Interpreting Therapeutic Response on Immune Cell Number and Spatial Distribution within the Tumor Microenvironment

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OracleBio
* OracleBio is a specialised 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

* Utilise 2 Image analysis platforms: [DEFINIENS](#) [indica labs](#)

* Our services help:
  - Demonstrate therapeutic proof of mechanism within tumor
  - Identify clinically translatable tissue biomarker / PD read-outs
  - Guide disease area selection for clinical cancer studies
  - Support development of a tissue based therapeutic CDx
• Expertise in utilizing sophisticated image analysis software for quantification of H&E / IHC / IF / ISH marker staining within cells and tissue.

• Example read-outs include numbers of positive cells / nuclei / spots, tissue morphological features, marker staining intensity, Histological scores etc.

• Deliver accurate and reproducible results, allowing for in-depth interpretation and more informed decision making.

• For studies requiring histology / IHC, OracleBio utilises a focused network of GCP/GLP accredited partner CROs in Europe.

• Over the past 4 years OracleBio has collaborated successfully in several pre-clinical / clinical studies with our IHC CRO partners.

• Via our Pathologist network, clinical and pre-clinical pathology review of H&E and IHC tissue slides or images to support Pharma R&D

• Clinical or pre-clinical Pathologist performed digital annotation of specific regions of interest (ROI) for analysis on tissue slide images
How we work with Clients

Histology images can be directly uploaded to a secure client folder on our cloud server (most popular and efficient approach)

Alternatively, send us your histology slides and we can generate whole slide images using our Digital Slide Scanner

We can also directly manage multi-part studies through established alliances with other CRO’s, combining image analysis with tissue sourcing, histology/IHC, digital pathology review & in life studies

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**Typical Study Workflow**

**Confirm Study Remit**
- Material to be sent
  - Images only, or Slides / Tissue Blocks?

**Study Requirements**
- IHC required as well as analysis? GCP/GLP?
- Marker staining for quantification: IHC (single / dual) / ISH; Fluorescence multiplex?
- Agree required data read-outs to be quantified across whole tissue section or specific ROI

**Develop & Confirm Analysis Algorithm**
- Algorithm work-up
  - Develop algorithm to detect agreed read-outs. Pathologist input required to define ROI?
  - Process 5~10% of study slides with developed algorithm and set up an online screen share meeting with client to review initial analysis & gain feedback
  - Make any algorithm amendments based on client feedback

**Process Study Images**
- Slide Processing
  - Review all study images to mark-up any artefacts on sections to be excluded from analysis
  - Process algorithm on all study slide images in batch mode to generate data
  - QC all images for accuracy of ROI overlay and cell stain detection

**Deliver Data**
- Data Deliverables
  - Image analysis overlay images showing ROI & marker staining quantification
  - Raw data image analysis csv files
  - A summary Excel data file for the whole study
  - A full report including analysis methodology, data, representative analysis images and where applicable, graphs and statistical analysis of group results

*OracleBio
Image Analysis Solutions*
Almost all immune system components within tumor tissue can be stained and quantified via histology image analysis.

Examples include:

- CD3 (T-cell)
- CD4 (helper T-cell)
- CD8 (cytotoxic T-cell)
- FoxP3 (regulatory T-cell)
- CD20 (B-cell)
- CD56 (NK Cell)
- CD80 (Dendritic cell)
- CD68 / CD163 (M1/M2 Macrophage)
- Checkpoint activating / inhibiting receptors & ligands
- Perforin
- Granulysin
- Arginase
3 Case Studies
Study outline

* 4 groups:
  - Vehicle
  - CB-1158 (arginase Inhibitor)
  - Anti CTLA-4
  - Anti CTLA-4 + CB-1158

* n=5 LLC xenograft tumors per group

* CD8+ T-cell IHC performed by histology partner

* Digital image analysis for quantification of CD8+ T-cells in viable tumor performed by OracleBio
1. Calithera Biosciences, USA

Data presented at EORTC, November 2015

* CB-1158 significantly increased CD8+ T-cell infiltrates in tumor when administered with anti CTLA-4, demonstrating potential to combine CB-1158 with other I-O therapeutic agents to enhance anti-tumor efficacy
Syngeneic allograft model with s.c. implanted CT-26 cells (colon cancer)

(A) Whole Section IHC, (B) Analysis ROI detection overlay, (C) Magnified area showing CD8 IHC in Brown & nuclei blue, (D) Detection of CD8 IHC (red overlay)
2. E-therapeutics, UK

Syngeneic allograft model with s.c. implanted CT-26 cells (colon cancer)

