After modeling the importance of DNA-PK inhibition under oxic and various low oxygen conditions using CB.17$^{DNA-PKcsw/t}$ and SCID/st$^{DNA-PKcsnull}$ mouse embryonic fibroblast cells *in vitro*, we used the clonogenic survival and resazurin reduction proliferation assays on HeLa human cervical carcinoma cells to explore the activity of these prodrugs. Stirred cell suspension and mouse liver microsome stability assays were used to explore cofactor requirements, metabolic kinetics and oxygen dependence of prodrug bioreduction.

The survival of CB.17 cells was similarly enhanced when radiation was administered under hypoxic conditions in both CB.17 and SCID/st cells with oxygen enhancement ratios (OERs) of 2.6 and 2.4 respectively. Hypoxic SCID/st cells were comparable in radiosensitivity to oxygenated CB.17 cells, indicating that suppression of DNA-PK activity acts as a radiosensitizer similar in potency to oxygen. Cell viability and clonogenic survival assays utilizing hypoxia activated DNA-PK inhibitors show that these agents have high selective toxicity to hypoxic versus oxic cells when treated for 6 h. Microsomal stability assays revealed the requirement for the cofactor NADPH in the bioreductive process and cell suspension assays confirm that the prodrug is selectively reduced under hypoxic conditions in H460 human lung carcinoma cells producing active DNA-PK inhibitor.

Our results indicate that chemical inhibition of DNA-PK increases sensitivity of both normoxic and hypoxic cells to ionizing radiation and suggest that inhibition of NHEJ may be a valid strategy to increase the radiation sensitivity of hypoxic cells. Further studies to assess the ability of hypoxia activated DNA-PK inhibitors to penetrate tumor tissue and reach hypoxic cell populations located distal to vasculature are in progress.

**508** POSTER

**Radiation-induced c-Met expression sensitizes lung cancer cells to c-Met antagonists**

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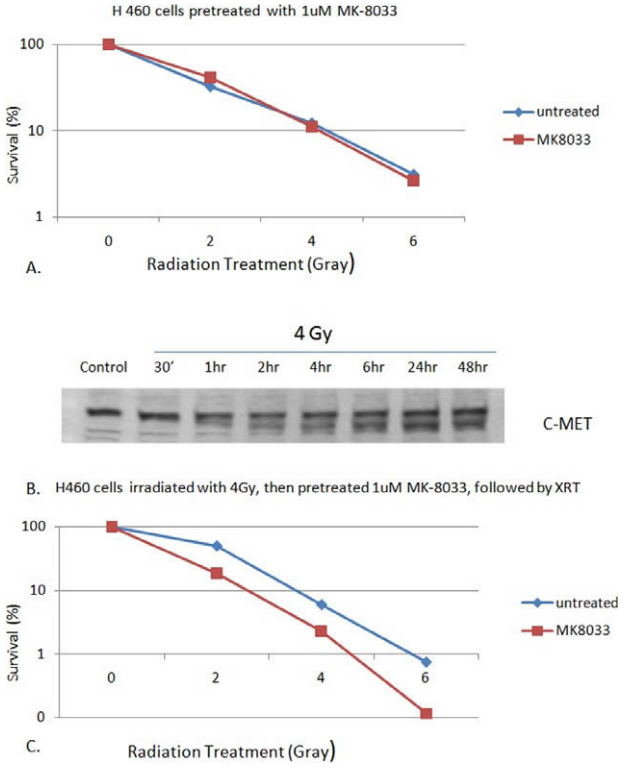
**Background:** Expression of the proto-oncogene product c-Met can protect cells from being killed by a variety of DNA-damaging agents, including radiation. Conversely, inhibiting c-Met can radiosensitize cells and inhibit several DNA-repair enzymes. Although c-Met overexpression correlates with poor prognosis in lung cancer, only about 10% of non-small cell lung cancer (NSCLC) tumors overexpress c-Met. We explored whether radiation itself could induce c-Met expression in NSCLC cells and whether combining radiation with the small-molecule c-Met inhibitor MK8033 would further radiosensitize those cells.




**Material and Methods:** We tested the effectiveness of combining ionizing radiation+MK8033 on the human NSCLC cell line H460, which does not endogenously express c-Met, with clonogenic survival assays. H460 cells were irradiated to 4 Gy with a $^{137}$Cs source (3.5 Gy/min), treated with vehicle control (DMSO) or 1 μM MK8033 for 1 hour, irradiated to 0, 2, 4, or 6 Gy, and incubated for 23 hours. Cells were then trypsinized, counted, replated, and incubated for 12 days, after which colonies were stained and counted. Plating efficiency and surviving fractions were calculated relative to those of unirradiated cells. c-Met protein was quantified by western blotting and quantified by Image Quant software.

**Results:** Pretreating unirradiated H460 NSCLC cells with 1 μM MK8033 for 1 hour did not radiosensitize those cells compared with unirradiated, untreated control cells (Fig 1A). Irradiation to 4 Gy, however, increased the expression of c-Met protein, which peaked at 24 hours and persisted for at least 48 hours(Fig 1B). The irradiated H460 cells were further radiosensitized by the addition of MK8033: the mean surviving fraction at 2 Gy ($SF_2$) of cells pretreated with MK8033 was 0.41, whereas the $SF_2$ for irradiated H460 cells that were then treated with MK8033 was 0.18 (Fig 1C).

**Conclusions:** We found that irradiation induced the expression of c-Met by NSCLC cells, which were radiosensitized when treated with the c-Met inhibitor MK8033. Because the current standard of care for unresectable NSCLC is radiation, the addition of c-Met inhibitors partway through the radiation cycle may prove useful for enhancing the therapeutic ratio in NSCLC.

**509** POSTER

**Bortezomib enhances radiosensitivity in solid tumor cells through down-regulation of CIP2A**

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**Introduction:** Bortezomib, a proteasome inhibitor, has been clinically approved in hematological malignancies. We have previously reported that down-regulation of phospho-Akt (P-Akt) plays a key role in determining the sensitivity of hepatocellular carcinoma cells to bortezomib-induced apoptosis (Cancer Res, 2008). In this study, we report that bortezomib sensitizes cancer cells to radiotherapy through down-regulation of cancerous inhibitor of protein phosphatase 2A (CIP2A).

**Material and method:** Human cancer cell lines, including SiHa (cervical cancer), and Huh-7, (hepatocellular carcinoma) were treated with radiation and/or bortezomib then evaluated for apoptosis, and signal transduction. Flow cytometry and Western blotting were performed for apoptosis and signal transduction analysis. Gene silencing was done by small interference RNA.

**Results:** Cancer cells, including SiHa and Huh-7, showed significant resistance to radiation-induced apoptosis (up to 10 Gy). The combination of bortezomib (starting at 250 nM) and radiation restored the sensitivity of cancer cells to radiation-induced apoptosis. Our data indicated that CIP2A played a key role in mediating the radiosensitizing effect of bortezomib. The combination of bortezomib and radiation down-regulated CIP2A and subsequently reduced phospho-Akt via up-regulation of protein phosphatase 2A activity. Knockdown of CIP2A by RNA-interference overcame apoptotic resistance to radiation in cancer cells, and ectopic expression of CIP2A in cancer cells abolished the radiosensitizing effect of bortezomib, indicating that inhibition of CIP2A mediates the combination effects.

**Conclusion:** Bortezomib sensitizes cancer cells to radiotherapy through down-regulation of CIP2A.

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