A NOVEL ALK-BASED COMBINATION THERAPY FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is the most frequent class of malignant primary brain tumors and among the most aggressive forms of cancer. Affected individuals present poor prognoses; the standard treatment has not evolved significantly in recent decades; and, unfortunately, most GBM patients relapse after a few months[1,2]; therefore, we urgently require novel drugs or the identification of novel drug combinations. In preclinical studies, anaplastic lymphoma kinase (ALK) inhibitors have been postulated as promising candidates for GBM treatment; however, clinical trials with ALK inhibitors failed to report significantly improved patient survival rates[3]. As ALK signaling might play an anti-apoptotic role in glioma cells[4] and promote resistance to cancer therapies, combining ALK inhibitors with other cytotoxic drugs may improve efficacy.

This project aims to identify optimal combinations of chemotherapeutic drugs with the ALK inhibitor crizotinib (CRZ) in 2D and 3D GBM models with the ultimate goal of developing novel nanotherapeutics[5] that support the targeted and controlled delivery of an efficacious drug combination to GBM patients.

To this end, we performed a high throughput combination screening of CRZ with the Prestwick library in the U87MG GBM cell. We treated cells for 72 h at 2.5 μM at the massive screening stage alone or combined with the IC_{25} concentration of CRZ (3.5 μM) (identified in previous cytotoxicity studies). We calculated Synergy scores using the Bliss model and set the cut-off for positive hits at a Bliss synergy score of ≥ 30 and a drug combination cytotoxicity of ≥ 50 % (compared to non-treated control). The analysis rendered a list of fourteen potential hits; however, the confirmation of candidates highlighted "Hit 13" as providing optimal synergism with CRZ. Combining different concentrations of both drugs and using the SynergyFinder web application (version 3.0), we identified an optimal CRZ:Hit13 ratio for enhanced synergism. We selected the 1:1 ratio at a concentration of 3.5 μM for the following assays and used those conditions to evaluate the anti-tumor potential of this combination in a more complex *in vitro* model. We cultured U87MG spheroids in ultra-low adherence U-shaped 96-well plates for 72 h before treatment for 72 h. We determined cytotoxicity by ATP measurement and by analyzing spheroid volume and cell death with propidium iodide staining with the Biotek Cytation 5 Cell Imaging Multimode Reader (Agilent). Surprisingly, we discovered that CRZ alone exerted a more potent cytotoxic effect in U87MG spheroids than monolayer cell cultures, allowing us to decrease the concentration of the drugs needed to exert potent anti-tumor effects.

In conclusion, we describe a list of candidate drugs that increase the anti-tumor effect of CRZ in GBM and report a database of FDA-approved drugs that could be assessed as potential therapeutic agents for this disease. Among the compounds, "Hit 13" synergizes with CRZ and, therefore, represents the most suitable candidate; this drug will be explored to develop novel combination nanotherapeutics for GBM treatment to increase the effectiveness of the combination therapy. Of note, further critical studies must establish more adequate concentrations of CRZ:Hit13 in more complex models of GBM and shed light on the molecular mechanism triggered by this combination therapy.

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