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## Modulating the tumor microenvironment to increase radiation responsiveness

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### Abstract

Radiosensitivity can be influenced both by factors intrinsic and extrinsic to the cancer cell. One of the factors in the tumor microenvironment (TME) extrinsic to the cancer cell that can affect radiosensitivity is oxygenation. Severely hypoxic cells require a 2–3 fold higher dose of radiation to achieve the same level of cell killing as do well-oxygenated cells. Other elements in the microenvironment that may influence tumor radiosensitivity are the response of stromal cells to radiation and the expression of factors such as vascular endothelial growth factor (VEGF) and hypoxia inducible factor-1 (HIF-1). There are currently several classes of agents that may increase tumor radiosensitivity by modulating the TME. Pre-clinical evidence indicates that inhibition of VEGF may increase local control after radiation. Several mechanisms have been postulated to explain this including radiosensitization of tumor endothelial cells, prevention of the establishment of new vasculature post-radiation, and increased oxygenation secondary to vascular normalization. Agents targeting HIF-1 also increase local control after radiation in pre-clinical models. This may occur via indirect inhibition of VEGF, which is a downstream target of HIF-1, or by VEGF-independent means. When combined with radiation, the EGFR inhibitor cetuximab improves local control and survival in patients with head and neck cancer. Pre-clinical data indicate that EGFR inhibitors can increase the intrinsic radiosensitivity of cancer cells. They can also improve tumor blood flow and oxygenation, which may increase extrinsic radiosensitivity. One of the pathways downstream of EGFR that may contribute to this effect is the PI3K/Akt pathway. Agents that directly inhibit this pathway improve blood flow and increase tumor oxygenation in pre-clinical models. The challenge remains to obtain clinical data from patients showing that modulation of the TME is an important mechanism by which biological agents can radiosensitize tumors and then to utilize this information to optimize therapy.

### Keywords

radiation; radiosensitization; vascular normalization; EGFR; VEGF; HIF; PI3 kinase; Akt; tumor microenvironment

### Introduction

Radiation therapy has been used for over a hundred years to treat patients with cancer; however, in spite of this long track record, it is difficult to achieve local control in many patients. To improve the efficacy of radiation therapy, it is important to understand mechanisms of radioresistance. Radiosensitivity can be influenced by both factors intrinsic and extrinsic to the cancer cell. Some investigators feel that the sensitivity of the cancer cell

itself is the predominant factor in determining the radiation response of a tumor.<sup>1</sup> As will be discussed later in this review, the activation of certain oncogenes may be associated with increased resistance to radiation. Factors extrinsic to a tumor cell can also contribute significantly to a tumor's response to radiation. There has been a great deal of recent interest in the role that host cells that reside in the stroma may play in the radiation response. Studies have shown that when identical tumors are implanted into mice with variable genetic backgrounds, there is an increased radiation response in tumors in mice with sensitive genetic backgrounds.<sup>2,3</sup> These studies suggest that the host component plays an important role in tumor response and is thus an important target in cancer therapy. In particular, some investigators feel that the vascular endothelium may play a major role in the radiation response.<sup>3-5</sup> Kolesnick and colleagues performed experiments suggesting that radiation-induced apoptosis in the vascular endothelium of the microvasculature supplying the gastrointestinal (GI) tract is responsible for radiation-related GI toxicity rather than direct damage to the stem cells lining the GI tract.<sup>4</sup> However, Ogawa et al. used isogenic tumor cell lines that were either competent or defective in DNA double-strand break repair and reached the conclusion that tumor cell radiosensitivity was the major determinant of tumor response in nude mice.<sup>6</sup> However, in severe combined immunodeficiency (SCID) mice, both tumor cell sensitivity and radiation-induced stromal damage played a role.<sup>6</sup> Therefore, the question of the relative importance of stromal versus tumor cells in the radiation response is unresolved.

Another factor extrinsic to the tumor cell that can influence radiosensitivity is hypoxia, which is commonly seen in human cancers.<sup>7</sup> Cancer cells also display increased glycolysis and CO<sub>2</sub> production, resulting in acidification of the tumor microenvironment.<sup>8</sup> This acidic and hypoxic microenvironment can make cells resistant to both radiation and chemotherapy.<sup>9</sup> Hypoxic cells require higher doses of radiation compared to oxic cells to achieve the same level of killing due to the requirement that oxygen be present during irradiation to elicit maximal DNA damage through the creation of free radicals.<sup>10</sup> The oxygen enhancement ratio (OER), the ratio of radiation doses that confer the same level of cell death without and with oxygen is 2.5–3 at a high fractional dose (10–20 Gy) and approximately 2 at a fractional dose of ~2 Gy.<sup>10,11</sup> Clinical data indicate that hypoxia is associated with a worse outcome following radiation in many tumors including head and neck cancers.<sup>12-14</sup>

There are currently several classes of agents that might alter the tumor microenvironment TME in a way that could increase tumor radiosensitivity. In this review, we discuss the preclinical and clinical data that exist for using these agents, focusing on their ability to alter the TME. The biological agents that are currently being tested with radiation may alter both the cancer cell's intrinsic radiosensitivity, and, by altering the TME, their extrinsic radiosensitivity. In this review we will briefly discuss the former but focus on the latter.

