The mTOR pathway in obesity driven gastrointestinal cancers: Potential targets and clinical trials

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ABSTRACT

The mechanistic target of rapamycin (mTOR) is a crucial point of convergence between growth factor signalling, metabolism, nutrient status and cellular proliferation. The mTOR pathway is heavily implicated in the progression of many cancers and is emerging as an important driver of gastrointestinal (GI) malignancies. Due to its central role in adapting metabolism to environmental conditions, mTOR signalling is also believed to be critical in the development of obesity. Recent research has delineated that excessive nutrient intake can promote signalling through the mTOR pathway and possibly evoke changes to cellular metabolism that could accelerate obesity related cancers. Acting through its two effector complexes mTORC1 and mTORC2, mTOR dictates the transcription of genes important in glycolysis, lipogenesis, protein translation and synthesis and has recently been defined as a central mediator of the Warburg effect in cancer cells. Activation of the mTOR pathway is involved in both the pathogenesis of GI malignancies and development of resistance to conventional chemotherapy and radiotherapy. The use of mTOR inhibitors is a promising therapeutic option in many GI malignancies, with greatest clinical efficacy seen in combination regimens. Recent research has also provided insight into crosstalk between mTOR and other pathways which could potentially expand the list of therapeutic targets in the mTOR pathway. Here we review the available strategies for targeting the mTOR pathway in GI cancers. We discuss current clinical trials of both established and novel mTOR inhibitors, with particular focus on combinations of these drugs with conventional chemotherapy, radiotherapy and targeted therapies.

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1. Introduction

The mechanistic target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine protein kinase belonging to the phosphatidylinositol-3 kinase (PI3K) related kinase superfamily [1]. It acts as a cardinal regulator of metabolism, energy homeostasis and nutritional status of the cell as well as coordinating signalling from growth factors such as mitogens, cytokines and hormones. mTOR exists as the catalytic core of its two known signalling complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), which are each comprised of distinct substrates and carry out different cellular functions [2]. mTORC1 is characterised by its exclusive partner raptor (rapamycin-insensitive companion of mTOR) [3] whereas mTORC2 is defined by its exclusive partner rictor (rapamycin-insensitive companion of mTOR) [4].

mTORC1 function is inherently dependent on amino acid levels as depletion of amino acids past a certain threshold renders mTORC1 completely refractory to all other signals and inputs, which prevents the cell engaging in energy costly anabolic processes when sufficient nutrients are not available [5]. This prevents the cell engaging in energy costly anabolic processes when sufficient nutrients are unavailable [6]. Upstream activation of mTORC1 is primarily mediated through activation of the GTPase Rheb (Ras homolog enriched in brain) by growth factors (eg. insulin, insulin-like growth factor 1 (IGF-1)) and amino acid dependent (specifically leucine [7]) translocation of inactive mTORC1 from the cytoplasm to the lysosomal membrane via the Regulator–Rag complex [8] (Fig. 1). The main substrates phosphorylated by activation of mTORC1 are ribosomal protein S6 kinases (S6K) and the eukaryotic translation initiation factor 4E (eIF4E) binding proteins 1–3 (4E-BP1–3), which drive cell proliferation, growth and cap-dependent protein synthesis [9]. mTORC1 signalling also promotes lipid synthesis [10], nucleotide synthesis [2] and suppresses autophagy [11]. In this respect mTORC1 is often described as a central driver of anabolic processes and an inhibitor of catabolic processes [12]. Additionally recent research has defined the role of mTORC1 signalling in angiogenesis whereby mTORC1 acts as the integration point of metabolic signals and signalling from vascular endothelial growth factor A (VEGF-A) [13]. mTORC1 has been shown to regulate HIF-1α expression and drives VEGF-A expression through activation of STAT3, 4E-BP1 and S6K1 all working in conjunction to drive angiogenesis under hypoxia [14]. mTORC2 has not been as thoroughly studied as its counterpart mTORC1, however it is known to phosphorylate Akt, protein kinase C (PKC), and SGK1 (serum and glucocorticoid-induced protein kinase) [15]. Therefore its role is seen to be more related to modulation of metabolism and cell survival through up regulation of Akt. While the upstream regulators of mTORC2 remain to be fully elucidated, it is seen to involve association with ribosomes in a PI3K-dependent manner and phosphorylation of Akt [16].

