Chapter 11
Potential Future Indication of Rapamycin Analogs for the Treatment of Solid Tumors

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Abstract The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that is a central component of a complex signaling pathway involved in cell growth and metabolism. Thus, mTOR is an attractive target for cancer therapy. Sirolimus and related mTOR inhibitors have proven clinical benefit in otherwise unselected patients with advanced lymphoma, neuroendocrine tumors, renal cell carcinoma, gastrointestinal stromal tumors, and certain neoplasms arising in patients with germline mutations in tumor suppressor genes within the mTOR pathway. Trials evaluating activity in earlier stages of disease and in combination are ongoing. Presently, clinical trials are underway to identify additional malignancies that respond to mTOR inhibitors. To date, the antitumor activity of mTOR inhibitors is limited to a subset of patients. Despite extensive clinical evaluation, no biomarkers have been identified in patients with sporadic cancers. This chapter reviews data from preclinical and clinical studies of mTOR inhibitors in four malignancies, sarcoma, endometrial, and gastric and bladder cancer, and discusses the biomarker of sensitivity and resistance studied in these settings. Future research will evaluate the optimal regimens, schedules, patient populations, and combination strategies for this novel class of agents.

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11.1 Introduction

The mTOR is a serine/threonine kinase that has been evolutionarily conserved from yeast to human and is a component of a complex signaling pathway involved in cell growth and metabolism. In normal cells, there are positive and negative regulators that control the activity of mTOR. Positive regulators, such as growth factors and their receptors (e.g., insulin-like growth factor 1 (IGF-1) receptor, human epidermal growth factor receptor (HER), and vascular endothelium growth factor receptor (VEGF)), transmit signals through the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)-v-akt murine thymoma viral oncogene homolog (AKT)-mammalian target of rapamycin (mTOR) pathway, while negative regulators such as phosphatase and tensin homolog in chromosome 10 (PTEN), TSC1 (hamartin), and TSC2 (tuberin) inhibit signals to this pathway.

In a number of in vitro cell line and in vivo murine xenograft models, aberrant pathway activation through oncogene stimulation or loss of tumor suppressors contributed to tumor growth, angiogenesis, metastasis, and resistance to standard cancer therapy. These features are relevant for the development of cancer therapeutics as aberrant pathway activation could increase sensitivity to agents that target mTOR [14].

As monotherapy, rapalogs have antitumor activity with mild toxic effects. Temsirolimus and everolimus are approved for the treatment of patients with metastatic renal cell carcinoma (RCC), and temsirolimus is also approved for mantle cell lymphoma (MCL). Everolimus is indicated in the treatment of advanced pancreatic neuroendocrine tumors. Multiple trials of single agents and combination regimens involving mTOR inhibitors are currently underway to identify new therapeutic indications and improve the use of these drugs through combinations with standard and other targeted agents. This chapter addresses the clinical development of first-generation mTOR inhibitors in settings in which there is preclinical and clinical evidence of antitumor activity: sarcoma, endometrial cancer, gastric cancers, and bladder cancer.

11.2 mTOR Inhibitors for the Treatment of Sarcoma

Sarcomas are a group of heterogeneous tumors that originate from mesenchymal tissue, such as the bone, cartilage, or connective tissue, as well as the muscle, adipose, peripheral nerves, and blood vessels [1, 69]. Currently, few options exist for the treatment of sarcomas. Standard therapy includes surgery, chemotherapy, and radiotherapy. Patients with unresectable, recurrent, and metastatic diseases are treated with chemotherapy and have poor prognoses.

Aberrant activity in several molecular pathways has been linked to the pathogenesis of various sarcoma subtypes. As a result of the frequent aberrant signaling observed within the PI3K pathway, pharmacological targeting the pathway has been investigated. All inhibitors of mTOR, including sirolimus, temsirolimus, everolimus,

and ridaforolimus, have been assessed for their safety and efficacy in patients with different sarcoma subtypes [44]. There are ongoing phase 2 trials for sirolimus, temsirolimus, everolimus, and ridaforolimus, and results of a phase 3 trial for ridaforolimus as maintenance therapy in sarcoma have been published recently.

