Exploring the multifaceted nature of mTOR signaling in cancer

Andrea Caldarola (S5379903)
Biomedical Sciences Master's Program, AY 2023-2024
Supervisor: Prof. Dr. C.F. Calkhoven
Research group: Gene Regulation In Ageing and Age-Related Diseases (ERIBA)

Abstract

The mechanistic target of rapamycin (mTOR) is a protein kinase that plays an important role in regulating various cellular processes related to growth, survival, and proliferation, including protein, nucleotide, and lipid synthesis, as well as autophagy.

mTOR mediates its regulatory functions as the catalytic subunit of two protein complexes: mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2). Many cancers present hyperactivation of mTORC1, which results in uncontrolled cellular growth and proliferation, and, consequently, tumor initiation and progression. The clinical use of rapalogs, the first generation of mTOR inhibitors, has so far proven challenging, owing to their limitations in effectively inhibiting mTORC1, as well as to the fundamental drawbacks associated with mTORC1 inhibition.

This essay presents recent advancements within the field of mTOR signaling in cancer, with a focus on research surrounding the development of enhanced new-generations of mTOR inhibitors, improved targeting of mTOR inhibitor-sensitive cancers, effective combination strategies, as well as mTOR-activating approaches. Based on these advancements, suggestions for areas to be focused on in future research are provided.

Table of Contents

Introduction

The mechanistic target of rapamycin (mTOR) is a serine/threonine protein kinase in the PI3K-related kinase (PIKK) family. The activation of mTOR signaling has a mainly anabolic function, as it promotes lipid, protein and nucleotide production, and suppresses protein catabolism by inhibiting autophagy and proteasomal degradation[1,2]. mTOR activation also inhibits apoptosis, promotes cytoskeleton remodeling, and shifts glucose metabolism from oxidative phosphorylation to glycolysis and the oxidative pentose phosphate pathway[1,2]. These processes, driven by mTOR activation, support cell survival, proliferation, and migration[1,2], which has led to its study within the field of cancer therapeutics.

mTOR acts as the catalytic subunit of two protein complexes: mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2)[1,2]. mTORC1 consists of three core components, namely mTOR, regulatory protein associated with mTOR (Raptor)[3,4] and mammalian lethal with Sec13 protein 8 (mLST8)[5], and two inhibitory subunits, proline-rich Akt substrate of 40 kDa (PRAS40)[6-8] and DEP domain containing mTOR interacting protein (DEPTOR)[9]. mTORC2, on the other hand, is comprised of mTOR, mLST8, and rapamycin insensitive companion of mTOR (Rictor)[10,11], along with DEPTOR[9], mammalian stress-activated protein kinase interacting protein 1 (mSin1)[12-14], as well as protein observed with Rictor 1 and 2 (Protor1/2)[15-17] as regulatory subunits.

Activation and downstream effects of mTOR signaling

Activation of mTORC1 is induced when stimulation through both growth factors and an increase in amino acids concentration is present, whereas the complex is inhibited when metabolic stressors, namely low ATP levels, hypoxia and DNA damage, are present (Figure 1A)[1,2].

A key role within mTORC1 activation is that of the Tuberous Sclerosis Complex (TSC), which acts as the major inhibitor of mTORC1. Many of the pathways activated by cellular receptors of growth factors, including PI3K/Akt, MAPK/ERK and Wnt signaling, as well as the TNFα pathway, result in the inhibition of the TSC, thereby activating mTORC1 signaling[18]. The TSC mediates its regulatory effect by inhibiting the GTPase Ras homolog enriched in brain (Rheb)[19-21], which is bound to the lysosomal membrane. An increase in amino acid concentration results in the activation of the lysosomal membrane-bound Rag GTPases, which can then recruit mTORC1 to the lysosomal membrane where it can be activated by Rheb if the TSC has been inhibited by growth factor signaling[22,23]. mTORC1 can also be activated by Rheb located on the membrane of the Golgi apparatus, as increases in amino acids also activate the Rab1A GTPase, which recruits mTORC1 to the Golgi[24]. Additionally, the activation of PI3K/Akt signaling results in the phosphorylation of mTORC1's inhibitory subunit PRAS40, as well as its dissociation from the complex, further activating mTORC1[7].

Signaling induced by metabolic stressors, including activation of 5' AMP-activated protein kinase (AMPK) due to cellular energy deficit or hypoxia and the p53 -mediated DNA damage response, result in direct inhibition of mTORC1 through phosphorylation[25], as well as inhibition through activation of TSC[26-28] and AMPK-independent inhibition of the Rag GTPases[29,30]. AMPK's direct effect on mTORC1 is mediated through the phosphorylation of its regulatory subunit Raptor, which induces binding of 14-3-3 proteins and thus inhibition of mTORC1[25].

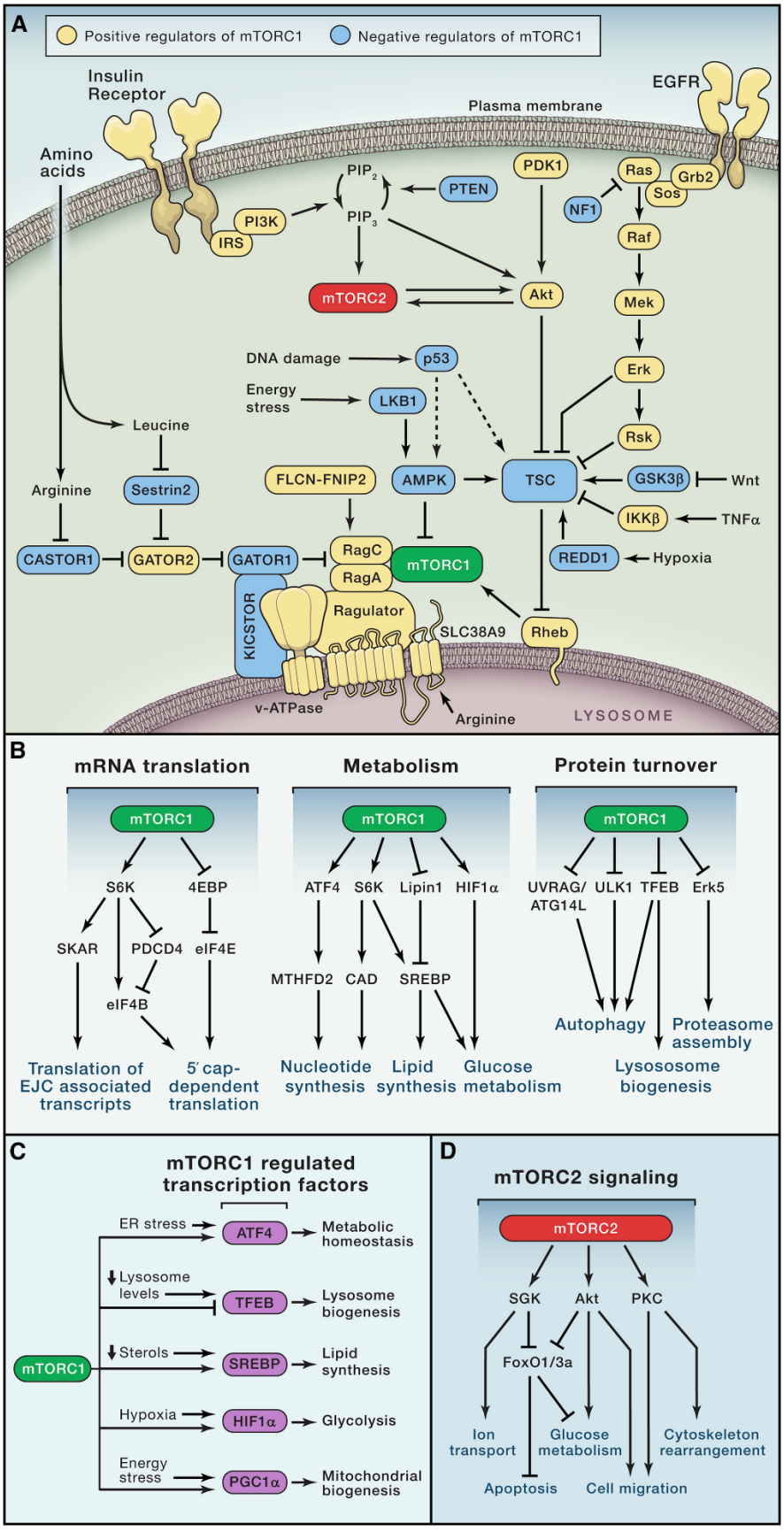

Figure 1. Activation and downstream effects of mTOR signaling.
A) Signaling pathways which regulate mTOR activation.
B) Main downstream pathways promoted by mTORC1 activation.
C) Transcription factors induced by mTORC1 signaling, and physiological stimuli which activate them.
D) Main downstream pathways promoted by mTORC2 activation.

From Saxton & Sabatini, 2017[1]

Like mTORC1, mTORC2 is activated by growth factor-related signaling (Figure 1A), specifically by stimulation through the insulin/insulin-like growth factor-1 (IGF-1) pathway through PI3K and Akt. When PI3K/Akt signaling is not active, mTORC2 is inactivated by its inhibitory subunit mSin1. mSin1 possesses a domain which can be bound by phosphatidylinositol (3,4,5)-trisphosphate, which is generated by activated PI3K[31]. This interaction relieves mSin1's inhibitory effect, thus activating mTORC2. mTORC2 is also activated through phosphorylation of mSin1 by Akt[32], which is itself a primary target of mTORC2[33]: a positive-feedback loop between Akt and mTORC2 activation is present.
Interestingly, mTORC2 has been shown to be activated by certain catabolic signals: mTORC2 is directly activated through phosphorylation by activated AMPK, and it is also activated by nutrient deprivation due to decreasing glutamine catabolites[34].

mTORC2 is regulated by mTORC1 due to the presence of a negative feedback loop within mTORC1 signaling: mTORC1 activates two negative regulators of insulin/IGF-1 signaling, growth factor receptor-bound protein 10 (Grb10)[35,36] and p70S6 Kinase 1 (S6K1)[37,38]. mTORC1 activity is likewise regulated by mTORC2 since, as previously mentioned, activated Akt inhibits the mTORC1-inhibiting TSC[18] and Akt is a primary target of mTORC2[33].
The interplay between the two mTOR complexes and the network of interactions that lead to their activation showcase the great complexity of mTOR signaling.

The two mTOR complexes promote cell survival, proliferation, and migration by phosphorylating effector proteins, through which the effects of mTOR activation on protein, lipid and nucleotide synthesis, as well as glucose metabolism and protein catabolism, are mediated (Figure 1B, 1C and 1D)[1,2].
In protein synthesis, mTORC1 exerts its influence by phosphorylating both eIF4E Binding Protein 1 (4EBP1)[39,40] and S6K1[41], which is also involved in mediating mTORC1's effects on lipid, nucleotide, and glucose metabolism. The phosphorylation of S6K1 triggers its activation through phosphorylation by phosphoinositide-dependent kinase 1 (PDK1), enabling S6K1 to then phosphorylate various substrates that promote mRNA translation initiation and to interact with S6K1 Aly/REF-like substrate (SKAR) to enhance translation efficiency[41,42,43]. Concurrently, the phosphorylation of 4EBP1 triggers its dissociation from the 5'cap-binding protein eIF4E, allowing for mRNA translation to begin[39,40].
In lipid synthesis, mTORC1 mediates the activation of sterol-responsive element binding protein (SREBP) transcription factors[44] both through activating phosphorylation of the aforementioned S6K1, which can phosphorylate SREBP1c to prevent its proteasomal degradation[45,46], as well as inhibitory phosphorylation of the SREBP-inhibiting Lipin1[47]. The activation of SREBPs results in increased expression of genes involved in fatty acid and cholesterol biosynthesis[44].
Regarding nucleotide synthesis, mTORC1 acts by activating through phosphorylation transcription factor 4 (ATF4) and S6K1[48-50]. Activated ATF4 stimulates expression of the mitochondrial tetrahydrofolate (mTHF) cycle enzyme methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), which is involved in purine synthesis[48], while S6K1 phosphorylates and activates carbamoyl-phosphate synthetase (CAD), which is an enzyme involved in pyrimidine synthesis[49,50].
Within the context of glucose metabolism, mTORC1 increases the translation of the transcription factor subunit hypoxia-inducible factor 1-α (HIF-1α), which, in turn, drives the expression of glycolytic enzymes[45].
The activation of SREBPs also enhances flux of glucose through the oxidative pentose phosphate pathway (PPP), as large amounts of NADPH is needed to drive the induced de novo lipid biosynthesis, and the ribose produced in this pathway is also needed for the synthesis of nucleotides[45].

