**Interleukin-2 receptor antagonists for pediatric liver transplant recipients:
A systematic review and meta-analysis of controlled studies**

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Abstract

Background: Interleukin-2 receptor antagonists (IL-2RA) are frequently used as induction therapy in liver transplant recipients to decrease the risk of acute rejection while allowing the reduction of concomitant immunosuppression. The exact association with the use of IL-2RA however is uncertain. *Methods*: We performed a systematic literature search for relevant studies. Random effects models were used to assess the incidence of acute rejection, steroid-resistant rejection, graft loss, patient death, and adverse drug reaction, with or without IL-2RA. *Results*: Six studies (2 randomized and 4 nonrandomized) met the elegibility criteria. Acute rejection at 6 months or later favored the use of IL-2RA significantly (relative risk [RR] 0.38; 95% confidence interval [CI] 0.22-0.66, p-value [p] = 0.0005). Although not statistically significant, IL-2RA showed a substantial reduction of the risk of steroid-resistant rejection (RR 0.32; CI 0.19-1.03, p = 0.0594). Graft loss and patient death showed a reductive tendency through the use of IL-2RA. *Conclusions*: The use of IL-2RA is safe and is associated with a statistically significantly lower incidence of acute rejection after transplantation and substantial reduction of steroid-resistant rejection, graft loss and patient death.

Introduction

Liver transplantation in children is used to heal diseases like biliary atresia, Alagille's syndrome, metabolic liver disease, neonatal hepatitis, hepatic malignancies, to name only a few. New immunosuppressive agents, such as rapamycine and monoclonal antibodies now try to improve the long-term outcome of transplant patients. According to the literature available there is insufficient evidence of new immunosuppressive concepts in children liver transplantations, for example due to the short follow-up time and the small size of studies (19-21). Only a few published controlled clinical studies report on the use of a new immunosuppressive agent called basiliximab (Simulect®) or daclizumab (Zenapax®). These are monoclonal antibodies targeting the interleukin-2 (IL-2) receptor (IL-2R). Initially they were approved for therapy of patients after renal transplantation. For a few years they have also shown positive effects in off-label use in patients after liver transplantation. Now both are used regularly in adult liver transplantation to spare concomitant medication as calcineurin inhibitors (CNI), so called cyclosporine A and tacrolimus, and steroids to reduce long lasting adverse drug reaction (ADR). These monoclonal antibodies are now frequently used in Europe (1) and in the US (2) as induction therapy in adult liver transplant recipients to decrease the risk of acute rejection. The two IL-2R antibodies (IL-2RA), daclizumab and basiliximab, were commercially available, but daclizumab has recently been withdrawn from the market for commercial reasons. IL-2RA specifically bind and block the IL-2R α-chain (which corresponds to CD25), which is present only on the surface of activated T-lymphocytes (3). The IL-2 signal is essential for the activation of lymphocytes; it induces second messenger signals to stimulate T cells to enter the cell cycle and proliferate, resulting in clonal expansion and differentiation. The commercially available IL-2RA are both monoclonal anti-CD25 immunoglobulin G (IgG) antibodies, but their structure and synthesis are different. Daclizumab is a humanized antibody built by total gene synthesis using oligonucleotides (4), whereas basiliximab is a chimeric murine-human antibody (5). The competitive block of IL-2R, and thereby of IL-2-mediated activation, lasts for 4 to 12 weeks, depending on the antibody and the administration protocol (3).

In the present study, we do an analysis of published controlled trials to examine the effect of IL-2RA in children after liver transplantation. We assume that the reduction of concomitant medication as CNI or steroids through the additional therapy with IL-2RA will reduce the long-term ADR such as kidney failure, disturbance of growth, diabetes mellitus and other metabolic disorders.

Material and methods

The methods of this meta-analysis were similar to our results on adult patients published in (6). The methods of literature search, the inclusion and exclusion criteria, outcome measures and methods of statistical analysis were definded in a protocol according to the recommendations in the Cochrane Handbook for Systematic Reviews of Interventions (7, Part 2). We also used the Preferred Items for Systematic Reviews and Meta-Analysis (PRISMA) (8) to structure this report.

Literature search
A systematic literature search was performed without language restrictions in December 2012 in the following databases: PubMed, Transplant Library and Cochrane Library. The following search terms were used: "liver transplantation," "interleukin 2 receptor inhibitor/antagonist," "basiliximab," "daclizumab," "zenapax," "simulect," "pediatric," "child," "children," and abbreviations thereof. The keywords were combined with Boolean operators.

Inclusion and exclusion criteria
All prospective, controlled pediatric studies and pediatric studies with prospective experimental

group and historical control group in which IL-2RA induction therapy in liver transplant recipients was compared with placebo or no add-on were included. A first nonsystematic review of the literature showed that in pediatric liver transplantation patients IL-2RA are used in addition to standard immunosuppression therapy concepts to reduce other immunosuppressive drugs, such as CNI and corticosteroids. We have therefore structured this meta-analysis into three separate comparisons as follows: In the first group IL-2RA is added to the experimental group and is compared to no add-on or placebo, while boths study arms got equal concomitant immunosuppressive medication. This group is referred to as the *IL-2RA only* comparison in the following. In the second group IL-2RA is additionally combined with delayed CNI in the experimental arm (*delayed CNI* comparison). The third group compared IL-2RA with a standard immunosuppressive protocol with reduced or even dropped steroids in the experimental arm (the *no/low steroids* comparison). Other immunsuppressive medication had to be the same in both treatment arms, e.g., mycophenolate mofetil (MMF).

