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 tumour 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, DepoVax™) in a cervical cancer model.

- Label immune cell populations cells 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:

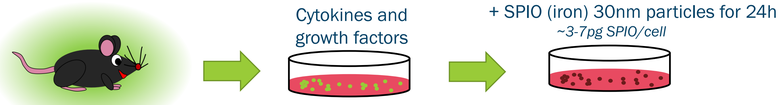
- **RECIST**[1]: Response Evaluation Criteria in Solid Tumours
  - Measures the changes in tumour volume over time
  - Traditional metric used for chemotherapy and radiotherapy
- **PERCIST**[2]: PET Criteria in Solid Tumours
  - Uses FDG-PET to measures tumour metabolic changes
- **irRC**[3]: Immune Related Response Criteria
  - Published in 2009
  - Accounts for a time-gap between treatment and tumour regression (some swelling allowed)

Neither PERCIST nor irRC can distinguish 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[5] 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** (FIESTA 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 quantitation (see Zoe O'Brien-Moran's poster)

Table 1: Treatment groups for CD8+ cell tracking

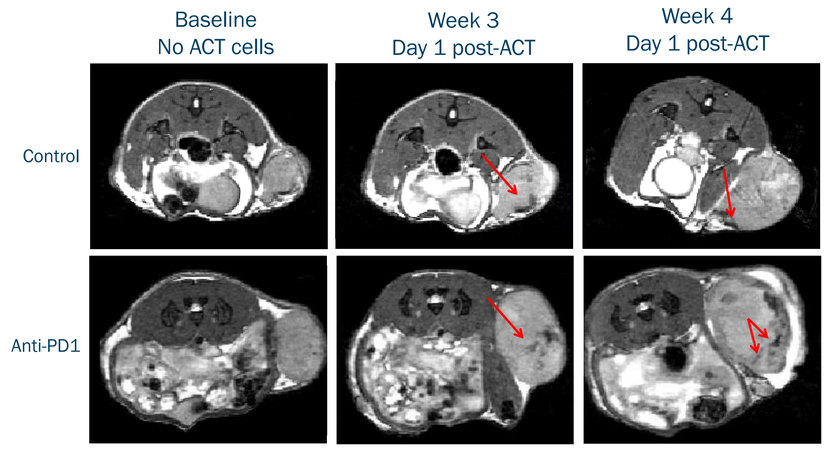
Group	# of mice	Treatment
1	6	Control; no Treatment
2	5	Anti-PD1 (200ug/day) days 7, 9, 11, 21 & 25
3	5	DPX (5ug); day 15
4	5	Anti-PD1 + DPX; days 7, 9, 11, 21 & 25 and day 15 (DPX)

5. Data analysis is done in VivoQuant, Rview, and Matlab. Regions of interest are drawn on the images to quantify voxel information

References: **1.** Therasse et al. (2000) New guidelines to evaluate the response to treatment in solid tumors. JNCI 92(3):205 **2.** Wahl RL et al (2009) From RECIST to PERCIST: Evolving considerations for PET response criteria in solid tumors J Nucl Med 50:122S **3.** Wolchok Jd et. al (2009) Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res*. **15** (23): 7412

CD8+ Migration and quantitation

- Collected scans without cells for a baseline level during week 3, 24h after cells transfer in week 3 and 24h after cells transfer in week 4.
- We observe an increase in dark voxels after adoptive cells transfer (ACT) in week 3 and 4.



- Tumour and lymph node volumes in relation to treatments.
- Lymph node swelling at the tumour site is an excellent biomarker for immune system activation
- Combination therapy of anti-PD1 + DPX results in the smallest tumour volumes and greatest lymph node swelling
  - Indicates strong immune system activation