## Targeting the Vasculature to Improve Radiation Response

The idea of targeting the tumor vasculature as cancer therapy goes back to Dr. Judah Folkman, who in 1971 first proposed the idea that this might be an effective cancer treatment.<sup>15</sup> The rationale for this idea was the finding that tumors required the development of a new network of blood vessels to sustain growth beyond 1 mm<sup>3</sup>.<sup>16,17</sup> The idea of targeting the vasculature as an adjunct to radiation therapy has also been proposed.

There have been two main strategies used to target tumor vessels, vascular disruptive agents (VDAs) and anti-angiogenic agents. VDAs target pre-existing tumor vessels<sup>17</sup> whereas the latter target the angiogenic process itself by inhibiting the action of factors that stimulate new blood vessel development.<sup>18</sup> ZD6126, an example of a VDA, disrupts the tubulin

cytoskeleton of endothelial cells, leading to their death. In preclinical studies, this agent has been shown to enhance the anti-tumor effects of radiation in large tumors (>1 g) but not small ones (<0.3 g).<sup>19</sup> Another VDA, OXi4503, was shown to reduce the radiation dose required to control 50% of the tumors (TCD50) from 52 Gy without the drug to 41 Gy with the drug.<sup>20</sup>

The other strategy to target tumor vasculature is to inhibit angiogenesis. As tumors grow, they outstrip their blood supply, leading to regions that are underperfused and poorly oxygenated. This stimulates the expression of the  $\alpha$  subunit of hypoxia-inducible factor-1 (HIF-1), a key transcription factor that plays an important role in cellular and systemic responses to hypoxia. HIF-1 $\alpha$  is upregulated across a broad range of cancers and regulates key features of tumor biology such as angiogenesis and glucose metabolism.<sup>21,22</sup> One of the genes upregulated by HIF-1 is vascular endothelial growth factor (VEGF), a potent mediator of angiogenesis that enhances endothelial cell survival, induces vasodilatation and regulates pericyte coverage.<sup>23</sup> Both HIF-1 $\alpha$  and VEGF have been tested as potential targets for improving the radiation response.

VEGF expression leads to hyperproliferation of blood vessels, which should improve oxygenation within tumors. However, VEGF also has another important property, that of increasing vascular permeability.<sup>24</sup> In fact, VEGF was originally discovered as a vascular permeability factor and termed VPF.<sup>25</sup> Perhaps because the supraphysiologic secretion of VEGF in tumors causes excessive leakiness or perhaps because there is not a coordinate expression of other key angiogenic factors, the vessels within a tumor appear dilated, saccular, tortuous and are poorly functional with sluggish blood flow. Therefore, in spite of high levels of VEGF, which should act as a compensatory mechanism, tumors continue to have regions of hypoxia.

Pre-clinical work has shown that VEGF can be induced in response to radiation and that inhibition of VEGF can increase tumor control after radiation. Gorski et al. showed that VEGF was induced after irradiation both in vitro and in vivo in several different cell lines.<sup>26</sup> Treatment of mice bearing xenografts with a neutralizing antibody to VEGF prior to irradiation led to a synergistic effect on the inhibition of tumor regrowth. The authors postulated that this was mediated via an effect on the endothelial cell rather than the tumor cells because in vitro anti-VEGF treatment potentiated radiation-induced lethality of human umbilical vein endothelial cells (HUVECs) but not tumor cells. Lee et al. also used an anti-VEGF antibody in a mouse xenograft model to show that VEGF inhibition in combination with radiation led to a delay in tumor regrowth, which was additive for LS174T colon carcinoma xenografts and greater than additive for U87 glioblastoma xenografts.<sup>27</sup>

