

the present study was to evaluate boronated EGF and the anti-EGFR MoAb cetuximab (IMC-C225), as molecular targeting agents for BNCT of the F98 rat glioma. The parental wildtype tumor, F98<sub>WT</sub>, was transfected with the gene encoding human EGFR to produce a receptor (+) glioma, designated F98<sub>EGFR</sub>. Boronated bioconjugates were produced by linking a heavily boronated polyamidoamine (PAMAM) dendrimer by means of heterobifunctional reagents to either EGF or cetuximab. Biodistribution studies were carried out in Fischer rats bearing intracerebral (i.c.) implants of F98<sub>EGFR</sub> or F98<sub>WT</sub> gliomas and the bioconjugates were administered by either direct intratumoral (i.t.) injection or convection enhanced delivery (CED). At 24 h following i.t. injection of boronated cetuximab (C225-G5-B<sub>100</sub>), the mean tumor boron concentrations in rats bearing either F98<sub>EGFR</sub> or F98<sub>WT</sub> gliomas were  $92.3 \pm 23.3$   $\mu\text{g/g}$  and  $36.5 \pm 18.8$   $\mu\text{g/g}$ , respectively. In contrast, uptake of the non-targeted boronated dendrimer (G5-B<sub>100</sub>) was  $6.7 \pm 3.6$   $\mu\text{g/g}$ . Based on its favorable *in vivo* uptake, C225-G5-B<sub>100</sub> was evaluated as a delivery agent for BNCT in F98<sub>EGFR</sub> glioma bearing rats. This was carried out at the Massachusetts Institute of Technology Nuclear Reactor (MITR) 24 h following CED of C225-G5-B<sub>100</sub> and 2.5 h after i.v. boronophenylalanine (BPA). The mean survival time (MST) of rats that received the bioconjugate, administered i.c. by CED, was  $45 \pm 3$  d compared to  $25 \pm 3$  d for untreated control animals. A further enhancement in MST to  $>60$  d was obtained by administering C225-G5-B<sub>100</sub> in combination with i.v. BPA ( $p < 0.001$ ). Similar studies were performed using boronated EGF (BD-EGF), administered by CED to F98<sub>EGFR</sub> glioma bearing rats. The MST of rats that received BD-EGF either alone or in combination with i.v. BPA were  $53 \pm 13$  d and  $>61 \pm 14$  d, respectively, compared to  $40 \pm 5$  d for BPA alone and  $31 \pm 4$  d for irradiated controls ( $p < 0.001$ ). These data are the *first* to demonstrate the efficacy of a boronated MoAb for BNCT of an i.c. glioma and are paradigmatic for future studies using combinations of low and high molecular weight <sup>10</sup>B delivery agents.

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POSTER

#### Radiosensitization of human prostate cancer by natural polyphenol inhibitor of Bcl-2/XL, (-)-gossypol, results in tumor regression

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Radioresistance markedly impairs the efficacy of tumor radiotherapy and involves anti-apoptotic signal transduction pathways that prevent radiation-induced cell death. The Majority of human prostate cancers overexpress Bcl-2 and/or Bcl-XL, the important negative regulators of apoptosis. Overexpression of Bcl-2 and Bcl-XL in prostate and other types of cancer cells has been shown to confer resistance to radiation and chemotherapeutic agents. (-)-gossypol, a natural product from cottonseed, has recently been identified as a potent small molecule inhibitor of both Bcl-2 and Bcl-XL. In the current study, we tested our hypothesis that (-)-gossypol may improve prostate cancer's response to radiation by inhibiting the anti-apoptosis activity of Bcl-2/XL and making cancer cells more sensitive to radiation therapy.

Our data show that (-)-gossypol inhibits tumor cell growth and induces apoptosis in human prostate cancer PC-3 cells with a high levels of Bcl-2/XL proteins, but has minimal effect on normal cells. In clonogenic assays, treatment of PC-3 cells with (-)-gossypol significantly reduced radiation resistance of PC-3, resulted in 10- and 20-fold reduction of colony formation at 8 Gy X-ray irradiation. Fluorescence resonance energy transfer (FRET) assay using Bcl-XL-CFP and Bax-YFP or Bad-YFP co-transfected DU-145 cells suggests that (-)-gossypol potentially blocks the interaction of Bcl-XL with Bax and Bad in live cells, in a time- and dose-dependent manner. The data support that (-)-gossypol induces apoptosis, at least in part, through inhibition of the anti-apoptotic protein Bcl-XL, although the interactions of (-)-Gossypol with other targets, either directly or indirectly, may also play a role and this is a subject of our further investigations.

