Specificity of Multimodal molecular MR and US imaging applied to kidney tumor xenografts

Marie Poirier-Quinot[1], Ludovic de Rochefort[1], Ingrid Leguerney[1,2], Sandra Robin[1,2], Xavier Violas[3], Luc Darrasse[1], Rose-Marie Dubuisson[1], Stéphanie Pitre-Champagnat[1], Philippe Robert[3], and Nathalie Lassau[1,2]

[1]*Univ Paris Sud, CNRS, UMR 8081, IR4M, Orsay, France,* [2]*IRCIV Gustave Roussy, Villejuif, France,* [3]*Guerbet, Research, Roissy CDG Cedex, France*

Purpose :

The development of biomarkers for early diagnosis of diseases and to evaluate the response to therapy is an active research area. To achieve this goal, the development of targeted contrast agents (CA) is of great interest to increase the specificity of imaging techniques [1]. One approach among the most sensitive in MRI is to use functionalized superparamagnetic iron oxides nano-objects which detection is in a range compatible with the *in vivo* receptor concentration [2]. Here, a multimodal molecular imaging protocol comprising US and MRI is developed. The specificity of contrast agents (µbubbles and iron oxide nanoemulsion) functionalized to target the same integrin (αvβ3) in mouse xenograft tumor model of kidney cancer is presented.

Material/Methods:

Animal model: 8 nude mice were xenografted with a human kidney tumor (3.10^6 cells A498). After 3 weeks, they had two imaging sessions, comprising US and MRI. US and MR αvβ3-targeted and non-targeted CA were injected in i.v. in the tail vein at 2 days intervals.

Imaging protocol: Dynamic contrast-enhanced DCE-US and dynamic susceptibility contrast DSC-MRI were applied.

Molecular US acquisitions were performed on a small animal dedicated system (Visualsonics VEVO-2100, 20MHz probe MS-250), using MicroMarker™ αvβ3-targeted and control (IgG) µbubbles, resolutions of 165 µm - lateral and 75 µm - axial. The CA wash-in step and its targeting is observed during and 10 minutes after injection of 50 µL of CA [3]. All imaging sequences, bolus acquisitions, and recordings of the ultrasound signal before and after destruction of the microbubbles were analyzed with the Vevo CQ software. The signal from targeted microbubbles linked to their receptors was evaluated. Targeted microbubbles were separated from freely circulating microbubbles using a destruction/replenishment approach [4]. The best match between pre- and post-destruction image frames was identified and subtracted from each other giving the differential targeted enhancement (DTE). DTE was then an indicator of the amount of microbubbles adherent to molecular endothelial receptor

Molecular MR acquisitions (Fig.1-a and -b) were then performed a clinical 1.5 T (Philips Achieva), using a conventional 23mm-diameter surface coil. Mice were anesthetized (isoflurane+O_2). Apparent diffusion coefficient (ADC) was quantified through a diffusion weighted sequence: 2D spin-echo-EPI, TR/TE=1.9s/73ms, 1 mm thick, 0.5 mm in plane resolution, 2 diffusion gradients (b=0/600 s.mm^{-2}). Dynamic Susceptibility Contrast (DSC)-MR was performed during one hour, through the repetition of T2* acquisitions (3D gradient echo multi-echo sequence - TR/TE/dTE = 90/5.9/9.7 msec, 0.3x0.3x0.5 mm^3 pixel size, 220 Hz/pix, Tacq = 4.2min) pre- and post- injection of 100 µmol Fe/kg USPIO-based nanoemulsion functionnalized with RGD binding αvβ3 (P4000), as well as a control nanoemulsion (P3999) Guerbet.

Data Analysis: MR data were co-localized onto the US 2D acquisition: the closest slice in the 3D MR-acquisition volume (ADC and T2* images) was registered by an expert radiologist. Tumor size was estimated from US and from MRI (manual segmentation). ROIs of the whole tumor, of hypo (cyan ROI)- and hyper- (purple ROI) vascularized area were drawn for regional analysis of: 1) ADC Fig 1a, 2) DTE measurement (US - fig 1c) and 3) relaxation rate R_2^* measurement. Fig 1b displays MR acquisitions pre- and post-injection of targeted CA. The mean ΔR_2^* ($R_{2\,pre}^* - R_{2\,post}^*$) was calculated, followed for 1 hour to quantify binding to the targeted receptor.

