Patient relevant preclinical *in vivo* models using image-guided small animal irradiation for drug discovery

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Introduction

- Radiotherapy is a primary, adjuvant or neoadjuvant treatment for a number of different cancers such as glioblastoma, breast, lung and prostate.
- Significant advances have been made in improving the delivery of ionizing radiation to provide precise dosing with reduced side effects to surrounding normal tissue using image guided micro-irradiation (IGMI).
- However in the preclinical setting the use of IGRT is less common with traditional irradiation studies utilising whole body irradiation with lead shielding attempting to focus the radiation to a specific area on the animal or simple single beam techniques.
- The development of the image-guided small animal radiation research platform (SARRP) allows the treatment of animal models of cancer more accurately and importantly, with planned protocols similar to those utilised in the clinic.
- Here we demonstrate the application of IGMI to treat subcutaneous xenograft tumours established from both cell lines and patient-derived material with little or no adverse effects, as well as the utilisation of the SARRP for *in vitro* screening.

SARRP Features

- The SARRP integrates cone beam computed tomography (CBCT) imaging (high resolution, low imaging dose and 3D reconstruction) with radiation treatment (X-ray).
- Irradiation & imaging takes place in a chamber that incorporates a gantry and robotic specimen stage enabling non-coplanar field arrangements and anterior-posterior/posterior-anterior irradiation (Figure 1).
- Image fusion options for easy target localization, dose planning and avoidance of normal organs at risk.
- High precision beam geometry to achieve conformal dose distributions and clinical quality.
- Open platform to enable the addition of other imaging modalities for future research.

Figure 1: External view of SARRP (left) and internal view showing robotic stage, rotating gantry and X-ray tube (right)

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Methods

•***In vitro* assay**: HCC-827 (NSCLC adenocarcinoma cell line with an activating EGFR mutation, del E746-A750) and HCC827-ER (Erlotinib resistant variant) were grown in T25 flasks and treated with irradiation. Cells were counted after 6 days.

•***In vivo* xenograft**: HCC-827 and HCC-827ER1 cells were implanted subcutaneously in nude mice (HsdOla:MF1-*Foxn1^{nu}*). Tumour measurements and body weights were taken 3 times weekly and treatment initiated when the tumours reached a mean volume of ~200mm^3 (n=5 per group). Erlotinib was dosed at 25mg/kg po QD.

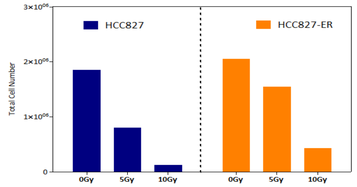
•***In vivo* patient-derived xenograft (PDX)**: We have established Caucasian non-small cell lung cancer (NSCLC) PDX models which are maintained subcutaneously *in vivo* in nude mice (HsdOla:MF1-*Foxn1^{nu}*) admixed with a human stromal cell component (bone marrow-derived human mesenchymal stem cells, ScienCell). Tumour measurements and body weights were taken 3 times weekly and dosing initiated in 2 models when the tumours reached a mean volume of ~200mm^3.

•***In vivo* Irradiation**: Mice were anaesthetised and transported to the SARRP where CBCT images were acquired. Using the MuriSlice software the isocenter of the tumour was identified and aligned with the central axis of the beam. Fractionated irradiation was administered with the SARRP (225 kV peak X-ray beams; dose rate of 2.5 Gy/min) using collimators of various diameters and a double beam (gantry position at 0° and 180°) under the guidance of the CBCT. A tolerability was performed initially to evaluate 3Gy/day × 5 days for 2 week.

In Vitro Assay

HCC-827 showed a dose-dependent kill effect in response to elevating levels of irradiation resulting in reduced cell number (Figure 2) . Similar the Erlotinib resistant line showed a dose response however the response to 5Gy was markedly less than in the wildt-ype parental line suggesting that the acquired resistance to Erlotinib bestowed some resistance to irradiation.

