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Combining angiogenesis inhibition and radiotherapy: A double-edged sword

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ABSTRACT

A large number of patients that undergo radiotherapy develop local failure. To improve the efficacy of treatment, there is an increasing interest in combining radiotherapy with novel targeted therapies. Inhibiting the growth of new tumor blood vessels, i.e. tumor angiogenesis, is such a targeted therapy. Growing tumors induce angiogenesis to ensure an adequate delivery of oxygen and nutrients and several angiostatic drugs have been approved for the treatment of cancer patients. Both pre-clinical and clinical studies have shown that radiotherapy can influence tumor angiogenesis and that angiogenesis inhibition can potentiate the effect of radiotherapy. Therefore, the combination of angiogenesis inhibition and radiotherapy holds a promising future in cancer treatment. However, the radiosensitizing effects of angiogenesis inhibition are transient and recent findings indicate that the effects of irradiation on angiogenesis depend on the dose and treatment schedule. This raises questions regarding the scheduling of both treatment modalities in order to achieve the optimal treatment efficacy with minimal toxicity. In this review the opportunities and pitfalls of combining angiostatic agents with radiotherapy are discussed. The lessons learned from (pre)clinical studies are summarized with an emphasis on scheduling and dosing of the combination therapy. Finally, the opportunities of ongoing clinical studies are discussed and opportunities to improve the combination of angiostatic drugs with radiotherapy are presented.

1. Introduction

More than half of all cancer patients undergo radiotherapy at some stage during their anticancer treatment (Bernier et al., 2004; Delaney et al., 2005). Despite technical advances that continue to improve the efficacy of radiotherapy, a large number of patients still develop local failures (Begg et al., 2011; Bernier et al., 2004). This resistance to therapy is in part related to the effects of the therapy on the tumor microenvironment including the vascular bed (Barcellos-Hoff et al., 2005). Recent pre-clinical studies describe that irradiation has direct effects on the tumor vasculature (Imaizumi et al., 2010; Sofia Vala et al., 2010). In addition, known side effects of radiotherapy, such as telangiectasia, capillary rupture and thrombosis, indicate local vascular dysfunction (Fajardo, 2005). All this points toward a functional relationship between radiotherapy and the vasculature. This is further exemplified by pre)clinical studies demonstrating that targeting the tumor vasculature can potentiate the effect of cytotoxic therapies, including radiotherapy (Bonner et al., 2006; Dings et al., 2007; Geng et al., 2001; Lima et al., 2011; Mauceri et al., 1998). Since the therapeutic efficacy of current angiogenesis inhibitors is limited due to rapid development of resistance, the combination of these agents with radiotherapy has opened new opportunities for cancer therapy (Nieder et al., 2006; Senan and Smit, 2007; Smith et al., 2000). However, recent studies have provided novel insights regarding the effects of radiotherapy on vascular cells and the importance of dose-scheduling when both treatment modalities are combined.

Here, we discuss the opportunities and pitfalls of combining angiostatic agents with radiotherapy. We summarize the current knowledge regarding the effects of irradiation on vascular cells and angiogenesis. In addition, the lessons learned from (pre)clinical studies are discussed with emphasis on the importance of scheduling and dosing of the combination therapy. Finally, ongoing clinical trials are evaluated and opportunities for further improvement of the combination of angiostatic drugs with radiotherapy are put forward.

2. Angiogenesis and cancer therapy

Angiogenesis is the growth of new blood vessels out of pre-existing capillaries. Because expanding tumors have a continuous and increasing need for oxygen and nutrients, tumor cells induce angiogenesis by secreting a variety of growth factors, including vascular endothelial growth factor (VEGF) (Griffioen and Molema, 2000; Potente et al., 2011). Once secreted, these factors activate the key cells of the angiogenesis process, i.e. the endothelial cells.

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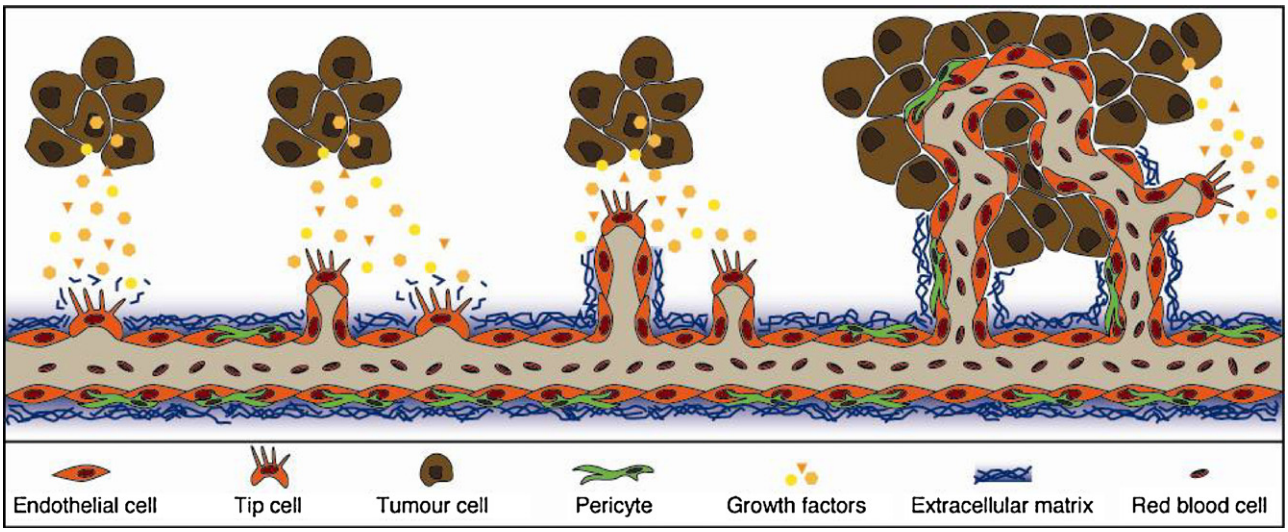

Fig. 1. Tumor angiogenesis. Schematic representation of the angiogenesis process. Under hypoxic conditions, tumor cells start to secrete pro-angiogenesis growth factor. These factors activate endothelial cells in nearby capillaries which start to degrade the extracellular matrix. Subsequently, endothelial cells with filopodia extensions guide proliferating and migrating endothelial cells into the direction of the growth factor gradient. Finally the tip cells connect adjacent sprouts forming a functional vascular network which leads to improved oxygenation of the tissue. The ongoing growth of tumor cells continuously induces new blood vessel formation.

