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 efficacy of adoptively transferred NK cell's anti-tumor response and clinically-approved reagents are not yet available. Labeling of hematological and non-hematological cancers [1-3]. Often, lack of knowledge on trafficking patterns and NK cell biology limits the purpose of this study is 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 (1.1x1.1mm² in-plane resolution, 2mm slice thickness, 16 echoes, 40 averages, ~42 minutes total scan time). A $^{19}$F reference vial (2.3x10¹⁶ $^{19}$F spins/mm³) was placed contralateral to the tumor for in-vivo quantification. T₁-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).

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.