

# New Radiosensitizing Regimens, Drugs, Prodrugs, and Candidates

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## Abstract

There have been significant advances over the last decade in the understanding of cellular, biochemical, and molecular effects of ionizing radiation combined with certain types of cytotoxic drugs and prodrugs, as well as new “targeted” biological agents in human tumor and normal cells. At the same time, new information has evolved regarding specific genetic and epigenetic changes found in certain human cancers, which result in alterations in ionizing radiation damage recognition and damage repair processes. As a result, novel targeting approaches for human tumor radiosensitization is an active area for translational and clinical research in radiation oncology. In this article, we review the current status of existing and new radiosensitizing regimens.

Cisplatin and 5-fluorouracil (5-FU) are currently the only radiosensitizing drugs in common clinical use as the standard of care to improve radiation therapy.<sup>1-5</sup> Cisplatin and 5-FU radiosensitize tumors to ionizing radiation and enable supra-additive therapeutic benefits, including increased survival. Cisplatin radiosensitization in radiation therapy is now a therapy of choice recommended by the National Cancer Institute (NCI) for high-risk, early stage, locally advanced cervical cancer. Similarly, the use of continuous-infusion 5-FU during preoperative or postoperative radiation therapy for stage II and III rectal cancer is also an NCI-recommended standard of care.

In addition to 5-FU, other base- or sugar-modified halonucleoside analog drugs and prodrugs with radiosensitizing activities are in clinical trials or will enter trials in the near future. These include gemcitabine (Gemzar, Lilly), capecitabine (Xeloda, Roche), fludarabine, and IPdR (5-iodo-2-deoxypyrimidinone-2'-deoxyribose). These halogen-modified nucleoside analog drugs or prodrugs are modified substrates for metabolic enzymes, have clinically important single-agent activities as antimetabolites, are incorporated into DNA, and are radiosensitizing in appropriate pretreatment and/or concurrent radiotherapy regimens.

Tumors may also be radiosensitized to ionizing radiation (IR) through inhibition of Rad51-catalyzed homologous DNA double-strand break repair (DSBR) by imatinib mesylate (Gleevec, Novartis), through inhibition of epidermal growth factor receptor (EGFR) signaling through medication by cetuximab (Erbix, Imclone) or gefitinib (Iressa, AstraZeneca), or through inhibition of vascular endothelial growth factor (VEGF)-mediated tumor angiogenesis by bevacizumab (Avastin, Genentech). Thus, enhancing IR-induced DNA breaks or inhibiting repair of DNA breaks or angiogenesis can increase tumor cell death and enhance the benefits of radiation therapy.

## Infused 5-FU and Oral Capecitabine

5-FU in combination with IR is in common use in first-line therapy for rectal, head and neck, esophageal, gastric, pancreatic, biliary, anal, and cervical cancers. For some tumors, 5-FU chemoradiotherapy improves survival rates. 5-FU chemoradiotherapy maximizes local control and also improves organ preservation, with excellent functional

## Keywords

Radiosensitization, drugs and prodrugs, biologics, new targets

outcomes for patients with head and neck, anal, and rectal tumors. Identification of advantages of continuous infusion of 5-FU, administered before and during radiation, has led to increased radiation sensitivity of tumor cells, increased therapeutic gain, and increased median survival of patients, as compared to bolus 5-FU treatment with radiation.

Cumulative data from 16 separate studies in rectal cancer patients, involving more than 1,000 patients, show infusional 5-FU treatment has a high overall survival, with local control rates of up to 96%.<sup>6</sup> In nonrandomized studies, preoperative chemoradiotherapy with infusional 5-FU in rectal cancer demonstrates superior results as compared with surgery or external beam radiation alone. Infused 5-FU is effective in reducing T3 rectal cancer tumor bulk, tumor downstaging in sphincter preservation, and decreasing rates of local failures and distant metastases. Acute, perioperative, and late complication rates remain similar to those commonly seen with radiation alone.<sup>7,8</sup> In addition, several studies show beneficial results from preoperative chemoradiotherapy with oral capecitabine, a prodrug of 5-FU, which parallels the continuous infusion of 5-FU in resectable rectal cancer and results in tumor downstaging in more than 40% of treated patients and local response rates near 80%, with acceptable toxicity profiles.<sup>9-11</sup> In general, treatment schedules that result in prolonged exposure to 5-FU drug or prodrug-based chemoradiation show improved downstaging, sphincter preservation, and improved median survival rates in rectal cancer patients.

Local control rates or complete responses as high as 98% have been obtained for head and neck cancers treated using continuous infusional 5-FU treatment and chemoradiotherapy in combination with cisplatin, as compared with standard radiation therapy.<sup>12-14</sup> However, these results were accompanied by high but manageable grade 3 and 4 toxicities in up to 93% of patients, with the need for feeding tube placement in a high percentage of patients. Bolus 5-FU did not appear as effective as continuous infusion regimens in chemoradiotherapy of head and neck tumors.<sup>6</sup>

In esophageal cancers, combined modality chemoradiotherapy using infusional 5-FU and cisplatin with radiation therapy has overall response rates between 50% and 97% and complete response rates as high as 91%, with an excellent 5-year survival (50%).<sup>15</sup> Aggressive combined modality therapies of esophageal carcinomas have resulted in excellent long-term survival rates of up to 50% at 4 years. Prolonged exposure to 5-FU-based chemoradiotherapy in combination with cisplatin (or paclitaxel) is both efficacious and tolerable.<sup>16</sup>

The tumor control rates in anal cancer patients treated with infusional 5-FU and mitomycin C (MMC) chemoradiotherapy range from 60% to 90% with radiation doses of 45–55 Gy; MMC improves disease-free and colostomy-free survival in advanced anal cancer.<sup>17</sup> Due to the toxicity of MMC, new studies testing cisplatin with 5-FU will determine improvements in local control rates and survival.<sup>18</sup>

Continuous infusion of 5-FU during radiation therapy in resectable gastric carcinoma produces recurrence-free

and 5-year survival rates of 43% for postoperative treatment regimens and tumor response rates of 58%.<sup>19</sup> Toxicity is significant but manageable and supports adjuvant 5-FU-based chemoradiotherapy as the treatment of choice following surgery.

Chemoradiotherapy with 5-FU is also the primary choice of treatment for locally advanced or unresectable pancreatic cancer. A combined modality approach using 5-FU, cisplatin, and radiation therapy provided a modest survival benefit to these patients.<sup>20-24</sup> The 1-year survival rate is between 18% and 66%, while the 2-year survival rate is between 13% and 37%, with better results in resected patients (59%). The use of a temporal chemotherapy dose density in pancreatic cancer patients has allowed delivery of 16% more chemotherapy and 13% more radiation therapy without an increase in toxicity, resulting in the 1-year survival rate increasing to 88%.<sup>25</sup>

### Continuous Administration of Infused 5-IUdR or Oral 5-IPdR With X-Ray Therapy

5-IUdR (5-iodo-2'-deoxyuridine) has been recognized as a radiosensitizing drug candidate since the early 1960s.<sup>26,27</sup> DNA incorporation is a prerequisite for radiosensitization of tumors by 5-IUdR, and the extent of radiosensitization correlates directly with the percentage of thymidine (TdR) replaced by 5-IUdR incorporated into tumor DNA.<sup>28,29</sup> Preclinical and phase I/II 5-IUdR clinical studies show synergistic benefits of intravenously infused 5-IUdR in combination with radiation in patients with newly diagnosed gliomas. Intravenous (IV) infusion of 5-IUdR for up to 4 days, combined with radiation therapy for 6 weeks, increased the mean survival of anaplastic astrocytoma patients from 2.0 years to 3.2 years, and 7 of 21 treated patients (33%) survived for 5 years and remained in remission.<sup>30</sup>

