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Systematic Review with Meta-Analysis: sirolimus- or everolimus-based immunosuppression following liver transplantation for hepatocellular carcinoma

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Abbreviations:

HCC: hepatocellular carcinoma

CNI: calcineurin inhibitor

mTOR: mechanistic target of rapamycin

RFS: recurrence-free-survival

OS: overall survival

NOS: Newcastle Ottawa Scale

RR: risk ratio

CI: confidence interval

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The guarantor of this manuscript is Sam E. Grigg. The concept and idea for this study originated with SEG, PJG and NYD. SG and GLS were responsible for abstract/title and full text screening, data extraction and quality assessment. SEG, PJG and NYD analysed and interpreted the data. SEG wrote the original manuscript. All authors reviewed and approved the final version.

Summary

Background

Calcineurin-inhibitor immunosuppressants (tacrolimus and ciclosporin) have been associated with an exposure related increase in tumour recurrence following liver transplantation for hepatocellular carcinoma (HCC). Conversely, mechanistic target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) have been suggested to reduce recurrence rates and improve survival in this patient group.

Aim

To clarify the potential benefit of mTOR-inhibitors in HCC transplant patients by comparing recurrence and survival outcomes with calcineurin-inhibitor-based immunosuppression.

Methods:

A systematic review and meta-analysis was performed. The inclusion criteria were observational or interventional studies reporting the effect of early-initiated (<6 months post-transplant) mTOR-inhibitor-based immunosuppression on survival or tumour recurrence in patients transplanted with HCC, compared to a control of calcineurin-inhibitor-based therapy.

Results

Meta-analysis demonstrated that compared with calcineurin-inhibitor controls, recurrence-free-survival was significantly increased with mTOR-inhibitor-based therapy at 1-year (Risk-Ratio (RR): 1.09, 95% confidence-interval (CI): 1.01-1.18) and 3-years (RR: 1.1, 95%CI: 1.01-1.21) post-transplant, with a non-significant increase at 5-years (RR: 1.15, 95%CI: 0.99-1.35). Overall survival was improved at 1-year (RR: 1.07, 95%CI: 1.02-1.12), 3-years (RR: 1.1, 95%CI: 1.02-1.19), and 5-years (RR: 1.18, 95%CI: 1.08-1.29). Recurrence-rate was lower in the mTOR-inhibitor arm (RR: 0.67, 95% CI: 0.56-0.82), with no significant increase in acute rejection (RR: 1.1, 95%CI: 0.94-1.28).

Conclusions:

mTOR-inhibitor-based immunosuppression may be a preferable option in patients transplanted with HCC. It improves recurrence-free-survival over at least three years and reduces the recurrence rate compared with standard calcineurin-inhibitor-based therapy, with no significant increase in the rate of acute rejection. Future research should clarify the effect in higher versus lower risk cohorts.

Keywords: mechanistic target of rapamycin inhibitor, everolimus, sirolimus, calcineurin-inhibitor, hepatocellular carcinoma, tumour recurrence, liver transplantation

1. Introduction

Liver transplantation for hepatocellular carcinoma (HCC) has increased markedly in the past decade. The most recent data suggest that HCC is the primary indication for transplantation in 14% of recipients in the USA, 25% in the United Kingdom, 20% in Australia and New Zealand, and 44% in China.[1-4]

A significant concern in this patient population is the risk of tumour recurrence post-transplant. Rates of recurrence are generally in the order of 15% although they can vary markedly with one systematic review reporting a range of 7 - 40%.[5] Once the tumour has recurred, the patient outcome is generally very poor with limited curative treatment options.[6]

Over the past twenty years, the most effective method to reduce the risk of recurrence has been the careful selection of transplant recipients. This has been facilitated by the introduction of the Milan criteria in 1996, which categorise patients based on tumour size, number and vascular invasion.[7] Since then, some centres have developed similar, but slightly expanded criteria, with later protocols incorporating biological markers.[8, 9]

Recently, there has been a focus on the role of immunosuppression in oncogenesis. Calcineurin inhibitors (CNIs), initially ciclosporin and now tacrolimus, have been implicated in promoting cancer cell proliferation and survival.[10] Consequently, there has been a push to identify an alternative option. The mechanistic target of rapamycin (mTOR) inhibitors are a class of immunosuppressants that have a different mechanism of action, and have been found to have anti-proliferative and anti-angiogenic effects.[11] The two main agents are sirolimus and everolimus. These drugs have been predominately used because of their low nephrotoxicity profile, however they have also been suggested to improve survival and reduce tumour recurrence rates in the subset of patients transplanted for HCC.[12]

The aim of this present study was to clarify this potential benefit of sirolimus and everolimus in HCC transplant patients by performing an updated systematic review and meta-analysis of comparative studies reporting tumour recurrence and survival outcomes with mTOR-inhibitor versus calcineurin-inhibitor-based immunosuppression.

2. Methods

2.1 Search strategy

A systematic search was conducted in the MEDLINE, EMBASE and Cochrane Central Register of Control Trials (CENTRAL) databases. Databases were searched from their inception until December 17, 2018. A structured search strategy was developed in conjunction with the hospital librarian. The Medical Subject Headings, Emtree and text terms used in the search included*: 'sirolimus', 'everolimus', 'rapamycin', 'rapamune', 'mammalian*

target of rapamycin inhibitors', 'mTOR inhibitors', 'hepatic transplantation' and *'liver transplantation'*. The specific search strategies adapted for each database can be found in the appendix (figure S1). There were no publication date or language restrictions implemented. Bibliographies of included studies were scanned to identify additional articles.

2.2 Inclusion and exclusion criteria

2.2.1 Inclusion:

Comparative observational or interventional study designs analysing the effect of early initiated (<6 months post-transplant) mTOR-inhibitor based immunosuppression on survival or tumour recurrence in patients transplanted with HCC, with a control arm of CNI based therapy.

2.2.2. Exclusion

Case reports, review articles, and paediatric studies (<18 years) were excluded. If two studies published data on the same cohort, the earlier study was only included if it reported on different endpoints.

2.3 Study selection

Results from each database were exported to the data screening and extraction tool Covidence, which is endorsed as the preferred platform used for Cochrane Reviews.[13] Title/abstract, and subsequent full-text screening was undertaken by two independent reviewers (SG and GS).

2.4 Outcome measures

The primary outcome of interest was recurrence free survival (RFS). Secondary endpoints were overall survival (OS), recurrence rate, overall mortality, recurrence-related mortality, acute rejection, and hepatic artery thrombosis.

2.5 Data extraction

Primary and secondary outcomes were extracted independently by two authors (SG and GS). The following demographic and clinical parameters were recorded by one author (SG): study characteristics (first author, year of publication, study design, country where study was performed, immunosuppression protocols, time from transplant to mTOR-inhibitor initiation, follow-up period), target and recorded mTOR-inhibitor and CNI trough levels, population

characteristics (age, sex, percentage beyond Milan criteria, tumour size, number of tumour nodules, vascular invasion, tumour stage, tumour grade, and alpha-foetoprotein levels), and adverse events.

