Summary and Conclusion
Glioblastoma is one of the volatile and most aggressive of the cranial tumors affecting 2% of the total tumor population worldwide. The model used in this study is the Male Wistar Rat. The etiology involves genes like \textit{c-Myc}, \textit{Bcl-2}, \textit{Akt} and \textit{PCNA}. The animal model was created by cranial implantation of C6 Glioma cell lines using a stereotaxic apparatus (xenograft development). The molecule that was to be analysed as an anti-tumor candidate was JQ1 (tert-butyl 2-((6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate) a member of the Triazolothienodiazepine family. The molecule has been proven to be a anti-proliferative agent. The activity of the compound against glioblastoma was first tested on C6 Glioma Cell line using the MTT Assay after which the molecule was carried forward for further \textit{in vitro} examination. The \textit{in vitro} studies involved the propagation of N2A cell lines and carrying out suitable assays and molecular studies, after JQ1 treatment. The \textit{in vivo} studies involved the segregation of experimental wistar rats in to Group 1 (Control), Group 2 (Tumor Control), Group 3 (Tumor Induced + 7mg/kg BW JQ1 for 30 days), Group 4 (Tumor Induced + 7mg/kg BW JQ1 for 15 days and 15 days on observation) and Group 5 (Drug Control).

The effect of JQ1 treatment for both \textit{in vitro} and \textit{in vivo} cases is as follows:

- The MTT assay doesn’t exhibit any immediate cytotoxicity for 24 hrs. Groups were split as Tumor control, JQ1 concentrations in the order 100nmol, 200nmol, 300nmol, 400nmol and 500nmol for 6hrs, 12hrs, 18hrs and 24hrs respectively with duplicates for each concentration/time. Though there was enough evidence to suggest anti-proliferation with cell concentration remaining the same 70% confluence in all treated wells.

- The RT-PCR results yielded a gradual decrease in \textit{c-Myc} expression upon increasing gradient of JQ1 concentration (100, 200, 300, 400, 500 nmol) as against Tumor Control.
cells in vitro, especially 400nmol demonstrating a physiological drop in expression. This confirmed c-Myc was the target.

- The in vitro RT-PCR results for PCNA, Bcl-2 and Akt correlated with the pattern displayed by c-Myc, with Akt still displaying a basal expression quantum as compared with other three for 400nmol and 500nmol JQ1 concentration

- The in vitro FACS analysis also was performed with increasing concentrations of JQ1 concentration 100-500nmol against a tumor control. As per the in vitro RT-PCR results, Cell Cycle entry into S-phase dropped by 50% at 400nmol JQ1 concentration strengthening the fact that 400nmol concentration causes crucial physiological change.

- Using the data from RT-PCR results 400nmol concentration of JQ1 was fixed for in vitro analysis in Immunocytochemistry, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM).

- The in vitro immunocytochemistry analysis displayed almost nil expression for c-Myc,PCNAand Bcl-2 for 400nmol JQ1 concentration whereas p-Akt still displayed basal expression post JQ1 treatment. The western blot analysis also agreed with immunocytochemistry pattern.

- The in vitro SEM analysis demonstrated a smooth cellular exterior for the JQ1 treated cells as compared with tumor control cells which displayed moon-like cratered surface.

- The in vitro TEM results showed a shrunken nucleus with marginated chromatin with convoluted cellular membrane as well as multiple autophagosomes in JQ1 treated cells. The treated cells also showed glycogen accumulation and few lipid droplets. The tumor control cells in contrast showed huge anaplastic nucleus and few autophagosomes.
- A significant DNA damage was found in 300, 400 and 500 nmol dosages of JQ1 respectively. This confirmed apoptosis.

- The confirmation of c-Myc suppression and subsequent deregulation of other associated oncogenes and altered metabolism and cell death prompted the further in vivo studies using the Male Wistar Rat as the animal model. The molecule JQ1 was analysed for BBB passage, which was qualitatively proved through detection in CSF and Brain using HPLC.

- The in vivo model was successfully created using stereotaxic apparatus and direct cranial implantation of C6 Glioma cells. There were 5 groups in total that were set up namely Control (Group 1), Tumor Control (Group 2), Tumor + JQ1 (7mg/kg BW) treated for 60 days (Group 3), Tumor + JQ1 (7mg/kg BW) treated for 15 days and then untreated observation (Group 4) and Drug Control (Group 5).

