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Phase II trial of the mTOR inhibitor, temsirolimus and evaluation of circulating tumor cells and tumor biomarkers in persistent and recurrent epithelial ovarian and primary peritoneal malignancies: a Gynecologic Oncology Group study

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Abstract

Purpose—Patients with persistent/recurrent epithelial ovarian cancer/primary peritoneal cancer (EOC/PPC) have limited treatment options. AKT and PI3K pathway activation is common in EOC/PPC, resulting in constitutive activation of downstream mTOR. The GOG conducted a phase II evaluation of efficacy and safety for the mTOR inhibitor, temsirolimus in EOC/PPC and explored circulating tumor cells (CTC) and AKT/mTOR/downstream tumor markers.

Methods—Eligible women with measurable, persistent/recurrent EOC/PPC who had received 1–3 prior regimens were treated with 25 mg weekly IV temsirolimus until progression or intolerable toxicity. Primary endpoints were progression-free survival (PFS) ≥6-months, tumor response, and toxicity. CellSearch® system was used to examine CTC, and AKT/mTOR/downstream markers were evaluated by archival tumor immunohistochemistry. Kendall's tau-b correlation coefficient

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Temsirolimus was provided by the Cancer Therapy Evaluation Program of the National Cancer Institute (NCI-CTEP). The study involves original work which was presented in part at the 41st Annual Meeting of the Society of Gynecologic Oncologists, San Francisco, CA, March 13–17, 2010.

CONFLICT OF INTEREST

The authors wish to report that there are no conflicts of interest.

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(r) and Cox regression modeling were used to explore marker associations with baseline characteristics and outcome.

Results—Sixty patients were enrolled in a two-stage sequential design. Of 54 eligible and evaluable patients, 24.1% (90%CI 14.9%–38.6%) had PFS ≥6 months (median 3.1 months), 9.3% (90%CI 3.7%–23.4%) experienced a partial response. Grade 3/4 adverse events included metabolic(8), gastrointestinal(8), pain(6), constitutional(5) and pulmonary(4). Suggested associations were between cyclin D1 and PFS ≥6 months, PFS or survival; positive CTC pre-treatment and lack of response; and high CTC expression of M30 and PFS ≥6 months/longer PFS.

Conclusions—Temsirolimus appears to have modest activity in persistent/recurrent EOC/PPC; however, PFS is just below that required to warrant inclusion in phase III studies in unselected patients. Cyclin D1 as a selection marker and CTC measures merit further study.

Keywords

Circulating tumor cells; ovarian clinical trial; AKT/PI3K pathway; mTOR inhibitor

INTRODUCTION

Ovarian cancer is the second most common gynecologic malignancy and the fifth leading cause of cancer death among American women[1]. Most women are diagnosed in advanced stages and undergo surgical debulking followed by platinum\taxane-based chemotherapy. Initial complete response to therapy can be achieved; however, relapse is common and it is almost universally fatal[2,3].

The mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase that is expressed in all mammalian cells[4]. mTOR is a central switch balancing input from growth factors such as receptor tyrosine kinases with input from nutrient availability sensors to coordinate a series of downstream genes such as the p70-S6 protein kinase (p70-S6K) and 4E-BP1 family of proteins[4]. For example, phosphorylation of p70-S6K leads to phosphorylation of the S6 ribosomal protein which induces protein synthesis at the ribosome, stimulating mTOR activity, and regulating cell growth, whereas phosphorylation of 4E-BP1 results in dissociation from the eukaryotic translation initiation factor 4E (eIF4E) and translation of cell growth control genes, including cyclin D1. In this way, constitutive action of the mTOR pathway via overexpression of its components or inactivating mutations of its inhibitors can lead to carcinogenesis via uncontrolled growth.

Recent studies, both in laboratory models and in clinical trials, have demonstrated the phosphatidylinositol-3-kinase(PI3K)/AKT/mTOR pathway is activated in cancers and its inhibition may be an effective strategy[5–14]. Approximately 40% of ovarian cancers have increased AKT2 activity[5,7]. Downstream of AKT, phosphorylated (*p*)mTOR and *p*GSK-3β correlate with AKT activation status in >80% of ovarian cancers, validating activation of the PI3K/AKT pathway[7]. Thus, pharmacologic agents that inhibit downstream targets of the PI3K/AKT signaling pathway such as mTOR might preferentially kill tumor cells having AKT dependence for survival not shared by normal cells[8]. Specific small molecule inhibitors including rapamycin or rapamycin analogues such as temsirolimus, everolimus (RAD001) and ridaforlimus (formerly deferolimus, AP23573) prevent mTOR activity by binding to the mTORC1 complex (mTOR and the FK506 binding protein-12) but not the mTORC2 complex[15]. Temsirolimus 25 mg weekly IV (flat dose) was selected for this trial due to a favorable pharmacokinetic profile, reduced immunosuppressive properties as compared to rapamycin[16], and extensive experience in phase I and phase II trials[10–12,17–18].

