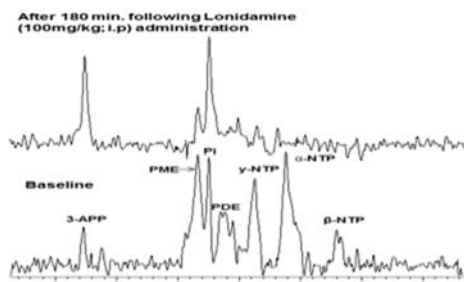


# Lonidamine sensitizes human breast cancer xenografts to Doxorubicin via metabolic modulations

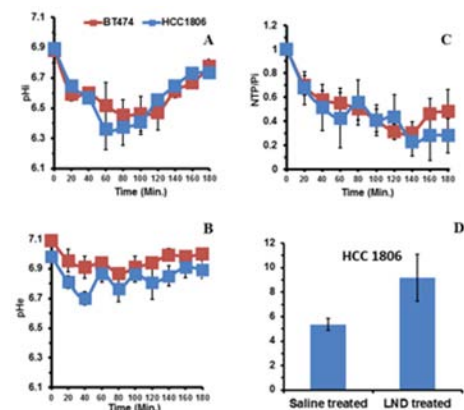
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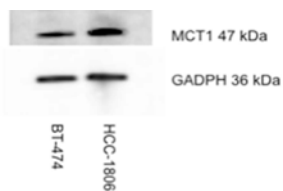
**Introduction:** Breast cancer is the second most common newly diagnosed cancer after skin cancer and second leading cause of cancer death among women (lung cancer is 1st) in the United States (1). Based on their molecular signatures, invasive breast cancers can be classified into 4 subtypes: luminal A and B (both of which are positive for hormone receptors), HER-2 and basal-like (triple negative) cancer. Treatment options for patients with breast cancer include surgery, chemo, radiation, hormone and targeted therapies. Chemotherapy of metastatic breast cancer is effective initially but cannot eradicate the tumor; most patients eventually develop drug resistance. The need for more effective breast cancer management motivates the search for new adjuvant agents that potentiate chemotherapeutic drugs while reducing drug resistance. By exploiting the natural tendency of breast and other cancers to convert glucose to lactate (Warburg effect), we demonstrate that a small



**Fig. 1.** *In vivo* localized (Image Selected *In vivo* Spectroscopy - ISIS) <sup>31</sup>P phosphorus magnetic resonance spectroscopy spectra of HCC1806 breast cancer xenograft grown subcutaneously in nude mice (lower) Baseline- and (upper) 180 min. post LND administration (100 mg/kg, i.p.).



**Fig. 2.** *In vivo* intracellular pH (pHi) (A), extracellular pH (pHe) (B), bioenergetics (NTP/Pi) (C) profile as a function of time (n=3) in response to LND (100 mg/kg; i.p.) administered at time zero. High resolution <sup>1</sup>H NMR of perchloric acid extract of HCC1806 tumors (n=3) 40 min. post LND administration in comparison with saline controls (n=2) (D). The values are presented as mean ± S.E.M.



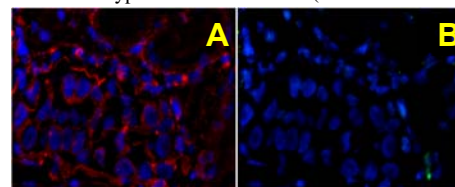
**Fig. 3.** Western blot for MCT-1 expression by human breast cancer cell lines.

molecule, lonidamine (LND) (2), can sensitize breast cancers to doxorubicin via selective intracellular acidification and suppression of high energy phosphate production in the tumor.