Another strategy to inhibit VEGF signaling is to block its function downstream at the level of the endothelial cell. Hess et al. used PTK787/ZK222584, an inhibitor of the VEGF receptor, and found that when this drug was given concurrently with radiation there was a greater delay in the regrowth of SW480 colon adenocarcinoma xenografts compared with either treatment by itself.<sup>28</sup> The same agent was also tested with radiation by Zips et al. in head and neck squamous cell carcinoma xenograft models.<sup>29</sup> Using different scheduling regimens, they found that if PTK787 was given before or during the course of fractionated radiation therapy, there was no beneficial effect; however, when given following the completion of the course of radiotherapy, there was a marked improvement in growth delay. Similar to these results, Williams et al. found that although the VEGFR inhibitor ZD6474 delayed tumor regrowth when given concurrently with radiation, a much more marked inhibition was noted if the drug was given after the radiotherapy had been completed (sequential treatment).<sup>30</sup>

More recently, the combination of radiation and cediranib (RECENTIN; AZD2171), a highly potent orally available inhibitor of VEGF receptor tyrosine kinase activity, has been investigated. In mouse xenograft models of human lung cancer and colon cancer cells, the combination resulted in greater tumor growth delay than either treatment alone.<sup>31–33</sup> One of these studies examined two different schedules of the drug, either once daily 2 hours prior to each fraction of radiation and daily thereafter or started immediately after the course of radiotherapy was completed.<sup>32</sup> Both regimens were effective in delaying tumor regrowth.

In theory, targeting the vasculature might lead to increased hypoxia, which would hinder the ability of radiation to kill cells; however, in reality the effect is much harder to predict. For VDAs that obliterate the vasculature, this is likely the case, so if one were to use radiation after administration of these agents, this might be counterproductive. The study using the VEGFR inhibitor ZD6474 that was discussed above found that this treatment was associated with decreased tumor perfusion.<sup>30</sup> The same group found that cediranib by itself resulted in a decrease in total vessel density and an increase in hypoxic fraction.<sup>31</sup> However, a review of the literature on the effects of anti-angiogenic agents on tumor oxygenation shows this to be far more complicated, with some drugs increasing hypoxia and others decreasing it.<sup>34</sup>

What is the explanation for such varied effects on tumor oxygenation by anti-angiogenic agents? The concept of “vascular normalization”, proposed by Rakesh Jain<sup>35</sup> may provide an explanation. According to this model, high levels of VEGF within tumors actually impede tumor oxygenation because the vessels function poorly. Paradoxically, inhibition of VEGF expression in these tumors can cause the vessels to remodel to become less permeable and less aberrant, resulting in decreased interstitial fluid pressure (IFP), improved blood flow and increased tumor oxygenation. Consistent with this model, Jain’s group showed that blocking VEGF signaling in mice with xenografts using DC101, a VEGF-receptor-2 antibody, decreased IFP by producing vessels that were more “normal” in appearance and in function with greater coverage by pericytes.<sup>36</sup> Furthermore, DC101 induced a hydrostatic pressure gradient across the vascular wall, which led to deeper penetration of the drugs into tumors. In a different study, the same group showed that this “normalization window” was accompanied by an improvement in oxygenation which was transient.<sup>37</sup> Lee et al. found that anti-VEGF antibody resulted in a significant decrease in vascular density and IFP in U87 xenografts accompanied by an improvement in tumor oxygenation. In contrast, in LS174 xenografts, the drug did not change tumor oxygenation although it did decrease IFP.<sup>27</sup>

The evidence discussed above supporting the concept of vascular normalization is pre-clinical. There are some data indirectly supporting vascular normalization in human patients. The most commonly used anti-VEGF agent in the clinic is bevacizumab (Avastin), an antibody with a half-life of 2–3 weeks that binds to VEGF to block VEGFR activation. Willett and colleagues reported on a clinical trial in which six patients with locally advanced adenocarcinoma of the rectum received bevacizumab alone, followed after 2 weeks by concurrent bevacizumab, 5FU and external beam radiation, then surgery.<sup>38</sup> Twelve days after the initial bevacizumab injection, IFP was reduced in four of four patients who had endoscopic measurements. The decrease in IFP was postulated to be due to vascular “normalization”, and this idea was supported by the fact that the fraction of vessels positive for  $\alpha$ -smooth muscle actin increased in four of five patients by day 12.

In a study of patients with recurrent glioblastomas treated with the VEGFR inhibitor cetiranib, Batchelor et al. used MRI techniques to show that the drug induced a decrease in relative vessel size as early as one day after the start of treatment which persisted to day 28.<sup>39</sup> By day 56 vessel size had started to increase, suggesting a closure of the structural vascular normalization window. Dynamic contrast-enhanced MRI (DCE-MRI) showed a

decrease in  $K_{trans}$  at days 1 and 28 that persisted to day 112.  $K_{trans}$  measures the rate of contrast agent between plasma and the extravascular extracellular space and is dependent upon both vascular permeability and vessel surface area. Therefore, this result is consistent with the idea that vascular permeability is decreased with cetiranib, although effects on vessel surface area confound these results.

