Published in final edited form as:
Curr Angiogenes. 2012 September ; 1(3): 169–179.

Combining Bevacizumab with Radiation or Chemoradiation for Solid Tumors: A Review of the Scientific Rationale, and Clinical Trials

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Abstract

Radiation therapy or the combination of radiation and chemotherapy is an important component in the local control of many tumor types including glioblastoma, rectal cancer, and pancreatic cancer. The addition of anti-angiogenic agents to chemotherapy is now standard treatment for a variety of metastatic cancers including colorectal cancer and non-squamous cell lung cancer. Anti-angiogenic agents can increase the efficacy of radiation or chemoradiation for primary tumors through mechanisms such as vascular normalization and augmentation of endothelial cell injury. The most commonly used anti-angiogenic drug, bevacizumab, is a humanized monoclonal antibody that binds and neutralizes vascular endothelial growth factor A (VEGF-A). Dozens of preclinical studies nearly uniformly demonstrate that inhibition of VEGF-A or its receptors potentiates the effects of radiation therapy against solid tumors, and this potentiation is generally independent of the type or schedule of radiation and timing of VEGF-A inhibitor delivery. There are now several clinical trials combining bevacizumab with radiation or chemoradiation for the local control of various primary, recurrent, and metastatic tumors, and many of these early trials show encouraging results. Some added toxicities occur with the delivery of bevacizumab but common toxicities such as hypertension and proteinuria are generally easily managed while severe toxicities are rare. In the future, bevacizumab and other anti-angiogenic agents may become common additions to radiation and chemoradiation regimens for tumors that are difficult to locally control.

Keywords

Bevacizumab; radiation therapy; anti-angiogenic therapy; glioblastoma; rectal cancer; pancreatic cancer

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

INTRODUCTION

The majority of solid tumors can be eradicated from where they originated with surgical resection, sometimes in combination with radiation or chemoradiation. Thus most patients who succumb to solid tumors die of metastatic rather than locoregional disease. However for some solid tumors located in difficult anatomic locations such as the brain, head and neck, retroperitoneum/pelvis, rectum, and pancreas, unresectable primary tumors or locoregional recurrence of resected tumors remain a significant source of morbidity and in some cases mortality. In the past few decades, we have witnessed an explosion in our understanding of tumor biology and in the more recent past, we have seen the fruits of this increased knowledge as targeted biological therapies. In 1971, Dr. Judah Folkman proposed in a *New England Journal of Medicine* article that inhibiting angio-genesis would be an effective strategy to treat human cancers [1]. This prediction was fully realized in 2004 with the approval of bevacizumab in combination with chemotherapy for the treatment of patients with metastatic colorectal cancer [2].

Bevacizumab is a humanized monoclonal antibody which binds and neutralizes vascular endothelial growth factor A (VEGF-A). Since 2004, bevacizumab has been FDA-approved for use in metastatic renal cell cancer, progressive glioblastoma, and metastatic non-small cell lung cancer [3]. FDA approval of bevacizumab for metastatic breast cancer was recently withdrawn. In addition to bevacizumab, three small molecule inhibitors with anti-angiogenic activity (sunitinib, sorafenib, and pazopanib) have been approved as single agents for the treatment of metastatic renal cell cancer and/or unresectable hepatocellular cancer. Unfortunately, these metastatic or locally advanced tumors eventually become resistant to these therapies, and prolongation in overall survival ranges from 0-5 months. Many other angiogenesis inhibitors including antibodies and small molecules are in various phases of clinical trials.

The use of bevacizumab as a biological enhancer of radiation or chemoradiation for primary tumors or isolated metastases has been much slower to progress into the clinic compared to the addition of bevacizumab to chemotherapy regimens for metastatic disease. This review will briefly present the relevant principles of radiation oncology and anti-angiogenic therapy, give an overview of preclinical studies examining anti-angiogenic therapies and radiation for solid tumors, and then focus on published clinical trials using this therapeutic strategy.

PRINCIPLES OF RADIATION ONCOLOGY

Radiation inhibits cancer cells primarily through damage of DNA. Radiation is administered to tumors either in the form of photons (i.e. x-rays and gamma rays) or particles (i.e. protons, neutrons, and electrons) [4]. Photons or particles can interact directly with DNA causing ionizations (high linear energy transfer) or they can interact with molecules such as water and oxygen and form free radicals that then interact with DNA (low linear energy transfer) [5]. This indirect damage has been estimated to contribute to over 80% of the overall radiation-associated cell lethality under normoxic conditions [6]. Ionizing radiation causes a variety of changes to DNA including DNA double stranded breaks, which are the

primary cause of cell inactivation and cell killing. In eukaryotic cells, DNA double-strand breaks can be repaired by homologous recombination repair (HRR) or nonhomologous end joining (NHEJ) [7]. Following exposure of cancer cell DNA to ionizing radiation, potential consequences include normal cell division, DNA damage induced senescence, DNA damage induced apoptosis, or mitotic-linked cell death.

The various manifestations of DNA damage can occur rapidly or manifest after many cell divisions. Cells may respond to DNA damage by initiating apoptosis within hours of radiation injury, or DNA damage may lead to death through abnormal chromosomal segregation during mitosis. Mitotic-linked cell death may be more important in cancer cells that in normal tissues, as the p53 pathway is commonly mutated in solid tumors [8]. Dysfunction of p53 related machinery prevents cells from initiating rapid apoptotic death in response to radiation, and predisposes to premature entry into M phase, before DNA damage is repaired [9]. Acute cell death by this mechanism is delayed in comparison to apoptosis, with histological stigmata of mitotic catastrophe occurring 2-6 days after radiation [10].

