MRI to monitor the evolution of a subcutaneously implanted lymphoma in a mouse preclinical model

MRI: A powerful tool for a non-invasive volume assessment and monitoring of tumor

Magnetic Resonance Imaging (MRI) is a first-class modality to monitor tumoral growth over time and treatment effects of novel therapies without the need for invasive procedures. This non-invasive modality offers several key advantages aligning with the 3Rs (Replacement, Reduction and Refinement) initiative:

- **Precision:** MRI provides accurate 3D volumetric measurements of irregular shaped tumors, frequently observed in cancer preclinical models.
- **Non-Invasive**: MRI allows for longitudinal studies in contemporaneous cohorts, minimizing variability and reducing the number of animals necessary to test novel therapeutics or medical devices.
- **Early Detection**: MRI has greater sensitivity in detecting small tumor to evaluate early effects of therapeutic interventions.
- **Quantitative**: MRI allows quantitative measurements for more detailed understanding of underlying pathology and allow for improved prognostic estimation.

These advantages make MRI an indispensable tool for preclinical research, providing valuable insights into tumor biology and the effectiveness of potential treatments.

Introduction:

Subcutaneous tissue inoculation in murine preclinical model is an efficient and cost-effective way to evaluate in vivo the response of tumors to novel therapies. The tumoral cells are implanted into the flank of a mice. Tumor is then usually assessed using a caliper, the current gold standard method to monitor the volume growth. The caliper has however some limitations. It cannot distinguish the tumor itself from the surrounding inflammatory or fibrotic tissue. It is also an invasive method that measures only the external surface of a tumor ignoring larger and deeper lesion. On contrary, MRI is a non-invasive approach that allows (1) a 3D assessment of the tumoral volume (2) and in vivo monitoring of lesional growth. This project aims at showing that MRI provides better accuracy and reliability compared to caliper measurements for assessing subcutaneous tumors, supporting its broader application in large-scale preclinical oncology studies.

Materials and Methods:

Experimental Design

- **Model:** Female murine model (*Mus musculus*, A20 on a BALB/c background; weight=20.41g±1.39)
- **Sample Size:** 10 mice

- **Surgery:** Subcutaneous implantation of approx. 2x2mm of tumoral segment from B lymphoma between dermis and peritoneum
- **MRI:** 1.5T MRI (Signal GE Explorer, General Electrics, Chicago, Illinois, USA)
- **Sequences:** 3D T1-weighted imaging DIXON; Dorsal, Sagittal and Transverse T2-weighted imaging DIXON
- **Imaging:** MRI data acquisition was performed at 5 time points (Baseline, D5, D10, D14 and D18) to monitor changes in tumoral volume
- **Gold Standard:** Electronic Digital Caliper (e.g., Spatial Resolution = 0.01mm)

Caliper Volumetrics

The volume of each tumor was calculated as an approximation of an ovoid (**Eq.1**) by estimating the radius as the average of the minor and major axes of the lesion.

$$v = \frac{4\pi}{3} . r^3 \qquad \textbf{Eq.1}$$

MRI Segmentation and Volumetrics

Each Tumor volume at D1, D4 and D8 were manually segmented by two MRI application engineers on the T2-weighted MRI sequence (**Fig.2**), using 3D Slicer (v5.8.1; available at: https://www.slicer.org/). This approach was privileged as automated algorithms do not perform well due to the irregular nature of tumor shape. The tumoral volumes were finally estimated by multiplying the number of pixels segmented by the dimensions of the voxel (0.4 x 0.4mm) in the image.

