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Emerging role of mTOR in the response to cancer therapeutics

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Abstract

The movement toward precision medicine with targeted therapeutics for cancer treatment has been hindered by both innate and acquired resistance. Understanding the molecular wiring and plasticity of oncogenic signaling networks is essential to the development of therapeutic strategies to avoid or overcome resistance. The mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) represents a highly integrated signaling node that is dysregulated in the majority of human cancers. Several studies have revealed that sustained mTORC1 inhibition is essential to avoid resistance to targeted therapeutics against the driving oncogenic pathway in a given cancer. Here we discuss the role of mTORC1 in dictating the response of tumors to targeted therapeutics and review recent examples from lung cancer, breast cancer, and melanoma.

The challenge of resistance to targeted cancer therapies

Genomic analysis of tumors combined with the rational development of drugs targeting common oncogenic drivers have paved the way for personalized and precision medicine approaches to cancer therapy. Patient stratification based on tumor genotype will improve the effective response rates of targeted therapeutics. However, despite this approach of matching the appropriate patients with the appropriate therapy, a lack of response to the given therapy is still common, indicating an innate resistance of these tumors. Furthermore, despite robust initial clinical responses in some cases, continued exposure to targeted therapeutics leads to the development of acquired resistance to the drug [1]. Signal rewiring and redundancy within oncogenic signaling networks can underlie both innate and acquired resistance of tumor cells to targeted therapeutics that hit a single component of the network, even if that component is the major driver of tumor growth. Therefore, to develop more effective therapeutic strategies and accurately predict sensitivity and resistance, downstream convergence points within the signaling network that dictate clinical response must be identified. Here, we discuss the mechanistic (or mammalian) target of rapamycin complex 1 (mTORC1) as a shared downstream effector of oncogenic signaling pathways and its role in the response of cancer cells to targeted therapeutics.

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Common oncogenic signaling pathways converge to activate mTORC1

The ability of organisms to adapt to fluctuations in nutrient availability is fundamental to the survival and growth of a species. Eukaryotic cells, particularly those within multicellular organisms, have the ability to detect nutrients present within the cell and its surroundings, and also sense systemic signals from growth factors, cytokines, and hormones. A small number of highly connected signaling nodes are required to integrate these diverse intracellular and extracellular signals and to coordinate an appropriate physiological response. mTORC1 sits at the convergence point of a vast signaling network that functions to integrate a wide array of signals in order to properly control cell, tissue, and organismal growth [2].

The mTOR Ser/Thr kinase exists within two physically and functionally distinct protein complexes, mTORC1 and mTORC2 (Figure 1, Box 1). For the purposes of this discussion, we will focus primarily on mTORC1. In response to growth-promoting signals from exogenous growth factors and endogenous nutrients, mTORC1 is activated and regulates a number of downstream targets to promote a shift from catabolic to anabolic metabolism. Activation of mTORC1 promotes the conversion of available nutrients into biomass through the stimulation of protein, lipid, and nucleotide synthesis, processes that are fundamental to cell growth and proliferation [3–9]. Given the great demand for nutrients and energy that accompanies the induction of these anabolic processes, the activation state of mTORC1 in normal cells is very tightly controlled.

There are two systems of small G proteins, the Rag and Rheb GTPases, which are key to the integrated control of mTORC1 by intracellular nutrients and exogenous growth factors (Figure 2). The coordinated actions of the Rag proteins, downstream of amino acids, and Rheb, downstream of growth factors, for regulation of mTORC1 on the surface of lysosomes have been reviewed in detail elsewhere [2, 10, 11]. In brief, the Rag GTPases are responsible for localization of mTORC1 to the lysosome when adequate levels of intracellular amino acids are present. While this is not sufficient to activate mTORC1, this translocation brings mTORC1 in proximity to Rheb, which in its GTP-bound state is a potent and essential activator of mTORC1 [12, 13]. Integration of the amino acid signal with growth factor signaling comes at the level of Rheb regulation at the lysosome. A complex of proteins comprised of the tuberous sclerosis complex (TSC) tumor suppressors (TSC1 and TSC2) and TBC1D7, referred to as the TSC complex, acts as a GTPase-activating protein (GAP) for Rheb, thereby converting Rheb to its GDP-bound state incapable of activating mTORC1. The TSC complex is acutely regulated by at least two of the major growth factor signaling pathways in cells, the PI3K-Akt and Ras-ERK pathways, through inhibitory phosphorylation events on TSC2 [14–18]. The Akt-mediated phosphorylation of TSC2 dissociates the TSC complex from Rheb at the lysosome, allowing Rheb to become GTP-loaded and activate mTORC1 [13]. While this mode of regulation closely couples mTORC1 activation by intracellular nutrients to external cues that signal the status of the tissue or organism, cancer cells lose this coupling through the activation of oncogenic signaling pathways.

Cancer cells exhibit dysregulated growth characterized by reduced dependency on exogenous growth factors. The genetic events underlying this property lead to constitutive

activation of mTORC1 in the majority of human cancers, across nearly all lineages [19]. Upstream activating mutations or amplifications in common oncogenes, such as receptor tyrosine kinases (RTKs), oncogenic fusion proteins, PI3K, AKT, RAS, and RAF, and loss of function mutations in common tumor suppressors such as PTEN, NF1, LKB1/STK11, and APC, all lead to aberrant activation of mTORC1 (Figure 2). The convergence of these oncogenic signaling pathways on mTORC1 and the large number of distinct genetic alterations that can lead to uncontrolled mTORC1 signaling in tumors, together with its key role in anabolic tumor growth, make mTORC1 both a promising target for cancer therapy and a major mechanism of resistance to targeted therapeutics.