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Study of the specific pathway effects of targeted agents in ovarian cancer is hampered by the lack of easy access to tissue for repeat sampling. Circulating tumor cells (CTC) can be detected in the blood of cancer patients using the CellSearch® system whereas they are absent in healthy controls[19]. Furthermore, the presence of CTC is an independent prognostic marker for several cancer types including breast[20–22], prostate[23,24] and colorectal cancers[25–27]. Although CTC have been enriched from the blood of ovarian cancer patients by flow cytometry[28,29], the prognostic/predictive value of CTC enumeration in epithelial ovarian cancer/primary peritoneal cancers (EOC/PPC) has not been assessed. Additionally, CTC can be queried for the effect of targeted agents[30].

In this phase II trial of the efficacy and safety of temsirolimus in persisent/recurrent EOC/PPC, we also explored the AKT/mTOR pathway and downstream targets including p70-S6K, 4E-BP1 and cyclin D1 in archival tumor, and also recovered and characterized CTC for enumeration and expression of potential biomarkers indicative of temsirolimus therapeutic effects, including *p*S6 ribosomal protein via immunofluorescent techniques[30] and apoptosis using M30, a biomarker indicating cytokeratin degradation[31].

MATERIALS AND METHODS

Patients

Women with recurrent/persistent EOC/PPC on review of the surgical pathology report and with RECIST measurable disease who had received 1–3 prior cytotoxic regimens were eligible. Patients must have had at least one prior platinum-based regimen and, if enrolled following primary treatment, must have had a platinum-free interval of <12 months. Cytostatic agents were allowed with primary treatment but not for recurrences. Patients must have had adequate bone marrow function with absolute neutrophil count ≥1,500/mcl, platelets ≥100,000/mcl as well as creatinine ≤1.5x institutional upper limit of normal (ULN), bilirubin ≤1.5xULN, SGOT and alkaline phosphatase ≤2.5xULN, neuropathy ≤ Common Criteria for Adverse Events (CTCAE) v3.0 grade 1, fasting cholesterol <350 mg/dl, and fasting triglycerides <400 mg/dl. Patients who had received radiation to more than 25% of marrow-bearing areas or a history of other invasive malignancies were excluded. This study was approved by the regulatory agencies and Institutional Review Boards at participating institutions. All patients provided written informed consent consistent with federal, state, and local requirements.

Treatment and Dose Modifications

Patients were treated by Gynecologic Oncology Group (GOG) member institutions with 25 mg weekly IV temsirolimus provided by the National Cancer Institute Cancer Therapy Evaluation Program (NCI-CTEP), in 28-day cycles until disease progression or toxicity. Subsequent cycles were delayed to a maximum of three weeks to allow bone marrow recovery and prophylactic granulocyte colony-stimulating factor (GCSF) was not allowed. Dose reductions to 15 mg IV (−1) and 10 mg IV (−2) were instituted with grade 4 thrombocytopenia. Treatment was discontinued if pneumonitis related to temsirolimus was suspected. Non-hematologic grade 2 or greater (peripheral neuropathy, renal and other) toxicities and grade 3 liver enzyme elevations required treatment delay for up to three weeks to allow recovery to ≤ grade 1 and one level dose reduction. Nausea, emesis, diarrhea, constipation, electrolyte abnormalities, hyperglycemia and hyperlipidemia were managed with supportive therapies without dose reduction.

Immunohistochemistry for AKT/ /mTOR/downstream biomarkers

Archival formalin-fixed and paraffin-embedded tumor were obtained from a previous surgery or biopsy and analyzed (see Supplemental Materials) by immunohistochemistry for

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*p*AKT (DAKO, clone 14-5, *p*S473), *p*mTOR (Cell Signaling, clone 49F9, pS2448), *p*p70-S6 (Cell Signaling, clone IA5, *p*T389), *p*4E-BP1 (Cell Signaling, clone 236B4, *p*T37/46), and cyclin D1 (DAKO, clone SP4) by blinded reviewers (KCQ, AKG).

Evaluation of Circulating Tumor Cells

CTC enrichment, enumeration and phenotypic characterization were performed (see Supplemental Materials) pre-cycle 1, 2 and 3 using the CellSearch® System (Veridex, Raritan NJ)[32].

Statistical Considerations and End Points

Response was assessed by physical examination every 4 weeks and by radiologic studies every other cycle. Activity by either RECIST tumor response or progression-free survival ≥6 months (PFS at 6 months) was considered sufficient to declare the drug worthy of further investigation in a phase III trial. The probability of a type I error (i.e. alpha) was controlled with a bivariate 2-stage design[33]. The probability of early termination when the agent is inactive was approximately 50%, depending in part on the degree of association between response and PFS at 6 months.