IV 5-IUdR has also been investigated with radiation therapy treatments in patients with glioblastoma multiforme (GBM). In a phase I/II GBM study with IV 5-IUdR, 47 patients with newly diagnosed GBM received radiation therapy and continuous IV infusion for up to 14 days prior to radiation. The clinical results and subset analysis showed dose-limiting toxicity and, thus, most patients did not receive the full drug dose. In all 47 total GBM radiation therapy patients, the median survival was 45 weeks. At 24 months posttreatment, there was a 57% increase in 5-IUdR patient survival. In the 28 of 47 ambulatory patients, 5-IUdR treatment increased the median survival from 50 weeks to 64 weeks. At 24 months, there was a 50% increase in 5-IUdR patient survival.<sup>31</sup> While IV 5-IUdR has demonstrated an efficacy and survival advantage in these forms of brain cancer in phase II clinical trials, there were significant and unacceptable gastrointestinal and myelotoxicities from 5-IUdR treatments. Intravenously administered 5-IUdR has a relatively short serum half-life, on the order of minutes. Thus, long continuous IV 5-IUdR drug infusions are necessary to achieve effective 5-IUdR incorporation into tumor cell DNA for DNA radiosensitization in radiation therapy. During long infusion periods, IV 5-IUdR produces significant toxicities,

especially due to 5-IUdR DNA incorporation and DNA damage in rapidly proliferating normal bone marrow cells and gastrointestinal cells.

5-IPdR is an orally available or injectable prodrug of 5-IUdR, originally synthesized as a potential antiviral pyrimidinone-based 5-IUdR prodrug for *in vivo* metabolism into the 5-IUdR pyrimidine nucleoside. 5-IPdR has significant antiviral as well as anticancer activities. The entire family of halogenated TdR analogs encompasses 5-FUdR, 5-CUdR, 5-BUdR and 5-IUdR, or their corresponding prodrugs, including 5-FPdR and 5-IPdR. These analogs have been recognized for single-agent antiviral or anticancer clinical activities, as well as for potential uses as radiosensitizing drugs.<sup>26,27</sup> IPdR has demonstrated promising results in preclinical studies.

5-IPdR is metabolically converted from 5-IPdR to 5-IUdR. This metabolism primarily occurs in the liver via the activity of human hepatic aldehyde oxidase enzyme. 5-IUdR is the active metabolite of 5-IPdR. Thus, 5-IPdR has similar mechanisms of drug action as 5-IUdR. However, in contrast to IV 5-IUdR, IV or oral 5-IPdR has a longer half-life in blood, superior pharmacokinetics, reduced toxic systemic side effects, 2.5-fold increased incorporation of 5-IUdR nucleotides into DNA, and significantly increased radiosensitization, as monitored in tumor xenografts. The tumor sensitizer enhancement ratio is 1.53 for oral 5-IPdR and 1.02 for IV 5-IUdR in human colorectal cancer xenografts. In xenografted high-grade glioma, the tumor sensitizer enhancement ratio is 1.31 for oral 5-IPdR and 1.07 for IV 5-IUdR.<sup>32</sup>

The pharmacokinetics of 5-IPdR in athymic nude mice and Rhesus monkeys show a similar 2-compartment pattern with a rapid peak plasma level within 15–30 minutes, followed by a slower elimination phase, which is 5-IPdR dose-dependent.<sup>33</sup> These pharmacokinetic data were used in the design of the pharmacokinetics study as part of the initial phase I trial. Investigational New Drug (IND)-related toxicology studies in rodents and ferrets used a once-daily times 14-day treatment schedule, similar to the phase I trial of oral 5-IPdR (ferrets). Using preclinical data, the starting dose for the phase I trial (85 mg/m<sup>2</sup> daily) equals about 0.1 maximum tolerated dose (MTD) in ferrets. The trial will determine pharmacokinetics and directly measure drug targeting as the percentage of IUdR–DNA incorporation in tumor and 2 normal tissues, oral mucosa, and peripheral granulocytes, in order to address the potential therapeutic gain in addition to establishing the MTD of oral 5-IPdR. Oral 5-IPdR will be given daily in the phase I study, planned for initiation in 2005 at the Ireland Cancer Center, Case Western Reserve University, Cleveland, Ohio. The NCI is sponsoring the phase I oral IPdR trial in combination with standard radiation for patients with solid tumors. This phase I oral IPdR clinical study is an investigator-initiated clinical trial.

5-IUdR is actively transported into cells, and its metabolism is dependent on the TdR salvage pathway.<sup>26</sup> Nuclear thymidine kinase phosphorylates 5-IUdR to 5-IdUMP, followed by

phosphorylation by thymidylate kinase to 5-IdUDP and subsequently by NDP kinase to 5-IdUTP, which competes with dTTP as a substrate for DNA polymerase-catalyzed incorporation of the nucleoside triphosphate into newly synthesized DNA. DNA incorporation of 5-IUdR is an absolute prerequisite for radiosensitization, and the extent of radiosensitization correlates directly with the percentage of TdR (5-methyl-UdR) replaced with 5-iodo-UdR in tumor cell DNA.<sup>28,34</sup>

The molecular mechanism of 5-IUdR radiosensitization is most likely related to the increased susceptibility of the iodonucleoside-substituted DNA to the effects of IR in clinical radiation therapy. A proposed biochemical mechanism of 5-IUdR-mediated radiosensitization includes a DNA incorporated, 5-iododeoxyuridine reacting with IR-induced hydrated electrons that at 1.8 Gy results in reactive uracyl radicals and halide (I<sup>-</sup>) ions, which also damage and break the unsubstituted complementary-strand DNA directly opposite or clustered around the incorporated drug.<sup>35</sup> Unrepaired or misrepaired DNA double-strands (ds) result in cell death.<sup>36,37</sup> Repair of IR DNA damage can also be reduced by pre-IR and/or continuous exposure to the halonucleoside drug.<sup>38</sup>

In DNA radiosensitizing regimens, xenografted human brain tumor cells continuously treated with 5-IPdR (1,500 mg/kg/day for 14 days) effectively replace about 1 in every 27 (3.7%) thymidine C-5 position methyl groups with a 5-iodo-group in the UdR base.<sup>39</sup> Normal liver, bone marrow, and intestine cell DNA in these xenograft models replace about 0.3%, 1.3%, and 3.3%, respectively, of TdR with 5-IUdR. In contrast, 5-IUdR (100 mg/kg/day for 14 days) was incorporated into brain tumor DNA and replaced about 1.4% of thymidine C-5 methyl groups with a 5-iodo group in the UdR base.

