Title
Exploiting the therapeutic potential of the PI3K-AKT-mTOR pathway in enriched populations of gynecologic malignancies

Permalink
https://escholarship.org/uc/item/01p8v6nv

Journal
Expert Review of Clinical Pharmacology, 7(6)

ISSN
1751-2433

Authors
Eskander, Ramez N
Tewari, Krishnansu S

Publication Date
2014-11-01

DOI
10.1586/17512433.2014.968554

Copyright Information
This work is made available under the terms of a Creative Commons Attribution License, available at https://creativecommons.org/licenses/by/4.0/

Peer reviewed
Exploiting the therapeutic potential of the PI3K-AKT-mTOR pathway in enriched populations of gynecologic malignancies

Ramez N Eskander & Krishnansu S Tewari

To cite this article: Ramez N Eskander & Krishnansu S Tewari (2014) Exploiting the therapeutic potential of the PI3K-AKT-mTOR pathway in enriched populations of gynecologic malignancies, Expert Review of Clinical Pharmacology, 7:6, 847-858, DOI: 10.1586/17512433.2014.968554

To link to this article: http://dx.doi.org/10.1586/17512433.2014.968554

Published online: 10 Oct 2014.

Article views: 179

View related articles

View Crossmark data

Citing articles: 6
Exploiting the therapeutic potential of the PI3K-AKT-mTOR pathway in enriched populations of gynecologic malignancies

Expert Rev. Clin. Pharmacol. 7(6), 847–858 (2014)

Ramez N Eskander1 and Krishnansu S Tewari*2
1Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of California Irvine Medical Center, Building 56 Room 260, 101 The City Drive South, Orange CA 92868 USA
2Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of California, Irvine, 101 The City Dr. South, Building 56 Room 264, 101 The City Drive South, Orange, CA 92868, USA
*Author for correspondence: Tel.: +1 714 456 7631
Fax: +1 714 456 6632
ktewari@uci.edu

Given the prevalence of phosphatase & tensin homolog mutations in histologic specimens harvested from patients with endometrial cancer, significant interest in systemic treatment with PI3K/Akt/mTOR inhibitors has emerged. Several Phase II trials have been completed studying mTOR inhibitors in advanced/recurrent endometrial cancer. The mTOR pathway also appears to be important in some cervical cancers. Finally, because clear cell carcinoma of the ovary and renal cell carcinoma have a shared histology, the potential for activity of mTOR inhibitors in clear cell cancer of the ovary is implicit. This article reviews the results of Phase II clinical trials of PI3K/Akt/mTOR pathway inhibitors in patients with endometrial cancer, and discusses the potential therapeutic landscape of mTOR inhibition in enriched populations in gynecologic cancers.

KEYWORDS: cervical cancer • clear cell ovarian cancer • endometrial cancer • mTOR inhibitors • PI3K/AKT/mTOR pathway • PTEN

Phosphatase & tensin homolog mutations in endometrial cancer

Endometrial carcinoma is the most common gynecologic malignancy in the USA, with an estimated 52,630 new cases and 8590 deaths projected in 2014 [1]. Most women (80–85%) present with early stage disease, and surgery in the form of hysterectomy is curative. Unfortunately, a proportion of patients will present with advanced disease or develop disease recurrence, with associated poor survival [2]. Currently, available cytotoxic therapies for the treatment of advanced stage, progressive or recurrent disease, have shown limited success. In the setting of metastatic disease recurrence, 5-year survival rates are less than 15%, with median survival ranging from 7 to 12 months [2]. The lack of effective treatment options once the disease has metastasized represents an unmet need in endometrial cancer care, and highlights the importance of investigating novel targeted therapies in an effort to improve oncologic outcomes [3,4].

Significant advancements in our understanding of the molecular mechanisms underlying endometrial carcinoma have been made in recent years (Table 1). Traditionally, endometrial carcinomas are categorized as either Type 1 or, less commonly, Type 2. Type 1 endometrial cancers are estrogen driven, associated with obesity, anovulation and metabolic syndrome, with an associated favorable survival when diagnosed in early stages. Conversely, Type 2 endometrial cancers are not estrogen driven, more commonly high grade and portent a poor prognosis due to metastatic potential. Phosphatase and tensin homolog (PTEN), a tumor suppressor, is altered or mutated in 46–83% of Type 1 endometrial cancer specimens, resulting in the activation of PI3K/protein kinase B (Akt)/mTOR signaling [5,6]. Furthermore, mutations of Kirsten rat sarcoma viral oncogene homolog (K-Ras) (10–20%), and PIK3CA (25–36%), encoding for the catalytic subunit p110α of PI3K, are frequently observed in endometrial cancer [5,7,8]. These
findings have collectively resulted in the exploration of the PI3K/Akt/mTOR pathway as a potential therapeutic target in patients with advanced stage or recurrent endometrial cancer in an effort to improve survival and quality of life.

The PI3K/Akt/mTOR pathway

Activation of the PI3K/Akt/mTOR pathway begins with growth factor receptor tyrosine kinase ligand binding, resulting in activation of PI3K (Figure 1). The primary role of activated PI3K is to convert phosphatidylinositol-4,5-bis-phosphate to phosphatidylinositol-3,4,5-triphosphate [9]. Accumulation of phosphatidylinositol-3,4,5-triphosphate at the cell surface then results in phosphorylation and activation of Akt, a protein serine–threonine kinase. The tumor suppressor PTEN, when functionally active, can dephosphorylate phosphatidylinositol-3,4,5-triphosphate, reversing Akt activation and inhibiting further downstream signaling [10]. Furthermore, PTEN mediates agonist-induced apoptosis via up-regulation of apoptotic machinery and the down-regulation of anti-apoptotic proteins [11]. However, in the absence of PTEN inhibition, Akt phosphorylates and inhibits the tuberous sclerosis complex (TSC), leading to mTOR activation. Activated mTOR subsequently forms 2 different multiprotein complexes, mTOR complex 1 and mTOR complex 2, associated with the regulatory associated protein of mTOR (raptor) [10]. Ultimately, phosphorylation and activation of two separate downstream signaling molecules, eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) and ribosomal protein S6 kinase 1, promotes the translation of proteins involved in cell growth, angiogenesis, proliferation and survival [9,12–14].

