Abstract: We have used a small animal irradiator/imager to deliver fractionated dose treatment and chemotherapy to orthotopically growing human cervix cancer xenografts in immune-deprived mice.

Methods: Irradiation treatment has been delivered to tumours of 5-8 mm diameter growing in the cervix of mice using an 8-beam protocol with imaging of the target immediately prior to each fraction. The treatment plan allows for a 1-2 mm margin around the imaged tumour target. The radiation treatment (225kVp X-rays) is being combined with cisplatin to match current external beam treatment procedures for cervix cancers. Tumour growth delay analysis is being performed using the imaging features of the irradiator to assess tumour size as a function of time during and after the treatment.

Results: There is limited response to single dose treatment with cisplatin (12 mg/kg) alone but no enhanced response when combined with fractionated irradiation. Ongoing experiments are testing daily 2Gy fractions combined with 5E1, an antibody that blocks SHH and IHH activity. Growth of nodal metastases in the aortic chain is also reduced by this Hh inhibitor. Normal tissues are being collected at the time of sacrifice of the mice for histological analyses (data not yet available).

Conclusions: This combination of a small animal irradiator/imager with an orthotopic xenograft model allows for preclinical studies with radiochemotherapy that can closely mimic clinical studies.

Cervix Orthotopic Xenograft Model

Small pieces of ME180 human cervix cancer xenografts were used for implantation into the cervix of NOD/SCID, NOD/Rag-1/-/gammaT null (NRG) mice or Rag22/gammaT null mice. Mice were monitored weekly by palpation for tumour growth. Tumors are imaged at ~4-5mm in diameter (see below) and assigned to the treatment groups. At sacrifice para-aortic lymph node metastases were assessed using the DsRed fluorescence marker in the ME180 cells then excised and metastasis confirmed with H&E staining.

In vivo cyclic hypoxia

Mice bearing orthotopic tumors were exposed to cyclic hypoxia (12x10min cycles of breathing 7%O2 or air; 4hr/day/2 wks) during their growth with or without receiving 5E1 for assessment of effects on lymph node metastases.

5E1 Treatment

5E1, an antibody that blocks the SHH and IHH ligands was administered by i.p injection (20 mg/kg/weekly) with cyclic hypoxia or fractionated irradiation. (see below).

Irradiation and Cisplatin Treatment.

Fractionated radiation treatment was given to the orthotopic xenografts using an image-guided small animal irradiation. (see Figure 1) using an 8-beam configuration with an 8mm collimator. Each animal was set-up individually for each treatment and imaged and adjusted to located the tumour in the beam. Fractionated treatment was delivered 3x/wk (5Gy fractions) or 5x/wk (2Gy fractions) with or without Cisplatin given weekly (6mg/kg) or 5 days/wk (2mg/kg/day). Tumor size was monitored 2x/wk using the imaging facility of the irradiator.

Conclusions

1) The combination of a small animal image-guided irradiator with an orthotopic xenograft model allows for preclinical studies with radiochemotherapy that can closely mimic clinical treatments.

2) The combination treatment with fractionated irradiation and the hedgehog pathway inhibitor (5E1) shows increased efficacy consistent with an additive effect of the two agents. This agent also significantly affects the development of lymphnode metastasis in the mice, further supporting the potential value of Hedgehog inhibitors for combination with radiation in the treatment of cervix cancer.

3) The treatments involving fractionated irradiation and cisplatin showed limited evidence for additive effects but the data is limited by toxicity associated with multiple doses of cisplatin combined with radiation. Further studies are required with lower doses more equivalent to current therapy doses (~ 6mg/kg/wk).