Published in final edited form as:

Lancet Oncol. 2011 November ; 12(12): 1109–1117. doi:10.1016/S1470-2045(11)70244-3.

Phase 1–2 study of docetaxel plus aflibercept in patients with recurrent ovarian, primary peritoneal, or fallopian tube cancer

Robert L Coleman, Linda R Duska, Pedro T Ramirez, John V Heymach, Aparna A Kamat, Susan C Modesitt, Kathleen M Schmeler, Revathy B Iyer, Michael E Garcia, Debbie L Millera, Edward F Jackson, Chaan S Ng, Vikas Kundra, Robert Jaffe, and Anil K Sood
 Department of Gynecologic Oncology and Reproductive Medicine (Prof R L Coleman MD, Prof P T Ramirez MD, A A Kamat MD, K M Schmeler MD, M E Garcia RN, D L Miller RN, Prof A K Sood MD), Department of Thoracic/Head and Neck Medical Oncology (J V Heymach MD), **Department of Diagnostic Radiology** (Prof R B Iyer MD, Prof C S Ng MD, V Kundra MD), **Department of Cancer Biology**, (Prof A K Sood), and **Center for RNAi and Non-Coding RNA** (Prof R L Coleman, Prof A K Sood), M D Anderson Cancer Center, Houston, TX, USA; Division of Gynecologic Oncology, University of Virginia, Charlottesville, VA, USA (L R Duska MD, S C Modesitt MD); **Department of Imaging Physics** (Prof E F Jackson PhD) and **Center for Reproductive Medicine and Biology** (Prof R Jaffe MD), **University of California, San Francisco, CA, USA**

Summary

Background—Biologically targeted therapies have been postulated as a viable strategy to improve outcomes for women with ovarian cancer. We assessed the safety, tolerance, pharmacokinetics, relevant circulating and image-derived biomarkers, and clinical activity of combination aflibercept and docetaxel in this population.

Methods—For the phase 1 (pharmacokinetic) study, eligible patients had measurable, recurrent or persistent epithelial ovarian, primary peritoneal, or fallopian tube carcinoma with a maximum of two prior chemotherapy regimens. Aflibercept was administered intravenously over three dose levels (2, 4, or 6 mg/kg; one dose every 21 days) to identify the maximum tolerated dose for the phase 2 study. Pharmacokinetics were assessed and dynamic imaging was done during a lead-in phase with single-agent aflibercept (cycle 0) and during combination therapy with intravenous docetaxel (75 mg/m²). Eligibility for the phase 2 study was the same as for phase 1. Patients were enrolled in a two-stage design and given aflibercept 6 mg/kg intravenously and docetaxel 75 mg/m² intravenously, every 3 weeks. The primary endpoint was objective response rate (ORR) as assessed by Response Evaluation Criteria in Solid Tumors version 1.0. The trial has completed enrolment and all patients are now off study. The trial is registered at ClinicalTrials.gov, number NCT00436501.

Correspondence to: Prof Robert L Coleman, Department of Gynecologic Oncology, University of Texas, MD Anderson Cancer Center, Houston, TX 77030, USA rcoleman@mdanderson.org.

Contributors RLC was the principal investigator (PI), wrote the clinical protocol and manuscript, and was responsible for all aspects of the trial report and conduct. LRD was the site PI for the University of Virginia and was responsible for overseeing the phase 2 enrollees at that location. LRD, PTR, AAK, SCM, and KMS were the highest enrollers onto the phase 2 trial. RBI was the trial's independent radiologist responsible for reviewing and adjudicating all RECIST declarations. MEG (phase 1 and 2) and DLM (phase 2) were research nurses for the trial and collected and submitted all clinical data. JVH and AKS performed the assays for circulating endothelial cells. EFJ, VK, and CSN did the dynamic imaging studies. RJ and AKS contributed the preclinical investigation to support the trial. AKS was the co-PI and provided oversight and coordination for all translational elements of the trial. All authors reviewed and contributed to the writing of the manuscript, images, tables, and figures.

Conflicts of interest All other authors declare no conflicts of interest.

Findings—From the phase 1 study, the recommended phase 2 doses of aflibercept and docetaxel were found to be 6 mg/kg and 75 mg/m², respectively. Log-linear pharmacokinetics (for unbound aflibercept) were observed for the three dose levels. No dose-limiting toxicities were noted. 46 evaluable patients were enrolled in the phase 2 trial; 33 were platinum resistant (15 refractory) and 13 were platinum sensitive. The confirmed ORR was 54% (25 of 46; 11 patients had a complete response and 14 had a partial response). Grade 3–4 toxicities observed in more than two patients (5%) were: neutropenia in 37 patients (80%); leucopenia in 25 patients (54%); fatigue in 23 patients (50%); dyspnoea in ten patients (22%); and stomatitis in three patients (7%). Adverse events specifically associated with aflibercept were grade 1–2 hypertension in five patients (11%), and grade 2 proteinuria in one patient (2%).

Interpretation—Combination aflibercept plus docetaxel can be safely administered at the dose and schedule reported here, and is associated with substantial antitumour activity. These findings suggest that further clinical development of this combination in ovarian cancer is warranted.

Funding—US National Cancer Institute, US Department of Defense, Sanofi-Aventis, Gynecologic Cancer Foundation, Marcus Foundation, and the Commonwealth Foundation.

Introduction

The need for biologically targeted therapies for women with epithelial ovarian cancer is largely driven by the unacceptable morbidity and mortality associated with this disease. Among the most promising avenues for therapy are drugs that target VEGF. Aflibercept is a novel VEGF-ligand-binding fusion protein that serves as a decoy receptor for VEGF binding at high affinity.¹ The structural features of this compound also provide strong, picomolar binding affinity for placental growth factor. The known interactions between placental growth factor and neuropilin-1 and neuropilin-2 provide additional potential mechanisms for regulation of tumour-associated vasculature.²

There has been limited assessment of aflibercept in patients with ovarian cancer. The initial clinical preparation of aflibercept was subcutaneously administered, which had limitations with regard to dose escalation.^{3–5} An intravenous solution was developed and two single-agent randomised phase 2 studies were done in patients with recurrent ovarian cancer.^{5,6} Results from these studies showed that in heavily pretreated patients, single-agent aflibercept could induce tumour response, delay progression, prevent reaccumulation of ascites, and substantially prolong the need for paracentesis. Up to now, there have been no studies of aflibercept in combination with chemotherapy in patients with ovarian cancer.

