TITLE: A Phase II Study of the Combination of Aflibercept (VEGF-Trap) plus Modified FOLFOX 6 in Patients with Previously Untreated Metastatic Colorectal Cancer

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SCHEMA

Registration

↓

mFOLFOX6 [a] + aflibercept[b]

Days 1 and 15[c]

↓

Response assessment q8 week

↓

Continue treatment as long as treatment criteria are met[d]

a. mFOLFOX6= oxaliplatin 85 mg/m^2 IV with leucovorin 400 mg/m^2 (levoleucovorin 200 mg/m2 if standard leucovorin is not available, omit leucovorin if neither standard or levoleucovorin is available) IV followed by 5FU 400mg/m^2 IV bolus over 5-15 minutes followed by 5FU 2400 mg/m^2 continuous IV infusion over 46 hours

b. Aflibercept dosing= 4 mg/kg IV

c. 1 cycle = 28 days

d. Discontinue treatment in the event of disease progression, unacceptable toxicity, intercurrent illness rendering the patient unacceptable for further treatment, or patient refusal

TABLE OF CONTENTS

1. OBJECTIVES

1.1. Primary Objectives

- To evaluate the progression free survival (PFS) of patients with untreated metastatic colorectal cancer (mCRC) receiving the combination of modified FOLFOX6 (mFOLFOX6) and aflibercept.

1.2. Secondary Objectives

- To evaluate the objective response rate (CR+PR) and the disease control rate (CR+PR+SD), as determined by RECIST 1.1 criteria, of patients with untreated mCRC receiving the combination of mFOLFOX6 and aflibercept.
- To evaluate overall survival of patients with untreated mCRC receiving the combination of mFOLFOX6 and aflibercept.
- To further characterize the safety and toxicity of the combination of mFOLFOX6 and aflibercept, including 60 day all-cause mortality.
- To describe patients with mCRC whose disease is rendered resectable as a consequence of therapy with the combination of mFOLFOX 6 and aflibercept.
- Biologic and radiographic correlative objectives
    - To assess the use of dynamic imaging modalities including DCE MRI and FDG-PET to evaluate changes in vascular permeability and FDG avidity and correlate with clinical efficacy (PFS, OS, and response by RECIST 1.1)
    - To evaluate circulating levels of VEGFA, PlGF, soluble VEGF-R2, CXCL12 and CXCR4 as potential biomarkers for efficacy of aflibercept.
    - To evaluate for the presence of VEGF single nucleotide polymorphisms (SNPs) and whether any SNP(s), when detected, may be predictive of efficacy and/or toxicity of aflibercept.
    - To assess microvessel density/tumor blood flow, capillary permeability and vessel normalization by tumor biopsy pre and post treatment with aflibercept.
    - To evaluate the presence of hypertension as a predictive biomarker for clinical efficacy of aflibercept

2. BACKGROUND

2.1. Colorectal cancer

Colorectal cancer (CRC) remains the 3rd leading cause of cancer death in the United States, with an estimated 142,000 new cases and 50,000 deaths in 2010 (1). Roughly 15% of patients

present with metastatic disease, and another 40-50% will develop distant metastases during their disease course (2). A small percentage of patients with limited metastatic disease can be cured with surgical resection (3), but for the majority of patients with metastatic colorectal cancer (mCRC), treatment is palliative. For decades, single-agent 5-fluorouracil (5-FU) was the only active treatment available. In the recent years, the addition of oxaliplatin or irinotecan to 5-FU, as well as the development of the novel monoclonal antibodies bevacizumab, cetuximab, and panitumumab, has provided extended survival for these patients, with a median survival of around 18-22 months (4-13).

2.1.1. Angiogenesis in CRC

Angiogenesis is necessary for normal organ development and differentiation, as well as adult blood vessel formation, and organ and tissue repair. Aberrant angiogenesis is associated with a variety of pathologic conditions, including cancer. For solid tumors to grow beyond 1-2 mm, neoangiogenesis is necessary to maintain adequate tumor tissue oxygenation (14). In CRC, the process of neovascularization plays an important role in cancer progression. Increased angiogenesis in the primary tumor has been associated with relapse, metastases, and overall poor prognosis in CRC patients (15-18).

2.1.2. VEGF

Vascular endothelial growth factor (VEGF) is a protein which is critical to angiogenesis (19, 20). The VEGF family of proteins consists of VEGF A-D as well as placental growth factor (PlGF) (21). VEGF-A in particular functions to activate endothelial cells and promote their survival, and thus plays a crucial role in tumorigenesis. The biologic effects of VEGF are mediated via its receptor tyrosine kinases, VEGFR1-3, which are expressed on the surface of vascular endothelial cells. VEGFR1 functions predominantly to upregulate macrophage and monocyte regulation and effects vascular permeability, and can also positively or negatively regulate VEGFR2 expression. VEGFR2 is the most active promoter of tumor angiogenesis. VEGFR3 is involved in development of lymphatic endothelial cells (21). Binding of VEGF to its receptor initiates a cascade of events promoting angiogenesis, such as endothelial cell proliferation and migration, and increased vascular permeability (22-24). Phosphorylated VEGFR2 is capable of activating numerous cell signaling pathways implicated in cancer growth, including the MAPK and PI3K/Akt pathways, Src, and HSP27 which promote proliferation, survival and chemotaxis in endothelial cells and ultimately produce the characteristic effects of VEGF on vessels, including increased vascular permeability and angiogenesis (21, 25). Overexpression of VEGF has been shown to induce activation of Akt, STAT3, and ERK and increases proliferation of vascular endothelial cells (25). Both VEGFA and VEGFR2 are upregulated in the setting of tissue hypoxia (21), which constitutes a potential mechanism for tumor resistance to anticancer therapy. VEGF pathway interruption can be achieved via various mechanisms, including soluble decoy receptors that prevent VEGF from binding to its normal receptors (26-28), small molecule VEGFR TKIs (29-31), and antibodies against VEGF (32-34) or its receptors (35). Blockade of VEGF-mediated signaling suppresses tumor growth *in vivo* (33). In preclinical studies, preventing VEGF from binding to its receptor was one of the most effective ways of blocking the VEGF signaling pathway (36).

2.1.3. Targeting VEGF in CRC

VEGF is expressed on about 50% of CRCs, with little to no expression on normal colonic mucosa. In CRC, VEGF expression has been correlated with tumor size, tumor stage, lymph node metastases, and distant metastases (37, 38), as well as depth of tumor invasion (39), and appears to be an adverse prognostic factor (38, 40). The efficacy of VEGF as a therapeutic target is well-established in this disease, but currently anti-VEGF monoclonal antibodies are the only approved method of VEGF inhibition. Bevacizumab, a humanized monoclonal antibody that binds to VEGF and prevents its interaction with its receptor, is the only commercially available anti-angiogenic therapy for mCRC. Addition of bevacizumab to fluoropyrimidine and oxaliplatin-based therapy improves outcomes in mCRC. In the E3200 trial, bevacizumab was shown to significantly prolong overall survival (13 vs 10 months) and improved response (9% vs 3%) when combined with FOLFOX4 as compared to FOLFOX4 alone in patients with previously treated mCRC (7). These results led to the phase III NO16966 trial, which investigated the role of bevacizumab versus placebo in the first-line setting when added to fluoropyrimidine and oxaliplatin-based chemotherapy with FOLFOX or XELOX for the treatment of mCRC (41, 42). The primary endpoint of the study was met, with a significant improvement in progression-free survival in patients receiving oxaliplatin-based therapy plus bevacizumab versus placebo (9.4 vs 8 months, respectively). In subset analysis of FOLFOX versus XELOX, the PFS benefit was only significant for XELOX, however, the results may have been affected by the discontinuation of chemotherapy before evidence of disease progression in a substantial number of patients. There was no significant overall survival benefit for the addition of bevacizumab versus placebo to chemotherapy (21.3 vs 19.9 months). Objective response rates were similar (47% vs 49%), and 8.4% of patients receiving bevacizumab were able to undergo attempted curative metastatectomy (42). Bevacizumab also improves outcomes when added to irinotecan plus fluoropyrimidine therapy. In a randomized phase III study of patients with untreated mCRC, the addition of bevacizumab to the combination of bolus 5FU, leucovorin and irinotecan (IFL) significantly improved overall survival to a median of 20.3 months versus 15.6 months with IFL alone, with an overall response rate of 44% (4). Interestingly, the even patients who did not have objective responses by RECIST on this study still benefitted as much from the addition of bevacizumab as those who had an objective response. The phase III BICC-C study showed a significant survival benefit for the addition of bevacizumab to FOLFIRI in the first line setting of mCRC with a median OS of 28 months, versus 23 months for FOLFIRI alone and 19 months for IFL plus bevacizumab (9, 43). While the toxicity profile of bevacizumab does not seem to overlap with chemotherapy, commonly observed toxicities included proteinuria and hypertension, and an increased rate of thrombotic events are also seen. Based on the results of these studies, bevacizumab is approved for the treatment of mCRC in the untreated or refractory setting, in combination with 5-FU based cytotoxic chemotherapy. The clinical benefit observed from anti-VEGF therapy with bevacizumab highlights the need for further investigation to discover novel methods of optimizing blockade of this pathway in mCRC, in order to continue to improve clinical outcomes.

2.2. 5-Fluorouracil, Leucovorin and Oxaliplatin

2.2.1. 5-Fluorouracil and Leucovorin

5-fluorouracil (5-FU) is a pyrimidine antimetabolite which is metabolized intracellularly into its active form. Its subsequent incorporation into DNA and RNA inhibits thymidylate synthase and thus interferes with DNA synthesis and replication. Leucovorin is a reduced form of folic acid which enhances the cytotoxicity of 5-FU by stabilizing the binding of 5-dUMP and thymidylate synthase. 5-FU and leucovorin have been the backbone of CRC chemotherapy for more than the past decade. Standard leucovorin is a racemic mixture of the d and l-isomers and only the l-isomer is active. A preparation containing the l-isomer, known a levoleucovorin is commercially available. The standard dose of levoleucovorin is 50% of the dose of standard leucovorin. It is unknown whether leucovorin is a necessary component of FOLFOX. Recent shortages of leucovorin have required administration of the regimen without leucovorin commonly both on study and in clinical practice. Given the short half-life of 5FU, infusional regimens appear to provide the maximal dose intensity when administered over 24-48 hours. Improvement in response, PFS, toxicity, and a trend toward improved OS were seen with 5-FUadministered as a short continuous infusion, versus bolus (44).

2.2.2. Oxaliplatin

Oxaliplatin is a platinum derivative with a 1,2-diaminocyclohexane carrier ligand. While its exact mechanism of action is unknown, oxaliplatin is thought to exert its cytotoxic effects through the formation of DNA adducts. Oxaliplatin is distributed extensively in the plasma, although the elimination of total platinum in the urine is slow, with approximately 33% eliminated in 48 hours. Fecal elimination accounts for a trivial amount of clearance. As a single agent, oxaliplatin has minimal activity in advanced CRC.

2.2.3. FOLFOX

Oxaliplatin and 5-FU appear to be at least additive in their antitumor activity, although the mechanism of synergy is unclear. The combination of 5FU, leucovorin and oxaliplatin (FOLFOX) was initially approved as second line therapy for patients who had failed first-line IFL, based on a 9% ORR versus 0% with 5FU/LV or oxaliplatin alone, as well as improved time to progression and palliation of symptoms (45). A subsequent study of the combination in the first-line setting versus 5FU/LV (46) resulted in significantly improved PFS (9 vs 6.2 m) and ORR (51% vs 22%). Although OS benefit was not significant, a trend toward improved OS was seen with FOLFOX, suggesting that this regimen was beneficial in untreated mCRC. The role of FOLFOX as frontline therapy for mCRC was further solidified by the Intergroup N9741 study in 2004, which showed a significant improvement in time to progression (8.7 vs 6.9 m), ORR (45% vs 31%), and OS (19.5 vs 15m) versus IFL (12, 46). There have been multiple variations in the

FOLFOX regimen, based on the dose and schedule of 5FU, LV and oxaliplatin. The FOLFOX6 regimen consists of oxaliplatin 100 mg/m2 over 120 minutes, followed by LV 400 mg/m2 over 2 hours, followed by 5-FU 400 mg/m2 bolus, then 5-FU 2400 mg/m2 continuous infusion over 46-48 h, repeated every 2 weeks. A modified version of this regimen is currently in use (modified FOLFOX6 or mFOLFOX6) which includes a decrease in the dose of oxaliplatin to 85 mg/m2 (as in the FOLFOX4 regimen). Oxaliplatin-related peripheral neuropathy, which is cumulative after 4-5 months of therapy, tends to be the treatment-limiting toxicity with this regimen. The OPTIMOX1 and 2 trials were conducted for this reason to evaluate the impact on toxicity and efficacy of a stop-and-go administration of oxaliplatin in patients with mCRC receiving FOLFOX. The OPTIMOX1 trial revealed oxaliplatin can be safely discontinued after 3 months of FOLFOX therapy, and safely re-initiated following a period of maintenance therapy with infusional 5FU/LV upon resolution of toxicity or at disease progression, with achievement of objective response or stabilization of disease in 69% of patients at reintroduction, a trend toward less neurotoxicity and no adverse impact on OS, PFS, or duration of disease control (47). The follow-up OPTIMOX2 trial confirmed that maintenance therapy is required, as it improves outcomes versus no therapy, during the oxaliplatin-free period (48)

2.3 Aflibercept (VEGF Trap)

Aflibercept is a novel recombinant human fusion protein comprised of domain 2 of VEGFR1 fused with domain 3 from VEGFR2, attached to the hinge region of the Fc(a) domain of human IgG1. Aflibercept binds to VEGF and prevents it from interacting with its receptors (VEGFR), thus inhibiting the growth of new blood vessels that supply tumors. Aflibercept binds VEGF A and B in the picomolar (pmol/L) range, and also binds placental growth factor (PlGF), although with lower affinity. The binding of aflibercept to these ligands in vivo to block tumor angiogenesis and vascular permeability is the anticipated mechanism of activity of aflibercept. Aflibercept would be expected to lead to reduced tumor growth by a dual mechanism: reducing the density of tumor vasculature and reducing the supply of available nutrients and tissue matrix components escaping from leaky tumor vessels.

2.3.1 Preclinical Studies

2.3.1.1 *In Vitro* Activity Studies

In vitro single-agent activity of VEGF Trap

In vitro binding assays of VEGF Trap revealed a binding affinity for VEGF of about 1 pM. VEGF Trap also showed increased binding affinity for VEGF-A (dissociation constant [Kd] of 0.5 pM for VEGF-A). When added to cultured endothelial cells, VEGF-Trap completely blocked VEGF-induced VEGFR2 phosphorylation when added at a 1.5-fold molar excess compared with the added VEGF, which is consistent with a very high-affinity binding to VEGF. In addition,

VEGF Trap also binds PlGF1 and 2 (49).

2.3.1.2 *In vivo* activity studies

In vivo pharmacology studies have indicated that treatment with aflibercept effectively inhibits tumor growth of a wide variety of rodent and human tumor cell lines implanted either subcutaneously or orthotopically in mice. Aflibercept treatment inhibited the growth of tumors representing a variety of tissue types including melanoma, glioma, rhabdomyosarcoma, Wilms tumor, neuroblastoma, Ewing sarcoma, lymphoma, and ovarian, pancreatic, prostate, mammary, gastric, and colon tumor tissues with a broad pharmacological index. Antitumor activity was observed from 2.5 to 40 mg/kg (49-52). Aflibercept treatment significantly decreased the vessel density in tumors, an indication that tumor-induced angiogenesis was inhibited. In order to compare the efficacy of this method of VEGF blockade with other known VEGF blockers, aflibercept was compared at equimolar doses to DC101, a well-characterized monoclonal antibody that targets VEGFR2, in a mouse melanoma model. The doses of DC101 required to inhibit tumor growth were much higher than that of aflibercept, and the dose of DC101 required to be equally efficacious accumulated at 60-fold higher serum levels than aflibercept, suggesting that a much lower dose could be potentially efficacious in humans (49, 52).

2.3.1.3 Pharmacokinetics

Pharmacokinetic (PK) parameters of aflibercept in the mouse, rat, and monkey are summarized in Table 1. Inhibition of tumor growth was observed in mouse xenografts tumor models with aflibercept doses $\geq$ 2.5 mg/kg. This dose corresponds to a free aflibercept C_{max} concentrations of 10 µg/mL, about 10-fold higher than bound aflibercept concentrations. This suggests that levels of free aflibercept in excess of bound aflibercept may be necessary for pharmacological activity. Accordingly, the target pharmacological exposure in humans is proposed to be a safely administered dose of aflibercept, where maximal bound levels of aflibercept are achieved and an excess of free aflibercept is sustained.

Table 1. Summary of nonclinical pharmacokinetics for aflibercept

IV Route						
Species	**Dose (mg/kg)**	**C_{max} (µg/mL)**	**AUC (µg.h/mL)**	**$t_{1/2}$ (h)**	**Clearance (mL/h/ Kg)**	**Volume (mL/kg)**
Mouse	1	19.5	306	48	3.31	226
Rat	1	20.8±2.4	463±65	54±9	2.21±0.34	168
Monkey	5	181.7±46.4	10235±1532	98±31	0.5±0.07	69.1
AUC=area under the curve						

Following IV administration, free aflibercept was characterized by a low clearance (0.5-3 mL/hr/kg) and low volume of distribution (69 to 226 mL/kg), and a long apparent elimination half-life (48 to 98 hours) in all animal species evaluated.

2.3.1.4 Toxicology

Preclinical toxicology was evaluated in rats after a single IV administration and SC administration for 13 weeks as well as in cynomolgus monkeys after SC and IV administration for 4 and 13 weeks and IV administration for 26 weeks.

Single administration
A single IV injection (30-minute infusion) of aflibercept at 0, 50, 150, or 500 mg/kg to rats resulted in lesions at the injection site (tail vein) and a moderate decrease in mean body weight gain in males at all dose levels. The Lethal Dose was above 500 mg/kg and the No-Observable Adverse Effect Level (NOAEL) was 150 mg/kg.

Repeated administration
In the 13-week rat study, SC administration of aflibercept 3 times per week was associated with morbidity and mortality at 1 and 2 mg/kg/administration. The most prominent microscopic findings were observed in the kidney (glomerular mesangial thickening and tubular protein casts) from 0.5 mg/kg/administration and were associated at 1 and 2 mg/kg/administration with increased serum BUN and urinary protein levels, and decreased serum protein levels. Renal toxicity of aflibercept observed in normal immunocompetent rats that produced antibodies to aflibercept, was due, at least in part, to the deposition of immune complexes in renal glomeruli. Microscopic changes in the femur (decrease and/or loss of metaphyseal bone trabeculae and metaphyseal capillaries, thinning of cortical bone) were observed at doses ≥1 mg/kg/administration. The NOAEL was determined to be 0.1 mg/kg/administration.

In the monkey studies, the main compound-related microscopic findings were observed in the bone, nasal cavity, kidney, ovary, and adrenal gland. In the bone, aflibercept-induced effects consisted mainly of thickening of the growth plate and osteocartilaginous exostoses observed on the axial and appendicular skeleton that correlated with hunched posture at clinical examination. In the nasal cavities, degeneration/regeneration of the respiratory and olfactory epithelium, and atrophy/loss of nasal septum and/or turbinates was often associated with hemorrhage and suppurative exudate. Histopathologic findings in the kidneys (increased glomerular mesangial matrix) were associated in a few animals with decreased serum total protein and albumin levels and increased serum BUN and urine protein and/or microalbumin levels. In the ovaries, the decreased number of maturing follicles, granulosa cells, and/or theca cells was associated with an overall inhibition of the female reproductive function. The histopathologic changes in the bone growth plate and ovary were considered related to the pharmacological activity of aflibercept. In the adrenals, a decreased vacuolation of adrenal zona fasciculata cells with cytoplasmic eosinophilia was observed. In addition, focal vascular proliferation/degeneration was noted in a range of organs, including in particular the digestive system, urinary bladder, heart, and brain of a few monkeys. Main clinical pathology changes consisted of slight increases in red

blood cell parameters, fibrinogen, globulin, BUN, and decreases in albumin. In addition, increased liver enzyme (GGT, AST, ALT, ALP) levels were noted in a few monkeys with portal inflammation and necrosis. Aflibercept administration also resulted in a decrease in sperm motility and increased incidence of abnormal spermatozoa morphology. Most aflibercept-related findings were noted from the lowest doses tested (1.5 to 3 mg/kg/administration). With the exception of osteocartilaginous exostoses and nasal cavity findings, aflibercept related findings were reversible within 5 months. Aflibercept was not highly immunogenic in monkeys treated by either the SC or IV route for 13 weeks, while the incidence of antibody response increased in monkeys treated for 26 weeks.

