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Review Article

Highlights from the 46th Annual Meeting of the American Society for Therapeutic Radiology and Oncology (ASTRO)

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Abstract

On October 3-7, 2004, The American Society for Therapeutic Radiology and Oncology (ASTRO) held its 46th Annual Meeting in Atlanta, Georgia, U.S.A. The meeting was devoted to the presentation of advances in the management of malignant diseases with radiation modalities. The educational elements of this program are targeted at oncologists of all disciplines (*i.e.*, surgical oncologists, medical oncologists, radiation oncologists), physicists, biologists, nurses and therapists, as well as all healthcare workers who are involved in the treatment of patients with malignant diseases. The program includes presentations on standard, investigational and experimental therapeutics, as well as intensity-modulated radiation therapy, treatment planning, alternative fractionation, molecular and radiation biology.

This year, considerable time was devoted to basic science and the opportunity for novel chemical agents to interact with radiation. Such abstracts primarily fell into three categories: radiosensitization, radioprotection and new diagnostic tools (especially agents that could delineate nodal areas that have historically been difficult to identify).

Radiosensitization strategies

Knox *et al.* (1) reported the results of 5 trials that assessed the role of Bexxa® (tositumomab and iodine I131-tositumomab) therapy for relapsed, refractory or transformed low-grade non-Hodgkin's lymphoma. A total of 250 patients were enrolled in these trials at several institutions, including the Memorial Sloan-Kettering Cancer Center, Stanford University, the University of Alabama and the University of Michigan. Patient-specific dosimetry was used to deliver a total-body dose of 75 cGy of radiation. Durable responses were achieved in 81 patients (32%). This long-term response population (LTRP) received a median of 3 prior regimens, with 43% receiving at least 4 prior therapies. This LTRP had multiple poor prognostic characteristics, including 89% with stage III/IV disease, 37% without response to previous therapy, 49% with bulky disease (> 5 cm), 41% with bone marrow involvement, 23% with transformed histology and 23% with elevation of lactate dehydrogenase (LDH). The complete response (CR) rate for the LTRP was 77%. The median duration of overall response was 47 months and the median duration of CR was not attained. Moreover, among those patients who achieved a CR, 56% continue to be followed in CR for over 9 years. Thus, the Bexxar® regimen appears to produce substantial clinical benefit in the form of long-term, durable responses in heavily pretreated patients with low-grade NHL. The findings are quite dramatic when one considers that in patients with low-grade NHL, the rate and duration of response following treatment tend to decrease with each successive (and more intense) regimen.

Fiveash *et al.* (2), from the University of Alabama, assessed targeted antibody therapy in the setting of radiation therapy against gliomas. mTRA-8 is a proapoptotic mouse antibody that induces apoptosis in tumor cells but not in normal cells. Effects are achieved via a caspase 8-dependent mechanism. *In vitro* toxicity of temozolomide (Temodar) and mTRA-8 was examined utilizing the clonogenic survival assay and the colorimetric MTS assay with D54MG glioma cells. Athymic nude mice were injected subcutaneously with D54MG cells. Eight treatment groups of 7 mice each were compared. The groups included untreated controls, mTRA-8 alone, radiotherapy alone, temozolomide alone, radiotherapy plus mTRA-8, radiotherapy plus temozolomide, mTRA-8 plus temozolomide and triple therapy (mTRA-8 + temozolomide +

radiotherapy). Tumor irradiation consisted of 5 fractions of 5 Gy on a cobalt machine. Mice received temozolomide via gavage (50 mg/kg on days 16, 18, 21, 23 and 25) and mTRA-8 (200 μg) was injected i.p. on days 15, 18, 22, 25, 29 and 32. *In vitro*, both MTS and CS assays indicated enhanced cytotoxicity of combination treatment over the individual treatments. Of the 56 tumors (7 x 8 groups), 8 demonstrated tumor regression, including all 7 tumors treated with mTRA-8 plus temozolomide plus radiotherapy. The mTRA-8 antibody alone had only minimal activity in these systems. With follow-up of over 160 days, the triple therapy-treated tumors have not grown and the mice are healthy. The initial results of the intracranial model show 8 of 9 animals surviving with triple therapy *versus* 6 of 9 treated with radiation plus temozolomide and 0 responders among untreated controls or animals treated with TRA-8 alone. These data are therefore consistent with the hypothesis that the mTRA-8 antibody can enhance the response to malignant glioma. In the context of triple therapy, no animal experienced recurrence. Even with i.p. administration, it appears that TRA-8 can augment the response of gliomas to chemotherapy and or radiation therapy.