All retrospective, noncontrolled pediatric studies and pediatric studies with multiorgan transplantation or retransplantation were excluded. Pharmacological studies that did not provide data on clinical outcome measures were excluded as well because of their very short follow-up time. The literature search strategy was designed and performed by two reviewers (N.D.C., A.D.G.). Publications were screened independently by two reviewers (N.D.C., A.D.G.). Disagreement and any discrepancies were resolved by discussion of all four reviewers.

Outcome measures

The primary outcomes analysed were acute rejection, steroid-resistant rejection, graft loss and patient death. Secondary outcomes were ADR namely renal dysfunction by need of dialysis or oliguria, de novo malignancy (excluding recurrence of hepatocellular carcinoma), post transplant lymphoproliferative disease (PTLD), infection complications, including fungal, viral and bacterial infections, new onset of metabolic and cardiovascular disorders such as arterial hypertension (HTN), hyperlipoproteinemia (HLP) and posttransplant diabetes mellitus (PTDM).

Study quality

The quality items assessed were blinding, randomization, allocation concealment, intention-to-treat (ITT) analysis, completeness of follow-up, and the method of handling missing values. Assessment was performed according to definitions stated in the Cochrane Handbook (7, Ch. 8). Quality of studies was assessed independently by two reviewers (N.D.C., A.D.G.) without blinding to journal and authorship. Furthermore, completeness of follow-up was defined as the number of patients that were not lost to follow-up. We reported completeness of follow-up as stated by the authors. Methods of handling missing values are stated as reported by the authors of the respective study.

Data extraction

All available data for the described outcome measures were extracted at all available timepoints from individual trials. In contrast to kidney transplants, it has been shown that morphological signs of rejection in protocol biopsies of transplanted livers without clinical correlates require no treatment and have no long-term ADR (9). Therefore, we only included treated acute rejections in the primary analysis, when the reported acute rejection was stratified into "treated" and "nontreated." When data on outcome measures were not provided or studies seemed to be duplicates, the authors were contacted to provide more data. Data extraction was performed by one reviewer (NDC) using a standardized form and checked by two reviewers (ADG, CR).

Data analysis

We expressed the results of dichotomous outcomes as relative risk (RR) with values smaller than 1 favoring IL-2RA. When no event was observed in both arms, we excluded it from the

corresponding comparison (7, Ch.16.9.3). We performed the analysis using a random effects model, as in case of doubt it makes more sense to use the more general approach (including the fixed-effects model as a special case), which will usually lead to more conservative results (10). For the random effects models the amount of residual heterogeneity (i.e., τ^2) was estimated by the restricted maximum likelihood (REML) method (11). Confidence intervals for τ^2 were obtained by the Q-profile method (12). The model parameters were estimated by way of weighted least squares, with weights equal to the inverse sum of the variance of the estimate and the estimate of the residual heterogeneity. Then Wald-type tests and confidence intervals were obtained for the parameter estimates (11). We analyzed heterogeneity among studies using Cochrane's Q test and calculating I^2 to measure the proportion of total variation due to heterogeneity beyond chance (13). We performed subgroup analyses for primary outcomes which had significant results. Subgroups and factors defined a priori were methodological quality of trial (i.e., randomized versus nonrandomized), comparison group, type of IL-2RA, type of CNI, and use of mycophenolate mofetil (MMF). For the primary analysis we pooled effect measurements from trials with different follow-up time; but timepoint of measurement (grouped by 6 months versus 12 months and later) was evaluated in a subgroup analysis. In some of the subgroups a valid analysis was not possible. In order to examine the influence of covariates affecting the direction and/or strength of the relation between dependent and independent variables we used the moderator test. For statistically significant results we calculated the number needed to treat (NNT) describing how many patients are needed to be treated with an intervention, here IL-2RA, to prevent one patient of having one additional bad outcome, here for example acute rejection. Publication bias was assessed using funnel plots (14) and tests for funnel plot asymmetry (11). The R environment for statistical computing (v. 2.11.0) (15) with packages "meta" (v. 2.5-0) (16), "metafor" (v. 1.4-0) (11), and "lme4" (v. 0.999375-37) (17) were used for all analyses.

Results

Literature search
Database searches yielded 325 entries (see Fig.1), of which 15 were excluded as duplicates. Of the remaining 310 publications that qualified for abstract review, 252 were excluded primarily because they were not controlled trials, the effect of IL-2RA was not investigated, they were not dealing with pediatric patients or they were not conducted in patients undergoing first liver transplantation. Also retrospective studies were excluded. The remaining 58 publications underwent full article review and 38 further publications were excluded. Most common reasons were retrospective studies, other comparator than IL-2RA, studies with adult patients, non controlled studies and reviews. A total of 20 trials qualified for inclusion in this review. 13 studies were excluded because of being duplicates, preliminary reports and follow-up reports of the included studies. One study was excluded because of having no reported follow-up time and the authors did not respond to our requests of further information. Six studies were eventually included for analysis (18-23). All trials were obtained as full-text puplications. In case of multiple reports on the same study we cited the most recent full-text publication as the index publication. Two authors of reports were contacted in order to resolve ambiguities. One author answered, the other author did not respond.

