolerance. *Transplantation* 2010; **90**: 405.

97. Li L, Talisetti A, Hsieh S, et al. A peripheral blood 12 gene-set for diagnosis of pediatric liver allograft tolerance. *American Journal of Transplantation* 2010; **10**: 11.
98. Li L, Wozniak L, Heish S, et al. A peripheral blood 13 gene-set for diagnosis of pediatric liver allograft tolerance. *Pediatric Transplantation* 2011; **15**: 60.
99. Manzia T, Angelico R, Gherardi D, et al. Long-term outcome and cost effectiveness of sustained immunosuppression withdrawal after liver transplantation. *Transplantation* 2014; **98**: 709.
100. Manzia T, Angelico R, Toti L, et al. The long term clinical outcome of liver transplant recipients who successfully withdrawn immunosuppression. *American Journal of Transplantation* 2013; **13**: 540.
101. Manzia TM, Angelico C, Iesari S, Ackenine K, Lerut J, Tisone G. Cost effectiveness of immunosuppression withdrawal after liver transplantation. *Transplantation* 2018; **102**(7): S121.
102. Manzia TM, Angelico R, Ciano P, et al. The long term follow-up immunosuppression free state in HCV liver transplant recipients. *American Journal of Transplantation* 2012; **12**: 197.
103. Manzia TM, Angelico R, Gherardi D, et al. Long-term outcome and cost saving of immunosuppression withdrawal after liver transplantation. *Transplantation* 2015; **99**(7): 130.
104. Manzia TM, Angelico R, Toti L, et al. Cost saving of immunosuppression withdrawal after liver transplantation in a spending review era. *Transplant International* 2015; **28**: 200.
105. Manzia TM, Angelico R, Toti L, et al. Predicting "operational tolerance" after liver transplantation. *Transplantation* 2010; **90**: 34.
106. Manzia TM, Toti L, Angelico R, Sorge R, Bellini I, Anselmo A. Weaning off immunosuppression after liver transplantation: Predictive factors and short-term results. *Liver Transplantation* 2010; **16**: S73.
107. Manzia TM, Toti L, Angelico R, et al. "Operational tolerance" in stable liver transplant recipients: Predictive factors and short-term results. *American Journal of Transplantation* 2010; **10**: 174.
108. Nafady Hego HA, Li Y, Ohe H, Sakaguchi S, Uemoto S, Koshiba T. Critical role of naive regulatory T cells in operational tolerance after pediatric living-donor liver transplantation. *Transplantation* 2010; **90**: 404.
109. Nafady Hego HA, Li Y, Sakaguchi S, Uemoto S, Koshiba T. Up regulation of FOXP3 expression in CD4+CD25+ IL-7R α low regulatory T cells in operational tolerance after pediatric living donor liver transplantation. *Transplantation* 2010; **90**: 9.
110. Nafady-Hego H, Li Y, Ohe H, et al. Upregulation of FOXP3 expression of donor specific CD4+CD25++IL-7low+ regulatory T cells in operationally tolerant recipients after pediatric living-donor liver transplantation. *American Journal of Transplantation* 2011; **11**: 392.
111. Nafady-Hego H, Li Y, Ohe H, et al. The value of CD127 to isolate and define CD4+CD25++ regulatory T cells in operationally tolerant recipients after pediatric liver transplantation. *Transplantation* 2012; **94**: 481.
112. Ohe H, Egawa H, Uemoto S, Koshiba T. Prope tolerance: A more realistic approach to improve long-term outcomes for pediatric living-donor liver transplantation. *American Journal of Transplantation* 2010; **10**: 347.
113. Ohe H, Koshiba T, Li Y, et al. Prope tolerance : A more realistic approach to improve long-term outcomes for pediatric living-donor liver transplantation. *Transplantation* 2010; **90**: 325.
114. Reding R, Pire A, Janssen M, Brunati A, De Magnée C, Bourdeaux C. Steroid-free tacrolimus-based immunosuppression promotes long-term prope/operational tolerance in pediatric liver transplantation. *Pediatric Transplantation* 2009; **13**: 63-4.
115. Scapa J, Wozniak L, Naini BV. Can't the liver be a little more tolerant: A clinicopathologic study of immunologic tolerant pediatric liver transplant recipients. *Laboratory Investigation* 2018; **98**: 648.
116. Schulz-Juergensen S, Marischen L, Wesch D, Kabelitz D, Kohl M, Burdelski M. Diagnostic value of markers of operational immune tolerance after pediatric liver transplantation. *Journal of Pediatric Gastroenterology and Nutrition* 2010; **50**: E46-E7.
117. Shaked A, Chang B, Guettouche T, et al. Potential application of serum miRNA signature for minimization of immunosuppression and diagnosis of rejection following liver transplantation.

