Biphasic clearance of depot vaccine antigen and substrate visualized using SPIO MRI

Kim Brewer[1,2], Kerry Lake[3], Nicole Pelot[3,4], Drew DeBay[3], Andrea Penwell[1], Genevieve Weir[1], Marc Mansour[1], and Chris Bowen[2,3]

[1]*Immunovaccine Inc., Halifax, NS, Canada,* [2]*School of Biomedical Engineering, Dalhousie University, Halifax, NS, Canada,* [3]*Institute for Biodiagnostics (Atlantic), National Research Council of Canada, Halifax, NS, Canada,* [4]*Electrical Engineering, Dalhousie University, Halifax, NS, Canada*

Introduction: Immunotherapies are a rapidly growing class of anti-cancer therapies aimed at enhancing the patient's own immune response. DepoVax™ is a novel liposome-in-oil-based vaccine platform developed by Immunovaccine Inc. that uses tumor-associated antigens (TAA) encapsulated in liposomes and suspended in oil. The oil acts as an adjuvant that greatly increases the potency of the vaccine and elicits a strong cytotoxic T-cell response [1-3]. Previously, work has been done by our group using MRI for non-invasive longitudinal assessment of tumor growth and immune response via the monitoring of volumetric changes in lymph nodes (LNs) adjacent (i.e. the inguinal LNs) or distant (the popliteal LNs) to the site of vaccination [4]. Due to its oil substrate, the DepoVax™ vaccine platform permits longitudinal visualization of the vaccine site on MRI. However, this does not reveal any information about the clearance time of the individual vaccine components, particularly the TAAs. By attaching superparamagnetic iron oxide (SPIO) to the TAA and then encapsulating it in liposomes, it is possible to visualize the biodistribution of the TAA over time and evaluate whether there is truly a slow clearance of the antigen from the depot site, resulting in a potentiated immune response. In this study, mice underwent a C3 (HPV16 tumor model) challenge to evaluate the longitudinal clearance of the DepoVax™ vaccine components using SPIO conjugated to the TAA or associated with the lipid.

Methods: 20 female C57BL/6 mice (4-6 weeks old) underwent C3 tumor cell implantation on Day 0, with $5x10^5$ cells implanted subcutaneously (s.c.) into the left flank. On Day 5 post-implantation, mice received either i) DepoVax™ with 5µg R9F (n=5), ii) DepoVax™ with 5µg SPIO-R9F (n=5), iii) DepoVax™ containing SPIO-lipid with 5ug R9F (n=5), or iv) PBS control injection (n=5). Vaccine formulations were delivered via a single 50µL s.c. contralateral immunization (right flank). MRI scans were performed between Days 5-8 and then weekly for 6 weeks to evaluate tumor progression/eradication as well as lymphatic response. Baseline scans were also performed prior to tumor challenge (Day -8) to allow proper comparison of anatomical structures, for a total of 7 MRI time points in the study. All data were acquired on a 3T magnet equipped with 21 cm ID gradient coil (Magnex Scientific, Oxford, UK) interfaced with a Varian DD Console (Varian Inc., Palo Alto, Ca). A 25mm ID quadrature transmit/receive RF coil (Doty Scientific, Col., SC), was used to image tumors, vaccination sites, and left & right inguinal lymph nodes simultaneously. Sagittal images were obtained using a 3D true-FISP (bSSFP) sequence (TR/TE = 8/4 ms, flip angle = 30°, 38.4x25.5x25.5 mm FOV with 256x170x170 matrix centred on the torso, $150\mu m^3$ isotropic resolution, 6 signal averages). Total scan time was 48 minutes per animal. LN volumetry was performed on each animal over the time course using a semi-automated segmentation algorithm (RView) [5].

Right and left inguinal lymph node volume was evaluated for every mouse image of adequate clarity from artefact, as well as tumor size and Depovax™ depot volume where applicable. For each segmented volume, the average pixel intensity was computed with RView and then scaled to the average pixel intensity of muscle tissue in the leg, which was used as a reference for SPIO-free tissue image intensity. Volumes were critically reviewed by a second party to identify any segmentations that required reevaluation. In order to appropriately account for varying iron concentration in sub-regions of the vaccine depot, a new formula based on two point estimates of transverse relaxation rate enhancement was developed to estimate iron content pixel-by-pixel

$$m_{FE} = V_{FE} \frac{1}{N_{ROI}} \sum_{ROI_{FE}} \ln\left(\frac{I_{DPV}}{I_{mus}} * \frac{I_{mus}}{I_{FE}}\right)$$

Where N_{ROI} was the number of pixels included in the segmented ROI, I_{FE} the intensity of an individual pixel with iron-labeled Depovax™, I_{mus} the measured muscle tissue intensity particular to the image being analyzed and I_{DPV}/I_{mus} a ratio between the intensities of unlabeled Depovax™ and muscle tissue which was constant across all images. Although the calculation for iron is not quantitative, it does allow longitudinal assessment of the changes.