Mason and colleagues from the M.D. Anderson Cancer Center (3) evaluated molecular targeting of Toll-like receptor 9 with CpG oligodeoxynucleotides (ODN) in order to achieve enhancement of tumor response to radiation. Oligodeoxynucleotides are synthetic DNA sequences containing unmethylated cytosine-guanine motifs which exert potent immunomodulatory and antitumor effects. CpG ODNs induce cytokines, activate natural killer (NK) cells and elicit vigorous T-cell responses that can lead to significant antitumor effects. The investigators tested CpG ODN-1826 for its ability to enhance tumor response to radiotherapy. In the experiment, mice bearing a syngeneic immunogenic sarcoma (FSA) received single-dose or fractionated irradiation to the tumor-bearing leg with or without CpG ODN. CpG ODN was administered subcutaneously (3 x 100 μg/mouse). The radiation schedule was either a single dose of 20 Gy or 10 fractions of 2 Gy each. Fractionated irradiation was delivered twice daily (6 h apart) for 10 doses in 5 days. The CpG ODN significantly enhanced the effect of single-dose and fractionated irradiation by factors of 2.49 and 6.91, respectively. Neither CpG ODN alone nor irradiation alone produced cures, whereas the combined approach cured 60% as a single dose and 44% in the fractionated regimen. As such, this novel approach offers great potential to improve the efficacy of clinical radiotherapy.

MacDonald *et al*. (4) evaluated PABA (*p*-aminobenzoic acid) as a radiosensitizer. The compound, which is water-soluble and widely available as a dietary supplement, has also been used as a component of sunscreens. It has found clinical application in the treatment of scleroderma and Peyronnie's disease. In this abstract, the authors sought to evaluate the combination of radiation, PABA and docetaxel in the murine 4T1 model. Towards this end, 4T1 tumor cells were subcutaneously injected into the flanks of 8 groups of BALB/c mice. Three days later, mice were randomly assigned to receive PABA (1 mg/day). A second randomization was performed to docetaxel (4 mg/kg on days 7, 14, 21, 28 and 35). At 14 days postimplantation, another randomization was carried out to radiation treatment (two 5-Gy fractions of Cobalt-60). At day 37, PABA treatment had minimal effect on tumor growth. PABA, docetaxel and radiation inhibited growth by 50% as compared to controls (p = 0.01), by 31% as compared to PABA and RT (p = 0.03), and by 48% as compared to PABA and docetaxel (p = 0.02). In these experiments, it was clear that the trimodal approach of PABA, irradiation and docetaxel efficiently inhibited tumor growth. Since PABA has a long-standing track record in other conditions (it is known to be quite safe), there is high translational potential for PABA regimens in clinical oncology.

Xavier *et al*. (5) were intrigued by studies suggesting that modulation of CHK1/CDC25A signaling pathways may increase radiosensitivity and they therefore examined the effect of PABA on the expression and stability of CDC25A in tumor cells *in vitro* and *in vivo*. Towards this end, mitochondrial dehydrogenase activity was assessed with a commercially available *in vitro* proliferation assay kit. cDNA array analysis was performed on tumor cells incubated in the presence or absence of PABA. Western blot analysis was carried out on whole-cell lysates derived from tumor cells that were incubated in the presence or absence of PABA. Finally, the effects of PABA on tumor radiosensitivity was assessed *in vivo* in both the chick embryo and murine tumor models. The results revealed that pretreatment of breast carcinoma cells with PABA enhanced the antiproliferative effects of ionizing radiation *in vitro* and the radiosensitivity of breast carcinoma tumors grown *in vivo*. Analysis of cDNA array data suggested that CDC25A gene expression was enhanced approximately 2-fold in both murine and human melanoma cells as compared to untreated cells. This increase in CDC25A expression was confirmed by Western blot analysis of whole-cell lysates derived from both melanoma and breast carcinoma cells incubated in the presence of PABA. Interestingly, pretreatment of breast carcinoma cells with PABA resulted in a reduction of radiation-induced degradation of CDC25A *in vitro*. These findings suggest that PABA may enhance the radiosensitivity of both breast cancer and melanoma *in vitro* and *in vivo*. The translational potential here is obvious.