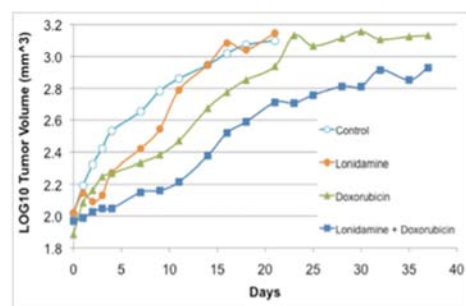
**Material and Methods:** A basal-like human breast cancer line, HCC1806, (referred to as triple negative breast cancer, TNBC) and a line, BT-474, that is positive for ER and over-expresses Her2/neu were implanted into female nude mice. Both lines were obtained from ATCC and were maintained in DMEM culture medium supplemented with 10% fetal bovine serum and 0.5% penicillin/ streptomycin at 37°C in 5% CO<sub>2</sub>. Cells in exponential growth phase were harvested; 10<sup>6</sup> HCC1806 cells suspended in 100 μL culture medium were inoculated in the flank of the mouse. Before inoculation of BT-474 cells, a 60-day release pellet was implanted subcutaneously around the neck region with a 10 gauge precision trocar. One week later, 10<sup>7</sup> BT-474 cells suspended in 200 μL of culture medium were inoculated in the flank of the mouse. HCC1806 is a rapid growing tumor, taking one week to become palpable (3 mm in diameter). In contrast, BT-474 grows much slower, taking about 2-3 weeks to become palpable. Tumors were measured twice a week by caliper and their volumes estimated by the formula:  $V = (axb^2)\pi/6$ , where a and b are two orthogonal diameters of the tumor, a>b. Breast tumor xenograft development (n=3), intracellular pH (pHi), extracellular pH (pHe) and bioenergetics (βNTP/Pi) measurements as well as lactate levels were determined on a 9.4 T horizontal-bore Varian MR spectrometer as described elsewhere (2). LND (100 mg/kg; i.p.) was injected intraperitoneally. High resolution NMR of tumor extracts was performed with a 9.4 T vertical-bore Bruker magnet. Four cohorts of age- and weight-matched animals were randomized to the different treatment groups with the same protocol as described in a previous publication (2). The pHi, pHe and bioenergetics data at time points following LND administration were compared by nonparametric Mann-Whitney statistical analysis and lactate by t-test analysis (SPSS 16). To assess the significance of treatment effects, maximum regrowth doubling time was calculated and used to calculate tumor growth delay.

**Results:** A representative localized <sup>31</sup>P MR spectrum of human HCC1806 xenografts before (Lower) and after (upper) LND administration is shown in Fig. 1. LND treated tumors exhibited a delta pHi of  $0.54 \pm 0.23$  (p < 0.05),  $0.44 \pm 0.14$  (p = 0.05) in HCC1806 and BT474, respectively (Fig. 2A), and a slight decrease in delta pHe of  $0.28 \pm 0.19$  (p = 0.05),  $0.22 \pm 0.07$  (p = 0.05) in HCC1806 and BT474, respectively (Fig. 2B). A remarkable depletion of high energy phosphate stores in tumors (βNTP/Pi normalized to the baseline) by  $77.0 \pm 0.09\%$  (p < 0.05) was observed in HCC1806 and  $70.0 \pm 0.12\%$  (p > 0.05) in BT474 following LND administration (Fig. 2C). An approximate 3-fold (p < 0.05) increase in lactate was detected in the LND treated HCC1806 tumor extracts compared to saline treated controls (Fig.

2D). Western-blot (Fig. 3) and immunostaining (Fig. 4) confirmed the expression of MCT-1 in tumor tissues. Treatment with LND increased breast cancer (HCC 1806) response to DOX (12 mg/kg, i.v.) (Fig. 5). Results of tumor growth delay following LND and DOX treatment are summarized in table 1. **Discussion:** LND exhibits a lack of dose-limiting myelotoxicity (bone marrow suppression) associated with typical antineoplastic agents. Data presented here suggest that LND potentiates responses of breast cancers to doxorubicin through intracellular acidification and perturbation of energy production of the tumor. **Acknowledgements:** This study is supported by grant 1-R01-CA-172820 01A1, 5-R01-CA129544 04; DoD: W81XWH-10-1-0320 and W81XWH-10-1-0604 **References:** (1). Cancer facts and figures, 2013. (2). Nath K et al. NMR Biomed 26(1); 98-105, 2013.



**Fig. 4.** Immunostaining of xenograft tissues of human BT-474 for MCT-1. **A:** overlay of MCT-1 (red) and nuclei (DAPI, blue). **B:** DAPI staining only. Positive staining of MCT-1 in cell membrane and cytosol surrounding the nuclei is evident.



**Fig 5.** Tumor volume vs. time for HCC1806 tumor xenografts treated with saline (control), LND (100 mg/kg, i.p.), doxorubicin (12 mg/kg, i.v.) and doxorubicin plus LND.

Treatment	Growth Delay (Days)	Doubling Time (Days)	log <sub>10</sub> cell kill
Control	0.0	2.0	0.00
Lonidamine	0.0	2.5	0.00
DOX	7.0	4.4	0.49
Lonidamine + DOX	23.0	4.2	1.70

**Table. 1.** Calculated growth delay, maximum regrowth doubling time and log<sub>10</sub> cell kill in mouse xenografts of HCC1806 human breast.