These cells constitute the inner lining of all blood vessels and after activation they produce proteases that degrade the basal membrane and underlying extracellular matrix components. Subsequently, the endothelial cells start to proliferate and migrate, thereby forming novel vascular sprouts that will eventually reassemble into a capillary bed (Fig. 1) (Griffioen and Molema, 2000; Potente et al., 2011). In tumors, the structure of this vascular bed is abnormal and irregular (Jain, 2005; Pries et al., 2009). Moreover, due to vessel leakiness and spatial disorganization, the oxygen supply inside the tumor is not homogeneous, resulting in continuous angiogenesis stimulation. Interfering with tumor angiogenesis has become a promising approach for cancer treatment, as it aims to block the supply of oxygen and nutrients to the tumor cells causing tumor growth arrest or even tumor regression (Verheul and Pinedo, 2007). In the past decade, several anti-angiogenesis drugs have been developed, some of which are FDA approved agents for different types of cancer, including the human monoclonal antibody targeting VEGF (bevacizumab), and small-molecule inhibitors of the VEGF-receptor kinase activity (sunitinib and sorafenib) (Gotink and Verheul, 2010). Unfortunately, so far most current angiostatic drugs have only a moderate effect on the overall patient survival rate (Allegra et al., 2011; Ebos et al., 2009; Escudier et al., 2010; Jain et al., 2006). This is related to rapid development of drug resistance which raises questions regarding potency of current angiostatic therapies (Griffioen, 2007; Potente et al., 2011; Thijssen et al., 2007). Consequently, novel therapies are being developed that target other angiogenic growth factors like placenta growth factor (PlGF) (Van de Veire et al., 2010) or galectin-1 (Dings et al., 2007; Thijssen et al., 2010). On the other hand, angiostatic therapy appears to improve the efficacy of most conventional therapies (Dings et al., 2011; Herbst et al., 1998; Nowak-Sliwinska et al., 2011; Shrimali et al., 2010; Teicher, 1998), including radiotherapy (Dings et al., 2007; Teicher et al., 1994). This has opened a new window of therapeutic opportunities for angiostatic drugs.

3. Rational behind combination of angiostatic drugs and radiotherapy

3.1. Effects of radiotherapy on endothelial cells and angiogenesis

The interaction between radiotherapy and angiogenesis is not well studied. Hlushchuk et al. observed that applying 3 Gy irradiation for four days to mammary carcinoma xenografts in nude mice caused a reduction in tumor vascularization (Hlushchuk et al., 2008). The irradiation predominantly damaged the immature vessels, whereas the more mature vessels appeared resistant to radiotherapy. While no sprouting angiogenesis was observed, four days after ending radiotherapy intussusceptive angiogenesis (vessel splitting) appeared which continued for at least two weeks. After nineteen days, a wave of sprouting angiogenesis was observed which was accompanied by tumor re-growth. The authors suggested that switching between sprouting and intussusceptive angiogenesis might represent an adaptive and protective tumor response to radiotherapy (Hlushchuk et al., 2008). In a more recent study it was also demonstrated that irradiation can inhibit ongoing angiogenesis without affecting the mature vessels. By inserting a matrigel plug in the skin of non-irradiated or locally pre-irradiated mice (single dose of 20 Gy), Imaizumi et al. demonstrated that irradiation inhibits de novo angiogenesis (Imaizumi et al., 2010). The treatment did not disrupt quiescent dermal vessels *in vivo*, nor did it induce apoptosis in those vessels. The latter was also observed performing *in vitro* studies on human umbilical vein endothelial cells (HUVEC) (Imaizumi et al., 2010). These results suggest that irradiation inhibits ongoing angiogenesis without affecting the established vasculature.

However, Geng et al. suggested that the irradiation effects on endothelial cells – and thus on angiogenesis – are dose-dependent. They administered low dose (2–3 Gy) or high dose (6 Gy) irradiation to xenograft tumors of either glioblastomas or melanomas. While increased tumor vascularization and blood flow was observed after a single treatment of 2–3 Gy, the treatment with 6 Gy caused a decrease in tumor vasculature and blood flow (Geng et al., 2001). Interestingly, at day seven the blood flow started to return in the high dose irradiated glioblastoma xenografts and was already greater than the initial blood flow in the high dose irradiated melanoma xenografts (Geng et al., 2001). While the latter result supports the observations by Hlushchuk et al., this study also suggests that low dose irradiation might directly potentiate tumor angiogenesis. This was supported by a study in which increased *in vitro* tube formation by HUVEC was observed after the cells were irradiated with 1 Gy (Vincenti et al., 2011). Low dose irradiation has also been shown to decrease the expression of anti-angiogenesis factors. For example, the change in expression of the miR-17-92 cluster has been studied in endothelial cells after

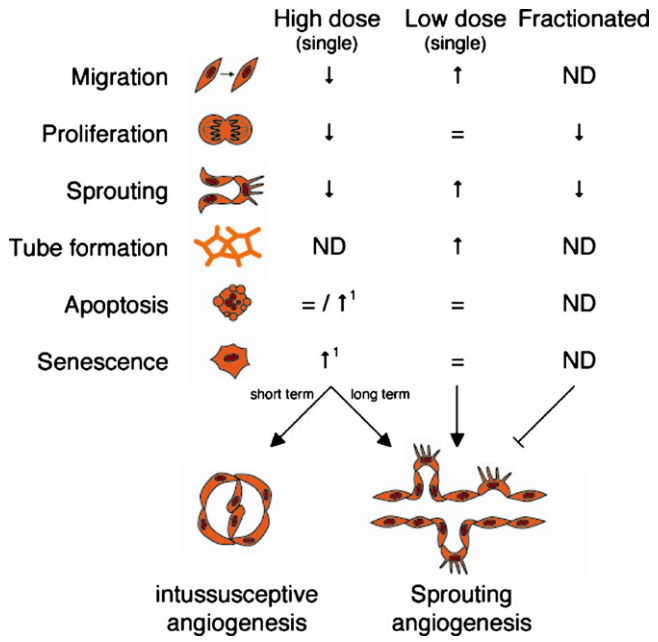

Fig. 2. Effects of irradiation on endothelial cells and angiogenesis. (1) Effect depends on the confluency of the cells. Confluent, i.e. quiescent, cells are apoptosis resistant and become senescent while sub-confluent, i.e. proliferating, cells go into apoptosis. ND = not determined.

