

INHIBITION OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) PATHWAY FOR THE TREATMENT OF CANCER

HIGHLIGHTS

- Vascular endothelial growth factor (VEGF) inhibition for the treatment of cancer is reviewed. Items in blue bold link to New Medicine's Oncology KnowledgeBASE ([nm/OK](#)) so subscribers may access the complete regularly updated [records](#) of all agents/targets/developers mentioned in this document.
- Bevacizumab (Avastin; Genentech/Roche), a monoclonal antibody (MAb), is the only currently approved VEGF inhibitor that selectively targets VEGF-A, while 3 other approved oral drugs, pazopanib (Votrient; GlaxoSmithKline), sunitinib (Sutent; Pfizer) and sorafenib (Nexavar; Onyx Pharmaceuticals) are orally available multi-targeted receptor tyrosine kinase inhibitors that include VEGF receptors among their targets.
- Worldwide sales of Avastin were \$6.2 billion in 2010. The recent FDA decision to rescind approval for advanced breast cancer in combination with standard treatments threatens up to \$1 billion in sales.
- Potential adverse effects (AE) of Avastin have come under increased scrutiny and may, along with unfavorable cost benefit analyses, pose challenges to its growth potential and continued widespread use.
- The clinical development program for Avastin provides a roadmap of the complexities and challenges of developing a drug with such wide ranging potential in cancer therapy; at least 1,000 clinical trials have been initiated, with about half of them ongoing.
- Avastin is also under investigation in combination regimens in about 78 trials with approximately 38 novel agents, 33 of which target more than 37 unique molecular moieties (Exhibit 1).
- Cancer drug developers are beginning to conduct trials with combinations of novel targeted agents. With over 600 distinct targeted agents having entered/completed clinical trials the possibilities are staggering.
- Increased focus on targeted cancer drugs and the high cost of treatment associated with their use underscore the pressing need for new methods (e.g., predictive biomarkers) to select specific drugs for specific patients.
- Most of the VEGF pathway inhibitors in clinical development are multitargeted, addressing several VEGF family members plus other targets (Exhibit 2); the most common targets are c-Kit, FLT3, and Met.
- In addition to the VEGF family, many other molecules play a role in angiogenesis such as Tie2 receptor and its ligand angiopoietin, neuropilin 1, metalloproteinase, heparinase, integrins, and ephrins, among others.

THE VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) FAMILY

Vascular endothelial growth factors (VEGF) represent a family of secreted polypeptides comprising seven members, VEGF-A, VEGF-B, placenta growth factor (PlGF), VEGF-C, VEGF-D, viral VEGF (also known as VEGF-E), and snake venom VEGF (also known as VEGF-F). **VEGF-A**, the first described member of this family, plays important roles in mammalian vascular development and in diseases involving abnormal growth of blood vessels. Other VEGF are also involved in the development of lymphatic vessels and disease-related angiogenesis. Members of the VEGF family bind cellular receptor tyrosine kinases **VEGFR1** (Flt-1), **VEGFR2** (KDR) and **VEGFR3** (Flt4) with high but variable affinities and selectivities.

Angiogenesis, from the Greek for birth of vessels, supports tumor growth and the establishment of metastases by providing a conduit for nourishment. Ideally, angiogenesis might be inhibited as a preventive strategy before tumors are established because angiogenesis is not vitally important in adults, and its inhibition might be accomplished without causing serious side effects. Current treatment with angiogenesis inhibitors, however, is mostly in patients with advanced disease.

Bevacizumab (Avastin; Genentech/Roche)

Avastin is the only currently approved VEGF inhibitor that selectively targets VEGFA. At least three other approved drugs, all orally available, target VEGF receptors. **Pazopanib** (Votrient; GlaxoSmithKline) is a multitargeted VEGFR inhibitor against VEGFR1, VEGFR2, and VEGFR3. Among other approved drugs, **sunitinib** (Sutent; Pfizer), a multitargeted tyrosine kinase inhibitor, targets VEGFR2 along with platelet-derived growth factor receptor beta (PDGFRB), c-Kit, fms-like tyrosine kinase 3 (FLT3) and ret proto-oncogene (RET). Similarly, **sorafenib** (Nexavar; Onyx Pharmaceuticals), another multitargeted tyrosine kinase inhibitor, acts against Raf, Braf, VEGFR2, VEGFR3, PDGFRB, FLT3, c-Kit and RET.

Regulatory Status of Avastin

The development and commercialization of Avastin is a blueprint of a successful clinical and marketing program in oncology. Although not par-

ticularly effective in any of its approved indications, the drug benefited from a well conducted clinical program at a time when molecularly targeted drugs were emerging as the antidote for the shortcomings of cytotoxic chemotherapeutics. However, Avastin was ineffective as monotherapy, providing the first signal that molecularly targeted approaches in cancer might not meet expectations. Avastin was approved in the USA in 2004 for first line treatment of advanced, metastatic colorectal cancer (CRC), in combination with a 5-FU-based chemotherapy regimen and went on to be approved for advanced colorectal, breast, lung and kidney cancer and relapsed glioblastoma. Eventually, Avastin became one of the most successful cancer drugs ever developed with global sales of \$6,210.5 million in fiscal 2010. Although USA sales were flat in 2010, sales in Japan were up 51% to \$600.8 million, mostly driven by continued strong uptake in CRC and non-small cell lung cancer (nscl) and sales in Latin America grew by 42%. In the third quarter of 2010, Avastin was introduced in China for first line treatment of metastatic CRC.