The design targeted 23 patients for entry to the first stage but allowed deviation to account for non-eligible patients. The cumulative targeted sample size for the second stage was 52 patients but was allowed to deviate as before. Advancement to second stage occurred with rules as detailed in the results section. With 54 patients accrued, the design required more than 8 patients with tumor responses or more than 13 patients with PFS at 6 months before declaring the agent worthy of phase III investigation. The design had approximately 10% power when the regimen had $\pi_r \leq 0.10$ and $\pi_s \leq 0.15$ where π_r and π_s are the probabilities of a patient having a response and being PFS at 6 months respectively. The null probabilities were obtained from an analysis of historical controls[34–44]. The design had approximately 90% power when $\pi_r = 0.25$ or $\pi_s = 0.35$, which were deemed to be minimally clinically significant values. The frequency/severity of adverse events were evaluated with CTCAEv3 and events deemed at least possibly related to the regimen were tabulated.

The correlations among the five tumor markers and three CTC measures, and associations between the eight biomarkers and six baseline characteristics (some data presented in Table S1 in Supplemental Materials) or two categorical measures of outcome were assessed with Kendall's or Spearman's correlation coefficient, Fisher's Exact Test or an Exact Chi-Square test.[45–48] Kaplan-Meier method and Cox proportional hazards models were used to examine associations between the eight biomarkers and PFS and overall survival (OS)[49,50]. Suggested associations were assessed by any test with $p<0.05$ for the purpose of hypothesis generation and to prioritize further testing. The statistical power of these exploratory biomarker assessments was low due to small sample sizes.

RESULTS

Of the 60 patients enrolled, six were excluded due to improper prior treatment (n=3), inadequate tests (n=1), no measurable lesions (n=1) and patient refused all treatment (n=1) leaving 54 evaluable for efficacy and toxicity. Twenty-five patients were accrued during the first stage, and more than two responses or more than five PFS at 6 months were required to open to a second stage. First stage response/PFS criteria were met after interim analysis with three responses and seven PFS at 6 months, and the study completed second stage accrual. Patient characteristics are provided in Table 1. Median age for the group was 62 years and 90.7% were Caucasian. All patients had previous surgery, and the majority had recurrent

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serous ovarian cancer. Up to three courses of cytotoxic chemotherapy were allowed and 25.9% of patients had three courses of prior chemotherapy.

Distribution of completed cycles as well as response and follow-up data are reported in Table 1. Median number of cycles was 2.5 with 3 patients receiving 10 or more cycles (range 1–24 cycles). Dose reductions per protocol were necessary in 27/54 (50%) of patients. There were no dose re-escalations. Partial responses were seen in 5 of patients (9.3%; 90% CI 3.7%–23.4%). PFS at 6 months was also used as an efficacy endpoint and 13 (24.1%; 90% CI 14.9%–38.6%) achieved this outcome. Three patients had clear cell histology of which one had a partial response lasting 4 months. Kaplan-Meier Curves for PFS and OS are shown in Figure 1A. Median PFS and OS were 3.2 and 11.6 months respectively. The longest PFS was 21 months in a 53 year old with stage IIIC platinum-resistant ovarian cancer who received 24 courses at 25 mg/week with no dose reductions/ delays. At 9.8 months of median follow-up, 44.4% of patients were alive, all with disease.

Adverse events (AE) encountered on study were as expected for mTOR inhibitors and are detailed in Table 2. Notable grade 3 AE consisted of 4/54 constitutional (all fatigue), 6/54 gastrointestinal (2 mucositis, 2 dehydration and 2 bowel obstruction), and 8/54 metabolic (2 hypokalemia, 2 hypertriglyceridemia, 2 liver enzyme elevations, one hypophosphatemia and one hyperglycemia). One grade 3 renal failure was reported. Study drug was discontinued in 3 patients because of grade 3 pulmonary infiltrates thought to be related to study drug and to represent interstitial pneumonitis. The grade 4 constitutional AE consisted of asthenia and fatigue while the grade 4 vascular AE consisted of a pulmonary embolus.

Immunohistochemistry assays were performed for pAKT^{S473}, pmTOR^{S2448}, pp70-S6K^{T389}, p4E-BP1$^{T37/46}$ and cyclin D1 in archival tumor to explore whether activation of the AKT/ mTOR signaling pathway was associated with measures of clinical outcome following temsirolimus treatment (Table 3, Figure 1B–1G). Cyclin D1 expression was observed in 42% of the archival tumors and appeared to be correlated with PFS ≥6 months (r=0.281) but not tumor response (Table 3), and to be associated with a reduced risk of progression (Figure 1B; hazard ratio [HR]=0.495, 95% confidence interval [CI]=0.260–0.943) and longer OS (Figure 1C).

CTC were enriched in 19/43 (44%), 11/38 (29%) and 14/31 (45%) in pre-cycle1, 2 and 3 blood specimens with counts ranging from 1–11, 1–84, and 1–190, respectively. Positive CTC pre-cycle 1 appeared to be associated with increasing disease (r=0.340) but not with PFS ≥6 months (Table 3) or shorter PFS (Figure 2A). Positive CTCs persisted in 8 patients (4 increasing disease; 4 stable disease). High M30 and pS6 were both defined as ≥75% positive CTC. Of the cases with positive CTC counts pre-cycle1, high M30 (a marker of apoptosis) was observed in 10/19 with levels ranging from 17–100% and was suggestively correlated with PFS ≥6 months (r=0.683; Table 3). Figure 2B is consistent with the dichotomized PFS findings but the log-rank test was not suggestive. Of the cases with positive CTC counts pre-cycle1, high pS6 expression was observed in 12/17 patients but did not appear to be associated with any measure of clinical outcome (Table 3 and Figure 2C). Landmark analyses did not indicate that changes in CTC counts were associated with PFS (Figure 2D) or OS; however, the sample sizes were small, giving the tests low power. Paired pre-cycle1 and 2 assessments were available for four patients for M30 (all increasing disease) and three patients for pS6 (2 increasing disease; 1 stable disease), and it was not possible to explore the changes in M30 and pS6 with tumor response.