Rapamycin and alternate rapalogs interfere with PI3K/Akt/mTOR signaling primarily via inhibition of the mTOR complex 1, preventing phosphorylation of 4E-BP1, S6 kinase 1, and other proteins leading to cell cycle arrest and decreased angiogenesis [10,15]. The propensity of PTEN mutations in endometrial cancer specimens and their subsequent aberrant activation of the PI3K/Akt/mTOR pathway provided the therapeutic rationale behind exploration of mTOR inhibitors (mTORi) as nonhormonal targeted therapies. Alternatively, PI3K and Akt specific inhibitors as well as dual PI3K/mTORi are currently being tested.

mTOR specific inhibitors in endometrial cancer

As detailed earlier, loss of PTEN protein function occurs in 46–83% of endometrial carcinomas leading to deregulation of the PI3K/AKT/mTOR signaling [16]. The clinical development of mTOR specific inhibitors in an effort to improve survival among patients with currently incurable endometrial cancer has been a priority over the last several years.

Four Phase II trials have investigated the use of single agent mTORi in the treatment of patients with recurrent endometrial cancer after failing prior therapy (Table 2). The earliest report was presented at the 2007 Annual Meeting of the American Society of Clinical Oncology [17]. A total of 45 patients with progressive endometrial cancer were studied in an open-label single arm design. Subjects were allowed up to two prior regimens of cytotoxic chemotherapy. Patients

Table 1. Molecular characteristics of endometrial cancer.

| Type I            | Type II           |
|-------------------|-------------------|
| ER/PR >90%        | 0–31%             |
| HER-2/neu (overexpression) | 3%       | >20%           |
| EGFR expression   | 46%               | 34%            |
| p53 mutation      | 5–10%             | 80–90%         |
| PTEN (loss of function) | 40–80% | 10–11%         |
| PIK3CA mutation   | 20–30%            | Rare           |
| p16 inactivation  | 10%               | 40%            |
| K-ras mutation (activation) | 13–26% | 0–10%          |
| E-cadherin (loss of expression) | 10–20% | 62–87%         |
| β-catenin (gain of function) | 25–38% | Rare           |

EGFR: Epidermal growth factor receptor; ER: Estrogen receptor; PR: Progesterone receptor.
received single agent AP23573 at a dose of 12.5 mg iv. every 5 days weekly, every other week for 28-day cycles (FIGURE 2D). The primary efficacy endpoint was clinical benefit response, defined as a complete or partial response or prolonged stable disease (defined as stable disease for at least 16 weeks) by modified RECIST guidelines [17]. Notably, 9 of 27 (33%) patients evaluable for response had clinical benefit response, including two partial responses. The most common adverse events reported included fatigue, anemia, mouth sores and nausea/vomiting [17].

The three remaining Phase II clinical trials enrolled a total of 224 evaluable subjects. The largest study that included 130 patients evaluated the activity of ridaforolimus in comparison to progestin or chemotherapy in 130 women with advanced endometrial cancer in an open-label, randomized Phase II trial [18]. An interim analysis of the first 114 patients treated demonstrated a median progression free survival (PFS) of 3.6 months for ridaforolimus compared to 1.9 months for progestin (hazard ratio [HR] = 0.53; one-sided p = 0.008) as assessed by independent radiology review. A stable disease rate of 35% was noted in the ridaforolimus arm [18].

As a result of the promising results achieved by ridaforolimus in preclinical and Phase I clinical trials involving different tumors, a prospective Phase III clinical trial, SUCCEED (Sarcoma Multi-Center Clinical Evaluation of the Efficacy of Ridaforolimus), was conducted to assess the effect of ridaforolimus on PFS and overall survival (OS) in patients with metastatic soft tissue or bone sarcomas [19]. Despite achieving its primary end point, a statistical improvement in PFS (17.7 weeks with study drug vs. 14.6 weeks with placebo; p = 0.0001), an OS advantage was not reached. Significant toxicities, including stomatitis (61%), infection (52%), fatigue (36%) and diarrhea (32%), were noted, with 6 (1.8%) treatment related deaths. Ultimately, after review of the risk-benefit profile, the drug was rebuked by both the US FDA and the EMA. Given the aforementioned developments, it is unlikely ridaforolimus will have a role in the treatment of advanced stage, metastatic or recurrent uterine cancer as a single agent. Additionally, identifying molecular markers predictive to response is doubtful, given the failure to do so in alternate disease sites.

Ridaforolimus and another mTORi, temsirolimus, were subsequently studied as single agents in chemotherapy-naïve and chemotherapy-treated patients with recurrent endometrial cancer (Table 2) [20,21]. Response rates ranged from 7–24% with a stable disease rate of 46–69%. A 7.3-month PFS was seen in the chemotherapy-naïve population treated with single agent temsirolimus in comparison to 3.2 months in subjects who received prior cytotoxic therapy [21]. Interestingly, the analysis of PTEN loss via immunohistochemistry and mutational analysis as well as molecular markers of the PI3K/Akt/mTOR pathway did not correlate with the clinical outcome or response. The most commonly reported toxicities included fatigue, pruritus, rash, mucositis, hematologic and metabolic abnormalities.

Temsirolimus is currently US FDA approved for the treatment of renal cell carcinoma. It is a derivative of sirolimus.
Importantly, temsirolimus was the first US FDA approved mTOR-targeted agent. The registration trial leading to approval of the drug in patients with advanced renal cell carcinoma was a Phase III study comparing temsirolimus monotherapy to IFN-α monotherapy and a combination of temsirolimus and IFN-α [22]. Patients who received temsirolimus alone had longer OS (HR for death, 0.73; 95% CI: 0.58–0.92; p = 0.008) and PFS (p <0.001) than did patients who received interferon alone. OS in the combination-therapy group did not differ significantly from that in the IFN-α alone group (HR, 0.96; 95% CI: 0.76–1.20; p = 0.70). Median OS times in the interferon group, the temsirolimus group and the combination-therapy group were 7.3, 10.9, and 8.4 months, respectively. Rash, peripheral edema, hyperglycemia and hyperlipidemia were more common in the temsirolimus group, whereas asthenia was more common in the interferon group. There were fewer patients with serious adverse events in the temsirolimus group than in the interferon group (p = 0.02) [22].

Currently, there are no Phase III clinical trials examining temsirolimus in endometrial cancer patients. If US FDA approval is requested for patients with advanced stage, metastatic or recurrent endometrial cancer, it is likely that investigators will have to demonstrate an analogous OS advantage. Additionally, if biologic markers predictive of response are identified, potential applications in the endometrial cancer arena may emerge. In patients with renal cell carcinoma, baseline expression of phosphorylated S6 or phosphorylated p70 S6 kinase was associated with objective response to temsirolimus in a small population of patients [23]. Certainly, sample expansion and validation in a larger prospective study is warranted.