mTORC1 prevents autophagy initiation by phosphorylating a key kinase in autophagosome formation, unc-51-like autophagy-activating kinase 1 (ULK1), thus impeding its activation by AMPK[51]. mTORC1 also phosphorylates the transcription factor EB (TFEB), inhibiting its nuclear translocation and thus its stimulating effect on the expression of genes related to lysosomal biogenesis and autophagy [52,53,54]. Moreover, mTORC1 influences protein turnover through the ubiquitin-proteasome system (UPS), as its inhibition has been shown to increases proteasome-dependent proteolysis, either by elevating protein ubiquitylation[55] or enhancing expression of proteasomal chaperones[56]. Interestingly, prolonged mTORC1 activation boosts proteasome activity, suggesting a compensatory mechanism to counteract the increase in protein synthesis mediated by mTORC1 signaling[57].

mTORC2, on the other hand, mediates its effects by phosphorylating members of the AGC protein kinase family, including PKCα, PKCδ, PKCζ, PKCγ and PKCε, which are involved in regulating cytoskeletal remodeling and cell migration[10,11,58-60]. As mentioned previously, Akt is a primary target of phosphorylation by mTORC2[33]. Once activated, Akt promotes cell survival, proliferation, and growth by inhibiting the FoxO1/3a transcription factors (which promote cell cycle arrest and apoptosis) and the TSC [26,61]. Another protein kinase of the AGC family phosphorylated by mTORC2 is serum- and glucocorticoid-induced protein kinase 1 (SGK1), which also inhibits the FoxO transcription factors and regulates ion transport, thereby promoting cell survival[62].

mTOR signaling and current mTOR-targeting approaches in cancer

The hyperactivation of mTOR signaling results in the promotion of processes associated with oncogenic cell growth, namely cell survival (through inhibition of apoptosis) and proliferation, as well as migration. The signaling pathways that activate mTOR signaling, namely the PI3K/Akt and the MAPK/ERK pathway are indeed often found to be hyperactivated in cancer through mutations in the genes encoding the pathway proteins[1,63]. mTOR signaling hyperactivation can also be induced by activating mutations within the individual components that lead to mTOR activation, such as the Rag GTPases, or inactivating mutations within its negative regulators, such as p53[1,63]. Mutations within the mTOR gene itself can be found at a rate between 12-4% in different cancers: endometrial carcinoma, melanoma, esophagogastric adenocarcinoma, colorectal adenocarcinoma, renal clear cell carcinoma, and non-small cell lung cancer[64]. mTOR has also been observed to be mutated in 3% of metastatic cancers in two cohorts[64].

This interest in mTOR lead to the development of mTOR inhibitors, the first of which are derivatives of the natural compound rapamycin named rapalogs (Figure 2). These compounds act by forming a complex with FK506-binding protein 1A (FKBP1A), which can then bind mTOR both by itself as well as within mTORC1, thus inhibiting the latter[65]. While rapalogs can only directly inhibit mTORC1, the binding of the rapalog-FKBP1A complex to mTOR prevents its participation in the mTORC2 complex, which translates into rapalogs having an inhibitory effect on mTORC2 signaling following prolonged treatment[66]. Two rapalogs, temsirolimus and everolimus, have been approved by the European Medicines Agency for use in advanced HR+, HER2- breast cancer; advanced pancreatic neuroendocrine tumors; advanced neuroendocrine tumors of gastrointestinal or lung origin; advanced renal cell carcinoma and mantle cell lymphomas[67,68].

However, rapalogs have seen limited success in clinical settings, which has been attributed to different factors. Firstly, rapalogs do not completely inhibit mTORC1 activity, as the phosphorylation of some substrates is not completely inhibited by rapalog treatment. Specifically, the phosphorylation of 4EBP1 is only partially affected by rapalog treatment[69-72], and this process has been observed to be among the most important in mTOR hyperactivation-driven tumorigenesis[73,74]. Secondly, rapalogs inhibit mTORC1's

phosphorylation of Grb10 and S6K1[35-38], which once activated negatively regulate PI3K/Akt signaling. Rapamycin has also been shown to activate Ras/MEK/ERK signaling by activating the proto-oncogene tyrosine-protein kinase Src and the epidermal growth factor receptor[75]. Furthermore, rapamycin has been shown to up-regulate expression and phosphorylation of the platelet-derived growth factor receptor beta, resulting in the activation of both PI3k/Akt and MAPK/ERK signaling[76]. Thus, inhibiting mTORC1 partially results in the upregulation of signaling that stimulates cell proliferation. Finally, rapalogs, by inhibiting mTORC1, induce autophagy, which can benefit tumors characterized by poor vascularization and low abundance of nutrients, such as pancreatic cancers[77].

Owing to the drawbacks of rapalogs and mTOR's central role in regulating biological processes associated with malignancy, an important research question to be investigated is thus:

How can mTOR-targeting therapeutic strategies be improved?

mTOR signaling in cancer has been extensively explored in recent years. Certain research findings have led to the identification of several approaches which may be the key to overcoming the limitations of the current based approved therapeutic strategies targeting mTOR. Firstly, improving mTOR targeting through new mTOR inhibitors (mTORi) with different mechanisms of action from rapalogs. Secondly, identifying cancer types which are most responsive to mTORi-based therapeutic approaches. Thirdly, combining mTORi with chemotherapeutic, immunotherapeutic or specific target-therapy compounds. Finally, hyperactivating mTOR in order to induce anti-cancer effects.

Advancements in mTOR inhibitors

To address some of the drawbacks that characterize rapalogs, a new generation of mTORi was developed: ATP-competitive mTORi (ATP-mTORi), which act by binding to the active site of mTOR (Figure 2)[1,63]. This distinct mechanism of action results in ATP-mTORi having different molecular effects from rapalogs. Firstly, ATP-mTORi are capable of binding mTOR both within mTORC1 and mTORC2, and thus inhibit both complexes[70,71]. Secondly, as competitive inhibitors ATP-mTORi act by obstructing the catalytic site, and thus have a stronger effect on the phosphorylation of substrates than rapalogs, which are allosteric inhibitors that bind to a more superficial region of mTOR's catalytic cleft[63]. In line with this, ATP-mTORi have been shown to be effective in inhibiting 4EBP1 phosphorylation[70,71], as opposed to rapalogs, resulting in greater anti-cancer effects and promising pre-clinical results[1,63]. ATP-mTORis have also been explored in clinical settings, though they have so far produced ambivalent results, demonstrating anti-cancer effects as well as toxicity issues. One example is Sapanisertib, which has shown clinical benefit when treating certain cohorts of advanced HR+, HER2- breast cancer patients. Specifically, Lim et al. reported a clinical benefit rate at 16 (CBR-16) weeks of 45% and an overall response rate (ORR) of 8% when combining sapanisertib with exemestane/fulvestrant in advanced HR+, HER2- breast cancer patients which had already been treated with everolimus and exemestane/fulvestrant, and found to be sensitive to everolimus[78]. The CBR-16 and ORR were reduced in patients who had been observed to be everolimus-resistant, respectively at 23% and 2%. Sàenz et al. observed an increase in median progression-free survival (PFS) when comparing treatment with fulvestrant alone (PFS: 3.5 months) to fulvestrant in combination with either weekly sapanisertib (PFS: 5.6 months) or daily sapanisertib (PFS: 7.2 months)[79]. However, treatment discontinuation due to adverse events was also observed more frequently in the combination therapy arms. No ATP-mTORi has been fully approved for clinical use by either the EMA or the FDA, however sapanisertib was granted fast track designation by the FDA for the treatment of patients advanced lung squamous cell carcinomas (LUSCs) with mutations in the nuclear factor erythroid 2-related

factor (NFE2L2) gene, and who have been already treated with chemotherapy and immune checkpoint inhibitor therapy. This followed the results of a clinical trial in which treatment of this patient group with Sapanisertib resulted in an ORR of 25% and a PFS of 8.9 months[80].

It has been hypothesized that the inhibitory effect that ATP-mTORis have on mTORC2 is detrimental to their overall clinical benefit. This is because Akt inhibition (as Akt is a primary target of mTORC2) results in induction of hyperglycemia[81] and the relief of a feedback inhibition mechanism mediated by Akt on the expression of receptor tyrosine kinases (RTK)[82]. Moreover, the enhanced anticancer effect have been found to be mostly mediated by their more complete inhibition of mTORC1, and thus to be independent of mTORC2[70,71]. This has led to the development of third generation mTORi known as bi-steric mTORi or Rapalinks (Figure 2). These compounds are composed of a rapamycin-like core moiety, which determines selectivity for mTORC1, fused to an ATP-mTORi in order to obtain a stronger inhibitory effect on mTORC1's catalytic activity than that of rapalogs[83]. This strategy has proven effective in pre-clinical data, with rapalinks showing effective inhibition of 4EBP1 phosphorylation and anti-cancer effects[84]. Another advantage of rapalinks is that they are less susceptible to the development of drug-resistance through mutations. This is because in the case of rapalogs and ATP-mTORis a mutation respectively in the FKBP12 binding domain or in the kinase domain of mTOR can prevent the binding of the compound to mTOR[85]. Rapalinks, on the other hand, can still inhibit mTORC1 after the development of a resistance mutation, as they bind to mTOR at two different sites. One of these compounds, RMC-5552, is currently being studied as a combination treatment with RAS(ON) inhibitors in patients with relapsed/refractory solid tumors[86]. This trial has shown promising initial results, with a partial response in 1/5 evaluable patients and stable disease in 3/5.

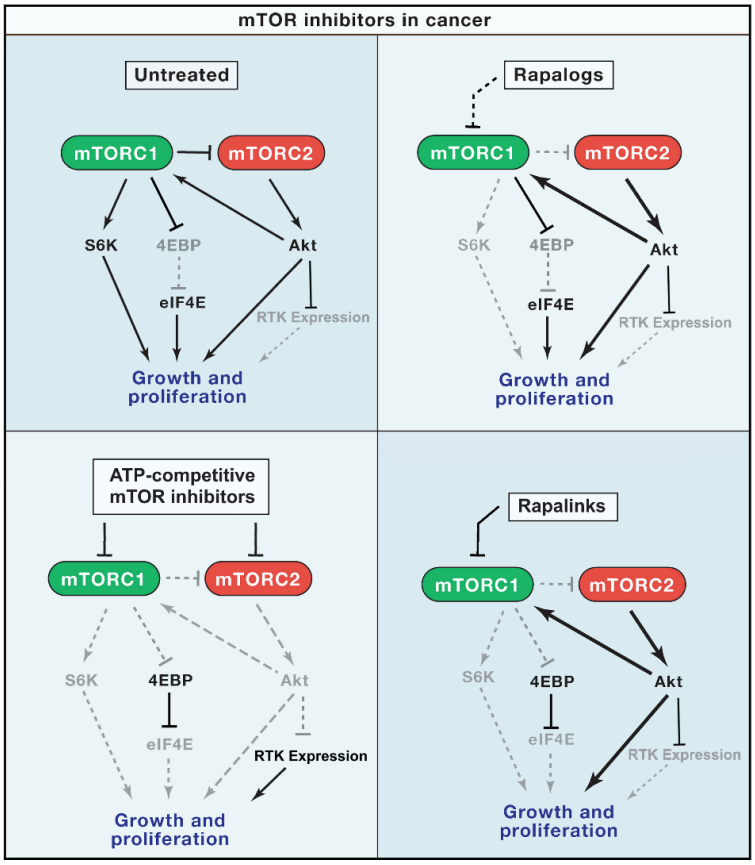

Figure 2. Effects of mTOR inhibitors on major mediators of cell growth and proliferation.
Pathway components active in each condition are indicated in black, inhibited and thus inactive components are indicated in gray.