Reproductive Toxicology

The SC administration of aflibercept at 25 mg/kg to adult female marmosets during the follicular or luteal phase inhibited antral follicle development and ovulation. When administered in the luteal phase, aflibercept induced functional luteal involutions. Single IV injections of aflibercept at 0.25, 1, 4, or 12.5 mg/kg, at defined stages of menstrual cycle to adult female stumptail macaques led at all doses to rapid dose-dependent and reversible suppression of ovarian function.

The effects of aflibercept on male and female fertility parameters were also evaluated in sexually mature cynomolgus monkeys treated at 3, 10 and 30 mg/kg/administration once a week for 15 weeks and then every other week up to Week 26. In females, the abrogation of ovarian function and follicular development was evidenced from 3 mg/kg/administration. Aflibercept-induced inhibition of ovarian function was reversible within 3 to 18 weeks after the last injection. In males, the main aflibercept-induced effects consisted of a decrease in sperm motility and an increase in incidence of spermatozoa morphological abnormalities from 3 mg/kg/administration. These effects were fully reversible after a 13 week recovery period.

The effects of aflibercept on embryofetal development were evaluated in pregnant rabbits treated IV at doses of 3, 15, or 60 mg/kg/administration on gestation Days 6, 9, 12, 15, and 18 (total of 5 administrations) during organogenesis. Aflibercept at 60 mg/kg/administration induced abortion, minimal to moderate maternal toxicity, and embryolethality. External, visceral (affecting mainly the heart and great vessels), and/or skeletal malformations were observed in fetuses from dams treated at 3 mg/kg/administration (corresponding to a plasma exposure of 1935 µg.h/mL, which is approximately 1.3 times the systemic exposure in patients at the recommended human dose). The visceral and skeletal malformations are likely related to the pharmacological activity of aflibercept.

Genotoxicity

No studies are planned to evaluate genotoxicity of aflibercept because genotoxicity assays are not applicable for biologics.

2.3.2 Clinical Experience with Aflibercept

Aflibercept has been administered IV and SC to healthy subjects, and IV and SC to oncology patients. Available information is described in this CIB. Additional studies sponsored by the National Cancer Institute – Cancer Therapy Evaluation Program (NCI-CTEP) are ongoing.

The PK profiling of aflibercept has been determined:
• in healthy subjects, after 1 hour single IV administration at doses of 1 to 4 mg/kg, and after single SC administration at 2 mg/kg.
• in cancer patients, after SC administration (single and repeated doses) at doses of 25 µg/kg once weekly to 800 µg/kg twice weekly.
• in cancer patients, after IV administration after 1 hour infusion at doses of 0.3 mg/kg to 7 mg/kg every 2 weeks, and up to 9 mg/kg every 3 weeks, following both single and repeated administrations.

Concentrations of free aflibercept, bound aflibercept, and anti-aflibercept antibodies were measured by ELISA. Concentrations of total aflibercept were derived from measurements of free and bound aflibercept.

No metabolism studies of aflibercept have been performed since it is a protein.

2.3.2.1Clinical Pharmacokinetics

The kinetics of aflibercept were determined in healthy subjects in two studies, PDY6655 and PDY6656. The primary objective of these studies was to evaluate the blood pressure effects of a single dose of aflibercept. In study PDY6656, the pharmacokinetics of aflibercept have been evaluated following a single IV administration at the doses of 1, 2, and 4 mg/kg versus placebo in four parallel groups of twelve healthy subjects. In study PDY6655, the absolute bioavailability of the aflibercept SC formulation based on free and bound aflibercept levels in healthy male subjects was determined following administration of a single dose of 2 mg/kg.

Mean plasma concentration-time profiles for study PDY6656 are shown in Figure 1.

The PK profile of free aflibercept was biphasic with concentrations detectable up to 35 days in a majority of subjects at a dose of 1 mg/kg and up to 42 days at the doses of 2 and 4 mg/kg. Free aflibercept was characterized by dose dependent clearance, and a volume distribution of about 6 L. Dose dependent clearance is consistent with a saturation of the rapid clearance pathway of binding endogenously produced VEGF. The elimination half-life of free aflibercept was about 5 days.

Bound aflibercept Cmax and AUC increased proportionally with doses between 1 and 2 mg/kg, then plateaued between 2 and 4 mg/kg, suggesting that free aflibercept was present in sufficient amounts to bind all endogenous VEGF at the higher dose levels. Median tmax of bound aflibercept increased with dose from 14 days at the dose of 1 mg/kg to 28 days at the dose of 4 mg/kg.

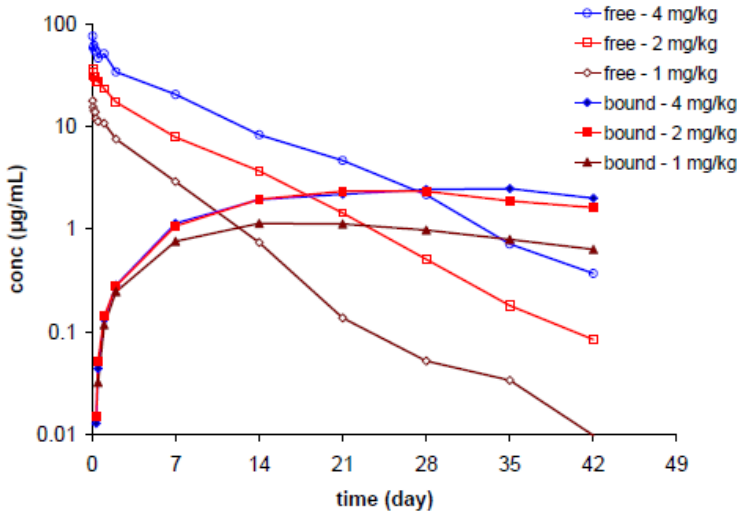
Free VEGF levels in plasma increased from below the limit of quantitation (15 pg/mL) during the two first weeks after infusion to a mean concentration of 48.7 pg/mL, 42 days after administration of the 4 mg/kg dose.

Following administration of 1, 2, and 4 mg/kg doses, free aflibercept levels remained in excess of bound aflibercept levels for approximately 10, 18, and 28 days, respectively.

Absolute bioavailability of free aflibercept SC was 54.9 %, with an elimination half-life of approximately 5 days, comparable to that estimated after IV infusion.

The current clinical development program is focused on IV administration.

Figure 1. Mean free and bound aflibercept concentration-time profiles (µg/mL)



The pharmacokinetics of aflibercept were studied in patients receiving aflibercept as an IV single agent during Cycle 1 (TED6115) and during repeated cycles (extension study TED6116). Patients received 2 doses IV, administered two weeks apart, in study TED6115. Patients enrolled in the extension study (TED6116) continued to receive aflibercept every other week at their assigned dose, or at a lower dose if toxicity was encountered.

Mean PK parameters of free aflibercept are summarized in Table 2.

Table 2. Mean (CV%) pharmacokinetic parameters of free aflibercept in study TED6115 (Cycle 1)

Dose (mg/kg)	No. of patients per dose	C_{max} (µg/mL)	C_{last} (µg/mL)	AUC_{last} (day*µg/mL)	AUC (day*µg/mL)	$t_{1/2}$ (day)	V_{ss} (L)	CL (L/day)
0.3	3	4.00 (9)	0.147 (38)	8.97 (15)	9.34 (15)	1.75 (21)	4.51 (29)	1.95 (42)
1.0	7	17.9 (31)	0.659 (116)	47.3 (47)	50.9 (54)	2.58 (50)	5.88 (22)	1.87 (51)
2.0	6	34.5 (11)	2.36 (71)	110 (30)	125 (35)	3.76 (42)	5.58(21)	1.13 (31)
3.0	7	48.7 (30)	4.06 (63)	184 (30)	226 (34)	6.18 (38)	7.74 (33)	1.14 (48)
4.0	7	97.4 (43)	11.0 (51)	209 (31)	293 (15)	5.51 (18)	7.88 (38)	1.10 (38)
5.0	4	86.8 (34)	9.63 (28)	326 (69)	428 (64)	7.43 (38)	9.89(31)	1.27 (65)
7.0	12	159 (21)	14.4 (55)	497 (44)	605 (46)	5.14 (37)	6.12 (29)	0.915 (39)

Free aflibercept exposure increased more than proportionally in the dose range 0.3 to 2 mg/kg, then approximately proportionally to dose in the dose range 2 to 7 mg/kg. The apparent elimination half-life increased with doses from 1.70 days at 0.3 mg/kg to 3.76 days at 2 mg/kg, reaching 5.14 days at 7 mg/kg. Mean clearance values of free aflibercept decreased from 1.95 L/day estimated at the dose of 0.3 mg/kg to 1.13 L/day at the dose of 2 mg/kg. These dose dependent values are in agreement with a saturation of the rapid clearance pathway of binding endogenously produced VEGF.

Mean PK parameters of bound aflibercept are summarized in Table 3.

Table 3. Mean (CV%) pharmacokinetic parameters of bound aflibercept in study TED6115 (Cycle 1)

Dose (mg/kg)	No. of patients per dose	T_{max}[a] (day)	C_{max} (µg/mL)	C_{last} (µg/mL)	AUC_{last} (day*µg/mL)
0.3	3	9.96 (7.03 – 9.98)	0.575 (5)	0.494 (16)	5.53 (5)
1.0	7	9.98 (7 – 14)	1.22 (29)	1.12 (38)	9.98 (30)
2.0	6	13.5 (9.01 – 13.9)	1.58 (32)	1.57 (32)	12.7 (41)
3.0	7	13.9 (7.08 – 23)	1.72 (23)	1.55 (34)	16.1 (38)
4.0	7	9.99 (3.04 – 14)	1.34 (45)	1.27 (49)	10.8 (38)
5.0	4	10.5 (6.98 – 21.8)	1.94 (25)	1.94 (25)	12.9 (18)
7.0	13	14 (7.02 – 27.9)	2.37 (37)	2.35 (38)	20.9 (26)

[a] Median and range

The concentrations of bound aflibercept increased with doses between 0.3 and 2 mg/kg, then plateaued between 2 and 7 mg/kg, suggesting that bound concentrations were limited by the endogenous production of VEGF. Free aflibercept levels remained in excess of bound aflibercept levels during the two weeks from the dose of 2 mg/kg.

Sources of pharmacokinetic variability

Intrinsic factors which could impact PK have not been evaluated to date; aflibercept is not metabolized by hepatic CYP enzymes and the renal elimination of aflibercept corresponds to approximately 1% of the glomerular filtration rate.

Extrinsic factors as sources of PK variability, such as cytotoxic agents given in combination with aflibercept, have been evaluated (Table 4). Pharmacokinetic data are presented in table 5.

Table 4. Clinical oncology studies assessing drug-drug interactions

Study no. Start/end dates	Study design	Route/schedule of administration
TCD6117 End date: 30-Oct-2008	Phase 1, open-label, multicenter, dose-escalation study of aflibercept in combination with standard doses of FOLFOX4 in patients with advanced cancer	IV, q2w
TCD6118 Ongoing	Phase 1, open-label, multicenter, dose-escalation study of aflibercept in combination with standard doses of irinotecan, LV, and 5-FU in patients with advanced cancer	IV, q2w
TCD6119 Ongoing	Phase 1, open-label, multicenter, dose-escalation study of aflibercept in combination with standard doses of docetaxel, cisplatin and 5-FU in patients with advanced cancer	IV, q3w
TCD6120 Ongoing	Phase 1, open-label, multicenter, dose-escalation study of aflibercept in combination with standard doses of docetaxel, then with standard doses of docetaxel/cisplatin in patients with advanced cancer	IV, q3w
TCD6121 Ongoing	Phase 1, open-label, multicenter, dose-escalation study of aflibercept in combination with standard doses of gemcitabine, then with standard doses of gemcitabine/erlotinib in patients with advanced cancer	IV, q2w

Table 5. Mean (CV%) pharmacokinetic parameters of free and bound aflibercept in combination studies.

			Free aflibercept (Cycle 1)			Bound aflibercept		
Dose (mg/kg)	Study # (regimen)	No of patients per dose	C_{max} (µg/mL)	C_{last} (µg/mL)	CL (mL/day/kg)	End of Cycle 1	Median of following cycles	Free/bound ratio from cycle 3
2	**TCD6117** q2w	3	32.5 (17)	2.29 (67)	14.1(36)	1.76 (13)	3.49 (58)	0.74 (60)
	TCD6118 q2w	4	35.4 (18)	2.03 (90)	11.9 (21)	2.01 (33)	4.21 (23)	0.94 (22)
	TCD6119 q3w	5	35.8 (13)	0.153 (31)	16.8 (22)	1.54 (14)	2.27 (16)	0.16 (22)
	TCD6120 q3w	7	43.0 (27)	0.451 (130)	19.1 (29)	1.61 (36)	2.49 (45)	0.27 (110)
4	**TCD6117** q2w	15	88.3(29)	19.1 (65)	8.42 (51)	1.68 (60)	3.04 (29)	1.77 (37)
	TCD6118 q2w	12	64.0 (36)	4.49 (74)	17.8 (42)	2.00 (19)	3.72 (29)	2.15 (49)
	TCD6119 q3w	9	85.7 (17)	1.16 (130)	16.6 (42)	1.32 (35)	2.65 (57)	1.01 (62)
	TCD6120 q3w	10	130 (36)	1.80 (100)	15.4 (30)	1.92 (22)	3.1 (36)	0.96 (57)
	TCD6121 q2w	18	90.3 (35)	4.95 (99)	13.84 (40)	1.62 (34)	3.22 (37)	1.96 (52)
5	**TCD6117** q2w	9	104 (26)	19.5 (100)	9.54 (45)	1.77 (43)	3.76 (36)	4.50 (57)
	TCD6118 q2w	10	94.8 (66)	6.83 (54)	16.4 (46)	1.67 (15)	3.70 (27)	2.58 (42)
	TCD6120 q3w	13	124 (42)	5.18 (58)	11.5 (42)	2.52 (25)	3.48 (22)	1.75 (53)
6	**TCD6118** q2w	12	125 (46)	7.02 (84)	15.6 (52)	1.83 (32)	3.57 (46)	3.50 (50)
	TCD6120 q3w	29	119 (24)	3.20 (79)	14.1 (32)	1.97 (25)	2.83 (34)	1.30 (57)
	TCD6121 q2w	11	115 (26)	11.5 (75)	15.4 (63)	1.95 (71)	3.41 (30)	4.98 (82)
7	**TCD6120** q3w	4	153 (18)	6.61 (94)	13.3 (42)	2.55 (25)	3.03 (29)	2.22 (66)
9	**TCD6120** q3w	3	198 (19)	6.10 (40)	10.6 (26)	2.36 (4)	2.86 (26)	2.15 (94)

TCD6117 used FOLFOX4, TCD6118 used irinotecan + LV + 5-FU, TCD6119 used docetaxel, cisplatin, 5-FU, TCD6120 used docetaxel and cisplatin, and TCD6121 used gemcitabine and erlotinib.

Free and bound aflibercept concentrations determined in combination studies are in the same order of magnitude of those observed in the monotherapy study TED6115, suggesting that cytotoxic combinations (FOLFOX 4, irinotecan, 5-FU, docetaxel, and gemcitabine) have no influence on the PK of aflibercept. Anti-aflibercept antibodies have been reported using ELISA methods. These findings are not unexpected, as aflibercept is a recombinant protein product. The immunogenicity testing with present methods showed that less than 1% of patients evaluated developed anti-aflibercept antibodies. Further assay development is ongoing and the implications and understanding of anti-aflibercept antibodies is under evaluation.

2.3.2.2 Clinical Efficacy

Phase I studies have a demonstrated safety in advanced solid tumors, as well as phase II efficacy data in second line mCRC including patients refractory to bevacizumab. Recent phase III data showed a survival benefit for the addition of aflibercept over placebo to second-line chemotherapy in mCRC.

2.3.2.2.1 Phase I Trials of Aflibercept

Aflibercept has been studied in as a single agent and in combination with chemotherapy in the phase I setting.

In a phase I study of patients with refractory advanced solid tumors, 47 patients were enrolled and received escalating doses of single-agent aflibercept from 0.3 to 7 mg/kg IV every 2 weeks (53). DLTs included grade 3 ALT elevation at 1 mg/kg, grade 3 dyspnea and arthralgia at 2 mg/kg, and grade 3 hypertension at 4 mg/kg. At the 7 mg/mg dose, DLTs included proteinuria (n=1) and rectal ulceration (n=1). The most common drug-related AEs were fatigue (63%), nausea (36%) and vomiting (27%), which were generally grade 1 and resolved with discontinuation of the drug. Toxicities commonly associated with antiangiogenic therapy that were seen with aflibercept included dysphonia (46%), hypertension (38%) and proteinuria (10%). The incidence of grade 3-4 hypertension increased with increasing doses of aflibercept (42% at 4 mg/kg, 75% at 5 mg/kg, and 46% at 7 mg/kg). Based on this toxicity profile, the recommended phase II dose of aflibercept was 4 mg/kg every 2 weeks as a single agent. Evidence of activity was observed, with 3 patients achieving confirmed PR and an additional patient with an unconfirmed PR. Correlative DCE MRI showed evidence of decreased tumor perfusion and vascularity with increasing dose levels of aflibercept.

TCD6117 (AVE0005A/1001) was an open-label, phase 1, dose-escalation, sequential-cohort study of the safety, tolerability, and pharmacokinetics of IV aflibercept administered every 2 weeks in combination with FOLFOX4 (oxaliplatin 85 mg/m2 on Day 1, leucovorin 200 mg/m2 on Day 1 and Day 2, 5-FU bolus/infusion 400/600 mg/m2 on Day 1 and Day 2 of a 2-week cycle) in patients with advanced solid malignancies. Thirty-two patients (male/female: 9/23, median age 59 years [range 25 to 79], ECOG performance status 0/1: 11/21) were enrolled at 3 aflibercept dose levels: 2 mg/kg (n = 4), 4 mg/kg (n = 18), and 5 mg/kg (n = 10). Primary tumors were mainly gastrointestinal (16, including 8 pancreas, 3 cholangiocarcinoma, and 3 gastric), breast (5), ovarian (4), and others (7). Most patients (75%) were heavily pretreated, with a median number of prior lines of chemotherapy of 3 (range 1 to 9). Patients received a median number of 3.5 treatment cycles (range 2 to 8), 7.0 (range 2 to 20), 5.5 (range 1 to 20) at the 2, 4, and 5 mg/kg dose levels, respectively.

2.3.2.2.2 Phase II Trials of Aflibercept in CRC

A second line, 2-stage phase II trial evaluated aflibercept monotherapy in two cohorts of previously-treated mCRC patients, a cohort of patients previously treated with bevacizumab and a cohort of bevacizumab-naïve patients (54). Aflibercept 4mg/kg IV every 2 weeks was administered. Among the bevacizumab naïve patients, the disease control rate (DCR = PR + SD > 16 weeks) was 29% and median PFS was 2 m; among bevacizumab pretreated patients, there was one partial response, with a disease control rate of 30% and median PFS of 3.4 m. Only the bevacizumab pretreated cohort met the prespecified response criteria to proceed with further trials. Aflibercept was well tolerated in these patients (54).

2.3.2.2.3 Phase III Trials of Aflibercept in CRC

Data were recently presented from a randomized phase III trial (VELOUR) in which patients with mCRC who had failed oxaliplatin-based therapy were randomized to receive FOLFIRI plus aflibercept 4 mg/kg or placebo every 2 weeks. 30% of patients had failed prior bevacizumab therapy as well. The primary endpoint of the trial was OS, which was met with a statistically significant improvement in the aflibercept arm (13.5m vs 12 m). PFS (6.9m vs 4.7 m) and ORR (20% vs 11%) were also significantly improved with the addition of aflibercept (55). These results were remarkably similar to the 2nd-line E3200 trial with FOLFOX and bevacizumab (7), further supporting the role of anti-VEGF therapy in this disease. Grade 3-4 AEs observed in VELOUR with >2% higher incidence with aflibercept versus placebo were diarrhea, asthenia/fatigue, stomatitis/ulceration, infections, hypertension, GI/abdominal pain, neutropenia/neutropenic complications, and proteinuria. AEs led to treatment discontinuation in 26.6% of patients receiving aflibercept, versus 12.1% of patients receiving placebo.

2.3.2.3 Clinical Toxicology

To date, aflibercept has been administered to 76 healthy subjects and approximately 2000 patients with advanced solid malignancies in clinical oncology trials. In company sponsored trials, doses have been administered up to 800 μg/kg twice weekly SC, 7 mg/kg every 2 weeks, and 9 mg/kg IV every 3 weeks. In company-sponsored Phase 1 and Phase 2 single agent trials >400 patients have been dosed with aflibercept for >2000 cycles, primarily on an every 2 week schedule. In company-sponsored Phase 1 combination trials, >200 patients have been dosed for >1000 cycles in either an every 2 or 3 week schedule. Phase 3 combination trials evaluating 4 mg/kg every 2 weeks and 6 mg/kg every 3 weeks of aflibercept are recently completed/ongoing.

