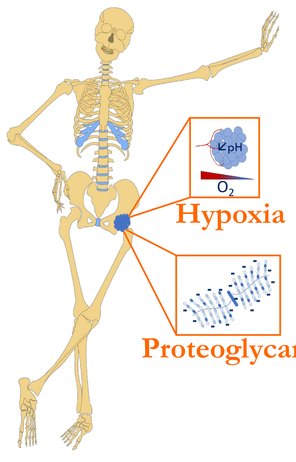
# Interest of *in vivo* CEST MRI to study chondrosarcoma tumour microenvironment

R. Autissier[1,2], E. Maubert[1], S. Schmitt[1], P. Auzeloux[1], C. Peyrode[1], F. Degoul[1], A. Traoré[2], J. M. Bonny[2], E. Miot-Noirault[1], L. Mazuel[1,2] & G. Pagès[2]

[1] *Université Clermont Auvergne, INSERM, UMR1240 IMoST, 63000 Clermont-Ferrand, France*

[2] *INRA, AgroResonance, UR370 QuaPA, 63122 Saint-Genès-Champanelle, France*

Contact: Roxane.Autissier@uca.fr




## Introduction:

Chondrosarcoma is a malignant cartilage tumour and represents the second most common primary malignant solid tumour of bone. It accounts for approximately 25% of all bone sarcomas (Bertoni *et al*. 2002). Poorly vascularized and rich in proteoglycans (PGs), chondrosarcomas are considered to be chemo- and radio-resistant with efficient treatment usually limited to surgical resection with large disease-free margins. If the hypoxic and PGs status of the tumour can be assessed by TEP imaging and scintigraphy, it requires however 2 separated exams.

In this context, we propose to develop **an MRI strategy based on Chemical Exchange Saturation Transfer (CEST)** to simultaneously **co-register both hypoxia (pH) and PGs content *in vivo***.

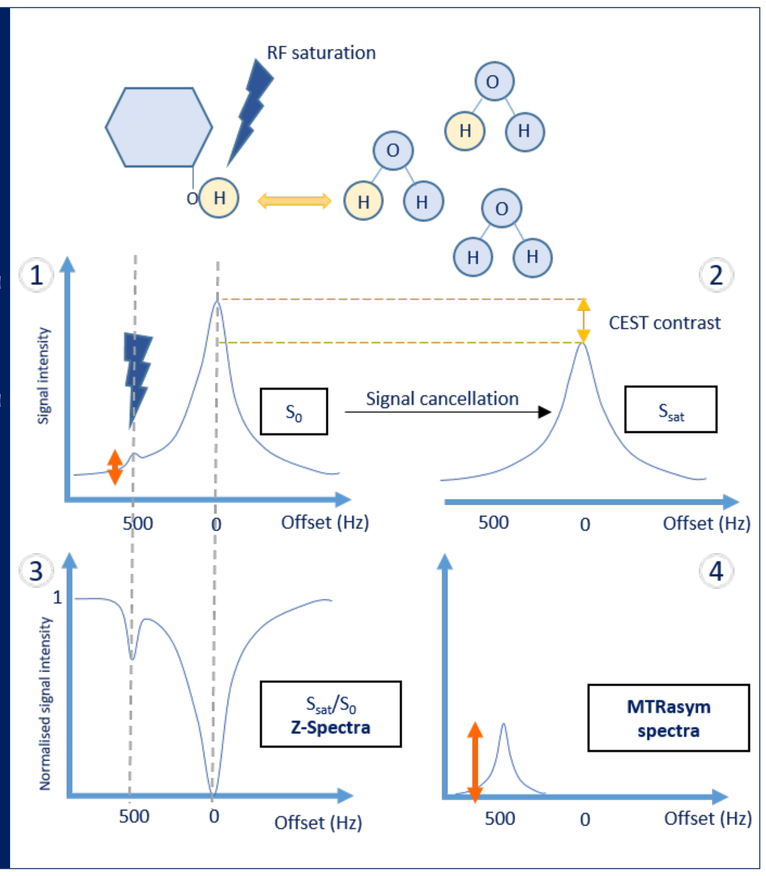
## Materials and Methods:

***In vivo model of chondrosarcoma:*** 5 week female nude NMRI mice (n=10) were implanted with rat chondrosarcoma SWARM xenograft (1mm$^3$ fragment) in scapula position. After 2 weeks growth (average tumour volume: 215,04 mm$^3$ ± 86,27), xenografts were characterized in terms of PGs content by $^{23}$Na MRI, $^{99m}$Tc-NTP 15-5 radiotracer (developed in UMR 1240 INSERM, Peyrode *et al*. 2011) and hypoxia by $^{18}$F-FMISO (Rajendran *et al.* 2015). Then, both tumour characteristics were evaluated by CEST MRI.

