In vivo Imaging of Tumor Hypoxia using ^{19}F MRS of Trifluoromisonidazole

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Introduction: Tumor treatment response and patient survival have been related to tumor hypoxia [1]. Thus, different methods are currently evaluated in their ability to measure tumor hypoxia *in vivo* [2-4]. Here, we investigate in two cancer animal models the extent and distribution of tumor hypoxia as a function of tumor growth and in response to changes in oxygenation using *in vivo* ^{19}F MRS/MRSI of Trifluoromisonidazole (TF-MISO).

Material and Methods: We studied tumors from the mouse mammary cancer MCa implanted in the right hind foot of C3H/He mice and the rat prostate cancer cells, R3327-AT, implanted in the right hind leg of athymic nu/nu mice [5]. *In vivo* ^{1}H MRI, ^{19}F MRS and ^{19}F MRSI experiments were performed on a Bruker 7T Biospec MR spectrometer when tumor volumes, as determined by caliper measurement, reached approximately 250 mm^3, 500 mm^3, and ≥700 mm^3 for R3327-AT leg tumors, and 150 mm^3, 250 mm^3, 600 mm^3 for MCa foot tumors. Each animal underwent between one and three MR studies. Following i.v. tail vein injection of 75 mg/kg TF-MISO, the animal was placed into the magnet using a customized animal holder and home-built MR coils adjusted to the different tumor sizes. The animal body temperature was maintained at 37ºC as described previously [5]. The different gases (air, carbogen or pure oxygen) with isoflurane mixed in were administered via a face mask. The water signal was shimmed to a line-width of 40-70 Hz, and ^{1}H MR-images were acquired to assess the tumor anatomy. ^{19}F MRS of the entire tumor and localized ^{19}F MR spectra were acquired after intratumoral TF-MISO levels reached steady state (between 1.5h and 4h post TF-MISO injection) and TF-MISO plasma levels had decreased to below MR detection (plasma half life of TF-MISO ~ 32 min, data not shown). The ^{19}F MR data were processed and analyzed using XsOsNMR. The TF-MISO signal was fitted in the time domain after applying a line-broadening factor of 20Hz to the FID and was quantified as described previously [5]. For *ex vivo* analysis, tumors were excised after the injection of the hypoxia marker pimonidazole (60 mg/kg) and OCT embedded. Sections of the fresh-frozen tumors were stained for pimonidazole (hypoxia), DAPI (nuclei) and hematoxylin/eosin (necrosis).

Results: Intratumoral TF-MISO levels reach a steady state after ~ 1.5 h postinjection and remain stable up-to 5h in both tumor models (Fig. 1). In R3327-AT leg tumors below 600 mm^3, the intratumoral TF-MISO concentration was significantly lower in animals breathing 100% O_2 than in animals breathing 21% O_2, whereas for tumors ≥700 mm^3, TF-MISO levels were low and not influenced by the change in applied oxygen percentage (Fig. 2, left panel). Intratumoral TF-MISO increased moderately in R3327-AT tumor-bearing animals breathing 21% O_2 with tumor growth for small to medium-sized tumors and dropped significantly for tumors ≥700 mm^3 (Fig. 2, left panel). In MCa foot tumors, the intratumoral TF-MISO concentration was unaffected by the change in oxygen percentage the animals were breathing (Fig. 2, right panel). In these tumors, the intratumoral TF-MISO concentration increased moderately from small to medium-sized tumors and leveled out for large tumors (Fig. 2, right panel). Fig. 3 displays a representative TF-MISO distribution across a large MCa foot tumor, with the TF-MISO signal being strongest in the central region of this tumor, indicating the ability to measure localized hypoxia. Pimonidazole and H&E staining of representative tumor tissue sections revealed that R3327-AT tumors develop large necrotic regions surrounded by a rim of hypoxic cells with tumor growth (Fig. 4, left panel). MCa tumors above 200 mm^3 revealed stronger overall staining of pimonidazole indicating the development of tumor hypoxia (Fig. 4, right panel).

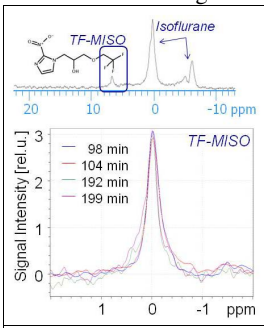

Fig. 1: Top: TF-MISO structure and representative *in vivo* ^{19}F MR spectrum.