Crane *et al*. (6) attempted to tackle a particularly refractory disease (*i.e.*, locally advanced pancreatic cancer) with a combination of radiation therapy, capecitabine and bevacizumab. Entry criteria included patients with locally advanced or medically inoperable pancreatic cancer. CT criteria were used to classify the patients. Bevacizumab (5 mg/kg i.v.) was administered 2 weeks prior to the start of irradiation (50.4 Gy to the primary and draining nodal groups), then every 2 weeks thereafter (2.5 mg/kg in the first 12 patients, then 5 mg/kg in the latter 12 patients). Capecitabine was administered continuously with radiation on days 14-52 (650 mg/m^2 p.o. b.i.d.

for the first 6 patients, then 825 mg/m^2 p.o. b.i.d. for the remaining patients). Patients with responding or stable disease were offered maintenance bevacizumab. Twenty-four patients have completed treatment. Grade 2 gastrointestinal (GI) toxicity (National Cancer Institute [NCI] Common Toxicity Criteria [CTC]) was seen in 9 patients and grade 3 GI toxicity was seen in 2 patients. Five patients had grade 2 hand-foot syndrome. There have been 3 grade 3 bleeding episodes related to bevacizumab. All patients completed radiotherapy, although 3 required treatment breaks. Only 1 patient required hospitalization during the course of the therapy. One of 12 patients treated at 2.5 mg/kg and 6 of 11 patients treated at 5 mg/kg bevacizumab achieved a partial response. Only 1 patient experienced objective tumor progression. Thus, treatment with this novel combination was relatively well tolerated and quite active against locally advanced pancreatic cancer. The researchers indicated that they have since continued to enroll patients at doses of 7.5-10.0 mg/kg of bevacizumab.

Investigators from the University of Michigan at Ann Arbor evaluated Smac peptidomimetic compounds as radiosensitizers in human cancer cell lines. Saito *et al*. (7) pointed out that IAPs (inhibitors of apoptotic proteins) comprise a family of well-conserved eukaryotic antiapoptotic proteins. Moreover, overexpression of IAPs is one of the mechanisms used by cancer cells to counteract the induction of apoptosis by ionizing radiation. Smac has been shown to promote apoptosis by directly binding to and displacing caspase 9 from XIAP (X-linked inhibitor of apoptosis protein) and other IAPs. Hence, Smac appears to function as an endogenous proapoptotic protein by directly inhibiting the function of IAPs. This group designed and synthesized a series of potent peptidomimetics with high affinity for the BIR3 domain of the XIAP protein by mimicking the conserved IAP-binding motifs in Smac and caspase 9 proteins. The efficacy of these peptidomimetics as radiosensitizers was gauged in the experiments presented. Standard clonogenic assays were performed on a series of well-established human cancer cell lines, including those derived from non-small cell lung cancer (NCI-H460 and NCI-H661), prostate cancer (PC-3) and ovarian cancer (A2780, SK-OV-3), in the presence of varying concentrations of Smac peptidomimetics such as SH-97 and SH-102. Preliminary results on the prostate cancer line (PC-3) suggested that potent Smac peptidomimetics act as effective radiosensitizers *in vitro*. An even greater enhancement of the radiosensitivity of NCI-H460 cells was observed with SH-97 and SH-102. These results suggest that Smac peptidomimetics are effective radiosensitizers in clonogenic assays in some, but not all, of the human cancer cell lines investigated. Accordingly, the use of potent and cell-permeable Smac peptidomimetics may represent a novel strategy to improve the therapeutic index of radiation therapy.

Sharma *et al.* (8) described the radiosensitization of prostate carcinoma cells by ON-01910, a protein kinase inhibitor and cell cycle modulator. The agent is a benzylstyrylsulfone that inhibits CDK1 kinase activity, targets the G2/M cell cycle checkpoint in tumor cells and demonstrates cytotoxicity against a broad range of tumor cell lines. In this study, prostate cancer cell lines (RM-1, PC-3), normal human fibroblasts and prostate epithelial cells were evaluated. Cells were incubated with ON-01910 for 5-18 h followed by irradiation (0-6 Gy). Alternatively, cells were incubated chronically with ON-01910 at very low concentrations (40-100 nM) and irradiated 24 h after exposure. Pulse incubation with ON-01910 (5-18 h) was cytotoxic, with drug-induced G2/M arrest and apoptosis in both RM-1 and PC-3 cells, which was further enhanced by irradiation. At 72 h following drug exposure, the apoptotic index in PC-3 cells was 54 ± 6%, 77 ± 7% and < 10%, respectively, in cells treated with ON-01910, ON-01910 + 6 Gy radiation and radiation (6 Gy) alone. In contrast, normal fibroblasts and prostate epithelial cells were arrested in both G1 and G2 phases of the cell cycle and exhibited only minimal apoptosis (< 5%). Immunoblot analyses demonstrated a reduction in cyclin B1 and Bcl-2 expression for both RM-1 and PC-3 cells following ON-01910 treatment. Chronic exposure (40-50 nM) resulted in decreased clonogenicity and apoptotic values which were significantly greater than in untreated controls or cells treated with radiotherapy alone. The survival fraction at 2 Gy (SF2) was 0.79 in controls, which decreased to 0.32 and 0.23, respectively, at 40 and 50 nM of ON-01910. It was concluded that pulse and chronic exposure to ON-01910 was associated with significant cytotoxicity in human and murine prostate cancer cell lines at concentrations that exhibited only minimal cytotoxicity against normal cells. The drug may therefore be an attractive agent for chemoradiation strategies against prostate cancer in view of the selective cytotoxicity combined with enhanced radiosensitization at low doses.