In summary there are a great deal of pre-clinical data suggesting that anti-angiogenic agents in combination with radiation may improve outcome. Several mechanisms have been proposed as to how this might happen. Vascular normalization is one means by which the efficacy of radiation therapy might be increased after anti-angiogenic therapy, via increased oxygenation. It remains to be seen whether this happens in patients and what the kinetics of this effect might be. If this occurs, then the optimal time to start anti-VEGF therapy would be prior to the start of radiation. Another potential mechanism by which anti-VEGF therapy might work is via radiosensitization of the endothelial cells. Alternatively, VEGF may be induced by stromal cells following radiation. This may allow for establishment of new vasculature, which anti-angiogenic therapy might prevent. Pre-clinical data indicating that delivering anti-angiogenic therapy after the completion of radiation therapy results in better control support this idea.<sup>29,30</sup> Whether these agents will work with radiation therapy in the clinic, and if so, which of these explanations is correct remains unknown. Currently, there are numerous trials listed with the National Cancer Institute (NCI) that are investigating the use of bevacizumab or other agents with anti-angiogenic properties in combination with radiation with or without standard chemotherapeutic agents. Therefore, the answers to these questions will be forthcoming.

## Anti-HIF Therapy

Another potential target that could influence radiosensitivity is HIF-1, which as previously discussed, is a master transcription factor activated in response to hypoxia. It consists of two subunits, termed  $\alpha$  and  $\beta$ .<sup>21,22</sup> The  $\alpha$  subunit is encoded by three different genes, HIF-1 $\alpha$ , which is expressed ubiquitously and HIF-2 $\alpha$  and HIF-3 $\alpha$ , which are tissue specific. The  $\beta$  subunit is constitutively expressed regardless of the level of oxygenation. Under normoxia there is continual degradation of the  $\alpha$  subunit, but this is inhibited under hypoxia. This allows the  $\alpha$  subunit to accumulate rapidly and then bind to the  $\beta$  subunit to form the active HIF-1 factor. This transactivates dozens of target genes including VEGF, glucose transporter 1 (glut1) and various glycolytic enzymes that help cells adapt to hypoxia. Moeller et al. have reported that following radiotherapy, HIF-1 $\alpha$  accumulates and upregulates the expression of cytokines that enhance endothelial cell radioresistance.<sup>40</sup> This group used YC-1,<sup>41</sup> an agent originally developed for circulatory disorders that inhibits platelet aggregation and vascular contraction and also targets HIF-1. In a subsequent study Moeller et al. found the net effect of HIF-1 blockade with YC-1 on tumor radioresponsiveness was highly dependent on treatment sequencing, with regimens using radiation first being significantly more effective than the alternative.<sup>42</sup>

More recently another group showed that treatment with YC-1 followed by radiation mitigated the effect of radiation therapy, while YC-1 treatment following radiation suppressed the post-irradiation upregulation of HIF-1 activity and subsequently resulted in delayed tumor growth.<sup>43</sup> This study indicated that sequencing determines whether a HIF1 inhibitor enhances or inhibits the therapeutic effect of radiation. YC-1 significantly suppressed HIF-1 activity, leading to a decrease in microvessel density and an increase in tumor hypoxia. This led the authors to suggest that the sequence of YC-1 followed by radiation was associated with a poorer outcome because it resulted in an increase in the radioresistant hypoxic fraction.

Two other agents that are HIF-1 $\alpha$  inhibitors that have been tested as radiosensitizers are TAS106 and S-2-amino-3-[4'-N,N-bis(2-chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride (PX-478). TAS106 has been reported to be helpful against radiation-resistant hypoxic cells in solid tumors.<sup>44</sup> PX-478 was found to enhance radiosensitivity in prostate cancer cell lines in both normoxic and hypoxic conditions.<sup>45</sup> Further studies demonstrated that the effect of PX-478 as a radiosensitizer was even more potent in vivo than in vitro, likely due to blunting of the tumor stromal response to radiation.<sup>46</sup> Specifically PX-478 blunted HIF-1 dependent angiogenic signaling, which was induced by ischemia caused by vascular damage due to radiation. Currently, PX-478 is being investigated in phase I clinical trials.