2. mTOR and obesity

Because the mTOR pathway is so central to the assimilation of signalling from growth factors, hormones and nutrients with cell growth and metabolism, there has been substantial research implicating it with obesity and cancer [17]. Obesity is a state of systemic chronic inflammation induced by excess adipose tissue accumulation when specific caloric needs exceed energy expenditure [18] and is one of the leading risk factors for development of cancer [19,20]. Current evidence suggests a strong association between incidence of obesity and specific gastrointestinal cancers such as colorectal, gastric, pancreatic and esophageal cancer [21]. It is well established that adipose tissue is an important endocrine organ involved in the production of numerous metabolic and inflammatory mediators such as free fatty acids, chemokines and adipocytokines [22,23]. Adipose-associated polypeptides such as leptin, adiponectin, insulin-like growth factors and ghrelin represent potential mechanisms promoting cancer development [24]. For example hyperinsulinemia and insulin resistance frequently occurs in most obese patients and is associated with a worse prognosis in multiple malignancies [25]. Insulin can stimulate the synthesis of IGF-1 [26], which exerts multiple mitogenic effects on cancer cells through activation of numerous signal pathways such as PI3K/Akt, MAPK and STAT3 [18]. These pathways contribute greatly to cancer initiation and progression and can all converge downstream on mTOR [27–29]. A growing body of research is showing that the mTOR pathway is heavily implicated in the initiation and progression of obesity driven gastrointestinal cancers [30]. Equally, increased signalling through mTOR is being implicated in the pathogenesis of obesity [17], and the development of insulin resistance in metabolic syndrome [31]. Both hyperinsulinemia and postprandial hyperinsulinemia have been shown to increase phosphorylation of S6K and inhibitory insulin substrate-1 [31]. Importantly mTOR signalling is required for adipogenesis as early studies showed that rapamycin inhibited both the proliferation and differentiation of human adipocytes [33]. Rapamycin was also shown to reduce obesity induced by a high fat diet in mice through long term inhibition of mTORC1 [34], however this effect, while beneficial, progresses to impaired glucose tolerance and insulin resistance [35].

3. mTOR and obesity related gastrointestinal cancers

3.1. Oesophageal cancer

Oesophageal cancer is rapidly increasing in incidence, particularly when compared to other malignancies and is characterised by low survival rates and poor prognosis [36]. Oesophageal adenocarcinoma (OAC) has one of the strongest associations with obesity, specifically visceral (abdominal) obesity, in terms of incidence and pathogenesis [37]. Barrett’s oesophagus (BO), a premalignant lesion associated with the development of OAC, is also associated with obesity and concomitant gastro-oesophageal reflux disease (GORD) [38]. This proposed mechanism between BO and visceral obesity is believed to be related, at least in part, to increased acidic reflex observed in obese patients [39]. Chronic exposure to bile acid and gastric reflux initiates the inflammatory processes crucial in the progression from BO to OAC, and this can activate mTOR through stimulation of I KKβ/TSC1 signalling in BO associated OAC [40]. Treatment of oesophageal cancer cells with mTOR inhibitors rapamycin and Bay-11–7082 was shown to effectively inhibit bile acid induced cell transformation and proliferation [40].

In a xenograft mouse model of oesophageal cancer both rapamycin induced and siRNA induced inhibition of mTOR were shown to decrease tumour size and mTOR expression [41]. The use of both agents was shown to have a greater anti-tumour effect than either agent alone. In OAC patients, overexpression of phosphorylated mTOR (p-mTOR) is significantly correlated with poorer overall survival [42].
In addition to its importance in OAC, mTOR is shown to be involved in the pathogenesis of oesophageal squamous cell carcinoma (OSCC). Increased expression and activation of mTOR was shown in a study examining mTOR expression in multiple resected OSCC tumours and OSCC cell lines [43]. Down regulation of the tumour suppressor PTEN significantly correlated with up regulation of mTOR in multiple OSCC cell lines [44]. A large patient cohort study of single nucleotide polymorphisms in mTOR revealed certain mTOR genotypes could increase risk of OSCC [32]. Interestingly when stratified by patient BMI there was a greater significant association between three specific mTOR SNPs (rs2295080, rs1057079, and rs1064261) and risk of OSCC in patients with a BMI 25 [45]. Studies such as these underpin the role of mTOR in the interaction between genetic and environmental risk factors in obesity related oesophageal cancers [46–49].

3.2. Gastric cancer

Obesity has long been a suspected risk factor for gastric cardia adenocarcinoma (GCC) while has been shown to be unrelated to gastric non-cardia adenocarcinoma (GNCC) [50,51]. While data is limited meta-analysis has shown no association between GNCC and obesity and this was not significant when adjusted according to patient sex [52]. A recent meta-analysis from the EPIC cohort showed that obesity as measured by BMI showed no association in terms of risk with GCC or GNCC, however when adjusted for waist circumference there was a higher risk of GCC. Therefore further investigation into the role of abdominal obesity and risk of GCC is warranted. There is mounting evidence that the mTOR pathway is deregulated in gastric cancer with specific genetic mutations in the PI3K/Akt/mTOR pathway frequently being observed regardless of GC subtype [53]. IHC analysis of GC patient samples showed that high expression of p-mTOR, specifically in the tumour cell cytoplasm, correlated with tumour stage, metastasis and overall survival [54]. Related studies have reported similar findings of mTOR expression in GCC having a positive correlation with tumour metastasis and invasiveness. One such study determined that mTOR is highly expressed in GCC tumour cells with relatively little expression in the surrounding normal gastric tissue [55]. Mouse studies of mTOR signalling in GC have shown that targeted inhibition of mTOR using everolimus can inhibit cell proliferation, tumour vascularisation and local tumour dissemination [56]. Upstream of mTOR, mutations and amplification of PI3K and Akt respectively are often observed in GC [58] and over activation of PI3K, Akt and elf-4E were significantly associated with lymph node metastasis [59].