**Combining mTOR specific inhibitors with hormonal therapy in endometrial cancer**

The use of hormonal therapy in the treatment of patients with endometrial cancer dates back nearly 40 years, following immunohistochemical data indicating that greater than 90% of well differentiated endometrioid type (Type 1) endometrial cancers express estrogen and progesterin receptors [24]. More recently, *in vitro* evidence has emerged indicating synergistic effects when mTORi are given concurrently with hormonal therapy [25,26]. It has been shown that PTEN loss and PI3K activating mutations may confer *in vitro* resistance to hormonal therapy [27]. In many models, this resistance has been overcome by the use of an mTORi. Additionally, there is reported crosstalk between hormone receptor signaling pathways and the PI3K/Akt pathway along several levels, with PI3K/Akt/mTOR activation resulting in tamoxifen resistance in breast cancer studies [25].

Given the theoretical benefits of combination therapy, two Phase II clinical trials were completed exploring the use of mTORi and hormonal therapy in patients with recurrent endometrial cancer (Table 3) [28,29]. Fleming *et al.* in Gynecologic Oncology Group (GOG) protocol 248 randomized patients with advanced, persistent or recurrent endometrial cancer to receive temsirolimus 25 mg iv, weekly or the combination of temsirolimus 25 mg iv, weekly plus megestrol acetate 80 mg bid for 3 weeks alternating with tamoxifen 20 mg bid for 3 weeks [27]. Unfortunately, the combination arm, which included both temsirolimus and hormonal therapy, was closed...
Table 3. Clinical studies with mTOR specific inhibitors in combination with hormonal agents or cytotoxic drugs in the treatment of endometrial cancer.

| Study (year) | N  | mTORi + agent | Prior therapy | RR (%) | SD (%) | PFS (months) | Common G ≥2 toxicities                                      | Ref. |
|-------------|----|----------------|---------------|--------|--------|--------------|----------------------------------------------------------|------|
| Fleming et al. (2014) | 22 | Temsirolimus 25 mg iv. weekly + megace 80 mg BID for 3 weeks alternating with tamoxifen 20 mg BID for 3 weeks | No prior hormonal therapy. One prior CT acceptable | NR    | NR     | Median 4.2 months | Fatigue, pneumonitis, edema, thrombocytopения, thromboembolic events | [28] |
| Slomovitz et al. (2010) | 28 | Everolimus 10 mg PO daily + letrozole 2.5 mg PO daily for 28-day cycles | Up to 2 prior CT allowed | 21%   | 14%    | NR           | Fatigue, nausea, stomatitis, hyperglycemia, | [29] |

Agent: Hormonal therapy; BID: Twice daily; CT: Chemotherapy; G: Grade; iv.: Intravenous; NR: Not reported; PFS: Progression free survival; PO: Orally; RR: Response rate; SD: Stable disease.

prematurely due to an unacceptable rate of venous thromboembolism (35% rate of DVT [n = 5] and nonfatal pulmonary embolus [n = 2]). Interim analysis indicated no difference in response rate between the two arms.

Analogously, the combination of another mTORi, everolimus and letrozole was investigated in an open-label, single-arm Phase II study enrolling patients with recurrent endometrial cancer who failed ≤2 prior chemotherapy regimens [29]. Everolimus was administered at a dose of 10 mg p.o. daily in combination with letrozole at a dose of 2.5 mg daily for 28-day cycles. A total of 28 patients were enrolled, with a confirmed objective response rate of 21%. The most common drug related toxicities were fatigue (50%), nausea (45%), stomatitis (45%), hypertriglyceridemia (27%) and hyperglycemia (27%).

Currently, everolimus (in combination with exemestane) is indicated for the treatment of postmenopausal women with advanced hormone receptor-positive, HER2-negative breast cancer after failure of treatment with letrozole or anastrozole. It is additionally approved for the treatment of adult patients with progressive neuroendocrine tumors of pancreatic origin with unresectable, locally advanced or metastatic disease, as well as for the treatment of adult patients with advanced renal cell carcinoma (RCC) after failure of treatment with sunitinib or sorafenib. It is also approved for the treatment of adult patients with renal angiomylipoma and TSC, not requiring immediate surgery as well as pediatric and adult patients with subependymal giant cell astrocytoma that requires therapeutic intervention but cannot be curatively resected [25,30–32].

Three prospective, double-blind, randomized registration trials in breast, pancreatic and RCC ultimately led to US FDA approval of everolimus for the aforementioned indications. All trials permitted cross-over and unblinding in the event that the therapeutic arm showed a survival advantage. Importantly, all three trials showed a significant improvement in PFS, with impressive HRs ranging from 0.33–0.45, without an OS advantage.

To date, no Phase III trial has been conducted investigating everolimus use in recurrent endometrial cancer patients, although with limited therapeutic options in this setting, thought should be given toward additional exploration of this agent.

Combining mTOR specific inhibitors with cytotoxic chemotherapy and/or anti-angiogenesis therapy

Recurrent, metastatic endometrial cancer is traditionally managed with systemic cytotoxic chemotherapy regimens or hormonal agents as detailed above. Unfortunately, response rates are poor as both toxicity and drug resistance limit the benefits derived from chemotherapy [33]. Importantly, however, preclinical evidence has suggested that the use of both mTORi, which are primarily cytostatic, and cytotoxic agents may exhibit synergistic anticancer effects [33]. In vitro studies, using endometrial cancer cell lines, demonstrated that the mTORi rapamycin potentiated the effects of paclitaxel and cisplatin through inhibition of cellular proliferation and induction of apoptosis [34–36].

In a Phase I clinical trial, Temkin et al. evaluated weekly temsirolimus and topotecan in the treatment of advanced and/or recurrent gynecologic cancer [33]. Six of the 15 (67%) enrolled subjects had recurrent uterine cancer. The regimen was not tolerable in women with a history of radiation due to hematologic toxicity. However, in those patients who were radiation naïve, a Phase II dose of 25 mg of temsirolimus with 1 mg/m² of topotecan given weekly, was recommended. A total of 9 of 11 patients (81.8%) enrolled in the trial were found to have stable disease at time of first evaluation.

Furthermore, the combination of carboplatin, paclitaxel and temsirolimus was studied in patients with advanced solid tumors in a dose escalation Phase I clinical trial [37]. Carboplatin, paclitaxel on day 1 with temsirolimus on day 1 and 8 was well tolerated, with 82% of the endometrial cancer patients exhibiting an objective partial response. More recently, the
targeted anti-angiogenic agent bevacizumab has been studied as a single agent in patients with recurrent or metastatic endometrial cancer [38]. Fifty-six patients were enrolled in the trial, of whom 52 were evaluable. Seven patients (13.5%) experienced clinical responses, with 40.4% surviving progression free for at least 6 months. Importantly, mTOR inhibition has also been shown to have anti-angiogenic effects in vitro, with temsirolimus exhibiting hypoxia inducible factor-1α inhibitory properties and decreasing VEGF expression, resulting in decreased angiogenesis [15,39].

