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Aflibercept

Prop INN

Antiangiogenic Agent
Vascular Endothelial Growth Factor Inhibitor

AVE-0005
VEGF $Trap_{R1R2}$
VEGF Trap

Recombinant fusion protein composed of the second Ig domain of VEGFR1 and the third Ig domain of VEGFR2 fused to the Fc region of human IgG_1

Des-432-lysine-[human vascular endothelial growth factor receptor 1-(103-204)-peptide (containing Ig-like C2-type 2 domain) fusion protein with human vascular endothelial growth factor receptor 2-(206-308)-peptide (containing Ig-like C2-type 3 domain fragment) fusion protein with human immunoglobulin G1-(227 C-terminal residues)-peptide (Fc fragment)], (211-211':214-214')-bisdisulfide dimer

CAS: 862111-32-8

EN: 303153

Abstract

The proangiogenic cytokine vascular endothelial growth factor (VEGF) is involved in several adult angiogenic pathologies, including the progression of solid tumor growth and age-related macular degeneration (AMD). Inhibition of VEGF signaling thus represents a potentially effective therapeutic strategy to block pathological angiogenesis in these indications. Several strategies to inhibit VEGF signaling are available, including direct targeting of VEGF, interference with VEGF binding to its receptors and inhibition of VEGF receptor (VEGFR) tyrosine kinase activity. A novel approach consisting of decoy VEGF receptors was designed and may optimize VEGF inhibition. From a series of recombinant VEGF decoy receptors, aflibercept (VEGF $Trap_{R1/R2}$) emerged as the most promising agent, exhibiting superior pharmacokinetics, high-affinity binding to VEGF and potent antiangiogenic effects in preclinical models of cancer and pathological ocular neovascularization. Moreover, the clinical efficacy and safety of aflibercept were demonstrated in these indications. Aflibercept is presently in clinical testing for the treatment of solid tumors, wet AMD and diabetic macular edema (DME).

Background

Aberrant angiogenesis is known to underlie several pathological conditions, including cancer and wet age-related macular degeneration (AMD), and one of the most validated approaches for inhibiting angiogenesis involves targeting the vascular endothelial growth factor (VEGF) pathway (1-7).

Strategies to inhibit VEGF signaling include agents that directly target VEGF, agents that interfere with VEGF binding to its receptors and agents that inhibit VEGF receptor (VEGFR) tyrosine kinase activity (8-17). Several VEGF inhibitors have been described and are under active development for the treatment of solid tumors and/or wet AMD, as shown in Tables I and II, respectively. The first antiangiogenic agent approved was bevacizumab (Avastin™; Genentech, Roche), a humanized monoclonal antibody that directly binds to and inhibits all isoforms of VEGF. Bevacizumab is marketed for the treatment of metastatic colorectal carcinoma in combination with intravenous 5-fluorouracil (5-FU)-based chemotherapy and registered for the treatment of non-small cell lung cancer (NSCLC) (18, 19). It also continues to undergo phase II or III testing for other types of cancers and wet AMD. Based on its success, researchers continue to search for more effective means to inhibit VEGF signaling, thereby enhancing antiangiogenic efficacy.

One novel approach to optimize VEGF inhibition comprises administration of decoy VEGF receptors. Several VEGF-blocking agents, referred to as VEGF Traps, were constructed at Regeneron, with aflibercept (VEGF $Trap_{R1/R2}$, AVE-0005) exhibiting the most favorable pharmacokinetic profile *in vivo* and emerging as the most promising development candidate. Aflibercept is a soluble recombinant decoy VEGF receptor that was created by fusing the second immunoglobulin (Ig) domain of VEGFR1 with the third Ig domain of VEGFR2 and then fusing this construct to the constant region (Fc) of human IgG_1. Aflibercept was shown to bind VEGF with an affinity that was about 800-fold higher than that of bevacizumab, and was chosen for further development as an antian-

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Table I: Antiangiogenic VEGF inhibitors under development for the treatment of cancer (from Prous Science Integrity®)

