MRS Pharmacodynamic Markers of a Novel Histone Deacetylase Inhibitor, LAQ824, in a Human Colon Carcinoma Model

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Introduction: LAQ824 is a novel anticancer drug that inhibits histone deacetylase (HDAC), resulting in growth inhibition, cell cycle arrest and apoptosis. The aim of this work was to develop a non-invasive and robust pharmacodynamic biomarker for target inhibition and tumor response following LAQ824 treatment.

Methods: Human HT29 (colon) carcinoma xenografts were examined using *in vivo* ^{31}P-MRS, pre- and after 2 days of LAQ824 treatment (25mg/kg i.p.). Controls were treated with vehicle alone (10% DMSO and 90% of 5% dextrose in water). Extracts of the tumors were also examined by *in vitro* ^{31}P and ^{1}H-MRS. Histone (H3) acetylation, heat shock protein 70 and c-Raf-1 expression were determined using Western blotting. Tumor microvessel density was assessed by immunohistochemical staining of CD31.

Results and Discussion: Significant tumor growth inhibition was observed in HT29 xenografts following 2 days of LAQ824 treatment when compared with vehicle-treated controls. Western blots of the excised tumors showed increased histone H3 acetylation in the LA824-treated group (Fig.1). These results confirm the expected inhibitory effect of LAQ824 on HDAC in HT29 xenografts. c-Raf-1 expression was found to decrease after LAQ824 treatment but the expression of Hsp70 remained unchanged after treatment (Fig.1). *In vivo*, the ratio of phosphomonoester (PME)/total phosphorus (TotP) signal was significantly increased (P=0.003) in LAQ824-treated HT29 xenografts and this ratio was found to correlate inversely with tumor response (r=-0.61, P=0.01). This PME increase is confirmed by the significant rises in phosphocholine (PC) (P=0.04) and phosphoethanolamine (P=0.02) levels observed in ^{1}H- and ^{31}P-MR spectra of LAQ824-treated tumor extracts when compared with controls. These results are consistent with findings from a recent study in a prostate cancer cell line showing that PC levels also increase following HDAC inhibition with a SAHA analogue (*Sankaranarayanapillai et al., Mol Cancer Ther 2006; 5: 1325-34*). Marked decreases in NTP, phosphocreatine (PCr) and an increase in inorganic phosphate (Pi) levels were also found in *in vivo* ^{31}P-MR spectra of LAQ824-treated tumors (Fig.2), where significant decreases in intracellular pH (P=0.02), β-NTP/TotP (P=0.001) and β-NTP/Pi (P=0.003) ratios and an increase in Pi/TotP ratio (P=0.0002) were observed. No significant changes were found in vehicle controls. This observation indicates that tumor bioenergetics are severely compromised following treatment. Elevated free choline (P=0.01), leucine (P=0.005), iso-leucine (P=0.005) and valine (P=0.04) levels and reduced glycerophosphocholine (P=0.02), glycerophosphoethanolamine (P=0.05), glutamate (P=0.04), glutamine (P=0.03), glucose (P=0.001) and PCr and creatine (P=0.05) levels were found in LAQ824-treated HT29 tumor extracts when compared with controls and these metabolic changes are consistent with impaired tumor bioenergetics. A marked reduction of CD31 staining was found in LAQ824-treated tumors indicating reduced vessel density in the LAQ824-treated group when compared with controls (Fig.3). The effects that we observed in tumor bioenergetics, the metabolic changes and the vascular changes are likely to be caused by tumor vessel shutdown following the vascular disruptive effect of LAQ824 on HT29 tumors.

Conclusions: Inhibition of HDAC by LAQ824 resulted in altered phospholipid metabolism and compromised tumor bioenergetics. The PC and phosphomonoester increases and metabolic changes associated with compromised tumor bioenergetics may have the potential to act as non-invasive pharmacodynamic markers for determining tumor response following treatment with LAQ824 or other HDAC inhibitors.

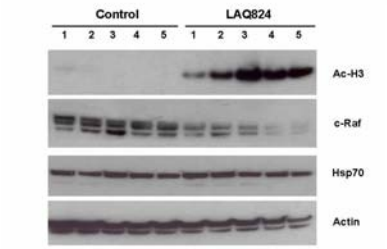

Fig. 1: Western blots for histone (H3) acetylation and c-Raf-1 and Hsp70 expressions are shown in HT29 xenografts following daily (x2) i.p. injection of vehicle and LAQ824.

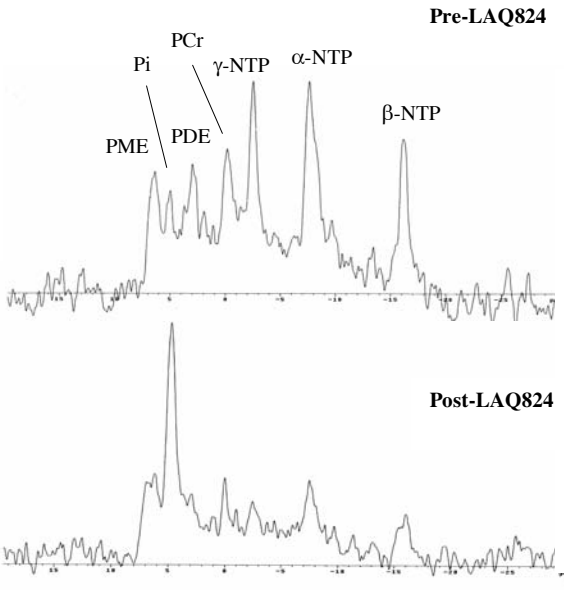

Fig. 2: *In vivo* ^{31}P-MR spectra of a HT29 tumor before and after 2 days of LAQ824 treatment. Peak assignments were as follows: phosphormonoesters (PME), phosphodiesters (PDE), inorganic phosphate (Pi), phosphocreatione PCr), nucleoside riphosphate (α-, β-, γ-NTP).

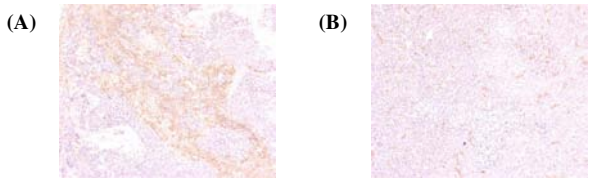

Fig. 3: Immunohistochemical staining of CD31 for endothelial cells are shown (blood vessels are in brown) in HT29 xenografts following daily (x2) i.p. injection of vehicle (A) and LAQ824 (B). Magnification, x4.

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