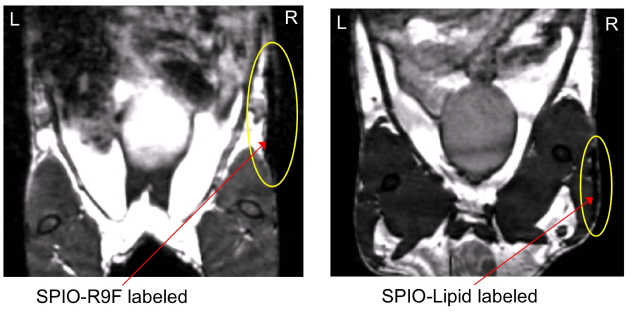

Figure 1 – MRI images showing vaccine sites labeled with either SPIO-R9F (left), or with lipid associated SPIO (right) in week 1 (immediately post-vaccination). Depot sites labeled with SPIO exhibit strong signal losses and are appear as black signal voids just under the skin.

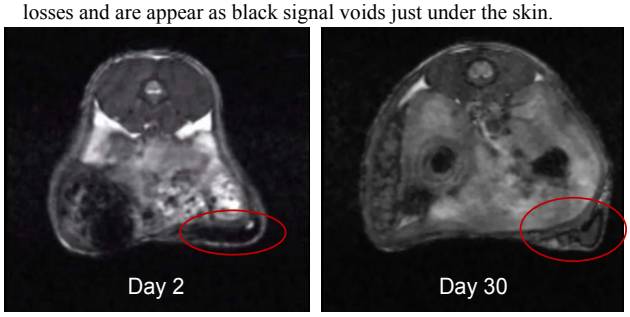

Figure 2 – MRI images showing vaccine site labelled with SPIO-R9F at two timepoints. The SPIO-R9F complex exhibited significant intensity changes over time, indicating antigen is being actively transported from the depot sites in a non-homogeneous manner.

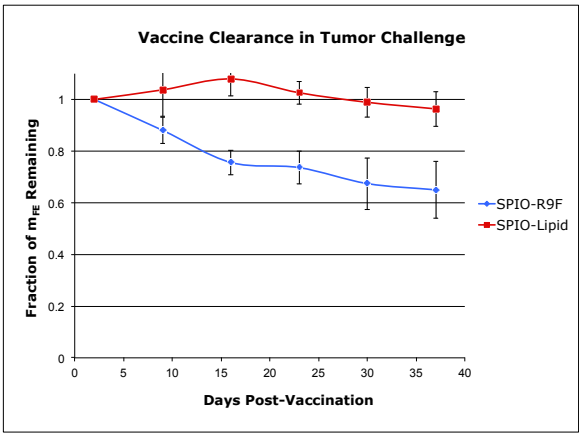

Figure 3 – Clearance curves of vaccine components over time. SPIO-R9F clears slowly but consistently with time, leaving approximately 60% of the antigen left after Day 35, whereas the amount of lipid remains constant over the course of the study. Error bars represent standard error.

Results: In the first week post-injection, the depot containing SPIO appeared as a uniform dark pouch with clearly defined boundaries between tissues for both SPIO-R9F and the SPIO-associated lipid (Figure 1). However, over the course of our study, the vaccine distributed to the surrounding tissue, and antigen was actively transported from the depot site, causing a greying of borders and pockets of differing intensity throughout for the SPIO-R9F images (Figure 2). When the mass of iron was calculated using the formula described above, the amount of iron was found to be relatively constant for the SPIO-associated lipid, indicating that the depot site was not losing significant amounts of oil over the course of the study. In contrast, the amount of iron for SPIO-R9F cleared slowly but consistently, decreasing by approximately 40% over 35 days (Figure 3).

Discussion and Conclusions: By conjugating SPIO to the TAA, we are able to visualize the biodistribution and clearance of the TAA over multiple weeks in response to a C3 tumor challenge. This supports the idea that DepoVax™ (as a depot vaccine) resides at the vaccine site for a prolonged period of time, increasing systemic exposure, resulting in an enhanced immune response.

References: [1] Daftarian et al.,Vaccine, 2006, [2] Mansour et al., JTM, 2008, [3] Karkada et al., J Immunother, 2010, [4] DeBay et al. Abstract #2722 ISMRM 2011 [5] http://rview.colin-studlholme.net