irradiation (Vincenti et al., 2011). This cluster is known to promote angiogenesis by decreasing the expression of the angiogenesis inhibitors trombospondin-1 (Tsp-1) and connective tissue growth factor (CTGF) (Heusschen et al., 2010; Urbich et al., 2008). Already 1 h after irradiation (1 Gy), the expression of the miR17-92 cluster in HUVEC was enhanced. This increased expression was still evident after 4 h, but declined after 8 h (Vincenti et al., 2011). More recently, Sofia Vala et al. presented data that further supported the pro-angiogenesis activity of low dose irradiation (Sofia Vala et al., 2010). By applying low dose irradiation (<0. 8 Gy) to cultured human lung micro-vascular endothelial cells (HMVEC-L) they demonstrated enhanced endothelial cell migration without effects on the proliferation or survival of the cells. Moreover, low dose irradiation activated the VEGF-receptor 2 (VEGFR2) and enhanced VEGF expression in conditions mimicking hypoxia (Sofia Vala et al., 2010). In a zebrafish model low dose irradiation accelerated angiogenic sprouting during embryogenesis leading to an irregularly shaped vasculature. The vascular pattern normalized after ten days suggesting that low dose irradiation has a short term and transient effect (Sofia Vala et al., 2010). Finally, in tumor bearing mice, low dose irradiation significantly promoted angiogenesis, accelerated tumor growth, and enhanced metastatic spread (Sofia Vala et al., 2010). The latter, i.e. induction of metastasis following radiotherapy, has been observed in several other studies as well (Camphausen et al., 2001; Kaplan and Murphy, 1949; Suit et al., 1970). Altogether, while high dose radiotherapy hampers angiogenesis, low dose radiotherapy appears to have a pro-angiogenesis effect both *in vitro* and *in vivo*. Thus, radiotherapy can directly affect angiogenesis and the effects depend on the dose and scheduling (Fig. 2). This readily applies to the clinical setting since patients frequently receive fractionated radiotherapy which consists of daily low doses of approximately 2 Gy. Moreover, since the pro-angiogenesis effects of low dose irradiation appear to be rapid and transient, daily administration of 2 Gy might repeatedly stimulate angiogenesis. In the event that the fractionated radiotherapy does not lead to complete tumor eradication, such a proangiogenesis effect might contribute to enhanced tumor re-growth and metastasis. However, until now clinical evidence for such a response is lacking.

Apart from direct effects, several studies have shown that irradiation can also indirectly influence angiogenesis by inducing the expression of pro-angiogenesis growth factors like VEGF by tumor cells or other cells that reside in the tumor stroma (Solberg et al., 2008; Tsai et al., 2007). For example, Nozue et al. found a significant increase in VEGF expression after radiotherapy in rectal carcinomas (Nozue et al., 2001). Macrophages in the stromal tissue have also been shown to enhance their VEGF expression after irradiation. Consequently, inhibiting the tumor-associated macrophages prior to radiotherapy increased the anti-tumor effect (Meng et al., 2010). Besides VEGF and several other pro-angiogenic factors (Allavena et al., 2008), macrophages also release nitric oxide (NO), which plays a role in the stabilization of hypoxia-inducible factor 1 (HIF1) (Li et al., 2007). HIF1, which becomes active in a hypoxic environment, promotes endothelial cells survival and angiogenesis (Semenza, 2003). Moeller et al. demonstrated that inhibition of HIF1 increased the radiosensitivity of the tumor, when it was applied after the irradiation (Moeller et al., 2004, 2005). In nude mice, tumors lacking HIF1 activity were also growing slower after irradiation than wild type tumors (Williams et al., 2005). The role of HIF1 expression during radiotherapy, influenced by cycling hypoxia and free radicals, has been extensively reviewed by Dewhirst et al. (2008). Irradiation also induces local inflammation and activates expression of pro-survival transcriptional factors, including nuclear factor kappa B (NF-κB). This again indirectly stimulates angiogenesis (Deorukhkar and Krishnan, 2010).

Besides stimulating angiogenesis through different pathways, irradiation also induces recruitment of bone marrow (BM) derived circulating cells, like endothelial progenitor cells (EPCs). However, Ahn et al. provided evidence that not the EPCs but CD11b positive myelomonocytic cells are involved in the revascularization of tumors through vasculogenesis. This was linked to matrix metalloproteinase-9 and inhibition of this protein enhanced the response of tumors to radiotherapy and inhibited vascularization of the tumor (Ahn and Brown, 2008). Apparently, irradiation can indirectly stimulates angiogenesis *via* effects on local cells and by recruitment of angiostimulatory cells from distant sites in the body.

Altogether, these data show that the resistance to radiotherapy might be partially caused by the effects of radiation on endothelial cells, stromal cells and angiogenesis. In addition, the effects of radiotherapy on angiogenesis are time- and dose-dependent. This highlights the importance to define the optimal time-point at which angiostatic therapy should be initiated during radiotherapy in order to overcome this mechanism of resistance. In addition, it illustrates the necessity to develop methods that monitor the effects of dose-scheduling irradiation on angiogenesis.

3.2. The vascular normalization window

While the induction of angiogenesis by radiotherapy might be inhibited using angiostatic drugs, disruption of the tumor vasculature is likely to hamper proper perfusion of the tumor tissue. Since poorly perfused, i.e. hypoxic, tumors are more resistant to radiotherapy (Overgaard, 2007; Overgaard and Horsman, 1996), it was anticipated that angiostatic therapy would negatively interfere with radiotherapy. Surprisingly, a study by Teicher et al. suggested a positive interaction between angiogenesis inhibition and radiotherapy. The authors observed that fractionated radiotherapy induced a stronger growth delay of subcutaneously injected lung carcinoma cells when mice were also treated with the angiogenesis inhibitor TNP-470 (Teicher et al., 1994). Similar effects were observed using a different tumor model in rats (Teicher et al., 1995). Combining irradiation with the angiogenesis inhibitor angiostatin also showed a significant reduction of tumor growth