Taxanes are a good partner in combination regimens with biologically targeted drugs, because of modulation of cytokines that drive neovascularisation and angiogenesis, and non-overlapping toxicities, such as neuropathy, myelosuppression, and hypersensitivity. These effects have been shown in metronomic (dose-dense) and bolus infusion schedules, with docetaxel seeming to have more potent effect than paclitaxel on endothelial-cell viability.⁷ Preclinical investigation of taxanes in combination with anti angiogenesis-targeted drugs in ovarian cancer models shows additive and, in some models, synergistic anti-tumour effects. Observations from retrospective studies support this interaction.^{8–10}

Based on these considerations and our preclinical data supporting substantial efficacy with these two drugs,¹¹ we initiated a phase 1–2 study to assess the safety, tolerance, and efficacy of combination aflibercept and docetaxel. We also examined the pharmacokinetic profile and surrogate circulating and imaging biomarkers.

Methods

Patients

This phase 1–2 study was approved by the National Cancer Institute's (NCI) central investigational review board (IRB) and the local IRBs at M D Anderson Cancer Center and the University of Virginia. The phase 1 study was activated on Jan 17, 2007, and closed to new patients on March 7, 2008. After toxicity observations, the phase 2 study opened on June 4, 2008, and closed to new patient entry on Sept 27, 2010. The last patient receiving study treatment was removed from the study on Aug 3, 2011.

Eligibility criteria (for phase 1 and 2) included: documented recurrent or persistent epithelial ovarian, primary peritoneal, or fallopian tube carcinoma; histological confirmation of the original primary tumour; measurable disease (for assessment by Response Evaluation Criteria in Solid Tumors [RECIST]¹²); performance status of 2 or lower; and no active infection requiring antibiotics. Any therapy directed at the malignant tumour, including biological, immunological, chemo therapy, or radiation therapy was to be dis-continued at least 3 weeks before registration. During the phase 1 study, at least one target lesion was required to be suitable for dynamic contrast-enhanced (DCE)-MRI and ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG)-PET. This determination was made by our study radiologists and generally represented lesions that could be reproducibly imaged, in areas with minimum movement, and larger than 1 cm in size. Intolerance to either procedure was an exclusion criterion for the phase 1 study. Allowable primary therapy included high-dose or maintenance regimens. Patients were allowed to receive one additional cytotoxic-chemotherapy regimen for management of recurrent or persistent disease, including retreatment with initial chemotherapy regimens. Prior treatment with docetaxel was allowed, provided there was no on-agent disease progression. No restriction was made for paclitaxel therapy.

Patients were considered ineligible if they had received radiation to more than 25% of marrow-bearing areas, had uncontrolled hypertension, uncompensated congestive heart failure, symptomatic coronary artery disease, or myocardial infarction within 6 months. Patients with a history of other invasive malignancies, with the exception of non-melanoma skin cancer, were excluded if there was evidence of another malignancy within the previous 5 years. Prior treatment with aflibercept was an exclusion criterion; however, prior treatment with other biological therapies, including bevacizumab, was not, as long as 3 weeks had transpired between the last dose and registration. All patients were required to provide written informed consent, consistent with all federal, state, and institutional investigational requirements, before receiving protocol therapy.

Procedures

Eligible patients enrolled in the phase 1 study were considered for three planned dose levels of aflibercept (Regeneron Pharmaceuticals, Tarrytown, NJ, USA; 2 mg/kg, 4 mg/kg, and 6 mg/kg) intravenously over 60 min, in combination with docetaxel (Sanofi-Aventis USA, Bridgewater, NJ, USA) 75 mg/m² by conventional infusion once every 21 days. Doses higher than 6 mg/kg were not pursued because of concerns for class-effect toxicity. Moreover, previous phase 1 studies of single-agent aflibercept have shown VEGF saturation above 2 mg/kg, and there is a lack of a clear dose–response relationship above 4 mg/kg when administered biweekly. Dose escalation followed a standard three-plus-three design, where up to three additional patients were enrolled at any dose level where a dose-limiting toxicity (DLT; haematological or non-haematological) was recorded. One or more DLTs following expansion required enrolment of three additional patients at the previous dose

level. The recommended phase 2 dose (RP2D) was defined as the dose level where, at most, one of six patients (if expanded) experienced a DLT, or dose level 3.

To evaluate aflibercept-specific effects on pharmacokinetic parameters and translational biomarkers, in phase 1 patients were given single-agent aflibercept in a lead-in manner (cycle 0). Webappendix p 8 outlines the treatment design and timing of the various tests and imaging. Specifically, in addition to baseline imaging and laboratory studies, blood was obtained at eight timepoints (baseline, 15, 30, 60, 90, 180, and 360 min, and 24 h) on day 1 of cycle 0, and daily for 4 days during week 1 and weekly (trough samples) through two complete cycles. Plasma was separated by centrifugation within 1 h and stored frozen. Aflibercept (unbound and bound) and endogenous VEGF were assessed in ELISA-based assays with a lower limit of quantification of 15.6 ng/mL, 43.9 ng/mL, and 15.0 pg/mL, respectively. The bound VEGF–aflibercept complex was measured using an antibody to human VEGF as the capture and an antibody to the human Fc domain as previously described.¹³ In biweekly dosing schedules, aflibercept has been reported to be most efficacious when the unbound fraction exceeds the bound fraction at the pretreatment trough; this relationship has not been explored in the every-3-week infusion schedule. Therefore, we designated dose level 1 as 2 mg/kg, since this is the lowest dose at which unbound aflibercept was in parity with bound aflibercept in the biweekly infusion schedule and would be expected to be safely administered. Data for plasma concentration of aflibercept over time were analysed using non-compartmental methods. The maximum plasma concentration ($C_{\max}$) and time to peak concentration ($T_{\max}$) were determined from a concentration–time curve for each patient. Area under the curve (AUC) to the last measured timepoint was calculated with the linear trapezoidal method and extrapolated to infinity (AUC_{0-t}) by adding the final measured plasma concentration divided by the terminal rate constant, which was derived from the slope of the natural-log-transformed concentrations and times on the terminal elimination phase of the decay curve. The half-life ($t_{1/2}$) of aflibercept was calculated by dividing 0.693 by the terminal rate constant (according to the equation for decay). Apparent total clearance of aflibercept from plasma (CL/F) was calculated by dividing the total dose by the AUC_{0-t} . Accumulation of aflibercept was estimated from the measured AUC0–24 hours at steady state, to the measured AUC0–24 hours after the first dose. Serum was also obtained at baseline and every 8 weeks for anti-aflibercept antibody formation.