Four rapalogs have shown activity in preclinical sarcoma models. Preclinical testing has indicated that sirolimus has single-agent antitumor activity in select sarcoma xenografts [27] and in combination with cytotoxic agents such as cyclophosphamide and vincristine [26]. Temsirolimus treatment was effective in inhibiting tumor growth in murine xenograft models of rhabdomyosarcoma cell lines [19]. The antitumor activity of temsirolimus was associated with a reduction of hypoxia-inducible factor-1 (HIF) 1α levels, VEGF protein expression, and microvessel density, suggesting suppressed tumor growth through an antiangiogenic mechanism. Everolimus has demonstrated antiproliferative activity against several tumor cell lines and in a broad range of human tumor xenografts [9]. In a mouse model of human gastrointestinal stromal tumors (GIST), everolimus inhibited protein translation and cell proliferation in tumor lesions [61]. Treatment with everolimus also decelerated tumor growth and prolonged life span in a mouse model of leiomyosarcoma [25]. Ridaforolimus reduced the rate of cell proliferation in vitro in a panel of 11 sarcoma cell lines and inhibited the rate of tumor growth in a leiomyosarcoma xenograft model [68].

Two phase 1 studies of ridaforolimus showed that 23 % (6/21) of patients with various sarcomas had a clinical benefit response. Two patients (15.4 %) treated with oral ridaforolimus had partial responses (liposarcoma and dendritic cell sarcoma), and another two (28.5 %) patients treated with intravenous ridaforolimus achieved partial responses (mixed Müllerian tumor and Ewing sarcoma) [45, 46]. Rapid and potent mTOR inhibition was observed in peripheral blood monocellular cells from all patients tested.

Three rapalogs were evaluated in phase 2 studies in sarcoma patients (Table 11.1). Temsirolimus as single agent and combination therapy with cixutumumab was evaluated in two phase 2 studies, and overall 11 partial responses were reported (undifferentiated fibrosarcoma of the thigh, leiomyosarcoma of the uterus, one in the IGF-1R-positive soft tissue sarcoma group, six in the IGF-1R-positive bone sarcoma group, and two in the IGF-1R negative group) [54, 64]. Everolimus was studied in a phase 2 study in patients with soft tissue sarcoma (STS) or bone sarcoma, but limited clinical efficacy was observed. Among 30 evaluable patients, efficacy was seen in 2/15 patients (13% arm I) and 4/15 patients (27% arm II) [60]. Everolimus has also been studied in combination with imatinib in patients with imatinib-resistant GIST [63]. Among 23 evaluable patients, four were progression-free at 4 months. An ongoing phase 2/3 clinical trial is further evaluating the benefit of combined treatment with everolimus and imatinib in patients with progressive GIST.

Ridaforolimus has been the rapalog most extensively tested in sarcoma. Two phase 2 trials in patients with advanced sarcomas enrolling over 300 patients have reported six partial responses (two osteosarcoma, one spindle cell sarcoma, one malignant fibrous histiocytoma, one liposarcoma, and one follicular dendritic cell

Table 11.1 Phase II and III trials in sarcoma

Agent	Phase	Clinical trial no.	Number of patients	Objective response rate (ORR, %)	Progression-free survival (PFS)	Overall survival (OS)	Reference
Temsirolimus	2	NCT00087074	41	5	2 months – median time to progression (TTP)	7.6 months	[54]
Cixutumumab + temsirolimus	2	NCT01016015	57 + 63	NA	A: 6.9 weeks B: 10.6 weeks C: 11.6 weeks median PFS	A: 18.9 B: 14.2 C: 14.7 months	[64]
Everolimus and imatinib	1–2	NCT00510354	Strata 1: 28 Strata 2: 47	NA	Strata 1: 17 % Strata 2: 37 % PFS at 4 months; 1.9 and 3.5 months median PFS	Strata 1: 14.9 Strata 2: 10.7 % median OS	[63]
Everolimus	2	NCT00767819	61	Arm 1: 13 % Arm 2: 27 %	NA	NA	[60]
Ridaforolimus	2	NCT01010672	212	CBR –28.8 %	15.3 weeks – median PFS	40 weeks – median OS	[11]
Ridaforolimus	1/2a	NCT00112372	147 (85 sarcoma)	24.5 % – all pts. 27.1 % – sarcoma pts.	12.1 % – all pts. 17.1 % –sarcoma pts.	NA	[47]
Ridaforolimus	3	NCT00538239	711	1.3 % decrease in target lesion size vs. a 10.3 % increase with placebo	Ridaforolimus arm: 17.7 % vs. placebo arm: 14.6 weeks – median PFS	Ridaforolimus arm: 90.6 weeks vs. placebo arm: 85.3 weeks – median OS	[15]