The most frequent TEAEs (all grades, regardless of relationship to study medication) observed across all doses were:

- overall incidence >25%: fatigue, nausea, diarrhea, vomiting, anorexia, hypertension, headache, constipation, mucosal inflammation, dysphonia, epistaxis, and abdominal pain
- overall incidence 10-25%: peripheral sensory neuropathy, pharyngolaryngeal

pain, temperature intolerance, abdominal pain upper, arthralgia, chills, dyspepsia, dyspnea, neuropathy peripheral, palmoplantar erythrodysesthesia syndrome, pain in extremity, back pain, bone pain, dehydration, gastroesophageal reflux disease, edema peripheral, skin discoloration, alopecia, dizziness, insomnia, and pyrexia. The profile of AEs reported is overall consistent with what has been reported with VEGF blockade and FOLFOX4. A dose of 4mg/kg of aflibercept was selected based on similar free and bound aflibercept levels in single agent and in combination with FOLFOX4 (56).

Overall, based upon treatment-related serious or severe AEs in clinical trials, the following toxicities may occur with IV aflibercept as a single agent or in combination with other drugs:

- **Hematological disorders and resistance:** anemia, thrombocytopenia, thrombotic microangiopathy; additionally in combination with cytotoxic chemotherapy: neutropenia including febrile neutropenia, neutropenic colitis, and sepsis
- **Cardiac disorders:** cardiac failure
- **Digestive toxicity:** abdominal pain, gastrointestinal hemorrhage, intestinal perforation, intestinal obstruction, enteric fistula, peritonitis, pneumatosis intestinalis, nausea, vomiting, diarrhea, constipation, mucosal inflammation or ulceration, stomatitis, hepatic enzymes increased
- **General disorders:** asthenia, fatigue, musculoskeletal pain, injection site reaction
- **Immune system disorders**: hypersensitivity
- **Metabolic disorders:** dehydration
- **Musculoskeletal:** arthralgia, myalgia, osteonecrosis
- **Nervous system:** headache, dizziness, encephalopathy (including reversible posterior leukoencephalopathy syndrome), cerebral ischemia, cerebral hemorrhage, cerebral venous thrombosis
- **Renal disorders:** proteinuria (including nephrotic syndrome), hematuria, renal failure
- **Respiratory disorders:** dysphonia, dyspnea, epistaxis, hemoptysis, pulmonary embolism, tracheo-esophageal fistula
- **Skin disorder** in combination with cytotoxic chemotherapy: palmar-plantar erythrodysaesthia syndrome, erythema
- **Vascular disorders:** hypertension (including malignant hypertension), deep vein thrombosis, and phlebitis

<u>Developmental/Reproductive Toxicity</u>

As angiogenesis is critical to fetal development, the inhibition of angiogenesis following administration of aflibercept is likely to result in adverse effects on pregnancy. There are no adequate and well-controlled studies in pregnant women using aflibercept. Women of childbearing potential should be advised to avoid becoming pregnant while receiving aflibercept, and advised to use effective contraception (including abstinence and double-barrier methods) during and up to a minimum of 6 months after the last dose of treatment. Developmental and

reproductive toxicity studies of aflibercept have not been performed thus far. Aflibercept has not been tested in breastfeeding women, however IgG1s are secreted into breast milk, and aflibercept should not be administered to women who are breastfeeding. No genotoxicity studies have been conducted with aflibercept.

If a patient becomes pregnant while receiving aflibercept, therapy should be discontinued immediately and the patient should be apprised of the potential hazard to the fetus and/or the potential risk of loss of pregnancy. Patients who discontinue aflibercept should also be counseled concerning the prolonged exposure following discontinuation of therapy (half-life of approximately 20 days) and the possible effects of aflibercept on fetal development.

Immunogenicity and Infusion Reactions
As of February 12, 2009, a total of 3923 serum samples from 1254 cancer patients treated with aflibercept (as monotherapy or in combination with cytotoxic chemotherapy) were assessed for immunogenicity using ELISA methods. To date, anti-aflibercept antibodies have been detected in 11 samples from 11 different patients (incidence: 0.9% across the 1254 patients in single and combination agent studies.)

As of 12 February 2009, 9 SAEs of hypersensitivity, all recovered, have been reported. Two of the 9 cases were not related to aflibercept but rather to additional medication (non-protocol paclitaxel in one case and protocol oxaliplatin in the second case). The remaining 7 cases of hypersensitivity were assessed by the Investigators as related to study treatment. The majority of the events were of moderate severity.

2.4 Correlative Studies Background

2.4.1 Laboratory/Biologic Correlative Studies

Specific Aim: To identify novel predictive markers of response to therapy in patients with untreated metastatic colorectal cancer receiving aflibercept. The tissue and blood correlative studies from this trial will include, but are not limited to the following listed below. Any leftover tissue or blood will be kept for future unknown research. Note that consent for future unknown research for the tissue banking and blood banking parts are optional to enroll on this study. .

Measurement of Serum VEGF, VEGFR, and P1GF (Cynthia Timmers, PhD)

There remains a critical need to identify biomarkers to predict which patients will benefit from anti-angiogenic therapy, and which patients will not respond. The majority of prior clinical trials conducted with bevacizumab have included biomarker studies. Previous studies have shown that plasma VEGFA may be a prognostic biomarker in mCRC, with high levels associated with worse OS than

low levels, but predictive value of VEGF was not observed for bevacizumab-based treatment (57). It was previously demonstrated that VEGF and VEGFR2 may be predictive for efficacy with bevacizumab in breast cancer patients, with high expression correlating with better PFS (58). Similarly, VEGFA and VEGFR2 were found to be potentially predictive of efficacy for pancreas cancer patients treated with bevacizumab, with high levels correlating with improved survival with treatment (59). To further investigate predictive markers, samples from several previously conducted trials were re-analyzed to evaluate potential predictive value of plasma VEGFA, using a novel ELISA-based assay favoring shorter isoforms $VEGFA_{121}$ and $VEGFA_{110}$. Results were presented at the 2011 ESMO meeting and showed a potential predictive value of high levels of VEGFA for efficacy with bevacizumab, with high plasma VEGFA levels correlating with improved PFS and OS following treatment with bevacizumab in pancreas, breast and gastric cancers, but this effect was not seen in CRC (60), suggesting that further investigation is needed to study these and other novel predictive biomarkers for efficacy in this disease. It was also hypothesized that certain VEGFA isoforms such as $VEGFA_{121}$ and/or $VEGFA_{110}$ may be driving predictive value and may vary in different tumor types, which may be a reason for the predictive value of VEGF observed in some cancers and not others on this (60).

Predictive biomarkers for aflibercept were investigated in a phase II study. In recurrent glioblastoma multiforme (GBM) patients treated with aflibercept, circulating VEGF levels were significantly decreased within 24 hours of treatment with aflibercept, and correlated with radiographic response on MRI. These data suggest that decreased levels of VEGF may predict response to therapy with aflibercept (61). PIGF levels initially decreased following aflibercept treatment, but increased significantly by day 28. Lower baseline levels of P1GF were also associated with improved response suggesting potential value as a predictive marker (61). These data suggest that VEGF and its receptor and P1GF may be potential predictive biomarkers for response to therapy with aflibercept. We will measure serum levels of VEGFA, VEGFR2, and P1GF during treatment, which will be correlated with clinical outcomes.

2.4.1.1 Measurement of Serum CXCL12 and CXCR4 (Cynthia Timmers, PhD)

Several studies have shown that chemokines (chemoattractant cytokines) are major regulators of cancer cell trafficking and adhesion, and are an important factor in organ-selective metastases. CXCR4 and its only known ligand CXCL12 (SDF1) have a known role in the metastatic spread of colorectal cancer (62). In preclinical models, CXCL12-induced CXCR4 activation results in migration and survival of colorectal cancer cells (63, 64) CXCR4 overexpression is associated with lymph node metastases in colon cancer (65) and its expression has been associated with adverse outcome including increased risk of recurrence, development of metastatic disease, and inferior survival (63, 65). Inhibition of CXCL12-CXCR4 signaling results in marked inhibition of solid tumor metastases in animal models (62, 66, 67).

There is evidence to suggest that VEGF upregulates the CXCR4 expression on vascular endothelial cells (VEC), and is synergistic with CXCL12-mediated VEC migration (68). Additionally, due to the mechanism of action of VEGF, tumor changes in blood flow/microvessel density, capillary permeability, and blood vessel normalization are potentially impacted by anti-VEGF therapies (68). Inhibition of VEGF may therefore lead to decreased expression of CXCR4. CXCL12/CXCR4 inhibition has been shown to decrease the growth of gastrointestinal tumors (68). We hypothesize that VEGF inhibition may alter chemokine expression and that pretreatment levels of chemokine expression may be prognostic or predictive for response to therapy with aflibercept. We will obtain serum measurements of CXCR4 and CXCL12 at various timepoints of treatment and correlate these levels with clinical outcomes to test this hypothesis.

2.4.1.2 Single nucleotide polymorphisms (SNPs) (Cynthia Timmers, PhD)

Previous attempts to identify tumor-related biomarkers that predict efficacy of bevacizumab (such as tumor expression of VEGF) have been largely unsuccessful- a possible reason being that angiogenesis is a host-related process. However, there is a significant amount of inherited genetic variability within VEGF and its receptor including multiple single-nucleotide polymorphisms (SNPs) (69, 70) which may have prognostic and/or predictive value. The VEGF gene is located on chromosome 6p12-p21 and is made up of 8 exons separated by 7 introns that exhibit alternative splicing to form a family of proteins (71, 72). There have been several polymorphisms identified in the VEGF gene, some of which are associated with variations in VEGF protein production (69, 73, 74). These polymorphisms include [-2578C>A,-1154G>A, and-634G>C (translation start site counted as +1) in the promoter or 5' untranslated region and +936C>T in the 3'-untranslated region]. VEGF gene polymorphisms have been associated with the development of cancers in which angiogenesis plays a critical role (75-78), and recent data shows that the presence of certain VEGF SNPs can be predictive of clinical outcome in breast and ovarian cancers (79-81). In a study of 446 of colorectal cancer patients who had undergone prior surgical resection for stage II or III disease, VEGF gene polymorphisms were shown to be an independent prognostic marker (82). Specifically, 3 genotypes were analyzed: -2578C>A, -634G>C, and +936C>T. On univariate analysis, all 3 had a survival effect. On multivariate analysis, patients with the -634G>C polymorphism, the G/C genotype or C/C genotype exhibited significantly superior OS and PFS compared with the G/G genotype. For patients with the +936C>T polymorphism, survival was significantly worse if the C/T or T/T genotype was present, versus the C/C genotype. The -2578C>A polymorphism was not significantly associated with outcome on multivariate analysis (82). Subsequent haplotype analysis evaluated the combined prognostic effect of the 3 polymorphisms. Haplotype CCC was associated with significantly improved survival versus CGC, and in turn CGC was associated with improved survival over AGT haplotype. These data

suggest a prognostic influence of certain VEGF gene SNPs in patients with CRC.

The predictive value of VEGF SNPs for response to anti-angiogenic therapy with bevacizumab was also recently demonstrated in breast cancer, where patients with the VEGF genotypes -2578 AA and -1154 AA had superior OS with bevacizumab treatment versus other VEGF genotypes. In addition, VEGF SNP genotypes -634 CC and -1498 TT were strongly associated with less grade 3-4 hypertension with bevacizumab therapy (70, 83). Recently presented preliminary data suggest that VEGF SNPs may have predictive value across a wide variety of cancers treated with bevacizumab including CRC (84). Patients with VEGF-2578 AA genotype had a trend toward improved clinical efficacy and genotypes -1498 TT and -634CC had a trend toward less grade 3-4 toxicities.

We will analyze VEGF SNPs in patients with mCRC treated with aflibercept to identify any SNPs that may be prognostic or predictive of clinical efficacy and/or toxicity. We will also compare our results to those of patients previously treated with bevacizumab.

2.4.1.3 Measurement and Quantification of Tumor Vasculature (Cynthia Timmers, PhD)

In preclinical mouse models, treatment with VEGF-Trap resulted in decline in tumor vascular patency and blood flow with a marked decrease in tumor blood vessel density by more than 70% in seven days, and decreased VEGFR2 and VEGFR3 expression in surviving vascular endothelial cells. In addition, the sprouting of new blood vessels was also inhibited (85). A second study in mice bearing human colorectal cancer xenograft tumors also showed that VEGF-Trap resulted in a decrease in tumor vasculature in just 3 days, and also induced a decrease in the caliber of tumor vessels and inhibited proliferation of vascular endothelial cells and formation of new blood vessels (25). We will measure the effect of treatment with aflibercept on tumor vasculature by immunohistochemistry performed on tumor biopsies pretreatment, and post–cycle 2 (tumor biopsies after cycle 2 will only be performed at Ohio State University Medical Center) with aflibercept. Results will also be correlated with measurements of tumor vasculature by DCE-MRI and serum markers of angiogenesis.

2.4.1.5 Hypertension

Hypertension is a well-known toxicity of anti-angiogenic therapy. Several studies have also shown that hypertension may be a predictive biomarker for efficacy of anti-angiogenic therapy (68, 70, 86-90) however this has not been shown in all studies (91) and warrants further investigation. We will characterize hypertension as a treatment-related toxicity according to CTCAE v. 4.0 and evaluate for correlation with clinical outcomes to determine if hypertension may be a predictive biomarker for efficacy of aflibercept in CRC.

2.4.2 Radiographic Correlative Studies (Dr. Michael Knopp)

2.4.2.1 DCE-MRI

Tumor treatment evaluation needs to address as the tumor are a truly three-dimensional, continuously evolving process and during their development and therapy, neoplastic lesions change their properties (i.e. neoangiogenesis, metabolism and necrosis). These changes can occur simultaneously in various parts of a tumor that leads to lesion heterogeneity. Three-dimensional detailed assessment with dynamic contrast enhanced MRI (DCE-MRI) is able to demonstrate changes in tumor vascularity and viability in all parts of a tumor. Furthermore, anti-angiogenic therapy can lead to early changes in tumor vasculature and tissue perfusion without corresponding changes in tumor size. This is a limitation of using traditional RECIST criteria alone to measure response to anti-angiogenic therapy. In clinical trials of bevacizumab, objective response lack of response RECIST or did not necessarily predict clinical outcome (92), highlighting the need for new imaging modalities to supplement the inadequacies of RECIST in this area. DCE-MRI provides the ability to measure effect of therapy on tumor heterogeneity, microcirculation, vascularity and viability and is a promising imaging modality both for the measurement of anti-angiogenic effects of therapy, and prediction of early response to treatment. The value of such an approach in cancer diagnosis and treatment evaluation has been extensively shown (93-97). Dose dependent blood vessel changes have been noted on DCE-MRI with anti-angiogenic therapy (68). A study in a GBM population treated with aflibercept demonstrated evidence of decreased vascular permeability on DCE-MRI, which correlated with decreased free VEGF levels and tumor response (98). We will perform DCE-MRI at week 0 (pre-therapy) and after 8 weeks (2 cycles) of therapy to evaluate changes in tumor vasculature with aflibercept therapy, and assess for relationships between DCE MRI findings and clinical outcomes. DCE-MRI will only be performed at sites that have the technology available. DCE-MRI is optional for participating sites that are unable to perform the imaging due to financial restrictions. We will also evaluate for relationships between circulating markers of angiogenesis (see Section 2.4.1) and changes in tumor vasculature observed on DCE-MRI.

2.4.2.2 FDG-PET

While the use of RECIST criteria is the standard approach to assessment of response to anticancer therapy, there are limitations to this method in the setting of anti-angiogenic therapy, when tumors can exhibit necrosis and changes in vascularity without changes in size. ^{18}FDG-PET is a functional imaging technique that relies on tumor uptake of radiolabeled tracer 18 fluorodeoxyglucose (^{18}FDG). FDG-PET is a widely-used imaging modality in the detection and monitoring of a variety of metastatic cancers, including colorectal cancer (99-102). ^{18}FDG is a radiopharmaceutical analog of glucose that is taken up by metabolically active tumor cells using a facilitated transport similar to that used by glucose. FDG-6-phosphate is effectively “trapped” within these cells, as it is not a substrate for the subsequent enzymatically-

driven pathways for glucose and the rate of dephosphorylation is slow. Increased tumor FDG uptake as measured by PET, although a function of proliferative activity, also reflects viable tumor cell number. Several factors can lead to changes in FDG uptake in a tumor, including alterations in tumor perfusion as a result of the effects of anti-angiogenic therapy on tumor vasculature (103), which makes PET scanning an attractive potential adjunct to traditional CT scan in the assessment of response to anti-angiogenic therapy. In fact, various PET techniques can be used to directly evaluate a variety of parameters relating to tumor neovasculature, including hemodynamic parameters (blood flow, blood volume) and tissue properties (glucose metabolism, hypoxia) (103). It has been demonstrated in several cancers that changes in tumor metabolism can be seen on PET after just a few weeks of effective therapy (104-107) and that these findings may predict clinical outcome.

In rectal cancer, decrease or loss in FDG avidity has been correlated with responses to anti-VEGF therapy (108), and in metastatic colon cancer patients treated with anti-angiogenic therapy, the use of FDG-PET along with DCE MRI was able to detect changes in tumor vasculature that predicted response to therapy after just 3 cycles of treatment in one case series (109), and in a second study, decreased FDG uptake on PET correlated with significantly improved progression-free survival in patients treated with FOLFOX plus bevacizumab (110). Integration of PET with conventional imaging techniques may therefore provide a useful adjunct to assessment of response to anti-angiogenic therapy in CRC (111). To further investigate the utility of FDG-PET in this setting, we will perform FDG-PET scans on all patients at week 0 (pre-therapy) and after 8 weeks (2 cycles) of therapy and correlate results with clinical efficacy and DCE MRI findings. FDG-PET will only be performed at sites that have the technology available. FDG-PET is optional for participating sites that are unable to perform the imaging due to financial restrictions.

2.5 Study Rationale

Colon cancer remains a therapeutic challenge despite advances in both our understanding of the disease as well as development of new biologic and targeted therapies. Angiogenesis and particularly VEGF have a known pathogenic role in colon cancer and are well-established as a valid therapeutic target in this disease. As bevacizumab is the only approved anti-VEGF therapy for mCRC, further investigation into anti-VEGF targeted therapy is warranted in an effort to improve outcomes in these patients. Aflibercept represents a novel approach to targeting VEGF with a different mechanism of action than bevacizumab (including a higher affinity for VEGF-A than bevacizumab), and has shown activity in patients refractory to bevacizumab. Aflibercept is safe and tolerable in phase I and II studies with a toxicity profile similar to bevacizumab. Results of the recent phase III VELOUR study with aflibercept + FOLFIRI as second line therapy, including a patient population in which 28% of patients had received prior bevacizumab,

were comparable to previously conducted studies of FOLFOX + bevacizumab as second line therapy. These results provide further validation of VEGF as a therapeutic target in mCRC and provide rationale for bringing the combination of aflibercept +FOLFOX to the first-line setting.

The rationale for the correlative science stems from the information provided above. There remains an unmet need to identify predictive biomarkers for anti-angiogenic therapy, in order to better select patients who are likely to benefit from treatment, and detect early in the treatment course those patients who will not respond. Our proposed work outlined above will investigate circulating markers of angiogenesis and SNPs that have been shown to be potential predictive markers for anti-angiogenic therapy, in patients treated with aflibercept. Our radiographic correlative studies are designed to further investigate the utility of DCE-MRI and FDG-PET as adjunctive imaging modalities and potential early predictors of response to anti-angiogenic therapy. Development of additional methods of response assessment, particularly those which may be able to predict early responders and spare non-responders the added toxicity of anti-angiogenic agents, is crucial in the setting of anti-angiogenic therapy, given the limitations of traditional RECIST criteria.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically confirmed adenocarcinoma of colorectal origin that is metastatic or locally advanced and unresectable.

3.1.2 Patients must have measurable disease, as defined by RECIST 1.1 criteria: one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$10 mm with spiral CT scan (CT scan slice thickness no greater than 5 mm) Malignant lymph nodes will be considered measurable if they are $\geq$15 mm in short axis. See Section 11 for the evaluation of measurable disease.

3.1.3 Patients must not have received any prior systemic therapy for metastatic or locally advanced CRC. Prior VEGF inhibitors are not allowed.

3.1.4 Prior adjuvant therapy for CRC including fluoropyrimidines either alone or in combination with oxaliplatin is allowed, provided that all therapy was completed $\geq$ 12 months from cancer recurrence, therapy duration was $\leq$ 6 months, and all prior toxicities have completely resolved (residual grade 1 neuropathy is allowed).

