The optimal immunosuppression management to prevent early rejection after liver transplantation - A systematic review of the literature and expert panel recommendations

Eleonora De Martin, MD, PhD[1], Maria-Carlota Londoño, MD, PhD[2], Juliet Emamaullee, MD, PhD, FRCSC, FACS[3], Jan Lerut, MD, PhD[4], Jonathan Potts, PhD, MRCP[5], Varuna Aluvihare, MD, MRCP[6], Michael Spiro, MBBS, BSc, FRCA, FFICM[5,7], Dimitri Aristotle Raptis, MD, MSc, PhD[5,8], Geoffrey McCaughan, MD, PhD[9]. *On behalf of the ERAS4OLT.org Working Group*

Affiliations

1. APHP, Hôpital Paul Brousse, Centre Hépato-Biliaire, INSERM Unit 1193, Hepatinov, Villejuif, France
2. Liver Unit, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi I Sunyer and Centro de Investigación en Red de Enfermedades Hepáticas y Digestivas, University of Barcelona, Barcelona, Catalonia, Spain
3. Department of Surgery, University of Southern California, Los Angeles, USA
4. Institute for Experimental and Clinical Research (IREC) Université catholique Louvain (UCL) Brussels, Belgium
5. Clinical Service of HPB Surgery and Liver Transplantation, Royal Free Hospital, London, UK
6. Transplant Hepatology Lead Institute of Liver Studies, King's College Hospital, London, UK
7. Department of Anesthesia and Intensive Care Medicine, Royal Free Hospital, London, UK
8. Division of Surgery and Interventional Science, University College London, UK
9. A.W. Morrow Gastroenterolgy and Liver Center, Sydney Medical School, University of Sydney, Centenary Institute, Australian National Liver Transplant Unit ,Sydney, Australia

Correspondance: Eleonora De Martin, 12-14 Av Paul Vaillant Couturier, 94800 Villejuif, France, eleonora.demartin@aphp.fr Tel. 01.45.59.64.33, Fax 01.45.59.36.96. Geoffrey McCaughan, RPAH Missenden Rd Sydney 2050 Australia, g.mccaughan@centenary.org.au Tel. +61 418212805 Fax +61 2 95155182

Protocol registration: CRD42021245586

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ctr.14614.

Conflict of interest: The authors declare no conflicts of interest.

Funding: This manuscript was prepared for the ERAS4OLT.org Consensus Conference 2022, which is partially funded by the International Liver Transplant Society (ILTS).

Word Count: 4753

Keywords: Immunosuppression, liver, transplantation, acute cellular rejection

Data statement: Data sharing is not applicable to this article as no new data were created.

This work was conducted in preparation for the ILTS - ERAS4OLT.org Consensus Conference on Enhanced Recovery for Liver Transplantation, January 2022, Valencia, Spain.

Abbreviations

ACR: Acute cellular rejection
AUC: Area under the concentration–time curve
BPAR: Biopsy-proven acute rejection
CI: Confidence interval
CyA: Cyclosporin
CNI: Calcineurin inhibitor
eGFR: Estimated glomerular filtration rat
MDRD: Modification of Diet in Renal Disease
MELD: Model for End-stage Liver Disease
MMF: Mycophenolate mofetil
MPA: Myco-phenolic acid
mTOR: mechanistic Target of Rapamycin
TAC: Tacrolimus
ULN: Upper limit of normal

Abstract

Background: The optimal immunosuppression protocol to prevent early acute cellular rejection (ACR) after liver transplantation (LT) avoiding prolonged hospitalization and early hospital re-admission is undefined.

Objectives: To identify the most suitable immunosuppression regimen for inclusion in ERAS programs in order to minimize early ACR after LT and to provide expert panel recommendations

Data sources: Ovid MEDLINE, Embase, Scopus, Google Scholar, and Cochrane Central.

Methods: Systematic review following PRISMA guidelines and recommendations using the GRADE approach derived from an international expert panel. Studies from January 2000 onwards focusing on early ACR were included. Rates of early renal dysfunction and infection were evaluated. CRD42021245586

Results: Thirty-seven studies met inclusion criteria; 23 randomized controlled trials, 14 retrospective or prospective observational comparative or non-comparative studies. Several sources of biases which potentially confound conclusions were identified: heterogeneity in immunosuppression protocols, higher serum tacrolimus levels than currently used in clinical practice, differences in the definition of ACR.

Conclusions:

Tacrolimus is the standard immunosuppression after LT and can be used in combination with other drugs such as corticosteroids and MMF, and in association with anti-IL2 receptor antibody (IL2Ra) induction. **(Quality of Evidence; Low | Grade of Recommendation; Strong).** Low dose or delayed introduction of tacrolimus in association with corticosteroids and MMF and/or anti-IL2Ra induction can be used to reduce acute kidney injury. **(Quality of**

Evidence; Low | Grade of Recommendation; Strong). Use of tacrolimus in association with corticosteroids and MMF and/or anti-IL2Ra induction does not lead to increased infection rates. **(Quality of Evidence; Low | Grade of Recommendation; Weak)**

Introduction

One of the most outstanding advances in the management of liver transplant (LT) recipients is the improvement of immunosuppression (IS). The increasing potency of immunosuppressive drugs has significantly decreased the incidence of rejection, steroid-resistant rejection, and rejection-related graft loss.[1] However, consequently patients are potentially more exposed to the side effects of IS, including renal dysfunction and opportunistic infections.

Early acute cellular rejection (ACR) has been reported in 5%-52% of LT recipients within the first year. This wide variation in the reported incidence of ACR is due to significant differences between studies, and lack of a standard definition of ACR (biopsy-proven or not, scoring systems, protocol or for-cause liver biopsies).[2] Acute cellular rejection usually responds to pulsed corticosteroids but it can prolong hospitalization and lead to early re-admission. In this sense, the optimal IS strategy is currently unknown but it should seek a balance between the minimum dosing required to prevent rejection whilst minimizing IS-related side effects, particularly renal dysfunction and opportunistic infections. The use of induction therapy aiming to delay and/or reduce the exposure to calcineurin inhibitors (CNI) seems to be an acceptable strategy as it has been proven to improve graft outcomes and 5-year patients' survival.[3] However, its implementation varies widely among different centres and countries, probably owing to the limitations of the available information, and more importantly due to the heterogeneity of LT candidates (MELD and Child-Pugh scores, renal function, the presence of clinical decompensation and/or hyponatremia at the time of transplant). These factors challenge the applicability of strict IS protocols and force a personalized approach, adapted to individual needs.

There are several issues that remain unanswered regarding the best IS regimen early after transplantation, including the need of induction therapy, the need of corticosteroids, and the best renal sparing regimen.[2] The aim of the present study is to provide evidence-based and expert recommendations on the optimal IS management to prevent early ACR after LT, whilst minimizing renal impairment and infectious complications, for inclusion in ERAS programs.

This work was conducted in preparation for the ILTS– ERAS4OLT.org Consensus Conference on Enhanced Recovery for Liver Transplantation, January 2022, Valencia, Spain.

Methods

Protocol and registration

The systematic review protocol was registered at PROSPERO (CRD42021245586).

Eligibility criteria

Outcomes of interest were early (< 90 days) ACR, infection and renal function. Eligible studies included adult (>18 years) primary LT recipients and original articles, comparative and non-comparative studies, retrospective or prospective. All of the included studies had early ACR as primary or secondary endpoint. Where the time interval to the rejection episode was not clearly stated, it was assumed that ACR was evaluated as an early outcome.

Exclusion criteria were: 1) conference abstracts, 2) non English articles, 3) studies published before the year 2000, 4) studies with randomization after 4 weeks post-LT, 5) studies focusing on HCV-positive patients, 6) studies without clear ethical approval.

Information sources

Databases searched was performed by professional academic librarians and included: Medline-Ovid, Embase, Scopus, Clinicaltrials.gov and Cochrane Central Register of Controlled Trials, Google Scholar from 2010 to present. The research date was 22nd March 2021. Two authors (EDM, GMC) determined the eligibility for each citations. Some of the studies were deleted and additional studies from the year 2000 not identified by the original research were included.

Search

The search string below was used and adapted to each database: (liver OR hepatic) AND (transplant OR transplants OR transplantation OR transplantations OR graft OR grafts) AND ("early rejection" OR "acute rejection" OR "cellular rejection") AND (immunosuppression OR immunosuppressive)

Study selection

Studies were screened for eligibility using Endnote v.10 then downloaded for full text review and inclusion by two reviewers independently. Additional studies were included by the expert review panel, even published after the year 2000, if they were relevant to the topic. Few studies were also eliminated as not compliant with the inclusion criteria.