***In vivo multimodal imaging:*** All acquisitions were performed on anesthetized mice (1.5% isoflurane in air/$O_2$ 70/30, v/v, mixture). *MRI acquisitions:* MRI images were acquired at 11.7 T using a 72-mm volume quadratic coil with surface reception. Diffusion weighted imaging (b=500s.mm$^{-2}$) was first performed to localize the tumour, then WASSR (B1=0,1µT for 1s, Δωsat=±1000Hz in 20Hz steps) and CEST Z-spectra ($B_1$=1.5µT for 4s, Δωsat=±2500Hz in 50Hz steps) were acquired based on a RARE sequence. $^{23}$Na MRI was recorded thanks to a FLASH sequence in axial plane. Data were analysed using an in-house program written on MathWorks®. After correction for $B_0$ inhomogeneities, the CEST maps were generated and Area Under the Curve (AUC) were calculated at given frequencies on Z-spectra asymmetries with MTRasym or three-offset measurement approaches (Jin *et al.* 2013). *Scintigraphic imaging:* $^{99m}$Tc-NTP15-5 (13MBq/animal) and $^{18}$F-FMISO (18MBq/animal) were administered intravenously then acquisitions were performed 30min or 4h after injection with 15min planar acquisition, respectively. Data were analysed using Interview$^{TM}$FUSION and VivoQuant$^{TM}$.

***Ex vivo analysis:*** the presence of PGs and hypoxia was assessed by Alcian blue and pimonidazole staining respectively.

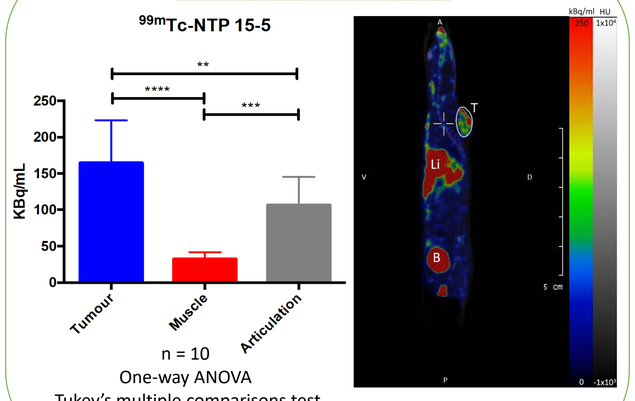
CEST MRI principle




## Results:

## 1/Evaluation of proteoglycans content

### Scintigraphic imaging:



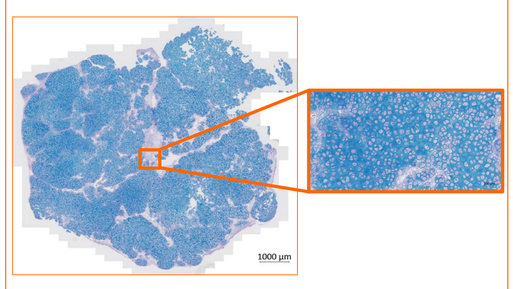




Tumour/Muscle ratio
4,953 ± 0,961

High accumulation of $^{99m}$Tc-NTP15-5 inside the tumour reflects the presence of PGs content

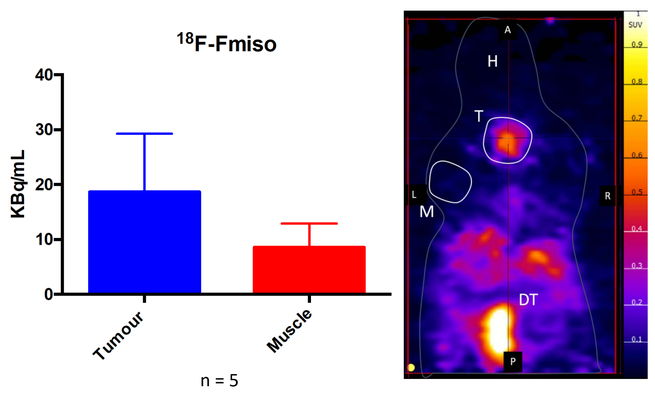
### Alcian blue staining of PGs:




High alcian blue staining illustrates the high PGs content

## 2/Evaluation of hypoxia (pH)

### Scintigraphic imaging:



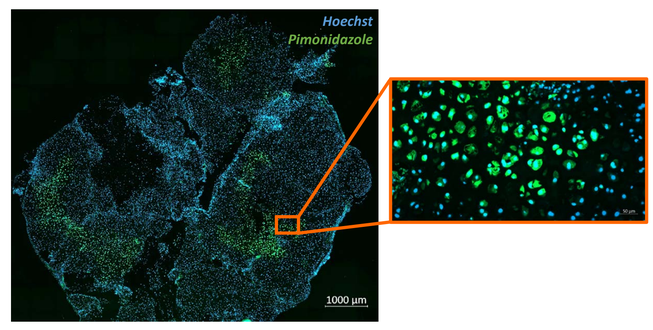




Tumour/Muscle ratio
2,168 ± 0,896

$^{18}$F-FMISO uptake inside chondrosarcoma reflects mild hypoxia

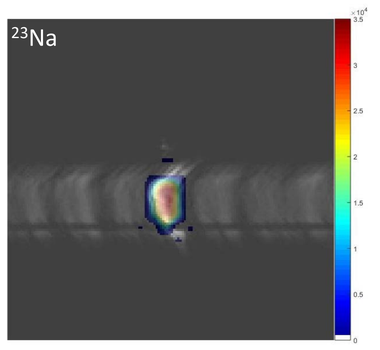
### Pimonidazole immunohistochemistry of hypoxia:




Pimonidazole staining confirms the presence of tumoural hypoxic areas

## CEST MRI

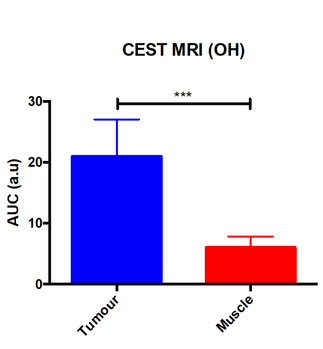
### $^{23}$Na MRI imaging:

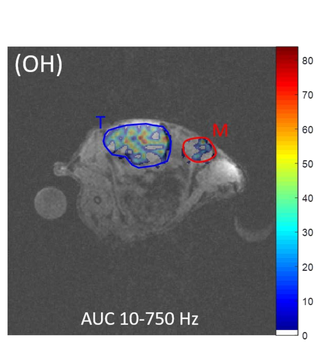



Representative $^{23}$Na image of chondrosarcoma

$^{23}$Na MRI reflects the presence of PGs

### CEST MRI - hydroxyls functions for PGs imaging:



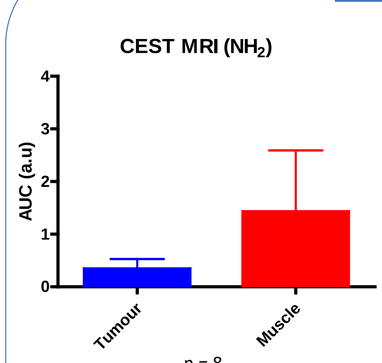



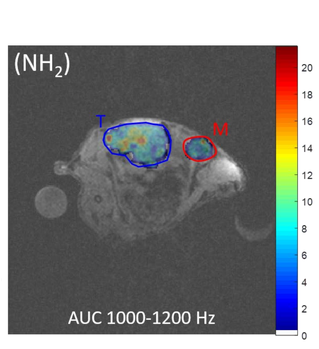

Tumour/Muscle ratio
4,066 ± 1,468

A significant increase in tumour AUC compared to muscle

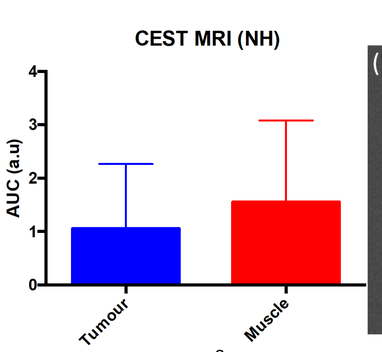
AUC of hydroxyls functions can confirm large presence of PGs inside the tumour

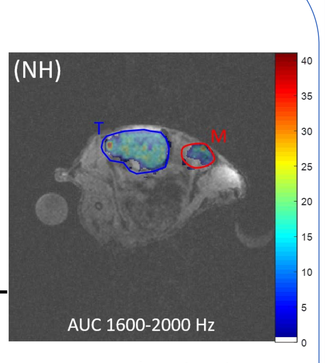
### CEST MRI - amines and amides functions for pH imaging:




Tumour/Muscle ratio
0,213 ± 0,182




Tumour/Muscle ratio
0,972 ± 1,166

No differences in AUC between tumour and muscle were observed for both amine and amide functions

Cartography of AUC between 1000-1200Hz (amine moiety) suggests regional changes in pH within chondrosarcoma

## Conclusion – Discussion:

Preliminary findings reveal potential of **CEST MRI as multimodal imaging approach**. In a unique acquisition , CEST MRI provides *in vivo* **quantitative data of extracellular matrix as well as a high resolution cartography of hypoxia.**

Perspectives are now to evaluate CEST MRI as an *in vivo* method to assess response to therapy.

Grants: "La Ligue contre le Cancer Auvergne-Rhône-Alpes". MRI and nuclear imaging were performed at AgroResonance and UMR 1240 INSERM respectively, in the context of IBISA labelled IVIA platform.