Adapted from Saxton & Sabatini, 2017[1]

Another avenue of research that has been investigated is improving mTORi through nanomedicinal approaches. This aims to improve the pharmacokinetic properties of mTORi, and thus enhance clinical benefit by increasing drug delivery of the compound to the tumor, thus increasing anti-cancer effects and decreasing adverse events. One example is nab-sirolimus, i.e. albumin-bound rapamycin in the form of nanoparticles, that has exhibited effective anticancer activity in pre-clinical studies[87-89], as well as positive clinical results. In a phase 2 clinical trial, treatment of malignant perivascular epithelioid cell tumors (PEComa) with nab-sirolimus resulted in an ORR of 39%, stable disease in 52% of patients and mostly manageable treatment-related adverse events[90]. Another study showed positive results in different nonhematologic malignancies, with 2/17 evaluated patients showing a partial response and 12/17 exhibiting stable disease[91]. Furthermore, the study found that nab-sirolimus effectively inhibited phosphorylation of 4EBP1, showcasing an improvement over rapalogs regarding this important drawback.

Identification of cancer subtypes sensitive to mTOR inhibitors

In order to improve mTOR targeting approaches, a clear strategy is to identify cancers which are most likely to be heavily affected by mTOR inhibition.

Cancers with mutations in genes that result in hyperactive mTOR signaling, such as activating mutations within mTOR itself[92,93], are the ones most likely to be sensitive to rapalog treatment. Mutations within the components of the TSC, such as TSC1 and TSC2, have been widely observed to determine mTORi sensitivity, with many examples of patients harboring TSC1/2 inactivating mutations exhibiting enhanced clinical benefits from mTORi therapy when applied to different kinds of cancers, including renal and urothelial carcinomas[93,94]. This was also observed in the aforementioned clinical trial in which PEComas were treated with nab-sirolimus: 89% of patients harboring a TSC2 mutation achieved a confirmed response, while only 13% of patients without a TSC2 mutation responded to the treatment[90].

The relation of mTOR expression levels to mTORi sensitivity has not yet been extensively studied, however it has been found to be associated with disease onset and progression in various types of cancers, including gastric, prostate, esophageal, liver, and ovarian cancers[95]. In some cases, mTOR overexpression correlates with a more aggressive phenotype and worse prognosis, however, this can vary between different cancer subtypes. For example, while high mTOR expression seems to be a positive prognostic marker in luminal breast cancers[96], it correlates with more aggressive triple negative breast cancer phenotypes[97]. High mTOR levels have been linked to disease recurrence in laryngeal and hepatocellular carcinomas[98,99]. In this context, it may be beneficial to use tumor transcriptomic data in order to find patients which may benefit from mTORi treatment.

In recent years, alterations in several genes that are not part of the mTOR signaling pathway have been found to impact sensitivity to mTORi. Firstly, hyperactivity of the MYC transcription factor has been shown to determine resistance to PI3K/mTOR inhibitors[100,101]. Indeed, deregulation of MYC has been shown to create a dependence on AMPK-related kinase 5 (ARK5) and inhibit mTORC1 signaling, thereby reducing the effectiveness of mTORi[102]. MYC status was also found to be significantly associated with insensitivity to treatment with everolimus in metastatic breast cancer patients[103]. A significant association between response to rapalog treatment and the cytoplasmic levels of metastasis-associated antigen 1 (MTA1), an adenosine triphosphate (ATP) synthase modulator, in breast cancer patients[104]. High expression of the pro-angiogenic growth-promoting factor Midkine (MDK) was observed in rapamycin-resistant stem-like tumor cells which originated from TSC-associated tumors[105]. Moreover, an association between the RNF43_p.G659fs mutation, detected in approximately 8% of patients with colorectal cancer, and increased

PI3K/mTOR inhibitor sensitivity was observed[106]. Loss of the mitogen-activated protein kinase 6 (MAPK6), whose overexpression has been found to be associated with worse prognosis in several cancers, was found to sensitize cancer cells to treatment with ATM-mTORi[107]. Similarly, the loss of Phosphatidylinositol 3-kinase C2 domain-containing subunit gamma (PIK3C2G), which has been observed to be frequently downregulated in pancreatic ductal adenocarcinoma (PDAC) as well as associated with more aggressive phenotypes of PDAC, was found to sensitize pancreatic cancer cells and tumors to everolimus[108].
Overall, these findings indicate that sensitivity to mTORi is affected by a cancer cell's reliance on mTOR signaling in order to sustain its proliferation, as activation of alternative proliferation pathways (e.g. through hyperactivity of MYC or MDK) resulted in insensitivity to mTORi.

Combinational approaches in mTOR-targeting therapies

Another avenue of research related to mTOR signaling in cancer is the exploration of combination treatments. Rapalogs have shown to be effective as part of a combination treatment in different advanced tumors[63]. Everolimus has been shown to be effective in combination with CDK inhibitors[109,110], chemotherapeutic agents[111,112], RTK-signaling-targeting monoclonal antibodies and inhibitors[111,113-116] in advanced cancers, as well as with aromatase inhibitors[110,113-115] in breast cancer. Similarly, temsirolimus has been shown to provide clinical benefit when used in combination with RTK-signaling-targeting monoclonal antibodies and inhibitors[117-119] and chemotherapeutic agents[120-122] for the treatment of advanced tumors.

The effectiveness of combination therapies using mTORi and chemotherapeutic agents can partially be attributed to relief of mTORC1 hyperactivity-dependent chemoresistance. Indeed, mTOR signaling has been shown to regulate the expression of the Fanconi anemia group D2 protein, with mTOR hyperactivity thereby promoting the repair of DNA double-strand breaks and resistance to DNA-damaging chemotherapeutic agents[123]. Furthermore, mTORi has been shown to restore sensitivity to adriamycin/cisplatin in a multidrug-resistant oropharyngeal carcinoma cell line[124]. Similarly, the combined treatment of chemoresistant epithelial ovarian cancer cells with cisplatin and a PI3K/mTOR inhibitor showed significantly enhanced anticancer effects compared to cisplatin[125]. Moreover, the Aurora-A kinase was observed to induce chemoresistance in endometrial cancer cells by activating mTOR signaling[126]. Similarly, the m6A RNA demethylase alkB homolog 5 (ALKBH5) was observed to mediate cancer stemness and chemoresistance in pancreatic cancer cells through indirect activation of mTOR signaling[127]. Finally, higher mTOR protein or expression levels have been observed to be associated with decreased sensitivity to chemotherapy respectively in patients with urothelial carcinoma[128] and acute lymphoblastic leukemia[129].

Many mechanisms known to be associated with immunotherapy resistance, including defective neoantigen presentation, T-cell resistance, increased levels of immunosuppressive cytokines and presence of inhibitory cell types, have been observed to be linked to the PI3K/AKT/mTOR pathway[130]. Another logical approach would thus be to investigate synergistic interactions between immunotherapeutic agents and mTORi. Targeting PI3K/AKT/mTOR signaling has been found to enhance the effectiveness of anti-PD1 and anti-CTLA4 immune checkpoint inhibitor (ICI) therapies in pre-clinical models of melanoma[131], non-small cell lung cancer[132] and breast cancer[133]. Recent clinical trials have mainly focused on combining ICIs with PI3K inhibitors for the treatment of advanced melanomas[134,135] and lymphomas[136], with preliminary results indicating that the combination can be used safely.

Interestingly, recent studies also suggest that inhibiting PI3K/AKT/mTOR signaling during the manufacturing process of chimeric antigen receptor (CAR) T-cells can lead to enhanced CAR T-cell production[63,137]. This is due to stimulation of the T-cell receptor during the CAR T-cell manufacturing process activating the PAM pathway, leading to T-cell differentiation and decreased CAR T-cell persistence[138]. In this context PI3K inhibitors have been shown to restrict T-cell differentiation, improve CAR T-cell persistence, reduce exhaustion markers, and result in an increase in production of CD8+ CAR-T cells, which results in increase tumor-targeting cytotoxicity[138-141].

Finally, combination therapies can be used to address the fundamental issues concerning mTOR-targeting therapeutical approaches.
As mentioned previously, inhibition of mTORC1 partially results in the upregulation of RTK-signaling. Combination therapies employing rapalogs and compounds such as trastuzumab and bevacizumab have been found to be clinically beneficial[111,113,118], and this may be partially due to the fact that these compounds mitigate the RTK signaling-upregulation drawback of rapalogs, producing a synergistic effect. Moreover, compounds capable of inhibiting both mTOR and PI3K (dual PI3K/mTOR inhibitors) have shown anti-cancer activity when used to treat several advanced solid tumors[63]. As these compounds address one of the limitations of mTORC1 inhibition, i.e. its activating effect on PI3K/Akt signaling, it may be possible to obtain a similar synergistic effect by combining Rapalinks, the new-generation of mTORC1 inhibitors, with PI3K inhibitors.
Another effect of mTORC1 inhibition is the induction of autophagy, which may limit their use in cancers which may benefit from this, such as poorly vascularized and nutrition-starved tumors, such as pancreatic cancers. This limitation could be addressed by combining mTORi with autophagy inhibitors. Indeed, the combination of rapalogs with the autophagy inhibitors chloroquine and hydroxychloroquine has shown some promising results in clinical trials when used to treat advanced solid cancers and melanomas[142,143].

Hyperactivating mTORC1 as a therapeutical strategy

A recent development within the greater research field of mTOR-signaling in cancer, is the observation of anticancer effects associated with hyperactivation of mTORC1.
Specifically, knockdown of the TSC component TSC1 was observed to enhance mTORC1 activity, mitochondrial respiration, ROS production and apoptosis, thereby reducing cell viability within Burkitt's lymphoma (BL) cell lines and reducing tumor growth in xenotransplanted tumors[144]. Within BL cells, TSC1 expression was found to be upregulated by MYC overexpression[144] (as BLs are MYC-driven cancers), which is consistent with previous finding of MYC-driven mTORi resistance: the upregulation of TSC1 results in broad inhibition of mTORC1 signaling, thus the cancer cells do not rely on mTORC1 signaling to promote growth and proliferation, leading to resistance to mTORi. Moreover, mTORC1 hyperactivation was found to sensitize multiple myeloma (MM) cells to treatment with proteasome inhibitors[145]. Similarly to what was observed by Hartleben et al. in BL cells[144], mTORC1 hyperactivation in MM cells led to mitochondrial stress and ROS production[145]. Thus, mTORC1 hyperactivity's effect on cell viability may be due to the induction of metabolic stress and impairment of stress adaptation. These findings hint at the possibility of hyperactivation of mTORC1 providing clinical benefits in patients with certain malignancies, such as MYC-driven or metabolic stress-sensitive cancers.

A function for mTORC1 hyperactivation has also been proposed in cancer immunotherapy, as TSC2-depleted melanoma cells have been observed to be more sensitive to cytotoxic T lymphocyte (CTL)-mediated cell death[146]. This was found to be caused by the inability of these cells to shift their mTOR

signaling balance towards mTORC2, as well as elevated expression of the TNF-related apoptosis-inducing ligand (TRAIL) receptors. Moreover, melanoma patients with a lower TSC2 expression:TRAIL signaling ratio were observed to be significantly more likely to respond to ICI therapy[146]. Of note, the promotion of anabolic metabolism mediated by mTORC1 is required for T-cell activation and expansion[1], which may result in further enhancement of T-cell-mediated responses in mTOR-hyperactivating approaches.

Discussion

Overall, recent advancements in mTOR signaling research, specifically within the development of advanced mTORi, improved targeting of mTORi-sensitive cancers, effective combination strategies, as well as mTOR hyperactivation, have opened new possibilities for improving cancer treatments.

Within the research areas of mTOR inhibitors, RapaLinks were designed to address the drawbacks of both rapalogs and ATP-mTORi. These compounds have shown promise in pre-clinical data[83,84], and merit further study in clinical settings. Rapalinks may also benefit from the use of nanomedicinal technologies in order to enhance drug delivery in advanced solid tumors treatment, which is likely to be one of the main applications of these compounds, as both rapalogs and ATP-mTORi have often been shown to have clinical benefits when used to treat this category of malignancies[63]. Thus, applying nanomedicinal technologies to next-gen mTORi should also be further explored in future research, for example in order to evaluate the effectiveness of Rapalinks on malignancies that benefit from the use advanced drug delivery platforms, such as pancreatic cancers, which are often poorly perfused and thus difficult to reach through conventional drug administration[147].