In December 2010, Avastin was faced with several challenges to its role in the treatment of advanced HER2-negative breast cancer in combination with standard treatments. In an unusual move, the FDA announced that it would seek to revoke the accelerated approval of Avastin for the treatment of advanced (metastatic) breast cancer (*more*), which could result in a loss in annual sales of \$1 billion. The FDA decision followed the recommendation of the its Oncologic Drugs Advisory Committee (ODAC), which voted 12-1 to remove the breast cancer indication from the Avastin label. In addition, European authorities recommended restricting the drug in breast cancer to use only in combination with paclitaxel, the regimen most often used in Europe, but not with docetaxel or capecitabine.

These decisions are based on the failure of Avastin to meaningfully extend survival in **four** separate trials in patients with breast cancer. Avastin was granted accelerated approval in 2008, based on results from a phase III clinical trial ([protocol ID: ECOG-2100; CAN-NCIC-E2100; NCCTG-E2100; NSABP-E2100; NCT00028990](#)) that treated locally recurrent or metastatic breast cancer in combination with paclitaxel in the first line setting. According to the original trial that sup-

Exhibit 1

Targets of Novel Agents* in Clinical Trials in Combination with Avastin

- Akt (protein kinase B, PKB)
- Alphanuβ3 (alpha-nu-β3, alpha-nu-β-3, anb3, alphavβ3, alpha-v-β3, avb3) integrin
- Alphanuβ5 (alpha-nu-β5, alpha-nu-β-5, anb5, alphavβ5, alpha-v-β5, avb5) integrin
- Angiopoietin-1
- Angiopoietin-2
- Aurora kinase
- c-Kit
- Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4, CTLA-4)
- EGF-like-domain, multiple 7 (EGFL7)
- Epidermal growth factor (EGF) receptor (EGFr, ErbB-1, ErbB1, HEr-1, HEr1)
- Fibroblast growth factor receptor (FGFr)
- Hepatocyte growth factor/scatter factor (HGF/SF or SF/HGF)
- HEr2/neu (HEr-2/neu, ErbB-2, c-erbB-2, ErbB2)
- Histone deacetylase (HDAC)
- hTR mRNA
- Insulin-like growth factor 1 receptor (IGF1r, IGF-1r, IGFIr, IGF-Ir)
- Integrin alpha 2 (ITGA2)
- Mammalian target of rapamycin (mTOR)
- Met [hepatocyte growth factor receptor (HGFr)/c-Met]
- Neuropilin 1 (NRP1, NRP-1)
- Phosphatidylinositol 3-kinase (PI3K)
- Phosphatidylinositol 3-kinase, class 2, alpha polypeptide (PIK3C2A), p110 alpha
- Platelet-derived growth factor receptor alpha (PDGFrA)
- Platelet-derived growth factor receptor beta (PDGFrB, PDGFr)
- Poly (ADP-ribose) polymerase (PARP)
- Protein kinase C (PKC) β (PKC-B)
- Ret proto-oncogene (RET), glial cell-line derived neurotrophic factor receptor (GDNFr)
- Src-kinase family (SKF)
- Telomerase
- Tie-2 (Tie2 or Tek)
- Toll-like receptor 9 (TLr9), CD289
- Tumor necrosis factor-related apoptosis-inducing ligand receptor 2 (TRAILr2), death receptor 5 (Dr5)
- Tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-r1), death receptor 4 (Dr4)
- Vascular endothelial growth factor (VEGF) receptor 1 (VEGFr1, Flt-1)
- Vascular endothelial growth factor (VEGF) receptor 2 (VEGFr2, VEGFr-2)/(FLK1, Flk-1)/KDR
- Vascular endothelial growth factor (VEGF) receptor 3 (VEGFr3, VEGFr-3) / (FLT4, Flt-4) / PCL

*Some of the novel agents are multitargeted

Source: New Medicine's Oncology KnowledgeBASE (nm/ok), March 19, 2011

ported approval, adding Avastin to paclitaxel increased the median progression-free survival (PFS) by 5½ months. According to results from recent trials, the AVADO trial ([protocol ID: BO17708; NCT00333775](http://clinicaltrials.gov/ct2/show/study?term=BO17708&rank=1)) and the RIBBON-1 trial ([protocol ID: AVF3694g; NCT00262067](http://clinicaltrials.gov/ct2/show/study?term=AVF3694g&rank=1)), the median PFS was between <1 month to 2.9 months, depending on the treatment group. Also, patients in the Avastin arm experienced more side effects than those treated with chemotherapy, and there was no survival benefit in favor of Avastin in any

of the trials. According to results from a phase III clinical trial ([GENENTECH-AVF2119g; GUMC-00299; MSKCC-01008; UAB-0028; UAB-F001009003; NCT00109239](http://clinicaltrials.gov/ct2/show/study?term=GENENTECH-AVF2119g&rank=1)) combining Avastin with capecitabine versus capecitabine alone in patients with refractory metastatic breast cancer, adding Avastin significantly enhanced response rates but did not result in a longer PFS (HR=0.98). Overall survival and time to deterioration in QoL were comparable in both treatment groups ([Miller KD, et al., JCO, 1 Feb 2005;23\(4\):792-9](http://www.jco.org/jco/article/23/4/792-9)).