3.3. Hepatocellular carcinoma

While hepatocellular carcinoma (HCC) can arise from a vast myriad of carcinogenic factors both obesity and non-alcoholic fatty liver disease (NAFLD) are established risk factors for the development of HCC [60,61]. Increased levels of both adiponectin and leptin has been observed in patients with cirrhotic HCC and non-cirrhotic HCC [62] and adiponectin level has been found to be predictive of overall survival in HCC patients.
Hypoadiponectinaemia can accelerate the formation of HCC [64] and concomitantly, adiponectin inhibits phosphorylation of mTOR and can prevent HCC tumourigenesis in nude mice [65]. Mutations in the mTOR pathway are seldom seen in HCC with mTOR activation in HCC principally being due to upstream ligand dependent receptor activation [66] namely the EGFR, IGF and PTEN signalling pathways [67]. Transcriptomic analysis of patient data sets has similarly revealed HCC subsets that have upstream over-expression of IGF2 and IGF1R in addition to mutations in PIK3CA, culminating in deregulated mTOR signalling [68]. Blockade of mTOR can enhance upstream inhibition of growth factors involved in HCC such as fibroblast growth factor receptor (FGFR). Combined inhibition of both FGFR and mTOR, using FGFR inhibitor BGJ398 and rapamycin, in an orthotropic model of HCC lead to a significant inhibition of tumour growth and prevented recruitment of vascular smooth muscle cells (VSMCs) and hepatic stellate cells (HSCs) into liver tumours [69].

3.4. Pancreatic cancer

While diabetes is a well established risk factor for pancreatic cancer (PC), there hasn’t been as clear a delineation between obesity and risk of PC with only a weak association being reported [70] or certainly a non-linear relationship [71]. However recent research has revealed a link between pro-inflammatory eicosanoid prostaglandin E2 (PGE2) signalling and the mTOR pathway in obesity associated pancreatic cancer [72]. PGE2 is an integral effector in the inflammatory milieu seen in obesity and is over activated in the progression of obesity-associated cancers [24]. In multiple pancreatic cell lines treatment with PGE2 resulted in enhanced signalling of the mTOR pathway via increased phosphorylation of S6K1 [72]. It is becoming increasingly evident that the mTOR pathway is intricately involved in the progression of PC with mTOR pathway genes found to be mutated in specific subsets of PC, as multiple mTOR pathway genes are mutated in specific subsets of PC [73]. Tissue microarray analysis revealed down-regulation of two critical upstream regulators of mTOR, TSC2 and PTEN, low expression of which correlated with poorer disease free and overall survival in PC patients [74]. A recent study of metastatic pancreatic ductal adenocarcinoma (PDAC) revealed a specific disease subset, whereby loss of PTEN or TSC1 haploinsufficiency facilitated development of PDAC through hyper activation of the mTOR pathway [75].

3.5. Colorectal cancer

Colorectal cancer (CRC) is the third most common cancer globally [36] and its incidence is consistently linked to incidence of obesity [76, 71]. Leptin signalling has been shown to be a driving factor in colon cancer cell proliferation and can induce phosphorylation of Akt and mTOR [77]. Expression of the leptin receptor (Ob-R) was strongly correlated with activation of the PI3K/Akt/mTOR pathway and downstream phosphorylation of mTOR [77]. Conversely activation under low adiponectin conditions was found to be a key mechanism in the promotion of proliferation and colorectal carcinogenesis [78].

Genes important to the development of CRC, such as APC, p53 and K-ras, lie upstream of mTOR and can mediate their oncogenic signalling in part through mTOR [79]. APC gene mutations are common in sporadic CRC and mTOR has been found to have a crucial role in APC-deficient colorectal cancer [80]. Loss of the APC gene is pivotal in the pathogenesis of CRC and mTORC1 activity is essential for the proliferation of APC
deficient enterocytes [81]. Using multiple genetic mouse models it was found that APC loss led to increased activity of eukaryotic elongation factor 2 (eEF2) [82], which is required for intestinal cell proliferation. In- found that APC loss led to increased activity of eukaryotic elongation

level and disease stage, lymph node involvement and recurrence [83].