Included studies
Table 1 shows the characteristics of the included studies. Three trials (18-20) compared IL-2RA to no treatment without modification of concomitant immunosuppressive medication (*IL-2RA only* comparison). Only one trial (21) compared IL-2RA in combination with delayed CNI to no treatment with standard immunosuppression (*delayed CNI* comparison). Two trials (22, 23) compared IL-2RA in combination with early discontinuation or reduction of steroids to no treatment with standard immunosuppression (*no/low steroids* comparison). One study (22) excluded patients

with a severe renal dysfunction and another trial (23) excluded children with autoimmune hepatitis. In two-thirds of the included studies basiliximab was used for induction (18, 19, 22, 23) and another two-thirds of the trials used tacrolimus as CNI (20-23). Two of the six studies used MMF (20, 21) and all but the experimental arms of the studies of *no/low steroids* comparison (22, 23) used steroids as concomitant medication. Most trials had a study duration of more than 12 months (18, 21-23).

Quality of included studies

Table 2 shows the quality assessment of the included studies. None of the studies were classified as blinded, whereas two of them did not report the status of blinding and were classified as „not stated". Two studies (21, 22) were prospective and randomized, a third study (20) was entirely prospective and three studies (18, 19, 23) had a prospective experimental group and historical control group. Of the randomized trials, allocation concealment was found to be adequate in none of the studies, but unclear in one study (22) and inadequate (21) in another. For the four nonrandomized studies (18-20, 23) allocation concealment was not applicable. ITT analysis was stated and performed in one study (22) and was assessed as adequate. In three further trials (18, 20, 23) ITT analysis was assumed and considered adequate because the authors reported on all patients at the endpoints of the study. One study (19) reported ITT analysis but it was assessed as inadequate. According to the definition given in (7, Ch. 16.2.1), the authors of that trial did a per-protocol (PP) analysis. In only one trial (21) we could not assess ITT analysis and it was therefore classified as „not stated". None of the authors stated how missing values were handled. Only two studies (22, 23) described completeness of follow-up. Table 3 summarizes the immunosuppressive therapy of included pediatric trials.

Primary ouctomes

Acute rejection. Reduction of acute rejection favored the use of IL-2RA (RR 0.38; CI 0.22–0.66; p = 0.0005; 6 trials; Fig. 2). The effect is also seen in the subgroup of randomized trials (RR: 0.31; CI 0.20–0.47; p < 0.0001; 3 trials), but is not statistically significant in nonrandomized studies (RR: 0.46; CI 0.18–1.18; p = 0.1039; 3 trials). The relative risk (RR) of all studies had a statistically significant heterogeneity (p=0.0126) which is due to the study of Gibelli et al. 2004 (19). Omitting the study by Gibelli et al. (2004) the risk reduction is larger (RR 0.30; CI 0.21–0.43; p < 0.0001; 5 trials). There is only the *IL-2RA only* comparison (RR: 0.44; CI 0.19–1.002; p = 0.0507; 3 trials) and the *no/low steroids* comparison (RR: 0.31; CI 0.12–0.84; p = 0.0211; 2 trials) to analyse; only the *no/low steroids* comparison was significant in reduction of acute rejection, favoring the use of IL-2RA. Stratifying studies by follow-up time showed a statistically significant reduction of acute rejections with IL-2RA at 12 months and later (RR 0.31; CI 0.21–0.45; p < 0.0001; 4 trials), but not at 6 months (RR 0.51; CI 0.16–1.66; p = 0.2654; 2 trials). Furthermore, subgroup analysis stratified by the type of IL-2RA used showed a statistically significant effect of both basiliximab (RR 0.44; CI 0.21–0.92; p = 0.0299; 4 trials) and daclizumab (RR 0.29; CI 0.18–0.47; p < 0.0001; 2 trials). The subgroup with daclizumab induction therapy got additional MMF as immunosuppressive concomitant medication, it showed a lower p-value and showed no statistically significant heterogeneity. Stratifying trials by type of CNI used showed a statistically significant effect of tacrolimus (RR 0.30; CI 0.19–0.46; p < 0.0001; 4 trials) but not for cyclosporine A (RR 0.53; CI 0.20–1.40; p = 0.1999; 2 trials). Finally we analysed the subgroup stratified by control group. Studies with retrospective control group showed no significant reduction of acute rejection (RR 0.46; CI 0.18–1.18; p = 0.1039; 3 trials) in comparison to these with prospective control group (RR 0.31; CI 0.20–0.47; p < 0.0001; 3 trials). The number needed to treat (NNT) is 3.6, which means that four children after liver transplantation have to be treated with IL-2RA in addition to standard immunosuppressive therapy to prevent one patient of having an acute rejection. Four studies defined acute rejection clinically and confirmed it by biopsy (18-21). Analysis of this subgroup showed a statistically significant reduction of acute rejection (RR 0.40; CI 0.21–0.76; p = 0.0052; 4

trials). One trial (22) used the term of an episode of acute rejection as outcome measurement and another study (23) did not state a definition. None of the trials were taking protocol biopsies.