mTOR inhibitors as single agent therapeutics

Due to the prevalence of mTORC1 activation in human cancers, there has been an on-going interest in mTORC1 inhibitors for the treatment of a wide variety of cancers, including solid carcinomas and sarcomas, as well as those of hematopoietic origins. These inhibitors include rapamycin and its analogs (rapalogs), which act through allosteric mechanisms, and more recently, mTOR kinase domain inhibitors, which inhibit both mTORC1 and mTORC2 (Figure 1, Box 2). While we await the outcome of trials using mTOR kinase inhibitors [20], rapalogs have already been tested in nearly one thousand clinical cancer trials, across most cancer lineages and genetic tumor syndromes (<http://clinicaltrials.gov>). With few exceptions, the results of monotherapy rapalog trials indicate that inhibiting mTORC1 alone is not sufficient to cause tumor regression in the majority of cases. A notable exception is results from trials on TSC and a related lung disorder, lymphangioleiomyomatosis (LAM), which occurs in TSC patients or sporadically through mutations in TSC2. Due to their central function in inhibiting mTORC1, loss of the TSC tumor suppressors leads to robust constitutive activation of mTORC1 in TSC and LAM lesions. The dependence of tumor growth on mTORC1 in these settings is evident, as rapalogs shrink tumors by approximately 50% and improve clinical outcomes [21–23]. Interestingly, extraordinary responders have been reported in cancer trials with rapalogs. There is some genetic evidence that such responses might be dictated by tumor-specific loss of function mutations in TSC1 or TSC2, as observed in cases of bladder and thyroid cancer [24, 25]. Solid tumors with rare activating mutations in mTOR, itself, have also been found to exhibit exceptional sensitivity to rapalogs [26, 27]. Reciprocally, a mutation in the FRB domain of mTOR (Box 2) was found to underlie acquired resistance to rapalogs in a case of anaplastic thyroid cancer that had a TSC2 mutation and exhibited an exquisite initial response [25]. It will be important to determine if such mutations arise in the tumors of TSC and LAM patients, who face prolonged use of rapalogs.

Given the key role for mTORC1 in driving anabolic metabolism in cancer cells, it is somewhat surprising that rapalogs are not more effective on their own. There are likely several contributing factors to their limited success [28]. First among these is that rapalogs are only partial inhibitors of mTORC1 (see Box 2). Furthermore, rapalogs have been found to elicit cytostatic rather than cytotoxic responses, arresting or delaying cells in the G1 phase of the cell cycle in eukaryotic cells from yeast to human [29]. Even the highly responsive tumors from TSC and LAM patients rapidly regrow upon removal of these drugs [21, 22], suggesting that the tumors shrink due to cytostatic and cell size effects rather than tumor cell

death. Rapalogs have a particularly strong anti-proliferative effect on lymphocytes, which underlies their common use as immunosuppressant agents [30]. These immunosuppressive properties could provide an additional mechanism, not intrinsic to the tumor cells, that limits the anti-tumor effects of rapalogs by hindering immune clearance of tumor cells in the tumor microenvironment. The molecular wiring of the mTOR signaling network might further prevent cytotoxic responses from rapalogs. Inhibition of mTORC1 relieves a large number of distinct negative feedback mechanisms that normally serve to attenuate upstream RTK, PI3K, and mTORC2 signaling, thereby promoting the activation of downstream cell survival pathways [31–39]. Finally, as discussed above, mTORC1 is most frequently activated in cancers through oncogenic events lying upstream (Figure 2). While mTORC1 is a shared downstream effector, it represents just one branch of the highly branched oncogenic signaling nodes most commonly activated in cancer, including RTKs, RAS, and PI3K. As such, mTORC1 inhibition alone is not sufficient to overcome the entire oncogenic program propagated from these pathways. However, as detailed below, accumulating evidence indicates that efficient mTORC1 inhibition might be essential for a given treatment to promote tumor regression.

Sustained mTORC1 signaling as a driver of resistance to targeted therapeutics

Multiple studies in distinct cancer lineages have revealed that mTORC1 inhibition, while not sufficient in most cases, is likely necessary to achieve a clinical response to drugs that target the primary oncogenic pathway in a given cancer. Using genetically-defined cancer cell lines, tumor models, and patient samples, these studies have all arrived at the same conclusion: sustained mTORC1 signaling following treatment with a targeted therapeutic is strongly associated with both innate and acquired resistance to that therapeutic [40]. Thus, while mTORC1 inhibitors appear to be limited in their use as single agent therapies, there is growing evidence that they may be effective in combinatorial approaches to cancer treatment, specifically when used with targeted therapeutics. Below we discuss three distinct cancer settings as examples where the role of mTORC1 in therapeutic resistance has been defined.

EGFR-mutant lung cancers

The epidermal growth factor receptor (EGFR) is an RTK important for many developmental and physiological processes, and it is frequently activated by mutation or amplification in a subset of human cancers [41]. Ligand binding to EGFR induces downstream stimulation of signaling pathways, including the RAS-RAF-MEK-ERK and PI3K-Akt pathways that, when improperly activated, promote tumor development and progression [41–43]. Activating mutations in *EGFR*, which cluster in its kinase domain, occur in up to 15% of human non-small cell lung cancer (NSCLC). In-frame deletions in exon 19 and the L858R mutation account for 85% of all oncogenic *EGFR* mutations and confer sensitivity to the tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib [41, 43]. However, despite multiple clinical trials showing robust initial response rates of *EGFR*-mutant tumors to TKIs, acquired resistance emerges in the majority of cases [41]. The most common mechanism of resistance in lung cancer is associated with a secondary mutation in EGFR itself (T790M), which

renders the activated kinase insensitive to these small molecule inhibitors [44, 45]. As such, second and third generation small molecule inhibitors and therapeutic antibodies have been designed to overcome this resistance at the level of EGFR. These include HKI-272 (neratinib), afatinib, and cetuximab [42, 46], and the pyrimidine-based EGFR TKIs AZD9291, CO-1686, HM61714 and WZ4002 [47–49]. However, activation of bypass signaling events through additional oncogenic mutations, for instance in other RTKs, leading to sustained Ras or PI3K signaling has emerged as a major mechanism of resistance to these next generation EGFR inhibitors in EGFR-mutant NSCLC [41, 50–52].

