The Role of mTOR Inhibitors in Hematologic Disease: From Bench to Bedside

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The mTOR pathway plays a central role in many cellular processes, such as cellular growth, protein synthesis, glucose, and lipid metabolism. Aberrant regulation of mTOR is a hallmark of many cancers, including hematological malignancies. mTOR inhibitors, such as Rapamycin and Rapamycin analogs (Rapalogs), have become a promising class of agents to treat malignant blood diseases—either alone or in combination with other treatment regimens. This review highlights experimental evidence underlying the molecular mechanisms of mTOR inhibitors and summarizes their evolving role in the treatment of hematologic disease, including leukemia, lymphoma, myeloma, immune hemocytopenia, and graft-versus-host disease (GVHD). Based on data presented in this review, we believe that mTOR inhibitors are becoming a trusted therapeutic in the clinical hematologist's toolbelt and should be considered more routinely in combination therapy for the management of hematologic disease.

Keywords: rapamycin, Rapalogs, mTOR pathway, malignant and benign hemopathies, graft-versus-host disease

The mammalian target of rapamycin (mTOR) is a downstream target of multiple signaling pathways involved in biological activities, including the familiar PI3K/Akt/mTOR signaling pathway, which plays an important role in cell growth, differentiation, metastasis and survival, and has become an important target of cancer treatment. Rapamycin (Rapa), the first mTOR inhibitor, was initially used as an immunosuppressive drug in the field of solid organ transplantation (1). In-depth studies of rapamycin and its analogs (Rapalogs), and of the mTOR signaling pathway, have led to the understanding that Rapalogs can not only induce tumor cell apoptosis, cell cycle arrest and signal transduction inhibition, but that they also affect gene transcription and epigenetic regulation. The mTOR signaling pathway is critical in normal myeloid and lymphoid development and function. Moreover, hyperactivation of mTOR is a hallmark of many hematological diseases and provides a strong rationale for the use of mTOR inhibitors (mTORi), such as Rapalogs. The primary focus of this review is to 1) review the literature on the regulation and outcome of the mTOR signaling pathways from a basic research perspective, and 2) summarize the use of mTOR inhibitors to target aberrant mTOR activation and signaling in hematological diseases, such as acute leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma,

multiple myeloma, GVHD, and Waldenström macroglobulinemia. We also provide our perspective on the evolution of this field, as the application and efficacy of mTOR inhibitors becomes more widely recognized in the field of blood disorders.

mTOR AND mTOR INHIBITORS

The mammalian target of rapamycin (mTOR) is a large (289 kDa), conserved serine protein kinase, belonging to the PI3K kinase family. mTOR can integrate the stimulatory signals from nutrients, growth factors and environmental pressure, and regulate cell growth, proliferation, differentiation, and cell cycle progression. In the immune system, mTOR can transmit and integrate signals from the immune microenvironment, and is considered a key regulator of immune metabolism and function (2). At present, it is believed that there are two dominant, upstream signaling pathways for mTOR, one is the PI3K/Akt/ mTOR pathway, which interacts with mTOR to positively regulate, and the other is the LKB1/AMPK/mTOR pathway, which has primarily been reported as a negative regulator of mTOR (3, 4).

mTOR exerts its activity *via* the formation of two catalytically distinct complexes, mTORC1 and mTORC2 (5). mTORC1 complex is composed of mLST8, PRAS40, Deptor, and Raptor.

mTORC2 complex is composed of Rictor, mSIN1, Protor, mLST8, and Deptor. In general, mTORC1 plays a more dominant role in regulating cellular anabolic processes, while the effect of the mTORC2 signaling pathway is primarily through Akt Studies have shown that mTORC1 signaling pathway plays an important role in the metabolism of glucose, lipids, nucleotides, and proteins as well as in the regulation of mitochondrial biogenesis (6). The downstream signaling molecules of mTORC1 are primarily P70S6K (p70 ribosomal S6 kinase) and 4E-BP1 (eukaryotic transcription initiation factor binding protein), by which protein translation and synthesis are increased (**Figure 1**). On the other hand, mTORC2 is primarily involved in the insulin signaling pathway to regulate glucose and lipid metabolism (7). Importantly, when mTORC1 is completely inhibited by mTOR inhibitors, mTORC2 signaling is preserved (8).

Several pharmacological agents have been developed which inhibit mTOR (mTOR inhibitors; referred to broadly here as mTORi), including Rapamycin and its analogs. Rapamycin, clinically named sirolimus (SRL), was a macrolide antibiotic discovered in the 1970s (9). SRL has many functional properties, including inhibition of yeast growth, anti-cancer, anti-aging and anti-atherosclerotic effects as well as immune regulation (10–12). In the context of immune regulation, SRL can inhibit T cell activation and proliferation, and restrain B cell activation and
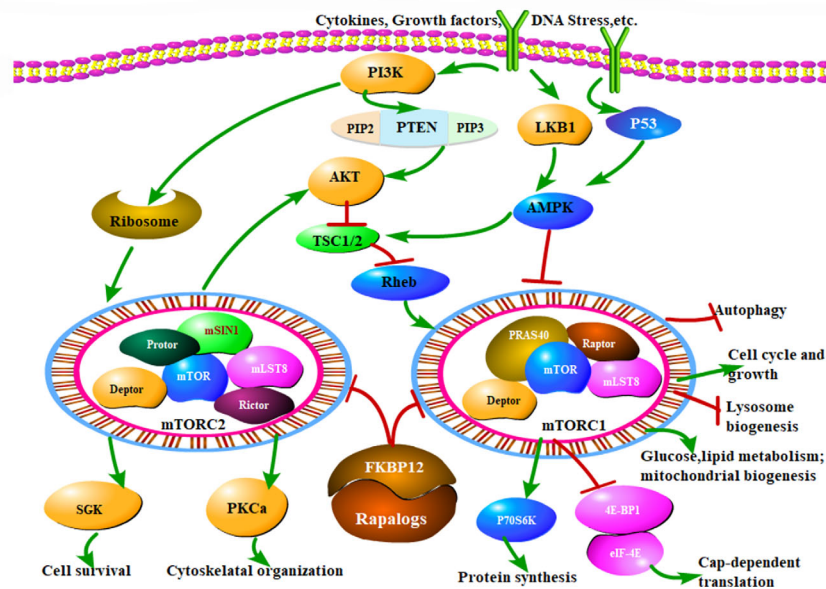

