Labeling of human peripheral blood mononuclear cells with a Fluorine-19 perfluorocarbon agent permits their *in vivo* detection using cellular MRI and allows for cancer vaccine formulation comparisons

Corby Fink[1,2], Jeffrey Gaudet[2,3], Paula Foster[2,3], and Gregory Dekaban[1,2]

[1]*Microbiology and Immunology, Western University, London, Ontario, Canada,* [2]*Robarts Research Institute, London, ON, Canada,* [3]*Medical Biophysics, Western University, London, ON, Canada*

Background: Cancer immunotherapy is an emerging research area that uses one's immune system to combat cancer. An example involves the *ex vivo* culturing of peripheral blood mononuclear cells (PBMC), which contain antigen presenting cells (APC), with tumor-specific antigen (Ag) to create an APC-based cancer vaccine. To be effective, tumor Ag-presenting APC must migrate to secondary lymphoid organs, such as the lymph node, post injection. Within secondary lymphoid organs, they present Ag to T cells and induce tumor Ag-specific T cell proliferation, forming the basis of cancer immunotherapy. Previous research has demonstrated that the quantity of tumor-Ag loaded APC reaching the lymph node is directly proportional to the therapeutic outcome of the cancer vaccine. Furthermore, knowledge of where else these therapeutic cells migrate to post-injection is imperative before translation into human studies. Fluorine-19 (^{19}F) MR imaging can be employed to longitudinally track and quantify the migration of therapeutic cells, in the form of PBMC, labeled with a FDA-approved ^{19}F agent *in vivo* and we propose that this imaging technique can be optimized such that we can detect differences in *in vivo* migration efficiencies between untreated PBMC and PBMC treated with APC-activating cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF). Here, our laboratory presents the first time human PBMC have been labeled with a ^{19}F cell labeling agent, verified both through nuclear magnetic resonance (NMR) and flow cytometry as well as presents the *in vivo* detection of migrated PBMC to the draining lymph node post injection in a mouse model using cellular MRI. The efficient labeling and detection of PBMC *in vivo* in combination with APC-based cancer vaccines being used clinically creates a scenario in which this imaging technique can be disseminated into the clinic.

Methods: PBMC were obtained from the peripheral blood of three healthy male volunteers (n=3) and cultured for 2 days at varying cell concentrations. Cells were labeled with a red fluorescent ^{19}F-perfluorocarbon emulsion at varying concentrations to assess for labeling efficiencies using flow cytometry on an LSRII as well as collection of a cell pellet for each cell and ^{19}F-perfluorocarbon concentration to quantify the average number of ^{19}F atoms per cell using NMR. Furthermore, PBMC were also labeled with a non-fluorescent ^{19}F agent (5.0mg/mL) for 48 hours, with GM-CSF (20ng/mL) added to some culture cells after 24 hours. $3x10^6$ ^{19}F-labeled PBMC, cultured with or without GM-CSF, were injected into the footpad of male nude mice. Two days post injection, mice were anesthetized and proton (^{1}H) and ^{19}F scans were conducted at 9.4T with a dual-tuned ^{1}H/^{19}F mouse body coil to quantify migration of ^{19}F-labeled cells to the draining popliteal lymph node. A 3D-balanced steady state free precession (bSSFP) sequence was employed with an image resolution of 1x1x1mm^3 for ^{19}F and 200x200x200μm^3 for ^{1}H, which equated to a scan time of less than 90 minutes. For *in vivo* quantification of ^{19}F-labeled cells, NMR was performed on a cell pellet of known cell number as well as measuring and comparing the signal in a region of interest to the signal in a reference of known concentration of ^{19}F atoms using Voxel Tracker software.