Drug	Source	Phase
Bevacizumab[1]	Roche/Genentech	R-2006
Aflibercept	Regeneron/sanofi-aventis	III
Axitinib	Pfizer	III
Pazopanib hydrochloride	GlaxoSmithKline	III
Sorafenib[2]	Bayer/Onyx Pharmaceuticals	III
Sunitinib malate[3]	Pfizer	III
Vandetanib	AstraZeneca	III
Vatalanib succinate	Novartis/Bayer Schering Pharma	III
Cediranib	AstraZeneca	II/III
BIBF-1120	Boehringer Ingelheim	II
Brivanib alaninate	Bristol-Myers Squibb	II
CP-547632	OSI Pharmaceuticals	II
Motesanib	Amgen	II
PI-88	Progen	II
Plitidepsin	PharmaMar	II
Squalamine lactate	Genaera	II
SU-14813	Pfizer	II
Telatinib	Bayer	II
TSU-68	Taiho	II
XL-647	Exelixis	II
XL-880	Exelixis	II
XL-999	Exelixis	II
AEE-788	Novartis	I/II
ABT-869	Abbott	I
CT-322	Adnexus	I
E-7080	Eisai	I
KRN-951/AV-951	Kirin Brewery/AVEO Pharmaceuticals	I
OSI-930	OSI Pharmaceuticals	I
PF-337210	Pfizer	I
PTC-299	PTC Therapeutics	I
RAF-265	Novartis	I
Tasquinimod	Active Biotech	I
TKI-258	Novartis	I
Veglin	VasGene Therapeutics	I
XL-184	Exelixis	I
XL-820	Exelixis	I
XL-844	Exelixis	I
ZK-304709	Bayer Schering Pharma	I
YN-968D1	Advenchen Laboratories	IND Filed
2C3	Peregrine Pharmaceuticals	Preclinical
GFB-204	Tigris Pharmaceuticals	Preclinical
KI-23057	Kirin Brewery	Preclinical

[1]Launched in 2004 for metastatic colorectal cancer
[2]Launched in 2005 for renal cell carcinoma
[3]Launched in 2006 for renal cell carcinoma and gastrointestinal stromal tumors

giogenic agent for the treatment of solid tumors, wet AMD and diabetic macular edema (DME) (20, 21).

Preclinical Pharmacology

Aflibercept was demonstrated to bind with high affinity to human $VEGF_{165}$ (K_D = 1 pM) and $VEGF_{121}$ (K_D approximately 1-10 pM); on the other hand, the K_D value for aflibercept binding to placental growth factor 2 (PGF2) was about 45 pM. Aflibercept also potently blocked the ability of VEGF to activate its receptor. The agent completely blocked VEGFR2 phosphorylation in cultured human umbilical vein endothelial cells (HUVEC) and inhibited VEGF-induced proliferation of NIH/3T3 cells stably transfected with a VEGFR2/TrkB chimeric receptor. The agent was also effective *in vivo* in completely blocking VEGF-induced acute hypotension in rats when administered at 25 mg/kg 24 h before or 5 mg/kg 1 or 3 days before a single bolus of $VEGF_{165}$ (10 μg); aflibercept had no effect, however, when administered 7 days before VEGF (21).

At a dose of 25 mg/kg s.c. twice weekly for 2-3 weeks, aflibercept significantly inhibited the growth of murine melanoma B16F10.9, human rhabdomyosarcoma A673 and rat glioma C6 tumors implanted s.c. in SCID mice. Analysis of tumor samples showed that treatment with aflibercept completely blocked tumor-associated angiogenesis. The efficacy of aflibercept was compared to the

Table II: Antiangiogenic VEGF inhibitors under development for the treatment of wet age-related macular degeneration (AMD) (from Prous Science Integrity®)