2.5 Quality assessment

Observational studies were assessed by two independent reviewers (SG and GS) using the Newcastle-Ottawa Scale (NOS). Any disagreements were resolved by consensus with a third author (NDY). The NOS is an assessment tool for non-randomised studies that grades quality based on selection of the study groups, comparability of the groups, and the ascertainment of exposure (case-control designs) or outcome (cohort designs).[14] Randomised controlled trials that addressed a different question (e.g. renal function with mTOR-inhibitors), and only included recurrence data in HCC transplant patients as a minor subset, were considered like a cohort study and assessed using the NOS. Randomised controlled trials specifically designed to investigate mTOR-inhibitors in HCC transplant patients were assessed using the Cochrane Risk of Bias tool.

2.6 Statistical analysis

2.6.1 Pooled analysis

Outcomes were reported as risk ratios (RR) with 95% confidence intervals (CI). Pooled RRs were calculated using the DerSimonian and Laird random-effects model, which accounts for variation in true effect sizes between studies, and gives a more conservative pooled estimate than the fixed-effects model.

2.6.2. Heterogeneity

Heterogeneity was assessed using the parameters Q, I^2, and tau^2 with respective P-values and 95% confidence intervals. A p-value less than 0.10 was considered statistically significant. Values of 25%, 50% and 75% for I^2 have been considered to indicate low, moderate, and high heterogeneity respectively.[15]

2.6.3 Publication bias

Evidence of publication bias was first assessed by inspecting the symmetry of selectivity funnel plots. As this method is largely subjective, Egger's regression coefficient was calculated when at least 10 primary studies were involved.[16] In an attempt to evaluate any

impact potential bias might have on the summary risk ratio, 'Trim and Fill' estimates were performed when there was not statistical heterogeneity.

All statistical analysis was performed using Mix 2.0 Pro software.[17]

2.7 Sensitivity and sub-analysis

A sensitivity analysis was undertaken for the primary endpoints (1, 3 and 5-year RFS) by repeating the analysis including only studies scoring >7 on the NOS, or randomised controlled trials with low risk of bias.

A sub-analysis was performed by stratifying studies by the specific mTOR-inhibitor used (sirolimus and everolimus). A further pre-defined sub-analysis was carried out by stratifying studies/datasets on the percentage of subjects outside Milan criteria. A recent review of nation-wide explant pathology reports in the USA demonstrated that 25% of transplant recipients had HCC beyond Milan criteria, rising to more than 30% in some regions.[18] Therefore, studies with <30% of subjects outside Milan criteria were included in the 'Inside Milan' subgroup, and those with >70% of subjects outside Milan criteria were included in the 'Outside Milan' subgroup. Studies with between 30-70% of subjects outside Milan criteria, or where it was not explicitly stated, were excluded from the sub-analysis.

3. Results

Results were reported in accord with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist.[19]

3.1 Search strategy

The initial search strategy retrieved 5145 studies. After the removal of duplicates (957), 4188 remained. Title and abstract screening identified 165 articles for full text review of which 142 studies were subsequently excluded for the following reasons: lack of relevant data (57), overlapping publications from the same trial (71), no CNI control arm (5), uncertain data on the denominator (number with HCC) in each group (5), initiation of mTOR-inhibitor therapy >6 months post-transplant (2), and an intervention arm containing mTOR-inhibitor based therapy *or* celecoxib (1). One further study had an intervention arm containing mTOR-inhibitors *and* another experimental anti-oncogenic agent, and was excluded due the potential

confounding effect. Therefore, 23 studies were included in the quantitative analysis.[20-42] Figure 1 illustrates the PRISMA flowchart of the retrieval process.

3.2 Study design, demographics, and outcomes

The main features of each study design are outlined in Table 1. There were 17 observational cohort studies,[20-24, 26, 27, 29-31, 34-36, 38, 39, 41, 42] and 6 randomised controlled trials.[25, 28, 32, 33, 37, 40] Of the 6 randomised controlled trials, only 2 were specifically designed to analyze RFS in HCC transplant patients.[28, 32] The remaining 4 primarily addressed the effect of mTOR-inhibitors on renal function, but included data on HCC recurrence.[25, 33, 37, 40] One paper[38] had an overlapping patient cohort with a more recently published study,[42] and was only included in the quantitative analysis when it reported unique endpoints.

Thirteen studies reported on sirolimus,[20-32] and 10 on everolimus.[33-42] The mTOR-inhibitors were most often used in combination with low dose CNIs, although there was no standard regimen. Table S1 (appendix) describes specific regimens, and target and recorded trough levels for sirolimus or everolimus, and ciclosporin or tacrolimus in each group.

Seventeen studies reported on the number of patients beyond Milan criteria.[20, 22-24, 26, 28-32, 34-40, 42] In 11 of these, Milan criteria were determined by liver explant pathology, whilst in the remaining 6, the method of assessment (explant vs. radiological) was not explicitly stated. Four studies only included patients outside the Milan criteria, whilst 12 had less than 50% beyond it. Two studies reported outcomes stratified by Milan criteria.[28, 30] Table S2 (appendix) details further important demographic and prognostic factors. The Milan criteria, tumour size, number of tumours, vascular invasion, cancer stage, grade, and alpha-foetoprotein levels have all been identified as indicators of tumour recurrence risk.[43]

3.3 Study quality assessment

Table S3 (appendix) displays the NOS for each included observational study. The median score was 6 (range 4 - 9). Points were most commonly lost due to a lack of description of any subjects lost to follow-up. Table S4 (appendix) details Cochrane's risk of bias tool for randomised controlled trials.

3.4 Pooled risk ratios: primary outcome (recurrence-free-survival)

Eleven studies[20, 21, 23, 24, 26, 28-30, 32, 34, 42] (1395 subjects) reported on 1-year RFS, 10 studies[21, 23, 26-30, 32, 34, 42] (1342 subjects) on 3-year RFS, and 5 studies[21, 26, 28-30] (968 subjects) on 5-year RFS. Meta-analysis demonstrated a significant improvement with mTOR-inhibitor therapy at 1 year (RR 1.09, 95% CI 1.01 - 1.18), and 3 years (RR 1.1, 95% CI 1.01 - 1.21) post-transplant, with a non-significant increase at 5 years (RR 1.15, 95% CI 0.99 - 1.35). Figure 2 shows the corresponding forest plots and Q, I^2 and tau^2 statistics. There was significant heterogeneity between studies at 1-year, but not the 3-year or 5-year timepoints. ($p = 0.08$, $p = 0.18$, $p = 0.12$ respectively).

3.5 Pooled risk ratios: secondary outcomes

Pooled risk ratios and heterogeneity statistics (Q, I^2, tau^2) for secondary endpoints are detailed in table 2. Associated forest plots are included in the appendix (figures S2 - S4).

3.5.1. Overall survival

Twelve studies[20-22, 26, 28-31, 34, 36, 39, 42] (5550 subjects) reported on 1-year, 11 studies[22, 26-31, 34, 36, 39, 42] (5422 subjects) on 3-year, and 8 studies[21, 22, 26, 28-31, 36] (5183 subjects) on 5-year overall survival. There was a significant survival advantage with mTOR-inhibitor therapy at 1-year (RR: 1.07, 95% CI: 1.02 - 1.12), 3-years (RR: 1.1, 95% CI: 1.02 - 1.19), and 5-years (RR: 1.18, 95% CI: 1.08 - 1.29) post-transplant. There was significant heterogeneity among pooled studies at 1 and 3-years ($p = 0.01$, $p = 0.02$ respectively), but not 5-years ($p = 0.08$).