CRC in the mTOR signalling pathway and that these correlated with depth of
tors p70s6K, and 4EBP1 in human CRC samples showed high activity of

chymal transition (EMT) and cell motility [84]. CRC metastasis was

mTOR signalling through RhoA and Rac1 regulated epithelial-mesen-
mTOR has also been implemented in CRC metastasis, as elevated
CRC revealed a signi

[81].

rapamycin, and this was effective at suppressing tumour development,
and in parallel did not affect normal intestinal proliferation or apoptosis [81].

Examinations of both the mRNA level and protein level of mTOR in
CRC revealed a significant relationship between high mTOR expression
level and disease stage, lymph node involvement and recurrence [83].
mTOR has also been implemented in CRC metastasis, as elevated
mTOR signalling through RhoA and Rac1 regulated epithelial-mesen-
chymal transition (EMT) and cell motility [84]. CRC metastasis was
completely inhibited in vivo upon inhibition of mTOR1 and mTORC2
[85]. Immunohistochemical analysis of mTOR and its downstream effectors
p70s6K, and 4E BP1 in human CRC samples showed high activity of the
mTOR signalling pathway and that these correlated with depth of
CRC infiltration [85].

While our understanding of the mTOR pathway in GI cancers is still
in its infancy, research in this area has established a strong enough link
to justify targeting mTOR in obesity associated GI cancers (Fig. 2).

4. Targetting the mTOR pathway in clinical trials

4.1. Clinical trials of rapamycin and rapalogos

The discovery of rapamycin (the first mTOR inhibitor) predated the discovery of mTOR itself. Originally approved as an immunosuppressant
[86], it was later discovered that rapamycin targeted mTOR and that it
had anti-proliferative effects, which resulted in the drug being investi-
gated as an anti-cancer agent [87]. Rapamycin’s unfavourable pharma-
cokinetics limited it’s use as a drug which drove the

adverse events pro-
inflammatory effects and reduced the risk of progression by 34% and the PFS was 1.7 months com-
pared to 1.4 months compared to BSC, indicating that combination of

beletorimod with other effective targeted therapies or chemo-
therapies may be a more promising strategy [102].

The adverse events profile for everolimus in gastric cancer was con-
sistent with that observed in other trials evaluating everolimus in other
cancers. Everolimus was suggested to have clinical activity in a subset of
patients in this study and extensive biomarker analysis of the GRANITE-
1 study is underway in an effort to identify this subset of GC patients [102].

4.2. Clinical trials of ATP competitive mTOR kinase inhibitors

Following on from the somewhat disappointing clinical efficacy of
rapalogos, ATP-competitive mTOR tyrosine kinase inhibitors (TKIs)
were developed. These compounds inhibit the catalytic site of the
mTOR kinase domain, giving them the advantage of targeting the kinase
activity of both mTORC1/2 thus blocking the feedback activation of the
PI3K/Akt signalling pathway [103]. This robust inhibition of mTORC2
dependent activation of Akt limits this form of resistance to mTOR in-
hibitors hopefully enhancing their efficacy. In a preclinical assessment
of the oral mTOR kinase inhibitor OSI-027, this compound was shown to selectively inhibit mTORC1 mediated phosphorylation 4E-BP1 and S6K1 as well as mTORC2 specific activation of Akt in multiple rapamycin sensitive and resistant in-vitro models [104]. Additionally it had superior efficacy compared to rapamycin in multiple colon cancer xenograft models [104]. OSI-027 was brought forward to a phase I clinical trial to assess its pharmacodynamic profile in a broad range of cancers and achieved substantial clinical effect [105]. However it was poorly tolerated with over a third of patients requiring dose reductions [105] and was discontinued due to lack of clinical efficacy in phase II trials [106].

One promising mTOR TKI, AZD2014, has shown dramatic anti-proliferative effects in preclinical studies of breast cancer [107] and HCC [108]. The first clinical trial of AZD2014 examined pharmacokinetics and pharmacodynamics in 56 patients across a range of malignancies [109]. The Mean Tolerated Dose (MTD) was established and there were partial clinical responses seen in both pancreatic and breast cancer patients [109]. Azd2014 has been brought forward to phase II clinical trials in Gastric adenocarcinoma in combination with paclitaxel (NCT02449655). Another similar mTOR TKI, INK128, has shown efficacy in preclinical studies of GI cancers such as PAC [110] and is being examined in phase I studies across a range of malignancies.

