Mammalian target of rapamycin inhibitors as the treatment for steroid-refractory acute graft-vs.-host disease after allogeneic hematopoietic stem cell transplantation: A systematic review and individual patient data meta-analysis

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Abstract. The prognosis of patients with steroid-refractory acute graft-vs.-host disease (SR-aGVHD) following allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains poor. Furthermore, the recommendations for second- and third-line therapeutic approaches are controversial. The present study aimed to analyze the efficacy and safety of mammalian target of rapamycin (mTOR) inhibitors in SR-aGVHD after allo-HSCT. The present study searched the PubMed and Embase databases for relevant publications from December 2001 to September 2024. The primary endpoints were overall response rate (ORR), complete response rate (CRR), chronic GVHD, overall survival (OS) and infection-related complications at any time after mTOR inhibitor therapy. The response rate at 1 month after mTOR inhibitor therapy was also assessed. The present meta-analysis included 5 studies involving 134 patients. The ORR and CRR at any time were 65% (95% CI, 44-81%) and 46% (95% CI, 22-72%) after mTOR inhibitor-based therapy for SR-aGVHD, respectively, with 1- to 3-year OS rate ranging from 36% (95% CI, 27-46%) to 30% (95% CI, 20-42%) The ORR and CRR after a 1-month treatment with mTOR inhibitors were 57% (95% CI, 34-78%) and 58% (95% CI, 13-93%), respectively. Transplant-associated thrombotic microangiopathy (TA-TMA) and cytopenia occurred in 36% (95% CI, 22-52%) and 43% (95% CI, 18-72%) of patients with SR-aGVHD, leading to treatment interruption with mTOR inhibitors in 10% (95% CI, 5-22%) and in 6% (95% CI, 2-13%) of patients, respectively. The highest CRR (78%) was observed in steroid-refractory patients treated with mTOR inhibitors combined with IL-2R antagonists, with an mTOR inhibitor serum trough level of 7-13 ng/ml, although a minimum therapeutic level was 4 ng/ml. Prolonged therapy was more likely to be effective. In conclusion, mTOR inhibitors exhibit potent activity against SR-aGVHD. However, their use may be associated with complications such as TA-TMA and cytopenia.

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Introduction

Despite the routine use of immunosuppressive prophylaxis, ~50% of patients develop acute graft-vs.-host disease (aGVHD) following allogeneic hematopoietic stem cell transplantation (allo-HSCT) (1). Among these, 14-36% of patients will develop severe aGVHD (2), leading to early post-transplant mortality (3,4). The non-relapse mortality rate by response days 14, 28 and 56 for those who do not achieve complete remission has been reported as ~49, 53 and 69%, respectively (4). The first-line treatment for aGVHD is systemic corticosteroid therapy; however, 35-50% of patients are refractory to corticosteroid treatment (5-7). The long-term prognosis for patients with steroid-refractory (SR)-aGVHD remains poor, with survival rates of 5-30% (8). Several second-line treatments for SR-aGVHD have already been developed, including mycophenolate mofetil (MMF), a selective inhibitor of the Janus kinase pathway (ruxolitinib), IL-2 receptor antagonists (IL-2RAs), antitumor necrosis factor (TNF-α) antibodies, extracorporeal photopheresis (ECP) and mesenchymal stromal cells (MSCs) (5,9-15). Consensus on the optimal second-line therapy for SR-aGVHD has not been achieved for decades due to the lack of randomized controlled trials (RCTs) for second-line treatments.

Sirolimus (rapamycin) and second-generation mammalian target of rapamycin (mTOR) inhibitors (temsirolimus and everolimus) inhibit T-cell proliferation, promote the production of regulatory T cells (Tregs) and suppress dendritic cell function, thereby exerting immunosuppressive effects (16). Further, mTOR inhibitors have been proven to maintain the graft-vs.-leukemia (GVL) effect (17). Besides their effects on Tregs, sirolimus and its analogs display direct antitumor activity against several malignancies, such as advanced renal cell carcinoma, multiple myeloma, non-Hodgkins lymphoma and myelodysplastic syndrome (18). Sirolimus exerts dose-dependent immunomodulatory effects on $CD8^+$ memory T cells *in vivo* exposed to viral and bacterial pathogens, such as acute lymphocytic choriomeningitis virus, modified vaccinia virus Ankara and *Listeria* (19-22). Therefore, in addition to its immunosuppressive properties, sirolimus has antifungal, antiviral and antineoplastic properties.

Sirolimus-based regimens for the prophylaxis of aGVHD have exhibited promising results over the past decade, including lower rates of aGVHD and treatment-related mortality in patients undergoing allo-HSCT (23-35). These regimens can markedly reduce the relapse rate and improve survival in patients with lymphoma undergoing allo-HSCT with reduced-intensity conditioning regimens (24). The use of sirolimus in the first-line therapy of aGVHD has been previously evaluated, demonstrating similar initial treatment efficacy as prednisone (36). However, the data on its potential role in SR-aGVHD are scarce. The present meta-analysis aimed to systematically review current evidence on the use of mTOR inhibitors for the treatment of SR-aGVHD.

Materials and methods

Inclusion/exclusion criteria. The present meta-analysis was performed on published papers with complete data. The severity of aGVHD was graded according to organ staging of aGVHD and overall aGVHD grading according to Keystone Consensus 1994 criteria (5). The inclusion criteria were as follows: i) Studies on patients with SR-aGVHD with no age, ethnicity or sex restrictions; ii) patients diagnosed with SR-aGVHD after allo-HSCT; iii) patients who received mTOR inhibitor-based therapy as treatment for SR-aGVHD; and iv) studies published in English. Reviews, case reports, animal models, cell lines, letters, duplicate publications and conference or meeting abstracts without available data were excluded. Each study was independently reviewed for inclusion and exclusion criteria by two investigators. Discrepancies were resolved by consensus.