Our *in vivo* studies using PC-3 xenograft models in nude mice show that orally administered (-)-gossypol has anti-tumor activity but achieves a much greater efficacy with tumor regression when used in combination with X irradiation. Combination therapy of (-)-gossypol 10 mg/kg, p.o. q.d.5  $\times$  4 weeks, with fractionated irradiation, 2 Gy q.d.5  $\times$  3 weeks, achieved 96% tumor growth inhibition (T/C = 3.4%) in tumors with initial size of 100 mm<sup>3</sup>, significantly more effective than either (-)-gossypol or radiation alone (T/C = 96% and 37%, respectively) ( $p < 0.01$ , n = 16). Similar results were observed with PC-3 tumors with initial sizes of 200 and 400 mm<sup>3</sup> at the start of radiation, whereas only combination therapy resulted in tumor regression. For PC-3 tumors with starting size of 200mm<sup>3</sup>, (-)-gossypol plus radiation achieved significant tumor growth delay (T-C = 54.5 days) as compared with (-)-gossypol or radiation alone (T-C = 0 and 8.5 days, respectively). *In situ* TUNEL-staining showed significantly more apoptotic cells induced in the tumors treated with (-)-gossypol plus radiation than either treatment alone. Anti-CD31 immunohistochemical

staining indicates that (-)-gossypol plus radiation significantly inhibited the tumor angiogenesis.

In summary, our results demonstrate that the natural polyphenol inhibitor of Bcl-2/XL, (-)-Gossypol, can radiosensitize prostate cancer *in vitro* and *in vivo* without augmenting toxicity. (-)-Gossypol may improve the outcome of current prostate cancer radiotherapy and represents a promising novel anticancer regime for molecular targeted therapy of hormone-refractory prostate cancer with Bcl-2/XL overexpression. Clinical trials are being planned.

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#### Selective inhibition of Ras, PI3-kinase and Akt isoforms can radiosensitize human carcinoma cell lines

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H-Ras activation has been shown to enhance survival after irradiation in many tumor cells. Human tumors more frequently have mutations in K-ras or demonstrate Ras activation due to signaling from receptor tyrosine kinases such as the EGF receptor (EGFR). The role of K-Ras in radiation resistance and the relative contributions of H- and K- and N-Ras signaling to EGF<sup>R</sup>-mediated promotion of cell survival are not as well defined. We have now examined the effects of selectively inhibiting the expression of individual Ras isoforms on downstream signaling and radiation survival using RNA interference (RNAi) in a panel of human tumor cell lines that differ in Ras status. We also used this technique to examine the contribution of PI3-kinase and Akt isoforms to radiation survival. Specific inhibition of oncogenic K-Ras expression in cells expressing K-Ras<sup>V12</sup> and specific inhibition of oncogenic H-Ras expression cells expressing H-Ras<sup>V12</sup> decreased clonogenic survival after irradiation. Inhibition of H- or N-Ras, but not K-Ras reduced clonogenic survival in cells with EGF<sup>R</sup>-activated Ras signaling. Inhibition of H-, K-, or N-Ras using siRNA decreased both phospho-Akt and phospho-p42/44 MAP kinase, however, pharmacologic inhibition of the MEK-ERK pathway by itself had little effect on survival while inhibition of PI3-kinase resulted in radiosensitization. Isoform-specific inhibition of PI3-kinases was carried out with siRNA. Combined inhibition of PI3-kinase p85 $\beta$  and either p110 $\alpha$  or p110 $\beta$  subunits had a greater effect on radiation survival than inhibition of individual subunits. Testing the contribution of Akt isoforms showed that Akt-1 was the most effective in down-regulating phospho-Akt and decreasing radiation survival. However, co-transfection of siRNA for Akt-1, -2 and -3 further decreased radiation survival. The effects of siRNA inhibition of Akt-1 were rescued by exogenous expression of mouse Akt-1. This study demonstrates that the activation of PI3K-Akt pathway is an important part of survival signaling by Ras, whether the activation results from mutation of *ras* or over-expression of EGF<sup>R</sup>. This study further indicates that specific inhibition of PI3-kinase and Akt isoforms can reverse survival signaling in tumor cells.