CBR clinical benefit response

sarcoma) [11, 47]. The pivotal Sarcoma Multicenter Clinical Evaluation of the Efficacy of Ridaforolimus (SUCCEED) was designed to determine whether oral ridaforolimus can be used to maintain disease stability in the metastatic setting [15]. Among 711 patients enrolled, ridaforolimus treatment led to a statistically significant improvement in progression-free survival (PFS) compared with placebo (median PFS, 17.7 versus 14.6 weeks). Median overall survival (OS) with ridaforolimus was 90.6 weeks versus 85.3 weeks with placebo. Single-agent ridaforolimus was associated with a 29 % clinical benefit rate and 2 % partial response rate. Adverse events (AE) more common with ridaforolimus included stomatitis, infections, fatigue, thrombocytopenia, noninfectious pneumonitis, hyperglycemia, and rash. These toxicities are as expected for mTOR inhibitors.

In conclusion, mTOR inhibition in sarcoma patients may induce stable disease and, in a subset of patients, partial responses. The rarity of complete responses in patients indicates a cytostatic rather than cytotoxic effect for mTOR inhibition except in a small and as yet undefined subset of patients.

11.3 mTOR Inhibitors for the Treatment of Endometrial Carcinoma

Endometrial cancers are the most common gynecologic cancers in developed countries and third most common cause of gynecologic cancer death [48, 49]. Endometrial carcinomas are classified as type I and type II, based on clinical features and pathogenesis. Type I endometrial cancers occur most commonly in pre- and perimenopausal women often with a history of endometrial hyperplasia and exposure to elevated levels of estrogen. Type I endometrial carcinoma has an endometroid histology and is characterized by the presence of progesterone receptors and a benign biological behaviour. Type II endometrial carcinomas comprises types with high-grade serous and clear cell histologies, reduced/lack expression of progesterone receptors and originate from the mucosa, independently of hormonal stimulation [49]. Surgery is the primary treatment for resectable disease. Chemotherapy and radiation may be offered to women with high risk of recurrence following surgery. Chemotherapy and hormonal agents may be offered in the setting of recurrent/metastatic disease [48, 49].

Activation of the PI3K pathway occurs frequently in endometrial carcinoma through mutations in the catalytic and regulatory subunits of PI3K (PI3KCA, PI3KR1) and PTEN, suggesting an important role of these genes in the tumorigenesis [17]. Preclinical studies with ridaforolimus demonstrated antiproliferative activity in endometrial tumor cell lines [68]. In a mouse PTEN heterozygous model, everolimus significantly reduced endometrial hyperplasia and the proliferation index and significantly increased apoptosis compared with control [42].

Three rapalogs, everolimus, temsirolimus, and ridaforolimus, have been evaluated for activity in patients with recurrent/metastatic disease with/without prior chemotherapy (Table 11.2). In total, six phase 2 single-agent and one combination

Table 11.2 Phase 2 trials in endometrial carcinoma

Agent	Phase	Clinical trial no.	Number of patients	Median duration of nonprogressive disease (months)	Median progression-free survival (PFS, months)	Median overall survival (OS, months)	Reference
Everolimus	2	NCT00870337	44	Response: 3.1 SD: 4.3	2.8	8.1	[59]
Everolimus	2	NCT00087685	35	4.5	NA	NA	[66]
Temsirolimus	2	NCT00072176	Arm 1: 33 (chemotherapy-naïve disease)	Response: 5.1 SD: 9.7	7.33	NA	[55]
			Arm 2: 27 (1 chemotherapy regimen)	Response: 4.9 SD: 3.8	3.25	NA	
Temsirolimus + hormone therapy	2	NCT00729586	20 (temsirolimus alone arm)	NA	NA	NA	[22]
Ridaforolimus	2	NCT00122343	45	Response: 29 SD: 4	Na	NA	[12]
Ridaforolimus	2	NCT00770185	35	SD: 53	NA	NA	[37]
Ridaforolimus	2	NCT00739830	64	NA	5.6	9.6	[56]

SD stable disease, *NA* not available

studies in patients with endometrial carcinoma have been reported. Among 44 patients with advanced endometrial cancer refractory to one or two chemotherapy regimens who received everolimus, there was a 36 % 3-month nonprogressive disease rate [59]. Four patients experienced partial responses. In a second trial, of 35 previously treated patients, the nonprogressive disease rate at 8 weeks was 43 %, and the median duration of nonprogressive disease was 4.5 months [66]. Median PFS was 2.8 months, and median OS was 8.1 months. The most common adverse events were anemia, fatigue, hypercholesterolemia, and lymphopenia. Thus, everolimus demonstrated some evidence of antitumor activity and acceptable tolerability in patients with chemotherapy-refractory advanced or metastatic endometrial cancer.

