Trifluoromisonidazole Detects Hypoxia - An *In Vivo* and *In Vitro* Multimodality Study

E. Ackerstaff[1], M. Suehiro[1], N. Kruchevsky[1], S. Carlin[1], E. H. Rosenfeld[1], P. Burgman[1], G. Yang[1], G. Torchon[1], O. Ouerfelli[1], P. B. Zanzonico[1], K. L. Zakian[1], C. C. Ling[1], and J. A. Koutcher[1]

[1]Memorial Sloan-Kettering Cancer Center, New York, NY, United States

Introduction: As the response of tumors to treatment and patient survival have been related to tumor hypoxia [1], different avenues are being currently investigated in their ability to detect and localize tumor hypoxia *in vivo* [2-5]. Here, we investigate the ability of Trifluoromisonidazole (TFmiso) to measure tumor cell hypoxia *in vivo* by ^{19}F MRS/MRSI and *in vitro* by ^{18}F-TFMISO uptake as a function of oxygen tension.

Material and Methods: We studied tumors from the rat prostate cancer cell line, Dunning R3327-AT, implanted in the right hind leg of athymic nu/nu mice [6]. To measure TFmiso uptake *in vivo*, ^{19}F MRS/MRSI (Fig. 1A) was performed following *i.v.* tail vein injection of 75 mg/kg TFmiso on a Bruker 7T Biospec MR spectrometer when tumor volumes reached ~ 250 mm^3, 500 mm^3, and ≥700 mm^3 [7]. Different gases (air - ~21% O_2, 100% O_2, or carbogen - ~95% O_2) plus isoflurane mixed in were administered via nosecone inhalation to change tumoral oxygen. The TFmiso signal was fitted and quantified [7]. We have developed the bimodality imaging MR/PET agent ^{18}F-TFmiso [8], allowing us to validate *ex vivo* the accumulation of TFmiso in hypoxic tumor areas and to quantify TFmiso uptake *in vitro*. For *ex vivo* analysis, tumors were excised after the injection of the hypoxia, immunohistochemical 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). Tumors from mice injected with ^{18}F-TFmiso also underwent ^{18}F digital autoradiography (DAR) prior to staining. To measure the TFmiso uptake *in vitro* as a function of oxygen tension, three prostate cancer cell lines, R3327-AT, PC-3, and LNCaP, were incubated with ^{18}F-TFmiso for 30 min, 60 min, and 120 min with 70 mM ^{18}F-TFmiso (1-3 µCi/ml) at 0%, 0.05%, 0.1%, 0.5%, 1%, and 21% O_2, the cells lysed at the end of incubation, and ^{18}F activity counted in the cell lysates, following previously published methods [9]. Where residual ^{18}F-activity permitted, the amount of protein-bound (i.e. TCA precipitable) ^{18}F-TFmiso as a fraction of total ^{18}F-TFmiso was determined.

Results: In R3327-AT leg tumors of ~500 mm^3, intratumoral TFmiso concentrations were significantly lower in animals breathing 100% O_2 than in animals breathing 21% O_2 (Fig. 1B). Intratumoral TFmiso decreased moderately in R3327-AT tumor-bearing animals breathing 21% O_2 with tumor growth (Fig. 1B). Intratumoral TFmiso was significantly lower for tumors ≥700 mm^3 than for small tumors (~250 mm^3, Fig. 1B). Fig. 1C shows the structure of ^{18}F-TFmiso. As can be seen by the spatial concordance of the ^{18}F-TFmiso distribution in the DAR image (Fig. 1, D1) and the pimonidazole staining of the same tissue section (Fig. 1, D2), TFmiso accumulates in hypoxic tumor cells in a manner similar to that of pimonidazole. Our *in vitro* studies demonstrated that ^{18}F-TFmiso uptake increased with decreasing [O_2] and increasing incubation time, with no specific uptake at 21% O_2.