Oxygen is the most important modifier of the biologic effect of radiation [11]. Survival curves for cancer cells exposed to radiation under hypoxic and normoxic conditions demonstrate that significantly greater doses are required during hypoxia for equivalent cell killing [12]. Oxygen increases the efficacy of radiation by forming DNA-damaging free radicals. The oxygen enhancement ratio is the ratio of radiation dose required for equivalent cell death in the absence of oxygen compared with the dose required in the presence of oxygen [13]. The oxygen enhancement ratio for different cells varies between 2.5 and 3.5, implying that about 3 times as much radiation is required under hypoxic conditions as compared to normoxic conditions for equivalent cell killing. This effect is of great significance in solid tumors, where up to 60% of advanced tumors will contain hypoxic or anoxic regions [14]. Clinically, hypoxia has been shown to predict poor prognosis after radiation therapy in numerous solid tumors including head and neck cancer, glioblastoma, and sarcoma [15-17]. In addition, radiotherapy delivered in a hyperbaric oxygen chamber was found to improve local control while causing greater damage to surrounding tissues in patients with head and neck cancer [18].

Radiation damages not only cancer cells but also tumor stroma and a margin of normal tissue surrounding the tumor. In early radiation experiments, it was noted that transplantable tumors had retarded growth when implanted into radiated tissues, and this observation was largely attributed to the disruption of capillary networks by radiation [19]. Damage to the microvasculature is a possible alternative or complementary hypothesis to the view that radiation-based tissue damage primarily targets the stem, or mesenchymal compartment of tissue. Mice receiving 15 Gy of whole body radiation typically die with denudation of the gastrointestinal (GI) mucosa, but basic fibroblast growth factor (bFGF or FGF-2) protects against this syndrome [20]. Supporting vascular damage as a precursor event to stem-cell death in this model, Paris *et al*., found that bFGF receptors are expressed by the alimentary vascular endothelium but not the stem-cell containing GI crypts [21]. Basic FGF treatment was found to reduce vascular endothelial apoptosis, with correlating reductions in GI denudation and death. Similar mechanisms have been found in murine models of radiation

pneumonitis [22], infertility induced by ovarian radiation [23], and central nervous system (CNS) radiation toxicity [24].

PRINCIPLES OF ANTI-ANGIOGENIC THERAPY

Cancer cells, like all living cells, cannot survive without a blood supply to provide oxygen and nutritional support. Angiogenesis, or the formation of new blood vessel from pre-existing vessels, is regulated by a balance between pro-angiogenic and anti-angiogenic factors [25]. VEGF-A is likely the most important factor regulating tumor angiogenesis [26], and exerts its effects primarily through two tyrosine kinase receptors VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1, KDR) [27]. VEGFR-2 is thought to mediate the vascular growth and permeability actions of VEGF-A [28]. Other important angiogenic pathways include the platelet-derived growth factor (PDGF) family, fibroblast growth factor (FGF) superfamily, angiopoietins (ANGs), TIE signaling, NOTCH, and Delta-like ligand 4 (DLL4) signaling [3]. Many of these pro-angiogenic pathways are over-expressed in tumors leading to a tumor vasculature that is structurally and functional abnormal. Compared to normal organ and tissue vessels, tumor vessels are leaky, tortuous, dilated, and have a haphazard pattern of interconnection [29]. This abnormal blood flow contributes to tumor acidosis, and high vessel permeability leads to increased tumor interstitial pressure.

It was initial hypothesized that targeting the tumor vasculature would circumvent typical therapeutic resistance because the target was genetically stable endothelial cells as opposed to genetically unstable cancer cells [30]. We now know that resistance to anti-angiogenic therapy is common. Anti-angiogenic therapy may result in an initial response followed by relapse in some patients or in lack of initial benefit for other patients. This suggests two modes of resistance: evasive resistance, where tumors adapt during treatment, and intrinsic resistance where counteracting mechanisms are pre-existing [31]. Non-mutation based mechanisms for resistance to anti-angiogenic therapy have been classified into four categories. First, a number of alternative angiogenic signaling cascades may allow tumors to circumvent blockade of a single pathway [32, 33]. Implicated ligands include platelet-derived growth factors (PDGFs), fibroblast growth factors (FGFs), ephrins A1 and A2, angio-poietin 1 and interleukin 8. Second, post-therapy hypoxia may result in recruitment of bone marrow-derived vascular progenitors and modulating cells that “rebuild” the tumor vasculature [34-37]. Implicated modulators include tumor associated macrophages, TIE2+ monocytes, VEGFR1+ hemangiocytes, and CD11b+ and CD45+ myeloid cells. Evidence supporting both of these mechanisms was found during clinical investigation of glioblastoma that recurred in patients treated with a small molecule inhibitor of VEGF receptors [38]. A vascular progenitor cell activator stromal-derived factor 1α (SDF1α) and the pro-angiogenic ligand FGF2 were low during tumor regression, but became elevated in the serum during recurrence. The third mechanism involves pericytes, the supporting cells of the vasculature that are found in close juxtaposition to endothelial cells [39]. Tumor vessels have poor pericyte coverage, but vessels surviving anti-angiogenic therapy have tight pericyte coverage, suggesting that endothelial cells can recruit pericytes to maintain pro-angiogenic survival signaling. Lastly, tumors may maintain nutritional support without angiogenesis through increased invasion and by co-opting pre-existing blood vessels as the tumor infiltrates normal tissue [40].