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DISCUSSION

A variety of targeted agents have been tested in the phase II setting in ovarian cancer patients with multiple prior chemotherapy regimens[51,52]. Of these, only vascular endothelial growth factor (VEGF) inhibitors such as bevacizumab have been actively included in subsequent phase III trials of untreated patients. Bevacizumab was shown to have a 21% clinical response rate, including complete responses, and a 40% PFS at 6 months in a phase II single agent study with similar (albeit 2 prior chemotherapeutic agents as compared to 3 in this study) persistent/recurrent EOC/PPC patients as those reported here[53]. A PFS advantage was recently reported for carboplatin/paclitaxel with bevacizumab versus placebo from cycles 2 through 22 in a GOG phase III trial[54]. Meanwhile, the search continues for other biologic agents with single agent, additive or synergistic activity in EOC/PPC.

Results of other targeted agents in persistent/recurrent EOC/PPC including GOG phase II evaluations of single agent bortezomib[55], trastuzumab[56], gefitinib[57], imatinib[58], sorafenib[59,60], and enzastaurin[61] have not reached the level of interest seen with bevacizumab[53,54,62]. Given the preclinical activity, the paucity of activity of targeted agents in EOC/PPC other than those targeting VEGF is perplexing[7]. In this study, the 9.3% response rate and 24.1% PFS at 6 months of the mTOR inhibitor temsirolimus in EOC/PPC is second only to bevacizumab in the GOG Phase II series. However, this response is below and the PFS is just at the threshold for the response/PFS endpoint and is insufficient to warrant addition to upfront regimens in a phase III study. We recognize that the study population was unselected and subgroups may show greater benefit if selected by specific response markers. Additionally, such as seen in metastatic renal cell cancers treated with temsirolimus, an overall survival benefit may exist even without differences in response rates[63]. One purpose of translational research in the context of phase II studies may be to identify appropriate selection markers for targeted agents. In addition, since mTOR inhibitors can synergize with chemotherapy in ovarian cancer models[13,14], phase II single agent activity may not be reflective of activity in combination with chemotherapy.

The current study suggested an association between cyclin D1 expression with high p4E-BP1 in tumors, a greater likelihood of being PFS ≥6 months, and overall longer PFS and survival following temsirolimus treatment. Several studies have shown that 4E-BP1 and cyclin D1 are downstream of mTOR activation[64–66], and that the anti-proliferative activity of mTOR inhibitors including temsirolimus are mediated by acceleration of cyclin D1 turnover, up-regulation of p27 expression, inhibition of cyclin-dependent kinase activation and retinoblastoma protein phosphorylation, as well as stabilization of 4E-BP1 binding to eIF4E preventing translation of genes stimulating cell growth including cyclin D1[64–70]. mTOR inhibitors also appear to exhibit anti-neoplastic activity via inhibition of translation of pro-angiogenic factors, pro-survival proteins, and proteases involved in tumor invasion and metastasis, and by disruption of metabolic processes that fuel tumor progression[64–70].

Temsirolimus was not active in >75% of persistent/recurrent EOC/PPC in this trial. Resistance to the targeted agent is a possible cause for the low response rate and proportion of women PFS at 6 months. For example, mTORC1 is sensitive whereas mTORC2 is resistant to rapamycin[64–70]. Indeed, development of rapid resistance to mTORC1 inhibition (with rapamycin) has been shown in glioblastoma mutliforme via reflex upregulation of *p*AKT^{S473}, an activating substrate of mTORC2[71]. Second generation inhibitors of mTOR, referred to mTOR kinase domain (mTORK) inhibitors, are now available that target rapamycin-resistant pathways by inhibiting the ATP site of both mTORC1 and mTORC2[68–70]. mTORK inhibitors have yet to be evaluated in EOC/PPC. Additionally, there is redundancy in signaling pathways and one site of inhibition may be insufficient to block cellular processes driving tumor progression. Funding was not available for a

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comprehensive analysis of the mTOR pathway in this multi-institutional phase II trial of single agent temsirolimus[72].