3.1.5 Patients who have received prior locoregional therapy for metastatic disease including surgical resection, microwave ablation, cytoreductive surgery with hyperthermic intraperitoneal chemotherapy, or radiation are eligible providing the measurable disease is clearly manifest and is outside of the radiation port or ablation field. Patients who have received liver directed treatments such as

yttrium-90 radioembolization or transarterial chemoembolization are eligible if their measurable disease is outside of the liver.

3.1.6 Age ≥18 years.

3.1.7 Life expectancy ≥ 12 weeks.

3.1.8 ECOG performance status 0-1 (see Appendix A).

3.1.9 Patients must have adequate organ and marrow function as defined below:

- hemoglobin	≥ 9 g/dL (blood transfusion permitted to attain this value)
- absolute neutrophil count	≥1,500/μL
- platelets	≥100,000/μL
- total bilirubin	≤1.5 X institutional ULN
- AST (SGOT)/ALT(SGPT)	≤2.5 X institutional ULN (may be ≤ 5x ULN if increase is due to metastatic disease)
- creatinine	≤ 1.5 x institutional ULN
OR	
- Creatinine Clearance	≥60 mL/min/1.73 m2 for patients with creatinine levels above institutional ULN
- urinalysis	UPCR < 1 or <1000 mg protein/24 hr
- Blood Pressure	< 160/90

3.1.10 Ability to understand and the willingness to sign a written informed consent document.

3.2 **Exclusion Criteria**

3.2.1 Patients may not be receiving any other investigational agents.

3.2.2 Patients with known or suspected brain metastases, carcinomatous meningitis, uncontrolled seizure disorder, active intracranial bleeding or active neurologic disorder are excluded.

3.2.3 Patients with an active second primary malignancy or history of malignancy within 5 years of enrollment are excluded, with the exception of non-melanoma skin cancers and cervical cancer which has been treated with curative therapy.

3.2.4 Grade ≥ 2 sensory neuropathy at the time of enrollment.

3.2.5 Major surgery within 4 weeks of enrollment. Any and all surgical incisions must be fully healed prior to study enrollment. *Note: Recent port placement

for chemotherapy administration is not considered an exclusionary surgical incision.

3.2.6 Female or male patients of reproductive capacity unwilling to use methods appropriate to prevent pregnancy are excluded. Effective contraception is required for at least 6 months following the last administration of aflibercept.

3.2.8 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, uncontrolled hypertension (BP $\geq$ 160/90), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements, or any condition that the PI feels would make the patient ineligible.

3.2.9 Positive pregnancy screening test with a minimum sensitivity of 25 IU/L of hCG within 72 hours of registration. Breastfeeding women are also excluded.

3.2.10 History of pulmonary embolus within 3 months or DVT within 4 weeks of enrollment. Patients on anticoagulation must be on a stable dose of warfarin with a therapeutic-range INR or on a stable dose of low molecular weight heparin.

3.2.11 Active congestive heart failure (NYHA class II-IV).

3.2.12 History of an arterial thrombotic vascular event including CVA, MI, unstable angina, coronary or peripheral arterial bypass graft, or TIA within 6 months.

3.2.13 Serious or non-healing wound, ulcer or bone fracture. If patient develops a serious or non-healing wound, ulcer or bone fracture after enrollment, but prior to study drug initiation, patient is not eligible to begin protocol therapy.

3.2.14 History of treatment-resistant peptic ulcer disease, erosive esophagitis, gastritis, or diverticulitis within 3 months.

3.2.15 History of GI perforation within 5 years or patient has a current or prior intestinal fistula.

3.2.16 Known chronic infectious disease including, but not limited to, HIV/AIDS

3.2.17 History of major hemorrhage including gastrointestinal bleeding (grade 2-4), pulmonary hemorrhage, or clinically significant hemoptysis (>1 tsp in 24 hours) within the last 5 years. Patients with underlying conditions that predispose to bleeding, such as bleeding diathesis, known esophageal varices, or tumor involving major vessels, are also excluded.

3.2.18 Inability to understand or comply with study protocol.

3.2.19 Known hypersensitivity to Chinese hamster ovary cell products or to recombinant human or murine antibodies, or any of the treatments in this protocol.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

OSU patients will be registered by the OSU research coordinator.

Subsite patients will have eligibility verified and will be entered on study centrally at The Ohio State University by the Subsite Coordinator. All subsites should call the Subsite Coordinator (Jennifer Sexton) at *614-366-5642,* to verify enrollment availability. The required registration forms (*Eligibility Checklist and Registration Form)* can be found in the Supplemental Forms Document.

Following registration, patients should begin protocol treatment within 5 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Subsite Coordinator should be notified of cancellations as soon as possible.

4.2 Registration Process

To register a subsite patient, the following documents should be completed by the research nurse or data manager and faxed or securely e-mailed to the Subsite Coordinator (Jennifer Sexton):

- Copy of all required laboratory tests per the protocol calendar. Screening tests must be within the specified window.
- Signed patient consent form
- HIPAA authorization form
- Eligibility Checklist (refer to Supplemental Forms Document)
- Registration Form (refer to Supplemental Forms Document)
- Source documents verifying every inclusion & exclusion criteria
- Additional source documents pertaining to verification of eligibility (emails, other signed forms, etc)

The Subsite Coordinator will email the research nurse or data manager to confirm receipt of the registration request and supporting documents. The research nurse or data

manager at the participating site should call the Subsite Coordinator if the email confirming receipt of the registration request is not received within 2 hours. To complete the registration process, the Subsite Coordinator will

- assign a patient study number
- register the patient on the study
- fax or securely e-mail the patient study number and dose information to the participating site
- call the research nurse or data manager at the participating site to verbally confirm registration.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Appropriate dose modifications for FOLFOX and aflibercept are described in Section 6. Reportable adverse events and for FOLFOX and aflibercept are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. For the purposes of this study, 1 treatment cycle = 28 days.

Agents will be administered in the following order on days 1 and 15 of each cycle:

1. Aflibercept 4 mg/kg as a 1-hour IV infusion, **followed by:**

2. mFOLFOX6

- **Oxaliplatin** 85 mg/m2 intravenously infused over 2 hours, **followed by**
- **Leucovorin** 400 mg/m2 (Or levoleucovorin 200 mg/m2. If leucovorin is not available due to drug shortages the regimen should be administered with the leucovorin omitted) IV over 2 hours. Alternatively, leucovorin may be administered (via separate infusion lines) concurrently with oxaliplatin, **followed by**
- **5-FU** 400 mg/m2 IV bolus over 5-15 minutes, then 2400 mg/m2 continuous IV infusion over 46 hours.

At the investigator's discretion, Ca++ and Mg++ (1g each) may be administered prior to oxaliplatin and again prior to 5-FU.

Instructions for the preparation, handling, and administration of aflibercept are provided in Appendix B. Treatment must be administered through a tunneled central line. For the evaluation, a cycle will be defined as 28 days.

5.2 General Concomitant Medication and Supportive Care Guidelines

- **Antiemetic prophylaxis:** symptomatic management of nausea is at the discretion of the treating investigator with the following recommended guidelines:

- Oxaliplatin is emetogenic. All patients receiving oxaliplatin should be pre-medicated with an acceptable antiemetic regimen. Patients may receive dexamethasone 10-20 mg IV as pre-treatment antiemetic unless there is a relative or absolute contraindication to corticosteroids. Other antiemetics may be used in addition to the suggested regimen, if clinically indicated.

- Patients should have anti-emetic available as needed.

- The use of additional antiemetics prophylactically or as needed should be prescribed and administered as needed and adjusted during the study at the discretion of the treating investigator.

- **Diarrhea Management:** Symptomatic management of diarrhea is at the discretion of the treating investigator. The following guidelines are recommended:

  - All patients should be instructed to take anti-diarrheal medications at the earliest sign of diarrhea or abdominal cramping after beginning the study medications. These signs can include (a) loose stool, (b) the occurrence of one or two more bowel movements than usual in one day, or (c) unusually high volume of stool. A low dose of anti-diarrheal medication may be required chronically while patients are receiving treatment.

  - If an anti-diarrheal medication is needed, the following options are suggested:

    - Loperamide: 4 mg at the first onset of diarrhea, then 2 mg every 2 hours around the clock until diarrhea free for at least 12 hours. Patient may take 4 mg every 4 hours during the night.

    - Diphenoxylate/Atropine: two tablets at the first onset of diarrhea, then two tablets four times a day until diarrhea-free for at least 12 hours.

    - If diarrhea continues >7 days, consider sending stool for C. difficile assay.

- **Management of hypertension:** The optimal choice of antihypertensive agents to control hypertension induced by aflibercept has not been established. Clinical management of hypertension should be at the discretion of the treating physician. Acceptable antihypertensives noted to be effective include angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, diuretics, and beta blockers.

- **Management of pharyngo-laryngodysesthesias:** Oxaliplatin may cause discomfort in the larynx or pharynx associated with dyspnea, anxiety, swallowing difficulty and
  is exacerbated by cold. Appropriate therapy includes use of anxiolytics, cold avoidance and monitoring.

- **Management of oxaliplatin hypersensitivity reactions:** Platinum hypersensitivity can cause dyspnea, bronchospasm, itching and hypoxia. Appropriate treatment includes supplemental oxygen, steroids, antihistamines, and epinephrine; bronchodilators and vasopressors may be required. Platinum hypersensitivity a rare but potentially life-threatening event and should be treated promptly according to institutional guidelines. Grade 3-4 oxaliplatin hypersensitivity occurs in 2-3% of patients receiving this agent and incidence increases with multiple courses of therapy.

- **Management of aflibercept infusion reactions:** Symptomatic and supportive treatment should be administered according to each participating institution's local procedures for treating infusional reactions to other protein therapeutic agents. See section 6.2.5 for information regarding the management of infusion reactions related to aflibercept.

- **Anticoagulation:** Patients requiring therapeutic anticoagulation should be carefully monitored while receiving therapy with aflibercept. For patients receiving anticoagulation with warfarin, INR must be kept in the therapeutic range and should be checked prior to every dose of aflibercept.

5.3 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse events(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- Patients may be removed from study to undergo surgical resection.

5.4 Duration of Follow Up

Patients will be followed every 3 months after removal from treatment until death. Adverse event reporting should continue for 30 days after last dose of protocol therapy. Patients removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.5 Criteria for removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.3 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

Additional cycles of therapy may be administered provided that the patient meets the following criteria on Day 1 of each cycle:

- ANC $\geq$ 1500/μL
- Platelets $\geq$ 75,000/μL
- Non-hematologic toxicity recovered to less than or equal to grade 1 (or tolerable grade 2 or baseline)
- No evidence of progressive disease
- If the initiation of a new cycle, or therapy during a cycle, is delayed for $\geq$ 4 weeks, treatment with FOLFOX and aflibercept should be discontinued.

• Subjects with adverse events that are manageable with supportive therapy may not require dose reductions (*e.g.*, nausea/vomiting may be treated with antiemetics, diarrhea may be treated with loperamide, electrolyte abnormalities may be corrected with supplements rather than by dose reduction). However, toxicity persists despite optimum supportive measures, dose reductions should be implemented.

• Subjects will be withdrawn from the study if they fail to recover to CTC Grade 0-1 or tolerable grade 2 (or within 1 grade of starting values for pre-existing laboratory abnormalities) from a treatment-related adverse event within 28 days OR they experience agent related adverse events requiring dose modification despite three previous dose reductions (i.e. would require a fourth dose reduction) unless the principal investigator agrees that the subject should remain in the study because of evidence that the patient is/may continue deriving benefit from continuing study treatment (i.e. patient has PR, CR, SD $\geq$ 3 months). The appropriate reduced dose will be determined after discussion with the principal investigator .

Table 6. Dose Levels for FOLFOX and Aflibercept

Agent	Initial dose	Level -1	Level -2	Level -3
Oxaliplatin	85 mg/m^2	65 mg/m2	50 mg/m2	40 mg/m2
5-FU bolus[a]	400 mg/m^2	discontinue	---	---
5-FU infusion[b]	2400 mg/m^2	1920 mg/m2	1600 mg/m2	1360 mg/m2
Aflibercept	4 mg/kg	2 mg/kg	discontinue	---

[a] Leucovorin (LV) dose is always 400 mg/m2 (Or if levoleucovorin is used the dose is 200 mg/m2) given prior to 5-FU bolus. If 5-FU bolus is held or discontinued, LV is also held or discontinued.
[b] 5-FU infusion is delivered continuously over 46-48 hours.

6.1 Dose Modifications for FOLFOX

In order for a treatment cycle to begin, all criteria in section 6.0 must be met.

6.1.1 Selected hematologic and nonhematologic adverse events (excluding neurotoxicity)

Toxicity NCI CTCAE v 4.0 Grade	**Note: Dose Modifications are based on AEs observed during a cycle (i.e., after day 1 of a cycle). Dose modifications must be based on the AE requiring the greatest modification. Skipped doses are not made up.**	
	Current cycle	**Next cycle**
No toxicity	Maintain dose level	Maintain dose level
Neutrophils (ANC)		
Grade 1 (ANC <LLN- 1500/mm^3)	Maintain dose level	Maintain dose level
Grade 2 (ANC <1500-1000/mm^3)	Maintain dose level	Maintain dose level
Grade 3 (ANC <1000-500/mm^3)	Skip 5FU and oxaliplatin. If toxicity resolves to ≤ grade 1 during the cycle, 5-FU and oxaliplatin may be resumed with omission of bolus 5-FU.	Discontinue bolus 5FU, and reduce oxaliplatin by 1 dose level or add Neulasta
Grade 4 (ANC <500/mm^3)	Skip 5-FU and oxaliplatin. If toxicity resolves to ≤ grade 1 during the cycle, 5-FU and oxaliplatin may be resumed with omission of bolus 5-FU and reduction of oxaliplatin by 1 dose level.	Discontinue bolus 5-FU and continue oxaliplatin at reduced dose level from previous cycle (or add Neulasta or reduce oxaliplatin by 1 dose level if treatment was not resumed in previous cycle).
Platelets[a]		
Grade 1 (Plt <LLN-75,000/ mm^3)	Maintain dose level	Maintain dose level
Grade 2 (Plt <75,000-50,000/ mm^3)	Omit bolus 5-FU and reduce oxaliplatin 1 dose level for remainder of current cycle	Resume 5FU and oxaliplatin at the previous dose levels.
Grade 3 (Plt <50,000-25,000/ mm^3)	Skip 5-FU and oxaliplatin. If plts recover to ≥ 75,000 during the cycle, 5-FU and oxaliplatin may be resumed with omission of bolus 5-FU.	Discontinue bolus 5-FU
Grade 4 (Plt <25,000)	Skip 5-FU and oxaliplatin. If plts recover to ≤ 75,000 during the cycle, 5-FU and oxaliplatin may be resumed with omission of bolus 5-FU and reduction of oxaliplatin by 1 dose level.	Discontinue bolus 5-FU and continue oxaliplatin at reduced dose level from previous cycle (or reduce oxaliplatin by 1 dose level if treatment was not resumed in previous cycle).
Febrile Neutropenia (ANC <1000	Skip 5FU and oxaliplatin. If fever	Discontinue bolus 5-FU and

and T ≥ 38.5°C)	and neutropenia resolve during the current cycle, 5-FU and oxaliplatin may be resumed with omission of bolus 5-FU and reduction of oxaliplatin by 1 dose level, provided ANC and platelets ≤ grade 1.	continue oxaliplatin at the reduced dose level from previous cycle (or reduce oxaliplatin by 1 dose level if treatment was not resumed in previous cycle).
Other hematologic toxicities (e.g. anemia, lymphopenia) do not require dose modification; however, red blood cell transfusion should be strongly considered for hemoglobin < 8 g/dL		
Diarrhea		
Grade 1	Maintain dose level	Maintain dose level
Grade 2	Reduce 5-FU 1 dose level for remainder of cycle. Oxaliplatin is not reduced.	Resume 5-FU at previous dose level. Maintain oxaliplatin dose.
Grade 3	Skip 5-FU and oxaliplatin. If diarrhea resolves to ≤ grade 1 during the current cycle, 5-FU is resumed at 1 lower dose level with omission of bolus 5-FU. Oxaliplatin is not reduced.	Discontinue bolus 5-FU and continue 5-FU at reduced dose from previous cycle (or reduce by 1 dose level if not resumed in previous cycle). Maintain oxaliplatin dose.
Grade 4	Skip 5-FU and oxaliplatin. If diarrhea resolves to ≤ grade 1 during the current cycle, 5-FU and oxaliplatin are resumed at 1 lower dose level with omission of bolus 5-FU.	Discontinue bolus 5-FU and continue 5-FU and oxaliplatin at reduced doses from previous cycle (or reduce by 1 dose level if not resumed in previous cycle).
Mucositis/Stomatitis		
Grade 1	Maintain dose level	Maintain dose level
Grade 2	Reduce 5FU one dose level for remainder of cycle.	Resume treatment at previous dose level.
Grade 3	Skip 5-FU and oxaliplatin. If mucositis resolves to ≤ grade 1 during the cycle, 5-FU and oxaliplatin are resumed at one lower dose level with omission of bolus 5-FU.	Discontinue bolus 5-FU and continue 5-FU at reduced dose from previous cycle (or reduce by 1 dose level if not resumed in previous cycle). Maintain oxaliplatin dose at previous (non-reduced) dose level.
Grade 4	Skip 5-FU and oxaliplatin. If mucositis resolves to ≤ grade 1 during the cycle, 5-FU and oxaliplatin are resumed at one lower dose level with omission of bolus 5-FU.	Discontinue bolus 5-FU and continue 5-FU and oxaliplatin at reduced doses from previous cycle (or reduce by 1 dose level if not resumed in previous cycle).
Nausea/Vomiting (despite antiemetics)		
Grade 1	Maintain dose	Maintain dose
Grade 2	Maintain dose	Maintain dose
Grade 3	Hold oxaliplatin and continue 5-FU. If toxicity resolves to ≤ grade	Continue oxaliplatin at reduced dose level from previous cycle. 5-

	1 during the cycle, resume oxaliplatin at 1 lower dose level for remainder of cycle	FU is not reduced.
Grade 4	Hold 5-FU and oxaliplatin. If toxicity resolves to ≤ grade 1 during the cycle, reduce 5-FU and oxaliplatin 1 dose level and omit bolus 5-FU for remainder of cycle.	Discontinue bolus 5-FU. Continue 5-FU and oxaliplatin at reduced dose levels from previous cycle (or reduce by 1 dose level of treatment not resumed in previous cycle).
Pulmonary Toxicity Grade ≥ 3 cough, dyspnea, hypoxia, pneumonitis, or pulmonary infiltrates	Hold all therapy until interstitial lung disease is ruled out.	Oxaliplatin may be resumed at the physician's discretion if interstitial lung disease is ruled out, infection has resolved, and dyspnea or hypoxia are resolved to ≤ grade 1. **If ILD is confirmed, all protocol therapy is discontinued.**
All other non-hematologic toxicities (except neurologic)*[b,c,]	Dose modifications for other nonhematologic adverse events at the start of subsequent cycles of therapy, and at time of retreatment are the same as recommended for nausea/ vomiting (above) with the following exception: **Omit bolus 5-FU and decrease infusion 5-FU by one dose level for other Grade>3 non-hematologic events**	
*Exceptions: alopecia, anorexia, fatigue, nausea if can be controlled by antiemetics, clinically insignificant metabolic/laboratory abnormalities.		

a Hemolytic Uremic Syndrome (HUS)/Thrombotic Thrombocytopenic Purpura (TTP): The hemolytic uremic syndrome should be suspected in individuals who experience unexplained severe hemolysis, hemoglobinemia and renal failure as demonstrated by an increase in serum creatinine. Patients suspected of experiencing HUS or demonstrating symptoms of TTP should have the following laboratory analyses conducted: creatinine, BUN, urinalysis with microscopic evaluation, CBC with differential and platelets, PT/PTT, Fibrinogen, Fibrinogen Degradation Products (FDP), Anti-thrombin III (ATIII), von Willenbrand Factor (VWF), anti-nuclear antibodies (ANA), rheumatoid factor (RhF), C3, C4, CH50, anti-platelet antibodies, platelet associated IgG, circulating immune complexes.
Oxaliplatin should be discontinued for any suspected occurrence of HUS or TTP.
b With any suspicion of veno-occlusive disease (VOD) of the liver (hyperbilirubinemia, ascites, unexplained weight gain, hepatomegaly, splenomegaly, esophageal varices or other sign of portal hypertension), chemotherapy must be held. If VOD is diagnosed clinically, chemotherapy must be discontinued.
c Dose modifications should only be applied for those untoward events (including but not limited to metabolic or vascular toxicities) that might bear some relationship to study treatment.

