2749 Radiosensitization of Lung Cancer Tumors with Pan-Caspase Inhibitor Z-VAD

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Radiosensitization of cancer cells in the absence of apoptosis is found to be associated with upregulation of autophagy, a type II programmed cell death. The present study examined the radiosensitizing effects of caspase inhibition on lung cancer cells *in vitro* and in an *in vivo* mouse model. Upon radiation treatment in conjunction with a pan-caspase inhibitor N-benzyloxycarbonyl-valyl-alanyl-aspartyl-fluoromethylketone (Z-VAD), H460 lung cancer cells were significantly radiosensitized in the clonogenic assay. The enhanced tumor cytotoxicity was associated with an overexpression of autophagic proteins ATG5-ATG12 and Beclin-1, and with an increase in punctate localization of GFP-LP3 characteristic of autophagosome formation. The combination therapy yielded promising results in a xenograft tumor mouse model, markedly slowing the rate of tumor growth and angiogenesis with minimal changes in body weight ratio. TUNEL assay further revealed that the combination treatment produced two-fold less apoptosis *in vivo* than with radiation alone. This study confirms our previous finding with a breast cancer mouse model (1) that the combination treatment consisting of radiation and Z-VAD is well tolerated *in vivo* and potentiates the cytotoxic effects of current radiation therapy. Further understanding and targeting of autophagy thus may contribute to the enhancement of tumor cytotoxicity in radiation therapy.

Author Disclosure: M. Hwang, None.

2750 A Novel Poly(ADP-ribose) Polymerase Inhibitor, ABT-888, Sensitizes Malignant Human Cell Lines to Ionizing Radiation Under Oxia and Hypoxia

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Purpose/Objective(s): Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme that recognizes and binds to DNA breaks, and facilitates the subsequent recruitment of DNA repair proteins, thereby coupling the sensing of DNA damage to the activation of DNA repair pathways. There has been much interest in the development of specific PARP inhibitors to potentiate radiotherapy and chemotherapy. Phase I clinical trials are ongoing, but it is unclear as to whether this agent is effective against hypoxic cells. We therefore tested the role for a novel PARP inhibitor, ABT-888, as a radiosensitizing agent in a preclinical context under oxia and hypoxia.

Results: Human prostate (DU-145, 22RV1) and non-small cell lung (H1299) cancer cell lines were used for all experiments. ABT-888 inhibited PARP activity *in vitro* (IC_{50} 41 nM) and *in vivo*. Clonogenic survival assays revealed that ABT-888 is able to radiosensitize malignant human cell lines (RER 1.38). Furthermore, in the presence of acute hypoxia, ABT-888 radiosensitized malignant cell lines down to survival levels similar to oxic sensitivity.

Conclusions: The study of molecularly-targeted agents with radiotherapy is of great interest, due to their potential for enhancing the therapeutic ratio. Our study is the first to our knowledge, which demonstrates that inhibition of PARP activity can radiosensitize hypoxic cancer cells. Future *in vivo* studies will examine the efficacy of this inhibitor and determine biomarker profiles of activity in xenograft preclinical models.

Author Disclosure: S.K. Liu, None; C. Coackley, None; R. Bristow, None.

2751 Panitumumab, a Fully Human Anti-EGFR Monoclonal Antibody, Augments Radiation Response in Xenograft Models of Upper Aerodigestive Tract Cancers

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Purpose/Objective(s): The potential value of modulating epidermal growth factor receptor (EGFR) signaling as a cancer treatment approach is reflected by the broad array of EGFR inhibitors currently under evaluation in clinical trials. Panitumumab, the first fully human anti-EGFR antibody in clinical development, has proven well tolerated in cancer patients with epithelial malignancies, and has recently received FDA approval for the treatment of metastatic colorectal cancer. In the current preclinical studies, we examine the activity of panitumumab in SCCHN and NSCLC xenograft models. In light of the central role of radiation in the treatment of upper aerodigestive tract cancers, we further investigate the interaction of panitumumab with radiation in these experimental systems.

