mTOR Inhibitors, with Special Focus on Temsirolimus and Similar Agents

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Abstract The mammalian target of rapamycin (mTOR) is a highly conserved serine/threonine kinase that belongs to the family of PI3K-related protein kinases (PIKKs). Dysregulation of mTOR signaling is associated with the development of cancers, including myeloid and lymphoid malignancies. Here, we will provide a brief overview of mTOR inhibitors and discuss the results obtained using these compounds in hematologic malignancies and especially in lymphomas. Moreover, mechanisms of drug resistance will be highlighted.

Keywords Everolimus (RAD001) · Lymphoid Malignancies · mTOR inhibitors · Rapamycin · Ridaforolimus (MK-8669) · Temsirolimus (CCI-779)

Introduction: Rapamycin and Rapalogs History

mTOR inhibitors comprise different compounds which have been developed starting from rapamycin, a macrolide antibiotic produced by the bacterium *Streptomyces hygroscopicus.* Rapamycin was isolated in a soil sample on Easter Island, also known as Rapa Nui, from where its name is derived [1] and firstly used as an antifungal agent [2]. However, shortly after, it was also shown to have strong immunosuppressive and antiproliferative properties due to its ability to inhibit mTOR [3–5]. Thus, FDA-approved Rapamycin use in transplantation to prevent allograft rejection and in coronary-artery stents to prevent restenosis in 1999 and 2003, respectively [6]. On the other hand, its application in cancer therapy started in the late 1990s, when several analogs of the drug, called rapalogs and including temsirolimus (CCI-779), everolimus (RAD001), and ridaforolimus (MK-8669), were developed with the aim to improve its pharmacokinetics and stability (Fig. 1) [7]. Temsirolimus was the first

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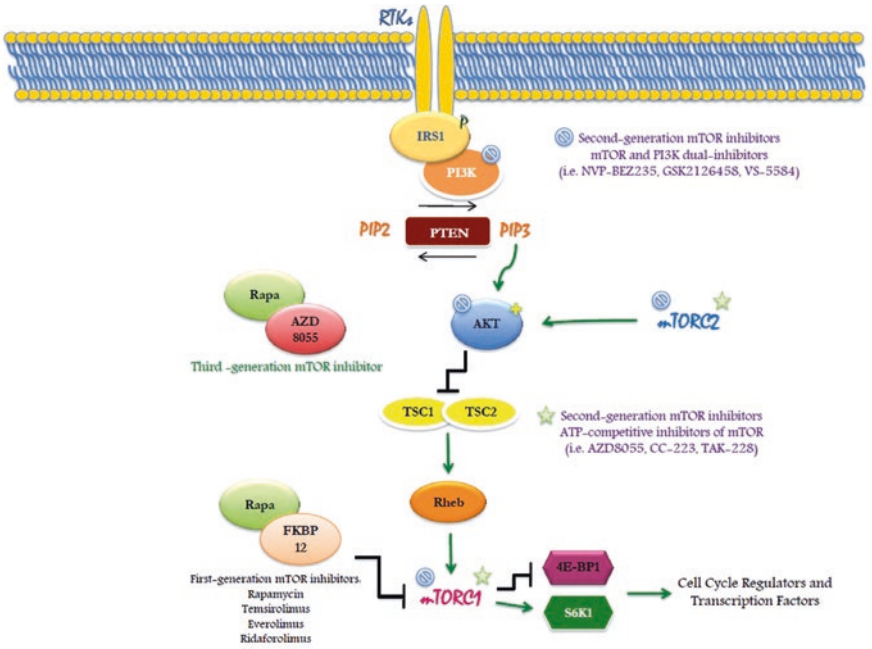

Fig. 1 Schematic representation of mTOR signaling pathways and mTOR inhibitors mechanisms of action. mTOR works through two distinct multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 has 4E–BP1 and S6 K1 as its two major substrates by which promote the translation of key cell cycle regulators and transcription factors. mTORC2 is a key regulator of Akt full activation via phosphorylation of Ser473. Rapamycin interaction with the intracellular receptor FKBP12 as well as new generation mTOR inhibitor has been shown. The third-generation mTOR inhibitor is a molecule in which rapamycin is cross-linked with a kinase inhibitor of mTOR. Abbreviations: mTOR mammalian target of rapamycin, RTKs Receptor tyrosine kinases, PI3K phosphoinositide 3-kinase, TSC tuberous sclerosis, Rapa Rapamycin

mTOR inhibitor to gain FDA authorization for any malignancy, having been approved for the treatment of advanced renal cell carcinoma [8]. Moreover, temsirolimus is the only mTOR inhibitor approved for the treatment of lymphomas and in particular it is registered for the treatment of relapsed and/or refractory mantle cell lymphoma (MCL) in the European Union and several other countries. To date, all these agents, and the so called second generation mTOR inhibitors, are being investigated alone or in combination in solid as well as in hematologic malignancies.