Data Analyses and Results

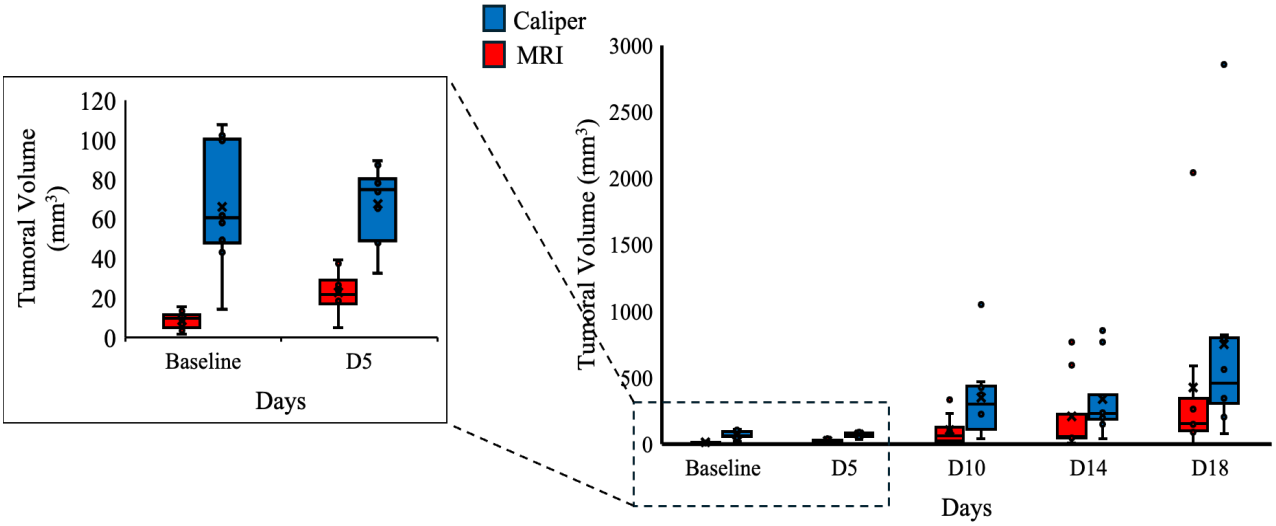

Fig. 1: *Tumoral volume (mm^3) calculated at different timepoints days using MRI and Caliper*. The tumoral volume increased overtime using both methods (i.e., MRI and Caliper) (ANOVA, $p<0.001$). The values calculated with the Caliper were greater than MRI at each timepoint (ANOVA, all: $p<0.05$).

