Review

Markers for Efficacy of Mammalian Target of Rapamycin Inhibitor

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Abstract. *Mammalian target of rapamycin (mTOR), an important sensor for growth factors, nutritional deprivation and other stresses in controling translation, plays a critical role in tumorigenesis. Several rapalogs exhibited antitumor activity clinically, with a modest average response rate, while a small subset of patients exhibited significantly greater clinical benefits. A better understanding of cellular mechanisms and the results of clinical studies can help identify an optimal biomarker to predict the efficacy of mTOR inhibitors. We discuss these potential markers in terms of selection of candidates, baseline expression, pathway inhibition and source of targeted protein.*

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase of PI3K (phosphatidylinositol 3-kinase) family and integrates the stimuli of nutrients, energy and stress to affect cell growth and proliferation through the regulation of mRNA translation (Figure 1) (1, 2). Two structurally and functionally distinct mTOR-containing complexes, referred to as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), have different sensitivities in mTOR signaling to rapamycin. mTORC1, consisting of mTOR, mammalian lethal with Sec13 protein 8 (mLST8), regulatory-associated protein of mTOR (raptor) and proline-rich protein kinase B (AKT) substrate 40 (PRAS40), can regulate cell growth. mTORC1 kinase activity is sensitive to rapamycin treatment (2-4). mTOR, mLST8 and rapamycin-insensitive companion of mTOR (rictor) form mTORC2, which can affect the actin cytoskeleton. The autophosphorylation of mTORC2 is rapamycin-insensitive (5).

mTORC1 is an important effector that senses the stimuli of growth factors or insulin *via* the phosphatidylinositol 3-kinases (PI3K) pathway. The recruitment of PI3K leads to activation of AKT through the phosphorylation of phosphoinositide-dependent protein kinase 1 (PDK1), which can be antagonized by phosphatase and tensin homolog (PTEN) (2). AKT phosphorylates PRAS40 and tuberous sclerosis complex 2 (tuberin) to promote mTORC1 expression (6). PRAS40, a substrate of mTORC1, can inhibit mTORC1 kinase activity by directly disrupting substrate binding. Once it is phosphorylated, PRAS40 can in turn relieve its inhibition of mTORC1 and activate the mTORC1 signaling cascade (7-9). Tuberous sclerosis protein 2 (TSC2) acts as a GTPase-activating protein and negatively-regulates the expression of the rat sarcoma viral oncogene homolog (RAS) homolog enriched in brain (RHEB), a member of RAS superfamily of GTP-binding proteins. AKT-dependent phosphorylation of TSC2 can inactivate GTPase (10, 11). Thus, GTP loading of RHEB activates mTORC1, resulting in the regulation of eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) and S6 kinase expression (12). Additionally, the RAS/mitogen-activated protein kinase (MAPK) pathway can sense the stimuli of growth factors to signal mTORC1. The activation of extracellular signal-regulated kinase (ERK), a RAS-dependent downstream protein, drives the phosphorylation of TSC2 to prevent inhibition of RHEB. ERK can mediate the mTORC1 expression either through the phosphorylation of TSC2 and RHEB or through direct activation of the raptor (13-16). Moreover, mTOR bridges the alteration of energy and translation *via* the liver kinase B1 (LKB1)-5' adenosine monophosphate-activated protein kinase (AMPK) pathway. Increasing of the AMP/ATP ratio stimulates the phosphorylation and activation of LKB1, which in turn can phosphorylate and activate AMPK. AMPK-dependent

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phosphorylation of TSC2 enhances the inhibition of mTORC1 (17, 18). Genotoxic stress is wired to TSC2-inducing mTORC1 inactivation through p53-modulated phosphorylation of sestrin1/2 and subsequent activation of AMPK (19). Regulated in development and DNA damage 1 (REDD1) is a sensor of hypoxia which controls the phosphorylation of TSC1/TSC2 complex and the subsequent modulation of mTORC1 (20).