Temsirolimus has been evaluated in two phase 2 trials. The first trial included patients who were chemotherapy naïve (group A) or who had received one prior line of chemotherapy for recurrent disease (group B) [55]. In the chemo-naïve group, four patients (14 %) had a confirmed partial response. In the chemotherapy-treated group, one patient had a confirmed partial response (4 %). Neither the loss of PTEN protein expression nor PTEN mutations evaluated from archival tumor specimens correlated with response. In the second trial, 3 of 21 previously treated patients had partial responses [22].

Ridaforolimus has been evaluated in two single-arm and one randomized phase 2 trials. In the first uncontrolled trial, there were two partial responses among 31 patients with endometrial carcinoma who had no prior chemotherapy [37]. In the second trial of 45 previously treated patients, 13 of 45 patients (29 %) had clinical benefit: 5 (11 %) with confirmed partial responses and 8 (18 %) with prolonged stable disease [12]. No correlation between PTEN protein expression and/or PIK3CA/AKT mutations and outcome was found. The interim report of the randomized phase 2 clinical trial comparing oral ridaforolimus with either hormonal therapy ($n=53$) or chemotherapy ($n=13$) [56] showed a median PFS of 3.6 months for patients receiving ridaforolimus compared to 1.9 months for those patients treated with hormonal therapy. No objective responses were reported for ridaforolimus. Ridaforolimus treatment was associated with higher toxicity rates, for hyperglycemia (19 %), fatigue, diarrhea, anemia, and mucositis. The results of these studies with ridaforolimus, everolimus, and temsirolimus suggest that mTOR inhibitors have consistent but modest single-agent clinical benefit in advanced and recurrent endometrial cancer.

11.4 mTOR Inhibitors in Gastric Cancers

Stomach cancer is the fourth most commonly diagnosed cancer and the second leading cause of cancer death worldwide [21]. Current management of localized gastric cancer is surgical resection with or without radiation and chemotherapy [40]. For patients with advanced unresectable disease and for patients that develop recurrent disease after surgery, chemotherapy may prolong survival and quality of life

[2]. However, long-term outcomes of patients with advanced gastric cancer are poor, and thus, there is a need for novel targeted agents that may confer a better survival benefit.

Preclinical studies have shown dysregulation of mTOR activity in gastric cancer cell models and suggest that mTOR is a rational therapeutic target [3]. Mutations in upstream regulators of the mTOR signaling pathway, such as EGFR, amplification of human epidermal growth factor receptor 2 (HER2), PI3K, and PTEN, have been observed in patient-derived gastric tumor samples [13, 74]. Overexpression of the mTOR downstream effectors elf-4E and 4E-BP1 was shown in gastrointestinal cancer cells and primary tumors [16]. Others have shown that expression of phosphorylated mTOR protein in human gastric carcinomas correlated with tumor progression and poor survival [28, 34, 50]. Oncogenic transformation in tumors occurs with dysregulation of the mTOR pathway [8]. In addition, pharmacological inhibition of the PI3K pathway may induce an antitumor effect. Treatment of gastric cancer cell lines with the mTOR inhibitors sirolimus or everolimus was associated with an antiproliferative effect and decrease in phosphorylation of ribosomal protein S6 kinase 1 (S6K1) and 4E-BP1 and a reduction of HIF-1α and VEGF [10, 23, 39]. Everolimus treatment resulted in G1 cell cycle arrest and inhibited the proliferation of gastric cancer cell lines [35]. Consistent with the antiproliferative effects observed in vitro, mTOR inhibitors alone or in combination with other agents significantly delayed tumor progression in xenograft models of gastric cancer [10, 34].

Currently, everolimus is the only mTOR inhibitor that has been investigated in phase 1/2 clinical trials of patients with advanced gastric cancer (Table 11.3). In phase 1 trials, objective responses were seen with single-agent everolimus and in combination with mitomycin. Everolimus 10 mg/day resulted in a partial response with duration of more than 4 months in a heavily pretreated patient with gastric cancer and liver metastasis [53]. In a trial of everolimus (5–10 mg/day) plus mitomycin C, 3 of 13 evaluable patients (23 %) experienced a partial response, and 3 patients had stable disease [57].