Material/Methods: Cellular interactions in response to EGFR inhibition by panitumumab and radiation have been examined. SCCHN lines SCC-1483 and SCC-1 and NSCLC line H226 were exposed to panitumumab, radiation (6 Gy), or the combination, and proteins involved in apoptosis and tumor proliferation were analyzed by western blot.

In addition, we evaluated the antitumor activity of panitumumab alone and in combination with radiation in the SCC-1483 xenograft model. Mice bearing SCC-1483 tumor xenografts (100–200 mm^3) were treated with panitumumab as a single agent to assess antitumor activity. We further examined the interactive impact of panitumumab in combination with radiation (twice weekly) in tumor xenografts. Following treatment, tumors were harvested and IHC staining for pEGFR and proliferating cell nuclear antigen (PCNA) was performed.

Results: *In vitro*, EGFR blockade was shown to augment radiation-induced apoptosis as measured by Poly (ADP-ribose) polymerase (PARP) expression. Additionally, panitumumab blocked radiation-induced EGFR phosphorylation and downstream signaling through pMAPK and pSTAT3. In SCC-1483 tumor xenografts, systemic administration of panitumumab as a single agent inhibited tumor growth in a dose-dependent fashion. In combination studies, the concurrent administration of 25 ug panitumumab and 2 Gy radiation resulted in potent inhibition of tumor growth when compared to either treatment modality alone. Additionally, combination therapy demonstrated markedly reduced PCNA tumor staining. In these animal studies to date, panitumumab was well tolerated without discernible systemic toxicity.

Conclusions: Panitumumab demonstrates significant antitumor activity in our xenograft model systems. Moreover, a significant interaction of panitumumab in combination with radiation is observed, both *in vitro* and *in vivo*. In light of the strong preclinical and clinical data supporting the use of EGFR inhibitors with radiation, these results provide further support for the clinical evaluation of panitumumab in combination with radiation in the treatment of patients with epithelial malignancies of the upper aerodigestive tract.

Author Disclosure: T.J. Kruser, None; E. Armstrong, None; S. Huang, None; R. Radinsky, Amgen, Inc., A. Employment; C. Starnes, Amgen, Inc., A. Employment; D. Freeman, Amgen, Inc., A. Employment; P.M. Harari, Amgen, B. Research Grant; AstraZeneca, Genentech, Bristol Myers, ImClone, C. Other Research Support; Amgen, AstraZeneca, Genentech, Bristol Myers, ImClone, D. Speakers Bureau/Honoraria; Amgen, AstraZeneca, Genentech, Bristol Myers, ImClone, F. Consultant/Advisory Board.

2752 WITHDRAWN

2753 Radiation Induces Invasiveness of Pancreatic Cancer Via Upregulation of Heparanase

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Purpose/Objective(s): Pancreatic cancer often shows resistance to radiation therapy, and randomized trails could not demonstrate benefit from radiation, revealing rather conflicting results. Moreover, some reports suggested worse outcome when radiation was applied. Although reported in the past, radiation-induced changes in invasive potential of pancreatic carcinoma, as well as the underlying molecular mechanisms remain obscure. Tumor invasion depends on enzymatic degradation of the extracellular matrix (ECM). Heparan sulfate (HS) glycosaminoglycan, the principal polysaccharide of the ECM is a key element responsible for ECM self-assembly and barrier properties. Mammalian heparanase is the predominant enzyme capabale of HS degradation. Heparanase is now recognized as an essential determinant of cancer aggressiveness, acting via breakdown of extracellular barriers for invasion and metastasis, as well as release of HS-bound angiogenic and growth factors, thus generating a supportive microenvironment for tumor progression. In human pancreatic carcinoma heparanase is expressed in correlation with reduced survival. We hypothesized that radiation affects pancreatic cancer aggressiveness via induction of heparanase.