Literature search. The literature search was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (37). Relevant publications from December 2001 to September 2024 were searched in the PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Embase (https://www.embase.com/) databases. The key words were 'mTOR inhibitor' or 'sirolimus' or 'rapamycin' or 'everolimus' and 'steroid refractory' or 'steroid resistant' or 'corticosteroid refractory' and 'acute graft vs. host disease' or 'aGVHD'. The search results were limited to humans and studies published in English. The search results included the overall response rate (ORR), complete response rate (CRR), chronic GVHD (cGVHD) incidence and overall survival (OS) rate in patients with SR-aGVHD treated with mTOR inhibitors.

Data extraction and quality assessment. All studies reported response rates. The main outcomes observed after treatment with mTOR inhibitors were ORR, CRR, cGVHD, OS and infection-related complications. Furthermore, the efficacy of this treatment was evaluated 1 month after treatment. Different studies had different time points. Therefore, the time frame for the evaluation of response rate at 1 month after treatment was prolonged; that is, the earliest studies evaluated it at 2 weeks, whereas the latest studies evaluated it at 6 weeks after treatment with mTOR inhibitors. All these studies were included in the present analysis. Incomplete data were recorded as 'not available'. A formal quality assessment was conducted using the Risk Of Bias In Non-randomized Studies of Interventions tool (https://github.com/mcguinlu/robvis) (Fig. 1).

Statistical analysis. All statistical calculations were conducted with the 'meta' package version 4.18-0 of R software (version 4.0.5; R Development Core Team). The present study assessed statistical heterogeneity between studies using the I^2 statistic and Cochran's Q-test. A random-effects model was used and a P-value <0.10 (38).

Results

In total, 5 studies on treatment with mTOR inhibitors (Fig. 2) for SR-aGVHD were identified from the database search results (39-43), which included 134 patients (Table I). mTOR inhibitors (sirolimus, n=99; everolimus, n=35) were only available as an oral formula; they were used as second-line therapy for 87 patients with SR-aGVHD after initiating steroid treatment at a dose of 2 mg/kg/day (n=53) (40,41,43) and 1 mg/kg/day (n=34) (42). However, 29 patients with SR-aGVHD received an mTOR inhibitor as an alternative therapy after the failure of second-line treatment (41,43). The second-line immunosuppressant therapies before administering mTOR inhibitors included anti-CD25 monoclonal antibodies (daclizumab or inolimomab; n=18) and others [anti-thymocyte globulin (ATG), n=5; anti-IL2, n=3; anti-TNF, n=2; and ECP, n=1] (41,43). Previously introduced immunosuppressive agents such as cyclosporine A and tacrolimus were maintained after administering mTOR inhibitors (41-43).

Administration of mTOR inhibitors. The present study data reported heterogeneity in the doses of mTOR inhibitors used by patients. A loading dose of sirolimus was administered in 4 studies (40-43). The loading dose was associated with weight and the combination of antifungal agents, such as voriconazole, itraconazole and posaconazole, which was followed by a maintenance dose adjusted to serum trough levels in the therapeutic range. mTOR inhibitors were indefinitely continued at the discretion of the treating physician (41-43) rather than for a fixed duration, as reported in the study by Benito *et al* (40). mTOR inhibitors were discontinued due to their adverse effects, unresponsiveness or progression of aGVHD (40).

Patients were divided into the following four groups based on the blood trough levels (target concentration) and the median

