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# Tryphostin (AG 879): A Potential Drug to Treat Glioblastoma.

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## Background

- Glioblastoma (GBM) is one of the most aggressive, primary-malignant tumors of the central nervous system, associated with high morbidity and mortality rates. Accounting for 45.2% of adult brain tumors, GBM has a 5.5% survival rate.[1] Despite various modern approaches including radio- and chemo-therapies and surgery, GBM remains incurable.[2]
- Normally, nerve growth factor receptors (NGFR's) regulate cell proliferation, survival, cell cycle progression, apoptosis, and cell migration by binding to nerve growth factor (NGF).
- NGFR expression is heightened in GBM.

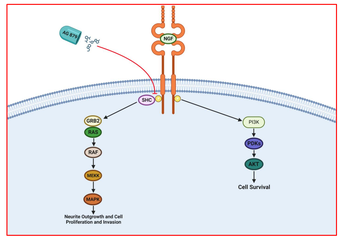



**Fig. 1.** NGFR signaling in cancer and AG 879 inhibition.

- Tumorigenic NGFR inhibits p53 transcription by directly interacting with its DNA binding domain and through direct association with MDM2, enhancing MDM2-mediated p53 ubiquitination and proteasomal degradation.
- Tryphostin AG 879 (also known as AG 879) is a known tyrosine kinase A (TrkA) inhibitor in the NGFR pathway.[3]
- AG879 inhibits phosphorylation at Y490 on TrkA activation domains, inhibiting neurite outgrowth and cell proliferation.[4]

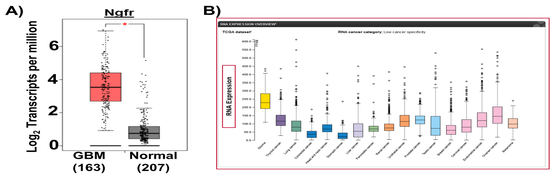
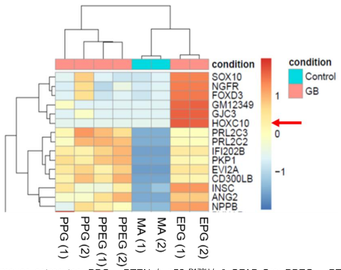



**Fig. 2. NGFR expression increased in GBM. (A)** Gene Expression Profiling Interactive Analysis (GEPIA) on NGFR expression in GBM. (**B**) TCGA Data of NGFR RNA expression in various types of cancer.

## Methods

- U251 and U118 cells were cultured in DMEM high-glucose media.
- Cells were split and seeded into 96-well plates to conduct MTT analysis.
- Cells were split and seeded into 10mm plates to conduct lysate collection and protein estimation.
- Lysates were also used to conduct Western Blot analysis.
- Gels were transferred and probed for β-actin and phospho-Akt.

## Results




MA – mouse astrocytes; PPG – PTEN $^{-/-}$; p53 $^{R172H+/-}$ & GFAP Cre; PPEG - PTEN$^{+/-}$; P53 $^{R172H+/-}$; EGFRvIII & GFAP Cre; EPG – EGFRvIII, p16 $^{-/-}$ & GFAP Cre.

**Fig. 3.** NGFR expression increased in EPG mouse syngeneic cell lines, which possess an EGFRVIII overexpression and tumor suppressor p16 deletion. RNA-seq analysis was done in MA, PPG, PPEG and EPG mouse GBM syngeneic cell lines.

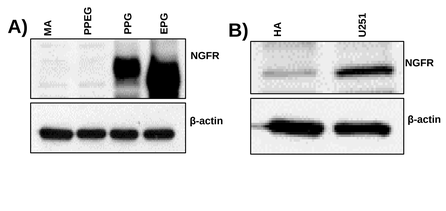



**Fig. 4. NGFR expression inside GBM cell lines.** NGFR expression was assessed in various models (**A**), including mouse astrocytes (MA), PPEG, PPG, and EPG. NGFR expression was heightened in PPG and EPG models relative to other models. β-actin was used as as a loading control. NGFR expression was also assessed in the human GBM cell line U251, using human astrocytes as a control (**B**). NGFR expression was heightened in cells possessing GBM. β-actin was used as as a loading control.