Genentech/Roche requested an FDA hearing on the use of Avastin in metastatic breast cancer, and in February the FDA scheduled a hearing for June 28-29, 2011, in front of the same ODAC members who previously rejected the drug. In its request, Genentech argued that the committee did not include enough breast cancer specialists and, therefore, could not properly evaluate the role of Avastin in this indication. The FDA has expressed willingness to review new clinical data that indicate the drug is effective in particular subgroups of women. During the first day of the hearing, witnesses chosen by Genentech and the FDA's drug division will be given equal time. ODAC members will make their recommendations on the second day. The final decision will be made by FDA Commissioner Margaret Hamburg, MD. Until this procedure is concluded, Avastin will remain approved in combination with paclitaxel for the first line treatment of HER2-negative metastatic breast cancer. If the FDA revokes the indication, oncologists could prescribe Avastin as an off-label drug for metastatic breast cancer. Off-label use of approved cancer drugs is routinely covered by many payors, especially if the use is supported by recommendations in [NCCN Clinical Practice Guidelines in Oncology](#) and NCCN Drugs & Biologics Compendium.

Side Effects of Avastin

The side effects profile of Avastin monotherapy is well established. The most common adverse effects (AE) that occur in >10% of treated patients and at least twice the rate reported in control arms of clinical trials are epistaxis (nosebleed), headache, hypertension, rhinitis, proteinuria, taste alteration, dry skin, rectal hemorrhage, lacrimation disorder, back pain and exfoliative dermatitis (Prescribing Information, Feb. 2011 revision). However, Avastin is used almost exclusively in cancer regimens in combination with chemotherapeutic and/or other targeted agents, which complicates its safety evaluation. For instance, in July 2008, Genentech informed healthcare professionals of reports of several cases of microangiopathic hemolytic anemia (MAHA) in patients with solid tumors treated with Avastin in combination with Sutent, an SAE not encountered in previous clinical settings..

Treatment with Avastin in combination with other drug therapies is associated with AE and serious AE (SAE) that range from mild to life threaten-

ing and may cause death. Across all studies, AE were the reason for discontinuation of Avastin in 8.4-21% of all patients. FDA labeling for Avastin includes a Boxed Warning for SAE, including gastrointestinal perforation (in up to 2.4% of treated patients), surgery and wound healing complications, and hemorrhage. Other potential SAE include non-gastrointestinal fistula formation, arterial thromboembolic events, hypertensive crisis, reversible posterior leukoencephalopathy syndrome, proteinuria and congestive heart failure (CHF).

The AE profile of Avastin contributed to the FDA decision to withdraw the accelerated approval in the first line treatment of metastatic breast cancer. The FDA concluded that, given its serious and life-threatening side effects, the risk-to-benefit ratio of Avastin is insufficient to justify its use in this setting.

In a related decision, UK's NICE declined to recommend Avastin in any combination for the first line treatment of advanced breast cancer and gave the drug a poor assessment ([more](#)). In addition, in December 2010, NICE declined to recommend the use of Avastin in combination with oxaliplatin and either fluorouracil plus folinic acid or capecitabine for the treatment of metastatic CRC ([more](#)).

The Boxed Warning and potential SAE associated with Avastin treatment underscore the frequent and potentially serious side effects of many of the molecularly targeted agents produced by biotechnology R&D. Although targeted drugs are much more selective than cytotoxic chemotherapeutics, they inhibit (or stimulate) targeted pathways that may play multiple roles in normal processes, in addition to their intended interference in malignant processes. Furthermore, virtually all drugs, even those with high specificity for their target, exert unintended effects.

In a number of recently published reports, investigators identified some of the more serious complications associated with Avastin-based combination regimens by a systematic review and meta-analysis of published randomized, controlled trials involving large patient populations.

According to researchers at Stony Brook University Medical Center, Avastin increases the risk

for severe proteinuria and renal damage. Based on data from 16 trials involving 12,268 patients with a variety of tumors, the incidence of high grade (Grade 3/4) proteinuria with Avastin was 2.2%. Compared with chemotherapy alone, Avastin combined with chemotherapy significantly increased the risk for high grade proteinuria (RR=4.79) and nephrotic syndrome (RR=7.78); higher doses of Avastin were associated with increased risk of proteinuria. Regarding tumor type, renal cell carcinoma (RCC) was associated with the highest risk for a cumulative incidence of 10.2%. There was no significant difference between platinum and non-platinum-based concurrent chemotherapy with regard to risk for high grade proteinuria ($p=0.39$) ([Wu S, et al., J Am Soc Nephrol, Aug 2010;21\(8\):1381-9](#)).

In the same population, the incidence of all grade hypertension in patients treated with Avastin was 23.6%; 7.9% was high grade (Grade 3/4) for an RR of 5.28 ($p<0.001$) in comparison with controls. Also, although not statistically significant, there was a trend suggesting that Avastin may increase the risk of hypertensive crisis (Grade 4) with an RR of 3.16. The increased risk of high grade hypertension was observed in patients treated with Avastin at 2.5 mg/kg/week (RR=4.78) and also at 5 mg/kg/week (RR=5.39). The risk of high grade hypertension may vary with tumor types, ranging from an RR of 2.49 in patients with mesothelioma to an RR of 14.80 in patients with breast cancer ([Ranpura V, et al., Am J Hypertens, May 2010;23\(5\):460-8](#)).

