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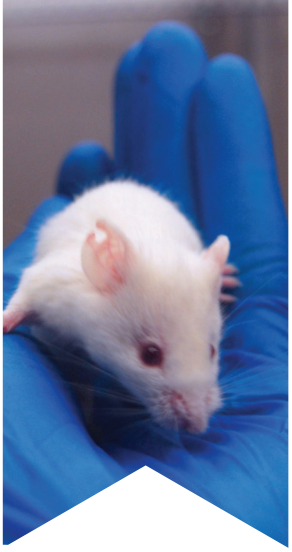

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# NMRD as a biomarker for tumours: a preclinical study

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## Introduction

Fast field cycling (FFC) NMR relaxometry is a low-field magnetic resonance technique which measures the dependence of the spin-lattice relaxation rate $\mathbf{R_1}$ (= $1/T_1$) on the magnetic field over a wide range of field strengths with just one instrument. The important information extracted from the relaxation dispersion curves (NMRD profiles) concerns molecular motions (molecular dynamics) described by means of spectral density $\mathbf{J(\omega) \propto R_1}$.

## *In vivo* application of FFC NMR to the study of tumours

Fast Field Cycling NMR relaxometry is a versatile technique that can be applied to a wide number of sectors including the biomedical sector. In the last few years, several publications about promising FFC studies performed on ex-vivo samples have been published [**1, 2, 3, 4**]. However, in 2018 the first in-vivo FFC study performed on tumour-bearing mice was successfully carried out showing encouraging results, [**5**] with the possibility to discriminate between not only healthy and disease tissues but also between different kinds of tumours.

## *In vivo* FFC reveals the role of intracellular water lifetime as a tumor biomarker

In [**5**] it is shown that there is a clear correlation between the rate of cellular water exchange and tumour activity. Furthermore, results show that there is also a correlation between relaxation rates and tumour types. In [**5**] a non-invasive technique based on magnetic resonance imaging (MRI) is presented which, using weighted proton resonance dispersion profiles at low fields, allows tumour development to be monitored.

## The *in-vivo* experiment

NMRD profiles were acquired on narcotized mice using a Stelar SPINMASTER FFC NMR relaxometer with a prototype set-up which allowed it to host a mouse (ca. 20gr). A 0.5T wide bore magnet was used with the implementation of a dedicated 11mm transmitter/receiver solenoid detection coil placed around the mouse's leg where the tumour cells were injected. In this study, 3 different kinds of mouse mammary adenocarcinoma cells were used: TS/A, 4T1, 168FARN. The 3 cell lines were selected because they have different aggressiveness and metastatic potential (i.e. 168FARN<TS/A<4T1). The size of the tumour was comparable to the size of the leg, in order to be sure that the NMR signal from inside the coil was from the tumour and not from the healthy tissue. During the acquisition, the values of R1 for 25 field points logarithmically distributed in the range [0.01-20MHz] were measured.

FIG. 1

*Anesthetized mouse in the probe, ready to be inserted inside the magnet for measurements. The white arrow indicates the detection ring coil around the tumour-bearing leg.*

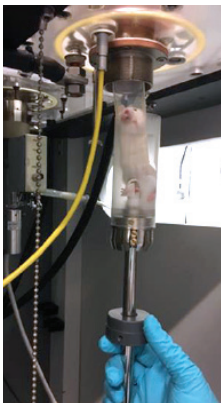

FIG. 2

*Anesthetized mouse in the probe, ready to be inserted inside the magnet for measurements. The white arrow indicates the detection ring coil around the tumour-bearing leg.*

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# The model

A tissue or an in-vitro cellular system may be considered as a two-compartment system, composed of:

- the **extracellular space** (comprising the intravascular volume) characterized by an averaged $\mathbf{R1_{ex}}$ value
- the **intracellular compartment** characterized by a more restricted mobility of the water molecules, with relaxation termed $\mathbf{R1_{in}}$
- $\mathbf{V_{ex}}$ and $\mathbf{V_{in}}$ being the respective volume fractions

Water molecules can cross the barrier between the 2 compartments mixing their relaxation rates. Therefore, the $\mathbf{t_{in}}$ (intracellular water residence time) and $\mathbf{t_{ex}}$ (extracellular water residence time) have to be inserted in the model. According to this bicompartmental model, the evolution time of $\mathbf{M_Z}$ (longitudinal magnetization) is dependent on the relationship between the absolute values of the "relaxation" term, $|\mathbf{R1_{in}} - \mathbf{R1_{ex}}|$, and an "exchange" term $|\mathbf{k_{in}} + \mathbf{k_{ex}}|$, (where $\mathbf{K_{in}} = \mathbf{1}/\mathbf{t_{in}}$ and $\mathbf{k_{ex}} = \mathbf{1}/\mathbf{t_{ex}}$) in a relationship that has been previously defined as the NMR "shutter-speed". In the intermediate-exchange region, the time evolution of $M_Z$ can be bi-exponential, but the relaxation rates obtained from the fitting of the data can be "contaminated" by the exchange occurring between the two compartments. Results showed that different intracellular water residence time tin and intra- and extra-cellular volumes were obtained from the fitting procedure, with values that change proportionally to the tumour aggressivity.

