Transplantation

Conversion from calcineurin to mammalian target of rapamycin inhibitors in liver transplantation: a meta-analysis of randomised controlled trials

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Abstract:	Context: Conversion to mammalian target of rapamycin inhibitors (mTORi) is often utilised in liver transplantation to overcome calcineurin inhibitor (CNI) nephrotoxicity but the evidence base for this approach is not well defined. Objective: To summarise the evidence, from randomised-clinical-trials (RCTs), for conversion from CNI to mTORi-based immunosuppression after liver transplantation. Data Sources: Databases and conference abstracts were searched up to August 2015. Study Selection: RCTs evaluating conversion from CNI to mTORi-based maintenance immunosuppression following adult liver transplantation. Data Extraction: Descriptive and quantitative information was extracted; summary mean difference (MD) and risk ratio (RR) estimates were synthesized under a random-effects model. Heterogeneity was assessed using the Q statistic and I2. Data synthesis: Ten RCTs, with a total of 1,927 patients, met the final inclusion criteria. Patients converted to mTORi had significantly better renal function at 1 year following randomisation compared to patients remaining on CNI (MD: 7.48 mL/min/1.73m2, 95%CI: 3.18-11.8). The risks of graft loss (RR: 0.77, 95%CI: 0.29-2.09, I2: 31%) and patient death (RR: 1.05, 95%CI: 0.63-1.73, I2: 0%) were similar for patients converted to mTORi and patients remaining on CNI. However, conversion to mTORi was associated with a higher risk of acute rejection (RR: 1.76, 95%CI: 1.33-2.34, I2: 0%) and study discontinuation due to adverse events (RR: 2.17, 95%CI: 1.38-3.44, I2: 63%) up to one year post-randomisation. Conclusions: Conversion from CNI to mTORi following liver transplantation is associated with improved renal function after one year but increases the risk of acute rejection and may be poorly tolerated.

Conversion from calcineurin to mammalian target of rapamycin inhibitors in liver transplantation: a meta-analysis of randomised controlled trials

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TEG: acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis

CJEW: research design; analysis and interpretation of data; drafting of manuscript

PG: analysis and interpretation of data; drafting of manuscript

JAB: research design; analysis and interpretation of data; drafting of manuscript

EEN: research design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis; study supervision

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List of abbreviations

CI: confidence intervals

CKD: Chronic Kidney Disease

CKD-EPI: Chronic Kidney Disease - epidemiology

CNI: Calcineurin Inhibitor

CrCl: Creatinine Clearance

EMBASE: Excerpta Medica Database

GFR: Glomerular Filtration Rate

ITT: Intention-to-Treat

MDRD: Modification of Diet in Renal Disease

mTORi: mammalian Target of Rapamycin inhibitor

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RCT: Randomised Controlled Trial

RCSEng: Royal College of Surgeons of England

RR: Risk Ratio

SMD: standardised mean difference

Abstract

Context: Conversion to mammalian target of rapamycin inhibitors (mTORi) is often utilised in liver transplantation to overcome calcineurin inhibitor (CNI) nephrotoxicity but the evidence base for this approach is not well defined.

Objective: To summarise the evidence, from randomised-clinical-trials (RCTs), for conversion from CNI to mTORi-based immunosuppression after liver transplantation.

Data Sources: Databases and conference abstracts were searched up to August 2015.

Study Selection: RCTs evaluating conversion from CNI to mTORi-based maintenance immunosuppression following adult liver transplantation.

Data Extraction: Descriptive and quantitative information was extracted; summary mean difference (MD) and risk ratio (RR) estimates were synthesized under a random-effects model. Heterogeneity was assessed using the Q statistic and I^2.

Data synthesis: Ten RCTs, with a total of 1,927 patients, met the final inclusion criteria. Patients converted to mTORi had significantly better renal function at 1 year following randomisation compared to patients remaining on CNI (MD: 7.48 mL/min/1.73m^2, 95%CI: 3.18-11.8). The risks of graft loss (RR: 0.77, 95%CI: 0.29-2.09, I^2: 31%) and patient death (RR: 1.05, 95%CI: 0.63-1.73, I^2: 0%) were similar for patients converted to mTORi and patients remaining on CNI. However, conversion to mTORi was associated with a higher risk of acute rejection (RR: 1.76, 95%CI: 1.33-2.34, I^2: 0%) and study discontinuation due to adverse events (RR: 2.17, 95%CI: 1.38-3.44, I^2: 63%) up to one year post-randomisation.

Conclusions: Conversion from CNI to mTORi following liver transplantation is associated with improved renal function after one year but increases the risk of acute rejection and may be poorly tolerated.

Introduction

The calcineurin inhibitors (CNIs) tacrolimus and ciclosporin are the principal components of maintenance immunosuppressive therapy following orthotopic liver transplantation and have made a major contribution to current long term transplant outcomes with 5-year graft survival approaching 70% (1, 2). However, CNIs are associated with a number of potentially serious side effects including nephrotoxicity, diabetes, hypertension, and neurotoxicity that contribute to morbidity and mortality following transplantation. Renal impairment is a particular problem following liver transplantation, with 10-20% of recipients progressing to stage 4 or 5 chronic kidney disease within 5 years of transplantation, with CNI therapy being a major contributing factor (3-5).

Mammalian target of Rapamycin inhibitors (mTORi) are a distinct class of immunosuppressive agents that have a different mode of action to that of CNIs although they bind to the same intracellular immunophilin as tacrolimus, namely FKBP12. The mTORi/FKBP12 complex binds to and inhibits the TORC1 complex, inhibiting proliferation of many cell types, including lymphocytes (6). The mTORi include sirolimus and the more recently introduced sirolimus analogue, everolimus, designed with the aim of improving oral bioavailability (7). The side effect profile of mTORi is different to that of CNI and includes impaired wound healing, mouth ulcers, skin rashes, arthralgia, diabetes, hyperlipidaemia and pneumonitis (8). Importantly mTORi do not share the same nephrotoxicity as CNIs which makes them an attractive alternative to CNIs for maintenance therapy after liver transplantation; although they do cause glomerular disease in some patients resulting in marked proteinuria (9). *De novo* use of mTORi after liver transplantation is avoided because of concerns relating to hepatic artery thrombosis and poor wound healing (10). Interest has focussed, instead, on the delayed introduction of mTORi to allow reduction or elimination of CNIs to preserve or improve renal function while maintaining adequate levels of immunosuppression. A number of randomised controlled trials (RCTs) have examined the

potential benefits of introducing either sirolimus or everolimus after liver transplantation using a variety of protocols that differ with respect to the timing of conversion to mTORi, whether CNI are eliminated or reduced and in the level of baseline renal function at the time of mTORi introduction. Such studies have given conflicting results on the efficacy and side effect profile of mTORi, but have led to an increasing recognition that mTORi have a potentially important role to play in preserving renal function after liver transplantation.

We have undertaken a systematic review and meta-analysis of randomised trials to assess the evidence base for conversion from CNI to mTORi-based maintenance immunosuppression after liver transplantation with a particular focus on preservation of renal function.

METHODS

Eligibility criteria, information sources and search strategy

A systematic literature search was performed using PubMed, EMBASE, the Cochrane Central Register of Controlled Trials and the Transplant Library at the Royal College of Surgeons of England (RCSEng) up to August 2015 using a predefined algorithm (Table S1) without language restrictions. Abstracts from conferences were searched for relevant publications using the algorithm implemented in the Transplant Library of the RCSEng (11). References included in pertinent systematic reviews were also screened.