Stratification of patients through genomic and transcriptomic data is likely to be the most effective strategy in assigning mTORi-based therapies to the patients who are most likely to respond to them. Thus, a key area of focus in future research should be the integration of large next-generation sequencing datasets of patients which have been treated with mTORi, in order to find alterations within tumor genomes or transcriptomes which create a dependency on hyperactive mTOR signaling and thus sensitize cancers to mTORi (such as RNF43_p.G659fs[106] and loss of PIK3C2G[108]), as well as alterations which determine independence from mTOR signaling and thus mTORi resistance (such as MYC[100-103] and MDK[105] hyperactivity). This data should then be used to select patients which will most benefit from mTORi-based therapies, as well as to find possible novel synergistic combination treatment approaches. For example, as MTA1 knockout was shown to increase sensitivity to mTORi, and high MTA1 expression was found to be correlated with mTORi resistance in breast cancer patients, a combination of novel MTA1 inhibitors and mTORi may provide clinical benefits to specific patients[104].

Using new-generation mTORi in order to sensitize chemo/immunoresistant cancers to respectively DNA damage-based chemotherapeutic agents or ICIs is also an area that merits further investigation as PI3K/Akt/mTOR signaling has been shown to have a strong link to the molecular mechanisms which mediate chemoresistance[123-127] and immunotherapy resistance[130]. Rapalogs have already been shown to provide clinical benefits when used in combination with chemotherapeutic agents[111,112,117-119], thus it's likely that a similar and possibly greater benefit may be gained by using Rapalinks. Ultimately, the effectiveness of Rapalinks may still be limited by the fundamental drawbacks of mTORC1 inhibition, namely the induction of RTK signaling[35-38,75,76] and autophagy[77]. Thus, it will be important to investigate the presence of synergistic effects between Rapalinks and compounds that can address their limitations, such as RTK-targeting inhibitors and monoclonal antibodies, PI3K inhibitors and autophagy inhibitors.

Finally, as shown by Hartleben et al.[144] and Darawshi et al.[145], studying hyperactivation of mTORC1 signaling within MYC-driven or metabolic stress-sensitive cancers is also an area of research which merits further investigation, as it may lead to development of new effective therapeutic approaches for specific patients. Since depletion of TSC2 was observed to sensitize melanoma cells to T cell-induced killing[146], combining hyperactivation of mTORC1 with immunotherapies may also be an interesting avenue of research. This observation, however, is at odds with previous findings, namely the observation of PI3K/Akt/mTOR signaling inhibitors having immunotherapy-enhancing effects[131-133]. This could be due to mTORC1 signaling regulating both immunosuppressive and immune-promoting mechanisms, and the balance between the two being shifted in favor of immune-promoting functions (such as promoting TRAIL receptor expression) when mTORC1 is hyperactivated. Hyperactivation of mTORC1 may be achieved through the development of novel inhibitors of the components of the TSC, TSC1 and TSC2. While hyperactivation of mTORC1 could hypothetically have a detrimental effect through the promotion of processes associated with malignancy, namely cell proliferation and migration, individuals harboring germline mutations which inactivate the components of the TSC mostly develop benign tumors such as angiomyolipomas and angiofibromas[148]. Thus, the clinical benefits of TSC inhibitors may well outweigh their possible drawbacks when used to treat cancers sensitive to mTORC1 hyperactivation.

In conclusion, while mTOR-targeting did not find immediate widespread success in cancer therapy, recent developments within the broader field of research on mTOR signaling in cancer present a range of promising strategies for improving mTOR-targeting approaches. Through the development of more advanced mTORi, improved targeting and novel combination strategies, as well as possibly unorthodox activating approaches, continuing research within this field will be essential in bringing us closer to finding more effective cancer therapies. While challenges and limitations persist, these developments have advanced our understanding of cancer and have brought us closer to the establishment of new clinically beneficial mTOR-targeting therapies.

Bibliography

1. Saxton, R. A., & Sabatini, D. M. (2017). mTOR Signaling in Growth, Metabolism, and Disease. Cell, 168(6), 960–976. https://doi.org/10.1016/j.cell.2017.02.004
2. Szwed, A., Kim, E., & Jacinto, E. (2021). Regulation and metabolic functions of mTORC1 and mTORC2. Physiological reviews, 101(3), 1371–1426. https://doi.org/10.1152/physrev.00026.2020
3. Kim, D. H., Sarbassov, D. D., Ali, S. M., King, J. E., Latek, R. R., Erdjument-Bromage, H., Tempst, P., & Sabatini, D. M. (2002). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell, 110(2), 163–175. https://doi.org/10.1016/s0092-8674(02)00808-5
4. Hara, K., Maruki, Y., Long, X., Yoshino, K., Oshiro, N., Hidayat, S., Tokunaga, C., Avruch, J., & Yonezawa, K. (2002). Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell, 110(2), 177–189. https://doi.org/10.1016/s0092-8674(02)00833-4
5. Kim, D. H., Sarbassov, D. D., Ali, S. M., Latek, R. R., Guntur, K. V., Erdjument-Bromage, H., Tempst, P., & Sabatini, D. M. (2003). GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. Molecular cell, 11(4), 895–904. https://doi.org/10.1016/s1097-2765(03)00114-x
6. Sancak, Y., Thoreen, C. C., Peterson, T. R., Lindquist, R. A., Kang, S. A., Spooner, E., Carr, S. A., & Sabatini, D. M. (2007). PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Molecular cell, 25(6), 903–915. https://doi.org/10.1016/j.molcel.2007.03.003
7. Vander Haar, E., Lee, S. I., Bandhakavi, S., Griffin, T. J., & Kim, D. H. (2007). Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nature cell biology, 9(3), 316–323. https://doi.org/10.1038/ncb1547
8. Wang, L., Harris, T. E., Roth, R. A., & Lawrence, J. C., Jr (2007). PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding. The Journal of biological chemistry, 282(27), 20036–20044. https://doi.org/10.1074/jbc.M702376200
9. Peterson, T. R., Laplante, M., Thoreen, C. C., Sancak, Y., Kang, S. A., Kuehl, W. M., Gray, N. S., & Sabatini, D. M. (2009). DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. Cell, 137(5), 873–886. https://doi.org/10.1016/j.cell.2009.03.046
10. Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Rüegg, M. A., Hall, A., & Hall, M. N. (2004). Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nature cell biology, 6(11), 1122–1128. https://doi.org/10.1038/ncb1183
11. Sarbassov, D. D., Ali, S. M., Kim, D. H., Guertin, D. A., Latek, R. R., Erdjument-Bromage, H., Tempst, P., & Sabatini, D. M. (2004). Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Current biology : CB, 14(14), 1296–1302. https://doi.org/10.1016/j.cub.2004.06.054
12. Frias, M. A., Thoreen, C. C., Jaffe, J. D., Schroder, W., Sculley, T., Carr, S. A., & Sabatini, D. M. (2006). mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. Current biology : CB, 16(18), 1865–1870. https://doi.org/10.1016/j.cub.2006.08.001
13. Jacinto, E., Facchinetti, V., Liu, D., Soto, N., Wei, S., Jung, S. Y., Huang, Q., Qin, J., & Su, B. (2006). SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell, 127(1), 125–137. https://doi.org/10.1016/j.cell.2006.08.033

14. Yang, Q., Inoki, K., Ikenoue, T., & Guan, K. L. (2006). Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. Genes & development, 20(20), 2820–2832. https://doi.org/10.1101/gad.1461206
15. Pearce, L. R., Huang, X., Boudeau, J., Pawłowski, R., Wullschleger, S., Deak, M., Ibrahim, A. F., Gourlay, R., Magnuson, M. A., & Alessi, D. R. (2007). Identification of Protor as a novel Rictor-binding component of mTOR complex-2. The Biochemical journal, 405(3), 513–522. https://doi.org/10.1042/BJ20070540
16. Thedieck, K., Polak, P., Kim, M. L., Molle, K. D., Cohen, A., Jenö, P., Arrieumerlou, C., & Hall, M. N. (2007). PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. PloS one, 2(11), e1217. https://doi.org/10.1371/journal.pone.0001217
17. Woo, S. Y., Kim, D. H., Jun, C. B., Kim, Y. M., Haar, E. V., Lee, S. I., Hegg, J. W., Bandhakavi, S., Griffin, T. J., & Kim, D. H. (2007). PRR5, a novel component of mTOR complex 2, regulates platelet-derived growth factor receptor beta expression and signaling. The Journal of biological chemistry, 282(35), 25604–25612. https://doi.org/10.1074/jbc.M704343200
18. Huang, J., & Manning, B. D. (2008). The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. The Biochemical journal, 412(2), 179–190. https://doi.org/10.1042/BJ20080281
19. Menon, S., Dibble, C. C., Talbott, G., Hoxhaj, G., Valvezan, A. J., Takahashi, H., Cantley, L. C., & Manning, B. D. (2014). Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. Cell, 156(4), 771–785. https://doi.org/10.1016/j.cell.2013.11.049
20. Inoki, K., Li, Y., Xu, T., & Guan, K. L. (2003). Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes & development, 17(15), 1829–1834. https://doi.org/10.1101/gad.1110003
21. Tee, A. R., Manning, B. D., Roux, P. P., Cantley, L. C., & Blenis, J. (2003). Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Current biology : CB, 13(15), 1259–1268. https://doi.org/10.1016/s0960-9822(03)00506-2
22. Sancak, Y., Peterson, T. R., Shaul, Y. D., Lindquist, R. A., Thoreen, C. C., Bar-Peled, L., & Sabatini, D. M. (2008). The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science (New York, N.Y.), 320(5882), 1496–1501. https://doi.org/10.1126/science.1157535
23. Kim, E., Goraksha-Hicks, P., Li, L., Neufeld, T. P., & Guan, K. L. (2008). Regulation of TORC1 by Rag GTPases in nutrient response. Nature cell biology, 10(8), 935–945. https://doi.org/10.1038/ncb1753
24. Fan, S. J., Snell, C., Turley, H., Li, J. L., McCormick, R., Perera, S. M., Heublein, S., Kazi, S., Azad, A., Wilson, C., Harris, A. L., & Goberdhan, D. C. (2016). PAT4 levels control amino-acid sensitivity of rapamycin-resistant mTORC1 from the Golgi and affect clinical outcome in colorectal cancer. Oncogene, 35(23), 3004–3015. https://doi.org/10.1038/onc.2015.363
25. Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., Turk, B. E., & Shaw, R. J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. Molecular cell, 30(2), 214–226. https://doi.org/10.1016/j.molcel.2008.03.003
26. Inoki, K., Zhu, T., & Guan, K. L. (2003). TSC2 mediates cellular energy response to control cell growth and survival. Cell, 115(5), 577–590. https://doi.org/10.1016/s0092-8674(03)00929-2
27. Feng, Z., Hu, W., de Stanchina, E., Teresky, A. K., Jin, S., Lowe, S., & Levine, A. J. (2007). The regulation of AMPK beta1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-AKT-mTOR pathways. Cancer research, 67(7), 3043–3053. https://doi.org/10.1158/0008-5472.CAN-06-4149