The informed development and evaluation of targeted agents would benefit from the availability of serially assayed non-invasive predictive biomarkers. CTC have been documented in breast cancers where their enumeration is prognostic[20–22,73], but have also been documented in colorectal cancers[25–27], prostate cancers[23–24], and other cancers[74–77] but not in healthy subjects or patients with non-malignant diseases[78]. CTC have been identified in ovarian cancers[28,29], but their relation to prognosis has not been reported. In the current study, the CellSearch® system was employed due to the improved accuracy and sensitivity compared with flow cytometry methods[19]. At least one tumor cell was identified from blood in up to 45% of patients and a suggested associated was seen between pre-cyle1 CTC and progressive disease

Studies to detect molecular events in CTC are accumulating for many malignancies[31,76,79]. We examined a selective marker of apoptosis using M30 which recognizes a caspase-cleaved epitope of cytokeratin-18[27] and *p*S6[2626] a substrate of p70-S6K. Suggested associations were observed pre-treatment between positive CTC and progressive disease, high M30 and PFS ≥6 months, and high *p*S6 with fewer prior regimens. In the absence of robust anti-tumor activity, it is not possible to definitively state that these associations are due to treatment and not disease predictors of prognosis. However, these exploratory findings support the continued development of CTC analysis and phenotypic characterization of CTC as translational endpoints in EOC/PPC. In the current study, serial CTC assessments did not appear to change notably following treatment; however, serial CTCs were not acquired in the majority of patients. Emphasis on the ability to phenotypically characterize CTC may increase enthusiasm for specimen submission. Additional CTC studies and further optimization and standardization of techniques (CellSearch® vs. other methods) are needed to define the clinical value of predictive/prognostic CTC assessments in EOC/PPC.

In conclusion, temsirolimus appears to have modest activity, but insufficient to justify further study in a phase III study in unselected EOC/PPC. Exploratory findings regarding cyclin D1 expression in tumors as a selection marker for mTOR inhibitor treatment and CTC measures in blood merit further study in this disease.

Research Highlights

- Single agent temsirolimus exhibited modest activity in unselected patients with persistent or recurrent epithelial ovarian/peritoneal cancer with 24% of women progression-free ≥6 months and 9% experiencing a partial response.
- Positive tumor expression of cyclin D1 appeared to be associated with high p4E-BP1 in tumors, a greater likelihood of being PFS ≥6 months, longer PFS and longer survival following temsirolimus treatment.
- Detectable circulating tumor cells pre-treatment appeared to be associated with lack of response to temsirolimus.

Supplementary Material

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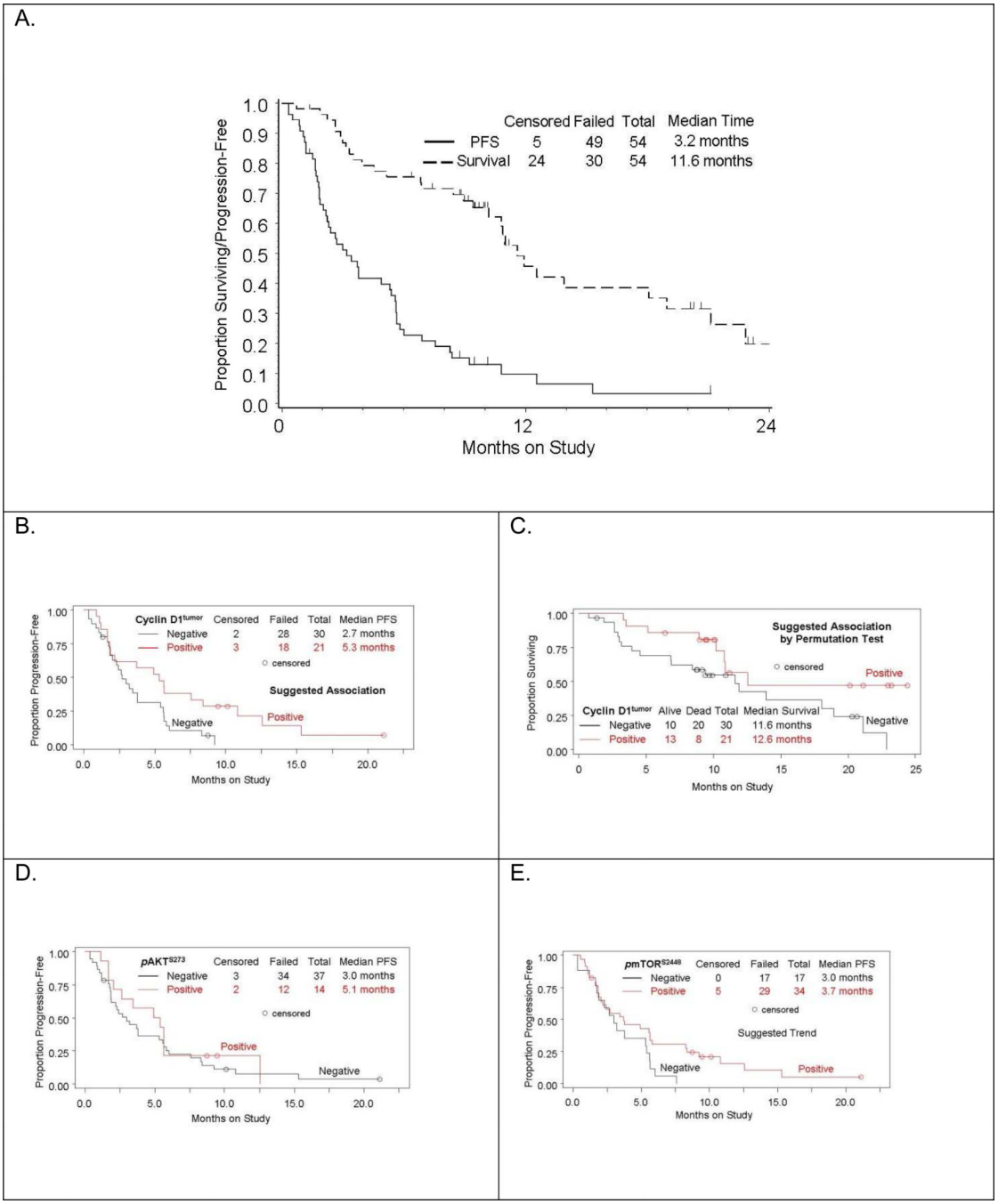

