Therapeutic Potential of Bevacizumab/Gemcitabine combination in Bladder Cancer:
In Vitro Studies

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The high failure rate of both invasive and nonmuscle invasive bladder cancer (BC) has motivated the testing of combination therapy to improve patient's outcomes. Bevacizumab is an antiangiogenic monoclonal antibody which binds to vascular endothelial growth factor A (VEGF-A) and stops the growth of blood vessels needed for tumor progression. It is used in the treatment of colorectal, breast, kidney and lung cancer in combination with chemotherapeutic agents. Bevacizumab is not yet approved for BC, but since these neoplasms express VEGF-A and its receptors the drug efficacy is anticipated. Gemcitabine (Gem) is a chemotherapeutic drug that alters DNA synthesis resulting in apoptosis of cancer cells and is being employed with relative success in BC patients.

In this study we intend to evaluate the efficacy of bevacizumab and Gem alone or in combination in BC, by performing in vitro assays with nonmuscle-invasive (5637) and invasive (HT1376 and T24) BC cell lines.

To confirm that our cell lines were bevacizumab targets, VEGF and HER2 expression was evaluated by Real-time PCR. MTT assay, which measures the mitochondrial activity and therefore the cell viability, was used to evaluate the drugs´ cytotoxicity. Drug effect in cell cycle and apoptosis induction was determined by Flow Cytometry.

It was observed that all three cell lines express high levels of VEGF and HER2, particularly, the nonmuscle-invasive 5637 cell line. Cytotoxic effect of bevacizumab alone was insignificant since the IC50 value for the three lines tested was ~5 mg/ml, a concentration 10 fold higher than the physiologically accepted for therapy (0,4 mg/ml). Also, no relevant perturbations on the cell cycle or apoptosis induction were observed up to non-physiological concentrations of bevacizumab. Gem alone was highly cytotoxic, with an IC50 value of ~10 ng/ml and its dose-response curve was not altered when combined with bevacizumab, suggesting that this drug interaction is not synergistic at the cytotoxic level. Preliminary experiments suggest that supernatants from BC cells treated with bevacizumab have decreased capacity to activate endothelial cells compared with nontreated BC cells.

The present studies are basic platforms to further assess the therapeutic synergism of bevacizumb and Gem in more complex systems, such as xenographs in mice models. We soon intend to move forward to in vivo studies, envisaging the design of clinical trials with the combination of these two agents.