

# Detection of Lung Metastases using Hyperpolarized $^3\text{He}$ MRI and Targeted Magnetic Nanoparticles – Histologic Validation and Detection Limits

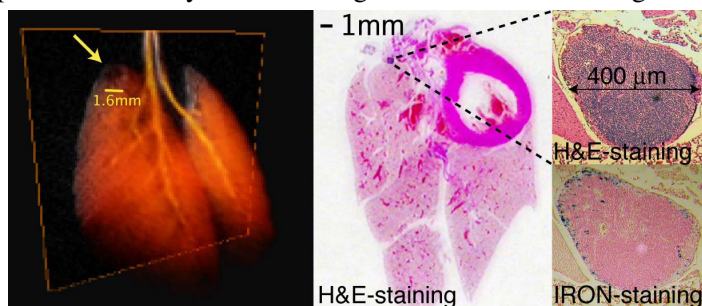
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**Introduction:** In cancer patients, lung metastases are highly correlated with poor treatment outcome. Hence early and sensitive detection of metastatic cancer cells in the lung is a critical objective. Recently we demonstrated a novel approach to this problem using hyperpolarized (HP)  $^3\text{He}$  MRI in combination with targeted iron oxide nanoparticles functionalized with lutenizing hormone releasing hormone (LHRH-SPIONs) [1, 2]. We now build on this initial work by applying the method to a larger number of animals (N=8) and two different models of human cancer (breast and prostate adenocarcinoma) that are well known to produce lung metastases. HP  $^3\text{He}$  lung images were directly compared to lung histology to validate that regions of  $^3\text{He}$  signal loss were the result of metastatic nodules that were targeted by the LHRH-SPIONs and to gain insights into the detection limits of the method.

**Methods:** Female and male BALB/c nude mice were inoculated at 9 weeks of age with MDA-MB-231 human adenocarcinoma and PC-3 human prostate cancer cells, respectively. After approximately 60 days, the mice received an IP injection of 100 mg/kg LHRH-SPIONs. The mice were then imaged 48hr later using a 3D radial gradient echo acquisition with an isotropic resolution of 156 $\mu\text{m}$ /pixel. Images were acquired at TE=1ms to show regional ventilation and TE=4ms for  $T2^*$  sensitivity. After imaging, the mice were sacrificed and the lungs fixed for histology. Histology slides were stained with H&E for histopathology examination and Prussian Blue to visualize iron from accumulated SPIONs.

**Results and Discussion:** In 7 of 8 mice,  $^3\text{He}$  images acquired at TE=4 ms showed remarkable signal voids. In 6 of the 7 mice, the signal voids were located on the upper right cranial lobe, while in one of them the void extended over the entire right lobe. These voids are either not apparent or remarkably smaller on the  $^3\text{He}$  images acquired at TE=1 ms, suggesting that the signal void is a  $T2^*$  effect rather than a ventilation deficit. Histopathology examinations revealed that the signal voids for all the animals arose from metastatic nodules that had been successfully targeted by the contrast agent. The smallest nodule detected in this study was 300 micrometer in diameter, which resulted in a 1.2 mm in diameter signal void on the TE=4ms images. In general, the region of signal loss was found to be 4-5 times bigger than the effective tumor size and it increases with echo time. Detailed examination of the histology slides (LHRH receptor assay) did reveal several small metastatic nodules (less than 10 micrometers in diameter) that did not reach a sufficient iron concentration to produce a readily visualized signal loss in the  $^3\text{He}$  images.



Top: 3D reconstruction of the lungs from a prostate tumor bearing mouse after the injection of LHRH- SPIONs. A signal loss of about 1.6 mm is observable in the long echo time image, in the right cranial lobe. Histological examination revealed a metastatic tumor of small size (400 micrometers) that had been targeted by the SPION-Particles.

Right: Examples of HP  $^3\text{He}$  images on a healthy mouse and on tumor models showing a metastatic nodule in the right cranial lobe

**Conclusions:** Hyperpolarized  $^3\text{He}$  MRI has been used to detect lung metastases targeted with SPION particles with high resolution and specificity in two different human cancer models and the location of signal voids shows excellent correlation with histology. Using this method, we were able to detect lung metastatic nodules as small as 300 $\mu\text{m}$ , but smaller nodules (<100 micrometers) may become detectable through optimized pulse sequence parameters and more sophisticated approaches to visualizing the signal voids. This method could also be used to detect and track labeled cancer cells to explore the oncogenic pathways of metastases in lungs. More generally, the ability to detect targeted cells using hyperpolarized gas MRI opens a promising new avenue to molecular imaging in the lung.

**References :** <sup>1</sup>R.T.Branca et al, ISMRM 16th annual meeting, 475. <sup>1</sup>J. Zhou et al., Biomaterials 2006; 27(9): 2001-2008.