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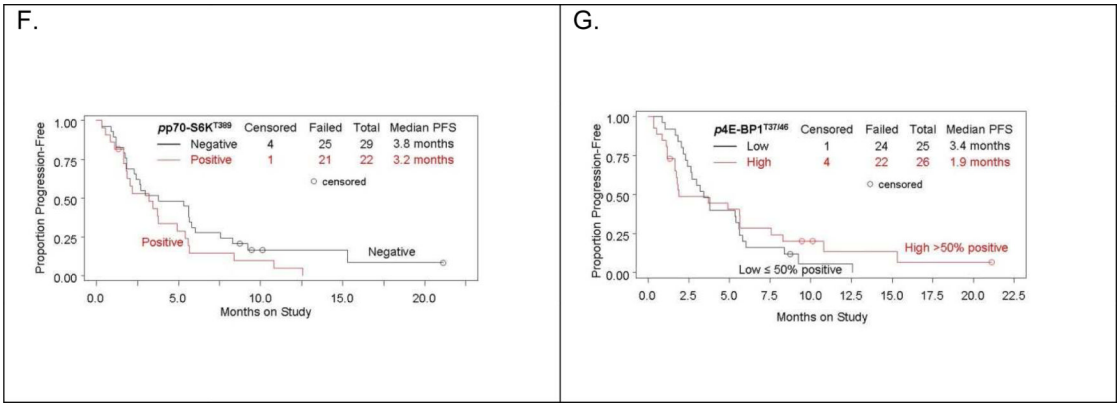

Figure 1.

Kaplan-Meier progression-free survival (PFS) and overall survival (OS) distributions for persistent/recurrent epithelial ovarian cancer or primary peritoneal cancer (A). Kaplan-Meier plots for progression-free survival (PFS)(B) and overall survival (OS)(C) by tumor expression of cyclin D1 categorized as negative or positive, and for progression-free survival by tumor expression of *p*AKT (D), *p*mTOR (E), *p*p70-S6K (F) categorized as negative or positive, or *p*4E-BP1 (G) categorized as low (<50% positive tumor cells) or positive (≥50% positive tumor cells) following temsirolimus treatment. Suggestive associations were assessed by logrank test with $p<0.05$.

