Inhibitors of cellular oxygen sensors – innovative tools for the treatment of Neuroblastoma?

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INTRODUCTION

Neuroblastoma is the most common solid malignant extracranial tumor of childhood originating from the sympathetic nervous system. Under standard therapy, the 5-year event-free survival rate remains below 50 % in high-risk neuroblastoma. Aggressive tumors grow faster than the supplying blood vessels, which leads to hypoxia in the tumor center, thus activating hypoxia related signalling pathways. Mammalian cells sense hypoxia via the oxygen-sensing enzymes prolyl-4-hydroxylase domain (PHD) 1-3 proteins and factor inhibiting HIF (FIH). Inhibition of the PHDs leads to the accumulation of the transcription factor subunit Hypoxia-inducible-factor-1α (HIF-1α; Fig. 1). Treatment with pharmacologic PHD inhibitors (PHIs) has reduced tumor mass and metastasis formation in other solid tumors. The study aims to investigate the effect of PHIs on neuroblastoma cells to explore the PHI potential as future treatment option.

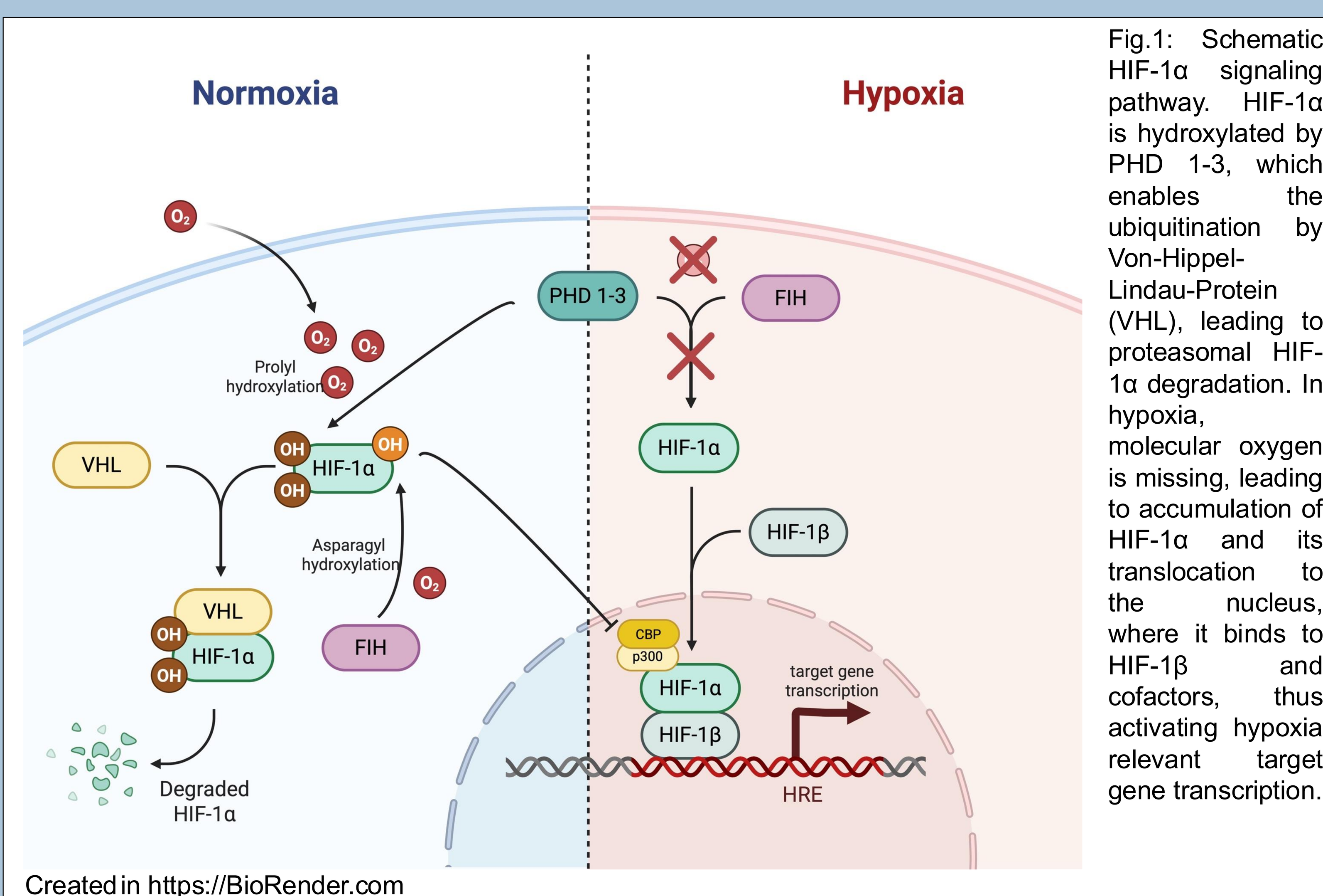

Fig.1: Schematic HIF-1α signaling pathway. HIF-1α is hydroxylated by PHD 1-3, which enables the ubiquitination by Von-Hippel-Lindau-Protein (VHL), leading to proteasomal HIF-1α degradation. In hypoxia, molecular oxygen is missing, leading to accumulation of HIF-1α and its translocation to the nucleus, where it binds to HIF-1β and cofactors, thus activating hypoxia relevant target gene transcription.