Masunaga and colleagues (9) combined boron neutron capture therapy (BNCT) with ZD-6126, a vascular targeting agent. ZD-6126 is a tubulin-binding drug which brings about disruption of the cytoskeleton of proliferating cells. Sodium borocaptate-10B was injected into squamous cell carcinoma (SCC VII)-bearing mice. Approximately 20 min later, ZD-6126 was administered. Gamma-ray spectrometry was used to measure the 10B concentrations in tumors and normal tissue. Pharmacokinetic analysis revealed that the combination with ZD-6126 greatly increased the 10B concentrations in tumors after 60 min following BSH (borocaptate sodium) injection and after 120 min following BPA (*p*-boronophenylalanine) injections. Although it was pointed out that the concentrations of 10B from BSH in normal tissues were also raised by combination therapy with BSH, this was not significant. Combination with ZD-6126 had almost no effect on the concentrations of 10B from BPA in normal tissues. The surviving fractions of total tumor cells and the micronucleus frequencies of both total and quiescent tumor cells were reduced and increased by combinations with ZD-6126, respectively (independent of whether BSH or BPA was employed). However, the degree of these changes in the surviving fractions and the

micronucleus frequencies was more pronounced in tumors from BSH-injected mice than from BPA-injected mice, and in quiescent tumor cells compared to total tumor cells independent of the 10B carrier used. The investigators concluded that combination with ZD-6126 was more promising in BSH-BNCT than in BPA-BNCT and more effective for enhancing the sensitivity of quiescent tumor cells compared to total tumor cells.

Radioprotection strategies

Several abstracts probed the role of radioprotective agents in conjunction with modern radiation treatment strategies. Investigators from the University of Kentucky (10) performed a randomized, double-blind, placebo-controlled trial of balsalazide in the prevention of acute radiation enteritis as a consequence of pelvic irradiation. Balsalazide is a salicylate that yields high concentrations of active 5-ASA in the distal colon and rectum. It does not possess the antigenic sulfa moiety which is present in sulfasalazine (the only other 5-ASA with demonstrated benefit in this setting). In this study, 100 patients were randomized to receive pelvic irradiation with or without the experimental drug. Patients suffering from either carcinoma of the uterine cervix or prostate cancer were eligible for the study. All patients received at least 45 Gy via the four-field technique to the pelvis; prostate cancer patients were often boosted to 65 Gy while cervix cancer patients could reach 75 Gy. All patients received either 2250 mg of balsalazide or an identical placebo twice a day. Side effects were scored with the aid of the NCI CTC scale. In general, all gastrointestinal symptoms were decreased among the patients taking the investigational preparation. Proctitis was prevented most effectively by the drug. No patient taking balsalazide experienced grade 4 diarrhea, whereas 2 control arm patients did. Most balsalazide patients gained weight, while the majority of control arm patients lost an average of 3 pounds during the course of therapy. Surprisingly, dysuria was also much lower among those taking the experimental agent. Acute radiation enteritis remains a debilitating side effect of radiation treatment. It is likely that this pilot study will spawn a multiinstitutional cooperative group study.