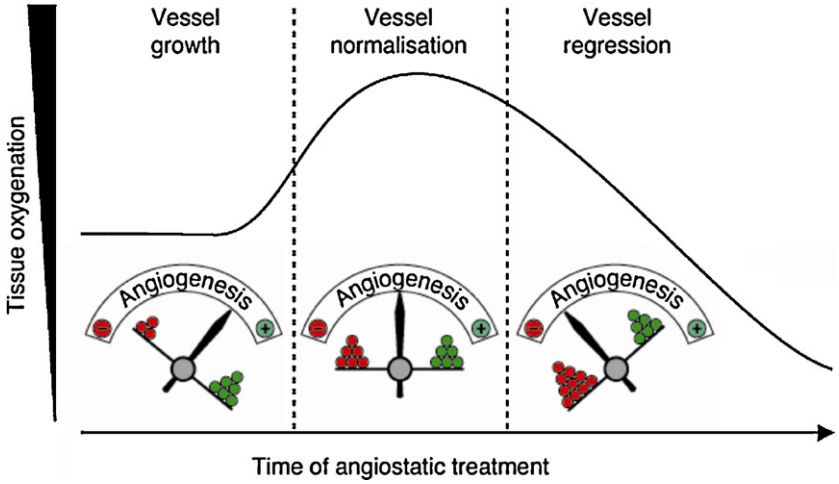

Fig. 3. Vascular normalization window. In the hypoxic tumor environment there is a surplus of angiogenesis growth factors which results in activation of tumor angiogenesis and vessel growth. During angiostatic treatment, the balance between pro- and anti-angiogenesis factors is restored. This caused vessel normalization and improved tissue oxygenation. Prolonged treatment results in an excess of angiostatic factors which will induce vessel regression and a decreased tissue oxygenation.

in mice (Gorski et al., 1998; Mauceri et al., 1998). In addition, the microvessel density (MVD) in tumors treated with combination therapy was decreased as compared to those receiving only radiotherapy (Mauceri et al., 1998). While this suggests increased tumor hypoxia and thus an impaired response to radiotherapy, the studies by Teicher et al. showed that anti-angiogenesis therapy actually increased tumor oxygenation (Teicher et al., 1994, 1995). Although the increased oxygenation could explain the enhanced response to radiotherapy, the mechanism by which angiogenesis inhibitors could decrease tumor hypoxia remained elusive.

One possible explanation involves the interstitial fluid pressure (IFP). In tumors a high IFP is frequently observed due to the poorly developed and leaky vessels in the angiogenic microenvironment (de Bock et al., 2011; Jain, 2005). Milosevic et al. demonstrated that in patients with cervical carcinoma, a high IFP in the tumor was associated with hypoxic tumors (Milosevic et al., 1998). Reducing the IFP using angiogenesis inhibitors might thus improve vessel function resulting in a better oxygen supply. However, other studies showed that elevated IFP in the tumor had no relationship with hypoxia (Lunt et al., 2008; Tufto et al., 1996). Nevertheless, Rofstad et al. demonstrated that elevated IFP in normoxic tumors was associated with elevated VEGF-A expression (Rofstad et al., 2010) and blocking VEGF-A might therefore still lower IFP and enhance oxygen supply. In line with this, Lee et al. described that treatment of human tumor bearing mice with a monoclonal antibody targeting human VEGF reduced the IFP and improves the response to irradiation (Lee et al., 2000).

Based on these and other observations the concept of 'vascular normalization' was introduced (Jain, 2005). Vascular normalization refers to the remodeling of a dysfunctional (tumor) vasculature to a more normal phenotype. It includes increased pericyte coverage of endothelial cells, production of a better basement membrane and decreased vessel dilatation (Tong et al., 2004; Winkler et al., 2004). It occurs when the misbalance between anti-angiogenesis and pro-angiogenesis factors in the tumor microenvironment is restored, e.g. by angiostatic therapy. In a therapeutic setting, vascular normalization is usually not a sustainable condition since continuous application of anti-angiogenesis drugs will shift the balance to the angiostatic side causing a lower blood vessel density and tumor hypoxia (Fig. 3). Therefore, the term 'normalization window' was coined which describes the transient period of vessel normalization with improved tumor perfusion and oxygenation that occurs during anti-angiogenesis therapy. This normalization window, together with the irradiation induced pro-angiogenesis signaling provides a rational to combine anti-angiogenesis therapy with radiotherapy.

4. Preclinical evaluation of the vascular normalization window

To benefit most from combination therapy it is important to determine at which time-point the vascular normalization window occurs and for how long it persists. Winkler et al. monitored tumor hypoxia in U87 glioma tumors in mice during treatment with a VEGFR2-blocking antibody. Tumor hypoxia decreased from the second day and was almost abolished on day five but started to return on day 8. In the same time frame an increase and decrease of vessel coverage by pericytes was observed, suggesting transient vessel normalization (Winkler et al., 2004). Vascular normalization was also observed in mice with an orthotopic glioblastoma, treated with the VEGFR-TKI cediranib (Kamoun et al., 2009). Two days after start of treatment, the vessel permeability and diameter was significantly decreased and normalization of perivascular cell coverage was observed. We also studied tumor oxygenation in three tumor models during treatment with different angiogenesis inhibitors. Treatment with bevacizumab or the angiostatic peptide anginex induced elevated oxygenation levels from days one to four (Dings et al., 2007). On day five, oxygenation levels decreased and dropped below those in control mice. In agreement with Winkler, pericyte coverage increased during elevated oxygen levels (Dings et al., 2007). Moreover, the anti-tumor effect improved when radiotherapy was applied within the window of increased oxygenation (Dings et al., 2007; Winkler et al., 2004). While these studies confirm that angiostatic treatment improves the response to radiotherapy, it remains difficult to translate these findings to the clinic. The tumor growth kinetics in patients differ from animal models, making it difficult to determine when the normalization window occurs in patients. In addition, easy non-invasive methods to repeatedly monitor tumor oxygenation in patients are lacking. Interestingly, Matsumoto et al. first used time domain electron paramagnetic imaging (EPRI) to generate pO_2 maps of the tumors in mice. They achieved a reliable oxygen resolution of approximately 1 mmHg (Matsumoto et al., 2006). Recently they published a study in which they used EPRI and magnetic resonance imaging (MRI) to measure the pO_2 and MVD in tumor bearing mice. Using these non-invasive methods they observed that treatment with sunitinib improved tumor oxygenation from days two to four, confirming the concept of vascular normalization (Matsumoto et al., 2011) In

a different study, the fluctuations in vasculature of the tumor were measured using T2*-weighted gradient-echo magnetic resonance imaging. It was suggested that these fluctuations were associated with altered blood flow and thus hypoxia (Baudelet et al., 2004). Future research should further focus on the development of tools to monitor tumor perfusion and oxygenation during angiostatic therapy to identify the optimal time point to start combination therapy.