A Phase I trial of liposomal doxorubicin, bevacizumab and temsirolimus in patients with advanced gynecologic and breast malignancies showed the regimen to be well tolerated with manageable toxicities. A 19% partial response rate was seen for all enrolled subjects [39]. Furthermore, GOG protocol 229G explored the combination temsirolimus and bevacizumab in a Phase II clinical trial of patients with recurrent or persistent endometrial cancer. Preliminary results were recently presented at the 2012 Annual Meeting of the Society of Gynecologic Oncology, with clinical response in 24.5% of patients and 47% surviving progression free for at least 6 months. The median OS was nearly 17 months in this pretreated population.

More recently, GOG protocol 86P was developed as a randomized Phase II study exploring the efficacy of cytotoxic chemotherapy in combination with either anti-angiogenic therapy or the mTORi temsirolimus. The study arms included: carboplatin + paclitaxel + bevacizumab with bevacizumab maintenance therapy; carboplatin + paclitaxel + temsirolimus with temsirolimus maintenance and ixabepilone + carboplatin + bevacizumab with bevacizumab maintenance. The primary objective of the study is to evaluate the hazard of progression or death in each of the 3 arms relative to historical controls. Patients with stage 3 or 4A (measurable disease required) as well as those with stage 4B or recurrent endometrial cancer (measurable or not) were allowed to enroll in the study. Importantly, patients were excluded if they received prior chemotherapy or targeted therapy and were required to have a GOG performance status of 0–2. The study recently closed to accrual and we await maturation of the results.

### PI3K specific inhibitors in endometrial cancer

In an analogous manner to other protein kinase inhibitors, the existing PI3K inhibitors bind competitively in the ATP-binding pocket of the catalytic domain [40]. This molecular strategy has enabled the development of both nonspecific and isoform-specific PI3K inhibitors [40].

Several PI3K specific inhibitors are in various stages of clinical development and therapeutic testing (Table 4). At the 2011 ASCO Annual Meeting, antitumor activity in a panel of preclinical models and in patients with advanced solid tumors in a Phase I dose escalation study was reported for BAY 80-6946 (copanlisib), a potent and highly selective reversible pan-class I PI3K inhibitor (FIGURE 2A) [41]. A total of 17 nondiabetic patients received BAY 80-6946 over 1 h iv. infusions at doses of 0.1 mg/kg (1), 0.2 mg/kg (3), 0.4 mg/kg (3), 0.8 mg/kg (8) and 1.2 mg/kg (2). Of the 17 subjects, 2 (11.8%) had endometrial cancer. One of the two subjects with endometrial cancer exhibited stable disease for 8 months while on treatment [41].

A subsequent presentation at the 2012 ASCO Annual Meeting evaluated BAY 80-6946 (BAY) in an expansion cohort of 23 nondiabetic patients with solid tumors [42]. Of the 23 subjects, 3 (13%) had endometrial cancer. Outcome results were not reported. Common grade 2–3 adverse events included hyperglycemia requiring insulin therapy, hypertension, and interstitial pneumonitis.

In addition to the above agents, other PI3K specific inhibitors are currently being investigated including BKM120 and XL147 (FIGURES 2B & 2E).

### Dual PI3K & mTORi in endometrial cancer

In an effort to improve therapeutic efficacy and avoid acquired resistance, dual PI3K/mTORi have been investigated in both the preclinical and clinical setting. In both in vitro and in vivo ovarian and prostate cancer models, the combination exhibited more activity than either single agent alone [43]. Furthermore, at the molecular level, combined mTOR inhibition prevented the rebound activation of Akt that is seen after treatment with rapamycin and its analogs and caused more sustained inhibition of Akt phosphorylation [43].

---

**Table 4. Clinical studies with PI3K-specific inhibitors as monotherapy for the treatment of endometrial cancer.**

| Study (year) | N | PI3Ki | Prior therapy | RR (%) | SD (%) | PFS (months) | Common G ≥2 toxicities | Ref. |
|--------------|---|-------|---------------|--------|--------|-------------|------------------------|-----|
| Patnaik et al. (2011) | 17 | BAY 80-6946 | NR | NR | 1 of the 2 subjects with EC exhibited SD for 8 months | NR | Hyperglycemia; fatigue; nausea; vomiting; alopecia; diarrhea; mucositis; anemia | [41] |
| Lotze et al. (2012) | 23 | BAY 80-6946 | NR | NR | NR | NR | Hyperglycemia; HTN; pneumonitis | [42] |

EC: Endometrial cancer; G: Grade; HTN: Hypertension; NR: Not reported; PFS: Progression free survival; PI3Ki: Phosphatidylinositol-3 kinase inhibitor; RR: Response rate; SD: Stable disease.
Currently, several Phase I and II clinical trials are exploring dual PI3K/mTORi for use in patients with advanced or recurrent endometrial carcinoma (Table 5).

Akt-specific inhibitors in endometrial cancer

As a downstream effector of the PI3K signaling cascade, Akt plays an essential role in tumor cell survival, proliferation and inhibition of apoptosis. Both in vitro and in vivo assays have illustrated a synergistic cytotoxic effect when the Akt inhibitor MK2206 was used in combination with epidermal growth factor receptor inhibitors as well as the cytotoxic agents doxorubicin, carboplatin, 5-FU, gemcitabine and docetaxel [44]. Exploration of Akt specific inhibitors in endometrial cancer is limited to an ongoing Phase II, two-arm PIK3CA mutation stratified clinical trial of MK-2206 in recurrent or advanced endometrial cancer [45].

Toxicities associated with PI3K, mTOR & AKT inhibitors in endometrial cancer

As with all novel therapies, the potential benefits of these agents must be weighed against their side effects. Recently completed Phase III clinical trials of mTORi in patients with RCC and hormone receptor positive advanced breast cancer confirmed that the side-effect profile associated with this class of agents is overall tolerable and manageable [10,25,46]. Primarily, metabolic abnormalities, including hyperglycemia and hyperlipidemia can be expected in patients treated with mTORi. Importantly, patients with endometrial cancers commonly harbor additional cardiovascular risk factors such as hypercholesterolemia, diabetes and hypertension, enhancing the clinical implications of the previously listed toxicities [47–49]. More commonly, however, patients treated with this class of agents experience thrombocytopenia, anemia, asthenia, diarrhea, rash and fever [50].

One of the more serious drug-class-specific toxicities encountered in Phase II and III clinical trials is interstitial pneumonitis [51,52]. However, recent data indicate that only a small fraction of patients (10%) will present with symptoms or require intervention due to pneumonitis while on therapy [53,54]. The majority of patients will experience symptom resolution with dose delays, dose reductions or administration of systemic steroids. For unclear reasons, everolimus and ridaforolimus carry a lower risk of pneumonitis when compared to temsirolimus.