Multiple independent studies in cellular and mouse models of EGFR-mutant NSCLC have demonstrated that sustained, or incompletely inhibited, mTORC1 signaling can contribute to TKI resistance. Studies in a genetically engineered mouse model (GEMM) of lung cancer driven by *EGFR*^{L858R+T790M} showed that a combination of HKI-272 (neratinib) and rapamycin was greatly superior to either drug alone in causing tumor regression [53]. The combination of afatinib and cetuximab has been found to overcome resistance to first generation TKIs in *EGFR*^{L858R+T790M}-induced lung cancer [54]. However, clinical trials using this drug combination showed a less than 30% patient response, with median response duration of less than 6 months [55, 56]. As observed in the human tumors, resistance to combined treatment with afatinib and cetuximab in both xenograft and *EGFR*^{L858R+T790M} GEMM tumors was associated with sustained mTORC1 signaling, and rapamycin could overcome this resistance [57]. Interestingly, sequencing of biopsies from resistant tumors revealed a frameshift mutation in TSC1 in one of the tumors, suggesting loss of function of the TSC complex leading to constitutive mTORC1 signaling. Knockdown of *TSC1* expression induced afatinib resistance in an otherwise sensitive *EGFR*^{L858R+T790M} NSCLC line, providing genetic evidence that sustained mTORC1 signaling is sufficient to cause resistance. An additional study found that rapamycin prevents lung tumor formation in an inducible *EGFR*^{L858R+T790M} GEMM and can increase progression-free survival after treatment with an EGFR TKI [58]. The importance of mTORC1 reactivation as a mechanism of acquired resistance has also been extended to *EGFR*^{L858R+T790M} cellular and mouse models treated with a combination of the pyrimidine-based EGFR inhibitor WZ4002 and a MEK inhibitor (trametinib) [52]. Taken together, these studies suggest that sustained mTORC1 signaling underlies the acquired resistance of EGFR-mutant NSCLC to treatments that effectively target EGFR.

PIK3CA-mutant breast cancer

It has been estimated that mutations leading to constitutive activation of the PI3K-Akt pathway occur in >70% of breast cancers [59], and activating mutations in *PIK3CA*, encoding the p110 α -isoform of class I PI3K, are particularly prevalent, accounting for ~30% of cases [60]. Drugs that selectively target the p110 α isoform (e.g., BYL719) or multiple isoforms of class I PI3K (e.g., GDC0941) are in clinical development [61], with some clinical trials using *PIK3CA* mutations to strictly stratify the patient population [62, 63]. While this patient stratification clearly increases response rates in such trials, non-responders are still common, indicating innate resistance of these tumors. A similar phenomenon has been observed in a large panel of breast cancer cell lines, where *PIK3CA* mutations are the best predictor of sensitivity to BYL719, but strongly resistant cell lines with these mutations

also exist [64]. While PI3K inhibitors properly block PI3K-Akt signaling in the resistant lines, these cells invariably exhibited sustained mTORC1 signaling. Furthermore, when BYL719-sensitive cell lines were selected for resistance, the resistant clones had reactivated mTORC1. Treatment with a rapalog was sufficient to sensitize the resistant cells to BYL719, and the combined treatment halted xenograft tumor growth, whereas either treatment alone had little to no effect [64]. Importantly, the status of a commonly used marker of mTORC1 signaling, phosphorylation of the ribosomal protein S6 (phospho-S6), appears to predict sensitivity or resistance in biopsies from breast cancer patients treated with BYL719. *PIK3CA*-mutant tumors that respond show inhibition of phospho-S6, while those with either innate or acquired resistance show continued presence of phospho-S6 with BYL719 treatment. Therefore, sustained mTORC1 signaling in *PIK3CA* mutant breast cancers treated with PI3K inhibitors is strongly associated with both innate and acquired resistance.

BRAF-mutant melanoma

The *BRAF* oncogene encodes a Ser/Thr kinase that is activated by somatic point mutations, most commonly V600E, resulting in constitutive activation of the RAF-MEK-ERK signaling pathway in a subset of human cancers [65–67]. B-RAF^{V600E} is the driver oncogene in over 50% of melanomas. There have been excellent advances in the development of RAF and MEK inhibitors to target this pathway, with FDA-approved drugs for treating metastatic *BRAF*-mutant melanoma including the MEK inhibitor trametinib and two RAF inhibitors, vemurafenib and dabrafenib [68–70]. However, despite robust initial responses to these agents, drug resistance eventually emerges [66, 67]. Accordingly, various mechanisms of resistance to *BRAF*-targeted therapies have been investigated [66, 67], with some studies pointing to a role for the establishment of alternative routes to mTORC1 activation [71, 72]. In a panel of melanoma cell lines, whether phospho-S6 levels were decreased or sustained corresponded to the respective sensitivity or resistance of the cells to RAF-MEK pathway inhibitors, whereas phospho-ERK levels were less correlated with the response [71]. Likewise, in xenograft tumor models and patient biopsies, the inhibition/activation of mTORC1 signaling also correlated with the sensitivity/resistance to RAF-MEK-targeting compounds. In melanoma patients, sustained phospho-S6 was associated with poor prognosis, and initial responders that became refractory to treatment showed reappearance of phospho-S6 in tumors. Furthermore, an independent study demonstrating that mutations in the NF1 tumor suppressor promote resistance to B-Raf inhibitors in mouse models of B-Raf mutant melanoma found that mTOR inhibitors can overcome this resistance [72]. Thus, mTORC1 activation through mechanisms that bypass RAF or MEK inhibition contributes to both innate and acquired resistance in melanoma. These studies provide evidence that mTORC1 inhibition is necessary for effective tumor response to RAF-MEK-targeted therapeutics in melanoma.

Mechanisms contributing to sustained mTORC1 signaling and therapeutic resistance

The implication of the findings from multiple distinct cancer lineages is that mTORC1 inhibition appears to be necessary to achieve a lasting clinical response to drugs targeting the primary oncogenes in a given cancer (Figure 3). It seems likely that sustained mTORC1