American journal of transplantation Conference: 2014 world transplant congress United states 2014; **14**(Supplement 3): 230.
118. Shaked A, Feng S, Punch J, et al. Early post-transplant immunosuppression (IS) withdrawal-Final outcomes of the ITN030ST AWISH Study. *American Journal of Transplantation* 2016; **16**: 269.
119. Shaked A, Feng S, Punch J, et al. Initial outcomes of ITN030ST: Early post-liver transplant immunosuppression withdrawal. *Liver Transplantation* 2012; **18**: S106.
120. Shaked A, Feng S, Punch J, et al. Initial outcomes of ITN030ST: Early post-liver transplant immunosuppression withdrawal. *American Journal of Transplantation* 2012; **12**: 197.
121. Shaked A, Feng S, Punch J, et al. Lessons learned from the ITN030ST immunosuppression withdrawal (ISW) trial in liver transplant recipients. *Liver Transplantation* 2011; **17**: S84.
122. Soyama A, Takatsuki M, Muraoka I, et al. Chronological evaluation of immune status by immune function assay (immuknow) for weaning of immunosuppression in living donor liver transplantation recipient. *Liver Transplantation* 2012; **18**: S201.
123. Takatsuki M, Eguchi S, Hidaka M, et al. Impact of peripheral blood CD4+ adenosine triphosphate activity in long-term living donor liver transplantation under weaning of immunosuppression. *Liver Transplantation* 2011; **17**: S207.
124. Takatsuki M, Okada S, Hidaka M, et al. Elective weaning of immunosuppression in living donor liver transplant recipients. *Transplant International* 2017; **30**: 453.
125. Takatsuki M, Soyama A, Torashima Y, et al. Weaning of immunosuppression in living donor liver transplant recipients. *Liver Transplantation* 2013; **19**(6): S90.
126. Taubert R, Danger R, Londono MC, et al. Operational tolerance causes a long lasting active immunoregulation within the graft. *Transplant International* 2014; **27**: 14.
127. Taubert R, Danger R, Londono MC, et al. Operational tolerance causes a long lasting active immunoregulation within the graft. *Journal of Hepatology* 2015; **62**: S248-S9.
128. Teisseyre J, Markiewicz-Kijewska M, Kalicinski P, Teisseyre M. Withdrawal of immunossupresion after liver transplantation-own experience. *Transplantation* 2012; **94**: 746.
129. Teisseyre J, Markiewicz-Kijewska M, Kalicinski P, Ismail H, Teisseyre M, Jankowska I. Immunosuppression withdrawal in children after liver transplantation-is it possible? *Liver Transplantation* 2014; **20**: S174.
130. Toti L, Manzia TM, Angelico R, et al. The Tor Vergata weaning of immunosuppression in liver transplant patients: The long-term follow-up. *Liver Transplantation* 2013; **19**(6): S208.
131. Tryphonopoulos P, Ruiz P, Weppler D, et al. Long term follow up of 22 operational tolerant liver transplant recipients. *Transplantation* 2010; **90**: 33.
132. Tryphonopoulos P, Ruiz P, Weppler D, et al. Clinical outcome of liver transplant recipients after successful immunosuppression withdrawal. *American Journal of Transplantation* 2010; **10**: 2.
133. Tryphonopoulos PT, Ruiz P, Weppler D, et al. Long term follow up of 30 operationally tolerant liver transplant recipients. *Transplantation* 2012; **94**: 130.
134. Uchida Y, Ohe H, Yoshizawa A, et al. Is it possible to wean immunosuppression in pediatric liver transplantation? The problem of long-term management. *Liver Transplantation* 2012; **18**: S82-S3.
135. Wozniak LJ, Li L, Hsieh S, et al. Validation of a transcriptional profile of tolerance in pediatric liver transplant recipients. *Transplantation* 2010; **90**: 402.
136. Yoshitoshi EY, Yoshizawa A, Kaneshiro M, Takada N, Okamoto S, Uemoto S. The long-term impact of immunosupression weaning in renal function of children after living-donor liver transplantation. *Transplantation* 2012; **94**: 223.
137. Yoshizawa A, Kaneshiro M, Uebayashi E, et al. The analysis of pathological and serological findings in the long-term pediatric liver transplantation: The achievement of clinical operational tolerance. *Transplantation* 2018; **102**(7): S896.
138. Yoshizawa A, Kaneshiro M, Ueda D, et al. The status of immunosuppressive therapy for the outpatients after pediatric liver transplantion: The achievement of clinical operational tolerance. *Pediatric Transplantation* 2017; **21**: 21.
139. Zarkhin V, Talisetti A, Cox K, Hurwitz M, Esquivel C, Sarwal M. HLA-G: A novel serological marker for liver transplant tolerance. *American Journal of Transplantation* 2010; **10**: 178.

140. Zhao X, Li Y, Waki K, et al. Propagation of vδ1 γ δ T cells and their skewed T cell receptor within accepted grafts after pediatric semi-allogeneic liver transplantation. *Transplantation* 2012; **94**: 56.