- As the study couldn’t establish any direct relation between c-Myc and PCNA nor justify any cross-talk, PCNA was removed from further in vivo analysis.

- The in vivo RT-PCR results for c-Myc, Bcl-2 and Akt gave a similar correlated expression as in in vitro studies yielding minimal expression for Group 1 and Group 3 animals as compared Group 2 and Group 4 animals.

- The in vivo immunohistochemistry analysis showed correlation between gene expression and protein signature, with Group 2 and Group 4 exhibiting higher protein expression as compared to Group 1 and Group 3. The western blot analysis also agreed with immunohistochemistry pattern.

- The TEM images of Group 3 tissues showed lesser lipid accumulation as compared to group 4 tissues but was more than the JQ1 treated C6 Glioma Cells in vitro. The
glycogen accumulation remained the same for both the treated groups (Group 3 and Group 4). Both the treated tissues displayed shrunken nucleus compared to the tumor control.

- The *in vivo* analysis of tumor mass displayed bigger tumor mass for Group 4 animals as compared with tumor mass of Group animals.

- The survival rate analysis demonstrated a 30% increase in the group that received complete treatment (Group 3) as against tumor control (Group 2) as against the Group 4 which survived 20% lesser than the Group 2 animals because of partial JQ1 treatment.

- The weight differences were as anticipated. All the animal groups started with an average weight of 200g. At the end of the experiment regime Group 1 and Group 5 exhibited a weight gain of 14%, Group 3 exhibited a weight gain of 3%, and group 2 and Group 4 displayed a weight loss of 8% and 21% respectively.

- Glucose, Lactate and Lipid were elevated in CSF of Tumor Groups (Group 2 and Group 4) as compared with Control and fully treated animals (Group 1 and Group 3) whereas the Total Protein profile remained the same across all groups.

- The Glucose, Lactate, Total lipid and Total Protein show an elevated profile in Group 2 and Group 4 animals compared to Group 1 and Group 3 animals for serum analysis

- Glucose, Total Protein, Urea, Uric Acid and Total Lipid (Cholesterol, Free Fatty Acid, Phospholipid, Triglycerides) showed a coordinated increase for brain tissue in Group 2 and Group 4 animals as compared Group 1 and Group 3 animals.

- The Group 2 and Group 4 animals also showed high activity for metabolic enzymes Creatinine Kinase (CK), Lactate Dehydrogenase (LDH), Glucose-6-Phosphate Dehydrogenase (G6PDH) and 5’Nucleotidase for brain tissue in Group 2 and Group 4
animals as compared to Group 1 and Group 3 animals, following suit with Biomolecular data.

- The brain tissue analysis for antioxidants didn’t display any change of antioxidant activity for Superoxide Dismutase (SOD), Catalase (CAT), Glutathione – S – Transferase (GST) and Glutathione Hydrogen Peroxide Oxidoreductase (GPX) contrary to general conception of reduced activity in tumor tissue.

- The histopathalogy indicates highly invasive tumor growth in tumor groups (Group 2 and Group 4) with numerous cells and normal or close to normal tissue phenotype for control (Group 1), treated (Group 3) and Drug controls (Group 5) after H&E staining.

- The results for Acute toxicity demonstrate partial cavitation and necrosis and Chronic toxicity demonstrate excessive cavitation and necrosis for wistar rat brains using JQ1 after H&E staining on liver sections as compared with normal liver sections of wistar rats.

- The results indicate a strong suppression of c-Myc expression due to inhibition and displacement of its upstream inducer BRD4 by the small molecule JQ1 and subsequent silencing of associated oncogenes like PCNA, Bcl-2, Akt and Cyclin D1. There is an apparent upregulation of Caspase 3, but pathological data also suggest involvement of Autophagosomes and Mitotic Catastrophe as contributors of cell death. There is also massive alteration in intra-cellular biochemistry.

The study concludes by mentioning JQ1 as a good anti-cancer agent against glioblastoma, given the drug dosage regime is clinically standardized especially when partial treatment by the molecule leads to aggressive tumorogenesis and faster death as compared to untreated subjects.
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