Ribosomal protein S6 kinase 1 (S6K1) and 4EBP1 are important downstream proteins of mTOR which control the translation (2). Activated S6K1 promotes the translation of 5' tract of oligopyrimidine (5'TOP)-containing mRNA that encodes ribosomal proteins and elongation factors through phosphorylation of 40S ribosomal protein S6 (21). In addition, S6K1 affects the phosphorylation of eukaryotic translation initiation factor 4B (eIF4B) acting as an RNA-binding protein. Phosphorylated eIF4B enhances ATPase activity and RNA helicase activities of eIF4A (22). 4EBP1, an mTOR substrate, binds to eIF4E to act as a repressor. Phosphorylated 4EBP1 dissociates with translation activator eIF4E and relieves the repression to eIF4E binding to eIF4G (23). In turn, the eIF4E/eIF4G complex, referred as eIF4F, can promote cap-dependent translation and encode proteins involved in the cell-cycle (24).

Dysregulation of mTOR/PI3K function has been previously shown to be responsible for malignant transformation, cell growth and proliferation (25). Mutations of encoding the catalytic subunit of PI3K (*PI3KCA*) are a common oncogenic alteration in human cancer (26). Acting downstream of the human epidermal growth factor receptor (HER) family, even tyrosine kinase inhibitors effectively and continuously suppress the phosphorylation of EGFR and HER2 with subsequent inhibition of downstream MAPK and c-Jun N-terminal kinases (JNK) signaling cascade, but AKT signaling can be re-activated by the re-phosphorylation and re-activation of HER3 through heterodimerization and transphosphorylation (27). Loss-of-function mutation in *PTEN* is also a trigger inducing oncogenic activation of the mTORC1 pathway. However, alterations of the mTOR repressors, TSC1 and TSC2, are uncommon in human cancer (6). In addition, oncogenic RAS can activate mTORC1 *via* a PI3K-independent pathway, MAPK cascade (13). The mutated v-Raf murine sarcoma viral oncogene homolog B1 (*BRAF*)-dependent activation of ERK can phosphorylate and inactivate LKB1 and downstream AMPK, with a subsequent increase in cell proliferation (28). Oncogenic mTORC1 controls the translation of cyclin D1, V-myc myelocytomatosis viral oncogene homolog (c-Myc), vascular endothelial growth factor and hypoxia-inducible factor 1 to regulate cell growth, angiogenesis and metastasis (29-33).

Rapamycin and its analogs bind to FK506-binding protein (FKBP12) and allosterically inhibit mTOR kinase leading to suppression of activation of mTORC1-targeted 4EBP1 and S6K1 and translation (34, 35). Prolonged rapamycin treatment can inhibit the activity of mTORC2 and its downstream AKT even though mTORC2 is rapamycin-insensitive (36). However, rapamycin-inactivated S6 kinase can relieve its inhibition to insulin receptor substrate-1 (IRS1) through feedback. Up-regulation of IRS-1 promotes AKT activity, which can attenuate the antitumor efficacy (37).

Several rapalogs, including everolimus, temsirolimus and ridaforolimus, have been shown to possess antitumor activity towards some types of cancer in pre-clinical and clinical studies (Table I) (38). In a randomized phase III study, temsirolimus prolonged overall (OS) and progression-free survival (PFS) among patients with metastatic renal cell carcinoma (RCC), compared to interferon treatment-alone and to combination therapy with interferon and temsirolimus. The median OS was 10.9, 7.3 and 8.4 months, respectively, among patients who received tensirolimus, interferon and combination therapy (39). A phase III double-blind study randomized a total of 410 patients with RCC, whose disease progressed on VEGF tyrosine kinase inhibitor to receive everolimus or placebo. The median PFS was 4 and 1.9 months ($p<0.0001$) in the everolimus and placebo groups, respectively (40).