5. Combination therapy

5.1. Lessons from pre-clinical studies

As illustrated above, effective combination therapy relies on proper dose-scheduling of both treatment modalities. In 1998 a study was conducted in mice to investigate different treatment schedules combining irradiation with the endogenous angiogenesis inhibitor angiostatin (Gorski et al., 1998; O'Reilly, 1997). Angiostatin administered concomitantly with irradiation for 2 days was more effective as compared to administration after irradiation (adjuvant). Continuation of angiostatin treatment for 14 days following the concomitant therapy had no additional effects (Gorski et al., 1998). Opposite effects were described by Zips et al. Here, fractionated radiotherapy (15×2 Gy) was combined with PTK787/ZK222584 which inhibits all three VEGF receptors. Both in the concomitant and neo-adjuvant setting no additional effects were observed compared to radiotherapy alone. In contrast, adjuvant treatment resulted in slower re-growth of human squamous cell carcinoma in tumors of mice (Zips et al., 2003). Lack of activity in a concomitant setting was also reported for a vascular-targeting agent that disrupts the endothelial tubulin cytoskeleton (Wachsberger et al., 2005). Treatment 1 h before radiotherapy even appeared to slightly enhance tumor growth (Wachsberger et al., 2005). In another study, administration of the VEGFR-2 inhibitor ZD64742 2 h before irradiation did delay tumor growth, albeit less than sequential administration which was applied 30 min after irradiation (Williams et al., 2004). In a study with fibrosarcoma tumors in mice, anti-VEGFR2 DC101 and anti-VEGF G6-31 were applied before or after single dose radiotherapy. The angiostatic agents were only radiosensitizing when delivered immediately prior to single dose radiotherapy (<1 h). Other schedules, applying the angiostatic drugs 24 h or 48 h prior to radiotherapy, or directly after the radiotherapy had no additional anti-tumor effect (Truman et al., 2010). The anti-tumor effect of blocking the delta-like 4 (Dll4)/Notch pathway after radiotherapy was studied in Dll4 expression or Dll4 non-expressing cancer cell lines as xenografts in mice. Administration of Dll4 antibody or the Notch-inhibitor DBZ after radiotherapy resulted in a supra-additive growth delay. This was due to nonfunctional tumor angiogenesis and extensive tumor necrosis (Liu et al., 2011).

Altogether, the effects of combination therapy are inconsistent or even conflicting. Interestingly, in all studies mentioned above, no side effects or normal tissue toxicity were described or observed. Vascular targeting agents are considered to have a low toxicity level, as they should only affect the angiogenic vessels. However, toxicities have been observed in the clinical setting when angiostatic drugs are combined with cytotoxic therapies as discussed later on. To decrease the toxicity, lower doses of the drugs and radiotherapy might be used. This might be feasible when both modalities are precisely scheduled. This altogether illustrates the complexity of studying the dose-scheduling effects on the efficacy of combination therapy. Overall, more studies suggest a beneficial effect of concomitant or adjuvant treatment as compared to neo-adjuvant administration. This lack of activity in the latter is most likely related to ineffective timing of radiotherapy. Prolonged neo-adjuvant therapy will cause vessel regression while brief neo-adjuvant therapy will not have induced vessel normalization at the time when radiotherapy is applied. Indeed, we observed an enhanced anti-tumor effect when radiotherapy was applied within the window of normalization as compared to either mono-therapy, concomitant therapy or prolonged neo-adjuvant treatment (Dings et al., 2007). Similar results were reported by Matsumoto et al. (2011) which further suggests that neo-adjuvant angiostatic therapy can enhance the anti-tumor effects of radiotherapy when the latter is scheduled precisely within the normalization window. This again demonstrates the importance of scheduling and a future challenge will be to translate this knowledge into clinical practice.

5.2. Lessons from clinical trials

Although valuable, the experiments in cultured tumor cells or tumor models in mice are only partly representative for slowly developing tumors in patients. Thus, clinical trials are essential to expand the knowledge regarding combination treatment and how to implement this therapy in a clinical setting. Several phase I clinical trials have been performed to study the effects of combining angiostatic drugs with (chemo)radiation. For example, in a phase I dose escalation study in patients with locally advanced pancreatic cancer, bevacizumab was combined with radiotherapy and capecitabine. The patients received bevacizumab 2 weeks before and every 2 weeks during radiotherapy. Only 1 of the 46 assessable patients displayed progressive disease (PD) while 9 showed partial response (PR) and the remaining patients minor response (MR) or stable disease (SD). The median survival from the start of the protocol was 11.6 months (Crane et al., 2006). Encouraging response rates were also observed when bevacizumab was combined with oxaliplatin, capecitabine and radiotherapy in patients with rectal cancer (Czito et al., 2007). All drugs were administered concomitantly with radiotherapy and of the 11 patients enrolled, 2 had a complete response (CR), 4 had PR and 5 had SD (Czito et al., 2007). In a recently published phase II study, rectal cancer patient also received bevacizumab combined with concomitant capecitabine and radiotherapy (Gasparini et al., 2012). Six out of the 43 patients had no residual cancer, a varying percentage of cancer cells was found in 22 patients. Of the 40 patients that underwent resection, negative circumferential margins were achieved in 38 patients (Gasparini et al., 2012). A slightly less but still considerable clinical benefit was observed in a phase I dose escalation study, where bevacizumab was added to chemoradiation in patients with poor-prognosis head and neck cancer. Of the included 43 patients, the response was assessable in 26 patients. The overall response rate was approximately 85% and almost 70% of patients had CR. The median overall survival was 10.7 months albeit that overall survival for patients which had received prior radiotherapy (9.2 months) was significantly lower as compared to those that were not previously radiated (40.1 months) (Seiwert et al., 2008).

