Targeting Phosphatidylinositol-4-5-Bisphosphate 3 Kinase (PI3K) and Protein Kinase C (PKC) activation in Diffuse Midline Glioma (DMG)


### PI3K activation is an oncogenic driver in DMG

**Difuse Midline Glioma (DIPG)** is an incurable childhood brain cancer. More than 80% of patients harbour a point mutation in Histone H3 which sees a substitution of lysine 27 for a methionine (K27M) in either H3F3A/H3.3 or H3F3B/H3.1 variants.

- H3K27M mutations result in global hypermethylation, promoting oncogenic transcription.

The RTK-AKT-P38K/mTOR pathway (Fig. 1) are altered in more than 2/3rd of DIPG patients, therefore, present an attractive therapeutic target. However, PI3K inhibitors have been notoriously ineffective due to their inability to cross the blood-brain barrier (BBB).

**Hypothesis:** Analysis of signal transduction pathway alterations following PI3K inhibition using Paxalisib, via phosphoproteomic profiling, will uncover DMG survival dependencies and reveal novel drug targets for combinational therapies.

### Phosphoproteomics identifies activated PKC

**Figure 2.** PKC is activated by PI3K inhibition.

a) Quantitative phospho-proteomic profiling was performed in biological triplicate using SU-DIPG-36 (K27M, ACRV1, G328E, PK3R1 M326I) treated with 1μM GDC-0084 for 6hrs and vehicle control (DMSO).

b) Treatment with paxalisib, further increased PKC substrate phosphorylation including downstream effector proteins MARCKS and MARKO1 to alter actin cytoskeleton remodeling.

c) Analysis using PhosTrack, Kinase enrichment, identified significant activation of the PKC signaling pathway.

### PK3K and PKC inhibition is highly synergistic

**Figure 3.** Synergistic treatment strategies targeting PKC signalling.

a) In a drug screen of over 12 compounds, the most synergistic drugs were those targeting PKC signalling, in particular enzastaurin or midostaurin (determined using Synergy Finder) in both

- H3K27M PI3K mutant or
- H3K27M PI3K wildtype DMG cell lines (determined using Bliss independence dose response analysis).

b) Treatment of DMG cells (SU-DIPG-VI and SU-DIPG-XIII) with paxalisib drives PKC substrate phosphorylation including MARCKS and MARKO1. Combined inhibition of PI3K with paxalisib and PKC with enzastaurin or midostaurin completely ablated PI3K/Akt, mTOR, PKC-B substrate phosphorylation.

### PKC activation is calcium dependent

**Figure 4.** Targeting PI3K induced Calcium modulation.

Treatment with the calcium chelator BAPTA-AM was able to prevent PKC activation after Paxalisib treatment (a). The combination of Paxalisib and BAPTA-AM or Gabapentin was able to induce synergetic effects on DIPG cell growth and survival (b-c). These calcium related effects were independent of the secondary PKC activation pathway, DAG, where paxalisib did not increase DAG levels (d).

### PKCβ knockdown increases paxalisib efficacy

**Figure 5.** PKCB Knockdown increases paxalisib efficacy.

PKCB was knocked down in SU-DIPG-VI (a) and SU-DIPG-XIII (b) cells using shRNA and significantly increased the efficacy of paxalisib as a single agent, highlighting its therapeutic potential of PI3K and PKC inhibition.

**Conclusions:** These data highlight the power of phosphoproteomic profiling to aid in the rational design of drug combination strategies. Pre-clinical DMG patient derived xenograft studies are currently under investigation to inform future clinical trials.