6 SUMMARY

Treatment options of advanced gastrointestial tumors are limited and unsatisfactory. Research and development of new innovative tumor therapies is therefore urgently needed.

The insulin-like growth factor receptor 1 (IGF-1R) is a new and interesting target for innovative and targeted cancer therapy. In many human tumors including gastrointestinal tumors the IGF-1R is overexpressed and/or over-activated, which has been demonstrated to contribute to tumorigenesis and tumor progession.

Histone deacetylases are recognized to be promising targets for molecular tumor therapy. The equilibrium of steady state acetylation of the histones is tightly controlled by antagonistic effects of histone acetyltransferases (HATs) and HDACs and is known to play an important role in the regulation of gene transcription. In a variety of tumors the activity of HDACs and HATs is dysregulated. Disruptions of the equilibrium of histone acetylation and deacetylation leads to an abberant expression of mitooncogenic genes thereby mediating tumor cell proliferation and tumorigenesis.

This study was aimed to evaluate the potency of therapeutic interventions by an inhibition of the activity of the IGF-1R and the histone deacetylase activity respectively in several gastrointestinal tumors, as this has not yet been tested .

In the first part of this work the antiproliferative effects of an IGF-1R inhibition in three gastrointestinal tumor entities, gastrointestinal neuroendocrine tumors (NET), hepatocellular carcinoma (HCC) and colorectal carcinoma (CRC), were investigated.

In vitro studies in cell line models revealed that inhibition of IGF-1R by the specific IGF-1R tyrosine kinase inhibitor NVP-AEW541 significantly inhibited cell growth of all three gastrointestinal tumor models in a time- and dose-dependent manner. Antineoplastic effects of NVP-AEW541 were also detected in primary cultures of human NET and CRC, which were investigated in order to have a clinically more relevant cell model as compared to commercially available cell lines. In addition, they form the basis for an individualized tumor therapy.

The antiproliferative effects of IGF-1R inhibition were found to be based on the induction of mitochondria-dependent apoptosis and an arrest of the cell cycle at the G1/S-checkpoint.

Western blot analyses revealed respective changes of the expression pattern of apoptosis- and cell cycle- relevant proteins. In this respect the proapoptotic protein Bax was upregulated while antiapoptotic Bcl-2 was suppressed. Moreover, the two cell cycle

inhibitors $p21^{Waf1/Cip1}$ and $p27^{Kip1}$ were overexpressed whereas the expression of the cell cycle promoter cyclin D1 was inhibited. Furthermore it could be demonstrated that IGF-1R inhibition causes downregulation of the Ras-Raf-MAPK- and the PI3-K signalling pathway.

The in-depth characterization of the signalling events and pathways which were causative for the observed antineoplastic effects of the IGF-1R inhibition provided a rational for combination therapies. Combination treatment proved that coapplication of IGF-1R inhibitors with conventional cytostatics result in additive to even overadditive growth inhibition of gastrointestinal tumor cells.

Since IGF-1R is known to interact with the epidermal growth factor receptor, the effect of targeting both the EGFR as well as IGF-1R by combination treatment was evaluated, too. In fact, the simultaneous inhibition of IGF-1R and EGFR enhanced the antiproliferative effect as compared to the effect of either agent alone.

The second part of this work was to evaluate the effect of histone deacetylase (HDAC) inhibition on the growth of gastrointestinal neuroendocrine tumors (NET) and cholangiocarcinoma (CCC) cells. The anti-HDAC investigations were mainly performed with the synthetic benzamide MS-275. Additionally, a part of the investigations was also performed with two other, naturally occurring HDAC inhibitors, namely trichostatin A and sodium butyrate.

All HDAC inhibitors exerted significant antiproliferative effects both in NET as well as in CCC cells by inducing apoptosis and interfering with cell cycle progression.

HDAC inhibitor-induced apoptosis was characterized by activation of caspase-3 and subsequent DNA fragmentation. HDAC inhibition resulted in a significant cell cycle arrest at the G_1-S checkpoint in all investigated cell lines. Interestingly, some of the investigated cell lines showed an additional G2/M arrest revealing cell type specific mode of action of HDAC inhibitors.

Investigations of the expression pattern of apoptosis- and cell cycle-relevant proteins confirmed the cell type specific differences. For instance, expression of antiapoptotic Bcl-2 was dowregulated both in NET as well as in CCC cells, while proapoptotic Bax was solely upregulated in CCC cells.

Combination treatment of HDAC inhibitors with conventional cytostatics or new, targeted anticancer agents respectively resulted in (over-)additive growth inhibitory effects. Especially combinations of the HDAC inhibitor MS-275 with the multi-kinase

inhibitor sorafenib or the proteasome inhibitor bortezomib were highly effective both leading to synergistic growth inhibition.

To conclude, the data of this work show that inhibition of the insulin-like growth factor receptor 1 (IGF-1R) and also those of histone deacetylases (HDAC) represent two novel promising approaches for the treatment of advanced gastrointestinal tumors. In light of the encouraging *in vitro*-data, these innovative therapeutic concepts should be evaluated *in vivo* and in clinical studies in the future.