All randomized controlled trials evaluating conversion from CNI to mTORi-based maintenance immunosuppression in adult isolated liver transplantation were considered. Studies were deemed eligible if they evaluated abrupt or slow conversion to mTORi, in first or subsequent liver transplant recipients, irrespective of time after transplantation and baseline renal function. Studies that were considered eligible included those where the intervention (conversion to mTORi) and reference (CNI continuation) groups received additional maintenance immunosuppression comprising antimetabolites (mycophenolate or azathioprine) and steroids. Observational and non-controlled studies, studies evaluating paediatric patients and animal studies were excluded (Figure 1). Detailed methodology on data extraction, on data synthesis and statistical analyses, and on assessment of trial methodological quality is presented as supplementary information. Analyses were performed in RevMan 5 (Cochrane Collaboration, 2010) and STATA 10 (STATA Corp., College Station, IL). All p-values are two tailed. The study is reported according to the PRISMA checklist (12).

Results

A total of 1,382 potentially relevant citations were identified (PubMed: 636, EMBASE: 508, Cochrane Central Register of Controlled Trials: 130, Centre for Evidence in Transplantation Library: 108). Following review of titles and abstracts and removal of duplicate publications, 42 potentially eligible articles were identified. Ten trials, including a total of 1,927 randomised patients, were selected for inclusion in the meta-analysis (Figure 1). Two randomised controlled trials were excluded: the study reported by Herlenius et al because it evaluated conversion from CNI to either sirolimus or mycophenolate mofetil without inclusion of a reference arm (13); and the study reported by Asrani et al because it evaluated *de novo* rather than delayed use of sirolimus, and reduction rather than cessation of tacrolimus (10).

All included studies were designed to evaluate the safety and efficacy of conversion from CNI to mTORi immunosuppression in adult liver transplant patients. Study design characteristics, immunosuppression regimens and reported outcomes for each trial are summarized in Table S2. The median sample size was 112 participants (IQR 41-271) and the median treatment duration was 12 months (min 12 months, max 72 months). All studies reported renal function, acute rejection, graft loss, patient survival and adverse events. Renal function was measured by radionuclide method in one trial (14) and estimated using Cockcroft-Gault (15-20), Chronic Kidney Disease Epidemiology Collaboration (21, 22) and MDRD formulae (23, 24) in the remaining studies (25, 26). Early conversion to mTORi (defined as ≤6 months after transplantation) was evaluated in 4 studies (16, 17, 21, 25), whereas 6 studies evaluated late conversion to mTORi (14, 15, 18-20, 26). Five studies, including 943 participants, examined conversion from CNI to everolimus (17, 20, 21, 25, 26), whereas the remaining 5 studies, including 984 participants, evaluated conversion from CNI to sirolimus (14-16, 18, 19). There was variation in baseline renal function, both within and

between studies, but the majority of patients had mild or moderate renal dysfunction at the time of randomisation (CKD stage II or III).

The risk of bias was evaluated using the Cochrane's Collaboration tool (Table S3). Allocation sequence generation was described in 9 studies, but allocation concealment was clearly reported only by Watson et al (14). Eight studies were open-label, whereas 2 studies did not report blinding parameters. Attrition was adequately reported in all studies and was generally low (<20%) and intention-to-treat analyses were reported in all trials. Table S5 shows the proportion of patients that failed to be randomised or discontinued the allocated treatment, for each study. At the meta-analysis level, there was no indication of small study effects, based on either funnel plot asymmetry or the Begg-Mazumbar statistic; we acknowledge that this conclusion is based on a limited number of studies.

Assessed outcomes and evidence synthesis

Renal function

Renal function at 1 year following randomisation was reported by all included studies. Because of variability in the reporting of this outcome (six studies reported GFR estimates whereas four studies reported CrCl measurements/estimates; Table S2), the standardised mean difference (SMD) between the mTORi and the CNI groups was calculated. In the ITT analysis, patients converted to mTORi had significantly better renal function at 1 year following randomisation compared to patients remaining on CNI (SMD: 0.40, 95% CI: 0.17-0.63, I^2: 78%; Figure 2A). Transformation of SMD into the GFR scale corresponded to a mean difference of 7.48 mL/min/1.73m^2 (95% CI: 3.18-11.8) between the two groups. When studies were stratified according to time of conversion to mTORi (early versus late conversion, defined as ≤6 months after transplantation), there was a non-statistically significant trend towards a more favourable GFR difference between mTORi and CNI groups in the early conversion trials (SMD: 0.53, 95% CI: 0.28-0.77, I^2: 69%) compared to late

conversion trials (SMD: 0.22, 95% CI: -0.06 to 0.49, I^2: 52%), with reduction in heterogeneity only for late conversion trials (Figure 2B). Trial stratification according to mTORi type (sirolimus versus everolimus) showed no significant subgroup differences (SMD: 0.44, 95% CI: 0.16-0.71, I^2: 70% for everolimus conversion trials versus SMD: 0.37, 95% CI: -0.04 to 0.77, I^2: 81% for sirolimus conversion trials; Figure 2C).

To further address heterogeneity for renal function at 1 year following conversion to mTORi, sensitivity analyses were performed excluding 2 trials evaluating CNI minimisation in the reference group (20, 25) or steroid elimination regimens (25). Overall, there was no change in heterogeneity compared to the original meta-analysis (SMD: 0.35, 95% CI: 0.12-0.58, I^2: 74% for GFR at 1 year). A recent study indicated that estimation of GFR using the MDRD formula may lead to incorrect interpretation of renal function in liver transplant patients; we have, therefore, performed additional sensitivity analysis excluding 2 trials that reported GFR estimates based on MDRD (25, 26) and a similar effect to the original meta-analysis was observed (SMD: 0.31, 95% CI: 0.09-0.53, I^2: 74%). Moreover, meta-regression analyses accounting for baseline GFR or CrCl estimates, showed that baseline renal function had no significant effect on the difference in renal function between mTORi and CNI groups at 1 year following randomisation (data not shown).

Acute rejection

All included studies contributed to the meta-analysis evaluating the association between conversion from CNI to mTORi based immunosuppression and acute liver allograft rejection (Table S4). All studies used the definition of biopsy proven acute rejection (BPAR) except those by Eisenberger et al and Shenoy et al (15, 19). Conversion to mTORi compared to CNI maintenance was associated with higher risk of reported acute rejection up to one year post-randomisation (RR: 1.76, 95% CI: 1.33-2.34, I^2: 0%; Figure 3). Analysis based on a definition of BPAR showed similar findings (RR: 1.77, 95% CI: 1.33-2.36, I^2: 0% for patients converted to mTORi). There was a higher risk of acute rejection following conversion to

mTORi both in sirolimus conversion trials (RR: 2.19, 95% CI: 1.36-3.54, I^2: 0%) and everolimus conversion trials (RR: 1.57, 95% CI: 1.10-2.23, I^2: 0%) and overall, subgroup analyses did not reveal statistically significant differences between subgroups (data not shown).