FIGURE 1 | The mTOR signaling pathway and Rapalog therapeutic mechanisms. mTOR modulates a variety of cellular activities through mTORC1 and mTORC2. MTOR can respond to extracellular stimuli, such as cytokines, growth factors and DNA stress, which mainly regulate cell growth, cell cycle, and other physiological activities through PI3K/Akt/mTOR pathway. Normally, TSC1/TSC2 forms a dimer complex, which is an inhibitor of small GTPase Rheb, and Rheb is a necessary stimulating protein for mTOR activation. Hence the mTORC1 activity is critically inhibited by TSC1/TSC2 complex. When Akt is activated, it can phosphorylate ser939 and thr1462 of TSC2 and inhibit the formation of TSC1/TSC2 complex, thus relieving the inhibition of Rheb, then activated Rheb sensitized mTOR function. Additionally, PI3K activates the ribosome to increase mTORC2 reaction. In a word, PI3K promotes the mTOR activates. Another important pathway of mTOR is regulated by AMPK. Firstly, AMPK directly phosphorylates Raptor to inhibit mTORC1 activity. Raptor is the downstream molecule of AMPK, and TSC2 is not involved in this process. Secondly, AMPK can also activate TSC1/TSC2 complex and inhibit mTORC1 activity as described above. Therefore, unlike the PI3K, AMPK restrains the mTOR function. Rapalogs can combine the FKBP12 and inhibit the kinase activity of mTORC1 and mTORC2, ultimately regulate various biological functions of cells, that is the mechanism of mTOR inhibitors. *Arrows indicate activation and bars represent inhibition.*

antibody production caused by antigens and cytokines (13). In the clinic, SRL is primarily used in the prevention of organ rejection after solid organ transplantation, as well as for the treatment of autoimmune diseases (14). With the development of Rapamycin analogs in recent years, Rapalogs, many studies have reported their therapeutic effects on hematological diseases (15).

To date, the most common Rapalogs are: Everolimus (Eve, RAD-001), Deforolimus (Def, Ridaforolimus), Zotarolimus (Zot), and Temsirolimus (Tem, CCI-779) (**Figure 2**). Everolimus is an mTOR inhibitor developed by Novartis (Switzerland), which is a 40-o-(2-hydroxyethyl) derivative of rapamycin, with the molecular formula $C_{53}H_{83}NO_{14}$, (958 kDa). In March 2009, the drug received accelerated approval from the FDA for the treatment of kidney cancer (16, 17). Deforolimus is a C40 derivative of Rapa, which is a semi-synthetic derivative designed by Computer-aided drug design (CADD) with molecular formula $C_{53}H_{84}NO_{14}P$ (990 kDa) (18). Def is still considered an investigational agent in the management of advanced solid tumors, such as sarcoma (19) and breast cancer (20). Only clinical study reported using Def for the treatment of the hematological malignancy lymphoma, and the overall response rate (ORR) was 50% (21). Zotarolimus is a Rapa derivative developed by Abbott and Medtronic. It is a C40 tetrazole substitute of Rapa, with molecular formula $C_{52}H_{79}N_5O_{12}$ (966 kDa). Compared with Rapa, Zot has a shorter half-life *in vivo*. The mechanism of Zot is similar to Rapa. Zot combines with FKBP12 to form a complex, which bind to mTOR protein kinase to form a trimer, inhibits the activity of mTOR by preventing its phosphorylation, and prevents cell cycle progression from G1 to S phase (22). It was designed for use as a coating in stents with phosphorylcholine as a carrier. Temsirolimus, also named 42-[3-hydroxy-2- (hydroxymethyl)-2-methylpropionate], is a Rapa derivative with molecular formula $C_{56}H_{87}NO_{16}$ (1030 kDa). It is a Rapa soluble esterified derivative officially approved by the US FDA in 2007, which inhibits the translation of several key proteins for cell cycle regulation. These effects lead to cell cycle arrest in the G1 phase, therefore also known as Cell Cycle Inhibitor-779 (CCI-779). In August 2007, FDA approved temsirolimus as a first-line treatment for advanced renal cancer. At present, it is marketed as a treatment of renal cancer, and is being investigated in the clinic for the treatment of breast cancer (23), lymphoma (24), lung cancer (25), and many other tumors (26).

APPLICATIONS OF mTORI IN HEMATOLOGICAL MALIGNANCIES

Acute Leukemia

Acute myeloid leukemia (AML) is a type of cancer of the bone marrow in which myeloid stem cells differentiate and accumulate as abnormal myeloblasts in the bone marrow, impair erythropoiesis/hematopoiesis and eventually migrate throughout the body. Although mTOR activation is frequently observed in AML blasts, the precise function and the downstream targets of mTOR in this disease are poorly understood. Feng et al. revealed that PFKFB3 was a novel downstream substrate of mTOR signaling pathway, and found that up-regulation of PFKFB3 *via* aberrant mTOR signaling was essential for AML cell survival. Moreover, PFKFB3 inhibitor PFK15 and rapamycin synergistically ablated AML cell proliferation (THP1 and OCI-AML3 cells) (27). Xu et al. found that PI3K/Akt/mTOR pathway was up-regulated in most AML cells. Rapa combined with etoposide, a chemotherapy, enhanced the effect of etoposide on primary AML cells; moreover, leukemia progenitor cells were more sensitive to Rapa than normal progenitor cells (28, 29). Brown et al. have found that rapamycin inhibited growth of B-precursor
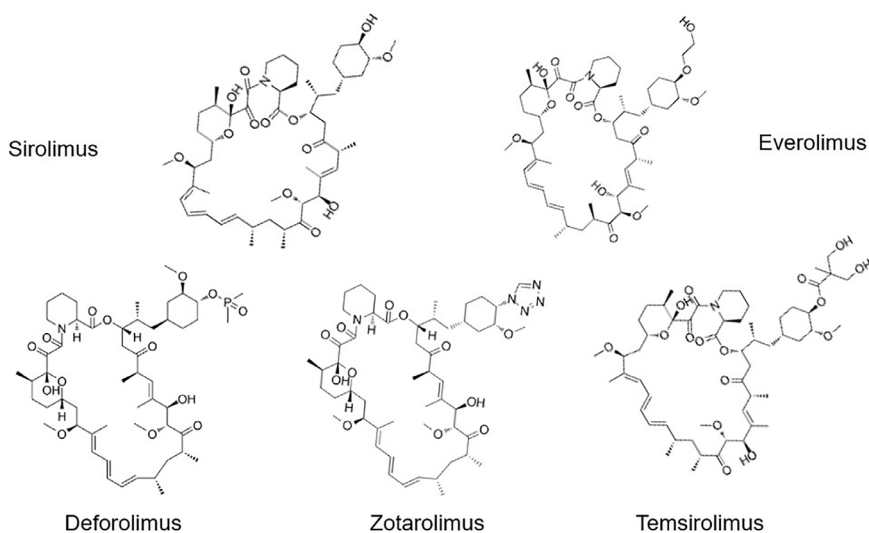

FIGURE 2 | Chemical structure of five mTOR inhibitors.