Steroid-resistant rejection. All trials reported data on steroid-resistant rejection. One study (22) reported about no steroid-resistant rejection in both arms, so that we excluded it from analysis. IL-2RA in addition to standard double or triple immunsuppressive therapy after liver transplantation in children did not reduce steroid-resistant rejection statistically significantly (RR 0.44; CI 0.19–1.03; p = 0.0594; 5 trials). If we exclude one of the older studies (19) with the most extreme effect from the analysis, we get a statistically significant reduction of steroid-resistant rejection without significant heterogeneity (RR 0.34; CI 0.14–0.79; p = 0.0123; 4 trials). Stratifying trials by randomization status (randomized subgroup: RR 0.18; CI 0.04–0.74; p = 0.0177; 2 studies (20, 21) and nonrandomized subgroup: RR 0.65; CI 0.24–1.78; p = 0.3971; 4 trials (18-19, 22-23)) and comparison did not show statistically significant effects (*IL-2RA only* comparison: RR 0.77; CI 0.30–1.98; p = 0.5894; 3 trials (18-20); *delayed CNI* comparison (21) and *low/no steroids* comparison (23) only one study each) except the subgroup of randomized studies. However, we saw a statistically significant reduction of steroid-resistant rejection in studies (18, 21, 23) with follow-up measurement at 12 months and later (RR 0.33; CI 0.12–0.89; p = 0.0281; 3 trials; Fig.3), but not for 6 months (RR 0.93; CI 0.18 –4.67; p = 0.9269; 2 trials (19, 20)). There was a statistically significant reduction in steroid-resistant rejection by using IL-2RA in subgroups using tacrolimus (RR 0.17; CI 0.05–0.57; p = 0.0041; 3 trials (20, 21, 23)) and daclizumab induction therapy combined with additional MMF dose and prospective control group (RR 0.18; CI 0.04–0.74; p = 0.0177; 2 trials (20, 21)).

Graft loss and patient death. Four studies (18, 21-23) reported data on graft loss and patient death. Neither graft loss (RR 0.65; CI 0.34–1.21; p = 0.1737; 4 trials) nor patient death (RR 0.61; CI 0.27–1.37; p = 0.2296; 4 trials) were statistically significantly reduced by using additional IL-2RA in combination to standard immunosuppressive medication in the observation period. In the forest plot one study (22) is prominently deviating from the remaining studies. After excluding this study from the analysis we saw a statistically significant result for reducing graft loss by the use of IL-2RA (RR 0.44; CI 0.21–0.92; p = 0.0298; 3 trials), but not for death (RR 0.42; CI 0.16–1.12; p = 0.0831; 3 trials). However, all analyses show a trend towards a lower incidence of graft loss and patient death in the experimental group using IL-2RA in addition to standard double-drug or triple-drug therapy.

Secondary outcomes
Due to limited data we were unable to do subgroup analyses except for the *IL-2RA only* comparison. Also it was not possible to collect enough data to analyse secondary outcomes, namely de novo malignancy, PTDM or HLP. Four studies reported on renal dysfunction (18, 20, 22); data analysis of this outcome showed a reductive tendency but no statistically significant reduction by using IL-2RA (RR 0.96; CI 0.60–1.54; p = 0.8683; 4 trials). Furthermore three studies (18, 19, 22) reported on new onset arterial hypertension (HTN). Analysis showed no significant reduction of HTN by using IL-2RA but a reductive tendency (RR 0.85; CI 0.60–1.21; p = 0.3731; 3 trials). Three studies (18, 20, 22) reported on PTLD but one of them (21) yielded no event in both arms, so that we excluded it from analysis. PTLD was not reduced by using additional IL-2RA therapy (RR 1.6; CI 0.20–12.67; p = 0.6587; 2 trials), on the contrary, it showed a higher RR in the experimental group. Two studies (18, 22) reported on infection complications and outcomes were also reported on subgroups named viral, bacterial and fungal infections. Additional IL-2RA therapy with standard immunosuppressive medication did not reduce infection complications statistically significantly (infection complications: RR 0.80; CI 0.60–1.07; p = 0.1363; 2 trials; viral infection: RR 1.06; CI 0.62–1.80; p = 0.8356; 2 trials; fungal infection: RR 1.15; CI 0.46–2.87; p = 0.7624; 2 trials; bacterial infection: RR 0.68; CI 0.34–1.37; p = 0.2838; 2 trials). Infection complications and

bacterial infection showed a reductive tendency but viral and fungal infection were more frequent in the experimental group. The subgroup analysis of AE of the *IL-2RA only* comparison showed no significant reduction by using IL2-RA in any of the secondary outcomes renal dysfunction, HTN or PTLD.

Discussion

The use of IL-2RA in addition to standard double-drug or triple-drug therapy statistically significantly lowers the risk of acute rejection in pediatric patients after liver transplantation. Acute rejection rate is reduced by two thirds through the use of IL-2RA (RR 0.38). These results are similar to those we found in our meta-analysis in adult liver transplant recipients (6). The relative risk of all studies has a significant heterogeneity which is introduced by the study of Gibelli et al. 2004 (19). Most of the subgroup analyses support a statistically significant reduction of acute rejection through the additional use of IL-2RA, all subgroup analyses showed a substantial reduction by at least a factor of two.

The use of IL-2RA in addition to standard double-drug or triple-drug therapy also shows a substantial reduction of steroid-resistant rejection after pediatric liver transplantation (RR 0.44). If we exclude one of the older and prominently deviating studies (19) from analysis, we get a statistically significant reduction of steroid-resistant rejection without significant heterogeneity. Subgroup analysis stratified by measurement time at 12 months and later, randomized subgroup, as well as a subgroup of only prospective controlled trials observed significant reduction of steroid-resistant rejection through the use of IL-2RA.

Although the risk of acute rejection is substantially reduced when IL2-RA is applied, we did not observe a statistically significant reduction in graft loss or patient death. Observed trends suggested that the patient collective may be too small to observe significant effects, but we see a clinical relevant reduction of patient death (RR 0.61) and graft loss (RR 0.65) by one third. These results are similar to those we found in adult liver transplantated patients (6).