Normal or tumor cells exposed to 5-IUdR or 5-IPdR, which incorporate 5-iodo-dTTP into DNA, and subsequent treatment with IR both accumulate DNA base modifications, single- and double-strand DNA breaks, and undergo DNA repair.<sup>40</sup> However, compared to untreated normal cells, untreated tumor cells often overexpress, at both the RNA and at the protein levels, the key enzymes of the DNA repair pathways catalyzing DNA base excision repair (BER), as well as homologous DSBR. IR exposure induces or super-induces the gene expression (as RNA) and the protein activities of the critical enzymes in both these DNA repair pathways, specifically Rad51 and Ref-1/Ape1.<sup>41</sup>

BER enzymes mediate short patch repair of single nucleotide DNA base damage or long patch repair of damaged DNA chains. DSBR enzymes mediate homologous DNA DSBR. In DSBR, the end of the dsDNA break is recessed and the Rad51 protein-coated DNA (referred to as the Rad51 protein-coated DNA filament) catalyzes the search for homology. DSBR enzyme-mediated homologous dsDNA break repair involves Rad51 protein monomers cooperatively binding and polymerizing on the single-strand end of the broken DNA copy. The IR broken DNA strand coated with Rad51 protein as a nucleoprotein filament is aligned on the homolo-

gous unbroken double-strand DNA copy. Subsequent DSBR occurs via a Rad51-mediated DNA strand exchange, which results in a highly accurate DNA double-strand repair.<sup>42</sup>

### Methoxyamine Inhibition of BER and Methoxyamine Potentiation of 5-IUdR or 5-IPdR DNA Breaks

Human tumor cells deficient in DNA mismatch repair (MMR) show greater 5-IUdR incorporation into DNA and greater 5-IUdR radiosensitization than genetically matched MMR-proficient cells<sup>43-45</sup> and suggested incorporated 5-IUdR removal by DNA repair. To identify which DNA repair pathway was involved in removing 5-IUdR from DNA, the sensitivity to 5-IUdR was measured in cells defective in XRCC1, a scaffolding protein directly associated with DNA polymerase, DNA ligase III, and poly(ADP-ribose) polymerase (PARP) during BER or single-strand break repair (SSBR) reactions. XRCC1 coordinates repair of SSBs induced as intermediates of BER<sup>46,47</sup> and modulates BER via Ape1, the human AP endonuclease.<sup>48</sup>

Cells with altered XRCC1 expression can be specifically sensitized to treatment with 5-IUdR.<sup>49</sup> The increased susceptibility of XRCC1 mutant cells to 5-IUdR-induced cytotoxicity and DNA damage also results in increased radiosensitization following treatment with 5-IUdR. However, DNA incorporation of 5-IUdR in XRCC1 mutant cells is equivalent to that in wild-type cells. Following 5-IUdR treatment, the differences in cytotoxicity and DNA damage evident in genetically defined tumor cell lines are attributed to a defect in DNA repair, rather than differential incorporation. Thus, DNA repair deficiencies associated with XRCC1 polymorphisms can predict more favorable outcomes.

XRCC1-mutant cells are hypersensitive to 5-IUdR treatment, 5-IUdR cytotoxicity, and radiosensitization. Cotreatment of human colon tumor or other cells with methoxyamine (MX) enhances 5-IUdR cytotoxicity.<sup>50</sup> MX treatment blocks single nucleotide BER, by reaction with the aldehydic C-1' position of the acyclic sugar left in the DNA abasic AP site, following the removal of the damaged nucleotide. The MX-adducted AP site is a stable intermediate, refractory to the lyase activity of DNA polymerase and to AP endonuclease cleavage in BER.

Chemical inhibition of BER by MX treatment also overcomes resistance to the chemotherapeutic methylating agent temozolomide (Temodar, Schering).<sup>51,52</sup> Increased 5-IUdR incorporation into DNA in cells lacking functional single-nucleotide BER can cause increased numbers of DNA breaks, left unrepaired following the removal of 5-IUdR. The presence of these DNA breaks may be explained by abasic sites left unrepaired within a topoisomerase II DNA cleavage site acting to inhibit topo II<sup>53</sup> and to significantly increase the enzyme-mediated DNA cleavage. These transient DNA breaks may be converted to permanent double-strand DNA breaks.

In human colon tumor cells, administration of MX significantly increases incorporation of 5-IUdR into DNA.<sup>54</sup> MX-adducted AP sites, although refractory to the action of

the single-nucleotide short patch BER pathway, can still be processed by the long-patch BER pathway.<sup>54</sup> Thus, DNA synthesis in long-patch BER can continuously accumulate 5-IUdR in DNA, causing cells to undergo futile and suicidal cycles of excision and incorporation of 5-IUdR, with increased cytotoxicity, due to the presence of 5-IUdR alone and the potential for greater radiosensitization. Combined treatment with 5-IUdR and MX results in a supra-additive effect on the IR-induced cell killing, in both MMR-deficient and MMR-proficient human colon tumor cells.<sup>54</sup> Since MX administered alone is not a drug, it does not have an effect on the IR-induced cytotoxicity. The combined treatment with 5-IUdR and MX is strongly synergistic in human colon tumors.<sup>54</sup>

BER has a critical role following DNA incorporation of certain modified nucleoside analog drugs, including 5-IUdR, fludarabine monophosphate (2-F-Ara-AMP) and others. Inhibition of BER by MX is also an important therapeutic strategy to further evaluate in 5-IPdR drug development as a radiosensitizer. MX treatment is also highly effective in eliminating temozolomide resistance in human tumor cells grown as xenografts in mice.<sup>55</sup> Preclinical toxicology, biodistribution, and pharmacokinetic studies of MX are being completed. The safety of MX in combination with temozolomide in human colorectal patients will be evaluated in a proposed phase I safety trial at the Ireland Cancer Center.

### Gemcitabine

Gemcitabine is 2',2'-difluorodeoxycytidine (dFdC). In a dFdC, 2 separate fluorine atoms are substituted, 1 for the H and another for the OH group, both of which are normally present at the C-2' position of the sugar in dC.<sup>56</sup> This substitution also alters the sugar conformation. The pyrimidine base in the drug gemcitabine is unmodified and an H atom is present at the C-5' position of dC and dFdC. There would only be a C-5' base modification in gemcitabine if the dC base were methylated by a cellular DNA methylase enzyme after incorporation of dFdCTP into cellular DNA.

Radiation-induced free radicals, principally from irradiation of water molecules, produce hydroxyl free radical attack of DNA. Approximately 1 out of every 4 of these hydroxyl radicals reacts with the deoxyribose sugar phosphate moiety with preferences for specific deoxyribose positions in the order, C-4'>C-3'>C-2'>C-1' or preference for the C-5' position linking the sugar with the phosphate group.<sup>57</sup>

The majority of the hydroxyl radicals, the remaining 3 of the 4, react relatively equally with the different DNA bases, but with preferences for specific positions. On the unmodified pyrimidine (dT, dG) bases in DNA, the attack preferentially occurs at the 5,6 double bond between the 2 carbon atoms. A different situation would be presented where there was a methyl group at the C-5' position of cytidine. Hypermethylation of the C-5' position of cytidine can occur in DNA in CpG islands of the promoters of silenced DNA repair and tumor suppressor genes.<sup>58</sup>

Certain deoxycytidine base analogs of clinical interest in radiotherapy, including 5-azacytosine and 5-aza-cytosinear-

abinoside, cannot be methylated. However, the nucleoside drugs, cytarabine (araC) or gemcitabine, when incorporated into DNA, can be considered as potential substrates for C-5' base methylation. There are several metabolic and mechanistic similarities between gemcitabine and cytarabine (Ara-C, 1-β-D-arabinofuranosylcytosine); however, gemcitabine appears to possess broader antitumor activities.<sup>54</sup> Cytarabine enhances the cytotoxicity of various different DNA damaging and breaking agents, possibly by inhibiting DNA repair, in combination treatments with etoposide, or with cisplatin, as well as in combination with IR. Gemcitabine has also been successfully used in combination with either cisplatin, carboplatin, 5-FU, etoposide, or paclitaxel in chemoradiotherapy. These drugs (and their analogs) also continue to be evaluated as radiosensitizers in radiotherapy trials by the Cancer Therapy Evaluation Program (CTEP) of the NCI.