Exhibit 2

Targets of Multitargeted VEGF Pathway Inhibitors in Clinical Trials

- Aurora A (Aurora-2)
- Axl
- Bcr-Abl
- Bcr-Abl (T315I mutation)
- BRaf (B-Raf)
- c-Kit
- Colony stimulating factor 1 receptor (CSF1r)
- CRAF (C-Raf, RAF1)
- Ephrin B4 (EphB4)
- Epidermal growth factor (EGF) receptor (EGFr, ErbB-1, ErbB1, HEr-1, HEr1)
- Fibroblast growth factor 2 (FGF-2, FGF2, FGF2, BFGF)
- Fibroblast growth factor receptor (FGFr)
- Fibroblast growth factor receptor 3 (FGFr3)
- Fms-like tyrosine kinase 3 (FLT3, FLT-3)
- HEr2/neu (HEr-2/neu, ErbB-2, c-erbB-2, ErbB2)
- HEr4 (ErbB4)
- Kinesin-like spindle protein (KSP), Eg5
- Lymphocyte-specific protein tyrosine kinase (Lck)
- Macrophage stimulating 1 receptor (MST1r); Ron
- Met [hepatocyte growth factor receptor (HGFr)/c-Met]
- P38 mitogen-activated protein kinase (p38 MAPK)
- Placental growth factor (PGF)
- Platelet-derived growth factor receptor alpha (PDGFrA)
- Platelet-derived growth factor receptor beta (PDGFrB, PDGFr)
- Protein kinase C (PKC)
- RAF
- Ret proto-oncogene (RET), glial cell-line derived neurotrophic factor receptor (GDNFr)
- Src-kinase family (SKF)
- Tie-2 (Tie2 or Tek)
- Vascular endothelial growth factor (VEGF) receptor 1 (VEGFr1, Flt-1)
- Vascular endothelial growth factor (VEGF) receptor 2 (VEGFr2, VEGFr-2) / (FLK1, Flk-1) / KDR
- Vascular endothelial growth factor (VEGF) receptor 3 (VEGFr3, VEGFr-3) / (FLT4, Flt-4) / PCL
- Vascular endothelial growth factor (VEGF, VEGF-A, VEGFA)

Source: New Medicine's Oncology KnowledgeBASE ([nm/ok](#)), March 19, 2011

The same researchers report that treatment with Avastin may significantly increase the risk of cardiac ischemic events. In the same population, the incidences of all grade and high grade arterial thromboembolic events in patients treated with Avastin were 3.3% and 2.0%, respectively for an RR of 1.44 ($p=0.013$) compared with controls, irrespective of dose level. In addition, significantly increased risks were observed in patients with RCC (RR=3.72; $p=0.029$) and CRC (RR=1.89; $p=0.001$). Notably, the risk of high grade cardiac ischemia with Avastin was higher than in controls with an RR

of 2.14 ($p=0.021$). However, the risk of ischemic stroke with Avastin was not significantly different from controls ([Ranpura V, et al., Acta Oncol, Apr 2010;49\(3\):287-97](#)).

Based on 12,294 patients with a variety of solid tumors participating in 17 randomized controlled trials, the risk of GI perforation in patients treated with Avastin was 0.9%, for an RR of 2.14 ($p=0.011$) and a mortality of 21.7%. Risk varied with dose and tumor type. The RR for patients treated with Avastin at 5 mg/kg and 2.5 mg/kg per week were 2.67 and 1.61, respectively. Higher risks were observed in patients with CRC (RR=3.10) and RCC (RR=5.67) ([Hapani S, et al., Lancet Oncol, Jun 2009;10\(6\):559-68](#)).

Avastin is also associated with an elevated risk of venous thromboembolism. Among 7,956 patients with a variety of advanced solid tumors from 15 randomized controlled trials, the risk of venous thromboembolism was significantly increased in patients treated with Avastin with an RR of 1.33 ($p<0.001$) compared with controls. The incidence of all grade and high grade venous thromboembolism in patients treated with Avastin were 11.9% and 6.3%, respectively. The risk was significantly increased for both all grade and high grade venous thromboembolism and varied according to type of cancer; it was 19.1% in patients with CRC, 14.9% in patients with nsclc, 7.3% in patients with breast cancer, and 3% in patients with RCC ([Nalluri SR, et al., JAMA, 19 Nov 2008;300\(19\):2277-85](#)).

It was also recently reported that adding Avastin to standard chemotherapy significantly increases the risk of death. According to a meta-analysis involving 10,217 patients with a variety of advanced solid tumors treated in 16 randomized clinical trials, the overall incidence of fatal AE with Avastin was 2.5% versus 1.7% with chemotherapy alone for an RR of 1.46 ($p=0.01$). This association varied significantly with chemotherapeutic agents ($p=0.045$) but not with tumor types ($p=0.13$) or Avastin doses ($p=0.16$). Avastin was associated with an increased risk of fatalities (3.3% versus 1.0%) in patients treated with taxanes or platinum agents (RR=3.49), but not when used in conjunction with other agents. The most common causes of fatalities were hemorrhage (23.5%), neutropenia (12.2%) and GI tract perforation (7.1%). The highest risk of death

was associated with taxanes, which represent the standard of treatment in advanced breast cancer ([Ranpura V, et al., JAMA, 2 Feb 2011;305\(5\):487-94](#)). These results, obtained from patients in clinical trials, are probably worse in the normal clinical setting.

Avastin has also been associated with the risk of congestive heart failure (CHF) in patients with metastatic breast cancer. According to a meta-analysis conducted by investigators at Dana-Farber Cancer Institute Harvard Medical School (Boston, MA) and the University Hospital del Mar Institut Municipal d'Investigació Mèdica (Barcelona, Spain), among 3,784 patients enrolled in randomized clinical trials, the overall incidence for high grade CHF in Avastin and placebo-treated patients were 1.6% and 0.4%, respectively. The relative risk of CHF in Avastin-treated patients was 4.74 ($p=.001$) compared to placebo. In subgroup analyses, there were no significant differences in CHF incidence or risk between patients treated with a low dose (2.5 mg/kg) versus a high dose (5 mg/kg) of Avastin or among patients treated with different chemotherapy regimens ([Choueiri TK, et al., JCO, 4 Jan 2011; epub ahead of print](#)). The increase in CHF risk, reported for the first time, may be caused in part by the same mechanism of action that inhibits angiogenesis. Although beneficial in preventing the formation of blood vessels that feed tumors, inhibition of VEGF may have a negative effect on the hearts of women with breast cancer that may have been compromised by previous cancer treatments.