Drug	Source	Phase
Bevacizumab	Various	II/III
Aflibercept	Regeneron/Bayer	II
AG-13958	Pfizer	II
AGN-211745/Sirna-027	Allergan/Sirna Therapeutics (Merck & Co.)	II
Bevasiranib sodium	Acuity Pharmaceuticals	II
Vatalanib succinate	Novartis	II
TG-100801	TargeGen	I
PRS-050	Pieris	Preclinical
VEGF121/rGel	Targa Therapeutics	Preclinical

anti-VEGF monoclonal antibody DC-101 in the murine B16F10 melanoma model. Results showed that higher doses of DC-101 were required to achieve comparable inhibition of tumor growth to aflibercept. In addition, the effective DC-101 dose of 40 mg/kg accumulated, such that circulating serum levels were about 60-fold higher than an equally effective dose (3.2 mg/kg) of aflibercept (2442 ± 272 μg/ml *vs.* 40 ± 8 μg/ml). Results suggest that a much lower dose of aflibercept may be used as compared to anti-VEGF monoclonal antibodies to achieve inhibition of tumor growth (21).

Aflibercept (25 mg/kg s.c. twice weekly) given to athymic mice starting 2 days after s.c. inoculation with pancreatic cancer cells caused significant growth inhibition of T3M4, COLO 357, PANC-1 and BxPC-3 tumors within 2-4 weeks postinoculation. The overall inhibition rates were 89% (at 2 weeks), 97% (at 5 weeks), 97% (at 6 weeks) and 92% (at 6 weeks) for the respective tumor types. Tumors from aflibercept-treated animals had markedly reduced tumor microvessel density as compared to controls. Analysis of T3M4 tumors from aflibercept-treated animals revealed decreases in the expression of VEGFR1, neuropilin-1 and neuropilin-2. Aflibercept was also shown to be effective in reducing intrapancreatic PANC-1 tumor growth and metastasis in nude mice when administered 3 weeks postinoculation in an orthotopic model (22).

A study using nude mice implanted intrarenally with cultured human neuroblastoma NGP-GFP cells showed that aflibercept (500 μg i.p. biweekly) inhibited tumor growth by 81% compared to controls. Treatment with aflibercept also caused dramatic regression of co-opted vascular structure evident during the early stages of tumor growth in this model. In contrast, daily i.p. treatment with A4.6.1, a humanized anti-VEGF monoclonal antibody (100 μg) or NX-1838, which targets human $VEGF_{165}$ (250 μg), only resulted in partial inhibition of growth (approximately 50%) (23).

Aflibercept (100 or 500 μg i.p. biweekly starting 1 week postinoculation for 5 weeks) was demonstrated not only to be effective in significantly reducing tumor weight (92.7% and 99.0%, respectively, at week 6), tumor vasculature and the incidence of lung metastases in athymic mice bearing intrarenal orthotopic human Wilms' (SK-NEP-1) tumors, but also eradicated mature, pre-existing vasculature in established tumors and caused marked regression of large and well-established tumors (79.3% reduction in tumor weight by day 36) and lung metastases (approximately 80% by day 36) when administered at 500 μg s.c. biweekly starting 5 weeks postimplantation (24, 25).

Analysis of the cellular effects of aflibercept (25 mg/kg i.p.) on tumor vessels was performed in 10-12-week-old RIP-Tag2 transgenic mice bearing spontaneous pancreatic islet tumors and treated on day 0 or days 0, 3 and 6 with the agent, and in wild-type C57BL/6 mice implanted s.c. with a 1 mm^3 piece of Lewis lung carcinoma and administered the agent twice daily for 7 days starting 4-6 days postimplantation. Treatment with aflibercept resulted in dramatic and early alterations in endothelial cells, pericytes and basement membrane of vessels in both tumor types. The endothelial fenestrations evident in RIP-Tag2 tumors disappeared and vascular sprouting, patency and blood flow in some vessels were arrested in these tumors as early as 24 h postdosing. At 7 days, a reduction of over 70% in vascular density was noted in RIP-Tag2 tumors and a reduction in VEGFR2 and VEGFR3 expression was observed in surviving endothelial cells. Endothelial fenestrations were not evident in Lewis lung tumors and aflibercept induced less tumor regression in this model, suggesting that the presence of these phenomena may predict vessel sensitivity to the agent. Aflibercept treatment also caused multiple alterations in pericytes in both tumor types, leading to degeneration, although to a lesser extent compared to endothelial cells; pericytes on surviving tumor vessels acquired a more normal phenotype. Degeneration of endothelial cells following treatment caused the appearance of basement membrane ghosts (26).