Circulating endothelial cells (CEC) and circulating endothelial precursors (CEP) are known to be mobilised in response to VEGF in murine models^{14,15} and in humans,^{16,17} and express VEGF receptor 2 (VEGFR2).¹⁸ We postulated that binding of VEGF with aflibercept would reduce this mobilisation, potentially providing an early marker of drug efficacy. We assessed CECs in whole blood at baseline, 48 h after cycle 0 (single-agent aflibercept), before cycle 1 (combination aflibercept and docetaxel), and subsequently with every other cycle. CECs were identified by flow cytometry, using antibodies CD45-PerCP, Flk-1-PE, CD31-APC, and CD117-FITC. We distinguished CEPs from mature CECs by the presence of the c-KIT ligand receptor CD117, as shown previously.¹⁹

Since anti-VEGF therapy can induce perfusion alterations in the tumour microenvironment, we assessed the effect of aflibercept on perfusion with dynamic radiographic imaging (DCE-MRI and ¹⁸F-FDG-PET). The analytical details for this assessment are provided in the webappendix p 2.

Once the RP2D was established, the phase 2 study was initiated to address the primary endpoint, objective response. Eligible patients were given aflibercept 6 mg/kg followed by standard premedication and docetaxel 75 mg/m², each intravenously over 60 min, once every 21 days. The infusion was sequenced to enable a blood draw to assess for anti-

aflibercept antibody and to clearly identify potential drug-induced hypersensitivity reactions. Actual bodyweight at cycle 1 was used to calculate the dose of aflibercept and was unchanged unless a 10% or more difference in weight at subsequent cycles was recorded. The maximum body-surface area used for docetaxel dose calculations was 2.0 m². Treatment cycles were 21 days for each compound and were continued until withdrawal of consent, evidence of disease progression, substantial side-effects precluding further administration, inability to tolerate the lowest doses because of toxicity, or confirmed complete RECIST response (radiographic confirmation was made two cycles after documented resolution of all previously documented disease).

Initial treatment modifications consisted of cycle delay, dose reduction, or both, depending on the severity and duration of aflibercept-related toxicity. The use of haemopoietic cytokines and protective reagents was restricted to patients who experienced recurrent neutropenic complications despite dosing and delay modifications. Treatment modifications were based on the absolute neutrophil count rather than the total white-blood-cell count. Subsequent cycles of therapy were not allowed until the absolute neutrophil count was at least 1500 cells per μ L (Common Terminology Criteria for Adverse Events [CTCAE] version 3; grade 1) and the platelet count was at least 100 000 per μ L. Therapy could be delayed for a maximum of 2 weeks; however, patients who did not have adequate counts after a 2-week delay were removed from the study. Patients experiencing a first occurrence of febrile neutropenia, documented grade 4 neutropenia persisting 7 days or longer, underwent a one-dose-level reduction (to 60 mg/m²) in docetaxel dose for subsequent cycles. For recurrent febrile neutropenia or recurrent documented grade 4 neutropenia persisting 7 days or more (after initial dose reduction), prophylactic growth factors were to be administered. Patients with grade 4 thrombocytopenia were also to undergo a one-dose-level reduction in docetaxel.

Grade 2 (or higher) peripheral neuropathy required a reduction of one dose level (to 60 mg/m²) in docetaxel and delay in subsequent therapy for a maximum of 2 weeks until recovery to grade 1. Patients were monitored for hypertension before each infusion. Patients with persistent or symptomatic hypertension did not receive an infusion until blood pressure was controlled (140/90 mm Hg or lower). Up to 4 weeks of medical management to reach control was allowed. If not achieved, the patient was to be removed from the study. Patients underwent urinalysis for proteinuria every other cycle of therapy. Urine protein-to-creatinine ratio was calculated; if greater than 3.5, therapy was to be withheld for up to 3 weeks. Longer delay or grade 4 proteinuria resulted in removal from the study. Patients experiencing any grade arterial thrombosis, or grade 3 or 4 venous thrombosis were removed from study. Grade 3 (or higher) elevations in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, or bilirubin required a reduction of one dose level and delay in subsequent therapy for a maximum of 2 weeks until recovery to grade 1. Non-haematological toxicities with a grade 2 or higher effect on organ function required a reduction of one dose level and delay in subsequent therapy, for a maximum of 2 weeks until recovery to grade 1 or pretherapy baseline. For patients with a severe hypersensitivity reaction, immediate termination of the infusion and administration of injectable steroids and epinephrine was recommended, as well as supportive care. Patients with grade 3 hypersensitivity reactions were allowed to undergo retreatment after standard docetaxel premedication at the discretion of the investigator. No dose escalations or re-escalations were allowed on this study and intolerance of a one-level dose reduction necessitated removal from the study.

Webappendix p 5 outlines the pretreatment and treatment study procedures, and the monitoring schedule used for the phase 1 and 2 studies. Standard RECIST criteria were followed to evaluate response and progression. All documented responses were required to

undergo confirmation by imaging, examination, or both, no sooner than 4 weeks after the initial documentation of response. In the absence of new symptoms, this response assessment was consistently done after two additional cycles of therapy. Progression-free survival (PFS) was calculated from study entry until documented disease progression, death, or date of last contact. Overall survival was defined from the date of study entry until death or date of last contact. CA-125 was not considered in assessing response or progression.