Two phase 2 single-agent studies have been reported in patients with advanced gastric cancer. In a recent phase 2 trial conducted in Japan, everolimus 10 mg/day was administrated to 53 patients with metastatic gastric cancer previously treated with one or two prior chemotherapy regimens [18]. Although no complete or partial responses were documented, 45 % of patients had a decrease in tumor size from baseline by independent radiologic review. Although median progression free survival was 2.7 months no complete or partial responses were obtained. At a median follow-up time of 9.6 months, median overall survival was 10.1 months. Everolimus monotherapy resulted in a promising disease control rate in patients with previously treated advanced gastric cancer [18].

A prospective, open-label, single-arm phase 2 trial (10 mg/day) evaluated the antitumor activity and the molecular determinants of responsiveness to everolimus 10 mg/day in heavily pretreated advanced gastric cancer patients ($n = 54$) [76]. Two patients (3.7 %) achieved partial response, and the disease control rate was 38.9 %. The high expression of pS6 (Ser240/Ser244) at baseline was significantly associated with higher disease control rate (DCR) and prolonged PFS [76].

Table 11.3 Phase II and III trials in gastric carcinoma

Agent	Phase	Clinical trial no.	Number of patients	Response rate or clinical benefit rate	Median progression-free survival (PFS, months)	Median overall survival (OS, months)	Reference
Everolimus	2	NCT00519324	54	56 % (95 %CI)	2.7	10.1	[18]
Everolimus	2	NCT00729482	54	18.4 (4-month PFS rate)	1.7	8.3	[76]
Everolimus + BCS vs. placebo + BSC	3	NCT00879333	648	4.5 – everolimus arm 2.1 – placebo arm	1.68 – everolimus arm 1.41 – placebo arm	5.39 – everolimus arm 4.34 – placebo arm	[72]
Paclitaxel + everolimus	3	NCT01248403	480	NA	NA	NA	Clinicaltrial.org

BSC best supportive care, *NA* not available

Results from these phase 2 trials led to two randomized double-blind, multicenter phase 3 studies. In the first study (GRANITE-1, gastric antitumor trial with everolimus-1), patients with confirmed advanced gastric cancer and disease progression after one or two lines of systemic chemotherapy were randomized 2:1 to oral everolimus 10 mg/day plus best supportive care (BSC) or placebo plus BSC. The primary endpoint was OS. A total of 656 patients were enrolled, and 439 were randomized to everolimus and 217 to placebo. Median OS was 5.39 months with everolimus versus 4.34 months with placebo (HR 0.90; 95 % CI, 0.75–1.08, $P=0.1244$). Median PFS per local investigator assessment was 1.68 months with everolimus versus 1.41 months with placebo. The response rates were 4.5 % with everolimus versus 2.1 % with placebo [72]. Everolimus monotherapy did not significantly improve OS in patients with advanced gastric cancer previously treated with one or two lines of systemic chemotherapy. The second phase 3 trial (RADPAC) is underway. It will evaluate paclitaxel monotherapy with or without everolimus in the second- or third-line setting [3]. The study has a target enrollment of 480 patients and the OS as the primary endpoint (NCT01248403).

11.5 mTOR Inhibitors in Bladder Cancer

Bladder cancer is the second most common malignancy of the genitourinary (GU) tract in men and is increasing in women [33] Greater than 90 % of bladder cancers diagnosed in western populations are transitional cell carcinomas of the urothelium (TCCU). TCCU is known to be sensitive to chemotherapy. The two first-line chemotherapy regimens for patients with locally advanced or metastatic urothelial carcinoma are a combination of gemcitabine and cisplatin (GC) or a four-drug combination of methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) [5]. In metastatic disease, chemotherapy is rarely curative and most patients with clinically localized cancers relapse after first-line therapy. The development of new therapies for treating patients with metastatic TCCU is a priority.

Aberrant activation of the PI3K-mTOR pathway may be involved in the progression of TCCU, as suggested by two recent studies [20, 70]. In one study, multivariate analysis showed that expression of pS6 and low PTEN expression correlated with shorter recurrence-free survival (RFS) in patients with high-risk non-muscle invasive TCCU [20]. Wu and colleagues reported that PTEN mutations are present in approximately 30 % of patients with TCCU and that the PI3K pathway regulated TCCU cell invasion [75]. In vitro and animal studies of everolimus and temsirolimus indicated antitumor activity in TCCU [38, 62]. These results suggest that the mTOR pathway is active in TCCU and provide a rationale for clinical trials targeting mTOR in this disease.

