Treatment of patient-derived NSCLC xenograft preclinical models using image-guided small animal irradiation

Nektaria Papadopoulou[1], Andrew Mckenzie[1], Lucy Page[1], Jason King[1], Yinfei Yin[1], Henry Li[2], Martin Page[1], Ian Wilson[3], Jean-Pierre Wery[2], and Rajendra Kumari[1*]

[1]PRECOS Ltd., a Crown Bioscience Company, Loughborough, UK; [2]Crown Bioscience Inc. Santa Clara, US, [3]Xstrahl Ltd, Camberley, UK

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Introduction

- Radiotherapy is a primary, adjuvant or neoadjuvant treatment for a number of different cancers such as lung, glioblastoma, breast and prostate. Image-guided micro-irradiation (IGMI) is widely used to treat cancer patients providing more accurate treatment plans 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) allows the treatment of animal models of cancer more accurately and importantly, with planned protocols similar to those utilised in the clinic.
- 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®). However, in many cases resistance emerges with secondary mutations in EGFR (T790M) or amplifications such as c-MET occurring.
- 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.

Methods

- ***In vivo* xenograft**: Caucasian non-small cell lung cancer (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). HCC827 and HCC827=ER1 cells were implanted subcutaneously in nude mice (ValidatedXeno™ in HsdOla:MF1-*Foxn1^{nu}*). Erlotinib was dosed at 25mg/kg po QD. Crizotinib was dosed at 50mg/kg po QD. 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 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.

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.

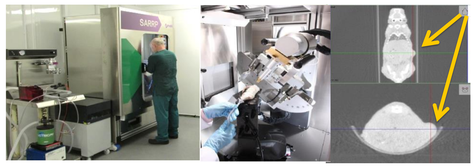
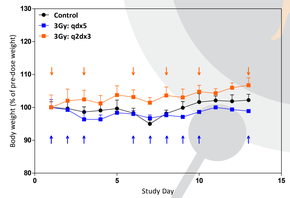


Figure 1: External view of SARRP (left), internal view showing robotic stage, rotating gantry and X-ray tube (middle), isocentre identification using MuriSlice software with CT imaging (right)

Results: Tolerability

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

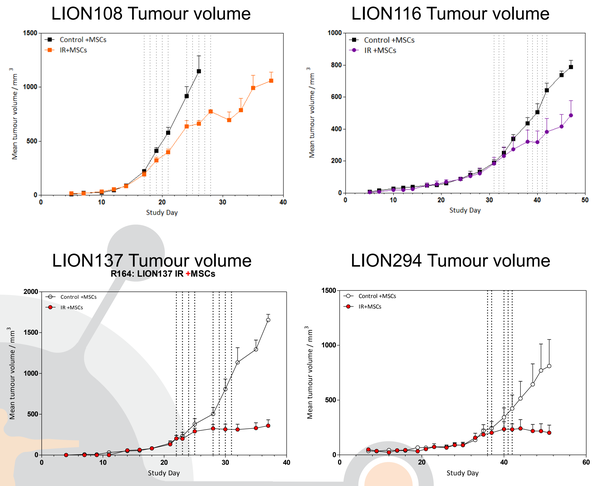
Figure 2: Tolerability: % body weight (arrows indicate dosing events)



Results: NSCLC PDX

- Mice bearing subcutaneous NSCLC PDX tumours showed different levels of sensitivity to irradiation treatment (Figure 6).
- Body weight measured during the study increased gradually as expected (data not shown) and no adverse effects were noted.

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



Results: Combination studies

- HCC827 NSCLC adenocarcinoma cell line harbours an activating EGFR mutation (del E746-A750) and was used to test the combination strategies that could be investigated using targeted agents and IGMI to overcome resistance.
- Mice bearing subcutaneous HCC827 xenograft tumours showed high sensitivity to Erlotinib treatment (25mg/kg po QD, p<0.001 Two way ANOVA) resulting in tumour regression (Figure 4A).
- Treatment with 2 cycles of 3Gy/day for 5 days using the SARRP also resulted in tumour regression (p<000.1).
- An Erlotinib resistant variant of HCC827 (HCC827-ER1) has been developed in our labs which has c-met amplification.
- The sensitivity to Erlotinib was reduced, the growth rate was higher and sensitivity to irradiation was reduced (Figure 4B).
- Crizotinib, a c-met inhibitor, was tested in the wildtype and resistant model alongside Erlotinib +/- IR.
- No effect with Crizotinib alone in wildtype model was seen (Figure 4A) whereas in HCC827-ER1 model there was a significant reduction (Figure 4B, p<0.001; ~60% tumour growth inhibition), which supported the role of c-Met amplification in the resistance mechanism.
- In the HCC827-ER1 model, the tumour regression induced by IR was lost , however in combination with Crizotinib this was restored. Interestingly, treatment with Erlotinib and IR also resulted in tumour regression

Figure 4: The effect of 3Gy/day irradiation on HCC827 & HCC-827ER1 subcutaneous xenografts in combination with Crizotinib and Erlotinib.

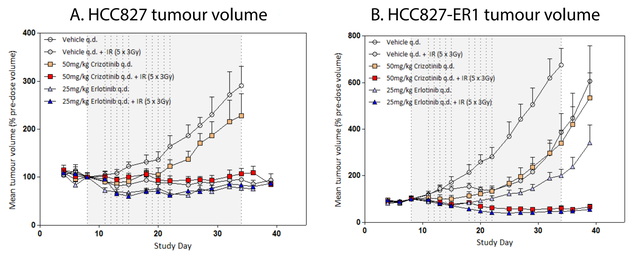


Table 1: Summary of Caucasian NSCLC PDX models

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	ND	Partial response
LU6429	137	SCC	p53	FGFR1 & FGFR2	Stable disease	ND	Complete response	Complete response
LU6432	192	SCC		ND	Resistant	ND	ND	Resistant
LU6483	289	SCC		ND	ND	ND	ND	Resistant
New	294	SCC	ND	ND	ND	ND	ND	Complete response

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 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, of in combination with new agents.

*Contact information: Dr. Rajendra Kumari, rajendra.kumari@precos.co.uk