In addition to these four adaptive mechanisms of anti-angiogenic treatment evasion, similar abilities may be pre-existent, as is likely in the numerous patients who demonstrate no response to anti-VEGF therapy in the clinical setting [31]. Alternative angiogenic signaling cascades may be responsible for continued angiogenesis, as suggested by elevated levels of bFGF seen in an analysis of advance breast cancer biopsies [41]. Some tumors such as pancreatic ductal adenocarcinoma, contain large avascular regions without necrosis, suggesting these tumors can survive under hypoxic conditions [42]. Other tumors, such as moderate grade astrocytoma may have already co-opted normal blood vessels through peri-vascular invasion at the time of tumor vascular targeting therapies [43].

Normal vasculature in organs and tissues is relatively quiescent and thus it was also originally hypothesized that targeting proliferating tumor vessels would result in minimal side effects. Anti-angiogenic agents such as bevacizumab generally have less side effects than cytotoxic chemotherapy but side effects do indeed occur [44]. Common but less severe toxicities include hypertension and proteinuria. Hypertension can be controlled with medications, and proteinuria resolves after cessation of bevacizumab. More serious side effects which occur sporadically include wound complications and ventricular dysfunction/ congestive heart failure. Life-threatening toxicities seen with bevacizumab occur rarely in patients and include hemorrhage, thrombosis, and GI perforation.

As tumors expand, there exists a critical balance between tumor angiogenesis and hypoxia. Tumor cells respond to hypoxic stress through multiple mechanisms, including stabilization of hypoxia inducible factor-1α (HIF-1α). Stabilized HIF-1α is then transported to the nucleus, where it forms a dimer with the constitutively expressed ARNT subunit [45] and consequently activates expression of at least 150 genes. These genes orchestrate adaptive responses including those mediating further tumor angiogenesis (e.g. VEGF-A) [46], invasion (e.g. c-Met) [47], and metastasis (e.g. FOXM1) [48, 49]. Sustained anti-VEGF-A therapy can ultimately lead to loss of tumor vessels and increased hypoxia [50]. There has been recent controversy on the effects of VEGF inhibition on primary tumor invasiveness and metastatic potential [51]. Casanovas and colleagues found that VEGFR-2 inhibition of RIP1-Tag2 mouse pancreatic endocrine tumors led to increase intratumoral hypoxia along with increased tumor invasiveness and liver metastases [52, 53]. One possible mechanism is via the stabilization of HIF-1α and the subsequent expression of genes that promote invasion, including c-MET [47]. Ebos *et al*., found that sunitinib (which targets VEGF and other pathways) increased liver and lung metastases for both experimental and spontaneous metastases [54]. This conflicts with other preclinical studies showing inhibition of metastases with VEGF inhibitors [55] as well as clinical studies demonstrating that bevacizumab as a single agent can prolong patient survival against metastatic renal cell cancer and other cancers [56, 57]. Clinical concern for increased invasiveness or metastasis following anti-VEGF-A therapy is focused on glioblastoma, where this phenomenon is commonly observed [58]. It is important to note that the vast majority of cancer patients treated with VEGF-A inhibitors have established metastatic disease and relatively few studies have examined the use of VEGF-A inhibitors in patients with primary tumors and no clinically evident metastases. For anti-angiogenic agents to be more broadly used in the neoadjuvant setting with radiation or chemoradiation, it is vital to determine under what

circumstances VEGF-A inhibitors may increase the invasiveness and metastatic potential of primary tumors.

INSIGHTS FROM PRECLINICAL STUDIES OF ANTI-ANGIOGENIC THERAPY AND RADIATION

There have been dozens of preclinical studies examining the combination of radiation and anti-angiogenic agents on solid tumors [59]. These studies examine several different agents including neutralizing antibodies to VEGF-A [60-62], neutralizing antibodies to VEGF receptors [63-65], and small molecule inhibitors of angiogenic factor signaling [66-68]. Investigators have used highly diverse approaches to the scheduling of therapy including the use of fractionated and non-fractionated radiation and the delivery of anti-angio-genic therapy before, during, and after radiation. Appropriate scheduling of therapy would ideally deliver radiation during periods of minimal tumor hypoxia. Unfortunately, measurements of tumor perfusion and oxygenation during anti-angiogenic therapy have not demonstrated a uniform result in all settings. As one example, DC101 increased hypoxia in mammary carcinoma xenografts [64], but produced a window of decreased hypoxia in orthotopic gliomas [65]. Despite this diversity of effects on tumor oxygenation, the addition of anti-angiogenic therapy to radiation has shown an additive or synergistic anti-tumor effect with nearly all studies. Some preclinical studies have demonstrated cure of the tumor with this combination strategy. For example, Lee *et al*., demonstrated that glioblastoma xenografts maintained regression for the 100 day duration of the study after six days of VEGF-A inhibition using the monoclonal antibody A.4.6.1 followed by radiation [62].