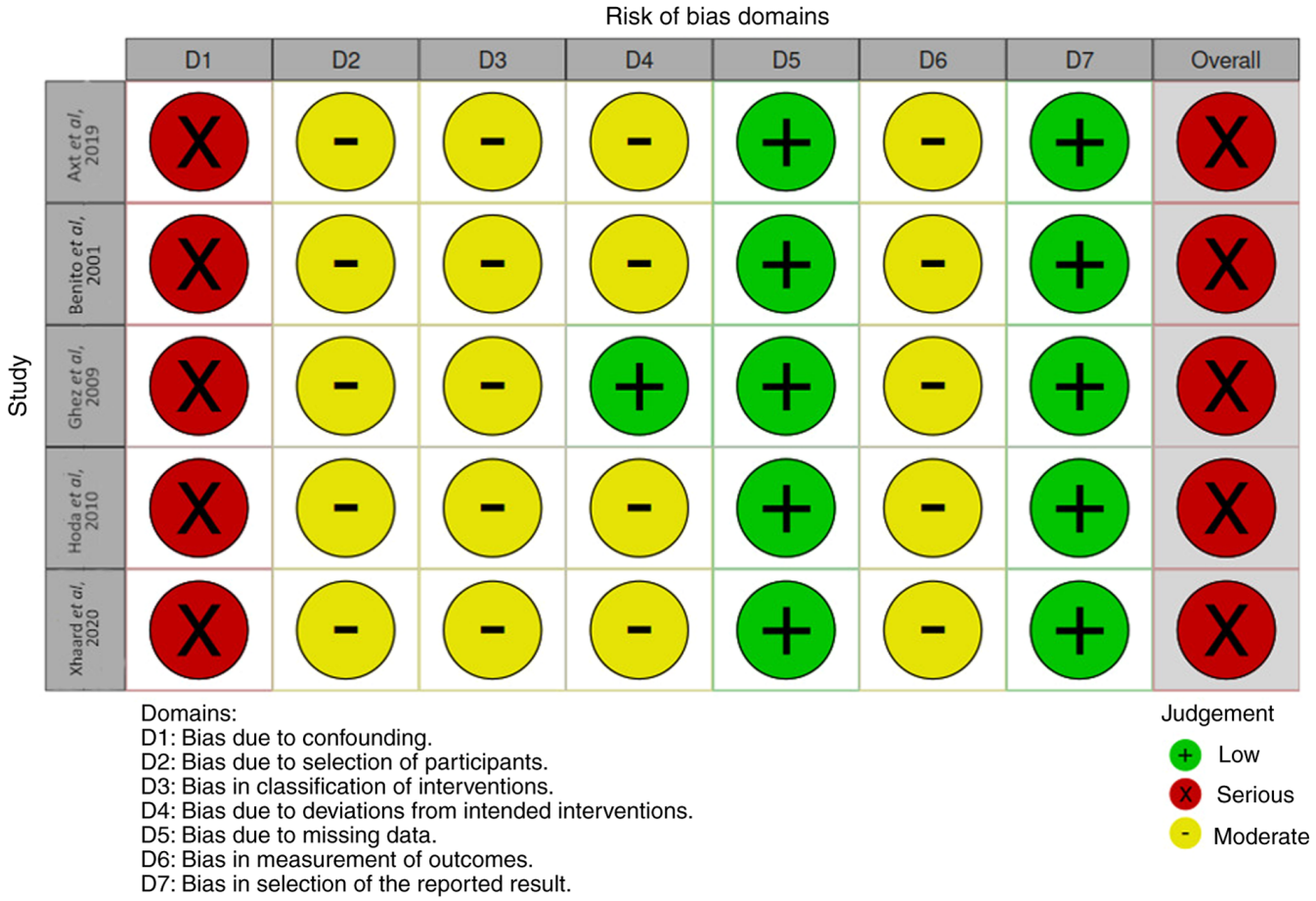

Figure 1. Risk of bias assessment of the included studies.

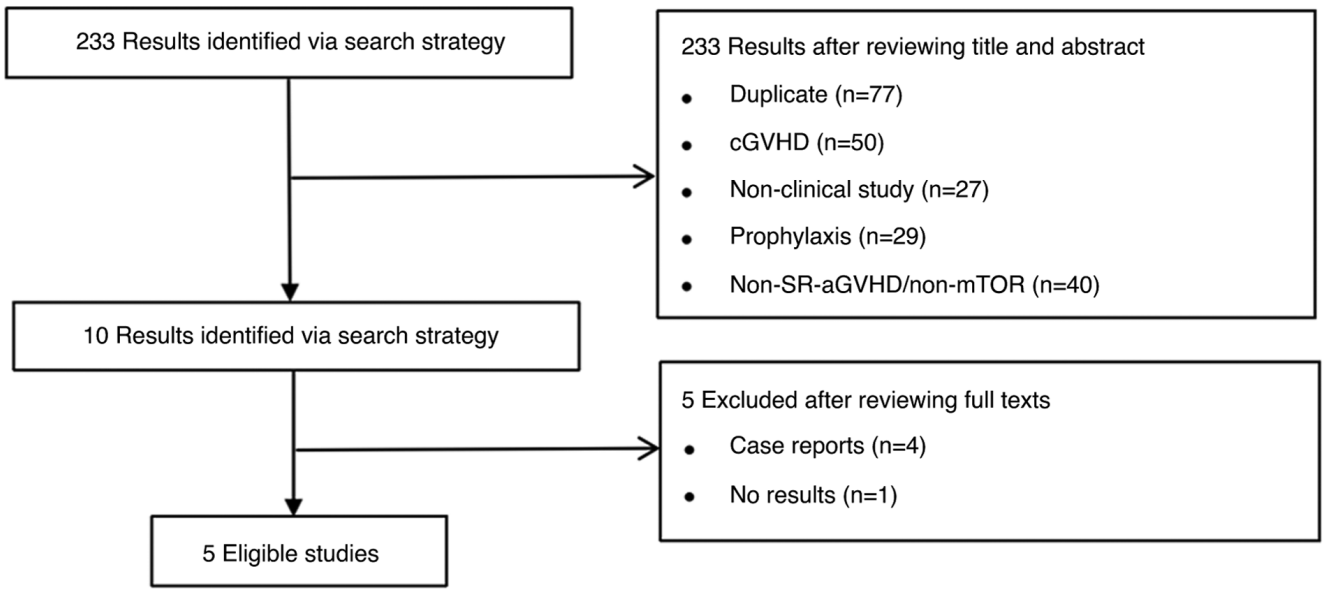

Figure 2. Flowchart for study selection. GVHD, graft-vs.-host disease; cGVHD, chronic GVHD; SR-aGVHD, steroid-refractory acute GVHD; mTOR, mammalian target of rapamycin.

duration of treatment with mTOR inhibitors: i) High target concentration (17-31.8 ng/ml) during a short course (14 days; range, 1-14 days) in the study by Benito *et al* (40); ii) appropriate target concentration (7-13 ng/ml) during the long course (61 days; range, 27-150 days) in the study by Ghez *et al* (41); iii) a wide range of target concentration (4-12 ng/ml) in the study by Hoda *et al* (42); and iv) and low target concentration (4-8 ng/ml) in 35 days with a wide range of 5-971 days

Table I. Main characteristics of five studies on mTOR inhibitors-based treatment.

