MRI-monitored Intra-arterial Delivery of SPIO-Labeled Natural Killer Cells to Hepatocellular Carcinoma

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Introduction: Image-guided, transcatheter, liver-directed therapies such as chemoembolization and radioembolization are widely used for the targeted treatment of hepatocellular carcinoma (HCC). Adoptive immunotherapy with natural killer (NK) lymphocytes is another promising approach for the treatment of HCC particularly because NKs can migrate to tumors and infiltrate the tumor body; however, conventional systemic IV administration may lead to an insufficient NK dose reaching the tumor. Quantification of NK dose delivered to tumors may be critical to optimize procedures or to predict response. There exists a critical need for high-resolution imaging methods to allow accurate visualization of *in vivo* NK delivery. We hypothesize that: a) transcatheter IA infusion allows for targeted NK delivery to HCC and b) iron oxide labeling methods allow for visualization and quantification of IA NK delivery with MRI.

Methods: 4.0×10^6 NK-92 cells (ATCC; Manassas, VA) were labeled overnight with 10 pg/cell of Texas Red iron oxide nanoparticles (GENOVIS AB, Sweden) using 4.5 mg/ml protamine sulfate as a transfection agent. Cell viability was measured before and after labeling using an automated cell counter. Labeling efficacy was measured using fluorescence microscopy with DAPI and Prussian blue iron staining. Phantom studies were performed using labeled NK cell concentrations from 10^6 to 10^4 cells/ml uniformly suspended in 1.0% agarose. Multi-echo GRE sequences implemented on a 7.0T MRI scanner (Bruker, Billerica, MA) with off-line MATLAB processing were used to generate T2* maps. With IACUC approval, 6 Sprague-Dawley rats were implanted with 4.0×10^6 McA-RH7777 HCC cells divided between the left lateral and median lobes to simulate a metastatic tumor. After 8 days of tumor growth, a 24G catheter (Terumo Medical Co., Somerset, NJ) was placed in the proper hepatic artery via laparotomy, and digital subtraction angiography (DSA) confirmed placement; the gastroduodenal artery was ligated permanently distal to the site of catheter puncture, and the common hepatic artery was temporarily ligated to control bleeding. TSE and GRE scans were performed before and after IA infusion of labeled NK cells. T2* measurements of tumor and normal liver were each compared pre- and post-infusion using a paired t-test. Rats were sacrificed and livers harvested; Prussian blue staining of tissue sections was used to identify labeled NK cell delivery. Percentages of cells within representative high-powered (20×) fields (%HPF) were used to quantify the amount of Prussian blue stained NK cells; tumor and normal liver tissues were compared by unpaired t-test.

Results: NK cell viability was >90% before and after labeling. Labeling efficacy was >95% (**Fig 1**). Phantom studies indicated a R2* relaxivity of 9.0×10^{-4} sec^{-1}/(cells/ml) for labeled cells (**Fig 2**). DSA confirmed successful catheter placement in each animal (**Fig 3**). Transcatheter NK infusions led to significant reductions in tumor T2* (mean±SD: pre 13.5±0.2 msec, post 6.4±2.6 msec, p=0.02) but no significant reductions in normal liver T2* (mean±SD: pre 5.2±0.4 msec, post 6.1±0.9 msec, p=0.10) during intra-procedural MRI scans (**Figs 4** and **5**). NK deposition attenuated tissue signal intensity within T2* scans. Histologic %HPF measurements were significantly higher in tumor (0.81%) than in surrounding normal liver tissues (0.08%) (p<0.01) (**Fig 6**).

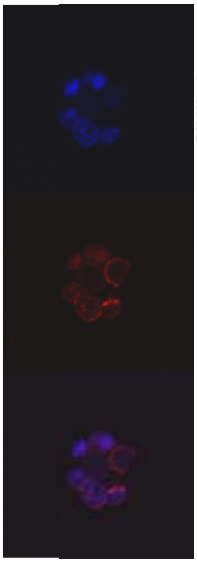

Fig 1. 40× view of NK lymphocytes labeled with Texas Red iron oxide nanoparticles. Blue DAPI-stained nuclei (**top**) surrounded by red fluorescent particles (**middle**) in cytoplasm; merged image at **bottom**.