Analysis of Ewing's sarcoma tumor samples taken from patients during diagnostic surgery revealed a significant correlation between VEGF expression and intratumoral microvessel density. Of the 34 samples analyzed, 18 expressed high levels of VEGF. Experiments performed *in vivo* using *nu/nu* mice injected s.c. with Ewing's sarcoma cells (RD-ES and A673) showed that treatment with aflibercept (2.5 or 25 mg/kg twice weekly starting on day 15 postinoculation and continuing for up to 4 weeks) significantly delayed the growth of RD-ES tumors, whereas only the higher dose significantly delayed A673 tumor growth (27).

Aflibercept treatment of athymic BALB/c *nu/nu* mice bearing i.p. human ovarian SK-OV-3, VEGF-overex-

pressing SK-OV-3 (25 mg/kg s.c. twice weekly starting 14 and 1 day postinoculation, respectively) or OVCAR-3 cancer xenografts (25 mg/kg s.c. twice weekly starting 14 days postimplantation and continued for 5 weeks) resulted in significant reductions in tumor burden (*e.g.*, about 56% for OVCAR-3), inhibition of ascites formation and induction of marked intratumoral vascular remodeling. Moreover, enhanced inhibition of tumor growth was observed in athymic mice inoculated i.p. with OVCAR-3 cells when aflibercept (10 mg/kg 3 times weekly for 4 weeks starting 2 weeks postinoculation) was combined with paclitaxel (10 mg/kg 3 times weekly on alternate days for 4 weeks): 97.7% *vs.* 55.7% and 54.8% for aflibercept and paclitaxel alone, respectively. In addition, combination treatment completely inhibited ascites formation as compared to the significant 96.4% and 85.5% reductions, respectively, observed with aflibercept and paclitaxel alone, and was also associated with intratumoral vascular remodeling and inhibition of metastasis. Mice treated with the combination survived significantly longer as compared to animals treated with either agent alone (129.9 ± 38.9 days *vs.* 49.4 ± 4.48 and 70.6 ± 23.5 days, respectively, postinoculation with no further treatment) (28, 29).

The growth of s.c.-implanted murine mammary (MA13/C), murine melanoma (B16), human pancreatic (BxPC-3) and human colon (HCT 116 and HT-29) tumors in mice was inhibited at both early and advanced stages following aflibercept treatment (2.5, 10, 25 and 40 mg/kg s.c. 2 or 3 times weekly for 3 weeks). Excellent therapeutic indices were obtained in all models. For example, a therapeutic index of > 16 was obtained in the B16 model, where log cell kill (*i.e.*, tumor growth delay/3.32 x tumor doubling time) values at the respective doses were 2.4, 3.9, 5 and 5.4. The agent was also particularly effective in inhibiting MA13/C tumor growth, with enhanced activity observed when given in combination with 5-FU (34-145 mg/kg i.v. once weekly). Combination therapy (40 mg/kg aflibercept + 90 mg/kg 5-FU) resulted in synergistic activity, with a cell kill of 2.7 log obtained compared to 1.4 and 1.3 log, respectively, for either agent alone (30).