Quality of studies and Recommendations Grading

The "Grading of Recommendations Assessment, Development and Evaluation" (GRADE) approach was used for grading quality of evidence and strength of recommendations.[4] The GRADE system was designed to provide a comprehensive and structured approach to rating the quality of evidence (QOE) for systematic reviews, and to grade the strength of recommendations for development of guidelines in health care. We applied the modified GRADE approach for QOE assessment derived from systematic reviews using estimates summarised narratively.[5] The QOE was rated separately for each outcome. The direction

and strength of recommendation was assessed individually by all authors and disagreements resolved by consensus.[6,7]

Results

Study selection

Overall, 37 studies met selection criteria. These included 23 RCTs, 14 retrospective or prospective observational comparative or non-comparative studies (**Figure 1**). The study characteristics are summarized in **Table 1**.

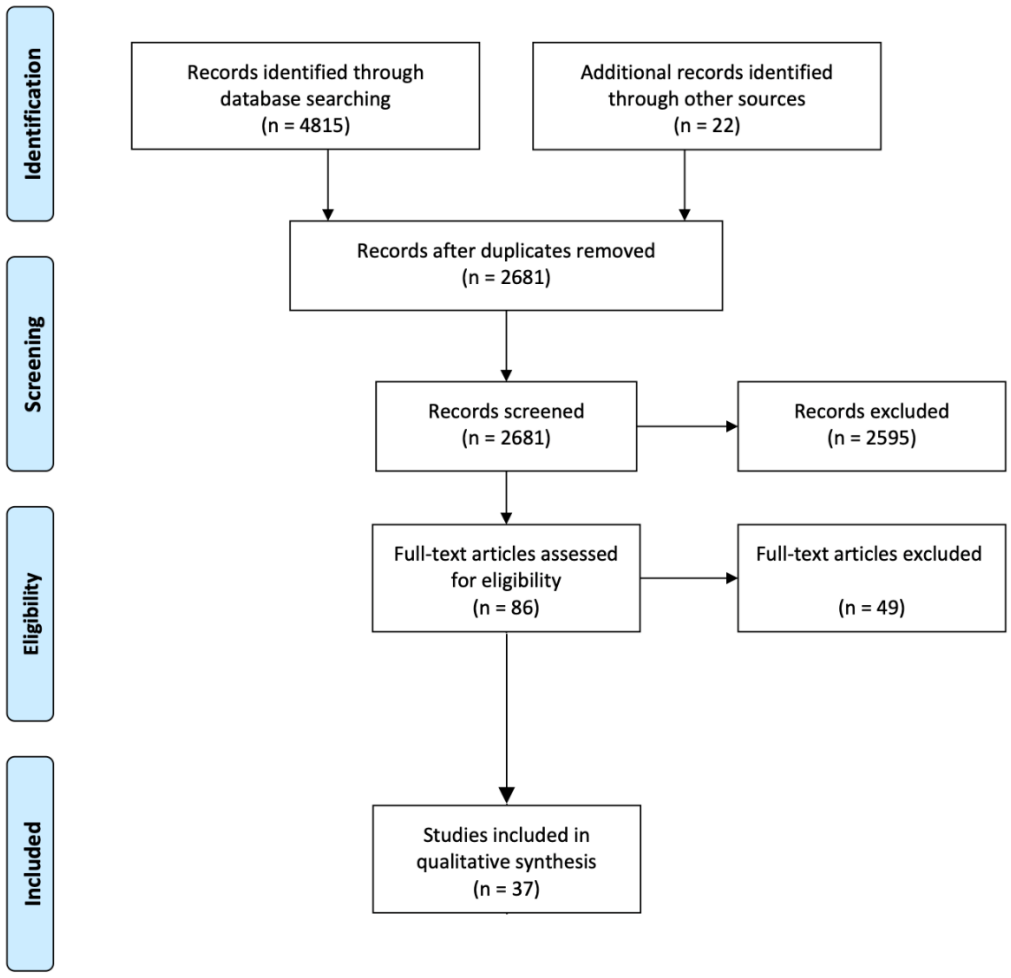

Figure 1. Flow diagram

Table 1. Study characteristics

	Study type	No. of patients	Main outcomes assessed
Asrani et al 2013	RCT	222 standard-dose TAC n=112	• ACR • Patient and graft survival • Hepatic artery thrombosis • Sepsis
Bajjoka et al 2008	Observational comparative retrospective	198 ATG n=118	• ACR • EGFR 12 months • Patient and graft survival • Bacterial Infection • CMV
Bari et al 2020	Observational comparative prospective	40 budesonide n=20 control n=20	• ACR • Steroid-resistant AR • Patient and graft survival • NODAT • Infections (Bacterial, Fungal,Viral)
Becker et al 2008	RCT	602 TAC+DAC n=305	• ACR • Patient and graft survival • Re-transplant • Renal function
Benitez et al 2010	RCT	37 ATG n=21 control n=16	• ACR • Patient and graft survival • Bacterial and CMV/fungal infection • Biliary Stricture • Neurotoxicity
Biselli et al 2009	Observational comparative retrospective	60 MMF+low dose CNI n=30	• ACR • EGFR 2 year • Gastrointestinal side effects • Infections
Boudjema et al 2011	RCT	195 standard-dose TAC n=100	• ACR • Renal dysfunction • Arterial hypertension • Diabetes
Calmus et al 2002	Observational non-comparative	101 basiliximab+triple therapy	• ACR • Infection
Calmus et al 2010	RCT	198 DAC+delayed TAC n=98	• ACR • Renal function • Patient and graft survival at 6 months
Castedal et al 2018	Observational comparative	238 steroid-free n=155 steroid based n=83	• ACR • One-year patient and graft survival • NODM
Cillo et al	RCT	140	• ACR • Renal function
Dannhorn et al 2014	Observational comparative retrospective	94 Prograf n=46 Adoport n=48	• ACR • Patient and graft survival • CMV viremia • Sepsis • Acute kidney injury
Hashim et al 2020	RCT	89 triple immunosuppression n=47 basiliximab+low dose steroids+MMF+TAC	• 6-month mortality • ACR, renal function, wound healing, infections, hepatic artery thrombosis
Hirose et al 2000	Observational non-comparative	32 DAC+steroids+MMF±CNI	• ACR
Iesari et al 2018	RCT	212 TAC monotherapy n=107	• Stable liver function without rejection • ACR, patient and graft survival, safety

Klintmalm et al 2014	RCT	260 basiliximab+belatacept high dose+MMF n=52 belatacept high dose+MMF n=51 belatacept low dose+MMF n=50	• Combined ACR, graft loss and death • ACR, renal function, HCV recurrence, cardiovascular and metabolic comorbidities, safety
Levitsky et al 2011	Observational comparative retrospective	140 alemtuzumab+TAC+MMF n=55 TAC+steroids n=85	• ACR • Patient and graft survival, infections, renal function, metabolic complications, malignancies and patients on monotherapy at 2 years
LLadó et al 2018	Observational non-comparative prospective	69 basiliximab+delayed TAC+MMF+steroids	• Renal function • ACR, creatinine, eGFR, dialysis
Lerut et al 2008	RCT	156 TAC+placebo = 78 TAC+steroids = 78	• ACR • CS resistant ACR • Bacterial , Fungal and Viral infection
McAlister et al 2001	Observational non-comparative prospective	56 Low-dose TAC+SRL	• ACR in SRL-TAC combination • Pharmacokinetics – interference SRL-TAC
Moenech et al 2007	RCT	110 TAC+steroids n=54 TAC+placebo n=56	• ACR • Patient and graft survival • Incidence of steroid side effects
Nashan et al 2009	RCT	58 TAC+MMF+steroids n=31	• BPACR • Pharmacokinetics of MMF • Renal function
Neuhaus et al 2002	RCT	381 basiliximab n=188 standard-dose TAC+steroids	• BPAR < 6 months • Analysis stratified HCV pos/neg pats • Composite endpoint : BPAR < 6 months death/ graft loss/ at 6-12 months
Neuberger et al 2009	RCT	n=183 low-dose TAC+steroids+MMF n=170	• BPAR • Renal function • Change in baseline GFR
O'Grady et al 2002	RCT	606 TAC+steroids+AZA	• Combined endpoint : death, re-transplantation (graft loss) and treatment failure for immunological reasons • Same and ACR (< and > 14 days), steroid resistant rejection, chronic rejection and change
Pageaux et al 2004	RCT	174 CyA+basiliximab+prednisolone n=90	• ACR • Patient and graft survival • Adverse events and metabolic complications
Pelletier et al 2013	RCT	100 steroid-free n=50	• ACR • Patient and graft survival • Bacterial Infection • New onset metabolic syndrome
Ramirez et al 2013	RCT	39 steroid-free n=19	• ACR • Patient and graft survival • Bacterial and CMV infection • New onset metabolic syndrome
Rodriguez-Peralvarez et al 2013	Observational comparative retrospective	493 Tac n=237 TAC+steroids or AZA or MMF n=89	• ACR • Patient and graft survival • Chronic renal impairment • Chronic rejection