4.3. Clinical trials of dual mTOR/PI3K inhibitors

As mTOR is heavily linked to the PI3K pathway in terms of cancer progression and resistance to mTOR inhibitors, this prompted the development of dual PI3K/mTOR inhibitors, which target the p110α, β, and γ isoforms of PI3K in addition to the catalytic sites of mTORC1/2 [90]. This dual targeted approach attempts to completely shut down the PI3K/Akt/mTOR pathway even in cancers that have over expression of this pathway [111]. In a recent study of CRC, mTORC2 was shown to be over-expressed in CRC cells and down-regulation of mTORC2 reduced proliferation of colon cancer cells and inhibited the formation of tumour xenografts in vivo. Combined inhibition of PI3K and mTORC1/2 by dual mTOR/PI3K inhibitor NVP-BEZ235 was shown to induce tumour regression in a mouse model of sporadic CRC [112]. Consistent with this, a more recent study also demonstrated the efficacy of NVP-BEZ235 and of an additional catalytic mTOR inhibitor, pp242, in human colon cancer cell line xenografts [113]. In addition to its effect in CRC, NVP-BEZ235 has recently been shown to highly effectively in attenuating growth of pancreatic cancer cells and work synergistically with gemcitabine to induce potent cytotoxicity in gemcitabine resistant pancreatic cancer cells [114].

Another highly selective dual mTOR/PI3K inhibitor VS-5584 has been shown to have significant efficacy in a rapalog resistant colorectal cancer xenograft model reducing both tumour growth and the number of functional tumour blood vessels [115]. More pertinent however is the preferential targeting of cancer stem cells (CSCs) by VS-5584, where it has been shown to exert a selective effect on CSCs in a broad range of cell lines, xenograft and patient tumour explant models [116]. VS-5584 has recently been granted orphan status for clinical development in mesothelioma and has entered into a Phase I dose escalation...
study in advanced non-hematologic malignancies and lymphoma (NCT01991938).

Several clinical trials are examining the efficacy of dual PI3K/mTOR inhibitors and while there is some encouraging early trial data, there is still evidence that some cancers may be intrinsically resistant to dual inhibition of PI3K/mTOR. A recent screening of multiple cancer cell lines found that KRAS mutations could confer resistance to dual PI3K/mTOR inhibitors [117]. This resistance was specifically linked to changes in the level of phosphorylation of 4E-BP1, and was absent in wild type KRAS tumours or PIK3CA mutated tumours [117]. Studies such as this highlight the need for reliable clinical biomarkers to assess efficacy of mTOR inhibitors.

### 4.4. Clinical trials of mTOR inhibitors in GI cancers in combination with other therapeutics

Many mTOR inhibitors have only modest clinical activity and rapid development of resistance is common. Consequently clinical trials are largely shifting in favour combining mTOR inhibitors with conventional chemotherapy and radiotherapy to improve outcomes and circumvent resistance. Due to the heterogeneity in mTOR signaling seen across multiple cancer types [59,75] it is critical to assess which subsets of cancer patients will benefit from addition of an mTOR inhibitor to their existing treatment regimens. Combining PI3K/AKT/mTOR pathway inhibitors with chemotherapy and radiotherapy could improve efficacy [114, 118–120] and potentially prevent tumour regrowth between doses of treatment.

#### 4.4.1. Clinical trials combining mTOR inhibitors and conventional therapies

Recent studies have demonstrated that dual PI3K/mTOR inhibitors can act as radiosensitisers and augment radiation-induced cytotoxicity in cancer cells. The dual PI3K/mTOR inhibitor BEZ235 was shown to have a synergistic effect with radiation in CRC cells by attenuating double strand break repair and sensitising CRC cells to radiation [121]. The same synergistic effect was seen in vivo where combination of BEZ235 and radiation decreased tumour size greater than either therapy alone. The expression of mTOR, elf4E, and S6 was also significantly decreased [121], indicating that combined mTOR targeting and radiotherapy could be particularly beneficial.

Preliminary clinical data holds promise that mTOR inhibitors can sensitize cancer cells to conventional chemotherapy. A phase I trial investigating the combination of everolimus and capecitabine showed encouraging results in a broad cohort of cancers, with the combination regime being safe and tolerable [122]. This study demonstrated clinical benefit in 39% of patients, with drug related adverse events being mainly of grade <2, however for future trials it may be advisable to screen patients for alterations in the PI3K/AKT/mTOR pathway as this could result in the greatest clinical benefit.

The combination of an mTOR inhibitor with capcitabine (5-fluorouracil) has demonstrated synergy in preclinical studies and safety in a phase I trial examining combination of everolimus and capcitabine in pancreatic cancer. A recent phase II trial further contested the feasibility and clinical efficacy of this combination in a cohort of pancreatic cancer patients and an acceptable toxicity profile was observed for 5 mg everolimus BID and capcitabine 1000 mg/m² for 14 days every 3 weeks [123]. Moderate clinical activity was achieved only in first line pancreatic cancer patients [123].

