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Tumor mutational analysis of GOG248, a phase II study of temsirolimus or temsirolimus and alternating megestrol acetate and tamoxifen for advanced endometrial cancer (EC): An NRG Oncology/Gynecologic Oncology Group study

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Abstract

Objective—Rapamycin analogs have reproducible but modest efficacy in endometrial cancer (EC). Identification of molecular biomarkers that predict benefit could guide clinical development.

Methods—Fixed primary tissue and whole blood were collected prospectively from patients enrolled on GOG 248. DNA was isolated from macro-dissected tumors and blood; next-generation sequence analysis was performed on a panel of cancer related genes. Associations between clinical outcomes [response rate (RR) 20%; progression-free survival (PFS) median 4.9

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ygyno.2016.02.025>.

months] and mutations (PTEN, PIK3CA, PIK3R1, KRAS, CTNNB1, AKT1, TSC1, TSC2, NF1, FBXW7) were explored.

Results—Sequencing data was obtained from tumors of 55 of the 73 enrolled pts. Mutation rates were consistent with published reports: mutations in PTEN (45%), PIK3CA (29%), PIK3R1 (24%), K-RAS (16%), CTNNB1 (18%) were common and mutations in AKT1 (4%), TSC1 (2%), TSC2 (2%), NF1 (9%) and FBXW7 (4%) were less common. Increased PFS (HR 0.16; 95% CI 0.01–0.78) and RR (response difference 0.83; 95% CI 0.03–0.99) were noted for AKT1 mutation. An increase in PFS (HR 0.46; 95% CI 0.20–0.97) but not RR (response difference 0.00, 95% CI –0.34–0.34) was identified for CTNNB1 mutation. Both patients with TSC mutations had an objective response. There were no statistically significant associations between mutations in PIK3CA, PTEN, PIK3R1, or KRAS and PFS or RR.

Conclusions—Mutations in AKT1, TSC1 and TSC2 are rare, but may predict clinical benefit from temsirolimus. CTNNB1 mutations were associated with longer PFS on temsirolimus.

1. Introduction

Endometrial cancer (EC) is the most common gynaecologic malignancy in the developed world and affects approximately 55,000 women in the United States each year [1]. Treatment options for advanced and recurrent endometrial cancer remain limited and there is a need for more efficacious, tolerable treatments [2].

The phosphoinositide 3-kinase (PI3K) pathway (Fig. 1) regulates key aspects of cancer biology including energy utilization, cellular proliferation, and survival (reviewed in [3]). Briefly, upon activation of receptor tyrosine kinases, PI3K phosphorylates the lipid phosphatidylinositol 4,5-bisphosphate (PIP2), creating phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 recruits the protein kinase AKT, to the membrane and AKT is phosphorylated and activated, in part, by mTOR complex 2 (mTORC2). Activated AKT phosphorylates and inhibits tuberous sclerosis complex 2 (TSC2) within the multi-protein TSC complex (which includes TSC1 and TSC2 gene products). The TSC complex indirectly inhibits mTOR complex 1 (mTORC1). Hence, PI3K-AKT signaling activates mTORC1, a key regulator of metabolism and cell growth processes. PTEN hydrolyzes PIP3 back to PIP2, deactivating the pathway. The PI3K pathway contains numerous negative feedback loops and is intricately networked with other signaling pathways including the RAS-ERK pathway. ECs harbor the highest rates of PI3K pathway alterations reported to date including PTEN (~50%), PIK3CA (~25%) and PIK3R1 (20%), and AKT (~5%) mutations [4–10].

The rapamycin analogs (rapalogs) directly bind and allosterically inhibit mTORC1, a downstream effector of PI3K signaling, and have reproducible but modest efficacy in EC [11–18]. Several studies have explored the association of molecular biomarkers and response to rapalogs in EC [19–21]. A combined analysis of three National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) protocols reported no association between clinical response rates and PTEN mutation or PIK3CA mutation. A Group d'Investigateurs Nationaux pour l'Etude des Cancers Ovariens (GINECO) report was consistent with this result and, in addition, reported an association of K-RAS mutation with a decreased progression-free and overall survival. More recently, CTNNB1 mutation has been reported

to show a trend towards association with PFS in a study of everolimus and letrozole. In tumors of cancers of other origin, TSC mutations have been associated with clinical response to a rapalog [22,23].

