Chapter 13

The PI3K/Akt/mTOR Pathway in Ovarian Cancer: Biological Rationale and Therapeutic Opportunities

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Additional information is available at the end of the chapter

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1. Introduction

Ovarian cancer is the most lethal cause of gynecological cancer deaths in the developing world and typically presents at an advanced stage when optimal debulking and platinum based-chemotherapy remain the cornerstone of management. Unfortunately, despite frequent initial responses to chemotherapy, these tumors almost invariably relapse. Thanks to recent large scale molecular profiling studies in ovarian cancer, such as the integrated genomic analyses performed by the Cancer Genome Atlas (TCGA) network, significant headway has been made in our understanding of the molecular pathogenesis of ovarian cancer[1]. However these advances have failed to translate into meaningful clinical benefit for patients. The only approved novel 'targeted' therapy to date in ovarian cancer is the anti-angiogenic antibody, bevacizumab, for which reliable predictive markers still elude us.

With the possible exception of the p53 signaling network, the PI3K/Akt/mTOR cascade is probably the most frequently altered signaling pathway in cancer, including ovarian cancer. First generation inhibitors of mTOR have demonstrated anti-tumor activity and are currently approved for the treatment of renal, pancreatic, breast and some brain cancers. In addition, a huge number of PI3K, Akt and second generation mTOR inhibitors are in early clinical trials.

We propose to provide a brief overview of the PI3K/Akt/mTOR signaling network and discuss the rationale for targeting this pathway in ovarian cancer. Preclinical data and results of recent clinical trials will be presented. In addition, some of the challenges facing the development of these inhibitors in ovarian cancer will be discussed, such as the need for predictive markers and quality tumor samples, drug resistance, managing toxicity, as well as trial design considerations in order to optimize the development of novel therapies against the PI3K pathway in ovarian cancer.

2. The PI3K/Akt/mTOR signaling pathway

The phophatidylinositol 3 Kinase (PI3K) pathway is a complex signaling network coordinating a number of direct upstream inputs from growth factors (EGF, heregulin, TGF, and others), tyrosine kinase receptors (IGF1R, EGFR, HER2…) or other membrane receptors such as Met as well as cross-talk with the Ras-Raf-Mek-Erk pathway via indirect input from Ras (Figure 1). PI3K is composed of a p110 catalytic subunit and a p85 regulatory subunit. The p110 subunit of PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to the active second messenger, PIP3 which recruits Akt to the plasma membrane, and results in a conformational change and activation of PDK1 and Akt proteins. Akt is a serine threonine kinase that regulates a huge number of downstream targets [2],[3], while the phosphatase and tensin (PTEN) analog protein acts as an endogenous pathway repressor by de-phosphorylating PIP3 back to PIP2. Akt controls critical cellular survival and metabolic processes by influencing some of the following:

1. Via downstream regulation of p53, NFκB (nuclear factor κB) or CREB (cAMP response element-binding protein), Akt promotes the transcription of genes involved in anti-apoptotic and proliferative responses such as XIAP (X-linked inhibitor of apoptosis protein), the apoptosis regulating protein Bcl-2, survivin and others[4].

2. Akt also phosphorylates proteins involved in cell cycle regulation and apoptosis thus promoting cell cycle progression and survival:

a. Phosphorylation of GSK3 inhibits proteosomic degradation of cyclin D1,

b. Phosphorylation of the cyclin-dependent kinase (CDK) inhibitors p21 and p27 commits them to nuclear export and removes their inhibitory effect on cyclin D and cyclin E,

c. Downregulation of the apoptotic effector, caspase 9.

3. In addition downstream signaling via mammalian target of rapamycin (mTOR) activates two key substrates 4EBP1 and p70S6K resulting in increased translation of target genes involved in angiogenesis (VEGF), or cell cycle progression (cyclin D1, c-Myc)[5].

In addition to activation via upstream input, the PI3K pathway can be 'intrinsically' activated due to i) gain of function mutations or amplifications in the p110 subunit of PI3K (*PIK3CA*), ii) mutations in the p85 subunit (*PIK3R*), iii) mutations or amplifications in one of the Akt isoforms (*AKT1, AKT2, AKT3*), or iv) due to loss of its negative regulator, *PTEN* via inactivating mutations, copy number loss or homozygous deletions.

While mTOR is probably the best described direct target of Akt, the mTOR complex is actually composed of two components, the mTORC1-Raptor complex primary coordinator of translational control via 4EBP1 and p70S6K[6]; and the mTORC2-Rictor complex whose function is less well described but likely regulates cell proliferation and survival in part by Akt activation via phosphorylation at Serine 473[7]. Importantly mTORC1 is sensitive to inhibition by rapamycin, while mTORC2 is not. In the presence of selective mTORC1 inhibition, mTORC2 can exert a positive feedback on Akt[8]. As discussed later, this positive feedback loop may have important implications regarding the emergence of resistance to first generation mTOR inhibitors (rapalogs) that exclusively target mTORC1, with no effect on mTORC2.

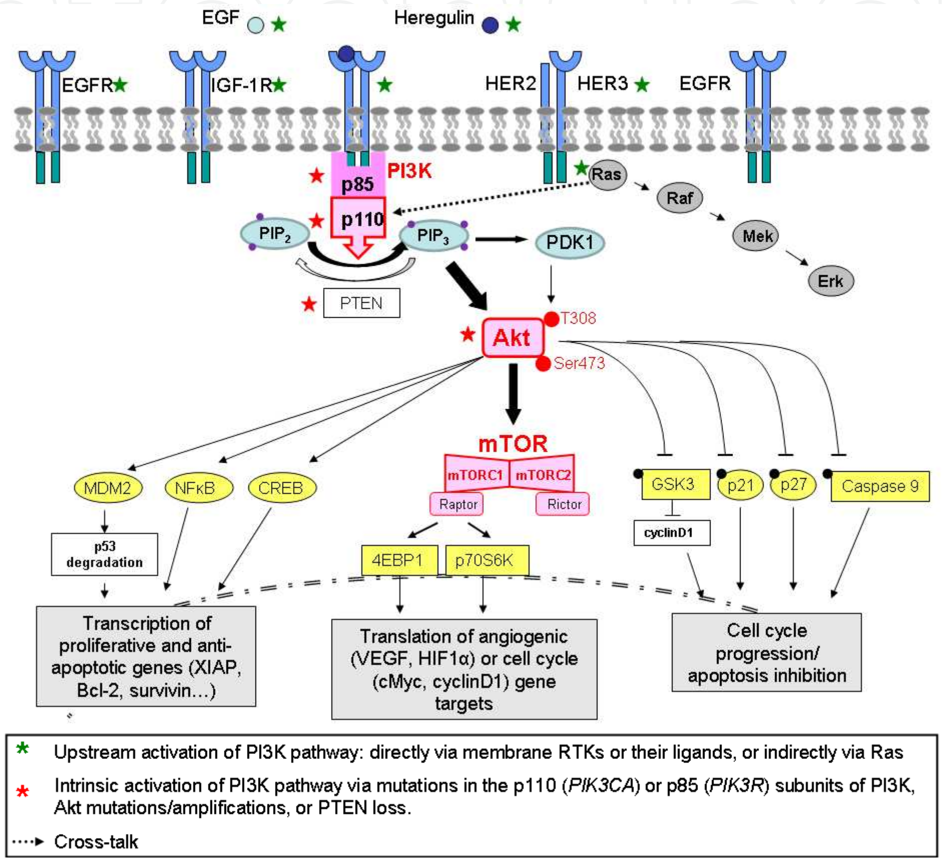

Figure 1. The PI3K/Akt/mTOR signaling pathway. This pathway is up-regulated in a significant proportion of ovarian cancers via either (i) direct upstream stimulation (growth factor receptors and their ligands), (ii) indirect activation via cross-talk with the Ras pathway, or (iii) intrinsically via activating genetic alterations in PI3K or Akt, or via loss of function in the tumor suppressor, PTEN.