Liver allograft loss and mortality

Three studies contributed to the meta-analysis evaluating the association between conversion from CNI to mTORi based immunosuppression and liver allograft loss (16, 17, 21), whereas in the remaining seven studies none of the liver allografts were lost within the first year post-randomisation (graft loss was censored for patient death with the exception of the Spare the Nephron study that reported a composite outcome of death and graft loss) (14, 15, 18-20, 25, 26). Patients converted to mTORi had similar risk of allograft loss compared to patients remaining on CNI (RR: 0.77, 95% CI: 0.29-2.09, I^2: 31%; excluding the Spare the Nephron trial did not change the RR significantly but eliminated heterogeneity). Overall, 12 patients in the mTORi group and 17 patients in the CNI group lost their graft within the first year post-randomisation. There were no reported allograft losses in late conversion trials but the data were too sparse to allow for sensitivity or meta-regression analyses.

All studies reported mortality up to 1 year post-randomisation. Overall, 38 (3.6%) patients in the mTORi group and 29 (3.4%) patients in the CNI group died within the first year post-randomisation. There were no differences in mortality between patients converted to mTORi and those remaining on CNI (RR: 1.05, 95% CI: 0.63-1.73, I^2: 0%). Risk ratios and heterogeneity were similar when trials were stratified according to time of conversion to mTORi or according to mTORi type.

Adverse events

Adverse events were reported by all studies included in the meta-analysis, although there were differences between studies in the nature and incidence of the reported adverse events.

The risk of study discontinuation due to adverse events up to 1 year post-randomisation was greater in patients converted to mTORi than in patients remaining on CNI (RR: 2.17, 95% CI: 1.38-3.44, I^2: 63%; Figure 4). Stratification by time of conversion showed that the risk of study discontinuation following conversion to mTORi was statistically significantly higher in late conversion trials (RR: 5.02, 95% CI: 2.91-8.68, I^2: 0%) compared to early conversion trials (RR: 1.57, 95% CI: 1.14-2.15, I^2: 42%). Risk ratios and heterogeneity did not change significantly if trials were stratified according to type of mTORi. Sensitivity analyses showed similar risk ratios for the overall and subgroup analyses but eliminated heterogeneity for early conversion trials (RR: 1.71, 95% CI: 1.34-2.17, I^2: 0%) and everolimus conversion trials (RR: 1.98, 95% CI: 1.45-2.71, I^2: 0%).

Reported adverse events along with risk ratio estimates and 95% CI up to one year post-randomisation are summarised in Figure 5. Compared to patients on CNI continuation, those converted to mTORi had a higher risk of hyperlipidaemia (4.7% and 26.5% respectively; RR: 4.81, 95% CI: 3.06-7.55, I^2: 0%); hypercholesterolaemia (4.9% and 22.8% respectively; RR: 4.18, 95% CI: 1.79-9.75, I^2: 57%); requirement for new statin therapy (7.4% and 16.1% respectively; RR: 1.77, 95% CI: 0.65-4.86, I^2: 8%); skin rash (3.4% and 23.1% respectively; RR: 5.58, 95% CI: 3.45-9.02, I^2: 0%); mouth ulceration (0.7% and 13.3% respectively; RR: 10.18, 95% CI: 4.26-24.33, I^2: 0%); proteinuria (1.0% and 4.1% respectively; RR: 3.19, 95% CI: 1.40-7.28, I^2: 0%); and oedema (9.0% and 20.1% respectively; RR: 2.08, 95% CI: 1.58-2.74, I^2: 0%). There was a non-statistically significant trend towards higher risk of infections in the mTORi conversion group (47.4%, compared to 38.0% of patients maintained on CNI; RR: 1.18, 95% CI: 0.98-1.43, I^2: 52%). Patients converted to mTORi had a lower risk of requiring renal replacement therapy (RR: 0.48, 95% CI: 0.21-1.11, I^2: 19%) that did not reach statistical significance. Heterogeneity was significant for studies reporting hypercholesterolaemia and this was eliminated for the three studies (17, 20, 26) evaluating conversion to everolimus (RR: 2.51, 95% CI: 1.39-4.54, I^2: 0%). Similarly, subgroup

analyses for infections showed similar risk ratios to the pooled analysis but heterogeneity was eliminated for everolimus and late conversion trials (data not shown).

Longer term outcomes

Longer term renal function (>1 year) was reported by only two of the included trials. The H2304 study showed that patients converted to mTORi had significantly higher renal function at 3 years following randomisation compared to patients remaining on CNI (ITT analysis, MD: 17.0 mL/min/1.73m^2, 95% CI: 13.5-20.6) (27); a similar trend was reported for an 'on-treatment' population by the PROTECT study at 3 years follow up (MD: 6.9 mL/min/1.73m^2, 95% CI: 1.7-12.3) (28). No differences were reported between patients remaining on CNI and those converted to mTORi in the three studies reporting allograft loss (18, 27, 28) and the two studies reporting patient death (27, 28) 3 years following randomisation (data not shown).

Discussion

The findings from this systematic review and meta-analysis of RCTs show that conversion from CNI to mTORi-based maintenance immunosuppression after liver transplantation is associated with a significant improvement in renal function at 12 months following conversion. Graft and patient survival were equivalent in patients converted to mTORi and those remaining on CNI, but recipients converted to mTORi had a higher risk of acute graft rejection. Moreover, discontinuation due to adverse events was more commonly observed in patients converted to mTORi.

A previous meta-analysis published in 2010 evaluated the use of sirolimus in patients with renal impairment after liver transplantation and concluded that conversion to mTORi was associated with a non-significant trend towards improved renal function (29). While several observational studies were assessed, only three RCTs (including a total of 86 patients) were available at that time for inclusion in the analysis (14, 15, 19). In the present study a further seven RCTs (2 evaluating sirolimus and 5 evaluating everolimus) were available for analysis (giving a total of 1,927 patients) enabling a more robust, direction-consistent estimate of the effect of CNI discontinuation on renal function. Given the observed marked heterogeneity (I^2: 78%) for trials reporting on the effect of mTORi conversion on renal function, caution is required with respect to the magnitude of the overall estimate for this outcome. Subgroup and sensitivity analyses reinforced the overall conclusion that conversion to mTORi was associated with improved renal function but did not eliminate heterogeneity. The present analysis showed that conversion to mTORi did not have an adverse effect on graft or patient survival compared to CNI continuation and minimal heterogeneity was observed for these outcomes.

It has been reported that conversion to mTORi and discontinuation of CNI without adequate antibody induction therapy increases the risk of acute rejection (17, 21). The present meta-analysis showed that conversion to mTORi is associated with a higher risk of acute rejection

although the cumulative sample size cannot support a well-powered subgroup analysis. Nevertheless in one of the studies the study arm examining conversion to everolimus and CNI elimination was discontinued because of a high incidence of biopsy proven acute rejection (21). While the present analysis did not show a difference in acute rejection between trials evaluating abrupt and tapered discontinuation of CNI, it has been suggested that tapered discontinuation is preferable, especially when mTORi conversion is introduced within the first few months of liver transplantation (17). CNI minimisation is an alternative strategy to CNI withdrawal after conversion to mTORi and may allow preservation of renal function without compromising efficacy of immunosuppression (30). Two of the RCTs included in the present analysis adopted this approach, one of which reported superior GFR in the mTORi group whereas the other showed equivalent renal function after one year (20, 25). Experience in renal transplantation suggests that there is enhanced nephrotoxicity when CNIs are combined with mTORi (31-33).