Anti-angiogenic therapy may enhance the efficacy of radiation therapy by at least two mechanisms, both of which are incompletely understood. The first mechanism focuses on the reduction of hypoxia to maximize death of cancer cells during the acute phase of radiation damage. Effective and persistent blockade of pro-angiogenic signaling eventually decreases tumor vascular density and oxygenation. Before this end result anti-angiogenic therapy has been observed to temporarily increase oxygenation and decrease interstitial fluid pressure in what has been termed “vascular normalization” [29]. Under the influence of tumor secreted cytokines, tumor vasculature is disorganized, leaky and prone to erratic blood flow [69]. This disorganization may be primarily due to a VEGF-A driven over-expression of “tip cell” behavior; or over-activated endothelial cells behaving as leading points of new blood vessels [70, 71]. Neutralization of VEGF-A reduces these abnormalities in preclinical tumor models [72]. Winkler *et al*., demonstrated a 4-6 day window following anti-VEGFR-2 treatment where radiation induced a greater than additive delay in tumor growth, and this window correlated with a maximum decrease in tumor hypoxia [65].

The second mechanism by which anti-angiogenic therapy enhances radiation damage focuses on the late phase death of cancer cells through maximizing damage to the tumor microvasculature. In some studies, synergistic delay in tumor growth has been observed regardless of whether the angio-genesis inhibitor is given immediately before or after radiation [73], and delivering radiation to xenografts under clamp-hypoxia did little to alter growth curves with and without angiogenesis inhibitors [62,74]. A diverse selection of tumor cell lines has been shown to increase VEGF-A secretion in response to radiation

[60,75,76]. Numerous angio-genesis modulating agents have been shown to alter endothelial cell clonogenic survival in response to radiation [60,61,77]. Xenograft tumors unable to secrete VEGF-A are significantly more radiosensitive *in vivo* [61].

A mechanism directly relating VEGF signaling to endothelial apoptosis in response to radiation is beginning to emerge. Radiation exposure leads to the generation of ceramide, which induces apoptosis in endothelial cells through p53 independent mechanisms [23]. Compared to tumors implanted into wild-type mice, tumors implanted into mice that are unable to generate ceramide grow twice as fast, demonstrate reduced endothelial apoptosis, and are highly resistant to radiation [78]. Anti-angiogenesis factors may act as radiation sensitizers through modulation of this ceramide-induced apoptosis mechanism. In VEGF-A treated endothelial cells, ceramide levels and apoptosis remain low in response to radiation. A combination of anti-VEGF-A antibody and radiation shown to work on tumors in control mice had no effect on tumors in mice unable to generate ceramide through both genetic and antibody-based manipulation [79].

CLINICAL TRIALS OF ANTI-ANGIOGENIC THERAPY AND RADIATION OR CHEMORADIATION

More than half of cancer patients will be treated with radiation during the course of their disease [80]. Thus drugs that radiosensitize tumors have the potential to benefit a large number of patients. Clinical trials combining bevacizumab with radiation or chemoradiation have shown significant promise in the treatment of CNS tumors, head and neck squamous cell cancer, pancreatic and colorectal adenocarcinoma and soft tissue sarcoma.

CNS Tumors

Several clinical trials combining anti-angiogenic agents with radiation have been performed for CNS tumors, primarily gliomas and glioblastoma multiforme (GBM). Table 1 summarizes 4 published clinical trials combining radiation or chemoradiation with bevacizumab for CNS tumors. Three trials targeted patients with newly diagnosed GBM while one trial treated patients with recurrent GBM or anaplastic glioma.

Based on the European Organization for Research and Treatment of Cancer (EORTC)/ National Cancer Institute of Canada (NCIC) randomized phase III trial of radiation alone versus radiation plus temozolamide followed by six cycles of temozolamide (TMZ) [81], adjuvant radiation plus TMZ has become the standard of care for newly diagnosed GBM following surgical resection and serves as a comparison arm for other adjuvant therapies. Lai *et al*., treated 70 newly diagnosed GBM patients with adjuvant radiation (60 Gy) and TMZ combined with biweekly bevacizumab followed by TMZ and bevacizumab for a maximum of 24 cycles or until progression [82]. For patients completing the 24 cycles, single-agent bevacizumab was continued until progression. These 70 patients were compared to a control cohort of 110 patients treated with standard radiation and TMZ. The median overall survival was 19.6 months in the bevacizumab group compared to 14.6 months in the radiation/TMZ group. Progression free survival was 13.6 months versus 7.6

months. Additional significant toxicities possibly attributable to bevacizumab included 4 wound infections, 4 GI bleeding or perforation events, and 2 CNS hemorrhages.

In a similar study by Narayana *et al*., 51 patient with newly diagnosed GBM were treated after surgery with radiation (60 Gy), TMZ, and bevacizumab followed by 6 cycles of TMZ and bevacizumab [83]. The median survival for these patients was 23 months and the median progression free survival was 13 months. Of note, 20 of 35 patients (57%) with relapse suffered a diffuse recurrence, defined as the presence of disease on MRI in more than 2 lobes. Toxicities that could be possibly attributed to bevacizumab included 1 pulmonary embolism and 2 deep vein thromboses. Using a more aggressive adjuvant chemotherapy regimen, investigators at Duke University Medical Center treated 75 newly diagnosed GBM patients after surgery with radiation (60 Gy), TMZ, and bevacizumab followed by TMZ, bevacizumab, and irinotecan for two week cycles for a total of 6-12 cycles [84]. The addition of irinotecan clearly increased toxicity given 23% of patients stopped adjuvant therapy due to toxicity. Median overall survival was 21.2 months and median progression-free survival was 14.2 months. There were 2 toxic deaths from adjuvant therapy, one from neutropenic sepsis and one from pulmonary embolism.