Clinical studies suggest that mTOR inhibitors have limited efficacy in unselected TCCU patients but may be active in a subset of patients with TCCU and tuberous

Table 11.4 Phase II trials in urothelial carcinomas

Agent	Phase	Clinical trial no.	Number of patients	Response rate or clinical benefit rate	Median progression-free survival (PFS, months)	Median overall survival (OS, months)	Reference
Everolimus	2	NCT00805129	45	20	3.3	10.5	[43]
Everolimus	2	NCT00714025	37	5	NA	NA	[65]
Everolimus	2	NCT00933374	27	19	2.7	6.5	[51]
Temsirolimus	2	Eudra-CT 2008-008478-30	15	NR	2.5	3.5	[24]

NR not reported, *NA* not available

sclerosis complex (TSC) mutations. Three studies of everolimus and temsirolimus have reported low response rates as single agents or in combination with chemotherapy in unselected patients (Table 11.4) [24, 51, 65]. Among 37 evaluable patients treated with single agent everolimus, one near-complete response, one partial response and several minor responses were seen and suggest that everolimus possesses biological activity in a subset of patients with bladder cancer. When whole-genome sequencing was used to investigate a complete and durable response in a patient with metastatic bladder cancer treated with everolimus, it showed a loss of function mutation in TSC1 (tuberous sclerosis complex 1), a regulator of mTOR pathway activation [31]. To maximize benefit from targeted agents such as everolimus, the preselection of patients based on molecular phenotype is required [43].

11.6 Biomarker Studies in Clinical Trials with mTOR Inhibitors

On the basis of results from clinical trials, it is clear that the activity of mTOR inhibitors is limited to a subset of patients. As a result, there has been considerable research activity to identify markers that might predict sensitivity or resistance to mTOR inhibitors. To date there are no validated markers. Reasons for lack of successful identification of predictive biomarkers are multiple and include lack of correlation between preclinical models and patients and the likelihood that biomarkers of sensitivity and resistance to mTOR inhibitors are multifactorial and context specific. Recently reported preclinical and clinical studies in sarcoma, gastric, endometrial, and urothelial carcinoma have evaluated a number of potential candidate predictive markers (Table 11.5). These markers include genetic mutations and abnormal protein expression of various PI3KCA pathway components.

Table 11.5 Results of biomarkers studies from clinical trials

	Marker evaluated – clinical studies	Results
Sarcoma	PD of ridaforolimus on p4EBP1 in surrogate normal tissue and tumor human specimens – phase 1 study [6]	Ridaforolimus induced a dose-dependent inhibition of p4EBP1 in PBMC, skin, and tumors that was associated with antitumor response
	p4EBP1 inhibition in PBMC [47]	No correlation between marker effect and antitumor activity
	IHC of archival/fresh tumor samples for p27 Kip1, FKBP12, PTEN, pAKT, pS6, p4EBP1, pelF4E [11] VEGF levels pre-/post-dosing in blood samples	No correlation between archival tumor markers and CBR Blood VEGF levels show no correlation with CBR
	pS6 levels in pre/post-temsirolimus treatment PBMC [54]	No significant relationship between pS6 and clinical outcomes
Endometrial	IHC protein expression for ER, PR, HER2, LKB1, PI3K, PTEN, pAKT, 4E-BP1, S6; FISH for PTEN [71]; DNA sequencing for KRAS, PIK3CA, PTEN, AKT1	The level of proteins expressions not predictive of response PTEN deletion/mutations are not predictive of everolimus treatment response. Patients with KRAS mutations may not benefit from everolimus treatment
	Mutational profiling on FFPE tumor samples by OncoCarta Panel v1.0 [36]: AKT1,2; BRAF, CDK4, EGFR, HER2, MET, HRAS, KRAS, NRAS, PDGFRA, PIK3CA, RET	No correlation with outcome (response rate or progression disease) and the presence-absence of mutations
	IHC protein expression for PTEN, mTOR, pAKT, pS6 [55] PTEN mutational status by sequencing	No correlation with clinical outcome (tumor response or stable disease)
	PTEN and pS6 expression by IHC and KRAS mutational analysis [41]	None of the biomarkers correlated with outcome
Gastric	S6K1, HER2, pAKT, HIF-2α, PTEN, cyclin D1, KI67, p53; mutations in PIK3CA and PTEN [72]	Results are not reported yet
	pS6, p4EBP1, pmTOR, and p6SK1 by IHC from biopsies at baseline prior to everolimus [76]	High expression of pS6 at baseline was significantly associated with higher DCR and prolonged PFS; the relative increase in mTOR was associated with prolonged PFS