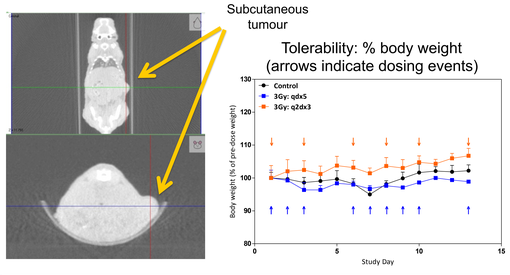
Figure 2: Dose dependent effect of irradiation on cell number of HCC827 and HCC-827ER1 cells



In vivo tolerability

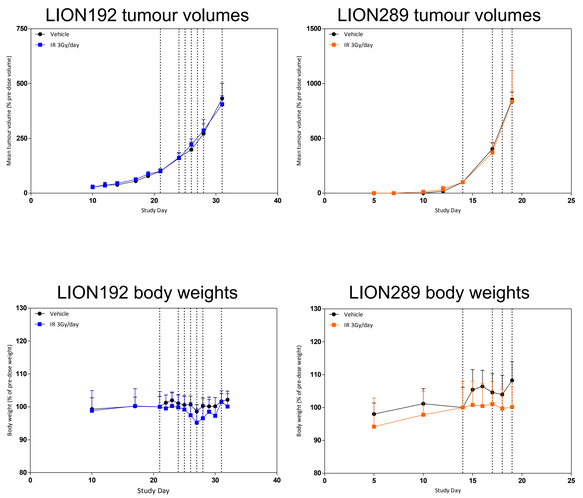
Mice bearing subcutaneous NSCLC PDX tumours tolerated 2 cycles of 3Gy/day for 5 days using the SARRP.

Figure 3: Identification of isocentre of subcutaneous tumours using the MuriSlice software following CT imaging (left) to enable irradiation treatment of 3Gy/day which resulted in no adverse effects or loss in body weight (right)



In vivo PDX models

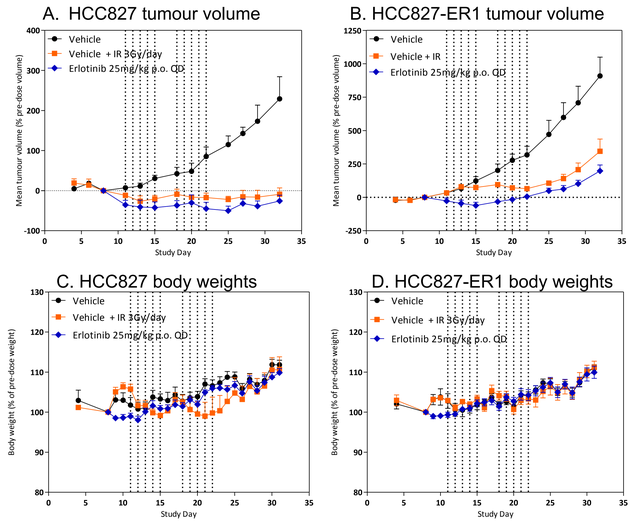
Mice bearing subcutaneous NSCLC PDX tumours showed low sensitivity to irradiation treatment in the 2 models tested (Figure 4). Body weight measured during the study increased gradually as expected for both models (bottom panel) and no adverse effects were noted.



In vivo efficacy to IGMI

Mice bearing subcutaneous HCC-827 xenograft tumours showed high sensitivity to Erlotinib treatment (25mg/kg po QD, p<0.001 Two way ANOVA) resulting in tumour regression (Figure 5A). Similarly treatment with 2 cycles of 3Gy/day for 5 days using the SARRP resulted in tumour regression (p<000.1). In HCC-827ER1 model the sensitivity to Erlotinib was reduced and growth rate higher (Figure 5B). The sensitivity to irradiation was also reduced. Body weight measured during the study increased gradually as expected for both models (bottom panel, Figure C&D).

Figure 5: The effect of 3Gy/day irradiation on HCC827 & HCC-827ER1 subcutaneous xenografts and body weights.



Conclusions

The SARRP platform allows the use of irradiation with anti-cancer agents in small animals with reduced side effects and improved outcome. This will allow these novel preclinical models to be used effectively for drug discovery programmes to identify promising treatment options for clinical testing of cancer patients using either radiotherapy alone, of in combination with new agents.

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