Schnitzbauer et al 2015	Observational non-comparative	101 TAC OD	• ACR • Renal function • Tacrolimus levels
Soliman et al 2007	Observational comparative retrospective	473	• ACR • Bacterial and CMV/Herpes infection • Patient survival •
Sterneck et al 2000	RCT	57 MMF n=28	• ACR • Bacterial and CMV infection • Patient and allograft survival
Trunečka et al 2010	RCT	475 OD Tacrolimus n=239 BD Tacrolimus n=236	• BPAR • BPAR at 12 months post LT • Incidence and time to ACR/BPAR • Steroid resistant rejection • Patient and graft survival at 24 weeks & 12
Trunečka et al 2015	RCT	TAC (0.2mg/kg),+MMF (1g BD until D14 then 0.5g BD) n=289 TAC (0.15-0.175mg/kg), MMF+basiliximab n=291	• Renal function (GFR by MDRD4) at week 24 • eGFR, GFR by iohexol clearance and creatinine clearance at week 24 • Graft loss or BPAR
Verna et al 2011	Observational comparative retrospective	229 baxiliximab+delayed CNI+MMF+steroids n=102	• 30 day and 1 year survival • Serum creatinine, graft survival, dialysis-free survival, ACR at 30 days and 1 year
Wiesner et al 2001	RCT	565 MMF n=278 AZA n=287	• Treated BPAR <6 months post LT • Graft loss (death/re-transplantation) within 1 year • Time to graft loss, use of anti-lymphocyte
Yoshida et al 2005	RCT	148 DAC+MMF+steroids+ delayed, low-dose TAC n=72	• GFR by MDRD and creatinine clearance up to 12 months

ACR: Acute Cellular Rejection, ATG: Anti-Thymocyte Globulin, AZA: Azathioprine, BD: bis die, BPACR: Biopsy-Proven Acute Cellular Rejection, CMV: Cytomegalovirus, CNI: Calcineurin Inhibitors, DAC: Daclizumab, EGFR: Estimated Glomerular Filtration Rate, EVL: everolimus, GFR: Glomerular Filtration Rate, HCV: Hepatitis C Virus, IS: immunosuppression, MMF: Mycophenolate Mofetil, MDRD: Modification of Diet in Renal Disease, NODAT: New Onset Diabetes After Transplantation, OD: once daily, RCT: Randomized Controlled Trial, SRL: Sirolimus, TAC: Tacrolimus.

Results of individual studies

In all of the following studies, three major outcomes were evaluated: early ACR, infection, and renal function. The results are displayed in **Table 2**.

Table 2. Study outcomes

	Acute rejection	Renal dysfunction	Infection
Asrani et al 2013	BPAR standard-dose TAC vs reduced-dose TAC + SRL 30 vs 26 (p=ns)	Adjusted mean decrease from baseline of creatinine clearance standard-dose TAC vs reduced-dose TAC + SRL -28.9 vs. -17.2 (p=0.027)	Any infection standard-dose TAC vs reduced-dose TAC + SRL 68.5% vs 79.6% (p=0.07) Sepsis
Bajjoka et al 2008	BPAR ATG vs control 16% vs 26% (p=0.08)	NA	CMV disease ATG vs Control 2.5% vs 11.3% (p<0.01) Overall infection ATG vs control 28% vs 51% (p <0.05)
Bari et al 2020	BPAR budesonide vs prednisone 5% vs 5% (p=1.00)	NA	Overall infection Budesonide vs prednisone 0% vs 30% (p=0.02)
Becker et al 2008	BPAR TAC+DAC vs TAC+MMF 19.7% vs 16.2% (p=ns)	TAC/DAC vs TAC/MMF 12.1% vs 9.4% (p=ns)	Bacterial sepsis TAC/DAC vs TAC/MMF 0.7% vs 3.4%, (p=0.02)
Benitez et al 2010	ACR episode ATG vs control 52.4% vs. 25% (p = 0.09)	Renal function was similar in both groups (data not shown)	Overall infections ATG vs control
Biselli et al 2009	MMF vs control 0% vs 10% (p=ns)	NA	MMF vs control 20% vs 3.3% (p=ns)
Boudjema et al 2011	reduced-dose TAC+MMF vs control 46 (46%) vs 28 (30%)	Control vs reduced-dose TAC + MMF 42 (42%) vs 23 (24%) HR [95% CI] 0.49 [0.29-0.81] p=0.004	NA
Calmus et al 2002	23 (22.8%)	32.7%	Overall infection 83 (82.2%) – 48 bacterial, 56 viral
Calmus et al 2010	delayed-TAC vs standard-dose TAC 20 vs 23 (p=ns)	Creatinine > 130, delayed TAC 22 (22.4%) vs standard-dose TAC 30 (29.7%), p=ns Mean eGFR delayed TAC 76.8 ±32 mL/min vs standard-dose TAC 66±.23.8 mL/min p=0.09	NA
Castedal et al 2018	steroid-free vs steroid-based 43 (27.7%) vs 23 (27.7%) (p=ns)	NA	NA
Cillo et al 2019	EVL vs standard-dose TAC 12 (12.9%) vs 2 (4.3%) (p=0.09)	eGFR-MDRD-4 median difference at 1 mo 20mL/min/1.73 m^2 (worse in standard-dose TAC group p<0.01	NA
Dannhorn et al 2014	Prograf vs Adoport 8/46 (17.4%) vs 9/48 (18.8%) (p=ns)	Prograf vs Adoport 26/46 (56.5%) vs 9/48 (29.2%) (p=ns)	Prograf vs Adoport 27/46 (58.7%) vs 26/48 (54.2%) (p=ns)
Hashim et al 2020	TAC+MMF+steroids vs basiliximab+ TAC+MMF+steroids lower doses 17% vs. 9.5% (p=0.15)	renal dysfunction TAC+MMF+steroids vs basiliximab+ TAC+MMF+steroids lower doses 19.14% vs 7.1% (p=0.004)	TAC+MMF+steroids vs basiliximab+ TAC+MMF+steroids lower doses 27.5% vs. 28.5% (p=0.37)
Hirose et al 2020	no CNI vs low dose CNI 100% vs 36%	NA	NA

Iesari et al 2018	ATG vs TAC 10% vs 24% (p=0.019) steroid-sensitive 11.9% vs. 10.3% (p=0.826)	eGFR ATG vs TAC 60 vs 77, p=0.032	NA
Klintmalm et al 2014	basiliximab+belatacept HD +MMF vs belatacept HD+MMF vs belatacept LD+MMF vs TAC+MMF vs	differences in cGFR between the belatacept and TAC were observed as early as month 1 and persisted through month 12 (15–34 mL/min/ 1.73 m2 higher in each belatacept group vs. the TAC groups at month 12)	basiliximab + belatacept HD + MMF vs belatacept HD + MMF vs belatacept LD + MMF vs TAC + MMF vs
Lerut et al 2008	TAC monotherapy vs TAC+steroids Early ACR 10/78 vs 16/28 (p=ns)	TAC mono vs TAC + steroids Mean early creatinine 1.53 vs 1.26	TAC mono vs TAC + steroids Early Bacterial 43/78 vs 13/78 (p=ns)
Levitsky et al 2011	alemtuzumab inductions + TAC+MMF vs TAC+steroids 20% vs. 30.3% (p=0.13)	creatinine: 1.3±0.6 mg/dL vs. 1.4±0.6 mg/dL (p=0.25) dialysis: 1.8% vs 0 (p=0.87)	alemtuzumab inductions + TAC+MMM vs TAC + prednisone 63.6% vs. 44.3% (p=0.03)
LLadó et al 2019	No rejection episodes	7.2%	36%
McAlister et al 2001	14%	“near normal “ for whole group	CMV infection 7%
Moenech et al 2007	TAC+steroids vs TAC+placebo 35.2% vs 48.2%, p=0.116	abnormal kidney function 2 vs 1, p=ns	CMV TAC-steroids vs TAC+placebo
Nashan et al 2009	standard-dose TAC +MMF+steroids Vs TAC low dose+MMF+steroids	NA	NA
Neuberger et al 2009	BPAR needing treatment standard-dose TAC+steroids vs low dose TAC+steroids+MMF vs DAC induction+steroids+MMF +TAC delayed introduction	GFR ml/min/1.73m² reduction -23.6 vs -21.22 vs -13.63 A vs C p=0.012; A vs B p=0.199 Hemodialysis	NA
Neuhaus et al 2002	At week 2 basiliximab vs placebo 13.3% vs 28% (p<0.001)	Similar kidney function. (data not shown)	basiliximab vs. placebo 80.3% vs 83.4% HCV neg 74.2% basiliixmab vs. 79.2% in placebo Serious inf:29.6 vs 30.6%
O’Grady et al 2002	TAC vs CyA No ACR 52% vs 41% p=0.009 One treatment response	TAC vs CyA renal support 18 vs 15%	TAC vs CyA infection treated 88 vs 86%