signaling will be found as a major mechanism of resistance to targeted therapeutics in many cancers. For instance, it was recently shown that activation of an alternate route to mTORC1 underlies the resistance of *PIK3CA*-mutant head and neck squamous cell carcinomas to a PI3K-p110 α inhibitor [73]. Identifying the most common, lineage-specific mechanisms leading to sustained mTORC1 signaling that are independent of the original therapeutic target is key to developing strategies for more effective combination therapies. A common mechanism will likely be through the upregulation, amplification, or oncogenic activation of upstream RTKs [73, 74]. In addition, oncogenic activation of the PI3K-Akt pathway in Ras or Raf-driven cancers, and vice-versa, will maintain mTORC1 signaling in the face of treatments that target these common oncogenic pathways. While TSC mutations occur in sporadic tumors, such as bladder and liver, they are rare in most cancers (<5%). However, given its central role in mTORC1 regulation, it is probable that more mutations affecting the function of the TSC complex will be identified in future genetic studies aimed at revealing the mode of innate or acquired resistance in different cancer settings. In the three cancers discussed above, loss of function of the TSC complex was shown to confer resistance to targeted therapeutics in cell-based models [57, 64, 71]. While mutations leading to increased signaling of the Rag GTPases to mTORC1, or activating mutations on mTOR itself, have been identified [26, 27, 75, 76], the vast majority of oncogenes and tumor suppressors influencing the activation state of mTORC1 signal through the TSC complex (Figure 2). Therefore, it is likely that, whether through direct mutation or genetic events affecting upstream components of the network, aberrant inhibition of the TSC complex will frequently underlie sustained mTORC1 signaling, and thus, therapeutic resistance.

The downstream consequences of sustained mTORC1 activation that contribute to its role in therapeutic resistance are not currently known. As mTORC1 drives a metabolic program to promote tumor cell growth [2–9, 28] it is possible that an inability to block these anabolic processes underlies resistance. A potential role for mTORC1 signaling in triggering immune checkpoint responses has also recently come to light. mTORC1 activation in NSCLC was found to promote expression of the immune checkpoint receptor PD-L1, thereby contributing to immune evasion [77]. Therefore, while mTOR inhibitors directly suppress T-cell activation [30], perhaps contributing to their limited use as monotherapies, sustained mTORC1 signaling in tumor cells might also exert an immunosuppressive, protective effect that is intrinsic to the tumor.

Concluding Remarks

The collective findings from the studies discussed above suggest a new paradigm in precision medicine to enhance and prolong tumor responses to targeted therapeutics. Patient stratification based on the identification of driver oncogenes remains a priority for selecting the primary molecular target and specific inhibitor. Where possible, tumor biopsies before treatment and again early on in the treatment, with assessment of markers of mTORC1 signaling, such as phospho-S6, would be powerful in predicting responders and non-responders. Those with sustained mTORC1 signaling could immediately move to combination therapy with an mTOR inhibitor. However, in therapeutic settings where most, if not all, patients eventually become refractory to the initial treatment, a frontline therapy that combines treatment with an mTOR inhibitor would prevent or delay the development of

resistance. The potential toxicity of such combinations, especially with the newer mTOR kinase inhibitors (Box 2), will be a major consideration. Based in part on preclinical studies such as those described above, many cancer trials combining either chemotherapies or specific targeted therapies with mTOR inhibitors are already underway (<http://clinicaltrials.gov>).

The past 20 years has seen tremendous progress in identifying the oncogenes and tumor suppressors that provide the genetic basis of cancer development. The use of therapeutic compounds targeting the effected proteins and pathways has uncovered the tremendous plasticity within oncogenic signaling networks that underlies the inevitable development of resistance. As illustrated here with mTORC1, understanding signal rewiring and resistance at the molecular level is essential to developing more effective treatment strategies for cancer (see Outstanding Questions).

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REFERENCES

1. Garraway LA, Janne PA. Circumventing cancer drug resistance in the era of personalized medicine. *Cancer Discovery*. 2012; 2:214–226. [PubMed: 22585993]
2. Dibble CC, Manning BD. Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. *Nat. Cell Biol*. 2013; 15:555–564. [PubMed: 23728461]
3. Robitaille AM, et al. Quantitative Phosphoproteomics Reveal mTORC1 Activates de Novo Pyrimidine Synthesis. *Science*. 2013; 339:1320–1323. [PubMed: 23429704]
4. Ben-Sahra I, et al. Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. *Science*. 2013; 339:1323–1328. [PubMed: 23429703]
5. Duvel K, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol. Cell*. 2010; 39:171–183. [PubMed: 20670887]
6. Porstmann T, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell Metab*. 2008; 8:224–236. [PubMed: 18762023]
7. Ricoult SJH, et al. Oncogenic PI3K and K-Ras stimulate de novo lipid synthesis through mTORC1 and SREBP. *Oncogene*. 2015; 35:1250–1260. [PubMed: 26028026]
8. Howell Jessica J, et al. A growing role for mTOR in promoting anabolic metabolism. *Biochem. Soc. Trans*. 2013; 41:906–912. [PubMed: 23863154]
9. Ben-Sahra I, et al. mTORC1 induces purine synthesis through control of the mitochondrial tetrahydrofolate cycle. *Science*. 2016; 351:728–733. [PubMed: 26912861]
10. Dibble CC, Cantley LC. Regulation of mTORC1 by PI3K signaling. *Trends Cell Biol*. 2015; 25:545–555. [PubMed: 26159692]
11. Bar-Peled L, Sabatini DM. Regulation of mTORC1 by amino acids. *Trends Cell Biol*. 2014; 24:400–406. [PubMed: 24698685]
12. Sancak Y, et al. Ragulator-Rag Complex Targets mTORC1 to the Lysosomal Surface and Is Necessary for Its Activation by Amino Acids. *Cell*. 2010; 141:290–303. [PubMed: 20381137]
13. Menon S, et al. Spatial Control of the TSC Complex Integrates Insulin and Nutrient Regulation of mTORC1 at the Lysosome. *Cell*. 2014; 156:771–785. [PubMed: 24529379]
14. Manning BD, et al. Identification of the Tuberous Sclerosis Complex-2 Tumor Suppressor Gene Product Tuberin as a Target of the Phosphoinositide 3-Kinase/Akt Pathway. *Mol. Cell*. 2002; 10:151–162. [PubMed: 12150915]