The mTOR Pathway and mTOR Inhibitors

mTOR is a downstream effector of the PI3K/AKT pathway (Fig. 1). mTOR works through two distinct multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [9] which are evolutionarily

conserved from yeast to mammals [10, 11]. These two complexes consist of unique mTOR-interacting proteins that determine their substrate specificity and localize them to different subcellular compartments, thus affecting their activation and function [12].

mTORC1 recruits substrates through the regulatory-associated protein of mTOR (RAPTOR) that are then further aligned to the catalytic cleft of mTOR. Rapamycin inhibits mTOR complex 1 (mTORC1) through the interaction with the intracellular receptor FKBP12 forming an inhibitory complex, which binds a region in the C terminus of TOR proteins [13, 14]. However, the exact mechanism of how this interaction with the FRB domain leads to inhibition of mTOR signaling remains to be defined. It has been proposed that rapamycin does not inhibit initial substrate recruitment but blocks correct alignment of some substrates to the catalytic cleft [15]. This could explain why rapamycin is more effective in blocking the phosphorylation and activation of ribosomal protein S6 kinase 1 (S6 K1) than that of eIF4E–binding protein 1 (4E–BP1). On the other hand, mTORC2 was identified as a rapamycin-insensitive entity, as acute exposure to rapamycin did not affect mTORC2 activity or Akt phosphorylation. However, subsequent studies have shown that, at least in some cell lines, prolonged exposure with rapamycin seems to prevent also mTORC2 assembly by progressive sequestration of the intracellular pool of mTOR and subsequently led to inhibition of AKT-signaling [16].

Temsirolimus (CCI-779), everolimus (RAD001), and ridaforolimus (MK-8669) are rapamycin analogs, called rapalogs, developed to overcome its limited pharmacological properties, such as poor water solubility and chemical stability [7] and to obtain drugs with improved pharmacokinetic (PK) properties and reduced immunosuppressive effects. However, they preserve the interactions with FKBP12 and mTOR maintaining a similar mechanism of action based on inhibition of mTORC1 and induction of cell cycle arrest in the G1 phase [17]. Unluckily, in clinical trials conducted in cancer patients they showed only limited benefits. Possible explanations could be not only due to the incomplete block of mTORC1 kinase towards its substrate 4E–BP1 and the rapalog's inability to effectively inhibit mTORC2, but also related to the existence of feedback loops as well as the activation of mechanisms outside the mTOR pathway [18].

Besides rapalogs, second generation mTOR inhibitors have been developed with the aim to have a more potent anticancer activity (Table 1). One class is represented by the so-called selective mTORC1/2 inhibitors which are small molecules working like ATP-competitive inhibitors of mTOR. In particular, they block the phosphorylation of all known downstream targets of both mTORC complexes without inhibiting other kinases. It seems that the greater anti-proliferative and pro-apoptotic effects of these molecules compared to rapamycin and observed in preclinical studies are linked to the complete block of 4E–BP1 phosphorylation and to the decreased protein expression of cyclin D1 and D3 as well as to a significant induction of p27 [19, 20]. Another class of small molecules able to inhibit mTOR is the mTOR and PI3K dual-inhibitors. With respect to the other compounds they do have the advantage to target all the three key enzymes,

Table 1 Second generation mTOR inhibitors

Compound	Company	Generic name	Phase	Disease	Mechanism of action
AZD8055	AstraZeneca		I–II	AST, GBM, HCC, Lymphomas	Selective mTORC1/2 inhibitors
CC-223	Celgene		I–II	AST, NSCLC, DLBCL	Selective mTORC1/2 inhibitors
MLN0128, INK128, TAK-228	Intellikine	Sapanisertib	I–II	AST, Lymphoma, ALL, MM, WM	Selective mTORC1/2 inhibitors
OSI-027	OSI pharmaceuticals		I	AST, Lymphomas	Dual PI3K/mTOR inhibitors
NVP-BEZ235	Novartis	Dactolisib	I–II	Breast, Renal, Prostate, GBM, Sarcoma, Pancreatic, Leukemia	Dual PI3K/mTOR inhibitors
Pf-05212384 (PKI-587)	Pfizer	Gedatolisib	I–II	Breast, Colorectal, AST, AML/MDS	Dual PI3K/mTOR inhibitors
XL147 (SAR245408)	Exelixis/ Sanofi-Aventis	Pilaralisib	I–II	Breast, lung, endometrial, GBM, lymphomas	Dual PI3K/mTOR inhibitors
XL765 (SAR245409)	Exelixis/ Sanofi-Aventis	Voxtalisib	I–II	Breast, lung, GBM	Dual PI3K/mTOR inhibitors
GDC-0980	Genentech	Apitolisib	I–II	Breast, renal, endometrial, colorectal, prostate, lymphomas	Dual PI3K/mTOR inhibitors

AST advanced solid tumors, *GBM* glioblastoma multiforme, *HCC* hepatocellular carcinoma, *NSCLC* non-small cell lung cancer, *DLBCL* diffuse large B-cell lymphoma, *AML/MDS* acute myeloid leukemia/myelodysplastic syndrome, *ALL* acute lymphoblastic leukemia, *MM* multiple myeloma, *WM* Waldenstrom Macroglobulinemia

PI3K, Akt, and mTOR. Thus, they potentially overcome the known feedback loops occurring with rapalogs and being active in tumors with alterations downstream of PI3K but upstream of mTOR [21]. Unluckily, the results in clinical trials are not consistent with the ones obtained in preclinical studies carried out in several types of cancers using these molecules [22].

