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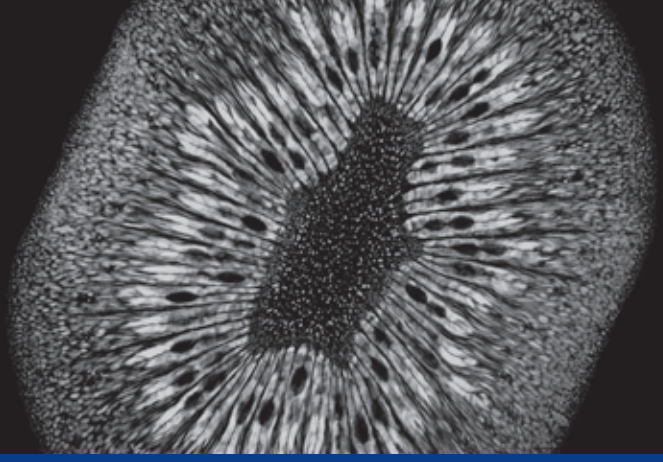
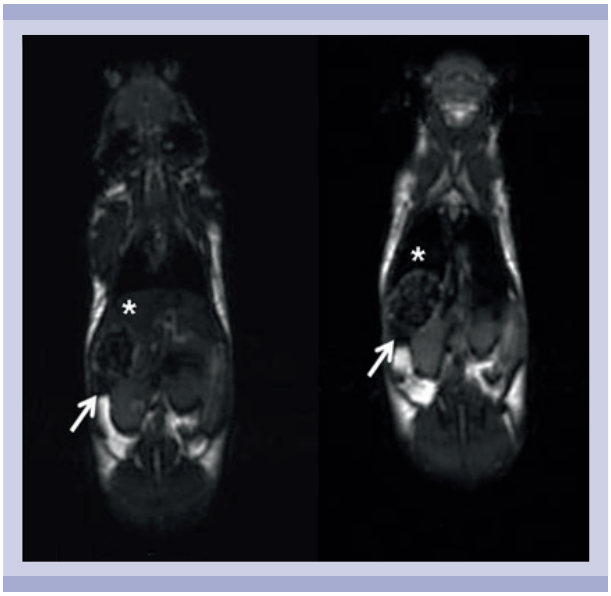


MRI of chronic lymphocytic leukemia



Magnetic resonance imaging (MRI) of chronic lymphocytic leukemia (CLL) by ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles

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Introduction

The efficacy of iron oxide nanoparticles as a macrophage monocytic phagocytic system (MMPS)–specific agent for liver, spleen and lymph node MRI has been demonstrated in experimental and clinical studies. Several studies have shown that these particles can significantly improve the detection and characterization of focal lesions within these organs[1]. Due to their size-dependent properties and their applicability in non-invasive imaging methods, these materials are promising candidates for research, diagnostic, and therapeutic applications in various fields such as cancer, neurodegenerative diseases (e.g. multiple sclerosis, stroke) as well as inflammatory diseases (e.g. rheumatoid arthritis, atherosclerosis)[2]. In our study we focused on a specific mouse xenogeneic transplantation model, NOD/LtSz-scid/scid (NOD/SCID), of chronic lymphocytic leukemia (CLL)[3]. CLL is a type of slow growing leukemia, characterized by a gradual increase in the number of B lymphocytes, first in the blood and bone marrow and, as the disease progresses, in the lymph nodes, liver and spleen. In particular, computed tomography (CT) is used as the first-line modality for imaging of lymphoid malignancies but, specifically for CLL, the role of CT has not yet been clearly defined[4]. In order to prevent the exposure of the CLL patient to irradiation, we aimed to establish a specific MRI method to better visualize the presence of the disease within the spleen using a pre-clinical setting.

Materials and methods

FeraSpin™ XS (Viscover™, *nanoPET Pharma* GmbH, Germany) consisting of USPIO nanoparticles, with a mean particle size of 10-20 nm, was used as MRI agent. Upon intravenous injection, FeraSpin XS nanoparticles circulate in the blood-stream and are taken up by macrophages, including those in the liver and spleen (reticulo-endothelial system, RES). Two groups of mice were injected with the imaging agent: one group of healthy NOD/LtSz-scid/scid (NOD/SCID) mice (NSG mice; n = 2) and another group of NOD/SCID mice injected with chronic MG0248-CLL cells (NSG-CLL mice; n = 4), which served as a xenogeneic mouse transplantation model[3]. Scanning was performed on a clinical 3 T MR scanner (Signa® EXCITE® HDxT, GE Healthcare, Milwaukee, USA) and the mice were positioned in a prototype coil (linear birdcage transmit/receive coil, 100 mm length, 55 mm diameter, tuned at 127.6 MHz, Flick Engineering Solutions B.V., Netherlands, General Electric Company) placed on a warm plate to prevent animal hypothermia. Imaging was performed on both animal groups before and 24 hours