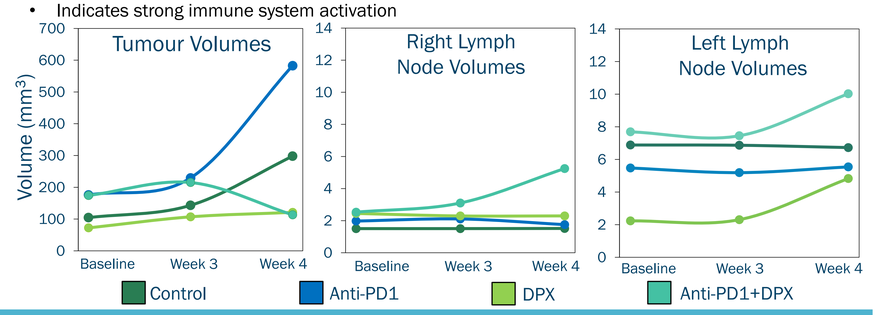
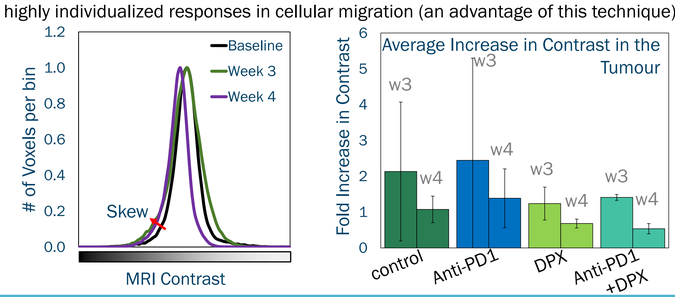


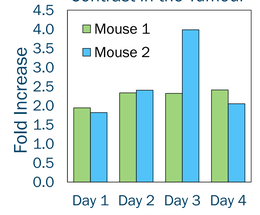
Image Analysis

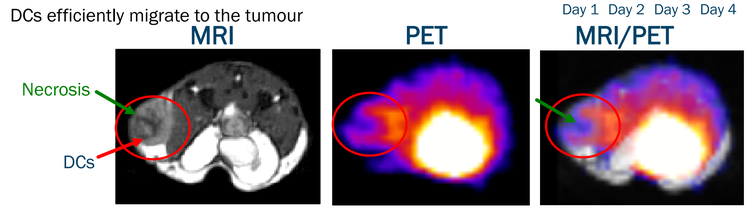
- Left: is a sample histogram showing the increased skewness of the voxels (cubic pixel) towards dark contrast (arrows) at week 3. The skewness to the left in week 1 indicates an increase in dark areas within the tumours (hopefully CD8+ cells, but may also be necrosis)
- Right: Average fold increase 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

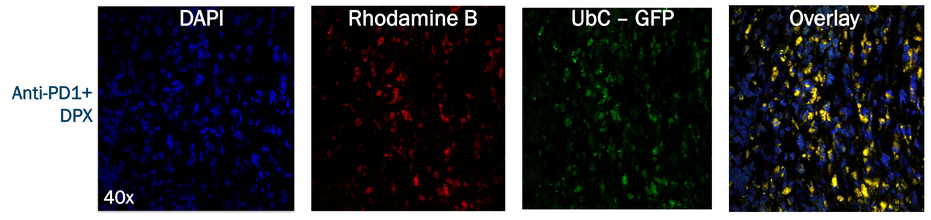
- The purpose of the study was to test different DC concentrations, SPIO loading, and cellular purity over one week
- We are using PET/MRI to distinguish between injected cells containing SPIO particles and tissue necrosis, which both have negative ("dark") contrast.
- Areas with no or little ^{18}F-FDG (fluorodeoxyglucose) radioactivity in the tumour indicates necrotic tissue and can be eliminated from analysis.
  - Shown below is necrotic tissue and DC-SPIO cells
- DCs efficiently migrate to the tumour



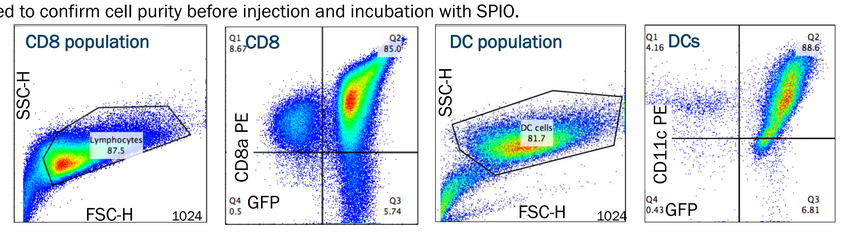


FACS and Immunohistochemistry

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 particle).



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 SPIO-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 MRI** pulse sequence to measure iron concentrations in each voxel.
  - Uses R_2* 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.

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