## Epidermal Growth Factor Receptor (EGFR) Inhibitors

The EGFR receptors (erb1/EGFR, erb2/HER2, erb3/HER3 and erb4/HER4) are members of a family of receptor tyrosine kinases implicated in the development of some types of cancer, especially non-small cell lung cancers and head and neck cancers. EGFR activation leads to an intracellular signaling cascade via Ras activation, which stimulates the extracellular signal pathway regulated kinase (ERK)/mitogen-activated protein kinase (MAP) kinase pathway (Fig. 1). A number of EGFR inhibitors including the monoclonal antibodies cetuximab (Erbix) and panitumumab (Vectoibix) and small molecule tyrosine kinase inhibitors gefitinib (Iressa) and erlotinib (Tarceva) have shown potential in the treatment of several types of human cancers and have been reviewed by us previously.<sup>47</sup> Radiation can increase the expression of EGFR, which has been implicated in increasing the radiation resistance of cancer cells. In a landmark randomized phase III trial, the combination of cetuximab and radiation was found to be superior to radiation only in the treatment of locally advanced head and neck squamous cell carcinoma with clear improvement in local control and survival favoring the combined modality group.<sup>48</sup>

Harari and colleagues found that the monoclonal antibody C225 enhanced the radiosensitivity of head and neck cancer cells as measured by clonogenic survival.<sup>49</sup> C225 also augmented radiation killing in human xenografts implanted in nude mice.<sup>50,51</sup> Early studies with gefitinib found that it inhibited growth factor production and angiogenesis in various cancer cells and enhanced the antitumor activity of ionizing radiation.<sup>52,53</sup> Investigators found that gefitinib radiosensitized cells in vitro perhaps by suppressing cellular DNA repair capacity.<sup>54,55</sup> Gefitinib not only inhibited radiation-induced activation of DNA-dependent protein kinase<sup>56</sup> but it also inhibited homology-directed recombination repair in human breast cancer cells.<sup>57</sup> Chinnayan et al. showed that erlotinib radiosensitized cells in vitro, an effect that might have occurred through a combination of increased apoptosis, cell cycle arrest and changes in DNA damage repair.<sup>58</sup>

We have investigated the effects of EGFR inhibition on the tumor microenvironment to see whether there might be effects that could affect subsequent therapy. Our previous work demonstrated that the small molecule EGFR inhibitors erlotinib and gefitinib decreased VEGF mRNA expression, decreased secretion of VEGF protein, and blunted HIF-1 $\alpha$  induction in response to hypoxia in SQ20B head and neck squamous cell carcinoma cells.<sup>59</sup> More recently we have shown that erlotinib treatment altered vessel morphology and decreased vessel permeability within human xenografts grown in nude mice.<sup>60</sup> Furthermore, we have found that erlotinib increased tumor blood flow measured by power Doppler ultrasound and decreased hypoxia and tumor O<sub>2</sub> saturation.<sup>60</sup> Others have shown that EGFR inhibition with C225,<sup>61</sup> or gefitinib<sup>62,63</sup> can lead to improved oxygenation in tumors. Solomon et al. used <sup>18</sup>F-FAZA PET scanning with the nitroimidazole FAZA to show this,<sup>63</sup> and Warburton et al. used EF5 flow cytometry.<sup>62</sup> Our results offer a mechanism for this phenomenon. We hypothesize that these changes in tumor physiology are an indirect effect

of EGFR inhibition, which causes decreased VEGF secretion by the tumor cells, leading to vascular normalization, improved blood flow, and improved oxygenation. We speculate that this may be part of the mechanism by which the drug may increase in vivo radiosensitivity, in addition to the other effects it has been noted to have in vitro.

## The Phosphatidylinositol 3-Kinase (PI3K)/Akt/mTOR Pathway

The PI3K/Akt pathway is commonly activated in human cancers<sup>64,65</sup> (Fig. 1). Phosphatidylinositol 3-kinases are a family of enzymes that phosphorylates the 3'-OH of the inositol ring of phosphatidylinositol. Phosphatidylinositol 3,4,5-trisphosphate (PIP3) is an important lipid second messenger generated by PI3K that plays a vital role in several signal transduction pathways.<sup>66,67</sup> PIP3 activates the serine/threonine kinase AKT by physically binding to it via a pleckstrin homology domain. This leads to translocation of Akt to the inner cell membrane, allowing it be phosphorylated on 2 specific residues, Ser473 and Thr308. Phosphatase and tensin homologue gene (PTEN) is a phosphatase that opposes the action of PI3K, thereby reducing the level of activated (phosphorylated) AKT. AKT controls protein synthesis and cell growth by phosphorylation of mammalian target of rapamycin (mTOR). The PI3K pathway, which is known to play a key role in controlling cell proliferation, growth and survival, is activated in many cancers.<sup>68</sup>