after administration of 100 µL/25 g mouse of FeraSpin XS, corresponding to a dose of 40 µmol Fe/kg body weight. In the healthy animal group (NSG mice) imaging was also performed 6 days and 14 days after imaging agent injection. Both qualitative and quantitative analyses were performed with a FIESTA (fast imaging employing steady state acquisition) sequence as described in literature[5]. Regions of interest (ROI) were defined and the signal intensities in the liver and spleen of each mouse were calculated. Histological analysis was performed according to standard techniques on the NSG and NSG-CLL mice, 28 days and 6 days post injection (p.i.) of FeraSpin XS, respectively. Immunohistochemistry of the spleen using anti-human-CD20 antibody was performed to demonstrate the human $CD20^+$ CLL cell aggregates. Perls' Prussian blue staining was conducted for the detection of iron and a quantitative assessment by estimation of iron-positive cells was performed using an Olympus BX4l microscope.

Results and discussion

NSG mice (control)

In the NSG mice, at 24 hours p.i. of FeraSpin XS, a strong decrease in signal intensity (SI) in the liver (80 %) and spleen (50 %) was observed (Fig. 1A and B). In order to investigate the time-dependent SI in the healthy animal group, we repeated the imaging acquisition at 6 days and 14 days after imaging agent administration. While the SI of the liver was found to increase and reach a baseline level at 14 days p.i., the SI of the spleen remained constant over the imaging time period. Additionally, we performed a histopathological analysis on the liver and spleen by Perls' Prussian blue staining at 28 days p.i. of FeraSpin XS (Fig. 1C). Only very few iron oxide nanoparticles were observed in both organs indicating that the nanoparticles are cleared from the liver and spleen in the healthy animal group within one month.

NSG-CLL mice (tumor)

In the NSG-CLL mice, 24 hours p.i. of FeraSpin XS, a significant decrease in SI (60 %) was observed in the liver but, in comparison, only a slight decrease in the SI (20 %) was observed in the spleen (Fig. 2A and B). Immunohistochemistry of the liver and spleen using the anti-human-CD20 antibody to reveal the focal aggregates of human $CD20^+$ CLL showed that these aggregates were localized only in the white pulp of the spleen (Fig. 2C) and close to the vessels within the liver (data not shown). Interestingly, iron oxide nanoparticles were detectable only in the red pulp (healthy area) of the spleen and were found to be absent in the white pulp, where the CLL cells were localized (Fig. 2D).

Our findings demonstrate that after FeraSpin XS administration, a significant initial decrease in SI is observed in the healthy liver and spleen (NSG mice), which is attributed to phagocytosis of iron oxide nanoparticles by the reticulo-endothelial cells. In the NSG-CLL mice, however, a reduced uptake of FeraSpin XS by the spleen is observed and is due to the presence of the CLL foci within the white pulp.