15. Inoki K, et al. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat. Cell Biol.* 2002; 4:648–657. [PubMed: 12172553]
16. Tee AR, et al. Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. *Proc. Natl. Acad. Sci. U. S. A.* 2002; 99:13571–13576. [PubMed: 12271141]
17. Roux PP, et al. Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. *Proc. Natl. Acad. Sci. U. S. A.* 2004; 101:13489–13494. [PubMed: 15342917]
18. Ma L, et al. Phosphorylation and Functional Inactivation of TSC2 by Erk. *Cell.* 2005; 121:179–193. [PubMed: 15851026]
19. Menon S, Manning BD. Common corruption of the mTOR signaling network in human tumors. *Oncogene.* 2008; (27 Suppl 2):S43–S51. [PubMed: 19956179]
20. Chiarini F, et al. Current treatment strategies for inhibiting mTOR in cancer. *Trends Pharmacol. Sci.* 2015; 36:124–135. [PubMed: 25497227]
21. Bissler JJ, et al. Sirolimus for Angiomyolipoma in Tuberous Sclerosis Complex or Lymphangioleiomyomatosis. *N. Engl. J. Med.* 2008; 358:140–151. [PubMed: 18184959]
22. Franz DN, et al. Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. *Ann. Neurol.* 2006; 59:490–498. [PubMed: 16453317]
23. Krueger DA, et al. Everolimus for Subependymal Giant-Cell Astrocytomas in Tuberous Sclerosis. *N. Engl. J. Med.* 2010; 363:1801–1811. [PubMed: 21047224]
24. Iyer G, et al. Genome Sequencing Identifies a Basis for Everolimus Sensitivity. *Science.* 2012; 338:221–221. [PubMed: 22923433]
25. Wagle N, et al. Response and Acquired Resistance to Everolimus in Anaplastic Thyroid Cancer. *N. Engl. J. Med.* 2014; 371:1426–1433. [PubMed: 25295501]
26. Grabiner BC, et al. A diverse array of cancer-associated mTOR mutations are hyperactivating and can predict rapamycin sensitivity. *Cancer Discovery.* 2014; 4:554–563. [PubMed: 24631838]
27. Wagle N, et al. Activating mTOR mutations in a patient with an extraordinary response on a phase I trial of everolimus and pazopanib. *Cancer Discovery.* 2014; 4:546–553. [PubMed: 24625776]
28. Li J, et al. Rapamycin: one drug, many effects. *Cell Metab.* 2014; 19:373–379. [PubMed: 24508508]
29. Heitman J, et al. Targets for Cell Cycle Arrest by the Immunosuppressant Rapamycin in Yeast. *Science.* 1991; 253:905–909. [PubMed: 1715094]
30. Pollizzi KN, Powell JD. Regulation of T cells by mTOR: The known knowns and the known unknowns. *Trends Immunol.* 2015; 36:13–20. [PubMed: 25522665]
31. Harrington LS, et al. The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. *J. Cell Biol.* 2004; 166:213–223. [PubMed: 15249583]
32. Shah OJ, et al. Inappropriate Activation of the TSC/Rheb/mTOR/S6K Cassette Induces IRS1/2 Depletion, Insulin Resistance, and Cell Survival Deficiencies. *Curr. Biol.* 2004; 14:1650–1656. [PubMed: 15380067]
33. Manning BD. Balancing Akt with S6K: implications for both metabolic diseases and tumorigenesis. *J. Cell Biol.* 2004; 167:399–403. [PubMed: 15533996]
34. O'Reilly KE, et al. mTOR Inhibition Induces Upstream Receptor Tyrosine Kinase Signaling and Activates Akt. *Cancer Res.* 2006; 66:1500–1508. [PubMed: 16452206]
35. Manning BD, Cantley LC. AKT/PKB Signaling: Navigating Downstream. *Cell.* 2007; 129:1261–1274. [PubMed: 17604717]
36. Dibble CC, et al. Characterization of Rictor Phosphorylation Sites Reveals Direct Regulation of mTOR Complex 2 by S6K1. *Mol. Cell. Biol.* 2009; 29:5657–5670. [PubMed: 19720745]
37. Hsu PP, et al. The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. *Science.* 2011; 332:1317–1322. [PubMed: 21659604]
38. Yu Y, et al. Quantitative Phosphoproteomic Analysis Identifies the Adaptor Protein Grb10 as an mTORC1 Substrate that Negatively Regulates Insulin Signaling. *Science.* 2011; 332:1322–1326. [PubMed: 21659605]