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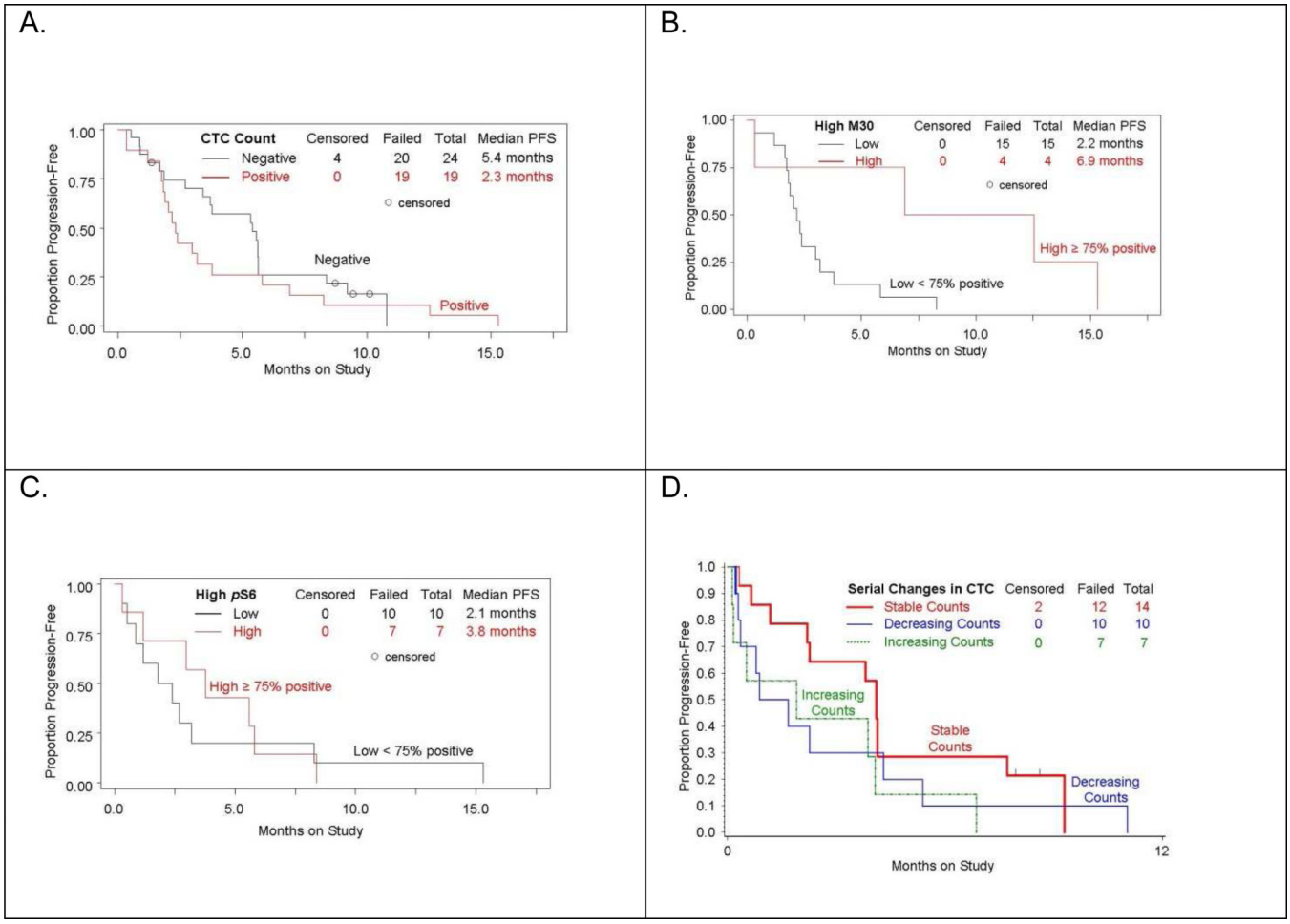

Figure 2.

Kaplan-Meier progression-free survival (PFS) distributions for pre-treatment circulating tumor cells (A), pre-treatment circulating tumor cell (CTC) expression of the apoptotic marker M30 (B) or pre-treatment CTC expression of *p*S6 (C), or change in CTC counts from pre-cycle 1 to pre-cycle 2 (D) following temsirolimus treatment. Circulating tumor cells (CTC) counts were categorized as negative or positive. M30 and *p*S6 were categorized as low (<75% positive CTC) or high (≥75% positive CTC). Serial changes in CTC were categorized as stable counts, decreasing counts or increasing counts. Suggestive associations were assessed by logrank test with $p<0.05$.

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Table 1

Characteristics of eligible and evaluable enrolled patients (n=54) as well as treatment cycles, response rates and follow up data.

Characteristic	Category	No	%
Age	20–29	2	3.7
	40–49	7	13.0
	50–59	15	27.8
	60–69	11	20.4
	70–79	16	29.6
	80–89	3	5.6
Race	Unspecified	1	1.9
	African American	4	7.4
	Caucasian	49	90.7
Ethnicity	Hispanic	0	0.0
	Non-Hispanic	41	75.9
	Unknown	13	24.1
Performance Status	0	37	68.5
	1	17	31.5
Site of Disease	Ovary	46	85.2
	Peritoneum	8	14.8
Cell Type	Adenocarcinoma, Unsp.	8	14.8
	Clear Cell Carcinoma	3	5.6
	Endometrioid Adenocarcinoma	4	7.4
	Serous Adenocarcinoma	39	72.2
Grade	1: Well differentiated	2	3.7
	2: Moderately differentiated	11	20.4
	3: Poorly differentiated	41	75.9
Prior Chemotherapy	1	16	29.6
	2	24	44.4
	3	14	25.9
Prior Radiation	No	50	92.6
	Yes	4	7.4
Prior Surgery	Yes	54	100.0
Response	Partial response	5	9.3
	Stable disease	22	40.7
	Increase disease	21	38.9
	Indeterminate	6	11.1
PFS > 6 Months	No	40	74.1
	Yes	13	24.1
	Unknown (Pt. Lost)	1	1.9
Cycles of Treatment	1	12	22.2
	2	15	27.8
	3	4	7.4
	4	9	16.7
	5	2	3.7
	6	7	13.0
	9	2	3.7
	10+	3	5.6
Alive	Without progression	5	9.3
	With progression	19	35.2
Dead	From disease	29	53.7
	From neither Rx nor disease	1	1.9

Proportion of patients with tumor responses was 9.3%. Approximate 90% marginal confidence interval (CI) was 3.7%, 23.4%. Proportion of patients with progression free survival (PFS) at 6 months was 24.1%. Approximate 90% marginal CI was 14.9%, 38.6%.

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Table 2

Grade and number of patients experiencing Common Criteria or Adverse Events (CTCAE) v3 reported adverse events(n=54).