We also looked at the possibility of reducing concomitant immunosuppressive medication when using IL-2RA because most published studies explored this effect. We could classify published studies into three different experimental immunosuppressive regimes, namely the *IL-2RA only* comparison (18-20), the *delayed CNI* comparison (21), and the *no/low steroids* comparison (22-23). Stratifying trials by comparison, there is only the *IL-2RA only* comparison and the *no/low steroids* comparison to analyse. The *no/low steroids* comparison shows a statistically significant reduction of acute rejection favoring the use of IL-2RA. In the analysis of other primary outcomes the number of studies in each comparison is too small, so that we find no statistically significant effect in any. We see a clinically relevant reductive effect in the *IL-2RA only* and *no/low steroids* comparisons of the risk of steroid-resistant rejection, patient death and graft loss through the additional use of IL-2RA in the experimental group.

Due to the limited amount of data we were unable to perform subgroup analyses except for the *IL-2RA only* comparison. Also it was not possible to collect enough data to analyse secondary outcomes, namely de novo malignancy, PTDM or HLP. Analysis of included studies shows a reductive tendency of renal dysfunction, new onset posttransplant arterial hypertension and infection complication especially bacterial infections in experiemtal group which uses IL-2RA in addition to standard immunsuppressive therapy. The subgroup analysis of the *IL-2RA only* comparison showed no statistically significant reduction by using IL2-RA in any of the secondary outcomes called renal dysfunction, HTN or PTLD.

Strengths and limitations

The main limitation of this review is the small number of randomized controlled trials, even

compared to trials in kidney transplantation (24), and our systematic review and meta-analysis of adult patients after liver transplantation (6). The low number of studies makes it difficult to acquire enough data to demonstrate statistical significance. Corresponding to our experience with studies of adult liver transplant recipients (6) we decided to include not only randomized trials but also nonrandomized controlled trials and studies with prospective experimental group and retrospective control group in this review. Half of them compared IL-2RA to no add-on. The other half explored the effects of reduced or delayed concomitant immunosuppression. Therefore, we decided to include those studies in order to increase the total number of included trials. We also allocated them to predefined comparisons of concomitant medication. Furthermore, we included and pooled studies that used a different type of IL-2RA, had different concomitant medication (type of CNI and MMF), or had different follow-up times. Because all these differences may be sources of heterogeneity, it was planned to explore differences of effect by performing subgroup analyses. Because of the small number of included studies some studies dominate the results as we have seen in analyses including Gibelli et al. 2004 (19) or Spada et al. 2006 (22). Both studies met the inclusion criteria. Due to the paucity of data on secondary outcomes we were only able to extensively analyze the primary endpoints. Another problem was the insufficient detailed reporting of outcomes; this was noticed most evidently regarding the adverse drug reaction of immunosuppression. Few studies give data on complications and ADR, but also these were measured or grouped differently in the various trials. We endeavored to overcome this limitation by grouping data on side effects into broader categories, but this may further limit the interpretation of the results. Although we attempted to minimize publication bias by searching for and including data from different databases. Nonetheless, this review and meta-analysis gives us a first impression of the evidence and the order of magnitude of the effect of using IL-2RA as an induction therapy in addition to standard double-drug or triple-drug therapy in pediatric liver transplant recipients. For further analysis we require more studies, but we do not expect more data to accumulate over the next years. In order to gain information on long-term effects of reduced or delayed concomitant immunosuppression, which is urgently needed in pediatric liver transplant recipients, more prospective controlled trials are needed.

Clinical implications

Four pediatric patients would need to be treated with IL-2RA to prevent one acute rejection (NNT ≈ 4). The risk reduction for acute rejection is higher than would be expected from experience with adult liver transplantation (6) which could be a result of differences in the pediatric metabolism. We have no evidence for a difference in effectiveness between basiliximab and daclizumab in reducing the risk of rejection. In conclusion, the use of IL-2RA reduces the risk of acute rejection without a significant increase of harmful effects. This effect may allow for reduction of coimmunosuppression to avoid the adverse drug reaction of CNI or steroids. Also we observe a substantial reduction of the risk of steroid-resistant rejection, patient death and graft loss by using IL-2RA in addition to standard double-drug or triple-drug therapy, and therefore we should value this result as clinical relevant.

Acknowledgement

Table 1. Characteristics of included trials stratified by the three pre-specified comparison groups

Trial (Author& Year)	Patient Subgroup[2]	Sample Size Exp	Cont	Age^ Exp	Cont	Sex (male) Exp	Cont	Typ of IL-2RA	Control Substance	CNI	MMF	Follow -up§
IL-2RA only comparison: IL-2RA vs placebo/no treatment												
Gangschow 2005		54	54	4.2 (0.3-8.9)"	matched	ns	ns	Bas	no	Cya	no	36
Gibelli 2004		28	28	3 (1.3-16)"	matched	ns	ns	Bas	no	Cya	no	6
Schuller 2005		18	12	3.95 ± 0.33	3.9 ± 0.26	9	4	Dac	no	Tac	yes	6
delayed CNI comparison: IL-2RA and delayed and/or reduced CNI vs placebo/no treatment and standard immunosuppressive co-medication												
Heffron 2003		61	20	6.8 ± 6.3	5.3 ± 6.6	24	7	Dac	ns	Tac	yes	24
no/low steroids comparison: IL-2RA and minimized steroids or no steroids vs placebo/no treatment and standard immunosuppressive co-medication												
Spada 2006	renal function	36	36	2.9 (1.5-4.3)"	2.8 (1.5-4.3)	18	15	Bas	no	Tac	no	12
Gras 2008	no auto-immun hepatitis	50	34	1.7 (0.4-14.0)"	2.0 (0.4-14.0)	27	16	Bas	no	Tac	no	36