Table S3: Characteristics of included studies: Chronological overview of reports

First Author Year (Country)	*Study ID (Cohort Overlap)*	*Study Population*	*Study Design*	*Control Group (IS Maintenance or Weaning)*	*DD/LD LT*	*Mean/median Age at LT (range)/years*[2]	*Liver Disease Etiology*	*Viral Status during ISW*	*IS Drug(s) at ISW Enrollment*	*Reason(s) for ISW*	*Pre-ISW Biopsy*	*ISW Schedule*	*Assessment of OT*	*Mean/median (range) FU/months*	*# Patients Included in Prognostic Analyses (Total)*
Takatsuki 2001 (Japan)	***"Kyoto"***	Pediatric	Monocenter Retrospective	No	LD	NR/1.1 (0.1 - 15.2)	non-autoimmune	no patients with viral liver disease etiology	TAC+CS	elective non-elective	NR	elective: doses gradually decreased over 3-6 mo non-elective: individually, depending on clinical course and indication	LFT	NR/23.5 (3 - 69) [OT]	63
Pons 2003 (Spain)	***"Murcia 1"***	Adult	Monocenter Prospective	No	DD	53/56 (35 - 65)[3]	non-autoimmune	no patients with viral liver disease etiology	CsA+CS	elective	Yes	10% decrements at 1 mo intervals, followed by prednisone withdrawal (over 2 mo)	LFT Biopsy	(17 - 24) [OT]	9
Pons 2008 (Spain)	***"Murcia 1"***	Adult	Monocenter Prospective	Yes	DD	48/NR ± 10[3]	non-autoimmune	no patients with viral liver disease etiology	CsA	elective	Yes	10% decrements at 1 mo intervals, followed by prednisone withdrawal (over 2 mo)	LFT Biopsy	(10 - 30) [OT]	12
Pons 2009 (Spain)	***"Murcia 1"***	Adult	Monocenter Prospective	No	DD	48/NR ± 9.5 (31 - 62)	non-autoimmune	no patients with viral liver disease etiology	CsA CsA+CS Other	elective non-elective	Yes (only elective cases)	elective: 10% decrements at 1 mo intervals, followed by prednisone withdrawal (over 2 mo) non-elective: over 1-6 mo	LFT Biopsy	53/48 (10-132)	20
Millán 2010 (Spain)[1]	***"Barcelona/Rome/Leuven"***	Adult	Monocenter Prospective	No	DD	45/47 (19 - 63)	non-autoimmune	<20% replicative viral disease	TAC CsA MMF Other	elective	NR	decreased in monthly intervals, complete discontinuation after 6-9 mo	NR	>12	24
Nafady-Hego 2010 (Japan)	***"Kyoto"***	Pediatric	Monocenter Retrospective	Yes	LD	[17/NR (7 - 28)] OT [11/NR (5 - 22)] nonOT	non-autoimmune	no patients with viral liver disease etiology	TAC Other	elective non-elective	NR	NR	LFT	88/NR ± 34	42

