In vivo assessment of tumor heterogeneity in Mouse at 1.5 T: comparison between a blood pool and a conventional extracellular contrast agents by tumoral 3D-microimaging

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Purpose: Tumor grade is based on a deep analysis of tissular architecture such as tissue heterogeneity, level of differentiation, mitotic activity, angiogenesis, amount of necrosis, degree of infiltration. The aim of this study is to characterize the tumoral tissular heterogeneity in a mouse breast tumor model by associating a non-specific and a rapid-clearance-blood-pool contrast agents in a contrast-enhanced dynamic MRI protocol. Distinguishing the different components requires a very high spatial resolution which is provided on a conventional body 1.5 T MRI unit by a High-Temperature Superconducting (HTS) surface coil. HES histology is used as the reference method.

Materials and Methods: Animal Model: MDA-MB-435 is a high grade human mammary tumor cell line isolated from a metastatic ductal adenocarcinoma (*1*). Tumoral induction was done on n=6 nude Mice 44±9 days before MR imaging. HTS surface Coil: a superconducting surface probe at 77 K was used for MRI, with an effective diameter of 12 mm, and an unloaded/loaded Qs of about 11000/9000 inside the magnet (*2*). MR sequences: MR imaging was conducted on a 1.5 T clinical magnet (Signa, GEMS). Two different T1w-sequences were performed: High Spatial Resolution (HSR) and isotropic High Spatial Resolution (isoT-HSR): see Table. Contrast Agents: P792 (Vistarem®, Guerbet, France, (*3*)) and Gd-DOTA (Dotarem®, Guerbet, France) were injected i.v. by manual bolus at 0.04 mmolGd/kg and 0.3 mmolGd/kg respectively. Each mouse received both products (randomized order), with respect of a one day delay between the two injections. Histology: HES standard coloration was done on histological slices parallel to axial MRI plan.

	HSR	isoT-HSR
Timing	during 1 hr p.i.	at 1 hr p.i.
Sequence	3D-SPGR	
TR/TE - FA	57/14 ms - 70 deg	
Acq. Time	6.9 min	15.6 min
FOV (mm^3)	30×15×8.4	30×15×3.6
Matrix	512×256×28	512×256×60
Voxel size (μm^3)	59×59×300	59×59×59

Table : Sequence parameters

Semi-quantitative analysis: Region of interest were placed in different area of tumor, in accordance with regions observed in histology, to compare the enhancement kinetics of both products.

Results: Histology revealed that all tumors were high grade carcinomas, poorly differentiated and with ~50% of necrosis. Some of tumors had micro-calcifications and micro-infiltrations. On the six Mice, four different regions were distinguished on histology: Alive Tumoral Tissue (ATT), Necrosis (N), Excaved Necrosis with no cells (EN) and well vascularized granulated Non-Tumoral Tissue (NTT) (Fig. 1). These regions were also delineated on enhanced-MRI, with a different pattern of enhancement between the two products: P792 distributes quickly in NTT via micro-vessels and diffuse slowly in the necrotic areas (N, EN), whereas Gd-DOTA accumulates in N and EN within the 10 first minutes (Fig. 2, 4). When tumor contains non tumoral well vascularized component, P792 allows a clear differentiation between this NTT region and N, whereas Gd-DOTA accumulates in both (Fig. 1). The isotropic sequence has a good SNR and allows depiction of tumoral micro-vessels when performed after a blood pool contrast agent injection (Fig. 3).

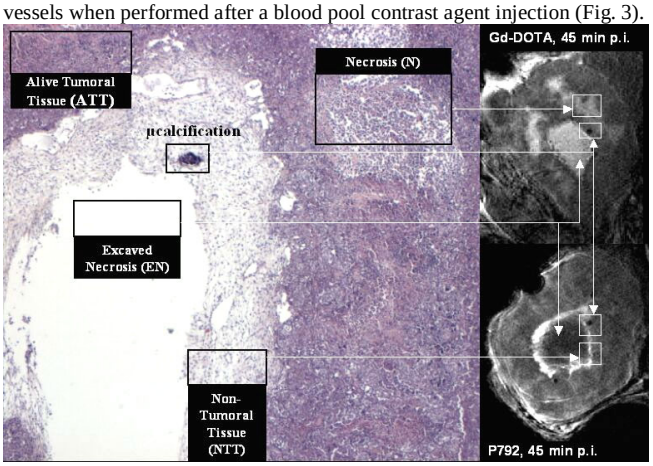

Figure 1 : Correlation between histology and enhanced-MRI (Mouse#188)

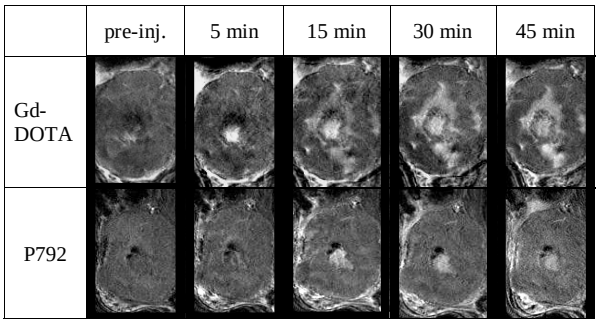

Figure 2 : Examples of enhancement kinetics with HSR (Mouse#181)

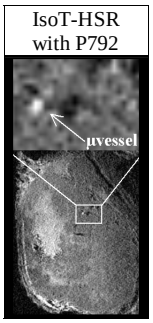

Figure 3 : Mouse#189

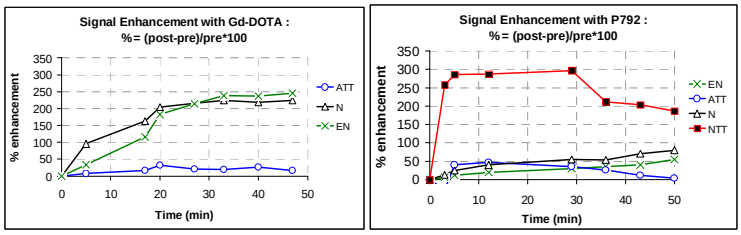

Figure 4 : Semi-quantitative analysis of enhancement after injection (Mouse#188)

Discussion: We observed a clear differentiation between the two products, according their molecular structure. N, EN: In this breast cancer model, the necrotic areas are characterized by a high interstitial volume, low cellular density and low vascularization. We demonstrated that these "necrotic lakes" are easily accessible to the small molecule at the early phase after injection (<20min) whereas the enhancement is delayed and progressive with the macromolecule. NTT: The rapid enhancement of P792 in the NTT demonstrates that such regions are well vascularized and are one major route of the contrast arrival. ATT: The low permeability of macromolecule and the high cell density limit their accumulation in the tumoral tissue, contrary to small molecule which diffuse freely through tumoral neo-vessels. The high resolution is a key point for tissue characterization: For example, visualization of tumoral micro-vessels with P792 is feasible with a 60 µm resolution, which makes possible quantitative compartmental analysis with an input function inside the tumor itself (perspective of this work). Finally, the 3D isotropic resolution, allowing multiplannar reconstruction, is a powerful tool for tumor architecture analysis and histology correlation.

Conclusions: This study shows that conjunction of very high spatial resolution (isotropic 60 µm voxels) with quantitative kinetic features is of great potential for tissue characterization. The comparison between two gadolinium chelates with different molecular size (non specific extracellular and rapid clearance blood pool contrast agents) allows a better description of tumoral heterogeneity. MRI microscopy with HTS surface coils and contrast media dynamic analysis open large perspectives for small animals imaging at 1.5 T.

References:

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