There is currently a trend towards early (≤6 months after transplantation) rather than late conversion to mTORi after liver transplantation before residual kidney function deteriorates and chronic kidney disease is established. Three out of the five most recent RCTs included in the present analysis evaluated early conversion to everolimus (the earliest being conversion at 10 days) and included recipients with relatively high baseline estimated GFR. Our meta-analysis showed that early versus late conversion to mTORi was associated with a trend towards better renal function at twelve months; however, our analysis was underpowered to exhibit a robust subgroup difference and, therefore, the evidence for the optimal time for conversion to mTORi is inconclusive. Nevertheless, it is notable that every one of the early conversion trials showed a statistically significant improvement in renal function 12 months after conversion to mTORi, whereas five out of the six trials evaluating late conversion to mTORi did not show a statistically significant difference in 12-month renal function between the CNI and mTORi groups.

Mammalian target of rapamycin inhibitors are associated with a number of well described side effects that may limit the ability of patients to tolerate them (8, 34, 35). Our analysis confirmed this, indicating that the risk of study discontinuation following mTORi conversion was twice that of patients maintained on CNIs and trial withdrawal due to adverse events was more likely after late conversion to mTORi. Withdrawal rates in patients converted to mTORi varied widely between RCTs from only 5% to as high as 55%. Overall, there was a substantial risk of adverse events following conversion to mTORi and this represents a significant barrier for their utility in preserving renal function after liver transplantation. Specifically, our analyses showed that conversion to mTORi is associated with an increased risk of hyperlipidaemia and hypercholesterolaemia, although the requirement for new statin therapy was not different to patients maintained on CNI. Limited data from retrospective studies suggested a beneficial effect of conversion to mTORi on management of hypertension (36), however, there was insufficient high-quality evidence to examine this association in our study. Conversion to mTORi also increased the risk of dermatological adverse events and mouth ulceration, but the rate of infections was similar to that of patients receiving CNI maintenance. Pooled analysis from two early and one late conversion trials did not confirm the known association of mTORi with poor wound healing. A significant drawback of treatment with mTORi is the development of proteinuria which may reach the nephrotic range, especially following exposure to high sirolimus concentrations (37); our analysis confirmed this association, although the reported proteinuria levels were usually mild or less often moderate whereas development of nephrotic range proteinuria was rare and occurred in the presence of significant pre-existing renal injury.

The majority of RCTs included in our meta-analysis were not powered to detect a difference in graft or patient survival and given the high rate of study withdrawals reported, the true effect of mTORi conversion on graft and patient outcomes is still to some extent uncertain. This is especially the case for long-term outcomes, given that only three of the ten studies

included in the meta-analysis reported outcomes beyond one year. Evidence from retrospective analyses suggest that sirolimus immunosuppression is associated with a significant graft and patient survival benefit after liver transplantation for hepatocellular carcinoma (38-40); only two trials examined outcomes following mTORi conversion in this subgroup of patients and, compared to patients maintained on CNI, reported a non-significant reduction in disease recurrence in the everolimus group (25) and significantly better graft/patient survival in the sirolimus group (16). Both studies, however, were underpowered to examine outcomes in patients undergoing liver transplantation for hepatocellular carcinoma and strong evidence on the utility of mTORi in this subgroup of patients is still lacking. Similarly, the evidence from cohort studies regarding the effect of mTORi on hepatitis C recurrence and fibrosis progression in patients undergoing liver transplantation for hepatitis C related cirrhosis is equivocal (36, 41, 42); although the study by Villamil et al (26) suggested that conversion to everolimus reduces liver fibrosis progression, further large randomised-controlled trials are needed to provide clear evidence as to optimal immunosuppression in this group of patients.

It is important to acknowledge some additional limitations of the present meta-analysis. Publication and language bias may be operating in any clinical field; however, the comprehensive search algorithm utilised herein, including the Cochrane Controlled Trials Registry and the Transplant Library at the RCSEng that are built from multiple large databases, enhanced the detection of smaller trials and we would, therefore, expect that incorporation of any unpublished evidence would not substantially alter the overall status of the evidence. This notion was supported by the observed consistency of the reported summary effects in small studies. Moreover, although randomised evidence is protected from selection bias, performance and detection bias could be potential confounders. An approach towards addressing this would be to exclude open-label studies. Unfortunately, all included studies were, by necessity, open-label trials and, therefore, trial exclusion was not an

available option. Finally, a number of study parameters could potentially interfere with our study results. The inclusion of trials examining conversion to either sirolimus or everolimus utilising heterogeneous treatment algorithms; the variation in baseline renal function between studies; and the distinct patient characteristics within individual trials might have contributed to the observed heterogeneity. Nevertheless, the all-inclusiveness and randomised nature of the analysed evidence limits potential sources of bias; alternative research designs, such as individual patient data meta-analysis, which may further address confounding lie beyond the scope of the present study.

In conclusion, the currently available randomised evidence indicates that conversion from CNI to mTORi following liver transplantation is associated with improved renal function at 12 months and this benefit is likely to be more pronounced when conversion occurs early after transplantation before irreversible kidney injury is established. In deciding the optimal immunosuppression strategy for their patients, clinicians should be alert to the increased risk of acute rejection following conversion to mTORi which is, however, treatable and has no effect on short-term graft and patient outcomes. Conversion to mTORi, especially when attempted late after transplantation, may be poorly tolerated and careful patient selection is important to maximise the benefits of this intervention. No firm conclusions can be drawn about the relative efficacy of different mTORi, and the relative advantages of CNI minimisation versus discontinuation.

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Figure Legends

Figure 1. Flowchart of included studies

Abbreviations: EMBASE, Excerpta Medica Database; CNI, Calcineurin Inhibitor; mTOR, mammalian Target of Rapamycin; RCT, randomised controlled trial.

Figure 2A: Mammalian target of rapamycin inhibitor versus calcineurin inhibitor; mean GFR up to 1 year post-randomisation

Figure 2B: Mammalian target of rapamycin inhibitor versus calcineurin inhibitor; mean GFR up to 1 year post-randomisation stratified by time post-transplant

Figure 2C: Mammalian target of rapamycin inhibitor versus calcineurin inhibitor; mean GFR up to 1 year post-randomisation stratified by type of mammalian target of rapamycin inhibitor sirolimus and everolimus

Abbreviations: GFR, Glomerular Filtration Rate; CNI, Calcineurin Inhibitor; mTOR, mammalian Target of Rapamycin

*5 patients in the mTOR inhibitor arm and 4 patients in the CNI arm of this study were excluded from the initial randomised population (by the authors of the study) because of missing post-baseline GFR data (forming the intention-to-treat population)

**1 patient randomised to receive mTOR inhibitor was excluded from the intention-to-treat analysis (by the authors of the study) because they did not receive the allocated intervention after randomisation

Figure 3. Mammalian target of rapamycin inhibitor versus calcineurin inhibitor; any rejection up to 1 year post-randomisation

Abbreviations: CNI, Calcineurin Inhibitor; mTOR, mammalian Target of Rapamycin

*5 patients in the mTOR inhibitor arm and 4 patients in the CNI arm of this study were excluded from the initial randomised population (by the authors of the study) because of missing post-baseline GFR data (forming the intention-to-treat population)

**1 patient randomised to receive mTOR inhibitor was excluded from the intention-to-treat analysis (by the authors of the study) because they did not receive the allocated intervention after randomisation

Figure 4. Mammalian target of rapamycin inhibitor (mTORi) versus calcineurin inhibitor (CNI); adverse events up to 1 year post-randomisation leading to study discontinuation

The safety population analysis is reported (all eligible studies reported adverse events for the safety population defined as the total number of patients that received at least one dose of the allocated intervention). Abbreviations: CNI, Calcineurin Inhibitor; mTOR, mammalian Target of Rapamycin

*4 patients in each arm of this study were excluded from the initial randomised population (by the authors of the study) because they did not receive the allocated intervention after randomisation

**1 patient in the mTOR inhibitor arm and 2 patients in the CNI arm of this study were excluded from the initial randomised population (by the authors of the study) because they did not receive the allocated intervention after randomisation

***1 patient in the mTOR inhibitor arm did not receive the allocated intervention after randomisation but was included in the safety population of the CNI arm (by the authors of the study) because the patient had been receiving CNI prior to randomisation

Figure 5. Pooled risk ratio estimates and 95% confidence intervals for adverse events up to 1 year following conversion to mammalian target of rapamycin inhibitor

*All studies reported graft loss censored for patient death apart from the Spare the Nephron study that reported a composite outcome of death and graft loss; excluding the Spare the Nephron trial did not change the risk ratio for graft loss significantly.