In a phase III study of 410 patients diagnosed with progressive advanced neuroendocrine carcinoma, median OS in the everolimus-treated group was significantly longer than that in the placebo group (11 months *vs*. 4.6 months; $p<0.001$) (41). Moreover, mTOR inhibitors have also shown their antitumor activity in breast cancer, non-small cell lung cancer, endometrial cancer, gastric cancer and non-Hodgkin's lymphoma (41-49).

Not all patients with cancer will respond positively to mTOR inhibitors, but a small group might obtain modest clinical benefits. It is appropriate to identify biomarkers that can enhance our ability to predict and monitor the clinical efficacy of such an intervention and guide our selection of candidates for mTOR-based therapies (50, 51). The tyrosine kinase inhibitor (TKI) of EGFR has shown great promise as a biomarker. In the Iressa Survival Evaluation in Lung Cancer (ISEL) study, EGFR TKI treatment failed to prolong a survival of all lung cancer patients. Non-smoking patients of Asian origin have shown a significant increase in survival on therapy with gefitinib (52, 53). The results of the Erlotinib in Previously Treated Non–Small-Cell Lung Cancer (BR21) study also showed a higher response rate to erlotinib among Asian patients, and a statically significant correlation between the expression of EGFR and better survival (54). As a first-line treatment of pulmonary adenocarcinoma in Asian non-smokers or former light smokers, gefitinib had a higher response rate than carboplatin-paclitaxel in all patients and mutation subgroups (43.0% *vs*. 32.2, $p<0.001$ in all patients; 71.2% *vs*. 47.3%, $p<0.001$ in the mutation-positive subgroup). *EGFR* mutation can be a positive biomarker to predict the responsiveness of pulmonary adenocarcinoma to gefitinib (55). Cetuximab, monoclonal antibody to EGFR, also exhibits similar potential. Colon cancer patients with mutated V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) responded poorly to

cetuximab-irinotecan plus fluorouracil and luecovorin (FOLFIRI), as compared to those with wild-type *KRAS*. The response rate was 59.3% and 43.2% in the cetuximab-FOLFIRI and FOLFIRI groups, respectively, in patients with wild-type *KRAS* tumor (odds ratio=1.91), and 36.2% and 40.2% in patients with mutant-*KRAS* tumor (odds ratio=0.8) (56). Thus, *KRAS* mutation can be a negative-predictive biomarker of response of colon cancer to EGFR-targeted therapies (57).

Potential Markers of mTOR Inhibitors Efficacy

Some potentially optimal biomarkers for efficacy of mTOR inhibitors were identified in the tumors, the skin or the peripheral mononuclear cells (PBMCs) in studies to predict response and survival (Table II) (45, 58-66). Higher levels of baseline phospho-AKT (pAKT) in patients with non-small cell lung cancer (NSCLC) were associated with poor PFS at an unadjusted alpha level of 5% (45). Among 13 patients with NEC, higher baseline phospho-mTOR (pmTOR) levels were correlated with tumor response (p=0.001). Increasing AKT expression (p=0.041) or reduced pmTOR expression (p=0.048) after 2-week temsirolimus treatment was correlated with an increase of time-to-progression (TTP) (59). However, pre-treatment expression of AKT failed to predict clinical benefit in patients with glioblastoma multiforme (GBM), NSCLC and RCC (58, 60, 65). In terms of downstream factors of mTOR, patients of small cell lung cancer (SCLC) with disease control had higher expressions of baseline S6 Kinase than those with progressive disease (p=0.0093), among patients who received everolimus treatment (65). In contrast, baseline expression of S6 kinase was not a marker of radiological response in GBM patients or of survival in SCLC patients (58, 65). In addition, higher expression of baseline tumor-derived phospho-S6K1 (pS6K1) that were noted in 71% of responding patients and 38% of non-responders showed a correlation with MRI response at the of continuous staining index cut-off of more than 200 among patients with GBM. However, inhibition of PBMC-derived S6K1 at 24 h after the first treatment (p=0.098) and before the fourth treatment (p=0.082) did not predict a radiological response in patients (59). Therefore, in terms of phospho-S6 (pS6), patients with RCC with higher expression obtained clinical response to temsirolimus (p=0.02). The median OS was 17.3 months in patients with high expression of pS6 and 9.1 months in patients with intermediate or low expression of pS6 (p=0.02) (60). The positive correlation between pS6 expression and clinical efficacy was reproducible in patients with sarcoma. Stable disease was significantly higher in patients with high expression (≥20% of tumor cells), as compared to patients with low expression (0-10% of tumor cells) (73% *vs*. 33%, $p \leq 0.05$) (64). More than 50% inhibition of S6 phosphorylation in tumor were correlated with a better Ki-67 response in patients with GBM tumors receiving mTOR-inhibitor therapy (61). However, no positive relation between pS6 and the clinical response was demonstrated in patients with NEC (59). pS6 inhibition was not associated with TTP among sarcoma patients (67). To determine the role of phospho-PRAS40 (pPRAS40), an increase in PRAS40 phosphorylation after 1-week of rapamycin treatment had a shorter TTP in GBM patients (p=0.049) (61). In a pre-clinical study, *PTEN* loss can predict the antitumor activity of mTOR inhibitor (68). However, patients with deletion of PTEN by fluorescence *in situ* hybridization (FISH) or expression of PTEN by immunohistochemistry (IHC) staining did not benefit from mTOR inhibitors (58). The grade of PTEN expression was not related to the effect of mTOR inhibition. The tumor response was not significantly different between the high PTEN expression group and the low expression group (58% *vs*. 33%, p=0.55) in patients with RCC (60). Additionally, some other factors including 4EBP1, phospho-4EBP1 (p4EBP1), and hypoxia-induced factor-1 alpha (HIF1 α) did not exhibit a significant correlation with clinical outcomes (63, 65).