ALL cell lines *in vitro*, as evidenced by apoptotic cell death. Mice with advanced ALL and were treated with rapamycin as a single agent exhibited enhanced survival compared to control-treated ALL-bearing animals (30). Avellino et al. reported SRL (+/- doxorubicin) can promote the apoptosis of primary pediatric ALL blast cells *in vitro*, indicating that rapamycin targets two pathways that are crucial for cell survival and chemoresistance of malignant lymphoblasts (PI3k/Akt and FKBP51/NF-kB) (31). In a phase I/II study of everolimus in combination with HyperCVAD in patients with R/R ALL, ORR was in 41% and significantly higher in first salvage patients as compared with second salvage or beyond. Moreover the inhibition of phosphorylation of S6K1 did not correlate with response, and the combination regimen did not increased toxicity than HyperCVAD alone (32). Thus, the combination of Rapalogs with some chemotherapies may be an effective regimen for AML and ALL.

Results of clinical trials using Rapalogs as a single agent have shown very limited response in AML patients, such as sirolimus (33), deforolimus (21), or everolimus (34). There are also reports of combination strategies; for example, SRL was combined with MEC (mitoxantrone, etoposide, cytarabine) to treat patients with relapsed, refractory, or untreated high-risk AML. The ORR to the combination regimen was 16%–47% (35–37), which suggested SRL and MEC may be an effective option as a treatment regimen for AML. However, Burnett et al. added everolimus to consolidation therapy in AML, and reported there was no difference in relapse-free survival and overall survival (38). This study suggests that the addition of everolimus to chemotherapy provides no benefit (38). Interestingly, Recher et al. found that some primary AML cells have innate Rapa-resistance, due to several mechanisms, including FKBP-12 mutations, high eIF-4/4E-BP1 ratio, defective regulation of p27kip1, and c-myc amplification (33). Taken together, combination of Rapalogs with other chemotherapies might be an effective regimen for patients with high-risk acute leukemia; however, stratification of patients who are likely to respond to these regimens, perhaps based on underlying mutation patterns, must be better understood.

Myelodysplastic Syndrome

Myelodysplastic syndrome (MDS) is a group of hematopoietic stem cell disorders, characterized by ineffective clonal hematopoiesis leading to blood cytopenias, and convey a variable risk of progression to AML (39). Activation of the mTOR pathway in CD33^{+} cells from MDS patients by the amino acid L-leucine in 5q- syndrome was reported by Yip BH et al. (40). Importantly, in primary cells from high-risk MDS patients, not only was mTOR activated but also its downstream targets, P70S6K and 4E-BP1. Treatment with Rapa significantly increased CD33^{+} cells apoptosis from high-risk MDS patients, but not cells from healthy donors or those patients with low-risk MDS (41). Mutated *GSTT1* gene was detected in some MDS patients and this mutation creates a sequence that is 63% homologous to human FKBP-rapamycin associated protein (FRAP). To examine the effect of mutant GSTT-1, two cell lines (K562 and HL-60) were stably transfected with the

mutant type *GSTT1* gene. Expectedly, rapamycin induced significant growth inhibition of those two cell lines, suggesting that rapamycin could be included as a potential therapeutic modality for high-risk MDS patients (42).

Platzbecker et al. (43) treated 19 MDS patients with SRL as a single agent, and demonstrated that SRL might have some activity in patients with more advanced MDS but lacked efficacy in low-risk MDS patients. Martin et al. (44) treated 20 MDS patients with temsirolimus at a weekly dose of 25 mg; however, only four (20%) reached the response assessment after 4 months without hematological improvement. Based on these this response assessment, they concluded that Temsirolimus has no beneficial effects in elderly MDS patients. However, because MDS is a highly heterogeneous disease, a more in-depth study on the therapeutic effect of Rapalogs in patients with MDS is warranted.

Chronic Myeloid Leukemia

CML is a hematopoietic disorder characterized by the malignant expansion of bone marrow stem cells, with the presence of a reciprocal translocation between chromosomes 9 and 22 resulting in activation of fusion gene BCR-ABL expression (45). It has been found that BCR/ABL, a common driver mutation in CML, mediates the expression of VEGF and its transcriptional activator HIF1 through PI3K and mTOR pathways (46). It also found that BCR/ABL regulates P70S6K and 4E-BP1 through mTOR activation (47–49). *In vitro*, Rapamycin (combined with celecoxib) induced cell cycle arrest and apoptosis of the CML cell line K562. As we know, tyrosine kinase inhibitors (TKIs) are the first line therapy for CML, implying that a combination of TKI with mTORi could enhance the antitumor effects of TKI treatment on CML cells (50). Several groups have, indeed, demonstrated this synergistic effect, combining imatinib with rapamycin (47, 51), or everolimus (52) to overcome TKI resistance *in vitro*, including cell line K562, murine CML model and CML patients samples. However, whether Rapalogs represent a new treatment opportunity for TKI resistant CML remains to be investigated in the clinic.

Myeloproliferative Neoplasms

MPNs often begin with an abnormal gene mutation or change in a stem cell in the bone marrow, resulting in hemocytopenia and splenomegaly, etc. Types of MPN include myelofibrosis, polycythemia vera and essential thrombocythemia (53). Bartalucci et al. found that mTOR signaling pathway was activated in MPN including myelofibrosis (MF), which was located downstream of JAK2 signaling pathway (54). Guglielmelli et al. (55) carried out treatment of patients with myelofibrosis with everolimus, resulting in 20% of patients' spleens shrinking > 50%, 40% of patients' spleens shrinking > 30%, and 15%–25% of patients with hematological reaction. In other studies (56, 57), JAK2/V617F mutated leukemia cell lines (HEL and SET2) and patients' samples of PV or PMF were sensitive to everolimus. Moreover, the combination of JAKs inhibitor (ruxolitinib) and everolimus, showed synergy in inducing cell-cycle arrest and blockade of cell proliferation (58), which is promising for MPN treatment.

Hodgkin Lymphoma

Aberrant activation of the PI3K/Akt/mTOR pathway is a hallmark of lymphomas, including Hodgkin lymphoma (HL) (59, 60) and non-Hodgkin lymphomas (NHLs) (61, 62). Abnormal activation of upstream or downstream molecules of mTOR can cause disease development. mTOR mutations have been described in diffuse large B-cell lymphoma (DLBCL) samples (63), and activated ABC-DLBCL cell lines expressed high level of S6K1, which is a downstream target of mTOR (64). mTOR directly mediates Cyclin D1 downregulation through glycogen synthase kinase (GSK)-3b in mantle cell lymphoma (MCL) (65). Based on these observations and preclinical data, clinical trial using Rapalogs have been carried out in patients with lymphoma (66).