Table 11.5 (continued)

	Marker evaluated – clinical studies	Results
Bladder	TMA for pS6, p4EBP1, PTEN using pretreatment FFPE samples; mutation screening for FGFR3, PIK3CA, HRAS, BRAF [43]	No clear association was seen between mTOR pathway marker expression and 2-month PFS; No correlation between mutational status and outcome
	Expression of plasmatic angiogenesis proteins (angiopoietin 1, PDGF-AB), PTEN expression, and PIK3CA mutational status [65]	Everolimus treatment induced a significant decrease of plasma angiopoietin 1, and PDGF. PTEN loss might be associated with everolimus resistance

4E-BP1 eukaryotic translation initiation factor 4E-binding protein 1, *AKT1,2* v-akt murine thymoma viral oncogene homolog 1, 2, *BRAF* v-Raf murine sarcoma viral oncogene homolog B1, *CDK4* cyclin-dependent kinase-4, *DNA* deoxyribonucleic acid, *EGFR* epidermal growth factor receptor, *ER* estrogen receptor, *FISH* fluorescence in situ hybridization, *FKBP12* FK506 binding protein-12, *HER2* human epidermal growth factor receptor-2, *HRAS* Harvey rat sarcoma viral oncogene homolog, *IHC* immunohistochemistry, *KRAS* Kirsten rat sarcoma viral oncogene homolog, *LKB1* liver kinase B1, *MET* hepatocyte growth factor receptor, *NRAS* neuroblastoma rat sarcoma viral oncogene homolog, *p27 Kip1* cyclin-dependent kinase inhibitor 1B, *p4EBP1* phosphorylated eukaryotic translation initiation factor 4E-binding protein 1, *pAKT* phosphorylated v-akt murine thymoma viral oncogene homolog PD pharmacodynamic effect, *PDGFRA* platelet-derived growth factor receptor, alpha, *pelF4E* phosphorylated eukaryotic initiation factor-4E, *PI3K* phosphoinositide 3-kinase, *PIK3CA* phosphoinositide 3-kinase catalytic domain, *PR* progesterone receptor, *pS6* phosphorylated ribosomal protein S6 kinase, 70 kDa, polypeptide 1, *PTEN* phosphatase and tensin homolog, *S6* ribosomal protein S6 kinase, 70 kDa, polypeptide 1, *VEGF* vascular endothelial growth factor

11.6.1 Sarcoma Biomarker Studies

Four clinical trials in sarcoma patients have evaluated a number of aberrant genetic and gene expression markers including protein markers such as phospho-4EBP1 (p4EBP1), phosphoribosomal s6 kinase of 70 kDa (pS6), PTEN, AKT, and VEGF in surrogate normal tissue and tumor human specimens. To date, two candidate markers have been identified: the level of pS6 expression was predictive of early tumor response to ridaforolimus, and p4EBP1 inhibition was induced in peripheral blood monocellular cells, skin and tumors and was associated with antitumor response [6, 29]. These results have not been confirmed in other studies. In a recent published phase 1/2a trial of the mTOR inhibitor ridaforolimus, no correlation was observed between inhibition of phosphoproteins or levels of circulating VEGF and antitumor activity in 147 patients with refractory or advanced malignancies and sarcoma [11, 47]. Lack of correlation may be due to the heterogeneity of sarcomas evaluated as well as the complexity of the mTOR pathway. Overall, no biomarkers to predict benefit in sarcoma patients have been identified to date.

11.6.2 Endometrial Biomarkers Studies

Presently, four clinical trials with endometrial cancer patients have evaluated various markers, including genetic mutations in upstream and downstream regulators of the mTOR pathway (Kirsten RAS (KRAS), AKT, PIK3, PTEN) and abnormal protein expression (estrogen receptor (ER), progesterone receptor (PR), HER2, p4EBP1, pS6, PTEN, AKT) in surrogate normal tissue and tumor human specimens. No marker has been found to correlate with clinical outcome. To date, two candidate markers have been identified in preclinical studies: PTEN mutant tumors were sensitive to mTOR inhibition [73], and miR-100 was an independent prognostic marker of OS [67].