Talisetti 2010 (USA)	***"Stanford"***	Pediatric	Multicenter Retrospective	Yes	both	1.4/NR (0.3 - 4.9) [OT] 3.4/NR (0.3 - 16) [nonOT]	non-autoimmune	no patients with viral liver disease etiology	TAC CsA	non-elective NR	NR	NR	LFT	91/NR (24 - 145) [OT]	62
Waki 2011 (Japan)	***"Tochigi"***	Pediatric	Monocenter Retrospective	No	LD	2.1/NR ± 2.6 [OT] 3.3/NR ± 4.1 [nonOT]	non-autoimmune	no patients with viral liver disease etiology	TAC+CS CsA+CS	elective	NR	gradual reduction over 2 yr	LFT	NR	52
Bohne 2012 (Spain, Italy, Belgium)	***"Barcelona/Rome/Leuven" NCT00647283***	Adult	Multicenter Prospective	Yes	NR	[NR/62 (27-73) [OT] NR/51 (25-71) [nonOT]]	non-autoimmune	<20% replicative viral disease	TAC CsA MMF AZA "No-CNI"	elective	Yes	Over 6 to 9 months: first MMF over 3 months then CNI 1/4 to 1/2 every 3 weeks	LFT Biopsy	NR	75
Feng 2012 (USA)	***"WISP-R" NCT00320606***	Pediatric	Multicenter Prospective	No	LD	1.0/0.6 (0.3 - 7.5)	non-autoimmune	no patients with viral liver disease etiology	TAC CsA	elective	Yes	stepwise over > 36 wk (decrease every 4 to 8 wk)	LFT Biopsy	NR/32.9 (IQR 1.0 - 49.9)	20
Ohe 2012 (Japan)	***"Kyoto"***	Pediatric	Monocenter Retrospective	Yes	LD	2.9/NR ± 3.7 [OT] 2.1/NR ± 2.9 [nonOT]	NR	NR	TAC CsA	elective non-elective	NR	elective: bid, qd, 4 times a wk, twice a wk, once a wk, twice a mo, once a mo, and stop (3-6 mo interval between each step)	LFT Biopsy	NR	134
Benítez 2013 (Spain, Italy, Belgium)	***"Barcelona/Rome/Leuven" NCT00647283***	Adult	Multicenter Prospective	No	NR	47/NR ± 10	non-autoimmune	<20% replicative viral disease	TAC CsA MMF AZA Other	elective	Yes	Over 6 to 9 months: first MMF over 3 months then CNI 1/4 to 1/2 every 3 weeks	LFT Biopsy	48.9/NR ± 7.7	98
García de la Garza 2013 (Spain)	***"Pamplona"***	Adult	Monocenter Prospective	No	DD	NR/57	non-autoimmune	<20% replicative viral disease	MMF CsA TAC SRL	elective	Yes	monthly reductions (6 - 10 fractions)	LFT	NR/14 (IQR 9-23) [OT]	24
Waki 2013 (Japan)	***"Tochigi"***	Pediatric	Monocenter Retrospective	No	LD	2.2/NR ± 2.7 [OT] 4.3/NR ± 4.6 [nonOT]	NR	NR	TAC+CS CsA+CS	elective	NR	NR	LFT Biopsy	NR	40
Lin 2015 (Taiwan)	***"Taipei"***	Pediatric	Monocenter Prospective	No	both	4.0/NR ± 4.8	non-autoimmune	<20% replicative viral disease	TAC Other	elective	Yes	stepwise reduced by half in 4-wk intervals	LFT Biopsy	41/NR ± 6.0	15
García de la Garza 2015 (Spain)	***"Pamplona"***	Adult	Monocenter Prospective	No	DD	NR/57	non-autoimmune	NR	MMF TAC	elective	Yes	monthly reductions (6 - 10 fractions)	LFT	NR/43.5 (31-60) [OT]	24
Bonaccorsi-Riani 2016 (Spain, Italy, Belgium)	***"Barcelona/Rome/Leuven" NCT00647283***	Adult	Multicenter Prospective	No	NR	NR	non-autoimmune	<20% replicative viral disease	TAC CsA MMF AZA	elective	Yes	doses gradually decreased by 1/4 to 1/2	LFT Biopsy	NR	68

								Other			within 3 wk over 6-9 mo				
Nafady-Hego 2016 (Japan)	***"Kyoto"***	Pediatric	Monocenter Retrospective	Yes	LD	[17/NR (7-28)] [OT] [11/NR (5-22)] [nonOT]	non-autoimmune	no patients with viral liver disease etiology	TAC TAC+CS Other	elective non-elective	NR	NR		88/NR ± 34 [OT]	43
Revilla-Nuin 2017 (Spain)	***"Murcia 1-3 & Pamplona"***	Adult	Multicenter Mixed	No	NR	53.6/54 (40-65) [OT] 54.2/57 (32-66) [nonOT]	<20% autoimmune	<20% replicative viral disease[4]	CsA TAC MMF SRL EVL Other	elective	Yes	≥10% decrements at 1 mo intervals	LFT Biopsy	54/60 (12 - 144) [OT] 47/36 (1 - 180) [nonOT]	47
Baroja-Mazo 2019 (Spain)	***"Murcia 3" ISRCTN15775356***	Adult	Monocenter Prospective	No	NR	58.6/59 (52-65) [OT] 62.4/63.5 (56-71) [nonOT]	non-autoimmune	<20% replicative viral disease[4]	CsA TAC TAC+CS Other	elective	Yes	≥10% decrements at 1 mo intervals	LFT Biopsy	NR	17
Perez-Sanz 2019 (Spain)	***"Murcia 3" ISRCTN15775356***	Adult	Monocenter Prospective	No	NR	58.6/59 (52-65) [OT] 62.4/63.5 (56-71) [nonOT]	non-autoimmune	<20% replicative viral disease[4]	CsA TAC TAC+CS Other	elective	Yes	≥10% decrements at 1 mo intervals	LFT Biopsy	NR	17
Levitsky 2020 (USA)	***"Chicago" NCT02062944***	Adult	Monocenter Prospective	No	both	[63.7 (47-76) [OT] 62.7 (44-67) [nonOT]]	non-autoimmune	<20% replicative viral disease	SRL	elective	Yes	slowly over 3-6 months (reduced every month by 50% of total dose until 0.5 mg daily for one month, then each month 0.5 mg every other day, twice wkly, finally once wkly)	LFT Biopsy	NR	15
Feng 2020 (USA, Canada)	***"iWITH" NCT01638559***	Pediatric	Multicenter Prospective	No	both	NR/1 IQR 1-2 [OT] NR/1 IQR 1-3 [nonOT]	non-autoimmune	no patients with viral liver disease etiology	TAC CsA	elective	Yes	reduced in 7 steps of 4 or 6 wk duration (36 - 48 wk total)	LFT Biopsy	«4 yr»	88

[1] This Barcelona study is attached to the multicenter "Barcelona/Rome/Leuven" study due to cohort overlap.