Statistical analysis

The study used a flexible, two-stage accrual design that allowed early termination if there was evidence that treatment was ineffective.²⁰ In its initial version, the first stage of accrual was to be 24 patients with the possibility to open a second stage (n=34; 58 total) if seven or more complete responses (CR) or partial responses (PR) were confirmed. The regimen would be considered of clinical interest if 24 or more responses were confirmed. However, after discussions with the NCI Cancer Therapy Evaluation Program, an amendment to the protocol was requested (April 7, 2009) to increase the minimum treatment-effect boundary (from 45% to 50%), while retaining the upper limit of inactivity at 30%. Under the amendment, the first stage of accrual called for 22 evaluable patients; if seven or more patients had a confirmed response, enrolment was expanded (by n=24) to 46 patients. The regimen would be considered active if 19 or more patients had a confirmed PR or CR. If the true response rate was 30%, the probability of designating the treatment as active would be 10%; if the true response rate was 50%, the probability of correctly classifying the treatment as active would be 90%. All analyses were done with JMP software version 8.0.2. The trial was registered with ClinicalTrials.gov, number NCT000436501.

Role of the funding source

This clinical trial was mainly funded by the US National Institutes of Health, which supplied the investigational drug, held the multicentre investigational new drug application, and was the primary contact for trial amendments and toxicity reporting. Funding from the US Department of Defense, the Marcus Foundation, the Gynecologic Cancer Foundation, and the Commonwealth Foundation supported the translational elements, pharma cokinetic testing, and dynamic imaging. Sanofi-Aventis supported some of the costs for the phase 2 multisite monitoring, oversight, and data collection. Regeneron was responsible for analysis and interpretation of pharmacokinetic data in phase 1. The corresponding author had access to all the data and the final decision to submit for publication.

Results

Nine consecutive patients completed cycle 0 and at least two cycles of combination therapy to be evaluable for all endpoints of the phase 1 study. Table 1 shows the demographics for this population. The median number of aflibercept courses administered was five (range 3–15). Three of the eight patients with platinum-resistant and taxane-resistant disease progressed during treatment with these two drugs; the remainder progressed within 6 months of completion. The median number of prior therapies was two (range 1–2); no patients had previously received an anti-VEGF agent.

Three patients per dose level were consecutively enrolled. The RP2D for aflibercept was established at 6 mg/kg based on the absence of an observed DLT in cycle 0 (single agent) or cycle 1 (combination therapy). Toxicities attributed to therapy are listed in table 2. The most common haematological toxicities in phase 1 were myelo suppression and anaemia. Non-haematological toxicities (grade 3) included headache, hypertension, fatigue, dyspnoea, and ulceration. One patient with hypertension and one with perianal ulceration had treatment discontinuation after four and 13 cycles, respectively.

The pharmacokinetic parameters for unbound and bound aflibercept are shown in figure 1 and webappendix p 7. VEGF binding was rapid and reached steady state about 1 week after infusion. Bound aflibercept values were comparable among the dose levels, suggesting saturation of endogenous VEGF. However, unbound aflibercept trough concentration at preinfusion was higher than the concentration of bound aflibercept only at dose level 3, suggesting that this dose was the most favourable to explore in phase 2. Clearance of unbound aflibercept was linear and mainly unchanged between the dose levels. No drug–drug interactions were observed in the limited pharmacokinetic assessment done during cycle 1, or in trough values through cycle 2 of combination therapy. Additionally, no anti-aflibercept antibodies were noted in any of the participants.

Of the nine evaluable patients, two PRs (22%) were confirmed by serial imaging (individual details for the responders are provided in webappendix p 6). Seven of the nine participants had CECs detected, including both patients with a confirmed response. Suppression of mobilised endothelial cells was observed in these two patients. All other patients had stabilisation of their baseline values with a spike coinciding with documented progression; however, a rise in value before clinical progression was not clearly observed (webappendix p 9).

All nine patients had ^{18}F -FDG-PET–CT performed at baseline and 48–72 h after receiving single-agent aflibercept. In six of nine patients, decreases in mean standardised uptake value (SUV_{max}) of designated target lesions were seen ranging from 2% to 19%. Despite patient selection for target lesions suitable for imaging, there was no obvious correlation between mean change in SUV_{max} from preinfusion to postinfusion and response in this study. However, DCE-MRI, which was obtained at the same timepoints, was more closely associated with clinical outcome. In both responding patients, and in the patient with a near PR who was removed for perianal abscess, the average K_{trans} value (microvascular permeability) of a target lesion decreased substantially (by >50%) at 48 h, returning to sub-baseline level before cycle 1. This was not noted in any other patient; however, two patients had non-evaluable scans due to motion artifacts.

After establishment of the RP2D, the initial phase 2 cohort was enrolled to assess for predefined clinical efficacy and toxicity. A total of 49 patients were enrolled in the trial; three were non-evaluable because of withdrawal of consent ($n=2$) or incomplete treatment for assessment ($n=1$). Table 1 outlines the clinical demographics of the 46 evaluable patients; their clinical characteristics were similar to those enrolled in the phase 1 study, including the proportion of platinum-sensitive patients (treatment-free interval [TFI] 6 months). The median TFI for platinum-sensitive patients was 10 months (IQR 9–12 months). Of the 33 platinum-resistant or taxane-resistant patients, 25 were primarily resistant and eight were secondarily resistant after platinum or taxane reinduction therapy for recurrent disease. 15 patients had progressed on these agents immediately before enrolment (refractory). Unlike the phase 1 population, five patients had been previously treated with bevacizumab.