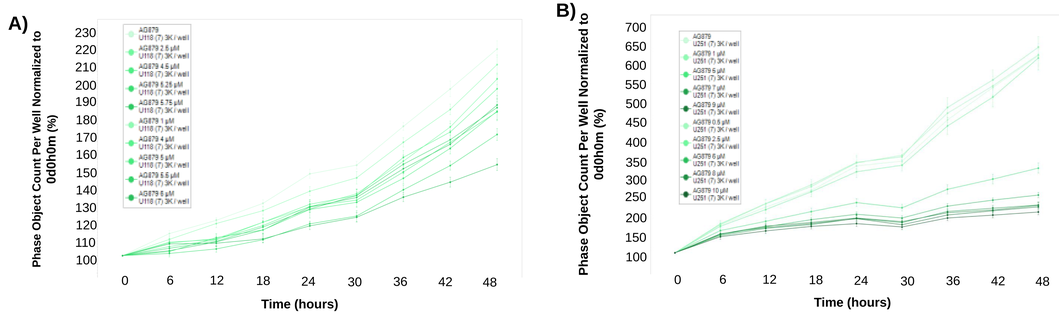



**Fig. 5. AG 879 inhibits U118 and U251 GBM cell growth in a concentration-dependent manner.** U118 (**A**) and U251 (**B**) GBM cell lines were treated with varying concentrations of AG 879 (portrayed by changing color density). Their growth was tracked over 48 hours using IncuCyte® S3 Live-Cell Analysis system, which highlighted the drug's dose-dependent inhibition.

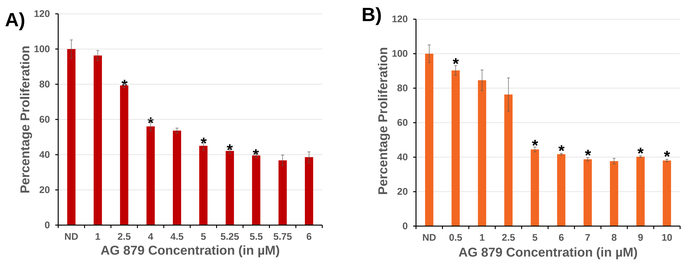



**Fig. 6. AG 879 shows dose dependent growth inhibition.** To assess the survivability of GBM cells against varying concentrations of AG 879, MTT analysis was used. Such values demonstrated dose-dependent inhibition caused by AG 879 in U118 (**A**) and U251 (**B**) GBM cell lines. From it, $IC_{50}$ values were identified. *P-values less than 0.05 were considered as statistically significant.

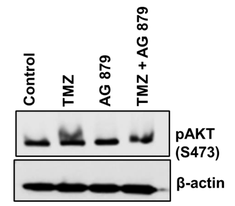



**Fig. 7. AG 879 and TMZ combination decreases AKT activation (cell survival).** U251 cells were treated with $IC_{25}$ of TMZ, AG 879 or combination of both for 72 hours and lysates were analyzed for AKT activation. β-actin serves as the loading control.

## Summary and Discussion

- In both human and mice GBM cell lines, NGFR expression is heightened compared to normal.
- AG 879 shows dose-dependent growth inhibition in both U251 and U118 GBM cell lines, as demonstrated through MTT analysis. This was further confirmed through IncuCyte® system analysis.
- Combination treatments of TMZ and AG 879 showed decreased proliferation of GBM cells compared to controls and individual drugs themselves.
- AG 879, a TrkA inhibitor, kills cancer cells by inhibiting phosphorylation of NGFR, which inhibits subsequent signaling cascades and prevents neurite outgrowth, cell proliferation, and invasion[4].
- In the future, research can identify the blood-brain barrier permeability of AG 879, mechanisms by which AG 879 elicits its anti-tumorigeneic potential, and further assess the possibility of combination treatments on mice.

## References

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