Everolimus has significant activity in relapsed/refractory (R/R) HL and is currently listed as a treatment option for HL in the National Comprehensive Cancer Network (NCCN) guidelines (67). Everolimus demonstrates single-agent activity in treating Hodgkin (67–71) and non-Hodgkin lymphoma (72–76), with responses ranging from 20% to 47%. In a small phase 2 study, 19 patients with R/R HL were treated with single-agent everolimus (10 mg/d), with an ORR of 47% (69). This was recapitulated in a larger multicenter study of 57 patients with an ORR of 42%, including five complete responses (CRs) and a median PFS of 9 months (68). The combination of sirolimus and HDAC inhibitor (vorinostat) is well-tolerated with encouraging activity in very heavily pretreated patients with Hodgkin lymphoma who are refractory to standard therapies. In 28 patients enrolled and accepted SRL and vorinostat scheme, the ORR was 57% (CR 32%+PR 25%) (77, 78). The emergence of effective new drugs in the treatment HL, including CD30 mAbs and checkpoint inhibitors, such as PD-(L)1 mAbs, may limit the broad application of mTORi in this setting. However, mTORi may be useful in later lines of therapy for difficult-to-treat, multidrug-exposed patients with R/R HL.

Non-Hodgkin Lymphomas

There are also clinical trials using Rapalogs to treat NHL, for example, temsirolimus is used for relapsed MCL with an ORR 38-47% (79, 80). A phase I b clinical trial investigated temsirolimus in association with R-CHOP (R-CHOP-T), or high-dose cytarabine plus rituximab (R-DHA-T), or fludarabine, cyclophosphamide plus rituximab (R-FC-T). Regarding efficacy, ORR during treatment period was 40% for R-CHOP-T including two patients who reached CR, 43% for R-FC-T including three patients who reached CR, and 47% for R-DHA-T including six patients who reached CR (81). In another study, Temsirolimus combined with bortezomib in DLBCL achieved an ORR of 31% (82). Yet another study investigated single-agent temsirolimus 25 mg weekly administrated in three groups of patients with relapsed aggressive and indolent lymphomas: group A (diffuse large B-cell lymphoma, transformed follicular lymphoma), group B (follicular lymphoma), and group C (chronic lymphocytic leukemia/small lymphocytic lymphoma, and other indolent lymphomas). Group A had an overall and complete response rate of 28.1% and 12.5%,

respectively, and median PFS of 2.6 months and median OS of 7.2 months. Group B had overall and complete response rates of 53.8% and 25.6%, respectively, and median PFS of 12.7 months; median OS has not yet been reached. Group C had a partial response rate of 11% with no complete responders (83). Everolimus has been used to treat R/R marginal zone lymphomas (MZLs) with an ORR of 25% (75). Temsirolimus and everolimus are not very effective in lymphoma, which may be due to inhibition of mTORC1 but not mTORC2, resulting in feedback activation of PI3K/Akt (84). As for chronic lymphocytic leukemia (CLL), Decker et al. found that Rapamycin mediates the arrest of B cells of CLL in G1 phase (85). Three cases of lymphoproliferative diseases after transplantation have been successfully treated with rituximab and Rapamycin (86). Clinical evidence indicate that everolimus has a positive effect in patients with lymphoplasmacytic lymphoma/Waldenström macroglobulinemia (LPL/WM) and ORR can reach 70% (87). Taken together, subtypes of lymphoma may respond differently to mTORi; additional studies are needed to clarify the benefit/risk ratio of mTORi use in these settings.

Multiple Myeloma

Multiple myeloma (MM) is a hematological malignancy characterized by abnormal production of immunoglobulins by malignant plasma cells (PCs). Previous studies have indicated that the PI3K/Akt/mTOR signaling pathway is aberrantly activated in MM cells (88, 89). MM blast cells secrete growth factor IL-6 and IGF-1 to activate PI3K/Akt, which induce abnormal expression of mTOR (90). Following curcumin treatment, mRNA and protein expression levels of mTOR were decreased, inducing apoptosis in MM cell lines, indicating a potential novel therapy for MM (91). Lamanuzzi A et al. (92) evaluated endothelial cells (ECs) from 20 patients with monoclonal gammopathy of undetermined significance (MGUS) and 47 patients with MM, and found higher activation of mTORC2 downstream effectors, suggesting a major role of mTORC2 in the angiogenic switch to MM. Inhibition of mTORC2 with lenalidomide and bortezomib exhibited a synergistic anti-angiogenic effect (92). Combining rapamycin with resveratrol has a synergistic effect in inhibition of myeloma cell line viability (93). Everolimus shows synergistic anti-myeloma effects with bortezomib through inhibition of the Akt/mTOR pathway in both the MM cell lines and MM-bearing mice model (94, 95).

In a clinical trial, 17 patients with R/R MM were administered single-agent everolimus; the ORR was 59% including eight patients with stable disease (SD), one with partial response (PR), one with minor remission (MR) (96). In another trial, 26 patients were administered everolimus and lenalidomide for 21 days with a 7 days break between cycles. The ORR was 65%, the median PFS was 5.5 months and median overall survival (OS) was 29.5 months (97). Temsirolimus has also been combined with lenalidomide in a phase I study in patients with relapsed MM. In total, 21 patients were enrolled, two patients (10%) achieved PR, and 15 patients (71%) had SD (98). In another phase I/II study evaluated temsirolimus in combination with

weekly bortezomib in R/R MM patients, 14 of 43 (33%) patients achieved ORR (99). There are currently multiple approved drugs to treat multiple myeloma in front-line therapy; however, there is still no cure for this serious disease. Thus, more studies are needed to evaluate the potential benefit of incorporating mTORi into late-line regimens in R/R MM patients.

APPLICATIONS IN NON-MALIGNANT HEMOPATHIES

Immune Thrombocytopenic Purpura

The pathogenesis of immune thrombocytopenic purpura (ITP) includes abnormal activation of autoreactive T and B cells, which leads to immune destruction of platelets, providing a theoretical basis for the application of rapamycin in ITP. Wang CY et al. (100) found that platelet autophagy was diminished in ITP patients, and platelet autophagy in ITP was regulated by the PI3K/Akt/mTOR pathway. Rapamycin induced autophagy and alleviated the destruction of ITP platelets (100). Rapamycin and prednisone were used to treat adult chronic ITP (101). After comparing the remission rates of rapamycin group and cyclosporine group, as well as the levels of Treg cells, IL-10, and TGF - β cytokines in responders, it was concluded that rapamycin combined with low-dose dexamethasone could not improve the remission rate, but the sustained remission time of those treated with Rapamycin was significantly longer than that of cyclosporine-treated patients, and the Rapa-responders' Treg cell level and Foxp3 mRNA expression level were significantly higher than those treated with cyclosporine.