GOG 248 was a randomized phase II study of temsirolimus (Torisel, Pfizer) versus temsirolimus and alternating megestrol acetate and tamoxifen for advanced endometrial cancer [12]. After the first stage of accrual randomization to the arm combining hormonal therapy with temsirolimus was stopped due to an excess of venous thromboses and no evidence of superior activity. Accrual to single agent temsirolimus continued and subjects on the combination arm were allowed to either stay on the assigned regimen or cross over to single agent temsirolimus. Next generation sequencing analysis was performed samples that were prospectively collected from patients enrolled in the study, with the objective of exploring the association of genetic biomarkers with clinical response.

2. Methods

2.1. Sample collection

Fixed primary tissue and whole blood were collected prospectively from patients enrolled on GOG 248. DNA was isolated from macro-dissected tumor and matched whole blood specimens, if available. ER/PR IHC was performed as previously published [12].

Genomic Analysis: Next-generation sequence analysis was performed on a panel of 504 genes (Oncopane_v2) with relevance in cancer by the Center for Cancer Genome Discovery at the Dana Farber Cancer Institute. Briefly, 200 ng isolated DNA was fragmented to 250 bp, purified and ligated to specific adaptors during library preparation. Each library was made with sample-specific barcodes, quantified using qPCR, and 12–13 libraries were pooled into equimolar concentrations to a total of 500 ng for gene enrichment using the Agilent SureSelect hybrid capture kit (Agilent Technologies, Santa Clara, CA). All enriched libraries were sequenced on a HiSeq 2500 (Illumina Inc, San Diego, CA) to $23 \cdot 10^6$ reads on average per sample, resulting into an average sequencing coverage of 231 fold with over 88% of exons covered at 30× or higher. The reads were de-convoluted (de-multiplexed) and aligned to the reference sequence b37 edition from the Human Genome Reference Consortium. Variant analysis was performed using cancer-specific variant discovery tools (GATK) [24], including MuTect [25] for Somatic base substitutions and Somatic Indel Detector for insertions and deletions.

Genes with known relevance to PI3K/mTOR signaling, candidate genes were identified to explore clinical associations. These included: PTEN, PIK3CA, PIK3R1, KRAS, CTNNB1, AKT1, TSC1, TSC2, NF1, NF2, FBXW7. The PIK3CA, AKT1, KRAS, and CTNNB1 mutations were limited to known activating mutations. All non-synonymous changes were included for the other genes. Mutations in PIK3CA were further categorized by domain location; mutations in PTEN were further categorized by projected impact on protein function. As associative studies were not separated by treatment arms, estrogen-related genes were not included in the analysis.

2.2. Association studies

Associations between clinical outcomes RR and PFS were independently evaluated with each candidate gene. RR and PFS were not separated by treatment arm. RR was defined using RECIST v1.0. Clopper-Pearson (Exact) confidence limits [26] were constructed and provide at least 95% coverage for the true difference in response rate. Hazard ratios were estimated in a proportional hazards model. Profile-likelihood 95% confidence intervals [27] were constructed. Given the exploratory nature of this analysis there was no adjustment for multiple testing. Additional tests of independence between genes and protein expression was performed using Fisher's exact test. The data analysis presented was generated using SAS/STAT software, Version 9.3 of the SAS System for Windows.

3. Results

All specimens were prospectively collected from GOG 248. For subjects with tissue available the response rate (RR) was 20% overall and median progression-free survival (PFS) was 4.9 months. Of 73 patients enrolled (51 on temsirolimus; 22 on combination), 14 had no tumor specimens available (13 on temsirolimus; 1 on combination) and 4 had insufficient tumor available for genomic analysis (3 on temsirolimus; 1 on combination) leaving 55 samples that form the basis of this manuscript (35 on temsirolimus; 20 on combination).