Figure 1

Figure 1

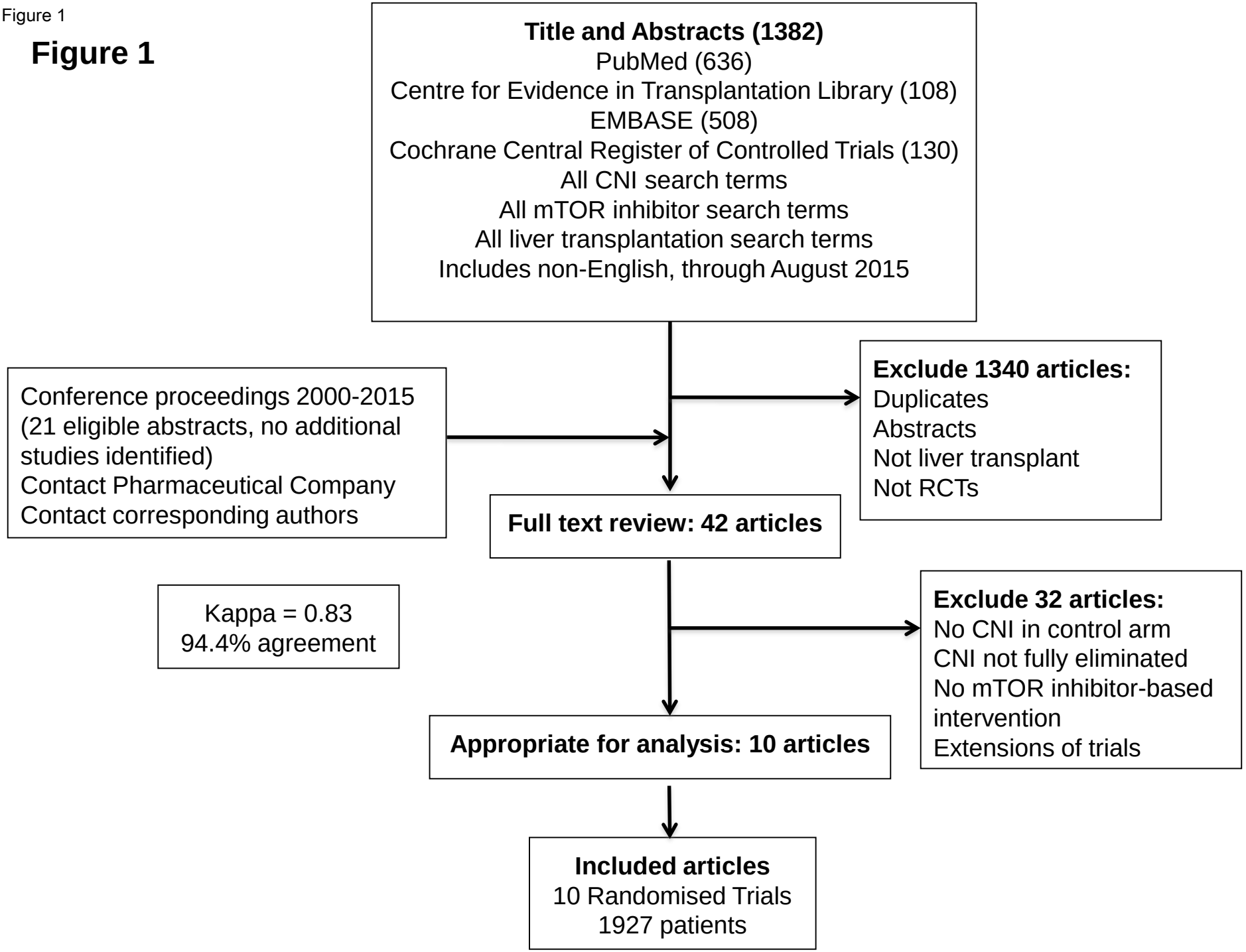
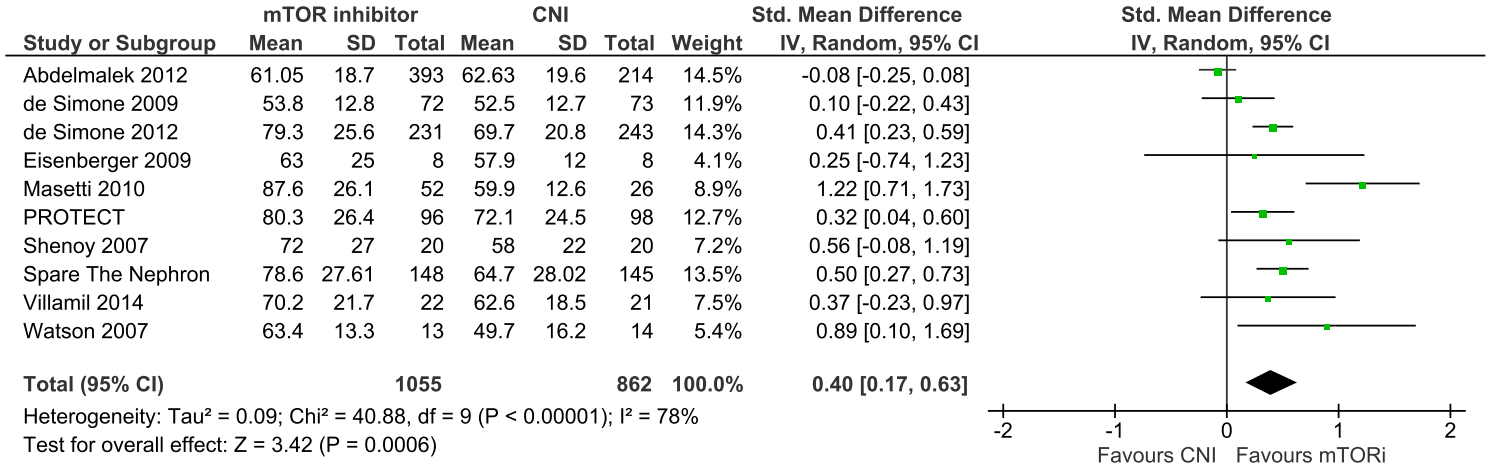


