The Aurora-A Inhibitor Alisertib is Synergistic with VEGFR Inhibitors in Glioblastoma Cell Lines

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Abstract

Glioblastoma is the most common primary malignant brain tumor in adults. The median survival of patients diagnosed is between 13-16 months (Lau et al., 2014). A serine-threonine kinase, Aurora-A, is crucial for mitosis and plays a role in the duplication of centrosomes, assembly of mitotic spindles and segregation of sister chromatids. Glioblastomas commonly overexpress Aurora-A, as well as vascular endothelial growth factor (VEGF). VEGF controls tumor angiogenesis and vasculogenesis. Drugs that inhibit Aurora-A and VEGF or its receptor may prevent cellular proliferation and neovascularization. Through colony formation assays and annexin V binding assays, we showed that the combined use of alisertib, an Aurora-A inhibitor, and a VEGFR inhibitor like cabozantinib or vandetanib synergistically inhibit proliferation and induce cell death of glioblastoma cells *in vitro* through apoptosis.

Background

- When used individually, both Aurora-A and VEGFR inhibitors have been found to prevent cellular proliferation (Van Brocklyn et al., 2014).
- They have been used separately in clinical trials.
- Aurora-A and VEGF receptors are highly overexpressed in glioblastoma.
- The inhibition of these pathways may be effective in preventing cellular proliferation and inducing cell death.

Methods

Colony Formation Assay

Cells were seeded at a density of 600 cells per plate in ninety 60-mm^2 plates and treated the following day with a range of concentrations of alisertib or VEGFR inhibitors, cabozantinib or vandetanib. After 3 days of treatment media was changed and cells were fixed and stained with Giemsa. Colonies of 20 cells or more were counted using a dissecting microscope. For IC_{50}s, a range of drug concentrations was chosen to find the concentration of drug at which 50% of colony formation was inhibited with respect to untreated controls. These IC_{50}s were then used to test for synergy between alisertib and VEGFR inhibitors using a 1:1 ratio of multiples of their respective IC_{50}s in various glioblastoma cell lines.

Apoptosis Assay

Cells were seeded at 1.25x10^5 cells/mL in 6-well plates and treated with alisertib (250nM), cabozantinib (10µM) or both drugs. After 24 hours of treatment cells were collected and an annexin binding assay was performed using an Alexa Fluor 594 annexin V conjugate and a Countess II FL cell counter per the manufacturers instructions.

Statistical Analysis

Synergy between two drugs can be seen when the effect of both drugs in combination is more than the additive effects of both drugs when used as single agents. To statistically test for synergy we will use the Chou-Talalay and Bliss independence models (Bliss, 1939; Chou, 1984).

Results

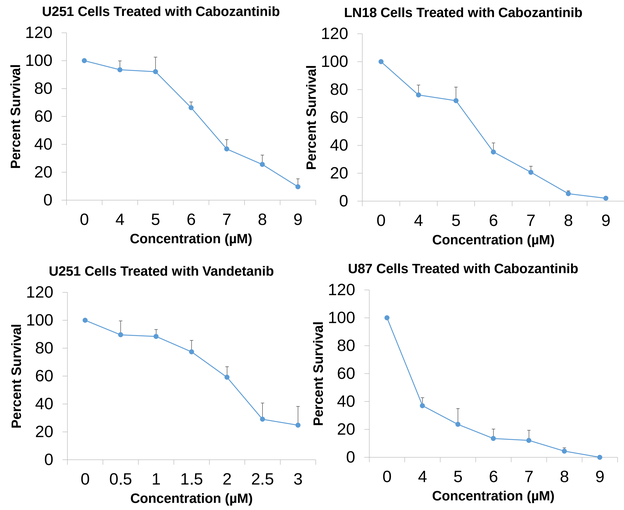

Figure 1. Colony formation assays were used to determine the IC_{50}s of cabozantinib and vandetanib in U251, LN18 and U87 cell lines and will be used for future colony formation assays to evaluate synergy between alisertib and these drugs.

