

## Anti-angiogenic therapy follow-up in mice brain glioma model with P04000, a new molecular imaging avb3-nanoemulsion of iron oxide.

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### Purpose:

Quantification of angiogenesis is a major clinical challenge for the characterization of tumor aggressiveness and evaluation of response to anti-angiogenic therapy. The avb3 integrin expressed by the tumor neovasculature is a preferred target that can be achieved with contrast agents vectored by the RDG motif. A new iron oxide nanoparticle based USPIO (P04000) is evaluated in terms of specificity and capacity to monitor antiangiogenic treatment in a brain tumor model in mice.

### Materials and Methods:

**Binding :** A competitive cell-binding assay was performed on HUVEC cells. avb3-binding affinity of RGD-targeted emulsion P04000 and non-targeted emulsion P03999 were tested. Fifty  $\mu$ L were incubated in duplicate in each well with 3 nM of  $^{125}$ I-echistatin solution for 2 hours incubation time at room temperature.

**In vivo imaging:** Nude mice NMRI nu / nu carriers brain tumor U87 3 mm in diameter were imaged during 120 min with a multi- gradient echo sequence ( TR 45 ms , TE 5 from 3 to 27 ms , 40 ° angle , Tacq 23 minutes) on a 2.35 T MRI (Bruker - Ettlingen) . The  $R_2^*$  maps were made with an homemade software (GOA) and the blind qualitative and quantitative analyzes ( $R_2^*$  average on overall tumor) were conducted by two different readers. The study is divided into two parts

**Proof of specificity:** Comparison between the test group (n = 6) injected with the USPIO contrast agent (P04000) functionalized with rgd motif to target avb3 integrin and control group (n = 6) injected with non targeted USPIO (P03999).

**Monitoring treatment :** Comparison of test group ( n = 6) treated with IP injection of bevacizumab (Avastin® ) at a dose of 5 mg/kg and control group ( n = 6) injected with saline . All animals were injected with the P04000 at a dose of 200  $\mu$ mol/kg.

### Results:

#### Binding: (fig 1)

Echistatin and RGD-targeted P04000 emulsion inhibited specific  $^{125}$ I-echistatin binding in a concentration-dependent manner with  $IC_{50}$  of, 0.6 nM, and 2.3 pM respectively. Non targeted-RGD emulsion P03999 did not displace specific  $^{125}$ I-echistatin binding.

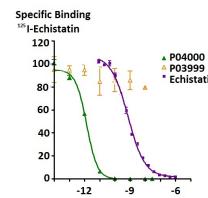


Fig 1

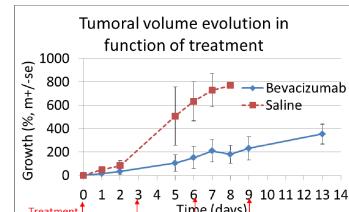


Fig 2

#### Proof of specificity: (fig 3, 4)

The contrast uptake in the tumor is continuing with targeted contrast agent while the reference product is eliminated

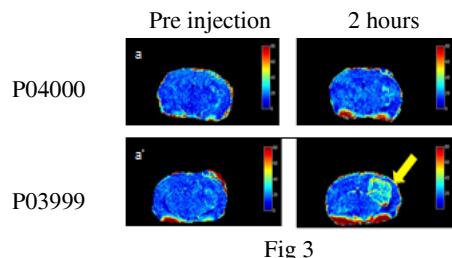


Fig 3

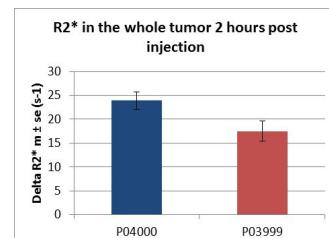


Fig 4

#### Monitoring treatment

##### (Fig 5)

No contrast uptake in bevacizumab treated group and persistent enhancement in the control group.

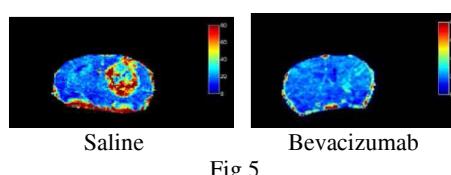


Fig 5

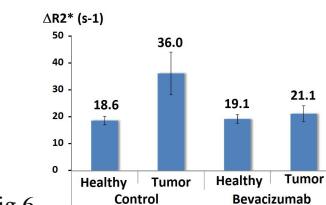


Fig 6

#### Discussion / Conclusion :

This study demonstrates the feasibility of molecular imaging with a high relaxivity contrast agent coupled to a high affinity rgd motif. The short diagnosis delay (2 hours) is allowed by stealth of the contrast agent (7 minutes half- life) while vascular compartment remaining agents require a longer delay [1]. The absence of binding post anti-angiogenic treatment contrast is consistent with the histologically observed extinction of the target (data not shown). The response to treatment 48 hours after a single injection of bevacizumab is earlier than the tumoral growth parameter (fig2). The next step of this work will be one hand to optimize the dose and diagnostic time and compare the response to treatment, in terms of earliness, to other potential techniques like DCE MRI and DWI . This MR contrast agent is able to early demonstrate the effect of anti-angiogenic therapy with feasible MRI sequence and data post processing in humans and in a compatible clinical imaging time.

#### References:

[1] Chen et al. Triblock copolymer coated iron oxide nanoparticle conjugate for tumor integrin targeting. *Biomaterials* 30 (2009) 6912–6919