There are three classes of PI3K: I, II and III. Class I PI3Ks are heterodimers composed of a catalytic and a regulatory subunit and are further subdivided into two subclasses: IA and IB. Class IA PI3K contains the p85 regulatory subunit that binds to receptor tyrosine kinases (RTK) and either an alpha, beta or delta p110 catalytic subunit (p110 $\alpha$ , p110 $\beta$  or p110 $\delta$ ). Additionally p110 is also a direct effector of Ras. Class IB PI3K is composed of the p110 $\gamma$  catalytic subunit complexed with the p101 regulatory protein. Class II consists of three members, PI3KC2 $\alpha$ , PI3KC2 $\beta$  and PI3KC2 $\gamma$ , while Class III contains only one member encoded by the gene VPS34.<sup>67,69,70</sup>

PI3K activation can occur via loss of PTEN, by Ras mutation or by increased expression of a growth factor receptor such as EGFR. There appears to be a relationship between PI3K/Akt pathway activation and radiosensitivity. The radioresistance of cells can be increased by the overexpression or activation of oncogenes such as epidermal growth factor receptor (EGFR)<sup>71</sup> and Ras<sup>72</sup> or loss of the tumor suppressor gene PTEN.<sup>73</sup> These mutations share in common the fact that they activate the PI3K-Akt pathway. Conversely, many groups have shown that blocking the Ras and/or PI3K pathway enhances the radiation response in vitro and in vivo.<sup>74-77</sup>

### PI3K inhibitors

LY294002 and wortmannin are the first generation PI3K inhibitors that inhibit most of the PI3Ks at low doses. Wortmannin, a fungal secondary metabolite, is a noncompetitive, irreversible inhibitor of PI3K with an IC<sub>50</sub> of 2–4 nM.<sup>78</sup> Wortmannin radiosensitizes cancer cells, however, it is biologically unstable and toxic to the liver.<sup>79</sup> Quercetin, a naturally occurring bioflavonoid, with an IC<sub>50</sub> of 3.8  $\mu$ M was shown to inhibit PI3K.<sup>80</sup> LY294002 the first synthetic PI3K inhibitor was based on quercetin.<sup>81,82</sup> LY294002 has been found to radiosensitize both in vitro<sup>77,79</sup> as well as in vivo.<sup>76</sup> However, LY294002 is unsuitable for human use due to toxicity.<sup>83</sup>

SF1126, a pan-PI3K inhibitor developed by Semafore Pharmaceuticals is a novel RGDS-conjugated LY294002 prod-rug. It was conjugated to RGDS purposely in order to have it bind to specific integrins within the tumor compartment, resulting in enhanced delivery of the active compound to the tumor vasculature and tumor. Indeed, it was shown to have

potent anti-tumor activity with a better pharmacokinetic and dynamic effect compared to its parent drug.<sup>84</sup> SF1126 also has radiosensitizing effect in glioma tumor models.<sup>85</sup>

Of a series of isoform-selective inhibitors of PI3K,<sup>86</sup> the pyridinylfuranopyrimidine inhibitor PI-103 was found to selectively inhibit p110 $\alpha$  and mTOR in malignant gliomas.<sup>87</sup> Chen et al. found that this inhibitor produced additive cytotoxic effects in combination with radiation therapy.<sup>88</sup> Their in vivo studies indicated a significant reduction in tumor growth rate induced by the combined treatment compared with each treatment modality alone. Prevo et al. showed that PI-103 enhanced the radiation sensitivity of tumor cells in vitro and resulted in the persistence of DNA damage as evidenced by elevated levels of  $\gamma$ H2AX foci.<sup>89</sup> This same group showed that the drug caused changes in the vessel morphology with increased pericyte coverage, consistent with vascular normalization.<sup>90</sup> This was accompanied by increased blood flow and decreased tumor hypoxia.

### Akt inhibitors

The Akt inhibitor 1L-6-hydroxymethyl-chiro-inositol 2(R)-2-O-methyl-3-O-octadecylcarbonate, has been tested against, HL60AR, an HL60 leukemia subclone with a constitutively activated PI3K/Akt pathway, which is resistant to multiple chemotherapeutic drugs and ionizing radiation.<sup>91</sup> At 20  $\mu$ M concentration the drug was able to inhibit Akt without affecting the PI3K activity and markedly reduced resistance of HL60AR cells to chemotherapeutic agents and ionizing radiation. Another novel Akt inhibitor 8-(1-Hydroxyethyl)-2-methoxy-3-(4-methoxy-benzyloxy)-benzo[c]chromen-6-one (Palomid 529 or P529) has been shown to have anti-cancer and radiosensitizing effects on PC3 prostate carcinoma cells and xenografts.<sup>92</sup>

### HIV Protease inhibitors

Our group and others have demonstrated that a class of drugs in common clinical use, the HIV protease inhibitors (HPIs), interferes with PI3K-Akt signaling and radiosensitizes a variety of tumor types.<sup>93-95</sup> These drugs have been widely used in HIV patients with well-characterized pharmacokinetics and documented toxicity profile.<sup>96</sup> A prominent side effect of HPI treatment is insulin resistance.<sup>97</sup> Akt plays a key role in the coordinated regulation of growth and metabolism by the insulin/IGF-signaling pathway.<sup>98</sup> Therefore, it was hypothesized that HPIs might block the PI3K-Akt signaling axis in tumor cells and hence might be used clinically as radiation sensitizers.<sup>93</sup> In this study, three HPIs, nelfinavir, amprenavir and saquinavir inhibited Akt phosphorylation at Ser473 and radiosensitized a variety of tumors.

