Multi-Modal Imaging to Evaluate the Effects of Novel TLR Agonist Adjuvants in Vaccine-Mediated Tumor Immunity

Deepak K Kadayakkara[1], Hyam I Levitsky[1], and Jeff W. M. Bulte[2,3]

[1]*Department of Oncology, Johns Hopkins School of Medicine, Baltimore, Maryland, United States,* [2]*Cellular Imaging Section, Institute for Cell Engineering, Johns Hopkins School of Medicine, Baltimore, Maryland, United States,* [3]*MRI research division, Johns Hopkins School of Medicine, Baltimore, Maryland, United States*

Target audience: This study is intended for MRI researchers interested in cell tracking, and scientists and clinicians interested in developing cancer vaccine adjuvants and understanding the underlying immunological mechanisms.

Purpose: The goal of this work is to non-invasively study the immunological mechanisms of cancer vaccines, and to evaluate the role of novel adjuvants in vaccine-mediated tumor protection. We evaluated Toll-like receptor (TLR) agonists as adjuvants in a GVAX cancer vaccine platform (1) consisting of irradiated tumor cells secreting granulocyte monocyte colony stimulating factor (GM-CSF). We used a SPIO-labeled tumor vaccine (or "magnetovaccine" (2)) in combination with adjuvants and quantified antigen delivery to local lymph nodes by MRI. Effector T cell responses were studied using antigen-specific transgenic T cells and BLI.

Methods: *Cell labeling and vaccination*: B16 melanoma cells stably expressing ovalbumin were irradiated and labeled with Molday Ion evergreen (Biopal, MA). Labeled cells were mixed with irradiated B78H1GM cells secreting GM-CSF to form a GVAX vaccine. TLR-4 and TLR-7 agonist adjuvants (Immune design, WA) were mixed with the GVAX vaccine, which was injected in the hind footpad. *MRI*: MRI was performed using a Bruker 11.7 T horizontal bore microimaging system equipped with a 23 mm volume coil. Images were acquired using a T2*-weighted GRE sequence at the level of the draining popliteal lymph nodes. Quantification was done by counting the dark pixels below a threshold in an ROI encompassing the lymph node as previously described (2). *BLI:* Transgenic $CD8^+$ T cells specific for ovalbumin and expressing luciferase were harvested from OT1-Luci transgenic mice, and injected one day prior to vaccination. The mice were imaged serially using an IVIS optical system (Caliper, MA). *Tumor therapy model*: A tumor challenge was given by injecting $1x10^5$ B16 cells subcutaneously. Mice were vaccinated on day 3, 10, and 17. Tumor size was monitored for 1 month.

Results: MRI showed that adjuvants decreased antigen delivery to lymph nodes, which was validated by flow cytometry (Fig. 1A-F). BLI showed T cells expanding in both GVAX alone and adjuvant treated mice (Fig. 1G). T cell expansion and accumulation was predominant in the draining popliteal lymph node in GVAX-treated mice. In adjuvant-treated mice, the signal was predominant in the spleen, liver and the vaccinated footpad, suggesting an extranodal expansion of ovalbumin-specific T cells. Tumor size measurements showed a better tumor therapeutic effect in the presence of adjuvants (Fig. 1H).

Discussion: Contrary to current expectations, TLR agonists reduced antigen capture and delivery to the lymph nodes. However, they induce a potent immune response and better tumor therapeutic effect. This study points to a novel mechanism of T cell priming and activation in the presence of TLR agonist adjuvants.

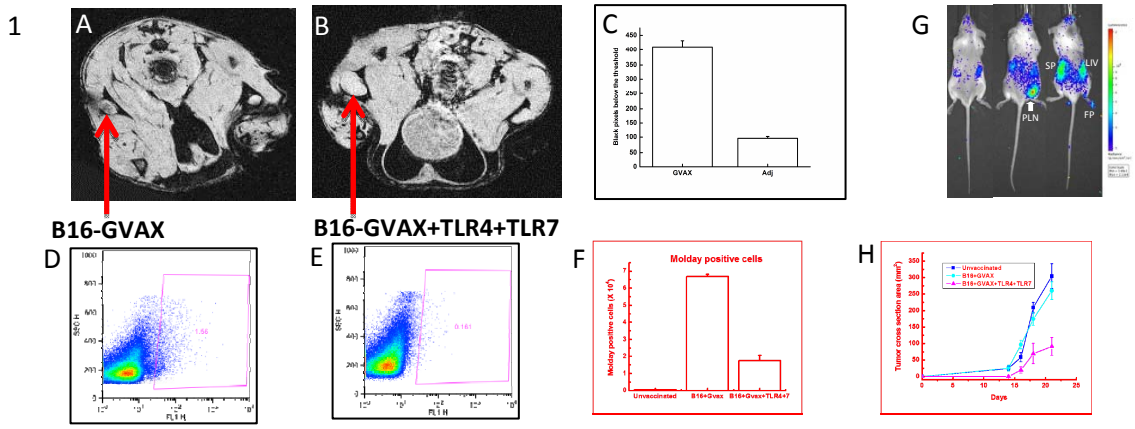

Figure 1: (**A,B**) Axial sections through popliteal lymph nodes for GVAX only and GVAX in combination with TLR agonist adjuvants on day 4 following vaccination. Dark pixels in the center of the node represent antigen-presenting cells containing SPIO. (**C**) Quantification of back pixels from Figs. 1 A and B. (**D,E**) Flow cytometry data validating MRI data for GVAX- and adjuvant-treated mice, respectively. (**F**) Quantification of the flow cytometry data containing Molday Evergreen-positive cells. (**G**) BLI of ovalbumin-specific $CD8^+$ T cells. Unvaccinated mice (left) do not show T cell expansion on day 4 following vaccination. Strong accumulation of T cells are seen in the draining popliteal node (PLN) in the GVAX-treated mice (middle), and strong accumulation is seen in spleen (SP), liver (LIV) and footpad (FP) in adjuvant treated mice (right) suggesting different patterns of T cell expansion and accumulation. (**H**) Tumor therapy experiments show better therapeutic effects with adjuvants in combination with GVAX (n=8 per group, $p<0.05$).

References: 1) Borrello I *et al. Hum gen ther* 1999;10(12):1983-91, 2) Long CM *et al.* Canc Res 2009;69(7):3180-7.

This work is supported by NIH U54 CA151838.