Pageaux et al 2004	steroids vs placebo 24.4% vs 38.1% p=0.03	NA	steroids vs placebo Infection 13.3% vs 8.3% CMV infection
Pelletier et al 2013	steroids+MMF+TAC vs	postoperative acute renal failure 24% vs 36% (p=0.19)	22/50 vs 52/50 (p=ns)
Ramirez et al 2013	steroids+basiliximab+MMF +TAC vs	NA	12/20 vs 12/19 bacterial infection (p=ns) no CMV either arm note :14/20 vs 11/19 HCV
Rodriguez-Peralvarez et al 2013	36.3% TAC monotherapy, 33.3% TAC+steroids, 34% TAC+AZA+/-steroids and 40% in TAC+MMF+/-steroids (p=0.85) TAC level <7ng/ml vs > >7ng/ml had moderate/severe rejection 41.2% vs 23.8% (p=0.004)	NA	NA
Schnitzbauer et al 2015	Once daily TAC+MMF+basilximab (no steroids)	mean creatinine 1.2mg/dl	NA
Soliman et al 2007	3 days ATG+CyA+steroids vs 10 days ATG+CyA+steroids	NA	bacterial and fungal (p=ns) death from infection + 10 days ATG (OR 8.7, p=< 0.0001) CMV and HSV (p=ns)
Sterneck et al 2000	MMF+CyA+steroids vs	NA	serious bacterial infection 2/28 vs 1/29 (p=ns)
Trunečka et al 2010	BPAR at 24 weeks BD vs OD 33.7% vs 36.3%	no difference in ΔCrCl from baseline to 12 months (p=0.86)	overall infections (BD) vs (OD)
Trunečka et al 2015	BPAR at 24 weeks in basiliximab/reduced TAC+MMF (12.1%) vs TAC+MMF (17.9%, p=0.016) and basiliximab/delayed TAC+MMF (16.8%, p=0.039)	week 24 GFR superior in basiliximab/reduced TAC/MMF and basiliximab/delayed TAC/MMF vs TAC/MMF 67.4 mL/min/1.73m2 (Arm 1) vs 76.4 (Arm 2), p=0.001 and 73.3 (Arm 3), p=0.047	NA
Verna et al 2011	BPAR basiliximab vs controls	30 day dialysis-free survival basiliximab vs controls 88% vs 94% (p=0.09)	no difference in sepsis-related deaths; 8% both groups
Wiesner et al 2001	BPAR MMF vs AZA 38.5% 47.7% (p=0.025)	NA	opportunistic infections MMF vs AZA 45.5% vs 43.2%
Yoshida et al 2005	no difference in BPAR	median GFR at 1 month DAC vs SOC 86.6 vs 70.1 (p<0.001)	CMV DAC vs SOC 40% vs 32% Non-CMV infections

Acute cellular rejection

Tacrolimus (TAC) vs Cyclosporine (CyA)

Calcineurin inhibitors form the basis of most IS regimens and several studies have assessed TAC vs CyA-based regimens. In the key original RCT by O'Grady et al [8], 301 LT recipients allocated to receive TAC were compared to 305 patients allocated to receive CyA. Target trough serum drug levels were 5-25 ng/mL (TAC) or 150-250 μg/mL (CyA). The absence of clinically significant ACR (52% in TAC vs 41% in CyA), as well as ACR requiring one successful treatment (19% vs 18%) or two successful treatments (8% vs 4%) were similar in both groups (O'Grady 2002). However significantly lower rates of death (p=0.04), and the following composite outcomes: death and re-LT (p=0.006), steroid-resistant rejection (p=0.009), and death, re-LT and immunological failure rates (p=0.001) were observed in the TAC arm.

Regarding the different TAC formulations, Trunečka et al. performed a double blind RCT comparing once daily (OD) slow release TAC (n=239) with BID dosing of the standard formulation (n=236).[9] Biopsy proven acute rejection (BPAR) at 24 weeks was similar in both groups 33.7% (BID) vs 36.3% (OD) p=0.512 and at 12 months 35.4% vs 37.9%, p=0.647[9]. Dannhorn et al compared in a single center retrospective observational study the outcomes of patients on TAC (Prograf, Astellas, Osaka, Japan)) (n=46) and patients on generic formulation of TAC (Adoport, Sandoz, Basel, CH)) (n=48).[10] The authors found no statistically significant difference in the frequency of a single episode of ACR (15% vs 18%).

Induction therapy

The impact of induction therapies on ACR onset was explored in 18 studies including 10 RCTs. Multiple induction therapies were assessed. Four studies evaluated the use of anti-thymocyte globulin (ATG).[11–14] In both studies from Bajjoka et al and Benitez et al. [12,13], the patients on ATG had a statistically non-significant higher ACR rate than patients on standard IS protocol ((16% vs 26%, p=0.08 and 52.4% vs. 25%, (p = 0.09), respectively). It should be noted that in the Benitez et al. paper the use of ATG was primarily aimed at inducing tolerance by rapid and early withdrawal of IS.[13] It clearly failed to do so.

In contrast, the more recent RCT of Lesari et al. found a lower histopathological rate of ACR in patients with ATG induction compared to patients on standard-dose TAC (10% vs 24%,

p=0.019), while clinical corticosteroid-sensitive (11.9% vs 10.3%, p=0.82) and corticosteroid-resistant ACR (2.1 vs 2.8, p=1) were comparable between the two groups.[14]

Five studies included patients treated with daclizumab (DAC). This drug is no longer in use hence the results of the studies can be found in Tables 1 and 2 but they are not detailed in this section.

Eight selected studies assessed the efficacy of basiliximab as induction therapy, (4 RCTs and 4 observational studies). In the early observational study of Calmus et al., ACR rate was 22.8% [15], while more recent reports showed a lower BPAR rate (4.9%) [16]. In the retrospective study of Verna et al., there was no statistically significant difference in BPAR in basiliximab vs control groups (26% vs 18%, p=0.13).[17] This was confirmed in the RCT of Hashim et al. that found ACR rate of 17% vs 9.5% (p=0.15) with and without basiliximab.[18] In the RCT of Neuhaus et al.[19] BPAR within 2 weeks after LT was lower in basiliximab group (13.3% vs 28%, p<0.001).[19] In the study of Klintmalm et al, basiliximab was used with belatacept.[20] ACR was higher in belatacept groups compared to the TAC groups (48%, 41.7%, 46.9% vs 15%, 38%). The study was interrupted due to the high side effects in the belatacept groups. The DIAMOND study, showed a lower ACR rate in patients treated with basiliximab and low dose of TAC.[21]

Levitsky et al. described the use of alemtuzumab and found no difference in ACR between patients with or without induction therapy (20% vs 30.3%, p=0.13).[22]

Low dose TAC-based IS regimens

The use of low dose of TAC was also part of some IS regimens. Low dose TAC was combined either with MMF or AZA in 6 studies. As previously described, in the RCT of Neuberger et al. there was no statistically significant difference in ACR including the group with low dose of TAC + MMF compared to the others.[23] This was also found in one RCT which compared LT recipients treated with standard-dose TAC (trough levels 10-15 ng/mL) to patients treated with reduced TAC (5-8 ng/mL) in combination with MMF and corticosteroids. In this study the incidence of BPACR at 6 months was 17% in both groups, with all episodes occurring within the first 12 weeks.[24] In addition one retrospective study confirmed the absence of statistically significant difference in MMF treated patients (0% vs 10%, p=ns).[25] Interestingly one RCT found a lower ACR rate with MMF+ low dose TAC (0.04 mg/kg twice a day to maintain trough levels ≤10 ng/mL) compared to the control (standard-

dose TAC 0.075 mg/kg twice a day to maintain trough levels ≥12 ng/mL) (30% vs 46%, HR [95%CI] 0.59 [0.37-0.94] p=0.024).[26] In the observational study of Rodriguez-Peralvarez et al. moderate to severe BPACR was not associated with the type of IS: 36.3% TAC monotherapy, 33.3% TAC + corticosteroids, 34% TAC + AZA +/- corticosteroids and 40% in TAC + MMF +/-corticosteroids (p=0.85).[27] In multiple logistic regression analysis moderate to severe BPACR was associated with lower TAC trough levels on the day of the protocol liver biopsy (OR=0.94; p=0.03). The authors suggested that TAC trough levels >7ng/ml may be sufficient to minimize the risk of ACR and 41.2% of patients with TAC level <7ng/ml had moderate/severe rejection compare to 23.8% over this threshold (p=0.004).[27]

MMF vs AZA

Two RCTs compared MMF to AZA. A small RCT did not report a statistically significant difference in ACR rates between patients treated with MMF and those receiving AZA on the background of CyA (p=0.06).[28] Wiesner et al. conducted the registration study of MMF in LT.[29] This international, multi-centre, double-blinded, RCT compared MMF (1g BD increased to 1.5g BD after 1 week) vs AZA (1-2mg/kg daily) in 565 liver transplant recipients receiving CyA and corticosteroid-based IS. The study identified a modest reduction in BPACR and graft loss only at 6 months in the MMF treated group (38.5% vs 47.7%, p=0.025).

mTOR inhibitors

A first single center feasibility study, showed a ACR rate of 14%.[30] In the RCT of Asrani et al. 222 patients were randomized at 48h post-LT.[31] There was no statistically significant difference in ACR in patients who received standard-dose of TAC (trough 7-15 ng/mL) and patients who received SRL (trough 4-11 ng/mL) and reduced dose of TAC (trough 3-7 ng/mL), p=0.6.[31] More recently, an open label RCT randomized patients to receive everolimus (EVL) at day 8 post-LT with progressive reduction (trough level <5 ng/mL) or withdrawal of TAC when EVL was stable >5 ng/mL or to continue TAC at 6-12 ng/mL. ACR in the EVL group was not statically different compared to controls (12.9% vs 4.3%, p=0.09).[32]