Recently, mTOR resistance mutations to both rapalogs and kinase inhibitors of mTOR have been identified. To overcome this resistance, a third generation mTOR inhibitors have been developed. This compound was called Rapalink in order to create a bivalent interaction that exploits the unique juxtaposition of two drug-binding pockets that contain rapamycin cross-linked with a kinase inhibitor of mTOR in the same molecule [23].

Pharmacokinetics of Rapalogs

Rapamycin and rapalogs have complex pharmacokinetics [24]. The use of rapamycin in cancer treatment has been largely limited by its intrinsic chemical stability. Thus, rapamycin chemical structure has been modified to increase its water solubility and bioavailability by adding a moiety at position C43. In particular an ester, an ether, or a phosphonate group creates temsirolimus, everolimus, and ridaforolimus, respectively (Fig. 2).

Rapamycin and its derivatives are substrates for the CYP3A4 pathway [25]. Temsirolimus is quickly metabolized through de-esterification in the liver to form its primary metabolite sirolimus. However, temsirolimus is not considered a prodrug for sirolimus, as both agents are pharmacologically active. Everolimus is also metabolized, mainly in the gut and liver, but even if six main metabolites have been

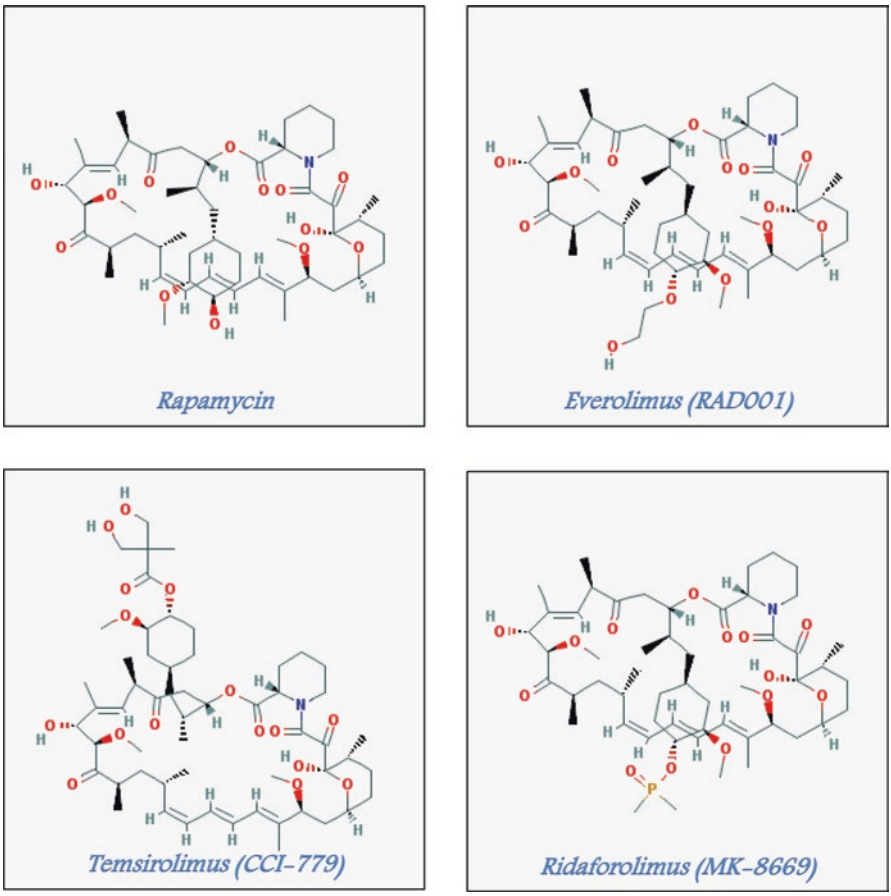

Fig. 2 Chemical structure of rapamycin and rapalogs

identified following its administration, everolimus is the main circulating component in human blood. As a result of their metabolism by isoenzymes of the CYP3A pathway, drugs that are substrates, activators, and inhibitors of these enzymes like rifampicin, anticonvulsants, and immunosuppressive compounds such as cyclosporine could potentially interact with rapalogs [26]. Moreover, due to the liver metabolism, both temsirolimus and everolimus require dose adjustments in patients with hepatic impairment while no correction is required in the presence of renal function alteration. Liver metabolism interferes also with the route of administration. Indeed, while intravenous (i.v.) rapalogs like temsirolimus and ridaforolimus display predictable pharmacokinetics with a high distribution volume and low interpatient variability, the pharmacokinetics of everolimus may be subjected to first-pass metabolism in the liver as well as influenced for absorption and bioavailability by the gastrointestinal tract (i.e. expression of ATP-binding cassette membrane transporters in the gut) [27].

Toxicity

The use of mTOR inhibitors as all the anti-cancer agents has been linked to the possibility of developing adverse events (AEs) that require specific management [28]. They can be directly mediated by the mTOR inhibitors antiproliferative effect [29] or driven by their ability to block a specific pathway [30].