Bottom: Representative expanded *in vivo* ^{19}F MR spectra demonstrating the stability of intratumoral TF-MISO after ~ 1.5 h postinjection.

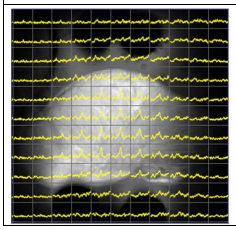

Fig. 3: Expanded spectral region of TF-MISO in localized ^{19}F MR spectra (2D weighted CSI, 1.56 mm x 1.56 mm in-plane, 3 mm slice) overlaid on ^{1}H MR image (3 mm slice) depicting a 580 mm^3 MCa foot tumor.

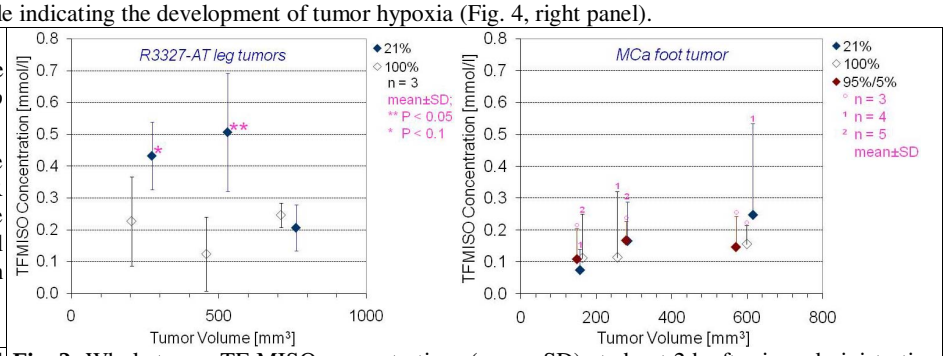

Fig. 2: Whole tumor TF-MISO concentrations (mean±SD) at about 2 h after i.v. administration of 75 mg/kg TF-MISO in dependence of tumor size and administered oxygen percentage. Anesthetized animals breathing either 21%O_2 (blue diamonds), 100% O_2 (open diamonds) or 95% O_2 / 5% CO_2 (red diamonds).

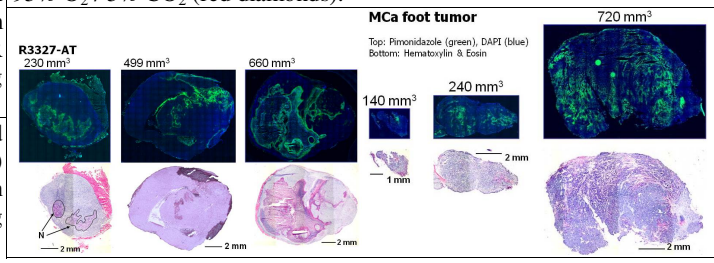

Fig. 4: Representative tissue sections stained for pimonidazole (green), DAPI (blue), and H&E (bottom panel), depicting hypoxia and necrosis for different tumor sizes and model.

Conclusions: In 2 tumor models, TF-MISO could reproducibly measure (i) whole-tumor hypoxia (ii) localization of tissue hypoxia with a spatial resolution of 1.56 x 1.56 x 3 mm^3 (iii) the effect of the breathing of different oxygen percentages on tumor hypoxia. Currently, we are working further on the quantification to account for B_1 variations across the MR coils. Our results suggest that hypoxia imaging by ^{19}F MR of TF-MISO may be useful in identifying tumors that can be successfully reoxygenated and, thus, sensitized for radiation therapy.

References: [1] Varlotto, J. and M.A. Stevenson, Int J Radiat Oncol Biol Phys, 2005. **63**(1): p. 25-36. [2] O'Donoghue, J.A., et al., Int J Radiat Oncol Biol Phys, 2005. **61**(5): p. 1493-502. [3] Rasey, J.S., et al., Int J Radiat Oncol Biol Phys, 1996. **36**(2): p. 417-28. [4] Salmon, H.W. and D.W. Siemann, Radiother Oncol, 2004. **73**(3): p. 359-66. [5] Procissi, D., et al., Clin Cancer Res, 2007. **13**(12): p. 3738-47.

Acknowledgments: Supported by NIH grants PO1 CA115675, R24 CA83084 and NCI P30 CA0874 (Cancer Center Support Grant). We like to thank Dr. D.C. Shungu and X. Mao for the software XsOsNMR.