6.1.2 Neurotoxicity

Paresthesias/Dysesthesias	**1-7 day duration**	**>7 day duration**
Grade 1: paresthesias/dysesthesias that resolve and do not interfere with function	Maintain dose	Maintain dose
Grade 2: paresthesias/dysesthesias interfering with function, but not ADL's	Maintain dose*	Decrease oxaliplatin 1 dose level*
Grade 3: paresthesias/dysesthesias with pain or with functional impairment that also interfere with ADL's	First episode: decrease oxaliplatin 1 dose level Second episode: stop oxaliplatin	Stop oxaliplatin
Grade 4: persistent paresthesias/ dysesthesias that are disabling or life-threatening	Stop oxaliplatin	Stop oxaliplatin

*Hold oxaliplatin for >grade 2 neurotoxicity. When ≤ grade 1, resume treatment with dose modifications. If > grade 1 toxicity persists after 4 weeks' hold, discontinue oxaliplatin. Continue 5-FU and aflibercept at previous doses while oxaliplatin is held.		
Laryngeal Dysesthesia		
Grade 1-2 (Mild, moderate)	Maintain dose and consider increasing duration of oxaliplatin infusion to 6 hours	Maintain dose and consider increasing duration of oxaliplatin infusion to 6 hours
Grade 3 (Severe)	At physician's discretion, either stop oxaliplatin or increase duration of infusion to 6 hours	Stop oxaliplatin
Patients may also discontinue oxaliplatin following multiple cycles even in the absence of dose-limiting neurotoxicity if, in the treating physician's judgement, neurotoxicity is likely to become problematic. Patients should continue to receive other protocol therapy and the oxaliplatin may be reintroduced subsequently.		

6.2 Dose Delays and Modifications for Aflibercept

Toxicity	**NCI CTCAE v 4.0 Grade**	**Note: Dose Modifications are based on AEs observed during a cycle (i.e., after day 1 of a cycle). Dose modifications must be based on the AE requiring the greatest modification. Skipped doses are not made up.**
		Action to be taken

Hypertension	Grade ≤ 2	Initiate antihypertensive drug therapy (see recommendation below) and close monitoring of blood pressure for further adjustment, as needed. No dose modification and no delay.
	Grade 3 (requiring more than one drug or more intensive therapy than previously)	Modify antihypertensive drug therapy (see recommendation below). Delay the administration of both FOLFOX **and** aflibercept for a maximum of 4 weeks, until recovery to blood pressure (BP) ≤ 150/100 or to systolic BP < 180 if diastolic BP < 90 for patients with known history of isolated systolic hypertension: • If BP is controlled within 4 weeks delay: -First episode: readminister FOLFOX **and** aflibercept at the same dose. - Second episode: readminister FOLFOX **and** aflibercept with Aflibercept reduced to dose level-1. -Third episode, discontinue aflibercept. • If BP is still uncontrolled despite appropriate anti hypertensive treatment and after 4 weeks delay: Administer FOLFOX **and** discontinue aflibercept for 1 cycle; the reintroduction of aflibercept at a dose reduced to dose level – 1 will be reconsidered at the time of the administration of the subsequent cycle (in combination with FOLFOX), only if BP is controlled at the time of re-administration. • In case of re-occurrence of grade 3 BP despite dose reduction of aflibercept, or if BP is still uncontrolled despite 1 omission of administration of Aflibercept, the patients will be permanently discontinued from aflibercept. FOLFOX may be continued at investigator discretion if he/she believes the patient is deriving clinical benefit.
	Grade 4	Permanently discontinue aflibercept. Seek cardiologist opinion.
Arterial thromboembolic events (e.g.: myocardial infarction, or stroke) **documented by appropriate tests**	Grade 3-4	Permanently discontinue aflibercept.
Hemorrhage[a]	Grade 3-4	Permanently discontinue study treatment.
GI perforation/fistula formation	Any grade	Permanently discontinue study treatment.
Reversible posterior leukoencephalopathy syndrome documented with appropriate tests	Any grade	Permanently discontinue study treatment.
Venous Thromboembolic events documented by appropriate tests	Grade 3	First episode: Treat with heparins **and** continue study treatment[b] Second episode despite appropriate anticoagulation: Permanently discontinue aflibercept
	Grade 4	Permanently discontinue aflibercept

Hypertriglyceridemia (Not an identified risk; however, observed in 2 patients receiving Aflibercept in this ongoing study: A Phase II Study of VEGF-Trap plus Modified FOLFOX 6 in Previously Untreated Patients with Metastatic Colorectal Cancer)	Grade $\leq$ 2	Medically manage per institutional guidelines. No dose modification and no delay.
	Grade 3	At first occurrence, omit Aflibercept that day and medically manage toxicity per institutional guidelines. If toxicity persists at next dosing time point (despite optimal medical management), hold until grade , < or = 2 and reduce Aflibercept by 1 dose level (table 6) for subsequent doses. If recurrent grade 3 occurs after dose reduction and despite optimal medical management, permanently discontinue aflibercept.
	Grade 4	At first occurrence, omit Aflibercept that day and medically manage toxicity per institutional guidelines. If toxicity persists at next dosing time point (despite optimal medical management), hold until grade , < or = 2 and reduce Aflibercept by 1 dose level (table 6) for subsequent doses. If recurrent grade 3 occurs after dose reduction and despite optimal medical management, permanently discontinue aflibercept.
Hypercholesterolemia (Not an identified risk; however, observed in 2 patients receiving Aflibercept in this ongoing study: A Phase II Study of VEGF-Trap plus Modified FOLFOX 6 in Previously Untreated Patients with Metastatic Colorectal Cancer)	Grade $\leq$ 2	Medically manage per institutional guidelines. No dose modification and no delay.
	Grade 3	At first occurrence, omit Aflibercept that day and medically manage toxicity per institutional guidelines. If toxicity persists at next dosing time point (despite optimal medical management), hold until grade , < or = 2 and reduce Aflibercept by 1 dose level (table 6) for subsequent doses. If recurrent grade 3 occurs after dose reduction and despite optimal medical management, permanently discontinue aflibercept.
	Grade 4	At first occurrence, omit Aflibercept that day and medically manage toxicity per institutional guidelines. If toxicity persists at next dosing time point (despite optimal medical management), hold until grade , < or = 2 and reduce Aflibercept by 1 dose level (table 6) for subsequent doses. If recurrent grade 3 occurs after dose reduction and despite optimal medical management, permanently discontinue aflibercept.

[a] In case of grade 3 hemorrhage, continuation of aflibercept may be considered depending on individual Benefit/Risk assessment.
[b] Based on investigator's judgement in assessing potential risk of extension and/or embolization in case of DVT

6.2.1 **Hypertension therapy recommendations**:

- If the patient is found to have asymptomatic grade 1 or higher hypertension during home monitoring, they should be seen within 24-48 hours so the blood pressure measurement can be confirmed and antihypertensive therapy initiated.
- *For patients without prior antihypertensive therapy*, at the time of the hypertensive episode the initiation of calcium-channel blockers should be considered as a first-intent treatment. A close monitoring of the BP should be initiated for further adjustment in treatment, as needed. Ultimately, antihypertensive treatment must be individualized based on the presence of comorbidity factors such as diabetes, cardiovascular or renal disease, additionally taking into

account the safety and the efficacy of any prior antihypertensive therapy received. In addition, oral and/or intravenous sodium intake should be carefully monitored in these patients.

- *For patients already under anti-hypertensive therapy* efforts should be done to optimize the existing therapy before adding other agents as required to control the BP.
- When hypertension is accompanied by signs or symptoms of end organ damage such as hypertensive retinopathy, kidney function abnormalities (like progressive proteinuria), or any signs or symptoms of cardiovascular morbidity or central nervous system (CNS) morbidity, treatment with aflibercept should be interrupted.

6.2.2 **Proteinuria:**

Determination and management of proteinuria:

Prior to each administration of aflibercept, perform UPCR and urinalyses.
Urinary protein creatinine ratio (UPCR) corresponds to the ratio of urinary protein and urinary creatinine concentrations (expressed in mg/dL). There is a high correlation between morning UPCR and 24-hour proteinuria in patients with normal or reduced renal function, UPCR demonstrated very good to excellent performance for the diagnosis of both abnormal and nephrotic proteinuria at all renal function levels. This ratio provides an accurate quantification of 24-hour urinary protein excretion.

UPCR to detect proteinuria, will be done on a random urine sample. If UPCR > 1, 24-hour urine collection to grade proteinuria will be performed. In addition, in case UPCR is greater than 2 or in case of proteinuria of renal origin (according to urinary protein electrophoresis) is associated with hematuria (microscopic or macroscopic), then a blood work-up in search for hemolytic anemia of microangiopathic origin should be initiated and a nephrologist consultation should be considered as detailed in Table 8.

This blood work-up could include schistocytes, haptoglobin, LDH and orosomucoïd whenever possible. Delay in availabilty of part of the results should not delay consultation to the nephrologist

Proteinuria should always be assessed taking into account the presence or absence of hematuria and the blood pressure status of the patient.

Table 8 summarizes the course of action with regard to aflibercept dosing, which will depends on the presence of hematuria and the level of 24-hour proteinuria results. Only one dose level reduction is permitted for aflibercept.

6.2.3 Reversible posterior leuko-encephalopathy (RPLS) or clinical symptoms related to vasogenic edema of the white matter:

Clinical presentations are variable and may include headache, altered mental status, seizure and cortical visual deficit. Hypertension is a risk factor. MRI scans are key to diagnosis and typically demonstrate vasogenic edema (hyperintensity in T2 and FLAIR images and hypodensity in T1 images) predominantly in the white matter of the posterior parietal and occipital lobes; less frequently, the anterior distributions and the gray matter may also be involved. RPLS should be in the differential diagnosis in patients presenting with unexplained mental status change, visual disturbance, seizure, or other CNS findings. RPLS is potentially reversible with early recognition

of symptoms and timely correction of the underlying causes, including control of BP and interruption of the offending drug, which are important in order to prevent progression to irreversible tissue damage.

6.2.4 Gastrointestinal perforation:

In case a patient reported abdominal pain or increase in severity of pre-existing abdominal pain, with or without associated symptoms (such as nausea, vomiting, constipation), he/she should be evaluated by a physician for possible gastro-intestinal perforation, as this has been reported with anti-VEGF agents.

6.2.5 Hypersensitivity reaction:

In case of hypersensitivity reaction, institutional treatment guidelines for this type of AEs, or the following proposed guideline in Table 4 can be applied.
For Grade ≥2 event, initiate anti-aflibercept Ab detection process.

Table 7. Management of Hypersensitivity Reactions

Symptom Severity	Intervention Recommendation
Mild-Moderate E.g., NCI CTCAE grade ≤ 2 cutaneous reaction, pruritus, flushing, rash, dyspnea, tachycardia, hypotension, anxiety, headache, myalgias, edema, nausea	Stop aflibercept infusion; Give diphenhydramine 50 mg IV and/or IV dexamethasone 10 mg; Resume aflibercept infusion after subject recovery.
Severe e.g., symptomatic bronchospasm, generalized urticaria, systolic BP ≤ 80 mm Hg, angioedema, anaphylaxis	Stop aflibercept infusion; Give IV diphenhydramine 50 mg and/or IV dexamethasone 10 mg and/or epinephrine as needed; Permanently discontinue aflibercept.

Table 8. Management of Proteinuria

	Aflibercept dosing for cycle n	During cycle n Repeat 24-h proteinuria as necessary[a]	Aflibercept dosing for cycle n +1	During cycle n + 1 Repeat 24-h proteinuria as necessary [a]	Aflibercept dosing for cycle n + 2
UPCR [0-1]	Administer aflibercept				
UPCR [1-2] Absence of hematuria	Administer aflibercept, then perform 24-h proteinuria: - if ≤3.5g/24-h	≤2g/24-h prior n+1 dosing :	Administer aflibercept		
		>2g/24-h prior n+1 dosing :	Omit dosing aflibercept	≤2g/24-h prior n+**2**dosing :	Resume aflibercept level –1[c]
				>2g/24-h prior n+**2** dosing :	Permanently discontinue aflibercept
	- if > 3.5g/24-h	≤2g/24-h prior n+1 dosing :	Administer aflibercept level -1[c]		
		>2 ≤ 3.5g/24-h prior n+1 dosing:	Omit dosing aflibercept	≤2g/24-h prior n+**2** dosing :	Resume aflibercept level –1[c]
				>2g/24-h prior n+**2** dosing :	Permanently discontinue aflibercept
		>3.5g/24-h prior n+1 dosing :	Permanently discontinue aflibercept		

Prior to cycle n aflibercept administration	Aflibercept dosing for cycle n	During cycle n Repeat 24-h proteinuria as necessary[a]	Aflibercept dosing for cycle n +1	During cycle n + 1 Repeat 24-h proteinuria as necessary [a]	Aflibercept dosing for cycle n + 2
UPCR [1-2] Presence of hematuria Or UPCR >2	Omit dosing aflibercept	Perform nephrologic work-up[b] and seek nephrologist opinion :			
		- TMA ruled out and ≤2g/24-h prior n+1 dosing :	Administer aflibercept		
		- TMA ruled out and >2 ≤ 3.5g/24-h prior n+1 dosing :	Omit dosing aflibercept	≤2g/24-h prior n+**2** dosing :	Resume aflibercept level –1[c]
				>2g/24-h prior n+**2** dosing :	Permanently discontinue aflibercept
		- TMA ruled out and >3.5g/24-h prior n+1 dosing :	Permanently discontinue aflibercept		
		- TMA diagnosed :	Permanently discontinue aflibercept, seek nephrologist opinion for continuation of chemotherapy		
Nephrotic syndrome	Permanently discontinue aflibercept, perform nephrologic work-up[b], seek nephrologist opinion for continuation of chemotherapy				

TMA: Thrombotic micro-angiopathy
a: Patients can be monitored with UPCR as necessary, however 24-hour proteinuria should be performed prior to make dosing decision.
b: 24-hour proteinuria, urinary protein electrophoresis, haptoglobin, orosomucoid, schistocytes and LDH
c: When a patient is already treated at dose level -1, aflibercept should be discontinued

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Serious AEs (SAEs), judged by the Investigator or Sponsor to be unexpected and reasonably associated with the use of the study drug(s), are reported directly to the FDA in accordance with applicable law, regulations and Study protocol with a copy submitted to Sanofi-Aventis. If the FDA does not require the sponsor (investigator) to submit SAEs that are unexpected and related, SAE reports should be sent to Sanofi-Aventis when discovered.

In addition, disease progression, or lack of efficacy, should be considered as expected and will, therefore, be excluded from expedited reporting requirements.

When investigational product is administered in combination with 1 or more approved anticancer agents, the expectedness determination should take into account the labeling of each specific marketed drug taken in combination based upon reference documents as defined in the protocol.

Preferred terms of comparable or lower specificity/severity should be considered expected. An event more severe than those included in this list will be considered unexpected.

7.1 Expected Adverse Events for Aflibercept

Table 9. Expected adverse events in treatment with intravenous aflibercept.

MedDRA SOC	Preferred Term
Blood and lymphatic system disorders	Anemia Thrombotic microangiopathy
Cardiac disorders	Cardiomyopathy (incl. cardiac failure)
Gastrointestinal disorders	Abdominal pain Diarrhea Enterocutaneous fistula Gastrointestinal hemorrhage (incl. fatal) Gastrointestinal perforation (incl. fatal) Intestinal obstruction Mucositis Nausea Peritonitis Rectal ulcer Stomatitis Vomiting
General disorders and administration site conditions	Asthenia (incl. fatigue and malaise Pyrexia
Immune system disorders	Drug hypersensitivity (incl. flushing, rigors)
Infections and Infestations	Abdominal abscess
Investigations	Hepatic enzyme increased (incl. ALT, AST, Alk. Phos: <5X ULN
Metabolism and nutrition disorders	Dehydration Hypokalemia Hypomagnesemia

	Hyponatremia
Musculoskeletal and connective tissue disorders	Arthralgia Osteonecrosis
Neoplasm benign, malignant and unspecified (including cysts and polyps)	Tumor hemorrhage
MedDRA SOC	**Preferred term**
Nervous system disorders	Cerebral hemorrhage (incl. fatal) Cerebral ischemia (incl. fatal) Encephalopathy (incl. Reversible Posterior Leukoencephalopathy Syndrome [RPLS]) Headache
Psychiatric disorders	Anxiety
Renal and urinary disorders	Proteinuria (incl. nephrotic syndrome) Renal failure
Reproductive system and breast disorders	Female genital tract fistula
Respiratory, thoracic, and mediastinal disorders	Dysphonia (hoarseness) Dyspnea Epistaxis Hemoptysis (incl. fatal) Pulmonary embolism (incl. fatal) Tracheo-esophageal fistula
Vascular disorders	Deep vein thrombosis Hypertension

Based upon treatment-related serious or severe AEs in clinical trials, the following toxicities may occur with IV aflibercept as a single agent or in combination with other drugs:
• **Hematological disorders and resistance:** anemia, thrombocytopenia, thrombotic microangiopathy; additionally in combination with cytotoxic chemotherapy: neutropenia including febrile neutropenia, neutropenic colitis, and sepsis
• **Cardiac disorders:** cardiac failure
• **Digestive toxicity:** abdominal pain, gastrointestinal hemorrhage, intestinal perforation, intestinal obstruction, enteric fistula, peritonitis, pneumatosis intestinalis, nausea, vomiting, diarrhea, constipation, mucosal inflammation or ulceration, stomatitis, hepatic enzymes increased
• **General disorders:** asthenia, fatigue, musculoskeletal pain, injection site reaction
• **Immune system disorders**: hypersensitivity
• **Metabolic disorders:** dehydration
• **Musculoskeletal:** arthralgia, myalgia, osteonecrosis
• **Nervous system:** headache, dizziness, encephalopathy (including reversible posterior leukoencephalopathy syndrome), cerebral ischemia, cerebral hemorrhage, cerebral venous thrombosis
• **Renal disorders:** proteinuria (including nephrotic syndrome), hematuria, renal failure
• **Respiratory disorders:** dysphonia, dyspnea, epistaxis, hemoptysis, pulmonary embolism, tracheo-esophageal fistula
• **Skin disorder** in combination with cytotoxic chemotherapy: palmar-plantar erythrodysaesthia syndrome, erythema
• **Vascular disorders:** hypertension, (including malignant hypertension), deep vein thrombosis,

and phlebitis

Note: aflibercept in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Adverse Event List for Commercial Agents

5-Fluorouracil
For a comprehensive list of adverse events please refer to the package insert. Section 8.2.1 provides an overview of toxicities related to 5-FU.

Leucovorin
For a comprehensive list of adverse events please refer to the package insert. Section 8.2.2 provides an overview of toxicities related to leucovorin.

Oxaliplatin
For a comprehensive list of adverse events please refer to the package insert. Section 8.2.3 provides an overview of toxicities related to oxaliplatin.

7.3 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4. A copy of the CTCAE version 4 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

7.4 Monitoring for Adverse Events

U.S. regulations require that a sponsor reports Serious Adverse Events (SAEs) occurring with use of its product in a clinical trial if it is unexpected, and felt to be related to use of the drug. During the conduct of Investigator Sponsored Trials (ISTs), where the investigator holds the Investigational New Drug (IND) application, all SAEs that occur will be evaluated by the investigator for reportability to FDA. An Adverse Event should be identified, Serious Adverse Event and Expectedness determined and causality assessed by the investigator using the definitions that follow.

7.4.1 Definitions

An **Adverse Event** is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have to have a causal relationship with the treatment. For marketed products in the U.S., a **Serious Adverse Event** is an adverse event occurring at any dose that results in any of the following outcomes:

a. Death
b. Life-threatening[1]
c. Persistent or significant disability/incapacity[2]
d. In patient hospitalization or prolongation of existing hospitalization
e. Congenital anomaly/birth defect

An event may not meet any of the above seriousness criteria but still be judged as medically serious. That is, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes (a-e) listed above. Some examples of this type of event are:

- blood dyscrasia without inpatient hospitalization
- convulsions without inpatient hospitalization
- intensive treatment in an emergency room or at home for allergic bronchospasm without inpatient hospitalization
- development of drug dependency
- drug abuse
- overdose with an associated serious event, or required intervention to prevent impairment/damage

An **Unexpected Adverse Event** is not listed in the current US Package Insert (USPI) or an event that may be mentioned in the USPI, but differs from the event because of greater severity or specificity.

Causality is a determination of whether there is a reasonable possibility that the drug may have caused or contributed to an adverse event. It includes assessing temporal relationships dechallenge/rechallenge information, association (or lack of association) with underlying diseases, and the presence (or absence) or a lack of one or more likely causes.

The Investigator must determine if an adverse event is in some way related to the use of the study drug. This relationship should be described as follows:

Unlikely: The event is clearly due to causes distinct from the use of the study drug, such as a documented pre-existing condition, the effect of a concomitant medication, a new condition which, based on the pathophysiology of the

1 The term "life-threatening" in the definition of "serious" refers to an event in which in the view of the initial reporter the patient was at immediate risk of death from the adverse experience as it occurred; it does not refer to an event which had it occurred in a more severe form, might have caused death.

