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P1-135: Autophagy inhibition sensitises glioblastoma cells to Src family kinase inhibitors Si306 and its prodrug

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Introduction
Glioblastoma (GBM) is among the most frequent and aggressive brain tumors characterized by infiltrating nature, high proliferation, and resistance to chemotherapy and radiation. GBM exhibit high expression of Src tyrosine kinase which regulates proliferation, survival, and invasiveness of tumor cells, making Src a potential target for GBM therapy. Numerous Src family kinase inhibitors (SFKI) were reported to induce autophagy, thus protecting cells from undergoing cell death. However, inhibition of autophagy was shown to sensitize cells to SFKI in several cancer types.

Material and Methods
Human GBM cell line U87 and its multidrug-resistant (MDR) counterpart U87-TxR were transfected with RFP-LC3, an autophagy marker. The ability of two SFKIs, pyrazolo[3,4-d]pyrimidines Si306 and its prodrug pro-si306, to induce autophagy in RFP-LC3-transfected GBM cells was evaluated by flow cytometry and fluorescent microscopy. Cell viability was assessed by MTT assay. The autophagy induction and autophagic flux were evaluated by Acridine orange assay, immunocytochemistry and immunoblotting. Cell proliferation rate was analyzed by CFSE assay. Cell death was detected by Annexin/Propidium Iodide assay. PARP-1 cleavage was assessed by immunoblotting.

Results and Discussions
SFKI treatment resulted in degradation of RFP-LC3 after 3 h treatment as well as in formation of RFP-LC3 puncta in GBM cells demonstrating autophagy induction. The effect of SFKIs on autophagy induction persisted after 48 h, as demonstrated by autophagy markers LC3 and p62. Inhibition of autophagy by Bafilomycin A1 sensitized both U87 and U87-TxR cells to Si306 and its pro-drug after 48 h. The anti-proliferative effect of Si306 and pro-Si306 was additionally increased after autophagy inhibition by Bafilomycin A1. Furthermore, while single SFKI treatments did not cause significant cell death, combination treatments with autophagy inhibitor induced necrosis in U87 and U87-TxR cells after 48 h. Detection of necrotic PARP-1 fragment further confirmed necrotic cell death.

Conclusion
Taken together, these data suggest that autophagy induced by Si306 and pro-Si306 has a protective role in GBM cells, and that autophagy modulation may be used to enhance the anticancer effects of SFKIs. In addition, as the ability of the SFKIs to induce autophagy was not diminished by the presence of the MDR phenotype makes these compounds promising for treatment of MDR cancers.