Bride et al. (102) conducted a multicenter prospective study (NCT00392951) on rapamycin as a single therapy for refractory autoimmune pancytopenia of 30 patients. The result showed that rapamycin had a significant effect on secondary multicellular hemocytopenia, such as secondary Hypogammaglobulinemia, Evans Syndrome (ES), and SLE (systemic lupus erythematosus), especially thrombocytopenia, with a CR rate of 67% (8/12). In this report, all children (12) with autoimmune lymphoproliferative syndrome (ALPS) achieved a durable CR, including rapid improvement in autoimmune disease, lymphadenopathy, and splenomegaly within 1–3 months of starting sirolimus. Double-negative T cells were no longer detectable in most, suggesting a targeted effect of sirolimus. Jasinski et al. (103) treated 17 patients with sirolimus, among which were 12 ITP and 5 ES cases. As a result, 73% of ITP patients achieved a CR by 3 months, while 50% of ES patients had a CR. Of the patients that achieved CR, 90% remained off all therapy for a median of 2 years.

Miano et al. (104) retrospectively analyzed primary ITP (10 cases) and ITP secondary to autoimmune lymphoproliferative syndrome (nine cases) who received sirolimus treatment, and had previously failed mycophenolate mofetil (MMF) therapy. As a result, 5/10 primary ITP patients (50%) and 8/9 (89%) secondary ITP patients responded to SRL respectively. Our center initialed a prospective, single-arm clinical trial, in which 86 patients were included and given SRL administration. The results showed that SRL treatment obtained an ORR of 85% at

the 3rd month without serious drug toxicity. After 12 months follow-up, the ORR remained at 65%. Importantly, in patients who responded, SRL treatment was associated with a reduction in the percentage of Th2, Th17 cells, and increase in the percentage of monocytic-Myeloid-Derived Suppressor Cells (M-MDSCs) and T regulatory cells (Tregs), indicating that SRL may re-establish peripheral tolerance. To date, this is the largest prospective study to date evaluating SRL as a rescue therapy in patients with R/R ITP (105). ITP is a heterogeneous disease with a complex pathobiology; based on our experience and others', we believe that mTORi can be effective in treating refractory ITP, but more randomized controlled trials (RCTs) are needed to support these observations.

Autoimmune Hemolytic Anemia

Sirolimus has benefited patients with primary autoimmune cytopenias, possibly by stimulating Tregs (105, 106). In the pathogenesis of AIHA, increased expansion or function of Treg cells can inhibit the over-activation of effector T cells and other immune cells, thus regulating the production of excess RBC antibodies (107, 108). Mqadmi et al. (109) used CD25 monoclonal antibody to treat C57/B16 mice to deplete $CD4^+CD25^+$ Treg cells in vivo, followed by injection allogeneic red blood cells into the abdominal cavity of C57/B16 mice to establish a murine AIHA model. These experiments showed that incidence of AIHA in Treg deficient mice increased from 30% to 90%. $CD4^+CD25^+$ Treg cells purified from the spleen of mice were re-injected into the diseased mice, leading to a reduction in hemolysis and autoantibodies. Thus, $CD4^+$ $CD25^+$ Treg cells play an important role in the pathogenesis of AIHA in animal models (110, 111).

Miano et al. reported that sirolimus was effective in five children with AIHA who did not respond to MMF previously. In their center, sirolimus is used as a rescue treatment, given at the dose of 2–3 mg/m^2 once a day, for at least 3 month (112). In refractory AIHA after intestinal transplant, four patients were reported who had marked improvement of hemolysis, after discontinuation of calcineurin inhibitor (CNI) and initiation of sirolimus (113). It was recommended sirolimus as the second-line treatment of AIHA in the case of failure to steroid treatment in 2016 of ASH (American Society of Hematology) meeting (114). Our center used sirolimus to treat the relapsed and refractory AIHA. Sixteen (16) patients were included and followed for at least 12 months; after 12 months, SRL was still effective for 9/12 patients (56.25%), with 7/9 patients achieving CR and 2/9 achieving PR. Five (5) of these patients had been discontinued with no relapse, and the longest withdrawal time without relapse was 24 months. The side effects of SRL were mild and tolerable to the patients. In conclusion, SRL is effective for R/R AIHA in adults, with mild side effects, and should be considered as frontline therapy for treatment of the disease (115).

Autoimmune Lymphoproliferative Syndrome

Autoimmune lymphoproliferative syndrome (ALPS) is mainly caused by mutations in FAS mediated apoptosis pathway (116, 117).

The pathogenesis of ALPS is that the double negative T cells (DNT), expressing CD3$^+$TCRαβ$^+$ CD4$^-$CD8$^-$, cannot be effectively removed due to apoptosis defects caused by FAS mutation, resulting in abnormal activation of immature T cell accumulation causes hepatosplenic lymphadenopathy. At the same time, over-activation of B lymphocyte leads to the increase of autoantibody production and the occurrence of autoimmunity (118). mTOR signaling activity was also enhanced in DNT cells, and mTOR inhibitors could specifically reduce DNT *in vivo*, indicating that the mTOR pathway is the main regulatory mechanism of lymphocyte proliferation and abnormal differentiation of ALPS (119). In 2006, the results of an experimental study in animals provided the first evidence that SRL can significantly improve the clinical symptoms of ALPS rats (120). Sirolimus has been described as useful in ALPS patients, in which it also reduced the count of DNTs (121).

Currently, there are some cases of successful application of sirolimus to control ALPS in adults and children (102, 122, 123). In one case-study, a 9-month-old female infant diagnosed ALPS with massive lymphadenopathy was treated with corticosteroids and splenectomy, but the curative effect was poor. Treatment with SRL (3 mg/m^2) was initiated and the symptoms of lymphadenopathy were relieved rapidly. After 6 weeks, lymphadenopathy decreased and blood cell count improved significantly (124). In another center, four steroid-resistant ALPS patients were treated with SRL for 4 weeks; all had a rapid complete or near complete response (123). SRL was used as single agent in 12 ALPS patients for at least 6 months. The results showed that SRL was effective in all ALS patients. It not only improved the decreased blood cells, but also improved the hyperplasia of lymphoid tissue (102). In a report, 16 ALPS patients were treated with SRL as the second or further line, after the multi-drug treatment failed. As a result, 12 (75%) patients responded effectively (125). Recently, IL-6 stimulation augmented mTOR activation in idiopathic multicentric Castleman disease (iMCD) patients was reported, furthermore, the degree of mTOR activation in iMCD was comparable to ALPS. This finding supports inhibition of mTOR activation as a novel therapeutic target for iMCD, and is currently under investigation in a clinical study (NCT03933904) (126).