Abbreviations: Exp – experimental group; Cont – control group; IL-2RA– interleukin-2 receptor antagonist; CNI – calcineurin inhibitor; MMF – mycophenolat mofetile; Bas – basiliximab; Dac – daclizumab; Cya – cyclosporine A; Tac – tacrolimus; ns – not stated; vs – versus.
^ - Age is given in mean ± standard deviation if available. " - Age is given in mean with (minimum – maximum).
§ - Length of follow-up, time is given in months.

Table 2. Summary of quality assessment of included trials

Trial (Author & Year)	Blinding	Randomized	Control Group	Allocation Concealment	ITT Analysis	Missing Values	Completeness of follow-up §		
							Exp (%)	Cont (%)	Month
IL-2RA only comparison: IL-2RA vs placebo/no treatment									
Gangschow 2005	no	no	historical	na	yes^ (ns)	ns	ns	ns	28-52
Gibelli 2004	no	no	historical	na	yes* (PP)	ns	ns	ns	6
Schuller 2005	no	no	concurrent+	na	yes^ (ns)	ns	ns	ns	6
delayed CNI comparison: IL-2RA and delayed and/or reduced CNI vs placebo/no treatment and standard immunosuppressive co-medication									
Heffron 2003	ns	yes	concurrent	inadequate	ns	ns	ns	ns	24
no/low steroids comparison: IL-2RA and minimized steroids or no steroids vs placebo/no treatment and standard immunosuppressive co-medication									
Spada 2006	ns	yes	concurrent	unclear	yes	ns	90	90	12
Gras 2008	no	no	historical	na	yes^ (ns)	ns	100	100	36

Abbreviations: ITT – intention-to-treat;, na – not applicable, ns – not stated; PP – per-protocol; Exp – experimental group; Cont – control group; vs - versus.

^ - ITT-analysis is assumed, because the author reported about at least one analysis with the total number of included patients.

* - author reported that ITT analysis was performed, but also stated conditions that must be met for patient to be included in analysis, such as "patient received at least one dose of medication" and/or "at least one follow-up available". § - as stated by authors or calculated from available data. + - prospective study.

Table 3. Immunosuppressive therapy of included pediatric trials

Trial (Author & Year)	IL2RA Type & Dosage	CNI Type, First Day of Therapy, Dosage & Target Level (tl)		Corticosteroids Dosage†		MMF Dosage
		Exp	**Cont**	**Exp**	**Cont**	
IL-2RA only comparison: IL-2RA vs placebo/no treatment						
Gangschow 2005	Basiliximab POD 0&4 i.v. 10mg (KG < 30kg) 20mg (KG > 30kg)	Cyclosporine A tl 150 - 200 µg/L after one year tl 80 - 100 µg/L		Prednisolone 60 mg/m² after one week 30 mg/m² thereafter weekly reduction about 5 mg/m² break off after one year		no
Gibelli 2004	Basiliximab POD 0&4 i.v. 10mg (KG < 30kg) 20mg (KG > 30kg)	Cyclosporine A 7 – 13mg/kg/d tl 850 – 1000 mg/dL	Cyclosporine A 5 – 7 mg/kg/d tl 850 – 1000 mg/dL	steroids		no
Schuller 2005	Daclizumab POD 0 & 14 i.v. 1mg/kgKG	Tacrolimus 0.2 mg/kg/d tl 10-12 ng/mL Start: POD 3	Tacrolimus 0.2 mg/kg/d tl 6-8ng/mL Start: POD 3	Methylprednisolone 20mg/kg Start: POD 0 fast reduction break off after 4th months		1200mg/m²/d Start: POD 14
delayed CNI comparison: IL-2RA and delayed and/or reduced CNI vs placebo/no treatment and standard immunosuppressive co-medication						
Heffron 2003	Daclizumab POD 0 i.v. 1mg/kgKG	Tacrolimus 0.15/mg/kg/d tl 10-14ng/mL Start: POD 7	Tacrolimus 0,15/mg/kg/d tl 10-14ng/mL Start: POD 0	Methylprednisolon: POD 0: 20mg/kg/d, POD 6: 0,3mg/kg/d		30mg/kg/d, p.o.
no/low steroids comparison: IL-2RA and minimized steroids or no steroids vs placebo/no treatment and standard immunosuppressive co-medication						
Spada 2006	Basiliximab POD 0 & 4 & POD 8-10 i.v. 10mg (KG < 35kg) 20mg (KG > 35kg)	Tacrolimus 0.04 mg/kg/d, p.o. tl 1th month 10-15 ng/mL tl 2th-3th month 10-15ng/mL tl 4th-6th month 6-8ng/mL thereafter 5-7ng/mL		Methyl-prednisolone i.o. 10mg/kg i.v.	Methylprednisolone i.o. 10mg/kg i.v. POD 1-6: 2mg/kg/d POD 7: 1mg/kg/d break off after 3th - 6th months maximum: 40mg	no
Gras 2008	Basiliximab POD 0 & 4 i.v. 10mg (KG < 35kg) 20mg (KG > 35kg)	Tacrolimus 0.2 mg/kg/d, p.o. Start: POD 0 tl 1th month 8-12 ng/mL thereafter 5-8 ng/mL		no	Methylprednisolone 10mg/kg/i.v. POD1 - 6: 2 mg /kg/d i.v. POD7 – 13: 1mg/kg/d p.o. POD14-20: 0.75 mg/kg/d POD 21-28: 0.5 mg/kg/d thereafter 0.25 mg/kg/d POD 90: 0.25mg/kg/d (cave: alternative therapy in 2th -6th months)	20 mg/kgKG/d (only the first 9 children took it)