2 A substantial disruption of a person's ability to conduct normal life functions.

condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug.

Possible: The event follows a reasonable temporal sequence from administration of the study drug or the event follows a known response pattern to the study drug *BUT* the event could have been produced by an intercurrent medical condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug or the event could be the effect of a concomitant medication

Probable: The event follows a reasonable temporal sequence from administration of the study drug and the event follows a known response pattern to the study drug AND the event cannot have been reasonably explained by an intercurrent medical condition *or* the event cannot be the effect of a concomitant medication

Definite: The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug and based on the known pharmacology of the study drug, the event is clearly related to the effect of the study drug

Unknown: Based on the evidence available, causality cannot be ascribed

7.5 Recording and Reporting

Information on the recording and reporting of adverse events is provided in Section 12.

7.6 Data Safety Monitoring Plan

The data and safety monitoring plan will involve the continuous evaluation of safety, data quality and data timeliness. Investigators will conduct continuous review of data and patient safety at their regular Disease Group meetings (at least monthly) and the discussion will be documented in the minutes. The PI of the trial will review toxicities and responses of the trial where applicable at these disease center meetings and determine if the risk/benefit ratio of the trial changes. Frequency and severity of adverse events will be reviewed by the PI and compared to what is known about the agent/device from the other sources; including published literature, scientific meetings and discussions with the sponsors, to determine if the trial should be terminated before completion. Serious adverse events and responses will also be reviewed by the OSUCCC Data and Safety Monitoring Committee (DSMC). The PI will also submit a progress report (biannually for Phase II) that will be reviewed by the committee per the DSMC plan. All reportable Serious Adverse Events (SAE) will also be reported to the IRB of record as per the policies of the IRB.

<u>For OSU</u>
All Serious Adverse events are to be submitted to the DSMC for their review.

Submissions are made via OnCore.

For Participating Sites
Participating sites will review patients enrolled at their institution for phase II investigator initiated studies quarterly. All reportable SAE will also be reported to the lead PI (at OSU) for review and reporting to the IRB and DSMC at OSUCCC. SAE's need to be faxed to OSU Medical Center utilizing SAE Submission Form (refer to Supplemental Forms Document). It is the responsibility of the OSU PI to obtain information on a quarterly basis on all patients on trial regardless of clinical trial site. These materials will be submitted to the OSUCCC DSMC by the OSU PI. Refer to section 12 for additional reporting requirements. Subsite institutions are required to report SAEs to their local IRBs per their institutional and IRB policies.

Regulatory Guidelines
This study will be performed in accordance with United States Investigational New Drug (IND) regulations (21 Code of Federal Regulations [CFR] 56), the guidelines of the International Conference on Harmonisation (ICH), and the most recent guidelines of the Declaration of Helsinki.
Institutional Review Board/Independent Ethic Committee Conduct of the study must be approved by an appropriately constituted institutional review board (IRB) or independent ethics committee (IEC). Approval is required for the study protocol, investigational drug brochure, protocol amendments, informed consent forms, patient information sheets, and advertising materials. No study drug will be shipped to a site until written IRB or IEC authorization has been received by the sponsor or its representative.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events associated with the investigational or commercial agents administered in this study can be found in Section 7.1.

8.1 Investigational Agents

8.1.1 Aflibercept

Other Names: AVE0005; VEGF Trap

Classification: antineoplastic agent (VEGF trap)

Molecular Formula: Aflibercept is a recombinant fusion protein consisting of human vascular endothelial growth factor (VEGF) receptor extracellular domains fused to the Fc portion of human immunoglobulin G1 (IgG1). Aflibercept contains portions of the extracellular domains of 2 different vascular endothelial growth factor receptors (VEGFRs): VEGFR1 (also known as Flt-1) and VEGFR2 (also known as KDR or Flk-1).

M.W.: 115

Mode of Action: Aflibercept is a specific antagonist that binds and inactivates circulating VEGF. Aflibercept was designed to prevent the growth of primary and metastatic tumors by blocking tumor angiogenesis and vascular permeability.

Description: Aflibercept is a fusion protein consisting of extracellular domains of human VEGF receptors (VEGFRs) fused to the Fc portion of human IgG1. Aflibercept contains portions of the extracellular domains of two different VEGFRs: VEGFR1 (Flt-1) and VEGFR2 (KDR or Flk-1). VEGFR1 and VEGFR2 are native receptors containing 7 Ig domains in the extracellular domain and an intracellular tyrosine kinase.

Aflibercept binds VEGF in the picomolar (pmol/L) range, and also binds placental growth factor (PlGF), although with lower affinity. The affinity constants (K_d) for binding to 2 human isoforms of VEGF, $VEGF_{165}$ and $VEGF_{121}$, are 0.50 pmol/L and 0.36 pmol/L, respectively. The K_d for human PlGF2 is 39 pmol/L. The binding of aflibercept to its ligands in vivo is expected to block tumor angiogenesis and vascular permeability.

How Supplied: Aflibercept is supplied as a clear, colorless solution for IV infusion. Excipients include sodium chloride, sodium citrate dihydrate, citric acid monohydrate, polysorbate 20, sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate, and water for injection.

Storage: The aflibercept concentrate for solution for infusion in its original unopened container under refrigerated conditions (2 to 8°C).

Stability: Shelf life of aflibercept is 36 months if properly stored under the above conditions.

Route*(s)* of Administration: Intravenous

Method of Administration: Multiple vials of aflibercept concentrate for solution for infusion may be required depending on the patient's weight and the intended dose. The necessary volume of aflibercept should be withdrawn from the vials and injected directly into the infusion bag. Aflibercept concentrate should be diluted for infusion with 0.9% sodium chloride solution or 5% dextrose by a healthcare professional. The dilution must be carried out under aseptic conditions. Any unused portion left in a vial must be discarded, as the investigational drug product does not contain any preservatives.

Potential Drug Interactions: No formal crossover design drug-drug interaction studies with antineoplastic agents have been conducted. However, the existing data of aflibercept in combination Phase 1 trials suggest that aflibercept does not affect the pharmacokinetics of 5-FU, oxaliplatin, gemcitabine, irinotecan, or docetaxel.

Patient Care Implications:

Pregnancy and lactation

Angiogenesis is critical to fetal development and the inhibition of angiogenesis following administration of aflibercept is likely to result in adverse effects on pregnancy. There are no adequate and well-controlled studies in pregnant women. All patients should be counseled regarding the potential risk of aflibercept to the developing fetus prior to initiation of therapy.

If the patient becomes pregnant while receiving aflibercept, she should be apprised of the potential hazard to the fetus and/or the potential risk of loss of pregnancy. Patients who discontinue aflibercept should also be counseled concerning the prolonged exposure following discontinuation of therapy (half-life of approximately 20 days) and the possible effects of aflibercept on fetal development.

No information is available regarding the safety of aflibercept in pregnant women and their fetuses, or in women who are breast feeding. It is not known whether aflibercept is excreted in human or animal milk and the potential for absorption and harm to the nursing infant is unknown. However, IgG1s are secreted into the breast milk of nursing mothers. The Investigator must make every effort to ensure aflibercept is not administered to women who are pregnant or breast feeding. Lactating women should be advised to discontinue nursing during aflibercept treatment and to not resume until at least 6 months following the last administration of aflibercept.

Prior to enrollment in a clinical trial of aflibercept, patients of childbearing potential must have a negative serum pregnancy test (within 7 days). In the event of pregnancy while on aflibercept treatment, aflibercept should be discontinued and the Sponsor informed immediately. Follow-up of the pregnancy will be mandatory until the outcome has been determined. Information related to the pregnancy must be given on a "Drug Exposure Via Parent-Data Collection" form that will be provided by the Sponsor. Effective contraceptive measures are required for 6 months following the last administration of aflibercept.

Overdose

There is no information on the safety of aflibercept given at doses exceeding 7 mg/kg every 2 weeks, or 9 mg/kg every 3 weeks.

Osteonecrosis of the jaw

For patients receiving aflibercept, good oral hygiene and regular dental follow-up is recommended. Patients should not undergo invasive dental procedures while receiving aflibercept without first consulting with the treating oncologist. If osteonecrosis develops, aflibercept should be permanently discontinued and the patient referred to an oral surgery specialist for further management.

Availability

Aflibercept is an investigational agent supplied to the investigators by Sanofi-Aventis.

8.2 Commercial Agents

8.2.1 **5-Fluorouracil**: please see package insert for additional information

Chemical Name: 2,4-Dihydroxy-5-fluoropyrimidine

Other Names: 5-FU, Adrucil

Classification: Antimetabolite

Molecular Formula: $C_4H_3FN_2O_2$ **Molecular Weight:** 130.08

Mode of Action: fluorouracil is a pyrimidine antagonist that interferes with nucleic acid biosynthesis. The deoxyribonucleotide of the drug inhibits thymidylate synthase, thus inhibiting formation of thymidylic acid from deoxyuridylic acid and thus interfering with the synthesis of DNA. It also interferes with RNA synthesis. 5FU is cell-cycle specific (S-phase).

How supplied: Commercially available in 500 mg/10mL ampoules and vials, and 1 gram/20 mL, 2.5 gram/50mL and 5 gram/100 mL vials.

Storage and stability: Stable for prolonged period of time at room temperature if protected from light. Inspect for precipitate; if apparent, agitate vial vigorously or gently heat to not greater the 140°F in a water bath. Do not allow to freeze.

Route of Administration: intravenous

Drug Administration: 5-FU will be administered at 400 mg/m2 IV bolus over 5-15 minutes, followed by 2400mg/m^2 continuous infusion over 46 hours.

Patient Care Implications: Reported Adverse Events and Potential Risks

Hematologic: Leukopenia, thrombocytopenia, anemia; can be dose limiting; less common with continuous infusion.
Dermatologic: Dermatitis, nail changes, hyperpigmentation, Hand-Foot Syndrome with protracted infusions, alopecia.
Gastrointestinal: Nausea, vomiting, anorexia, diarrhea, can be dose limiting; mucositis occasionally dose limiting; severe, cholera-like diarrhea which can be fatal when given with leucovorin.
Neurologic: Cerebellar Syndrome (headache and cerebellar ataxia).

Cardiac: Angina, noted with continuous infusion.
Ophthalmic: Eye irritation, nasal discharge, watering of eyes, blurred vision.

Drug Interactions: Chronic administration of cimetidine may decrease the clearance of 5-FU. Within individuals, interferon alpha may decrease 5-FU clearance in a dose and schedule-dependent manner. Dipyridamole 57 increases 5-FU clearance and lower C_{pss} during continuous infusion. No pharmacokinetic interaction between 85 mg/m_2 oxaliplatin and 5-FU/LV has been observed in patients treated every 2 weeks. Increases of 5-FU plasma concentrations by approximately 20% have been observed with doses of 130 mg/m_2 oxaliplatin dosed every 3 weeks. Leucovorin enhances the cytotoxicity of 5-FU by forming a more stable tertiary complex with thymidylate synthase. Concomitant administration of 5-FU with warfarin has been reported to result in increased INR/prolonged prothrombin time. **Patients receiving both drugs should be followed with weekly INRs**.

Note: 5-FU in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

Availability: Commercially available

8.2.2 **Leucovorin:** please see package insert for additional information.

Other Names: Folinic acid, Citrovorum factor, or 5-formyl-5,6,7,8-tetrahydrofolic acid

Classification: Chemically reduced derivative of folic acid

Mode of Action: Leucovorin can enhance the therapeutic and toxic effects of fluoropyrimidines used in cancer therapy, such as 5-fluorouracil. 5-Fluorouracil is metabolized to fluorodeoxyuridylic acid, which binds to and inhibits the enzyme thymidylate synthase (an enzyme important in DNA repair and replication). Leucovorin is readily converted to another reduced folate, 5,10-methylenetetrahydrofolate, which acts to stabilize the binding of fluorodeoxyuridylic acid to thymidylate synthase and thereby enhances the inhibition of this enzyme.

How Supplied: Leucovorin calcium for injection is supplied in sterile, single-use vials.

Storage and Stability: Store at 25°C (77°F); excursions permitted to 15-30°C (59°-86°F). Protect from light.

Route of Administration: Intravenous

Drug Administration: Leucovorin 400mg/m 2 in 250cc D5W is to be given over 2 hours. Leucovorin can be administered following oxaliplatin, or at the same time as oxaliplatin in separate bags using a Y-line.

Patient Care Implications: Known potential toxicities include nausea, diarrhea, thrombocytosis, rash, hives, pruritus, headache, and wheezing

Drug Interactions: May potentiate the toxic effects of fluoropyrimidines (5-fluorouracil) therapy, resulting in increased hematologic and gastrointestinal (diarrhea, stomatitis)
adverse effects. Monitor closely.

Availability: Commercially available

8.2.2.1 **Levoleucovorin:** please see package insert for additional information.

Other Names: Fusilev, or (6S)-N-{4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6pteridinyl)methyl] amino]benzoyl}-L-glutamate pentahydrate .

Classification: Chemically reduced derivative of folic acid

Mode of Action: Levoleucovorin can enhance the therapeutic and toxic effects of fluoropyrimidines used in cancer therapy such as 5-fluorouracil. 5-fluorouracil is metabolized to 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP), which binds to and inhibits thymidylate synthase (an enzyme important in DNA repair and replication). Levoleucovorin is readily converted to another reduced folate, 5,10-methylenetetrahydrofolate, which acts to stabilize the binding of FdUMP to thymidylate synthase and thereby enhances the inhibition of this enzyme.

How Supplied: Levoleucovorin Each 50 mg single-use vial of Fusilev for Injection contains a sterile lyophilized powder consisting of 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol. 50 mg vial of freeze-dried powder – NDC 68152-101-00.

Storage and Stability: Store at 25° C (77 °F) in carton until contents are used. Excursions permitted from 15-30° C (59-86 °F). Protect from light.

Route of Administration: Intravenous

Drug Administration: Levoleucovorin 200mg/m 2 in 250cc D5W is to be given over 2 hours. Leucovorin can be administered following oxaliplatin, or at the same time as oxaliplatin in separate bags using a Y-line.

Patient Care Implications: Known potential toxicities include nausea, vomiting, diarrhea, stomatitis, Abdominal Pain, Asthenia/Fatigue/Malaise , Anorexia/Decreased Appetite, Dermatitis, Alopecia,

Drug Interactions: Folic acid in large amounts may counteract the antiepileptic effect of phenobarbital, phenytoin and primidone, and increase the frequency of seizures in susceptible children. It is not known whether folinic acid has the same effects. However, both folic and folinic acids share some common metabolic pathways. Caution should be taken when taking folinic acid in combination with anticonvulsant drugs. Preliminary human studies have shown that small quantities of systemically administered leucovorin enter the CSF, primarily as its major metabolite, 5-methyltetrahydrofolate (5-MTHFA). In humans, the CSF levels of 5-MTHFA remain 1-3 orders of magnitude lower than the usual methotrexate concentrations following intrathecal administration.
Fusilev increases the toxicity of 5-fluorouracil.

Availability: Commercially available

8.2.3 **Oxaliplatin:** Please see package insert for additional information

Chemical Name: Trans-l-1,2-diaminocyclohexane oxalatoplatinum

Other Names: Eloxatin

Classification: Platinum Analogue

Molecular Formula: $C_8H_{12}N_2O_4Pt$ **Molecular Weight:** 359.3

Mode of Action: Oxaliplatin undergoes nonenzymatic conversion in physiologic solutions to active derivatives via displacement of the labile oxalate ligand. Several transient reactive species are formed, including monoaquo and diaquo DACH platinum, which covalently bind with macromolecules. Both inter and intrastrand Pt-DNA crosslinks are formed. Crosslinks are formed between the *N7* positions of two adjacent guanines (GG), adjacent adenineguanines (AG), and guanines separated by an intervening nucleotide (GNG). These crosslinks inhibit DNA replication and transcription. Cytotoxicity is cell-cycle nonspecific.

How Supplied: Powder for solution for infusion:

ELOXATIN is supplied in clear, glass, single-use vials with gray elastomeric stoppers and aluminum flip-off seals containing 50 mg or 100 mg of oxaliplatin as a sterile, preservative-free lyophilized powder for reconstitution. Lactose monohydrate is also present as an active ingredient.

NDC 0024-0596-02: 50 mg single-use vial with green flip-off seal individually packaged in a carton.

NDC 0024-0597-04: 100 mg single-use vial with dark blue flip-off seal individually packaged in a carton.

Concentrate for solution for infusion:
ELOXATIN is supplied in clear, glass, single-use vials with gray elastomeric stoppers and aluminum flip-off seals containing 50 mg, 100 mg or 200 mg of oxaliplatin as a sterile, preservative-free, aqueous solution at a concentration of 5 mg/ml. Water for Injection, USP is present as an inactive ingredient.

NDC 0024-0590-10: 50 mg single-use vial with green flip-off seal individually packaged in a carton.

NDC 0024-0591-20: 100 mg single-use vial with dark blue flip-off seal individually packaged in a carton.

NDC 0024-0592-40: 200 mg single-use vial with orange flip-off seal individually packaged in a carton.

Storage and Stability: Powder for solution for infusion:
Store under normal lighting conditions at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) [see USP controlled room temperature].

Concentrate for solution for infusion:
Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F). Do not freeze and protect from light (keep in original outer carton).

Route of Administration: Intravenous

Drug Administration: ELOXATIN 85 mg/m^2 IV infusion in 500 mL 5% Dextrose injection, USP (D5W) given over 120 minutes. Leucovorin 400 mg/m^2 IV infusion in D5W can be given at the same time in separate bags using a Y-line. Premedication with antiemetics, including 5-HT$_3$ blockers with or without dexamethasone, is recommended. When oxaliplatin is administered with 5-fluorouracil, the oxaliplatin infusion should precede that of 5-fluorouracil. Ensure the infusion lines are adequately flushed with 5% Dextrose between administration of the two drugs.

Patient Care Implications: Reported Adverse Events and Potential Risks
Allergy/Immunology: Allergic/Hypersensitivity reaction (including drug fever)
Auditory: Middle ear/hearing (ototoxicity, mild), inner ear/hearing (mild hearing loss).
Blood/Bone Marrow: decreased hemoglobin, hemolysis (e.g., immune hemolytic anemia, drug related hemolysis), decreased leukocytes, decreased platelets, neutropenia.

Cardiovascular (Arrhythmia): Sinus tachycardia, supraventricular arrhythmias (SVT/atrial fibrillation/flutter), ventricular arrhythmias
(PVCs/bigeminy/trigeminy/ventricular tachycardia).
Cardiovascular (General): Edema, hypertension, phlebitis (superficial), thrombosis/embolism (including pulmonary embolism).
Coagulation: DIC (disseminated intravascular coagulation).
Constitutional Symptoms: Fever (in the absence of neutropenia, where neutropenia is defined as AGC <1.0 x 109L), weight loss, fatigue (lethargy, malaise, asthenia).
Dermatology/Skin: Erythema or skin eruptions, alopecia, hand-foot skin reaction, injection site reaction, rash/desquamation
Endocrine: Hot flashes/flushes
Gastrointestinal: Anorexia, constipation, dehydration, dysphagia, diarrhea, esophagitis, odynophagia (painful swallowing), gastrointestinal reflux, enteritis, ascites (not otherwise specified), intestinal obstruction, stomatitis/pharyngitis (oral/pharyngeal mucositis), taste disturbance (dyspepsia), nausea, vomiting, colitis, ileus, typhlitis.
Hematologic: Single-agent oxaliplatin produces only mild myelosuppression with minimal to severe neutropenia, anemia, or thrombocytopenia. In combination, more grade 3/4 neutropenia or thrombocytopenia may be noted.
The hemolytic uremic syndrome (HUS) should be suspected in individuals who experience the following unexplained severe hemolysis, hemoglobinemia, and renal failure as demonstrated by an increase in serum creatinine. Patients suspected of experiencing HUS should have a thorough laboratory evaluation. **Oxaliplatin should be discontinued for any suspected occurrence of HUS.**
Hemorrhage: CNS hemorrhage/bleeding, hemoptysis, hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, melena/GI bleeding, rectal bleeding/hematochesia, other (hemorrhage NOS).
Hepatic: Increase alkaline phosphatase, increased bilirubin, increase GGT, hepatic enlargement, increase SGOT (AST), increase SGPT (ALT)
Infection/Febrile Neutropenia: Febrile neutropenia (fever of unknown origin) without clinically or microbiologically documented fever (ANC <1.0 x 10^9L fever >38.5°C), infection (documented clinically or microbiologically) with grade 3 or 4 neutropenia (ANC<1.0 x 109L), infection with unknown ANC, infection without neutropenia.
Metabolic/Laboratory: Acidosis (metabolic or respiratory), hyperuricemia, hypokalemia, hypophosphatemia, hyponaturemia,

hypomagnesemia, hypocalcemia.
Musculoskeletal: Involuntary muscle contractions.
Neurology: Ataxia (incoordination, including abnormal gait), insomnia, mood alteration (depression, anxiety), neuropathy cranial (ptosis), vertigo, acute sensory neuropathy induced or exacerbated by cold (including acute laryngopharyngeal dysesthesias, Lhermitte's sign, upper extremity paresthesias), chronic peripheral neuropathy.
Ocular/Visual: Conjunctivitis, vision abnormalities including blindness, optic neuritis, papilledema, hemianopsia, visual field defect, transient blindness.
Pain: Abdominal pain or cramping, arthralgia (joint pain), bone pain, chest pain (non-cardiac and nonpleuritic), headache (including migraine), Myalgia (muscle pain including cramps and leg cramps).
Pulmonary: Pulmonary fibrosis, cough, Dyspnea (shortness of breath, hiccoughs (hiccups, singultus), pneumonitis/pulmonary infiltrates (including eosinophilic pneumonia, interstitial pneumonitis, and interstitial lung disease), laryngospasm.
Renal/Genitourinary: Increased creatinine, renal failure, urinary retention, hemolytic uremic syndrome (HUS).

