

# Anti-angiogenic therapy in a murine liver cancer: Complementary assessment with MR-Elastography and Diffusion-Weighted

## MR imaging

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## PURPOSE:

Early detection of changes in the vascularity and cellularity of a tumor could represent a significant advance in treatment management using anti-angiogenic agents. Some insight into these features can be obtained using Magnetic Resonance (MR) imaging techniques, such as Diffusion-Weighted (DW) MR imaging which provides information about tissue architecture including cell density and necrosis<sup>1</sup>, and MR-Elastography (MRE) capable of quantifying in-vivo mechanical properties. MRE has shown its potential to provide new structural information characterizing tumor vasculature in animal models<sup>2</sup>. We aim to investigate the potential value of MRE and DW MR imaging in the detection of microstructural changes induced by an anti-angiogenic therapy (Sorafenib, Nexavar®, BAY 43-9006, Bayer, Leverkusen, Germany) in a human liver cancer cell line (HepG2, p53-wild-type) implanted in immune-deficient mice<sup>3</sup>.

## METHODS:

Eighteen 5 week old nude female mice were implanted subcutaneously with 4 million HepG2 cells in a suspension of 100  $\mu$ L. Once the tumor volume reached  $\sim$ 250 mm<sup>3</sup>, the 18 mice were split into 3 groups: 6 mice were treated orally for 8 days with the anti-angiogenic therapy (2.5 mg / 100  $\mu$ L of Sorafenib (Nexavar)), 6 mice treated with the vehicle suspension, and, finally, 6 mice –acting as controls– were not treated at all. Animals were scanned one day prior (day 0, D0) and on the final treatment day (day 8, D8) in a horizontal 7T MR scanner (Bruker, Pharmascan), with the exception of the 6 control mice which were scanned only at D0. We recorded: (i) T<sub>2</sub> weighted images (104  $\mu$ m in plane resolution), (ii) DW MR images (6 b-values: 0, 150, 300, 500, 750 and 1000 s/mm<sup>2</sup>, 300  $\mu$ m in plane resolution), and (iii) steady state MRE images with 3 vibration frequencies ( $\omega$ ) of 800, 900 and 1000Hz (300  $\mu$ m in plane resolution)<sup>4</sup>. The average apparent diffusion coefficient (ADC) and the shear modulus ( $|G^*|$ ) were calculated within a ROI encompassing the entire surface area of the tumor. The frequency evolution of  $|G^*|$  was fit to the power law model<sup>5</sup>  $|G^*| \sim |\omega|^y$ . Finally, all tumors (treated and vehicle at D8, and control at D0) were excised for histological analysis.

Note: Due to technical issues, of the 6 mice of the vehicle group only 2 mice underwent the MRE at D8.

## RESULTS:

ADC showed a trend towards increased values after treatment for the Sorafenib group ( $P=0.16$ ) and apparently no change for the vehicle group (D0  $P=0.75$ ) (Fig.1).

At D8, no significant change in stiffness ( $|G^*|$ ), measured at 1000 Hz, was observed ( $P=0.84$ ) for the Sorafenib group, while a decrease was observed for the 2 vehicle mice (Fig.2). Statistical tests could not be run for the vehicle group due to the low number of animals.

The study of frequency dependence (800-1000Hz) revealed a trend towards a decrease of the power law behavior of tumors treated with the anti-angiogenic agent ( $P=0.28$ ), while no change was observed for the vehicle group ( $P=0.85$ ) (Fig.3).

Histological analysis characterized the efficiency of both treatments by comparing the micro-structure of the tissue obtained at D8 (for the both groups) and at D0 for the control group:

- An emergence of necrosis (0-20 % haemorrhagic necrosis of the surface area of the tumor in the control group, 25-50 % and 5-25% of a mixed of haemorrhagic/ischemic necrosis for the Sorafenib group and the vehicle group, respectively)
- A significant decrease ( $P=0.02$ ) of the percentage of proliferative cells in the Sorafenib group ( $52 \pm 10\%$ ) compared to the control group ( $68 \pm 5\%$ ), while no significant difference was observed for the vehicle group ( $63 \pm 10\%$ ).
- No significant difference ( $P=0.41$ ) in the cellularity was observed between the control group ( $6641 \pm 485$  mm<sup>2</sup>), the Sorafenib group ( $6931 \pm 543$  mm<sup>2</sup>) and the vehicle group ( $646 \pm 744$  mm<sup>2</sup>).