3.5.2 Recurrence rate

Eighteen studies[20, 21, 23-26, 28, 31-37, 40, 41] (5812 subjects) reported on recurrence rate. There was variable follow-up between included studies (table 1). Meta-analysis demonstrated significantly lower recurrence with mTOR-inhibitor based immunosuppression (RR: 0.67, 95% CI: 0.56 - 0.82), with no significant heterogeneity amongst pooled studies ($p = 0.47$).

3.5.3 Overall mortality

Nine studies[21, 22, 26, 28, 31, 34, 37-39] (5445 subjects) reported on overall mortality at study end. Pooled analysis showed significantly lower mortality with mTOR-inhibitor therapy (RR: 0.69, 95%CI: 0.51 - 0.93). There was significant heterogeneity between included studies ($Q = 21.89$ [$p = 0.01$], $I^2 = 63\%$, $tau^2 = 0.11$).

3.5.4 Recurrence related mortality

Six studies (1059 subjects) reported on recurrence related mortality.[22, 26, 28, 33, 39, 41] Meta-analysis found a significant reduction in the mTOR-inhibitor treated patients (RR: 0.5, 95%CI: 0.31 - 0.81), with no significant heterogeneity (p = 0.22).

3.5.5 Acute rejection

Nine studies (1549 subjects) reported on acute rejection, with pooled analysis demonstrating no significant difference between the mTOR-inhibitor and CNI groups (RR: 1.1, 95%CI: 0.94 - 1.28).[20-23, 28, 36, 37, 40, 42] There was no significant heterogeneity between included studies (p = 0.95).

3.5.6 Hepatic artery thrombosis

Three studies (849 subjects) reported on the incidence of hepatic artery thrombosis.[21, 22, 28] Meta-analysis showed no significant difference between mTOR-inhibitor and CNI based therapy (RR: 0.9, 95% CI: 0.25 - 3.34), with no significant heterogeneity amongst pooled studies (p = 0.45).

3.6 Sensitivity and sub-analysis

3.6.1 Sensitivity analysis

Sensitivity analyses involving higher quality studies were performed for RFS at 1-year[20, 21, 23, 26, 28-30, 42] (RR: 1.1, 95% CI: 1.01 - 1.19), 3-years[21, 23, 26, 28-30, 42] (RR: 1.12, 95% CI: 1.02 - 1.23), and 5-years[21, 26, 28-30] post-transplant (RR: 1.15, 95% CI: 0.99 - 1.35). The magnitudes of the summary effects were preserved, and there was no significant difference in these pooled risk ratios compared to those calculated from all studies. Forest plots are displayed in the appendix (figure S5).

3.6.2 Sub-analysis: stratified by sirolimus and everolimus

Table 3 details pooled risk ratios from the meta-analyses of primary and secondary endpoints stratified by the type of mTOR-inhibitor used (sirolimus vs. everolimus). Forest plots are contained in the appendix (figure S6 - S12). There were fewer studies using everolimus at every endpoint, except recurrence rate. For each endpoint, there was no significant difference in the pooled risk ratios from the sirolimus and everolimus subgroups.

3.6.3 Sub-analysis stratified by Milan criteria.

For the 'Inside Milan' subgroup, there was a significant RFS advantage with mTOR-inhibitors at 3-years (RR: 1.13, 95% CI: 1.03 - 1.23), and a non-significant increase at 1-year (RR: 1.05, 95% CI: 0.98 - 1.11). Figure 3 displays the corresponding forest plots. For the 'Outside Milan' subgroup, there was no significant difference in RFS between mTOR-inhibitors and CNIs at both 1-year (RR: 1.06, 95% CI: 0.94 - 1.19), and 3-years (RR: 0.95, 95% CI: 0.83 - 1.1). Figure 4 shows the associated forest plots. There were insufficient data to include 5-year RFS in this analysis. Meta-analysis outcomes of secondary endpoints stratified by Milan criteria are reported in table 3, with forest plots contained in the appendix (S13 - S15). At each endpoint, there was no significant difference between the pooled risk ratios from the 'Inside Milan' and 'Outside Milan' subgroups.

3.7 Publication bias in primary endpoint (RFS)

Subjective examination of the selectivity funnel plots for 1-year and 3-year RFS (figures 5a, 5b) shows data points lying reasonably symmetrical around the pooled risk ratios. Comparatively, the funnel plot for 5-year RFS (figure 5c) appears more asymmetric. Egger's regression test for publication bias was non-significant at 1 and 3-years ($p = 0.33$, $p = 0.73$ respectively), and was not performed for 5-year RFS due to an insufficient number of primary studies. 'Trim and fill' corrected pooled risk ratios were calculated for 3-year (RR: 1.09, 95% CI: 0.99 - 1.2), and 5-year RFS (RR: 1.07, 95% CI: 0.98 - 1.16).

4. Discussion

As HCC becomes one of the most common indications for transplantation worldwide, there is a pressing need to try and maximise patient outcomes by minimising the risk of tumour recurrence. Optimising immunosuppression choice through the use of mTOR-inhibitor based regimens has been identified as one option.[12, 44] This present study is the most comprehensive and updated systematic review and meta-analysis comparing the effect of mTOR-inhibitor versus standard immunosuppression protocols on survival and tumour recurrence.

Pooled analysis demonstrated a significant 10% recurrence-free-survival advantage with mTOR-inhibitor therapy over at least 3 years. Similarly, there was also a clear benefit in overall survival with an 18% improvement by 5 years. It is difficult to ascertain whether this effect is directly due to the anti-oncogenic characteristics of mTOR-inhibitors, or indirectly due to a reduction in CNI levels. The mTOR protein has been observed to play a role in HCC

pathogenesis[45], and pre-clinical studies of sirolimus and everolimus in human HCC cell lines and xenograft models showed promising reductions in tumour growth.[11, 46] Conversely, CNIs have been linked with cancer cell proliferation[10], and an exposure-related increase in tumour recurrence.[47] mTOR-inhibitors have also been suggested to positively impact on non-HCC factors such as renal function,[48] and the risk of de novo malignancies,[49] which may contribute to the more sustained statistical benefit in overall survival, although these effects were not the focus of the primary studies or this review.