The effect of nelfinavir often appeared greater in vivo than in vitro, leading us to hypothesize that the drug may have effects on the TME that increase radiosensitivity. We have shown that nelfinavir decreases HIF-1 $\alpha$  and VEGF expression both in vitro and in vivo<sup>95,99</sup> and that it improves tumor oxygenation in A549 lung carcinoma xenografts.<sup>95</sup> We have recently found that nelfinavir can increase blood flow in tumors (Fig. 2). Thus, our results with nelfinavir are very similar to our results with erlotinib.<sup>60</sup> We hypothesize that the drug leads to decreased VEGF secretion by tumor cells, resulting in vascular normalization. Consistent with this idea, Qayum et al. found that nelfinavir treatment led to vessels that were more regular with increased interbranch length and reduced tortuosity, consistent with normalization.<sup>90</sup>

### mTOR inhibitors

The mTOR pathway is downstream of the Akt pathway (Fig. 1) and is an attractive target for cancer therapy. Rapamycin was the first mTOR inhibitor to be discovered. The other mTOR inhibitors currently in clinical development as anticancer agents include temsirolimus



(CCI779), everolimus (RAD001) and deforolimus (AP23573).<sup>100,101</sup> Rapamycin has been reported to radiosensitize both in vitro and in vivo soft tissue sarcoma cell lines and xenografts.<sup>102</sup> However, in another study, rapamycin and RAD001 radiosensitized vascular endothelial cells but failed to sensitize glioma cells.<sup>103</sup> Still another study demonstrated no direct radiosensitizing effects in glial cells treated with rapamycin in vitro, but a strong radiosensitizing effect in vivo.<sup>104</sup> Hence, the role of mTOR inhibitors in radiosensitization is still an open one.

## Conclusion

It is apparent that we now have access to a variety of agents with radiosensitizing abilities that can be used in the clinic. These include agents that inhibit either growth factors or growth factor receptors such as EGFR and VEGFR. These factors are often overexpressed in tumors or are released in response to radiation. Inhibitors are also available that can inhibit pathways downstream of the receptor. Inhibition of a single factor may affect multiple downstream events. These agents may affect the intrinsic radiosensitivity of tumor cells, but there is growing evidence that they can influence the microenvironment of the tumor to increase response to radiation. For example, inhibition of EGFR can lead to decreased VEGF secretion by tumor cells and alteration of tumor oxygenation in preclinical models. However, clinical data from patients showing that modulation of the tumor microenvironment is an important mechanism by which these agents radiosensitize are sparse. A major challenge remains to obtain this information non-invasively in patients and then to determine whether the sequencing of these agents with respect to radiation therapy can be optimized.