The evolving role of metformin in endometrial cancer therapeutics

Metformin belongs to the biguanide class of drugs and has been long used for the treatment of Type 2 diabetes. Recently, interest in the anticancer properties of metformin has emerged as epidemiologic studies in patients with breast and prostate cancer have shown a survival advantage in patients taking this medication [55–58]. Additionally, patients with endometrial cancer who are obese or have diabetes have been shown to have compromised survival and increased recurrence rates [59]. This adverse oncologic outcome associated with metabolic syndrome in patients with endometrial cancer may be a direct result of the metabolic and endocrine effects of obesity.

Preclinical studies conducted on breast, prostate, lung and endometrial cancer cell lines have shown metformin to be a potent inhibitor of cell proliferation and to potentiate the effects of cytotoxic chemotherapeutic agents. On a molecular level, metformin functions as both an insulin sensitizer and also inhibits hepatic gluconeogenesis. Specifically, metformin inhibits complex 1 activity in the mitochondria, leading to activation of the downstream target AMP-activated protein kinase (AMPK), which regulates cellular pathways controlling proliferation, including the mTOR pathway. In the presence of metformin, AMPK is phosphorylated and activated by liver kinase B1. This subsequently results in inhibition of mTOR related signaling. In elegant cell line studies, metformin mediated AMPK activation has been shown to inhibit cellular proliferation via mTOR inhibition and decrease in phosphorylation of the downstream target S6 [60]. AMPK independent mechanism, including inhibition of the regulator complex and regulated in development and DNA damage responses 1 upregulation, may also explain mTOR pathway inhibition.

Given the above, incorporation of metformin in endometrial cancer therapeutics is under active investigation. GOG protocol 286B is a prospective randomized Phase II/III study examining the impact of adding metformin to cytotoxic chemotherapy on

Table 5. Clinical trials with dual PI3K/mTOR inhibitors in the treatment of endometrial cancer.

| Manufacturer | Drug       | Trial design                  | Eligibility                                      | Ref. |
|--------------|------------|-------------------------------|-------------------------------------------------|------|
| Genentech    | GDC-0980   | Single arm open label         | Recurrent or persistent EC                       | [71] |
| Pfizer       | PF-04691502 PF-05212384 | Randomized Phase II non-comparative study | Recurrent or persistent EC (failed ≥1 prior platinum containing CT) | [72] |
| Daiichi Sankyo Inc. | DS-7423 | Phase I open label study      | Advanced solid tumor refractory to other treatment | [73] |
| Novartis     | BEZ235     | Phase I study                 | Advanced solid tumor refractory to other treatment | [74] |

CT: Chemotherapy; EC: Endometrial cancer.
PFS and OS in patients with measurable, advanced stage, persistent or recurrent endometrial cancer [61]. The study arms include: carboplatin + paclitaxel + metformin with maintenance metformin and carboplatin + paclitaxel + placebo with maintenance placebo. Notable translational objectives include assessment of the correlation between various metabolic factors (body mass index, diabetes status, fasting insulin and glucose levels) and response, as well as the contribution of metformin transporter expression on treatment outcomes. Given the tremendous cost savings of metformin (~$1 per day) and favorable toxicity profile, the results of this study may be practice changing.

Metformin has also been studied in combination with traditional mTORi as described earlier. In a Phase I clinical trial, patients with advanced stage solid tumors who had exhausted standard treatment options were treated with weekly intravenous temsirolimus and daily oral metformin [62]. Eleven patients were enrolled. Dose-limiting toxicities were observed in all patients at the initial dose level of 25 mg weekly of temsirolimus and 500 mg metformin orally twice daily. Toxicities included grade 4 pneumonitis, persistent grade 3 fatigue and thrombocytopenia requiring dose delays. The maximum tolerated dose was 20 mg temsirolimus weekly and 500 mg orally daily of metformin. One patient with head and neck cancer experienced a partial response. Five patients had stable disease including a patient with melanoma who had stable disease for 22 months.

Cervical cancer & mTOR signaling

Cervical cancer is caused by specific subtypes of the HPV that express viral oncogenes, E6 and E7. Oncogenic HPV E6 also causes rapid degradation of TSC, resulting in TORC1 activation and downstream mTOR signaling [57]. HeLa cells have been shown to be defective in the tumor suppressor liver kinase B1, which inhibits mTOR via TSC2 stimulation. mTOR pathway activation has been observed in cervical cancer cell lines and in an immunodeficient mouse xenograft model. Preclinical efficacy of mTOR inhibition by rapamycin and RAD001 was evident by virtue of decreased mTOR activity in vivo and a significant decrease in xenograft tumor burden [63].

In addition to the impact of oncogenic HPV infection on mTOR signaling, a relatively high prevalence of PIK3CA mutations have been found in cervical cancer, further suggesting a role for mTORi in this disease. In a Phase I study of 15 patients found to have PIK3CA mutations in their cervical cancers, 5 were treated with agents targeting the PI3K/AKT/mTOR pathway, of whom 2 had a partial response [64]. In another Phase I study, which included two women with advanced/recurrent squamous cell carcinoma of the cervix, patients were treated with weekly temsirolimus (25 mg on days 1, 8, 15, 22) and topotecan (1 mg/m² on days 1, 8, 15), and one of the women with cervical cancer experienced stable disease for 3 months. This combined regimen was not tolerated in patients who had previously received pelvic radiation therapy [65].

Finally, single agent temsirolimus (25 mg iv. weekly every 28 days) was studied in a Phase II trial of 33 women with advanced stage cervical cancer. One patient experienced a partial response (3.0%) and 19 patients had stable disease (57.6%) with a median duration of response of 6.5 months (range, 2.4–12.0 months) [66]. Six-month PFS was 28% (95% CI: 14–43%) and the median PFS was 3.52 months (95% CI: 1.81–4.7) [66]. No grade 4–5 adverse events were observed.

Clear cell carcinoma of the ovary & mTOR inhibition

Clear cell carcinoma accounts for 1–12% of epithelial ovarian carcinomas in the USA. Unfortunately, advanced-stage clear cell carcinomas do not respond as well to platinum-based chemotherapy as do the more common high-grade serous tumors. In a 2010 meta-analysis of 8000 patients on seven international front-line trials using platinum-based regimens, the median OS of the 221 women with stage III/IV clear cell carcinoma was 21.3 months versus 40.8 months for women with serous carcinomas [67]. The results are worse in the setting of recurrent disease where it does not seem to matter whether the disease is platinum sensitive or platinum resistant using conventional definitions.