Early enthusiasm for mTOR-inhibitors was tempered by a 2002 USA Food and Drug Administration 'black box' warning on *de novo* use of sirolimus post-transplantation, which was based on a phase II trial terminated prematurely partly due to increased rates of hepatic artery thrombosis.[50] Since then, many observational studies and multiple large trials have used mTOR-inhibitors in the early post-transplant period, and found no evidence for this association.[28, 51] This is reinforced in the present study, as the pooled analysis demonstrated no increased risk of hepatic artery thrombosis, although most primary studies elected to avoid mTOR-inhibitor treatment in the first week post-transplant. There was also no evidence for an increased rate of acute rejection, suggesting immunosuppressive efficacy was not compromised. There were insufficient data to perform a meta-analysis of graft loss, with only two studies explicitly reporting this outcome.[25, 37]

Three earlier meta-analyses have previously investigated this topic.[52-54] In contrast to this review, they only included studies using sirolimus based therapy. The first two were published in 2012 and are limited by the small number of pooled primary studies, with a maximum of three used in the quantitative meta-analysis of recurrence and survival endpoints.[52, 53] Moreover, the study by Menon et al has several errors, which hinder interpretation of the results.[55] The most recent meta-analysis by Zhang et al[54] involves an updated list of 11 studies.[54] However, it also contains notable mistakes in data extraction that make its conclusions unreliable.[56] In this context, an updated, accurate meta-analysis was needed.

A further strength of this present review is that it retrieved papers using both sirolimus or everolimus, and used a broader search strategy to minimise the risk of missing any relevant primary studies. A random-effects model was used in the quantitative analysis, as each primary study operated under slightly different conditions. By contrast, a fixed-effects model

assumes each study is functionally identical and that there is one common true effect. Previous meta-analyses on this topic have often switched to the less conservative fixed-effects model if I^2 was less than 50%, or the Q statistic was non-significant.[52, 54] This practice has been strongly discouraged, as it is argued the decision for which model to use should be based on the author's understanding of the primary studies, rather than the outcome of a heterogeneity statistical test, which is often under powered.[57]

In the current review, there was little evidence for heterogeneity between studies for two of the primary endpoints (3 and 5-year recurrence-free-survival). Conversely the Q and I^2 statistic suggested moderate heterogeneity in the pooled analyses of 1-year recurrence-free-survival, and 1 and 3-year overall survival. This could be partially explained by differences in individual study design. There was no consistent mTOR-inhibitor or CNI regimen, and different combinations of immunosuppressive agents were used. For example, sirolimus or everolimus could be administered as a monotherapy, although it was most commonly used in conjunction with reduced dose CNIs. Additionally, there was no consensus on the target trough levels. Nevertheless, this scenario likely reflects real-life clinical practice, where there is no fixed immunosuppressive protocol between centres and patients. Future randomised controlled trials may address the role of mTOR-inhibitor monotherapy in HCC patients, although this has been associated with higher rates of acute rejection.[48] A further contributing factor to any heterogeneity was the slight differences in population characteristics. Whilst age and gender were relatively consistent across studies, there was variation in the number of participants beyond Milan criteria, as well as other reported risk factors.

Pre-specified sub-analyses were used to explore differences in clinical characteristics amongst included studies. Everolimus is a close derivative of sirolimus, in which a 2-hydroxyethyl group has been introduced at position 40.[58] They are often considered to be interchangeable, and there are no clinical studies directly comparing their efficacy in HCC transplant patients. In this study, a sub-analysis stratified by the use of sirolimus versus everolimus did not suggest a greater effect of one over the other.

The SILVER trial suggested that the survival benefit of sirolimus was greater for low risk (all within Milan criteria) versus high risk patients.[28] The authors hypothesised that the anti-oncogenic properties of mTOR-inhibitors are more effective against early stage tumours. The present review attempted to clarify this finding by performing a sub-analysis stratified by the

percentage of patients beyond Milan criteria. Despite the pre-defined stratification point of 30%, the majority of included cohorts had either 0% or 100% of subjects outside Milan criteria (figures 3 and 4). There was no statistical difference between the pooled risk ratios of the 'Inside' and 'Outside Milan' criteria groups. However, statistical power for detecting differences in subgroups is often low, and should not in itself be used to conclude that the effect between subgroups is equivalent.[59] Informal comparison suggests a notably greater benefit at 3-year recurrence-free-survival in the 'Inside Milan' sub-group. As it has important implications for the use of mTOR-inhibitors in targeted populations, future primary studies should be specifically designed to explore this effect in low versus high risk patients by stratifying for selection criteria, as well as other prognostic factors such as microvascular invasion, tumour differentiation and alpha-foetoprotein levels.[60] Sub-analyses of these other risk factors were prohibited by a lack of comparable data (Appendix, table S2). Notably, with the advent of precision medicine, the efficacy of mTOR-inhibitors, and subsequent tailoring of immunosuppressive regimens, may be associated with the genomic and molecular pathogenesis of the cancer.

The most obvious criticism of this meta-analysis is the inclusion of observational cohort designs. However, the argument that systematic reviews of interventions should categorically be limited to randomised controlled trials suffers from the assumption that we would better off with a set of poor-quality randomised trials, than with a set of high-quality, low bias observational studies. Theoretical and empirical evidence suggest excluding cohort studies a priori is imprudent.[61] In this review, the risk of confounding is minimised by comparability in key risk factors. In fact, the main selection criteria for mTOR-inhibitors was based on poor renal function, which does not affect the chance of recurrence, and is likely to favour the comparator (CNIs) with regards to overall mortality. Moreover, the placebo effect is nullified by virtue of the objective outcome. Due to these conditions, the meta-analysis is likely to increase the precision of the estimate. The subsequent results were similar in the sensitivity analysis using only the studies judged to be higher quality.

Interpretation of certain endpoints (recurrence rate, overall mortality, recurrence related mortality, acute rejection) is limited by the differences in follow-up between included studies. Accordingly, greater emphasis was placed on time-insensitive outcomes (recurrence-free and overall survival). An alternative method to analyse time-to-event data is to calculate and pool hazard ratios, although this was not possible due to the data presentation in some cohort

studies.[62] Notably, the use of dichotomous effect measures can alter the estimate of recurrence-free-survival differences. Confidence intervals for 1 and 3-year recurrence free survival ranged up to 20%, and so future research will likely have an important impact on the precision. Unfortunately, few studies provided detailed data beyond 5-years. An exception was the SILVER trial, which reported follow-up until 8-years post-transplant, and found no survival benefit at this time-point.[28]

5. Conclusion

This meta-analysis provides an up to date evaluation of several tumour-related endpoints in HCC patients treated with mTOR-inhibitors compared with calcineurin-inhibitor immunosuppression post-transplant. Pooled-analysis indicated that mTOR-inhibitor based immunosuppression improved recurrence-free-survival over at least 3 years, and overall survival up to 5 years compared to conventional CNI-based protocols, without compromising rates of acute rejection or hepatic artery thrombosis. Therefore, mTOR-inhibitors may be a promising immunosuppressive option in HCC transplant patients, although there is not current evidence for a sustained benefit longer term. As some centres push to relax selection criteria, a concerted effort to further clarify the effect in higher risk patients as well as specific genomic and molecular cancer profiles would be valuable.

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Table 1. Characteristics of included studies: ordered by mTOR-inhibitor used and year of publication.