## CONCLUSIONS:

The structural biomarker ADC was not affected by the therapy at D8 probably due to the counteracting effects of the haemorrhagic / ischemic necrosis and the unchanged cellularity. The decrease in stiffness  $|G^*|$  measured at 1000 Hz after the administration of the vehicle suspension was attributed to the emergence of necrosis related to the tumour growth. The stable mechanical behaviour of the Sorafenib group may be due to counteracting effects originating from the emergence of necrosis (reducing the stiffness) and change in the micro-structure of vasculature which could lead to an increase in stiffness. Characterisation of the vasculature by immune-histology to assess the mean vessel density (using CD31 staining) and the vascular permeability (using  $\alpha$ -SMA staining) are under process.

The change in mechanical dispersion properties showed a trend towards a decrease of the power law behaviour in the Sorafenib group, which was not observed in the vehicle group. This is most likely due to the therapy-induced spatial alterations of the vascular bed.

**CLINICAL IMPLICATION:** Early tumor response to anti-angiogenic treatment is very complex. Many confounding effects occur which lead to counteracting processes rendering (in this particular case) the ADC measurement insensitive to the induced cell death. Apparently, only the biomechanical dispersion properties are sensitive to changes induced by the anti-angiogenic treatment. More data and deeper theoretical analysis are necessary to understand the mechanism of why dispersion properties seem to carry this information.

**REFERENCES:** (1)Padhani AR, Miles KA. Radiology 2010;256(2):348–364. (2) Juge, L. and al. (2012) Radiology 264, 436-444 (3) Okamoto K and al. Int J Oncol 2008; 33:733-742.(4) Sinkus and al.. (2005). Magn Reson Imaging, 23, 159-165. (5) Bagley R. L. & Torvik P. J. (1983). J. Rheol., 27, 201.

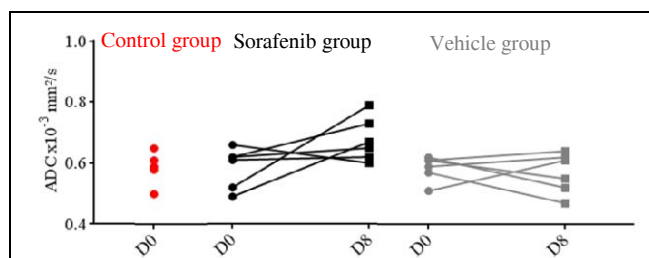


Fig.1: ADC  $\times 10^{-3}$  mm<sup>2</sup>/s measured on 6 b-values from 0 to 1000 s/mm<sup>2</sup> showed a trend toward an increase after anti angiogenic treatment (black) while no change was observed after the vehicle treatment (grey).

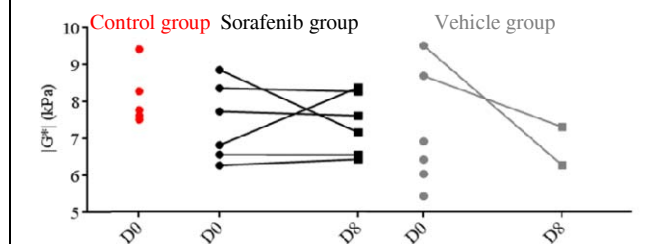


Fig. 2:  $|G^*|$  (kPa) measured at 1000 Hz did not change after treatment with the Sorafenib (black) while a decrease was observed for the 2 vehicle mice scanned (grey).

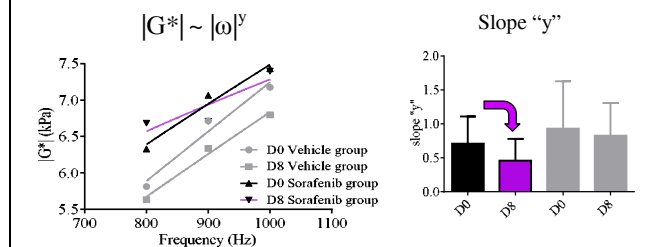


Fig.3: The study of frequency dependence revealed a trend toward a decrease of the power law behavior in tumors treated with Sorafenib, not visible in the vehicle group.