Utilizing Histology Image Analysis to Improve Drug Response Interpretation within the Tumor Microenvironment

Dr. Lorcan Sherry, CSO
**Company overview**

* OracleBio is a specialized CRO providing histopathology digital image analysis services to support
  - Pre-clinical and clinical Pharma R&D studies
  - Companion diagnostics development
  - Digital pathology review and biomarker research

* We utilise Definiens Tissue Studio® and Indica Labs HALO® + Hypercluster® image analysis platforms

* Management has extensive experience (>50 years) within Pharma R&D
  - AstraZeneca, Schering-Plough, Merck & Co

* Clients within USA, Asia and Europe covering large & small Pharma, Biotech & Academic Institutes
Utilizing histology image analysis to:

1. Quantify immunotherapy response on immune cell populations within the tumor microenvironment

2. Identify drug PD / target modulation biomarkers in xenograft tissue to support early clinical translational research

3. Select appropriate xenograft models for drug testing based on tissue region specific target expression
Quantifying immunotherapy response within xenograft tissue

Humanized LNCAP cell derived xenograft

* Image analysis can be utilised to quantify immunotherapy effects on:
  - Number of immune cells present within tumor and tumor microenvironment
  - Spatial distribution of immune cells within tumor tissue
  - Distance (proximity) of immune cells to tumor regions

Tissue courtesy of oncotest

OracleBio
Image Analysis Solutions

www.oraclebio.com
Number of immune cells within tumor & tumor microenvironment

Humanized LNCAP cell derived xenograft

CD8+ immune cells (brown); IHC by Aquila Histoplex
Number of immune cells within tumor & tumor microenvironment

Humanized LNCAP cell derived xenograft

<table>
<thead>
<tr>
<th>Analysis Parameter</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor ROI Area (mm$^2$)</td>
<td>24.5</td>
</tr>
<tr>
<td>Number of CD8+ cells in Tumor ROI</td>
<td>278</td>
</tr>
<tr>
<td>Number of CD8+ cells per mm$^2$ Tumor ROI</td>
<td>11</td>
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</table>
Spatial distribution of immune cells within tumor tissue

Humanized LNCAP cell derived xenograft

Spatial distribution of CD8+ cells within Tumor

- Region 1: 167
- Region 2: 48
- Region 3: 43
- Region 4: 20
- Region 5: 0

Legend:
- Tumor
- Non-Tumor
* Based on proposed drug MoA, evaluate specific innate/adaptive immune cell populations or other cell types/biomarkers:
  * CD3, FoxP3, CD4, CD8, CD20, CD25, CD56, CD68, CD163, Ki67, PD-1, PDL-1 etc
Identifying drug PD / target modulation biomarkers in xenograft tissue

Myeloma Xenografts

Quantitative analysis of tumours ex vivo to confirm MoA and identify target modulation biomarkers

Vehicle Group 1
Group 2
Group 3

Days (after start of treatment)

Tumour volume (mm³)
Target modulation biomarker p53

p53 IHC tissue image

Analysis ROI overlay

Original p53 IHC (magnified area)

p53 nuclei IHC detection & stain intensity classification

<table>
<thead>
<tr>
<th>Slide name</th>
<th># Nuclei Positive</th>
<th>Viable Tumour Area (µm²)</th>
<th>Positive Nuclei per mm² Viable Tumour</th>
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</thead>
<tbody>
<tr>
<td>G3-33 Level 2</td>
<td>22,621</td>
<td>84,855,926</td>
<td>267</td>
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</table>

Stats: Mann-Whitney non-parametric test

Slide name # Nucleus Positive Viable Tumour Area (µm²) Positive Nuclei per mm² Viable Tumour
G3-33 Level 2 22,621 84,855,926 267

Viable Tumour Area

- **Viable Tumour**
- **Necrotic Tumour**
- **Artefact**
- **White Space / Host Tissue**

**IHC Intensity**
- **+ve Low**
- **+ve Med**
- **+ve High**
Target modulation biomarker Caspase 3

CC3 IHC

Analysis ROI overlay

CC3 detection (yellow overlay)

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<tr>
<th>Slide name</th>
<th>ROI name</th>
<th>CC3 Marker Area (µm²)</th>
<th>No Stain Area (µm²)</th>
<th>% Marker Area</th>
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<tr>
<td>G3-33 Level 1</td>
<td>Tumour</td>
<td>15,160,638</td>
<td>57,448,708</td>
<td>20.88</td>
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Stats: Mann-Whitney non-parametric test

Identify drug target modulation biomarkers in CDX models

Further evaluate in appropriate PDX models

Potential utility as target modulation biomarkers in early clinical PK/PD studies

Pre-clinical - Clinical Translational Strategy
Utilizing image analysis for xenograft model selection

**Aim:** To select xenograft models with the highest drug target IHC staining **within Tumor tissue ROI** using a Tissue Multi Array (TMA) collected from different patient-derived xenografts.

Mammary PDX TMA cores IHC stained for Target

Image Analysis ROI Overlay

- **Viable Tumour**
- **Non-Tumour**
- **White Space**
- **Artefact**

In collaboration with oncotest
Utilizing image analysis for xenograft model selection

Original IHC Stained Image

Formation of cells in Viable Tumour ROI

Classification of cells in Viable Tumour ROI

- Nuclei
- Cytoplasm
- Membrane

- Negative
- Low Intensity
- Medium Intensity
- High Intensity
Utilizing image analysis for xenograft model selection

- Approach provides valuable information on drug target or biomarker region specific tissue expression levels in xenograft tissue.
- Provides rapid screen of xenograft samples to allow selection of the most appropriate model for oncology R&D programmes.

Average Histological Score per PDX

<table>
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<tr>
<th></th>
<th>MAXF 401</th>
<th>MAXF 449</th>
<th>MAXF 508</th>
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<th>MAXF 713</th>
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<td>245</td>
<td>10</td>
<td>164</td>
<td>103</td>
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Utilizing histology image analysis can support:

* An in-depth understanding of immunotherapy treatment on immune cell populations within the tumor microenvironment

* Identification of tissue based target modulation / PD biomarkers, which may support selection of translational read-outs for use in early clinical studies

* Detailed characterization of target expression across multiple xenograft models to better guide appropriate model selection for oncology R&D programmes