3. Relevance of PI3K/Akt/mTOR signaling in ovarian cancer

The PI3K/Akt/mTOR pathway is frequently deregulated in ovarian cancer. Array Comparative Genomic Hybridization (aCGH) studies on 93 ovarian tumors have identified this pathway as the most frequently altered in ovarian cancer [9]. Copy gains in the genes encoding both the p110α (*PIK3CA*) and p110β (*PIK3CB*) subunits of PI3K were associated with a poor prognosis in patients with ovarian cancer. Expression levels of both p110α and pAkt were analyzed in over 500 ovarian cancer tumors and associated with decreased survival. Activation of the pathway as measured by Akt or mTOR phosphorylation levels is almost ubiquitous in ovarian cancer and an independent negative prognostic marker [10-12].

Interestingly, the type of PI3K/Akt/mTOR molecular alteration appears to be histological subtype specific (Table 1). There is mounting evidence that ovarian cancer is a highly heterogeneous disease with marked differences in molecular profile, histology, prognosis and chemosensitivity depending on the subtype [1],[13],[14]. The most common subtype (70%) high grade serous ovarian cancer (HGSOC) is characterized by almost universal p53 mutations (95-97% of cases) and marked genomic instability resulting in frequent somatic copy number alterations (amplifications or deletions)[13]. In HGSOC, oncogenic mutations are rare, but amplifications of the p110 subunit of PI3K (*PIK3CA)* have been described in 20% of cases, amplifications of one of the *AKT* isoforms (*AKT 1*, *AKT2* or *AKT3*) occur in 15% to 20%, while *PTEN* deletions have been described in 5%[15],[16] (Table 1). Finally *RICTOR* or *RAPTOR* amplifications have also been reported [1]. Rare but potentially relevant mutations in HGSOC include activating *PIK3CA* mutations (3%), or loss of function *PTEN* mutations (1%) [17]. Mutations have also been described in the p85α subunit of PI3K (*PIK3R1*, 4%), resulting in loss of its negative regulation on the p110 subunit and constitutive kinase activity[18]. In summary, 40 to 50% of HGSOC may have constitutive PI3K signaling. In a significant proportion of HGSOC, hyperactive PI3K/Akt/mTOR pathway may also be attributable to upstream deregulations in receptor tyrosine kinases (RTKs) or cross-talk with the Ras/Mek/Mek/Erk pathway. Indeed, amplifications or mutations in RTKs such as *ERBB3*, *ERBB2*, *EGFR* or *IGF1R* have been described with frequencies of 1% to 9% [1],[17]. Similarly, the ras pathway is often altered in HGSOC by amplifications in *KRAS* (11%), *MAPK* (20%), loss of the tumor suppressor *NF1* (8%), or less frequent mutations in *KRAS*, *NRAS*, or *BRAF*.

Whereas individual mutations remain an infrequent event in HGSOC, they are much more prevalent in the rarer subtypes such as low grade serous, mucinous, endometrioid or clear cell ovarian cancer. For example, 20% of endometrioid and 35% of clear cell ovarian tumors display *PIK3CA* mutations[19],[20]. In addition, while *PTEN* loss of function mutations are rare in ovarian cancer in general, they are well documented in up to 20% of endometrioid tumors and *PTEN* deletion occurs in 20% of endometrioid and clear cell ovarian cancers[21]. Low grade mucinous and serous subtypes do not tend to demonstrate intrinsic activation of PI3K effectors, however they frequently exhibit *KRAS* mutations, or amplifications/mutations in *ERBB2[22],[23]*.

Importantly intrinsic activation of the pathway (via *PIK3CA* mutations and *PTEN* loss) has been shown to initiate ovarian tumors in mice and inhibition of PI3K/mTOR in these models delayed tumor growth and prolonged survival, thus providing critical proof of concept for the pathologic relevance of this pathway in OC and its potential as a therapeutic target[24],[25]. Whether amplifications of pathway members actually activate PI3K signaling and confer comparable sensitivity to pathway inhibitors remains to be established. Similarly, while cross-talk with Ras may result in PI3K activation, it is unlikely that this also results in PI3K pathway dependence, however as discussed later, alterations in KRAS may be relevant with regards to predicting benefit from dual PI3K-Ras inhibition.

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High grade serous ovarian cancer is exquisitely chemosensitive, with response rates to first-line platinum-based chemotherapy of 75%, but almost invariably relapses with acquired resistance. The rarer subtypes tend to respond poorly to platinum chemotherapy with response rates of only 15% to 30%. Thus both acquired and de novo chemotherapy resistance remains a significant clinical challenge in ovarian cancer. Increased phosphorylation of mTOR has been described in cell lines with acquired cisplatin resistance, and Akt signaling has been implicated in primary platinum resistance[12]. Inhibitors of Akt or mTOR were shown to restore chemo-sensitivity in vitro and in xenograft models [26],[27]. These data suggest a potential role for inhibitors of the PI3K pathway in modulating chemotherapy sensitivity and justify their use in combination with conventional cytotoxics.

Ovarian cancer histological subtype	Intrinsic PI3K pathway activation	PI3K activation via upstream membrane RTKs	PI3K activation via cross-talk with ras
High grade serous (70%)	**Amplifications**: *PIK3CA* (17-20%) AKT1 (3%) AKT2 (6-12%) AKT3 (8%) RICTOR (6%) RAPTOR (4%) **Deletions:** PTEN (7%)	**Amplifications:** *ERBB3* (4%) ERBB2 (3%) IGF1R (4%)	**Amplifications:** *MAPK* (25%) KRAS (11%) **Deletions:** NF1 (8%)
	Mutations: *PIK3CA* (3%) *PIK3R1* (4%) *PTEN* (1%)	**Mutations:** *EGFR* (4-9%) *ERBB2* (1%)	**Mutations:** *NF1* (4%) *KRAS* (1-5%) *NRAS* (1%) *BRAF* (1%)
Clear cell	**Deletions** *PTEN* (20%)	**Amplifications** *ERBB2* (14%)	
	Mutations: *PIK3CA* (33%)		
Endometrioid	**Deletions** *PTEN* (20%)		
	Mutations: *PIK3CA* (20%) *PTEN* (20%)		
Mucinous		**Amplifications:** *ERBB2* (18%)	**Mutations:** *KRAS* (40-60%)
Low grade serous		**Mutations:** *ERBB2* (15%)	**Mutations:** *KRAS* (40%) *BRAF* (1%)

Table 1. Molecular alterations according to ovarian cancer subtype that could contribute to PI3K pâthway activation either directly (deregulated PI3K members) or indirectly via alterations in upstream RTKs or Ras pathway members.

4. Results of clinical trials targeting the PI3K/Akt/mTOR pathway in ovarian cancer

The frequent PI3K/Akt alterations demonstrated in vivo in tumors from patients with ovarian cancer, combined with the evidence for dependence on this oncogenic pathway in preclinical models provide a robust biological rationale for investigating the benefit of targeting PI3K, Akt or mTOR in ovarian cancer. However as detailed throughout this chapter, the intrinsic complexity of this signaling network may limit the anti-tumor potential of inhibiting a single effector along the pathway.