Pneumonitis

Pneumonitis, or interstitial lung disease (ILD), is a potential complication of mTOR inhibitors [31]. The reported incidence varies widely as a result of a non uniform diagnostic criterium and active surveillance. Two main mechanisms for the pathophysiology of mTORi-induced ILD have been proposed. First, a directly toxic effect has been suggested since pulmonary toxicity appears to be a dose-related effect. Alternatively, an immunological origin is suggested by the high numbers of CD4+ T cells and eosinophils found in the BAL fluid of patients with ILD. In particular, three mechanisms are proposed: exposition of cryptic antigens, delayed-type hypersensitivity reaction and cytokine production. The diagnosis of mTORi-induced ILD is often difficult as clinical, radiological and pathological features are nonspecific and often are not distinguishable from respiratory infections. Thus, ILD should be a diagnosis of exclusion and diagnostic work up cannot be limited to x-ray or CT-scan but needs to include bronchoalveolar lavage (BAL) and pulmonary function tests (PFT). The onset typically occurs within 2–6 months after treatment initiation. The most common symptoms of ILD are nonspecific and include dyspnea, (dry) cough, fever, fatigue, hypoxia and occasionally hemoptysis. PFTs should be performed prior to starting mTOR inhibitor therapy to confirm a normal baseline

organ function. mTOR inhibitors should be avoided in patients with significant pulmonary fibrosis or severe chronic obstructive pulmonary disease. The optimal management of ILD is essential to balance the risk of iatrogenic morbidity with the maximum efficacy using mTORi in treating cancer patients.

Metabolic Adverse Events

Hyperglycemia and hyperlipidemia are the metabolic AEs registered in patients treated with mTOR inhibitors [32].

Mammalian target of rapamycin (mTOR) inhibitors are associated with a high incidence of hyperglycemia, ranging from 13% to 50%. In particular, Grade 3 to 4 hyperglycemic events occurred in 12% of patients treated with everolimus, and in 11% of patients treated with temsirolimus. The pathophysiology of mTOR inhibitor-induced hyperglycemia and new-onset diabetes mellitus (NODM) is complex and linked to the interaction between mTOR downstream target S6 K1 with growth factors, hormones, and nutrients.

mTOR inhibitors directly act on pancreatic β-cells with a reduction in glucose-stimulated insulin secretion. On the other hand, they also seem to improve peripheral insulin resistance. Preclinical data in muscle cells showed that long-term rapamycin treatment is able to promote β-oxidation of fatty acids while diminishing basal glucose transport and glycogen synthesis [33].

A similar mechanism has been proposed for hyperlipidemia. In primary cultures of rat hepatocytes, rapamycin has been shown to affect glucose uptake and glycogen synthesis switching the metabolic preference to fatty acids as a metabolic fuel, thus, stimulating lipolysis and producing high serum levels of fatty acids [34]. Another pathophysiologic mechanism through mTOR inhibitors that may cause hyperlipidemia is an impaired lipid clearance via inhibition of insulin-stimulated lipoprotein lipase (LPL) and a significant reduction in the fractional catabolic rate of very LDL apoB100 (a triglyceride-rich lipoprotein).

Levels of lipids and glucose (preferably fasting) should be performed before starting and regularly during treatment with mTOR inhibitors. In the case of onset of metabolic AEs management strategies are similar for all causes of diabetes and hyperlipidemia. Interventions such as diet, exercise, and specific drugs (lipid-lowering agents, oral antihyperglycemic agents or insulin) should be initiated based on lipids and glucose levels.

Hematological Toxicities

An alteration in the IL-10-dependent inflammatory auto-regulation seems to be responsible of mTOR inhibitor-related anemia. In particular, it may promote disruptions in iron homeostasis and gastrointestinal iron absorption as well as effects

on erythroid progenitor cell differentiation and/or erythropoietin receptor-mediated proliferation [35]. Anemia is generally mild, dose-dependent, and reversible upon discontinuation of treatment. The onset is generally within a month of initiation and is sustained throughout treatment. If detected, other causes of anemia have to be screened (i.e. occult blood in stools and vitamin B12 and folate levels). Oral or intravenous iron supplementation and erythropoiesis-stimulating agents should be effective for managing mTOR inhibitor-associated anemia, if not treatment needs to be discontinued.

Thrombocytopenia and leucopenia/neutropenia have been reported with mTOR inhibitor therapy. These AEs frequently occur simultaneously and usually resolve spontaneously. Complete blood counts should be performed routinely. Management is similar to that used for chemotherapy related-hematological toxicities. Grade 3 or higher neutropenia or thrombocytopenia may require temporary interruption of mTOR.

mTOR Inhibitors Associated Stomatitis (mIAS)

The incidence of mIAS varies widely (2–78%). As reported across multiple mTOR inhibitor clinical trials, grade 3/4 toxicities occur in up to 9% of patients. mIAS typically presents as distinct, painful, ovoid, superficial ulcers surrounded by a characteristic erythematous margin and due to a direct toxic effects of mTOR inhibitors on oral and nasal mucous membranes [36]. It resembles recurrent aphthous ulceration not only in clinical presentation but also in response to therapy.