Among all phase 2 patients, 307 treatment cycles were administered (median 6, range 1–22). All patients are now off study. Causes for treatment discontinuation were progression ($n=29$), CR ($n=11$), withdrawal of consent ($n=3$), and toxicity ($n=3$). Table 2 lists the documented haematological and non-haematological adverse events attributable to either drug. Overall, the regimen was well tolerated. There were no vascular thrombotic events or intestinal perforations observed in the study cohort; however, one patient with a remote history of a treated maxillary cutaneous fistula had reappearance after 18 cycles of therapy and chose to withdraw from the study. She remains disease-free 12 months off treatment. One patient reported confusion and was assessed at an outside facility. Despite a negative

neurological evaluation and imaging study, her local physician designated the event as a non-vascular transient ischaemic attack, possibly related to the study drug. Adverse events specifically associated with aflibercept were grade 1–2 hypertension in five patients (11%) and grade 2 hypotension in one patient (2%).

All patients had measurable disease and were evaluable for treatment effect. With a median follow-up of 24 months (range 1.6–32), 17 patients have died: 16 due to disease and one from other causes. Objective responses according to RECIST were confirmed in 25 of 46 patients (54%, 95% CI 39–69). 11 patients (24%) achieved a CR, including four patients who have not developed recurrent disease after a median of 18.4 months (range 5.2–31.1) of surveillance. One CR occurred among the five patients who had previously received bevacizumab. 14 patients had a partial response, and 11 had stable disease. Responses (three CRs and 12 PRs) were confirmed in 15 of 33 (45%) of patients resistant or refractory to platinum and taxane, and in ten of 13 (77%) of platinum-sensitive patients. Figure 2 shows a waterfall plot of the maximum percent change from baseline in measurable target lesions. The overall median duration of response was 6.0 months (range 1.8–27; eight cycles). Median progression-free and overall survival was 6.4 months (95% CI 5.1–10.3) and 26.6 months (13.1–inestimable), respectively (figure 3). In an exploratory analysis of factors affecting PFS—including age, number of prior regimens, primary or secondary platinum or taxane resistance, platinum-resistant versus platinum-sensitive recurrent disease, and prior bevacizumab exposure—only response to platinum and previous exposure to bevacizumab were independently associated. We recognise that lack of statistical power might have overlooked other important relationships among these covariates. Further details are provided in the webappendix p 2.

Discussion

The key findings from this study are that aflibercept and docetaxel can be safely administered together, and the combination is associated with substantial clinical activity, meeting our pre-specified benchmarks for further investigation (panel). Tolerance of repeated infusions (up to 22 cycles) was shown with no unexpected severe adverse events; in particular, there were no intestinal perforations, vascular thrombotic events, and no patients with reversible leukoencephalopathy. In addition, some of our translational correlates seemed to show changes in association with observed response. For example, target lesions assessed by DCE-MRI seemed to undergo early perfusion effects 48 h after administration of single-agent aflibercept in eventual responders. This is consistent with a previous phase 1 study of single-agent aflibercept that used DCE-MRI.¹³ Similarly, suppression of mobilised CECs seemed to correspond to ongoing response. However, in view of the small sample size, both of these associations require further exploration and validation. Additionally, although we did not exclude patients with prior bevacizumab therapy, we did observe one complete responder in that subgroup of five patients. The importance of prior anti-VEGF therapy on subsequent response is unknown and is a crucial issue for future trials because of the growing prevalence of its use.

We previously reported that aflibercept was effective in preclinical models of ovarian cancer and had additive, and in some models, synergistic activity with taxanes.^{1,21,22} These data corroborate other results that have convincingly showed that taxanes, regardless of schedule, and anti-VEGF therapy have synergistic activity in various solid tumours.^{23–31} At clinically relevant doses, docetaxel produces serum concentrations that are associated with in-vitro tumour-cell apoptosis and antiangiogenic properties, such as endothelial-cell proliferation, migration, and capillary-tube formation.^{32,33} The mechanisms responsible for an antiangiogenesis effect are varied and include induction of thrombospondin-I, an inhibitor of VEGF ligand–receptor interaction, modulation of VEGF secretion, endothelial-cell

apoptosis, regulation of local cytokines, and, most recently, suppression of regulatory T cells.³⁴⁻⁴⁵ However, the antiangiogenesis effect of docetaxel can be abrogated by local production of VEGF and basic fibroblast growth factor (bFGF),³² which supports further combination of taxane and anti-VEGF-based therapy.

Aflibercept was constructed to optimise one-to-one high-affinity binding of all VEGF ligands of VEGFR1 and VEGFR2, including placental growth factor.¹ When this study was initiated, an intravenous preparation had just been manufactured and single-agent dose-escalation studies were being initiated, assessing the tolerance of biweekly infusion. Although a maximum tolerated dose above 8 mg/kg was suggested by these trials, doses above 2 mg/kg were associated with unbound-to-bound aflibercept ratios higher than 1, suggesting saturation of endogenous VEGF-A.⁴ A randomised, double-blind, phase 2 study of intravenous aflibercept monotherapy (2 mg/kg or 4 mg/kg every 2 weeks) was done in 218 women with platinum-resistant advanced ovarian cancer (mainly third-line and fourth-line therapy).⁵ Response rates (by RECIST) were 7% and 4% for the 4 mg/kg and 2 mg/kg dose, respectively; resolution of ascites was identified in 29%. Disease stabilisation at 5 months was 15%. The overall adverse effects profile was consistent with systemic VEGF blockade, with hypertension being the most common grade 3–4 adverse event (16%) and bowel perforation rare (1.8%).

We initiated the present study to assess an every-3-week schedule of aflibercept. To minimise bias that concomitant chemotherapy might have on aflibercept and to assess potential early biomarkers of aflibercept efficacy, we designed the trial with a lead-in segment of single-agent aflibercept (cycle 0). Docetaxel has been studied on a 3-week schedule in patients with platinum-resistant ovarian cancer and is considered an active salvage regimen (response rate 22%, median PFS 2.1 months) in patients progressing on or within 6 months of paclitaxel therapy.⁴⁶ Additionally, single-agent docetaxel was studied in patients with platinum-sensitive disease, with a response rate of 33% (95% CI 17–55).⁴⁷ To minimise the likelihood of a type 1 error in our treatment population, we designed the phase 2 trial to exclude a 30% response rate, while targeting a 50% response. Our observed responses in 25 of 46 (54%) of enrolled patients met these targets and warrants further investigation (panel).