Abbreviations: i.o. – intraoperative; POD – postoperative day; Tac – tacrolimus; tl – target level; g/d - gramme pro day; mg/kg/d – milligramme pro kilogramme body weight pro day; kgKG – kilogamme body weight; Exp – experimental group; Cont – control group; na - not applicable (drugs were not used), ns – not stated (drugs were used but not specified), vs – versus.

† - All trials used methylprednisolone intraoperatively. In the postoperative period they used methylprednisolone or prednisolone.
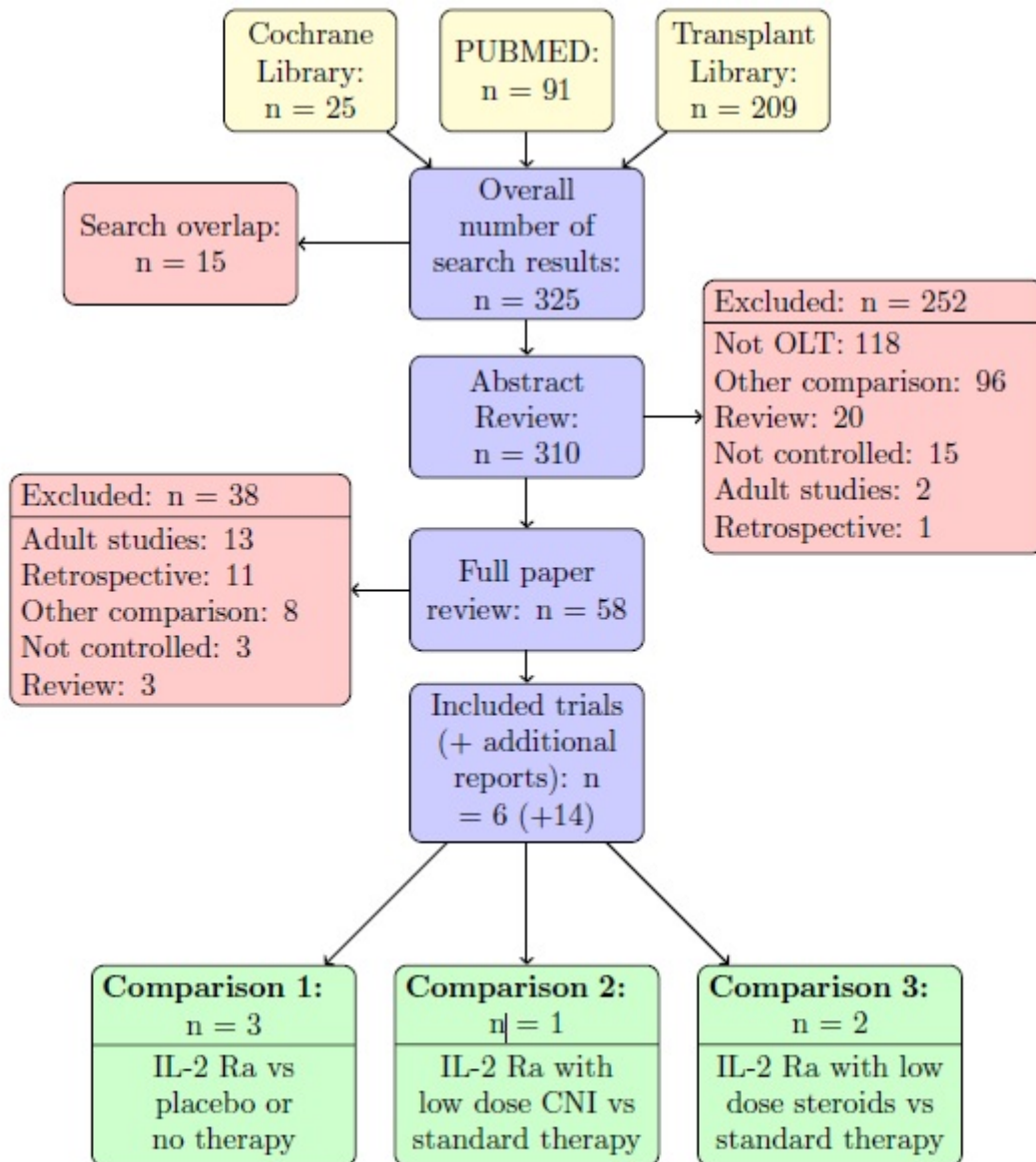
Fig. 1. Flow chart of systematic review. IL-2 RA - interleukin-2 receptor antagonist (Corresponding PRISMA[8]) Abbreviations: CNI - calcineurin inhibitor; OLT - orthotopic liver transplantation
(150.8mm x 165.9mm)