First author, year	Axt *et al*, 2019	Benito *et al*, 2001	Ghez *et al*, 2009	Hoda *et al*, 2010	Xhaard *et al*, 2019
(Refs.)	(39)	(40)	(41)	(42)	(43)
Study design	Retrospective	Prospective	Retrospective	Retrospective	Prospective
Number of patients	15	21	22	34	42
Second-line treatment, n	15	21	1	34	31
Third-line treatment, n	0	0	18	0	11
Beyond third-line treatment, n	0	0	3	0	0
mTOR inhibitor administration					
Sirolimus, n	15	21	22	34	7
Everolimus, n	0	0	0	0	35
Loading dose of sirolimus	NA	15 mg/m^2, 1 day	2-4 mg/m^2/day, days 1-5	6 mg/day (range, 3-8 days)	4-8 mg/day, days 1-2
Loading dose of everolimus	NA	NA	NA	NA	1-2 mg, bid
Maintenance dose of sirolimus	NA	4-5 mg/m^2/day	2-4 mg/day	1-2 mg/day	NA
Blood trough levels (range), ng/ml	NA	17.0-31.8	7-13	4-12	4-8
Median treatment length (range), days	NA	NA (1-14)	61 (27-150)	NA	NA
Second-line treatment (range), days	NA	NA (1-14)	NA	NA	35 (5-971)
Third-line treatment (range), days	NA	NA	NA	NA	19 (7-238)
Initiation time after aGVHD (range), days	NA	37 (19-78)	34 (7-177)	80 (9-255)	12 (4-144)
HLA matching, n					
MRD	NA	2	8	12	NA
mMRD	NA	4	0	0	NA
MUD	NA	7	5	22	NA
mMUD	NA	8	9	0	NA
aGVHD grade, n					
I	0	0	0	2	0
II	0	0	6	15	7
III	15	10	12	8	24
IV	0	11	4	8	NA
Organ involvement of aGVHD, n (%)					
Skin	NA	16 (76.2)	22 (100.0)	13 (38.2)	12 (28.6)
Gut	NA	10 (47.6)	7 (31.8)	27 (79.4)	27 (64.3)
Liver	NA	13 (61.9)	2 (8.0)	4 (11.8)	5 (11.9)
Response, n (%)					
ORR	8 (53.3)	12 (57.0)	20 (90.9)	26 (76.0)	NA
Second-line treatment	NA	NA	20 (90.9)	NA	15 (48.5)
Third-line treatment	NA	NA	NA	NA	3 (27.0)
Refractory to IL-2RAs	NA	NA	17 (94.0)	NA	NA
Refractory to others	NA	NA	NA	NA	3 (27.0)
CRR	NA	NA	19 (86.0)	NA	NA
Second-line treatment	NA	5 (28.0)	NA	15 (44.0)	13 (42.0)
Third-line treatment	NA	NA	NA	NA	1 (9.0)
Refractory to IL-2RAs	NA	NA	14 (78.0)	NA	NA
Refractory to others	NA	NA	NA	NA	1 (9.0)
ORR at 1 month	NA	12 (57.0)	NA	NA	NA
CRR at 1 month	NA	5 (23.8)	19 (86.0)	NA	NA
Median time to CR, days (range)	NA	NA	8 (5-15)	NA	NA
Second-line treatment, days (range)	NA	NA	NA	NA	16.5 (10-51)
Outcomes					
1-year overall survival rate, %	NA	33	NA	44	42
3-year overall survival rate, %	NA	NA	41	NA	32

Table I. Continued.

First author, year	Axt *et al*, 2019	Benito *et al*, 2001	Ghez *et al*, 2009	Hoda *et al*, 2010	Xhaard *et al*, 2019
Median follow-up, months	NA	8.7	6.8	7.0	12.0
Adverse events, n (%)					
Cytopenias	NA	5 (23.8)	14 (65.0)	NA	NA
Interruption due to cytopenias	NA	2 (9.5)	0 (0.0)	NA	3 (10.0)
TA-TMA	NA	10 (47.6)	10 (45.0)	7 (21.0)	NA
Interruption due to TA-TMA	NA	2 (9.5)	5 (22.5)	NA	2 (7.0)
Hyperlipidemia	NA	14 (66.7)	4 (18.0)	15 (44.1)	NA
Infection, n (%)					
Bacteria	NA	8 (38.1)	13 (60.0)	NA	28 (66.7)
Viruses	NA	3 (14.3)	NA	NA	24 (57.1)
Cytomegalovirus reactivation	NA	NA	9 (40.9)	NA	14 (33.3)
Fungi	NA	3 (14.3)	5 (22.7)	NA	11 (26.2)
cGVHD incidence, %	NA	90.0	52.9	44.0	NA

mTOR, mammalian target of rapamycin; GVHD, graft-vs.-host disease; aGVHD, acute GVHD; cGVHD, chronic GVHD; CRR, complete response rate; IL-2RAs, IL-2 receptor antagonists; NA, not available; ORR, overall response rate; TA-TMA, transplant-associated thrombotic microangiopathy; bid, twice a day; MRD, matched related donor; mMRD, mismatched related donor; MUD, matched unrelated donor; mMUD, mismatched unrelated donor.

in second-line therapy and 19 days (range, 7-238) in third-line therapy (43) (Table I).

Response after treatment with mTOR inhibitors. The ORR was 65% (95% CI, 44-81%; Fig. 3A) in patients with SR-aGVHD treated with mTOR inhibitors. Only 1 study was included in the ORR analysis at 1 month after treatment (40). The ORR at 1 month was 57% (95% CI, 34-78%; Fig. 3B). A single study was excluded from the CRR analysis after treatment, due to insufficient data (39). The CRR was 46% (95% CI, 22-72%; Fig. 3C). A total of 2 studies were included in the CRR analysis at 1 month after treatment (40,41). The CRR at 1 month was 58% (95% CI, 13-93%; Fig. 3D).

Overall, 2 studies in the meta-analysis included 29 patients using mTOR inhibitors as a third-line treatment for SR-aGVHD (41,43). Ghez *et al* (41) examined the optimal response and reported a 78% CRR within 8 days (range, 5-15 days), which was markedly higher compared with the 9% reported in the study by Xhaard *et al* (43). It was also higher compared with that for mTOR inhibitors used as second-line therapy (27% of CRR within 16.5 days; range, 10-51 days) in the study reported by Xhaard *et al* (43). Response was observed within at least 9 days in the study by Benito *et al* (40). In the study by Hoda *et al* (42), the median organ-specific time-to-best responses were as follows: i) Skin within 4 weeks (range, 1-8 weeks); ii) gastrointestinal organs within 4.5 weeks (range, 1-6 weeks); and iii) liver within 3 weeks (range, 2-4 weeks).

