MR Visualization of Depot Vaccines and Immune Activation for Cancer Therapies

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Introduction Depot vaccine formulations are unique in their ability to reside at the site of injection with prolonged latencies, enhancing the potentiation of immune response. Immunovaccine Inc. has developed a novel liposome-in-oil-based vaccine platform, DepoVax™, in which tumor associated antigens (TAA) are encapsulated in liposomes and suspended in oil. The oil acts as an adjuvant that greatly increases the potency of peptide-based cancer vaccines and elicits a strong cytotoxic T-cell response [1-3]. Conveniently, the oil substrate allows MRI visualization of DepoVax™ at the depot site, and permits non-invasive longitudinal assessment of the clearance time and biodistribution of the vaccine over weeks. Additionally, MRI can observe evidence of immune response through volumetric changes in lymph nodes (LNs), either systemic, or local to the vaccination or tumor implantation sites. The combined visualization of vaccine biodistribution over time, surrogate indicators of immune response measured in lymph nodes, and the resulting tumor elimination, makes MRI a powerful tool permitting non-invasive longitudinal investigation of the efficacy and mechanism of action for new cancer vaccines. In this study, mice underwent a C3 (HPV16 tumor model) tumor challenge to evaluate the therapeutic efficacy of Depovax™ and characterize the immune response *in vivo*. For the first time using MRI, elimination of established tumors was observed in 6 of 7 vaccinated mice, with corresponding volumetric changes in the inguinal LN draining the depot vaccine. As significantly, no non-specific immune reaction to the oil adjuvant in the vehicle control (free of TAA) was observed in LNs, since adjuvants historically have problems in this regard. The information provided by MRI in this study is critical for evaluating the biodistribution of novel depot vaccines like DepoVax™, and studying the mechanism of action and presence of appropriate immune response.

Methods 21 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™ (n=7), ii) a peptide-free vehicle control vaccine (n=7) or iii) no injection (n=7) as an unvaccinated control. Vaccine formulations were delivered via single 50µL s.c. contralateral immunization (right flank). MRI scans were performed on Day 5 and then weekly for 5 weeks to evaluate tumor progression/eradication as well as immune response. Baseline scans were also performed prior to tumor challenge (Day -8) to allow proper comparison of anatomical structures, for a total of 6 MRI time points in the study. All scans were performed 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 and poplitial LNs 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, 150µm^3 isotropic resolution, 6 signal averages). Total scan time was 48 minutes per animal. Tumor and LN volumetry was performed on each animal over the time course using a semi-automated segmentation algorithm (RView) [4]. Intra- and inter-group statistical comparisons were made via repeated measures ANOVA and 2-way ANOVA, respectively.

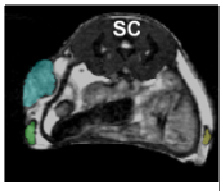

Figure 1. Example segmentation showing tumor (blue), left and right inguinal lymph nodes (green & yellow) SC = spinal cord.

Results & Discussion MRI revealed 100% tumor eradication in 6/7 mice in the DepoVax™ group by week 4, while all mice in non-Depovax™ groups continued to bear tumors to the end of the study (Figure 2, 3A). The one Depovax™ mouse that maintained a small tumor mass throughout the study displayed poor visualization of the injection site, very inconsistent with that of other mice within its group, casting suspicion on the injection accuracy in this case and suggesting a role for injection monitoring with MRI. A statistically significant tumor progression between weeks 3-5 in the control group ($p<0.0001$) and between weeks 4 and 5 in the vehicle control group ($p=0.0132$) was seen. Inter-group comparison at week 5 indicated statistically greater tumor volumes in both control ($p=0.0004$) and vehicle control ($p=0.0153$) groups compared to the Depovax™ group. Direct *in vivo* visualization of Depovax™ persistence over this longitudinal study (green arrows, Figure 2) corroborates earlier, indirect evidence suggesting a time scale of weeks to months for depot clearance. Immune response facilitated by Depovax™ is seen via enlargement of right inguinal LN (main vaccine draining site, suggestive of effector T-cell upregulation) within 1 week of injection, with a 2-fold increase in volume and sustained enlargement over the duration of the study (Figure 3B). Vehicle control did not elicit the same response, with no statistically significant changes in right LN volume between weeks proceeding vaccination. Inter-group comparisons show that right inguinal LNs in the Depovax™ group were 1.6 times larger than in the vehicle control group at the half-way point of the study. This suggests enhancement of vaccine induced immune activity in the presence of Depovax™ that cannot be attributed to the vehicle control alone.

Conclusions We are able to visualize Depovax™ and its elicited immune response for multiple weeks. A clear correlation with right LN enlargement (proxy for immune response to vaccine) and tumor eradication has been shown.

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

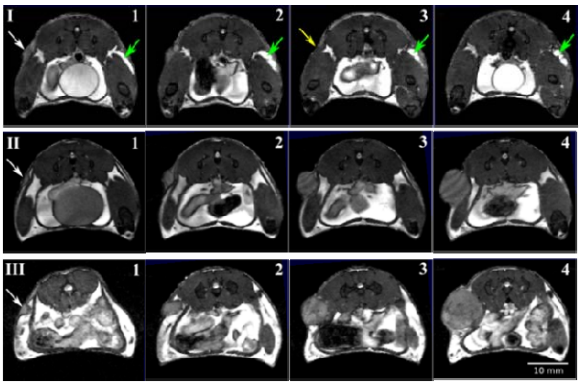

Figure 2. Image time series of Depovax™ (I), vehicle control (II) and control (III) mice over first 4 weeks (columns 1-4) of tumor challenge. White arrows show tumor implant sites, green arrows show depot injection sites. Yellow arrow (Depovax™ group, week 3) indicates complete tumor eradication while clear tumor progression is seen in the non-Depovax™ groups.

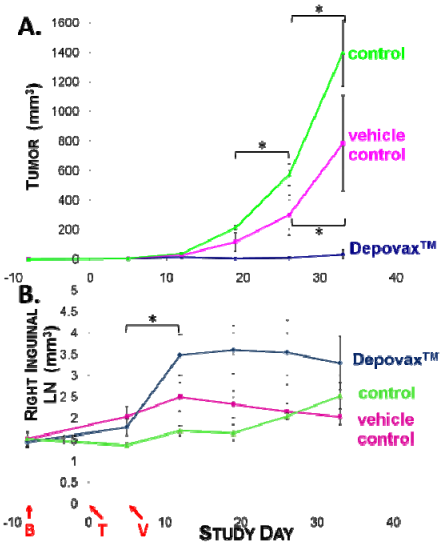

Figure 3. A. Tumor growth curves showing mean tumor volume (±SE) for each group at each MRI timepoint. **B.** Right inguinal LN volume (±SE) at each MRI timepoint for Depovax™, control and vehicle control groups. B=baseline, T=tumor implantation, V=vaccination.