28. Brugarolas, J., Lei, K., Hurley, R. L., Manning, B. D., Reiling, J. H., Hafen, E., Witters, L. A., Ellisen, L. W., & Kaelin, W. G., Jr (2004). Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. Genes & development, 18(23), 2893–2904. https://doi.org/10.1101/gad.1256804
29. Kalender, A., Selvaraj, A., Kim, S. Y., Gulati, P., Brûlé, S., Viollet, B., Kemp, B. E., Bardeesy, N., Dennis, P., Schlager, J. J., Marette, A., Kozma, S. C., & Thomas, G. (2010). Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. Cell metabolism, 11(5), 390–401. https://doi.org/10.1016/j.cmet.2010.03.014
30. Efeyan, A., Zoncu, R., Chang, S., Gumper, I., Snitkin, H., Wolfson, R. L., Kirak, O., Sabatini, D. D., & Sabatini, D. M. (2013). Regulation of mTORC1 by the Rag GTPases is necessary for neonatal autophagy and survival. Nature, 493(7434), 679–683. https://doi.org/10.1038/nature11745
31. Liu, P., Gan, W., Chin, Y. R., Ogura, K., Guo, J., Zhang, J., Wang, B., Blenis, J., Cantley, L. C., Toker, A., Su, B., & Wei, W. (2015). PtdIns(3,4,5)P3-Dependent Activation of the mTORC2 Kinase Complex. Cancer discovery, 5(11), 1194–1209. https://doi.org/10.1158/2159-8290.CD-15-0460
32. Yang, G., Murashige, D. S., Humphrey, S. J., & James, D. E. (2015). A Positive Feedback Loop between Akt and mTORC2 via SIN1 Phosphorylation. Cell reports, 12(6), 937–943. https://doi.org/10.1016/j.celrep.2015.07.016
33. Sarbassov, D. D., Guertin, D. A., Ali, S. M., & Sabatini, D. M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science (New York, N.Y.), 307(5712), 1098–1101. https://doi.org/10.1126/science.1106148
34. Kazyken, D., Magnuson, B., Bodur, C., Acosta-Jaquez, H. A., Zhang, D., Tong, X., Barnes, T. M., Steinl, G. K., Patterson, N. E., Altheim, C. H., Sharma, N., Inoki, K., Cartee, G. D., Bridges, D., Yin, L., Riddle, S. M., & Fingar, D. C. (2019). AMPK directly activates mTORC2 to promote cell survival during acute energetic stress. Science signaling, 12(585), eaav3249. https://doi.org/10.1126/scisignal.aav3249
35. Hsu, P. P., Kang, S. A., Rameseder, J., Zhang, Y., Ottina, K. A., Lim, D., Peterson, T. R., Choi, Y., Gray, N. S., Yaffe, M. B., Marto, J. A., & Sabatini, D. M. (2011). The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. Science (New York, N.Y.), 332(6035), 1317–1322. https://doi.org/10.1126/science.1199498
36. Yu, Y., Yoon, S. O., Poulogiannis, G., Yang, Q., Ma, X. M., Villén, J., Kubica, N., Hoffman, G. R., Cantley, L. C., Gygi, S. P., & Blenis, J. (2011). Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. Science (New York, N.Y.), 332(6035), 1322–1326. https://doi.org/10.1126/science.1199484
37. Harrington, L. S., Findlay, G. M., Gray, A., Tolkacheva, T., Wigfield, S., Rebholz, H., Barnett, J., Leslie, N. R., Cheng, S., Shepherd, P. R., Gout, I., Downes, C. P., & Lamb, R. F. (2004). The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. The Journal of cell biology, 166(2), 213–223. https://doi.org/10.1083/jcb.200403069
38. Shah, O. J., Wang, Z., & Hunter, T. (2004). Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. Current biology : CB, 14(18), 1650–1656. https://doi.org/10.1016/j.cub.2004.08.026
39. Brunn, G. J., Hudson, C. C., Sekulić, A., Williams, J. M., Hosoi, H., Houghton, P. J., Lawrence, J. C., Jr, & Abraham, R. T. (1997). Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin. Science (New York, N.Y.), 277(5322), 99–101. https://doi.org/10.1126/science.277.5322.99

40. Gingras, A. C., Gygi, S. P., Raught, B., Polakiewicz, R. D., Abraham, R. T., Hoekstra, M. F., Aebersold, R., & Sonenberg, N. (1999). Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes & development, 13(11), 1422–1437. https://doi.org/10.1101/gad.13.11.1422
41. Holz, M. K., Ballif, B. A., Gygi, S. P., & Blenis, J. (2005). mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. Cell, 123(4), 569–580. https://doi.org/10.1016/j.cell.2005.10.024
42. Dorrello, N. V., Peschiaroli, A., Guardavaccaro, D., Colburn, N. H., Sherman, N. E., & Pagano, M. (2006). S6K1- and betaTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth. Science (New York, N.Y.), 314(5798), 467–471. https://doi.org/10.1126/science.1130276
43. Ma, X. M., Yoon, S. O., Richardson, C. J., Jülich, K., & Blenis, J. (2008). SKAR links pre-mRNA splicing to mTOR/S6K1-mediated enhanced translation efficiency of spliced mRNAs. Cell, 133(2), 303–313. https://doi.org/10.1016/j.cell.2008.02.031
44. Porstmann, T., Santos, C. R., Griffiths, B., Cully, M., Wu, M., Leevers, S., Griffiths, J. R., Chung, Y. L., & Schulze, A. (2008). SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell metabolism, 8(3), 224–236. https://doi.org/10.1016/j.cmet.2008.07.007
45. Düvel, K., Yecies, J. L., Menon, S., Raman, P., Lipovsky, A. I., Souza, A. L., Triantafellow, E., Ma, Q., Gorski, R., Cleaver, S., Vander Heiden, M. G., MacKeigan, J. P., Finan, P. M., Clish, C. B., Murphy, L. O., & Manning, B. D. (2010). Activation of a metabolic gene regulatory network downstream of mTOR complex 1. Molecular cell, 39(2), 171–183. https://doi.org/10.1016/j.molcel.2010.06.022
46. Dong, Q., Majumdar, G., O'Meally, R. N., Cole, R. N., Elam, M. B., & Raghow, R. (2020). Insulin-induced de novo lipid synthesis occurs mainly via mTOR-dependent regulation of proteostasis of SREBP-1c. Molecular and cellular biochemistry, 463(1-2), 13–31. https://doi.org/10.1007/s11010-019-03625-5
47. Peterson, T. R., Sengupta, S. S., Harris, T. E., Carmack, A. E., Kang, S. A., Balderas, E., Guertin, D. A., Madden, K. L., Carpenter, A. E., Finck, B. N., & Sabatini, D. M. (2011). mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. Cell, 146(3), 408–420. https://doi.org/10.1016/j.cell.2011.06.034
48. Ben-Sahra, I., Hoxhaj, G., Ricoult, S. J. H., Asara, J. M., & Manning, B. D. (2016). mTORC1 induces purine synthesis through control of the mitochondrial tetrahydrofolate cycle. Science (New York, N.Y.), 351(6274), 728–733. https://doi.org/10.1126/science.aad0489
49. Ben-Sahra, I., Howell, J. J., Asara, J. M., & Manning, B. D. (2013). Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. Science (New York, N.Y.), 339(6125), 1323–1328. https://doi.org/10.1126/science.1228792
50. Robitaille, A. M., Christen, S., Shimobayashi, M., Cornu, M., Fava, L. L., Moes, S., Prescianotto-Baschong, C., Sauer, U., Jenoe, P., & Hall, M. N. (2013). Quantitative phosphoproteomics reveal mTORC1 activates de novo pyrimidine synthesis. Science (New York, N.Y.), 339(6125), 1320–1323. https://doi.org/10.1126/science.1228771
51. Kim, J., Kundu, M., Viollet, B., & Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nature cell biology, 13(2), 132–141. https://doi.org/10.1038/ncb2152
52. Martina, J. A., Chen, Y., Gucek, M., & Puertollano, R. (2012). MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. Autophagy, 8(6), 903–914. https://doi.org/10.4161/auto.19653

53. Roczniak-Ferguson, A., Petit, C. S., Froehlich, F., Qian, S., Ky, J., Angarola, B., Walther, T. C., & Ferguson, S. M. (2012). The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. Science signaling, 5(228), ra42. https://doi.org/10.1126/scisignal.2002790
54. Settembre, C., Zoncu, R., Medina, D. L., Vetrini, F., Erdin, S., Erdin, S., Huynh, T., Ferron, M., Karsenty, G., Vellard, M. C., Facchinetti, V., Sabatini, D. M., & Ballabio, A. (2012). A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. The EMBO journal, 31(5), 1095–1108. https://doi.org/10.1038/emboj.2012.32
55. Zhao, J., Zhai, B., Gygi, S. P., & Goldberg, A. L. (2015). mTOR inhibition activates overall protein degradation by the ubiquitin proteasome system as well as by autophagy. Proceedings of the National Academy of Sciences of the United States of America, 112(52), 15790–15797. https://doi.org/10.1073/pnas.1521919112
56. Rousseau, A., & Bertolotti, A. (2016). An evolutionarily conserved pathway controls proteasome homeostasis. Nature, 536(7615), 184–189. https://doi.org/10.1038/nature18943
57. Zhang, Y., Nicholatos, J., Dreier, J. R., Ricoult, S. J., Widenmaier, S. B., Hotamisligil, G. S., Kwiatkowski, D. J., & Manning, B. D. (2014). Coordinated regulation of protein synthesis and degradation by mTORC1. Nature, 513(7518), 440–443. https://doi.org/10.1038/nature13492
58. Gan, X., Wang, J., Wang, C., Sommer, E., Kozasa, T., Srinivasula, S., Alessi, D., Offermanns, S., Simon, M. I., & Wu, D. (2012). PRR5L degradation promotes mTORC2-mediated PKC-δ phosphorylation and cell migration downstream of Gα12. Nature cell biology, 14(7), 686–696. https://doi.org/10.1038/ncb2507
59. Li, X., & Gao, T. (2014). mTORC2 phosphorylates protein kinase Cζ to regulate its stability and activity. EMBO reports, 15(2), 191–198. https://doi.org/10.1002/embr.201338119
60. Thomanetz, V., Angliker, N., Cloëtta, D., Lustenberger, R. M., Schweighauser, M., Oliveri, F., Suzuki, N., & Rüegg, M. A. (2013). Ablation of the mTORC2 component rictor in brain or Purkinje cells affects size and neuron morphology. The Journal of cell biology, 201(2), 293–308. https://doi.org/10.1083/jcb.201205030
61. Guertin, D. A., Stevens, D. M., Thoreen, C. C., Burds, A. A., Kalaany, N. Y., Moffat, J., Brown, M., Fitzgerald, K. J., & Sabatini, D. M. (2006). Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. Developmental cell, 11(6), 859–871. https://doi.org/10.1016/j.devcel.2006.10.007
62. García-Martínez, J. M., & Alessi, D. R. (2008). mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). The Biochemical journal, 416(3), 375–385. https://doi.org/10.1042/BJ20081668
63. Glaviano, A., Foo, A. S. C., Lam, H. Y., Yap, K. C. H., Jacot, W., Jones, R. H., Eng, H., Nair, M. G., Makvandi, P., Geoerger, B., Kulke, M. H., Baird, R. D., Prabhu, J. S., Carbone, D., Pecoraro, C., Teh, D. B. L., Sethi, G., Cavalieri, V., Lin, K. H., Javidi-Sharifi, N. R., … Kumar, A. P. (2023). PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Molecular cancer, 22(1), 138. https://doi.org/10.1186/s12943-023-01827-6
64. Peng, Y., Wang, Y., Zhou, C., Mei, W., & Zeng, C. (2022). PI3K/Akt/mTOR Pathway and Its Role in Cancer Therapeutics: Are We Making Headway?. Frontiers in oncology, 12, 819128. https://doi.org/10.3389/fonc.2022.819128