Altogether, the outcomes on tumor response and patient survival appear predominantly positive. On the other hand, as discussed before, one should be aware of potential toxicities when an additional drug is added to conventional treatment schedules. In the previously discussed phase I trials, adding bevacizumab was generally well tolerated. In the study on locally advanced pancreatic cancer, bleeding duodenal ulcers (3 patients) and duodenal perforation (1 patient) were observed in the 30 first patients (Crane et al., 2006). These bleedings were tumor associated and might be attributed to bevacizumab. For the remainder of the study, patients with duodenal involvement were excluded. In the study in rectal cancer patients it was concluded that a dose of 15 mg/kg bevacizumab on day 1 followed by oxaliplatin and capecitabine during radiotherapy had an acceptable toxicity profile, with mainly grade II diarrhea and fatigue (Czito et al., 2007). The addition of

bevacizumab did not appear to increase the incidence of the toxicity related side effects compared to chemotherapy alone. In the trial with poor-prognosis head and neck cancer patients, a reduction of chemotherapy dosing was necessary, because of neutropenia (Seiwert et al., 2008). Possible toxicities related to bevacizumab were two deep venous thromboses, a fatal stroke and two fatal bleeding events, although the direct relation with bevacizumab was not established (Seiwert et al., 2008). In contrast, a direct effect of bevacizumab on gastrointestinal (GI) perforation has been described extensively in the literature. In a meta-analysis of 3 phase II and 14 phase III trials, it appeared that high dose bevacizumab (5 mg/kg/week) induced a risk of 6.95% of GI perforation in patients with colorectal cancer (Hapani et al., 2009). Low dose bevacizumab (2.5 mg/kg/week) induced a much lower incidence of GI bleeding (2.86%). In patients with renal cell carcinoma and ovarian cancer, also a high incidence of GI perforation (resp. 5.67% and 5.4%) was observed, whereas in other cancer types (i.e. pancreatic cancer, breast cancer) a much lower incidence (0.63%) was seen (Han and Monk, 2007; Hapani et al., 2009). Not only bevacizumab, but also angiogenic TKIs seem to induce GI perforation (Walraven et al., 2011), also when administered after radiotherapy (Peters et al., 2008). Because of this potentially lethal side effect, careful patient monitoring is still required when angiostatic agents are added to chemoradiation protocols. In addition, it should be considered to exclude patients of angiostatic therapy that have a medical history of GI ulceration or recent GI bleeding. Furthermore, although other side effects are generally well tolerated, addition of bevacizumab to (chemo)radiation should be closely monitored for toxicities. This necessity is further illustrated in a phase II trial, in which the effect of vandetanib on survival was compared with placebo in small cell lung cancer patients. Vandetanib (ZD6474), a TKI of VEGFR and epidermal growth factor receptor (EGFR), was orally administered at 300 mg/day after CR or PR to chemo(radio)therapy. Vandetanib was continued until disease progression or intolerable toxicity was observed, or for a maximum of 2 years. Surprisingly, vandetanib failed to show any efficacy in both overall survival and progression free survival compared to placebo. Furthermore, while less thromboembolisms and hemoptysis were observed, patients treated with vandetanib were more likely to display severe toxicities such as elevated liver enzymes, diarrhea and fatigue (Arnold et al., 2007). Lack of additive effects was also described in a phase III trial, which compared radiotherapy plus thalidomide with radiotherapy alone in patients with multiple brain metastases from an extracranial primary tumor (Knisely et al., 2008). Of 183 patients who underwent whole-brain radiotherapy (WBRT), 90 received thalidomide. The addition of thalidomide did not result in a survival benefit for patients with multiple, large or midbrain metastases. Although no novel toxicities were observed, 48% of the patients receiving thalidomide discontinued the drug because of side effects (Knisely et al., 2008). Comparable results combining thalidomide and irradiation were described by Atkins et al., in 2008 (Atkins et al., 2008).

These results show that toxicity is an important issue when combination therapy is applied in the clinic. Moreover, the efficacy of combination therapy appears to depend on the type of angiostatic drug. Thus far, the results of the (pre)clinical trials demonstrate mostly promising results. However, these studies have also made evident that proper dose-scheduling of both treatment modalities is one of the key delimiters for obtaining the best therapeutic results with minimal toxicity. Unfortunately, the clinical trials that have studied different dose-schedules are scarce. However, the vascular normalization window has been studied in patients. The VEGFR-TKI cediranib induced vascular normalization already at day 1 of treatment, which endured until day 28, in patients with glioblastoma (Batchelor et al., 2007). The technique to measure the 'vascular normalization index' (VNI) using MRI in recurrent glioblastoma has already been optimized and has been shown to correlate with patient outcome after a single dose of cediranib (Emblem et al., 2011). Besides cediranib, it also has been demonstrated that bevacizumab induced vascular normalization 12 days after treatment in patients with rectal carcinoma (Willett et al., 2004). Future studies and trials should focus on proper dose-scheduling of both treatment modalities. Given the diversity of different tumor types and the (increasing) number of available angiostatic agents it will be a challenge to determine which combination therapy has the best efficacy for specific patients.

5.3. Lessons to be learned

Currently, a variety of phase II/III clinical trials are being performed, combining (chemo)radiation with different angiostatic drugs (Table 1). Most of these trials use the two main types of angiostatic drugs that are FDA approved, i.e. (1) monoclonal antibodies targeting growth factors or growth factor receptors, e.g. bevacizumab, or (2) small molecule inhibitors of TKIs, e.g. sunitinib, sorafenib. In the future, this list might be expanded with small molecule inhibitors of the serine/threonine kinase mTOR (temsirolimus, everolimus) (Kim et al., 2008; Kirova et al., 2010; Schiewer et al., 2012). While the ongoing trials provide valuable insights in the efficacy of combination therapy they only give limited information regarding the optimal dose-scheduling protocols. No clinical trials are currently performed which compare different schedules of the combination therapy for the efficacy and toxicity. The setup of the ongoing studies is mostly too diverse to permit a direct comparison of therapeutic outcome. Only a few studies allow comparison of different scheduling regimes, e.g. two phase III trials combining radiotherapy with bevacizumab as either a neo-adjuvant/concurrent treatment or as concurrent/adjuvant treatment. Since both trials enroll patients with newly diagnosed glioblastoma multiforme that will receive comparable chemoradiation treatment, the efficacy of differential bevacizumab scheduling might be compared. The same is true for two phase II trials for patients with locally advanced rectal cancer. In these trials comparable chemoradiation therapy is combined with bevacizumab either administered concurrent or neo-adjuvant. However, since there is still no clear clinical rational for administration of angiostatic agents in a neo-adjuvant, concurrent, or adjuvant setting it would be worthwhile to design a clinical trial which includes different treatment schedules within the same patient population. Not only efficacy but also toxicity should be closely evaluated in such a trial.