A life threatening constellation of renal failure, anemia, and thrombocytopenia, has been reported in oxaliplatin trials. Also reported on oxaliplatin trials but with the relationship to oxaliplatin still undetermined: tongue paralysis, anemia, aphasia, abnormal hepatic dysfunction, hyporeflexia, anxiety, depression, dysarthria, insomnia, increase sweating, rhinitis, epistaxis, gout, pancreatitis, idiopathic thrombocytopenia (5 cases), thrombocytopenia associated with hemolytic anemia (2 cases).

Note: Oxaliplatin in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

Availability: Commercially Available

8.3 Agent Ordering

Aflibercept ordering will be completed through the Sanofi-Aventis IST Portal. Fluorouracil, leucovorin and oxaliplatin are commercially available.

8.4 Agent Accountability

Agent Inventory Records – The investigator, or a responsible party designated by

the investigator, must maintain a careful record of the inventory and disposition of all agents received.

9. CORRELATIVE STUDIES

9.1. **Collection of Specimen(s).** Patients are required to have tissue available before enrolling on the study. A fresh biopsy is only required if there is insufficient material for analysis. Repeat tumor biopsies after 8 weeks of therapy are optional and **will only be performed at the Ohio State University Medical Center**. In addition to tumor tissue, whole blood, plasma and serum will be collected at various timepoints outlines below.

9.1.1. Blood and Tissue Requirements:

- A whole blood sample should be collected prior to treatment on Cycle 1 Day 1 for subsequent DNA extraction for SNP analysis. Whole blood (approximately 5 mL) should be collected in an EDTA tube, aliquoted into two 1.5-2.0 mL cryovials and stored at -70°C or lower until shipping.

- A 10mL whole blood sample will be collected to obtain a plasma sample for all patients on C1D1 (prior to treatment), week 3, week 9, week 13 and 4 weeks after the patient goes off treatment. Whole blood should be collected in an EDTA tube (lavender top), inverted 8-10 times and placed on ice until centrifugation at 1200g for 10 minutes at +4°C. This should be done within 1 hour of collection to limit degradation and lysis. Plasma supernatant should be carefully aspirated without disturbing the buffy coat layer. About 4-5 mL of plasma can be obtained. Aliquot plasma into 4 properly labeled 1.5-2.0 mL cryovials (1 to 1.25 mL per tube). Store at -70°C or lower until shipping. Detailed instructions for this procedure are provided in the Laboratory Manual (provided separately).

- Additionally, one 10 mL red top or serum separator tube (SST) should also be collected at the same time points listed above (weeks 1, 3, 9, 13 and 4 weeks after going off treatment). This tube should be inverted five times and then allowed to sit at room temperature until a clot has formed (60 minutes for red top tubes or 30 for SST). Tubes should then be centrifuged at 1200g for 10 minutes at room temperature or +4 °C. Serum can be either poured or pipetted off into 4 properly labelled 1.5-2.0 mL cryovials (1 to 1.25 mL per tube) and stored at -70°C or lower until shipping. Detailed instructions for this procedure are provided in the Laboratory Manual (provided separately).

- An archival tissue block verified to contain tumor or a minimum of 10 slides cut at 4-5 microns (unstained and placed onto poly-l-lysine-coated or plus (+)

slides) will be required for IHC. An additional H&E stained 4-micron slide will also be required if sending slides.

- Fresh core biopsies are required upon study entry if sufficient archival material in not available. In addition, patients treated at The Ohio State University may undergo an optional biopsy after 8 weeks of treatment. This biopsy can be performed up to 7 days prior to C3D1. Biopsies will be performed preferably under image-guidance, although biopsy method will be chosen based on feasibility for each individual patient and tumor location. Four to five core needle biopsies will be taken depending on the size of the tumor. These cores should be immediately placed in 10% neutral buffered formalin, processed using standard operating procedures and embedded in paraffin. Detailed instructions for this procedure are provided in the Laboratory Manual (provided separately). Blocks should be labeled with the protocol number, subject number, date and institution.

9.1.2. Shipping of Specimen(s):

Specimens to be collected and prepared in this study include whole blood, plasma, serum and archival FFPE blocks or slides (FFPE core biopsies if required). Samples should be shipped in batches according to instructions listed below along with the "RESEARCH SPECIMEN REQUEST FORM" (included in the laboratory manual).

- All frozen specimens should be packed in dry ice in styrofoam shippers with enough dry ice to ensure that the samples will not thaw within a 48-hour period. A minimum 5 kg of dry ice should be used. Dry ice should be placed along the bottom of the styrofoam container. Each set of frozen specimens should be placed in a plastic resealable bag, grouped according to the subject. A paper towel should be wrapped around the samples. Each plastic bag for each patient should be identified with the following information: protocol number, institution name, patient study ID number. Before plastic bags are placed into the styrofoam box a layer of paper towels should be placed on top of the dry ice. The plastic bag should then be placed into the box, and additional dry ice should be placed along its sides. The styrofoam container should be sealed with strapping tape and placed into a sturdy cardboard mailing box. Copies of all completed "RESEARCH SPECIMEN REQUEST FORMS" (included in laboratory manual) should be included in a plastic resealable bag and placed on top of the Styrofoam container. The box should subsequently be sealed but in a way that will permit carbon dioxide to escape as the dry ice sublimates. Frozen specimens should be shipped overnight by carrier of choice on Monday, Tuesday, or Wednesday. Shipments should not be made on Thursday or Friday. Deliveries on Weekends and holidays will not be accepted.

- FFPE core biopsies should be placed in a resealable plastic bag (separate bags per patient) and can be shipped with the frozen samples but on the outside of

the styrofoam container. If not shipping with the frozen samples, place bags in a cardboard box and place in a padded envelope. These specimens with the specimen submission form can be sent by regular US mail or UPS ground.

- FFPE slides should be packaged in a slide holder and shipped in a well cushioned box. These specimens with the relevant pathological report/s can be sent by regular US mail or UPS ground.

Blood and Tissue Specimens should be shipped to the following address:

Cynthia Timmers, Ph.D.
Room 460B Biomedical Research Tower
460 West 12th Avenue
Columbus, Ohio 43210
Tel. (614)-366-9041
Cynthia.timmers@osumc.edu

9.2 Biologic Correlative Studies

9.2.1 Measurement of serum levels of VEGF, VEGFR, P1GF, CXCL12 and CXCR4

We will use a very sensitive ELISA kit from R&D Systems (Minneapolis, Minnesota, USA) to measure serum levels of the PIGF, VEGFA and it receptor VEGFR2 and also CXCL12 and its receptor CXCR4. We will correlate the serum levels of these proteins before and after treatment with any clinical responses observed. Any differences in the serum levels of these proteins will be statistically analyzed. We will collect these samples on the same schedule as DCE-MRI scans so that our values may be correlated with DCE-MRI analysis.

9.2.2 Relationship between hypertension and clinical efficacy

Blood pressure will be measured weekly for the first 6 weeks and at each therapeutic visit and will be graded according to NCI CTCAE v. 4.0. Results will be collected, analyzed, and correlated with response as outlined in Section 13.

9.2.3 SNP analysis

To evaluate genetic markers as potential predictive biomarker for treatment outcome, genomic DNA will be extracted from whole blood collected in EDTA tubes at baseline (week 1 pre-treatment). DNA will be purified using the Promega Maxwell 16 Blood DNA Purification kit on the Maxwell 16 automated extraction instrument. and quantified using PicoGreen fluorescence using Molecular Probes Quant-IT broad range dsDNA kit. Concentration will be based on the standard curve provided in the Quant-IT kit.

Single nucleotide polymorphisms (SNPs) in VEGFA, VEGFR1, VEGFR2, IL8 and CXCR2 will be assessed using TagMan SNP Genotyping Assays. Genotypes will be determined using the TaqMan Genotyper Software.

9.2.4 Measurement and quantification of tumor microvessel density

Immunochemistry and quantitative image analysis will be performed as quantitative measure of ongoing tumor associated angiogenesis. This assessment will be performed on tumor biopsies pretreatment and after 8 weeks of therapy. For these studies, anti-CD31 antibody will be used. CD31 is a cell surface glycoprotein on endothelial cells that mediate adhesion between vascular cells and their extracellular matrix and is involved in transmitting the signals from VEGF. It is strongly expressed by all endothelial cells and weakly by leukocytes. CD31 has been used successfully as a marker for microvessel density in our laboratory. This is the approach that will be used to stain sections of tumor biopsies taken from patients that have been treated with aflibercept and FOLFOX. Tumor sections (4-5 microns) will be stained with rat anti-mouse platelet endothelial cell adhesion molecule-1 antibody (PECAM-1, CD31; 5ug/ml). After applying the biotinylated secondary antibody, the avidin biotin peroxide complex (ABC) method using Vectastatin kit (Vector Laboratories) will be used for visualization and quantitation. Staining of blood vessels with CD31 is suitable for identification of angiogenesis in tumor biopsy samples. The stained slides will then be quantified using the Image J software provided by NIH.

9.3 Radiographic Correlative Studies

We are in the process of developing a trial-specific standard imaging manual that will be distributed to all participating sites. All DCE-MRI and FDG-PET images must be obtained according to the Imaging Protocol outlined in Appendix C. DCE-MRI and FDG-PET will only be performed at sites that have the technology available. DCE-MRI and FDG-PET are optional for participating sites that are unable to perform the imaging due to financial restrictions. Images will be uploaded to a central database and reviewed centrally at OSUMC by Dr. Knopp and his group.

9.3.1 DCE MRI

We will obtain DCE MRI images at weeks 0, and after 8 weeks +/- 1 week of treatment (after Cycle 2- see study calendar). Patients enrolled at sites where this technology is unavailable and/or patients with contraindications to MRI imaging, will not undergo this part of imaging. Dr. Michael Knopp and his group will be directly involved in the development and the conduct of the radiographic correlates for this study in collaboration with our group.

Patients will be imaged with conventional T2- and T1-weighted sequences prior to contrast agent application and with T1-weighted sequences after contrast agent application for tumor localization and volumetry. In addition, dynamic contrast

enhanced (DCE) images will be acquired to demonstrate tumor heterogeneity, microcirculation, vascularization and viability. DCE MRI will be carried out using a standard, commercially available, FDA approved contrast agent (Gd-chelate) and a power injector (Spectris, MedRad, Indianola, PA). All patients will be imaged on a 3 Tesla MR system (Achieva; Philips, Cleveland, OH) using a surface coil. Images will be acquired with a 3D fast spoiled gradient echo sequence covering the lesion with a time resolution in the range of seconds. For patients treated at OSU, the core lab of the Wright Center of Innovation will perform the lesion tracking assessment, uni-dimensional lesion measurement (RECIST) as well as 3-D volume segmentation.

The DCE-MRI data analysis will be performed using in-house developed software based on the IDL environment (Interactive Data Language; ITT, Boulder, CO). Regions of interest (ROIs) will be drawn on dynamic imaging data sets including tumor and artery. The ROIs for each time point will be co-registered to compensate for subject motion during scans. The ROI placement will be carried out by an experienced reader and confirmed by a radiologist. The tumor time-enhancement curves will be quantitatively analyzed. The adjusted Brix's model-based pharmacokinetic parameters (Amp, k_{ep}, k_{el}) will be determined by using the MINPACK-1 method for fitting the tracer kinetics equation to the tissue time-signal intensity curves

9.3.2 FDG-PET

^{18}FDG-PET will be performed using whole-body technique at week 0 (pre-therapy) and after 8 weeks +/- 1 week of treatment (after cycle 2). Patients enrolled at sites where this technology is unavailable, will not undergo this part of imaging. It is crucial that the ^{18}FDG-PET studies are acquired and processed using the same protocol at all sites. Subjects must fast 4-6 hours before the procedure, but can freely drink water, in order to diminish physiologic glucose uptake and reduce serum insulin levels to near basal level. Blood sugar cannot exceed 200 mg/dL at the time of the ^{18}FDG-PET study. Subjects should be injected with approximately 10-20 mCi of ^{18}FDG and remain still during an ^{18}FDG uptake period of at least 60 minutes. The ^{18}FDG uptake period of the post week 8 (cycle 3) PET scans should be within 15 minutes of the uptake period of the baseline PET scan. After the ^{18}FDG uptake period, two or three dimensional whole body emission scans encompassing the region between the earlobes and the proximal thighs will be obtained in several bed positions (usually 6-8) using a PET/CT system for several minutes (usually 3-5) per bed position (in accordance with the manufacturer's recommendations). The total scanning time for each patient usually ranges from 20-45 minutes. PET/CT may be performed with or without oral or IV contrast, but should be performed consistently for the baseline and post- week 8 scan. The PET projection data are corrected for random coincidences, scatter, and attenuation in accordance with manufacturer's recommendations. Transaxial images will be reconstructed into at least 128 x 128 pixel images with a pixel size according to manufacturer's recommendations, preferably of 5 mm or less. The reconstructed PET/CT images will be displayed on a computer workstation so that transaxial, sagittal, and coronal

images can be simultaneously viewed. All scans should be performed on calibrated PET/CT scanners and each subject should have the baseline and post-cycle 2 scans on the same scanner. Coincidence imaging using hybrid SPECT/PET systems is unacceptable. For each subject's PET scan, the following parameters must be recorded during the PET procedure: assay time, ^{18}FDG assay dose, injection time, residual time, residual dose, PET emission scan start time, subject weight (measured on the day of the scan). All clocks used to record time must be synchronized with the PET scanner computers. The ^{18}FDG-PET scans at baseline and at follow-up will be interpreted by an experienced nuclear medicine physician at each participating site who will be responsible for image interpretation. The images will be interpreted together with pertinent clinical and CT findings. Interpreting the PET scan in this fashion mimics the usual clinical situation, in which this information is incorporated into the interpretation, especially in the case of an equivocal scan finding that may be easily explained by the CT scan result (e.g., anatomic variation of the bowel or bladder) or clinical information (e.g., increased uptake at a site of recent surgery or biopsy). In addition, all PET/CT images will be sent to the Ohio State University for central analysis. Images obtained at baseline and cycle 3 will be compared to DCE-MRI images obtained at the same time points for combined evaluation of the effects of anti-angiogenic therapy and correlation with RECIST response by CT scan.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. In the event that the

patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. All study visits and evaluations have a +/- 3 day window.

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 13	Continued Treatment	Follow Up[o]	Off Treatment
Aflibercept mFOLFOX 6[b]		X		X		X		X		X		X		X	X[n]		
Informed consent	X																
Demographics	X																
Medical history	X																
Concurrent meds	X	X----------													----------X		
Physical exam	X	X[q]		X		X		X		X		X		X	X[n]		X
Vital signs[l]	X	X	X	X	X	X	X	X		X		X		X	X[n]		X
Height	X																
Weight	X	X		X		X		X		X		X		X	X[n]		X
Performance status	X	X		X		X		X		X		X		X	X[n]		X
CBC w/diff, plts PT/INR[c]	X	X[q]		X		X		X		X		X		X	X[n]		X
Serum chemistry[d]	X	X[q]		X		X		X		X		X		X	X[n]		X
Urine studies[e]	X	X[q]		X		X		X		X		X		X	X[n]		
LDH/ Phosphorous	X																
EKG	X																
Chest X ray		As Medically Indicated															
Adverse Event Evaluation		X ----------													----------X		X
Tumor measurements	X	Tumor measurements are repeated every 8 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.															X
Radiologic evaluation[m]	X	Radiologic measurements should be performed every 8 weeks.															X
B-HCG[f]	X																
CEA	X	CEA measurements to be performed every 4 weeks															X

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 13	Continued Treatment	Follow Up[o]	Off Treatment
Lipid Studies[p]	X					X				X				X	X[p]		X
Whole blood for SNP analysis		X															
Serum and plasma		X		X						X				X			
Assessment for resectability[g]										X							
DCE-MRI[i]	X									X							
FDG-PET[i]	X									X							
Tumor biopsy[j, k]	X									X							

a: Patients will continue to receive therapy on study until any of the following: disease progression, unacceptable toxicity, intercurrent illness, death, or refusal/withdrawal of consent to receive treatment.

b: Aflibercept will be administered at a dose of 4 mg/kg intravenously every 2 weeks. mFOLFOX 6 is administered intravenously every 2 weeks and consists of oxaliplatin 85 mg/m^2 plus leucovorin 400 mg/m^2 given together over 2 hours, followed by bolus 5-FU 400 mg/m^2 over 5-15 minutes, followed by continuous infusion 5-FU 2400 mg/m^2 over 46 hours.

c: For patients requiring therapeutic anticoagulation with warfarin, PT/INR should be assessed prior to each aflibercept infusion.

d: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.

e: Urinalysis for hematuria, protein and creatinine measurements. 24-hour urine collection for quantitative protein determination should be obtained for a urine protein/creatinine ratio >1.

f: Serum pregnancy test (women of childbearing potential). This must be obtained within 7 days of treatment initiation.

g: For patients with limited hepatic metastatic disease only, at each interval radiographic evaluation, a determination of possibility of R0 resection of all metastatic disease should be considered

h. Two 10mL tubes of blood will be obtained for purification of serum and plasma for pharmacodynamic correlates pre-dose on day 1 of weeks 1, 3, 9 and 13 and at the time of progression or when patient goes off study.

i: DCE-MRI and FDG-PET will be obtained at week 0 (pre-therapy) and after 8 weeks +/- 1 week (2 cycles) of therapy at sites with the technology available. DCE-MRI and FDG-PET are optional for participating sites that are unable to perform the imaging due to financial restrictions.

j: Patients are required to have paraffin embedded tissue available before enrolling on the study. A biopsy is only required if there is insufficient material for analysis. The tissue will be used for correlative studies that include measurement of tumor vascularity and SNPs.

k: At 8 weeks post-treatment, patients will have the option to undergo tumor biopsy (OSU patients only) to assess for changes in tumor vascularity.

l. Blood Pressure will be checked at home by the patient with two separate measurements at least 1 minute apart on C1D8, C1D22 and C2 Day 8 and the lower reading should be reported to the study coordinator by phone. If the patient has a home blood pressure monitoring device (wrist cuffs are allowed) they should bring it in on their 1st visit (for calibration), otherwise one will be provided to them.

m. Radiologic evaluation to include CT scan of Chest, Abdomen, and Pelvis
n. Patients may continue on treatment past 13 weeks so long as criteria in section 5.3 has not been met. Treatment schedule will remain the same as weeks 1-13. Physical exams will only be required to be done monthly. All other assessments will be every 2 weeks on treatment days.
o. After being taken off treatment, patient will be followed every 3 months until death. Adverse event reporting should continue for 30 days after last dose of protocol therapy. Patients removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. After removal from treatment, follow up may be done by phone, if needed. Radiologic evaluation frequency should occur per local standard of care. Patients are not required to continue to have radiologic evaluations per protocol schedule.
p. Lipid studies are to be performed at baseline and then monthly during protocol therapy and are to include LDL-cholesterol, HDL-cholesterol, and Triglycerides
q. If pre-study physical exam and labs were performed within 3 days prior to C1D1, these assessments do not need to be repeated on C1D1.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 8 weeks.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, Version 1.1 [58]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with aflibercept and mFOLFOX6

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least two cycles of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 2 will also be considered evaluable.)

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥10 mm with spiral CT scan (CT scan slice thickness no greater than 5 mm) Malignant lymph nodes will be considered measurable if they are ≥15 mm in short axis.

Note: Tumor lesions that are situated in a previously irradiated area will not be considered measurable.

Non-measurable disease. All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with ≥10 to <15mm short axis as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Target lesions. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as ‘present’, ‘absent’,

or in rare cases 'unequivocal progression'. In addition, it is possible to record multiple nontarget lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

11.1.3 **Methods for Evaluation of Measurable Disease**

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and ≥10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next (described in greater detail in Appendix C). If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected

instances.

Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).

Incomplete Response/ Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new

lesions is also considered progression).