Reference	Study design	Subjects (n)		mTORi used	Outside Milan criteria (%)		Time from LT to mTORi initiation	Follow up (days unless stated)		
		mTORi	CNI		mTORi	CNI		Overall	mTORi	CNI
Zhou[19] 2008	Retrospective cohort	27	46	SRL	100	100	1mo	499 +/- 41 (range, 282 – 847)	NS	NS
Zimmerman[20] 2008	Retrospective cohort	45	52	SRL	NS	NS	< 2 weeks	NS	Mean = 36mo	Mean = 25mo
Chinnakotla[21] 2009	Retrospective cohort	121	106	SRL	20.0	35.1	Day 1	NS	Median = 50mo	Median = 45mo
Vivarelli[22] 2010	Retrospective matched cohort	31	31	SRL	45.1	45.1	Median = 17 days (1 - 129)	751 +/- 373	718 +/- 300	785 +/- 436
Feng[23] 2012	Retrospective cohort	11	11	SRL	100	100	14 - 47 days (mean = 28 +/- 10)	12mo +/- 3 (range, 7 - 18)	NS	NS
Teperman[24] 2013	RCT	44‡	43‡	SRL	NS	NS	4 - 12 weeks	Median = 519§	NS	NS
Zhao[25] 2014	Retrospective cohort	94	71	SRL	52	37	NS	Pts from 2005 - 2012, follow-up rate = 96%	NS	NS
Bhangui[26] 2016*	Retrospective cohort	21‡	21‡	SRL	NS	NS	4 - 12 weeks	Median = 30mo (5 - 91)§	NS	NS
Geissler[27] 2016	RCT	252	256	SRL	34.9	36.7	4 - 6 weeks	NS	3-year enrolment, 5-year follow up)	3-year enrolment 5-year follow up
Xu[29] 2016	Retrospective cohort	62	80	SRL	48.4	50	15 - 30 days	NS	NS	NS
Yanik[30] 2016	Retrospective cohort	234	3702	SRL	11	5	Median days = 6 (5 - 15)	Median = 2.8 years (max = 11)	NS	NS
Shen[28] 2016	Retrospective cohort	26	30	SRL	30.1	50	<6mo	Median = 35mo (4 - 141)	NS	NS
Lee[31] 2017†	RCT	20	22	SRL	100	100	1 month	3-year follow-up period	NS	NS
Masetti[32] 2010	RCT	28‡	16‡	EVR	NS	NS	Day 10	21.8mo +/- 9.2§	22.2mo +/- 9.1§	21.1mo +/- 9.3§
Houssel[33] 2013†	Retrospective cohort	16	46	EVR	100	100	1 - 24mo, <6mo in 68.8%	NS	NS	NS
Cholongitas[34] 2013	Retrospective cohort	21‡	22‡	EVR	29	36	Median = 6mo§	NS	Median = 48mo (12 - 76)§	Median = 49mo (6 - 133)§

Ferreiro[35] 2014	Retrospective cohort	21	31	EVR	95.2	71	2 weeks	NS	41mo (+/- 3.9 - 9.4)	64mo (+/- 12.7 - 212)
Junge[36] 2015†	RCT	136‡	67‡	EVR	8.1	16.4	30 days	3 year follow-up period	NS	NS
Misas[37] 2017†¶	Prospective and retrospective cohort	69	138	EVR	23.5	27.5	15 - 21 days	Up to 3 years. Median not provided	NS	NS
Thorat[38] 2017	Prospective and retrospective cohort	37	29	EVR	All within UCSF	All within UCSF	4 - 21 days	Mean = 46mo (36 - 60)	NS	NS
Jeng[39] 2017	RCT	56‡	62‡	EVR	7.1, no data in 21.4	11.3, no data in 19.4	30 +/- 5 days	93% completed 12mo study§	92.3% completed 12mo study§	93.7% completed 12mo study§
Rodríguez-Perálvarez[41] 2018	Prospective and retrospective cohort	64	128	EVR	23.4	27.3	15 - 21 days	NS	Median = 34mo	Median = 60.7mo
Manzia[40] 2018	Retrospective matched cohort	24‡	25‡	EVR	NS	NS	Day 1	NS	30mo§	24mo§

Abbreviations: EVR = everolimus, LT = liver transplantation, Mo = months, mTORi = mTOR inhibitor, NS = not stated, RCT = randomised controlled trial, SRL = sirolimus, TAC = tacrolimus, UCSF = University of California San Francisco criteria

† = Conference abstract (note: Lee 2017 and Junge 2015 have RCT protocol published previously)

‡ = Participants listed are HCC patients of an extended cohort in the study

§ = Data are for extended cohort, not just HCC subset of patients

¶ = Misas has overlapping data with Rodríguez-Perálvarez. Only used endpoints not mentioned in Rodriguez.

Table 2. Pooled risk ratios (mTOR-inhibitor versus CNI based therapy) and heterogeneity statistics for secondary endpoints. The number of studies and total participants pooled for each endpoint is documented.

Secondary endpoints.					
			Heterogeneity statistics		
	studies (subject)	Pooled RR (95% CI) of mTORi vs. CNI	Q (p-value)	I^2 (95% CI)	tau^2 (95% CI)
1-year OS	12 (5550)	1.07 (1.02 - 1.12)	25.72 (0.01)	57% (19 - 78)	0 (0 - 0.01)
3-year OS	11 (5422)	1.1 (1.02 - 1.19)	21.5 (0.02)	53% (8 - 76)	0.01 (0 - 0.02)
5-year OS	8 (5183)	1.18 (1.08 - 1.29)	12.67 (0.08)	45% (0 - 76)	0.01 (0 - 0.02)
Recurrence rate	18 (5812)	0.67 (0.56 - 0.82)	16.78 (0.47)	0% (0 - 50)	0 (0 - 0.18)
Overall mortality	9 (5445)	0.69 (0.51 - 0.93)	21.89 (0.01)	63% (25 - 82)	0.11 (0.02 - 0.29)
Recurrence related mortality	6 (1059)	0.5 (0.31 - 0.81)	6.98 (0.22)	28% (0 - 82)	0.1 (0 - 0.59)
Acute rejection	9 (1549)	1.1 (0.94 - 1.28)	2.73 (0.95)	0% (0 - 65)	0 (0 - 0)

Hepatic artery thrombosis	3 (849)	0.9 (0.25 - 3.34)	1.58 (0.45)	0% (0 - 90)	0 (0 - 35.63)

Abbreviations: CNI = calcineurin inhibitor, mTORi = mTOR-inhibitor, OS = overall survival, RR = risk ratio

Table 3. Pooled risk ratios in subgroups (1) stratified by mTOR-inhibitor, and (2) stratified by Milan criteria. The number of studies and total participants pooled for each endpoint is documented. The P-value tests for statistical difference between subgroups risk ratios.