(A) Whole Section IHC, (B) Analysis ROI detection overlay, (C) Magnified area showing CD8 IHC in Brown & nuclei blue, (D) Detection of CD8 IHC (red overlay)

% CD8 Staining in Viable Tumor

% Tumor Volume

(Mean % change from Baseline)

Vehicle | CPI
---|---
\*P<0.05

Vehicle | CPI
---|---
\*P<0.05
2. **E-therapeutics, UK**

Syngeneic allograft model with s.c. implanted CT-26 cells (colon cancer)

- **A** Whole Section IHC
- **B** Analysis ROI detection overlay
- **C** Magnified area showing CD8 IHC in Brown & nuclei blue
- **D** Detection of CD8 IHC (red overlay)

**Impact** - Tissue based evidence for mechanism of action associated with change in tumor volume
The primary efficacy endpoint was histopathological regression from CIN2/3 to either CIN1 or normal pathology.

Secondary read-outs included IHC plus digital image analysis quantification of immune cells and biomarkers within collected cervical tissue samples.
* For patients treated with VGX-3100 and showing histopathological regression, CD8+ infiltrates in non-lesional (non-CIN2/3) mucosa significantly increased in both the epithelial and stromal compartments.

* Tissue IHC image analysis data generated aided the interpretation of VGX-3100 response in the Phase 2b trial.

Quantifying Immune Cell infiltration and Spatial Distribution within the Tumor Microenvironment
Evaluating immune cell spatial context within tumor

* Evaluations around MoA of Immunotherapies has driven a change in how we utilise image analysis.

* Cell type, density & location/distribution

* Spatial context analysis:
  * Distances to tumor interface
  * Cell-to-cell proximity
  * Cell clustering

* Increase understanding of:
  * Cell relationships within the tumour, at the interface & microenvironment
  * Cell to cell interactions and functions
Evaluating immune cell spatial context within tumor

- **Immunoscore®**: quantify the density of CD3+ T-cells and CD8+ cytotoxic T-cells within the tumor core and invasive margin.

- A strong lymphocytic infiltration, associated with good clinical outcome, has been reported in many different tumors including colorectal cancers.

*By evaluating the immune contexture of the tumor core and invasive margin, the Immunoscore® can aid the clinical decision-making process for colon cancer patients.*
Evaluating immune cell spatial context within tumor

Prognostic influence of tumour-infiltrating immune cells in gastric cancer critically depends on their cell-to-cell distance. (Feichtenbeiner et al Cancer Immunol Immunother, 2014)

Distance to the nearest cell indicated by:

- **Red** arrows FoxP3+ to FoxP3+
- **Blue** arrows CD8+ to CD8+
- **Green** arrows CD8+ to FoxP3+
- **Orange** arrows FoxP3+ to CD8+

*FoxP3 TILs must be located within a distance between 30 and 110 µm of CD8+ T cells to positively impact on prognosis*
Baseline density and location of T cells at the invasive tumor margin and inside tumors in metastatic melanomas have predictive value in the treatment outcome of patients receiving therapies that block the PD-1/PD-L1 axis.
Immune Cell Tumor Border Proximity Analysis
Immune Cell Tumor Border Proximity Analysis
Immune Cell Tumor Border Proximity Analysis

HALO™ Infiltration Histogram
CD8 densities around interface

HALO™ Infiltration Histogram
FoxP3 densities around interface
Immune Cell Tumor Infiltration Analysis
Nearest Neighbor plots – distances between CD8 & FoxP3 cells

Nearest Neighbor FoxP3 to CD8
Active Region

Nearest Neighbor FoxP3 to CD8
Inactive Region

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Active ROI's</th>
<th>Inactive ROI</th>
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<tbody>
<tr>
<td>Total Cells</td>
<td>12007</td>
<td>14780</td>
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<tr>
<td>Average Neighbor Distance (μm)</td>
<td>40.67</td>
<td>192.77</td>
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<tr>
<td>Number of Unique Neighbors</td>
<td>4692</td>
<td>1770</td>
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</tbody>
</table>
Cell to cell spatial relationships

**Multiplex IF:**
CD8 (Red), CD4 (Green), FoxP3 (Blue), Nuclei (White)

FoxP3 cells <30µm (dark blue), or >30µm (light blue), distance from CD4 (red)
Utilising Histology image analysis to quantifying Immune / inflammatory cells within the tumor microenvironment can guide and impact R&D:

- Build evidence for therapeutic **Proof of Mechanism**
- Guide **disease area selection** for clinical studies
- Identify clinically **translatable tissue biomarker** read-outs
- Further **characterise therapeutic activity** using spatial correlations to elucidate immune cell patterns in the tumor microenvironment
- Support development of a tissue based therapeutic **Companion Diagnostic**