When the patient also has measurable disease, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare. In such circumstances, the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration"*. Every effort should be made to document the objective progression even after discontinuation of treatment.

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

11.1.6 **Progression-Free Survival**

PFS is defined as the duration of time from start of treatment to time of documented progression or death whichever occurs first.

11.1.7 **Overall Survival**

Time from study initiation to time of death will be followed and documented for all patients.

11.1.8 **Response Rate**

Response rate is defined as the rate of complete response + partial response as defined by RECIST criteria.

11.1.9 **Disease Control Rate**

Disease control rate is defined as the rate of complete response + partial response + stable disease, as defined by RECIST criteria.

12. DATA/SAE REPORTING & REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and additional instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 **Serious Adverse Event Reporting**

12.1.1. Serious Adverse Events Reportable to the FDA

In the context of an Investigator Sponsored Trial being conducted under an IND, CFR 312.32 states the FDA needs to be informed of unexpected and related SAEs (SUSARs) as soon as possible or within 15 calendar days. In addition, the investigator must notify the FDA of any unexpected, fatal or life-threatening experience associated with the use of the drug as soon as possible, or within 7 calendar days by telephone or facsimile.

Subsite institutions are NOT permitted to report directly to the FDA or Sanofi-Aventis. Subsite institutions must report all SAEs to the Principal Investigator and the Subsite Coordinator within 24 hours of knowledge of the event. A brief description may be initially sent via secure email or fax. A complete report is then required to be submitted with SAE Submission Form (refer to Supplemental Forms Document) via secure email or fax to the Principal Investigator and Subsite Coordinator within 3 days. Follow up reports must be submitted per section 12.1.6. Subsite institutions are required to submit SAEs to their local IRBs as per their institutional and IRB policies.

When the principal investigator has determined that a Serious Adverse Event requires reporting to the FDA (unexpected and possibly related to study drug), the following actions

must be completed:

12.1.2 Actions towards FDA:

1. The Ohio State University will be the ONLY entity reporting SAEs to the FDA. Subsite institutions will NOT report SAEs the FDA. They should send SAEs to OSU for review and reporting to the FDA. Refer to section 12.1.1 for details.

2. **Telephone the FDA immediately** (day of awareness), in the case of reportable death or life-threatening events.

3. **Complete FDA Form MedWatch 3500A**.

4. **Send the completed MedWatch 3500A form to the FDA** (preferably by fax at 1-800-FDA-0178) within the timelines mentioned above.

5. **Attach the photocopy of all examinations**, medical notes and records related to the Serious Adverse Event and document the dates these were made. For laboratory results, include the laboratory normal ranges. For hospitalizations, Admission H&P, Discharge Summary, Consultative reports, etc. could be very helpful. In the case of a Fatal event, provide an autopsy report, when it becomes available.

12.1.3 Actions towards Sanofi-Aventis [Aflibercept]:

The Ohio State University will be the only entity reporting SAEs to Sanofi-Aventis. Subsite institutions will report SAEs to OSU, which will in turn report to the Sanofi-Aventis. Refer to section 12.1.1 for details.

Concurrently provide a copy of the information sent to FDA to sanofi-aventis by fax (908-203-7783) or email USPVMailbox@sanofi-aventis.com within 24 hours after the FDA submission (initial and follow-up information)

For IST contracts of recently approved new chemical/biological entities include the following:

- In addition to SUSARs, all SAEs shall be sent to sanofi-aventis on an ongoing basis.

- Results of any relevant complementary exams performed to obtain the final diagnosis of any SAE (e.g. hospital discharge summary, autopsy report, consultations, etc.), will be made available to sanofi-aventis upon request.

12.1.4 Actions towards co/sub-investigators (participating on this protocol and IRBs:

The principal investigator is responsible for providing all Serious Adverse Events (on MedWatch form 3500A) submitted to the FDA to the OSU IRB and co/sub-investigators

(via Dear Investigator Letter) participating on this protocol. This applies to initial and follow-up information. The Subsite Coordinator will be responsible for submitting subsite SAEs the OSU IRB. All SAEs will be sent to the subsite co-investigators by the Subsite Coordinator. Subsite institutions are required to submit SAEs to their local IRBs as per their institutional and IRB policies.

12.1.5 Actions towards other manufacturers (as relevant):

For reportable Serious Adverse Events (completed MedWatch form 3500A) that have occurred in patients treated with products other than of sanofi-aventis, the principal investigator must also transmit the information to the manufacturer of the suspect product within 24 hours.

12.1.6 Follow-up Reports

- The investigators should take all appropriate measures to ensure the safety of the subjects. Notably they should follow-up to determine the outcome of any Adverse Events (clinical signs, laboratory values or other, etc.) until the patient has recovered, abnormal values have returned to normal or until progression has been stabilized. This may imply that follow-up will continue after the subject has left the study and that additional investigations may be necessary.

- In the case of a Death or a Life-threatening event, the investigator should provide a follow-up report, with positive or negative findings within 5 days of the awareness of the initial observation.

- Any reportable Serious Adverse Events brought to the attention of the Investigator at any time after cessation of the trial and considered by him/her to be reasonably associated with medication administered during the period should also be submitted to the FDA. A copy should be provided to sanofi-aventis, if a sanofi-aventis product was involved or the appropriate manufacturer of another product within 24 hours after FDA submission (see above).

- The MedWatch form 3500A providing follow-up information about a reportable SAE must be submitted to the FDA as soon as possible or within 15 calendar days. As with the initial submission to the FDA of the MedWatch form 3500A, the principal investigator is also responsible for providing all follow-ups for reportable Serious Adverse Events (on MedWatch form 3500A) to the IRB and co/sub-investigators (via Dear Investigator Letter) participating on this protocol.

12.1.7 Multi-center Coordination

Principal Investigator Responsibilities:

The principal investigator is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the principal investigator. There will be only one version of the protocol, and each participating institution will use that document. The principal investigator is responsible for assuring that all participating institutions are using

the correct version of the protocol.
• The principal investigator is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements are the responsibility of the Principal investigator.
• The principal investigator is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
• The principal investigator will be responsible for the review of and timely submission of data for study analysis.

12.1.8 Coordinating Center Responsibilities (The Ohio State University)
• Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
• Prior to the activation of the protocol at each participating institution, documentation of IRB approval must be submitted to the Coordinating Center. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
• The Coordinating Center is responsible for central patient registration. The Eligibility Worksheet and requested supporting documentation will be faxed by the collaborating site to the OSU Subsite Coordinator. Patients will not begin treatment until a confirmation of eligibility by the OSU Subsite Coordinator has been performed and registration is received from the coordinating institution (OSU). A registration number will be given to each patient and will be used as a unique identifier on all patient materials and data sheets submitted thereafter. Every effort will be made to ensure that collaborating sites are notified of protocol eligibility and patient registration information within approximately 48 hours.
• The Coordinating Center will maintain documentation of all SAE reports including ones from collaborating sites. Participating institutions will report all serious adverse events in accordance with Sections 7 & 12 Adverse Event Reporting of the protocol.
• The Coordinating Center will provide Case Report Folders (CRFs) to all sites. The collaborating sites are responsible for the preparation of data for patients enrolled at their site. All data requested in the CRF must be provided to the principal investigator upon request. Source documents and research records for patients enrolled on the study must be made available for monitoring purposes upon request by the principal investigator.

13. STATISTICAL CONSIDERATIONS

13.1 **Study Overview**

This is a single arm single stage phase II study to evaluate the clinical efficacy of treatment with FOLFOX + aflibercept in patients with untreated metastatic colorectal cancer. The primary goal of this study is to assess the proportion of patients who are alive and progression-free at 15 months after treatment the combination of aflibercept plus mFOLFOX6 relative to the historical comparator of FOLFOX alone in the first line treatment of mCRC. In a secondary manner we will also evaluate response rates and tolerability and toxicity associated with this regimen as well as correlative endpoints such as angiogenic markers and how changes in these markers correspond to clinical outcomes.

Primary Endpoint: The primary endpoint to be evaluated in this trial is the 15-month

progression-free proportion, as measured by the proportion of patients alive and progression-free at 15 months from initiation of therapy. All eligible patients who have begun treatment will be included for evaluation of the primary endpoint. In addition, those patients who go on to undergo surgery will be excluded from the calculation of the primary endpoint to avoid potential bias. Based on prior experience and rates, we expect about 10% of these patients will be able to undergo surgery. These patients will be analyzed separately from the primary endpoint evaluation.

13.2 **Sample Size, Accrual, and Study Duration**

A maximum of 70 eligible patients will be enrolled on this trial and treated with the combination of aflibercept and mFOLFOX6. We anticipate that the annual accrual rate across all participating sites will be about 40 patients; therefore, the accrual period is expected to be approximately 21 months. These estimates are based on previous clinical trial experience in this patient population and practice history as well as the fact that patients will be accrued at the other participating institutions. The primary endpoint analyses will require 62 evaluable patients; the total number of 70 eligible patients reflects the overaccrual to account for about 10% of patients who are expected to be able to undergo surgery and thus excluded from the primary endpoint evaluation to avoid potential bias. Final efficacy analyses will begin after all patients have been followed for at least 15 months or until they have a defined event of interest (disease progression and/or death).

13.3 **Study design and primary endpoint analysis plans**

Based on historical data (41, 42), we would expect that treatment with FOLFOX alone in this setting would lead to a median progression-free survival (PFS) of around 8 months. Assuming that PFS is exponentially distributed, our null hypothesis is that the proportion of patients who are alive and progression-free at 15 months with FOLFOX alone (i.e. historical control) is at most 25%. We would consider this combination regimen of aflibercept+FOLFOX6 to be clinical promising if the true rate was 40% or higher. With these assumptions and constraining our one -sided type I error rate to 5% and type II error rate to 20%, this single stage phase II study design will require 62 evaluable patients. In other words, we will have at least 80% power to determine that this regimen is promising if in fact the true 15-month PFS rate is 40% or higher. As discussed above, we expect about 10% of patients to be able to undergo surgery. Since these patients have a much different clinical outcome than would be reflective of the treatment regimen itself, they will be excluded from our primary endpoint evaluation. However, as described below under secondary endpoint analyses, these patients will be evaluated in terms of toxicity and tolerability and for other secondary outcome measures.

Decision Rule: In the final analysis of all 62 evaluable patients, we will need to observe 22 or more patients (~35%) who are progression-free and alive at 15 months to determine that this regimen is promising. Otherwise, if 21 or fewer of these 62 evaluable patients are alive and progression-free at 15 months, we will consider this regimen to not warrant further study in this patient population.

Primary endpoint analysis: The proportion of patients who are alive and progression-free at 15 months will be evaluated and analyzed using all patients who received at least one day of treatment on study. The proportion will be calculated as the number of patients who are alive and progression-free at 15 months divided by the total number of evaluable patients (again, excluding those who go on to surgery prior to disease progression). Assuming that the number

of treatment successes (alive and progression-free) is binomially distributed, proportion estimates along with their corresponding exact 95% confidence intervals will be calculated.

13.4 Analysis of secondary endpoints

In addition, several secondary clinical and correlative endpoints will be evaluated in these subjects.

- ***Objective response rate (ORR) and percentage of patients able to undergo surgery***: each of these dichotomous outcomes will be evaluated across all evaluable patients and summarized as a proportion with corresponding 95% confidence interval in a manner similar to that described for the primary endpoint. Overall response rate will be defined as the proportion of patients who achieve a partial response (PR) or complete response (CR) based on RECIST 1.1 criteria (112) divided by the total number of evaluable patients. The proportion of patients who are able to undergo surgery will be calculated in a similar manner, where the number of patients who go on to surgery will be divided by the total number of evaluable patients. This latter categorization of patients (ability to undergo surgery) will also be considered in conjunction with the primary endpoint analysis as a treatment success outcome but in a secondary manner and not used in the decision rule associated with the primary endpoint. In this secondary set of analyses, the treatment success proportion will be calculated with those able to undergo surgery specifically included in the numerator of the proportion.
- ***Progression-free and overall survival:*** In addition to looking at the dichotomized outcome of PFS rate at 15 months, we will also evaluate progression-free survival as a time-to-event outcome. PFS will be defined as the time from study entry to the time of progressive disease and/or death. Patients will be censored at their last evaluation for these analyses if they were alive and progression-free at that time. In addition, those who go on to surgery or other treatment prior to disease progression will be censored at that timepoint. Overall survival will also be calculated for these patients, where overall survival will be defined as the time from study entry to time of death due to any cause. These time-to-event outcomes will be evaluated using the methods of Kaplan and Meier.
- ***Toxicity and Tolerability***: As per NCI CTCAE v4.0, the term toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. The maximum grade for each type of toxicity will be recorded for each patient, and frequency tables will be reviewed to determine toxicity patterns. In addition, we will review all adverse event data that is graded as 3, 4, or 5 and classified as either “unrelated” or “unlikely to be related” to study treatment in the event of an actual relationship developing. The incidence of severe (grade 3+) adverse events or toxicities will be described. We will also assess tolerability of the regimens through assessing the number of patients who required dose modifications and/or dose delays. In addition, we will also capture the proportion of patients who go off treatment due to adverse reactions or even those who refuse further treatment for lesser toxicities that inhibit their willingness to continue participation on the trial. These tolerability measures will be assessed within each of the treatment arms and we will explore differences in these measures between the arms. All patients who have received at least one dose of any of the therapeutic agents in a treatment arm will be evaluable for toxicity and tolerability.

- ***Levels of circulating VEGF, VEGFR, PIGF, CXCL12 and CXCR4:*** these markers will be evaluated before and after initiation of therapy. Baseline levels as well as change in these levels will be quantitatively summarized and graphically explored, in particular in terms of how they relate to clinical outcomes of interest. Analyses exploring differences in these angiogenic markers in relation to clinical outcomes will be largely hypothesis-generating. If deemed clinically active, we do expect at least 22 patients to have a positive clinical outcome as defined by the primary endpoint decision rule. Two-sample t-tests will be used to assess differences in these markers (baseline and after 8 weeks of treatment) between positive vs. negative clinical outcome groups. With limited numbers of patients we will primarily look for patterns of difference using graphical analyses; these can include scatterplots of baseline vs. change in markers with those with positive vs. negative clinical outcomes reflected through different plotting characters to identify potential patterns of interest.

- ***Identification of SNPs and correlation with response to treatment***. SNP markers will also be summarized in an exploratory manner in relation to clinical outcomes of interest. Cross tabulation tables and bar graphs will be used to assess potential patterns of allelic expression of certain SNPs between those with positive vs. negative clinical outcomes. SNP-based expression will also be explored in relation to time-to-event outcomes; given the limited numbers of patients, these analyses will be primarily hypothesis-generating.

- ***Measurement of tumor vascularity pre and post-treatment with aflibercept***: this will be analyzed and summarized in a manner similar to that described above for the angiogenic marker evaluations and their relationship to clinical outcomes of interest.

- ***Correlation between hypertension as a toxicity and response to treatment in patients in which it is observed***: incidence of hypertension along with severity score (i.e. grade) will be evaluated in relation to whether or not patients had a positive vs. negative clinical outcome. In particular, we will assess the percentage of those who had any treatment-related hypertension between the positive vs. negative clinical outcome patients, where they hypothesis is that treatment-related hypertension is reflective of the agent hitting its target.

- DCE-MRI and FDG-PET and correlation with response, clinical outcome, and serum biomarkers of angiogenesis: Imaging outcomes (positive vs. negative; max SUV) will be evaluated in relation to positive vs. negative clinical outcomes of interest as well as change in angiogenic markers from baseline to 8 weeks into treatment. These relationships will be summarized quantitatively as well as explored graphically. Overall, factors that may affect the incidence of a positive clinical outcome (i.e. progression-free and alive at 15 months) will be explored using a logistic regression model, with change in VEGF (or another angiogenic marker) from baseline to 8 weeks into therapy and with imaging outcome.

13.5 Reporting and Exclusions

13.5.1 Evaluation of toxicity. All patients will be evaluable for toxicity from the time of their first treatment with FOLFOX and aflibercept.

13.5.2 Evaluation of the primary endpoint. Patients must have received at least 2 cycles of therapy and have undergone radiographic response assessment to be considered evaluable for the primary endpoint of PFS. However, patients who are removed from study due to progressive disease or death prior to completion of 2 cycles of therapy will

be considered evaluable for the primary endpoint. Patients who are considered non-evaluable for the primary endpoint will be replaced. Patients removed from study to undergo surgery will not be evaluable for primary endpoint and will be replaced.

13.5.2 Evaluation of response. All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B

Instructions for preparation, handling, and administration of aflibercept

Multiple vials of aflibercept concentrate for solution for infusion may be required depending on the patient's weight and the intended dose. The necessary volume of aflibercept should be withdrawn from the vials and injected directly into the infusion bag.

Aflibercept concentrate should be diluted for infusion with 0.9% sodium chloride solution or 5% dextrose by a healthcare professional. The dilution must be carried out under aseptic conditions. Any unused portion left in a vial must be discarded, as the investigational drug product does not contain any preservatives.

Infusion bags made of the following materials may be used:

- PVC containing DEHP
- Polyolefin (PVC free DEHP free)

The final concentration of the diluted solution can range between 0.6 mg/dL and 8 mg/dL. This concentration allows for:

Polypropylene syringes may be used.

Chemical and physical stability of the diluted aflibercept solutions in polypropylene syringe or infusion bags has been demonstrated for up to 24-hours under refrigerated conditions (2° to 8°C) or for up to 8 hours at room temperature (approximately 25°C).

From a microbiological point of view, the product should be used immediately. In-use storage times and conditions are the responsibility of the user. Dilution under aseptic conditions should be applied.

Diluted solutions of aflibercept should be administered using infusion tubing made of the following materials:

- PVC containing DEHP
- DEHP-free PVC containing TOTM
- polypropylene
- polyethylene lined PVC
- polyurethane

The infusion sets must contain a 0.2 μM polyethersulfone inline filter. PVDF or Nylon filters should not be used.

Infusion can be conducted by gravity, with an IV infusion pump, or with a syringe pump using

administration sets made of the above materials.

The aflibercept IV dose should be infused over 1 hour. The infusion should not exceed 2 hours at ambient temperature (approximately 25° C).

Parenteral investigational drug products must be inspected visually for particulate matter and discoloration prior to administration.

APPENDIX C

Imaging Protocol for DCE-MRI and FDG-PET

See PDF attachment

APPENDIX D

Blood Pressure Log

Instructions: *Clinical Research Coordinator*

The Clinical Research Coordinator will provide one log to the subject prior to the C1D8, C1D22, and C2D8, circling for the patient the correct upcoming study visit, and entering the date for the patient that the blood pressures should be completed. The patient will take the log home, and record their blood pressure at home using either their home blood pressure monitoring device, or the device provided to them by the study. If the subject is using a home device, the device must be calibrated at the study clinic prior to use. Wrist cuffs may be used.
Prior to allowing the study subject to leave the clinic, the study subject and Clinical Research Coordinator should discuss a date and time that either the patient will call the coordinator, or the coordinator will contact the patient to receive the blood pressure recordings. The Clinical Research Coordinator will indicate the discussed plans on the log below. The patient should be asked to bring the log back with them when they return to their next study visit.

Blood Pressure Log

Instructions: *Study Patient*

Dear Study Patient,
Please sit resting for 5 minutes prior to taking your blood pressure. Sitting upright, with both feet on the floor if you are able, please follow the instructions on your blood pressure cuff to take a reading. The blood pressure measurement should be recorded in the space labeled *Blood Pressure Measurement #1* below, along with the time of the measurement in the space labeled "*Time*".
After at least one minute after the first blood pressure has been taken, a second blood pressure should be taken following the same directions. The second measurement should be recorded with the time as well in the space labeled "Blood Pressure Measurement #2".

Study Visit:	**C1D8**	**C1D22**	**C2D8**
Date:	____ / ____ / _____	____ / ____ / _____	____ / ____ / _____
Time #1:	_____ : _____ AM / PM	_____ : _____ AM / PM	_____ : _____ AM / PM
Blood Pressure Measurement #1:	_____ / _____	_____ / _____	_____ / _____
Time #2	_____ : _____ AM / PM	_____ : _____ AM / PM	_____ : _____ AM / PM
Blood Pressure Measurement #2:	_____ / _____	_____ / _____	_____ / _____

After you have completed both measurements, please contact your Clinical Research Coordinator at the time indicated above with this information, or, be sure to have this information available if the Coordinator is scheduled to contact you. Be sure to remember to bring this log with you to your next study visit.
If you have any questions about these instructions, please do not hesitate to contact your Coordinator at the contact listed below.

On the following date: _____

your Study Coordinator, _____, will collect the blood

pressure information you have recorded above. He/she

□ **Will call you** at Phone # (_____) _____ - _____

□ **Would like you to call him/her** at Phone # (_____) _____ - _____

Please have the table above completed and available for review during the phone call.