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#### Radiation sensitization of lung cancer through inhibition of MDM2

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**Background:** MDM2 interacts with p53 and reduces its transcriptional activity and stability. Inhibition of MDM2 using antisense oligonucleotides results in radiosensitization by up-regulating p53, p21 and bax or down-regulating bcl-2. The purpose of this study was to define additional mechanisms of radiosensitization via inhibition of MDM2 and determine whether inhibition of MDM2 enhances the cytotoxic effects of radiation upon lung cancer and its tumor vasculature.

**Methods:** Antisense oligonucleotides against MDM2 were used to downregulate MDM2 expression. H460 lung cancer cells were transiently transfected with either a control oligonucleotides or the antisense oligonucleotides, using Lipofectin. Western Blotting was used to verify specific attenuation of the target gene expression in the transfected cells. They were then treated with or without 3Gy of radiation. At various time points following irradiation, H460 cells were assayed for their survival by clonogenic assay; for apoptosis by flow cytometry of stained apoptotic cells; and for cell senescence by quantification of beta-galactosidase-expressed cells. Biological effects of inhibiting MDM2 were determined using H460 xenografts. H460 cells were injected into the hind limb of nude mouse to establish the mouse model for lung cancer. Mice bearing tumors were treated with seven i.p. injections of antisense oligonucleotides at the daily dose of 10mg/kg. They were treated with nothing else or with 2Gy  $\times$  5

radiotherapy. Tumor volumes and blood flow were determined by measuring the tumor size and power Doppler, respectively.

**Results:** Transient transfection of H460 cells with antisense oligonucleotides has specifically reduced the expression of MDM2 induced by radiation, by Western analysis. In addition, elevation of p53 and attenuation of survivin expression were detected in the transfected H460 cells. Clonogenic assay suggests that inhibition of MDM2 greatly decreased cell viability following irradiation. There is a dramatic increase of apoptotic and senescent cells following the treatment with antisense oligonucleotides plus irradiation. Mice bearing H460 xenografts had significant delay of tumor growth ( $p < 0.001$ ) after being treated with antisense oligos plus radiotherapy, compared with mice treated with either antisense oligos alone or radiotherapy alone. A four-fold reduction of blood flow was also detected in these treated tumors.

**Conclusion:** Inhibition of MDM2 has significantly decreased cell viability and increases apoptosis and senescence of H460 lung cancer cells, following irradiation. Combination of radiotherapy and inhibition of MDM2 through the antisense approach results in improved tumor control by radiotherapy in a mouse model of lung cancer. Further investigation is needed to confirm the advantage of this combined therapy.

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#### Enhancement of radioresponse by phosphatidylinositol 3-kinase inhibitor in radioresistant murine hepatocarcinoma

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**Background:** The objective of this study was to explore whether phosphatidylinositol 3-kinase (PI3K) inhibitor, wortmannin, could potentiate the antitumor effect of radiation in vivo, particularly in radioresistant murine tumor.

**Materials and Methods:** Murine hepatocarcinoma (HCa-I), a highly radioresistant tumor with TCD 50 of higher than 80 Gy, was transplanted in C3H/HeJ mice. Tumor-bearing mice were treated with wortmannin, 25 Gy radiation, or both. Wortmannin was intraperitoneally administered 1 mg/kg daily for 14 days. Tumor response to the treatment was determined by a tumor growth delay assay. To explore the mechanism underlying interaction between the drug and radiation, the level of apoptosis and regulating molecules were examined.

**Results:** In tumor growth delay assay, the drug increased the effect of tumor radioresponse with an enhancement factor (EF) of 1.9. Combined treatment of 25 Gy radiation with wortmannin increased radiation induced apoptosis in tumor additionally; peak apoptotic index was 1.1% in radiation alone, 1.3% in drug alone and 1.9% in the combination treatment group. Interestingly, combined treatment resulted in significant level of necrosis in tumor, suggesting that both tumor and its vasculature were attacked. Analysis of apoptosis regulating molecules with Western blotting showed significant upregulation of p53, p21 in the combination treatment group comparing to those in either radiation alone or drug alone group.

**Conclusion:** In murine hepatocarcinoma, the antitumor effect of radiation could be potentiated by use of wortmannin. The mechanism seems to involve both the increase of induced apoptosis in tumor and damage of tumor vasculature as well. Wortmannin in combination with radiation therapy may have potential benefit in cancer treatment.