Corticosteroid-free IS

The possibility of corticosteroid-free IS regimens was assessed in five RCTs, one retrospective study. In a first prospective, randomized, double-blind, placebo-controlled trial,

all patients were randomized on day 7 to receive either prednisone (n=90) or placebo (n=84) after 7-day blinded oral steroid tapering. The incidence of BPACR was significantly higher in CyA monotherapy group (38.1% versus 24.4%, p=0.03).[33] Another prospective double-blind and placebo-controlled RCT, TAC was used without induction therapy and all patients received methylprednisolone for the first 14 days, thereafter methylprednisolone (n=56) or placebo (n=54) for 6 months. Patients on methylprednisolone arm experienced a non-statistically significant higher ACR rate (48.2% vs 35.2%, p=0.116).[34] In the large study of Lerut et al, including 156 patients of TAC monotherapy observed no difference in rates of ACR (7.6% vs 8.9%) but a higher corticosteroid resistant ACR rate (20.5% vs 12.7%) at 3 months in the TAC monotherapy group.[35] In the RCT of Pelletier et al, patients on TAC + MMF with or without corticosteroids experienced a similar rate of ACR (14/50 vs 20/50, respectively, p=ns).[36] This was also confirmed in the RCT of Ramirez et al., in which only one patient in each group experienced ACR.[37] The retrospective study of Castedal et al. also did not report differences in ACR in corticosteroid-free or corticosteroid-based regimens (27.7% vs 27.7%, p=ns).[38]

One pilot study compared the use of budesonide (n=20) to the use of prednisone (n=20) and found no difference in BPAR between the two regimens (5% vs 5%, p=1).[39]

Renal function

TAC vs CyA

There was no difference in renal function in patients treated with TAC compared to patients treated with CyA. Need for renal support was reported in 18% vs 15% of patients in the O'Grady study.[5] Moreover similar Δ creatinine clearance from baseline to 12 months after LT was found in patients with slow release OD or BD standard-dose TAC, p=0.86).[9] No difference in renal function was observed in patients treated with Prograf or Adoport.[10]

Induction therapy / reduced or delayed TAC

Regarding the use of ATG as induction therapy, renal outcomes were reported in the study of Benitez et al.,[13] which stated similar results in both study groups whilst in the study of Iesari et al.,[14] the eGFR at 1 week was significantly lower in the ATG treated LT recipients (60 vs 77, p=0.032).

The studies including DAC regimens, excepted the study from Yoshida et al.,[43] found no difference in renal function in patients with and without DAC.[23,40,41] In studies assessing basiliximab IS regimens, results regarding renal function are heterogeneous. In the study of Hashim et al. renal dysfunction in the first six months post-transplant was reduced in the basiliximab group when compared to the non-basiliximab group (7.1% and 19.1% respectively, p=0.004).[18] Similarly, the RCT of Trunečka et al. observed higher GFR in patients on basiliximab + reduced TAC and basiliximab + delayed TAC when compared to patients on TAC+MMF (67.4 mL/min/1.73m2 (Arm 1) vs 76.4 (Arm 2), p=0.001 and 73.3 (Arm 3), p=0.047.[21] Klintmalm et al found that the differences in eGFR between the belatacept-treated patients and TAC-treated patients were observed as early as month one sustained until month 12 (15–34 mL/min/ 1.73 m2 higher in each belatacept group vs. the TAC groups at month 12).[20] On the other hand, Verna et al identified no difference in mean serum creatinine at 30 or 90 days after LT.[17] In the RCT of Boudjema et al., the use of MMF + TAC low dose was associated with better renal function compared to TAC standard dosing (24% vs 42%, HR [95%CI] 0.49 [0.29-0.81], p=0.004).[26]

Corticosteroid-free immunosuppression

One RCT of a corticosteroid-free regimen reported on renal function, and no difference in postoperative acute renal failure was observed between corticosteroid-free and corticosteroid-containing regimens (24% vs 36%, p=0.19).[36] Conversely, the all-inclusive study of Lerut et al. reported that the mean serum creatinine in the early post-LT period was significantly higher in the TAC monotherapy arm (1.53 mg/dL vs 1.26, p=0.01). It should be noted that in this study patients with pre-transplant renal dysfunction were included.[35]

mTOR inhibition

In the study of Asrani et al. adjusted mean decrease of creatinine clearance from baseline was greater in the TAC arm than in the SRL arm (-28.9 μmol/l vs. -17.2; p=0.027).[31] Similarly, better renal function was found in the EVL group (median difference in eGFR-MDRD-4 at 1 month 20mL/min/1.73 m^2, worse in standard-dose TAC group p<0.01).[32]

Infection

TAC vs Cya

In the O'Grady study there was no difference in occurrence of infections in patients on TAC compared to patients on CyA (88% vs 86%).[8] No difference in infectious side effects has been identified between the different TAC formulations: OD vs BD 39.2% vs 33.3% [9] and Prograf vs Adoport 58.7% vs 54.2%.[10]

Induction therapy

The results regarding infection rates in the studies using ATG as induction therapy are heterogeneous. Bajjoka et al. found a lower incidence of infection in ATG treated patients compared to controls (38% vs 51%, $p<0.05$) as well as the incidence of CMV disease (2.5% vs 11.3%, $p<0.01$).[12] Benitez et al. found no difference between groups with total infection of 52.3 in ATG treated patients and of 81 in controls, p=ns.[13] In the study of Soliman et al. there was also no difference in bacterial, fungal, CMV or HSV infection. The studies assessing basiliximab as induction therapy found no difference in terms of infection rate compared to the control groups.[17–20,37,42]

MMF vs AZA

The use of MMF did not increase incidence of infection compared to control (20% vs 3.3%, p=ns).[25] No difference was found in infection incidence in patients treated with MMF compared to AZA. In the study of Sternek et al, serious bacterial infections were reported in 2/28 vs 1/29 patients (p=ns).[28] Weisner et al. described 45.5% of opportunistic infections in MMF groups compared to 43.2% in AZA group.[29]

mTOR inhibitors

In the study of Asrani et al. there was also no difference in infection development (68.5% vs 79.6%, p=0.07) while sepsis was higher in patients in the SRL group (7.2% vs 20.4%, p=0.006).[31]

Corticosteroid-free IS

Studies assessing corticosteroid-free IS have not reported differences in infection rates when compared to corticosteroid-containing regimens.[34–37]

Quality of evidence

The summary of findings for the main outcomes, including the quality of evidence (QOE) assessment according to the GRADE approach are summarised in **Table 3**.

Table 3. Summary of Findings leading to the Quality of Evidence Assessment according to the GRADE approach

Summary of Findings									
Number of studies			Effect from comparative studies	Limitations	Inconsistency	Indirectness	Imprecision	Publication Bias	Quality of Evidence (GRADE)
RCT	Observational comparative	Observational non-comparative							
Outcome 1: EARLY ACUTE REJECTION									
23	9	5	No difference in intervention group in most of the studies	Very serious[a]	Very serious[b]	Serious[c]	Not serious[d]	Not likely	Low ●●○○
Outcome 2: RENAL FUNCTION									
18	3	4	No difference in intervention group in all studies	Very serious[a]	Very serious[b]	Serious[c]	Not serious[d]	Not likely	Low ●●○○
Outcome 3: INFECTION									
15	7	3	Heterogeneous in intervention group across studies	Very serious[a]	Very serious[b]	Serious[c]	Serious[e]	Not likely	Low ●●○○

a. Rated very serious due to differences in the definition and evaluation of outcomes and in the immunosuppression protocols used which bias all studies
b. Rated very serious due to the wide differences is results across studies
c. Rated serious due to the wide differences in terms of population, interventions and outcomes across studies
d. Rated not serious due to the good simple size and number of events
e. Rated serious due to the small simple size and number of events

Recommendations

Tacrolimus is the standard based immunosuppression after LT and can be used in combination with other drugs such as corticosteroids and MMF and in association with anti-IL2 receptor antibody (IL2Ra) induction. **(Quality of Evidence; Low | Grade of Recommendation; Strong).**

Low dose or delayed introduction of tacrolimus in association with corticosteroids and MMF and/or anti-IL2Ra induction can be used to reduce acute kidney injury. **(Quality of Evidence; Low | Grade of Recommendation; Strong).**

Use of tacrolimus in association with corticosteroids and MMF and/or anti-IL2Ra induction does not lead to increased infection rates. **(Quality of Evidence; Low | Grade of Recommendation; Weak)**

Evidence to recommendation framework according to the GRADE approach are summarized in Table 4a, 4b and 4c.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ctr.14614.