4.1. mTOR inhibitor monotherapy in ovarian cancer (Table 2)

The first inhibitors of the pathway to enter the clinic were rapamycin analogs that bind to the FK506 binding protein-12 of the MTORC1 complex and prevent mTOR activity. Rapamycin was used for years as an immunosuppressant to prevent rejection in solid organ transplants and hematological malignancies; its toxicity profile is therefore well described with main side effects consisting of edema, hypertension, renal toxicity, hematologic toxicity, and hypertriglyceridemia and hypercholesterolemia. In addition, rarer but potentially more concerning side effects included interstitial lung disease, risk of secondary lymphoma, and reactivation of latent infections[28]. Rapamycin analogs with less immunosuppressive properties, such as temsirolimus, everolimus and ridaforolimus have shown activity in a number of tumor types.

A phase II trial of temsirolimus at a flat dose of 25mg IV weekly in patients with ovarian cancer progressing after 1-3 previous regimens met its first stage response and PFS criteria at interim analysis with three responses and seven PFS at 6 months and pursued accrual through the second stage[29]. At final analysis, with 54 evaluable patients, grade 3-4 toxicities were as expected for mTOR inhibitors, mainly gastrointestinal (10%), metabolic (15%), and study drug was discontinued in 6% for interstitial pneumonitis. Unfortunately, objective responses were only seen in 9.3% (5/54) and 6 months PFS was 24% thus the study failed to meet its efficacy endpoint. Exploratory analyses were conducted in order to identify potential predictive markers. Phosphorylated-Akt, p-mTOR, p-p70-S6K, and cyclinD1 were measured in archival tumor samples as surrogates for activation of the PI3K pathway; only cyclinD1 levels were weakly correlated with PFS>6 months (r=0.28). The authors concluded that observed activity was insufficient to justify a phase III trial of temsirolimus in unselected patients with ovarian cancer. As discussed later in the chapter; these negative results may be explained by i) the lack of patient selection, ii) the cytostatic rather than cytotoxic effect of mTOR inhibitors (mTORi) and iii) the fact that these agents may require combinations with chemotherapy or other targeted agents to achieve a robust anti-tumor effect. The trial just fell short of its PFS efficacy endpoint (>24% PFS at 6 months), had the study limited enrollment to clear cell and endometrioid histologies known to show frequent PI3K alterations, the results may have been different.

4.2. mTOR inhibitors in combination with chemotherapy in ovarian cancer (Table 2)

Given the implication of mTOR and Akt in chemo-resistance and the preclinical studies suggesting an additive benefit with chemotherapy, studies have investigated mTORi-cytotoxic combinations. A phase I study of weekly topotecan (1mg/m2 days 1, 8 and 15) and temsirolimus 25mg days 1, 8, 15 and 22 on a 28 day schedule was conducted in 15 patients with gynecological malignancies including 7 patients with ovarian cancer. Dose limiting toxicities were myelosuppression and although efficacy was not a primary objective, 8 of 11 patients had stable disease at first evaluation and one patient with clear cell histology was still progression free at 6 months[30].

A phase Ib dose escalation study of temsirolimus (T) and pegylated liposomal doxorubicin (PLD) in advanced breast and gynaecological malignancies identified T 15mg and PLD 40mg/m2 as the maximum tolerated dose (MTD)[31]. The most frequent grade 3-4 adverse events were fatigue (5%), nausea (16%), mucositis (21%), rash (11%) and hand-foot syndrome (21%). The mean PFS was 4.9 months and the authors concluded that the combination warranted further study.

Two other phase I studies of rapalogs in combination with chemotherapy (temsirolimus plus carboplatin/paclitaxel[32] and everolimus plus weekly paclitaxel[33]) have been conducted with grade 3-4 neutropenia being the major DLT (at 89% and 56%, respectively) as well as fatigue and mucositis. These studies included a small number of patients with advanced ovarian cancer and responses were described (3 of 6 patients with ovarian cancer had a PR to temsirolimus plus carboplatin and paclitaxel). However given the small numbers and the combination with chemotherapy, no robust conclusions may be drawn regarding the added value of the mTOR inhibitor.

These early studies have begun to establish the feasibility and safety of mTORi-cytotoxic combinations, randomized trials will be required to investigate efficacy. In the interim, a number of non-randomized phase I and II studies are ongoing (Table 4). Given the heterogeneity of ovarian cancer, non-randomized phase II studies may require a degree of patient selection by molecular alteration or even histology in order to enrich the trial for potential responders and make the patient population more uniform with regards to natural disease course and chemosensitivity. Indeed studies recruiting patients with both high and low grade tumors with marked differences in tumor growth rates and responsiveness to chemotherapy may mask any benefit from the addition of the mTOR inhibitor. For example, a phase II trial of temsirolimus plus carboplatin and paclitaxel as adjuvant treatment is ongoing for patients with stage III or IV clear cell ovarian cancer (NCT01196429).

4.3. mTOR inhibitors in combination with anti-angiogenics in ovarian cancer (Table 2)

Finally, given the activity of VEGF inhibitors in ovarian cancer and the fact that downstream mTOR targets include angiogenic genes, there is A biological rationale for using mTOR and VEGF inhibitors in combination. A phase II trial of temsirolimus and bevacizumab in ovarian cancer has been conducted[34]. Thirty one (31) patients were evaluable for toxicity and 25 for efficacy. Adverse events included fatigue, mucositis, hypertension and neutropenia. In addition one grade 4 rash and 6% colonic perforations (2/31) were reported. While the confirmed PR rate is only 12% in the first 25 evaluable patients (all in platinum-resistant patients), the 6 months PFS rate of 56% (14/25) met efficacy criteria to justify progression to second stage accrual. Updated results are awaited. It is noteworthy that the study only enrolled patients who had not been exposed to anti-angiogenics; the previously reported RR of 15-21% in early trials of bevacizumab monotherapy among heavily pretreated patients with ovarian cancer raises the possibility that temsirolimus may be adding little anti-tumor effect to bevacizumab alone[35],[36]. A randomized phase II study is ongoing comparing bevacizumab alone to bevacizumab and everolimus in patients with recurrent ovarian cancer (NCT00886691, Table 4). Patients will be stratified according to their platinum-free interval or prior treatment with bevacizumab. This study should provide valuable insight into the potential additive benefit of this combinatorial strategy.

Reference	Phase	Treatment	N, total enrolled	N, ovarian cancer	Selected toxicities	Efficacy
Behbakht et al	II	Temsirolimus, 25mg IV D1, 8, 15, 22 Q28 days	54	54	G3-4 GI (10%), metabolic (15%), pulmonary (6%)	RR=9% 6 month PFS=24%
Temkin et al	I	Temsirolimus IV 25mg D 1, 8, 15, 22 + topotecan 1mg/m2 IV D1, 8, 15 Q28 days	15	7	G3-4 neutropenia and thrombocytopenia	RR=0 One SD for 6 months
Boers-Sonderen et al	Ib	MTD= temsirolimus IV 15mg D1, 8, 15, 22 + PLD IV 40mg/m2 D1 Q28 days	20	NA	G3-4 fatigue (5%), nausea (16%), mucositis (21%), vomiting (16%), rash (11%), hand-foot syndrome (21%)	NA
Kollsmannberger et al	I	MTD= temsirolimus IV 25mg D1 and 8 + carbo AUC5 IV D1 + Pac IV 175mg/m2 D1 Q 21 days	39	6	G3-4 neutropenia (89%), thrombocytopenia (21%), pulmonary (5%)	RR= 50% (3/6) SD=50% (3/6)
Campone et al	I	Everolimus PO 30mg daily + Pac 80mg/m2 D 1, 8, 15 Q 28 days	16	3	G3 neutropenia, anemia, thrombocytopenia, mucositis, fatigue	NA
Morgan et al	II	Temsirolimus IV 25mg D 1, 8, 15, 22 + Bev 10mg/kg D1 and 15 Q 28 days	31	31 evaluable for toxicity and 25 evaluable for efficacy	G3-4 fatigue (13%), mucositis (13%), HTN (6%), neutropenia (10%), rash (3%), colonic perforation (6%)	RR=12% 6month PFS 56%

Abbreviations: N: number of patients; IV: intravenous; D: day; Q: every; G3-4: grade 3-4; RR: response rate; PFS: progression-free survival; SD: stable disease; MTD: maximum tolerated dose; PLD: pegylated liposomal doxorubicin; NA: information not available; carbo: carboplatin; pac: paclitaxel; PO: per os.