Stereotactic radiotherapy allows a higher biological dose of radiation to be delivered to a more precise volume, and is emerging as a treatment for isolated brain metastasis and CNS tumor recurrence. Investigators at Memorial Sloan-Kettering Cancer Center treated 24 recurrent GBM and anaplastic glioma patients who either recurred or progressed despite prior chemoradiation [85]. Only two patients underwent debulking operations at the time of recurrence. Patients received bevacizumab every 14 days for 2 cycles followed by bevacizumab with stereotactic radiation (30 Gy) in 5 fractions. Bevacizumab continued until treatment failure for a median of 14 doses. Three patients discontinued therapy due to toxicity, with one intratumoral hemorrhage, one bowel perforation, and one wound dehiscence. One patient experienced lower GI bleeding 3 weeks after discontinuing therapy owing to tumor progression. Median progression-free survival was 7.3 months for recurrent GBM patients and 7.5 months for anaplastic glioma patients. Median overall survival was 12.5 months for recurrent GBM patients and 16.5 months for anaplastic glioma patients. These results compare favorably to historic progression-free survival rates of 9 and 13 weeks and overall survival rates of 25 and 47 weeks for recurrent GBM and anaplastic glioma, respectively [86].

Head and Neck Cancer

The addition of bevacizumab to chemoradiation for head and neck cancers has been described in four published studies (Table 2). In a phase I trial published in 2008, 43 patients with poor prognosis head and neck squamous cell carcinoma were treated with escalating doses of bevacizumab, fluorouracil and hydroxyurea [87]. Patients were included for either previously irradiated recurrent disease (67.4%) or an estimated 2-year survival <10%. Full-dose radiation ranged from 50.4 to 70 Gy depending on primary tumor site. Dose escalation went to 10mg/kg of bevacizumab after reduction of 5-fluorouracil and hydroxyurea. Late toxicities included 5 fistulae (11.6%) and 4 cases of ulceration/necrosis (9.3%). Mucocitis and neutropenia were also frequently observed. Five deaths occured during treatment

including one stroke, two hemorhages, and one case of sepsis. Median overall survival was 10.7 months.

Two phase II studies were published in 2011 examining bevacizumab and chemoradition for head and neck squamous cell carcinoma. In a randomized phase II trial, 26 patients with locally advanced head and neck squamous cell carcinoma were treated with hydroxyurea, continuous 5-fluorouracil and twice-daily radiation. Patients were randomized 2:1 to receive bevacizumab [88]. The study was halted after progression according to RECIST criteria was observed in four of five patients with T4 disease receiving bevacizumab and no progression was observed in the 2 control group patients with T4 disease. Two-year survival was 89% in the control group compared to 58% in the bevacizumab arm. Hainsworth *et al*., reported a phase II trial that added both bevacizumab and erlotanib to chemoradiation for 60 patients with head and neck squamous cell caricinoma [89]. Tumors were most commonly oropharyngeal (60%) and advanced stage (3% II, 23% III, 73% IV). Induction chemotherapy included 6 weeks of paclitaxel, carboplatin, bevacizumab, and continuous 5-fluorouracil followed by 7 weeks of chemoradiation including paclitaxel, bevacizumab, erlotanib, and radiation (68.4 Gy). This therapy was fairly intense, and three patients withdrew before radiation, one patient was unable to complete radiation due to toxicity, and 72% of patients required treatment interruption. After completion of therapy, 65% of patients had a partial response, and 30% had no clinically detectable tumor on follow-up CT scan and endoscopy. Estimated 3-year progression-free survival was 71% and the 3-year overall survival was 82% comparing favorably to the same chemoradiation regimen without targeted therapies. Bevacizumab potentially contributed to one death from cerebrovascular compromise.

There has been one phase II multi-institution trial examining the use of bevacizumab in nasopharyngeal carcinoma. This trial treated 44 paients with nasopharyngeal carcinoma (11% stage IIb, 55% III, 33% IV) with bevacizumab, cisplatin, and intensity-modulated radiation therapy (70 Gy) followed by additional bevacizumab, cisplatin, and 5-fluorouracil [90]. As opposed to earlier studies, no grade 3-4 hemorrhages were observed, which the authors attributed to greater precision acheivable with intensity-modulated radiation therapy. Two-year progression-free survival was 74.7% and two-year overall survival was 90.9%.