Acquired Aplastic Anemia and Pure Red Cell Aplasia

Acquired aplastic anemia is characterized by a hypoplastic, fatty bone marrow with profound reductions in hematopoietic stem/progenitor cells that lead to defective mature blood cell production and peripheral pancytopenia (127). In clinical observation, it was found that the IFN-γ level in serum and bone marrow of was increased in ~30% of AA patients increased, while cytotoxic T cells (CD8$^+$ T cells) production of IFN-γ promoted CD34$^+$ pluripotent hematopoietic progenitor cell apoptosis (128). The attack of CD34$^+$ cells by CD8$^+$ T cells is the basis of AA, the effect of CD8$^+$ T cells can be eliminated by SRL mediated CD4$^+$CD25$^+$ Treg cells, which inhibit the process and promote the proliferation of hematopoietic stem cells (129, 130). Rapamycin ameliorated

this phenotype in an immune-mediated AA mice model and inhibited the proliferation of T cells by preventing cell cycle transition from G0 to G1 phase (131). Reports also show that rapamycin can significantly enhance autophagy of Bone Marrow Stromal Cells (BMSCs). Rapamycin can inhibit the adipogenic differentiation of BM-MSCs in AA patients in a dose-dependent manner, and the inhibition rate can reach 50%-85%. Rapamycin can reduce the ratio of adipose tissue to bone marrow hematopoietic tissue, so as to improve the hematopoietic microenvironment of bone marrow. However, SRL may also promote the apoptosis of MSC; accordingly, rapamycin should be strictly controlled in clinical treatment (132). He et al. reported two case of AA responsive to sirolimus (1 mg/d) combined with cyclosporine (2 mg/kg/d) (133). In fact, controversial results are also presented. A study observed 35 AA patients received h-ATG/CsA/sirolimus, and the ORR at 3 months was 37% and 51% at 6 months, at last, it concluded that sirolimus (2 mg/day in adults and 1 mg/m^2/day in children) did not improve the response rate in patients with severe AA when compared to standard h-ATG/CsA treatment (134).

Sirolimus may not directly stimulate the growth and differentiation of red blood cells, but it can antagonize the inhibitory effect of PRCA patients' serum on red blood cells, and may play a role by inhibiting the components in serum (135). In 2007, ASH meeting, Sirolimus (1 mg/day) successfully treated a refractory case of congenital PRCA was reported (136). Reportedly, three patients of multi-resistant PRCA treated with SRL (2 mg/day), all responded well to SRL, and natural killer cell (CD16$^+$CD56$^+$) count was decreased after long-term administration of sirolimus (137). In 2018, 21 patients with refractory/relapsed acquired PRCA administered with SRL, and 76.2% of the patients was responsive and 42.9% was complete response (138). At present, there is still a need for multi-center and large-scale RCT to verify the positive role of rapamycin in acquired AA and PRCA.

Graft-Versus-Host Disease

SRL has been widely used to inhibit graft rejection after solid organ transplantation; however, increasing evidence demonstrate that SRL plays an active role in the prevention and treatment of graft-versus-host disease (GVHD) following hematopoietic stem cell transplantation (HSCT). GVHD is an exaggerated inflammatory reaction mediated by donor lymphocytes against host tissues. The conditioning regimen damages host tissues and causes release of pro-inflammatory soluble factors such as TNFα, IFN-γ, IL-1, IL-6, and nitric oxide. Increased levels of these factors lead to activation of host antigen presenting cells (APCs). Host APCs migrate into lymphoid tissue and subsequently activate donor immune cells, including T, B, and NK cells. This process elicits a complex cascade of both lymphocyte subsets and soluble inflammatory mediators. Lastly, these soluble and cellular mediators synergize to amplify local tissue injury and further promote inflammation and target tissue destruction (139, 140). Coenen JJ et al. (141), reported rapamycin, not cyclosporine, permits thymic generation and peripheral preservation of CD4$^+$CD25$^+$FoxP3$^+$ T cells. Treatment with cyclosporine led to

a reduced generation of CD4^{+}FoxP3^{+} T cells in GVHD mice model, whereas prolonged rapamycin treatment allowed for thymic generation of those cells. As peripheral tolerance induction is a prerequisite for successful treatment outcome after stem cell transplantation (SCT), this may challenge the use of cyclosporine as standard drug of choice (141).

In 2008, Armand et al. (142) found that sirolimus combined with calcineurin inhibitor prevented GVHD in lymphoma patients after bone marrow transplantation. The OS of patients in sirolimus group was significantly prolonged (3-year OS, 66% for sirolimus group vs. 38% for no sirolimus group). In 2016, Armand and his colleagues (143), again reported a multicenter randomized trial comparing sirolimus, tacrolimus and methotrexate to standard method (tacrolimus, methotrexate and mycophenolate mofetil) in lymphoma patients after SCT. As a result, there was no difference in 2-year OS, PFS, relapse, and chronic GVHD. However, the sirolimus-containing arm had a significantly lower incidence of grade II-IV acute GVHD. In 2019, another multi-center, randomized, phase 3 trial was reported. After HSCT, standard GVHD prophylaxis regimen (cyclosporine and mycophenolate mofetil) or the triple-drug combination regimen (cyclosporine, mycophenolate mofetil, and sirolimus) was used to test the efficacy of SRL. Consistent with previous findings, the incidence of II-IV aGVHD in the three-drug group (26%) was lower than that in the standard group (52%) at 100 days. The new discoveries were as following: The Non Recurrent Mortality (NRM) rate (16%) of the three drug groups was lower than that of the standard group (32%) (p=0.021) at 4 years; The OS rate in the three drug groups was higher than that in the standard group (p=0.035); The PFS rates in the three drug groups were higher than those in the standard group (p=0.045); The incidence of Grade III to IV aGVHD and chronic GVHD was of no differences (144). A meta-analysis included five RCTs to assess the efficacy and safety of sirolimus-based GVHD prophylaxis in patients after allogeneic HSCT, as the result, SIR was observed to significantly decrease the incidence of Grades II to IV

aGVHD; However, the incidence of Grades III to IV aGVHD and chronic GVHD was not decreased. SRL significantly increased sinusoidal obstructive syndrome, Moreover, SRL did not improve event-free survival and overall survival (145).

In addition to the above reports on the prevention of GVHD by SRL, the following is the story of using sirolimus to treat GVHD. Pidala et al. (146) initiated a multicenter randomized phase II trial to assess the CR/PR rates of SRL versus prednisone to treat patients with acute GVHD of standard risk. A total of 127 patients were enrolled and randomized. The day 28 CR/PR rates were similar for sirolimus 64.8% vs. 73% for prednisone. Interestingly, the day 28 rate of CR/PR with prednisone (≤0.25 mg/kg/day) was significantly lower than sirolimus (31.7% vs. 66.7%; P<0.001). Importantly, SRL was associated with reduced steroid exposure and hyperglycemia, reduced grades 2 to 3 infections, improvement in immune suppression discontinuation and patient-reported quality of life. For chronic GVHD treatment, Carpenter PA et al. (147), claimed that the two-drug (prednisone/sirolimus) and three-drug combination (prednisone/sirolimus/CNI) did not differ in rates of 6-month CR and PR. In other words, the results are the same whether CNI is not added to the two-drug regimen. Importantly, the two-drug regimen is easier to administer and is better tolerated. In our center, sirolimus was used to treat steroid- resistant/steroid- dependent extensive cGVHD. A total of 27 patients were enrolled and given sirolimus combined with cyclosporine or tacrolimus to observe the clinical efficacy and adverse events. Following the 6-month follow-up, the ORR was 55.6%. At the 1-year follow-up, there were five cases of CR and 11 cases of PR, ORR was 59.3%, PFS-12 reached 62.9% (17/27), and OS-12 was 100% (148).