Experiments performed *in vitro* using human glioblastoma cells (U-87MG) demonstrated that aflibercept can inhibit radiation (2-20 Gy)-induced VEGF secretion. Aflibercept (2.5 and 10 mg/kg every third day for up to 3 weeks starting when tumors reached 4-5 mm) also improved fractionated radiotherapy (3 x 5 Gy on days 0, 1 and 2 concurrently with aflibercept or 1 week after the start of aflibercept on days 7, 8 and 9) in athymic mice bearing s.c. U-87MG tumors. Tumor growth inhibition was slightly better in animals receiving aflibercept 1 week prior to radiation. Intratumoral vessels in treated animals were shorter and straighter and a lower vessel density was observed compared to untreated controls. Moreover, the percent of metabolically inactive tumor was higher in tumors from animals treated with the higher aflibercept dose in combination with radiation as compared to untreated tumors (31).

A study using C57BL/6 mice subjected to chemical- or intrastromal suture-induced corneal injury demonstrated the efficacy of systemically administered aflibercept (12.5 mg/kg s.c. on days 5 and 12 or 1, 2.5 or 10 mg/kg s.c. on days 0, 4, 7 and 10 postinjury, respectively) in suppressing corneal neovascularization. In the chemical injury model, it significantly delayed neovascularization for at least 30 days after the last injection compared to controls. In addition, treatment decreased corneal edema and infiltration of polymorphonuclear cells (PMNs). Further experiments using rats with suture-induced corneal injury revealed that subconjunctival administration of the agent (5, 25, 50 or 100 μg/eye on days 0, 3 and 6 postinjury) also significantly and dose-dependently inhibited corneal neovascularization (82-97%) and inflammation (32, 33).

Inhibition of angiogenesis and lymphangiogenesis and enhancement of graft survival (78% *vs.* 40%) were observed in mice bearing allogeneic (C57BL/6 to BALB/c) and syngeneic (BALB/c to BALB/c) normal-risk corneal transplantations and treated with aflibercept (12.5 mg/kg i.p. on days 0, 3, 7 and 14 post-transplantation). The agent was also effective (25 mg/kg i.p. on days 0, 4, 7 and 14 post-transplantation) in improving graft survival in mice bearing high-risk transplants (*i.e.*, intrastromal corneal placement of sutures for 6 weeks to induce vascularization followed by penetrating allogeneic keratoplasty 3 weeks later). At day 14, graft survival rates were 46% *vs.* 10% in controls, and whereas by day 21 and week 8 all control grafts were rejected, survival rates of 31% and 23%, respectively, were obtained in aflibercept-treated animals (34, 35).

Aflibercept given s.c. (25 mg/kg 1 day before and on days 2, 5, 8 and 11 postinjury) or as a single intravitreal (i.v.t.) injection (4.92 μg immediately after laser injury) significantly suppressed choroidal neovascularization (CNV) in mice with laser-ruptured Bruch's membrane. Experiments were also performed using transgenic Rho/VEGF mice who express VEGF in photoreceptors, which results in extensive subretinal neovascularization by postnatal day 21. Aflibercept (25 mg/kg s.c. on postnatal days 7, 10, 13, 16 and 19) significantly reduced the total average area of neovascularization per retina. VEGF is known to cause breakdown of the blood-retinal barrier (BRB), such as is seen in diabetes, and aflibercept (25 mg/kg s.c.) significantly decreased VEGF-induced BRB breakdown in C57BL/6 mice injected i.v.t. with recombinant VEGF and treated with the agent the day after VEGF injection, and in double transgenic rho/rtTA-TRE/VEGF mice with doxycycline-inducible photoreceptor expression of VEGF and treated with the agent 1 day before and 1 day after doxycycline injection (36).

Treatment of rats injected with Matrigel into the subretinal space with aflibercept (12.5 mg/kg s.c. on days 2 and 6 or days 10, 13 and 16) completely prevented choroidal neovascularization (CNV; CNV index = 0 *vs.* 211,353 ± 146,000) and caused significant regression of existing CNV (CNV index = 37,097 ± 45,868 *vs.* 244,500 ± 225,208), such that levels were significantly less than those of untreated controls at day 10 (37, 38).