Tumor-derived and Non-tumor-derived Markers

Tumor-derived factors are frequently studied targets. pmTOR, pS6K1, pS6, pAKT and PTEN expressions are significantly associated with clinical outcomes (58-60, 62, 64). PBMCs are an alternative source of studied proteins, but changes in PBMC-derived pS6 and pS6K1 are not predictive (67). Even in a preclinical study, everolimus exhibited its consistent inhibition of 4EBP1 and S6K1 in the tumors, skin and PBMCs. A reduction of 4EBP1 positively-correlated with an increased formation of 4EBP1-eIF4E complex in all three tissues. A decrease in S6K1 activity was positively associated with de-phosphorylation of its downstream ribosomal S6 in tumor extract. However, pS6 was only detected in tumor extract, but not in skin and PBMCs extract in control animals (69). A phase 1 study of patients with advanced solid tumors who received everolimus showed a significant inhibition of pS6 (p<0.001) and p-eIF4G (p<0.001). Skin tissues demonstrated a significant reduction of p4EBP1 (p<0.001) compared to tumors (p=0.058). There was a consistent correlation between a decrease in p4EBP1 and an increase in 4EBP1-eIF4E complex formation in all three tissues. Phosphorylation of AKT was significantly increased in the tumors (p=0.006) and the skin (p<0.001). A significant decrease in pS6 was only demonstrated in tumors; immunoblotting failed to detect a low level of baseline pS6 in the skin and PBMCs (66). In patients with head and neck squamous cell carcinoma, alterations of S6, pS6, 4EBP1, p4EBP1, AKT and pAKT were demonstrated in tumors and PBMCs. There was a significant temsirolimus-induced reduction of pS6 in the tumors and PBMCs (p=0.008). However, the significant reduction of p4EBP1 demonstrated in the tumors was not reproduced in PBMCs (70). The detection and expression of mTOR cascade in the tumors, the skin and PBMCs varied in different types of cancer. The tumor seems to be an optimal source of studied markers.