Detailed mutational and associative analyses were limited to genes that are commonly altered in endometrial cancer as well as genes that encode proteins that comprise the PI3K/mTOR pathway. Overall, mutation rates were consistent with published reports (Table 1, Sup. Table 1, Sup Fig. 1) ([28]). Mutations in PIK3CA, the gene that encodes the catalytic subunit of PI3K, were identified in 16 cases. Of those, the majority were known protein activating mutations located within the kinase domain. Five mutations were identified in the adaptor binding domain (ABD), a region in which PIK3CA mutations have been commonly identified in endometrial cancer, but less commonly in other cancers in which PIK3CA mutations have been reported [8]. Thirteen tumors had mutations in PIK3R1, which encodes the regulatory subunit of PI3K. These mutations were predominantly insertions or deletions with associated frameshifts. Mutations in PTEN, the lipid phosphatase that inhibits PI3K pathway activation, were identified in 25 samples. PTEN mutations were further categorized by location and type of mutation. The majority of mutations are predicted to result in protein truncation and resultant loss of function. Additionally, 8 samples had point mutations that are known to impact phosphatase activity. Two activating mutations in AKT1, the predominant effector kinase of PI3K signals, were identified. NF, TSC1, TSC2 and FBX7 have been reported to regulate PI3K and mTOR signals. Mutations that would result in protein frameshift were identified. Some of these alterations have been identified as potential germline variants; these were included in the analysis recognizing that the functional impact of these genetic alterations remains speculative (Sup Table 1). No mutations were identified in NF2. Nine and ten activating mutations were identified in K-RAS and CTNNB1 genes respectively.

Clinical results have been previously reported [12]. As in the full clinical group, the majority of patients included in this analysis had high grade tumors, were in their 60's at enrollment, had good performance status and had previously received chemotherapy (Sup. Table 2).

The RECIST responses and median PFS by mutation and treatment arm are tabulated (Sup Tables 3 and 4). Given the limited size of the clinical study and the early termination of the combination arm, associative analyses were performed on the overall response rate and PFS in the study, not by specific treatment arm. Differences in response and progression/death hazard ratios with confidence intervals are plotted in Figs. 2 and 3, respectively. Overall, confidence intervals are wide, due to the limited numbers of patients or events in each subpopulation. There was no significant difference in outcome detected between patients with or without common mutations in PI3K pathway (e.g. PTEN, PIK3CA, PIK3R1); between patients with or without K-RAS mutations; nor between patients with or without the less common mutations (NF, TSC1/2, FBXW7). AKT1 mutations were associated with a higher rate of response and longer PFS. CTNNB1 mutations were associated with longer PFS; an association with response was not detected.

For further hypothesis generation, the molecular features and clinical outcomes were summarized (Fig. 4). The table denotes objective response and PFS of 15 months, a duration chosen as clinically meaningful as it was the median overall survival (OS) seen with paclitaxel/doxorubicin/cisplatin (TAP) when used as frontline therapy for metastatic disease [29]. There were three patients who had both an objective response and PFS for 15 months. Two patients had both AKT1 and CTNNB1 mutation; one was treated on the temsirolimus arm and one was treated on the temsirolimus with hormones arm. The third patient had a PIK3CA kinase domain mutation and was treated on the temsirolimus arm. Of the 6 patients with objective response (OR), 4 had an activating CTNNB1 mutation.

Patients with any PTEN mutation appear equally distributed between patients with clinical benefit and those without. PTEN truncation mutations are present in both sets of patients; there were no PTEN hotspot or other mis-sense mutations among the patients with OR or 15 months PFS. Of the 9 patients with K-RAS mutation, there was one patient with an OR. There were two patients identified with mutations in TSC1 and TSC2 that could impact TSC complex stability and function; both had an OR.