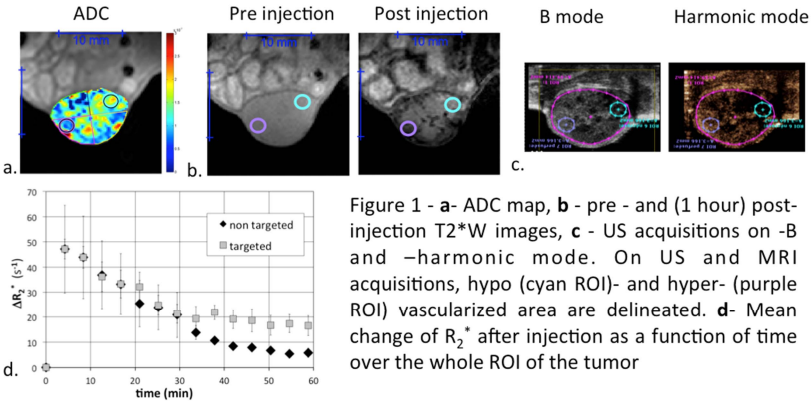

Figure 1 - **a**- ADC map, **b** - pre - and (1 hour) post-injection T2*W images, **c** - US acquisitions on -B and –harmonic mode. On US and MRI acquisitions, hypo (cyan ROI)- and hyper- (purple ROI) vascularized area are delineated. **d**- Mean change of R_2^* after injection as a function of time over the whole ROI of the tumor

Results: US and MRI tumor sizes correlated well ($R = 0.99$, $p < 0.001$ Pearson test). ADC mean value for all tumors was estimated at $1.65 \times 10^{-3} \pm 0.3 \times 10^{-3}$ mm^2.s^{-1}. The same structure could be distinguished on the ADC map (Fig 1a) and the US mode B (Fig 1c), hypo-echogenic signal regions on B mode corresponded to high ADC values (such as liquid regions) and hyper-echogenic regions to low ADC values.

The ADC map (Fig 1a) and molecular US and MR images (Fig 1b and c) highlighted that regions with elevated ADC were correlated to low perfused regions, and that regions with low ADCs to high perfused regions. Fig 1b illustrated a high signal homogeneity (pre-injection), which remained highly heterogeneous 1 hour post-injection of the targeted emulsion with structure similar to the ones visible on ADC and US images. As shown in Fig. 1d providing the mean decay curve for ΔR2* both for the targeted (gray square) and non-targeted (back diamond) emulsions, 1 hour post-injection, MR signal became significantly specific such that it can be assimilated to a quantitative index of αvβ3 (error bars were the standard error of mean).

On US acquisitions, DTE values (mean ± standard error of mean) were of 260.5 ± 65.9 for the non targeted CA and 2601 ± 488 for the targeted one. DCE-US targeted and non-targeted CA gave significantly different contrast modifications ($p < 0.01$, paired student's *t*-test). On DSC acquisitions, after one hour, targeted CA ΔR_2^* of 16.5 ± 3.9 min^{-1} (mean ± standard error of mean) was significantly different from the one of non-targeted CA, 5.8 ± 1.8 min^{-1}.

Discussion:

We presented a multimodal protocol involving US and MRI molecular imaging with contrast agents functionalized to target the same integrin (αvβ3). The specificity of each targeted CA was shown on kidney tumor xenografts in mice. Histology is needed to further confirm the specific fixation of αvβ3 *in vivo*. Multimodal molecular imaging of targeted CA and various imaged-derived biomarkers, such as ADC, for preclinical oncology may be used to follow-up the response to treatment. The use of clinical imaging set-up here could allow the fast transfer of this methodology into the clinics.

REFERENCES [1]. Kircher, et al., Radiology 2012, 263(3): p. 633. [2] Poirier-Quinot, et al., Future science 2013. [3] Lavisse et al. Invest Radiol 2008. [4] Willmann JK et al. Radiology. 2008