Table 1. IC_{50}s of VEGFR inhibitors in glioblastoma cell lines

Cell Line	Cabozantinib IC_{50} (µM)	Vandetanib IC_{50} (µM)
U251	6.5	2.25
LN18	5.5	—
U87	3.5	—

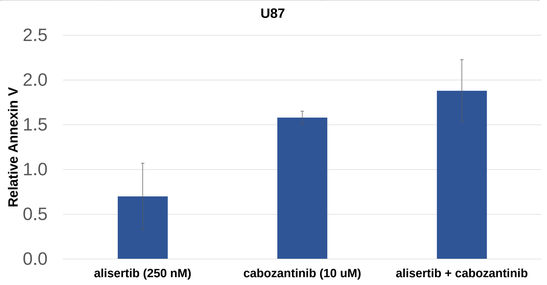

Figure 2. An annexin V binding assay was used to test for synergistic induction of apoptosis by alisertib and cabozantinib in U87 cells. Results are expressed as the fold increase in the percent of apoptotic cells with respect to untreated controls. When used in combination a concentration of alisertib that caused no increased apoptosis on its own potentiated the effects of cabozantinib.

Results

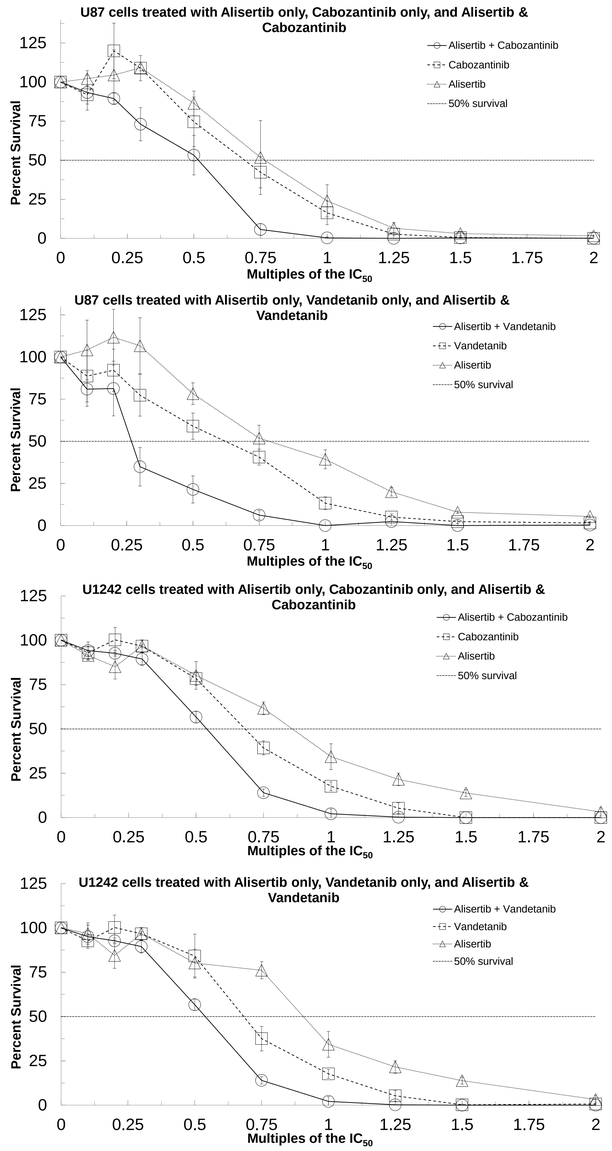

Figure 3. Colony formation assays were performed in U87 and U1242 cells to test for synergy between alisertib and VEGFR inhibitors, cabozantinib and vandetanib. Percent survival is expressed with respect to untreated controls and drug concentrations are presented as multiples of the IC_{50}s.

Acknowledgements and References

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Conclusion

In both cell lines tested (U87 and U1242) the combination of alisertib and a VEGFR inhibitor was found to be synergistic by Bliss analysis. In U87 cells a concentration of alisertib that caused no apoptosis on its own was found to potentiate the induction of apoptosis caused by cabozantinib. This data provides rationale for further *in vitro* and *in vivo* studies and may lead to the use of these drug combinations against glioblastoma in future clinical trials.