Table 4a Acute cellular rejection outcome

Question: What is the optimal immunosuppression management to prevent early rejection after liver transplantation?			
Decision domain	**Judgement**		**Reason for Judgement**
	Yes	No	
Balance between desirable and undesirable outcomes (estimated effects), with consideration of values and preferences (estimated typical) *Given the best estimate of typical values and preferences, are you confident that the benefits outweigh the harms and burden or vice versa?*	✓		TAC based IS in combination with other IS drug(s) leads to a reduction in ACR rate and consequently reduced hospital stay and early hospital readmission. The harms associated with the side effects of IS should be taken into account and the ultimate goal is the minimal IS efficacious dose
Confidence in the magnitude of estimates of effect of the interventions on important outcomes (overall quality of evidence for outcomes) *Is there high, moderate or low-quality evidence?*		✓	Quality of evidence is very low due to a high degree of heterogeneity in the studies
Confidence in Values and Preference, and their Variability *Are you confident about the typical values and preferences and are they similar across the target population?*	✓		We are confident that an IS regimen of TAC with other drugs (permitting deployment of low-dose /delayed introduction) can reduce both ACR rate and IS associated side effects.
Resource implications *Are the resources worth the expected net benefit from following the recommendation?*	✓		The resources required to prevent ACR (cost of IS drugs) are lower compared to the cost of prolonged hospitalization or hospital re-admission with minimal differences across individuals

Overall Quality of Evidence: ***Low***			
Recommendation: ***Strong, for the use of tacrolimus in association with other drugs to prevent ACR***			

Table 4b Renal function outcome

Question: What is the optimal immunosuppression management to prevent early rejection after liver transplantation?			
Decision domain	**Judgement**		**Reason for Judgement**
	Yes	No	
Balance between desirable and undesirable outcomes (estimated effects), with consideration of values and preferences (estimated typical) *Given the best estimate of typical values and preferences, are you confident that the benefits outweigh the harms and burden or vice versa?*	✓		Minimization of renal impairment can be obtained by reduced/delayed dosing of TAC. The potential harms (ACR) should be taken into account
Confidence in the magnitude of estimates of effect of the interventions on important outcomes (overall quality of evidence for outcomes) *Is there high, moderate or low-quality evidence?*		✓	Quality of evidence is very low due to the high heterogeneity of the studies

Confidence in Values and Preference, and their Variability *Are you confident about the typical values and preferences and are they similar across the target population?*	✓		We are confident that IS regimen associating TAC with other drugs (permitting delayed / low-dose deployment) can reduce renal impairment rate .
Resource implications *Are the resources worth the expected net benefit from following the recommendation?*	✓		The resources required to prevent renal impairement (sparing IS drugs) are lower compared to the cost of prolonged hospitalization or hospital re-admission with minimal differences across individuals
Overall Quality of Evidence: ***Low***			
Recommendation: ***Strong, for the use of tacrolimus in association with other drugs to prevent renal dysfunction***			

Table 4c Infection outcome

Question: What is the optimal immunosuppression management to prevent early rejection after liver transplantation?			
Decision domain	**Judgement**		**Reason for Judgement**
	Yes	No	

Balance between desirable and undesirable outcomes (estimated effects), with consideration of values and preferences (estimated typical) *Given the best estimate of typical values and preferences, are you confident that the benefits outweigh the harms and burden or vice versa?*	✓		Reduced/delayed dosing of TAC can lead to reduction in infection and consequently reduced length of stay. The potential harms (ACR) should be taken into account
Confidence in the magnitude of estimates of effect of the interventions on important outcomes (overall quality of evidence for outcomes) *Is there high, moderate or low-quality evidence?*		✓	Quality of evidence is very low due to the high heterogeneity of the studies and small sample size
Confidence in Values and Preference, and their Variability *Are you confident about the typical values and preferences and are they similar across the target population?*		✓	We are not confident that one IS regimen is optimal or superior in preventing infection but lower IS exposure will lower infection risk.
Resource implications *Are the resources worth the expected net benefit from following the recommendation?*	✓		The resources required to prevent infection (sparing IS drugs) are lower compared to the cost of prolonged hospitalization or hospital re-admission with minimal differences across individuals
Overall Quality of Evidence: ***Low***			
Recommendation: ***Weak, for the use of tacrolimus in association with other drugs to prevent infection***			

Discussion

Calcineurin inhibitors are the cornerstone of the immunosuppressive therapy in LT. The two major maintenance immunosuppressive drugs used in LT are CyA and TAC. The greater efficacy of TAC has been shown by the randomized controlled, open label UK-Ireland trial published by O'Grady et al.[8] and has been confirmed by several other similar studies and by the meta-analysis about the use of both CNI published by Mc Alister et al.[43] The TMC study demonstrated the superiority of TAC in combination with corticosteroids and AZA.[8] This study is of great value for several reasons: a) the large number of included, randomized patients; b) the small number of participating centers; c) the standardized protocol for drug dosing and concomitant medications; d) the use of protocol biopsies reported using Banff scoring; e) the clear definition of rejection and treatment failure of rejection; f) the robust adherence to the study inclusion (79% of patients followed-up for 12 months; drop out n=4); g) data processing and analysis independent of investigators and sponsors; and last but not least, h) the choice of the most relevant primary (clinical) endpoint combining patient survival, retransplantation and immunological treatment failure at 6 and 12 months post-LT. The latter point is very important because most IS studies only look at rejection and/or CNI-related toxicity.

Interestingly, the incidence of early ACR in the first three months was not different between TAC and CyA which is the main reason for the low quality of the evidence that TAC is more efficacious than CyA in preventing ACR. It is important to note that the incidence of infection and renal failure were similar for both regimens. This leads to our recommendation being graded as strong.

Fifteen similar RCTs containing TAC or CyA, corticosteroids and AZA (9 studies) or MMF (4 studies) were subsequently assessed in a meta-analysis including 3813 LT recipients.[43] This meta-analysis showed that TAC reduced the number of recipients with ACR and corticosteroid-resistant ACR in the first year post-LT.[43]

It should be noted that many of these studies used relatively high trough serum TAC levels compared to current practice, and the incidence of ACR varied widely, clearly showing the

huge heterogeneity in these clinical trials which also contributed to our level of evidence graded as low.

Delayed introduction with induction therapies

Twelve RCTs, four observational studies, three retrospective studies evaluated delayed CNI introduction in association with induction therapies. Overall delayed introduction of TAC was not associated with an increase in ACR and in some cases it was associated with a reduction in ACR rates compared to standard TAC based regimens.[21] The strength of this observation is supported by the good number of RCTs including a significant number of patients. However, several limitations need to be highlighted. These studies are heterogeneous in design, primary and secondary outcomes as well as in IS regimens. Some reports used CyA rather than TAC based regimen and antibody induction therapies included T cell depleting therapies with ATG or anti-IL2Ra therapies. In early studies the target trough levels of TAC were 10-20 ng/mL during the first 4 weeks and 5-15 ng/mL at later time points.[19,23,40,41] These are much higher levels than most centers would currently recommend. For all these reasons the grade of evidence is weak. More recent reports confirmed that delayed introduction of TAC does not increase the frequency of ACR episodes.[14,18,44] Finally, in a meta-analysis including 18 studies with a total of 2.961 patients, reduction of ACR favored the use of anti-IL2Ra and this effect was seen in both randomized and non-randomized studies included in the analysis.[42] However, stratifying trials by the time of measurement there was no difference in ACR rate at 3-6 months.

CNI low doses in association with other immunosuppressive drugs

Dual or triple therapy comprised of CNI and antimetabolite drugs with/without corticosteroids constitutes standard maintenance immunosuppression following LT in most centers.

The use of low dose of TAC in association with MMF was explored in three RCTs [23,24,26] in two observational studies.[25,27]
In all the studies apart from the RCT from Boudjema and colleagues there was no statistically significant difference regarding BPACR or clinically suspected ACR in patients treated with low doses of TAC compared to patients treated with standard-dose of TAC. The meta-analysis of 5 RCTs comparing TAC trough levels showed no difference in BPACR in

LT recipients treated with reduced TAC concentration (6-10 ng/mL) and patients with standard-dose TAC (>10 ng/mL) within the first month post-LT.[45]

Data comparing AZA and MMF in liver allograft recipients are sparse. The RCT of Wiesner et al. identified a modest but non-significant reduction in BPAR and graft loss at 6 months in the MMF treated group.[29] When adjusted for graft loss, there was a less marked reduction in the 6-month incidence of ACR alone but a significant decrease in corticosteroid-resistant ACR in those receiving MMF. However there are no data showing a reduction in rates of ACR when MMF is used instead of AZA on a background of TAC therapy. For this reason the grade of evidence is low. Despite this MMF has substituted AZA in most current IS protocols.

In the two RCTs exploring the use of mTOR inhibitors even if there was no statistically significant difference regarding ACR between patients with and without mTOR inhibitors there was an higher rate of side effects in the mTOR arms[32,33]. Therefore, we do not recommend their administration within the first post-transplant month.