Table 2. Completed clinical trials of mTOR inhibitors in ovarian cancer

While the evidence for clinical activity of mTOR inhibitors in ovarian cancer remains quite limited, especially compared to endometrial cancer where efficacy has been more encouraging, a number of phase II trials of mTOR inhibitors alone or in combination with conventional cytotoxics or targeted therapies are currently ongoing. These should help clarify the role mTOR inhibitors may have in the management of patients with ovarian cancer (Table 4).

4.4. Akt inhibitors

Targeting Akt upstream from mTOR may produce a more effective knock-down of signal transduction and a number of Akt inhibitors have therefore been generated. These include ATP-competitive inhibitors, allosteric inhibitors, peptide-based inhibitors and lipid-based inhibitors (reviewed in Stronach et al[37]). Akt inhibitors are still in early stages of clinical development and two compounds have been specifically tested in ovarian cancer (Table 3).

The most mature inhibitor in clinical development is the lipid-based inhibitor, perifosine, it interferes with the cell membrane recruitment of Akt (thus preventing activation). However early data in phase I and II trials in other tumor types were disappointing with frequent gastrointestinal toxicity and a lack of meaningful activity[38]-[41]. Given the suggestion that the narrow therapeutic window of perifosine may limit its clinical usefulness, combination trials with conventional cytotoxics have been conducted in order to improve the therapeutic index. Preclinical studies have shown that perifosine inhibited ovarian cancer cell proliferation, motility and angiogenesis and potentiated paclitaxel sensitivity in vitro and in vivo[42], [43]. On this basis, a phase I trial of perifosine and docetaxel in platinum and taxane resistant ovarian cancer was conducted[42]. Perifosine was given at a loading dose of 100mg every 6 hours for 4 doses followed by a daily dose according to dose level (50, 100 or 150mg daily) in combination with docetaxel 75mg/m2 day 1 every 3 weeks. Twenty one patients were enrolled including 11 at the MTD level of perifosine 150mg. No DLTs were observed, frequent adverse events included nausea, vomiting, anorexia, constipation and fatigue. With regards to efficacy at the MTD (N=11), there was one PR in an endometrioid ovarian cancer with a loss of function *PTEN* mutation (R130Q) and one SD maintained for 4 months in a PI3K mutated clear cell tumor. Two other patients without apparent PI3K alterations achieved SD while two patients with KRAS mutations progressed quickly. The investigators also performed pharmacodynamic studies using reverse phase protein array (RPPA) to detect changes in total and phosphorylated markers in pre-treatment versus day 7 tumor biopsies and functional imaging studies using FDG-PET scans. Bcl2 and ERK2 levels were increased by treatment suggesting that the low response rate may be in part explained by perifosine induced increases in alternate signalling pathways. However FDG-PET responses at one week correlated with inhibition of S6 phosphorylation raising the possibility that FDG-PET may serve as an early surrogate indicator of Akt inhibition.

GSK795 is an oral ATP-competitive pan-Akt inhibitor in early stages of development and a small phase I pharmacodynamic and pharmacokinetic study was conducted in order to characterize the relationship between AKT inhibition by GSK795 and downstream effects in patients with advanced platinum resistant ovarian cancer[44]. Twelve patients were enrolled. The only toxicities were grade 2 anorexia (18%) and vomiting (18%). FDG metabolism decreased in the majority of tumors but there was no dose response relationship. Among 5 patients treated at the higher dose levels, paired pre- and post-treatment tumor biopsies demonstrated downregulation in pAkt and in the tumor proliferative marker, Ki67. Two patients have achieved >6 months PFS with objective tumor regressions of 26% and 11%, respectively.

In addition to the aforementioned inhibitors, Akt isoform specific inhibitors are being developed, however the distinct functions of each of these isoforms and their relevance to different tumor types or individual tumor genetic background is still poorly understood. Studies of AKT isoform knockouts provide some insight into their relative roles: AKT1 loss is associated with impaired fetal development and increased fetal mortality; AKT2 loss leads to diabetes and AKT3 loss results in defective central nervous system development[45].

Reference	Phase	Treatment	N, total enrolled	N, ovarian cancer	Selected toxicities	Efficacy
Fu et al	I	MTD Perifosine orally 150mg/day + docetaxel, 75mg IV D1 Q21 days	21	21	Nausea, vomiting, anorexia, fatigue	At MTD (N=11) PR in 1 PTEN null, SD 3/11.
Gungor et al	I	GSK795 25, 50 or 75mg orqlly/day	12	12	G2 anorexia (18%), vomiting (18%)	16% SD for 6 mo (2/12) with tumor shrinkage of 26% and 11%

Table 3. Completed clinical trials of Akt inhibitors in ovarian cancer

4.5. PI3K inhibitors

The PI3K inhibitors, LY290002 and wortmannin have been used for years as tools in preclinical experiments to demonstrate the biological relevance of PI3K and explore its potential as a therapeutic target in cancer. However, the micromolar IC50 (50% inhibitory concentration) and off-target effects of these agents have limited their clinical applicability. Less toxic PI3K inhibitors are just entering phase II stages of clinical development (reviewed in Kurtz et al[46]). BKM120 is an oral selective PI3K inhibitor with an IC50 for the PI3K kinase of 35nM. A dose escalation phase I trial has shown that the drug is well tolerated at the MTD of 100mg once a day with rash, hyperglycemia, diarrhea and mood alterations in over a third of patients[47]. BKM120 demonstrated dose dependent inhibition of FDG activity and downregulation in p-S6 in skin biopsies. The only response was in a KRAS mutated breast

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cancer patient, and 7 patients had stable disease for more than 8 months. Five of these 7 patients had either PTEN loss or PI3K mutation. GDC0941 is an oral selective class I PI3K inhibitor that showed evidence of clinical activity in 3 patients enrolled in a phase I trial, including one ovarian cancer (PTEN negative) patient who remained on study for 5 months with a FDG-PET response, >50% decrease in pS6 staining in paired biopsies, and 80% decrease in CA-125[48]. XL147 is another selective PI3K inhibitor which was well tolerated in a phase I trial with rash as the main DLT. An associated trial of XL147 in combination with carboplatin and paclitaxel demonstrated that the combination was feasible with no evidence of PK interactions or overlapping toxicities and dose expansion cohorts are ongoing in ovarian cancer[49].

5. Challenges of PI3K/Akt/mTOR pathway inhibitors

Despite a strong preclinical rationale, clinical trials of novel agents targeting the PI3K/Akt/mTOR pathway in ovarian cancer have been disappointing. Given the complexity and redundancy of the PI3K signaling network, combined targeting may be required. The fact that all the trials conducted to date enrolled an unselected patient population may have diluted objective activity in a subset. It is therefore crucial that efforts are made to uncover resistance mechanisms, develop rationale combinatorial strategies, identify predictive biomarkers, and explore novel trial designs.

