# Molecular NMR and EPR in vivo detection of cell death using specific phosphatidylserine-targeted iron oxide particles.

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## Purpose:

The aim of the study was to develop a new molecular marker for non invasive diagnosis and monitoring of cell death in order to evaluate the efficacy of anti-cancer treatments in vivo. The phosphatidylserine-targeted peptide E3, isolated by phage-display (1), was coupled to pegylated ultrasmall particles of iron oxide (USPIO). USPIO particles are used as negative contrast agent for magnetic resonance imaging (MRI) due to strong T2 and T2* effects and besides this method they can also be quantitatively detected by electron paramagnetic resonance (EPR) (2).

## Methods and Results:

Transplantable liver tumors (TLT) were implanted in the gastrocnemius muscle of NMRI mice and tumor cell death was induced by x-ray irradiation. Accumulation of the intravenously administered USPIO-E3 particles in treated and untreated TLT tumors was compared to the accumulation of control particles (ungrafted USPIO and USPIO grafted to a scrambled peptide) ex vivo by X- band EPR, and in vivo by L-band EPR and by T2-weighted MRI. In irradiated tumors was greater accumulation of targeted USPIO particles compared to control particles or compared to accumulation of targeted particles in untreated tissues. MRI and X-band EPR were also used to compare accumulation of USPIO-E3 in three different tumor models presenting different degrees of radiosensitivity (fibrosarcoma FsaII is less sensitive than hepatocarcinoma TLT which is less sensitive than lymphoma EL4) in order to evaluate the ability of this new biomarker to distinguish between different levels of tumor cell death.

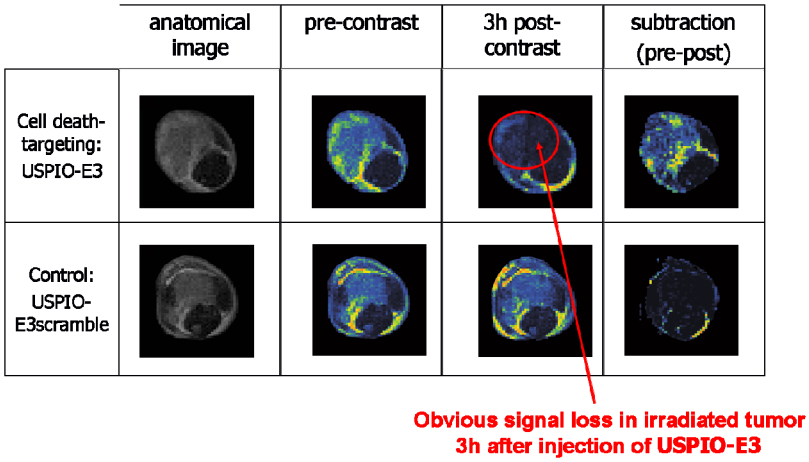


Fig. 1: Axial slices of irradiated tumors as obtained by T2-weighted MRI

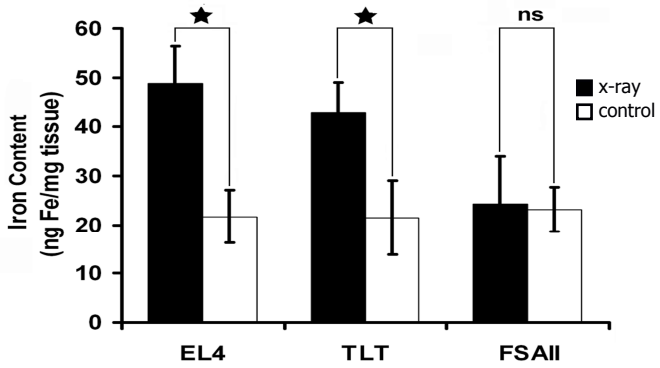


Fig. 2: Ex vivo quantification of the iron content in different tumor models by X-band

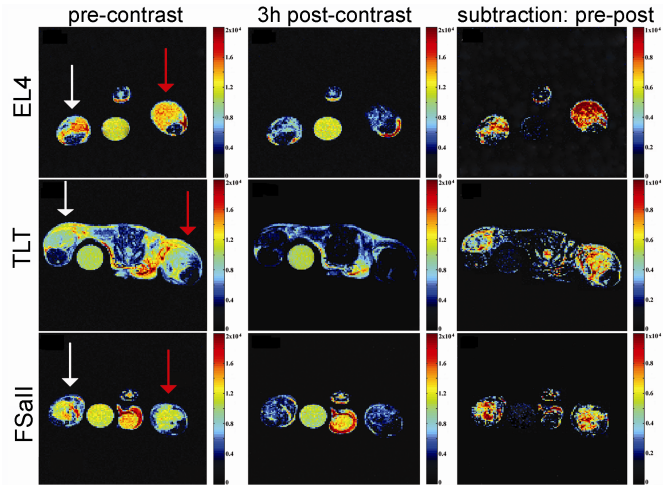


Fig. 3: Axial images of different irradiated tumor models as obtained by T2-weighted MRI. The red arrow indicates the irradiated tumor (right paw) and white arrow the untreated control tumor (left paw).

## Conclusion:

The major finding of the present investigation is that functionalization of the surface of iron oxide particles with the E3 hexapeptide allows the sensitive detection and mapping of tumor cell death after cytotoxic treatment. This molecular targeted system should be evaluated further as a potential biomarker of tumor response to treatment.

**References:** (1)Laumonier C. et al. J. Biomol. Screen. 2006;11:537-545, (2) Ianonne A. et al. Invest. Radiol. 1992;27:450-455.