Immunohistochemical stains of PTEN, phosphorylated AKT, estrogen and progesterone receptors were also performed on patient samples and are depicted in Fig. 4. As previously reported, there were no significant associations with protein expression and clinical response on trial [12]. Exploratory associative analyses were performed between mutation status and protein expression. Using Fisher exact test between each gene (mutation vs no-mutation) and IHC expression result (present or absent), an association of PTEN mutation and the presence of PR expression was identified (two-sided $P < 0.001$) (data not shown).

4. Discussion

We report the results of genetic analysis of samples from participants enrolled on GOG-0248 and the association with clinical outcome. Targeted next generation sequencing was utilized

for the genetic analysis; this technology allows for full gene analysis (i.e. is not limited to “hotspot” mutations) as well as the parallel evaluation of multiple cancer-related genes. Mutations in PIK3CA, the gene that encodes the catalytic subunit of PI3K, and mutations in PTEN were not associated with objective response or prolonged progression-free survival in our study. This result is consistent with other reports in the literature. Combined analysis of the three National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) studies, each of which evaluated a rapalog as single-agent therapy in advanced endometrial cancer [19], showed no significant association between clinical response rates and PTEN mutation and PIK3CA mutation. Similarly, associative studies in a GINECO led study of everolimus detected no association with PIK3CA or PTEN mutation with clinical benefit (stable disease and objective response) [21].

With collection of whole gene sequencing data in this study, we further evaluated the type and location of mutations in both PIK3CA and PTEN. The distribution of PIK3CA mutations in endometrial cancer is distinct from cancers of other histological origin [8]. In addition to the common activation mutations found in the helical and kinase encoding domains of PIK3CA, in endometrial cancer, mutations are also enriched in the adaptor binding encoding domain. The adaptor binding protein domain interfaces with the regulatory subunit of PI3K, but its encoding domain is rarely evaluated in hot-spot mutation assays. There were no significant associations between the specific domain mutations and clinical benefit in these studies. However, while there were objective responses in patients with activating mutations in the kinase domain, there were none among patients with ABD mutations. This may be an area that warrants further exploration in pre-clinical and clinical studies. PTEN mutations were further categorized as mis-sense mutations or truncation mutations. The vast majority of the mis-sense mutations occurred at R130, which sits adjacent to the catalytic pocket; mutations at this location disrupt the ability of PTEN to hydrolyze PIP3 [30]. Interestingly, while truncation mutations in PTEN were found in patients with and without clinical benefit, there were no R130 mutations identified in the patients with RR or PFS > 15 months, perhaps suggesting different clinical effects of PTEN protein loss as compared to catalytic activity.

We identified two patients with activating AKT1 mutations; both patients had an objective response and PFS of 15 months, resulting in a statistically significant association of AKT1 mutation with RR and PFS in this study. Moreover, copy number analysis identified a single patient with high AKT1 copy number (data not shown). This patient also had an objective response. While the numbers remain small, given this binary result as well as the biological rationale, these data strongly support that AKT1 mutation may be a predictive marker for clinical benefit to temsirolimus in endometrial cancer. AKT1 mutation data was reported for the GINECO trial with everolimus; one AKT1 mutation was reported in a patient who was categorized as having clinical benefit (defined as stable disease or objective response) suggesting further support of these data [21].

In this study we identified a single patient with a mutation in TSC1 and a single patient with a mutation in TSC2; both patients had an objective response. Mutations in TSC have been identified as predictive markers for rapalog response in tumors of other histological origin. The TSC complex is a multi-protein complex and includes TSC1 and TSC2 proteins

and, through RHEB, activates mTOR. Germline mutations in TSC1 and TSC2 were identified as the causative mutation in tuberous sclerosis, a clinical syndrome associated with neurological deficits as well as tumors of the brain, kidney, and skin [31]. Studies of everolimus have demonstrated activity in subependymal giant cell astrocytoma tumors and renal angiomyolipomas that are associated with tuberous sclerosis [32,33]. In addition somatic mutations of TSC in bladder cancers and an anaplastic thyroid cancer have been associated with clinical response to everolimus [22,23]. Functional studies were not performed for this analysis. The identified TSC2 mutation is located in the GAP-like domain of the protein and has been described in patients with a clinical diagnosis of tuberous sclerosis [34]. The TSC1 mutation is predicted to result in a frameshift near the end of the protein. Speculatively, this change could result in de-stabilization of the TSC complex, but functional data on this specific mutation was not found in the literature (<http://chromium.liacs.nl/LOVD2/TSC/home.php>) [35].