Rectal Cancer

The standard treatment in the United States for non-metastatic rectal adenocarcinoma that invades into the muscularis propria (T3) or beyond or has evidence of lymph node metastases, based on MRI or endorectal ultrasound, is preoperative radiation combined with 5-fluorouracil or capecitabine [91]. Preoperative chemoradiation followed by surgical resection significantly reduces local recurrence compared to surgery alone. The complete pathologic response rate after this neoadjuvant chemoradiation regimen is about 13-14% and local recurrence rates are generally <10% [92,93]. For patients with metastatic colorectal cancer, the addition of bevacizumab improves overall survival in patients receiving chemotherapy by about 4-5 months [94]. Thus combining bevacizumab with preoperative chemoradiation for nonmetastatic rectal cancer appears to be a logical therapeutic strategy with goals of increasing the complete pathologic response rate and decreasing local recurrence. As a benchmark for further investigation, a randomized trial of preoperative

chemoradiation using capecitabine alone versus capecitabine with oxaliplatin showed pathologic complete response rates of 13.9% and 19.2%, respectively (p=0.09) [93].

Seven studies have been published on the addition of bevacizumab to chemoradiation for colorectal cancer (Table 3). In a phase I trial, Czito *et al*., treated 11 patients with external beam radiation therapy (50.4 Gy) with concurrent escalating doses of bevacizumab, capecitabine and oxaliplatin [95]. Bevacizumab was administered at a fixed dose of 15 mg/kg on day 1 of radiation, and 10 mg/kg on days 8 and 22. Dose escalation was halted due to severe GI symptoms and the recommended phase II doses were capecitabine 625 mg/m^2 twice per day and oxaliplatin 50 mg/m^2 weekly. After surgical resection, two of the 11 patients were found to have a complete pathologic response. Willett *et al*., performed a phase I/II clinical trial of 32 patients with stage T3 or greater rectal adenocarcinoma [96,97]. Patients received 5-fluorouracil, bevacizumab 5-10 mg/kg, and 50.4 Gy. All 32 patients underwent surgery 7-10 weeks after chemoradiation, and a complete pathologic response was found in five patients (15.6%). Surgical complications included wound infection (5 patients, 15.6%), anastamotic leak (2 patients; 6.2%), abscess (2 patients), and delayed healing (2 patients). Five-year actuarial overall survival was 95% and local control was 91.7%.

Three phase II trials have now been completed examining the role of bevacizumab with standard chemoradiation for rectal cancer. Crane *et al*., treated 25 patients with 50.4 Gy of radiation combined with capecitabine and bevacizumab (5 mg/kg) [98]. Surgery was performed on all patients about 7 weeks after neoadjuvant treatment, and there were 3 major wound complications requiring further surgery. Eight patients (32%) had a complete pathologic response. Only one person had a local recurrence after a median follow-up of 22.7 months. In a similar study, 61 rectal cancer patients were treated with biweekly bevacizumab (5 mg/kg) and capecitabine during radiation (50.4 Gy) [99]. Fifty-eight patients proceeded to surgery, with a complete pathologic response observed in 8 patients (13.3%). Surgical complications included delayed wound healing (18 patients), infection (12 patients) and anastamotic leak (7 patients), with 6 patients requiring reoperation. A study in Canada examined 42 patients with locally advanced or low rectal adenocarcinoma [100]. Patients received their first dose of bevacizumab (5 mg/kg) two weeks before radiation. Patients then received 45 Gy of radiation over 5 weeks with an elective boost of 5.4 Gy. During radiation, patients received additional bevacizumab, capecitabine, and oxaliplatin. Seven patients (17%) had severe bleeding episodes, nearly all of which were potentially related to bevacizumab. Sixteen patients (38%) required interruption or discontinuation of preoperative therapy. Three patients were clinically unable to undergo surgery and 1 elected not to undergo surgery after a complete clinical response. Of the 38 patients undergoing surgery, a complete pathologic response was found in 18.4% of patients. Severe surgical complications included bleeding (1 patient), pelvic infection (6 patients), delayed healing (3 patients) and anastamotic leak (2 patients). Four patients required reoperation for complications. This 11% rate of reoperation is not outside of the 6-13% range [93,101] seen in similar clinical trials without bevacizumab.

Thus it is feasible but perhaps not beneficial to deliver bevacizumab with standard chemoradiation for rectal cancers. The complete pathologic response rates do not appear to

be significantly improved over chemoradiation without bevacizumab. Most of these studies do not have long enough follow-up to assess any improvements in local control rates. Bevacizumab can increase the incidence of bleeding and thrombotic events during chemoradiation. Wound and anastamotic complication are not uncommon after low anterior resection or abdominal perineal resections for rectal cancers, and there may be a concern that bevacizumab could increase these complications.

One additional study has explored the use of bevacizumab with chemoradiotherapy for 22 patients with colorectal cancers that were deemed unresectable [102]. Tumor location was varied with 41% rectal and 59% colon cancer. Patients underwent a 67.2 Gy split course of radiation, with daily capecitabine, amifostine, and bevacizumab. During treatment two patients had interruptions due to hypertension, and one died from a perineal infection. At 18 months follow-up, 17 patients were alive, 14 with no evidence of disease. Of 19 evaluable patients, 13 (68.5%) had a complete response and 4 (21.1%) had a partial response. Thus further studies are needed to determine how well the combination of bevacizumab with an aggressive chemoradiation regimen can locally control unresectable colorectal cancers.