39. Rodrik-Outmezguine VS, et al. mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. *Cancer Discovery*. 2011; 1:248–259. [PubMed: 22140653]
40. Kelsey I, Manning BD. mTORC1 Status Dictates Tumor Response to Targeted Therapeutics. *Sci. Signaling*. 2013; 6:pe31–pe31.
41. Chong CR, Janne PA. The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat. Med. (N. Y., NY, U. S.)*. 2013; 19:1389–1400.
42. Ciardiello F, Tortora G. EGFR Antagonists in Cancer Treatment. *N. Engl. J. Med*. 2008; 358:1160–1174. [PubMed: 18337605]
43. Sharma SV, et al. Epidermal growth factor receptor mutations in lung cancer. *Nat. Rev. Cancer*. 2007; 7:169–181. [PubMed: 17318210]
44. Kobayashi S, et al. EGFR Mutation and Resistance of Non-Small-Cell Lung Cancer to Gefitinib. *N. Engl. J. Med*. 2005; 352:786–792. [PubMed: 15728811]
45. Pao W, et al. Acquired Resistance of Lung Adenocarcinomas to Gefitinib or Erlotinib Is Associated with a Second Mutation in the EGFR Kinase Domain. *PLoS Med*. 2005; 2:0225–0235.
46. Kwak EL, et al. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc. Natl. Acad. Sci. U. S. A*. 2005; 102:7665–7670. [PubMed: 15897464]
47. Zhou W, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. *Nature*. 2009; 462:1070–1074. [PubMed: 20033049]
48. Walter AO, et al. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M-mediated resistance in NSCLC. *Cancer Discovery*. 2013; 3:1404–1415. [PubMed: 24065731]
49. Cross DAE, et al. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discovery*. 2014; 4:1046–1061. [PubMed: 24893891]
50. Engelman JA, et al. ErbB-3 mediates phosphoinositide 3-kinase activity in gefitinib-sensitive non-small cell lung cancer cell lines. *Proc. Natl. Acad. Sci. U. S. A*. 2005; 102:3788–3793. [PubMed: 15731348]
51. Wheeler DL, et al. Understanding resistance to EGFR inhibitors—impact on future treatment strategies. *Nat. Rev. Clin. Oncol*. 2010; 7:493–507. [PubMed: 20551942]
52. Tricker EM, et al. Combined EGFR/MEK Inhibition Prevents the Emergence of Resistance in EGFR-Mutant Lung Cancer. *Cancer Discovery*. 2015; 5:960–971. [PubMed: 26036643]
53. Li D, et al. Bronchial and Peripheral Murine Lung Carcinomas Induced by T790M-L858R Mutant EGFR Respond to HKI-272 and Rapamycin Combination Therapy. *Cancer Cell*. 2007; 12:81–93. [PubMed: 17613438]
54. Regales L, et al. Dual targeting of EGFR can overcome a major drug resistance mutation in mouse models of EGFR mutant lung cancer. *J. Clin. Invest*. 2009; 119:3000–3010. [PubMed: 19759520]
55. Janjigian YY, et al. Dual Inhibition of EGFR with Afatinib and Cetuximab in Kinase Inhibitor-Resistant EGFR-Mutant Lung Cancer with and without T790M Mutations. *Cancer Discovery*. 2014; 4:1036–1045. [PubMed: 25074459]
56. Pirazzoli V, et al. Afatinib plus Cetuximab Delays Resistance Compared to Single-Agent Erlotinib or Afatinib in Mouse Models of TKI-Naïve EGFR L858R-Induced Lung Adenocarcinoma. *Clin. Cancer Res*. 2016; 22:426–435. [PubMed: 26341921]
57. Pirazzoli V, et al. Acquired Resistance of EGFR-Mutant Lung Adenocarcinomas to Afatinib plus Cetuximab Is Associated with Activation of mTORC1. *Cell Rep*. 2014; 7:999–1008. [PubMed: 24813888]
58. Kawabata S, et al. Rapamycin prevents the development and progression of mutant EGFR lung tumors with the acquired resistance mutation T790M. *Cell Rep*. 2014; 7:1824–1832. [PubMed: 24931608]
59. Ebi H, et al. PI3K regulates MEK/ERK signaling in breast cancer via the Rac-GEF, P-Rex1. *Proc. Natl. Acad. Sci. U. S. A*. 2013; 110:21124–21129. [PubMed: 24327733]
60. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene*. 2008; 27:5497–5510. [PubMed: 18794884]
61. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat. Rev. Cancer*. 2009; 9:550–562. [PubMed: 19629070]

62. Juric D, et al. Abstract CT-01: BYL719, a next generation PI3K alpha specific inhibitor: Preliminary safety, PK, and efficacy results from the first-in-human study. *Cancer Res.* 2012; 72:CT-01–CT-01.
63. Fritsch C, et al. Characterization of the Novel and Specific PI3K α Inhibitor NVP-BYL719 and Development of the Patient Stratification Strategy for Clinical Trials. *Mol. Cancer Ther.* 2014; 13:1117–1129. [PubMed: 24608574]
64. Elkabets M, et al. mTORC1 Inhibition Is Required for Sensitivity to PI3K p110 α Inhibitors in PIK3CA-Mutant Breast Cancer. *Sci. Transl. Med.* 2013; 5:196ra199–196ra199.
65. Davies H, et al. Mutations of the BRAF gene in human cancer. *Nature.* 2002; 417:949–954. [PubMed: 12068308]
66. Salama AKS, Flaherty KT. BRAF in Melanoma: Current Strategies and Future Directions. *Clin. Cancer Res.* 2013; 19:4326–4334. [PubMed: 23770823]
67. Holderfield M, et al. Targeting RAF kinases for cancer therapy: BRAF mutated melanoma and beyond. *Nat. Rev. Cancer.* 2014; 14:455–467. [PubMed: 24957944]
68. Flaherty KT, et al. Improved Survival with MEK Inhibition in BRAF-Mutated Melanoma. *N. Engl. J. Med.* 2012; 367:107–114. [PubMed: 22663011]
69. Chapman PB, et al. Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. *N. Engl. J. Med.* 2011; 364:2507–2516. [PubMed: 21639808]
70. Hauschild A, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet.* 2012; 380:358–365. [PubMed: 22735384]
71. Corcoran RB, et al. TORC1 Suppression Predicts Responsiveness to RAF and MEK Inhibition in BRAF-Mutant Melanoma. *Sci. Transl. Med.* 2013; 5:196ra198–196ra198.
72. Maertens O, et al. Elucidating Distinct Roles for NF1 in Melanomagenesis. *Cancer Discovery.* 2013; 3:338–349. [PubMed: 23171796]
73. Elkabets M, et al. AXL Mediates Resistance to PI3K α Inhibition by Activating the EGFR/PKC/mTOR Axis in Head and Neck and Esophageal Squamous Cell Carcinomas. *Cancer Cell.* 2015; 27:533–546. [PubMed: 25873175]
74. Niederst MJ, Engelman JA. Bypass Mechanisms of Resistance to Receptor Tyrosine Kinase Inhibition in Lung Cancer. *Sci. Signaling.* 2013; 6:1–6.
75. Bar-Peled L, et al. A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science.* 2013; 340:1100–1106. [PubMed: 23723238]
76. Okosun J, et al. Recurrent mTORC1-activating RRAGC mutations in follicular lymphoma. *Nat. Genet.* 2016; 48:183–188. [PubMed: 26691987]
77. Lastwika KJ, et al. Control of PD-L1 expression by oncogenic activation of the AKT/mTOR pathway in non-small cell lung cancer. *Cancer Res.* 2015; 76:227–238. [PubMed: 26637667]
78. Wullschlegel S, et al. TOR Signaling in Growth and Metabolism. *Cell.* 2006; 124:471–484. [PubMed: 16469695]
79. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell.* 2012; 149:274–293. [PubMed: 22500797]
80. Sarbassov DD, et al. Phosphorylation and Regulation of Akt/PKB by the Rictor-mTOR Complex. *Science.* 2005; 307:1098–1101. [PubMed: 15718470]
81. García-Martínez Juan M, Alessi Dario R. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem. J.* 2008; 416:375–385. [PubMed: 18925875]
82. Facchinetti V, et al. The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. *EMBO J.* 2008; 27:1932–1943. [PubMed: 18566586]
83. Ikenoue T, et al. Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. *EMBO J.* 2008; 27:1919–1931. [PubMed: 18566587]
84. Guertin DA, Sabatini DM. The Pharmacology of mTOR Inhibition. *Sci. Signaling.* 2009; 2:pe24–pe24.
85. Benjamin D, et al. Rapamycin passes the torch: a new generation of mTOR inhibitors. *Nat. Rev. Drug Discovery.* 2011; 10:868–880. [PubMed: 22037041]