Another important issue to be addressed relates to patient-tailored medicine. Especially the introduction of targeted therapies has raised awareness that the response to therapy is most likely associated with the presence of the drug target in a specific patient. Thus, diagnostic tools are being developed to determine the availability – and thus the potential responsiveness – of drug targets. While this readily applies to the before mentioned angiostatic agents, it is especially relevant in the case of combination therapy. For example, the exact occurrence and timing of the vascular normalization window during angiostatic therapy in patients is still not well studied. Thus, a future challenge will be to develop rapid non-invasive methods to monitor, e.g. tumor oxygenation during neo-adjuvant angiostatic therapy. Similarly, monitoring the expression of pro-angiogenesis growth factors during radiotherapy could provide a rational to start concurrent or adjuvant angiostatic therapy. Moreover, it will help to decide which angiostatic agent is most likely to have a beneficial effect. Resolving these issues will eventually improve the therapeutic efficacy of the combination therapy of angiostatic drugs and radiotherapy.

Table 1
Current clinical trials with combination of angiostatic drugs and radiotherapy.

Angiostatic drug	Phase	Disease[1]	Scheduling[2]	Radiotherapy Rth[3]	Chemotherapy	Neo/adj to surgery
Bevacizumab						
NCT00570531	II	EC (locoregional)	conc	25 × *x* Gy	Paclitaxel/cisplatin/5-FU	neo
NCT00354679	II	ES (LA)	neo/conc	30 × *x* Gy	Cisplatin/irinotecan	neo
NCT01332929	I	Brain metastases	neo/conc	15 × 2 Gy or 10 × 3 Gy		
NCT00817284	II	GBM	neo/conc	30 × 2.0 Gy	Temozolomide *vs* irinotecan	
NCT00805961	II	GBM (first line treatment)	conc/adj	30 × 2 Gy	Temozolomide/everolimus	
NCT00590681	II	GBM (ND)	adj	30–33 × 1.8–2.0 Gy	Temozolomide	adj
NCT01186406	II	GBM (ND)	conc/adj	Standard *R*th for 6.5 weeks	Gliadel/temozolomide	adj
NCT00884741[a]	III	GBM (ND)	conc/adj	30 × *x* Gy	Temozolomide	
NCT00943826[a]	III	GBM (ND)	neo/conc	30 × 2 Gy	Temozolomide	
NCT01102595	II	GBM (unresectable)	neo/conc	30 × 2 Gy	temozolomide	
NCT01022918	II	GBM (unresectable)	neo/adj	X × *x* Gy	Temozolomide/irinotecan	
NCT00597402	II	GBM and gliosarcomas	conc/adj	33 × *x* Gy	Temozolomide/irinotecan	
NCT01209442	II	GBM	conc/adj	60 Gy in 2 weeks	Temozolomide	
NCT01013285	II	GBM or gliosarcoma (ND)	conc/adj	30 × *x* Gy	Temozolomide	
NCT01443676	II	GBM (eldery patient)	conc	Unknown		
NCT00369122	II	Cervical cancer (LA)	conc	45 Gy in 25 fractions	Cisplatin	
NCT00545792	II	Gynecological cancer (recurrent)	conc	Standard *R*th		
NCT00703976	II	HNSCC (LA)	conc	35 × 2 Gy	Cetuximab/pemetrexed	
NCT00281840	II	HNSCC (stage III/IV)	conc/adj	40 × *x* Gy	docetaxel	neo
NCT01004874	II	Malignant glioma (grade IV)	conc/adj	Standard *R*th for 6.5 weeks	Temozolomide/topotecan	adj
NCT00782756	II	Malignant glioma (ND)	neo/conc/adj	3 × *x* Gy/week for 2 weeks	Temozolomide	
NCT01478321	II	High grade recurrent malignant glioma	conc/adj	25 × *x* Gy	Temozolomide	
NCT00387374	II	NCSLC (stage IIIB/IV unresectable)	adj	10 × *x* Gy	Carboplatin/paclitaxel	
NCT00896181	II	NPC (advanced)	neo/conc	30–35 × *x* Gy	Doxatel/cisplatin/5-FU/carboplatin	
NCT00408694	II	NPC (Stage IIB-VB)	conc/adj	33 × *x* Gy	Cisplatin/5-FU	
NCT00334815	I/II	NSCLC (stage III irresectable)	conc	35 × *x* Gy	Cisplating/etoposide	
NCT00402883	II	NSCLC (stage III)	conc/adj	35 × 1.8 Gy	Premetrexed/Carboplatin	
NCT00578149	II	NSCLC (stage III)	conc/adj	35 × *x* Gy	Paclitaxel/carboplatin	
NCT00307723	I/II	pancreatic cancer	conc	35 × *x* Gy	Oxaliplatin/5-FU	
NCT00336648	II	Pancreatic cancer	conc/adj	28 × 1.8 Gy	Gemcitabine	neo
NCT00460174	II	pancreatic cancer (localized)	neo	15 × 2.4 Gy	Gemcitabine	neo
NCT00557492	II	Pancreatic cancer (potentially resectable)	conc/adj	10 × 3 Gy	Gemcitabine	neo
NCT00349557	II	Prostate cancer (high risk)	conc/adj	IMRT	Bicalutamide	
NCT00321685	II	RC (LA nonmetastatic)	conc	28 × 1.8 Gy	Capecitabine/oxaliplatin (FOLFOX)	neo (adj)
NCT00113230[b]	II	RC (LA)	conc	28 × 1.8 Gy	Capecitabine	neo
NCT01434147	II	RC (LA)	neo	25 × 1.8 Gy	Capecitabine/oxaliplatin	neo
NCT00557713	II	RC (LA)	neo/conc	28 × 1.8 Gy	Oxaliplatin/capecitabine	
NCT00842686[b]	II	RC (LA)	neo/conc	28 × 1.8 Gy	Capecitabine	neo
NCT00865189	II	RC (LA)	neo/conc	35 × *x* Gy	FOLFOX/5-FU	neo
NCT01043484	II	RC (localized)	conc	25 × 1.8 Gy	Capecitabine	neo
NCT00308516	II	RC (stage II/III)	conc/adj	28 × 1.8 Gy	FU/FOLFOX6	adj
NCT01481545	II	RC (poor risk)	conc/adj	25 × 1.8 Gy		
NCT00356031	II	Sarcoma	neo	30 × *x* Gy		neo
NCT00308529	II	SCLC (LA)	neo/conc/adj	34 × 1.8 Gy	Irinotecan/carboplatin	
NCT00193375	II	SCLC (limited stage)	adj	*X* × *x* Gy	Irinotecan/carboplatin	
NCT00387699	II	SCLC (limited stage)	conc/adj	15× twice daily *x* Gy	Cisplatin/etoposide	
Bevacizumab/erlotinib						
NCT00393068	II	EC (operable)	conc	25 × *x* Gy	5-FU/paclitaxel/carboplatin	
NCT00720356	II	GBM or gliosarcoma (ND)	conc/adj	30 × *x* Gy	temozolomide	
NCT00140556	I	HNSCC/NPC	conc	70 Gy in 7 weeks	Cisplatin	
NCT00392704	II	HNSCC (LA)	neo/conc	35 × *x* Gy	Paclitaxel/carboplatin/5-FU	
NCT00280150	I/II	NSCLC (stage III)	conc	74 Gy in 35 fractions	carboplatin	neo
NCT00614653	I	Pancreatic cancer	conc	28 × 1.8 Gy	Capecitabine	
NCT00735306	I/II	Pancreatic cancer	conc	28 × 1.8 Gy	Raltitrexed/oxaliplatin/5-FU	neo
NCT00307736	I/II	RC (LA)	conc	3 cycles of 10 × *x* Gy	5-FU	
NCT00543842	I/II	RC (LA)	conc	28 × 1.8 Gy	Capecitabine	
Bevacizumab/cetuximab						
NCT00703976	II	HNSCC (LA)	conc/adj	35 × 2 Gy	Pemetrexed	
NCT01262859	II	HNSCC (LA)	neo/con	35–37 × 2 Gy	Cisplatin	neo
NCT00968435	II	HNSCC (Stage III/IV)	neo/conc	70 Gy in 33 fractions	Cisplatin	