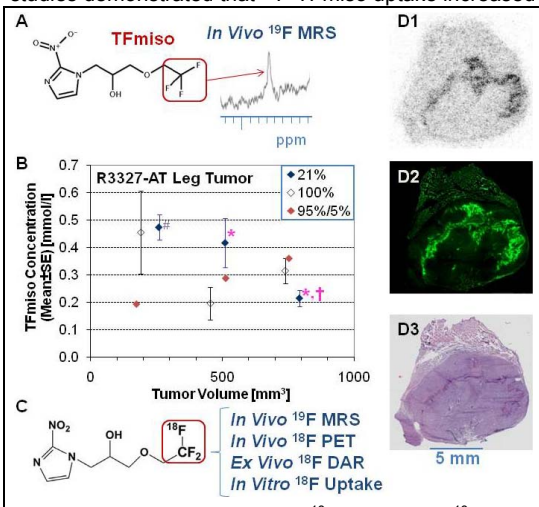

Fig. 1: A The structure of TFmiso and a representative *in vivo* ^{19}F MR spectrum. **B** Whole-tumor TFmiso concentrations at ~ 2 h after *i.v.* administration of 75 mg/kg TFmiso as a function of tumor size and % administered oxygen. Anesthetized animals breathing either 21%O_2 (blue diamonds, n=5, #n=6), 100% O_2 (open diamonds, n=5) or 95% O_2 / 5% CO_2 (red diamonds, n=1). *P<0.1, 21% *vs.* 100%; †P< 0.05, 21% large *vs.* small tumors. **C** ^{18}F-TFmiso. **D** ^{18}F-TFmiso (**D1**) and pimonidazole (**D2**) distribution, as well as necrosis (darkest purple areas in **D3**) in a representative R3327-AT leg tumor (269 mm^3), demonstrating the concordance of TFmiso- and pimonidazole-positive tumor areas in the peri-necrotic areas.

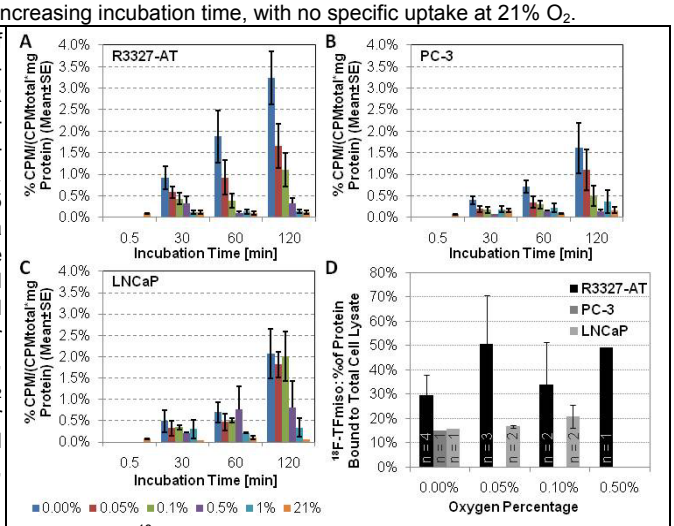

Fig. 2: A-C ^{18}F-TFmiso uptake/ mg cellular protein for R3327-AT, PC-3, and LNCaP. **D** In all cases, less than 50% of intracellular ^{18}F-TFmiso is protein bound after 120 min incubation time.

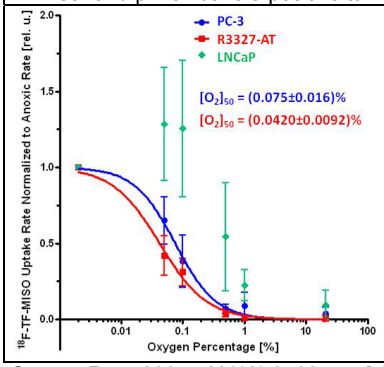

Fig. 3: The uptake rate normalized to the anoxic rate for ^{18}F-TFmiso and the $[O_2]_{50}$ were not significantly different between R3327-AT (n=5) and PC-3 (n=5). $[O_2]_{50}$ could not be dcalculated for LNCaP due to large variations for the small number of experiments (n=3).

Discussion: TFmiso could reproducibly measure whole-tumor hypoxia and the effect of the breathing of different oxygen percentages on tumor hypoxia. Similar to the hypoxia marker pimonidazole, TFmiso is taken up preferentially at [O_2] < 1% with the highest uptake in anoxic cells. The uptake and intracellular protein binding of TFmiso is to some extent cell line dependent, which may be explained by differences in nitroreductase activities among various cell lines [5]. **Conclusion:** Hypoxia imaging by multimodality imaging of TFmiso 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):25-36. [2] O'Donoghue, J.A., *et al.*, Int J Radiat Oncol Biol Phys, 2005. **61**(5):1493-502. [3] Rasey, J.S., *et al.*, Int J Radiat Oncol Biol Phys, 1996. **36**(2):417-28. [4] Salmon, H.W. and D.W. Siemann, Radiother Oncol, 2004. **73**(3):359-66. [5] Ljungkvist, A.S., *et al.*, Radiat Res, 2007. **167**(2):127-45. [6] Procissi, D., *et al.*, Clin Cancer Res, 2007. **13**(12):3738-47. [7] Ackerstaff, E. *et al.*, Proc ISMRM 2009. #196. [8] Suehiro, M., *et al.*, J Labelled Comp Radiopharm, 2009. 52(Suppl 1):S3. [9] Burgman, P., *et al.*, Nucl Med Biol, 2005. **32**(6):623-30. **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, Mihai Coman (deceased) and Sean Burke for their technical assistance.