Impact factors for response to mTOR inhibitors. The highest ORR (94%) and CRR (78%) were observed in refractory patients with SR-aGVHD receiving mTOR inhibitors as third-line treatment combined with IL-2RAs (41). The response rate was markedly higher compared with that of the other second-line treatments, including ATG, anti-IL2, anti-TNF and ECP (27% of ORR and 9% of CRR) (43). The response was also higher compared with that for mTOR inhibitors alone (15-76% ORR and 28-44% CRR) as the second-line treatment against SR-aGVHD (9,40,42,43).

No notable difference in CRR (42 vs. 44%) was observed between patients with SR-aGVHD treated with mTOR inhibitors as second-line salvage therapy refractory to 2 mg/kg of glucocorticoids (43) and those with treatment failure using 1 mg/kg of steroids (42). CRR did not vary markedly according to the organ involvement and overall aGVHD grade at the time of salvage with mTOR inhibitors (42,43). Regarding the initiation time of mTOR inhibitors after aGVHD, the CRR was similar (44 vs. 42%) between the prolonged (42) and timely initiation (43) [80 days (range, 9-255) vs. 12 days (range, 4-144 days)]. The CRR was markedly different (28 vs. 86%) even for a similar initiation time [37 days (range, 19-78 days) vs. 34 days (range, 7-177 days)] (40,41) (Table I), suggesting no impact of initiation time on the complete response to mTOR inhibitors.

Pharmacokinetic influence on response to mTOR inhibitors. The best response, including the highest CRR of 78% and the shortest time to CR within 8 days (range, 5-15 days), was observed in the study by Ghez *et al* (41), indicating the possible synergistic effect of anti-CD25 antibodies and rapamycin at an appropriate target concentration (7-13 ng/ml). The worst response of mTOR inhibitors in the study by Xhaard *et al* (43) might be attributed to lower serum trough levels (target concentration, 4-8 ng/ml). The CRR (42%) in the low-dose (target concentration, 4-8 ng/ml) group (43) was similar to that in the 4-12 ng/ml blood trough level group (44%) after administering mTOR inhibitors as second-line therapy (42). Definitive results

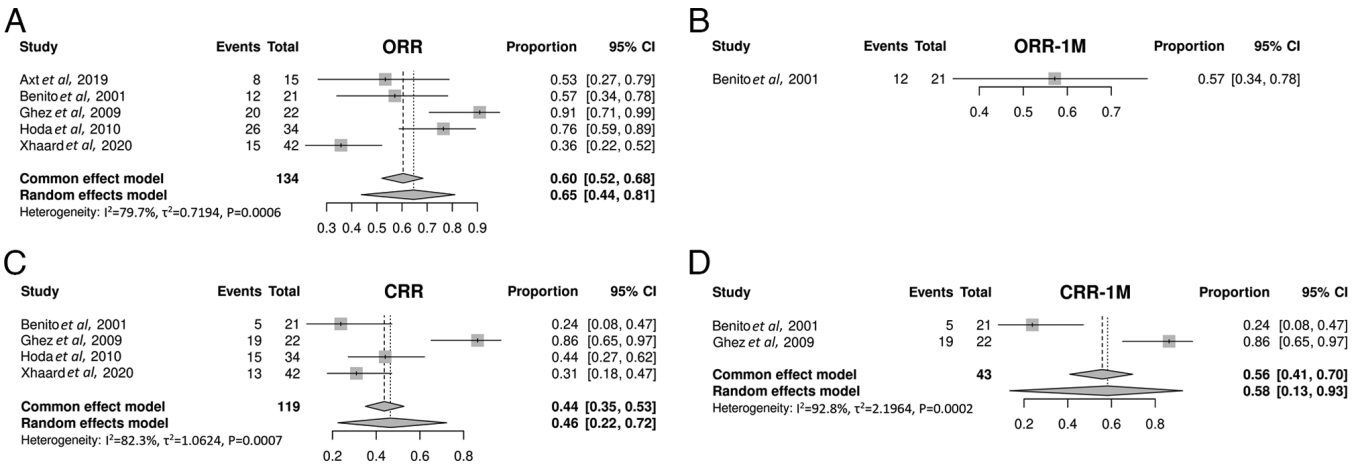

Figure 3. Response to mTOR inhibitor treatment in patients with steroid-refractory acute graft-vs.-host disease. Forest plots of (A and B) ORR and (C and D) CRR recorded (A and C) at any time and (B and D) at 1 month post-treatment. ORR, overall response rate; CRR, complete response rate; 1M, 1 month; mTOR, mammalian target of rapamycin.

of other studies also confirmed that the therapeutic levels were achieved above the target concentration of 4 ng/ml (42,43). Considering the worst response at the lower serum trough levels (target concentration, 4-8 ng/ml) (43), 7-13 ng/ml should be recommended as an appropriate serum trough level in the future. Dose escalation was usually permitted based on drug toxicity and response, with 21 patients achieving high blood trough levels (target concentration, 17-31.8 ng/ml). However, in the study by Benito *et al* (40), rapamycin had to be discontinued early due to its inefficacy and toxicity; this reflected that higher-dose mTOR inhibitors did not enhance the curative effect but had more toxicity. The CR to mTOR inhibitors (41-43) was markedly improved in the long-course group (no fixed duration) compared with that in the short-course group (14 days) (40). The time needed to achieve CR after administering mTOR inhibitors was at least 1-2 weeks, with an extensive range and a maximum time of 8 weeks (40-42). Therefore, prolonged therapy was more likely to be effective. The dosing should be considered while analyzing the response and toxicity in the future.