86. Sarbassov DD, et al. Prolonged Rapamycin Treatment Inhibits mTORC2 Assembly and Akt/PKB. *Mol. Cell.* 2006; 22:159–168. [PubMed: 16603397]
87. Choo AY, et al. Rapamycin differentially inhibits S6Ks and 4E–BP1 to mediate cell-type-specific repression of mRNA translation. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:17414–17419. [PubMed: 18955708]
88. Thoreen CC, et al. An ATP-competitive Mammalian Target of Rapamycin Inhibitor Reveals Rapamycin-resistant Functions of mTORC1. *J. Biol. Chem.* 2009; 284:8023–8032. [PubMed: 19150980]
89. Feldman ME, et al. Active-Site Inhibitors of mTOR Target Rapamycin-Resistant Outputs of mTORC1 and mTORC2. *PLoS Biol.* 2009; 7:0371–0383.
90. Kang SA, et al. mTORC1 Phosphorylation Sites Encode Their Sensitivity to Starvation and Rapamycin. *Science.* 2013; 341:1236566–1236561–1236566–1236569. [PubMed: 23888043]
91. Liu Q, et al. Kinome-wide Selectivity Profiling of ATP-competitive Mammalian Target of Rapamycin (mTOR) Inhibitors and Characterization of Their Binding Kinetics. *J. Biol. Chem.* 2012; 287:9742–9752. [PubMed: 22223645]

Box 1**Differential regulation and function of mTORC1 and mTORC2**

The Ser/Thr kinase mTOR exists in two major protein complexes in cells, mTORC1 and mTORC2, which are distinct in their regulation, susceptibility to different classes of inhibitors, and the downstream substrates that mTOR phosphorylates as a component of each complex (Figure 1) [78, 79]. Both complexes contain mTOR and a protein that associates with its kinase domain, called mLST8. It is believed that the functional differences between mTORC1 and mTORC2 stem from the other core components: Raptor for mTORC1 and a complex between Rictor and mSIN1 for mTORC2. The activation status of mTORC1 is acutely responsive to many different signals, including exogenous growth factors, endogenous nutrients (amino acids), and energy (ATP), and phosphorylation of its downstream substrates is also responsive to these cues [2]. Although the mechanism is not completely understood, growth factors also stimulate mTORC2 activity toward a subset of its downstream targets. However, other mTORC2 targets are phosphorylated constitutively [80–83]. The phosphorylation state of specific downstream targets in cells and tissues is a reliable readout of mTORC1 and mTORC2 activity. It is important to note that phospho-mTOR antibodies (e.g., phospho-S2448), often widely used to analyze clinical samples, do not distinguish between mTORC1 and mTORC2, and the functional relevance of the majority of the phosphorylation sites on mTOR remains unknown. Although mTORC1 and mTORC2 can both phosphorylate a regulatory sequence, referred to as the hydrophobic motif, which is highly conserved within the AGC family of protein kinases, they regulate a strictly non-overlapping subset of kinases from this family. For instance, mTORC1 phosphorylates the hydrophobic motif T389 on S6K1, whereas mTORC2 phosphorylates Akt on S473 within this motif. The determinants of differential substrate specificity for these and other structurally distinct targets of mTORC1 and mTORC2 have not been fully elucidated, but secondary binding motifs and differential subcellular localization of the downstream targets are likely to contribute.

Box 2**The complexity of rapamycin and other mTOR inhibitors**

Rapamycin (or sirolimus) and its analogs (e.g., everolimus, temsirolimus), collectively referred to as rapalogs, are all highly specific allosteric inhibitors of mTOR with the same mechanism of action [28, 84, 85]. Rapalogs bind to the intracellular protein FKBP12, and this drug-protein complex binds to the FKBP12-rapamycin binding (FRB) domain of mTOR, which is highly conserved and lies just N-terminal to the kinase domain. Some key features of this inhibition are important to note: 1) Binding of the FKBP12-rapalog complex to the FRB domain only occurs when mTOR is free of a complex or within mTORC1. Low nanomolar doses of rapalogs lead to rapid inhibition (within minutes) of mTORC1 but not mTORC2. 2) Higher doses or prolonged exposure (several hours or days) to rapalogs can also sequester free mTOR away from mTORC2 and block assembly of this complex, leading to its inhibition [86]. The influence of rapalogs on mTORC2 stability and activity varies greatly between different cell types and tissues. While it is currently unknown what underlies these differences, one could speculate that the rate of mTORC2 turnover might play a major role, with settings exhibiting higher turnover rates being most sensitive to rapalog-mediated mTORC2 inhibition. 3) Although rapalogs are highly specific, it is now well recognized that they only partially inhibit mTORC1 [87–89]. The sequence context of a given site phosphorylated by mTORC1, even distinct sites on the same downstream substrate, appears to play a particularly important role in dictating its sensitivity to rapalogs [90]. For instance, S6K1-T389 phosphorylation is highly sensitive to rapalogs in all settings, whereas another canonical downstream target 4E-BP1 has inhibitory sites directly phosphorylated by mTORC1 (T37 and T46) that are largely resistant. Therefore, if a cellular process is found to be resistant to rapalogs, one cannot conclude that it is occurring in an mTORC1-independent manner. Reciprocally, if a process is found to be rapalog sensitive, then the effects discussed above on mTORC2 should be considered, especially when using higher doses or longer durations of rapalog treatment.

