A DOSE RESPONSE STUDY OF RAF/MEK INHIBITOR AVUTOMETINIB AND FAK/PYK2 INHIBITOR DEFACTINIB IN GLIOBLASTOMA

Emely Morales Colon, MS[1] ; Lilia Kucheryavykh, PhD[2]
Universidad Central del Caribe, Bayamón, Puerto Rico[1,2]

BACKGROUND

Glioblastoma (GBM) is a rapid brain tumor. By 2022, GBM had a 5-year survival rate of 6.8% [1-2]. The current standard of includes chemotherapy, radiation, and surgery.

It was shown then GBMs up-regulate Raf/MEK and FAK/Pyk2 signaling [3-6]. Raf/MEK signaling is involved in the regulation of tumor cell proliferation and represents a promising target for treatment of cancers [4]. Avutometinib is a new RAF/MEK inhibitor that regulates cell cycle progression, apoptosis, or differentiation. Defactinib (VS-6063) is a FAK inhibitor that reduce cell migration, proliferation, and survival [3]. Targeting the molecular pathways of PYK2/FAK and RAF/MEK represent promising avenue for a therapeutic intervention

We **hypothesize** that the use of Avutometinib and FAK/Pyk2 inhibitor Defactinib will reduce GBM cell proliferation and tumor growth.

PURPOSE

The purpose of the study was to determine effective doses of Avutometinib and Defactinib on glioblastoma cell viability.

METHOLOGY

Treatments

Cells were treated with Defactinib (5μM, 10 μM and 13nM) or with Avutometinib (5μM, 1μM and 500nM) and a combination for a period of 72 hours at 37C.

Live/Dead Assay

The percentage of live and dead cells were assessed with trypan blue exclusion assays, followed by a live/dead assay (Invitrogene). The cells were stained with calcein (green fluorescence, for live cells) and propidium iodide (red fluorescence, for apoptotic cells) and analyzed with a fluorescent microscope.

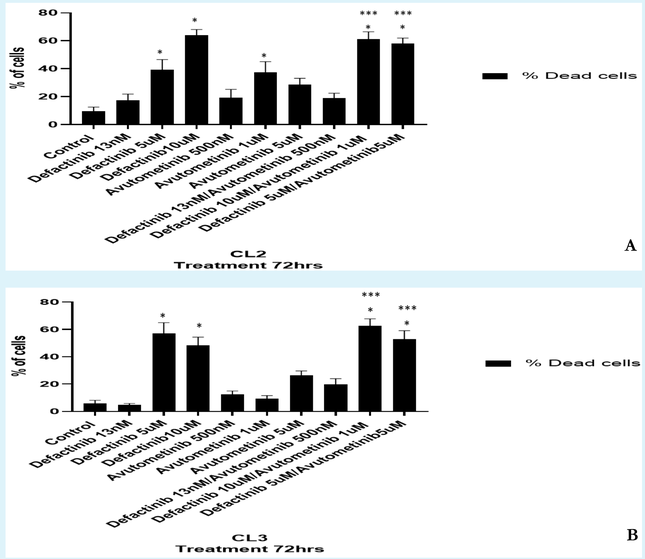
Cell Cycle Analysis

Flow cytometry cell cycle analysis was performed for primary glioma cell lines CL2 and CL-3. The Curnex Guava Cell cycle Kit was used as per manufacture instructions.

Statistical Analysis

Statistical analyses were achieved by using GraphPad Prism version 9.5.1(San Diego, CA, USA). Data wre express as means with SEM t/-SD. Significant differences among groups were conducted using a Two-way ANOVA. A p-value <0.05 was considered significant.

RESULTS



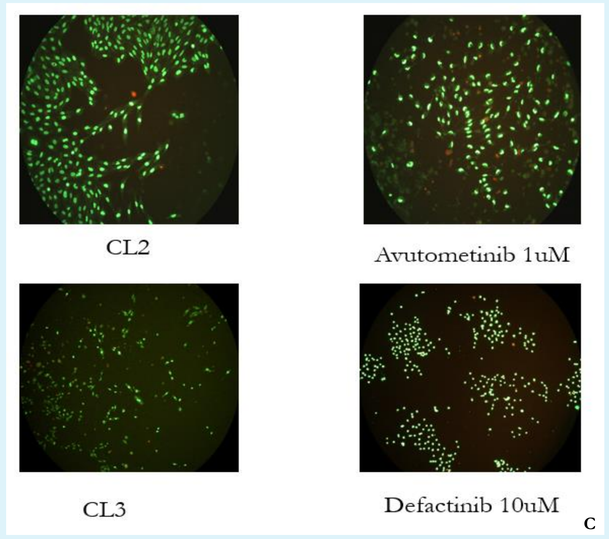
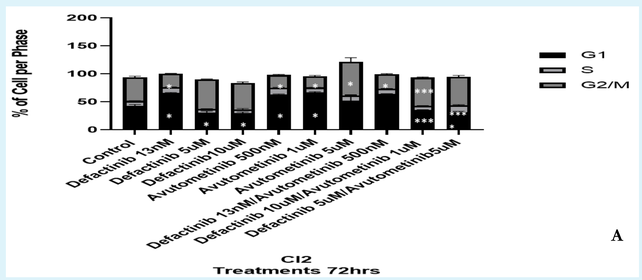
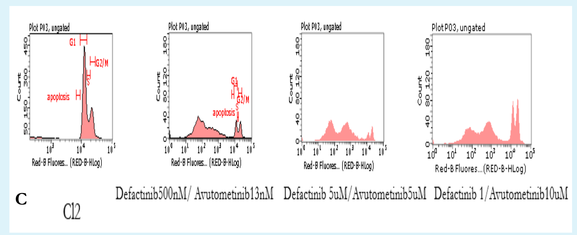