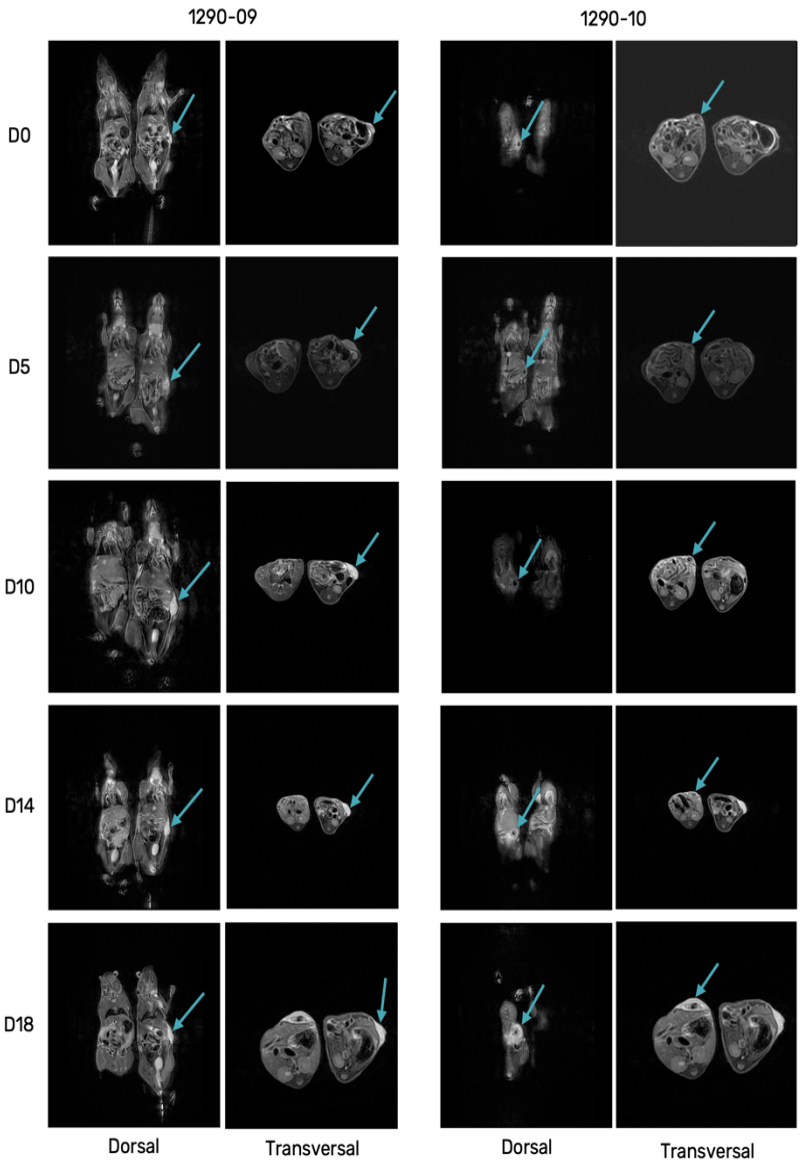

Fig. 2. *Dorsal and Transversal T2-weighted MRI at each imaging point.* MRI was able to monitor overtime the growth of a subcutaneous implanted tumor appearing in hypersignal on T2-weighted MRI sequence. The tumor is represented by the blue arrow.

Interpretation of Results

Differences in tumoral volumetrics were observed between the Caliper and MRI. Several reasons can explain these results. First, Caliper offers an approximation of the tumoral volume (i.e., sphere). Second, the visible mass measured with Caliper included all tissues directly surrounding the tumor. On the contrary, MRI was able to estimate an accurate 3D volume of the tumor. This imaging modality was also able to distinguish the tumoral mass from the surrounding tissues (i.e., fat, inflammation and dysplastic content) at each timepoint until the end of the study (D18) using a T2-weighted imaging

DIXON sequence. This result supports the use of MRI to distinguish the tumoral mass from the surrounding oedema/inflammation.

Relevance to Cancer Research

MRI is a powerful, non-invasive imaging modality that can play a crucial role in cancer research by enabling precise and reproducible in vivo measurement of tumoral volume. This is particularly valuable in early-phase drug development, where accurate, longitudinal monitoring of tumor growth or regression is essential for assessing the efficacy of novel therapeutic agents. MRI can capture detailed anatomical and functional information, including tumor vascularity, necrosis, and diffusion characteristics, provides a multidimensional view of tumor biology. It also offers high soft tissue contrast, which is particularly beneficial for visualizing tumors in anatomically complex regions (e.g., brain, liver, or pancreas).

MRI allows researchers to monitor in vivo tumor progression over time within the same animal. This not only enhances the statistical power of the preclinical studies but also aligns with the 3Rs by minimizing the number of animals needed. MRI datasets can also be retrospectively post-processed, allowing researchers to extract new insights (e.g., texture analysis, volumetric changes, or radiomics) even after the completion of the study. This adds significant value and flexibility to data interpretation, supporting both hypothesis-driven and exploratory analyses.

Conclusion

MRI and the associated post-processing methods to measure tumoral volume is a more sensitive, reliable and reproducible approach to assess the tumoral volume in longitudinal studies compared to the currently gold standard, the Caliper. MRI also lowers the need for additional animal cohorts for intermediate euthanasia, by enabling precise, non-invasive monitoring of tumoral progression over time. These not only enhance study efficiency and data quality but also aligns with the principles of the 3Rs in animal research.

By integrating MRI into cancer research workflows, scientists gain a robust, scalable, and ethically responsible tool to evaluate tumor response, monitor disease progression, and support the translation of preclinical findings into clinical trials. Given its accuracy, longitudinal capability, and ethical advantages, MRI should be considered the new standard for preclinical oncology studies.

This white paper provides a foundation for further studies that can refine the experimental design, optimize the treatment regimen, and expand on the use of MRI in oncology preclinical research.