Prophylactic strategies, including oral hygiene and avoiding injury to the epithelium of the oral cavity, are recommended. Topical high-potency corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs) and anesthetics can be used to promote healing and reduce pain, but severe resistant mIAS could require systemic corticosteroids. Moreover, if grade 2 or higher mIAS restricts oral intake of nutrients, in such cases, mTOR inhibitor dose reduction/discontinuation may be considered.

Mechanisms of Resistance

Mutations of mTOR

Similarly to what happen in patients treated with kinase inhibitors acquired resistance mutations have been reported in cells exposed to mTORC1 inhibitors [23]. The MCF-7 breast cancer cell line was exposed to high concentrations of either

rapamycin or a second-generation mTOR inhibitor (AZD8055) for 3 months. Subsequent deep sequencing of the emerged resistant colonies revealed clones harbored mutations located in the FKBP12–rapamycin-binding domain (FRB domain) or in the kinase domain. The clinical relevance of these mutations is supported by a case report of a patient who acquired an identical mTOR mutation after relapse while under treatment with everolimus [37] as well as by their observation in untreated patients.

Mutations that conferred resistance to ATP-competitive inhibitors of mTOR did not alter binding of the drug to mTOR but generated a hyperactive state of the kinase that can affect both mTORC1 and mTORC2. On the other hand, some of the identified hyper-activating mutations of mTOR are associated with increased sensitivity to rapamycin, suggesting that cancer cells harboring such mutations have an mTOR-dependent proliferation pattern. Interestingly, in a case report of a primary refractory HL, damaging mutations of the *TSC2* gene was considered responsible to the increased mTOR pathway activation and, thus, to the impressive clinical response observed using everolimus [38].

Genetic and Functional Heterogeneity

Genetic tumor heterogeneity is a well know concept in cancer biology. Both immunohistochemical staining and genome sequencing have been demonstrated that cancer cells displaying a high mTOR activity coexist with cancer cells having low mTORC1 activity in the same tumor. This observation has also been extended to primary tumor and distant metastases [39]. Moreover, genetic tumor heterogeneity has been reported for proteins that belong to signaling pathways upstream to mTOR such as PI3K/AKT and Ras/Raf/MEK/MAPK pathways.

Along with genetic alterations, a functional heterogeneity has been described for downstream effectors of mTOR like S6 K1 and 4E–BP1. An in vitro study on human colorectal cancer demonstrated that phosphorylation of S6 K1 and 4E–BP1 rarely occurs in the same cancer cell but rather shows mutual exclusivity [40]. Thus, since rapalogs do not block mTORC1-mediated 4EBP1 phosphorylation of cancer cells with a phospho-S6^{low}/phospho-4E-BP1^{high} pattern might be intrinsically resistant to rapalogs despite the presence of mTORC1 activity.

Finally, mTORC activity could be affected by micro-environmental conditions like oxygen levels [41] and pH values [42]. In both cases a downregulation of mTORC1 activity is registered, thus, cancer cells xhibit an mTORC1-independent growth and are therefore resistant to mTORC1 inhibition. Of note, hypoxia not necessarily leads to mTORC1 inhibition. For example, tumor cells harboring low levels of Ataxia Telangiectasia Mutated (ATM) protein, display a paradoxically elevated mTORC1 activity in hypoxic tumor regions. In particular, ATM is the

driver of a cascade comprising HIF1α and REDD1 which inactivates mTORC1 activity in a TSC1/TSC2 dependent mechanism [43].

Alternative Proliferation Pathways

There is a complex network of regulatory feedback loops responsible for limiting the proliferative signals transmitted by upstream effectors once mTORC1 is activated. Thus, once mTORC1 is inhibited, these negative feedback loops are stopped and alternative proliferation pathways like PI3K/AKT and RAS/RAF/MEK/MAPK are free to contrast the anticancer efficacy of rapalogs. This concept has been demonstrated in the preclinical setting, in which some data showed that blocking AKT or MAPK potentiated the anticancer efficacy of rapalogs [44].

Molecular Mechanisms of mTOR Activation in Lymphomas

Aberrant activation of the mTOR pathway is a marker of more aggressive disease and poorer prognosis in both Hodgkin (HL) and non-Hodgkin lymphomas (NHLs). As already discussed, this condition can be related to mTOR specific biology but it is often linked to alterations in key upstream pathway(s) [45–47].

For example, in a subset of MCL, mTOR directly mediates Cyclin D1 downregulation trough glycogen synthase kinase (GSK)-3β [46], while other authors described PTEN inactivating phosphorylation as the key mechanism responsible for the PI3K/Akt/mTOR pathway activation. Moreover, a similar mechanism has been described in HL too [48].

Activated B-cell DLBCL (ABC-DLBCL) cell lines activate S6 K1, a downstream target of mTOR, independently from Akt either through up-regulation of PIM2 or through activation by B cell receptor (BCR) signaling components [47]. Conversely, loss of PTEN has been described to correlate with the PI3K/Akt/mTOR pathway activation in germinal center B-cell-like DLBCL (GCB-DLBCL). Of note, mTOR mutations have been described in DLBCL samples [49]. Instead, phosphorylation of Akt is common in T cell lymphoma [50].