#### 4.4.2. Clinical trials of mTOR inhibitors and targeted therapies

The use of mTOR inhibitors, to both increase the efficacy and overcome resistance of targeted therapies, has been supported substantially by preclinical data. Ongoing phase I and II trials are examining combinations of mTOR inhibitors and multi-targeted TKIs such as imatinib, neratinib and pazopanib in a broad range of cancers. A phase I trial in gastrointestinal stromal tumours combining everolimus and imatinib in 31 patients with imatinib refractory disease showed disease stabilisation in 8 patients and partial response in 2 patients [124]. This could indicate that mTOR inhibition may be resensitising certain patients to imatinib.

A recent phase I study examined the combination of temsirolimus and neratinib, a pan-human epidermal growth factor (HER) TKI, in 60 patients with advanced solid tumours including colorectal and pancreatic cancer. The combination of neratinib and temsirolimus was tolerable across a range of malignancies with the best overall response including two complete responses, six partial responses and 27 cases of stable disease [125]. Results from this trial indicate that evaluation of this combination in GI cancers with significant HER2 and PI3K/mTOR pathway activation is warranted.

Inhibition of mTOR is known to have a direct anti-angiogenic effect through regulation of HIF-1α [126]. Therefore combinations mTOR inhibitors with anti-angiogenic therapies have been put forward as rational treatment strategies. A phase II trial combining everolimus with the anti-VEGF monoclonal antibody bevacizumab in metastatic colorectal cancer showed encouraging results with minor responses reported in 16% of patients and a further 30% achieving disease stability [127]. This combination was shown to be tolerable and had modest clinical activity however recent phase I trials combining mTOR inhibitors with anti-angiogenic TKIs have shown conflicting results. A phase I study of the combination of temsirolimus and pazopanib (a pan-VEGF receptor

| mTOR inhibitor | Patients | Drug combination | Phase | Trial Number |
|----------------|----------|-----------------|-------|--------------|
| Everolimus     | Pancreatic cancer | gemcitabine BYL719 and exemestane 5-FU | II    | NCT00569963 |
|                | Pancreatic neuroendocrine tumors | Octreotide acetate with or without bevacizumab Pasireotide | II    | NCT01229943 |
|                | Germ cell | VEGFR/PDGFR dual inhibitor X-82 | II    | NCT01784861 |
|                | Metastatic pancreatic cancer | Cetuximab and Capcitabine | II    | NCT01077986 |
| Rapamycin      | Pancreatic cancer | Metformin | II    | NCT02048384 |
| Sirolimus      | Pancreatic cancer | Vismodegib | I     | NCT01537107 |

| mTOR inhibitor | Patients | Drug combination | Phase | Trial Number |
|----------------|----------|-----------------|-------|--------------|
| Temsirolimus   | Metastatic pancreatic cancer | Metformin | I     | NCT00097647 |
| Sirolimus      | Pancreatic cancer | NCT00499486 |
inhibitor) in advanced solid tumours including CRC showed high levels of grade 3 and higher toxicities as doses far less than the approved dose of each drug as a single agent [128]. Overlapping mTOR inhibitor and VEGFR TKI toxicities could account for the unfeasibility of this combination and further research is required to understand these potential interactions and evaluate alternate treatment strategies to circumvent these toxicities.

In spite of certain negative trial results, many pharmaceutical companies are moving forward with trials combining mTOR inhibitors with targeted therapies, as this remains one of the more promising avenues to improve clinical efficacy of available therapeutics and overcome resistance.

### 5. Novel pathway crosstalk and potential new targets of the mTOR pathway

Recent studies have uncovered multiple novel upstream regulators of the mTOR pathway highlighting the extensive crosstalk between mTOR and signalling pathways such as Hedgehog, WNT, Notch and Hippo [129]. These “non classical” inputs of the mTOR pathway could reveal promising novel targeting opportunities in GI cancers where this crosstalk is driving tumour progression through the mTOR pathway.

#### 5.1. Hippo pathway

The Hippo pathway controls organ size by promoting apoptosis and inhibiting proliferation through its main downstream effector Yes-associated protein 1 (YAP1) [130]. Yap1 controls the transcription of genes that govern proliferation and induce apoptosis [131]. Coordination between Hippo and mTOR pathways was hypothesised to occur given the function of YAP1 in cell proliferation, which cannot be sustained without coordinate cell growth modulation by mTOR [131]. A recent study has reported a molecular mechanism through which the Hippo pathway can regulate cell growth by modulating mTORC1 and mTORC2 through the positive control of YAP1 [132]. YAP is responsible for the transcription of the microRNA mir-29, which in turn inhibits the translation of PTEN. This down-regulation of PTEN by YAP1 leads to increased PI3K signalling and subsequent increased activation of mTORC1 and mTORC2. This crosstalk is also implemented in the progression of HCC through another key activator of the Hippo pathway, the transcriptional coactivator TAZ. Knockdown of TAZ in HCC cell lines attenuated cancer cell growth via inactivation of the mTOR pathway and expression of TAZ mRNA was associated with HCC tumour size [133].