Results/Discussion: The ability of human PBMC from healthy volunteers to label with this ^{19}F agent after 48 hours of culture was examined using two techniques. First, NMR spectroscopy on a PBMC pellet of known cell number revealed that regardless of the cell concentration or ^{19}F concentration used in culture, PBMC labeled on the order of magnitude of 10^{11} ^{19}F atoms per cell on average, with a tendency for cells to label with an increased amount of ^{19}F when in more dense cell cultures (Fig.1). Fluorescent ^{19}F-labeled PBMC were also subjected to phenotyping studies such that individual cell lineages can be measured for their labeling efficiency with this ^{19}F agent as measured using the red fluorescent dye formulated into the ^{19}F perfluorocarbon emulsion. APC lineages within PBMC that are or can be activated using GM-CSF, such as dendritic cells and B lymphocytes, all label with a high efficiency with this red fluorescent ^{19}F agent, regardless of the cell or ^{19}F concentration employed in culture (Fig.2). Lastly, ^{19}F-labeled PBMC migration to the draining popliteal lymph node can be detected in a mouse model 48 hours post footpad injection. The amount of ^{19}F loading, as outlined in Figure 1, allowed for the detection and quantification of $1.01x10^5$ and $1.29x10^5$ ^{19}F-labeled cells within the popliteal lymph nodes of a representative mouse (Fig.3). This migration corresponds to approximately 4% of originally injected cells. As this migration to secondary lymph nodes can be quantified, this non-invasive imaging technique, with optimization, could serve as a surrogate marker to compare between the efficacies of different cell-based cancer vaccine formulations. For example, we could use this imaging technique to observe if treatment of PBMC with GM-CSF enhances migration to the lymph node and thus, creates a more potent vaccine with increased therapeutic benefits.

Significance: The study represents the first time that human PBMC isolated from peripheral blood were labeled with a ^{19}F-based cell labeling agent, as verified by both NMR and flow cytometry. Moreover, we demonstrated that cell lineages within PBMC that are important with respect to antigen presentation all label with high efficiency regardless of which cell or ^{19}F cell labeling agent concentration used. As a result of this efficient labeling, we were able to detect the migration of these labeled cells to the draining lymph node two days post footpad injection. As APC formulations have been used clinically in a prostate cancer vaccine and PBMC cell migration can be detected in a mouse model, application of this non-invasive imaging technique in the clinical to assess the effectiveness of such vaccines becomes feasible.

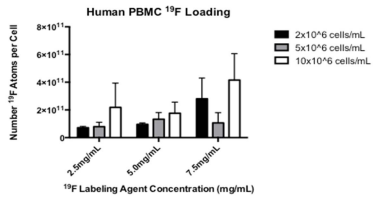

Figure 1. Human PBMC can be labeled efficiently with a ^{19}F Cell labeling agent. Cell pellets of a known number of human PBMC were collected after culturing for 48 hours *ex vivo* with various cell and ^{19}F labeling agent concentrations. ^{19}F-NMR was performed to measure the average number of ^{19}F atoms per culture condition.

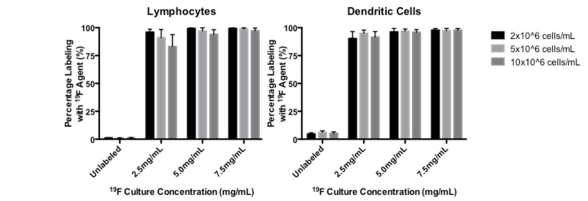

Figure 2. Human PBMC can efficiently be labeled with red fluorescent ^{19}F agent as assessed by flow cytometry. PBMC can be separated into specific cell lineages using cell surface markers such as CD11c for dendritic cells and CD3/CD19 for lymphocytes and individual cell lineage labeling efficiencies with a ^{19}F agent can be determined. All lineages label with high efficiency with the fluorescent ^{19}F after 48 hours in culture regardless of the cell or labeling agent concentrations employed.

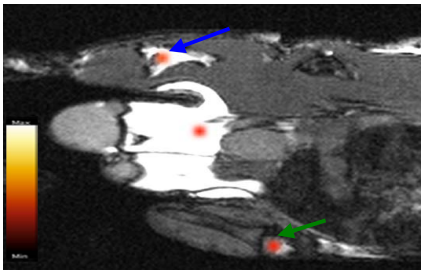

Figure 3. ^{19}F cellular MRI can be used to detect human ^{19}F-labeled cells in the popliteal lymph nodes of a nude mouse 48 hours following injection. ^{19}F/^{1}H MRI overlay images were collected 48 hours post footpad injection of $3x10^6$ PBMC labeled with a ^{19}F agent (5.0mg/mL) for 2 days and treated with human GM-CSF (20ng/mL) during the last 24 hours of culture. The ^{19}F signal, rendered in "hot-iron" colour scale, was able to detect **$1.01 \pm 0.88 \times 10^5$ ^{19}F-labeled cells** (**blue** arrow) in the left popliteal lymph node and **$1.29 \pm 0.3 \times 10^5$ ^{19}F-labeled cells** (**green** arrow) in the right popliteal lymph node, which was pretreated 3 hours prior to footpad injection with 300ng human IL-1β to investigate if this can improve migration.