Compared to serous tumors, clear cell tumors have low levels of chromosomal instability and a high frequency (up to 40%) of PIK3CA mutations [68]. Histologically, clear cell carcinoma of the ovary and RCC are similar. In addition, both neoplasms share many gene expression profiles as detailed by prior investigators [69,68]. This suggests that therapies active in one histologic subtype may be active in another tumor of similar histology independent of organ or origin. Examples of this paradigm can be seen in the activity of etoposide plus cisplatin for neuroendocrine tumors of the lung and the cervix and in the activity of various chemotherapy triplets ( vincristine, actinomycin-d, cyclophosphamide, cisplatin, vinblastine, bleomycin, and bleomycin, etoposide, cisplatin) in malignant germ cell tumors of the testis and ovary.

As mTORi have activity in advanced RCC and due to the relatively high prevalence of PIK3CA mutations in clear cell ovarian carcinoma, the GOG has launched a frontline single-arm Phase II trial (GOG protocol 268) to evaluate the combination of carboplatin, paclitaxel, and the mTORi, temsirolimus, followed by consolidation temsirolimus for stage III/IV clear cell ovarian cancer [70].

Summary

The PI3K/Akt/mTOR pathway is being targeted as a novel treatment strategy in patients with advanced or recurrent endometrial cancer. However, despite evidence of meaningful clinical response, appropriate dose, duration and timing of therapy remain unclear and represent areas of active clinical investigation. In addition, a role for mTORi in both cervical cancer and clear cell cancer of the ovary is gradually emerging.

Analogous to alternate targeted therapeutic strategies, preliminary results related to oncologic outcomes using mTORi in endometrial cancer patients were not as promising as anticipated. This is likely multifactorial, and may be related to crosstalk and negative feedback loops across parallel signaling pathways. This hypothesis has catalyzed investigation of combination regimens, as reviewed previously, with progressive interest in...
PI3K/Akt/mTOR inhibition in conjunction with traditional chemotherapeutics, hormonal drugs and anti-angiogenic agents. This principle of combination therapy may help circumvent the primary challenge posed by parallel signaling.

In addition to the aforementioned, our understanding of predictors of response continues to evolve as we develop drugs targeting the cellular PI3K/Akt/mTOR mechanism. It is unclear if activating mutations in PIK3CA and/or loss of PTEN activity is more predictive of response. Conversely, the contribution of KRAS activating mutations to resistance remains an area of active investigation. Furthermore, the role of PI3K as a critical regulator of glucose uptake and metabolism is becoming progressively more evident, with potential implications in a patient population that is commonly obese, with significant metabolic derangements.

Finally, identifying an appropriate drug dose is likely more complex with pathway specific targeting agents. Unlike traditional cytotoxic drugs, the pharmacologic dose (maximum tolerable dose on clinical studies) may not equate to the biologically effective/optimal dose. Moving forward, the incorporation of pharmacodynamic studies as translational end points in mTORi clinical trials is critical and may help confirm or refute pathway inhibition, informing treatment response.

Expert commentary

With advancements in pharmacologic drug discovery, additional PI3K/Akt/mTOR pathway inhibitors will be identified and incorporated into clinical research for women struggling with gynecologic malignancies. Furthermore, novel therapeutic drug combinations, joining targeted pathway inhibitors, cytotoxic chemotherapeutic agents and hormonal agents may emerge. Many of these agents have been described earlier.

Moreover, the active investigation of metformin as a cost–effective mTOR inhibitor is ongoing and may lead to promising advances in this patient population.

Five-year view

As our understanding of cancer genomics improves, the ultimate goal will be to develop a more personalized approach to cancer treatment, identifying targeted agents (PI3K/Akt/mTOR pathway inhibitors) that are more likely to provide a meaningful response or cure based on patient/cancer gene signatures and cancer-induced protein expression. The traditional paradigm in the clinical trials arena has been to study large populations with a single tumor type that may or may not express a relevant target for the drug under investigation. We predict that within 5 years’ time, a new platform for studying novel therapies will emerge through which enriched populations representing multiple tumor types, all expressing the same predictive biomarker (e.g., aberrant mTOR signaling in endometrial, cervical and clear cell ovarian cancer), can be enrolled onto a single Phase III randomized clinical trial with clinical and translational endpoints.