Gemcitabine has clinical antitumor activity in pancreatic, lung, ovarian, bladder, and breast cancer.<sup>59</sup> Gemcitabine was initially approved for pancreatic carcinoma, based on improved overall quality of life.<sup>60,61</sup> Gemcitabine has also been licensed for the treatment of non-small-cell lung cancer (NSCLC).<sup>62</sup> The standard regimen administers gemcitabine between 800 and 1,250 mg/m<sup>2</sup> IV once a week for 3 weeks out of a 4-week cycle. However, recent studies in NSCLC patients, using a twice-weekly (50 mg/m<sup>2</sup>/week or 100 mg/m<sup>2</sup>/week) regimen for 6 weeks, concurrent with 60–66 Gy of conformal thoracic radiation, found increased radiation therapy radiosensitization.<sup>63</sup> The overall complete and partial response was 88% and the median survival was 18 months. The twice-weekly gemcitabine dosing schedule was based on preclinical results from Lawrence et al,<sup>64</sup> showing the radiation-sensitizing effect of gemcitabine is lost 48–72 hours after the drug is administered. One-year overall survival and disease-free survival was improved in locally advanced NSCLC with acceptable toxicities.

Another phase II study used concurrent chemoradiation with biweekly gemcitabine after induction chemotherapy with cisplatin and gemcitabine in unresectable stage III NSCLC.<sup>65</sup> Three cycles of cisplatin (100 mg/m<sup>2</sup>, d1) and gemcitabine (1,250 mg/m<sup>2</sup>, d1 and 8) were administered every 3 weeks, followed by concurrent chemoradiation (gemcitabine 50 mg/m<sup>2</sup> Monday and Thursday, and radiotherapy 68.4 Gy, 1.8 Gy daily). Of 22 patients initially entered into this phase II study (Group A), unacceptable toxicity was detected and the study reduced the induction cisplatin dose (70 mg/m<sup>2</sup>) and the concurrent chemoradiotherapeutic gemcitabine dose (35 mg/m<sup>2</sup>). Another 34 patients (33 eligible, Group B) were then entered into the study. The response of Group A patients was 40% partial response and 18% complete response. The Group B response to treatment was 54% partial response and 12% complete response. For Group A, median survival was modest (11.8 months), but 6 (27%) patients were alive at a minimum follow-up of 35 months. Survival data for Group B is not yet available. Nonhematologic toxicity of chemoradiation was substantial and similar in both groups. This treatment was feasible in patients with unresectable

stage III NSCLC. Long-term results suggest selected patients could benefit in this approach.

Another phase II study determined the feasibility, toxicity, response rate, local control, distant metastases, and survival of concurrent chemotherapy with etoposide and cisplatin using 3D conformal radiotherapy and maintenance chemotherapy with cisplatin and gemcitabine in unresectable NSCLC.<sup>66</sup> A total of 23 patients with unresectable NSCLC entered into the trial and 18 (78%) completed treatment. Radiotherapy was given to a total dose of 66.6 Gy plus cisplatin (20 mg/m<sup>2</sup> d1–5, d29–34) with 2 cycles of cisplatin and etoposide chemotherapy (etoposide 90 mg/m<sup>2</sup> d1–3, d29–31). Following concurrent chemoradiation, all patients received gemcitabine, 900 mg/m<sup>2</sup> d1 and 8, with carboplatin or cisplatin, 70 mg/m<sup>2</sup> d2, every 21 days for 4 cycles. All 23 patients received non-coplanar 3D conformal radiotherapy using 4–6 fields. Two patients (9%) had stage II, 9 (39%) had stage IIIa, 9 (39%) stage IIIb, and 3 (13%) had stage IV disease. The response rate was 64% with 2 complete responses and 13 partial responses. At a minimum follow-up of 12 months, overall survival was 69% at 1 year. Actual local progression-free survival and actual distant metastases-free survival were 65% and 74% at 1 year, respectively. Concurrent therapy with cisplatin and etoposide with 3D conformal radiotherapy and maintenance chemotherapy with cisplatin and gemcitabine is a well tolerated regimen and has a good efficacy.

Sixty-nine patients with locally advanced head and neck cancer were enrolled in a phase I study of aggressive chemoradiotherapy with concurrent gemcitabine (dose-escalated from 50–300 mg/m<sup>2</sup>), paclitaxel (100 mg/m<sup>2</sup>), and 5-FU (600 mg/m<sup>2</sup>/day for 5 days) in conjunction with twice-daily radiation (1.5 Gy) delivered on an alternating week schedule (TGFX).<sup>67</sup> The clinical outcome and late toxicity of TGFX in a subset of patients, previously irradiated to the head and neck, was determined. Twenty-nine previously irradiated patients with recurrent or second primary head and neck cancer were treated with the TGFX regimen. Twelve patients underwent attempted surgical resection prior to chemoradiotherapy, of which 10 were left with no measurable disease. Patients with measurable disease were treated to a median dose of 72 Gy, while those with no measurable disease were treated to a median dose of 61 Gy. The cumulative radiation dose ranged from 74.4 Gy to 156.4 Gy, with a mean and median of 125.7 Gy and 131.0 Gy, respectively. The median follow-up was 19.1 months for all patients and 48.1 months for living patients. At 2 years, the overall survival was 37%, cause-specific survival was 39%, and locoregional control (LRC) was 54%. In patients with measurable disease at the time of treatment, the 2-year overall survival and locoregional control (LRC) are 26% and 45%, respectively, compared with 2-year overall survival and LRC of 58% ( $P=.09$ ) and 70% ( $P=.31$ ) for those with no measurable disease. Measurable disease and radiation dose were highly significant for overall survival and LRC on multivariate analysis. Of 14 patients assessable for late toxicity, 3 developed

grade 4–5 toxicity, 8 developed grade 2–3 toxicity, and 3 developed grade 0–1 toxicity. Six patients developed soft tissue complications: grade 5 in 1, grade 4 in 2, and grade 3 in 3. Six patients developed swallowing complications, of which 3 were gastrostomy-tube-dependent. Aggressive reirradiation with chemotherapy in locally advanced head and neck cancer provides a chance for long-term cure at the expense of acute and late toxicity. Attempted surgical resection prior to chemoradiation improved disease control and survival.

A phase II study was performed to confirm safety and efficacy of gemcitabine and radiation therapy in patients with localized adenocarcinoma of the pancreas that were deemed unresectable by computed tomography (CT) scans and/or endoscopic ultrasound and confirmed by surgical evaluation.<sup>68</sup> Patients received gemcitabine at 600 mg/m<sup>2</sup> IV weekly, with concurrent radiotherapy of 180 cGy/day, 5 days/week, for a total of 5,040 cGy (part A). Approximately 4 weeks after completion of combination therapy, patients without disease progression received gemcitabine, 1,000 mg/m<sup>2</sup> on days 1 and 8 of a 21-day cycle, for 6 cycles (part B). A total of 32 patients were enrolled in the trial; 28 were evaluable and 4 never received treatment. The median follow-up was 7.8 months. Twenty-four (85%) patients completed part A of treatment. Six patients (21%) had a partial response, 16 (57%) had stable disease, and 4 (14%) had progressive disease. The median time to progression was 6.3 months. The 1-year survival time was 31%. Median survival time was 7.9 months. Thus, gemcitabine and radiation therapy had moderate toxicities and could be safely administered. An Inter-group phase III study comparing gemcitabine alone versus gemcitabine and radiation therapy is ongoing.

