

# Cancer Stem Cell Activity in Mantle Cell Lymphoma Is Inhibited by TLR9 Activation

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Patients with Mantle Cell Lymphoma (MCL) often respond to initial cytotoxic therapy, but invariably relapse. This clinical pattern suggests that standard therapies fail to eliminate a population of chemoresistant cells responsible for tumor regrowth and disease relapse. Studies in myeloid leukemias and multiple myeloma have suggested that cancer stem cells (CSC) are relatively quiescent and that this property promotes drug resistance. Therefore, the induction of CSC proliferation may increase their susceptibility to anti-cancer treatment. Unmethylated CpG DNA sequences induce the activation and differentiation of normal B cells through toll like receptor 9 (TLR9), and synthetic CpG oligonucleotides (ODN) are currently undergoing clinical trials in MCL and other B cell non-Hodgkin's lymphomas. We initially examined the effects of TLR9 agonists on the clonogenic growth of the human MCL cell lines Granta 519, Jeko-1, and Rec-1 and found that the unmethylated CpG ODN 2006 (5ug/mL, 48 hours) significantly inhibited the ability of each of these cell lines to form colonies in methylcellulose ( $p < 0.01$ ). We previously demonstrated that clonogenic CSC in multiple myeloma and Hodgkin's lymphoma may be identified based on the relative activity of the intracellular detoxification enzyme aldehydedehydrogenase (ALDH). Similarly, each of the MCL cell lines contained small populations (0.5-2.3%) of ALDH+ cells that were enriched for *in vitro* clonogenic growth upon primary and secondary plating in methylcellulose. Following treatment with CpG ODN 2006, the population of ALDH+ cells was significantly reduced in each cell line ( $p < 0.05$ ). The self-renewal potential of normal and cancer stem cells is lost through the process of differentiation, and treatment of the MCL cell lines with CpG ODN 2006 resulted in the loss of CD19 and CD20 expression and induction of the plasma cell antigen CD138 by FACS. Similarly, RT-PCR for regulators of B cell differentiation revealed decreased CD19 expression coupled with increased transcription of BLIMP-1, XBP-1, and CD138. We hypothesized that the plasma cell differentiation induced by TLR9 agonists may improve the efficacy of anti-tumor agents with known activity against plasma cells, specifically the proteasome inhibitor bortezomib. Treatment with CpG ODN 2006 or bortezomib (10-100nM, 48 hours) alone induced apoptosis in a small percentage of cells within each MCL line, but the combination of these agents synergistically increased the proportion of Annexin V+ cells. CpG treatment in combination with bortezomib also significantly ( $p < 0.01$ ) decreased clonogenic potential of all three cell lines in an *in vitro* colony forming assay, while treating cells with CpGs and other chemotherapies including daunorubicin, etoposide, and steroid dexamethasone had no combinatorial effects. In summary, cellular activation by CpG ODN 2006 reduces MCL clonogenicity and stem cell function through the induction of differentiation. In turn, plasma cell maturation appears to increase the sensitivity of MCL to bortezomib and suggests a novel mechanism for the anti-tumor activity of CpG ODN.