5.1. Resistance

5.1.1. Feedback loops via MTORC2 or IRS1

Compensatory feedback loops may allow escape from blockade of a single effector of the pathway. Early on, paradoxical increases in pAkt were identified in preclinical models and in tumors from patients treated with mTOR inhibitors[8]. As illustrated in Figure 2, rapalogs suppress MTORC1 but do not affect the other subunit of mTOR, MTORC2. MTORC2 is a positive regulator of Akt, and selective inhibition of MTORC1 results in compensatory increase in Akt phosphorylation at Serine 473[50]. Rapalog-induced rebound Akt activation has been proposed as one of the mechanisms accounting for resistance to first generation inhibitors in the clinic. In addition, although the function and downstream effectors of MTORC2 are less well described, it is reasonable to expect that complete abrogation of the whole mTOR complex may be required to achieve a robust anti-tumor effect. As a result, mTORC1/mTORC2 dual inhibitors have been developed such as DS3078a, INK128, AZD8055, OSI027 and AZD2014 (reviewed in [51]).

Another postulated compensatory escape route from mTOR inhibition is via insulin growth factor 1 receptor (IGF1R, see Figure 2)[52]. Insulin receptor substrate-1 is normally under basal negative regulation via phosphorylation by mTOR; mTOR inhibition prevents IRS-1 phosphorylation thus allowing IRS-1 to complex with IGF1R and promote Akt signaling[53] thereby generating another positive feedback loop accounting for resistance.

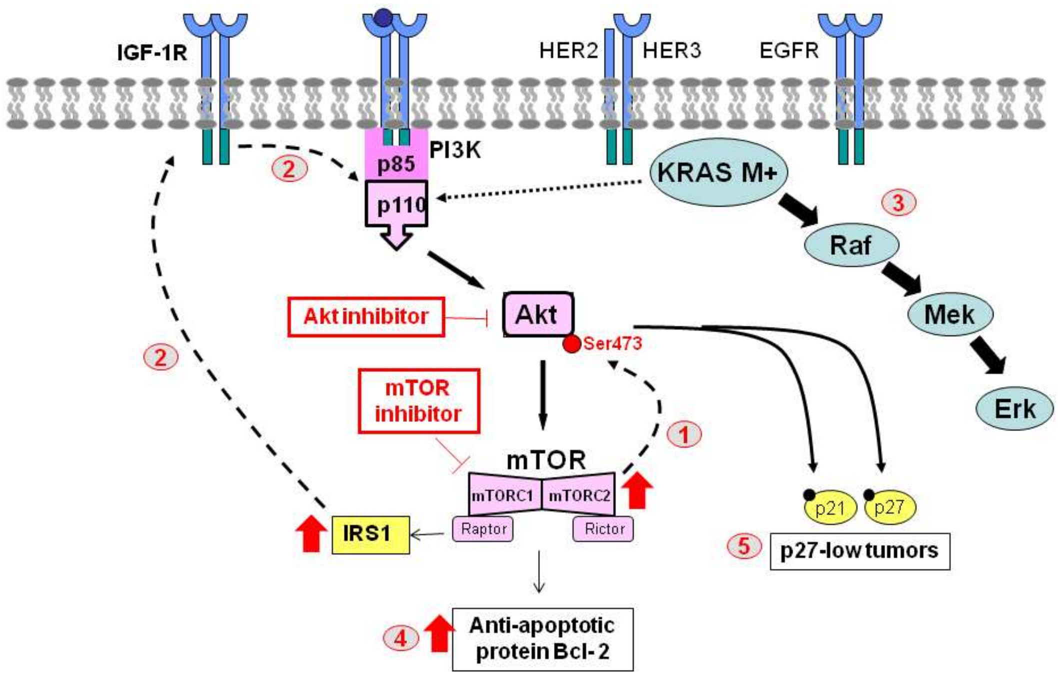

Figure 2. Proposed mechanisms accounting for resistance to inhibitors of the PI3K pathway. (1) Selective blockade of MTORC1 by rapalogs increases MTORC2 and results in positive feedback activation of pAkt. (2) Inhibition of mTOR removes the basal inhibition of IRS1, now free to bind to and activate IGF1R and promote PI£K activation. (3) In the presence of constitutive activation of KRAS, abrogation of the PI3K pathway alone does not inhibit cancer cell growth. (4) A dysfunctional apoptotic pathway (high bcl2, high survivin...) may lead to resistance to the pro-apoptotic effects of PI3K apthway inhibitors. (5) One downstream effect of Akt inhibition is cell cyle arrest via increase in the cdk inhibitors, p21 or p27; p27 low tumors may be resistant to PI3K pathway inhibitor induced cell cycles arrest.

5.1.2. The Ras pathway: KRAS/BRAF mutations and compensatory increases in Erk signaling

Interactions with parallel pathways may also allow escape from PI3K inhibition. Akt has been shown to be phosphorylated via cross-talk with Ras. Thus, in KRAS mutant tumors primarily driven by a constitutively upregulated Ras pathway, PI3k pathway inhibitors alone are unlikely to be effective. This hypothesis is supported by studies demonstrating that KRAS or BRAF mutated tumors are insensitive to mTOR inhibitors. Using a panel of cell lines including ovarian cancer, PI3K mutated tumors were shown to be sensitive, while dual PI3K and KRAS or BRAF mutated tumors were resistant to everolimus[54]. Importantly, they also demonstrated that knock-down of the KRAS mutation in these cells restored everolimus sensitivity in vitro and in vivo. In the presence of KRAS or BRAF mutations, tumors may exhibit 'oncogenic addiction' to an alternate survival pathway, e.g. Ras-Raf-Mek-Erk. This illustrates the fact that sensitivity to PI3K transduction inhibitors may require not only pathway activation but also demonstration of pathway dependence.

In addition to reactivating Akt, rapalogs have been reported to cause treatment induced increases in Mek/Erk signalling. In mice models and human tumors, everolimus increased Erk1/2 activation in post treatment tumor samples, suggesting the existence of crosstalk between the PI3K/mTOR and Mek/Erk signal transduction cascades[55]. Selective targeting of one pathway may simply result in compensatory upregulation in the other, and vice versa.

5.1.3. Dysfunctional apoptotic machinery

Even in tumor types such as renal cell or pancreatic neuroendocrine cancers where mTOR inhibitors have demonstrated sufficient clinical benefit to justify FDA approval, objective tumor responses are sporadic[56]. Some researchers have hypothesized that tumor shrinkage in response to mTOR inhibitors requires a functional apoptotic machinery. Majumder et al demonstrated that rapamycin-resistant SKOV3 ovarian cells have an activated PI3K pathway but upregulated levels of the anti-apoptotic protein, bcl2, and bcl2 knock-down using siRNA restored rapamycin sensitivity[57]. In line with this preclinical data, the Phase I trial of the Akt inhibitor perifosine reported compensatory increases in bcl2 in post treatment tumor biopsies[42].

5.1.4. Cell cycle dependent kinase (cdk) inhibitors

One of the major anti-tumor effects of PI3K blockade is to activate the cdk inhibitors p27 and p21, allow their nuclear translocation where they interact with, and inhibit cdks, thereby promoting cell cycle arrest. p27-null cells are resistant to rapamycin in vitro, some therefore postulate that tumors that have very low levels of p27 may therefore be less responsive to PI3K/Akt inhibition[58].