In locally advanced bladder carcinoma, radiation therapy, either alone or in combination with chemotherapy, is the treatment of choice for patients whose disease is not amenable to surgery.<sup>69</sup> A study was carried out to evaluate the efficacy and toxicity of neoadjuvant chemotherapy with gemcitabine and carboplatin, followed by concurrent chemoradiotherapy in locally advanced bladder carcinoma. Twenty-two patients initially received 4 cycles of gemcitabine and carboplatin, after which the response evaluation was performed with CT scanning and ultrasonography. Patients with complete response and partial response received 2 more cycles, followed by radical radiotherapy with weekly gemcitabine as a radiosensitizer. Those who had stable disease or progressive disease were given concurrent chemoradiotherapy after 4 cycles. All patients had a histopathological subtype of transitional cell carcinoma. Eleven patients (50%) had T3 lesion, 7 (31%) had T4, and 4 (18%) had T2. Out of 22 patients, 6 (27%) showed a complete response, and a partial response was seen in 9 patients (40%). Four patients (18%) had stable disease, whereas 3 patients showed progression of disease. Two cases out of 4 having stable disease showed tumor regressions after concurrent chemoradiotherapy. No grade III/IV toxicity was seen. Thus, neoadjuvant chemotherapy with gemcitabine and carboplatin, followed by concurrent chemoradiotherapy, appears to be a tolerable and effective treatment to

achieve significant tumor response in locally advanced bladder carcinoma.

Locally advanced bladder cancer patients have low survival rates after radical cystectomy. Postoperative radiotherapy (PORT) improved this survival, but this improvement was not satisfactory. A prospective randomized trial included 59 locally advanced bladder cancer patients.<sup>70</sup> Patients who underwent radical cystectomy and had P3b, P4a, grade 3 pathology and/or pelvic nodal involvement were randomized into 2 groups: Group I (27 patients) received PORT consisting of 45 Gy/30 fractions/5 weeks. Group II (32 patients) received adjuvant chemoradiotherapy in the form of 2 courses of gemcitabine at 1 g/m<sup>2</sup> on day 1 and day 8 and cisplatin at 70 mg/m<sup>2</sup> on day 2, the same regimen of PORT as in group I, followed by another 2 courses of gemcitabine and cisplatin. The toxicities of both chemotherapy and radiotherapy were evaluated, and the primary endpoint was disease-free survival. Only 3 Group II patients (9%) could not complete their prescribed regimen. Chemotherapy was tolerated in most of the patients with grade 1/2 toxicities. Grade 3/4 anemia were observed in 10% of the cycles. Patients were followed up for 3–17 months. The 1-year disease-free survival rate for Group I patients was 59 ± 15%, while that of Group II patients was 64 ± 11%, with no statistically significant difference ( $P=.4$ ). Adjuvant chemoradiotherapy using gemcitabine, cisplatin, and PORT was tolerable with minimal severe toxicities. Given the high-risk patient population, the 1-year disease-free survival is considered encouraging, but longer follow-up is required to assess any benefit to postoperative chemoradiotherapy compared with PORT alone in these high-risk bladder carcinoma patients.

### Fludarabine and <sup>125</sup>I-armed Tositumomab Radioimmunotherapy

Fludarabine is an adenine-base-modified fluoronucleoside drug analog with significant activity in hematologic malignancies and in preclinical solid tumor models. IV fludarabine phosphate (2-F-Ara-AMP, 9-β-D-arabinosyl-2-fluoradenosine monophosphate) is the most active single-agent drug for the treatment of chronic lymphocytic leukemia.<sup>71–73</sup> Fludarabine is also active against indolent non-Hodgkin lymphoma (NHL)<sup>74,75</sup> but appears to have certain limitations in single-agent activity against common solid tumors. Fludarabine is metabolically dephosphorylated to 2-F-Ara-A and then rephosphorylated to 2-F-Ara-dAMP and eventually to 2-F-Ara-dATP in the cell nucleus, where it is incorporated into cellular DNA and can be a DNA chain terminator.<sup>76</sup>

In preclinical studies, fludarabine in combination with IR has demonstrated radiosensitizing activity.<sup>77,78</sup> Fludarabine radiosensitization is being clinically studied in head and neck carcinomas, but its potential radiosensitizing activity has not been systematically studied in NHL. Phase I studies of fludarabine chemoradiotherapy were performed in patients with intermediate to locally advanced head and neck squamous cell carcinoma (HNSCC).<sup>79</sup> Fludarabine was

administered by IV daily 3–4 hours before the last 10 fractions of a standard radiation fractionation regimen (70 Gy in 7 weeks). The main objective was to determine the MTD of fludarabine in IR regimens. Twenty-eight patients with stage T2–T4 disease were treated with fludarabine doses started at 7.5 mg/m<sup>2</sup>/day and increased by steps of 2.5 mg/m<sup>2</sup>/day. Fludarabine in increasing doses to radiation did not result in increased intensity or duration of skin or mucosal toxicity, as compared to radiation alone. At a daily dose of 17.5 mg/m<sup>2</sup>, 2 patients out of 5 (40%) developed grade 4 neutropenia, and this dose was set as the MTD. All patients developed fludarabine dose-dependent lymphocytopenia. The plasma F-ara-A concentration peaked after 30-minute infusion in a dose-dependent fashion and reached an average peak concentration of approximately 2 μM for doses of 15 mg/m<sup>2</sup> and higher. Thus, fludarabine can be safely administered concurrently with radiation at a daily dose of 15 mg/m<sup>2</sup> during the final 2 weeks of radiotherapy. A phase II trial will evaluate the potential role of concurrent fludarabine chemoradiotherapy in the treatment of moderately to locally advanced HNSCC.

Radioimmunotherapeutic (RIT) treatment with tositumomab (Bexxar, GlaxoSmithKline) <sup>131</sup>Iodine-conjugated-anti-CD20 immunoglobulin (Ig) G, a radiolabeled monoclonal antibody that binds to the target antigen CD20 on normal and cancerous B-lymphocytes in NHL patients, has recently shown highly promising clinical results.<sup>80</sup> Tositumomab radioimmunotherapeutic regimens are indicated for the treatment of patients with CD20-antigen-positive, follicular NHL, with and without transformation, where disease is refractory to antibody treatment with rituximab (Rituxan, Genentech; a chimeric monoclonal anti-CD20 IgG), and the patient has relapsed following chemotherapy.

Tositumomab preferentially targets and delivers cytotoxic <sup>131</sup>Iodine radiation to B-lymphocytes, inducing breaks in genomic DNA in B-lymphocytes of tositumomab-treated NHL patients. Tositumomab can be given in up to 4 outpatient visits over 1–2 weeks and is specifically dosed based on an individual's drug clearance rate of unconjugated tositumomab, allowing the delivery of a predetermined amount of antibody-bound radiation to each patient.

Tositumomab was initially evaluated and intended as a single-course treatment of RIT, and it appears unlikely that the initial trial with fludarabine monophosphate followed by tositumomab was designed to take full advantage of a potential radiosensitizing regimen. The potential consequences and safety of multiple courses of tositumomab RIT as a therapeutic regimen, or combination of RIT regimens in specifically designed fludarabine chemoradiosensitizing regimens for NHL, have not been evaluated.