| | $V_{ex}$ | $t_{ex}$ |
|---|---|---|
| MUSCLE LEG | 0.14±0.02 | 1.24±0.25 |
| 4T1 | 0.22±0.08 | 0.68±0.20 |
| TS/A | 0.20±0.02 | 0.99±0.19 |
| 168FARN | 0.15±0.01 | 1.12±0.32 |

TAB. 1:
*Values of extracellular volume fraction (Vex) and intracellular water residence time for healthy tissue and 3 different types of tumours. (Adapted from [5]).*

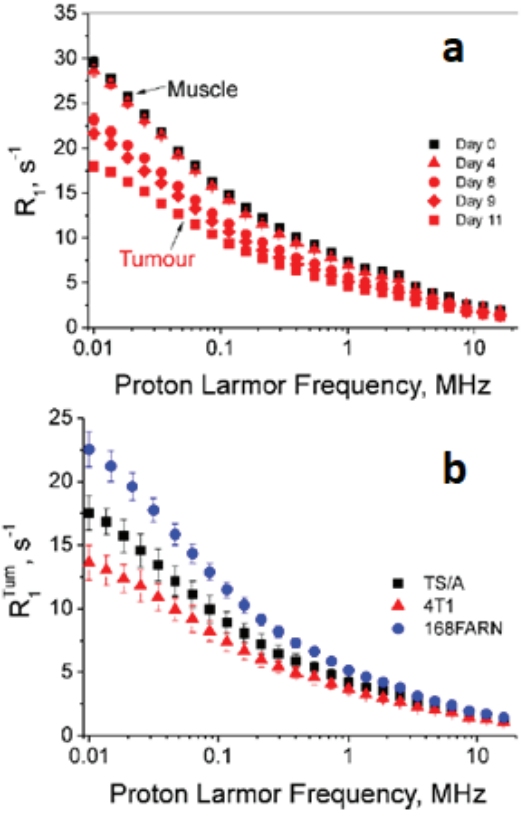
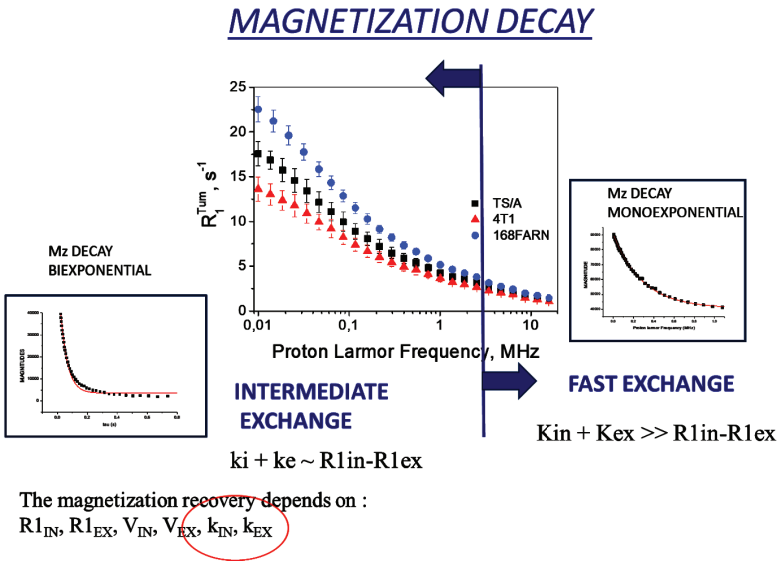



FIG. 4:

a) *NMRD profile of a mouse leg tissue: before (day 0) and after the intramuscular injection of 1 million 4T1 cells.*

b) *NMRD profiles of the tumour tissues grown on hind-limbs: 4T1 (red filled triangle), TS/A (black filled circle) and 168FARN (blue filled circle) acquired 11±2, 13±3 and 25±1 days after intramuscular injection, respectively. $R1^{tum}$ is the averaged relaxation rate normalized to the tumour mass fraction compared to the whole hind-limb (Adapted from [5]).*