CNV was completely or almost completely prevented by treatment with aflibercept (3 or 10 mg/kg i.v. weekly or 50, 250 or 500 μg/eye i.v.t. biweekly starting 1 week before laser injury) in adult cynomolgus monkeys with laser-induced retinal lesions. In addition, treatment with a single i.v.t. dose of aflibercept (500 μg at 2 weeks following laser injury) induced rapid reduction and regression of established active CNV. The grade 4 lesion frequency rate dropped from 44% to 0% by day 14 in animals treated with the agent. Aflibercept was considered to be well tolerated, since weekly administration for up to 13 weeks at doses of 50, 250 and 500 μg/eye i.v.t. was only associated with mild, transient ocular inflammation in the anterior chamber and vitreous (39, 40).

Two studies using a mouse model of oxygen-induced retinopathy characterized by pathological neovascularization showed that aflibercept given i.p. (25 mg/kg every other day on postnatal days 13-17, starting 12-24 h after mice returned to room air from hypoxic conditions) and i.v.t. (starting 2 days after return to room air from hypoxia) almost completely prevented the vascular abnormalities (*e.g.*, vascular tufts penetrating the inner limiting membrane, chaotic sprouting of vessels on the retinal surface) seen in untreated mice. Systemic treatment with the agent i.p. did not completely block retinal angiogenesis, since appropriate, normal regrowth of vessels in superficial, intermediate and deep layers was observed at postnatal day 19 (41, 42).

Aflibercept administered s.c. and i.v.t. inhibited retinal vascular permeability and leukostasis in mice with streptozotocin (STZ)-induced diabetes. S.c. administration of the agent (12.5 mg/kg starting 2-4 weeks after diabetes induction) significantly attenuated dye extravasation and significantly inhibited leukostasis compared to untreated mice at both 2 and 4 weeks after the induction of diabetes. Similarly, aflibercept administered i.v.t. (3 μg) significantly decreased dye extravasation on days 2 and 7 compared to untreated diabetic controls. Results suggest that aflibercept may be effective as a treatment for diabetic retinopathy and DME (43, 44).

Results from preclinical studies conducted in nonhuman primates suggest that aflibercept can inhibit ovarian function and may therefore prove effective in the treatment of polycystic ovary syndrome (POS), ovarian hyperstimulation syndrome and endometriosis, and in regulating fertility.

The effects of aflibercept (25 mg/kg s.c. on days 0, 2, 4, 6 and 8 of the follicular phase) on thecal angiogenesis and follicular development were examined in marmosets. The treatment significantly suppressed thecal angiogenesis, resulting in a significantly decreased thecal thickness. Reductions in granulosa cell proliferation and follicular diameter, impairment of antral follicular and ovulatory follicular development and significant increases in atresia were also observed with treatment. In addition, while aflibercept increased VEGF expression in the granulosa and theca of secondary and tertiary follicles, VEGFR1 and VEGFR2 expression was downregulated in thecal endothelial cells (45).

Results from two studies demonstrated that single i.v. injections of aflibercept can reversibly suppress ovarian function in the macaque. When administered during the midfollicular (0.25, 1 or 4 mg/kg) or late follicular (1 mg/kg) stages, aflibercept rapidly suppressed serum estradiol and inhibin B levels and increased serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels. In addition, ovulation and the formation of a functional corpus luteum were not evident, consistent with the increased concentrations of progesterone observed. Ovarian activity returned to normal when plasma free aflibercept levels decreased to < 1 mg/l. Animals treated with the agent during the midfollicular phase exhibited dose-dependent delays in ovulation (23 ± 0.7, 30 ± 1.4 and 43 ± 0.8 days postdosing for the respective doses *vs.* 7.2 ± 0.4 days in controls). A single i.v. injection of aflibercept was also effective in suppressing ovarian function at all stages of the luteal phase. Treatment during the early luteal phase (0.25, 1 or 4 mg/kg i.v.) resulted in significant suppression of serum progesterone and estradiol levels and increases in LH and FSH; no significant changes in inhibin A were detected with treatment. Reduced progesterone levels were sustained throughout the luteal phase with the two highest doses; reductions in progesterone with the lowest dose were transient, with menstruation occurring at the expected time. Aflibercept administered during the midluteal phase (1 mg/kg i.v.) also significantly suppressed progesterone levels (46, 47).