Materials/Methods: Two pancreatic cancer cell lines were used to investigate the effects of radiation on tumor aggressiveness. Sub-cytotoxic radiation was delivered at dose escalation. We applied Matrigel invasion assay, reverse transcription-polymerase chain reaction, Western blot hybridization, Immunofluorescent staining and enzymatic activity inhibition to study the role of heparanase in radiation-induced invasiveness.

Results: 10 Gy irradiation resulted in 6-fold increase in invasive potential of pancreatic carcinoma *in vitro*. This effect was abolished in the presence of N-acetylated glycol split heparin (100 NA,RO.H), a specific inhibitor of heparanase. We found specific and dose-dependent effect of irradiation on the levels of heparanase mRNA and protein, expressed by pancreatic carcinoma cell lines. These results were confirmed by immunofluorescent detection of increase in intracellular heparanase following radiation.

Conclusions: Our results suggest that heparanase gene induction by radiation represents a novel pathway underlying pancreatic cancer progression to a more aggressive phenotype. Radiation-induced invasiveness may be responsible, at least in part, for the limited benefit of radiation therapy in pancreatic cancer patients. Our data indicate that the concomitant use of heparanase inhibitors during radiotherapy could be a promising approach to improve treatment of pancreatic cancer.

Author Disclosure: A. Meirovitz, None; T. Peretz, None; B. Casu, None; I. Vlodavsky, None; M. Elkin, None.

2754 Prostate-Related Natural Health Products (Dietary Supplements) Modify Clinical Efficacy

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Purpose/Objective(s): Many individuals electively take prostate-specific health products (also known as dietary supplements) to alleviate symptoms linked with poor prostate health. Most supplements are hormonally-derived natural compounds that do not require FDA regulation. Moreover, the active ingredients are formulated with radical scavengers, antioxidants or modulators of stress-response pathways to enhance potency. A prostate cancer diagnosis or clinical evidence of elevated disease risk factors further increases use of such supplements. However, the impact of these agents on evidence-based radiotherapy practise is at best poorly understood. The purpose of this study was to determine if prostate-specific dietary supplements change the growth rate or radiosensitivity of normal and tumor prostate cell lines, producing a potential change in therapeutic ratio thereby altering the efficacy of radiation therapy.

Materials/Methods: Three well-known prostate-specific dietary supplements were purchased from commercial sources available to patients: Trinovin (red clover, biochanin A, formononetin, daidzein, genistein [phytoestrogen]), Provelex (lycopene, soy, saw palmetto, quercetin [phytoestrogen], selenium) and ProstateRx (saw palmetto). The cell lines used were RWPE-1 cells (normal prostate epithelial cell line), PC3 cells (grade IV prostatic adenocarcinoma) and DU145 cells (grade II prostatic carcinoma, not detectably hormone sensitive). Toxicity was assessed in cell proliferation assays (MTT) and radiosensitivity by conventional clonogenic assays (0.5–4 Gy). Cell cycle kinetics were assessed by the BrdU/PI pulse-labeling technique and DNA repair by measuring the disappearance of gammaH2AX foci.

Results: Cell growth for the malignant PC3 and DU145 cell lines was not affected by any of the prostate dietary supplements [Provelex (2 ug/ml), Trinovin (10 ug/ml) and ProstateRx (50 ug/ml)]. The radiosensitivity of PC3 and DU145 cells was also unaffected. However, Trinovin (10 ug/ml) and ProstateRx (6 ug/ml) inhibited growth of normal prostate cells by 20% and 40% respectively, but more importantly increased radiosensitivity after single low-dose radiation exposures (30% decrease in cell survival compared with radiation alone). The reduction in proliferation reflected a change in cell cycle kinetics. These data indicate that normal prostate cells are markedly more sensitive to growth inhibition effects of some hormone-based dietary supplements compared with tumor cells, and this can affect the radiosensitivity of normal prostate cells.