In life for life

• Patient derived xenograft (PDX) models sustain tumour heterogeneity and genetic integrity of the original patient sample when passed 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 HuPIMP® platform (Table 1), are maintained subcutaneously in vivo in nude mice (Hsd1Blr/Slc-Fas−/−) admixed with a human stromal 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 beam; dose rate of 2.5 Gy/min) using collimation of various dimensions and a double beam (aperture position 0° and 180°) under the guidance of the CBCT. A tolerability was performed in vivo to evaluate 3Gy/day for 5 days to 2 week which showed no adverse effects or bodyweight loss.

Results: NSCLC PDX

• 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 high precision beam geometry to achieve conformal dose distributions 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 isocenters, 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: Combination studies

• 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, LU6426 and LU6483 showed no response demonstrating resistance to treatment.

• Two models LU6426 and LU6442 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). 100%

Table 1: Summary of characterisation of Caucasian NSCLC PDX models

<table>
<thead>
<tr>
<th>Hullace ID</th>
<th>LION number</th>
<th>Sub-type</th>
<th>Known Mutations</th>
<th>Amplifications</th>
<th>FGFR inhibitor</th>
<th>EGFR inhibitor</th>
<th>Ctx response</th>
<th>IR response</th>
</tr>
</thead>
<tbody>
<tr>
<td>LU6425</td>
<td>LION-108</td>
<td>AOC</td>
<td></td>
<td>FGFR1, c-met</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Partial response</td>
<td>Partial response</td>
</tr>
<tr>
<td>LU6426</td>
<td>LION-116</td>
<td>SCC</td>
<td>FGFR2</td>
<td>FGFR1 &amp; FGFR2</td>
<td>Partial response</td>
<td>ND</td>
<td>Complete response</td>
<td>Complete response</td>
</tr>
<tr>
<td>LU6429</td>
<td>LION-117</td>
<td>SCC</td>
<td>FGFR1 &amp; FGFR2</td>
<td>FGFR1 &amp; FGFR2</td>
<td>Stable disease</td>
<td>ND</td>
<td>Complete response</td>
<td>Complete response</td>
</tr>
<tr>
<td>LU6432</td>
<td>LION-118</td>
<td>SCC</td>
<td>FGFR2</td>
<td>FGFR1 &amp; FGFR2</td>
<td>ND</td>
<td>Resistant</td>
<td>Complete response</td>
<td>Complete response</td>
</tr>
<tr>
<td>LU6483</td>
<td>LION-119</td>
<td>SCC</td>
<td>FGFR2</td>
<td>FGFR1 &amp; FGFR2</td>
<td>ND</td>
<td>Resistant</td>
<td>Complete response</td>
<td>Complete response</td>
</tr>
<tr>
<td>LION294</td>
<td>LION-120</td>
<td>SCC</td>
<td>FGFR2</td>
<td>FGFR1 &amp; FGFR2</td>
<td>ND</td>
<td>Resistant</td>
<td>Complete response</td>
<td>Complete response</td>
</tr>
</tbody>
</table>

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.