Table 1 (*Continued*)

Angiostatic drug	Phase	Disease[1]	Scheduling[2]	Radiotherapy Rth[3]	Chemotherapy	Neo/adj to surgery
Endostar						
NCT01158144	II	NSCLC (LA)	conc	30–33 × 2 Gy	Paclitaxel/carboplatin	
Endostatin						
NCT01218594	II	NSCLC (LA)	neo/conc/adj	30–33 × 2 Gy	Doxatel/cisplatin	
NCT01211002	IV	NSCLC (LA)	conc	30–33 × 2 Gy	Etoposide/cisplatin	
Sorafenib						
NCT00822848	I	Soft tissue sarcoma	neo/conc	28 Gy in 8 fractions	Epirubicin/Ifosfamide	neo
NCT00610246	I	cancer (not eligable for curative treatment)	neo/conc/adj	*X* × *x* Gy		
NCT00544817	II	GBM (post-surgical)	adj	30 × 2.0 Gy	Temozolomide	
NCT00892658	I	HCC	neo/conc/adj	3 fractions in 2 weeks		
NCT01328223	II	HCC (advanced)	conc/adj	23–24 × 2.0–2.5 Gy		
NCT00892424	I/II	Liver metastasis (unresectable)	neo/conc	3 × *x* Gy/week for 2 weeks		
NCT00609934	I/II	RCC with bone metastasis	conc/adj	10 × 3 Gy		
SU5416						
NCT00023725	I/II	Soft tissue sarcoma	neo/conc/adj	25 × *x* Gy		neo
NCT00023738	I/II	Soft tissue sarcoma	conc/adj	2 cycles of 11 × *x* Gy	Doxorubicin/ifosfamide/dacarbazine	neo/adj
NCT01308034	I	Non GIST Sarcomas	conc	30 × *x* Gy		
Sunitinib						
NCT01100177	II	GBM (ND)	neo/conc	30 × 2 Gy		
NCT01498835	I	Soft tissue sarcoma	conc	50.4 Gy in 28 fractions		
NCT00753727	I/II	Soft tissue sarcoma	neo/conc	28 × 1.8 Gy		
NCT00631527	I	Prostate cancer	conc	40 fractions of *x*Gy	Hormone therapy	
Thalidomide						
NCT00049361	II	Brain metastases (ND)	conc/adj	15 × *x* Gy	Temozolomide	
NCT00033254	III	Brain metastases	conc/adj	15 × 2.5 Gy		
Vandetanib						
NCT00745732	I/II	NSCLC	neo/conc	15 × 3 Gy or 33–35 × 2 Gy		

[a+b] Studies with a similar setup that allow comparison of different scheduling protocols.

[1] RCC = renal cell carcinoma; LA = locally advanced; HNSCC = head and neck squamous cell cancer; NSCLC = non-small cell lung cancer; EC = esophageal cancer; RC = rectal cancer; ND = newly diagnosed; GBM = glioblastoma; NPC = nasopharyngeal cancer; HCC = hepatocellular carcinoma; SCLC = small cell lung cancer.

[2] Scheduling of angiostatic drug to radiotherapy neo = neoadjuvant; conc = concurrent; adj = adjuvant.

[3] Radiotherapy is applied at a frequency of 5 days/week unless indicated otherwise. When the dose applied is unknown, this is indicated with × Gy.

6. Summary

Preclinical and clinical observations have shown a functional relationship between radiotherapy and the tumor vasculature. While radiotherapy can influence angiogenesis, the inhibition of angiogenesis can potentiate the effects of radiotherapy. With more than 50% of cancer patients receiving radiotherapy and an increasing number of angiostatic agents appearing in the clinic, the combination of both therapies holds a promise for future treatment strategies. Already, several (pre)clinical trials combining both therapies have been performed and many clinical trials are ongoing. While most studies demonstrate promising results there is still room for improvement. To achieve this, several issues regarding optimal dosing and scheduling have to be resolved. One highly important aspect is the development of non-invasive methods that can be used to monitor oxygenation and pro-angiogenesis signaling in tumors both prior to therapy as well as during therapy. This will increase the efficacy and reduce the toxicity when both treatment modalities are combined. Ultimately, this should lead to a better and more patient specific treatment of cancer.

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