Pancreatic Cancer

Three studies have combined bevacizumab with chemoradiation for pancreatic cancer. As in rectal cancer, investigations focused on bevacizumab as a biological modifier of chemoradiation for advanced disease. The prognosis for this disease remains poor, and median survival for those with metastatic disease is 3-6 months. Bevacizumab combined with chemoradiation has primarily been investigated in patients who have locally advanced or unresectable disease without overt metastases. In this patient population, complete pathologic response to chemoradiation is rare and median survival with chemoradiation is a little over a year [103]. The ability of preoperative chemoradiation to increase the likelihood of margin-negative (R0) resection is promising [104] but has yet to be measured by randomized prospective trials [105]. In a phase I trial, escalating doses of bevacizumab (starting at 5mg/kg every 2 weeks) started 2 weeks before a 50.4 Gy course of radiation, with escalating capecitabine (starting at 650 mg/m^2 bid) starting 2 weeks into radiation. In the first 30 patients, there were 3 bleeding events and 1 perforation at the site of the tumor. These events led to a reduction in total number of bevacizumab doses and exclusion of patients with tumors invading through the duodenum. The most commonly observed toxic events were vomiting, gastritis and diarrhea, with 19 patients (40%) requiring reductions in capecitabine and 4 patients (8%) requiring interruption of radiation. The authors considered these events comparable to chemoradiation without bevacizumab. Twenty percent of assessable patients had a radiographic response, and 4 patients (8%) were able to receive margin negative surgery, all of which occurred without perioperative complication. Median survival for the entire phase I cohort was 14.4 months.

In the subsequent phase II trial, 82 patients received 50.4 Gy with twice daily capecitabine (825mg/m^2), and bevacizumab (5 mg/kg) on days 1, 15 and 29 of chemoradiation [98]. Twenty-nine percent of patients required dose modifications with 80% reporting grade 3 or greater toxicities, most commonly GI side effects. Ten patients underwent laparotomy, 8 underwent resection, 5 cases had negative margins, and the remaining three had

indeterminate margins. For the entire cohort, median survival was 11.9 months. In addition to GI symptoms, 5 bleeding events (6.1%) were noted, all of which occurred after chemoradiation at sites unrelated to radiation of the tumor. Five deaths were related to treatment, with bevacizumab possibly contributing to one sudden death, one intraperitoneal infection and one colonic perforation. Toxic events significantly correlated with delivery of radiation to volumes more than 5 cm away from tumor.

Another phase II study at Northwestern enrolled patients with nonmetastatic pancreatic tumors to evaluate response rate, survival and toxicity [106]. Twenty-eight patients were treated with gemcitabine (1000mg/m^2, 7 doses), bevacizumab (10mg/kg, every 2 weeks, 5 doses) and radiation 36 Gy (15 fractions during middle cycle of treatment). One patient withdrew after 2 cycles for non-study related reasons. The most common toxicities were leucopenia and nausea, with no grade 4 or 5 toxicities observed. One GI perforation occurred during an endoscopic procedure. National Comprehensive Cancer Network criteria were used to describe pre-chemoradiotherapy resection status [107], with 7 (24%) resectable, 10 (34%) borderline and 12 (41%) unresectable cases. Three patients (43%) in the resectable group and 3 (30%) in the borderline group received complete resection of their tumor, with 2 (33% of resections) complete pathologic responses observed. Among these six patients, the only major surgical complication was one intrabdominal infection. For the entire cohort, median overall survival was 11.8 months and median progression-free survival 9.9 months. No statistical difference in survival outcomes was noted based on baseline resectability ($p = 0.75$ between unresectable and resectable). Surgical resection also did not change overall survival statistically ($p=0.27$) but there may have been insufficient power to see such a difference.

Thus it is feasible to add bevacizumab to standard chemoradiation regimens for nonmetastatic pancreatic cancer. Certain bevacizumab-related complications such as bleeding and bowel perforation can occur. It is difficult to determine from these studies whether the addition of bevacizumab significantly increases the rate at which unresectable tumors become resectable and there does not appear to be a significant increase in overall survival.

Soft Tissue Sarcoma

One study has combined radiation with bevacizumab for the treatment of resectable soft tissue sarcomas [108]. Twenty patients with intermediate-or high-grade sarcomas, ≥5cm in size, received bevacizumab for 2 weeks followed by 6 weeks of bevacizumab and radiation therapy (50 Gy). Tumor samples, blood samples, and perfusion CT scans were obtained before, during, and after neoadjuvant treatment. The combination of bevacizumab and radiation treatment was well tolerated with only 4 patients having grade 3 toxicities (hypertension, liver function test elevation). Bevacizumab and radiation resulted in ≥80% pathologic necrosis in 9 of 20 tumors (45%), which is over double the historical rate with radiation alone. Median microvessel density (MVD) decreased 53% after bevacizumab alone ($p<0.05$), and following combination therapy, median tumor cell proliferation decreased by 73%, apoptosis increased 10.4 fold, and blood flow, blood volume, and permeability surface area as determined by perfusion CT scans decreased by 62-72%

($p<0.05$). Analysis of gene expression microarrays of untreated tumors identified a 24-gene signature for treatment response. MVD and circulating progenitor cell levels at baseline and reduction in MVD and plasma soluble c-KIT with bevacizumab also correlated with good pathologic response ($p<0.05$). After a median follow-up of 20 months, only 1 patient had a local recurrence.