Figure 2A

Figure 2A



Study or Subgroup	mTOR inhibitor Mean	SD	Total	CNI Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Abdelmalek 2012	61.05	18.7	393	62.63	19.6	214	14.5%	-0.08 [-0.25, 0.08]
de Simone 2009	53.8	12.8	72	52.5	12.7	73	11.9%	0.10 [-0.22, 0.43]
de Simone 2012	79.3	25.6	231	69.7	20.8	243	14.3%	0.41 [0.23, 0.59]
Eisenberger 2009	63	25	8	57.9	12	8	4.1%	0.25 [-0.74, 1.23]
Masetti 2010	87.6	26.1	52	59.9	12.6	26	8.9%	1.22 [0.71, 1.73]
PROTECT	80.3	26.4	96	72.1	24.5	98	12.7%	0.32 [0.04, 0.60]
Shenoy 2007	72	27	20	58	22	20	7.2%	0.56 [-0.08, 1.19]
Spare The Nephron	78.6	27.61	148	64.7	28.02	145	13.5%	0.50 [0.27, 0.73]
Villamil 2014	70.2	21.7	22	62.6	18.5	21	7.5%	0.37 [-0.23, 0.97]
Watson 2007	63.4	13.3	13	49.7	16.2	14	5.4%	0.89 [0.10, 1.69]
Total (95% CI)			**1055**			**862**	**100.0%**	**0.40 [0.17, 0.63]**

Heterogeneity: Tau² = 0.09; Chi² = 40.88, df = 9 (P < 0.00001); I² = 78%

Test for overall effect: Z = 3.42 (P = 0.0006)

