Project updates and thoughts and findings on the use of patient xenotransplant models

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Update

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BP & DR; Gallipolli Foundation, 50K

Publications arising from my lab in 2016/17

Squamous Cell Carcinoma

Squamous cell carcinoma is a common and significant clinical problem.

Approximately 1500 Australians die each year as a result of cutaneous or head and neck SCC.

5-year survival from head and neck cancers
Reasons for the lack of new curative therapies

- Drug resistant disease
- Experimental models don’t mimic human cancers (85% of new drugs fail in clinical trial)

Tumours are complex

- Tumours between patients differ greatly
- Cells within a single tumour differ greatly

Conclusion

*Better preclinical models of HNSCC will improve translation of lab findings into successful clinical trials.*
We are establishing a HNSCC PDX model at TRI as a platform for

- Prioritising lead molecules for clinical trial
- As a tool to examine tumour behavior (e.g., metastasis, clonal evolution)
- For use with mice with a “humanized” immune system

<table>
<thead>
<tr>
<th>Age</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>&lt;50</td>
<td>1 (3)</td>
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<tr>
<td>&gt;50</td>
<td>30 (97)</td>
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<table>
<thead>
<tr>
<th>Gender</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>Male</td>
<td>27 (87)</td>
</tr>
<tr>
<td>Female</td>
<td>4 (13)</td>
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<table>
<thead>
<tr>
<th>Site</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>Tongue</td>
<td>7 (22)</td>
</tr>
<tr>
<td>Parotid</td>
<td>6 (20)</td>
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<tr>
<td>Oral Cavity</td>
<td>10 (32)</td>
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<tr>
<td>Naso/Oropharynx</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Skin</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Primary</td>
<td>24 (78)</td>
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<tr>
<td>Metastasis</td>
<td>4 (13)</td>
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<tr>
<td>Recurrence</td>
<td>3 (9)</td>
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<th>Smoking status</th>
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<tr>
<td>Ex-smoker</td>
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<tr>
<td>Current smoker</td>
<td>15 (48)</td>
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<tr>
<td>Non-smoker</td>
<td>5 (16)</td>
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<table>
<thead>
<tr>
<th>Histological Subtype</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>Well differentiated</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Moderately Differentiated</td>
<td>13 (42)</td>
</tr>
<tr>
<td>Poorly Differentiated</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Not stated</td>
<td>5 (16)</td>
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</table>
biopsy from SCC of head and neck patient at the time of operation

Sample washed in chlorhexidine for 1 min

Sample incubated with penicillin, gentamicin, streptomycin & amphotericin B overnight in 37 degrees

Sample kept for histology

RNA/DNA for downstream analysis

PDX pipeline (n=47)
Progress to date

• All sample collection complete (n=47)
• All tumours have been passaged through mice
• All samples for genomic analysis collected
• Sequencing is underway
• Rachel is writing up her thesis
• Manuscript to be completed upon completion of genomic analyses.
Findings for 1st generation PDX tumours

i) approximately 40% of HNSCC samples will generate PDX tumours which take between 6 & 44 weeks to develop,

ii) well differentiated HNSCC do not form PDX tumours and,

iii) there is a profound loss of tumour cells in the 14 day period following implantation that is accompanied by an increase in apoptosis.

Conclusion: there is a massive selection pressure placed on PDX tumours following implantation.
Studies to validate the use of expanding PDX tumours in 2\textsuperscript{nd} generation mice

1. Histopathology

Findings:
1. 2\textsuperscript{nd} generation tumours establish quickly (4-8 weeks)
2. Histopathology is similar to tumour of origin in 95\% of cases
Studies to validate the use of expanding PDX tumours in 2\textsuperscript{nd} generation mice

2. Sequencing

High depth resequencing of cancer panel (45 genes, > 700 fold depth)
What next?

1. Show the model truly reflects the tumour from which it was derived
2. Use the model to prioritise drug leads for human clinical trial
Conclusions so far

• PDX can be established
• 1\textsuperscript{st} and 2\textsuperscript{nd} generation PDX of HNSCC recapitulate the histopathology of the founder lesion
• 1\textsuperscript{st} generation mice take about 4 months
• 2\textsuperscript{nd} generation mice take 4-6 weeks

Considerations

• Established cell lines are convenient and quick but lack tumour complexity
• Skin painting models lack pathological relevance
• Transgenic/knockout models generally lack tumour complexity
• PDX models have their place but are difficult to work with and selection pressure is evident
• UV-irradiation is gold standard for cutaneous SCC
• 4NQO models are variable and long term but may be more tractable than PDX for HNSCC