Other impact factors for response to mTOR inhibitors. The present study demonstrated that all baseline characteristics including organ damage, grading of mTOR inhibitor rescue treatment, time to start mTOR inhibitors after steroid treatment or initial dose of glucocorticoids, did not influence the achievement of CR after mTOR inhibitor treatment, except for the prior and combined therapy, serum trough level and treatment course (Fig. 3). This was similar to the report from a single center (42). mTOR inhibitors (oral only) displayed notable intestinal absorption even in patients with gastrointestinal aGVHD (40-42).

Adverse events after treatment with mTOR inhibitors

Transplant-associated thrombotic microangiopathy (TA-TMA). TA-TMA occurred in 36% (95% CI, 22-52%) of patients with SR-aGVHD. The incidence of TA-TMA varied, which was likely due to the lack of a consensus definition for TA-TMA (44). Treatment with mTOR inhibitors was interrupted in 10% (95% CI, 5-22%) of the patients due to the persistence of TA-TMA. Furthermore, the median time to TA-TMA onset after rapamycin use was 24 days, with a range of 12-60 days. TA-TMA also occurred in certain patients at the start of rapamycin treatment (41). Therapy was successfully managed by reducing the dose or discontinuing cyclosporine A or tacrolimus. TA-TMA was resolved to a certain extent after interrupting treatment with mTOR inhibitors (Fig. 4A and B) (41-43).

Myelosuppression. The myelotoxic effect of rapamycin was observed in 43% (95% CI, 18-72%) of patients. Also, 6% (95% CI, 2-13%) of patients discontinued mTOR inhibitor treatment due to cytopenia. The median decrease in absolute neutrophil count was 57% and the median decrease in platelet count was 61% within 30 days of sirolimus treatment initiation (42). The blood cell counts were restored upon discontinuing treatment with mTOR inhibitors (Fig. 4C and D) (40).

Hyperlipidemia. Hyperlipidemia attributable to rapamycin was also a frequent complication (40-42), observed in 42% of patients (95% CI, 21-66%). No treatment interruption was required (Fig. 4E).

Infection. A total of 3 studies (40,41,43) included in the meta-analysis investigated the incidence of infections after mTOR inhibitor treatment. The incidence of bacterial, viral and fungal infections was 57% (95% CI, 43-70%; Fig. 5A), 34% (95% CI, 10-69%; Fig. 5B) and 22% (95% CI, 15-32%; Fig. 5C), respectively. The cytomegalovirus (CMV) reactivation rate was 36% (95% CI, 25-48%; Fig. 5D).

cGVHD and survival. A total of 3 studies on mTOR inhibitors were included to explore cGVHD (40-42). The cGVHD incidence after treatment with mTOR inhibitors was 88% (95% CI, 32-99%; Fig. 6A). Overall, 4 studies on mTOR inhibitors were enrolled to examine survival (40-43). A single study (39) was excluded as it did not report OS data. Of these, 3 studies on mTOR inhibitors were included in the 1-year OS analysis (40,42,43). The 1-year OS rate for treatment with mTOR inhibitors was 36% (95% CI, 27-46%; Fig. 6B). Only 2 studies on mTOR inhibitors were included in the 3-year OS analysis (41,43). The 3-year OS rate for mTOR inhibitors was 29% (95% CI, 19-41; Fig. 6C).