Study	Experimental Events	Total	Control Events	Total	RR	95%-CI	W(random)
Spada 2006	4	36	11	36	0.36	[0.13; 1.04]	14.1%
Gangschow 2005	9	54	29	54	0.31	[0.16; 0.59]	20.9%
Gibelli 2004	16	28	19	28	0.84	[0.56; 1.27]	25.5%
Heffron 2003	14	61	15	20	0.31	[0.18; 0.52]	23.3%
Schuller 2005	3	18	8	12	0.25	[0.08; 0.76]	13.2%
Gras 2008	0	50	3	34	0.10	[0.01; 1.83]	3.0%
Random effects model		**247**		**184**	**0.38**	**[0.22; 0.66]**	**100%**

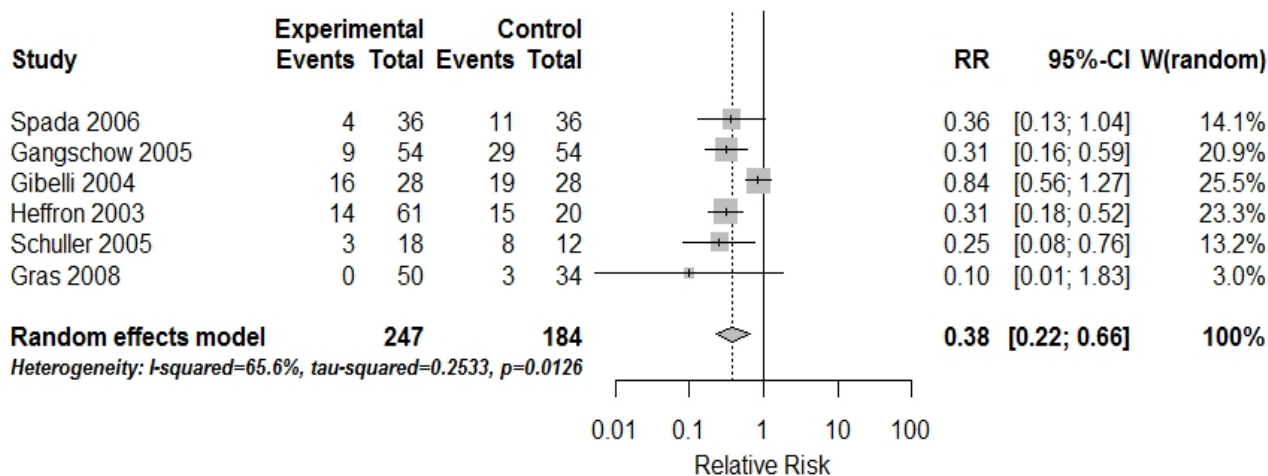*Heterogeneity: I-squared=65.6%, tau-squared=0.2533, p=0.0126*

Fig. 2. Forest plot of acute rejection of all included studies. The forest plot shows a reduced relative risk of acute rejection for pediatric patients which have used IL-2RA (experimental group). The result is significant, but also shows significant heterogeneity (p = 0.0126). Abbreviations: RR - relative risk; 95%-CI - 95% confidence interval; p – p value for test of heterogeneity; Experimental - experimental group, Control - control group.
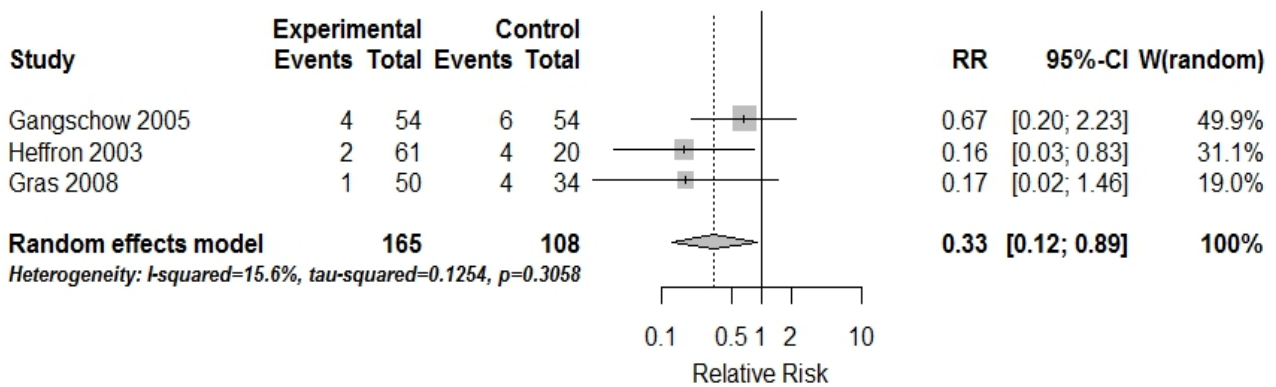
(210mm x 73.8mm)

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Fig. 3. Forest plot of steroid-resistant rejection stratified by follow-up measurement, here follow-up 12 months and later. The forest plot shows a reduced relative risk of steroid-resistant rejection for pediatric patients which have used IL-2RA (experimental group). The result is significant and shows no significant heterogeneity (p = 0.3058). Abbreviations: RR - relative risk; 95%-CI - 95% confidence interval; p – p value for test of heterogeneity; Experimental - experimental group, Control - control group.

(210mm x 65.8mm)

Figure Legends

Figure 1: Flow chart of systematic review. IL-2 RA - interleukin-2 receptor antagonist (Corresponding PRISMA[8]) Abbreviations: CNI - calcineurin inhibitor; OLT - orthotopic liver transplantation

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