The combination of aflibercept and docetaxel seems to be safe and active in patients with recurrent ovarian cancer. The level of activity and tolerability compare favourably to either drug individually, and strongly suggest that the combination could be developed into a viable option for this cohort of patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported in part by grants from the US National Institutes of Health (CA083639, CA098258) and Department of Defense (W81XWH-10-1-0158), the Gynecologic Cancer Foundation, the Marcus Foundation, the Commonwealth Foundation, the Betty Anne Asche Murray Distinguished Professorship (AKS), and the Ann Rife Cox Chair in Gynecology (RLC). Sanofi-Aventis supported some of the costs of the phase 2 monitoring, oversight, and data collecting.

RLC received research support from Sanofi-Aventis for some aspects of the phase 2 trial. RJ received research funding from Regeneron to support some of the preclinical experiments and preparation of preliminary data to support the current study.

References

1. Holash J, Davis S, Papadopoulos N, et al. VEGF-trap: a VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci USA*. 2002; 99:11393–98. [PubMed: 12177445]
2. Kim ES, Serur A, Huang J, et al. Potent VEGF blockade causes regression of coopted vessels in a model of neuroblastoma. *Proc Natl Acad Sci USA*. 2002; 99:11399–404. [PubMed: 12177446]
3. Tew WP, Gordon M, Murren J, et al. Phase 1 study of aflibercept administered subcutaneously to patients with advanced solid tumors. *Clin Cancer Res*. 2010; 16:358–66. [PubMed: 20028764]
4. Moroney JW, Sood AK, Coleman RL. Aflibercept in epithelial ovarian carcinoma. *Future Oncol*. 2009; 5:591–600. [PubMed: 19519199]
5. Tew WP, Colombo N, Ray-Coquard I, et al. VEGF-Trap for patients (pts) with recurrent platinum-resistant epithelial ovarian cancer (EOC): preliminary results of a randomized, multicenter phase II study. *Proc Am Soc Clin Oncol*. 2007; 25 abstr 5508.
6. Vergote I, Amant F, Advani S, et al. Intravenous aflibercept (VEGF Trap) in advanced ovarian cancer patients with recurrent symptomatic malignant ascites: main efficacy and safety results of a phase II, randomized, double-blind, placebo-controlled study. *Proc Eur Soc Gynecol Oncol*. 2009; 16 abstr 4.
7. Grant DS, Williams TL, Zahaczewsky M, et al. Comparison of antiangiogenic activities using paclitaxel (taxol) and docetaxel (taxotere). *Int J Cancer*. 2003; 104:121–29. [PubMed: 12532428]
8. Cohn DE, Valmadre S, Resnick KE, et al. Bevacizumab and weekly taxane chemotherapy demonstrates activity in refractory ovarian cancer. *Gynecol Oncol*. 2006; 102:134–39. [PubMed: 16527339]
9. Hurt JD, Richardson DL, Seamon LG, et al. Sustained progression-free survival with weekly paclitaxel and bevacizumab in recurrent ovarian cancer. *Gynecol Oncol*. 2009; 115:396–400. [PubMed: 19804901]
10. O'Malley DM, Richardson DL, Rheaume PS, et al. Addition of bevacizumab to weekly paclitaxel significantly improves progression-free survival in heavily pretreated recurrent epithelial ovarian cancer. *Gynecol Oncol*. 2011; 121:269–72. [PubMed: 21315428]
11. Hu L, Hofmann J, Holash J, et al. Vascular endothelial growth factor trap combined with paclitaxel strikingly inhibits tumor and ascites, prolonging survival in a human ovarian cancer model. *Clin Cancer Res*. 2005; 11:6966–71. [PubMed: 16203789]
12. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst*. 2000; 92:205–16. [PubMed: 10655437]
13. Lockhart AC, Rothenberg ML, Dupont J, et al. Phase I study of intravenous vascular endothelial growth factor trap, aflibercept, in patients with advanced solid tumors. *J Clin Oncol*. 2010; 28:207–14. [PubMed: 19949018]
14. Takahashi T, Kalka C, Masuda H, et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med*. 1999; 5:434–38. [PubMed: 10202935]
15. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res*. 1999; 85:221–28. [PubMed: 10436164]
16. Kalka C, Masuda H, Takahashi T, et al. Vascular endothelial growth factor (165) gene transfer augments circulating endothelial progenitor cells in human subjects. *Circ Res*. 2000; 86:1198–202. [PubMed: 10864908]
17. Kalka C, Tehrani H, Laudenberg B, et al. VEGF gene transfer mobilizes endothelial progenitor cells in patients with inoperable coronary disease. *Ann Thorac Surg*. 2000; 70:829–34. [PubMed: 11016318]
18. Peichev M, Naiyer AJ, Pereira D, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood*. 2000; 95:952–58. [PubMed: 10648408]