	CTCAEv3 Grade*				
	1	**2**	**3**	**4**	**5**
Leukopenia	14	14	0	0	0
Thrombocytopenia	18	5	0	0	0
Neutropenia	6	12	1	0	0
Anemia	20	19	3	0	0
Other Hematologic	0	2	0	0	0
Allergy/Immunology	1	0	0	0	0
Auditory/Ear	1	0	0	0	0
Cardiac	3	1	1	0	0
Coagulation	2	1	0	0	0
Constitutional	23	15	4	1	0
Dermatologic	24	10	1	0	0
Nausea	19	2	2	0	0
Vomiting	2	3	1	0	0
Gastrointestinal	16	19	6	0	0
Genitourinary/Renal	4	0	1	0	0
Hemorrhage	5	0	0	0	0
Infection	0	5	2	0	0
Lymphatics	3	1	1	0	0
Metabolic	17	12	8	0	0
Musculoskeletal	2	0	1	0	0
Neurosensory	6	2	0	0	0
Other Neurological	5	1	0	0	0
Ocular/Visual	1	0	0	0	0
Pain	13	7	6	0	0
Pulmonary	17	5	4	0	0
Sexual/Reproductive	1	0	0	0	0

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	CTCAEv3 Grade*				
	1	2	3	4	5
Vascular	0	0	0	1	0

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Table 3

Relationship between pre-treatment biomarkers[†] and tumor response[‡] or proportion experiencing progression-free survival (PFS) ≥6 months.

Biomarkers[†]	Tumor Response[‡]			PFS ≥6 months	
	No		Yes		
	ID+NE	SD	PR+CR	No	Yes
Tumor Expression					
***p*AKT**					
Negative	19 (51.4)	14 (37.8)	4 (10.8)	27 (75.0)	9 (25.0)
Positive	5 (35.7)	8 (57.1)	1 (7.1)	11 (78.6)	3 (21.4)
***p*mTOR**					
Negative	10 (58.8)	14 (41.2)	1 (5.9)	15 (88.2)	2 (11.8)
Positive	14 (41.2)	16 (47.1)	4 (11.8)	23 (69.7)	10 (30.3)
***p*p70-S6K**					
Negative	13 (44.8)	13 (44.8)	3 (10.3)	20 (69.0)	9 (31.0)
Positive	11 (50.0)	9 (40.9)	2 (9.1)	18 (85.7)	3 (14.3)
***p*4E-BP1**					
Low (≤ 50% +)	9 (36.0)	14 (56.0)	2 (8.0)	20 (80.0)	5 (20.0)
High (> 50% +)	15 (57.7)	8 (30.8)	3 (11.5)	18 (72.0)	7 (28.0)
Cyclin D1				**Suggested Association** *	
Negative	15 (50.0)	13 (43.3)	2 (6.7)	**25 (86.2)**	**4 (13.8)**
Positive	9 (42.9	9 (42.9)	3 (14.3)	**13 (61.9)**	**8 (38.1)**
CTC Count	**Suggested Association** *				
Negative	**7 (29.2)**	**14 (58.3)**	**3 (12.5)**	17 (73.9)	6 (26.1)
Positive	**12 (63.2)**	**6 (32.6)**	**1 (5.2)**	15 (78.9)	4 (21.1)
CTC Expression					
M30				**Suggested Association** *	

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Biomarkers†	Tumor Response‡			PFS ≥6 months	
	No		Yes		
	ID+NE	SD	PR+CR	No	Yes
Low (< 75% +)	10 (66.7)	4 (26.7)	1 (6.7)	**14 (93.3)**	**1 (6.7)**
High (≥ 75% +)	2 (50.0)	2 (50.0)	0 (0.0)	**1 (25.0)**	**3 (75.0)**
***p*S6**					
Low (< 75% +)	5 (50.0)	4(40.0)	1(10.0)	8 (80.0)	2 (20.0)
High (≥ 75% +)	3 (42.9)	2 (28.6)	2 (28.6)	6 (85.7)	1 (14.3)

Number of cases (row percentages)

†Immunohistochemistry assays were performed to examine expression of *phosphorylated* (*p*)AKT^{S273}, *p*mTOR^{S2448}, *p*p70-S6K^{T389}, *p*4E-BP1$^{T37/46}$, and cyclin D1 in archival tumor. Circulating tumor cells (CTC) were enriched and characterized using the CellSearch® system (Veridex, Raitan NJ) for enumeration and expression of the apoptotic markers M30 and pS6.

‡Tumor response was categorized as no for increasing disease and not evaluable (ID+NE) plus stable disease (SD) versus yes for partial response and complete response (PR+CR).

*Suggested Kendall's tau-b correlations (r) were observed between cyclin D1 and PFS ≥ 6 months (r=0.281); and positive CTC and progressive disease (versus not) (r=0.340). The correlation between M30 and PFS ≥ 6 months was high (r=0.683). Fisher's Exact Test suggested an association between M30 and PFS ≥ 6 months (odds ratio = 42; 90% CI 1.8 to 1150).

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