OPTIMIZATION OF DEMETHYLATING THERAPY FOR IDH1 MUTANT GLIOMAS
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BACKGROUND: Mutations in Isocitrate Dehydrogenase 1 (IDH1) are found in the majority of grade II and III gliomas and the subsequent progressive GBMs. The mutant IDH1 enzyme produces 2-hydroxyglutarate (2-HG), an “oncometabolite” that inhibits α-ketoglutarate dependent histone and DNA demethylases resulting in characteristic hypermethylation of genomic DNA and suppression of cellular differentiation as a key mechanism of tumor progression and maintenance. METHODS: To accurately test therapeutics for IDH1 mutant gliomas, we developed a patient derived IDH1 mutant anaplastic astrocytoma model which produces 2-HG and exhibits characteristic DNA hypermethylation. This IDH1 mutant glioma grows only as a xenograft and not in cell culture. We measured tumor growth using a flank model in 5-azacytidine treated vs. untreated animals, passaging both sets of tumors from animal to animal in order to increase the exposure time to the drug. Tumor size was measured for treated vs. untreated mice and tumors were analyzed for gene expression, differentiation markers and promoter DNA methylation. Methylation status was by pyrosequencing analysis of bisulfate-converted genomic DNA at five genetic loci, with subsequent studies assessing global gene promoter methylation status. RESULTS: Long term administration of 5-azacytidine resulted in reduction of DNA methylation of promoter loci, induction of glial differentiation, reduction of cell proliferation and significantly reduced tumor growth. Nearly complete tumor regression was observed by 14 weeks, resulting in a sustainable response and significant survival benefit. When therapy was discontinued, tumors did not show any signs of re-growth for at least an additional 8 weeks. Statistical analyses were performed using a student t-test for comparisons between the treatment groups. Following one cycle of 5-azacytidine treatment, GFAP expression was strongly increased and protein levels were maintained throughout subsequent cycles. 5-azacytidine treatment significantly reduced the fraction of Ki-67 positive cells in a time dependent manner and loss of DNMT1 expression in vivo following one treatment cycle. CONCLUSIONS: Using this genetically and molecularly accurate model we have demonstrated the preclinical efficacy and mechanism of action of the FDA approved demethylating drug 5-azacytidine in vivo. The goal of this work is to optimize treatment efficacy and to precisely define the molecular alterations responsible for successful therapy. Long term administration of 5-azacytidine resulted in tumor regression acting through the expected mechanism of reversal of DNA methylation, and promoting a more normal cellular differentiation process. Optimization of demethylating therapy shows promise for IDH1 mutant gliomas. SECONDARY CATEGORY: Tumor Biology.