5.2. Combinatorial strategies

Given the presence of redundant pathways and the adaptive capacity of cancer cells, drug combinations are increasingly being investigated in an effort to abrogate both primary and acquired resistance to PI3K pathway inhibitors. Different approaches include targeting the same pathway at different levels (vertical combinations) or aiming for different pathways (horizontal combinations).

5.2.1. Vertical combinations

With membrane growth factor receptor inhibitors

Activation of the PI3K pathway can be attributable to upstream activation via membrane receptor kinases, and preclinical data suggest that concurrent inhibition of mTOR and EGFR may result in synergistic anti-tumor effect. Studies are investigating the benefit of dual mTOR/EGFR blockade[59]. A completed phase I trial showed that the combination of everolimus, bevacizumab and panitumumab was well tolerated, and three patients with ovarian cancer achieved prolonged disease control for 11 to >40 months[59]. In addition, mTOR inhibition may induce IRS1 expression and promote Akt activation via IGF1R thus attenuating the anti-tumor effects of rapalogs[60]. The addition of IFG1R antibodies to mTOR inhibitors has been shown to improve growth inhibition in vitro[52]. Studies investigating concurrent IGF1R/mTOR targeting have shown that treatment is feasible with an acceptable toxicity profile and encouraging activity in other tumor types[61] and studies using this approach are ongoing in ovarian cancer (Table 4).

Treatment type	Phase	Experimental treatment	Prior treatment	Selection criteria (biomarker vs allcomers)	Secondary endpoints	Clinical trial.gov identifier
PI3K inhibitor	I	BKM120 + Olaparib (PARP inhibitor)	First line platinium-based CT	All comers	MTD for the combination, safety, PK, efficacy. PD markers of PI3K inhibition, determination of BRCA1 IHC, BRCA1 promoter hypermethylation and BRCA1/2 somatic mutation status	NCT01623349
AKT inhibitor	I	GSK2141795	Not specified	All comers	PK and PD by FDG/PET	NCT01266954
	II	MK-2206	Platinum resistant	*P13K* or *AKT* mutation or low PTEN expression	RR, PFS and OS, toxicities of MK-2206, explore the association between select biomarkers and response to MK-2206, to explore the development of feedback loop activation and target inhibition with MK-2206.	NCT01283035
	I	Perifosine + docetaxel	Not specified	All comers	Tumor response	NCT00431054
	I/II	GSK2110183 + carbo+pac	Platinum resistant, "/>2 prior lines of CT	All comers	Phase I : safety and tolerability Phase II : overall RR	NCT01653912
1st generation MTOR inhibitor	I	Sirolimus + ALVAC(2)-NY-ESO-1 vaccine	Not specified	Tumor expression of NY-ESO-1 or LAGE-1	Safety, effectiveness of sirolimus on enhancing vaccine efficacy, antibody titers, NY-ESO-1 specific CD8+ and CD4+ frequency and function, PFS.	NCT01536054
	II	Temsirolimus	Taxane based treatment, <3 prior CT	All comers	PFS, rate and duration of stable diseases, cancer antigen 125 (for ovarian cancer), overall survival, safety and toxicity, quality of life, rate and duration of stable diseases	NCT01460979
	II	Temsirolimus + carbo + pac	Refractory to standard treatment	All comers	MTD, toxicity, RR, PK.	NCT00408655
	I	Everolimus + PLD + carbo	One prior platinum/ taxane-CT	All comers	MTD for the combination, safety/tolerability, anti-tumor activity	NCT01281514
	I	Ridaforolimus + carbo + pac	<4 prior CT lines	All comers	MTD, preliminary efficacity, toxicity	NCT01256268
	II	Adjuvant Temsirolimus + carbo + pac followed by maintenance temsirolimus	First line	Clear cell histology only	PFS at 12 months, median PFS, OS, toxicity and RR. mTOR signaling pathway by IHC.	NCT01196429
1st generation mTOR inhibitor in combination with	II	Everolimus + bevacizumab	Previously treated	All comers	PFS at 6 months, complete response + partial response + stable disease	NCT01031381

Treatment type	Phase	Experimental treatment	Prior treatment	Selection criteria (biomarker vs allcomers)	Secondary endpoints	Clinical trial.gov identifier
antiangiogenic therapy	II	Temsirolimus + bevacizumab	Previously treated	All comers	RR, PFS at 6 months, OS, duration of response, TTP. No specific biomarker objectives specified but blood and tumor collected on all	NCT01010126
	I	Temsirolimus + Cediranib (VEGFR 2 inhibitor)	<2 prior line of CT for recurrent disease	All comers	MTD , response rate, clinical benefit	NCT01065662
	II	Everolimus +/- bevacizumab randomised trial	Platinum-based CT. Stratification according to platinum resistant vs. not, measurable disease vs. not and prior bevacizumab vs. not	All comers	PFS, tolerability, OS, RR, CA-125 response.	NCT00886691
mTOR or Akt inhibitor + IGF1R inhibitor	IB	MK-2206 (Akt inhibitor) or ridaforolimus + IGF1R Ab (dalotuzumab), non randomized study	Previously treated. Platinum resistance required for MK-2206 arm	All comers	Number of participants with dose limiting toxicities, number of participants whose best response is a partial response (PR) or complete response (CR)	NCT01243762
mTOR inhibitor in combination with Notch pathway inhibitor	I	Ridaforolimus + MK-0752	<3 prior CT lines	All comers	Number of participants with dose limiting toxicities, AUC for the ridaforolimus + MK-0752 doublet	NCT01295632

Abbreviations: PARP : poly-ADP-ribose polymerase ; CT : chemotherapy ; MTD : maximum tolerated dose, PK : pharmacokinetic ; PD : pharmacodynamic ; BRCA : breast cancer susceptibility gene ; IHC : immunohisotchemistry ; FDG/PET : fluorodeoxyglucose positron emission tomography ; RR : response rate ; PFS : progression-free survival ; OS : overall survival ; carbo : carboplatin ; pac : paclitaxel ; NY-ESO-1 : cancer-testis antigen-1 ; LAGE-1 : cancer-testis antigen-2 ; PLD : pegylated liposomal doxorubicin ; TTP : time to progression ; VEGFR : vascular endothelial growth factor receptor ; IGF1R : insulin-like growth factor receptor ; AUC : area under the curve.

Table 4. Ongoing trials of PI3K pathway inhibitors in ovarian cancer

Combined PI3K-mTOR or Akt-mTOR inhibition

As previously discussed, positive feedback loops generated by selective mTOR inhibition may result in paradoxical activation of Akt via mTORC2 and account for early resistance.

Dual MTORC1 and MTORC2 inhibitors have therefore been developed and shown to result in greater anti-tumor activity than rapalogs in preclinical studies[62]. Another strategy involves co-targeting mTOR as well as upstream PI3K in order to overcome the positive feedback loops via Akt. In addition, simultaneous targeting of several effectors of the PI3K pathway may improve the likelihood of completely shutting down the signaling cascade. A combination of everolimus and the PI3K inhibitor, PI-103 blocked rebound rapalog induced Akt activation and resulted in greater cell cycle arrest than either treatment alone in ovarian cancer cells[63]. NVP-BEZ235 is a novel agent that is both an ATP-competitive PI3K inhibitor and an inhibitor of both mTORC1 and mTORC2. Studies in ovarian cancer cell lines and mouse models have suggested that this drug caused cell cycle arrest and apoptosis, and prolonged survival of mice with established ovarian tumors[64]. A phase I trial of ridaforolimus with the Akt inhibitor MK2206 is ongoing and a dose expansion cohort in ovarian cancer is planned (NCT01295632). Other studies are exploring the benefit of inhibiting further downstream effectors such as p70S6 in combination with everolimus (NCT01115803).