Figure 1. Defactinib and Avutometinib affect cell viability at combinatorial treatment of 1/10μM and 5/5 10μM. Trypan Blue assay performed on GBM cells lines CL2 and CL3 (A, B) in 72hrs of treatment. Cells were treated with Avutometinib (VS-6063, 5μM, 1μM and 500nM), Defactinib (5μM, 10 μM and 13nM) and in combination. Live/Dead assay was performed at 72hrs of treatment (C). The number dead cells were analyzed using GraphPad Prism version 9.5.1. All data are presented as the mean t/- SEM.(n=3) with significant differences from controls(*),Defactinib(**) and Auvutometinib(***) are shown($p<0.05$).Tukey's multiple comparisons test was used to determine significance among the groups.





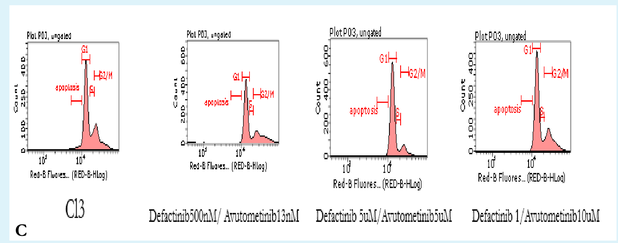


Figure 2:Defactinib cause cell cycle arrect at G2/M and Avutometinib affects the transition from G1 to S phase in glioma cells. Cell cycle distribution on GBM cells upon 72 hours of treatment with Defactinib (5μM, 10 μM and 13nM) and Avutometinib (5μM, 1μM and 500nM).and in combination (A and B) .Diagrams of distribution of cells at cell cycle phases are shown (C). The cell cycle distribution was analyzed using GraphPad Prism version 9.5.1. All data are presented as the mean t/- SEM (n=3) with significant differences from controls(*),Defactinib(**) and Auvutometinib(***) are shown($p<0.05$).Tukey's multiple comparisons test was used to determine significance among the groups.

CONCLUSION

- Avutometinib at 1-5 μM and Defactinib 5-10 μM represent the maximum cell toxicity in primary GBM cell lines.
- Combinatorial treatments does not affect the percentage of death cells comparing with Defactinib monotherapy.
- Avutometinib restricts cell cycle transition at G1-S at a concentration of 1μM.
- Defactinib at concentration of 5μM causes cell cycle arrest at G2/M.
- Combinatorial treatments of 5μM Avutometinib/5μM Defactinib and 1μM Avutometiniv/10 μM Defactinib showed restriction of cell cycle at G1-S transition and G2/M arrest.

REFERENCES

1. National Brain Tumor Society. (2022, September 15). *Glioblastoma awareness day*. National Brain Tumor Society. Retrieved April 30, 2023, from https://braintumor.org/events/glioblastoma-awareness-day/
2. Ratini, M. (2022, April 20). *What is glioblastoma?* WebMD. Retrieved April 30, 2023, from https://www.webmd.com/cancer/brain-cancer/what-is-glioblastoma
3. Alza, L., Näger, M., Visa, A., Cantí, C., & Herreros, J. (2020). FAK Inhibition Induces Glioblastoma Cell Senescence-Like State through p62 and p27. *Cancers*, *12*(5), 1086. https://doi.org/10.3390/cancers12051086
4. McCubrey, J. A., Steelman, L. S., Chappell, W. H., Abrams, S. L., Wong, E. W., Chang, F., Lehmann, B., Terrian, D. M., Milella, M., Tafuri, A., Stivala, F., Libra, M., Basecke, J., Evangelisti, C., Martelli, A. M., & Franklin, R. A. (2007). Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. *Biochimica et biophysica acta*, *1773*(8), 1263–1284. https://doi.org/10.1016/j.bbamcr.2006.10.001
5. Nuñez RE, Del Valle MM, Ortiz K, Almodovar L, Kucheryavykh L. Microglial Cytokines Induce Invasiveness and Proliferation of Human Glioblastoma through Pyk2 and FAK Activation. Cancers (Basel). 2021 Dec 7;13(24):6160. doi: 10.3390/cancers13246160. PMID: 34944779; PMCID: PMC8699228.
6. Rolón-Reyes K, Kucheryavykh YV, Cubano LA, Inyushin M, Skatchkov SN, Eaton MJ, Harrison JK, Kucheryavykh LY. Microglia Activate Migration of Glioma Cells through a Pyk2 Intracellular Pathway. PLoS One. 2015 Jun 22;10(6):e0131059. doi: 10.1371/journal.pone.0131059. PMID: 26098895; PMCID: PMC4476590.

FUNDING: This research was funded by the National Institutes of Health, grant 1SC1GM122691 & Puerto Rico Science Technology and Research TRUST Grant R021