#### 5.2. Hedgehog pathway

The Hedgehog (HH) pathway is essential for growth and development and is implicated in the pathogenesis of multiple GI cancers. Specifically in oesophageal cancers over activation of the HH pathway correlates with lymph-node metastasis [134,135]. A recent study has revealed crosstalk between the mTOR and Hedgehog pathways in OAC whereby S6K1 phosphorylates the HH transcription factor Gli1 independent of any upstream signalling from the HH pathway [136]. Gli1 is an established oncogene [137] and could be contributing to the development of OAC via the mTOR pathway. Combinations of mTOR inhibitors and HH inhibitors in OAC cells revealed greater growth inhibition than either therapeutic alone [136]. Equally a novel synthetic lethality has been identified in rhabdomyosarcoma, whereby combination treatment with Gli1/2 inhibitor GANT61 and PI3K/mTOR inhibitor PI103 reduced tumour growth in an in vivo model of rhabdomyosarcoma through caspase-dependent apoptosis [138].

#### 5.3. Notch pathway

Notch signalling is an integral pathway to cellular proliferation, differentiation and development [139]. Binding of the cell surface ligands delta and jagged to the notch receptors on an adjacent cell initiates a signal transduction pathway that culminates in the Notch intracellular domain (NICD) translocating to the nucleus and promoting target gene expression. A recent study in rat hepatoma cells showed that activation of mTORC1 by notch signalling promoted hepatic lipogenesis via hyper activation of the key component of mTORC1, raptor [140]. This hyper activation of raptor was independent of an increase in mRNA levels of raptor and shows that notch signalling is capable of promoting mTORC1

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**Table 4**

Clinical trials of mTOR inhibitors in combination with chemotherapy and targeted therapies in liver cancers.

| mTOR inhibitor | Patients | Drug combination | Phase | Trial Number |
|---------------|---------|-----------------|-------|-------------|
| Temsirolimus | Advanced | Bevacizumab & | II | NCT01010126 |
| Metastatic colorectal cancer | Bevacizumab & | | | |
| | Irinotecan & | | | |
| | Cetuximab | | | |
| | I | NCT00467194 |
| Sirolimus | Unresectable | Bevacizumab | I | NCT00467194 |
| Metastatic colorectal cancer | Bevacizumab | | | |
| | Cetuximab | | | |
| | I | NCT0139138 |
| Everolimus | Colorectal cancer | Panitumumab & | I/II | NCT01139138 |
| | | Cetuximab & | | |
| | | Irinotecan | | |
| | | I/II | | |
| | | Irinotecan | | |
| | | I | NCT00478634 |

**Table 5**

Clinical trials of mTOR inhibitors in combination with chemotherapy and targeted therapies in Oesophageal, Gastric and Colorectal cancers.

| mTOR inhibitor | Patients | Drug combination | Phase | Trial number |
|---------------|---------|-----------------|-------|-------------|
| Everolimus | Colorectal cancer | Panitumumab & | I/II | NCT01139138 |
| | | Cetuximab & | | |
| | | Irinotecan | | |
| | | I/II | | |
| | | Irinotecan | | |
| | | I | NCT00478634 |
| Metastatic colorectal cancer | Cetuximab & | | | |
| | Bevacizumab | | | |
| | I | NCT0139138 |
| Oesophageal cancer | Paclitaxel & | | | |
| | Carboplatin | | | |
| | Cetuximab | | | |
| | I | NCT01490749 |
| Gastric cancer | MitomycinC | | | |
| | Paclitaxel | | | |
| | LDE225 | | | |
| | III | NCT01248403 |
| | Paclitaxel | | | |
| | Carboplatin | | | |
| | I | NCT01514110 |
| Imatinib resistant gastrointestinal stromal tumors | Imatinib | | | |
| | I | NCT01275222 |
| Metastatic gastric cancer | Cisplatin; 5-FU; | | | |
| | Leucovorin & | | | |
| | Capecitabine | | | |
| | I/II | NCT00632268 |
| Esophageal cancer | TS-1 & Cisplatin | | | |
| | I | NCT01096199 |
signalling via increased interaction of mTOR and raptor as well as increased assembly of mTORC1. However further research is required to define the molecular mechanism by which this hyper activation of Notch signalling increases mTOR-raptor interactions and where along this pathway there are potential targets.

6. Moving forward

Research into the role of the mTOR pathway in cancer is rapidly developing and as a result there has been considerable efforts invested in bringing mTOR targeting agents to the clinic. While rapalogs have high specificity for mTOR, their propensity for the development of resistance means they may only be suited to combination therapy for the majority of cancers.