Clinical Development of Avastin

Avastin is one of the most extensively evaluated anticancer drugs in the clinic because of its novel mechanism of action (i.e., inhibition of angiogenesis), which is considered to be universally applicable to the treatment of cancer. At least 1,000 clinical trials with Avastin have been initiated worldwide, and about half of them are currently ongoing. The clinical development program with Avastin represents a roadmap of the complexities of evaluating a cancer drug with such a wide ranging potential. The large number of potential clinical indications, the lack of meaningful patient selection options, and the many potential combinations with approved and novel agents, create an incredibly broad opportunity for success or failure. The clini-

cal development of Avastin addresses nearly every malignancy/clinical indication and nearly every potential combination with other approved drugs. The Avastin development program illustrates the massive commercially driven drug evaluation program that is feasible when annual revenues from approved indications are exceptionally high and there exists a unique opportunity of extensive off-label use of the drug based on marginally favorable clinical trial results that would not support full marketing approval of the drug for a given indication. In breast cancer alone, where reported results have been marginal at best, about 90 clinical trials, including 30 phase III trials, are currently ongoing of the approximately 182 such trials that were initiated to date with Avastin in combination with various approved drugs in numerous breast cancer indications.

A cancer indication in late stage clinical development with Avastin is ovarian cancer. About 69 clinical trials have been initiated with Avastin in combination with various anticancer agents, with 36 currently ongoing; 8 of these are phase III trials. In February 2011, Roche reported that OCEANS, a phase III clinical trial ([protocol ID: AVF4095g; NCT00434642](#)) evaluating Avastin in combination with carboplatin and gemcitabine followed by a maintenance regimen of Avastin monotherapy until disease progression in women with recurrent, platinum-sensitive ovarian cancer, met its primary endpoint of PFS that was longer in patients treated with Avastin and chemotherapy followed by the continued use of Avastin alone, compared to women treated with chemotherapy alone. No new safety findings were observed and AE were consistent with those seen in previous pivotal trials. No details were released. Full data from the OCEANS trial will be submitted for presentation at an upcoming conference.

According to the results from the OCEANS trial, continued maintenance with Avastin increases PFS but the gains may not justify the treatment's high costs. Investigators at Ohio State University College of Medicine (Columbus, OH); and the University of Alabama at Birmingham, AL, conducted a cost-effectiveness analysis comparing the three arms of the Gynecologic Oncology Group clinical trial ([protocol ID: GOG-0218; NCT00262847](#)) with paclitaxel plus carboplatin with or without

Avastin and the chemotherapy combination with Avastin maintenance. Actual and estimated costs of treatment plus the potential costs of complications were established for each treatment regimen. Progression-free survival (PFS) and bowel perforation rates were obtained from reported trial results (Burger RA, *et al.*, ASCO10, Abs. LBA1). In this trial, PFS in patients treated with Avastin and chemotherapy and then Avastin administered for up to 10 months afterward was 14.1 months, 3.8 months longer compared to 10.3 months for chemotherapy alone. PFS in a third group of women treated with Avastin during chemotherapy only (i.e., no follow up maintenance with Avastin as a single agent) was 11.2 months, which was not statistically significant.

For the 600 patients entered into each arm, at the baseline estimates of PFS and the rate of bowel perforation, the cost of simple chemotherapy was \$2.5 million, compared with \$21.4 million for chemotherapy plus Avastin, and \$78.3 million for chemotherapy plus Avastin maintenance. These costs led to an incremental cost-effectiveness ratio (ICER) of \$479,712 per progression-free life-year saved (PF-LYS) for chemotherapy plus Avastin and \$401,088 per PF-LYS for chemotherapy plus Avastin maintenance. Although treatment with maintenance Avastin led to improved PFS, it was associated with both direct and indirect costs and was not cost effective. However, when the cost of Avastin was reduced by 25% of baseline, the ICER of chemotherapy plus maintenance Avastin fell below \$100,000 per PF-LYS. Therefore, the cost effectiveness of Avastin in the adjuvant treatment of ovarian cancer is primarily dependent on drug costs ([Cohn DE, *et al.*, J Clin Oncol, 7 Mar 2011; epub ahead of print](#)).

Avastin in Combination with Novel Agents

Avastin is also under clinical investigation in combination with about **38** novel agents in about 78 trials, representing 7.8% of all clinical trials with the drug. At least **32** of these drugs are targeting over **36** unique molecular moieties (Exhibit 1); several of these drugs are multi-targeted.

The Avastin combinations are part of an extensive program to combine approved targeted drugs with experimental drugs to address multiple pathways and are a harbinger of the future as developers gear up to initiate trials of combinations of experimen-

tal drugs. The goal is to disrupt multiple pathways driving tumor growth. This new wave of clinical trials will resemble the efforts of the past 10 years in which targeted agents were combined with approved cytotoxics in thousands of trials and were responsible for almost all of the approved indications of targeted agents. This approach, however, seems to have reached the point of minimal return. It is difficult to predict the scope of this new wave of clinical trials combining novel targeted therapeutics because more than 600 such drugs have already been evaluated in early trials, and many in preclinical stages are waiting in the wings. Thousands of molecular abnormalities have been linked to cancer and little is known about how different pathways interact with each other *in vivo*. The Avastin clinical program involving combinations of novel targeted agents illustrates the enormity of this task. To date, the drug has been or is undergoing evaluation with novel agents targeting more than 36 unique moieties.

