In-vivo tracking of ^{19}F-labeled natural killer cells with MRI in lymphoid tumor model

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Target Audience: Researchers and clinicians interested in immunotherapeutic cell-tracking with ^{19}F MRI.

Purpose: Tumor-specific immunotherapy is emerging as a novel treatment paradigm for patients with metastatic tumors incurable with conventional therapies. Natural killer (NK) cells are important innate immune effector cells shown to have anti-tumor effects against hematological and non-hematological cancers [1-3]. Often, lack of knowledge on trafficking patterns and NK cell biology limits the efficacy of adoptively transferred NK cell's anti-tumor response and clinically-approved reagents are not yet available. Labeling of various immune cells in-vivo with fluorinated compounds has allowed for detection and cell-tracking via ^{19}F MRI [4-5], but to date NK cells have not been studied with this approach. The goals of this study are to determine the trafficking pattern of ^{19}F-labeled NK cells in-vivo and how they mediate their anti-tumor effects in a humanized mouse model of pediatric cancers.

Methods: ***Animals:*** Two healthy mice and one lymphoma tumor-bearing mouse were used for this study. Mice were anesthetized with either 1.5% isoflurane (Iso) or ketamine/xylazine (Ket/Xyla) (2mg/10g Ket, 0.2mg/10g Xyla), monitored with a respiration pad and maintained at 36°C using a warm-air blower. ***Cells:*** Human NK (hNK) and mouse NK cells isolated from healthy donor peripheral blood mononuclear cells were cultured ex-vivo for 2 weeks. Mouse NK cells were initially used due to ease of availability. NK cells were incubated for 24 hours in a commercially available perfluoropolyether (PFPE) tracer agent (Celsense Inc., Pittsburgh PA). hNK cells were subsequently washed and injected intravenously into immunodeficient mice. ***MR:*** NMR was performed on a 9.4T Varian UI-400 (Agilent Technologies, Santa Clara, CA) spectrometer to verify successful uptake of the PFPE agent into NK cells. Imaging was performed on a 4.7T Varian small animal MRI system using a volume quadrature coil tunable to ^{19}F (187.9MHz). Coronal ^{19}F images were acquired using a spin-echo sequence (1.1x1.1mm^2 in-plane resolution, 2mm slice thickness, 16 echoes, 40 averages, ~42 minutes total scan time). A ^{19}F reference vial (2.3·10^{16} ^{19}F spins/mm^3) was placed contralateral to the tumor for in-vivo quantification.T_1-weighted GRE ^{1}H images were also acquired to visualize anatomy.

Results and Discussion: A dose-dependent response of ^{19}F signal was shown in mouse NK cells in duplicate (**Fig. 1**). NMR spectra of the separated cells and its supernatant confirmed that ^{19}F signal originated from within the hNK cells, rather than the supernatant (**Fig. 2**). ^{19}F-labeled hNK cells were then injected into an immune deficient mouse and ^{19}F signal accumulation was noted intra-tumor and on the periphery of the tumor (arrows in **Fig 3**). However, ^{19}F background signal contamination was observed (arrowheads in **Fig. 3**) due to Iso anesthesia. Iso was compared to Ket/Xyla to determine the extent to which Iso impeded interpretation of ^{19}F images. The results point to a strong background ^{19}F signal from Iso contamination (**Fig. 4**).

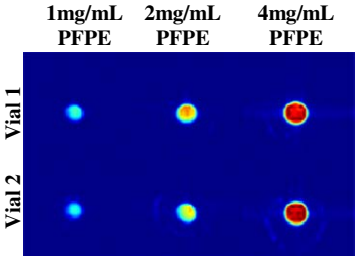

Fig. 1: *MR images showing dose-dependent signal of ^{19}F-labeled mouse NK cells.*

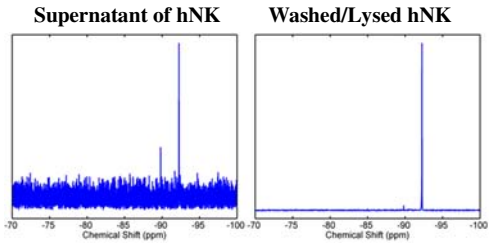

Fig. 2: *^{19}F NMR spectroscopy of PFPE in (left) supernatant of hNK cells, and (right) hNK cells post-washing and lysis.*

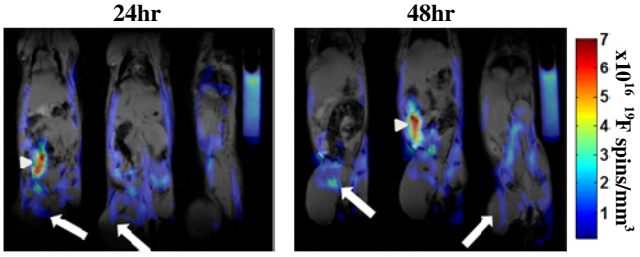

Fig. 3: *^{19}F signal in a lymphoid tumor (arrows) bearing mouse 24 hours (left) and 48 hours (right) following hNK injection. ^{19}F signal contamination noted (arrowheads).*

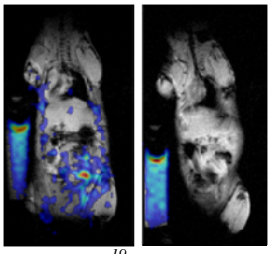

Fig. 4: *^{19}F signal in mice anesthetized with (L) Iso and (R) Ket.*

Conclusions: ^{19}F labeling of natural killer cells has been confirmed in-vitro, both with imaging and spectroscopic analysis. hNK infusion into a lymphoid tumor-bearing mouse showed that hNK cells can be successfully detected in-vivo, but there is some variance in the ^{19}F signal in the region of tumor. Given confounding fluorine signal contamination from Iso gas anesthesia, future studies will utilize Ket/Xyla anesthesia to eliminate background fluorine. NK cells can be labeled with ^{19}F and detected in-vitro and in-vivo, but background fluorine signals can interfere with detection by MRI.

References: [1] Miller et al. *Blood*. 2005. 105(8):3051-3057. [2] Ruggeri et al. *Science*. 2002. 295(5562):2097-100. [3] Murphy et al. *Imm. Rev.* 2001. 181(1): 279-289. [4] Srinivas et al. *MRM*. 2007. 58:725-734. [5] Helfer et al. *Cytotherapy*. 2010. 12:238-250.