In vivo Fast Field Cycling Relaxometry reports on the extra- and intracellular localization of iron oxide particles in tumour models.

Maria Rosaria Ruggiero¹, Simona Baroni¹, Valeria Bitonto¹, Smeralda Rapisarda², Silvio Aime³, Simonetta Geninatti Crich¹

Università degli studi di Torino, Torino, Italy

Introduction
The relationship "immune system/tumour" is considered an important hallmark of cancer¹. Tumour associated macrophages (TAMs) adopt an anti-inflammatory phenotype and secrete factors to promote angiogenesis and tumor invasion. The use of Ultra Small Iron Oxide nanoparticles (USPIO) has been already proposed to the TAM detection generating contrast in T2-weighted images independently of extra and intracellular localization of the NPs. While, T₁ at different fields appear dependent on localization, especially at low field, of the NPs allowing an unambiguous TAM quantification.

Methods
In vitro studies have been carried out on a murine monocyte-derived macrophage cell line (J774) to evaluate the relaxivity changes due to the intracellular localization of ferumoxytol, a clinical negative contrast agent. T₁ were acquired on a FFC relaxometer able to switch over a large range of field strengths (0.01-20MHz). In order to have a mouse, the commercially available relaxometer (Stelar, Mede, Italy) has been modified with the implementation of a 40 mm 0.5T Field Cycling magnet and a dedicated solenoid detection coil placed around the anatomical region of interest². The tumour xenografts were prepared by injecting three tumour cell lines (B16 melanoma, 4T1 and 168FARN breast carcinoma) in the hindlimb muscle.

Results/Discussion
The relaxivity peak at ca. 8-10 MHz observed in water on ferumoxytol is shifted to lower magnetic field strengths (at 0.5-1 MHz) when the NPs were entrapped in macrophages (Fig.1). For in vivo model, the selected types of tumours (168FARN, 4T1 and B16) are characterized by different amount of necrotic zones and macrophages infiltrating the tumor stroma. Ferumoxytol was injected at a dose of 0.5 mmol/kg of Fe. The profile obtained 3h and 24h after the injection were significantly different. The profile observed at 24h displays a bell-shaped profile with a maximum around 0.4-0.5 MHz similar to one found for ferumoxytol labelled macrophages. This finding clearly indicated the intracellular localization of ferumoxytol as confirmed by histological analysis by the Pearls assay.

Conclusions
The measured T₁ at different field immediately reports on the intra- or extra-cellular localization of the investigated contrast agent. This information could be open new horizons for cell tracking applications. Despite the herein used prototype FFC-NMR, FFC has recently been applied to MRI, largely thanks to the work of the Lurie group at Aberdeen University where two prototype human whole-body sized FFC-MRI scanners have been built³.

References

Acknowledgement
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 668119