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#### Modulation of tumor hypoxia in response to treatment with ionizing radiation and the VEGFR inhibitor PTK787/ZK222584

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**Background:** Tumor hypoxia represents a major obstacle for tumor radiation response. We previously reported a cooperative tumor growth delay effect of the combined treatment modality using the novel VEGF-receptor tyrosine kinase inhibitor PTK787/ZK222584 with ionizing radiation (IR). On the molecular level we demonstrated that PTK787/ZK222584 counteracts IR-induced VEGF receptor- and PI3K/Akt pathway-activation and further decreases an apoptotic threshold in endothelial cells by inducing degradation of PKB/Akt protein. The purpose of this study was to assess the combined treatment effect of IR and angiogenesis inhibitor (IoA) on the level of tumor hypoxia.

**Material and methods:** Growth delay experiments after a fractionated course of IR given concomitantly with PTK787/ZK222584 were performed against spontaneously growing MMTV/r-neu driven murine mammary tumors and against isogenic but ectopic allograft tumors in nude mice. We

assessed tumor hypoxia with serial <sup>18</sup>F-misonidazole positron emission tomography (PET) before, during and after different treatment regimens using an unique small animal PET facility with high resolution and determined tumor cell apoptosis and microvessel density by immunohistochemistry. Three-dimensional vessel morphology was analysed with mercox casting followed by electron microscopy.

**Results:** Combined treatment exerted a tumor model-specific cooperative growth delay effect against both the allograft and the spontaneously growing mammary tumor model with an increased treatment response in the spontaneous tumor model. PET-based analysis of treatment-dependent changes of tumor hypoxia suggests that IR counteracts an increase of tumor hypoxia as induced by treatment with the VEGF-receptor inhibitor alone. Low levels of tumor hypoxia after a fractionated course of IR and PTK787/ZK222584 correlated with increased tumor cell apoptosis, reduced microvessel density and extensive vessel destruction.

**Conclusions:** Important for clinical considerations PTK787/ZK222584 does not impair tumor oxygenation during a fractionated regimen of ionizing radiation. This work gives new insights into the combined treatment effect of IR and IoA on the level of the tumor microenvironment and supports the rational of this combined treatment modality for clinical application.

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#### In vivo efficacy of photodynamic therapy in xenografts of retinoblastoma

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**Background:** Retinoblastoma is the most frequent ocular tumor in the young children. Today, 95% of these tumors are cured by chemotherapy and radiotherapy in developed countries. However, these treatments frequently lead to the development of secondary tumors, notably for patients presenting a constitutive alteration of the RB1 gene.

Photodynamic Therapy (PDT) is based on the use of non toxic photosensitizers activated with a visible non ionising Laser light. It represents a conservative approach for the treatment of retinoblastoma, and must contribute to reduce the incidence of secondary tumors.

To study the *in vivo* efficacy of this therapy in these tumors, 2 human retinoblastoma xenografts, derived from surgical specimens, were established in *nude* mice and used to evaluate the phototoxic effect of mTHPC (Foscan®), a well known photosensitizer.

**Material and methods:** Two models of human retinoblastoma xenografted into *nude* mice were used (Rb-102-FER, Rb-109-LAK).

Mice were randomly separated into 4 groups (8 mice per group): a control group, an irradiated group, a Foscan®-treated group and a Foscan®-treated-irradiated group. Foscan® was injected intraperitoneally at a dose of 0.3 mg/kg. For light treatment, mice were anaesthetized. A 75J/cm<sup>2</sup> of green light (514nm wavelength) at a fluence rate of 100 mW/cm<sup>2</sup> was delivered to the tumors following opening of the skin. Irradiations were made 24h to 48h after Foscan® injection, when the photosensitizer has accumulated into the tumoral tissue. Injections and irradiations were repeated for 3 cures.

**Results:** In the control group the tumor volume doubling time was of 10.5 days for Rb-102-FER and of 7.5 days for Rb-109-LAK with few intermouse variation. No phototoxicity nor toxicity was observed in any group. Compared to the other groups, each Foscan®-treated-irradiated tumor presented a higher regression rate after irradiation even if a transient tumor size regression could also be observed for irradiated mice. Tumor regressions were transient after one irradiation but significant higher growth delays were observed along cures for Foscan®-treated-irradiated mice. Interestingly, 1 mouse treated (Rb-109-LAK) presented a complete tumor regression after the second irradiation, without regrowth until the end of experiment.

**Conclusions:** Our first results suggests that PDT is a non toxic therapy that could be efficient for retinoblastoma and may represent an alternative therapeutic approach for the conservative treatment of these tumors.