Corticosteroid-free immunosuppression

Corticosteroid-free IS has been studied on a background of TAC therapy. Data from 3 RCTs [36,37] indicated no difference in ACR rates. These studies used BPACR but had small numbers. The two double-blind RCT showed contradictory results, of note both studies were not completely corticosteroid-free as steroids were continued until day 14 after LT before being withdrawn and one was CyA-based while the other was TAC-based.[34] A larger all-inclusive study of TAC monotherapy indicated there was no difference in rates of ACR but a higher steroid-resistant ACR rate at 3 months in the TAC monotherapy arm.[35]
Two important meta-analyses indicated that if corticosteroids were not used and the background TAC regime remained the same then ACR episodes were increased.[46,47] However there was a suggestion that if additional agents were used to replace corticosteroids then ACR episodes actually decreased. Thus if corticosteroids are not part of the additional IS to TAC then either MMF or IL2Ra induction should be considered to minimise ACR episodes.

Immunosuppression strategies and renal function

Several randomized clinical trials [12,18,20,23,48] have evaluated the impact of induction therapy in combination delayed and/or low dose TAC + MMF and corticosteroids on renal function. These studies have important limitations: 1) between 5% and 37% of the patients included in the studies discontinued study treatment, 2) low dose TAC did not always translate into low TAC through levels, 3) there was no clear definition and sometimes no data on the incidence of acute kidney injury. Furthermore some studies excluded pre-existing renal dysfunction. A systematic review and meta-analysis about the use of IL2Ra, did show a lower incidence of renal failure.[42]

The study of Boudjema et al.[26] showed a lower incidence of renal dysfunction and better GFR levels in patients receiving low-dose TAC. These results suggest that the use of delayed introduction and/or low doses of TAC can lead to a reduction in early renal dysfunction even if the evidence is low due to the mentioned limitations.

The impact of early introduction of mTOR inhibitors on renal function showed a lower incidence of acute kidney injury but a significant proportion of the subjects participating in these studies were not eligible to be randomized. Moreover we do not recommend their use early post-transplant as part of an ERAS protocol, considering the high rates of side effects of these drugs.

Immunosuppression strategies and infection

In most of the studies there was no statistically significant difference in bacterial, fungal or viral infection rate.[8,9,17–20,28,29,34–37,48] A meta-analysis did not identify associations between corticosteroid-free regimens and development of infections.[46] The meta-analysis of Sgouaris et al. determined that CMV infection favored corticosteroid-free arm.[47] Therefore we cannot recommend one IS regimen over another in regard of infection, but one should keep in mind that higher IS load goes along with more infectious complications.

Limitations

The main limitation of our conclusions is the heterogeneity of studies and the lack of consistency in trial definitions, interpretations (e.g. definition and modality of diagnosis of ACR, cortico-resistant ACR, renal dysfunction) and evaluation. Immunosuppressive drugs changed over the time and the definition of ACR was different varying from biopsy-proven in some studies to clinically suspected in others. Therefore data from the included studies are difficult to compare as there have been few truly randomized double blinded placebo controlled trials. As the quality of evidence is often scarce we based our grade of recommendation predominantly on expert opinion.

Conclusion

Tacrolimus is the standard based immunosuppression after LT and can be used in combination with other drugs such as corticosteroids and MMF and in association with anti-IL2 receptor antibody (IL2Ra) induction. **(Quality of Evidence; Low | Grade of Recommendation; Strong).**

Low dose or delayed introduction of tacrolimus in association with corticosteroids and MMF and/or anti-IL2Ra induction can be used to reduce acute kidney injury. **(Quality of Evidence; Low | Grade of Recommendation; Strong).**

Use of tacrolimus in association with corticosteroids and MMF and/or anti-IL2Ra induction does not lead to increased infection rates. **(Quality of Evidence; Low | Grade of Recommendation; Weak)**

The prevention of ACR in the early post-operative period is specifically important in the context of enhanced recovery and early discharge. It's relevance to overall graft and patient survival is however less certain in the post-HCV era of transplantation, where the consequences of over-immunosuppression may be more significant. The data suggest that TAC based immunosuppression, in conjunction with other therapies currently reduces the incidence to <20%. However, minimizing other consequences of IS should be evaluated in future studies. Future research should focus on a new IS protocol combining multiple drugs

with as main aim a composite outcome including cardiovascular comorbidity, cancer risk, acute kidney injury and chronic rejection which are key to reduce long term morbidity.

ERAS4OLT.org Group Authorship

Claus Niemann, San Francisco, CA, USA, Joerg-Matthias Pollok, London, UK, Marina Berenguer, Valencia, Spain, Pascale Tinguely, London, UK, Adrian Gustar, Milano, Italy, Camila Hidalgo, London, UK.

Authorship

All authors qualify for authorship as per the International Committee of Medical Journal Editors (ICMJE) guidelines.

Author contributions

EDM: data analysis/interpretation, drafting article, critical revision of article, **MCL**: data analysis/interpretation, drafting article, critical revision of article, **JE**: data analysis/interpretation, drafting article, critical revision of article, **JL**: data analysis/interpretation, drafting article, critical revision of article, **JP**: data analysis/interpretation, drafting article, critical revision of article, **VA**: data analysis/interpretation, drafting article, critical revision of article, **MS/DAR**: project inception study protocol design, supervision of literature screening, methodological support, critical revision of article, **GMCC** : data analysis/interpretation, drafting article, critical revision of article.

References

1. Di Maira T, Little EC, Berenguer M. Immunosuppression in liver transplant. *Best Pract Res Clin Gastroenterol*. 2020;46-47:101681. doi:10.1016/j.bpg.2020.101681
2. Lerut JP, Gondolesi GE. Immunosuppression in liver and intestinal transplantation. *Best Pract Res Clin Gastroenterol*. 2021;54-55:101767. doi:10.1016/j.bpg.2021.101767
3. Cai J, Terasaki PI. Induction immunosuppression improves long-term graft and patient outcome in organ transplantation: an analysis of United Network for Organ Sharing registry data. *Transplantation*. 2010;90(12):1511-1515. doi:10.1097/TP.0b013e3181fecfcb
4. Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol*. 2011;64(4):383-394. doi:10.1016/j.jclinepi.2010.04.026
5. Murad MH, Mustafa RA, Schünemann HJ, Sultan S, Santesso N. Rating the certainty in evidence in the absence of a single estimate of effect. *Evid Based Med*. 2017;22(3):85-87. doi:10.1136/ebmed-2017-110668
6. Andrews J, Guyatt G, Oxman AD, et al. GRADE guidelines: 14. Going from evidence to recommendations: the significance and presentation of recommendations. *J Clin Epidemiol*. 2013;66(7):719-725. doi:10.1016/j.jclinepi.2012.03.013
7. Andrews JC, Schünemann HJ, Oxman AD, et al. GRADE guidelines: 15. Going from evidence to recommendation-determinants of a recommendation's direction and strength. *J Clin Epidemiol*. 2013;66(7):726-735. doi:10.1016/j.jclinepi.2013.02.003
8. O'Grady JG, Burroughs A, Hardy P, Elbourne D, Truesdale A, UK and Republic of Ireland Liver Transplant Study Group. Tacrolimus versus microemulsified ciclosporin in liver transplantation: the TMC randomised controlled trial. *Lancet Lond Engl*. 2002;360(9340):1119-1125. doi:10.1016/s0140-6736(02)11196-2
9. Trunečka P, Boillot O, Seehofer D, et al. Once-daily prolonged-release tacrolimus (ADVAGRAF) versus twice-daily tacrolimus (PROGRAF) in liver transplantation. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2010;10(10):2313-2323. doi:10.1111/j.1600-6143.2010.03255.x
10. Dannhorn E, Cheung M, Rodrigues S, et al. De novo use of generic tacrolimus in liver transplantation - a single center experience with one-yr follow-up. *Clin Transplant*. 2014;28(12):1349-1357.
11. Soliman T, Hetz H, Burghuber C, et al. Short-term versus long-term induction therapy with antithymocyte globulin in orthotopic liver transplantation. *Transpl Int Off J Eur Soc Organ Transplant*. 2007;20(5):447-452. doi:10.1111/j.1432-2277.2007.00463.x
12. Bajjoka I, Hsaiky L, Brown K, Abouljoud M. Preserving renal function in liver transplant recipients with rabbit anti-thymocyte globulin and delayed initiation of calcineurin inhibitors. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2008;14(1):66-72. doi:10.1002/lt.21309
13. Benítez CE, Puig-Pey I, López M, et al. ATG-Fresenius treatment and low-dose tacrolimus: results of a randomized controlled trial in liver transplantation. *Am J Transplant*. 2010;10(10):2296-2304. doi:10.1111/j.1600-6143.2010.03164.x
14. Iesari S, Ackenine K, Foguenne M, et al. Tacrolimus and Single Intraoperative High-dose of Anti-T-lymphocyte Globulins Versus Tacrolimus Monotherapy in Adult Liver

