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. Clongenic 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.

**646** POSTER

**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 additively; 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.

**647** POSTER

**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 $^{18}$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.

**648** POSTER

**In vivo efficacy of photodynamic therapy in xenografts of retinoblatoma**

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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.
Photodymanic 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 anaesthesized. A 75J/cm* of green light (514nm wavelength) at a fluence rate of 100 mW/cm* 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 retinoblatoma and may represent an alternative therapeutic approach for the conservative treatment of these tumors.