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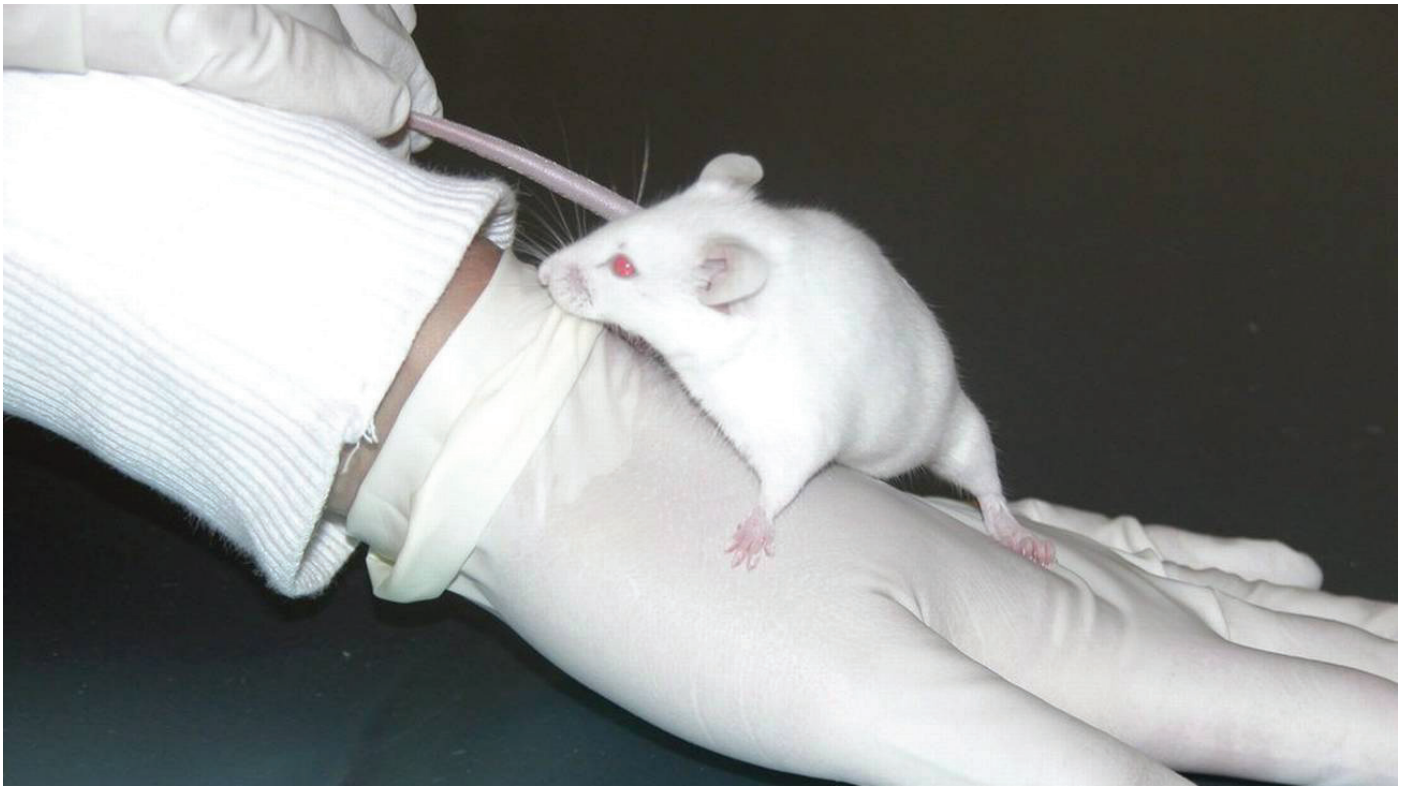
## Conclusions

The following important points are worth mentioning:

1. The field dependence of tissue water proton $\mathbf{T_1}$ at low magnetic fields reveals new diagnostic information.
2. The intracellular water lifetime acts as a molecular biomarker reporting on cellular metabolism and expression of membrane transporters.
3. The limitation of the absence of spatial resolution will be partially overcome using a surface coil (in collaboration with Stelar) or using the FFC-MRI scanner built by D. Lurie and L. Broche **[6]**.
4. Decrease of tin indicates that the water exchange rate across the membrane is a distinctive hallmark that differentiates muscle (representative of healthy cells) and tumour cells.



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## REFERENCES:

(1) Broche, L. M., Ashcroft, G. P., & Lurie, D. J. (**2012**). *Detection of osteoarthritis in knee and hip joints by fast field-cycling NMR.* Magnetic resonance in medicine, 68(**2**), 358-362.

(2) Broche, L. M., Ismail, S. R., Booth, N. A., & Lurie, D. J. (**2012**). *Measurement of fibrin concentration by fast field-cycling NMR.* Magnetic resonance in medicine, 67(**5**), 1453-1457.

(3) Ross, P. J., Broche, L., Davies, G. R., & Lurie, D. J. (**2017**, October). *A Fast Field-Cycling MRI system for clinical applications.* In 34th Annual Congress of the European Society for Magnetic Resonance in Medicine and Biology (ESMRMB): ESMRMB 2017.

(4) Ross, P. J., Broche, L. M., & Lurie, D. J. (**2015**). *Rapid field-cycling MRI using fast spin-echo.* Magnetic resonance in medicine, 73(**3**), 1120-1124.

(5) Ruggiero, M. R., Baroni, S., Pezzana, S., Ferrante, G., Geninatti Crich, S., & Aime, S. (2018). Evidence for the Role of Intracellular Water Lifetime as a Tumour Biomarker Obtained by In Vivo Field-Cycling Relaxometry. Angewandte Chemie, 130(**25**), 7590-7594.

(6) Broche, L. M., Ross, P. J., Davies, G. R., MacLeod, M. J., & Lurie, D. J. (**2019**). A whole-body Fast Field-Cycling scanner for clinical molecular imaging studies. Scientific reports, 9(**1**), 10402.

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