METHODS

Five different neuroblastoma cell lines SHEP-2, CHLA-20, CHLA-90, LAN-1 and COG-N-519 were treated with different PHIs (FG-4592, AKB-6548, GSK1278863, BAY85-3934) that are currently used in the clinic for treatment of renal anemia as well as with the pan inhibitor DMOG. HIF-1α stabilization as well as protein levels of the HIF-1α target genes PHD 2 and PHD 3 were detected using immunoblotting. Moreover, cell proliferation was assessed using a BrdU-assay. Cell survival and confluency were observed every eight hours for seven days in live-cell imaging in near-infrared-(NIR)-transfected neuroblastoma cell lines. These cells constitutionally express a fluorescent protein used as a parameter for cell vitality.

RESULTS

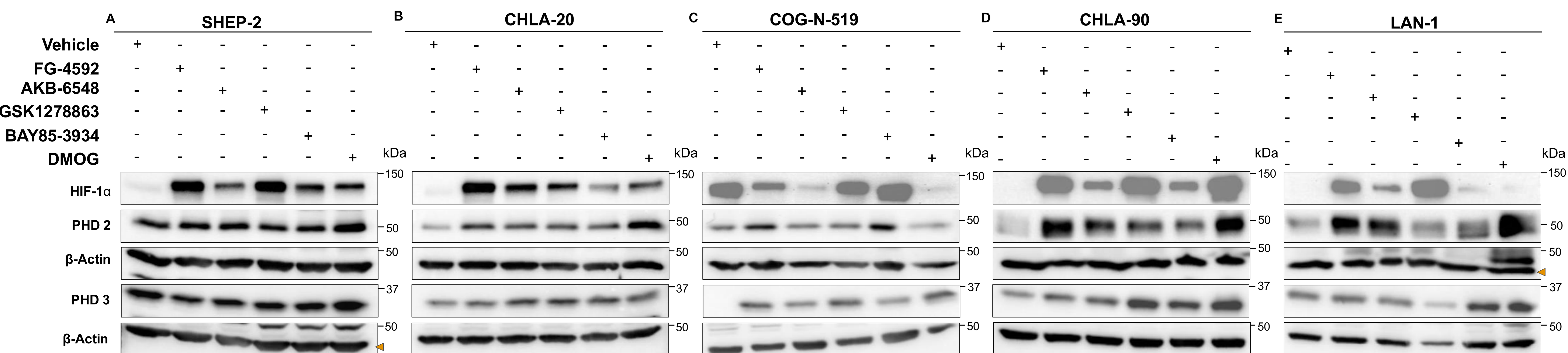

Fig. 2: Representative images of immunoblot analysis showing HIF-1α (120kDa) stabilisation as well as PHD 2 (46kDa) and PHD 3 (27kDa) induction in (**A**) SHEP-2, (**B**) CHLA-20, (**C**) COG-N-519, (**D**) CHLA-90 and (**E**) LAN-1 cells after treatment with the described PHIs for 24 hours (Vehicle (DMSO) 100µM, FG-4592 100µM, AKB-6548 100µM, GSK1278863 100µM, BAY85-3934 10µM, DMOG 1000µM). β-Actin (42kDa) was used as loading control.