Dual PI3K/mTOR inhibitors appear to have the widest profile of activity as these have multiple targets in the mTOR pathway however the severity of overlapping toxicities that these agents will have with other tyrosine kinase inhibitors is currently unknown and this may restrict their use with other targeted therapies. Available data on second-generation ATP-competitive mTOR kinase inhibitors demonstrates that their dual targeting of mTORC1 and mTORC2 could overcome issues with resistance to rapalogs and have higher single agent activity. However further genomic profiling of responsive tumours is necessary to be able to implement this in the most clinically relevant way.

While significant progress has been made in the development of new agents to target mTOR, how to best evaluate mTOR inhibitors in the clinical setting remains to be fully elucidated. Therefore there is a pressing need to develop biomarkers able to assess the efficacy and predict the response of mTOR inhibitors in patients. Currently it is possible to monitor activity of mTOR by analysing the phosphorylation status of S6K and 4E-BP1 and some clinical trials have successfully used blood and tumour samples from patients undergoing treatment with mTOR inhibitors to detect a decrease in S6K and 4E-BP1 phosphorylation [141]. However these may not be robust enough to become companion diagnostic tools as 4E-BP1 has been shown to contain rapamycin resistant phosphorylation sites [142]. Development of biomarkers must also address the discord in mTOR expression between primary and metastatic tumours, and considerable intratumoural heterogeneity of mTOR signalling between vascularised and hypoxic regions of tumours.

Furthermore, mTOR inhibitors may bring additional challenges in the clinical setting due to the complexity of their metabolic effects and their immunosuppressive potential especially in light of the fact that rapamycin is approved to prevent allograft rejection. These potential issues need to be clarified in both preclinical and clinical trials as they could greatly influence the cancer patients’ course of treatment.

Recent genomic, proteomic and metabolomic studies of mTOR in cell lines have revealed a wealth of information on novel cross talk between mTOR signalling and other pathways in GI cancers with Hippo, Hedgehog and Notch signalling pathways identified as upstream regulators of mTOR pathway [132,136,129]. Further investigation into the multiple pathways that converge on mTOR will reveal valuable information not only on the regulation of mTOR but also may provide new novel targets in this signalling network. Despite the challenges that need to be addressed in further studies on targeting mTOR, this area of research holds great promise in terms of potential clinical benefit and will likely have an important role in the treatment of obesity associated gastrointestinal cancers.

**Abbreviations**

- mTOR: mechanistic target of rapamycin
- GI: gastrointestinal
- PI3K: phosphatidylinositol-3 kinase
- raptor: regulatory-associated protein of mTOR
- rictor: rapamycin-insensitive companion of mTOR
- Rheb: Ras homolog enriched in brain
- IGF-1: insulin-like growth factor 1
- eIF4E: eukaryotic, initiation factor 4E
- 4EBP1-3: 4E binding proteins 1–3
- S6K: S6 kinases
- VEGF-A: vascular endothelial growth factor A
- STAT3: signal transducer and activator of transcription 3
- PKC: protein kinase C
- SGK1: serum and glucocorticoid-induced protein kinase
- Akt: protein kinase B
- MAPK: mitogen-activated protein kinase
- OAC: oesophageal adenocarcinoma
- BO: Barrett's oesophagus
- GORD: gastro-oesophageal reflux disease
- IKKβ: IκB kinase β
- TSC1: tuberous sclerosis 1
- p-mTOR: phosphorylated mTOR
- OSCC: oesophageal squamous cell carcinoma
- GCC: gastric cardia adenocarcinoma
- GNCC: gastric non-cardia adenocarcinoma
- HCC: hepatocellular carcinoma
- NAFLD: non-alcoholic fatty liver disease
- EGFR: epidermal growth factor receptor
- PTEN: phosphatase and tensin homolog
- PI3KCA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
- FGFR: fibroblast growth factor receptor
- VSMCs: vascular smooth muscle cells
- HSCs: hepatic stellate cells
- PGE2: prostaglandin E2
- PC: pancreatic cancer
- PDAC: pancreatic ductal adenocarcinoma
- CRC: colorectal cancer
- Ob-R: leptin receptor
- eEF2: eukaryotic elongation factor 2
- EMT: epithelial–mesenchymal transition
- Grb10: growth factor receptor bound protein 10
- TKIs: tyrosine kinase inhibitors
- CSCs: cancer stem cells
- BSC: best supportive care
- HER: human epidermal growth factor
- YAP1: Yes-associated protein 1
- HH: hedgehog
- NCID: Notch intracellular domain

**Conflicts of interest**

The authors declare that they have no competing interests.

**Transparency Document**

The Transparency document associated with this article can be found, in the online version.

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