1. Sub-group analysis: stratified by mTOR-inhibitor					
	Sirolimus		Everolimus		
	studies, subjects	Pooled RR (95% CI) of mTORi vs. CNI	studies, subjects	Pooled RR (95% CI) of mTORi vs. CNI	P-value for difference
1-year RFS	9 (1167)	1.12 (1.02 - 1.22)	2 (228)	1 (0.89 - 1.13)	0.32
3-year RFS	8 (1114)	1.12 (0.99 - 1.26)	2 (228)	1.06 (0.91 - 1.25)	0.72
5-year RFS	5 (968)	1.15 (0.99 - 1.35)	0	N/A	N/A
1-year OS	8 (5204)	1.09 (1.03 - 1.16)	4 (246)	1.03 (0.95 - 1.1)	0.39
3-year OS	7 (5076)	1.1 (1 - 1.21)	4 (246)	1.12 (0.95 - 1.3)	0.92
5-year OS	7 (5131)	1.16 (1.07 - 1.26)	1 (52)	N/A	N/A
Recurrence rate	9 (5009)	0.69 (0.53 - 0.88)	9 (803)	0.63 (0.44 - 0.91)	0.79
Overall mortality	5 (4933)	0.63 (0.44 - 0.92)	4 (512)	0.83 (0.48 - 1.44)	0.41
Recurrence related mortality	3 (900)	0.47 (0.23 - 0.96)	3 (159)	0.48 (0.19 - 1.2)	0.97
Acute rejection	5 (984)	1.11 (0.95 - 1.31)	4 (565)	0.96 (0.57 - 1.62)	0.62
Hepatic artery thrombosis	3 (849)	0.9 (0.25 - 3.34)	0	N/A	N/A
2. Sub-group analysis: stratified by Milan criteria					
	'Inside Milan criteria' (<30% subjects outside)		'Outside Milan criteria' (>70% subjects outside)		
	studies, subjects	Pooled RR (95% CI) of mTORi vs. CNI	studies, subjects	Pooled RR (95% CI) of mTORi vs. CNI	P-value for difference
1-year RFS	3 (556)	1.05 (0.98 - 1.11)	6 (459)	1.06 (0.94 - 1.19)	0.91
3-year RFS	3 (556)	1.13 (1.03 - 1.23)	4 (364)	0.95 (0.83 - 1.1)	0.16
5-year RFS	1 (292)	N/A	1 (226)	N/A	N/A
1-year OS	5 (4713)	1.06 (1 - 1.12)	4 (377)	1.06 (0.94 - 1.2)	1

3-year OS	5 (4713)	1.12 (1.01 - 1.24)	3 (304)	1.06 (0.89 - 1.28)	0.72
5-year OS	3 (4455)	1.15 (1.03 - 1.28)	2 (268)	1.36 (0.84 - 2.2)	0.57
Recurrence rate	5 (4426)	0.66 (0.44 - 0.99)	7 (250)	0.77 (0.52 - 1.13)	0.71

Abbreviations: CNI = calcineurin inhibitor, mTORi = mTOR-inhibitor, OS = overall survival RFS = recurrence-free-survival, RR = risk ratio

* P-value level of significance, <0.05

Figure titles and legends

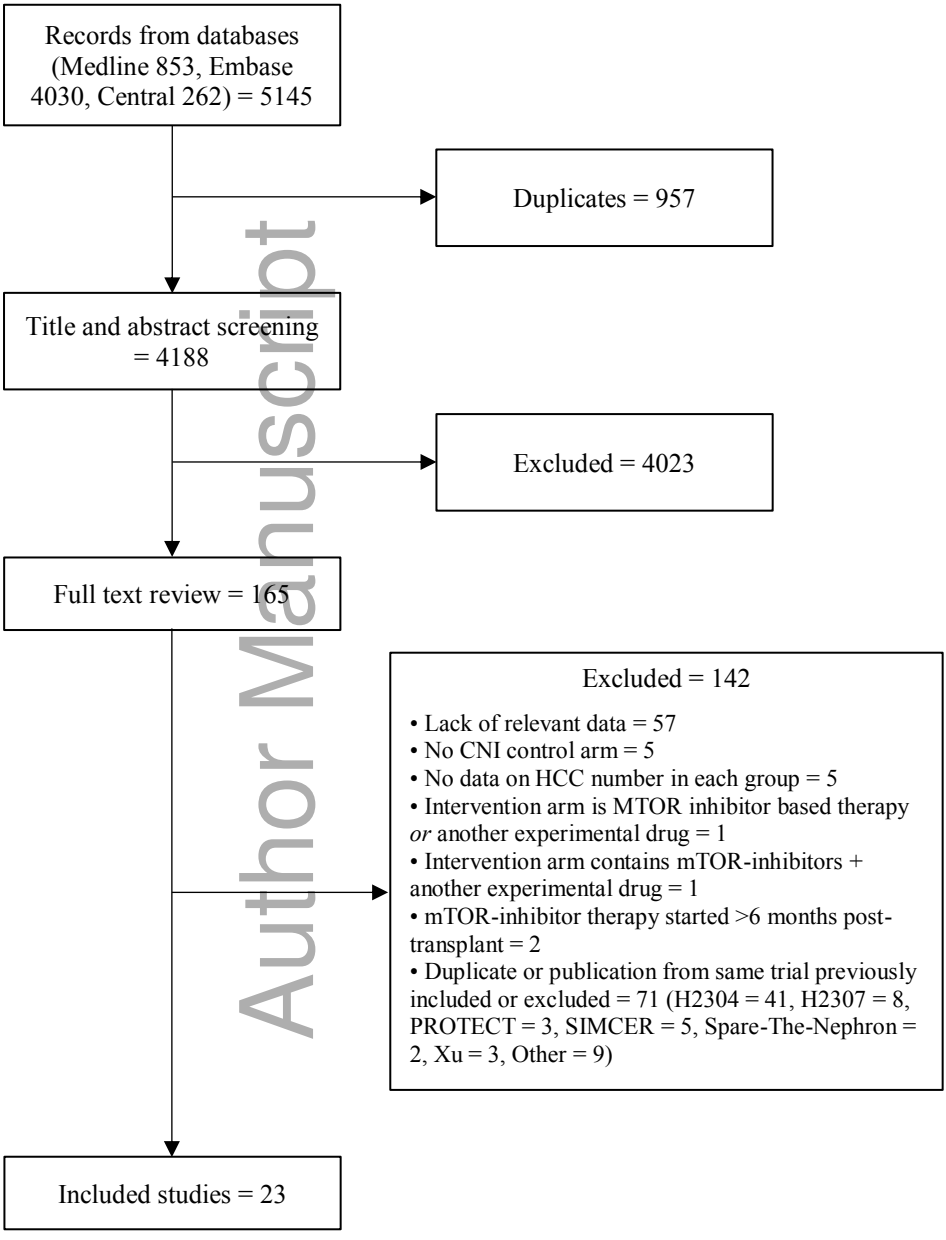
Figure 1. *PRISMA flow diagram of study retrieval process*

Figure 2. *Forest plots of 1, 3 and 5-year recurrence-free-survival with mTOR-inhibitor versus CNI-based immunosuppression***.** CNI (calcineurin inhibitor), EVR (everolimus), mTORi (mTOR-inhibitor), RR (risk ratio), SRL (sirolimus)

Figure 3. *Forest plots of 1-year and 3-year recurrence-free-survival with mTOR-inhibitor versus CNI-based immunosuppression for the 'Inside Milan' group (sub-group analysis).* CNI (calcineurin inhibitor), EVR (everolimus), mTORi (mTOR-inhibitor), RR (risk ratio), SRL (sirolimus)