[2] [age at study] if age at LT not reported

[3] corrected months to years

[4] Alberto Baroja-Mazo, personal communication 20200706

AZA, azathioprine; CNI, calcineurin inhibitor; CS, corticosteroid; CsA, cyclosporine A; DD, deceased donor; EVL, everolimus; FU, follow-up; IQR, interquartile range; IS, immunosuppression; ISW, immunosuppression withdrawal; LD, living donor; LFT, liver function tests; LT, liver transplantation; MMF, mycophenolate mofetil; mo, months; NR, not reported; OT, operational tolerance; SRL, sirolimus; TAC, tacrolimus; wk, weeks; wkly, weekly; yr, years

Table S4: Included conference abstracts[§]

First Author Year	Journal	Title	Study Population
Feng 2014[47]	Transplantation	High percentage of PD-1+ CD4+ T cells in a subset of tolerant pediatric liver recipients	pediatric
Ramaswami 2014[42]	Transplantation	Operationally tolerant pediatric liver transplant recipients exhibit features of immune activation and exhaustion	pediatric
Ramaswami 2014[44]	Journal of Immunology	Pediatric liver transplant recipients with operational tolerance exhibit features of immune activation and exhaustion	pediatric
Danger 2015[43]	American Journal of Transplantation	Hepatocyte-related miR-193a-3p is over-expressed in allograft biopsy from liver tolerant recipients	adult
Ramaswami 2015[46]	Transplantation	Pediatric liver transplant recipients with operational tolerance exhibit features of immune activation and exhaustion	pediatric
Burrell 2017[45]	American Journal of Transplantation	Tolerant pediatric liver transplant (LT) recipients (TOL) from WISP-R (NCT00320606) split into two phenotypically distinct groups by PD1 expression on CD4+ T cells at baseline, prior to immunosuppression withdrawal (ISW)	pediatric
Chandran 2017[38]	American Journal of Transplantation	Subclinical histologic findings are observed in 25% of stable adult liver transplant recipients (ALTRs) screened for immunosuppression withdrawal (ISW): OPTIMAL (NCT02533180)	adult
Chruscinski 2017[39]	Transplant International	Results of litmus (NCT 02541916): The liver immune tolerance biomarker utilization study	adult
Chruscinski 2018[40]	American Journal of Transplantation	Results of litmus (nct 02541916): The liver immune tolerance bio marker utilization study	adult
Chruscinski 2018[48]	Transplantation	Results of LITMUS (NCT 02541916): The liver immune tolerance bio marker utilization study	adult
Gu 2018[36]	Transplantation	Liver transplant tolerance induced by severe or consistent infection revealed the association with T cell exhaustion	pediatric
Sanchez-Fueyo 2018[37]	Journal of Hepatology	Prevalence of subclinical histological lesions and tolerance biomarkers in long-term adult liver transplant recipients considered for immunosuppression withdrawal	adult
Vionnet 2019[41]	American Journal of Transplantation	Identification of adult liver transplant recipients eligible to participate in an immunosuppression withdrawal trial employing non-invasive assessments of allograft status	adult

[§] The data issued from conference abstracts is not peer-reviewed.

Table S5: Included registered trials

Principal Investigator Year	**Trial ID** ***Trial Name***	**Title**	**Study population**
Sanchez-Fueyo 2011	NCT01445236	Pilot Study of Immunosuppression Drug Weaning in Liver Recipients Exhibiting Biomarkers of High Likelihood of Tolerance	adult
Sanchez-Fueyo 2015	NCT02498977 *LIFT*	Liver Immunosuppression Free Trial	adult
Markmann 2015	NCT02533180 *OPTIMAL*	Evaluation of Donor Specific Immune Senescence and Exhaustion as Biomarkers of Tolerance Post Liver Transplantation	adult
Levy 2015	NCT02541916 *LITMUS*	Liver Immune Tolerance Marker Utilization Study	adult
Chandran 2016	NCT02743793 *ALLTOL*	A Cohort Study of Operationally Tolerant Allograft Recipients	adult