SUMMARY

Several dozen preclinical studies demonstrate that adding anti-angiogenic therapy to radiation for solid tumors improves the efficacy of the radiation. The mechanisms by which angiogenesis inhibitors augment the effects of radiation in tumors remain controversial and require further elucidation. Anti-angiogenic agents such as bevacizumab may counteract the over-expression of VEGF-A in tumors, thus “normalizing” a highly disordered and leaky tumor vasculature and actually improving tumor blood flow and oxygenation [29]. Alternatively anti-angiogenic agents may augment the effects of radiation injury on tumor vasculature [23].

Bevacizumab and other anti-angiogenic agents are currently part of standard medical treatment regimens for patients with metastatic colorectal cancer, lung cancer, and renal cancer [70]. While anti-angiogenic therapies are being fairly rapidly incorporated into chemotherapy regimens for metastatic solid tumors, their use in combination with radiation or chemoradiation for primary tumors has been more measured. There are now a handful of phase I and phase II clinical trials which have examined the addition of bevacizumab to radiation or chemoradiation to enhance local control for certain tumors types including glioblastoma, metastatic brain lesions, head and neck cancer, rectal cancer, pancreatic cancer, and sarcoma. Many of these trials show encouraging results in terms of local control and toxicity profile. However, there are no phase III randomized trials to definitively demonstrate that adding bevacizumab or other anti-angiogenic agents to radiation or chemoradiation improves outcomes such as local control or overall survival. In the future, bevacizumab and other anti-angiogenic agents may become common additions to radiation and chemoradiation regimens for tumors that are difficult to locally control.

Acknowledgments

None declared.

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Table 1

Clinical Trials of Bevacizumab for CNS Tumors

Study	Phase	Number of patients	Presentation	Radiation technique	Radiation dose/fractions	Bevacizumab dose	Chemotherapy	Surgery	Median PFS (mo)	Median OS (mo)
Lai 2011	II	70	Newly diagnosed GBM	Conformal	60 Gy/30	10 mg/kg	Temozolamide	3% biopsy 57% subtotal resection 40% total resection	14.2	19.6
Narayana 2011	II	51	Newly diagnosed GBM	Conformal	59.4 Gy/33	10 mg/kg	Temozolamide	11% biopsy 18% subtotal resection 71% gross resection	13	23
Desjardins 2011	II	75	Newly diagnosed GBM	Conformal	59.4 Gy/33	10 mg/kg	Temozolamide and irinotecan	46% subtotal resection 53% gross resection	14.2	21.2
Gutin 2009	I	25	Recurrent GBM and anaplastic gliomas	Stereotactic	30 Gy/5	10 mg/kg	None	Only 2 patients with resection	7.3-7.5	12.5-16.5

Abbreviations: PFS, progression-free survival; OS, overall survival

Table 2

Clinical Trials of Bevacizumab for Head and Neck Tumors

Study	Phase	Number of patients	Presentation	Radiation dose/fractions	Bevacizumab dose	Chemotherapy	Surgery	Outcome
Seiwert 2008	I	43	Previously radiated or poor prognosis HNSCC	50.4-70 Gy/35	2.5-10 mg/kg	5-fluorouracil and hydroxyurea	None	Median OS 10.7 mo
Salama 2011	II	26 (19 received bevacizumab)	T4N0-1 HNSCC	60 Gy/30	10 mg/kg	5-fluorouracil and hydroxyurea	16% had prior tumor resection	Early termination due to high rate of local progression
Hainsworth 2011	II	60	HN SCC (73% stage IV)	68.4 Gy/38	15 mg/kg	Paclitaxel, carboplatin, 5-fluorouracil, erlotinib	None	95% PR or CR 3-yr PFS 71% 3-yr OS 82%
Lee 2011	II	44	Nasopharyngeal carcinoma	70 Gy/33	15 mg/kg	Cisplatin	None	2-yr OS 90.9% 2-yr PFS 74.7%

Abbreviations: HNSCC, head and neck squamous cell carcinoma; OS, overall survival; PFS, progression-free survival; PR, partial response; CR, complete response

Table 3

Clinical Trials of Bevacizumab for Colorectal Cancer

Study	Phase	Number of patients	Presentation	Radiation dose/fractions	Bevacizumab dose	Chemotherapy	Surgery	Pathologic complete response
Czito 2007	I	11 rectal	T3/T4 or N+	50.4 Gy/28	10-15 mg/kg	Capcitabine, oxaliplatin	9 LAR, 2 APR	18%
Willett 2010	I/II	32 rectal	T3/T4	50.4 Gy/28	5-10 mg/kg	5-fluorouracil	8 APR, 24 LAR	16%
Crane 2010	II	25 rectal	T3/T4 or N+	50.4 Gy/28	5 mg/kg	Capcitabine	6 APR, 18 LAR 1 local excision	32%
Velenik 2011	II	61 rectal	T3/T4 or N+	50.4 Gy/28	5 mg/kg	Capecitabine	70% sphincter preserving	13%
Kennecke 2011	II	42 rectal	Partially fixed T3/T4 or N2	50.4 Gy/28	5 mg/kg	Capcitabine, oxaliplatin	19 LAR, 18 APR 1 proctocolectomy	18%
Koukourakis 2009	II	9 rectal 13 colon	Unresectable tumor	67.2 Gy/15*	5 mg/kg	Amifostine, capecitabine	None	NA

Abbreviations: LAR, low anterior resection; APR, abdominoperineal resection; NA, not applicable

* Biological equivalent dose; conformal hypofractionated (3.4 Gy/fraction × 15) split course accelerated radiation