Using Molecular Imaging for Monitoring Cancer Immunotherapy
Success in a Cervical Cancer Model

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Introduction

Problem: Increasingly, clinical experience with immunotherapies indicates that traditional metrics for tumor evaluation may not be sufficient, necessitating more robust, immunotherapy specific, pre-clinical methods for therapy evaluation and optimization.

Goal and solution: Use Magnetic resonance imaging (MRI) with positron emission tomography (PET) to longitudinally track labeled immune cells following checkpoint inhibitor immunotherapy treatments (anti-PD1, DPX) in a cervical cancer model.

- Label immune cell populations with superparamagnetic iron oxide (SPIO) and track their migration.

Background:

- Tumours in response to immunotherapy often swell before shrinking, making use of traditional biomarkers difficult.
- Checkpoint inhibitors function by inhibiting the tumour’s ability to evade the immune system.
- Molecular imaging is an attractive tool for monitoring immunotherapy outcome because it provides longitudinal, individual insight into biological relationships between tumours & the immune system.

Current Biomarkers:

- RECIST: Response Evaluation Criteria in Solid Tumours
- Measures the changes in tumour volume over time
- Traditional metric used for chemotherapy and radiotherapy
- PERCIST: PET Criteria in Solid Tumours
- Uses FDG-PET to measure tumour metabolic changes
- CD8a: Immune Related Response Criteria
- Published in 2009
- Accounts for a time-gap between treatment and tumour regression (some swelling allowed)
- Neither PERCIST nor RECIST account for distinguishing between pseudo-tumor progression and immune-related patterns, impeding their reliability, additionally their use as prognostic biomarkers not well understood

Methods

1. Implant C57BL/6 and C57BL/6- Ubc-GFP mice with 5x10^3 C3 cervical cancer cells. Both groups also receive the same treatments (see table 1 below)

2. Collect lymph nodes or bone marrow from Ubc-GFP C57BL/6 mice and isolate the immune cells type of interest (CD8+) and culture in vitro for ~9 days. Label with SPIO 24h before injection.

3. Inject cells intravenously 24h before Day 1 imaging.

4. Image each mouse using a balanced steady-state free precession (PIESTA or BSSFP) pulse sequence to obtain anatomical detail and qualitatively locate cells. Then a TurboSPI pulse sequence for deriving iron concentration per voxel and cell quantification (see Zoe O’Brien-Moran’s poster)

5. Data analysis is done in VivoQuant, FACS & FISH, Review.

Table 1: Treatment groups for CD8+ cell tracking

<table>
<thead>
<tr>
<th>Group</th>
<th># of mice</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Control; no Treatment</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>Anti-PD1 (200ug/day) days 7, 9, 11, 21 &amp; 25</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>DPX (5ug); day 15</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Anti-PD1 + DPX; days 7, 9, 11, 21 &amp; 25 and day 15 (DPX)</td>
</tr>
</tbody>
</table>

IF: Tumours were extracted at the end of the study from control and anti-PD1+DPX mice, frozen, sectioned and stained with DAPI. Injected cells from the adoptive transfer are GFP+ and Rhodamine B labeled (SPIO particles).

FACS: Used to confirm cell purity before injection and incubation with SPIO.

Conclusions and future directions

- The migration of cytotoxic CD8+ T cells can be observed with MRI
- Attempts to quantify mice in groups results in highly variable data, in part due to the difficulty in delineating between SPID0-labeled cells and necrosis, but also due to individual differences in cell recruitment/migration
- PET can be used to deconvolute between necrotic tissue and SPIO-labeled immune cell populations
- Limitations: Available in limited quantities from the hospital PET facility and signal decays during the day

FUTURE DIRECTION (see Zoe O’Brien-Moran’s poster)

- Track and quantify cellular migration of regulatory T cells in a cervical cancer model.
- Use a TurboSPI/PET pulse sequence to measure iron concentrations in each voxel.
- Uses Rb+ maps: Presence of iron shows a distinct signal, necrosis doesn’t.
- Data obtained for CD8+ and DC cells but not yet analyzed.
- Compile cellular migration data from multiple cancer models to develop advanced in vivo understanding of immunotherapies to improve their implementation & combination.

References

1. Therease et al. (2006) New guidelines to evaluate the response to treatment in solid tumors. JNCI 98(23): 1715

CD8+ Migration and quantification

Image Analysis

- Left: A sample histogram showing the increased skewness of the voxels (cubic pixel) towards dark contrast (arrows) at week 3. The skewness to the left in week 1 indicates an increase in dark areas within the tumours (hopefully CD8+ cells, but may also be necrosis)
- Right: Average fold increase in MRI dark contrast of all treatment groups in comparison to a week 3 post-implant baseline scan. Large error bars are potentially due to necrotic tissue, but also highly individualized responses in cellular migration (an advantage of this technique).

DC Migration in control mice

- The purpose of the study was to test different DC concentrations, SPID0 loading, and cellular purity over one week
- We are using PET/MRI to distinguish between injected cells containing SPID0 particles and tissue necrosis, which both have negative ("dark") contrast.
- Areas with no or little H_{2}^{15}FDG (fluorodeoxyglucose) radioactivity in the tumour indicates necrotic tissue and can be eliminated from analysis.
- Shown below is necrotic tissue and DC-SPID0 cells
- DCs efficiently migrate to the tumour

FACs and Immunohistochemistry

CD8 population

Mouse 1

Mouse 2

Week 3

Week 4

Week 3

Week 4

Baseline

Mouse 1

Mouse 2

FACs

GFP

CD8a PE

Rhodamine

Overlay

CD8 population

baseline

Week 3

Week 4

Mouse 1

Mouse 2

Week 3

Week 4

Week 3

Week 4

Week 3

Week 4

Week 3

Week 4

Week 3

Week 4

Week 3

Week 4

Week 3

Week 4

Week 3

Week 4

Week 3

Week 4

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