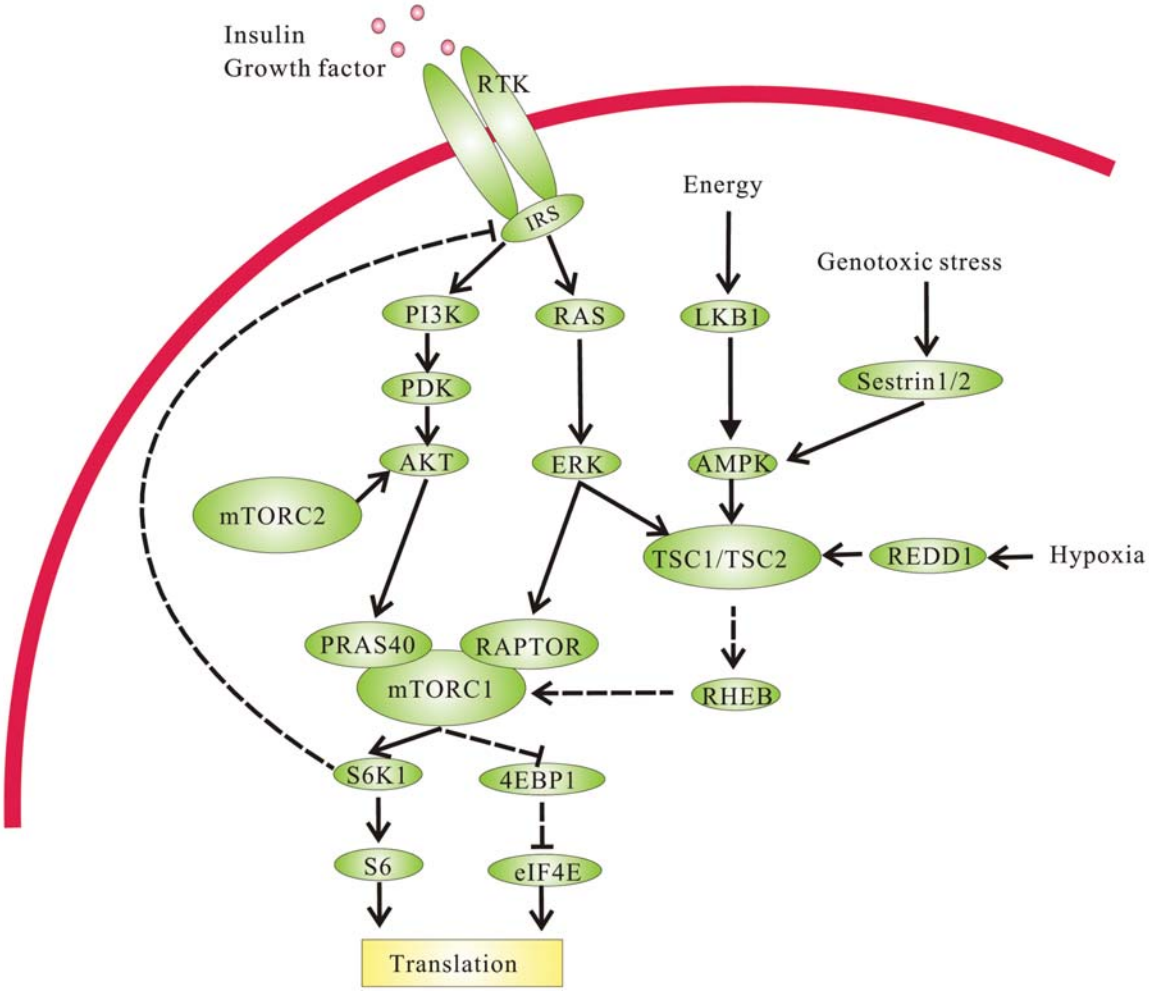

Figure 1. *Pathways involving mammalian target of rapamycin. RTK, Receptor tyrosine kinase; IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3-kinase; PDK, phosphoinositide-dependent protein kinase; mTORC1, mammalian target of rapamycin complex 1; mTORC2, mammalian target of rapamycin complex 2; RAPTOR, regulatory-associated protein of mTOR; PRAS40, proline-rich AKT substrate 40; S6, ribosomal protein S6; S6K1, kinase 1; 4EBP1, eukaryotic translation initiation factor 4E binding protein 1; eIF4E, eukaryotic translation initiation factor 4E; LKB1, liver kinase 1; AMPK, AMP-activated protein kinase; TSC1, tuberous sclerosis 1; TSC2, tuberous sclerosis 2; REDD1, regulated in development and DNA damage 1; RHEB, RAS homolog enriched in brain; ERK; AKT, proline-rich protein kinase B; RAS, the rat sarcoma viral oncogene homolog.*