Recently, a study using tositumomab following chemotherapy as a first-line treatment of NHL was reported<sup>80</sup> as a long-term follow-up of 35 patients who received IV fludarabine monophosphate at 25 mg/m<sup>2</sup>/day for 5 days, every 5 weeks for 3 cycles, followed in 6 to 8 weeks by <sup>131</sup>Iodine-armed

tositumomab therapy. At the time of enrollment, 97% of the patients had stage III/IV disease.

This sequential fludarabine and tositumomab radiotherapy produced long-term, durable, and complete remissions in the majority of patients. Thirty-five patients (89%) responded to fludarabine (3 complete responses, 28 partial responses). Following tositumomab therapy, all 35 patients had a response, including 83% complete responses. With a median follow-up of 4.4 years, median progression-free survival was not reached, and 72% of the patients that achieved a complete response remained in complete response. Complete responses occurred in 29 of 35 patients, and partial responses were observed in 6 of 35 patients. The median progression-free survival exceeded 4.4 years posttreatment, and most patients (20 of 29) who achieved a complete response after fludarabine and tositumomab treatment remained in complete response.

The principal adverse event associated with the sequential treatments of fludarabine monophosphate with tositumomab RIT was significant depression of blood cell counts, with grade 4 neutropenia, thrombocytopenia, and anemia in 34%, 29%, and 3% of patients, respectively. Sixteen patients (46%) received growth factors or transfusions, but there were no serious infections. Four patients (12%) developed elevated thyroid-stimulating hormone levels, and 2 patients (6%) became positive for human antimouse antibody. A major concern with RIT is the development of secondary acute myeloid leukemia or myelodysplastic syndromes. The frequency of this adverse event was comparable to patients who did not receive RIT.<sup>81,82</sup> None of the patients developed secondary cancers, such as myelodysplastic syndromes or acute myeloid leukemia. Fludarabine followed by tositumomab appears to be a highly effective and well-tolerated regimen for the initial therapy of advanced follicular NHL.

### **Inhibition of Overexpressed VEGF With Bevacizumab or VEGF Antisense Oligonucleotide and IR-Induced VEGF-Mediated Tumor Angiogenesis and Apoptosis**

VEGF is a critical growth factor for tumor angiogenesis and is important to regulate in radiosensitizing regimens. VEGF acts at various stages of the multistep angiogenic process, including acting as a paracrine growth factor in some tumors.<sup>83</sup> Increased VEGF protein expression is found in a variety of tumors, particularly in those tumors resistant to radiotherapy. These include malignant gliomas and melanomas, where overexpression is associated with poor prognosis. Radiation therapy stimulates production of VEGF in tumor cells.<sup>84</sup> Several groups of investigators observed enhanced chemotherapy and radiation therapy by antiangiogenic agents in tumor-bearing mice.<sup>85–89</sup> The mechanisms underlying the enhancement of the radiation response by antiangiogenic agents include a decrease in tumor oxygenation, a decrease in vascular density and, possibly, radiosensitization of endothelial cells. Kozin et al<sup>90</sup> demonstrated anti-VEGFR2 IgG antibody administration in combination with fraction-

ated radiotherapy significantly decreased, by a factor of 1.3–1.7, the dose of radiation necessary to locally control 50% of 2 different human tumor xenografts.

VEGF promotes and prolongs the survival of human endothelial cells, which is associated with increased expression of the antiapoptotic bcl-2 protein.<sup>91-92</sup> Enhanced endothelial cell survival is associated with a dose-dependent increase in bcl-2 expression and a decrease in expression of the processed forms of caspase-3.<sup>92</sup> VEGF induces bcl-2 by 5.2-fold. Overexpression of bcl-2 is associated with inhibition of apoptotic cell death, in the absence of VEGF, in human umbilical vein endothelial cells. Therefore, a likely mechanism of action of a VEGF antagonist during the radiation response is through the regulation of apoptosis.

Antibodies that target and inhibit specific VEGF receptors and antisense oligonucleotide drugs that inhibit all VEGF isoform activities are currently under clinical investigation. Bevacizumab is a recombinant humanized monoclonal IgG antibody which binds to and inhibits the VEGF A isoform of human VEGF with high affinity and has recently been approved as a first-line treatment for metastatic colorectal cancer. When bevacizumab is given in combination with irinotecan, 5-FU, and leucovorin, which is one of the standard regimens for colon cancer, bevacizumab prolongs survival by about 5 months.<sup>93</sup>

VEGF antisense (VEGF-AS; Veglin, VasGene Therapeutics) is a new and potentially interesting antiangiogenic drug candidate. It is a specific VEGF antisense thiophosphate substituted oligonucleotide that inhibits the production of all VEGF isoforms (A, C, D). In phase I studies, VEGF-AS is well tolerated and appears to be showing antitumor activity.<sup>94</sup> Another antiangiogenic inhibitor drug candidate is the small-molecule inhibitor SU5416 (SUGEN), a potent and selective inhibitor of the Flk-1/KDR receptor that blocks tyrosine kinase catalysis and inhibits tumor vascularization and growth.<sup>95</sup> The VEGF-survival phenotype in endothelial cells is mediated by VEGF binding to the Flk-1/KDR receptor.

Inhibition of VEGF activity is hypothesized to enhance radiation and chemotherapy response in patients overexpressing VEGF. To test this hypothesis, a phase I clinical trial was initiated to evaluate the feasibility of integrating bevacizumab into a contemporary treatment program of preoperative chemotherapy and radiation therapy, followed by surgery for patients with primary and nonmetastatic rectal cancer.<sup>96</sup> VEGF is overexpressed in rectal cancer and is associated with disease progression and inferior survival. Nine patients with clinical stage T3 or T4 NX M0 rectal cancer completed a treatment program of preoperative bevacizumab (5 mg/kg; 6 patients) or (10 mg/kg; 3 patients) on day 1, followed by concurrent administration of progressive disease (5 mg/kg or 10 mg/kg; d15, 29, 43) with 5-FU radiochemotherapy (225 mg/m<sup>2</sup> every 24 hours: d15–52) using radiation therapy (50.4 Gy in 28 fractions on d15–52). Surgery was performed 7 weeks after completion of bevacizumab, 5-FU, and radiation therapy. All 6 patients at a dose of 5 mg/kg of bevacizumab

completed the protocol without dose-limiting toxicity and underwent resection without complication. Three patients completed a dose of 10 mg/kg bevacizumab without dose-limiting toxicity, and 1 patient underwent resection without complication. Two patients were scheduled for surgery. Endoscopic responses just prior to surgery showed no visible macroscopic tumor in 6 of 7 evaluable patients. Similarly, pathologic evaluation demonstrated limited microscopic disease in the surgical specimens of 6 of 7 evaluable patients. These results indicated safety of bevacizumab in combination with neoadjuvant 5-FU and radiation therapy with surgery in the treatment of patients with locally advanced rectal cancer, in addition to significant clinical activity.