Figure 2B

Figure 2B

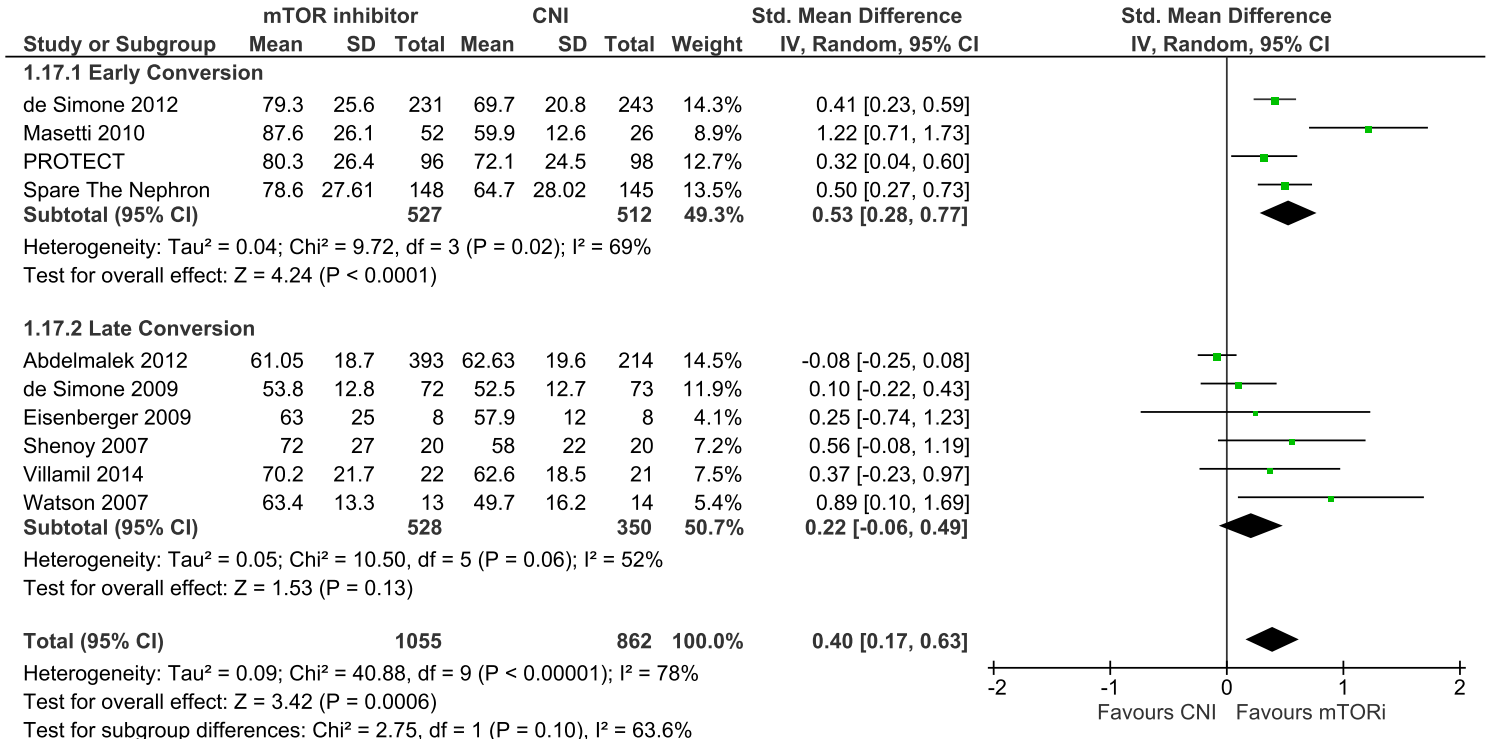


Figure 2C

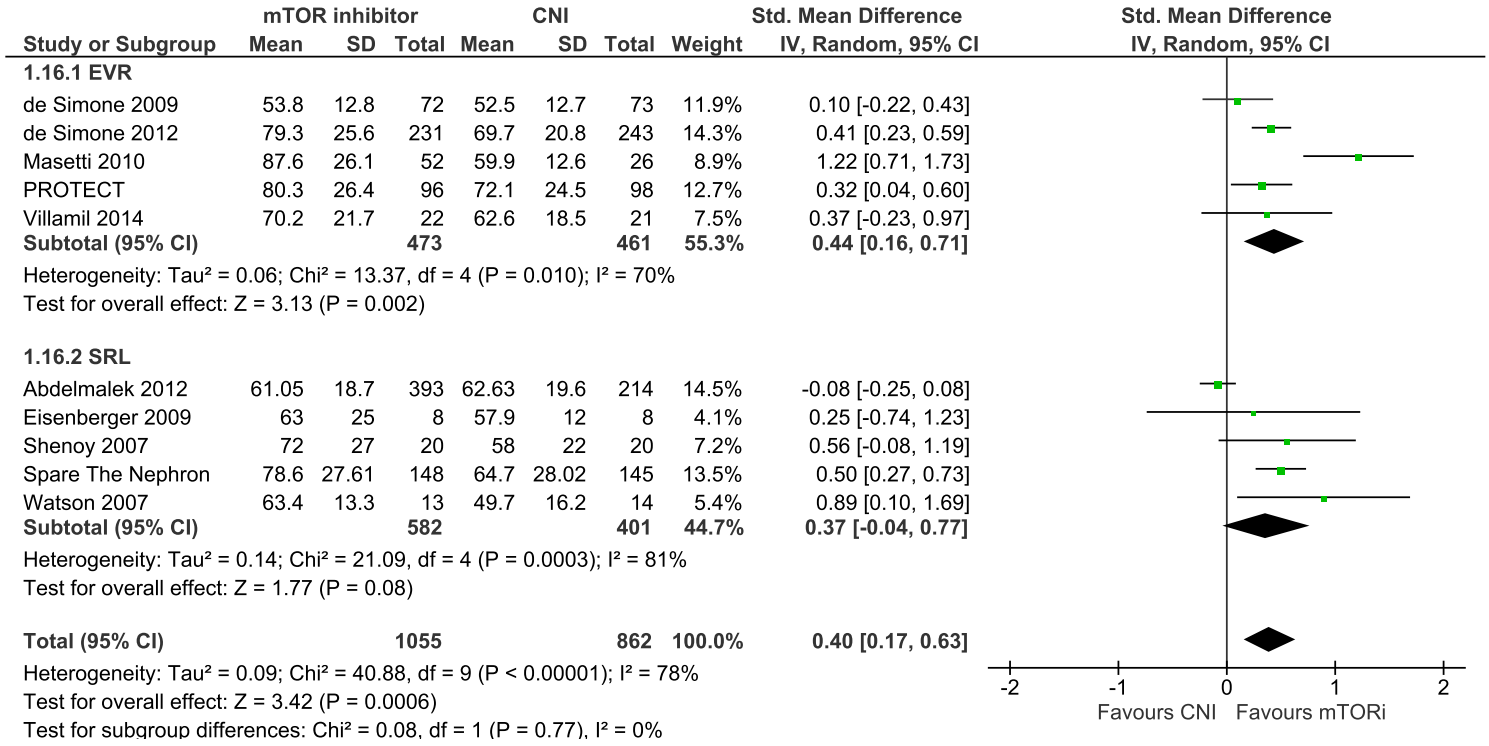

mTOR inhibitor
CNI
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
1.16.1 EVR
de Simone 2009 53.8 12.8 72 52.5 12.7 73 11.9% 0.10 [-0.22, 0.43]
de Simone 2012 79.3 25.6 231 69.7 20.8 243 14.3% 0.41 [0.23, 0.59]
Masetti 2010 87.6 26.1 52 59.9 12.6 26 8.9% 1.22 [0.71, 1.73]
PROTECT 80.3 26.4 96 72.1 24.5 98 12.7% 0.32 [0.04, 0.60]
Villamil 2014 70.2 21.7 22 62.6 18.5 21 7.5% 0.37 [-0.23, 0.97]
Subtotal (95% CI) 473 461 55.3% 0.44 [0.16, 0.71]
Heterogeneity: Tau² = 0.06; Chi² = 13.37, df = 4 (P = 0.010); I² = 70%
Test for overall effect: Z = 3.13 (P = 0.002)
1.16.2 SRL
Abdelmalek 2012 61.05 18.7 393 62.63 19.6 214 14.5% -0.08 [-0.25, 0.08]
Eisenberger 2009 63 25 8 57.9 12 8 4.1% 0.25 [-0.74, 1.23]
Shenoy 2007 72 27 20 58 22 20 7.2% 0.56 [-0.08, 1.19]
Spare The Nephron 78.6 27.61 148 64.7 28.02 145 13.5% 0.50 [0.27, 0.73]
Watson 2007 63.4 13.3 13 49.7 16.2 14 5.4% 0.89 [0.10, 1.69]
Subtotal (95% CI) 582 401 44.7% 0.37 [-0.04, 0.77]
Heterogeneity: Tau² = 0.14; Chi² = 21.09, df = 4 (P = 0.0003); I² = 81%
Test for overall effect: Z = 1.77 (P = 0.08)
Total (95% CI) 1055 862 100.0% 0.40 [0.17, 0.63]
Heterogeneity: Tau² = 0.09; Chi² = 40.88, df = 9 (P < 0.00001); I² = 78%
Test for overall effect: Z = 3.42 (P = 0.0006)
Test for subgroup differences: Chi² = 0.08, df = 1 (P = 0.77), I² = 0%
-2
-1
0
1
2
Favours CNI
Favours mTORi

Figure 3

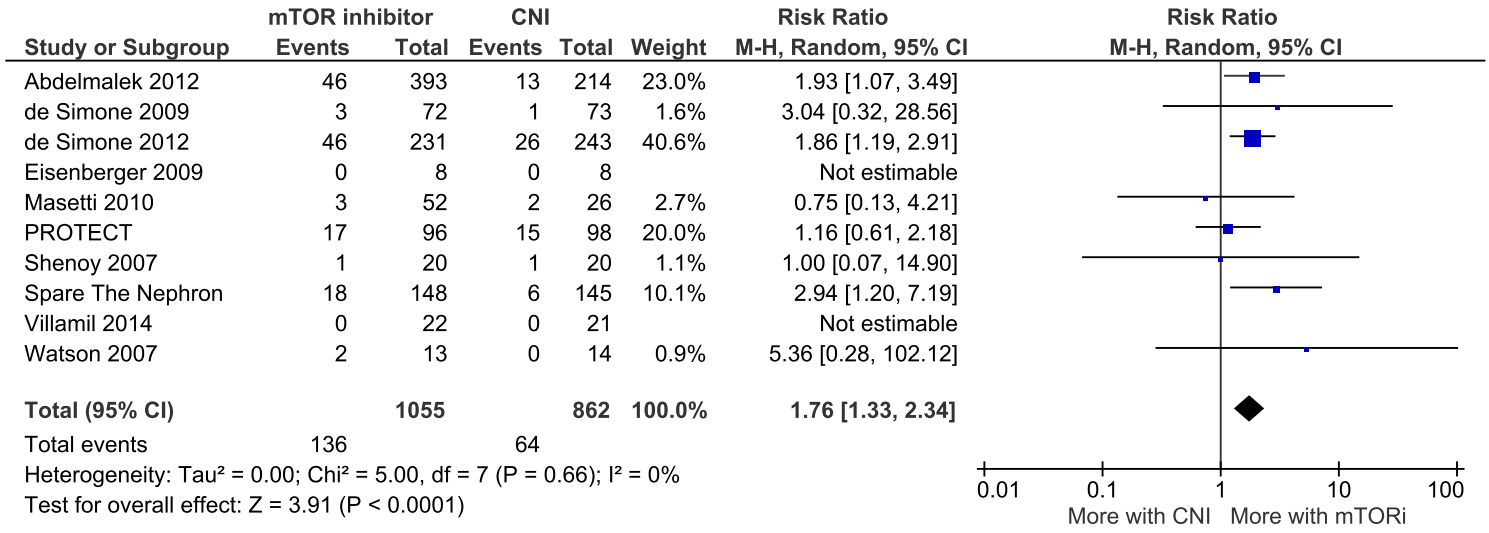

mTOR inhibitor
CNI
Risk Ratio
Risk Ratio
Study or Subgroup
Events
Total
Events
Total
Weight
M-H, Random, 95% CI
M-H, Random, 95% CI
Abdelmalek 2012 46 393 13 214 23.0% 1.93 [1.07, 3.49]
de Simone 2009 3 72 1 73 1.6% 3.04 [0.32, 28.56]
de Simone 2012 46 231 26 243 40.6% 1.86 [1.19, 2.91]
Eisenberger 2009 0 8 0 8 Not estimable
Masetti 2010 3 52 2 26 2.7% 0.75 [0.13, 4.21]
PROTECT 17 96 15 98 20.0% 1.16 [0.61, 2.18]
Shenoy 2007 1 20 1 20 1.1% 1.00 [0.07, 14.90]
Spare The Nephron 18 148 6 145 10.1% 2.94 [1.20, 7.19]
Villamil 2014 0 22 0 21 Not estimable
Watson 2007 2 13 0 14 0.9% 5.36 [0.28, 102.12]
Total (95% CI) 1055 862 100.0% 1.76 [1.33, 2.34]
Total events 136 64
Heterogeneity: Tau² = 0.00; Chi² = 5.00, df = 7 (P = 0.66); I² = 0%
Test for overall effect: Z = 3.91 (P < 0.0001)
0.01
0.1
1
10
100
More with CNI
More with mTORi