65. Sabatini, D. M., Erdjument-Bromage, H., Lui, M., Tempst, P., & Snyder, S. H. (1994). RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell, 78(1), 35–43. https://doi.org/10.1016/0092-8674(94)90570-3
66. Lamming, D. W., Ye, L., Katajisto, P., Goncalves, M. D., Saitoh, M., Stevens, D. M., Davis, J. G., Salmon, A. B., Richardson, A., Ahima, R. S., Guertin, D. A., Sabatini, D. M., & Baur, J. A. (2012). Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science (New York, N.Y.), 335(6076), 1638–1643. https://doi.org/10.1126/science.1215135
67. European Medicines Agency. Afinitor product page. https://www.ema.europa.eu/en/medicines/human/EPAR/afinitor accessed on 03/11/2023.
68. European Medicines Agency. Torisel product page. https://www.ema.europa.eu/en/medicines/human/EPAR/torisel accessed on 03/11/2023.
69. Choo, A. Y., Yoon, S. O., Kim, S. G., Roux, P. P., & Blenis, J. (2008). Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. Proceedings of the National Academy of Sciences of the United States of America, 105(45), 17414–17419. https://doi.org/10.1073/pnas.0809136105
70. Feldman, M. E., Apsel, B., Uotila, A., Loewith, R., Knight, Z. A., Ruggero, D., & Shokat, K. M. (2009). Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS biology, 7(2), e38. https://doi.org/10.1371/journal.pbio.1000038
71. Thoreen, C. C., Kang, S. A., Chang, J. W., Liu, Q., Zhang, J., Gao, Y., Reichling, L. J., Sim, T., Sabatini, D. M., & Gray, N. S. (2009). An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. The Journal of biological chemistry, 284(12), 8023–8032. https://doi.org/10.1074/jbc.M900301200
72. Kang, S. A., Pacold, M. E., Cervantes, C. L., Lim, D., Lou, H. J., Ottina, K., Gray, N. S., Turk, B. E., Yaffe, M. B., & Sabatini, D. M. (2013). mTORC1 phosphorylation sites encode their sensitivity to starvation and rapamycin. Science (New York, N.Y.), 341(6144), 1236566. https://doi.org/10.1126/science.1236566
73. Hsieh, A. C., Costa, M., Zollo, O., Davis, C., Feldman, M. E., Testa, J. R., Meyuhas, O., Shokat, K. M., & Ruggero, D. (2010). Genetic dissection of the oncogenic mTOR pathway reveals druggable addiction to translational control via 4EBP-eIF4E. Cancer cell, 17(3), 249–261. https://doi.org/10.1016/j.ccr.2010.01.021
74. She, Q. B., Halilovic, E., Ye, Q., Zhen, W., Shirasawa, S., Sasazuki, T., Solit, D. B., & Rosen, N. (2010). 4E-BP1 is a key effector of the oncogenic activation of the AKT and ERK signaling pathways that integrates their function in tumors. Cancer cell, 18(1), 39–51. https://doi.org/10.1016/j.ccr.2010.05.023
75. Chaturvedi, D., Gao, X., Cohen, M. S., Taunton, J., & Patel, T. B. (2009). Rapamycin induces transactivation of the EGFR and increases cell survival. Oncogene, 28(9), 1187–1196. https://doi.org/10.1038/onc.2008.490
76. Li, Q. L., Gu, F. M., Wang, Z., Jiang, J. H., Yao, L. Q., Tan, C. J., Huang, X. Y., Ke, A. W., Dai, Z., Fan, J., & Zhou, J. (2012). Activation of PI3K/AKT and MAPK pathway through a PDGFRβ-dependent feedback loop is involved in rapamycin resistance in hepatocellular carcinoma. PloS one, 7(3), e33379. https://doi.org/10.1371/journal.pone.0033379
77. Palm, W., Park, Y., Wright, K., Pavlova, N. N., Tuveson, D. A., & Thompson, C. B. (2015). The Utilization of Extracellular Proteins as Nutrients Is Suppressed by mTORC1. Cell, 162(2), 259–270. https://doi.org/10.1016/j.cell.2015.06.017

78. Lim, B., Potter, D. A., Salkeni, M. A., Silverman, P., Haddad, T. C., Forget, F., Awada, A., Canon, J. L., Danso, M., Lortholary, A., Bourgeois, H., Tan-Chiu, E., Vincent, S., Bahamon, B., Galinsky, K. J., Patel, C., Neuwirth, R., Leonard, E. J., & Diamond, J. R. (2021). Sapanisertib Plus Exemestane or Fulvestrant in Women with Hormone Receptor-Positive/HER2-Negative Advanced or Metastatic Breast Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research, 27(12), 3329–3338. https://doi.org/10.1158/1078-0432.CCR-20-4131
79. García-Sáenz, J. Á., Martínez-Jáñez, N., Cubedo, R., Jerez, Y., Lahuerta, A., González-Santiago, S., Ferrer, N., Ramos, M., Alonso-Romero, J. L., Antón, A., Carrasco, E., Chen, J., Neuwirth, R., Galinsky, K., Vincent, S., Leonard, E. J., & Slamon, D. (2022). Sapanisertib plus Fulvestrant in Postmenopausal Women with Estrogen Receptor-Positive/HER2-Negative Advanced Breast Cancer after Progression on Aromatase Inhibitor. Clinical cancer research : an official journal of the American Association for Cancer Research, 28(6), 1107–1116. https://doi.org/10.1158/1078-0432.CCR-21-2652
80. Paik, P. K., Fan, P. D., Qeriqi, B., Namakydoust, A., Daly, B., Ahn, L., Kim, R., Plodkowski, A., Ni, A., Chang, J., Fanaroff, R., Ladanyi, M., de Stanchina, E., & Rudin, C. M. (2023). Targeting NFE2L2/KEAP1 Mutations in Advanced NSCLC With the TORC1/2 Inhibitor TAK-228. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer, 18(4), 516–526. https://doi.org/10.1016/j.jtho.2022.09.225
81. Hagiwara, A., Cornu, M., Cybulski, N., Polak, P., Betz, C., Trapani, F., Terracciano, L., Heim, M. H., Rüegg, M. A., & Hall, M. N. (2012). Hepatic mTORC2 activates glycolysis and lipogenesis through Akt, glucokinase, and SREBP1c. Cell metabolism, 15(5), 725–738. https://doi.org/10.1016/j.cmet.2012.03.015
82. Chandarlapaty, S., Sawai, A., Scaltriti, M., Rodrik-Outmezguine, V., Grbovic-Huezo, O., Serra, V., Majumder, P. K., Baselga, J., & Rosen, N. (2011). AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer cell, 19(1), 58–71. https://doi.org/10.1016/j.ccr.2010.10.031
83. Rodrik-Outmezguine, V. S., Okaniwa, M., Yao, Z., Novotny, C. J., McWhirter, C., Banaji, A., Won, H., Wong, W., Berger, M., de Stanchina, E., Barratt, D. G., Cosulich, S., Klinowska, T., Rosen, N., & Shokat, K. M. (2016). Overcoming mTOR resistance mutations with a new-generation mTOR inhibitor. Nature, 534(7606), 272–276. https://doi.org/10.1038/nature17963
84. Lee, B. J., Mallya, S., Dinglasan, N., Fung, A., Nguyen, T., Herzog, L. O., Thao, J., Lorenzana, E. G., Wildes, D., Singh, M., Smith, J. A. M., & Fruman, D. A. (2021). Efficacy of a Novel Bi-Steric mTORC1 Inhibitor in Models of B-Cell Acute Lymphoblastic Leukemia. Frontiers in oncology, 11, 673213. https://doi.org/10.3389/fonc.2021.673213
85. Wagle, N., Grabiner, B. C., Van Allen, E. M., Amin-Mansour, A., Taylor-Weiner, A., Rosenberg, M., Gray, N., Barletta, J. A., Guo, Y., Swanson, S. J., Ruan, D. T., Hanna, G. J., Haddad, R. I., Getz, G., Kwiatkowski, D. J., Carter, S. L., Sabatini, D. M., Jänne, P. A., Garraway, L. A., & Lorch, J. H. (2014). Response and acquired resistance to everolimus in anaplastic thyroid cancer. The New England journal of medicine, 371(15), 1426–1433. https://doi.org/10.1056/NEJMoa1403352
86. Burris, H.A., Ulahannan, S.V., Haura, E.B., Ignatius Ou, S.I., Capasso, A., Munster, P.N., Kitai, H., Wang, Z., Hayes, J., Tao, L., Wong, S., Yang, Y.C., Jiang, J., Bitman, B., Singh, M., Gustafon, W.C, Rosen, N., & Schram, A.M. (2022). The bi-steric mTORC1-selective inhibitor RMC-5552 in tumors with activation of mTOR signaling: Preclinical activity in combination with RAS(ON) inhibitors in RAS-addicted tumors, and initial clinical findings from a single agent phase 1/1b study. (Abstract). Journal of Clinical Oncology, 40(16):3098.

87. Trieu, V., Yim, Z., Cordia, J., Yang, A., & Beals, B. (2007). Nanoparticle albumin-bound (nab) rapamycin as an anticancer agent.
88. Trieu, V., Ran, S., Volk, L., Stutzman, A., D'Cruz, O., & Desai, N. (2009, May). CNS safety, antitumor activity, and antiangiogenic activity of nab-rapamycin (ABI-009). In CANCER RESEARCH (Vol. 69). 615 CHESTNUT ST, 17TH FLOOR, PHILADELPHIA, PA 19106-4404 USA: AMER ASSOC CANCER RESEARCH.
89. Kennecke, H. F., Rahman, M., Yip, S., Woods, R., Schaeffer, D., & Tai, I. (2011). Effect of nab-rapamycin versus rapamycin in colorectal cancer cell lines and associations with KRAS and PI3K mutations. Journal of Clinical Oncology, 29(15_suppl), e13532-e13532.
90. Wagner, A. J., Ravi, V., Riedel, R. F., Ganjoo, K., Van Tine, B. A., Chugh, R., Cranmer, L., Gordon, E. M., Hornick, J. L., Du, H., Grigorian, B., Schmid, A. N., Hou, S., Harris, K., Kwiatkowski, D. J., Desai, N. P., & Dickson, M. A. (2021). nab-Sirolimus for Patients With Malignant Perivascular Epithelioid Cell Tumors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 39(33), 3660–3670. https://doi.org/10.1200/JCO.21.01728
91. Gonzalez-Angulo, A. M., Meric-Bernstam, F., Chawla, S., Falchook, G., Hong, D., Akcakanat, A., Chen, H., Naing, A., Fu, S., Wheler, J., Moulder, S., Helgason, T., Li, S., Elias, I., Desai, N., & Kurzrock, R. (2013). Weekly nab-Rapamycin in patients with advanced nonhematologic malignancies: final results of a phase I trial. Clinical cancer research : an official journal of the American Association for Cancer Research, 19(19), 5474–5484. https://doi.org/10.1158/1078-0432.CCR-12-3110
92. Wagle, N., Grabiner, B. C., Van Allen, E. M., Hodis, E., Jacobus, S., Supko, J. G., Stewart, M., Choueiri, T. K., Gandhi, L., Cleary, J. M., Elfiky, A. A., Taplin, M. E., Stack, E. C., Signoretti, S., Loda, M., Shapiro, G. I., Sabatini, D. M., Lander, E. S., Gabriel, S. B., Kantoff, P. W., … Rosenberg, J. E. (2014). Activating mTOR mutations in a patient with an extraordinary response on a phase I trial of everolimus and pazopanib. Cancer discovery, 4(5), 546–553. https://doi.org/10.1158/2159-8290.CD-13-0353
93. Kwiatkowski, D. J., Choueiri, T. K., Fay, A. P., Rini, B. I., Thorner, A. R., de Velasco, G., Tyburczy, M. E., Hamieh, L., Albiges, L., Agarwal, N., Ho, T. H., Song, J., Pignon, J. C., Barrios, P. M., Michaelson, M. D., Van Allen, E., Krajewski, K. M., Porta, C., Pal, S., Bellmunt, J., … Signoretti, S. (2016). Mutations in TSC1, TSC2, and MTOR Are Associated with Response to Rapalogs in Patients with Metastatic Renal Cell Carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research, 22(10), 2445–2452. https://doi.org/10.1158/1078-0432.CCR-15-2631
94. Iyer, G., Hanrahan, A. J., Milowsky, M. I., Al-Ahmadie, H., Scott, S. N., Janakiraman, M., Pirun, M., Sander, C., Socci, N. D., Ostrovnaya, I., Viale, A., Heguy, A., Peng, L., Chan, T. A., Bochner, B., Bajorin, D. F., Berger, M. F., Taylor, B. S., & Solit, D. B. (2012). Genome sequencing identifies a basis for everolimus sensitivity. Science (New York, N.Y.), 338(6104), 221. https://doi.org/10.1126/science.1226344
95. Marques-Ramos, A., & Cervantes, R. (2023). Expression of mTOR in normal and pathological conditions. Molecular cancer, 22(1), 112. https://doi.org/10.1186/s12943-023-01820-z
96. Beca, F., Andre, R., Martins, D. S., Bilhim, T., Martins, D., & Schmitt, F. (2014). p-mTOR expression is associated with better prognosis in luminal breast carcinoma. Journal of clinical pathology, 67(11), 961–967. https://doi.org/10.1136/jclinpath-2014-202320
97. Wang, J., Zhu, X., Xu, X., Guo, L., Shen, G., Liu, X., Chang, C., Wang, B., Yang, H., & Wang, M. (2017). PIK3CA mutations and downstream effector p-mTOR expression: implication for prognostic factors and therapeutic targets in triple negative breast cancer. International journal of clinical and experimental pathology, 10(7), 7682–7691.