In December 2010, the FDA has issued a draft guidance document, [Guidance for Industry Codevelopment of Two or More Unmarketed Investigational Drugs for Use in Combination](#) intended to assist pharmaceutical companies in the codevelopment of two or more novel (not previously marketed) drugs (excluding vaccines, gene or cellular therapies, or blood products) to be used in combination to treat a variety of diseases, including cancer. The guidance provides recommendations and advice on how to address certain scientific and regulatory issues that arise during such codevelopment.

Various strategies in designing clinical trials combining novel targeted agents are under consideration. Companies with deep pipelines in oncology are, initially, combining their own agents that have successfully completed phase I trials. Large drug companies are collaborating with boutique developers, as they attempt to cherry-pick promising novel agents shown to enhance the performance of their own drugs in preclinical studies. Additionally, large companies are collaborating with each other in order to maximize their own and each other's assets. These types of deals usually involve agents initially evaluated by their developers in phase I clinical trials, which are then to be further developed in combination jointly by the companies. In June 2009, Merck & Co. and AstraZeneca en-

tered into a collaboration to clinically investigate a combination of **MK-2206** (Merck) and **AZD6244** (ARRY-886; AstraZeneca) based on preclinical evidence suggesting that combined administration of these compounds might significantly enhance their anticancer properties. In December 2010, Merck KGaA and sanofi-aventis signed an agreement to jointly conduct early stage human trials with **MSC1936369B** (Merck) and **SAR245409** and **SAR245408** (sanofi-aventis).

The pursuit of targeted drugs combination regimens will bolster the demand for preclinical services to identify optimal combinations and accelerate demand for *in vitro* evaluations for optimal patient selection for clinical trials. The many possible combinations of agents targeting hundreds of molecular markers will create a significant opportunity for companies in the *in vitro* testing (IVT) sector.

NOVEL INHIBITORS OF THE VEGF PATHWAY

The success of Avastin in the marketplace has also spurred the development of many drugs targeting VEGF family members. To date, more than 100 agents have been investigated preclinically or clinically; 41 have been evaluated in clinical trials during the past 3 years and 34 are currently in active clinical development. The mechanism of action of most of these agents is different from that of Avastin. Avastin, a monoclonal antibody (MAb), blocks extracellular VEGF and prevents it from activating its receptors, while most of the other agents are either MAb that target the various VEGF receptors on the surface of cancer cells or small molecules that enter the cell and block tyrosine kinase activity. Many of these agents are multi-targeted, which further complicates direct comparisons with Avastin.

To date, VEGF pathway inhibitors have been used indiscriminately in patients selected by traditional disease status characteristics, without consideration for the molecular profile of tumors. This approach is based on the notion that angiogenesis attributed to the expression of VEGF family proteins is universal, and blockade of these proteins would provide benefit in most solid tumors. After it became apparent that monotherapy with VEGF inhibitors did not provide a meaningful outcome and combi-

nations with standard cytotoxics were suboptimal, establishing biomarkers of response and resistance became a priority. There is an urgent need to select the patients who are most likely to benefit from such high cost therapies. Another emergent priority is the identification of new targets to prevent the invariable escape from therapies that target specifically or primarily the VEGF pathway.

Currently there are no validated biomarkers for selecting patients for antiangiogenic therapy or identifying those harboring escape pathways that confer resistance to antiangiogenic approaches. Potential biomarkers associated with VEGF inhibitor resistance, measured at baseline, include VEGF polymorphisms. In addition, mechanistically based biomarkers obtained during treatment, including physiologic changes such as increases in blood pressure or circulating angiogenic molecules or collagen IV, may be associated with response. Biomarkers associated with VEGF pathway inhibition would require prospective evaluation before they could be usefully applied in patient selection or the development of clinical regimens ([Jain RK, et al., Nat Rev Clin Oncol, Jun 2009;6\(6\):327-38](#)).

Methods of biomarker evaluation are lacking in part because it is not clear, based on mechanisms of action of VEGF pathway inhibitors, which pharmacodynamic parameters or molecular biomarkers are associated with the effectiveness or lack of effectiveness of anti-VEGF approaches. Unlike Avastin, most of the VEGF pathway inhibitors in clinical development are multitargeted, addressing several VEGF family members plus other targets (Exhibit 2); the most common targets are c-Kit, FLT3, and Met. Assessing the effectiveness of these agents is complicated by the fact that multitargeted inhibitors do not display the same affinities for all of their putative targets. Therefore, it may be more effective to combine highly potent and selective inhibitors each addressing a unique target. Such a strategy guarantees maximum inhibition of the intended targets and allows the design of treatment options based on optimal dose and schedule regimens.

In addition to the members of the VEGF family, many other molecules that play a role in angiogenesis have been identified, including the Tie2 receptor and its ligand angiopoietin, neuropilin 1 (NRP1), metalloproteinase (MMP), heparinase, various in-

tegrins and ephrins, among others. Angiogenesis promoters represent a potentially rich source for targets to inhibit tumor growth and/or metastasis. Most of these angiogenic factors undergo a variety of alternative splicing events that result in different protein isoforms. VEGF itself is spliced into at least six different isoforms of 121, 145, 165, 183, 189 and 206 amino acids; VEGF₁₆₅ being the predominant species. Some of these isoforms are preferentially expressed in tumors.

VEGF Inhibitors in Late Stage Clinical Development

Currently, development of 27 of the 34 VEGF pathway inhibitors has advanced to phase II trials with 14 of these drugs undergoing phase III evaluation. The phase III trials are evaluating these drugs in a variety of advanced solid tumors including breast cancer, CRC, gastric cancer, gastrointestinal stromal tumors (GIST), glioblastoma multiforme (GBM), hepatocellular carcinoma (HCC), medullary thyroid cancer (MTC), nscl, ovarian cancer, pancreatic cancer, prostate cancer, and RCC, as well as advanced acute myeloid leukemia (AML).