Summary of Clinical Trials

Based on the encouraging preclinical in vitro and in vivo data [51–53] clinical trials using rapalogs have been carried out in hematological malignancies and, in particular, in lymphoproliferative disorders.

Temsirolimus

Temsirolimus has been widely investigated in hematological malignancies alone or in combinations. In lymphoma setting, it has been firstly used as single agent in a phase II trial at 250 mg/m^2 weekly in 34 patients with relapsed MCL. The overall response rates (ORR) was 38% with 1 (3%) complete response (CR) and 12 (35%) partial response (PR). The median time-to-progression in all patients was 6.5 months and the duration of response for the 13 responders was 6.9 months. Hematological toxicities were the most common adverse events (AEs) with thrombocytopenia occurring in all patients and being the most frequent cause of dose reductions even if usually resolving in 1 week. Hyperglycemia, increased triglycerides, mucositis, and fatigue were also registered [54]. A lower dose of 25 mg/m^2 weekly has been evaluated in a subsequent clinical trial with the aim to reduce the previous registered events. The ORR was similar (41%) and severe thrombocytopenia was less common (100% vs. 39%) [55]. The encouraging results of these phase II trials (RR of around 40%) pave the way for a large randomized phase III trial [56] in relapsed/refractory MCL patients. The higher doses in the temsirolimus arm (175 mg weekly for 3 weeks followed by 75 mg weekly) were significantly better than the investigator's choice both in ORR (22.2% vs 2%) and progression free survival (PFS), but the results were poorer than those reported in the phase II trial. However, data were considered consistent enough to obtain the European license for this indication. Recently, it has been published another phase III trial in relapsed/refractory MCL patients in which the standard of care temsirolimus has been used as a control arm compared to ibrutinib [57]. The primary efficacy analysis showed a significant improvement in PFS and a safer profile for patients treated with ibrutinib (median PFS 14.6 months vs 6.2 months). Moreover, an independent review committee-assessed overall response rate (ORR) was significantly higher for ibrutinib (71.9% vs 40.4%; $p < 0.0001$) with a CR rate of 18.7% vs 1.4%, respectively. Median treatment duration was 14.4 months for ibrutinib and 3.0 months for temsirolimus. Safety profile was favorable for the ibrutinib arm too. Reported grade 3 or higher treatment-related adverse events were lower with 94 (68%) versus 121 (87%) patients involved. Moreover, less patients discontinued treatment due AEs in the ibrutinib arm (25.5% vs 6.5%). Single agent temsirolimus has also been investigated in relapsed/refractory (Rel/Ref) primary CNS Lymphoma (PCNSL). A relatively high RR (54%) was observed but PFS (median PFS 2.1 months) was comparable with other studies [58]. Of note, treatment-associated mortality was considerable (13.5%). The authors interpretation is that frequent administration of steroids before response assessment as well as compromised condition of enrolled patients could be potential confounding factors for response evaluation and outcome. The most common AEs ≥3 grade were hyperglycemia (29.7%), thrombocytopenia (21.6%), infection (19%), anemia (10.8%), and rash (8.1%). Interestingly, neither drug nor its main metabolites were found in the CSF except in one patient in the 75-mg cohort who had 2 ng/ml of temsirolimus.

Temsirolimus has been combined with different drugs in different settings.

Combination of temsirolimus and bortezomib has been assessed in heavily pretreated Multiple Myeloma [59] and B-Cell Non-Hodgkin Lymphoma [60] patients. In both studies, the enrolled subjects received i.v. bortezomib (1.6 mg/m2) weekly on days 1, 8, 15, and 22 along with i.v. temsirolimus (25 mg) weekly on days 1, 8, 15, 22, and 29 every 35 days. Fourteen of 43 (33%) MM patients had a PR or better. Moreover, the authors noted a difference in bortezomib-responsive versus refractory patients to previous treatment with bortezomib suggesting that the combination might not completely overcome resistance or re-sensitize MM cells that are resistant to the proteasome inhibitor. On the other hand, the ORR in the Lymphoma setting was 31% (12 of 39 patients; 3 CR and 9 PR) while the median PFS was 4.7 months. Although the patients with Diffuse Large B-Cell Lymphoma (DLBCL) had a low ORR, 2 heavily pretreated patients achieved a CR after 2 cycles of therapy and both maintained remission for 7 months after the completion of protocol therapy. The underlying genetic heterogeneity of DLBCL has been suggested by the authors as presumably responsible for the wide variation observed in responses. There were no unexpected toxicities from the combination. AEs were generally manageable and similar with those reported with temsirolimus and bortezomib alone, in both studies.

The incorporation of temsirolimus in the doublet rituximab/bendamustine has been recently reported in a phase I study of Rel/Ref FL and MCL [61] showing promising preliminary activity especially in MCL along with a safety profile. An objective response was observed in 14/15 patients (93%), including 5 CR (33%; all MCL). Ongoing studies are assessing the temsirolimus combination with Rituximab and DHAP in patients with Rel/Ref DLBCL (NCT01653067) [62] and temsirolimus plus lenalidomide in relapsed NHLs (NCT01076543).