5.2.2. Horizontal combinations

With Mek inhibitors

Given the evidence that oncogenic activation of the ras pathway may be associated with resistance to mTOR inhibitors even in the presence of PI3K oncogenic mutations, targeting both PI3K and Ras pathways simultaneously is worthy of investigation. In a mouse model of ovarian cancer driven by PTEN loss and KRAS mutation, simultaneous blockade of both PI3K and Mek signalling using pharmacological inhibitors resulted in significant tumor regressions and prolonged survival compared to monotherapy[65]. A phase I study comparing the tolerability and efficacy of dual PI3K and Mek targeting to either treatment alone showed that the combination significantly increased the risk of Grade 3-4 toxicity from 18% to 54% ($p=0.001$), but all patients with alterations in the PI3K pathway and a KRAS or BRAF mutation had tumor regressions with dual targeting[66].

With chemotherapy

One of the earliest explored strategy has been the combination of novel inhibitors with chemotherapy. There has been the theoretical concern that the cytostatic effects of these drugs may in fact antagonize the cell cycle dependent effects of chemotherapy. Preclinical studies in ovarian cancer have indeed suggested that PI3K inhibitor-induced G1 arrest undermined the cytotoxic effects of agents such as cisplatin, paclitaxel, gemcitabine and topotecan that are primarily effective in the S or G2 phase of the cell cycle[67]. However preliminary data from non-randomized studies of mTOR inhibitors in combination with chemotherapy have reported objective response rates comparable to those expected for chemotherapy alone, thus providing indirect evidence for a lack of antagonism. Randomized studies will be required to rule out any antagonism between PI3K inhibitors and conventional cytotoxics.

With anti-angiogenics

Pro-angiogenic factors such as HIF1α and VEGF are downregulated by inhibition of PI3K signaling. This may explain the activity of mTOR inhibitors in HIF1α-driven clear cell renal

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cancer. Given the putative anti-angiogenic effects of PI3K pathway inhibitors and the known activity of the VEGF antibody, bevacizumab in ovarian cancer, there is a rationale for targeting multiple angiogenic regulators at once in an effort to shut down angiogenesis completely. In fact, clear cell ovarian cancers with their reported angiogenic signature and increased HIF1α signaling[68] may be particularly suited to a therapeutic strategy combining traditional anti-angiogenics with PI3K pathway inhibitors.

5.3. Biomarkers

In light of the heterogeneity of ovarian cancer, predictive as well as pharmacodynamic (demonstrating target downregulation) biomarkers are desperately needed in order to select patients most likely to respond. In addition biomarkers would be useful to identify the subset of patients who may benefit from specific combinations. One question is whether sensitivity can be predicted on the basis of activation status of pathway members.

5.3.1. Constitutive PI3K activity: PIK3CA mutations and PTEN loss of function

The main intrinsic effectors of the pathway that have been studied in preclinical and clinical models have been PTEN loss, and PIK3CA activating mutations. Early studies in cell lines including ovarian cancer demonstrated greater anti-proliferative activity of PI3K pathway inhibitors in PTEN-null or PIK3CA mutated cells[69]-[71], Di Nicolantonio et al, showed in cell lines and in 43 patient tumor samples that PIK3CA mutations sensitized cancer cells to everolimus, but co-existing KRAS or BRAF mutations predicted resistance[54]. More recent clinical and preclinical studies have reported contradictory correlations between PI3K mutations or PTEN loss and response to inhibitors[72],[73]; in particular, a significant number of PI3K mutated tumors fail to respond, while a proportion of tumors lacking PI3K and PTEN alterations respond. This is in contrast to the much stronger association between activating mutations and response to other targeted agents such as EGFR, BRAF or ALK inhibitors. Studies in tumor types with frequent PTEN mutations, such as melanoma have not demonstrated significant responses to mTOR inhibitors suggesting that patient selection on the basis of PTEN loss alone may not identify responders[74]. In a pooled analysis of 3 trials of mTOR inhibitors in endometrial cancers, MacKay et al found no correlation between PIK3CA mutation or PTEN loss and response[75]. However a recent report by Janku and colleagues suggested that PI3K mutations did preferentially identify responders[76]. They conducted mutational analyses on 140 patients with breast and gynecological malignancies (including 60 with ovarian cancer) enrolled in phase I trials of PI3K/Akt/mTOR inhibitors. They demonstrated that the response rate was higher among patients with PIK3CA mutated tumors (RR=30% versus 10%). However these results should be interpreted in light of the fact that all responders were included in a trial of temsirolimus, bevacizumab and liposomal doxorubicin. Given the known activity of bevacizumab and liposomal doxorubicin in ovarian cancer and the fact that half the responding patients had never been previously exposed to liposomal doxorubicin, mutations may simply correlate with prognosis, or with an improved response to treatment in general.

In conclusion, if trials of PI3K/mTOR inhibitors had limited enrolment to PTEN null or PI3K mutated tumors a significant proportion of responding patients would have been missed. In light of the imperfect association between PI3K mutations or PTEN loss and response to PI3K pathway inhibitors, most ongoing trials are enrolling an unselected patient population; unfortunately, most of these studies do not appear to be collecting archival tumor samples for detailed molecular analyses (Table 4).

5.3.2. pAkt and stathmin

The level of phosphorylated Akt has been identified as a read-out for activation of the PI3K pathway and thus a potential biomarker for responsiveness to PI3K inhibitors. An in vitro and in silico study using a panel of cell lines and xenograft models treated with PI3K pathway inhibitors showed that pAkt correlated with efficacy, and KRAS or BRAF mutations with resistance; neither PTEN loss nor PIK3CA mutations correlated with response[77]. Udai et al analyzed PI3K signaling output in patient tumor samples by measuring phosphorylation of 3 effectors downstream of PI3K, ie pAkt, p p70S6K and pGSK3beta[78]. No correlation was found between the presence of genomic alterations in PI3K or PTEN and activation of the pathway as measured by phosphorylated downstream targets. In a study of 17 well-characterized ovarian cancer cell lines, the majority failed to respond to Akt inhibitors despite Akt phosphorylation[79]. A high level of pAkt may not only reflect PI3K pathway intrinsic activation, but also result from cross-talk with Ras or other upstream signals.

In addition to being a non-specific measure of PI3K signal transduction, pAkt is a labile phosphorylated tumor marker, its stability is affected by pre-analytical factors such as tissue acquisition, ischemic time and fixation method[80],[81], In an effort to identify more stable biomarkers, Saal et al developed a gene expression signature of PI3K pathway activation and Stathmin, a regulator of microtubule dynamics was an accurate marker of the gene signature. Stathmin can be easily measured by immunohistochemistry and is increasingly being used as a surrogate marker for activation of the PI3K pathway[82].

5.3.3. KRAS/BRAF

As previously discussed a number of preclinical studies have demonstrated that KRAS and BRAF mutations confer resistance to inhibitors of the PI3K pathway[54],[77]. Intringingly, in a pooled molecular analysis of patients treated with PI3K/Akt/mTOR inhibitors in phase I trials, Janku et al reported 2 objective responses in patients with co-existing PI3K and KRAS or BRAF mutation[76]. Genomic analyses of tumors and cell lines has established that a subset of ovarian cancers have co-existing Ras and PI3K/Akt amplifications or mutations. This easily identifiable subset may benefit from coordinated inhibition of both pathways, and a trial combining a Mek inhibitor with a PI3K/mTOR inhibitor in ovarian cancer patients harboring KRAS/BRAF and PI3K/Akt genomic alterations is warranted.