In this study CTNNB1 mutations, which result in constitutively active β -catenin protein and ligand-independent Wnt pathway activation, were associated with improved PFS. Four of ten patients with this mutation achieved PFS of 15 months; two of these patients also had objective response. Of note, 3 of the 4 patients with this clinical benefit were treated in the temsirolimus only arm. An association of CTNNB1 mutation with OS has been reported in a study of everolimus and letrozole [16]. Our data may suggest that the potential predictive association with CTNNB1 mutation and treatment response may be due to mTOR inhibition, rather than hormonal manipulation. Alternatively, it remains possible that CTNNB1 is associated with a relatively indolent clinical course, though a recent publication suggests otherwise [36,37]. The signaling relationship between Wnt and mTOR pathways remains an emergent area of study. Further pre-clinical evaluation of the interaction of these signaling pathways in the context of endometrial cancer and mTOR inhibition is warranted.

In summary, we report the correlative studies from GOG-0248 a clinical study of temsirolimus with or without hormonal therapy. The association of RR and PFS with AKT1 mutations and objective responses in two patients with TSC1 and TSC2 mutations suggest that mutations that proximally activate mTOR may predict sensitivity to rapalogs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Conflict of interest

Andrea Myers reports grant funding from the Susan Smith Executive Fund for the conduction of the study and is currently a full time employee at Novartis. Ginny Filiaci reports grants from National Cancer Institute, during the conduct of the study.

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HIGHLIGHTS

- Mutational analysis of tumors from patients treated with temsirolimus is completed
- Associative analyses of PI3K/mTOR pathway gene mutations and outcomes are reported
- Downstream mutations in the pathway may predict clinical benefit from temsirolimus

Fig. 1.

Schematic of the PI3K/mTOR signaling pathway. Figure legend: upon receptor tyrosine kinase (RTK) stimulation, PI3K phosphorylates the lipid moiety PIP2, creating PIP3. AKT is recruited to the membrane, where it is phosphorylated and activated by the mTORC2 complex and PDK. AKT phosphorylates and inhibits the TSC complex, which permits the activation of mTORC1 by RHEB GTPase. The RTK pathway also activates the RAS-ERK pathway and feeds into TSC1/2 regulation. PTEN is a phosphatase and converts PIP3 back to PIP2, inhibiting PI3K signals.

Fig. 2.
Difference in proportion responding between mutation status groups with exact 95% confidence intervals.

Fig. 3. Progression-free survival hazard ratio estimate (mutation vs. no mutation) and profile likelihood 95% confidence limits.

Fig. 4. Summary of clinical and molecular results by individual patient, ordered by clinical benefit. T = temsirolimus arm; TTM = temsirolimus, tamoxifen, megace arm; EG1, EG2, EG3 = endometrial grades 1, 2, 3; ADC = adenocarcinoma; MUC = mucinous; MXD = mixed histology; UPS = uterine papillary serous; CC = clear cell; SQM = squamous; UDF = undifferentiated.

Table 1

Number and rates of genetic mutations in analyzed samples.

Gene	N	%
PTEN	25	45.5
PTEN - point mutation (including R130)	10	18.2
PTEN - truncation	17	30.9
PTEN - R130 missense	8	14.5
PIK3CA	16	29.1
PIK3CA - ABD	5	9.1
PIK3CA - helical	1	1.8
PIK3CA - kinase	9	16.4
PIK3CA - other	3	5.5
PIK3R1	13	23.6
CTNNB1	10	18.2
KRAS	9	16.4
NF1	5	9.1
AKT1	2	3.6
FBXW7	2	3.6
TSC1	1	1.8
TSC2	1	1.8