In summary, SRL has demonstrated clinical benefit in both the prevention and treatment of GVHD and may be preferred to other regimens for patients after HSCT. There is currently extensive clinical investigation of Rapalogs in the GVHD setting (**Table 1**).

TABLE 1 | Current recruiting Rapalog clinical trial landscape in hematologic diseases.

	Registration Number	Rapalogs	Phase	Disease	Status	Interventions
1	NCT03963024	Sirolimus	1	GVHD	Recruiting	Sirolimus
2	NCT03225417	Sirolimus	1/2	GVHD	Recruiting	Sirolimus + Ixazomib + Tacrolimus
3	NCT02891603	Sirolimus	1/2	GVHD	Recruiting	Sirolimus + Pacritinib + Tacrolimus
4	NCT03128034	Sirolimus	1/2	GVHD	Recruiting	Sirolimus + CsA + MMF
5	NCT03192397	Sirolimus	2	GVHD	Recruiting	Sirolimus + MMF +PTCY
6	NCT03970096	Sirolimus	2	GVHD	Recruiting	Sirolimus + Tacrolimus + MTX+ATG
7	NCT01903473	Sirolimus	2	GVHD	Recruiting	Sirolimus + Treg infusion + Low dose IL-2
8	NCT02722668	Sirolimus	2	GVHD	Recruiting	Sirolimus + MMF + ATG
9	NCT03246906	Sirolimus	2	GVHD	Recruiting	Sirolimus + CsA + MMF vs. Sirolimus + CsA + PTCY
10	ChiCTR2000029921	Sirolimus	2	GVHD	Recruiting	Sirolimus + CNI
11	NCT02583893	Sirolimus	2	AML	Recruiting	Sirolimus + Mitoxantrone + Etoposide + Cytarabine
12	NCT03878524	Sirolimus, Everolimus	1/2	MDS, PMF	Recruiting	Sirolimus for MDS; Everolimus for PMF
13	NCT03697408	Everolimus	1/2	cHL	Recruiting	Everolimus + Itacitinib
14	NCT03190174	Nab-rapamycin	1/2	cHL	Recruiting	Nab-rapamycin + Nivolumab
15	NCT02693535	Temsirolimus	2	NHL, MM	Recruiting	Temsirolimus
16	ChiCTR1900020657	Sirolimus	2	ITP	Recruiting	Sirolimus + Dexamethasone

PTCY, Post-Transplantation Cyclophosphamide; MMF, mycophenolate mofetil; MTX, methotrexate; ATG, Antithymocyte globulin; CNI, Calcineurin Inhibitor; CsA, ciclosporin; PMF, Primary myelofibrosis; cHL, classical Hodgkin lymphoma; Nab-rapamycin, Nanoparticle albumin-bound rapamycin.
The data are from https://www.clinicaltrials.gov/in America and www.chictr.org.cn in China. This table only includes the recruiting trails, and the "active not recruiting" and "completed" are excluded.

TABLE 2 | Clinical efficacy and safety of mTOR inhibitors in hematological disease.

Author (Ref)	Disease(s)	Patients (n)	Drugs and Schedules	Clinical Response	Adverse events (AEs)
Kasner MT. 2018 (36)	Relapsed, refractory, or untreated high-risk AML	51	Sirolimus 12 mg d1, 4 mg d2-9; Mitoxantrone 8 mg/m², 5days; Etoposide 100 mg/m², 5 days; Cytarabine 1 g/m², 5 days.	ORR was 47% (33% CR, 2% CRi, 12% PR), Blood p70S6 as a predictive biomarker for clinical response.	No significant AEs.
Perl AE. 2009 (37)	Relapsed, refractory, or untreated secondary AML	27	Sirolimus 12 mg d1, 4 mg d2-9; Mitoxantrone 8 mg/m², 5 days; Etoposide 100 mg/m², 5 days; Cytarabine 1 g/m², 5 days.	ORR was 22% (15% CR, 7% PR), p70S6 as a biomarker for SRL response.	Neutropenic fever, Bacteremia, Diarrhea, Transaminase/bilirubin, elevation, Pneumonia
Daver N. 2015 (32)	Relapsed/ Refractory ALL	24	HyperCVAD combined with continuous oral everolimus, starting on day 0 of Cycle 1, at a dose of either 5 or 10 mg/day.	ORR was 41% (33% CR, 8% PR and NR 59%), Everolimus inhibited phosphorylation of S6RP, but did not correlate with response.	Mucositis, Myelosuppression, Hyperglycemia, Transaminitis
Guglielmelli P. 2011 (55)	MPN	39	Everolimus in 3 dose-escalating cohorts at 5.0, 7.5, and 10.0 mg daily for 3 months.	Response rate was 60%, whereas CCDN1 mRNA, phospho-p70S6K level, and WT1 mRNA were identified as possible biomarkers.	Stomatitis, Hypertriglyceridemia, Skin disorders, Musculoskeletal, Hypercholesterolemia
Johnston PB. 2012 (68)	Hodgkin lymphoma	57	Everolimus 10 mg/day until disease progression or unacceptable toxicity.	The ORR and DCR were 42.1% and 77.2%, including CR 8.8%. The median time to response was 57 days, Median PFS was 9.0 months.	Fatigue, Thrombocytopenia, Cough, Rash, Pyrexia, Anemia, Dyspnea, Back pain, Diarrhea, Stomatitis, Pneumonitis.
Janku F. 2014 (77)	Refractory Hodgkin lymphoma	28	Sirolimus 1–5 mg/day q28 days; HDAC inhibitor vorinostat 100–400 mg/ day q28 days.	The ORR was 57% with 9 CRs (32%) and 7 PRs (25%).	Thrombocytopenia, Anemia, Transaminitis.
Tessoulin B. 2020 (81)	Relapsed MCL	41	15, 25, and 50 mg dose level plus RCHOP, or RDHA, or RFC	The ORR was 40% in RCHOP-T arm, 43% RFC-T arm, and 47% in RDHA-T arm.	Blood count disorders, metabolism troubles, biologic investigations, and infections.
Smith SM 2010 (83)	Relapsed DLBCL, FL and CLL	89	Single-agent temsirolimus 25 mg weekly	Group A (DLBCL) had an ORR of 28.1% and median PFS of 2.6 months. Group B (FL) had ORR of 53.8% median PFS of 12.7 months; Group C (CLL) had ORR of 11%.	Mild and/or reversible myelosuppression and mucositis
Ghobrial IM. 2010 (87)	WM	50	Everolimus 10 mg/day until progression.	The ORR was 70%, with a PR of 42% and 28% MR. CR 0.	Anemia, Leukopenia, Neutropenia, Pneumonia, Hypercholesterolemia, Hyperglycemia.
Yee AJ. 2014 (97)	Relapsed/ refractory multiple myeloma	26	Lenalidomide 15 mg; Everolimus 5 mg for 21 days with a 7-day rest period.	The ORR was 65% (1 CR + 4 PR + 10 MR). The median PFS was 5.5 months and median OS was 29.5 months. Phosphorylated p70S6K at Thr389 as biomarker.	Fatigue, Neutropenia, Thrombocytopenia, Neuropathy, and diarrhea, all of which were manageable with supportive care and dose-reductions.
Hofmeister CC. 2011 (98)	Relapsed/ refractory multiple myeloma	21	Lenalidomide 15–25 mg/d d1-d21; Temsirolimus 15–20 mg once per week during a 28-day cycle.	Two patients (10%) achieved PR and 15 patients (71%) had SD.	Fatigue, Neutropenia, Anemia, Hypophosphatemia, Hypokalemia, Rash, Hypokalemia, Hypophosphatemia, Anorexia, Nausea, Taste alterations.
Ghobrial IM. 2011 (99)	Relapsed/ refractory multiple myeloma	20	Temsirolimus 15 or 25 mg; Bortezomib at 1.3 or 1.6 mg/m² once a week, with dose escalation until dose-limiting adverse events were recorded.	14 of 43 (33%) patients achieved ORR.	Thrombocytopenia, Lymphopenia, Neutropenia Leucopenia, Anemia, Diarrhea.