Figure 4

Figure 4

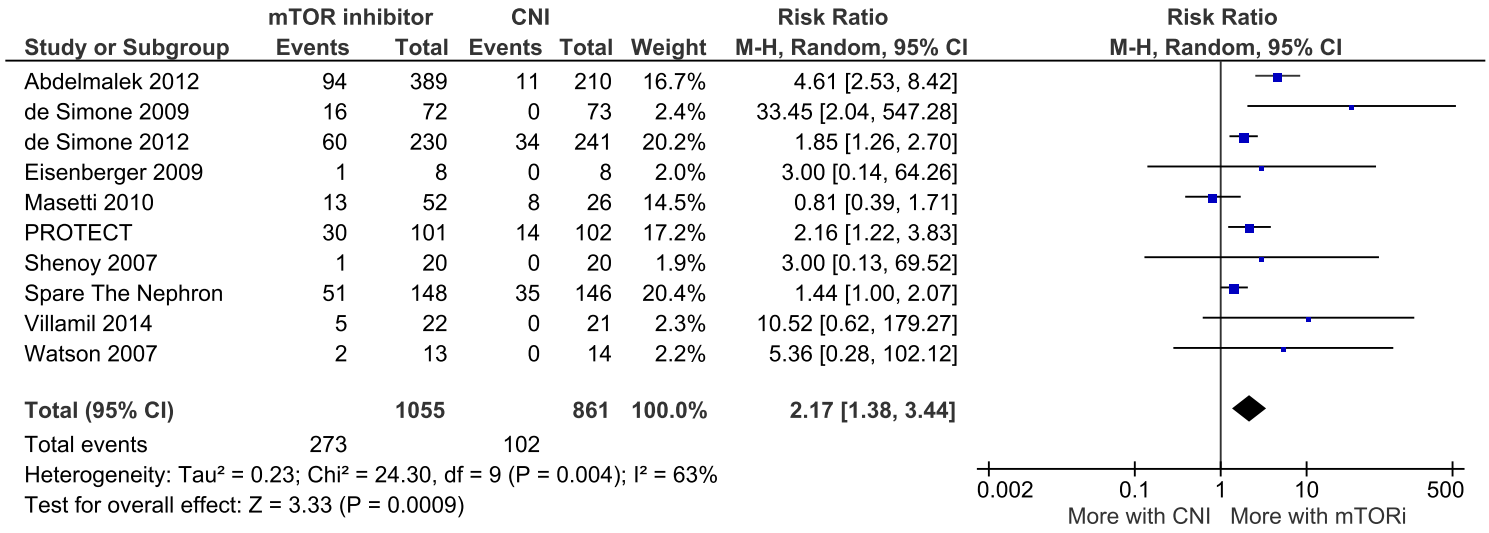
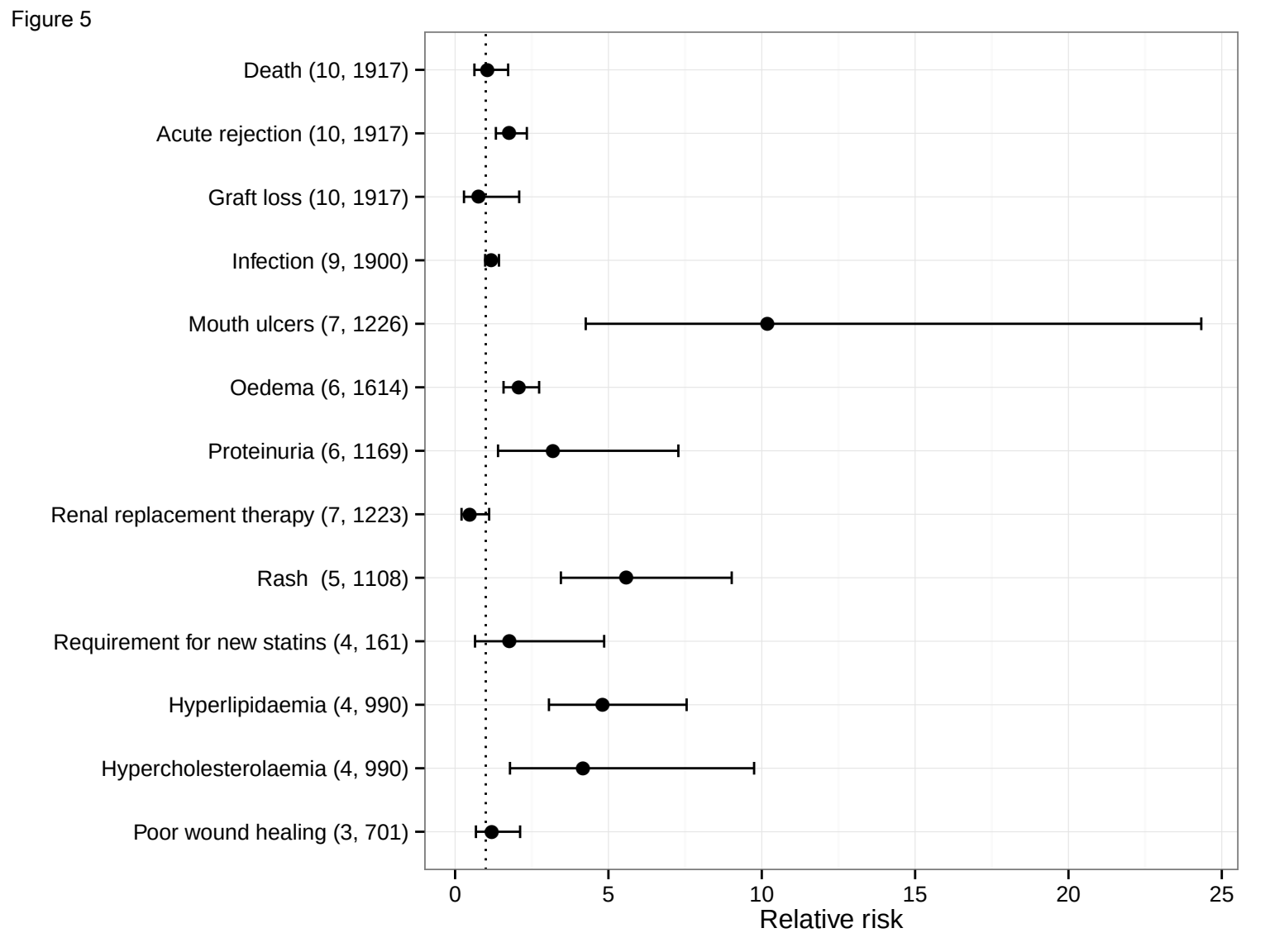


Figure 5


Death (10, 1917)
Acute rejection (10, 1917)
Graft loss (10, 1917)
Infection (9, 1900)
Mouth ulcers (7, 1226)
Oedema (6, 1614)
Proteinuria (6, 1169)
Renal replacement therapy (7, 1223)
Rash (5, 1108)
Requirement for new statins (4, 161)
Hyperlipidaemia (4, 990)
Hypercholesterolaemia (4, 990)
Poor wound healing (3, 701)
0
5
10
15
20
25
Relative risk

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