A variety of second-generation mTOR inhibitors have been developed in recent years that directly target the ATP-binding pocket of the mTOR kinase domain and, therefore, completely inhibit both mTORC1 and mTORC2. While one cannot distinguish between effects on mTORC1 versus mTORC2 without employing additional genetic approaches, these compounds have played a critical role in uncovering functions of mTORC1 that are more resistant to rapalogs, such as its promotion of protein synthesis or inhibition of autophagy [88–90]. Importantly, mTORC2-specific inhibitors are not currently available. Due to their mode of action, mTOR kinase domain inhibitors are not as specific and have various off-target effects on other protein and lipid kinases, including members of the phosphoinositide-3 kinase (PI3K) family and related protein kinases, which are evolutionarily related to mTOR [91]. However, reasonably selective tool compounds (e.g., torin1) and clinical stage drugs (e.g., AZD-2014, MLN0128/INK128, OSI-027, and GDC-0349) have been developed. Many cancer trials are underway, or just completed, with this second generation of mTOR inhibitors as monotherapies, as well as those that target both mTOR and PI3K isoforms [20]. While rapalogs have their limitations, as

discussed above, they have excellent pharmacokinetic and pharmacodynamic properties. An important outstanding question is, if similar properties can be achieved with mTOR kinase inhibitors, leading to widespread and complete inhibition of both mTORC1 and mTORC2, will these drugs display superior anti-tumor activity and can the treatments be tolerated at the effective doses.

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Outstanding Questions Box

- Will highly selective mTOR kinase inhibitors, which fully inhibit both mTORC1 and mTORC2, have an effective therapeutic window that is tolerated and superior to rapalogs?
- How widespread is the role of sustained mTORC1 activity in the development of resistance to targeted therapeutics across cancer lineages?
- Can biomarkers of mTORC1 activation, such as phospho-S6, be used as a predictor of resistance early on in treatment with targeted therapies?
- Will treatments starting with an mTOR inhibitor combined with an oncogene-targeting therapeutic increase response rates and delay the development of resistance?
- Are there effective dosing schemes with such combination therapies that will be reasonably well tolerated?
- Do the immunosuppressive effects of mTOR inhibitors, such as rapalogs, limit their use in cancer treatment, alone or in combination with chemotherapies, targeted therapies, or immunotherapies?
- How do mTOR inhibitors differentially influence tumor and stromal cells, including immune cells, in the tumor microenvironment, and how do these effects impact the sensitivity or resistance of tumors to these inhibitors or combination therapies?
- What are the most common physiological or genetic events leading to alternative activation of mTORC1, and thus resistance, in tumors with distinct anatomical locations and primary oncogenic drivers?
- Are there molecular properties of the oncogenic signaling network that underlie an inherent ability to rewire in response to specific inhibitors leading to therapeutic resistance?
- What are the downstream functions of mTORC1 that must be inhibited to achieve a clinical response to targeted therapeutics?
- Are there other shared effectors regulated downstream of common oncogenic signaling pathways that, like mTORC1, contribute to the development of resistance to targeted therapeutics in different cancer settings?

Trends Box

The movement toward precision medicine for cancer therapy has been hindered by innate and acquired resistance to targeted therapeutics.

Common oncogenes and tumor suppressors act in a signaling network that converges on mTORC1, leading to its aberrant activation in the majority of tumors.

While mTOR inhibitors are effective as monotherapies in some tumor settings, such as tuberous sclerosis complex, they are generally not sufficient to achieve anti-tumor responses in most sporadic cancers.

Evidence from multiple cancer lineages have demonstrated that sustained mTORC1 signaling following treatment with a targeted therapeutic is strongly associated with both innate and acquired resistance to that drug.

There is growing evidence that mTOR inhibitors are effective in combination with other targeted therapeutics to achieve prolonged anti-tumor responses and delay resistance.

Figure 1. The two mTOR complexes: components, regulation, substrates, and inhibition

The Ser/Thr kinase mTOR exists in two structurally and functionally distinct complexes, mTORC1 and mTORC2. The core essential components of each complex are shown. The activation state of mTORC1 is responsive to both nutrients and growth factors and is acutely sensitive to inhibition by rapalogs. Growth factors can also increase mTORC2 activity, but not all of its functions are growth factor-stimulated (Box 1). Rapalogs do not directly inhibit mTORC2, but can lead to dissociation and inhibition of the complex over time (Box 2). Both mTORC1 and mTORC2 are equally sensitive to mTOR kinase inhibitors. mTOR phosphorylates distinct downstream substrates within mTORC1 and mTORC2 and a few of the best established targets are shown (Box 1).

Figure 2. A network of oncogenes and tumor suppressors converge on the regulation of mTORC1

Some of the most common oncogenes (depicted in green), including receptor tyrosine kinases (RTKs), oncogenic fusion proteins formed by chromosomal translocations, PI3K, Akt, Ras and Raf, and tumor suppressors (depicted in red), including PTEN, NF1, and LKB1, lie within signaling pathways that share mTORC1 regulation as a downstream target. In normal cells, growth factors bind to and stimulate RTKs and G protein-coupled receptors (GPCR), which can activate mTORC1 through either the PI3K-Akt or Ras-Erk signaling pathways. These pathways converge to inhibit the TSC complex (TSC1, TSC2, and TBC1D7), resulting in the accumulation of GTP bound Rheb, which is a potent and essential activator of mTORC1. Other pathways including LKB1-AMPK and Wnt signaling also impinge on regulation of the TSC complex and Rheb. A parallel pathway exists for amino

acid sensing that involves the Rag family of GTPases, which act as a heterodimer of RagA or B with Rag C or D, and are regulated by different upstream sensors of amino acids. Oncogenic mutations within this network are common in cancer, resulting in aberrant activation of mTORC1 signaling and its promotion of anabolic processes underlying cell growth and proliferation.

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Figure 3. The role of sustained mTORC1 in resistance to targeted therapeutics

A simple model of the response and progression of a tumor treated with a therapeutic targeting the initial driving oncogenic pathway. Inhibitor A blocks Oncogene A (Onc-A), which attenuates signaling to multiple downstream effectors, including mTORC1. If the Onc-A pathway is the primary, non-redundant route to mTORC1, a robust anti-tumor response will be observed. Acquired resistance can develop due to bypass activation of a parallel pathway through Oncogene-B (Onc-B) resulting in sustained mTORC1 signaling in the presence of Inhibitor-A. This resistance can be overcome through combination therapy with an mTOR inhibitor in settings of both innate (i.e. initial non-responsive) and acquired resistance.