Another topic of interest was protection against radiation-induced lung damage. Carpenter *et al*. (11) had previously shown that intrathecal injection of the human MnSOD (manganese superoxide dismutase) transgene in HA-MnSOD-PL (hemagglutinin epitope-tagged MnSOD-plasmid/liposome) prior to irradiation (*e.g*., 1 day before radiation) prevents radiation-induced damage to the murine lung. At this year's meeting, they reported on an inhalation method for delivery of MnSOD to the lungs. In their experiments, they employed an HA-MnSOD plasmid which contained the human MnSOD transgene. Ultrasonic nebulization was used to deliver the agent via inhalation over a 2-min period. Expression of MnSOD in the mouse lung was determined by RT-PCR using primers that were specific for the human MnSOD transgene. Mice either received a single fraction of irradiation (20 Gy) or fractionated radiation (*i.e*., 500 cGy/day x 10 fractions over a 12-day period). MnSOD-PL was administered either 24 h before the single fraction, or twice a week or daily in the fractionated experiments. The results revealed increased expression of the MnSOD-PL transgene, as demonstrated by a significant increase in MnSOD biochemical activity. RT-PCR demonstrated a 30-fold increase in expression of mRNA for the human MnSOD transgene. Mice that inhaled MnSOD had significantly increased survival compared to controls that were merely irradiated ($p < 0.05$). The investigators thus proved that MnSOD-PL could be efficiently administered to the lungs via inhalation. These data are consistent with the notion that inhalation of MnSOD-PL by lung cancer patients may protect the normal pulmonary structures from radiation-induced damage during and after treatment. Naturally, these results (although quite provocative) are a long way from finding clinical application.

Stickney *et al*. (12) evaluated two novel hormones (HE-2100 and HE-3204) as agents that could protect against thrombocytopenia and neutropenia associated with whole-body irradiation (WBI). The system selected for evaluation was a nonhuman primate model of myelosuppression and thrombocytopenia from ionizing radiation. The hormones were administered either as 5 daily or once-weekly (through day 22) subcutaneous injections 2-4 h after sublethal (4.00 Gy) total-body irradiation (Cobalt 60). Blood counts were measured daily. HE-2100 induced a decrease in the number of days of severe neutropenia (< 500 cells/μl) in the sublethally irradiated primates from 7 days (vehicle) to 2 days at the highest dose ($p = 0.019$). Once-weekly injections of HE-3204 substantially reduced the number of days of severe neutropenia (absolute neutrophil count < 500 cells/μl) from a median of 10.5 days in the control group (n=2) to a median of 1.5 days (n=4). By day 22 of the study, the treated group had recovered while the control group had continued severe neutropenia. Thus, it appears that this novel class of adrenal steroid hormones substantially reduces myelosuppression induced by whole-body sublethal ionizing radiation. Further efficacy studies will now be pursued in humans.

Investigators from the Fox Chase Cancer Center in Philadelphia (13) speculated that the use of nonsteroidal antiinflammatory drugs (NSAIDs) confers a survival advantage upon irradiated prostate cancer patients. The authors drew upon a database of 1,206 patients with localized prostate cancer who were treated with 3-dimensional conformal radiation therapy. Within this group, 232 patients had used NSAIDs in the past. Based on prostate cancer parameters, the patients were stratified into three risk groups (Table I). Pretreatment with NSAIDs was

Table I.

Risk group	Gleason score	Prostate-specific antigen (PSA)	T stage
Low	2-6	< 10	1
Intermediate	7	10-20	2
High	8-10	> 20	3

associated with significant reductions in distant metastases and secondary malignant neoplasms. Moreover, the use of NSAIDs was associated with a statistically significant survival advantage. Indeed, the 10-year overall survival rates were 91% *vs*. 68% ($p < 0.0001$) for those who used NSAIDs compared to those who did not. The impact of NSAIDs was not preserved on multivariant analysis, however. The role of cyclooxygenase type 1 (COX-1) and 2 (COX-2) inhibition continues to be an exciting area of oncological research. The drugs may enhance apoptosis and inhibit angiogenesis. Nevertheless, the COX inhibitors have recently been called into question with regard to their safety profile. As such, caution must be exercised. However, the study by Nguyen *et al.* certainly allows the generation of an hypothesis that should be rigorously studied in prospective trials.

