



In life for life

Application of small animal image-guided irradiation to preclinical *in vivo* models such as patient-derived xenografts to inform on combination strategies

Abstract:

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Introduction

- Patient derived xenograft (PDX) models sustain tumour heterogeneity and genetic integrity of the original patient sample when passaged *in vivo* and are highly predictive of clinical efficacy. As such PDX models are widely used for preclinical efficacy testing of anti-cancer agents.
- Lung cancer is the largest cancer killer with poor 5-year survival rate. Non-small cell lung cancer (NSCLC) patients that have activating mutations in the EGFR gene are treated with epidermal growth factor receptor (EGFR) inhibitors e.g. Erlotinib (Tarceva[®]) and Gefitinib (Iressa[®]).
- Radiotherapy is a primary, adjuvant or neoadjuvant treatment for a number of different cancers including lung. Image-guided micro-irradiation (IGMI) is widely used to treat cancer patients providing more accurate treatment plans, tumour targeting and reduced side effects.
- 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, Xstrahl Ltd) allows the treatment of animal models of cancer more accurately and importantly, with planned protocols similar to those utilised in the clinic.
- In this study we report the application of SARRP to treat subcutaneous PDX tumours to demonstrate sensitivity and resistance.

Methods

- ***In vivo* xenograft:** Caucasian NSCLC PDX models, known as Lung In Oncology (LION) and part of our HuPrime[®] platform (Table 1), 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 ~200mm³.
- ***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 dimensions 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 x 5 days for 2 week which showed no adverse effects or bodyweight loss.

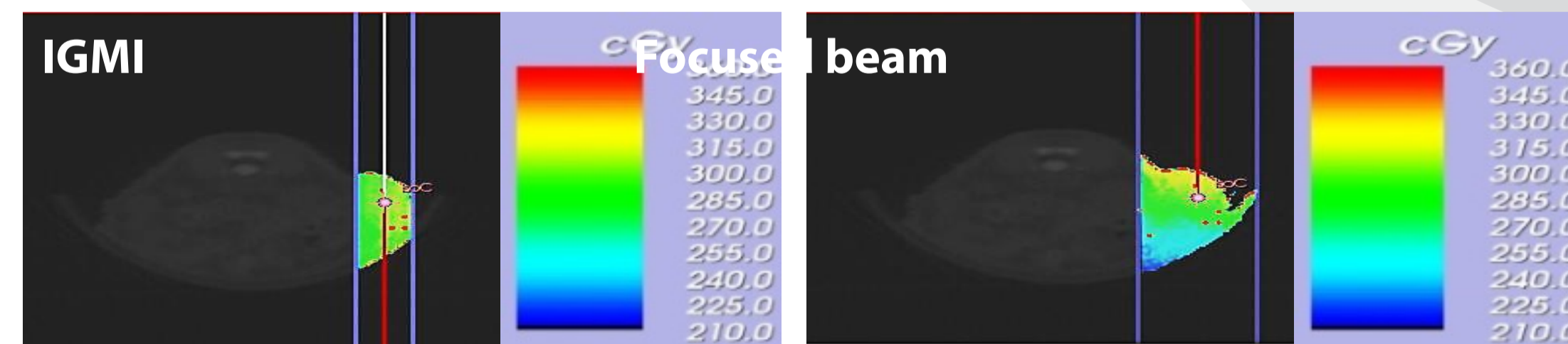
Background: SARRP features

- 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.
- SARRP delivers a uniform dose from multiple angles to a subcutaneous tumour thereby reducing normal tissue exposure whilst maintaining total dose (figure 2).

Figure 1: External view of SARRP (left), internal view showing robotic stage, rotating gantry and X-ray tube (middle), isocentre identification using MuriSlice software with CT imaging (right)



Figure 2: IGMI (left) delivers a uniform dose from multiple angles compared to focused beam (right). Using a 5x5 collimator and parallel opposed beams focused on isocentre, each beam delivers 50% of total dose allowing a more uniform coverage than using a single beam (shown by the uniform green colouring) and decreases the amount of damage to the surrounding tissue.



Results: NSCLC PDX

- Mice bearing subcutaneous NSCLC PDX tumours showed different levels of sensitivity to irradiation treatment (Figure 3).
- Body weight measured during the study increased gradually as expected and no adverse effects were noted with 2 cycles of 3Gy/day for 5 days.
- A summary of responders versus non responders is described in Table 1.
- In summary, two SCC models, LU6429 and LION294 showed complete response resulting in stabilisation of growth.
- In comparison two SCC models, LU6432 and LU6483 showed no response demonstrating resistance to treatment.
- Two models LU6426 and LU6425 showed partial response with a slower growth rate following treatment.