Comparing trial outcomes with these agents in specific indications with the performance of Avastin is difficult because most are multi-targeted and it is difficult to relate a target with the drug's performance. However, 5 of these drugs primarily target the VEGF pathway. Several failures of large randomized phase III clinical trials with these drugs underscore the challenges facing developers in this sector. In 2010, two phase III clinical trials (HORIZON II and HORIZON III) with **cediranib** (Recentin) under development by AstraZeneca, that evaluated the drug in combination with standard chemotherapy, failed to meet their primary objective in chemotherapy-naïve patients with advanced CRC.

In March 2011, sanofi-aventis and Regeneron Pharmaceuticals reported that according to results from the phase III VITAL clinical trial ([protocol ID: EFC10261; AVE0005; EudraCT 2007-000819-29; NCT00532155](#)) evaluating **afibercept** (VEGF Trap) as a second line treatment in patients with locally advanced or metastatic non-squamous nscl, adding afibercept to docetaxel did not meet the pre-specified criteria for the primary endpoint

of improvement in OS compared with a regimen of docetaxel plus placebo (HR=1.01).

Selected VEGF Pathway Inhibitors in Preclinical Development

Many VEGF inhibitors have been evaluated in early research and preclinical studies, and several are being currently pursued with the aim of improving the effectiveness of these agents.

Among agents in active preclinical development are MAb targeting VEGF-C and VEGF-D under evaluation by **Circadian Technologies**, which controls exclusive worldwide rights to an intellectual property portfolio focused around VEGF C and D. Key therapeutics in Circadian's portfolio, including **VGX-100**, VGX-200, and VGX-300, which block VEGF-C, VEGF-D, and VEGFr3, respectively, are alternative stimulators of VEGFr2 that may block blood vessel growth in tumors resistant to anti-VEGF-A therapy. When used in combination with drugs like Avastin, they may completely shut down angiogenesis mediated by VEGFr2, resulting in greater clinical efficacy. VEGF-C and VEGF-D are the only known proteins to bind and activate VEGFr3, which drives lymphatic vessel and tumor-associated blood vessel growth. In addition to internal product development programs, Circadian has licensed rights to parts of its IP portfolio to ImClone Systems, which is developing an **antibody-based drug** targeting VEGFr3 for the treatment of solid tumors.

PhiloGene is pursuing a different approach, exploiting an isoform of VEGF-A that appears to be antiangiogenic. Initially it was determined that VEGF was spliced into at least six different isoforms comprised of 121, 145, 165, 183, 189 and 206 amino acids, with the most common being VEGF165. Most of these isoforms are preferentially expressed in tumors, and their angiogenic effects principally are mediated by activation of VEGFr2. Subsequently and serendipitously, an additional spliced variant, VEGF165b, was identified. VEGF165b is formed by distal splice site selection in the terminal exon of VEGF and predicts an open reading frame encoding an alternative COOH-terminal sequence but the same number of amino acids in the mature protein. It predicts the translation of a protein of the same length as VEGF165, but with a different sequence and possibly a different mechanism of ac-

tion. The high degree (96%) of homology between VEGF165b and VEGF165 may explain why it had not been discovered earlier; VEGF165b would not have been distinguished from VEGF165 in most nucleic acid and protein assays. Conditioned media from cells expressing synthetic, recombinant VEGF165b inhibited VEGF165-mediated endothelial cell proliferation and migration *in vitro* and vasodilatation *ex vivo* ([Woolard J, et al., Cancer Res, 1 Nov 2004;64:7822-7835](#)).

VEGF165b is expressed in normal cells and tissues and circulates in human plasma. It inhibits angiogenesis and is downregulated in tumors. VEGF165b binds VEGFr2 with the same affinity as VEGF165 but does not activate it or stimulate downstream signaling pathways. Moreover, VEGF165b prevents VEGF165-mediated VEGFr2 phosphorylation and signaling in cultured cells, and in two different *in vivo* angiogenesis models, rabbit cornea and rat mesentery, VEGF165b is not angiogenic but, rather, inhibits VEGF165-mediated angiogenesis. VEGF165b-expressing tumors grow significantly more slowly than VEGF165-expressing tumors, indicating that a switch in splicing from VEGF165 to VEGF165b may inhibit tumor growth. Administration of recombinant human VEGF165b inhibits colon carcinoma tumor growth and tumor vessel density in nude mice, and directly inhibits *in vitro* such angiogenic activities as endothelial sprouting, orientation, and structure formation. Radiolabeled VEGF165b injected IV was taken up significantly by tumors without any AE noted on liver function or hemodynamics. These results suggest that regulation of VEGF splicing may be a critical switch from an antiangiogenic to a proangiogenic phenotype ([Rennel ES, et al., Eur J Cancer, Sep 2008;44\(13\):1883-94](#)).

While many current therapeutic approaches are based on removal of all forms of VEGF, PhiloGene's approach aims to restore the balance between the pro-angiogenic and antiangiogenic forms of VEGF, which may result in a more effective and safer strategy for treating both retinopathy and cancer.