Alfieri *et al.* (14) evaluated a purported radiation damage protector (Ex-RAD™, ON-01210) which theoretically is a benzylstyrylsulfone analogue. The report was presented by scientists from the Albert Einstein College of Medicine in New York in conjunction with Onconova Therapeutics. Ex-RAD™ is an orally available compound which is thought to prevent and ameliorate damage related to exposure to ionizing radiation. In general, benzylstyrylsulfone analogues are believed to serve as inhibitors of protein kinases such as cyclin-dependent kinases (CDKs). Inhibition of the latter enzymes can arrest cell cycling at several cell cycle checkpoints. In the experimental model used, Ex-RAD™ was evaluated in the context of total-body irradiation (TBI) of C57BL/6 mice. The mice were anesthetized and subjected to TBI (6.5-9.8 Gy, Philips MG 30 @ 81 cGy/minute). Orthotopically implanted MCF7 mammary carcinoma xenografts (125 mg) were evaluated for single-dose (15 Gy) studies for tumor *versus* normal tissue protection. Maximum protection was observed after TBI when the drug was administered 24 h prior to irradiation. Following exposure to 8 Gy, > 75% of animals survived in the Ex-RAD™-treated group compared to < 20% of the untreated controls ($p < 0.05$). Radiation protection continued to be observed with doses up to 9.8 Gy. No radiation protection was observed in the irradiated mammary carcinoma within the treated cohorts. Serum concentrations of Ex-RAD™ ranged from 100 to 210 μg/ml within 0.5-2 h (peak at 1 h) after 1.25 mg/kg (i.p.) and decreased biphasically to 70 μg/ml at 6 h. Tissue accumulation of the experimental agent was predominantly within the kidney, liver and intestine. The authors (supported by a U.S. Army Medical Research grant) concluded that Ex-RAD™ could represent a first-in-class, effective, orally available drug to prevent damage from ionizing radiation.

Kohl and colleagues from the Henry Ford Hospital in Detroit (15) evaluated the protective effect of ramipril, an inhibitor of angiotensin-converting enzyme (ACE), on radiation-induced normal tissue damage. Skin damage was assessed using a semiquantitative scale, scored 1-5, which included moderate erythema with dry skin, dry desquamation, moist desquamation and full thickness skin loss. Two groups were evaluated in a rodent model (radiotherapy alone and radiotherapy plus ramipril). Ramipril was administered at a dose of 2.5 mg/kg/day. Radiation-induced skin damage was consistently lower in the radiation + ramipril arm compared to animals treated with radiation alone. The protective effect was observed by day 30 and lasted until day 90. In the same group of animals, muscle damage was evaluated by measuring leg contraction and the data also showed a consistently significant ($p < 0.001$) decrease in radiation-induced retraction within the radiation + ramipril group (50%) compared to radiation alone (30%). The effect of ramipril on radiation-induced (8 Gy x 2) tumor growth delay was also tested in a murine model. A549 lung adenocarcinoma xenografts were grown intramuscularly in right hind legs of athymic mice and tumor size was recorded. There was tumor growth delay in the radiation alone group of approximately 8 days compared to no treatment (additional time to reach 3 times the initial tumor volume relative to untreated controls). Tumor growth delay with radiation + ramipril was extended from radiation alone by another 8 days, giving an approximate growth delay of 16 days compared to the untreated controls. These results were confirmed independent of whether ramipril was administered during or after the radiation treatment. In all experiments, the radiation + ramipril group showed significantly smaller tumor size at the last two time points when compared to radiation alone. The ACE inhibitor was thus able to protect against both acute (*e.g.*, skin) and late (*e.g.*, muscle) radiation-induced tissue damage, at least in the model studied. The effect was sustained despite alterations in fractionation schedules. There is also a possibility that ramipril can enhance the likelihood of tumor control; however, this will clearly require corroboratory experiments. Since ramipril is safely prescribed in the setting of hypertension, it is likely that we will see this drug formally tested in clinical trials soon.

Advances in diagnostic agents

Shih *et al.* (16) attempted to develop an approach for more accurate mapping of nodal disease in locally advanced prostate cancer. The issue has become particularly relevant with the publication of RTOG study 94-13, which demonstrated a disease-free survival advantage as a function of comprehensive irradiation of the pelvic lymph nodes for patients with intermediate-risk prostate cancer. Towards this end, the investigators sought to evaluate Combidex® (ferumoxtran-10), a novel contrast agent comprised of lymphotropic ultra-small, superparamagnetic iron oxide particles. Methodologically, the authors assessed 21 prostate cancer patients with pathologically confirmed node-positive disease. A total of 66 pathological nodes were identified by MRI with Combidex®. Of these, 20 were in the para-aortic region and 46 were in the pelvis. The position of each of these nodes was mapped to a common template based upon relationships to skeletal and or vascular anatomy. All 20