Financial & competing interests disclosure

This article was supported by an institutional NIH T-32 training grant (Ruth L Kirschstein, NRSA Institutional Training Research Grant, 2T32 CA-060396-11). The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Endometrial cancer is the most common gynecologic malignancy for which there are no US FDA approved nonhormonal biologic targeted agents.
- The tumor suppressor gene, phosphatase and tensin homolog (PTEN), is altered or mutated in 46–83% of endometrial cancer.
- PTEN mutations lead to aberrant signaling of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/protein kinase B (Akt)/mTOR pathway.
- mTOR inhibitors (mTORi) have demonstrated promising clinical activity in patients with advanced or recurrent endometrial cancer in Phase II trials.
- Combining mTORi with hormonal therapy or with cytotoxic and anti-angiogenic drugs may represent a therapeutic strategy in advanced and recurrent endometrial cancer.
- PI3K-specific inhibitors and Akt-specific inhibitors have also been developed and studies are underway combining these agents with mTORi in patients with advanced or recurrent endometrial cancer.
- Patients treated with mTORi may develop hyperglycemia and hyperlipidemia, and may also experience thrombocytopenia, anemia, asthenia, diarrhea, rash and fever.
- The diabetes drug metformin also inhibits mTOR signaling and is being studied by the Gynecologic Oncology Group in protocol 286B, a randomized Phase III clinical trial combining metformin with chemotherapy in women with advanced or recurrent endometrial cancer.
- The PI3K/Akt/mTOR pathway is an important pathway in some cases of cervical carcinoma.
- Shared histology may govern response to specific therapeutics, suggesting that clear cell ovarian cancer may respond to mTORi just as renal cell carcinomas do.
References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014;64:9-29
2. Obel JC, Friberg G, Fleming GF. Chemotherapy in endometrial cancer. Clin Adv Hematol Oncol 2006;4:456-68
3. Dellinger TH, Monk BJ. Systemic therapy for recurrent endometrial cancer: a review of North American trials. Expert Rev Anticancer Ther 2009;9:905-16
4. Aghajanian C, Sill MW, Darcy KM, et al. Phase II trial of bevacizumab in recurrent or persistent endometrial cancer: a Gynecologic Oncology Group study. J Clin Oncol 2009;27:2259-65
5. Kong D, Suzuki A, Zou TT, et al. Genotype-dependent efficacy of a dual PI3K/mTOR inhibitor, NVP-BEZ235, and an mTOR inhibitor, RAD001, in endometrial carcinomas. PLoS One 2012;7:e37431
6. Mountzios G, Pectasides D, Bournakis E, et al. Developments in the systemic treatment of endometrial cancer. Crit Rev Oncol Hematol 2011;79:278-92
7. Shoji K, Oda K, Kashiyama T, et al. PTEN1 is frequently mutated in primary endometrial carcinomas. Nat Genet 1997;17:143-4
8. Mourtzios G, Pectasides D, Bournakis E, et al. Developments in the systemic treatment of endometrial cancer. Crit Rev Oncol Hematol 2011;79:278-92
9. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2002;2:489-501
10. Diaz-Padilla I, Duran I, Clarke BA, Oza AM. Biologic rationale and clinical activity of mTOR inhibitors in gynecological cancer. Cancer Treat Rev 2012;38:767-75
11. Wang LE, Ma H, Hale KS, et al. Roles of genetic variants in the PI3K and RAS/RAF pathways in susceptibility to endometrial cancer and clinical outcomes. J Cancer Res Clin Oncol 2012;138:377-85
12. Lee DF, Hung MC. All roads lead to mTOR: integrating inflammation and tumor angiogenesis. Cell Cycle 2007;6:3011-14
13. Liu D, Hou P, Liu Z, et al. Genetic alterations in the phosphoinositide 3-kinase/akt signaling pathway confer sensitivity of thyroid cancer cells to therapeutic targeting of Akt and mammalian target of rapamycin. Cancer Res 2009;69:7311-19
14. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. Nature reviews Drug discovery 2009;8:627-44
15. Guba M, von Breitenbuch P, Steinbauer M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med 2002;8:128-35
16. Tewari KS, Monk BJ. American Society of Clinical Oncology 2011 Annual Meeting update: summary of selected gynecologic cancer abstracts. Gynecol Oncol 2011;122:209-12
17. Colombo N, McMeekin S, Schwartz P, et al. A phase II trial of the mTOR inhibitor AP23573 as a single agent in advanced endometrial cancer. J Clin Oncol 2007;25:5516
18. Oza AM, Poveda A, Clamp AR, et al. A randomized phase II (RP2) trial of ridaforolimus (R) compared with progestin (P) or chemotherapy (C) in female adult patients with advanced endometrial carcinoma. J Clin Oncol 2011; 29(Suppl; abstr 5009)
19. Demetri GD, Chawla SP, Ray-Coquard I, et al. Results of an international randomized phase III trial of the mammalian target of rapamycin inhibitor ridaforolimus versus placebo to control metastatic sarcomas in patients after benefit from prior chemotherapy. J Clin Oncol 2013;31:2485-92
20. Mackay H, Welch S, Tsao MS, et al. Phase II study of oral ridaforolimus in patients with metastatic and/or locally advanced recurrent endometrial cancer: NCIC CTG IND 192. J Clin Oncol 2011; 29(Suppl; abstr 5013)
21. Oza AM, Eilin L, Tsao MS, et al. Phase II study of temsirolimus in women with recurrent or metastatic endometrial cancer: a trial of the NCIC Clinical Trials Group. J Clin Oncol 2011;29:3278-85
22. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 2007;356:2271-81
23. Rini BI. Temsirolimus, an inhibitor of mammalian target of rapamycin. Clin Cancer Res 2008;14:1286-90
24. Thigpen JT, Brady MF, Alvarez RD, et al. Oral medroxyprogesterone acetate in the treatment of advanced or recurrent endometrial carcinoma: a dose-response study by the Gynecologic Oncology Group. J Clin Oncol 1999;17:1736-44
25. Basela J, Campone M, Piccart M, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 2012;366:520-9
26. Beeram M, Tan QT, Tekmal RR, et al. Akt-induced endocrine therapy resistance is reversed by inhibition of mTOR signaling. Ann Oncol 2007;18:1323-8
27. Fleming GF, Filiaci VL, Hanjani P, et al. Gynecologic Oncology Group. Hormone therapy plus temsirolimus for endometrial carcinoma (EC): gynecologic oncology group trial #248. J Clin Oncol 2011;29
28. Fleming GF, Filiaci VL, Marzullo B, et al. Temsirolimus with or without megestrol acetate and tamoxifen for endometrial cancer: a gynecologic oncology group study. Gynecol Oncol 2014;132:585-92
29. Slomovitz BM, Lu KH, Johnston T, et al. A phase 2 study of the oral mammalian target of rapamycin inhibitor, everolimus, in patients with recurrent endometrial carcinoma. Cancer 2010;116:5415-19
30. Yao JC, Shah MH, Ito T, et al. Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med 2011;364:514-23
31. Krueger DA, Care MM, Holland K, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. N Engl J Med 2010;363:1801-11
32. Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 2008;372:449-56
33. Temkin SM, Yamada SD, Fleming GF. A phase I study of weekly temsirolimus and topotecan in the treatment of advanced and/or recurrent gynecologic malignancies. Gynecol Oncol 2010;117:473-6
34. Shafer A, Zhou C, Gehrig PA, et al. Rapamycin potentiates the effects of paclitaxel in endometrial cancer cells through inhibition of cell proliferation and induction of apoptosis. Int J Cancer 2010;126:1144-54
35. Campone M, Levy V, Bourbouloux E, et al. Safety and pharmacokinetics of paclitaxel and the oral mTOR inhibitor everolimus in advanced solid tumours. Br J Cancer 2009;100:315-21
36. Bae-Jump VL, Zhou C, Bossijs JJF, Gehrig PA. Synergistic effect of rapamycin and cisplatin in endometrial cancer cells. Cancer 2009;115:3887-96
37. Kollmannsberger C, Hitre H, Siu LL, et al. Temsirolimus in combination with carboplatin and paclitaxel in patients with advanced solid tumors: a NCIC-CTG, phase I, open-label dose-escalation study (IND 179). Ann Oncol 2012;23:238-44

38. Aghajanian C, Sill MW, Darcy KM, et al. Phase II trial of bevacizumab in recurrent or persistent endometrial cancer: A Gynecologic Oncology Group study. J Clin Oncol 2011;29:2259-65

39. Moroney JW, Schlumberger MP, Helgason T, et al. A phase I trial of liposomal doxorubicin, bevacizumab, and temsirolimus in patients with advanced gynecologic and breast malignancies. Clin Cancer Res 2011;17:6840-6

40. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. Oncogene 2008;27:5497-510

41. Parnai A, Appleman LJ, Mount JM, et al. A first-in-human phase I study of intravenous PI3K inhibitor BAY 80-6946 in patients with advanced solid tumors: results of dose-escalation phase. J Clin Oncol 2011;29(Supp; abstr 30S5)