Baseline Expression and mTOR Inhibition

Baseline pmTOR, S6K, pS6K1, S6, pS6, PTEN and pAKT exhibited significant correlations with clinical efficacy. Their endogenous expressions suggest sensitivity to mTOR inhibition and their potential as biomarkers (58-60, 62, 64, 65). Treatment-specific alterations of pS6, pAKT and pPRAS40 were associated with TTP (59, 61). Not all downstream targets were significantly suppressed, however (70). No consistent associations were demonstrated between mTOR inhibition and clinical benefits (58, 67). Additionally, the dose of everolimus also impacted the repression of mTOR signaling. eIF4G was completely inhibited only at doses ≥50 mg (66). In addition, mTOR inhibition can reactivate AKT and MAPK pathways, which may attenuate the mTOR efficacy (37, 71). The role of pathway inhibition in the prediction of response remains controversial.

Conclusion

mTOR inhibition plays a potential role in treatment of some cancer types. Robust selection of markers will help in the selection of good patient candidates based on disease type. Many issues will impact on the validation of markers although mTOR signaling can be detected in tumor, skin and PBMCs in animal study (69). Several lines of evidence suggest an association between tumor-derived proteins and clinical benefit (58-60, 62, 64). PBMCs may be an alternative source of markers for the pharmacodynamic study of mTOR inhibition.

Table I. *Clinical outcomes of therapy with inhibitors of mammalian target of receptor.*

Study (Ref.), agent	Disease	No. of patients	Objective response rate (%)	Progression free survival (months)
Phase III				
Hudes *et al*., 2007 (39)	RCC			
Temsirolimus		209	8.6	3.8
Interferon		207	4.8	1.9
Temsirolimus+interferon		210	8.1	3.7
Motzer *et al*., 2008 (40)	RCC			
Everolimus		272	1	4
Placebo		138	0	1.9
Hess *et al*., 2008 (48)	MCL			
Temsirolimus		54	22	4.8
Temsirolimus		54	6	3.4
Investigators' choice		54	2	1.9
Yao *et al*., 2011 (42)	P-NET			
Everolimus		207	5	11
Placebo		203	2	4.6
Phase II				
Ansell *et al*., 2011 (76)	MCL			
Temsirolimus plus rituximab		69 (ITT)	59	9.7 (TTP)
Baselga *et al*., 2010 (44)	Breast cancer			
Everolimus plus letrozole		138	68.1	NA
Letrozole alone		132	59.1	NA
Chan at al., 2005 (43)	Breast cancer			
Temsirolimus		54	7.4	NA
Temsirolimus		55	10.9	NA
Witzig *et al*., 2011 (49)				
Everolimus	Aggressive lymphoma (total)	77	30	3
DLBCL	47	30	NA	
MCL	19	32	NA	
FL Gr 3	8	38	NA	
Others	3	0	NA	
Ghobrial *et al*., 2010 (77)	WM			
Everolimus		50	70	Not reached
Rizzieri et al, 2008 (78)	Hematological malignancies			
Deforolimus		52	10	NA
Okuno *et al*., 2010 (79)	Sarcoma			
Temsirolimus		41	5	2 (TTP)
Doi *et al*., 2010 (47)	Gastric cancer			
Everolimus		53	0	2.7

ITT, Intent to treat; NA, not applicable; TTP, time to progression. RCC, renal cell carcinoma; MCL, mantle cell lymphoma; P-NET, pancreatic neuroendorine tumor; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; WM, Waldenström macroglobulinemia.