Pharmacokinetics and Metabolism

Preliminary pharmacokinetic studies in mice demonstrated a C_{max} and AUC for aflibercept of 16 μg/ml and 36.28 μg.days/ml following an s.c. injection of 4 mg/kg (21).

The pharmacokinetics and ocular distribution of aflibercept (500 μg i.v.t.) were determined in rabbits. Maximum vitreal concentrations of free aflibercept were about 500 μg/ml at 0.25-6 h postinjection and the agent was eliminated from the vitreous with a $t_{1/2}$ of about 4.5 days. Aflibercept was also detected in the retina and choroid, where it was eliminated with a similar half-life. At 10 days postinjection, peak plasma total aflibercept levels of 1.6 μg/ml were measured, and at week 4, vitreal free aflibercept levels continued to be 10-fold higher than those of excess bound aflibercept. These results suggest that ocular production of VEGF would be completely suppressed for more than 6 weeks postinjection (48).

Safety

Preliminary results from an ongoing phase I trial suggested good safety and tolerability for aflibercept (i.v. every 2 weeks) in combination with FOLFOX4 (5-FU/leucovorin/oxaliplatin) in patients with advanced solid tumors. To date, 6 patients have received 19 cycles of combination treatment including 2 doses of aflibercept (2 and 4 mg/kg). Of these patients, 3 experienced reversible

and manageable grade 3 hypertension (n=2) and neutropenia (n=2). No grade 4 adverse events, dose-limiting toxicities (DLTs) or the development of anti-aflibercept antibodies were reported. Mean free aflibercept clearance was 17.1 mg/kg/day (49).

Clinical Studies

An open-label, dose-escalation phase I trial conducted in 14 patients with relapsed or refractory solid tumors or lymphoma examined the safety of aflibercept (25, 50, 100 and 200 μg/kg s.c. as a single dose followed 4 weeks later by 6 weekly doses). An apparent elimination $t_{1/2}$ of approximately 17 days was calculated. The agent appeared to be well tolerated, with no grade 3 or 4 adverse events observed and no anti-aflibercept antibodies detected. Grade 1 and 2 adverse events reported included reversible proteinuria, fatigue and constipation. One patient each with renal cell carcinoma and colon cancer had stable disease (50).

A phase I trial conducted in 38 patients with relapsed or refractory solid tumors demonstrated the safety and tolerability of s.c. aflibercept administered first as one or two doses followed 4 weeks later by 6 weekly or twice weekly doses (0.025, 0.05, 0.1, 0.2 and 0.4 mg/kg weekly and 0.8 mg/kg weekly or twice weekly). The maximum tolerated dose (MTD) was not reached in this study. Possible aflibercept-related grade 3 and 4 adverse events reported included hypertension (n=2), proteinuria (n=1), afebrile neutropenia (n=1) and pulmonary embolism (n=1); with the exception of hypertension, these events were not dose-related. No anti-aflibercept antibodies were detected. Patients receiving 0.8 mg/kg weekly and twice weekly displayed plasma levels of the agent similar to those associated with antitumor activity in preclinical models. Of the 35 evaluable patients, no objective partial or complete responses were seen. However, 17 patients, including 8 who received 0.8 mg/kg weekly or twice weekly, had stable disease for at least 10 weeks. These patients subsequently entered a long-term extension study (51).