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6. Practical issues: Samples and trial design

6.1. Access to quality ovarian cancer samples

As the data to date suggest that there is insufficient evidence to select patients for trials of PI3K inhibitors on the basis of specific molecular alterations, it is imperative that future trials enrolling unselected patient populations include parallel biological studies in an effort to uncover candidate biomarkers. Biological assays must be reproducible, robust and require access to high quality tumor samples. As such, pre-analytical variables must be controlled for as much as possible by following standardized sample collection, fixation, processing and storage procedures. When dealing with paraffin-embedded tissue, markers of the PI3K Akt pathway may be particularly susceptible to artefactual loss[80]. In fact, the optimal fixative for in depth genomic analyses is unlikely to be formalin, and may therefore require a shift in routine practice from paraffin to fresh frozen or RNAlater for sample storage.

6.2. Access to post-treatment samples

6.2.1. At relapse

It is likely that clonal evolution and treatment selection pressure will lead to important genomic and/or phenotypic modifications in the tumor in the interval between diagnosis and relapse. An increasing number of phase I and II trials are therefore requesting optional biopsies of metastatic disease and the vast majority of patients are willing to consent this procedure. A study of patients enrolled in phase I trials at our institution revealed that 84% of patients who were proposed optional tumor biopsies consented to the procedure, including sequential pre- and post-treatment biopsies[85]. All procedures were performed using an 18-gauge needle under ultrasound or computed tomography scanning and were associated with low minor complication rates (9/145 tumor biopsies). In 70% of the cases the biopsy met quality criteria for ancillary molecular (RNA and DNA) analyses. Access to samples from relapsed disease is likely to be particularly relevant to high grade ovarian cancer, where the initial disease is exquisitely chemosensitive and repeat profiling of the chemoresistant recurrence may reveal a completely different molecular profile.

6.2.2. Residual disease post-chemotherapy

The molecular characterization of ovarian cancer clones surviving after chemotherapy could identify targets for novel agents designed to eradicate chemoresistant residual disease. As discussed above, the combination of PI3K/Akt/mTOR inhibitors with chemotherapy may not be optimal because of the risk of cumulative toxicities as well as the theoretical risk that these inhibitors may antagonize the cytotoxic effects of chemotherapy. A more attractive approach may be sequential, where primarily chemosensitive ovarian cancer is treated with chemotherapy followed by PI3K inhibitors if indicated by the molecular profile of the residual resistant clones. Although recent trials using such an approach with erlotinib or olaparib after response to platinum based treatment were disappointing, neither trial selected the maintenance treatment on the basis of the profile of residual disease.

6.3. Surrogate tissue

Any effort to sample relapsed disease in ovarian cancer patients invariably faces the challenge of access to tumor. Recurrences tend to be limited to the abdominal cavity with diffuse carcinomatosis which can be difficult to biopsy safely. This is a critical need for more easily accessible surrogate tumor samples which would allow for serial tumor sampling throughout the disease course, to identify both predictive and pharmacodynamic markers. Possibilities include circulating tumor cells, ascites and circulating DNA.

Serial sampling of circulating tumor cells (CTCs) has been shown to provide useful prognostic and/or predictive information in a number of tumor types such as breast and prostate cancer[86],[87]. In the temsirolimus trial, CTCs were detected in 45% of patients before cycle 1 and found to correlate weakly with progressive disease, however no significant change in CTC levels were observed with treatment[29].

Udai et al demonstrated the feasibility of profiling the PI3K pathway from ascites in patients with advanced ovarian cancer: they successfully measured PI3K and PTEN mutations, amplifications and losses as well as PI3K signaling output in ascitic samples by ELISA for phosphorylated proteins[78]. Finally, cancer mutations have been identified by deep sequencing of circulating plasma DNA from patients with advanced ovarian cancer, providing another example of a non-invasive "liquid biopsy"[88].

1) Standardized quality ovarian cancer sample collection protocols at diagnosis and surgery optimized for comprehensive molecular studies.
2) Sequential biopsies for post-treatment/resistant tumor molecular profiling.
3) Studies investigating the feasibility and translational research value of surrogate tissue samples: ascites, circulating tumor cells, circulating DNA

Table 5. Sample-related considerations to enhance the development of PI3K pathway inhibitors in ovarian cancer

6.4. Novel trial designs

Conventional endpoints such as RECIST response may not be appropriate for inhibitors of the PI3K pathway that may result in disease stabilization rather than objective tumor shrinkage. Single arm phase II trials offer little data regarding activity of a novel drug: patient numbers are small, heterogeneous and comparisons with historical controls are intrinsically unreliable. A number of subtle deviations from traditional trial designs could help improve the likelihood that novel PI3K inhibitors make a successful transition from preclinical testing through early and late phase trials. Various strategies are outlined in table 6.

http://dx.doi.org/10.5772/54170

• Randomized placebo controlled phase II trials instead of single arm phase II. • Randomized discontinuation design: After an initial run-in phase where all patients receive the experimental agent, patients with stable disease are randomized to placebo versus continued drug. This model may be particularly suited to slower growing Type I ovarian cancers where the distinction between treatment induced disease stabilization and natural disease course may be difficult to make. • When evaluating tumor response on imaging, percentage tumor shrinkage as a continuous variable could be used, rather than categorical RECIST where an arbitrary cut-off of 30% decrease to define response may be more suited to conventional cytotoxics. • Metabolic response on functional imaging by FDG/PET. • Using each patient as internal control for evidence of drug activity: the ratio of time to progression (TTP) on experimental drug to TTP on last treatment (TTP_{n+1}/TTP_n), where $TTP_{n+1}/TTP_n \geq 1.3$ would suggest drug activity[89];

Table 6. Suggested modifications to the traditional trial design adapted to testing PI3K pathway inhibitors and other novel therapies

7. Conclusion

The PI3K pathway is emerging as an important and viable therapeutic target. However evidence for efficacy in ovarian cancer remains limited and predictive biomarkers to identify the patients most likely to benefit from this approach are desperately needed. Given the complexicity of the PI3K pathway and its cross-talk with other signaling networks, inhibiting a single member of the pathway may be insufficient to abrogate oncogenic signaling and result in meaningful tumor control. A number of resistance mechanisms to PI3K pathway inhibitors have been identified. Primary resistance may be attributable to co-existing KRAS or BRAF mutations; therefore concurrent PI3K and Mek inhibition in dual PI3K/KRAS mutated ovarian cancer may be worthy of investigation. In addition, treatment induced compensatory increases in alternate pathways (via IGF1R, MTORC2/Akt and others) may allow escape from selective mTOR targeting; response could be improved by appropriately designed combinatorial strategies. This suggests that abrogating adaptive escape pathways will require truly individualized treatment, selected on the basis of on-treatment tumor biopsies to identify the culprit compensatory pathways. A number of trials are ongoing exploring the benefit of combinations, unfortunately few are including correlative biological studies. Finally, for decades, ovarian cancer was treated as a uniform disease, a greater understanding of the biology of epithelial ovarian tumors has encouraged the initiation of a few histology-specific trials. The successful transition of novel PI3K pathway inhibitors from bench to the bedside of patients with ovarian cancer will depend on a greater integration of translation research in trial development. Efforts must be made to include comprehensive molecular profiling both at baseline and sequentially throughout the disease course, and studies investigating the usefulness of novel surrogate tumor markers such as ascites or circulating DNA will likely be essential.

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