para-aortic nodes were situated within 2.5 cm of the anterior surface of the lumbar vertebrae and 95% of pelvic nodes were within 3.5 cm of the bony surface of the sacrum or pelvic girdle. No positive nodes were identified inferior to the superior pubic rami. Nodal metastases mapped much more tightly relative to the vascular anatomy. They were found mostly in the right external iliac region, left external iliac region and para-aortic region. The latter were observed along the inferior 12 cm of the aorta and extending an additional 13 cm below the level of the aortic bifurcation. All were found within 1.2 cm of the aorta. Thirty-one pelvic metastases were seen along the external iliac vessels over a length extending from 2 cm superior to the common iliac bifurcation to 11 cm inferior. Fourteen nodes were found along the internal iliac vessels and 89% of all 66 nodes were within 2.5 cm of a major vessel. This group from Massachusetts General Hospital proposed that pelvic nodes can be confidently assumed to be encompassed by the distal 2 cm of the common iliacs prior to the bifurcation along with the proximal 11 cm of the external iliac vessels and the proximal 4 cm of the internal iliac vessels. These data will need to be confirmed, but clearly represent a major advance for the definition of pelvic anatomy in anticipation of intensity-modulated radiation therapy (IMRT). Hopefully, such a rigorous anatomic definition can facilitate dose escalation and simultaneously avoid excessive irradiation of the small bowel (which may be excluded from more tightly defined target volumes).

Taylor and colleagues from the U.K. (17) used MRI along with ultra-small superparamagnetic iron oxide particles to delineate a reference target volume for pelvic lymph node irradiation. The topic is particularly germane since IMRT strategies are predicated upon accurate target volume delineation. Since pelvic lymph nodes are poorly visualized with CT and MRI, blood vessels (with a 1-2 cm margin) are often used as surrogate markers to identify nodal regions. Invariably, a significant volume of small bowel is irradiated when treating the pelvic nodes due to the uncertainty of the precise nodal position. Combidex® is prone to localize in macrophages, thereby causing normal lymph nodes to appear black on T2-weighted images. This study used Combidex® to determine the distribution of pelvic lymph nodes in relation to anatomic structures. Twenty women with gynecological tumors had a preoperative MRI with Combidex® administration. Among the nodal areas outlined were the common iliac, external iliac, internal iliac and presacral chains. Five clinical target volumes (CTV) were generated for each patient by expanding blood vessels by 3, 5, 7, 10 and 15 mm. Nodal contours were overlaid on every CTV and analyzed for coverage. Planning target volumes (PTV) were generated by adding a margin of 1 cm to the aforementioned CTV. The volume of bowel, bladder and rectum within each CTV and PTV was measured to aid in the selection of the appropriate margin for each nodal group. Over 1,200 nodal contours were evaluated. The mean number of nodes per patient was 61 and the mean short axis diameter was 3.6 mm. Nodal coverage was 56%, 76%, 88%, 94% and 99% of nodes, respectively, by 3-, 5-, 7-, 10- and 15-mm vessel expansion. The mean volume of bowel within the PTV was 96 cc (15.4%) with a 3-mm vessel margin, 121 cc (19.4%) with a 5-mm margin, 146.9 cc (23.7%) with a 7-mm margin, 190 cc (30.8%) with a 10-mm margin and 266 cc (42.9%) with a 15-mm margin. It was clear from this work that MRI with Combidex® constitutes an innovative technique to localize and stage lymph nodes. It has enabled the production of reference CT images which delineate the pelvic lymph nodes. As such, guidelines have now been developed for pelvic nodal irradiation with IMRT using a modified 7-mm margin with proposed adjustments to improve coverage of specific target nodal groups. These guidelines enable reduction in the volume of irradiated bowel by almost 25% without compromising tumor coverage. Implementation of these guidelines could theoretically lead to enhanced tumor control (if dose-escalation strategies can be pursued with impunity) or less morbid radiation treatment if conventional doses are used.

Haas-Kogan *et al*. (18) presented data from the University of California at San Francisco on the expression levels of epidermal growth factor receptor (EGFR) in malignant gliomas as they relate to the response to OSI-774 (erlotinib), an EGFR inhibitor with activity against brain tumors. Eligible patients included those with stable or progressive malignant gliomas who were treated with escalating doses. The level of EGFR expression was assessed by immunohistochemistry (IHC) and EGFR gene amplification was evaluated by fluorescence *in situ* hybridization techniques (FISH). Despite the fact that patients were treated at varying dose levels, Table II demonstrates a significant association between expression levels of EGFR as measured by IHC and response to treatment ($p = 0.04$). A strong association was noted between diffusely positive protein expression as measured by IHC and EGFR gene amplification. These data, although limited, are important because no study to date has established a correlation between response to anti-EGFR treatment and the expression of EGFR.