98. Marioni, G., Staffieri, A., Lora, L., Fermo, S., Giacomelli, L., La Torre, F. B., Favaretto, N., Valentini, E., Manzato, E., & Blandamura, S. (2012). mTOR expression and prognosis in elderly patients with laryngeal carcinoma: uni- and multivariate analyses. Oral oncology, 48(6), 530–534. https://doi.org/10.1016/j.oraloncology.2012.01.002
99. Guerrero, M., Ferrín, G., Rodríguez-Perálvarez, M., González-Rubio, S., Sánchez-Frías, M., Amado, V., Pozo, J. C., Poyato, A., Ciria, R., Ayllón, M. D., Barrera, P., Montero, J. L., & de la Mata, M. (2019). mTOR Expression in Liver Transplant Candidates with Hepatocellular Carcinoma: Impact on Histological Features and Tumour Recurrence. International journal of molecular sciences, 20(2), 336. https://doi.org/10.3390/ijms20020336
100. Ilic, N., Utermark, T., Widlund, H. R., & Roberts, T. M. (2011). PI3K-targeted therapy can be evaded by gene amplification along the MYC-eukaryotic translation initiation factor 4E (eIF4E) axis. Proceedings of the National Academy of Sciences of the United States of America, 108(37), E699–E708. https://doi.org/10.1073/pnas.1108237108
101. Muellner, M. K., Uras, I. Z., Gapp, B. V., Kerzendorfer, C., Smida, M., Lechtermann, H., Craig-Mueller, N., Colinge, J., Duernberger, G., & Nijman, S. M. (2011). A chemical-genetic screen reveals a mechanism of resistance to PI3K inhibitors in cancer. Nature chemical biology, 7(11), 787–793. https://doi.org/10.1038/nchembio.695
102. Liu, L., Ulbrich, J., Müller, J., Wüstefeld, T., Aeberhard, L., Kress, T. R., Muthalagu, N., Rycak, L., Rudalska, R., Moll, R., Kempa, S., Zender, L., Eilers, M., & Murphy, D. J. (2012). Deregulated MYC expression induces dependence upon AMPK-related kinase 5. Nature, 483(7391), 608–612. https://doi.org/10.1038/nature10927
103. Bhin, J., Yemelyanenko, J., Chao, X., Klarenbeek, S., Opdam, M., Malka, Y., Hoekman, L., Kruger, D., Bleijerveld, O., Brambillasca, C. S., Sprengers, J., Siteur, B., Annunziato, S., van Haren, M. J., Martin, N. I., van de Ven, M., Peters, D., Agami, R., Linn, S. C., Boven, E., … Wessels, L. F. A. (2023). MYC is a clinically significant driver of mTOR inhibitor resistance in breast cancer. The Journal of experimental medicine, 220(11), e20211743. https://doi.org/10.1084/jem.20211743
104. Wang, T., Sun, F., Li, C., Nan, P., Song, Y., Wan, X., Mo, H., Wang, J., Zhou, Y., Guo, Y., Helali, A. E., Xu, D., Zhan, Q., Ma, F., & Qian, H. (2023). MTA1, a Novel ATP Synthase Complex Modulator, Enhances Colon Cancer Liver Metastasis by Driving Mitochondrial Metabolism Reprogramming. Advanced science (Weinheim, Baden-Wurttemberg, Germany), 10(25), e2300756. https://doi.org/10.1002/advs.202300756
105. Tang, Y., Kwiatkowski, D. J., & Henske, E. P. (2022). Midkine expression by stem-like tumor cells drives persistence to mTOR inhibition and an immune-suppressive microenvironment. Nature communications, 13(1), 5018. https://doi.org/10.1038/s41467-022-32673-7
106. Fang, L., Ford-Roshon, D., Russo, M., O'Brien, C., Xiong, X., Gurjao, C., Grandclaudon, M., Raghavan, S., Corsello, S. M., Carr, S. A., Udeshi, N. D., Berstler, J., Sicinska, E., Ng, K., & Giannakis, M. (2022). RNF43 G659fs is an oncogenic colorectal cancer mutation and sensitizes tumor cells to PI3K/mTOR inhibition. Nature communications, 13(1), 3181. https://doi.org/10.1038/s41467-022-30794-7
107. Cai, Q., Zhou, W., Wang, W., Dong, B., Han, D., Shen, T., Creighton, C. J., Moore, D. D., & Yang, F. (2021). MAPK6-AKT signaling promotes tumor growth and resistance to mTOR kinase blockade. Science advances, 7(46), eabi6439. https://doi.org/10.1126/sciadv.abi6439
108. De Santis, M. C., Gozzelino, L., Margaria, J. P., Costamagna, A., Ratto, E., Gulluni, F., Di Gregorio, E., Mina, E., Lorito, N., Bacci, M., Lattanzio, R., Sala, G., Cappello, P., Novelli, F., Giovannetti, E., Vicentini, C., Andreani, S., Delfino, P., Corbo, V., Scarpa, A., … Martini, M. (2023). Lysosomal lipid

switch sensitises to nutrient deprivation and mTOR targeting in pancreatic cancer. Gut, 72(2), 360–371. https://doi.org/10.1136/gutjnl-2021-325117

109. Bautista, F., Paoletti, X., Rubino, J., Brard, C., Rezai, K., Nebchi, S., Andre, N., Aerts, I., De Carli, E., van Eijkelenburg, N., Thebaud, E., Corradini, N., Defachelles, A. S., Ducassou, S., Morscher, R. J., Vassal, G., & Geoerger, B. (2021). Phase I or II Study of Ribociclib in Combination With Topotecan-Temozolomide or Everolimus in Children With Advanced Malignancies: Arms A and B of the AcSé-ESMART Trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 39(32), 3546–3560. https://doi.org/10.1200/JCO.21.01152
110. Bardia, A., Hurvitz, S. A., DeMichele, A., Clark, A. S., Zelnak, A., Yardley, D. A., Karuturi, M., Sanft, T., Blau, S., Hart, L., Ma, C., Rugo, H. S., Purkayastha, D., & Moulder, S. (2021). Phase I/II Trial of Exemestane, Ribociclib, and Everolimus in Women with HR+/HER2- Advanced Breast Cancer after Progression on CDK4/6 Inhibitors (TRINITI-1). Clinical cancer research : an official journal of the American Association for Cancer Research, 27(15), 4177–4185. https://doi.org/10.1158/1078-0432.CCR-20-2114
111. Toi, M., Shao, Z., Hurvitz, S., Tseng, L. M., Zhang, Q., Shen, K., Liu, D., Feng, J., Xu, B., Wang, X., Lee, K. S., Ng, T. Y., Ridolfi, A., Noel-Baron, F., Ringeisen, F., & Jiang, Z. (2017). Efficacy and safety of everolimus in combination with trastuzumab and paclitaxel in Asian patients with HER2+ advanced breast cancer in BOLERO-1. Breast cancer research : BCR, 19(1), 47. https://doi.org/10.1186/s13058-017-0839-0
112. Jun, T., Hahn, N. M., Sonpavde, G., Albany, C., MacVicar, G. R., Hauke, R., Fleming, M., Gourdin, T., Jana, B., Oh, W. K., Taik, P., Wang, H., Varadarajan, A. R., Uzilov, A., & Galsky, M. D. (2022). Phase II Clinical and Translational Study of Everolimus ± Paclitaxel as First-Line Therapy in Cisplatin-Ineligible Advanced Urothelial Carcinoma. The oncologist, 27(6), 432–e452. https://doi.org/10.1093/oncolo/oyab075
113. Ballhausen, A., Wheler, J. J., Karp, D. D., Piha-Paul, S. A., Fu, S., Pant, S., Tsimberidou, A. M., Hong, D. S., Subbiah, V., Holley, V. R., Huang, H. J., Brewster, A. M., Koenig, K. B., Ibrahim, N. K., Meric-Bernstam, F., & Janku, F. (2021). Phase I Study of Everolimus, Letrozole, and Trastuzumab in Patients with Hormone Receptor-positive Metastatic Breast Cancer or Other Solid Tumors. Clinical cancer research : an official journal of the American Association for Cancer Research, 27(5), 1247–1255. https://doi.org/10.1158/1078-0432.CCR-20-2878
114. Schmid, P., Sablin, M. P., Bergh, J., Im, S. A., Lu, Y. S., Martínez, N., Neven, P., Lee, K. S., Morales, S., Pérez-Fidalgo, J. A., Adamson, D., Gonçalves, A., Prat, A., Jerusalem, G., Schlieker, L., Espadero, R. M., Bogenrieder, T., Huang, D. C., Crown, J., & Cortés, J. (2021). A phase Ib/II study of xentuzumab, an IGF-neutralising antibody, combined with exemestane and everolimus in hormone receptor-positive, HER2-negative locally advanced/metastatic breast cancer. Breast cancer research : BCR, 23(1), 8. https://doi.org/10.1186/s13058-020-01382-8
115. Curigliano, G., Martin, M., Jhaveri, K., Beck, J. T., Tortora, G., Fazio, N., Maur, M., Hubner, R. A., Lahner, H., Donnet, V., Ajipa, O., Li, Z., Blumenstein, L., & Andre, F. (2021). Alpelisib in combination with everolimus ± exemestane in solid tumours: Phase Ib randomised, open-label, multicentre study. European journal of cancer (Oxford, England : 1990), 151, 49–62. https://doi.org/10.1016/j.ejca.2021.03.042
116. Motzer, R. J., Hutson, T. E., Glen, H., Michaelson, M. D., Molina, A., Eisen, T., Jassem, J., Zolnierek, J., Maroto, J. P., Mellado, B., Melichar, B., Tomasek, J., Kremer, A., Kim, H. J., Wood, K., Dutcus, C., & Larkin, J. (2015). Lenvatinib, everolimus, and the combination in patients with metastatic renal cell

carcinoma: a randomised, phase 2, open-label, multicentre trial. The Lancet. Oncology, 16(15), 1473–1482. https://doi.org/10.1016/S1470-2045(15)00290-9

117. Kaley, T. J., Panageas, K. S., Pentsova, E. I., Mellinghoff, I. K., Nolan, C., Gavrilovic, I., DeAngelis, L. M., Abrey, L. E., Holland, E. C., Omuro, A., Lacouture, M. E., Ludwig, E., & Lassman, A. B. (2020). Phase I clinical trial of temsirolimus and perifosine for recurrent glioblastoma. Annals of clinical and translational neurology, 7(4), 429–436. https://doi.org/10.1002/acn3.51009
118. Hobday, T. J., Qin, R., Reidy-Lagunes, D., Moore, M. J., Strosberg, J., Kaubisch, A., Shah, M., Kindler, H. L., Lenz, H. J., Chen, H., & Erlichman, C. (2015). Multicenter Phase II Trial of Temsirolimus and Bevacizumab in Pancreatic Neuroendocrine Tumors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 33(14), 1551–1556. https://doi.org/10.1200/JCO.2014.56.2082
119. Inwards, D. J., Fishkin, P. A., LaPlant, B. R., Drake, M. T., Kurtin, P. J., Nikcevich, D. A., Wender, D. B., Lair, B. S., & Witzig, T. E. (2014). Phase I trial of rituximab, cladribine, and temsirolimus (RCT) for initial therapy of mantle cell lymphoma. Annals of oncology : official journal of the European Society for Medical Oncology, 25(10), 2020–2024. https://doi.org/10.1093/annonc/mdu273
120. Tasian, S. K., Silverman, L. B., Whitlock, J. A., Sposto, R., Loftus, J. P., Schafer, E. S., Schultz, K. R., Hutchinson, R. J., Gaynon, P. S., Orgel, E., Bateman, C. M., Cooper, T. M., Laetsch, T. W., Sulis, M. L., Chi, Y. Y., Malvar, J., Wayne, A. S., & Rheingold, S. R. (2022). Temsirolimus combined with cyclophosphamide and etoposide for pediatric patients with relapsed/refractory acute lymphoblastic leukemia: a Therapeutic Advances in Childhood Leukemia Consortium trial (TACL 2014-001). Haematologica, 107(10), 2295–2303. https://doi.org/10.3324/haematol.2021.279520
121. Mascarenhas, L., Chi, Y. Y., Hingorani, P., Anderson, J. R., Lyden, E. R., Rodeberg, D. A., Indelicato, D. J., Kao, S. C., Dasgupta, R., Spunt, S. L., Meyer, W. H., & Hawkins, D. S. (2019). Randomized Phase II Trial of Bevacizumab or Temsirolimus in Combination With Chemotherapy for First Relapse Rhabdomyosarcoma: A Report From the Children's Oncology Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 37(31), 2866–2874. https://doi.org/10.1200/JCO.19.00576
122. Trivedi, N. D., Armstrong, S., Wang, H., Hartley, M., Deeken, J., Ruth He, A., Subramaniam, D., Melville, H., Albanese, C., Marshall, J. L., Hwang, J., & Pishvaian, M. J. (2021). A phase I trial of the mTOR inhibitor temsirolimus in combination with capecitabine in patients with advanced malignancies. Cancer medicine, 10(6), 1944–1954. https://doi.org/10.1002/cam4.3672
123. Shen, C., Oswald, D., Phelps, D., Cam, H., Pelloski, C. E., Pang, Q., & Houghton, P. J. (2013). Regulation of FANCD2 by the mTOR pathway contributes to the resistance of cancer cells to DNA double-strand breaks. Cancer research, 73(11), 3393–3401. https://doi.org/10.1158/0008-5472.CAN-12-4282
124. Gaur, S., Chen, L., Yang, L., Wu, X., Un, F., & Yen, Y. (2011). Inhibitors of mTOR overcome drug resistance from topoisomerase II inhibitors in solid tumors. Cancer letters, 311(1), 20–28. https://doi.org/10.1016/j.canlet.2011.06.005
125. Deng, J., Bai, X., Feng, X., Ni, J., Beretov, J., Graham, P., & Li, Y. (2019). Inhibition of PI3K/Akt/mTOR signaling pathway alleviates ovarian cancer chemoresistance through reversing epithelial-mesenchymal transition and decreasing cancer stem cell marker expression. BMC cancer, 19(1), 618. https://doi.org/10.1186/s12885-019-5824-9