(Continued)

TABLE 2 | Continued

Author (Ref)	Disease(s)	Patients (n)	Drugs and Schedules	Clinical Response	Adverse events (AEs)
Feng YM. 2020 (105)	Relapsed/ refractory ITP	86	Sirolimus 2–4 mg/day.	By the third month, 40% CR and 45% PR, whereby (ORR) of 85%. Percentage of Th2, Th17 cells, M-MDSCs, and Tregs as biomarker associated with response.	Hyperlipidemia, Hypertransaminase, Canker sores, Skin rash, Arthralgia.
Miano M. 2015 (125)	ALPS, ALPS-related syndrome, and autoimmune cytopenia.	16	Sirolimus 2–3 mg/m^2/day, at least 3M	12/16 children ORR75%, 11 with CR (69%), 1 with PR (6%).	Patients did not show an increased incidence of opportunistic infections with mild headache being the most common side effect.
Long ZB. 2018 (138).	Refractory/ relapsed acquired PRCA	21	Sirolimus 1–3 mg/day, at least 6M.	The ORR was 76.2%, with a CR of 42.9%. Tregs as biomarker associated with response.	Pneumonia, elevation of creatinine, mild elevation of transaminase, oral mucositis, sinus tachycardia, and elevation of triglyceride and cholesterol.
Wang L. 2015 (145)	Acute GVHD and chronic GVHD	395	(TAC, SRL and MTX or MMF) TAC: -3 days, 0.02 mg/kg/day, 5–10 ng/ml 100 days tapered; SRL: 2 mg qd (-3 days ~ +80 days), 3–12 ng/ml; MTX: +1 day (15 mg/m^2),+3 days,+6 days, +11 days (10 mg/m^2); or MMF: +0 day - +30 days (15 mg/kg tid), 30–40 days (15 mg/kg bid), in the absence of GVHD, tapered off by day 96 to day 150.	SRL significantly decrease the incidence of Grades II to IV aGVHD, but has no effect in decreasing cGVHD. SRL did not improve EFS and OS.	Sirolimus increased in the incidence of SOS and TMA.
Pidala J, 2020 (146).	Acute GVHD	58	Prednisone 2 mg/kg/day; SRL was given as a loading dose (6 mg for those aged >12 years, 5 mg/m^2 for those aged ≤ 12 years), at least 56 days (10–14 ng/ml until acute GVHD resolution, then 5–10 ng/ml after resolution until at least day 56).	Day 28 CR/PR rates were similar for sirolimus 64.8% vs. 73% for prednisone. The day 28 rate of CR/PR with prednisone ≤0.25 mg/kg/day was significantly higher for sirolimus than prednisone (66.7% vs. 31.7%). No differences were detected in DFS, relapse, NRM, and OS.	Hyperglycemia was lower in the sirolimus group. The rate of TMA within 6 months was higher in the sirolimus group vs the prednisone group.

SRL, Sirolimus; CR, Complete remission; CRi, complete remission with incomplete hematologic recovery; PR, Partial remission; ORR, Overall response rate; DCR, Disease control rate; MR, Minimal response; SD, Stable disease; HDAC, Histone deacetylase; MDSC, Myeloid derived suppressor cells; SOS, Sinusoidal obstruction syndrome; TMA, Thrombotic Microangiopathy; TAC, tacrolimus; MTX, methotrexate; MMF, Mycophenolate mofetil; DFS, Disease-free Survival; NRM, Non-relapse Mortality; OS, Overall Survival.

CONCLUSIONS AND FUTURE DIRECTIONS

Rapalogs, as inhibitors of mTOR, have demonstrated clinical value, not only in the regulation of abnormal immune responses found in benign hematologic diseases, but also for the treatment of hematological malignancies (**Table 2**). However, there are still some challenges in the application of Rapalogs. First, at present, most of the studies of Rapalogs are case reports of three-line or further line drugs, which are not widely used in large-scale populations, so there remains a lack of evidence—effectiveness and long-term safety of Rapalogs need to be further studied. Whether SRL can be used as a more advanced drug in the treatment paradigm(s) needs to be evaluated by more extensive clinical research. Second, it is necessary to improve the efficacy and safety of Rapalogs, establishing the proper dosing regiments, maintaining appropriate blood concentrations model to optimize the current treatment. Third, many cytotoxic drugs are limited in the pediatric setting. The question arises, can Rapalogs demonstrate an advantage over current therapies in convenience, safety, and efficacy on pediatric patients? Finally, in clinical studies to date, we found that there are inevitably some patients that do not responded to Rapalogs, irrespective of primary disease. Can we achieve precise treatment? Before treatment, can we accurately stratify patients which are likely responders to Rapalogs? Perhaps by detecting the activity of mTOR, specific mutations responsible of driving mTOR activity or a deeper prognostic biomarker? Additional work is required in this exciting and evolving area.

AUTHOR CONTRIBUTIONS

YF wrote the original draft and revised the manuscript. XC, KC, ZZ, ZW, SY, and XZ edited this review. XZ and YF: funded the work. All authors contributed to the article and approved the submitted version.

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