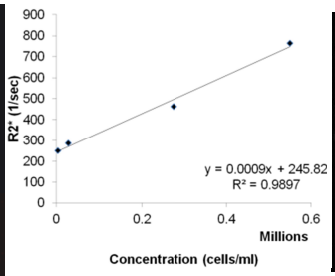

Fig 2. R2* relaxivity curve. Phantom studies generated R2* vs. known labeled cell concentrations. R2* relaxivity calculated to be 9.0×10^{-4} sec^{-1}/(cells/ml) by linear regression.

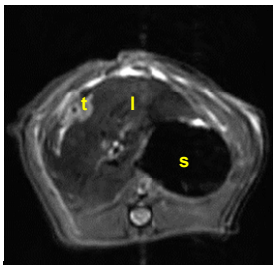

Fig 4. MRI TSE anatomical image. Transverse slice with largest tumor diameter shown: **t = tumor; l = liver; s = stomach.**

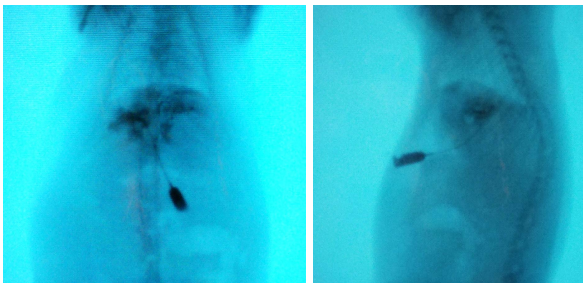

Fig 3. DSA of hepatic artery branches. Coronal view (**left**); sagittal view (**right**). Catheter was placed in the proper hepatic artery and contrast injected – note significant enhancement of the large median and left lateral lobes of the liver.

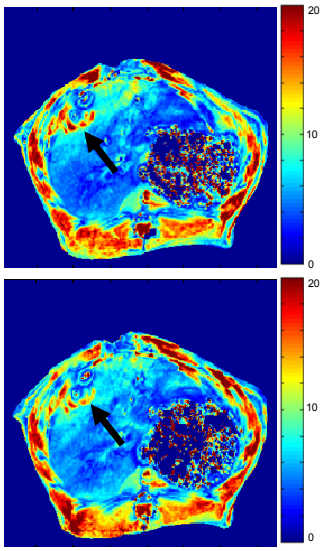

Fig 5. MRI T2* maps. Transverse slices with largest tumor diameter shown. Corresponding slice to TSE image in Fig 4. **Black arrow** indicates tumor. Colorbar gives T2* values in msec. Pre-infusion T2* map (**top**) indicates larger area of higher T2* values in the tumor compared to post-infusion T2* map (**bottom**); normal liver parenchyma remains unchanged.

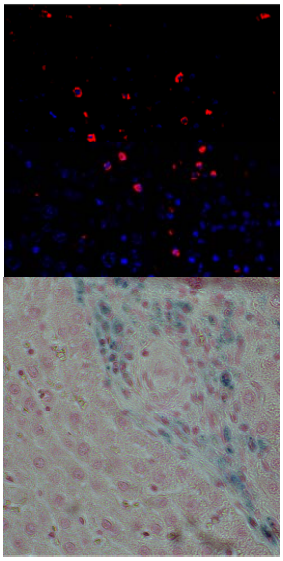

Fig 6. Microscopy. 40× fluorescence microscopy (**top**): NK cells (blue nuclei surrounded by Texas Red nanoparticles) infiltrating tumor tissue (blue nuclei only). 20× light microscopy (**bottom**): Prussian blue iron stain demonstrates labeled NK cells in tumor. Fluorescence and light microscopy views are of corresponding sections. Majority of NK cells were located in the vascular rim of the tumor compared to in the tumor core or in the normal liver parenchyma.

Conclusions: Transcatheter infusion permitted selective delivery of NK cells to HCC. The intra-hepatic distribution of iron oxide labeled NK cells was quantitatively visualized with MRI. Increased sample sizes are required to demonstrate a strong correlation between histological NK cell and MRI measurements. In the future, we will perform studies to assess relationships between therapeutic outcomes and the delivered NK dose, comparing IV and IA administration routes. Clinicians could one day use these methods to adjust patient-specific therapeutic regimens during adoptive immunotherapies for the treatment of HCC. *In vivo* quantification of the delivery of therapeutic agents is a powerful and exciting concept that offers great potential to address many unmet needs in both pre-clinical and translational research settings.