Glioblastoma (GBM) is characterized by an aggressive clinical course, therapeutic resistance and striking molecular heterogeneity. GBM remains incurable with a median survival of 12-15 months post-surgery even with standard of care chemotherapy and radiation. Tumor suppressor p53 (TP53) is frequently mutated in cancer and along with its downstream effectors is inactivated in more than half of all cancers. The remaining 50% of tumors have TP53 wild-type status. However, TP53 function is frequently attenuated in these cancers by other mechanisms including amplification and overexpression of its key negative regulator MDM2. Inhibition of the protein-protein interaction between p53 and MDM2 is still a novel concept for cancer therapy. MDM2 inhibitors are designed to restore and maintain p53 activity in p53 wild-type tumors.

Methods

Cytotoxicity assays

YOYO-1-cytotoxic dye was used to measure cell death following treatment with the BI-907828 with 2 selected BTSC lines (BT48 and BT301). Cells were plated as for viability assays. Apoptosis profiles were generated by using fluorescent dyes and real-time cell monitoring using a live cell imaging system. BI-907828 was added after 24 hours. Temozolomide was used at 10μg/mL as a control for all cell lines. YOYO-1 was added after 5 days of treatment for a period of 8 hours. Percent death was calculated as the percent green fluorescence over the percent total cell occupancy.

In vivo experiments

100,000 dissociated cells from BT48 and BT67 were implanted into the right striatum of female SCID mice. Tumors were allowed to establish for several weeks. For pharmacokinetic/pharmacodynamic (PK/PD) studies, 3 mice from each cell line were randomized as a pre-treatment group and the remaining mice were treated with a single oral dose of 50 mg/kg BI-907828 and further randomized for collection at various time points post dosing. Plasma, brain and thig/h/kg muscle samples were collected from n=3 mice per time point. For Kaplan Meier studies, treatment conditions were: 1. Vehicle po: 2. BI-907828 at 15 mg/kg weekly (q7d) po 3. BI-907828 at 50 mg/kg every 14th day (q14d) po 4. Temozolomide at 50 mg/kg weekly (q7d) po 5. Combinatorial treatment with temozolomide at 30 mg/kg weekly (q7d) po and BI-907828 at 15 mg/kg weekly (q7d) po. Treatments were started at day 50 and day 60 post-xenografts for BT48 and BT 67 respectively and performed for the duration of the study. Animals were sacrificed when experimental endpoints according to animal care guidelines were reached. Representative images of brains from each group were cryo-sectioned and stained with H&E and anti-human nucleolin antibody to detect human animal care guidelines were reached. Representative images of brains from each group were selected for each cell line for 5 day reads and a longer time point read. IC50s were generated in dose response curves using Alamar Blue® assays. BI-907828 was added after 24 hours. Temozolomide was used at 10ug/mL as a control for all cell lines. YOYO-1 was added after 5 days of treatment for a period of 8 hours and fluorescence measurement. The lower panels show the results obtained for the 4 hour time point.