19. Capillo M, Mancuso P, Gobbi A, et al. Continuous infusion of endostatin inhibits differentiation, mobilization, and clonogenic potential of endothelial cell progenitors. *Clin Cancer Res.* 2003; 9:377–82. [PubMed: 12538491]
20. Desai NP, Trieu V, Hwang LY, et al. Improved effectiveness of nanoparticle albumin-bound (nab) paclitaxel versus polysorbate-based docetaxel in multiple xenografts as a function of HER2 and SPARC status. *Anticancer Drugs.* 2008; 19:899–909. [PubMed: 18766004]
21. Byrne AT, Ross L, Holash J, et al. Vascular endothelial growth factor-trap decreases tumor burden, inhibits ascites, and causes dramatic vascular remodeling in an ovarian cancer model. *Clin Cancer Res.* 2003; 9:5721–28. [PubMed: 14654557]
22. Huang J, Frischer JS, Serur A, et al. Regression of established tumors and metastases by potent vascular endothelial growth factor blockade. *Proc Natl Acad Sci USA.* 2003; 100:7785–90. [PubMed: 12805568]
23. Tillmanns TD, Lowe MP, Schwartzbert LS, et al. A phase II study of bevacizumab with nab-paclitaxel in patients with recurrent, platinum-resistant primary epithelial ovarian or primary peritoneal carcinoma. *Proc Am Soc Clin Oncol.* 2010; 28 abstr 5009.
24. Labidi SI, Bachelot T, Ray-Coquard I, et al. Bevacizumab and paclitaxel for breast cancer patients with central nervous system metastases: a case series. *Clin Breast Cancer.* 2009; 9:118–21. [PubMed: 19433393]
25. Reynolds C, Barrera D, Jotte R, et al. Phase II trial of nanoparticle albumin-bound paclitaxel, carboplatin, and bevacizumab in first-line patients with advanced nonsquamous non-small cell lung cancer. *J Thorac Oncol.* 2009; 4:1537–43. [PubMed: 19887966]
26. Hurt JD, Richardson DL, Seamon LG, et al. Sustained progression-free survival with weekly paclitaxel and bevacizumab in recurrent ovarian cancer. *Gynecol Oncol.* 2009; 115:396–400. [PubMed: 19804901]
27. Gonzalez-Cao M, Viteri S, Diaz-Lagares A, et al. Preliminary results of the combination of bevacizumab and weekly paclitaxel in advanced melanoma. *Oncology.* 2008; 74:12–16. [PubMed: 18536525]
28. Link JS, Waisman JR, Nguyen B, et al. Bevacizumab and albumin-bound paclitaxel treatment in metastatic breast cancer. *Clin Breast Cancer.* 2007; 7:779–83. [PubMed: 18021479]
29. Cohn DE, Valmadre S, Resnick KE, et al. Bevacizumab and weekly taxane chemotherapy demonstrates activity in refractory ovarian cancer. *Gynecol Oncol.* 2006; 102:134–39. [PubMed: 16527339]
30. Gerber HP, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. *Cancer Res.* 2005; 65:671–80. [PubMed: 15705858]
31. Woodward EJ, Twelves C. Scheduling of taxanes: a review. *Curr Clin Pharmacol.* 2010; 5:226–31. [PubMed: 20406166]
32. Sweeney CJ, Miller KD, Sissons SE, et al. The antiangiogenic property of docetaxel is synergistic with a recombinant humanized monoclonal antibody against vascular endothelial growth factor or 2-methoxyestradiol but antagonized by endothelial growth factors. *Cancer Res.* 2001; 61:3369–72. [PubMed: 11309294]
33. Hotchkiss KA, Ashton AW, Mahmood R, et al. Inhibition of endothelial cell function in vitro and angiogenesis in vivo by docetaxel (Taxotere): association with impaired repositioning of the microtubule organizing center. *Mol Cancer Ther.* 2002; 1:1191–200. [PubMed: 12479700]
34. Albertsson P, Lennernas B, Norrby K. On metronomic chemotherapy: modulation of angiogenesis mediated by VEGF-A. *Acta Oncol.* 2006; 45:144–55. [PubMed: 16546859]
35. Belotti D, Vergani V, Drudis T, et al. The microtubule-affecting drug paclitaxel has antiangiogenic activity. *Clin Cancer Res.* 1996; 2:1843–49. [PubMed: 9816139]
36. Chen CA, Ho CM, Chang MC, et al. Metronomic chemotherapy enhances antitumor effects of cancer vaccine by depleting regulatory T lymphocytes and inhibiting tumor angiogenesis. *Mol Ther.* 2010; 18:1233–43. [PubMed: 20372107]
37. Damber JE, Vallbo C, Albertsson P, et al. The anti-tumour effect of low-dose continuous chemotherapy may partly be mediated by thrombospondin. *Cancer Chemother Pharmacol.* 2006; 58:354–60. [PubMed: 16333676]

38. Dreys J, Fakler J, Eisele S, et al. Antiangiogenic potency of various chemotherapeutic drugs for metronomic chemotherapy. *Anticancer Res.* 2004; 24:1759–63. [PubMed: 15274352]
39. Hata K, Osaki M, Dhar DK, et al. Evaluation of the antiangiogenic effect of Taxol in a human epithelial ovarian carcinoma cell line. *Cancer Chemother Pharmacol.* 2004; 53:68–74. [PubMed: 14569416]
40. Hu L, Hofmann J, Holash J, et al. Vascular endothelial growth factor trap combined with paclitaxel strikingly inhibits tumor and ascites, prolonging survival in a human ovarian cancer model. *Clin Cancer Res.* 2005; 11:6966–71. [PubMed: 16203789]
41. Klauber N, Parangi S, Flynn E, et al. Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and taxol. *Cancer Res.* 1997; 57:81–86. [PubMed: 8988045]
42. Lau DH, Xue L, Young LJ, et al. Paclitaxel (Taxol): an inhibitor of angiogenesis in a highly vascularized transgenic breast cancer. *Cancer Biother Radiopharm.* 1999; 14:31–36. [PubMed: 10850285]
43. Pasquier E, Honore S, Pourroy B, et al. Antiangiogenic concentrations of paclitaxel induce an increase in microtubule dynamics in endothelial cells but not in cancer cells. *Cancer Res.* 2005; 65:2433–40. [PubMed: 15781659]
44. Wang J, Lou P, Lesniewski R, et al. Paclitaxel at ultra low concentrations inhibits angiogenesis without affecting cellular microtubule assembly. *Anticancer Drugs.* 2003; 14:13–19. [PubMed: 12544254]
45. Lau D, Guo L, Gandara D, et al. Is inhibition of cancer angiogenesis and growth by paclitaxel schedule dependent? *Anticancer Drugs.* 2004; 15:871–75. [PubMed: 15457127]
46. Rose PG, Blessing JA, Ball HG, et al. A phase II study of docetaxel in paclitaxel-resistant ovarian and peritoneal carcinoma: a Gynecologic Oncology Group study. *Gynecol Oncol.* 2003; 88:130–35. [PubMed: 12586591]
47. Katsumata N, Tsunematsu R, Tanaka K, et al. A phase II trial of docetaxel in platinum pre-treated patients with advanced epithelial ovarian cancer: a Japanese cooperative study. *Ann Oncol.* 2000; 11:1531–36. [PubMed: 11205459]

Panel: Research in context**Systematic review**

We designed this clinical trial based on our observations in preclinical orthotopic ovarian cancer models with aflibercept, alone and in combination with chemotherapy. A systematic review of the literature was done through a search of PubMed and proceedings from national meetings, using the search terms: “anti-VEGF therapies”, “taxanes as anti-angiogenesis agents”, “ovarian cancer treatment”, “metronomic therapy”, and “aflibercept” (or “VEGF trap” or “AVE005” as alternatives). To examine the potential biomarkers, we also did a systematic search using the terms: “dynamic imaging (PET/MRI/CT) in cancer therapy”, “circulating biomarkers”, “circulating endothelial cells”, and “circulating nucleic acids”, and assessed which biomarkers might be informative and hypothesis generating. There were no date restrictions. Only articles published in English were included.