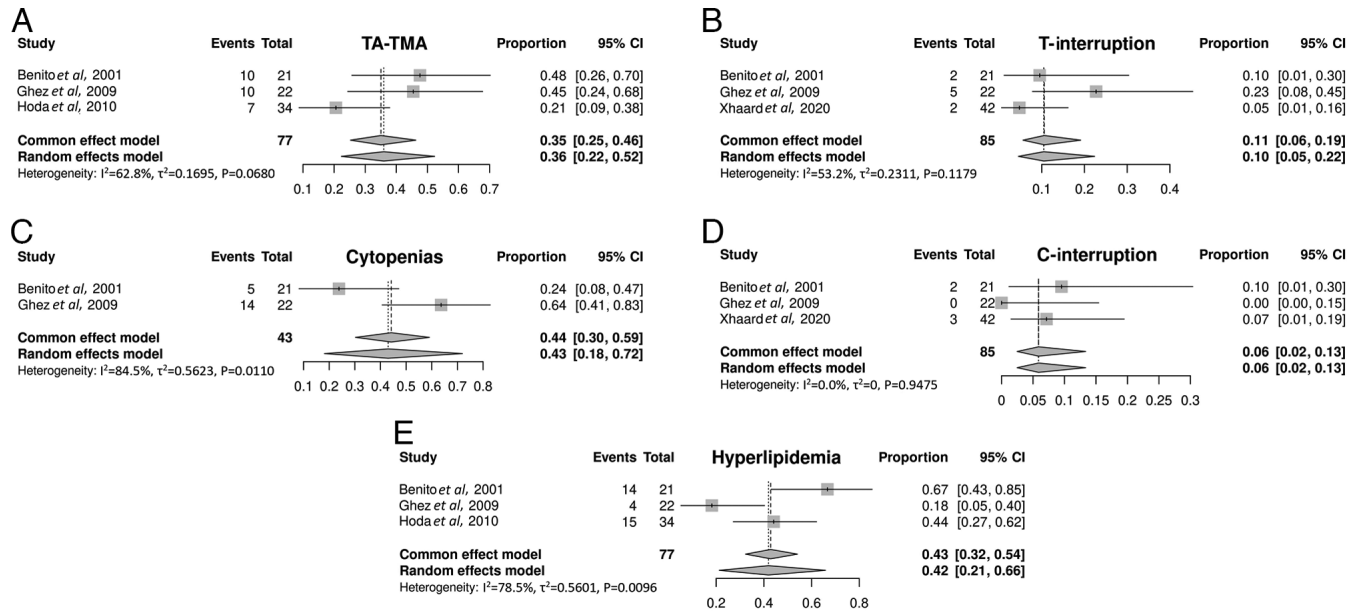

Figure 4. Adverse events following mTOR inhibitor therapy in patients with steroid-refractory acute graft-vs.-host disease. Forest plots of incidence of (A) TA-TMA, (B) interruption due to TA-TMA, (C) cytopenias, (D) interruption due to cytopenias and (E) hyperlipidemia. mTOR, mammalian target of rapamycin; TA-TMA, transplant-associated thrombotic microangiopathy; T, TA-TMA; C, cytopenias.

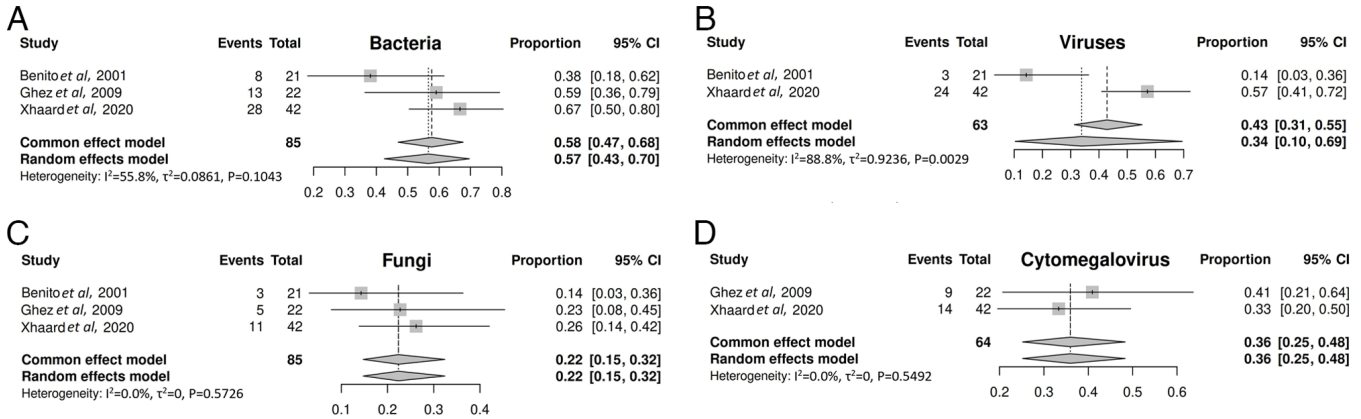

Figure 5. Incidence of infections after treatment with mTOR inhibitors in patients with steroid-refractory acute graft-vs.-host disease. Forest plots of incidence of (A) bacterial, (B) viral and (C) fungal infection and (D) CMV reactivation rate. mTOR, mammalian target of rapamycin; CMV, cytomegalovirus.

Discussion

The present systematic review summarized the current literature, including 5 studies on the use of mTOR inhibitors for the treatment of patients with SR-aGVHD. Furthermore, the present study was novel in demonstrating the feasibility of using mTOR inhibitors for SR-aGVHD therapy. The present study data evaluated 134 patients and the outcomes revealed that mTOR inhibitors used as salvage therapy exerted notable activity against SR-aGVHD, with a marked complete remission rate of 46%, a partial remission rate of 18% and 1- to 3-year OS from 36 to 29% in patients with SR-aGVHD. mTOR inhibitors induced markedly higher CRR compared with the CD52 antibody alemtuzumab (27.8%; 5/18) (45), tocilizumab (an IL-6 receptor antagonist; 33.3%; 2/6) (46), α1-antitrypsin (35%; 14/40) (47), etanercept (a recombinant human soluble TNF-α receptor fusion protein; 30.7%; 4/13) (48) and begelomab (anti-CD26 monoclonal antibody; 10.7%; 3/28) (49), compared with the preliminary results from a small series of patients with SR-aGVHD. The findings on mTOR inhibitor treatment were compared with the present meta-analysis results evaluating other treatments in patients with SR-aGVHD, achieving a lower CRR with ruxolitinib (56%) (11) and basiliximab (55%), which was identified as the most effective among the IL-2RAs for SR-aGVHD (13). mTOR inhibitors were not notably enhanced compared with ECP (50) and MSCs (50), with pooled CRRs for SR-aGVHD of 69.0 and 73.1% in the meta-analysis, respectively. Non-conventional treatments such as ECP and MSCs had the potential for SR-aGVHD therapy but were less feasible due to higher technical requirements and costs.

The highest CRR (78%) was achieved in patients receiving mTOR inhibitors as salvage therapy for SR-aGVHD and undergoing allo-HSCT. This was probably due to both the potential