Table S6: Summary of Results from Pediatric Studies

Study ID	First Author Year	Successful ISW	# Graft Loss	Prognostic Biomarkers	Biomarker Details	Numerical Evidence for Positive Association		Prognostic Clinical Parameters	Parameter Details	Numerical Evidence for Positive Association		Non-Prognostic Biomarkers	Biomarker Details	Non-Prognostic Clinical Parameters	Parameter Details
						OT	non-OT			OT	non-OT				
"Kyoto"	Takatsuki 2001	24/63 (38%)	0									Serum AST/ALT/GGT		HLA mismatch	
														ABO compatibility of graft	
														history of rejection	
	Nafady-Hego 2010	24/42 (57%)	0	Serum total bilirubin	mean ± SD [U/L]	0.8 ± 0.3	0.6 ± 0.2	time from LT to ISW	mean ± SD [yr]	14 ± 3	10 ± 5	Blood cell subpopulations	WBC, lymphocytes, CD4+	sex	
														ABO compatibility of graft	
														liver disease etiology	
	Ohe 2012	84/134 (63%)	0					HLA mismatch	HLA-A mismatch (% patients)	75	89	Blood cell subpopulations	WBC	HLA mismatch	HLA-DR
								HLA mismatch	HLA-B mismatch (% patients)[§]	94	89	Serum total bilirubin		ABO compatibility of graft	
								history of rejection	absence of early rejection (% patients)	83	60	Serum AST/ALT/GGT		liver disease etiology	
								IS regimen	tacrolimus trough levels in 1 wk post-LT; mean ± SD [ng/ml]	17 ± 12	11 ± 5			recipient age at LT	
								time from LT to ISW	reported "FU after LT"; mean ± SD [d][$]	4725 ± 1102	4135 ± 1099			sex	
														Donor age	
	Nafady-Hego 2016	25/43 (58%)	0	Serum total bilirubin	mean ± SD [U/L]	0.9 ± 0.3	0.6 ± 0.2	time from LT to ISW	mean ± SD [yr]	13.3 ± 2.9	9.9 ± 4.8	Blood cell subpopulations	WBC, lymphocytes, CD4+	sex	
														ABO compatibility of graft	

"Stanford"	Talisetti 2010	18/62 (29%)	0					recipient age at LT	mean (range) [yr]	1.4 (0.3 - 4.9)	3.4 (0.3 - 16)			sex liver disease etiology ABO compatibility of graft Donor age sex mismatch D/R
"Tochigi"	Waki 2011	18/52 (35%)	1					sex	% male	11.1	48.5			lymphocytotoxic crossmatch HLA mismatch recipient age at LT liver disease etiology history of rejection Donor age Postoperative complications (biliary, vascular) Cold or warm ischemia time PELD score
	Waki 2013	17/40 (43%)	0	Anti-HLA antibodies Anti-HLA antibodies Anti-HLA antibodies Anti-HLA antibodies Anti-HLA antibodies Anti-HLA antibodies	post-LT class I; % patients post-LT class II; % patients post-LT HLA-B; % patients post-LT HLA-C; % patients post-LT HLA-DQ; % patients post-LT HLA-DR; % patients	47 27 20 33 7 20	95 67 67 76 43 57	sex	% male	11.8	52.2	Anti-HLA antibodies Anti-HLA antibodies Anti-HLA antibodies	pre-LT post-LT HLA-A post-LT HLA-DP	history of rejection liver disease etiology HLA mismatch Lymphocytotoxic crossmatch time from LT to ISW Donor age Cold or warm ischemia time PELD score

"WISP-R" (NCT00320606)	Feng 2012	12/20 (60%)	0	Liver histology	C4d score; median (IQR)	6.1 (5.1-9.3)	12.5 (9.3-16.8)	time from LT to ISW	median (IQR) [mo]	100.6 (71.8-123.5)	73.0 (57.6-74.9)	Anti-HLA antibodies	HLA mismatch
				Liver histology	no portal inflammation; percent (95CI)	91.7 (61.5-99.8)	42.9 (9.9-81.6)					DSA	history of rejection
												Liver histology	Rejection activity index, hepatocyte apoptosis, central fibrosis, interface activity, steatosis	sex
														recipient age at LT
														IS regimen
														liver disease etiology
"Taipei"	Lin 2015	5/15 (33%)	0					time from LT to ISW	mean ± SD [yr]	2.25 ± 0.88	4.56 ± 1.96			recipient age at LT
								liver disease etiology	Metabolic: parenchymal	3:2	1:9			HLA mismatch
														sex
"iWITH" (NCT01638559)	Feng 2020	33/88 (38%)	0	Liver histology	mild portal inflammation; OR (95CI)	0.36 (0.14-0.90)						DSA	class II present at trial entry	recipient age at LT
				Liver histology	load of leukocytes (CD45+); OR (95CI)	0.97 (0.95-0.99)						Liver histology	load of CD4+ cells	time from LT to ISW
				Liver histology	load of APC:lymphocyte pairs; OR (95CI)	0.82 (0.74-0.92)						Allograft gene expression	*[CDHR2, MIF, PEBP1, SOCS1, TFRC]*	DD/LD LT
				Liver histology	load of infiltrating monocytes/ macrophages (MAC387+); OR (95CI)	0.91 (0.85-0.97)						Allograft gene expression	T-cell mediated rejection gene set	HLA mismatch