VEGF is upregulated by the loss or mutation of tumor suppressor genes, such as p53, and activation of oncogenes, such as ras, v-src, and HER2.<sup>97</sup> In addition, VEGF expression is particularly sensitive to oxygen tension and is rapidly upregulated by hypoxia, which is found in most tumors, possibly due to abnormal vascular supply.<sup>98</sup> Hypoxic tumor cells are extremely radioresistant. Significant effort has been placed on identifying and testing drugs and treatment regimens that may render hypoxic cells more sensitive to the effects of IR. This includes tirapazamine (Sanofi-Synthelabo) or efaproxiral (Efaproxyn, Allos Therapeutics). Tirapazamine is a small-molecule prodrug activated by intracellular reductases to a cytotoxic radical that, under hypoxic conditions, produces base damage, DNA single-strand breaks, DNA double-strand breaks, and chromosome aberrations.<sup>99-102</sup> However, in aerobic cells, the presence of oxygen causes oxidation of the radical to the nontoxic precursor. Efaproxiral is a synthetic small-molecule modifier of hemoglobin that enhances the diffusion of oxygen from hemoglobin in hypoxic tumor tissues by reducing the hemoglobin oxygen binding affinity.<sup>103</sup> Efaproxiral is administered shortly before radiation therapy.

The cotranscription factor and DNA BER enzyme Redox effector factor-1/apurinic/apyrimidinic endonuclease (Ref-1 or Ape1), is a critical component of the hypoxia-inducible transcriptional complex formed on the VEGF gene's hypoxic response element.<sup>104</sup> The presence of Ape1 in the complex is required for the apparent high affinity association between HIF-1 and its DNA recognition sequence. Recently it was shown that concurrent inhibition of Ape1 endonuclease activity by antisense oligonucleotides in pancreatic tumor cell lines (Panc-1 and MiaPaCa-2) and gemcitabine treatment resulted in a 2-log enhancement of gemcitabine toxicity in Panc-1 cells.<sup>105</sup> It will be important to determine whether the sensitization of some pancreatic cells to gemcitabine is due to downregulation of VEGF expression via Ape1-bound transcriptional complex such as HIF-1. It will also be important to continue to evaluate the antitumor activity of broadly reactive VEGF inhibitor drugs, especially including an isoform-independent VEGF antisense oligonucleotide drug. Potential synergistic combinations may occur with other radiosensitizing antisense oligonucleotides, including Rad51 antisense oligonucleotide,

Ref-1/Ape1 antisense oligonucleotide, or with other antiapoptotic antisense oligonucleotides, including oblimersen sodium (Genasense, Genta), a bcl-2 antisense oligonucleotide currently in clinical trials.

### **Inhibition of Overexpressed Tumor Rad51 With Imatinib Mesylate and Rad51 Antisense Oligonucleotide; Radiation Therapy-Induced Rad51-Mediated Tumor DNA DSB and Blockage of Tumor Cell Apoptosis**

Human Rad51 DNA repair enzyme catalyzes homologous DSB and is overexpressed in tumors and superinduced by IR. Elevated levels of de novo Rad51 mRNA and protein occurs in a wide variety of tumors and tumor cell lines.<sup>106</sup> Overexpression of Rad51 protein in tumors is associated with increased p21/WAF-1 expression, a decreased apoptotic response, and increased radioresistance in vitro.<sup>107-110</sup> In addition to the several-fold overexpression of Rad51 in tumor cells relative to the low expression in normal cells, IR treatment significantly increases the expression of Rad51 RNA and Rad51 protein-mediated DNA repair activity in both tumor and normal cells.<sup>111,112</sup> Rad51 levels also increased under certain hypoxic conditions in tumor cell lines (A. Vallega, unpublished experiments). Hypoxic induction of the expression of Rad51 in tumor cells may be associated with the binding of the hypoxia-inducible transcriptional complex that may also bind Ref-1/Ape1 to a hypoxia response element (HRE) associated with the human Rad51 gene.

Ribozyme or antisense oligonucleotides inhibit the expression of Rad51 mRNA and protein, and radiosensitizes breast and brain tumor cells in vitro.<sup>113-115</sup> Overexpression of Rad51 is also associated with increased resistance to DNA damaging and DNA breaking agents, including chlorambucil, nitrogen mustard, caffeine, cisplatin, and MMS.<sup>116-124</sup> Taken together, these data support the hypothesis that Rad51 overexpression can alter DNA double-strand repair and apoptotic responses. Thus, Rad51 is an important drug target for potentially increasing the radiosensitivity of tumors. Rad51-targeted drugs that specifically and preferentially inhibit IR-super-induced Rad51 (in tumors overexpressing Rad51 prior to irradiation) include imatinib mesylate and Radvac (Rad51 antisense oligonucleotide, Colby Pharmaceuticals), a drug candidate in development. These Rad51 inhibitor drugs may both specifically inhibit homologous DSB, release tumor Rad51 mediated blocks to apoptosis, and increase irradiated tumor cell death. Certain Rad51-specific antisense oligonucleotides appear to have single-agent activity and can cause human tumor xenograft regression even without combination with radiation or without combination with other DNA damaging drugs (A. Vallega, unpublished experiments).

Rad51 protein monomers cooperatively load onto the single-strand ends of dsDNA breaks and form a nucleoprotein filament, which catalyzes the search for the homologous (non-broken) DNA copy.<sup>125</sup> Rad51 protein monomers polymerize

on the DNA phosphate backbone, and each Rad51 monomer subunit spans approximately 3 consecutive nucleotides. DNA in the Rad51 nucleoprotein filament is extended to approximately 150% of its usual length. This Rad51 protein-coated DNA is the active species in homologous DNA repair. Assembly of the Rad51 protein monomers on broken DNA can be negatively regulated by wild type, but not mutant, p53 or BRCA2 proteins. Normal p53 and BRCA2 directly bind to Rad51 at or near sites that can block polymerization of the Rad51 monomers on DNA.<sup>126-131</sup>

Data from Conilleau et al<sup>132</sup> suggest the Rad51 tyrosine 315 residue is located on the Rad51 protein monomer at a key regulatory site in a position where it would effect or abut the next Rad51 monomer that binds on the DNA backbone. Rad51 tyrosine 315 phosphorylation by c-Abl tyrosine kinase would positively regulate the ability of Rad51 protein monomers to appropriately form (bind, load, polymerize) on broken DNA. Imatinib mesylate inhibition of Rad51 tyrosine 315 phosphorylation would inhibit the activity of nonphosphorylated Rad51 protein monomers, inhibiting formation of active Rad51-DNA nucleoprotein filaments and inhibiting Rad51-mediated homologous repair of IR-induced dsDNA breaks, therefore increasing cell radiosensitivity and cell death. In summary, imatinib mesylate can inhibit DNA break repair and increase tumor radiotherapy due to the inability of the tumor cell to appropriately phosphorylate Rad51 protein at tyrosine 315 following administration of the drug.

Russell et al<sup>133</sup> showed imatinib mesylate inhibits Rad51 and increases the radiosensitivity of 2 human glioma cell lines but not in a nonimmortalized normal human fibroblast cell line. Exposure of both glioma cell lines to radiation resulted in an increase in Rad51; imatinib mesylate treatment alone reduced Rad51. When glioma cells were pretreated with imatinib mesylate, radiation-induced Rad51 expression and nuclear Rad51 DNA repair foci were significantly reduced. Radiosensitivity was enhanced in these glioma cells after imatinib mesylate pretreatment of the cells. These data indicate that imatinib mesylate can enhance radiation-induced glioma tumor cell killing and suggest a mechanism via inhibition of Rad51 in the treated tumor cells. In contrast to the glioma cell lines, imatinib mesylate treatment under these conditions did not have an effect on Rad51 expression or Rad51 DNA repair foci formed in the normal fibroblast cells, nor did imatinib mesylate treatment increase radiosensitivity of the normal cell line. Thus, Rad51 may be regulated differently in the glioma and normal cell lines, and Rad51 may be targeted to selectively enhance the radiosensitivity of brain tumor cells, but not normal cells. A more recent study of the combination of imatinib mesylate and concomitant IR also showed increased radiosensitivity in primary human glioblastoma cells in culture.<sup>134</sup> A clinical trial is currently enrolling patients to test the effects of imatinib mesylate either with or without radiation therapy in the treatment of children with newly diagnosed or recurrent gliomas.