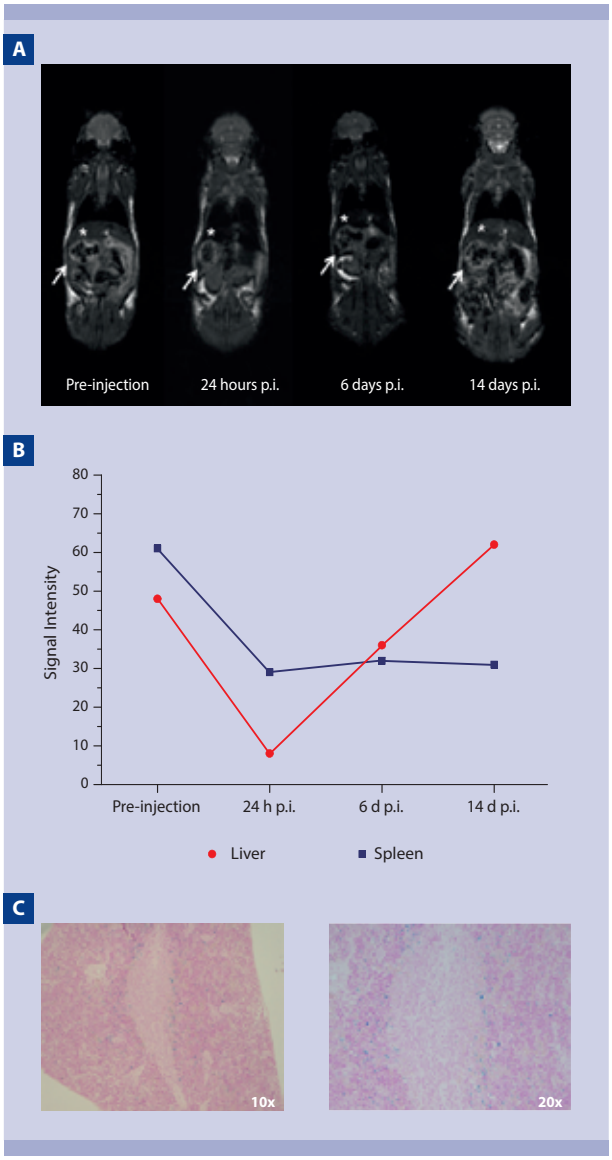

Figure 1: NSG mice (control)
A. Coronal MR images before, 24 hours, 6 days, and 14 days after FeraSpin XS administration. Signal intensity changes are observed in the liver (asterisk) and spleen (arrow). **B.** Time-dependence of SI in the liver and spleen before and after FeraSpin XS administration. **C.** Histopathological analysis of the healthy spleen by Perls' Prussian blue staining 28 days after FeraSpin XS administration reveals only few nanoparticles present.

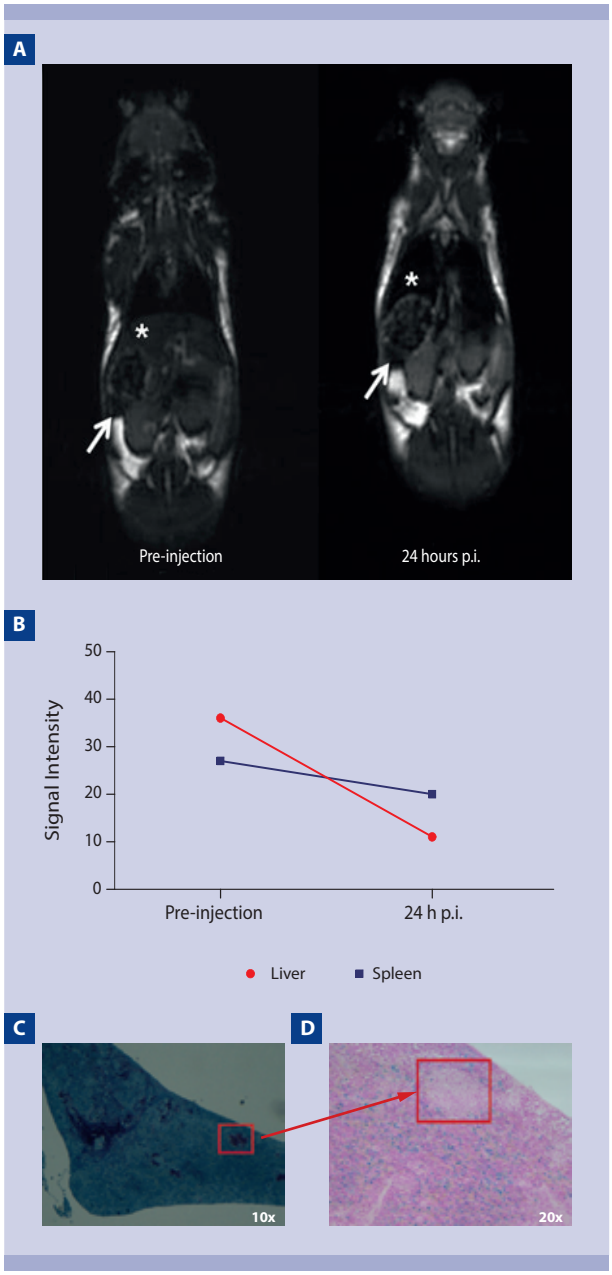

Figure 2: NSG-CLL mice (tumor)
A. Coronal MR images before and 24 hours after FeraSpin XS administration. Signal intensity changes are observed in the liver (asterisk) and spleen (arrow). **B.** Time-dependence of SI in the liver and spleen before and after FeraSpin XS administration. **C.** Immunohistochemistry with anti-human-CD20 antibody reveals that CLL cells are localized in focal aggregates in the white pulp of the spleen. **D.** Histopathological analysis of the spleen by Perl's Prussian blue staining 6 days after FeraSpin XS administration shows that iron oxide nanoparticles are present only in the red pulp of the spleen; no nanoparticles are present in the white pulp where the CLL cells are localized.

Conclusion

This report demonstrates the suitability of the MRI method to detect the presence of a non-solid tumor, such as chronic lymphocytic leukemia, within the spleen. The clearance of iron oxide nanoparticles within the spleen after 28 days p.i. in the healthy group of mice offers the potential to apply this technique for the MR imaging follow-up of CLL mouse models in order to evaluate the response to therapy.

Acknowledgements

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Viscover™ Product	Order No.
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FeraSpin™ S, 1 x 5 injections	130-095-166
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FeraSpin™ M, 1 x 5 injections	130-095-168
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FeraSpin™ XL, 5 x 5 injections	130-095-173
FeraSpin™ XXL, 1 x 5 injections	130-095-174
FeraSpin™ XXL, 5 x 5 injections	130-095-175

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