Figure 3: The effect of 3Gy/day irradiation on six PDX lines (dotted line indicates dosing events)

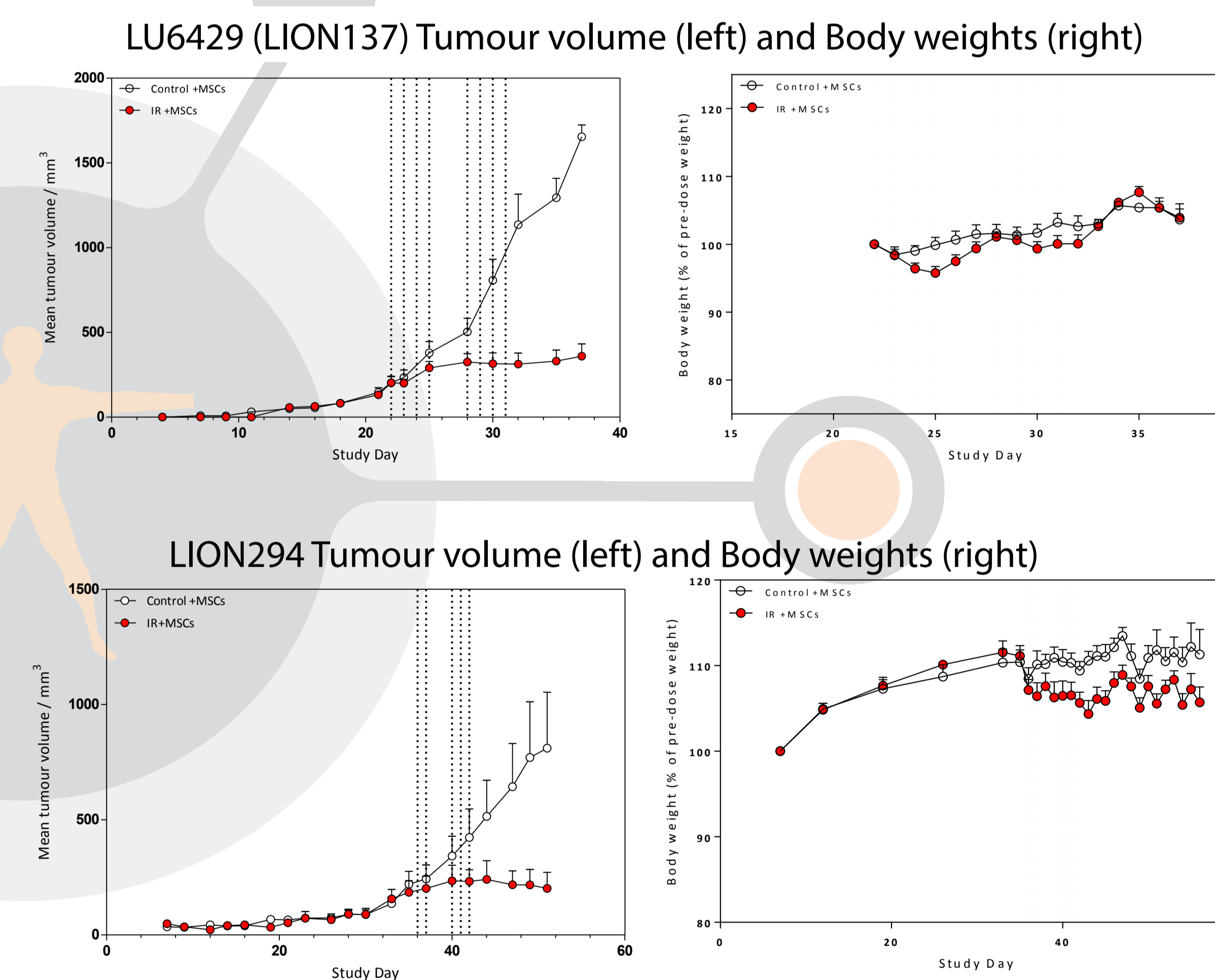
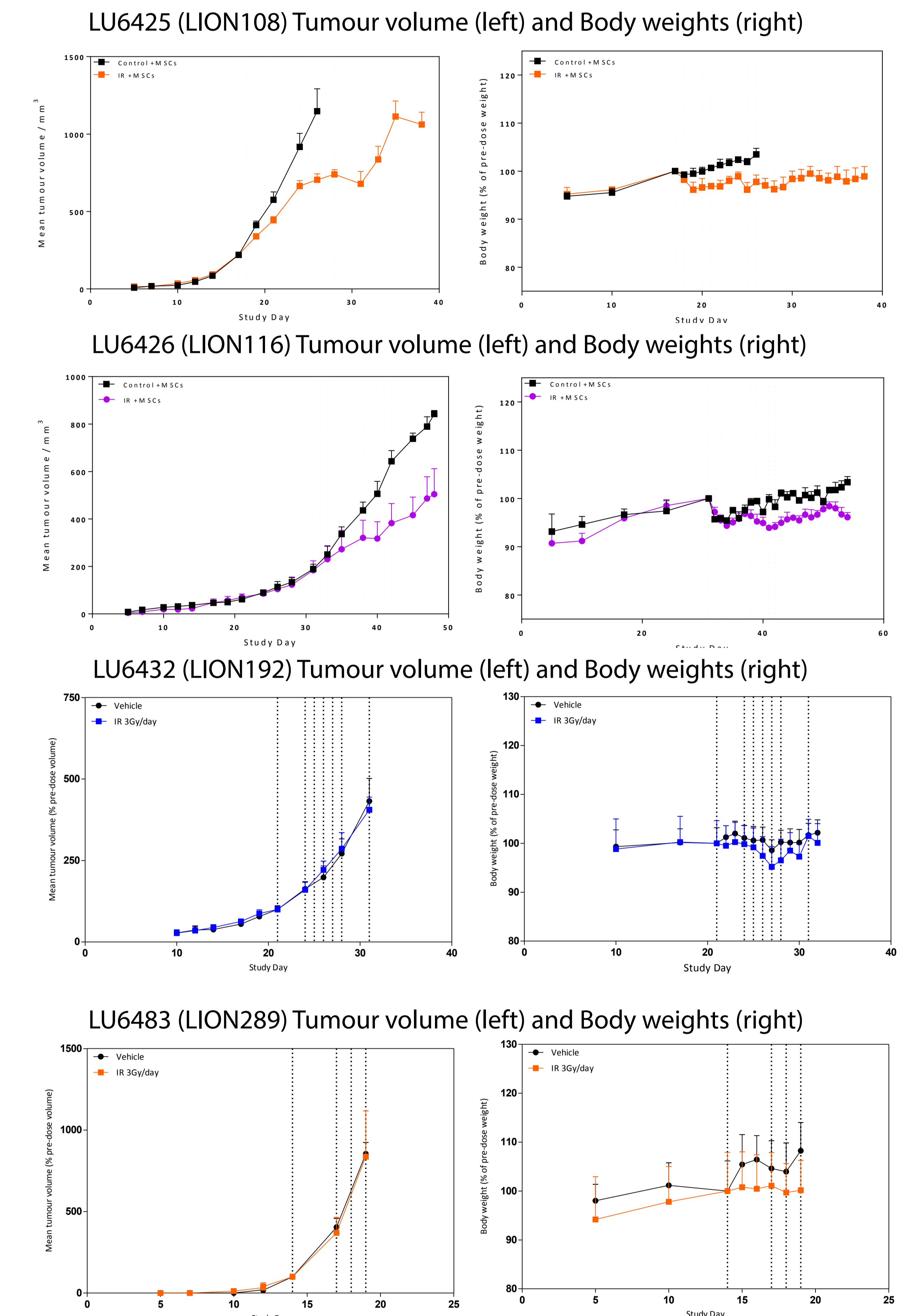


Table 1: Summary of characterisation of Caucasian NSCLC PDX models

HuBase ID	LION number	Sub-type	Known Mutations	Amplifications	FGFR inhibitor	EGFR inhibitor	CTx response	IR response
LU6425	108	ADC		FGFR1, c-met	Resistant	Resistant	Resistant	Partial response
LU6426	116	SCC	p53	FGFR1 & FGFR2	Partial response	ND	Partial response	Partial response
LU6429	137	SCC	p53	FGFR1 & FGFR2	Stable disease	ND	Complete response	Complete response
LU6432	192	SCC		ND	Resistant	ND	Resistant	Resistant
LU6483	289	SCC		ND	ND	ND	Complete response	Resistant
New	294	SCC	ND	ND	ND	ND	ND	Complete response

Results: Combination studies



Conclusions

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

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