Figure 4. *Forest plots of 1-year and 3-year recurrence-free-survival with mTOR-inhibitor versus CNI-based immunosuppression for the 'Outside Milan' group (sub-group analysis).* CNI (calcineurin inhibitor), EVR (everolimus), mTORi (mTOR-inhibitor), RR (risk ratio), SRL (sirolimus)

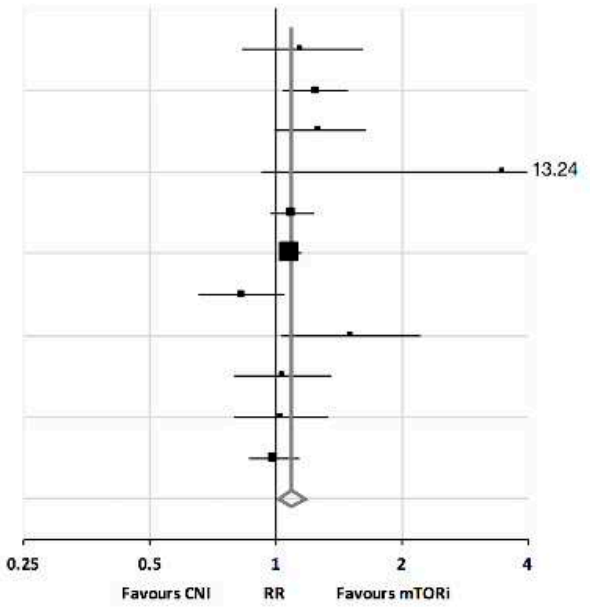
Figure 5. *Selectivity funnel plots of (a.) 1-year, (b.) 3-year and (c.) 5-year recurrence-free-survival.* Effect size [$\log_e$(RR)] is measured on the *x*-axis, and within-study variance on the *y*-axis. Each study is represented by a single equally sized circle. Contour lines marking statistical significance are integrated into the plot.



1-year recurrence free survival

Author (year)	mTORi	mTORi based: events (total)	CNI based: events (total)	Weight %	RR (95% CI)
Zhou (2008)	SRL	19 (27)	28 (46)	4.16%	1.16 (0.83; 1.62)
Zimmerman (2008)	SRL	42 (45)	39 (52)	11%	1.24 (1.04; 1.48)
Vivarelli (2010)	SRL	28 (31)	22 (31)	6.63%	1.27 (0.99; 1.64)
Feng (2012)	SRL	7 (11)	2 (11)	0.31%	3.5 (0.92; 13.24)
Zhao (2014)	SRL	87 (94)	60 (71)	16.85%	1.1 (0.98; 1.23)
Geissler (2016)	SRL	233 (252)	218 (256)	23.55%	1.09 (1.02; 1.16)
Xu (2016)	SRL	38 (62)	59 (80)	7.3%	0.83 (0.66; 1.05)
Shen (2016)	SRL	21 (26)	16 (30)	3.32%	1.51 (1.03; 2.22)
Lee (2017)	SRL	17 (20)	18 (22)	6%	1.04 (0.79; 1.36)
Houssel (2013)	EVR	14 (16)	17 (20)	6.31%	1.03 (0.79; 1.34)
Rodríguez-Perálvarez (2018)	EVR	53 (64)	107 (128)	14.58%	0.99 (0.87; 1.13)
Synthesis		**559 (648)**	**586 (747)**	**100%**	**1.09 (1.01; 1.18)**

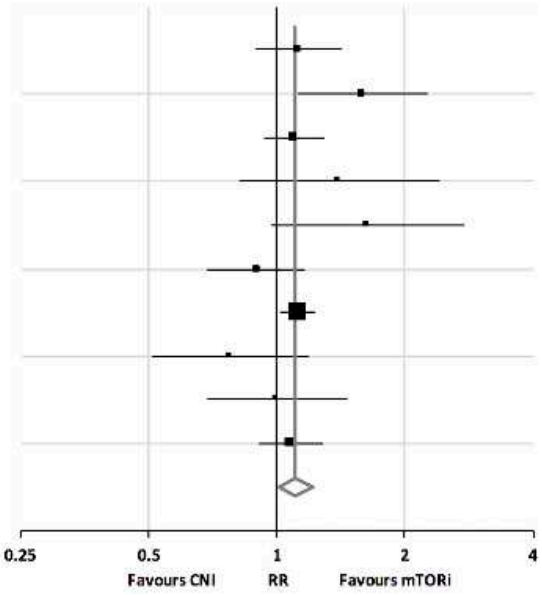
Heterogeneity: Q = 16.76 (Q-df = 4.76, p = 0.08), I^2 = 40% (95% CI 0 - 71), t^2 = 0.01 (95% CI = 0 - 0.02)



3-year recurrence free survival

Author (year)	mTORi	mTORi based: events (total)	CNI based: events (total)	Weight %	RR (95% CI)
Zimmerman (2008)	SRL	35 (45)	36 (52)	10.53%	1.12 (0.88; 1.43)
Vivarelli (2010)	SRL	27 (31)	17 (31)	5.83%	1.59 (1.12; 2.25)
Zhao (2014)	SRL	77 (94)	53 (71)	17.03%	1.1 (0.93; 1.29)
Bhangui (2016)	SRL	14 (21)	10 (21)	2.63%	1.4 (0.82; 2.4)
Shen (2016)	SRL	17 (26)	12 (30)	2.83%	1.63 (0.97; 2.75)
Xu (2016)	SRL	36 (62)	52 (80)	8.99%	0.89 (0.68; 1.17)
Geissler (2016)	SRL	203 (252)	185 (256)	27.09%	1.11 (1.01; 1.23)
Lee (2017)	SRL	12 (20)	17 (22)	4.11%	0.78 (0.51; 1.19)
Houssel (2013)	EVR	12 (16)	15 (20)	4.99%	1 (0.68; 1.46)
Rodríguez-Perálvarez (2018)	EVR	49 (64)	91 (128)	15.98%	1.08 (0.9; 1.28)
Synthesis		**482 (631)**	**488 (711)**	**100%**	**1.1 (1.01; 1.21)**

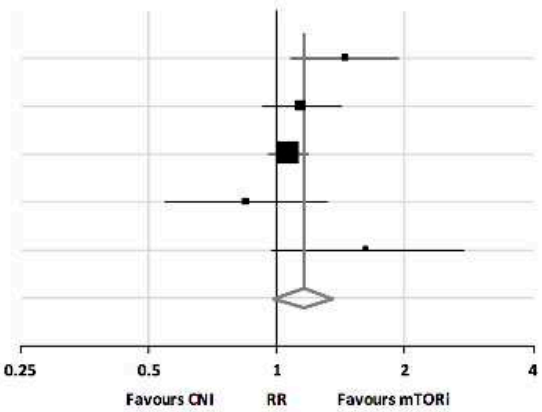
Heterogeneity: Q = 12.64 (Q-df = 1.64, p = 0.18), I^2 = 29% (95% CI 0 - 66), t^2 = 0.01 (95% CI = 0 - 0.03)