			Liver histology	load of CD4+ cells	history of rejection sex

§ only by multivariate analysis

$ only by univariate analysis

ALT, alanine aminotransferase; APC, antigen-presenting cell; AST, aspartate aminotransferase; D, donor; d, days; DD, deceased donor; DSA, donor-specific antibodies; FU, follow-up; LmlGGT, gamma glutamyltransferase; HLA, human leukocyte antigen; IQR, interquartile range; IS, immunosuppression; ISW, immunosuppression withdrawal; L, liter; LD, living donor; LT, liver transplantation; ml, milliliters; mo, months; ng, nanograms; OR, odds ratio; PELD, Pediatric End-stage Liver Disease; R, recipient; SD, standard deviation; U, units; WBC, white blood cells; wk, weeks; yr, years; 95CI, 95% confidence interval

Table S7: Summary of Results from Adult Studies

Study ID	First Author Year	Successful ISW	# Graft Loss	Prognostic Biomarkers	Biomarker Details	Numerical Evidence for Positive Association		Prognostic Clinical Parameters	Parameter Details	Numerical Evidence for Positive Association		Non-Prognostic Biomarkers	Biomarker Details	Non-Prognostic Clinical Parameters	Parameter Details
						OT	non-OT			OT	non-OT				
"Murcia 1"	Pons 2003	3/9 (33%)	0									Endothelial cell chimerism	in recipients with a different sex than their donor		
	Pons 2008	5/12 (42%)	0									Blood cell subpopulations	% γδ TCR cells	sex	
												Blood cell subpopulations	% Treg	recipient age at LT	
												PBMC gene expression	*FoxP3*	IS regimen	
												Blood cell subpopulations	% NK cells	history of rejection	
												Blood cell subpopulations	% B cells	time from LT to ISW	
												Blood cell subpopulations	% CD8+ CD28- cells		
												Serum AST/ALT/GGT			
	Pons 2009	8/20 (40%)	0									Serum AST/ALT/GGT		sex	
														recipient age at LT	
														IS regimen	
														history of rejection	
														time from LT to ISW	
"Barcelona/Rome/Leuven" (NCT00647283)	Millán 2010	13/24 (54%)	0	Immuno-assay	low percentage of CD8-CD69-IL2+ cells upon lymphocyte proliferation	NR	NR								
				Immuno-assay	low secreted IFNγ upon lymphocyte proliferation	NR	NR								

	Bohne 2012	33/75 (44%)	0	Allograft gene expression	*[CDHR2, MIF, PEBP1, SOCS1, TFRC]*; OR (95CI) (and 4 additional gene signatures)	49 (7-343) (89% SN, 86% SP, 80% PPV, 92% NPV, 13% ER) and (80% SN, 100% SP, 100% PPV, 85% NPV, 9.5% ER) AUC 0.83		time from LT to ISW	median (range) [mo]	143 (52-212)	86 (36-215)	Blood cell subpopulations	% Treg
				PBMC gene expression	*[NCR1, PDGFRB, PSMD14]* or *[SLAMF7, KLRF1, CLIC3, PSMD14, ALG8, CX3CR1]*	AUC 0.76 AUC 0.71		recipient age at LT	median (range) [yr]	62 (27–73)	51 (25–71)	Liver histology	
				Iron status	serum ferritin; mean (range)	185.5 (26-864)	73.5 (3-304)	IS regimen	% patients with CNI	66.7	91.3		
				Iron status	serum hepcidin 25								
				Liver histology	hepatocytic iron								
				Blood cell subpopulations	% NK cells								
				Blood cell subpopulations	% γδ TCR cells								
	Benítez 2013	41/98 (42%)	0	Serum GGT	mean ± SD [U/L]	50 ± 70	27 ± 28	recipient age at LT	mean ± SD [yr]	50 ± 10	45 ± 11	Anti-HLA antibodies	class I + class II
								sex	% male	83	58	Serum total bilirubin	
								time from LT to ISW	mean ± SD [mo]	131 ± 47	83 ± 40	Serum AST/ALT/GGT	
								IS regimen	% patients with CNI	56	79		
	Bonaccorsi-Riani 2016	25/68 (37%)	0	PBMC gene expression	*FoxP3*	up	down					PBMC gene expression	*CD3* *MAN1A1* *PRF1* *Toag-1*

	liver disease etiology
	history of rejection
	HLA mismatch
	Donor age
	IS regimen trough levels
	Renal function (creatinine, GFR)
	sex

