**Bortezomib Treatment Reduces Tumor Blood Flow and Perfusion as Measured by Dynamic Contrast-Enhanced 1H MRI**


1Memorial Sloan-Kettering Cancer Center, New York, NY, United States, 2Shandong Cancer Hospital and Institute, Jinan, Shandong, China, People's Republic of, 3Deceased

**Introduction:** The proteasomes inhibitor Bortezomib possesses, clinically and pre-clinically, anti-angiogenic and anti-tumor properties [1] and appears to selectively interfere in the hypoxia pathway [2]. Our study aims to determine biomarkers characterizing treatment response by investigating in a colorectal cancer model the effects of Bortezomib on the tumor vasculature in vivo and on the tumor hypoxia response ex vivo.

**Materials and Methods:** The preclinical tumor model was the human colorectal cancer xenograft model HT29-9HRE-TKeGFP, containing a hypoxia-inducible dual reporter fusion gene (HSV1-TK and eGFP) under the control of a hypoxia response element (HRE), implanted in the right flank of athymic nu/nu mice. Tumor blood flow/perfusion was evaluated by dynamic contrast-enhanced magnetic resonance imaging (DCE MRI) before and after treatment with Bortezomib (Fig. 1). DCE MRI experiments were performed using a home-built, solenoidal 1H MR coil on a Bruker 7T BioSpin MR spectrometer (Bruker, Germany). T2-weighted DCE MRI was performed at ~4.3 s temporal resolution and ~117 µm x 117 µm in-plane resolution. The contrast agent Gd-DTPA was injected via the tail vein after 2 min of acquisition followed by 20 min of dynamic acquisition. After the pre-treatment DCE MRI, the animals were randomly assigned to one of 3 groups: (i) untreated controls, treated with (ii) 1 dose (2.0 mg/kg) or (iii) 2 doses (1.5 mg/kg administered 24 h apart) of Bortezomib (Fig. 1). Each animal underwent 3 sequential DCE MRI experiments (day 0, 1, and 2) with spin density MR images facilitating tumor slice alignment of baseline DCE MRI with subsequent DCE MRI at 24 h and 48 h. The time-signal curves, obtained by DCE MRI, were normalized with respect to the initial 2 min of acquisition without contrast agent, fitted voxel-by-voxel using the Hoffman model [3] and Akep maps were generated for the corresponding tumor slices for all 3 time points. The Akep value is considered an approximate measure of vascular flow/perfusion [3]. To quantify the perfusion changes due to Bortezomib treatment, the median Akep value of each tumor was calculated from whole-tumor Akep histograms. For ex vivo evaluation of tumor perfusion and hypoxia, the perfusion marker Hoechst 33342 and the hypoxia marker pimonidazole were administered after the 48 h DCE MRI, followed by tumor excision. Pimonidazole and Hoechst 33342 distribution, and the endogenous hypoxia markers eGFP and CA-9 were assayed in tumor tissue sections by fluorescence microscopy.

**Results:** The MRI slices of the tumors for the 24 h and 48 h DCE MRI could be realigned reproducibly with the baseline DCE MRI, although better alignment was associated with smaller changes of tumor size (data not shown). Within the control group, whole-tumor median Akep values remained constant during the 3 days of the experiments, whereas Bortezomib treatment interrupted tumor blood flow/perfusion as evident from representative Akep maps obtained at 24 h in the 1-dose group and at 24 h and 48 h in the 2-dose group (Fig. 2). Ex vivo, central regions of tumors treated with 2 doses of Bortezomib had lower Hoechst 33342 and pimonidazole staining, and expressed less hypoxia-induced eGFP and CA-9 than control tumors, whereas in the tumor rim pimonidazole staining and CA-9 expression appeared to be increased (Fig. 4). The ex vivo staining patterns of Hoechst 33342, pimonidazole, eGFP and CA-9 after 1 dose of Bortezomib (Fig. 4) reflect a recovery towards patterns observed in controls, matching the behavior of tumor perfusion in vivo (Fig. 2, 3).

**Conclusion:** Our data suggest that Bortezomib treatment modifies the tumor micro-environment by decreasing tumor perfusion, as noninvasively detected by DCE MRI and supported by reduced Hoechst 33342 staining. The hypoaxia response in central regions of the tumor and an increased hypoxia response in the tumor rim in response to Bortezomib treatment.