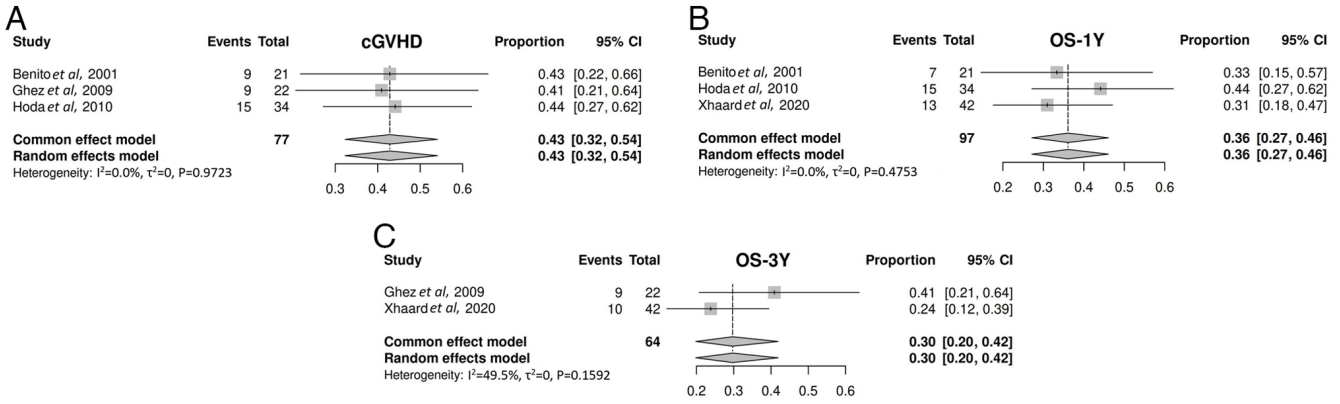

Figure 6. Forest plots of incidence of (A) cGVHD, (B) 1-year OS rate and (C) 3-year OS rate after treatment with mTOR inhibitors in patients with SR-aGVHD. mTOR, mammalian target of rapamycin; OS, overall survival; GVHD, graft-vs.-host disease; SR-aGVHD, steroid-refractory aGVHD; cGVHD, chronic GVHD; Y, year.

synergistic effect of mTOR inhibitors plus IL-2RAs (16) and an appropriate rapamycin dose (serum trough levels, 7-13 ng/ml). Due to the limited sample size, the present study conclusions should be drawn with caution although the outcome was promising. In the future, a prospective RCT can be designed for verification and *in vitro* experiments can be conducted to explore the possible synergistic effects of the two drugs used in combination. However, mTOR inhibitor treatment was interrupted with a high incidence of TA-TMA (22.5%) upon cyclosporine A prophylaxis due to the cumulative toxicity of mTOR inhibitors and calcineurin inhibitors (41). Although sirolimus also exhibited synergistic effects with tacrolimus, the lower serum trough levels (4-8 ng/ml) were associated with suboptimal efficacy (42% of CRR) (35). However, this regimen demonstrated a lower rate of mTOR inhibitor discontinuation (7%) (43).

The preliminary results of the study by Benito *et al* (40) indicated that sirolimus was extremely toxic at high serum trough levels (17-31.8 ng/ml), leading to frequent TA-TMA (47.6%) and cytopenia (23.8%). Besides the appropriate dose, the present study data emphasized the importance of prolonged treatment with mTOR inhibitors, as the T-cell response was also associated with the treatment duration (19). Switching CNI to MMF should be managed first for the long-term response of patients to rapamycin in the future (41). A total of 6% of the patients with SR-aGVHD after treatment with mTOR inhibitors developed severe cytopenia leading to treatment interruption (Fig. 4D). The hematological toxicity might limit the use of the combination with ruxolitinib and methotrexate.

The non-relapse mortality of SR-aGVHD is high (60-85%) (17), with an estimated 2-year survival rate of 17% (51) and a 4-year survival rate of 15% (52). Compared with the aforementioned findings, mTOR inhibitor salvage therapy depended on notably increased survival in SR-aGVHD, with 1- to 3-year OS ranging from 36 to 29%. Infection-related mortality (46%) was high in patients with SR-aGVHD (52). The incidence of bacterial infections (56%) and CMV reactivation (35%) after mTOR inhibitor-based therapy for SR-aGVHD was significantly lower compared with that after anti-cytokine treatment for SR-aGVHD (74 and 65%, respectively) (52). This might be because rapamycin enhanced the pathogen-specific $CD8^+$ T-cell response (21) and improved CMV-specific T-cell function (53) and the outcome of CMV complications (54).

Ruxolitinib offers a novel immunotherapeutic option for TA-TMA complicated by GVHD but is associated with cytopenia and CMV reactivation (55.6 and 33.3%, respectively) (55). Although ruxolitinib can also preserve GVL/graft-vs.-tumor (GVT) function (56), it is less favorable compared with sirolimus, which has antifungal, antiviral and antineoplastic properties. The incidence of infectious events can reach up to 73% using other immunosuppressive agents (particularly MMF), especially in the context of impaired GVL/GVT function, despite no increase in the incidence of TA-TMA or cytopenia (57).

In summary, the present study results confirmed that mTOR inhibitors displayed potent activity against SR-aGVHD and may be used alone or in combination with other targeted therapies (such as anti-CD25 monoclonal antibodies and ATG), despite biases in non-controlled studies, heterogeneity in the quality of studies and variability among patients receiving immunosuppressive agents. However, the generalizability of the present meta-analysis was limited by multiple factors, primarily heterogeneity stemming from variations in study designs, conduct and analysis processes, thereby compromising the accuracy of the present study findings. Furthermore, several biases might have been introduced, including disparities in medical resources across different time periods or regions. Furthermore, most studies included in the meta-analysis were retrospective with relatively small sample sizes. Therefore, the efficacy and safety of mTOR inhibitors in patients with SR-aGVHD require validation in further prospective RCTs comparing these inhibitors with other treatments. Therapeutic strategies of combined treatment regimens with a balance between efficacy and side effects are key to maximizing therapeutic efficacy while minimizing drug side effects. Future studies should also focus on the impact of rapamycin and other immunosuppressive agents on T-cell response.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YG and ZL confirm the authenticity of all the raw data. YG curated the data, provided project administration, validated the data and wrote the original draft. ZL devised the methodology, provided project administration and wrote the original draft. YiqZ searched the literature data and assisted with data analysis. ZP, JS, YinZ and SC contributed to data collection and analysis. ZG curated the data, conducted the investigation, reviewed the manuscript and acquired funding for the present study. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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