Selective acidification and de-energization of WM983B melanoma xenografts and sensitization to doxorubicin following lonidamine administration

Kavindra Nath[1], David S Nelson[1], Daniel F Heitjan[1], Rong Zhou[1], Dennis B Leeper[2], and Jerry D Glickson[1]
[1]*University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [2]*Thomas Jefferson University, Pennsylvania, United States*

Introduction: WM983B is an established human melanoma xenograft cell line model that expresses the V600D, E, K, BRAF mutation. This characteristic, expressed in about 50% of human melanoma, has been exploited with limited success by treatment with agents that specifically target the mutant BRAF signaling pathway; when used alone, these targeted signaling pathway inhibitors yield a transient response (1). Here, we extend to the WM983B model our use of lonidamine (LND; 100 mg/kg; i.p.), an inhibitor of the monocarboxylate transporter (MCT) that blocks cellular export of lactic acid and also inhibits transport of pyruvate into mitochondria, thereby decreasing intracellular pH (pHi) and inhibiting tumor energy production. We have shown that in other human melanoma models LND sensitizes melanomas to treatment with the nitrogen mustard, melphalan (2). Here we demonstrate that decreasing the pHi and bioenergetics status (βNTP/Pi) of WM983B xenografts with LND increases the intracellular activity of doxorubicin (DOX) presumably by increasing uptake of this antineoplastic agent. Treatment with LND plus DOX could be combined with targeted BRAF inhibition.

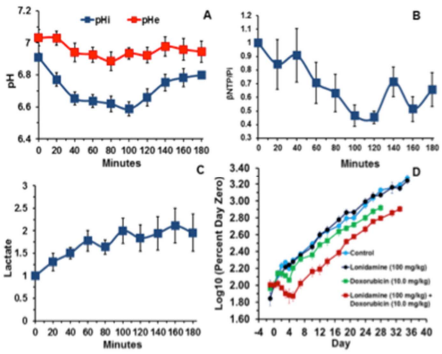

Figure. 2. (**A**). The intracellular pH (pHi) (n=7), extracellular pH (pHe) (n=5) profile as a function of time. **(B).** The changes of NTP/Pi (ratio of peak area) relative to baseline (n=7) **(C).** Change in tumor lactate as a function of time. Area under the curve was compared to baseline at each time points and was normalized to baseline levels as a function of time in response to LND (100 mg/kg; i.p.) administered at time zero. **(D).** Tumor Growth Delay experiment performed on 4 cohorts.

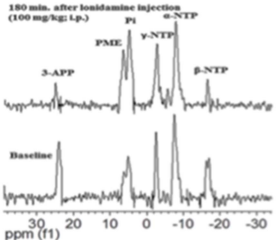

Figure. 1. *In vivo* localized (Image Selected In vivo Spectroscopy - ISIS) ^{31}Phosphorus magnetic resonance spectroscopy spectra of WM983B human melanoma xenograft grown subcutaneously in nude mice (lower) pre- and (upper) 180 min. post LND administration (100 mg/kg, i.p.).

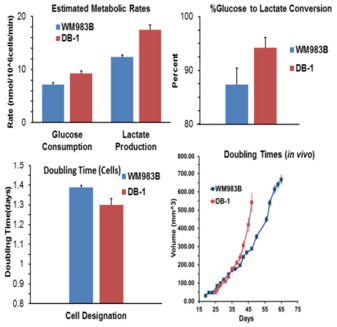

Figure. 3. (**A**). Estimated metabolic rates as glucose consumption and lactate production in DB-1 and WM983B cells. **(B).** Percent of glucose to lactate conversion in DB-1 and WM983B cells **(C).** Doubling times of DB-1 and WM983B cells (D). Doubling times of DB-1 and WM983B melanoma xenografts.