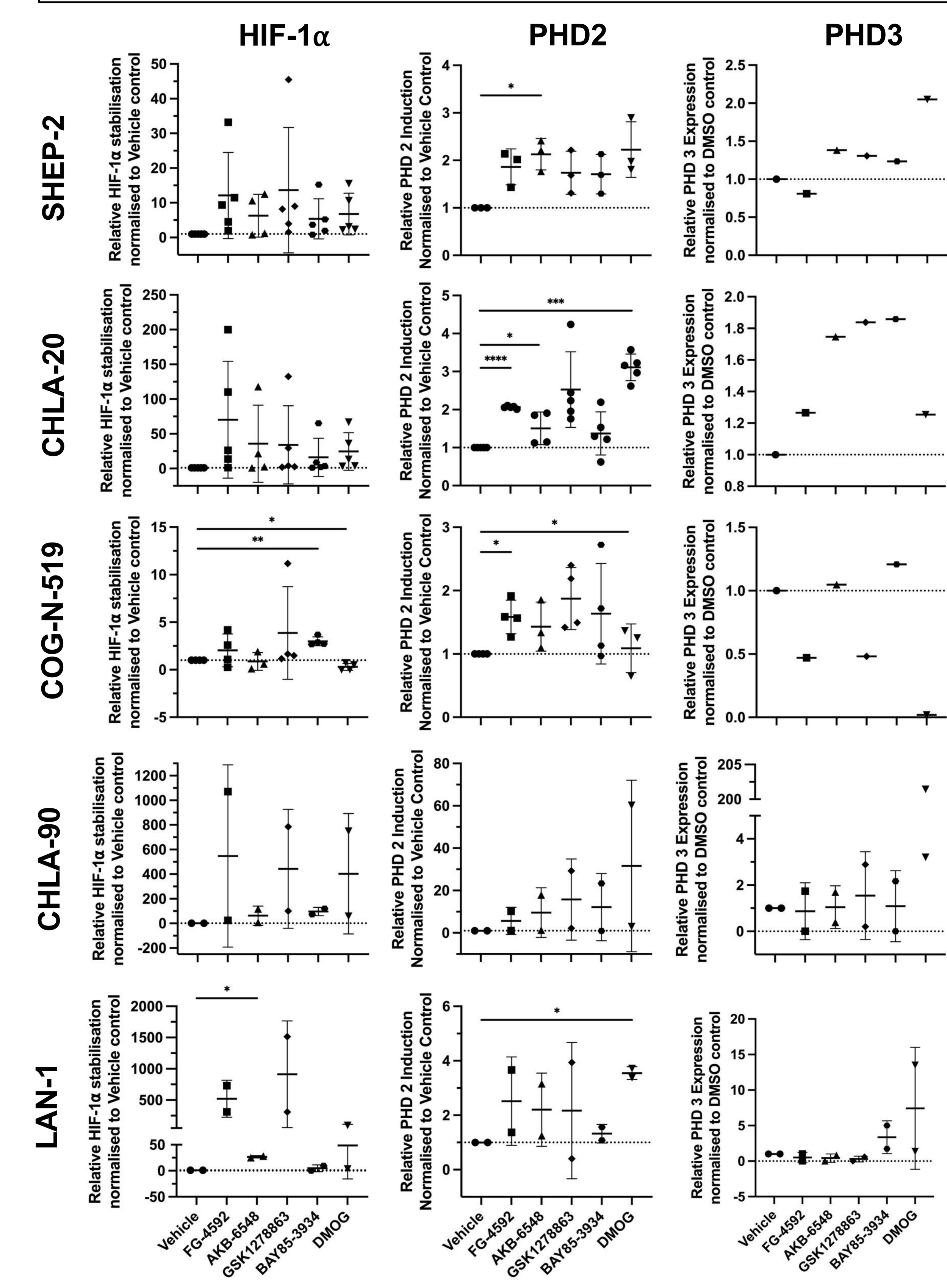

Fig. 3: Quantification of Immunoblots analysis shown in Fig. 2. Band intensities were measured and normalised to the respective loading control before normalising to vehicle control for each cell line.

