**Single agent and synergistic activity of the “first in class” dual PI3K/BRD4 inhibitor SF1126 with Sorafenib in hepatocellular carcinoma**

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**Supplementary Methods**

Chau Talalay synergy study

Hep 3B, Hep G2, SK-Hep1 and Huh7 cells (1 x104 cells/well) were plated in 96-well plates in DMEM medium containing 10% FBS and 2 mM L-glutamine. Cells were incubated overnight and treated with vehicle (acidified water or DMSO) or serial dilutions of SF1126 or Sorafenib for 48 hr. CCK-8 (Dojindo Molecular Technologies, Gaithersburg, MD) was added and plates were incubated at 37ºC in 5% CO2 for 3-6 hours. Colorimetric signals were read as absorbance at 450 nm. IC50 values were calculated from dose-response curves by plotting absorbance values normalized as a percentage of control against the log of the drug dose using GraphPad Prism (GraphPad Software, La Jolla, CA). Synergy studies were performed by treating cells with either SF1126 or Sorafenib alone or in combination at 0.25X, 0.5X, 1X, 2X, and 4X the respective IC50 values followed by the CCK-8 assay as described above. Isobolograms and Combination Index values were calculated according to the Chou-Talalay method using CompuSyn software (ComboSyn, Inc., Paramus, NJ).

Chromatin immunoprecipitation (ChIP) studies

JQ1 is well known for downregulating MYC transcription, hence we used this inhibitor as a positive control. In brief, cells were treated with SF1126 (10 μM), JQ1 (1 μM), or vehicle control for 24 hours and then cross-linked with 1.1% formaldehyde, washed with PBS and frozen at -80°C. Antibody-conjugated beads were prepared by blocking 50 μL of protein A/G agarose beads with 0.5% BSA (w/v) followed by incubation with 6.25 μg of BRD4 antibody, 5 μg of normal rabbit IgG, or 5 μL of tri-methyl histone H3 (Lys4) antibody. Cross-linked cells were lysed with lysis buffer 1 (50 mM HEPES pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, and 0.25% Triton X-100), washed with lysis buffer 2 (10 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA pH 8.0 and 0.5 mM EGTA pH 8.0) and sonicated in lysis buffer 3 (10 mM Tris-HCl pH 8, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% Na-deoxycholate, and 0.5% N-lauroylsarcosine) for 10 cycles for 30 seconds each on ice (18 W) with 60 seconds on ice between cycles. Sonicated lysates were supplemented with Triton X-100 to 1% and cleared. Aliquots were reverse-crosslinked and digested with RNase A overnight and purified with QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) for quantification of input chromatin. Sonicated, cleared chromatin (15 μg) was incubated overnight at 4°C with antibody-conjugated agarose beads. Beads were washed three times with wash buffer (50 mM Tris-HCl pH 8, 140 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.1% Na-deoxycholate, and 0.1% SDS), one time with was buffer with 500 mM NaCl, one time with LiCl wash buffer (20 mM Tris pH 8.0, 1 mM EDTA, 250 mM LiCl, 0.5% NP-40, and 0.5% Na-deoxycholate) and one time with TE. Chromatin was eluted in elution buffer (50 mM Tris-HCl pH 8, 10mM EDTA, and 1% SDS), reverse cross-linked and digested with RNase A overnight and then purified as above.

Legends to Supplementary Figures

Supplementary Table S1. Chau Talalay Synergy studies on HCC cells: Huh-7 and SK-Hep1 cells were treated with either SF1126 or Sorafenib alone or in combination at 0.25X, 0.5X, 1X, 2X, and 4X the respective IC50 values followed by the CCK-8 assay as described in Methods and used to calculate the Combination Index values at the IC50, IC75, and IC90 using the Chou-Talalay method.

Supplementary Figure S1. Annexin-V FITC analysis of SF1126 and Sorafenib treated HCC cells: (A) Fig. shows total no. of apoptotic cells in Hep 3B, Hep G2, SK-Hep1 and Huh7 cells treated with different conc. of SF1126 and Sorafenib either alone or in combination. (B) Fig. illustrates the FACS analysis data of SK-Hep 1 cells treated with different conc. of SF1126 and sorafenib either alone or in combination. Data is shown as mean ± SEM, \*P <0.05, \*\*P <0.01 and \*\*\*P <0.001 vs. vehicle, t test.

Supplementary Figure S2. SF1126 and Sorafenib inhibit cell cycle progression by inducing apoptosis. A & B, 1 x 104 Huh-7 (A) and SK-Hep-1 (B) cells were cultured overnight and were treated with SF1126 or Sorafenib alone (at 1, 5 & 10 μM conc.) and SF1126 + Sorafenib together (1:1; at 0.5, 2.5 & 5 μM concentrations each) for 24 hrs.Cell Death was assayed using Cell Death ELISA (Roche applied Science, Penzburg, Germany) (measuring the amount of cleaved DNA–histone complexes (nucleosomes) in the cytoplasm(P = 0.05). C & D, Caspase-3 activity assay of Huh-7 (C) and SK-Hep-1 (D) cells. 2 x 106 cells were cultured for overnight and were treated with SF1126 or Sorafenib alone (at 1, 5 & 10 μM concentrations) and SF1126 + Sorafenib together (1:1; 0.5, 2.5 & 5 μM conc. each) for 24 hrs. Caspase-3 activity was assayed using Caspase-3 activity assay (Roche applied Science, Penzburg, Germany. Data is shown as mean ± SEM, \*P <0.05, \*\*P <0.01 vs. DMSO, t test.