Deregulation of the PI3K/AKT/mTOR pathway signaling plays a significant role in endometrial cancer biology. Tumor DNA from 73 patients enrolled on three phase 2 trials of either temsirolimus or ridaforolimus was analyzed for mutations using the Sequenom technology and OncoCarta v 1.0 mutation panel [36, 37]. A mutation in at least one gene (PIK3, KRAS, MET, NRAS, AKT, and EGFR) was identified in 32 patients (44 %), and 9 patients (12 %) had more than one mutation. No significant correlation was seen in individual trials or within the pooled data set of three studies between the presence/absence of any mutation and response rate (RR) and early progression disease (PD) [36].

Another recent study aimed to determine whether the expression of various tumor biomarkers of the mTOR pathway correlated with tumor response to everolimus in metastatic recurrent endometrial cancer [71]. Thirty-six blocks were available for analysis of ER, PR, HER2, liver kinase B1 (LKB1), PI3K, PTEN, pAKT, 4EBP1, and S6 expression by immunohistochemistry, PTEN deletion by FISH, and mutational status of KRAS, PIK3, PTEN, and AKT1 genes. Twelve of 34 evaluable patients had partial response or stable disease, and 22 had progressive disease (PD). No marker of protein expression or gene mutation correlated with response to everolimus [71]. None of four patients with KRAS mutations responded to treatment and median PFS and OS were shorter, suggesting that these patients may not derive benefit from everolimus treatment [71].

11.6.3 Biomarker Studies in Gastric Cancer

In preclinical studies, two candidate markers, p4EBP1 and pS6, were reported as having potential predictive value. Cell proliferation in 3 of 8 cell lines was effectively inhibited by everolimus. Based on in vitro and in vivo results, the investigators concluded that phosphorylation of 4E-BP1 may be a predictive biomarker of everolimus sensitivity in gastric cancer [52]. In another study, investigators evaluated tumor samples from patients enrolled on a phase 2 trial of everolimus. They reported that high expression of pS6 (Ser240/244) may be a potential predictive biomarker for everolimus [76]. These correlations require further clinical validation.

A recent study has undertaken a comprehensive investigation of genomic copy number alterations in gastric cancer. The results of this study showed that genomic amplifications in receptor tyrosine kinase such as HER2 and KRAS components define five distinct gastric cancer molecular subgroups [16, 58]. The HER2 results are intriguing as a recent phase 3 demonstrated that the addition of trastuzumab to chemotherapy improved outcomes in patients with metastatic gastric cancer who overexpressed HER2, a feature found in 20 % of patients [4]. Other studies have shown that loss of PTEN, a negative regulator of the PI3K/AKT/mTOR pathway, may mediate trastuzumab resistance in breast cancer patients [7]. Taken together, the data provide a foundation to evaluate the combination of mTOR inhibitors and trastuzumab in HER2-positive gastric cancer and, perhaps, mTOR and MEK inhibitor combinations in other genetically defined subtypes of gastric carcinoma.

11.6.4 Biomarker Studies in Transitional Cell Carcinoma of the Urothelium

In a recent study, a patient with metastatic bladder cancer enrolled in a phase 2 trial achieved a durable and ongoing complete response to everolimus [31, 32]. Of the 13 everolimus-treated patients who underwent targeted exon sequencing, three (23 %) possessed non-sense TSC1 mutations, and two had minor treatment responses. Eight (89 %) of nine patients with tumor progression had wild-type TSC1. Patients with TSC1-mutated tumors continued to receive everolimus longer than those with wild-type tumors (7.7 versus 2 months). Sanger sequencing of an additional 96 high-grade bladder tumors found five tumors (6.2 %) containing TSC1 alterations. Thus, everolimus appears to be an active agent in TCCU harboring TSC1 mutations, although this represents a relatively small portion of patients with TCCU [30, 31]. The genotyping stratification of patients based on the presence of predictive molecular biomarkers such as TSC1 in clinical trials of mTOR inhibitors may ultimately improve the outcome for patients with advanced bladder cancer [43].

11.7 Conclusion

mTOR inhibitors appear to have antitumor activity in a subset of patients with bone and soft tissue sarcomas and carcinomas of stomach, endometrium, and urothelium. To date, however, the level of activity and the numbers of patients have been insufficient to result in marked improvements in survival in phase 3 trials conducted in unselected patients. In these disease settings, like others where mTOR inhibitors have been evaluated, the key challenges will be to identify markers of sensitivity such as the TSC mutations in TCCU patients and build on that activity by identifying active combinations that will lead to substantial improvements in patients' outcomes.

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