Everolimus

Like temsirolimus, the oral drug everolimus has been used as single agent in Rel/Ref aggressive and indolent NHLs [53, 63–65] as well as HL [66]. Recently, a phase II study has been carried out using oral single-agent everolimus in relapsed/refractory indolent lymphomas, mostly chronic lymphocytic leukemia (CLL) and follicular lymphoma (FL) [67]. Eligible patients received oral everolimus 10 mg daily on a 28 day-cycle schedule. The ORR in all 55 patients was 35% (19/55) with 4% (2/55) CRu, and 31% (17/55) PR; 36% (20/55) had stable disease. The median time to response was 2.3 months (range, 1.4–14.1) and the median DR was 11.5 months (95% CI, 5.7–30.4). The ORR was higher in FL (61%) than in CLL/SLL (19%). The median PFS and OS were 7.2 months and 29.4 months, respectively. Everolimus was well-tolerated with modest hematologic toxicity. Of note, two patients died of sepsis related to the drug. Thus, the authors concluded by suggesting further studies with mTORC1 inhibitors such as everolimus as single agent, and in

combination with other agents. The addition of alemtuzumab to everolimus in rel/ref CLL has been published recently too, but based on their results (33% partial responses, no complete responses) no further development of this regimen was recommended by the authors [68]. Another phase II trial evaluated the activity and safety of everolimus in Rel/Ref marginal zone lymphomas (MZLs) [69]. Thirty patients received everolimus for six cycles or until dose-limiting toxicity or progression. Twenty-four out of 30 patients were evaluable and a relevant proportion experienced side effects, resulting in dose reduction (9 patients) and/or early treatment discontinuation (10 patients). ORR was 25% (1 CR and 5 PRs). Moreover, one toxic death due to treatment-related pneumonia was recorded. Thus, due to the moderate antitumor activity and the observed toxicity, it seems that single agent everolimus has limited therapeutical space in this indolent setting. Of note, it has also been carried out a phase III trial of everolimus in monotherapy as maintenance (PILLAR-2; NCT00790036) providing 1 year of adjuvant everolimus to poor-risk (IPI] ≥3) in DLBCL patients who had achieved a CR with R-chemo. No differences have been observed in the 2-yr DFS rate (78% vs 77%) even if it seemed that everolimus had a trends toward OS and DFS in selected patient subgroups (males and IPI 4/5). However, also in this setting the responses were modest, transient and in some cases toxicity was relevant [70].

Conversely from what happened in CLL/SLL, the combination of everolimus with other drugs seems to be promising. Based on the encouraging results of the preclinical data [71] showing that combining panobinostat with the mTOR inhibitor everolimus inhibited panobinostat-induced mTOR activation and enhanced panobinostat antiproliferative effects in HL cell lines, a combination of these two drugs has been carried out in a phase I trial [72]. ORR 43% with CR 15% while the dose-limiting toxicity was thrombocytopenia (grade3/4 64%). Similarly, after a phase I trial, a phase II study of everolimus in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) as a first-line treatment for patients with peripheral T-cell lymphoma (PTCL) has been published [73]. Five (5) mg everolimus per day from day 1 to 14 every 21 days for a total of six cycles has been administered. A difference in the CR rate among subtypes was observed and was associated with PTEN loss evaluated by immunohistochemistry. Objective response rate was very high (90%; CR (n = 17) and PR (n = 10)).

Another combination has been tested in a phase I/II trial in which everolimus has been added to rituximab with or without bortezomib in Rel/Ref Waldenström's Macroglobulinemia (WM) [74]. Forty-six patients have received six cycles of both the combinations followed by maintenance with everolimus until progression. Thirty-six (78%) of the 46 patients enrolled received full dose therapy (FDT) of the three drugs. Promising results that deserve to be assessed in future trials on a larger randomized trial has been showed with an ORR of 89% (32/36 patients) with two CR (6%) and 19 PR or better response (53%). No dose-limiting toxicities have been observed in the phase I of the trial. No unexpected toxicities was recorded. Moreover, of note, 98% of registered patients had previously received rituximab, and 57% had received bortezomib.

Ridaforolimus

Only two clinical trials need to be cited on Ridaforolimus. In the first one drug has been investigated in a phase II clinical trial as monotherapy in 55 patients with Rel/Ref hematological malignancies. Drug was used as 30-min infusion on days 1–5 of a 2 weeks cycle. Of note best response was PR and it was observed only in two subsets of hematological malignancy, 29% in agnogenic myeloid metaplasia (AMM) and 33% in MCL. The most frequent grade 3/4 AEs were similar to those observed with other mTOR inhibitors, in particular mouth sores (15%), thrombocytopenia (15%), hyponatremia (7%) and hypokalemia (6%) [75]. On the other hand, the second one is a phase I study in which ridaforolimus is evaluated in combination with vorinostat in patients with advanced solid tumors or lymphoma (NCT01169532).