5-year recurrence free survival

Author (year)	mTORi	mTORi based: events (total)	CNI based: events (total)	Weight %	RR (95% CI)
Zimmerman (2008)	SRL	35 (45)	28 (52)	17.9%	1.44 (1.07; 1.94)
Zhao (2014)	SRL	68 (94)	45 (71)	25.33%	1.14 (0.92; 1.42)
Geissler (2016)	SRL	183 (252)	175 (256)	38.84%	1.06 (0.95; 1.19)
Xu (2016)	SRL	21 (62)	32 (80)	10.19%	0.85 (0.55; 1.31)
Shen (2016)	SRL	17 (26)	12 (30)	7.74%	1.63 (0.97; 2.75)
Synthesis		**324 (479)**	**292 (489)**	**100%**	**1.15 (0.99; 1.35)**

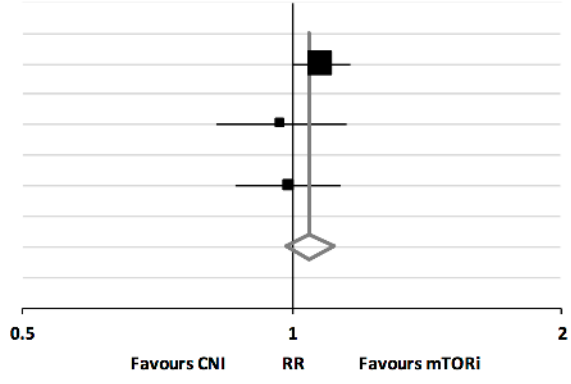
Heterogeneity: Q = 7.27 (Q-df = 1.27, p = 0.12), I^2 = 45% (95% CI 0 - 80), t^2 = 0.01 (95% CI = 0 - 0.06)



1-year recurrence free survival (Inside Milan)

Author (year)	% >Milan (mTORi : CNI)	mTORi based: events (total)	CNI based: events (total)	Weight %	RR (95% CI)
Geissler (2016)	0 : 0	138 (146)	128 (146)	67.24%	1.08 (1; 1.16)
Xu (2016)	0 : 0	28 (32)	36 (40)	13.05%	0.97 (0.82; 1.15)
Rodríguez-Perálvarez (2018)	23.4 : 27.3	53 (64)	107 (128)	19.71%	0.99 (0.87; 1.13)
Synthesis		**219 (242)**	**271 (314)**	**100%**	**1.05 (0.98; 1.11)**

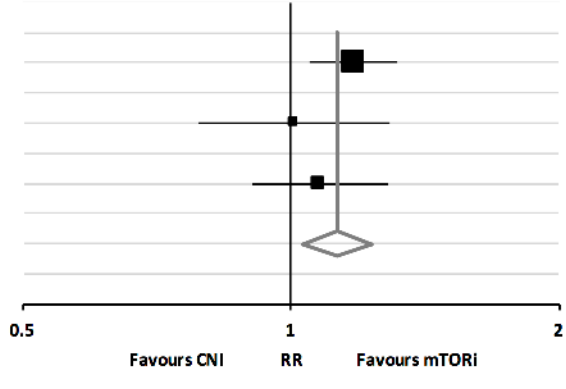
Heterogeneity: Q = 2.03 (Q-df < 0, p = 0.36), I^2 = 1% (95% CI = 0 - 90), t^2 = 0 (95% CI = 0 - 0.11)



3-year recurrence free survival (Inside Milan)

Author (year)	% >Milan (mTORi : CNI)	mTORi based: events (total)	CNI based: events (total)	Weight %	RR (95% CI)
Geissler (2016)	0 : 0	128 (146)	109 (146)	61.79%	1.17 (1.05; 1.31)
Xu (2016)	0 : 0	25 (32)	31 (40)	12.69%	1.01 (0.79; 1.29)
Rodríguez-Perálvarez (2018)	23.4 : 27.3	49 (64)	91 (128)	25.52%	1.08 (0.9; 1.28)
Synthesis		**202 (242)**	**231 (314)**	**100%**	**1.13 (1.03; 1.23)**

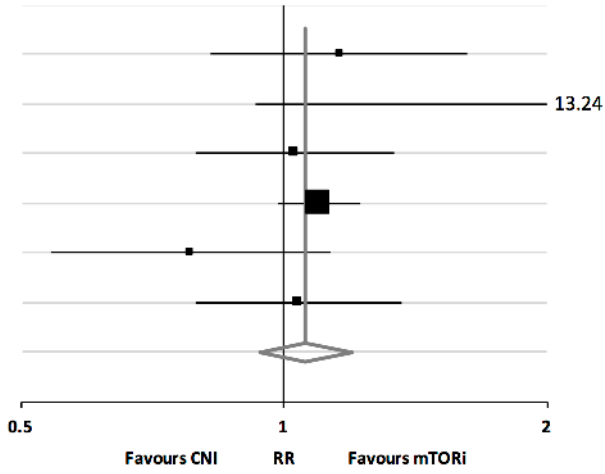
Heterogeneity: Q = 1.55 (Q-df < 0, p = 0.46), I^2 = 0% (95% CI = 0 - 90), t^2 = 0 (95% CI = 0 - 0.17)



1-year recurrence free survival (Outside Milan)

Author (year)	% >Milan (mTORi : CNI)	mTORi based: events (total)	CNI based: events (total)	Weight %	RR (95% CI)
Zhou (2008)	100 : 100	19 (27)	28 (46)	10.96%	1.16 (0.83; 1.62)
Feng (2012)	100 : 100	7 (11)	2 (11)	0.81%	3.5 (0.92; 13.24)
Houssel (2013)	100 : 100	14 (16)	17 (20)	16.63%	1.03 (0.79; 1.34)
Geissler (2016)	83 : 85.5	95 (106)	90 (110)	46.31%	1.1 (0.98; 1.22)
Xu (2016)	100 : 100	17 (30)	29 (40)	9.48%	0.78 (0.54; 1.13)
Lee (2017)	100 : 100	17 (20)	18 (22)	15.81%	1.04 (0.79; 1.36)
Synthesis		**169 (210)**	**184 (249)**	**100%**	**1.06 (0.94; 1.19)**

Heterogeneity: Q = 6.38 (Q-df < 0, p = 0.27), I^2 = 22% (95% CI = 0 - 80), t^2 = 0.01(95% CI = 0 - 0.04)



3-year recurrence free survival (Outside Milan)

Author (year)	% >Milan (mTORi : CNI)	mTORi based: events (total)	CNI based: events (total)	Weight %	RR (95% CI)
Houssel (2013)	100 : 100	12 (16)	15 (20)	13.19%	1 (0.68; 1.46)
Geissler (2016)	83 : 85.5	75 (106)	76 (110)	62.08%	1.02 (0.86; 1.22)
Xu (2016)	100 : 100	17 (30)	29 (40)	14.14%	0.78 (0.54; 1.13)
Lee (2017)	100 : 100	12 (20)	17 (22)	10.59%	0.78 (0.51; 1.19)
Synthesis		**116 (172)**	**137 (192)**	**100%**	**0.95 (0.83; 1.1)**

Heterogeneity: Q = 2.73 (Q-df < 0, p = 0.43), I^2 = 0% (95% CI = 0 - 85), t^2 = 0 (95% CI = 0 - 0.16)