Transplantation: One-year Results of an Investigator-driven Randomized Controlled Trial. *Ann Surg*. 2018;268(5):776-783.
15. Calmus Y, Scheele JR, Gonzalez-Pinto I, et al. Immunoprophylaxis with basiliximab, a chimeric anti-interleukin-2 receptor monoclonal antibody, in combination with azathioprine-containing triple therapy in liver transplant recipients. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2002;8(2):123-131. doi:10.1053/jlts.2002.30882
16. Schnitzbauer AA, Ayik C, Ulrich F, Bechstein WO, Monch C. Delayed bottom-up and amended simple method of dosing with once-daily tacrolimus application to achieve stable trough levels in liver transplantation. *Ann Transplant*. 2015;20:1-6.
17. Verna EC, Farrand ED, Elnaggar AS, et al. Basiliximab induction and delayed calcineurin inhibitor initiation in liver transplant recipients with renal insufficiency. *Transplantation*. 2011;91(11):1254-1260.
18. Hashim M, Alsebaey A, Ragab A, Soliman HE, Waked I. Efficacy and safety of basiliximab as initial immunosuppression in liver transplantation: A single center study. *Ann Hepatol*. 2020;19(5):541-545.
19. Neuhaus P, Clavien PA, Kittur D, et al. Improved treatment response with basiliximab immunoprophylaxis after liver transplantation: results from a double-blind randomized placebo-controlled trial. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2002;8(2):132-142. doi:10.1053/jlts.2002.30302
20. Klintmalm GB, Feng S, Lake JR, et al. Belatacept-based immunosuppression in de novo liver transplant recipients: 1-year experience from a phase II randomized study. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2014;14(8):1817-1827. doi:10.1111/ajt.12810
21. TruneČka P, Klempnauer J, Bechstein WO, et al. Renal Function in De Novo Liver Transplant Recipients Receiving Different Prolonged-Release Tacrolimus Regimens-The DIAMOND Study. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2015;15(7):1843-1854. doi:10.1111/ajt.13182
22. Levitsky J, Thudi K, Ison MG, Wang E, Abecassis M. Alemtuzumab induction in non-hepatitis C positive liver transplant recipients. *Liver Transpl*. 2011;17(1):32-37.
23. Neuberger JM, Mamelok RD, Neuhaus P, et al. Delayed introduction of reduced-dose tacrolimus, and renal function in liver transplantation: the “ReSpECT” study. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2009;9(2):327-336. doi:10.1111/j.1600-6143.2008.02493.x
24. Nashan B, Saliba F, Durand F, et al. Pharmacokinetics, efficacy, and safety of mycophenolate mofetil in combination with standard-dose or reduced-dose tacrolimus in liver transplant recipients. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2009;15(2):136-147. doi:10.1002/lt.21657
25. Biselli M, Vitale G, Gramenzi A, et al. Two yr mycophenolate mofetil plus low-dose calcineurin inhibitor for renal dysfunction after liver transplant. *Clin Transplant*. 2009;23(2):191-198. doi:10.1111/j.1399-0012.2009.00965.x
26. Boudjema K, Camus C, Saliba F, et al. Reduced-dose tacrolimus with mycophenolate mofetil vs. standard-dose tacrolimus in liver transplantation: a randomized study. *Am J Transplant*. 2011;11(5):965-976.

27. Rodríguez-Perálvarez M, Germani G, Papastergiou V, et al. Early tacrolimus exposure after liver transplantation: relationship with moderate/severe acute rejection and long-term outcome. *J Hepatol*. 2013;58(2):262-270. doi:10.1016/j.jhep.2012.09.019
28. Sterneck M, Fischer L, Gahlemann C, Gundlach M, Rogiers X, Broelsch C. Mycophenolate mofetil for prevention of liver allograft rejection: initial results of a controlled clinical trial. *Ann Transplant*. 2000;5(1):43-46.
29. Wiesner R, Rabkin J, Klintmalm G, et al. A randomized double-blind comparative study of mycophenolate mofetil and azathioprine in combination with cyclosporine and corticosteroids in primary liver transplant recipients. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2001;7(5):442-450. doi:10.1053/jlts.2001.23356
30. McAlister VC, Peltekian KM, Malatjalian DA, et al. Orthotopic liver transplantation using low-dose tacrolimus and sirolimus. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2001;7(8):701-708. doi:10.1053/jlts.2001.26510
31. Asrani SK, Wiesner RH, Trotter JF, et al. De novo sirolimus and reduced-dose tacrolimus versus standard-dose tacrolimus after liver transplantation: the 2000-2003 phase II prospective randomized trial. *Am J Transplant*. 2014;14(2):356-366.
32. Cillo U, Saracino L, Vitale A, et al. Very Early Introduction of Everolimus in De Novo Liver Transplantation: Results of a Multicenter, Prospective, Randomized Trial. *Liver Transpl*. 2019;25(2):242-251.
33. Pageaux GP, Calmus Y, Boillot O, et al. Steroid withdrawal at day 14 after liver transplantation: a double-blind, placebo-controlled study. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2004;10(12):1454-1460. doi:10.1002/lt.20291
34. Moench C, Barreiros AP, Schuchmann M, et al. Tacrolimus monotherapy without steroids after liver transplantation--a prospective randomized double-blinded placebo-controlled trial. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2007;7(6):1616-1623. doi:10.1111/j.1600-6143.2007.01804.x
35. Lerut J, Mathys J, Verbaandert C, et al. Tacrolimus monotherapy in liver transplantation: one-year results of a prospective, randomized, double-blind, placebo-controlled study. *Ann Surg*. 2008;248(6):956-967. doi:10.1097/SLA.0b013e31819009c9
36. Pelletier SJ, Nadig SN, Lee DD, et al. A prospective, randomized trial of complete avoidance of steroids in liver transplantation with follow-up of over 7 years. *HPB*. 2013;15(4):286-293.
37. Ramirez CB, Doria C, Frank AM, Armenti ST, Marino IR. Completely steroid-free immunosuppression in liver transplantation: a randomized study. *Clin Transplant*. 2013;27(3):463-471.
38. Castedal M, Skoglund C, Axelson C, Bennet W. Steroid-free immunosuppression with low-dose tacrolimus is safe and significantly reduces the incidence of new-onset diabetes mellitus following liver transplantation. *Scand J Gastroenterol*. 2018;53(6):741-747.
39. Bari K, Shah SA, Kaiser TE, et al. Safety and Efficacy of Budesonide for Liver Transplant Immune Suppression: Results of a Pilot Phase 2a Trial. *Liver Transpl*. 2020;26(11):1430-1440.
40. Becker T, Foltys D, Bilbao I, et al. Patient outcomes in two steroid-free regimens using tacrolimus monotherapy after daclizumab induction and tacrolimus with

mycophenolate mofetil in liver transplantation. *Transplantation*. 2008;86(12):1689-1694. doi:10.1097/TP.0b013e31818fff64
41. Calmus Y, Kamar N, Gugenheim J, et al. Assessing renal function with daclizumab induction and delayed tacrolimus introduction in liver transplant recipients. *Transplantation*. 2010;89(12):1504-1510. doi:10.1097/TP.0b013e3181db8cf0
42. Goralczyk AD, Hauke N, Bari N, Tsui TY, Lorf T, Obed A. Interleukin 2 receptor antagonists for liver transplant recipients: a systematic review and meta-analysis of controlled studies. *Hepatol Baltim Md*. 2011;54(2):541-554. doi:10.1002/hep.24385
43. McAlister VC, Haddad E, Renouf E, Malthaner RA, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus as primary immunosuppressant after liver transplantation: a meta-analysis. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2006;6(7):1578-1585. doi:10.1111/j.1600-6143.2006.01360.x
44. Llado L, Gonzalez-Castillo A, Fabregat J, et al. Efficacy and Safety of Delayed Prolonged-Release Tacrolimus Initiation in De Novo Hepatitis C Virus-Negative Orthotopic Liver Transplant Recipients: A Single-Center, Single-Arm, Prospective Study. *Ann Transplant*. 2019;24:36-44.
45. Rodríguez-Perálvarez M, Germani G, Darius T, Lerut J, Tsochatzis E, Burroughs AK. Tacrolimus trough levels, rejection and renal impairment in liver transplantation: a systematic review and meta-analysis. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2012;12(10):2797-2814. doi:10.1111/j.1600-6143.2012.04140.x
46. Segev DL, Sozio SM, Shin EJ, et al. Steroid avoidance in liver transplantation: meta-analysis and meta-regression of randomized trials. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2008;14(4):512-525. doi:10.1002/lt.21396
47. Sgourakis G, Radtke A, Fouzas I, et al. Corticosteroid-free immunosuppression in liver transplantation: a meta-analysis and meta-regression of outcomes. *Transpl Int Off J Eur Soc Organ Transplant*. 2009;22(9):892-905. doi:10.1111/j.1432-2277.2009.00893.x
48. Yoshida EM, Marotta PJ, Greig PD, et al. Evaluation of renal function in liver transplant recipients receiving daclizumab (Zenapax), mycophenolate mofetil, and a delayed, low-dose tacrolimus regimen vs. a standard-dose tacrolimus and mycophenolate mofetil regimen: a multicenter randomized clinical trial. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2005;11(9):1064-1072. doi:10.1002/lt.20490