Another phase I trial in 16 patients with relapsed or refractory solid tumors reported manageable safety and tolerability for s.c. aflibercept (0.3, 1 and 2 mg/kg on days 1 and 15). Examination of aflibercept plasma concentrations showed that peak levels increased approximately proportionately with doses. The MTD was not reached. The most common adverse events reported among the 10 patients receiving 0.3 and 1 mg/kg were fatigue (n=9), pain (n=4) and constipation (n=4). Two patients in these groups developed grade 3 dose-limiting arthralgia/fatigue/voice changes and non-dose-limiting alanine aminotransaminase (ALT) elevations which were related to aflibercept treatment. No anti-aflibercept antibodies were detected. Of the 15 evaluable patients, 1 patient (1 mg/kg) with metastatic renal cell carcinoma had stable disease for over 6 months and a minor response was observed in another patient (1 mg/kg) with advanced uterine leiomyosarcoma (52).

The safety and pharmacodynamics of i.v. aflibercept (0.3, 1, 2, 3 and 4 mg/kg every 2 weeks) were investigated in a phase I study in 30 patients with advanced solid tumors. Results from 27 patients showed that tumor lesions at 24 h postdosing from those patients receiving doses of 2 mg/kg or higher exhibited dose-related reductions in tumor vascular perfusion and permeability. Maximum bound aflibercept concentrations were achieved with doses of 2 mg/kg or greater; free aflibercept levels were higher than bound aflibercept levels throughout the 2-week dosing intervals at these higher doses (53).

The safety and efficacy of i.v. aflibercept (0.3, 1 or 3 mg/kg as a single dose followed by 4 weeks of observation and 3 subsequent doses 2 weeks apart) were examined in a multicenter, randomized, placebo-controlled phase I trial in 25 patients with neovascular AMD. The MTD was concluded to be 1 mg/kg, since 2 of 5 patients receiving 3 mg/kg developed DLTs of grade 4 hypertension (n=1) and grade 2 proteinuria (n=1); all other patients at this dose level were subsequently withdrawn. The majority of other drug-related adverse events reported were mild to moderate. A significant reduction in excess retinal thickness and volume was observed in the two higher dose groups at day 15 (mean percent decrease = 10%, 66% and 60% at 0.3, 1 and 3 mg/kg, respectively, *vs.* 12% on placebo) and in the 1 mg/kg dose group at day 71 (mean percent change = –63.3% *vs.* –5.6% and +47.1% on placebo and 0.3 mg/kg, respectively). No significant changes in visual acuity were observed (54).

A phase I trial conducted in 21 patients with neovascular AMD demonstrated the safety, tolerability and efficacy of a single i.v.t. injection of aflibercept (0.05-4 mg). Patients were followed for 12 weeks. The agent was well tolerated, with no serious adverse events or ocular inflammation reported. Pooled data from all patients revealed rapid and dramatic decreases in excess foveal thickness that were sustained for at least 6 weeks postinjection. In addition, visual acuity was improved (55, 56).

The safety, tolerability and efficacy of i.v. aflibercept (0.3 mg/kg x 4) were investigated in a randomized, placebo-controlled study in 9 patients with DME (foveal thickness = 250 μm or greater; best-corrected visual acuity = 20/40 or less). One patient on placebo did not complete the study. The agent was well tolerated and not associated with hypertension, proteinuria, ocular adverse events or anti-aflibercept antibodies. Three patients receiving aflibercept had an improvement in best-corrected visual acuity of 2 lines and a reduction in excess foveal thickness and leakage. The decreases in excess foveal thickness occurred following each dose of the agent and were sustained, with mean change in foveal thickness of –42% *vs.* +32% on placebo (57).

Aflibercept continues to undergo phase II/III development for advanced ovarian cancer, phase II development for recurrent or metastatic gynecological soft tissue sarcoma, metastatic breast cancer, metastatic or unresectable renal cell cancer, temozolomide-resistant malignant gliomas and advanced or metastatic platinum- and

erlotinib-resistant NSCLC, and phase I testing for relapsed or refractory advanced solid tumors and non-Hodgkin's lymphoma (NHL). Additional phase I and II trials are under way for the treatment of wet AMD and DME (58-71).

Sources

Regeneron Pharmaceuticals, Inc. (US); developed in collaboration with sanofi-aventis for oncology and with Bayer for ocular applications.

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