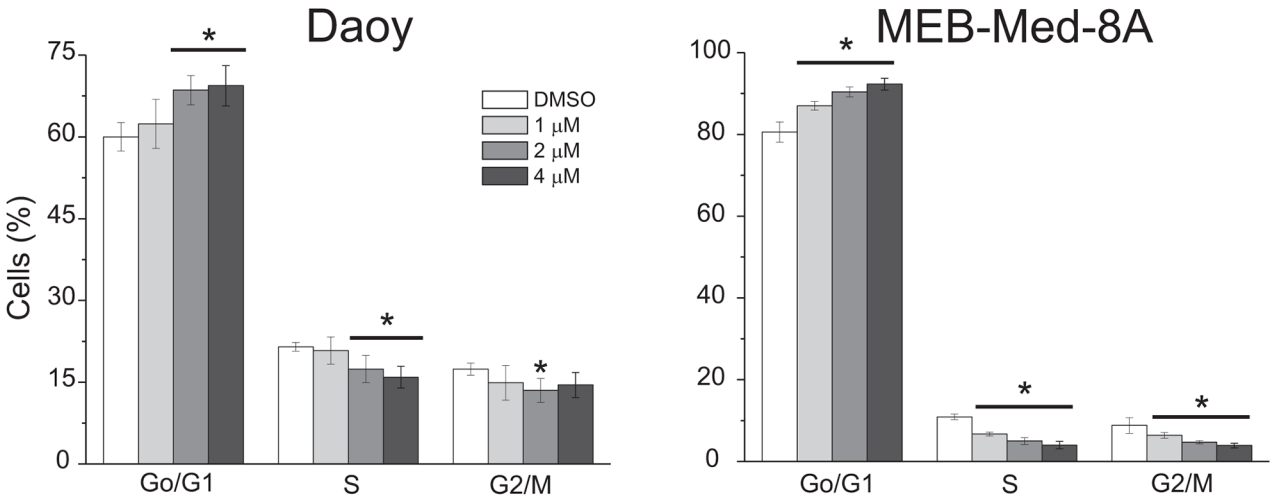
# The anti-neoplastic activity of Vandetanib against high-risk medulloblastoma variants is profoundly enhanced by additional PI3K inhibition

## SUPPLEMENTARY MATERIALS AND METHODS

### Cell cycle analysis

Daoy ($2\times10^5$/well) and MEB-Med-8A ($3\times10^5$/well) cells respectively were plated in 6-well cell culture dishes. After 48h of treatment with 1, 2 and 4 μM of Vandetanib the cells were exposed to 16 nM Hoechst 33342 and incubated for 45 min at 37°C. Both floating and attached cells were harvested and analyzed by flow cytometry (Navious, Beckman Coulter). Dead cells were stained by Propidium Iodid (PI). After gating on live cells, single cells were gated using width and area parameters from Hoechst 33342. The area parameter histogram was used to determine the percentage of cells in $G_1$, S and $G_2$M phases.




**Supplementary Figure 1: Vandetanib induces a $G_0/G_1$-phase cell cycle arrest in medulloblastoma.** Daoy and MEB-Med8A cells were treated with increasing concentrations of Vandetanib (1, 2 and 4 µM) for 48h. Subsequently cell cycle distribution was determined by Hoechst 33342 staining. The vehicle DMSO served as control. Statistically significant differences from control are marked by an asterisk (*p<0.05). The data shown represent five independent experiments.

**Supplementary Table 1: Inhibitory concentrations (IC50 in nmol) for MKI targets [1, 2, 3]**

| Drug | VEGFR-1 | VEGFR-2 | VEGFR-3 | EGFR | c-RET | PDGFR-α | PDGFR-β | c-kit |
|---|---|---|---|---|---|---|---|---|
| Vandetanib | >1000 | 40 | 110 | 50 | 130 | >1000 | >1000 | >10000 |
| Sunitinib | 10 | 10 | 10 | >10000 | 37 | 5 | 10 | 13 |
| Sorafenib | 57 | 90 | 20 | >10000 | 2 | 50 | 50 | 68 |

Protein binding 95-99%

**Supplementary Table 2: Expression of Vandetanib target proteins in medulloblastoma cell lines**

| Receptor | MEB-Med-8a | D283 Med | Daoy | D341 Med |
|---|---|---|---|---|
| VEGFR-2 * | + | +++ | +++ | + |
| VEGFR-3 * | ++++ | +++ | +++ | +++ |
| EGFR ** | - | - | ++++ | - |

(- not detectable, + = low; ++++ = high)

## REFERENCES

1. Marmé D, Norbert F. (2008). Tumor Angiogenesis Basic Mechanisms and Cancer Therapy.
2. Gross-Goupil M1, François L, Quivy A, Ravaud A. Axitinib: a review of its safety and efficacy in the treatment of adults with advanced renal cell carcinoma. Clin Med Insights Oncol. 2013; 7:269-277.
3. Kumar R1, Crouthamel MC, Rominger DH, Gontarek RR, Tummino PJ, Levin RA, King AG. Myelosuppression and kinase selectivity of multikinase angiogenesis inhibitors. Br J Cancer. 2009; 101:1717-1723.