However, not all proteins involved in the mTOR pathway can be detected in all three tissues. There were some discrepancies among tumor, skin and PMBCs in terms of expression and pharmacodynamic profiles of mTOR pathways, although the degree of mTOR inhibitor-induced inhibition in the tumor was correlated to that in the skin (66, 70). More studies must elucidate the correlation between skin-derived markers and clinical efficacy, but tumors currently seem to be a better source of studied biomarkers for mTOR inhibitor.

Baseline expressions of targeted proteins have been frequently studied. There was a disparity between dose and pharmacodynamic markers (66). Once it was determined that an active mTOR pathway is not critical in tumor growth, pathway inhibition failed to be an ideal marker of growth-inhibitory effects (7). Although mTOR blockade-specific compensatory alternations of pAKT and pPRAS40 can be a potential predictor of TTP, feedback alternations impact on the predictive role of mTOR inhibition (59, 61). Baseline expressions of targeted proteins may be more feasible than pathway inhibition.

Tumors, the skin and PBMCs are common sources of studied markers. However, expression of eIF-4E in the histological

Table II. *Markers studied for response to therapy with mammalian target of rapamycin inhibitors.*

Marker	Disease	Source of markers	End point	*p*-Value	Reference
PTEN					
Deletion	GBM	Tumor	MRI response *vs.* no response, 50% *vs.* 73%	0.17	58
Expression	GBM	Tumor	MRI response *vs.* no response, 73% *vs.* 95%	0.14	58
Expression					
Low *vs.* high	RCC	Tumor	*Clinical response, 33% *vs.* 58%	0.55	60
Positive	RCC	Tumor	ORR Temsirolimus *vs.* interferon, 8% *vs.* 7%	1	63
	RCC	Tumor	Median OS Temsirolimus *vs.* interferon, 11.3 *vs.* 7.1 months	(HR, 0.81)	63
	RCC	Tumor	Median PFS Temsirolimus *vs.* interferon, 5.7 *vs.* 3.8 months	(HR, 0.66)	63
Negative	RCC	Tumor	ORR Temsirolimus *vs.* interferon, 20% *vs.* 10%	(0.3006)	63
	RCC	Tumor	Median OS Temsirolimus *vs.* interferon, 10.7 *vs.* 8.3 months	(HR, 0.35)	63
	RCC	Tumor	Median PFS Temsirolimus *vs.* interferon, 5.8 *vs.* 4.3 months	(HR, 0.99)	63
Akt					
Higher	GBM	Tumor	MRI response *vs.* no response, 71% *vs.* 58%	0.39	58
	SCLC	Tumor	OS	> 0.2	65
pAkt					
Increasing	NEC	Tumor	Better TTP	0.041	59
Expression	NSCLC	Tumor	Significant correlation between pAKT expression and PFS at alpha level of 5%		45
Higher	GBM	Tumor	MRI response *vs.* no response, 35% *vs.* 58%	0.15	58
Higher	SCLC	Tumor	Poor OS	0.063	65
Higher	RCC	Tumor	Clinical response	0.07	60
pmTOR					
Higher	NEC	Tumor	Better tumor response	0.01	59
Decreasing	NEC	Tumor	Better TTP	0.048	59
4EBP1					
SCLC	Tumor	OS	>0.2	65	
p-4EBP1					
SCLC	Tumor	OS	>0.2	65	
S6K					
Higher	SCLC	Tumor	Better disease control	0.0093	65
	GBM	Tumor	MRI response *vs.* no response, 76% *vs.* 77%	0.99	58
	SCLC	Tumor	OS	> 0.2	65
pS6K					
Higher	GBM	Tumor	MRI response *vs.* no response, 71% *vs.* 38%	0.04	58
SCLC	Tumor	OS	> 0.2	65	
Inhibition 24 hours after first treatment	GBM	PBMC	Radiological response	0.98	58
Inhibition pre-fourth dose treatment	GBM	PBMC	Radiological response	0.82	58
pS6					
High level [b]HE (≥ 20% of tumor cells) vs.	RCC	Tumor	Clinical response[a]	0.02	60