Ohnishi et al<sup>114</sup> showed that specific Rad51 thiophosphate-modified antisense DNA oligonucleotide treatments inhibit Rad51 protein expression and enhanced the radiosensitivity of malignant mouse gliomas *in vivo* and human gliomas *in vitro*. Rad51 antisense oligonucleotide treatment of gliomas in mice specifically inhibited Rad51 and increased radiosensitization, as evidenced by a significantly improved radiotherapeutic ratio and increased survival. Rad51 antisense oligonucleotide inhibited the level of Rad51 mRNA by more than 95% and reduced the Rad51 protein expression by about 70%. Treatment of mouse 203G glioma cells with 100 nM of Rad51 antisense oligonucleotide significantly enhanced the radiation-induced cell kill compared to control cells treated with either control sense oligonucleotide or control scrambled oligonucleotide.

In this study, glioma cells implanted in the cisterna magna of mice were treated with Rad51-specific antisense oligo or control (scrambled) oligo with 10 animals in each group. The survival time of the tumor-bearing mice treated with the scrambled control oligo compared to the mice treated with Rad51 antisense test oligo was markedly prolonged. Ohnishi et al showed none of the 10 irradiated (6 Gy, whole body) mice were surviving at 12 days posttreatment with the scrambled control oligo, and 5 of 10 irradiated mice were still surviving at 35 days. The combination of Rad51 antisense oligo and IR consistently extended the survival time of the glioma-bearing mice for significantly longer periods than could be achieved with radiation therapy alone. Thus, specific inhibition of Rad51 with Rad51 antisense oligonucleotide can radiosensitize malignant mouse gliomas and human gliomas treated with Rad51 antisense oligonucleotide specific for human Rad51-increased radiation sensitivity.

### Inhibition of Epidermal Growth Factor Receptor Signaling With Cetuximab and Gefitinib

Tumors overexpressing the epidermal growth factor receptor (EGFR, erbB-1, or HER1) are often observed in malignant epithelial cancers and are associated with poor prognosis.<sup>135</sup> There are 2 known classes of EGFR inhibitor drugs with radiosensitizing potentials. The first is an IgG specifically targeted against an EGFR-specific antigenic determinant, and the second is a small-molecule tyrosine kinase inhibitor targeted against the intracytoplasmic catalytic domain of EGFR.<sup>136</sup> These drug candidates have been developed and tested in preclinical and clinical trials.

Preclinical data from studies using a combination of an EGFR inhibitor drug in combination with IR in tumor cells grown in culture, or cells grown as tumor xenografts established in nude mice, demonstrate enhanced tumor cell kill, tumor regression, or tumor growth delay in comparison to either modality (radiation therapy or drug) alone.<sup>136</sup> In preclinical studies, Huang et al<sup>137</sup> suggested the *in vivo* antitumor activity associated with radiation therapy in combination with the anti-EGFR monoclonal antibody cetuximab could be related to altered DNA repair after exposure to radiation or altered

expression of tumor angiogenesis factors, including VEGF or factor VIII.

Advanced phase III clinical trial data exist for cetuximab in combination with radiation therapy in head and neck cancer.<sup>138</sup> The preliminary results confirm that cetuximab-mediated inhibition of EGFR signaling augments therapeutic radiation outcomes for HNSCC patients. Patients were randomized to receive radiation therapy plus weekly cetuximab therapy (n=211) or radiation therapy alone (n=213) for 6–7 weeks. The percentage of patients who achieved locoregional control at 1 year and at 2 years following treatment was 69% and 56%, respectively, in cetuximab-treated patients. This is compared with 59% and 48% for those treated with radiation alone. Likewise, the percentage of patients alive at 2 and 3 years posttreatment was 62% and 57% for the combined radiation and cetuximab-treated patients versus 55% and 44% for those treated with radiation therapy alone. Both the duration of locoregional control and the duration of survival were statistically significant (log rank  $P=.02$  for both endpoints). With a minimum follow-up of 24 months and a median follow-up of 38 months, the median overall survival for the cetuximab-treated patients was 54 months, compared with 28 months for patients treated with radiation therapy alone. The clinical benefit was achieved with minimal enhancement in the overall toxicity profile. The incidence of grade 3/4 mucositis was similar in both treatment groups (55% in patients receiving radiation plus cetuximab and 52% in patients who received radiation alone). Three percent of patients in the radiation therapy plus cetuximab arm and 0% of patients in the radiation therapy-alone arm experienced grade 3/4 infusion reactions, and grade 3/4 skin reaction occurred in 34% of patients receiving radiation plus cetuximab, compared with 18% of radiation therapy-alone patients. The results from this study form the basis for future trials combining EGFR inhibitor drugs with ionizing radiation in other epithelial malignancies that overexpress EGFR.

Bianco et al<sup>139</sup> demonstrated that concurrent treatment of nude mice bearing established human colon tumor xenografts with the combination of radiation therapy and gefitinib, an EGFR-specific small molecule tyrosine kinase inhibitor drug, significantly improved survival, compared with control radiation therapy-treated or control gefitinib-treated mice. In the radiation therapy plus gefitinib group, 10% of mice were alive and tumor-free after 26 weeks following treatment. The combined radiation therapy and gefitinib treatment had a significant antiangiogenic effect, as determined by immunohistochemical monitoring of neovessels within the human colon tumors grown as xenografts in nude mice. The expression of transforming growth factor alpha, VEGF, and basic fibroblast growth factor was inhibited by combined radiation therapy and gefitinib treatment in tumor xenografts. This study provided the preclinical rationale for evaluating cancer patients for the combination of radiation therapy and gefitinib. Several clinical trials exploring the use of gefitinib in combination with IR are currently being investigated in patients with gliomas, NSCLC, and head and neck tumors.

Gefitinib has been approved in the United States for use as monotherapy for the treatment of patients with locally advanced or metastatic NSCLC after failure of both platinum-based and docetaxel chemotherapies.<sup>140</sup> The effectiveness of gefitinib is based on objective response rates. Thus far, no controlled trials have demonstrated a clinical benefit such as improvement in disease-related symptoms or increased survival.

## Conclusions

Oncology patients significantly benefit from increased acceptance and clinic uses of radiosensitizing regimens with either cisplatin or fluorouracil. Base- or sugar-modified halonucleoside analog drugs and prodrugs with radiosensitizing activities are in current clinical trials or will enter trials in the near future. These include gemcitabine, capecitabine, fludarabine, and IPdR. These halogen-modified nucleoside analog drugs, or prodrugs, which have clinically important single-agent activities as antimetabolites, are incorporated into DNA and have radiosensitizing activities.

Tumors may also be radiosensitized to IR by imatinib mesylate inhibition of Rad51-catalyzed homologous DNA DSB, by cetuximab- or gefitinib-mediated inhibition of EGFR signaling, or by bevacizumab inhibition of VEGF-mediated tumor angiogenesis. Enhancing IR-induced DNA breaks, inhibiting repair of DNA breaks, or angiogenesis increases tumor cell death and enhances radiation therapy.

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