

# EVALUATION OF DANUSERTIB EFFECTS ON CELL VIABILITY AND CITOMORPHOLOGICAL PARAMETERS IN CANCER STEM CELL LINES FROM GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is a grade IV astrocytoma and the least successfully treated solid tumor: current therapies provide a median survival of 12-15 months after diagnosis, due to the high recurrence rate. Glioma stem cells (GSCs) are believed to be the real driving force of tumor initiation, progression and relapse. Better therapeutic strategies GSC-targeted are needed.

Danusertib (Dan) is a small molecule with strong activity against Aurora kinases (AURK), a protein kinases family (AURKA, B, C) overexpressed in a variety of human cancers and correlated, also in GBM, with chromosomal instability, tumor aggressiveness and poor prognosis.

The aim of this study is to evaluate *in vitro* the effects of Dan on four GSC lines, previously characterized by a genetic-genomic approach. GBM2 and G179 showed a loss, while G166 and GliNS2 a gain of AURK genes.

At first the analysis of the expression levels of genes coding for AURK highlighted that AURKA and AURKB genes were upregulated in all our GSC lines, while AURKC was downregulated in G179 and upregulated in GliNS2. Moreover generally there was no correlation between AURK genes expression levels and the CNAs detected through the a-CGH analysis.

Then we investigated the effect of Dan exposure on cell viability, proliferation and cytomorphological parameters, by means of different assays (MTT, trypan blue and clonogenic assays, mitotic index and ploidy determination, morphological analysis). Results showed that response to Dan exposure was heterogeneous among GSC lines. Cell viability and proliferation were significantly reduced in GBM2 and G179, while G166 and GliNS2 were resistant. The analysis of cell and nuclear morphology in GBM2 and G179 highlighted the presence of large multinucleated cells and an increase in the number of polymorphic nuclei and micronuclei. Moreover conventional cytogenetics evidenced a significant increase in ploidy in GBM2 and G179.

At last we verified the genomic DNA integrity through the evaluation of the DNA Integrity Number (TapeStation, Agilent).

Mutational analysis of AURK and the study of chromosome segregation errors are in progress in order to deeply understand the heterogeneous response to Dan treatment on our GSC lines.