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Poster # 381

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Macrophage tracking using multi-modality 3D imaging in xenografts

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Abstract

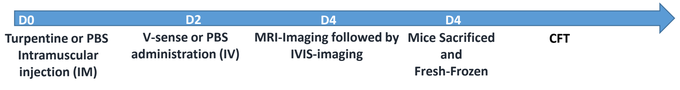
A common approach to immunotherapy is to promote the accumulation of specific bone marrow–derived myeloid cells into tumors for anti-tumor activity. While the pro- and anti-tumor effects of differentiated myeloid cells are complex, several novel therapeutic approaches are focused on recruiting macrophages to tumors and transforming their pro-tumor phenotypes of macrophages to anti-tumor phenotypes.

Research efforts to better understand and manipulate this type of macrophage biology is critical for developing and testing therapeutics. These experimental models commonly utilize imaging approaches, such as *in vivo* molecular imaging or histological approaches to visualize macrophages in the context of tumors. These imaging modalities provide different advantages in their ability to characterize macrophage distribution, tissue localization and kinetics. *In vivo* imaging, such as MRI and fluorescence provide strong temporal insights and general distribution in target tissues, but are limited by sensitivity and resolution. Fluorescence microscopy and histology provide substantially higher resolution and sensitivity regarding tumor-associated macrophages (TAM) localization and cellular morphology, but lack temporal resolution and only samples a small portion of target tissue. Cryofluorescence tomography (CFT) is an attractive new modality in which an entire animal or tissue can be imaged in 3D with high-resolution and increased sensitivity compared to nuclear *in vivo* imaging approaches.

Materials & Methods

We designed a multi-modality imaging study enabling the utilization of MRI, FLI and CFT for *in vivo* and *ex vivo* visualization of macrophages in tumor bearing animals. In this study, mice bearing xenografts were administered V-Sense (VS-1000H NIR, Celsense, Inc., Pittsburgh, PA), a perfluorocarbon emulsion containing fluorine-19 (^{19}F) and a NIR fluorophore. When administered intravenously (IV), V-Sense is preferentially taken up by cells of the recticuloendothlium system, including Kupffer cells and macrophages, especially in inflamed tissues (e.g. tumors), thus enabling imaging using both MRI and fluorescence imaging. Immediately following MRI, animals underwent FLI and were then frozen for CFT.

Study Timeline



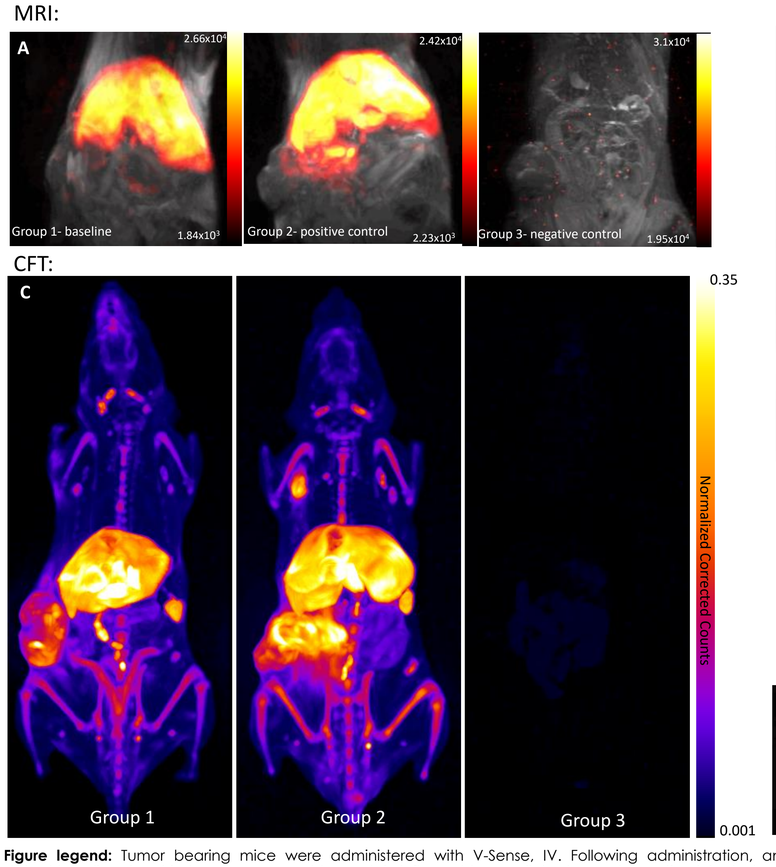


Figure legend: Tumor bearing mice were administered with V-Sense, IV. Following administration, animals were imaged using MRI to evaluate the ability to detect baseline signal in tumor compared to acutely inflamed tumors (intra-tumor, turpentine-injected animal as a positive control) (A). Liver uptake is visible in both V-sense administered animals, no uptake was detected in baseline tumors and little uptake was visible in the positive control animal (A). Following MRI, animals were imaged using IVIS to evaluate tumor uptake (B). Cy7 signal was detected in both baseline tumor and the positive control tumor, with higher uptake visible in the acutely inflamed tumor (B). Following IVIS, animals were taken down and immediately frozen whole and imaged using Cyofluorescence tomography (C). Reconstructed 3D images demonstrate strong, clear V-Sense signal in both liver and tumor, in agreement with IVIS and MRI, but also detects signal in bone marrow and lymph nodes (C). When compared to MRI, CFT was more sensitive in detecting baseline V-Sense signal, and provided higher resolution (D)

Results

^{19}F signal was evident in mouse tumors following acute inflammation, but not detected in the non-inflamed tumors, indicative for macrophage recruitment. However, when imaged using both FLI and CFT, V-Sense signal was detected in tumors of both groups (1 and 2). CFT proved to be the most sensitive, revealing V-Sense signal not only in the tumor and liver but also in several other organs, such as lymph nodes and bone marrow that were undetected with MRI or FLI. The biodistribution of the probe is consistent with previous *ex vivo* tissue studies (Ahrens, et al. 2011). No ^{19}F signal was detected in the control group (group 3- PBS, vehicle).

Discussion

We conducted a multi-modality imaging study using V-Sense, a perfluorocarbon compound that is dual labeled with ^{19}F and Cy7, and was shown to preferentially be uptaken by macrophages *in vivo* (Khurana et al. 2018). The results of this study demonstrate the added value of a multi-modality imaging approach using V-Sense, in which low resolution *in vivo* imaging and high-resolution 3D CFT imaging can complement each other, providing rich, multi-resolution layers of immunological information in the same subjects. Here, we provide a POC using V-Sense, a ^{19}F, Cy7 dual labeled compound. MRI and IVIS were conducted to gain spatio-temporal information, while CFT was conducted to complement the low resolution of MR and IVIS and to increase the sensitivity.

References

Ahrens et al. *BioTechniques.* 2011 April, 50:229-234

Khurana et al. *Magn Reson Med.* 2018 April, 79(4):1972-1980