Table II.

EGFR status	Negative	Focally +: weak	Focally +: strong	Diffusely +	Total
Responders to OSI-774	1		2	4	8
Nonresponders	12	7	5	7	31

References

1. Knox, S., Meredith, R.F., Coleman, M. et al. *Tositumomab and iodine I 131 (Bexxar therapeutic regimen) produces long-term durable responses in heavily pretreated patients with relapsed, refractory, and transformed low grade non-Hodgkin's lymphoma (NHL).* 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 149.

2. Fiveash, J.B., Gillespie, G.Y., Buchsbaum, D.J. *Enhancement of glioma radiation therapy and chemotherapy response with targeted antibody therapy against death receptor 5.* 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 74.

3. Mason, K.A., Neal, R., Hunter, N.R. et al. *Molecular targeting of Toll-like receptor 9 with CpG oligodeoxynucleotides (ODN) for enhancemnt of tumor radioresponse*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 2010.

4. MacDonald, S.M., Caunt, M., Brooks, P.C. et al. *para-Amino benzoic acid (PABA), a radiation chemotherapy sensitizer*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 2022.

5. Xavier, S., Roth, J., Caunt, M. et al. *para-Amino benzoic acid (PABA) modulates expression and stability of CDC25A in tumor cells and enhances radiosensitivity in vitro and in vivo*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 2038.

6. Crane, C.H., Ellis, L.M., O'Reilly, M. et al. *RhuMab VEGF (bevacizumab) with concurrent radiotherapy and capecitabine in locally advanced pancreatic cancer: An active, well tolerated regimen*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 34.

7. Saito, N.G., Sun, H., Nikolovska-Coleska, Z. et al. *Use of Smac peptidomimetic compounds as effective radiosensitizers of human cancer cell lines*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 2035.

8. Sharma, A.K., Mohan, S., Alfieri, A. et al. *Radiation sensitization of prostate carcinoma cells by ON 01910, a novel protein kinase inhibitor and cell cycle modulator*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 2003.

9. Masunaga, S., Sakurai, Y., Nagata, K. et al. *Applicability of combination with the vascular targeting agent ZD6126 in boron neutron capture therapy*. 46th Annu Meet Am Soc Ther Radiol Oncol - ASTRO (Oct 3-7, Atlanta) 2004, Abst 2059.

10. Jahraus, C.D., Bettenhausen, D., Sellitti, M. et al. *Randomized double-blind placebo controlled trial of balsalazide in the prevention of acute radiation enteritis as a consequence of pelvic radiotherapy*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 202.

11. Carpenter, M.T., Agarwal, A., Epperly, M. et al. *Inhalation delivery of hemagglutinin epitope-tagged manganese superoxide dismutase-plasmid/liposome (HA-MnSOD-PL) complexes to the lung protects against fractionated irradiation lung damage*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 71.

12. Stickney, D.R., Dowding, C., Reading, C. et al. *Protection of rhesus macaques from whole body radiation induced severe neutropenia and thrombocytopenia by novel hormones HE2100 and HE3204.* 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 2023.

13. Nguyen, K.H., Eisenberg, D.F., Hanlon, A.L. et al. *NSAID use confers a survival advantage for prostate cancer patients treated with radiation therapy*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 2182.

14. Alfieri, A.A., Liu, L., Sharma, A. et al. *Radiation damage protection by the benzyl styryl sulfone analog, Ex-Rad*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 2052.

15. Kohl, R., Brown, S.L., Zhu, G. et al. *Protective effect of ramipril, an inhibitor of angiotensin converting enzyme, on radiation-induced normal tissue damage without tumor protection.* 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 2001.

16. Shih, H.A., Harisinghani, M., Zeitman, A. et al. *Mapping of nodal disease in locally advanced prostate cancer: Rethinking the clinical target volume for pelvic node radiation based on vascular rather than boney anatomy*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 1013.

17. Taylor, A., Rockall, A.G., Usher, C. et al. *Delineation of a reference target volume for pelvic lymph node irradiation using MR imaging with ultrasmall supermagnetic iron oxide particles*. 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 156.

18. Haas-Kogan, D.A., Prados, M.D., Jelluma, N. et al. *Expression levels of EGFR in malignant gliomas are associated with response to treatment with the EGFR inhibitor OSI-774.* 46th Annu Meet Am Soc Ther Radiol Oncol – ASTRO (Oct 3-7, Atlanta) 2004, Abst 32.