126. Wu, J., Cheng, Z., Xu, X., Fu, J., Wang, K., Liu, T., Wu, C., Kong, X., Yang, Q., Yan, G., & Zhou, H. (2019). Aurora-A Induces Chemoresistance Through Activation of the AKT/mTOR Pathway in Endometrial Cancer. Frontiers in oncology, 9, 422. https://doi.org/10.3389/fonc.2019.00422
127. Zhang, Y., Liu, X., Wang, Y., Lai, S., Wang, Z., Yang, Y., Liu, W., Wang, H., & Tang, B. (2022). The m6A demethylase ALKBH5-mediated upregulation of DDIT4-AS1 maintains pancreatic cancer stemness and suppresses chemosensitivity by activating the mTOR pathway. Molecular cancer, 21(1), 174. https://doi.org/10.1186/s12943-022-01647-0
128. Winters, B. R., Vakar-Lopez, F., Brown, L., Montgomery, B., Seiler, R., Black, P. C., Boormans, J. L., Dall Era, M., Davincioni, E., Douglas, J., Gibb, E. A., van Rhijn, B. W. G., van der Heijden, M. S., Hsieh, A. C., Wright, J. L., & Lam, H. M. (2018). Mechanistic target of rapamycin (MTOR) protein expression in the tumor and its microenvironment correlates with more aggressive pathology at cystectomy. Urologic oncology, 36(7), 342.e7–342.e14. https://doi.org/10.1016/j.urolonc.2018.03.016
129. Khanna, A., Bhushan, B., Chauhan, P. S., Saxena, S., Gupta, D. K., & Siraj, F. (2018). High mTOR expression independently prognosticates poor clinical outcome to induction chemotherapy in acute lymphoblastic leukemia. Clinical and experimental medicine, 18(2), 221–227. https://doi.org/10.1007/s10238-017-0478-x
130. O'Donnell, J. S., Massi, D., Teng, M. W. L., & Mandala, M. (2018). PI3K-AKT-mTOR inhibition in cancer immunotherapy, redux. Seminars in cancer biology, 48, 91–103. https://doi.org/10.1016/j.semcancer.2017.04.015
131. Peng, W., Chen, J. Q., Liu, C., Malu, S., Creasy, C., Tetzlaff, M. T., Xu, C., McKenzie, J. A., Zhang, C., Liang, X., Williams, L. J., Deng, W., Chen, G., Mbofung, R., Lazar, A. J., Torres-Cabala, C. A., Cooper, Z. A., Chen, P. L., Tieu, T. N., Spranger, S., … Hwu, P. (2016). Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy. Cancer discovery, 6(2), 202–216. https://doi.org/10.1158/2159-8290.CD-15-0283
132. Lastwika, K. J., Wilson, W., 3rd, Li, Q. K., Norris, J., Xu, H., Ghazarian, S. R., Kitagawa, H., Kawabata, S., Taube, J. M., Yao, S., Liu, L. N., Gills, J. J., & Dennis, P. A. (2016). Control of PD-L1 Expression by Oncogenic Activation of the AKT-mTOR Pathway in Non-Small Cell Lung Cancer. Cancer research, 76(2), 227–238. https://doi.org/10.1158/0008-5472.CAN-14-3362
133. Yan, C., Yang, J., Saleh, N., Chen, S. C., Ayers, G. D., Abramson, V. G., Mayer, I. A., & Richmond, A. (2021). Inhibition of the PI3K/mTOR Pathway in Breast Cancer to Enhance Response to Immune Checkpoint Inhibitors in Breast Cancer. International journal of molecular sciences, 22(10), 5207. https://doi.org/10.3390/ijms22105207
134. Jin J, Rohatgi A, Gabrielle A, Rose A, Wilson M, Bruno TC, et al. Interim results from a phase I/II study of duvelisib PI3Kδγ inhibitor and nivolumab in patients with advanced unresectable melanoma who have progressed on anti-PD1 therapy. (Abstract). J Clin Oncol. 2023;41(16):9540.
135. Tawbi HA, Peng W, Phillips S, Milton DR, Amaria RN, Diab A, et al. Safety results from phase I/II study of the PI3Kβ inhibitor GSK2636771 (G) in combination with pembrolizumab (P) in patients (pts) with PD-1 refractory metastatic melanoma (MM) and PTEN loss. (Abstract). J Clin Oncol. 2020;38(15):e22000.
136. Geoffrey Shouse, Tanya Siddiqi, Leslie L. Popplewell, Alexandra Muir, Ivana Melgar, Kirsten Orand, Brandy Gonzalez, Sandrine Puverel, Lili Wang, Adam S Kittai, Lu Chen, Matthew S. Davids, Alexey V Danilov; A Phase I Trial of PI3Kαδ Inhibitor Copanlisib in Combination with Nivolumab in Patients with Richter's Transformation (RT) or Transformed Non-Hodgkin Lymphoma (tNHL). Blood 2022; 140 (Supplement 1): 6633–6634. doi: https://doi.org/10.1182/blood-2022-160157

137. Chandrasekaran, S., Funk, C. R., Kleber, T., Paulos, C. M., Shanmugam, M., & Waller, E. K. (2021). Strategies to Overcome Failures in T-Cell Immunotherapies by Targeting PI3K-δ and -γ. Frontiers in immunology, 12, 718621. https://doi.org/10.3389/fimmu.2021.718621
138. Zheng, W., O'Hear, C. E., Alli, R., Basham, J. H., Abdelsamed, H. A., Palmer, L. E., Jones, L. L., Youngblood, B., & Geiger, T. L. (2018). PI3K orchestration of the in vivo persistence of chimeric antigen receptor-modified T cells. Leukemia, 32(5), 1157–1167. https://doi.org/10.1038/s41375-017-0008-6
139. Stock, S., Übelhart, R., Schubert, M. L., Fan, F., He, B., Hoffmann, J. M., Wang, L., Wang, S., Gong, W., Neuber, B., Hückelhoven-Krauss, A., Gern, U., Christ, C., Hexel, M., Schmitt, A., Schmidt, P., Krauss, J., Jäger, D., Müller-Tidow, C., Dreger, P., … Sellner, L. (2019). Idelalisib for optimized CD19-specific chimeric antigen receptor T cells in chronic lymphocytic leukemia patients. International journal of cancer, 145(5), 1312–1324. https://doi.org/10.1002/ijc.32201
140. Dwyer, C. J., Arhontoulis, D. C., Rangel Rivera, G. O., Knochelmann, H. M., Smith, A. S., Wyatt, M. M., Rubinstein, M. P., Atkinson, C., Thaxton, J. E., Neskey, D. M., & Paulos, C. M. (2020). Ex vivo blockade of PI3K gamma or delta signaling enhances the antitumor potency of adoptively transferred CD8+ T cells. European journal of immunology, 50(9), 1386–1399. https://doi.org/10.1002/eji.201948455
141. Funk, C. R., Wang, S., Chen, K. Z., Waller, A., Sharma, A., Edgar, C. L., Gupta, V. A., Chandrakasan, S., Zoine, J. T., Fedanov, A., Raikar, S. S., Koff, J. L., Flowers, C. R., Coma, S., Pachter, J. A., Ravindranathan, S., Spencer, H. T., Shanmugam, M., & Waller, E. K. (2022). PI3Kδ/γ inhibition promotes human CART cell epigenetic and metabolic reprogramming to enhance antitumor cytotoxicity. Blood, 139(4), 523–537. https://doi.org/10.1182/blood.2021011597
142. Haas, N. B., Appleman, L. J., Stein, M., Redlinger, M., Wilks, M., Xu, X., Onorati, A., Kalavacharla, A., Kim, T., Zhen, C. J., Kadri, S., Segal, J. P., Gimotty, P. A., Davis, L. E., & Amaravadi, R. K. (2019). Autophagy Inhibition to Augment mTOR Inhibition: a Phase I/II Trial of Everolimus and Hydroxychloroquine in Patients with Previously Treated Renal Cell Carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research, 25(7), 2080–2087. https://doi.org/10.1158/1078-0432.CCR-18-2204
143. Rangwala, R., Chang, Y. C., Hu, J., Algazy, K. M., Evans, T. L., Fecher, L. A., Schuchter, L. M., Torigian, D. A., Panosian, J. T., Troxel, A. B., Tan, K. S., Heitjan, D. F., DeMichele, A. M., Vaughn, D. J., Redlinger, M., Alavi, A., Kaiser, J., Pontiggia, L., Davis, L. E., O'Dwyer, P. J., … Amaravadi, R. K. (2014). Combined MTOR and autophagy inhibition: phase I trial of hydroxychloroquine and temsirolimus in patients with advanced solid tumors and melanoma. Autophagy, 10(8), 1391–1402. https://doi.org/10.4161/auto.29119
144. Hartleben, G., Müller, C., Krämer, A., Schimmel, H., Zidek, L. M., Dornblut, C., Winkler, R., Eichwald, S., Kortman, G., Kosan, C., Kluiver, J., Petersen, I., van den Berg, A., Wang, Z. Q., & Calkhoven, C. F. (2018). Tuberous sclerosis complex is required for tumor maintenance in MYC-driven Burkitt's lymphoma. The EMBO journal, 37(21), e98589. https://doi.org/10.15252/embj.201798589
145. Darawshi, O., Muz, B., Naamat, S. G., Praveen, B., Mahameed, M., Goldberg, K., Dipta, P., Shmuel, M., Forno, F., Boukeileh, S., Pahima, H., Hermann, J., Raab, M. S., Poos, A. M., Weinhold, N., Rosenbluh, C., Gatt, M. E., Palm, W., Azab, A. K., & Tirosh, B. (2022). An mTORC1 to HRI signaling axis promotes cytotoxicity of proteasome inhibitors in multiple myeloma. Cell death & disease, 13(11), 969. https://doi.org/10.1038/s41419-022-05421-4

146. Lin, C. P., Traets, J. J. H., Vredevoogd, D. W., Visser, N. L., & Peeper, D. S. (2023). TSC2 regulates tumor susceptibility to TRAIL-mediated T-cell killing by orchestrating mTOR signaling. The EMBO journal, 42(5), e111614. https://doi.org/10.15252/embj.2022111614
147. Greene, M. K., Johnston, M. C., & Scott, C. J. (2021). Nanomedicine in Pancreatic Cancer: Current Status and Future Opportunities for Overcoming Therapy Resistance. Cancers, 13(24), 6175. https://doi.org/10.3390/cancers13246175
148. Henske, E. P., Jóźwiak, S., Kingswood, J. C., Sampson, J. R., & Thiele, E. A. (2016). Tuberous sclerosis complex. Nature reviews. Disease primers, 2, 16035. https://doi.org/10.1038/nrdp.2016.35