42. Lotze MT, Appleman LJ, Ramanathan RK, et al. Phase I study of intravenous PI3K inhibitor BAY 80-6946: Activity in patients (pts) with advanced solid tumors and non-Hodgkin lymphoma treated in MTD expansion cohorts. J Clin Oncol 2012;30(Supp; abstr 3019)

43. Mazzuolletti M, Bortolin F, Brunelli L, et al. Combination of PI3K/mTOR inhibitors: antitumor activity and molecular correlates. Cancer Res 2011;71:4573-84

44. Hirai H, Sootome H, Nakatsuru Y, et al. Identification of PI3KCA mutations in women with recurrent, unresectable, locally advanced or metastatic carcinoma of the cervix. A trial of the NCIC Clinical Trials Group (NCIC CTG IND 199). Gynecol Oncol 2013;128:265-74

45. Akt inhibitor mk2206 in treating patients with recurrent or advanced endometrial cancer. Available from: https://clinicaltrials.gov/ct2/results?term=NCT01307631&Search=Search

46. Brugarolas J. Renal-cell carcinoma–mTOR pathway-directed therapy in gynecologic cancers

47. Friedenreich CM, Biel RK, Lau DC, et al. Case-control study of the metabolic syndrome and metabolic risk factors for endometrial cancer. Cancer Epidemiol Biomarkers Prev 2011;20:2384-95

48. Friberg E, Osinski N, Mantzoros CS, Wolk A. Diabetes mellitus and risk of endometrial cancer: a meta-analysis. Diabetologia 2007;50:1365-74

49. Friberg E, Mantzoros CS, Wolk A. Diabetes and risk of endometrial cancer: a population-based prospective cohort study. Cancer Epidemiol Biomarkers Prev 2007;16:276-80

50. Hess G, Herbrecht R, Romagueria J, et al. Phase III study to evaluate temsirolimus compared with investigator’s choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. J Clin Oncol 2009;27:3822-9

51. Rodriguez-Pascual J, Cheng E, Maroto P, Duran I. Emergent toxicities associated with the use of mTOR inhibitors in patients with advanced renal carcinoma. Anticancer Drugs 2010;21:478-86

52. Bellmunt J, Sczylrik C, Feingold J, et al. Temsirolimus safety profile and management of toxic effects in patients with advanced renal cell carcinoma and poor prognostic features. Ann Oncol 2008;19;1387-92

53. Maroto JP, Hudes G, Dutcher JP, et al. Drug-related pneumonitis in patients with advanced renal cell carcinoma treated with temsirolimus. J Clin Oncol 2011;29:1750-6

54. Duran I, Siu LL, Oza AM, et al. Characterisation of the lung toxicity of the cell cycle inhibitor temsirolimus. Eur J Cancer 2006;42:1875-80

55. Poole CD, Halcox JP, Jenkins-Jones S, et al. Omega-3 fatty acids and mortality outcome in patients with and without type 2 diabetes after myocardial infarction: a retrospective, matched-cohort study. Clin Ther 2013;35:40-51

56. Currie CJ, Poole CD, Jenkins-Jones S, et al. Mortality after incident cancer in people with and without type 2 diabetes: impact of metformin on survival. Diabetes Care 2012;35:299-304

57. Libby G, Donnelly LA, Donnan PT, et al. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. Diabetes Care 2009;32:1620-5

58. Evans JI, Donnelly LA, Emslie-Smith AM, et al. Metformin and reduced risk of cancer in diabetic patients. BMJ 2005;330:1304-5

59. Chia WM, Newcomb PA, Tremblay-Dietz A, Hampton JM. Obesity, diabetes, and other factors in relation to survival after endometrial cancer diagnosis. Int J Gynecol Cancer 2007;17:441-6

60. Cantrell LA, Zhu C, Mendivil A, et al. Metformin is a potent inhibitor of endometrial cancer cell proliferation–implications for a novel treatment strategy. Gynecol Oncol 2010;116:92-8

61. Paclitaxel and carboplatin with or without metformin hydrochloride in treating patients with stage III, IV, or recurrent endometrial cancer. Available from: https://clinicaltrials.gov/ct2/results?term=NCT02065687&Search=Search

62. MacKenzie MJ, Ernst S, Johnson C, Winquist E. A phase I study of temsirolimus and metformin in advanced solid tumours. Invest New Drugs 2012;30:647-52

63. Molinolo AA, Marsh C, El Diniai M, et al. mTOR as a molecular target in HPV-associated oral and cervical squamous carcinomas. Clin Cancer Res 2012;18:2558-68

64. Janku F, Wheler JJ, Westin SN, et al. PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations. J Clin Oncol 2012;30:777-82

65. Temkin SM, Yamada SD, Fleming GF. A phase I study of weekly temsirolimus and topotecan in the treatment of advanced and/or recurrent gynecologic malignancies. Gynecol Oncol 2010;117:473-6

66. Tinkler AV, Ellard S, Welch S, et al. Phase II study of temsirolimus (CCI-779) in women with recurrent, unresectable, locally advanced or metastatic carcinoma of the cervix. A trial of the NCIC Clinical Trials Group (NCIC CTG IND 199). Gynecol Oncol 2013;128:265-74

67. Yan D, Kaye S. Ovarian clear cell adenocarcinoma: a continuing enigma. J Clin Pathol 2007;60:355-60

68. Zorn KK, Bonome T, Gangi L, et al. Gene expression profiles of serous, endometrioid, and clear cell subtypes of ovarian and endometrial carcinomas. Clin Cancer Res 2005;11:6422-30

69. Mackay HJ, Brady MF, Oza AM, et al. Prognostic relevance of uncommon ovarian histology in women with stage III/IV epithelial ovarian cancer. Int J Gynecol Cancer 2010;20:945-52

70. Temsirolimus, carboplatin, and paclitaxel as first-line therapy in treating patients with newly diagnosed stage iii-iv clear cell ovarian cancer. Available from: https://clinicaltrials.gov/ct2/results?term=NCT01307631&Search=Search
71. A study of GDC-0980 in the treatment of recurrent or persistent endometrial carcinoma. Available from: https://clinicaltrials.gov/ct2/results?term=NCT01196429&Search=Search

72. A study of two dual PI3K/mTOR inhibitors, PF-04691502 and PF-05212384 In patients with recurrent endometrial cancer. Available from: https://clinicaltrials.gov/ct2/results?term=NCT01420081&Search=Search

73. Safety and tolerability of DS-7423 in subjects with advanced solid malignant tumors. Available from: https://clinicaltrials.gov/ct2/results?term=NCT01364844&Search=Search

74. A study of BEZ235 in adult japanese patients with advanced solid tumors. Available from: https://clinicaltrials.gov/ct2/results?term=NCT01195376&Search=Search