Antiangiogenic and antineoplastic effects of novel thienyl-based tyrosine kinase inhibitors in hepatocellular carcinoma cells

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Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer death in the world, accounting for more than 80% of global primary liver cancers. In recent years, significant progress has been made in surgical treatment, interventional therapy, and radiotherapy for early HCC. However, advanced, and metastatic HCC are still lacking effective medical treatment options. Thus, new systemic treatments are urgently needed. Applying an orchestrated set of *in vitro* and *in vivo* methods we here characterized the antineoplastic and antiangiogenic mode of action of two newly synthesized tyrosine kinase inhibitors for innovative medical HCC treatment.

A cell free kinase assay was used determine the kinase inhibitory potency and specificity of the novel inhibitors, termed Thio-Iva and Thio-Dam**.** Crystal violet staining and real-time cell growth measurement (iCelligence) were used to determine antiproliferative effects of Thio-Iva and Thio-Dam in hepatocellular Huh-7 and SNU-449 cancer cells. Flow cytometry was employed to evaluate cell cycle arresting effects. Induction of reactive oxygen species (ROS) was determined by fluorescence microscopy with the ROS sensitive dye CellROX Orange. Apoptosis induction was measured with Caspase 3 activity ELISA. Western blots were used to confirm apoptosis and cell cycle arrest on the protein level. Antiangiogenic effects were studied in tube formation assays with endothelial EaHy.926 cells. Finally, the antiangiogenic and antineoplastic effects of the novel compounds were confirmed *in vivo* by performing chorioallantoic membrane assays (CAM assays).

Thio-Iva showed multi kinase inhibitory activity with most pronounced effects on VEGFR-2 that was inhibited by ~90 %. Thio-Iva and Thio-Dam displayed pronounced antiproliferative effects in hepatocellular HUH-7 and SNU449 cancer cells with IC_{50} concentrations in the submicromolar or low micromolar range. Flow cytometry analysis revealed a pronounced cell cycle arrest in the G2-M phase of the cell cycle and a corresponding Cyclin B1 suppression in HCC cell lines after treatment with Thio-Iva and Thio-Dam. The novel compounds were found to effectively inhibit capillary tube formation of endothelial EaHy.926 cells *in vitro*, pointing towards antiangiogenic effects of the novel compounds. Antiangiogenic and antineoplastic effects of Thio-Iva and Thio-Dam were confirmed in systemic measurements employing *in ovo* testing of tumor bearing fertilized chicken eggs (CAM assays). Notably, the novel compounds were even more powerful in reducing HCC tumor growth *in ovo* than the clinically relevant TK inhibitor Sorafenib.

Here, we could demonstrate the pronounced antineoplastic and antiangiogenic properties of the two novel tyrosine kinase inhibitors Thio-Iva and Thio-Dam in HCC cells. The novel compounds were shown to effectively attack HCC cells at cellular processes and features that are acquired during carcinogenesis and that are referred to as hallmarks of cancer. Our results show that Thio-Iva and Thio-Dam may provide new and valuable options for the treatment of hepatocellular carcinoma in the future.