Table II. *continued*

Table II. *continued*

Marker	Disease	Source of markers	End point	*p*-Value	Reference
[c]LE of (0-10% of tumor cells)	Sarcoma	Tumor	Stable disease, 73% *vs*. 33%	≤0.005	64
HE *vs*. dIE and LE	RCC	Tumor	Median OS		
17.3 *vs*. 9.1 months	.02	60			
Higher	NEC	Tumor	Better response	.097	59
>50% inhibition	GBM	Tumor	Higher Ki-67 response	< 0.0047	61
Inhibition	Sarcoma	PBMC	TTP	NS	67
HIF1α-					
Positive	RCC	Tumor	ORR	0.7521	63
			Temsirolimus *vs*. interferon, 13% *vs*. 11%		
	RCC	Tumor	Median OS	HR, 0.74	63
			Temsirolimus *vs*. interferon, 9.5 *vs*. 6.3 months		
	RCC	Tumor	Median PFS	HR, 0.98	63
			Temsirolimus *vs*. interferon, 5.5 *vs*. 4.6 months		
Negative	RCC	Tumor	ORR	0.2123	63
			Temsirolimus *vs*. interferon, 10% *vs*. 4%		
	RCC	Tumor	Median OS	HR, 0.79	63
			Temsirolimus *vs*. interferon, 10.7 *vs*. 7.2 months		
	RCC	Tumor	Median PFS	HR, 0.72	63
			Temsirolimus *vs*. interferon, 4.5 *vs*. 3.7 months		
P-PRAS40					
Induction	GBM	Tumor	Shorter TTP	0.049	61

*Clinical response included partial response, minor response and stable disease ≥4 weeks. PTEN, Phosphatase and tensin homolog; GBM, glioblastoma multiforme; MRI, magnetic resonance imaging; RCC, renal cell carcinoma; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; SCLC, small-cell lung cancer; NEC, neuroendorine tumor; AKT, proline-rich protein kinase B; TTP, time-to-progression; NSCLC, non-small cell lung cancer; pmTPR, phosphorylated mammalian tarted of rapramycin; 4EBP1, eukaryotic translation initiation factor 4E binding protein 1; pS6K, phosphor-S6 Kinase; PBMC, peripheral blood mononuclear cell; pS6, phospho-S6; HIF1α, hypoxia-induced factor 1 alpha; P-PRAS40, phospho-proline-rich AKT substrate 40.

tumor-free margins had shown a significant correlation with poor outcomes among head and neck cancer patients (72). In an animal study, mTOR inhibition delayed the time of tumor development in a minimal residual disease model that mimicked the mTOR-positive tumor margins (73). Several active clinical trials to study the role of mTOR inhibitor in adjuvant treatment of head and neck cancer patients will clarify the correlation between markers from the surgical margin and efficacy of adjuvant therapy.

More studies are necessary to settle the controversial issues. Subcellular location of AKT played an important role in pathology and survival. There was an association between nuclear AKT expression and disease-specific survival (62). Human papillomavirus (HPV) plays a critical role in tumorigenesis of head and neck squamous cell carcinoma (HNSCC) (74). A tissue microarray study suggested the correlation between p16 expression and expression of phosphorylated eIF-4E. There was no association between expression of p16 and phosphorylated S6 or 4EBP1. HPV can activate eIF-4E through an mTOR-independent pathway (75). However, a preclinical study suggested mTOR activation with subsequent elevation of pS6 in HPV-associated HNSCC. The mTOR blockade significantly inhibited tumor growth in mice (11). Therefore, more research is needed to elucidate the role of the mTOR pathway in tumorigenesis in HPV-positive HNSCC.

From our review, baseline expression of tumor-derived pS6 seems to be a candidate of efficacy of mTOR inhibition. Overall, more studies are needed to optimize the condition and source of studied proteins and to select a protein as a better biomarker.

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