Measuring lymph node swelling using MRI to act as a biomarker for tumour suppression

Kim Brewer[1,2], Drew DeBay[3], Kerry Lake[3], Iulia Dude[3,4], 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]Physics, University of Waterloo, Waterloo, ON, Canada

Introduction: Traditional anti-cancer therapies such as chemotherapy and radiation therapy often lack the specificity and effectiveness necessary for complete tumor elimination. As such, a new class, known as immunotherapies, have been growing in popularity. Immunotherapies are aimed at potentiating the body's own immune response by either directly injecting pre-primed cytotoxic cells or via vaccination. Immunovaccine Inc. has developed a novel vaccine delivery system, DepoVax[TM], which uses the prolonged clearance latencies inherent in depot vaccines to enhance a potentiated immune response. DepoVax[TM] encapsulates the tumor-associated antigens (TAA) in liposomes, which are then suspended in oil. The oil acts as an adjuvant, which improves the potency of the peptides and aids in eliciting a strong cytotoxic T cell response [1-3]. Previously, our group used MRI to assess the efficacy of DepoVax[TM] in vivo by evaluating longitudinal volume changes in tumor and at inguinal and popliteal lymph nodes (LN) [4]. We found that DepoVax[TM] provided total tumor suppression in 6 out of 7 mice and that there was significant enlargement of the vaccine draining (inguinal) LN at weeks two and three post-vaccination. The current work was aimed at further investigating whether the increase in the draining lymph node volume could potentially be used as a biomarker for successful vaccination (i.e. a vaccination that had successful tumor suppression). We again used mice challenged with C3 tumor cells (HPV16 model) and vaccinated with either DepoVax[TM] containing the TAA or a buffered salt solution (PBS) control. To assess whether LN enlargement was specific to DepoVax[TM]-based vaccines, or if other types of immunotherapeutic vaccines elicit this response, a third group of mice were vaccinated with a commonly used [3] conventional oil emulsion vaccine containing the TAA, and compared to the PBS control. To evaluate the prospect of using this volumetric characterisation of immune response (right LN enlargement) as a biomarker/prognostic indicator of vaccine efficacy (accompanying tumor suppression), we performed LN volumetry to evaluate changes using receiver operating characteristic (ROC) curves.

Methods: Two different tumor challenge studies were performed, one with DepoVax[TM] and one with conventional emulsion (CE). A total of 46 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 vaccine formulations via a single 50μL s.c. contralateral immunization into the right flank. For the DepoVax[TM] challenge, injections contained either i) DepoVax[TM] with 5μg R9F (n=22), or ii) PBS injection (n=12) as control. For the CE challenge, mice received either i) conventional emulsion with 5μg R9F (n=9) or ii) PBS injection (n=3) as control. MRI scans were performed on Day 5 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 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 centered on the torso, 150μm³ isotropic resolution, 6 signal averages). Total scan time was 48 minutes per animal. Tumor and LN volumetry was performed on each animal over the 7-week time course using a semi-automated 3D segmentation algorithm (RView) [5].

Receiver operating curves (ROC) were calculated from volumetric estimates of vaccine-draining LN (right inguinal) for each week of the study. For ROC analysis, tumor suppression was defined as successful if the final tumor volume was less than the mean tumor volume of the unvaccinated group by one standard deviation (SD); i.e. < ~370mm³. The false positive and true positive rates were then calculated by varying the % increase over baseline of right lymph node volume (the ROC decision criteria) for each week. The false positive rate (FPR) was FP/(FP+TN) and the true positive rate was TP/(TP+FN).

Results and Discussion: LN volumetry revealed a marked enlargement in right (vaccine draining) inguinal LNs in mice immunized with either DepoVax[TM] (Figure 1) or conventional emulsion, compared to their unvaccinated PBS control counterparts. These volume increases were significant between 8 and 15 days post-vaccination, with observed volumetric increases ranging from 100-1000% in vaccine draining right inguinal LNs (Figure 1). These observations agree well with our previous reports [4] using a similar tumor challenge design.

ROC curves generated for both day 8 post-vaccination and day 15 post-vaccination, yielded a FPR of only ~5% and a TPR of ~80% (using ROC decision criteria of approximately 75% volumetric LN increase). These results were true for both mice groups vaccinated with DepoVax[TM] (Figure 2) or conventional emulsion (Figure 3).

Conclusions: These results indicate that volumetric lymph node changes in response to vaccination in nodes draining the injection site may have a powerful predictive value for indicating whether tumor suppression will occur. The ROC curves indicate that this potential diagnostic predictor of accuracy has impressive specificity and sensitivity. This would allow non-invasive diagnostic scans to be used to calculate a biomarker for testing the efficacy of immunotherapeutic vaccines in preclinical models.

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
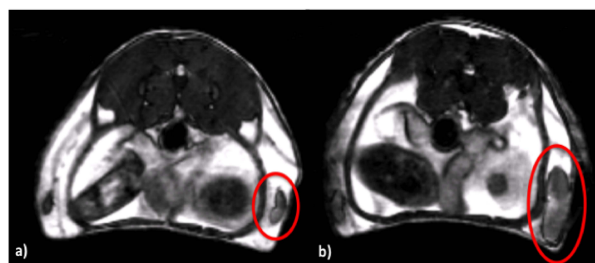
Figure 1 – Representative images of right inguinal LN enlargement (red circle). a) baseline and b) day 15 post-vaccination images reveal an approximate ~200% increase in LN volume for this Depovax[TM]-injected mouse.
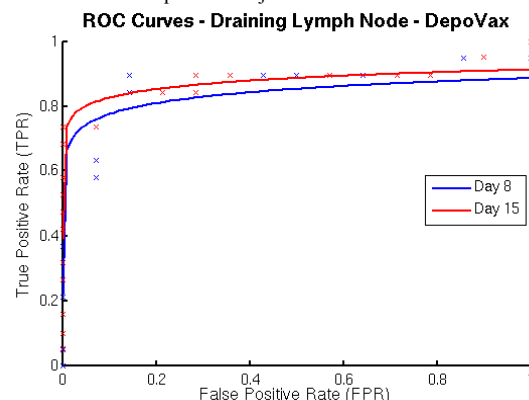

Figure 2 – Receiver operating curves for DepoVax[TM] on days 8 and 15 post-vaccination in tumor challenge.
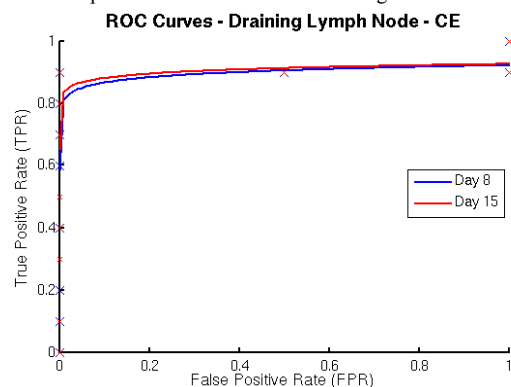

Figure 3 – Receiver operating curves for conventional emulsion (CE) on days 8 and 15 post-vaccination in tumor challenge.