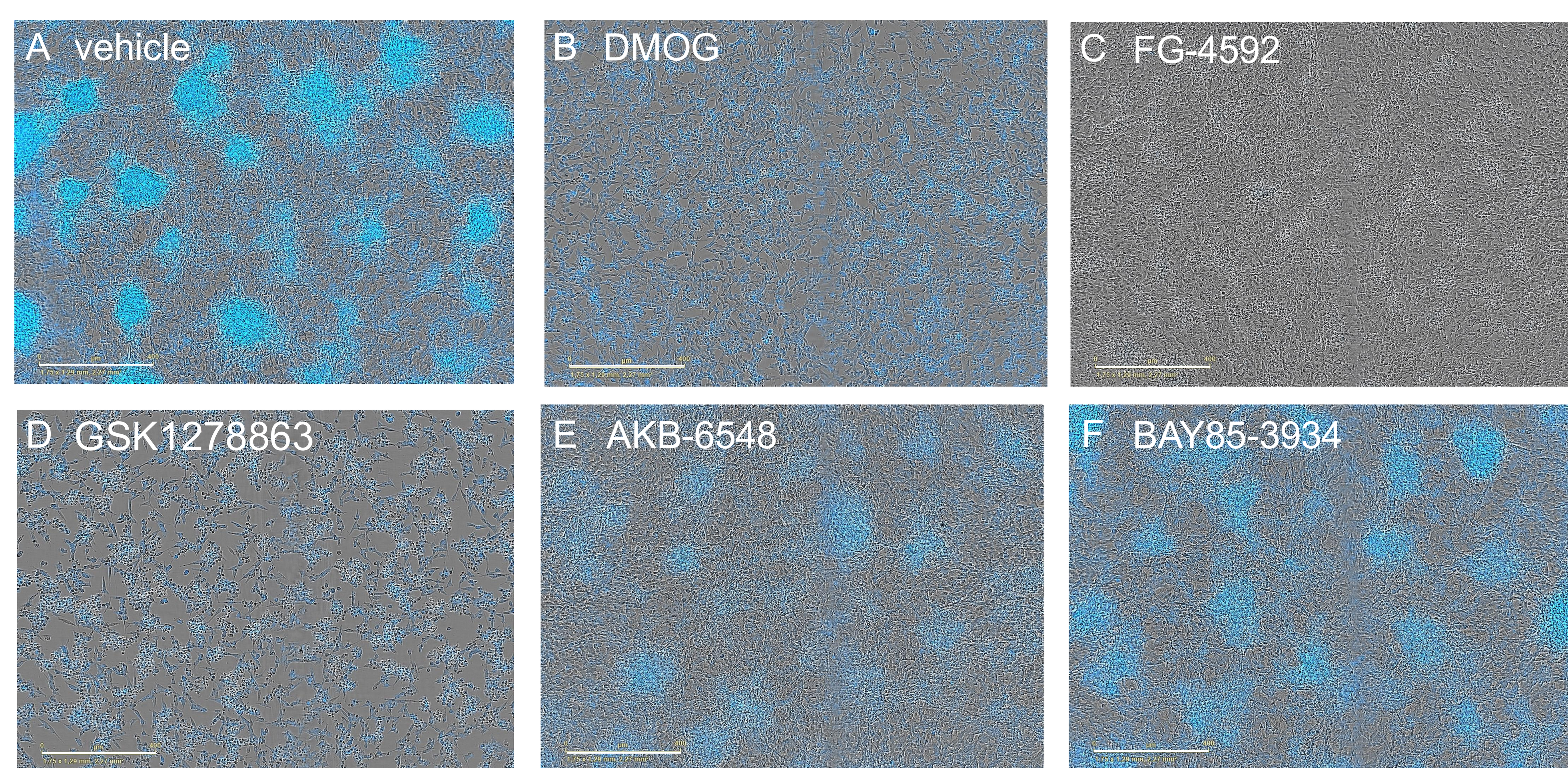

Fig. 4: Life cell imaging of NIR-expressing CHLA-20 cells treated with different PHIs. Representative microscopic images of CHLA-20 cells treated with (**A**) Vehicle, (**B**) DMOG, (**C**) FG-4592, (**D**) GSK1278863 (**E**) AKB-6548, (**F**) BAY85-3934. Cells were treated for 160 h before NIR Fluorescence intensity (blue) was assessed. Scale represents 400 µm.