Interpretation

Antiangiogenesis targeting, particularly with anti-VEGF agents, is becoming a viable therapeutic strategy in ovarian cancer care. The present study investigated this approach in women with recurrent, measurable, and mainly platinum-resistant or platinum-refractory disease. To our knowledge, this is the first phase 1–2 study combining docetaxel and aflibercept in recurrent ovarian cancer. The observed linear pharmacokinetics, lack of drug–drug interactions or new safety concerns, and clinical activity favour further development in randomised clinical trials, as is planned. Clearly, some patients benefit greatly from this therapeutic strategy; identifying them prospectively is the promise of efficacy biomarkers. Our pilot observations with dynamic imaging support further investigation of this type of non-invasive biomarker. A CT perfusion study by the Gynecologic Oncology Group is currently underway (NCT01167712).

Figure 1. Dose–response curves for (A) bound aflibercept and (B) unbound aflibercept

A log-linear dose–response was observed for each of the three dose levels. Aflibercept binding to VEGF was rapid and reached asymptote around 7 days after infusion, for all three dose levels. The concentration at this asymptote (dotted line) represents binding of all endogenous VEGF and saturation of the complexed fraction. The concentration at which this saturation is reached is reproduced on the unbound aflibercept dose–concentration graph (B) to show saturation kinetics over time. Only dose level 3 (6 mg/kg) produced an unbound-to-bound ratio of greater than 1 at day 21 on the 3-week intravenous schedule, signifying VEGF binding throughout the treatment cycle.

Figure 2. Waterfall plot of the maximum percent change from baseline in measurable target lesions as defined by RECIST criteria

Solid line represents the minimum increase in the sum of target lesions designating progressive disease as best response; dotted line represents the minimum change in the sum of target lesions designating a response. All measures between these two are classified as stable disease. RECIST=Response Evaluation Criteria in Solid Tumors.

Figure 3. Kaplan-Meier curves for (A) progression-free survival and (B) overall survival for all evaluable patients enrolled in the phase 2 trial

Table 1

Demographic characteristics for the phase 1 and phase 2 treatment populations

	Phase 1 (n=9)	Phase 2 (n=46)
Age in years, median (range)	59 (49–71)	58 (38–80)
Ethnic origin		
African-American	1	1
Asian	1	0
White	7	43
Hispanic	0	2
Primary cancer		
Fallopian tube	1	2
Ovarian	8	39
Peritoneal	0	5
Histology		
Adenocarcinoma	0	3
Clear cell	1	4
Endometrioid	0	1
Mixed	0	5
Serous	6	31
Transitional cell	2	0
Unclassified	0	2
Chemosensitivity at enrolment		
Primary platinum or taxane resistant	3	25
Secondary platinum or taxane resistant	5	8
Platinum sensitive	1	13
Prior bevacizumab	0	5

Data are number of patients, unless otherwise specified.

Table 2

Adverse events in the phase 1 and phase 2 cohorts

	Grade in phase 1 cohort (n=9)				Grade in phase 2 cohort (n=46)			
	1	2	3	4	1	2	3	4
Haematology								
Lowered haemoglobin	3	1	..	..	11	8	..	..
Lowered platelets	3	1	..	..	10	2	..	..
Lowered leucocytes	1	4	4	..	2	11	21	4
Neutropenia	..	1	..	8	..	1	9	28
Prolonged PTT	2	..	1	..	3	..	..	..
Chemistry								
Lowered magnesium	3	..	..	..	19	..	..	1
Lowered potassium	2	..	..	..	4	..	..	..
Raised ALT	1	..	..	..	2	2	..	..
Raised AST	4	..	..	..	6	1	..	..
Raised alkaline phosphatase	..	..	..	..	11	..	..	..
Raised creatinine	..	..	..	..	5	..	1	..
Other								
Hypertension	2	3	2	..	2	3	..	..
Fatigue	3	2	4	..	1	14	21	2
Dyspnoea	2	1	3	..	6	14	10	..
Nail changes	1	1	..	..	6	4	..	..
Infection, nail beds	..	..	..	..	..	5	..	..
Watery eyes	1	..	..	..	19	3	..	..
Voice changes	5	..	..	..	11	..	..	..
Ulceration	..	1	1	..	..	..	..	..
Gastrointestinal fistula	..	..	..	..	..	..	1*	..
Infection, other	..	3	..	..	..	5	..	..
Hypotension	..	..	..	..	..	1	..	..
Neuropathy, sensory	1	1	..	..	8	9	1	..
Hand or foot reaction	1	1	..	..	5	1	..	..

	Grade in phase 1 cohort (n=9)				Grade in phase 2 cohort (n=46)			
	1	2	3	4	1	2	3	4
Nose bleeds	3	1	..	..	..	20	2	..
Transient ischaemic attack	..	..	..	..	..	..	1	..
Alopecia	5	4	..	..	11	24	..	..
Stomatitis	5	3	..	..	10	23	3	..
Proteinuria	..	..	..	..	..	1	..	..
Headache	2	4	1	..	10	8	1	..

Data are number of patients. PTT=partial thromboplastin time, ALT=alanine aminotransferase, AST=aspartate aminotransferase.

* This patient had reappearance of a previously closed maxillary cutaneous fistula.