SUPPLEMENTAL RESULTS

Long-term treatment of HCT116.hCG.Luc orthotopic tumors: *in vivo monitoring and survival*

To confirm our previous results in a second colon cancer cell line, thirty-one mice were intracecally implanted with HCT116.hCG.Luc. Two weeks later, mice were randomized according to bioluminescence signal and treatments with (1) control vehicle, (2) FOLFOX-like, (3) topotecan, (4) topotecan+pazopanib, (5) FOLFOX-like+topotecan, (6) FOLFOX-like+pazopanib, (7) FOLFOX-like+topotecan+pazopanib (n=4-5) were initiated. As pazopanib monotherapy did not show any effect in our previous results, this group was not included in this experiment. Furthermore, UFT was replaced by a combination of UFT+Folinic acid+oxaliplatin, in order to closely mimic the clinical standard therapy of FOLFOX. This experiment lasted 28 weeks (supplemental Figures 4 and 5). At this endpoint, eight mice (2 mice on topotecan, 4 mice on topotecan+pazopanib, and 2 mice on FOLFOX-like+topotecan+pazopanib) were still alive with very limited tumor burden. FOLFOX-like, as well as the combination of FOLFOX-like+pazopanib did not significantly delay the increase in bioluminescence signal compared to controls (supplemental Figure 4), but did reduce the absolute strength of bioluminescence signal (supplemental Figure 5A). In contrast, all groups treated with a regimen containing oral topotecan showed a delayed increase and reduced maximum level in bioluminescence. Animals treated with topotecan alone, FOLFOX-like+topotecan, topotecan+pazopanib and FOLFOX-like+topotecan+pazopanib showed a significantly reduced signal compared to control animals ($P<0.05$). Among these groups, no significant difference in bioluminescence was observed. These results were confirmed by quantification of secreted β-hCG (supplemental Figure 5B). β-hCG-levels were lower in animals treated with

topotecan-containing regimens. However, due to high standard errors, β-hCG values did not reach significance. None of the treatment regimens resulted in weight loss. In the group treated with FOLFOX-like+topotecan+pazopanib, two mice died very early (week 8). Necropsy revealed very small tumor burden but severe anemia. The remaining number of mice was not sufficient to perform statistical analysis for this group.

Control animals showed a median survival of 52.5 days after tumor implantation. Survival was significantly prolonged in animals treated with topotecan alone (162 days, P = 0.0025), FOLFOX-like+topotecan (113 days, P = 0.0025) and topotecan+pazopanib (>196 days, P=0.0062) (supplemental Figure 5D,E). Among these treatment regimens, no significant difference in survival was observed.

Long-term treatment of HCT116.hCG.Luc orthotopic tumors: postmortem analysis

Endpoint necropsy and single organ bioluminescence results are shown in supplemental Figure 5C. All control animals had lymph node, liver, diaphragmatic and peritoneal metastases, 1 of 4 mice also had lung metastases, and 2 of 4 control mice had malignant ascites. All treatment groups receiving topotecan (as mono- or combination therapy) showed reduced hepatic, pulmonary, lymph node and peritoneal metastasis. Once again, this result was most pronounced in liver metastasis: Of a total of 13 mice treated with control vehicle or regimens without topotecan, 7 mice (=54%) had liver metastases. In striking contrast, none of of the 18 mice treated with topotecan mono- or combination therapy showed hepatic metastases.