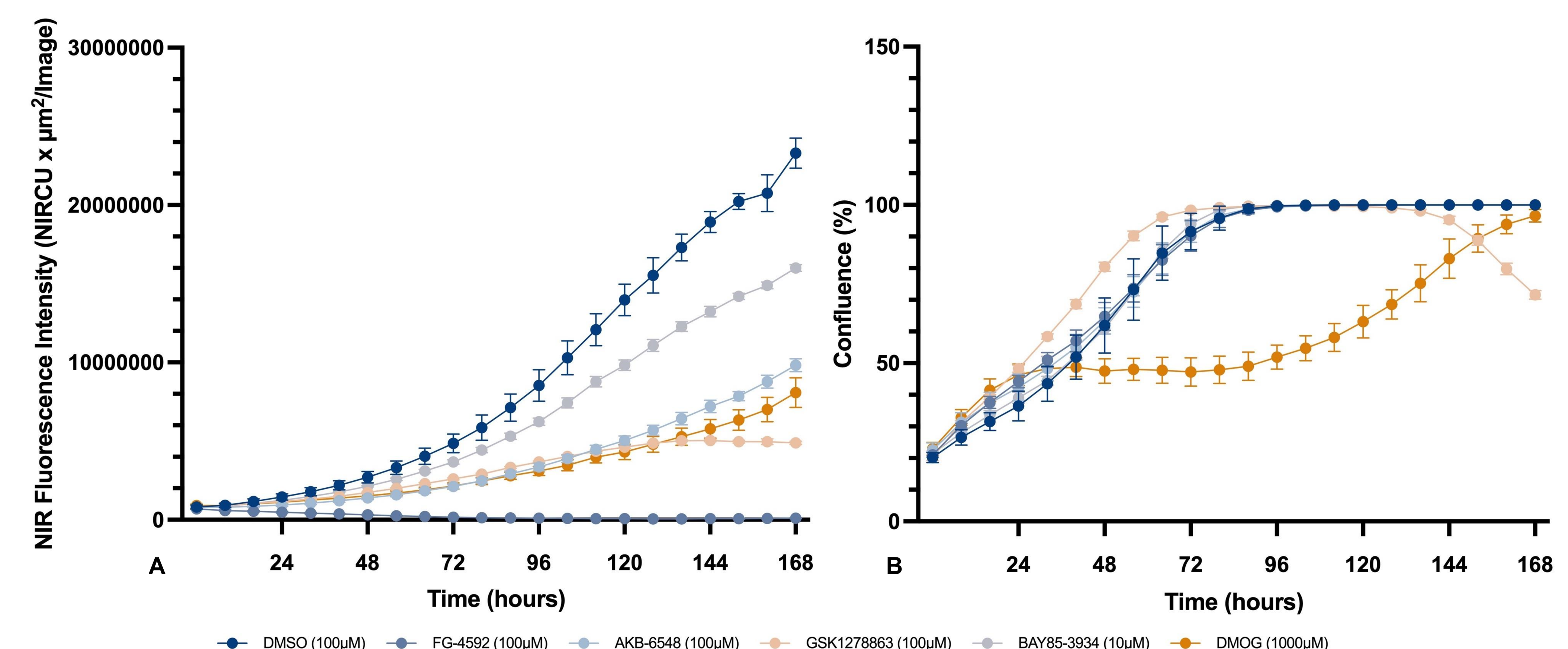

Fig. 5: Cell growth displayed as (**A**) NIR fluorescence and (**B**) confluency in CHLA-20 cells after single treatment with different PHIs. The data was assessed every 8 hours for 7 days.

SUMMARY AND CONCLUSIONS

- All tested PHIs stabilize HIF-1α, but to various degrees and cell line dependent
- PHD2 induction strongly varies depending on the cell line
- GSK1278863 reduces proliferation in life cell imaging after five days, while DMOG slows down cell growth, but does not inhibit it
- FG-4592 treatment decreases fluorescence rapidly, nevertheless cells continue to proliferate
- These contradictory results will be further investigated

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