A theranostic probe to image choline kinase expression and inhibition in a breast cancer model

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TARGET AUDIENCE. Clinicians and basic scientists interested in preclinical cancer imaging and molecular imaging.

PURPOSE. Heightened Choline Kinase (ChoK) activity has been reported in nearly 40% of human breast cancers, indicating that a large cohort of patients can benefit from therapies designed to exploit aberrant choline metabolism[1]. ChoK is an oncogene whose overexpression provides cancer cells with growth factors and biosynthetic precursors. With the development of ChoK inhibitors, MRS has been implemented to validate treatment efficacy. We have developed Near Infrared Fluorescence (NIRF) imaging agents, such as JAS239, that act as competitive inhibitors of ChoK[2] and allow for the assessment of ChoK expression complementary to the metabolite levels detected by MRS. Here we employ JAS239 as a companion diagnostic for evaluating ChoK-targeted treatments.

METHODS. Human breast cancer cell lines (MCF7) modified to overexpress ChoK (CK+) or an empty vector (EV) were provided by Drs. Zaver Bhujwalla and Tariq Shah[3]. Mice were inoculated with CK+ and EV cells on the left and right mammary fat pads, respectively. When tumors reached ~200 µL, 20 nmoles JAS239 in saline was injected i.v. At 24 h, the left and right fat pads were surgically exposed and imaged for NIRF (Ex. 745 nm, Em. 820 nm) using an IVIS Spectrum. A second cohort of mice were inoculated with a mouse-adapted human-derived triple-negative breast cancer cell line (4175-Luc+) and xenografts were allowed to grow to 200 µL. Baseline MRS was performed on a 9.4T horizontal bore magnet with T_2-weighted anatomical images used to plan the MRS voxel. A 3x3x3 mm^3 voxel was placed within the tumor and a spectrum was acquired using a PRESS sequence with the following parameters: TR=3000 ms, TE1=12.68 ms and TE2=10.01 ms, number of averages=128, complex points=4096 and spectral width=4000 Hz resulting in an acquisition time of 6 min 24 sec. Water suppression was performed using the VAPOR technique. An unsuppressed water spectrum was acquired to serve as a concentration reference. In one set of experiments, mice were injected i.p. with saline or 2 mg/kg of the ChoK inhibitor MN58b for 5 consecutive days. Post-treatment MRS and NIRF imaging were performed as described above. In a second set of experiments, mice bearing MDA-MB-231 tumors were treated with therapeutic doses of JAS239 (4 mg/kg for 5 days) and imaged at 7 days. MR spectra were analyzed in Mnova Lite 5.2.5 software and total choline (3.2 ppm) was quantified relative to unsaturated water (4.7 ppm). Optical imaging data was processed with LivingImage software (Perkin Elmer). ROIs were drawn around tumors and the Average Radiance Efficiency was measured.

RESULTS.

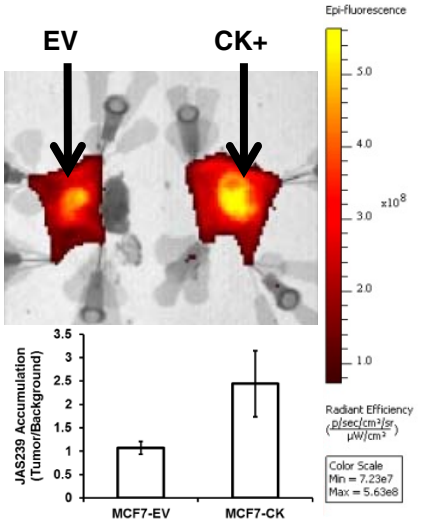

Figure 1. A significant 2.5-fold increase in JAS239 fluorescence in CK+ tumors compared to control EV tumors. Values represent mean ±SEM for 9 mice. $p < 0.05$.

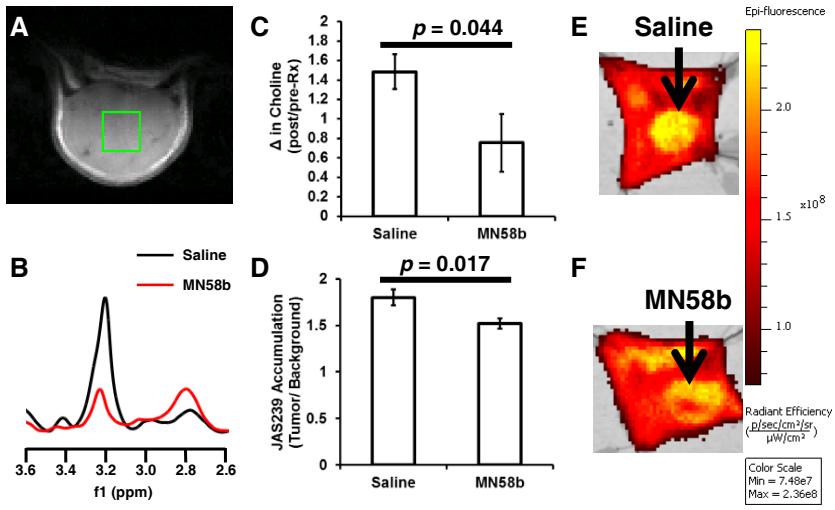

Figure 2. (A) Representative voxel selection in a T_2-weighted image of a 4175-Luc+ tumor and (B) ^{1}H spectra of saline or MN58b-treated tumors expanded to show choline region. (C) MN58b significantly lowered total choline (3.2 ppm) levels. (D) MN58b caused significant reduction in NIRF when (E) saline-treated tumors were compared to (F) ChoK-inhibited tumors. Values represent mean ±SEM of at least 4 mice per cohort.

DISCUSSION. By measuring the accumulation of JAS239 via NIRF imaging, we were able to distinguish tumors with high (CK+) vs. moderate (EV) ChoK levels. JAS239 was capable of delineating tumor boundaries even in EV tumors (Fig 1). To test the use of this imaging probe in conjunction with an established ChoK inhibitor, triple-negative breast tumor models were treated with MN58b or saline. MRS was used to measure a significant reduction in total choline in response to ChoK inhibition (Fig 2A-C). NIRF imaging showed that JAS239 accumulation was also reduced following treatment (Fig 2D-F), providing an additional method capable of distinguishing MN58b-treated from saline-treated tumors. Treatment with therapeutic doses of JAS239 (4 m..kg for 5 days) caused a significant reduction in tCho levels in MDA-MB-231 tumors as well as significant tumor growth delay (not shown).

CONCLUSION. JAS239 was able to identify ChoK-overexpressing tumors that may respond better to ChoK-targeted therapeutics. In conjunction with MRS, NIRF imaging can overcome some of the complications faced when validating ChoK inhibitors *in vivo*.

REFERENCES 1. Ramirez de Molina, A. *et al.*. *Oncogene* **21**, 4317-4322 (2002). 2. Arlauckas, SP. *et. al. Mol Can Therapeutics*, 3(9); 2149–58. 3. Shah, T. *et al.*. *NMR in Biomed* **23**, 633-642 (2010).