"Pamplona"	García de la Garza 2013+2015	15/24 (63%)	0	Immuno-assay	Lymphocyte proliferation / Stimulation Index; median (IQR)	7.5 (2.1-23)	41.7 (19-65)	time from LT to ISW	median (IQR) [mo]	156 (101-182)	71 (51-88)	Blood cell subpopulations	% Treg	liver disease etiology
				Blood cell subpopulations	% NK cells; median (IQR) borderline p=0.07	14.0 (9.9-20.7)	8.3 (6.0-13.7)					Blood cell subpopulations	% B cells	sex
												Blood cell subpopulations	CD4+, CD8+, lymphocytes	IS regimen
												Immuno-assay	cytokine production (IL-2, IL-4, IL-6, IL-10, IFNγ, TNFα)	
"Murcia 3" (ISRCTN15775356)	Revilla-Nuin 2017*	24/47 (51%)	NR	Blood cell subpopulations	(only prospective patients, n=14) ICOS+ cells [% of Treg]	<20%	>20%	time from LT to ISW	mean/median (range) [yr]	10.8/12 (4-18)	6.1/5 (4-14)			sex
														recipient age at LT
														IS regimen
														liver disease etiology
	Baroja-Mazo 2019	5/17 (29%)	NR					time from LT to ISW	mean/median (range) [yr]	9/9 (6-12)	6/5 (4-14)	Blood cell subpopulations	% CD39+ and CD73+ Tregs	sex
												PBMC gene expression	*A2AR* *A2BR* *ADA* *ICER*	recipient age at LT
												Serum ATP		IS regimen
												Serum ADO		liver disease etiology
												Serum sCD73		

	Perez-Sanz 2019	5/17 (29%)	NR	PBMC gene expression	*FEM1C SENP6*	AUC 0.967 AUC 0.933	time from LT to ISW	mean/median (range) [yr]	9/9 (6-12)	6/5 (4-14)	PBMC gene expression	*HELIOS NCR1 PSMD14 PDGFRB ERBB2*			
				PBMC miRNA	miR95 miR31	AUC 0.867 AUC 0.967					PBMC miRNA	miR24 miR146a miR155			
											Blood cell subpopulations	Vδ1:Vδ2 γδ TCR			
											Blood cell subpopulations	% Treg			
											Blood cell subpopulations Iron status	% ICOS+ Treg			
											Blood cell subpopulations	% γδ TCR cells			
											methylation of *FoxP3*-TSDR				
"Chicago" (NCT02062944)	Levitsky 2020	8/15 (53%)	0	Blood cell subpopulations	% "tolerogenic DC" (HLADR+ CD11c+ ILT3+ ILT4+)	p<0.01					Blood cell subpopulations	% Treg	recipient age at LT	[age at study]	
				Blood cell subpopulations	% naive CD8+	p<0.01					Blood cell subpopulations	% Breg	time from LT to ISW	NS trend	
				Blood cell subpopulations	% TEMRA+ CD8+	p<0.05					DSA		sex		
				Blood cell subpopulations	% Eomes+ CD8+	p<0.05					Liver histology	load of FoxP3+ CD4+ cells	Renal function (creatinine, GFR)		
				Allograft gene expression	*[CDHR2, MIF, PEBP1, SOCS1, TFRC]*	88% SN, 83% SP, 88% PPV, 83% NPV					Liver histology	load of APC:lymphocyte pairs	Hypertension		
				Allograft gene expression	*ABHD4 PYCR2 SIRPA*	up	down				Blood cell subpopulations	% NK cells	Diabetes		
				PBMC gene expression	*ABHD4 PYCR2 SIRPA*	down	up				Blood cell subpopulations	CD3+, CD4+, CD8+, CD14+, CD19+			
				Allograft gene expression	*NOP9*	down	up				Serum AST/ALT/GGT				

			PBMC gene expression	*NOP9*	down	up
			Liver histology	load of CD4+ cells; median (IQR)	178 (168-205)	85 (69-158)

* This report also includes patients from other cohorts ("Pamplona", "Murcia 1", "Murcia 2")

ADO, adenosine; ALT, alanine aminotransferase; APC, antigen-presenting cell; AST, aspartate aminotransferase; ATP, adenosine triphosphate; AUC, area under the curve; Breg, regulatory B-cell; CNI, calcineurin inhibitor; DC, dendritic cell; DSA, donor-specific antibodies; ER, overall error rate; GFR, glomerular filtration rate; GGT, gamma glutamyltransferase; HLA, human leukocyte antigen; IQR, interquartile range; IS, immunosuppression; ISW, immunosuppression withdrawal; L, liter; LT, liver transplantation; mo, months; PBMCNK, natural killer; NPV, negative predictive value; NR, not reported; NS, non-significant; OR, odds ratio; PBMC, peripheral blood mononuclear cells; PPV, positive predictive value; sCD73, soluble CD73; SD, standard deviation; SN, sensitivity; SP, specificity; TCR, T-cell receptor; Treg, regulatory T-cell; TSDR, T-cell–specific demethylated region; U, units; yr, years; 95CI, 95% confidence interval