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

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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 ^{1}H MR coil on a Bruker 7T BioSpin MR spectrometer (Bruker, Germany). T_1-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 microcopy.

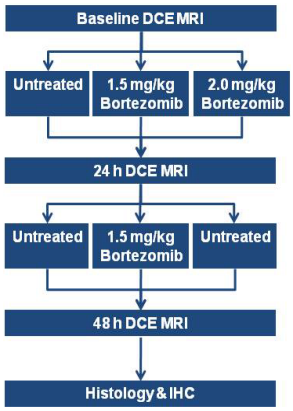

Figure 1: Experimental Scheme / Time Line

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).

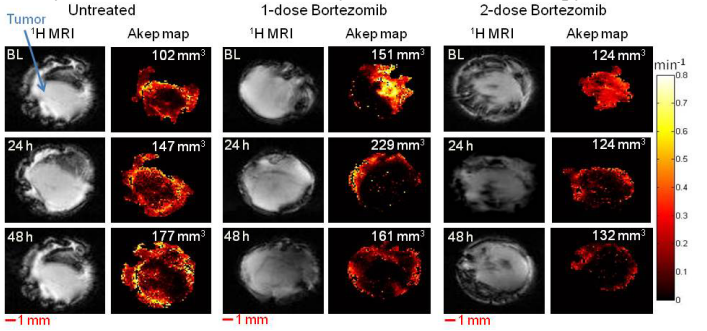

Figure 2: Representative tumor slices of tumors in the untreated controls and each treatment group. (117 µm x 117 µm in-plane resolution)

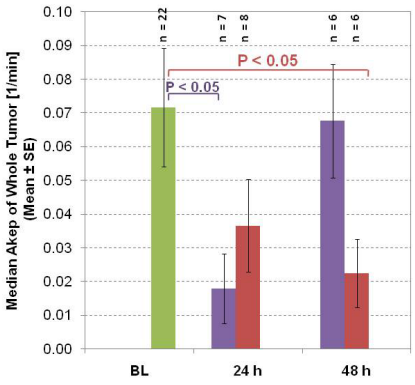

Figure 3: Comparison of median AKep values obtained from whole tumors in the untreated group and the two treatment groups: Green: average of all untreated mice at day 0 (BL); Purple, 1 dose of 2.0 mg/kg Bortezomib administered after day 0 DCE MRI (BL); Red, 2 doses of 1.5 mg/kg Bortezomib administered after BL and 24 h DCE MRI respectively. (Unpaired, two-tailed T-test assuming unequal variances.)

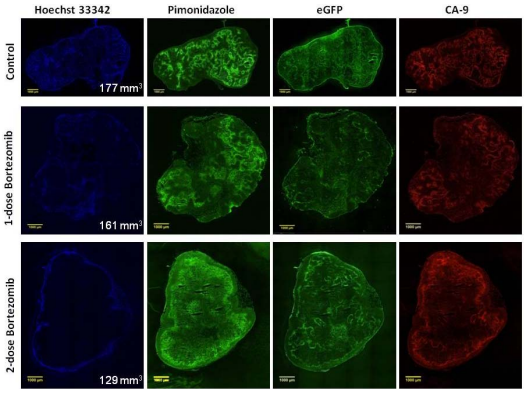

Figure 4: Representative distributions of Hoechst 33342, pimonidazole, eGFP and CA-9 in control (top) and treated (1-dose, center; 2-dose, bottom) HT29-9HRE-TKeGFP tumors.

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 *ex vivo* data indicate a suppression of the hypoxia response in central regions of the tumor and an increased hypoxia response in the tumor rim in response to Bortezomib treatment.

References: [1] Daniel KG, *et al.* (2005) Curr Cancer Drug Targets 5,529-41, [2] Birle, DC, *et al.* (2007) Cancer Res 67,1735-43, [3] U. Hofmann *et al.* (1995) Magn Reson in Med 33, 506-514. **Acknowledgements:** Supported by NIH grants PO1 CA115675 (Ling), RO1 CA56909 (Li), R33 CA109722 (Li), R24 CA83084 (SAIRP) and NCI P30 CA0874 (Cancer Center Support Grant).