Investigators at Roche generated **TAvi6**, a novel tetravalent IgG-like bispecific antibody based on Avastin with 2 disulfide-stabilized scFv (LC06) fused to the C-terminus of the heavy chain, which

targets VEGF-A and Ang-2. LC06, a human MAb targeting Ang-2, is also under development as an antiangiogenic agent. The activity of sTAvi6 is identical to that of the parental antibodies bevacizumab and LC06 in biochemical and cellular assays. In the orthotopic KPL-4-003 xenograft, tumor growth inhibition (TGI) was 79% for bevacizumab, 39% for LC06, 90% for the combination, and 91% for TAvi6. In the SC Colo205-009 xenograft, TGI was 66% for Avastin, 50% for LC06, 78% for the combination, and 87% for TAvi6. TAvi6 suppressed growth of tumors refractory to first line treatment with bevacizumab (Colo205 xenograft) and suppressed metastasis to the lung significantly in the KPL-4 xenograft. In the SC Calu-3 xenograft there was a strong inhibition of angiogenesis according to *in vivo* and *ex vivo* imaging and an advantage of TAvi6 compared to the antibody combination. In the VEGF-induced cornea-pocket assay, TAvi6 completely shut down angiogenesis (Scheuer W, et al, AACR-NCI-EORTC10, Abs. 468).

Vascular Disrupting Agents (VDA)

Angiogenesis inhibitors prevent the formation of new vessels but have little effect on the pre-existing vasculature of established tumors. Vascular disrupting agents (VDA), or endothelial disrupting agents, differ from antiangiogenesis drugs in that they target endothelial cells and pericytes within the established tumor vasculature, causing it to collapse, depriving the tumor of blood and oxygen and leading to tumor ischemia and necrosis. Drugs putatively described as VDA that have entered clinical trials defy classification. Most are targeted cytotoxics, while the mechanism of others remains obscure. Theoretically, VDA represent an attractive way to combat developed tumors but to date, clinical evaluation of these drugs has not yielded definitive effectiveness data. The development of several agents thought to act as VDA has been abandoned after disappointing clinical trial results.

ASA-404 (DMXAA), one of the earliest so-called VDA, underwent extensive clinical investigation but was abandoned after it failed in phase III trials. The antitumor action of ASA-404 involved both direct effects in the form of endothelial cell apoptosis, and indirect effects through the induction of cytokines within the tumor microenvironment. ASA-404 simultaneously targets at least

two cell types within the tumor microenvironment, vascular endothelial cells, and macrophages. In murine tumors, ASA404 decreases tumor blood flow, increases vascular permeability and increases vascular endothelial apoptosis in the short term and induces an increase in tumor concentrations of TNF and a number of other cytokines over time ([Baguley BC and McKeage MJ, Future Oncol, Oct 2010;6\(10\):1537-43](#)).

VB-111, under development by **Vascular Biogenics (VBI)**, represents an interesting approach to VDA. VB-111 was created using VBI's tissue and condition-specific expression vascular targeting system (VTS) platform, which is directed to areas undergoing angiogenesis, where it destroys newly formed blood vessels feeding solid tumors without harming healthy tissues. VB-111 consists of a non-replicating adenovirus vector (Ad-5, E1 deleted), which contains a modified murine pre-proendothelin promoter, the Vascular Targeting System (VTS), and a fas and human tumor necrosis factor (TNF) receptor chimeric transgene. The modified promoter specifically expresses the fas chimera transgene in tumor vessel endothelium, leading to targeted apoptosis. By debulking the tumor mass, VB-111 may enhance the activity of standard anti-cancer agents and may be a potential candidate for neoadjuvant therapy. Unlike other antiangiogenesis approaches designed to block specific cellular steps in processes leading to new blood vessel production or cellular proliferation, VB-111 destroys existing vascular endothelial cells. VB-111 may be effective both as a monotherapy and neoadjuvant therapy and in combination with chemotherapy or radiotherapy. It may be applicable to many different types of solid tumors where vascularization is a key driver of growth and metastasis.

In preclinical studies in multiple tumor models, VB-111 was tumor-tissue specific and did not significantly damage normal tissues or blood vessels. *In vitro*, VB-111 induced a dose-dependent apoptotic death in HUVEC but not in non-endothelial cells, confirming its specificity. In *in vivo* studies using the Lewis lung metastasis mouse model, VB111 was safe and active only in metastatic lesions and, with one injection, reduced tumor burden of lung metastases by 90% in a dose-dependent manner. IN this same animal model, the addition of VB-111 enhanced the activity of Sutent. An

additive effect was also observed when mice were treated with VB-111 and doxorubicin. VB-111 was similarly efficacious in other tumor models. Overall, VB-111 was found to be safe and tissue specific with a dual mode of action as an angiogenic inhibitor and VDA (Breitbart A, *et al.*, AACR10, Abs. 1369). Based on these preclinical results, VB-111 entered clinical trials in November 2007.

In a completed phase I clinical trial ([protocol ID: GT-111001; NCT00559117](#)) VB-111 was administered as a single IV infusion at escalating doses ranging from 1×10^6 to 3×10^{12} viral particles (VP) in 6 successive cohorts. Between November 2007 and August 2009, 27 patients enrolled at two cancer centers. VB-111 was well tolerated; no DLT

\geq Grade 3 were observed. As expected from the specificity of VB-111 the Fas chimeric transgene was not expressed in the blood. Disease stabilized on day 56 in one of the 15 patients in cohorts 1 to 5. Among the 12 patients in cohort 6, disease stabilized in 4 on day 56, and there was a PR in one patient with papillary thyroid carcinoma that persisted for 12 months post dosing. VB-111 was safe and well tolerated in patients with advanced metastatic cancer at a single administration of up to 3×10^{12} VP. MTD has not been reached (Trionzi P, *et al.*, AACR10, Abs. 1361). Currently, the drug is in phase II clinical trials in relapsed glioblastoma multiforme (GBM) and advanced differentiated thyroid cancer.

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