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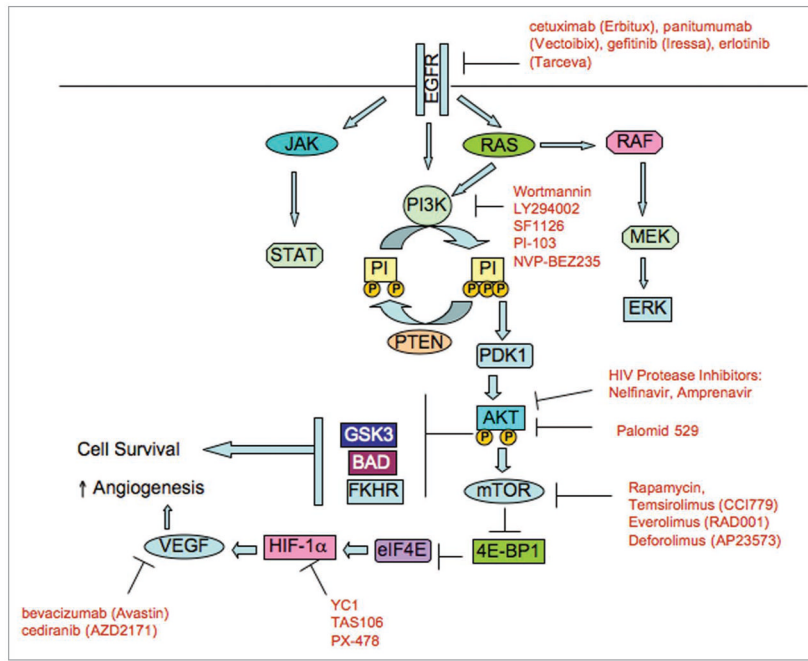
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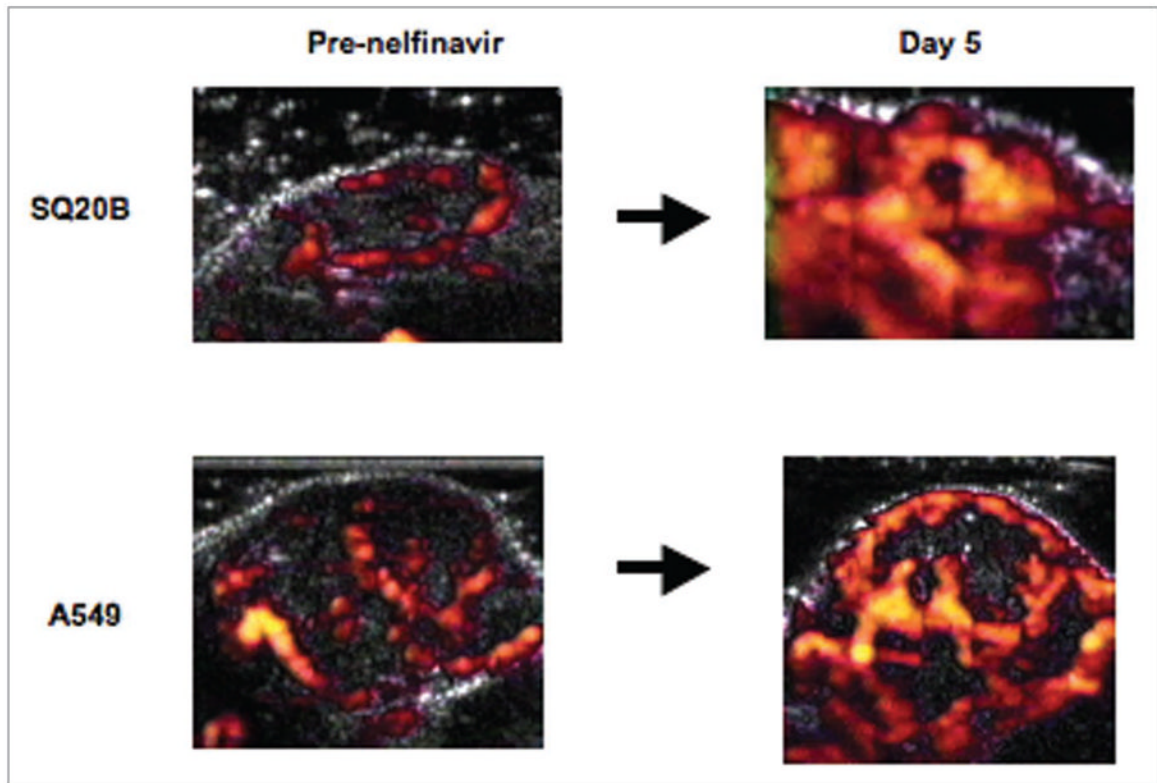
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**Figure 1.** Signaling pathways that can be inhibited to increase the radiation response of tumors. Activation of growth factor receptors including EGFR leads to upregulation of numerous downstream pathways. One of these, the PI3K/Akt pathway, has been implicated in radioresistance and in the regulation of hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) and vascular endothelial growth factor (VEGF) expression. Inhibition of the PI3K/Akt pathway has been shown to increase the intrinsic radiosensitivity of cancer cells. However, recent evidence suggests that inhibition of the EGFR/PI3K/Akt pathway can also increase the extrinsic radiosensitivity of tumors by altering the tumor microenvironment, perhaps by improving blood flow and tumor oxygenation.





**Figure 2.**

Xenograft blood flow response to nelfinavir treatment. Nude mouse was injected subcutaneously in the flank with SQ20B head and neck cancer cells or A549 lung cancer cells to form xenograft. When tumor reached a size of ~1 cm in diameter, mouse was anesthetized and injected with microbubble contrast agent, then power Doppler ultrasound was performed. Mouse was then started on nelfinavir-containing diet (80 mg/kg/day). Repeat Doppler measurement was performed at day 5. Doppler flow images are shown pre-nelfinavir (A) and at day 5 (B). Details regarding xenograft production and Doppler imaging can be found in Cerniglia et al.<sup>60</sup> and Pore et al.<sup>95</sup>