Material and Methods: WM983B human melanoma cells were obtained from the Wistar Institute and were grown in DMEM supplemented with 25 mM glucose, 2 mM glutamine, 10 mM HEPES, 100 Units/mL penicillin, 100 µg/mL streptomycin, and 10% FBS. 10 x 10^6 cells/ml, and inoculated subcutaneously in each mouse as a 0.1 mL suspension. pHi (n=7), extracellular pH (pHe) (n=5), βNTP/Pi (n=7) and steady-state levels of tumor lactate (n=5) estimation were measured by ^{31}P MRS (pH and bioenergetics) and ^{1}H MRS (Hadamard-selective multiple quantum coherence transfer pulse sequence), respectively, as described in our previous publication (2). Treatment response was measured by tumor growth delay analysis; four cohorts of five age- and weight-matched animals were randomized to the following treatment groups: cohort 1 (sham treated control) was infused intravenously (i.v.) with PBS and given appropriate sham intraperitoneal (i.p.) injections of tris/glycine buffer; cohort 2 was infused i.v. with PBS 40 min after LND administration i.p. (100 mg/kg); cohort 3 was injected i.p. with tris/glycine buffer and infused i.v. with doxorubicin (10 mg/kg delivered in ~10 sec) in PBS; cohort 4 was injected i.p. with LND and after 40 min, doxorubicin (10 mg/kg) was infused i.v. The data of pHi, pHe, βNTP/Pi and steady-state lactate (3) of tumors at different times following LND administration were compared using one-way ANOVA with Bonferroni and Tukey multiple comparisons. Growth Delay data analysis was performed as described in previous publication (2). We measured the glucose and lactate concentrations in the DB-1 and WM983B melanoma cells using a YSI 2300 STAT Plus Glucose & Lactate Analyzer calibrated with the YSI 2747 dual standard (10 mM glucose, 5 mM lactate). We made the assumption that the cells were in the logarithmic growth phase and that the metabolic activity in the whole flask was proportional to the characteristic activity of the cell line and to the number of cells in the flask.

Table 1. Estimated growth delay, doubling time (Td), log10 cell kill, and proportion surviving (with nonparametric bootstrap 95% confidence interval) by treatment arm.

	Parameter				
Treatment	Delay (days)	Td (days)	$\log_{10}$ cell kill	Percent surviving	95% CI
Lonidamine	0.5	3.2	0.05	91	(8, 329)
Doxorubicin	-0.9	4.6	-0.06	114	(15, 396)
Lonidamine + Doxorubicin	10.3	10.3	0.30	53	(9, 204)

Results: *In vivo* ^{31}P MRS (Fig. 1) demonstrates that WM983B human melanoma xenografts in immunosuppressed mice treated with LND, 100 mg/kg, i.p.) exhibited a sustained and tumor-selective decrease in pHi from 6.91 ± 0.03 to 6.59 ± 0.05 ($p < 0.001$). pHe exhibited a smaller non-significant decrease from 7.03 ± 0.05 to 6.89 ± 0.06 ($p > 0.05$) (Fig. 2A). Tumor bioenergetics also decreased 55 ± 0.05% ($p > 0.05$) relative to the baseline level and normalized to baseline values (Fig. 2B). The integrated intensities of the steady-state lactate levels of tumors normalized to baseline peaked at 100 min ($p > 0.05$) following LND administration (Fig. 2C). The effects of treatment with LND + DOX were evaluated by tumor growth delay experiments (Fig. 2D). LND + DOX produced a growth delay of 10.3 d (tumor doubling time 10.3d, $\log_{10}$ cell-kill = 0.30, percent surviving = 47%) compared to growth delays of LND alone of 0.5 d and DOX alone of -0.9 d (Fig. 2D and Table 1). WM983B cells have lower metabolic activity and growth characteristics in terms of glucose consumption, lactate production and doubling times respectively (Fig. 3).

Discussion: WM983B human melanoma xenografts treated with LND showed less decrease in pHi and bioenergetics and increase in lactate than DB-1 melanomas reported previously. Treatment with LND sensitized the tumors to Dox to a lesser extent than DB-1 melanoma xenografts. The WM983B tumors are less glycolytic compared to DB-1 tumors, exhibiting less lactate production. Since acute acidification has been reported to enhance the activity of platinum compounds (3) and alkylating agents such as nitrogen (N)-mustards (2, 4-7), we have evaluated the effect of LND-induced acidification on, doxorubicin, one of the most potent commonly used antineoplastic agent. We found that while LND alone and doxorubicin alone had minimal effects on melanoma growth, the combination of these agents administered according to a schedule based on NMR measurement of tumor pHi, substantially decreased the growth rate of this highly malignant tumor. This could provide a method for systemic therapy of this deadly cancer. **Acknowledgements**: NIH grants R01-CA129544 and R01-CA172820. Jeff Roman for performing *in vitro* experiments and Meenhard Herlyn from Wistar Institute, Philadelphia, USA for providing cells. **References:** (1). Flaherty KT, et al. Cancer 2014 Jun 1;120(11):1695-701. (2). Nath K et al. NMR Biomed 26(1); 98-105, 2013. (3) Atema A, et al. Int J Cancer 1993; 166-172. (4) Canter RJ, et al. Ann Surg Oncol 2004; 265-273. (5) Jahde E, et al. Cancer Res 1989; 2965-2972. (6) Kuin A, et al. Br J Cancer 1999; 793-801. (7) Wong P, et al. Clin Cancer Res 2005; 3553-3557.