Second Generation mTOR Inhibitors

AZD8055 is a first-in-class dual mTORC1/mTORC2 inhibitor. In preclinical models it was shown to prevent the mTORC2-mediated AKT activation observed with rapalogs [76]. In a phase I study of 49 patients with advanced solid tumors or lymphomas (NCT00731263) [77]. MTD was 90 mg BID. The most frequent AEs were elevated transaminases (22%) and fatigue (16%). Interestingly, metabolic AEs like hypercholesterolemia nor hypertriglyceridemia were not registered as observed with other mTORC1/mTORC2 inhibitors [78, 79]. The best response was SD in 7 patients for ⩾4 months.

The results of Part A of a phase I/II study on the dual mTORC1/mTORC2 kinase inhibitor CC-223 in 28 pretreated patients with advanced solid tumors or MM has been recently published [78]. The MTD was 45 mg/d, although 11.1% of patients at the MTD required dose reductions and 55.6% required interruptions. Hyperglycemia was the most common grade 3 AE (18%). Substantial pS6 K1 (>70%), p4E–BP1 (>40%), and pAKT (>50%) inhibition was observed at ≥30 mg CC-223, although pS6 K1 and pAKT inhibition was more complete than p4E–BP1 inhibition. Additionally, preliminary evidence of inhibition of pS6 K1, p4E–BP1, pAKT, and proliferation marker Ki-67 was observed in paired tumor biopsies in 2 patients. The authors reported one PR (3.6%) lasting 220 days in 1 patient with breast cancer and 8 patients (29%) with SD (>100 days in 5 patients), including 2 patients with tumors exhibiting molecular abnormalities associated with mTORC pathway activation. Part B focused on dose expansion into parallel cohorts of selected tumor types (MM, DLBCL, and selected solid tumors) is ongoing (NCT01177397).

TAK-228, another dual mTORC1/mTORC2 kinase inhibitor, has been tested in a phase I study including 39 patients with MM (31), NHL (4), and WM (4) [79]. Drug has been administered once daily (QD) at 2, 4, 6, or 7 mg, or QD for 3 days on and 4 days off each week (QDx3d QW) at 9 or 12 mg, in 28-day cycles. Cycle 1

DLTs occurred in 5 QD patients (stomatitis, urticaria, blood creatinine elevation, fatigue, and nausea and vomiting) and 4 QDx3d QW patients (erythematous rash, fatigue, asthenia, mucosal inflammation, and thrombocytopenia). The MTDs were determined to be 4 mg QD and 9 mg QDx3d QW. Thirty-six patients (92%) reported at least one drug-related toxicity; the most common grade ≥3 drug-related toxicities were thrombocytopenia (15%), fatigue (10%), and neutropenia (5%). Of the 33 response-evaluable patients, one MM patient had a minimal response, one WM patient achieved PR, one WM patient had a minor response, and 18 patients (14 MM, 2 NHL, and 2 WM) had SD. Authors concluded saying that further studies including combination strategies need to be carried out.

Preliminary data on BEZ235, a dual PI3-Kinase/mTOR inhibitor in adult patients with RR acute leukemia showing a single-agent anti-leukemic efficacy most pronounced in ALL, with an overall response rate of 30% and a sustained molecular remission in one patient. Since results of PK analysis and assessment of PD markers associated with PI3K signaling did not correlate with response the authors concluded that a more comprehensive genomic analysis may help to identify a subset of patients likely to benefit from treatment with dual PI3K-mTOR inhibitors (NCT01756118) [80].

CC-115, a novel inhibitor of mTOR kinase and DNA-PK, was evaluated in primary CLL cells in vitro and in seven Rel/Ref CLL patients and one SLL patient harboring ATM deletions/mutations enrolled in a larger phase I clinical trial, including 110 additional patients with solid tumors (NCT01353625) [81]. All but one patient had a decrease in lymphadenopathy, resulting in one iwCLL partial response (PR) and three PRs with lymphocytosis. Moreover, the encouraging preclinical data on the ability of CC-115 to revert CD40-mediated resistance to chemotherapy or venetoclax as well as to overcome Idelalisib resistance makes this compound attractive for further combination studies in the clinical setting.

Summary

The PI3K/AKT/mTOR signaling pathway plays a central role in cell growth proliferation and survival controlling different processes in protein synthesis and angiogenesis. Deregulation of this pathway is commonly found in several types of tumors.

Currently, two mTOR inhibitors, everolimus and temsirolimus, are approved by the European Medicines Agency (EMA) and the US Food and Drug Administration to treat cancer patients in clinical practice.

Unluckily the promising results obtained in the preclinical settings using rapalogs did not translate into the expected benefits in clinical trials because response to mTOR inhibitors is not durable and patients ultimately progress because of various mechanisms of resistance. The so-called “second generation mTOR inhibitors” are small molecules developed with the aim to overcome rapalogs weaknesses. However clinical trials results do not seems to differ a lot from those obtained with rapalogs.

Common and serious mTOR inhibitors related side effects include non-infectious pneumonitis, metabolic disorders, hematological and mucosal toxicities. They require specific management in order to balance risk and benefit related to the specific treatment.

Looking forward correlative or translational sub-studies are needed to clearly and quickly identify biomarkers of response and emerging drug resistance in order to maximize the benefit linked to mTOR inhibitors treatment. Moreover, future approaches may consider combinational strategies as a way to overcome such resistance and therefore improve efficacy of mTOR targeting agents in the clinical context.

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