treatment results only in low pCR rates, which is reflected by a moderate induction of apoptosis. IM-induced apoptosis is executed via the intrinsic (mitochondrial) pathway of caspase activation, which is regulated by the BCL-2 protein family. Overexpression of antiapoptotic BCL-2 proteins is frequently observed in a wide range of tumor entities and is associated with resistance. Here we have studied the therapeutic potential of a prototypic BH3 mimetic in GIST in vitro.
Methods: The BH-3 mimetic ABT-263, an inhibitor of BCL-2, BCL-XL and BCL-W, was used alone and in combination with anticancer agents(IM, 17-AAG, nutlin-3 and doxorubicin) in GIST models. Cell viability was measured using SRB assays; induction of apoptosis was analysed by annexin-V/7AAG staining and measurement of activated caspase 3/7. Expression of BCL-2 proteins and caspase-dependent protein cleavage was detected by immunoblotting (IB).
Results: Expression of pro- and antiapoptotic BCL-2 family members varied in the examined cell lines with high levels of BCL-2 in GIST882, GIST430 and GIST48B. ABT-263 given alone only moderately reduced viability with IC50s ranging between 500 nM (GIST48B) and 10 μM (GIST48). In IM-sensitive GIST882 combination of IM 1μM with of ABT-263 100 nM led to a significant increase of PARP cleavage (15-fold, compared to 9- and 1.5-fold for IM and ABT-263 alone) as measured by IB. Synergistic effects were also seen in cytometric assays (42% apoptotic cells for combinational treatment compared to 16% and 1% for respective single treatment). Combining ABT-263 with the MDM2 inhibitor nutlin-3 in IM-resistant cell lines GIST48, GIST430 and GIST48B led to an 11-, 15- and 19-fold activation of caspases 3 and 7, respectively, as compared to monotherapy with nutlin-3 (2-, 1- and 4-fold, respectively) or ABT-263 (each 2-fold). Combining ABT-263 with doxorubicin or 17-AAG failed to reveal a positive or negative interaction.
Conclusion: Antagonizing BCL-2 proteins with pharmacologic BH3 mimetics in GIST is a promising strategy to enhance apoptosis induced by several active agents, including IM, 17-AAG and nutlin-3. This provides a first rationale for clinical application of this strategy. Individual profiling of GIST-specific expression of BCL-2 family proteins may guide the selection of patients with the most benefit.

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Potential therapeutic effect of oxidative stress modulators in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, often diagnosed at an advanced stage when the most potentially curative strategies are no longer effective. Several known environmental risk factors for HCC development lead to generation of reactive oxygen species (ROS), promoting oxidative stress. On the other hand, taking in account that mitochondria are the main site for ROS production, it is conceivable that it may have a relevant role in carcinogenesis. Besides, neoplastic cells have a higher mitochondrial membrane potential than normal cells, which may also be explored in the development of new approaches to treat HCC.
The aim of this study was the evaluation of the therapeutic efficacy of new compounds targeting the mitochondria, such as dequalinium (DQ), a lypophilic cation, and the natural bioactive compounds, vitamin C (ascorbic acid, AA, and dehydroascorbic acid, DHA) and epigallocatechin-3-gallate (EGCG), a green tea polyphenol, as in monotherapy and/or in association with conventional antitumoral therapies.
For this purpose, we used the well-established HCC cell line, HUH-7, maintained in culture in absence and presence of increasing concentrations of the DQ, EGCG, AA and DHA, either in monotherapy or in combination with the conventional anticarcinogenic drugs, 5-Fluorouracil and doxorubicin. The antiproliferative effect was assessed by the Alamar Blue assay and cell death analysis performed by morphological studies and flow cytometry. In order to evaluate the involvement of oxidative stress and mitochondria in the observed cytotoxicity, the intracellular ROS accumulation was studied using the fluorescent probes DCFH2-DA and DHE; the mitochondrial membrane potential was determined using the fluorescent probe JC1.
The results obtained suggested that DQ and EGCG as single agents had an antiproliferative and cytotoxic effect in a dose and time dependent manner, while AA and DHA, when used alone, only showed a modest effect under the mentioned test conditions. However, when they were used in combination, a synergistic antiproliferative and pro-apoptotic effect could be observed. These compounds induced cell dead preferentially by apoptosis which may be related with the higher mitochondrial membrane potential depolarization and mediated by the observed increase in ROS production (especially superoxide).
This study suggested that DQ and natural bioactive compounds may constitute a new therapeutic option for HCC. However, new drugs associations, as well as new administration schemes, should be tested in order to improve the therapeutic efficacy in HCC.

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The role of MNK kinase inhibition in combination with mTOR inhibition in the proliferation of renal cancer cells

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Mitogen-activating protein kinase signal-integrating kinases (MNKs) are the sole kinases of eukaryotic initiation factor 4E (eIF4E) at serine 209. MNK1 is activated through MAPK signaling. eIF4E is an oncogene over-expressed in several cancers. It regulates the synthesis of proteins involved in progression to the malignant phenotype, including cyclin D1, VEGF and MDM2. The oncogenic activity of eIF4E can be dependent on its phosphorylation at serine 209. eIF4E is additionally regulated by binding to 4E-BP under the control of mTOR and it is already a therapeutic target in renal cancer via the mTOR inhibitors temsirolimus and everolimus. Inhibition of mTOR can result in an increase in eIF4E phosphorylation in some model systems. We have investigated the anti-proliferative effects of MNK inhibition in renal cancer cell lines both alone and in combination with mTOR inhibition.
Renal cancer cell lines were treated with CGP57380 (a MNK kinase inhibitor) or rapamycin or both. Western blotting was used to assess eIF4E and 4E-BP phosphorylation and downstream targets of eIF4E phosphorylation such as cyclin D1. The effects of treatment on proliferation and survival were assessed and assays of cell cycle analysis and apoptosis performed.
The proliferation of renal cancer cell lines is sensitive to inhibition of MNK kinases. This is predominantly due to cell cycle arrest and consistent with a reduction in cyclin D1 at both the protein and RNA level. Cells which showed sensitivity to mTOR inhibition showed an additive effect to the addition of MNK kinase inhibition.
Inhibition of eIF4E phosphorylation results in a reduction in proliferation in both VHL mutant and VHL wild-type renal cancer cell lines. There was an additive effect of the addition of rapamycin. The MNK kinases are worthy of further investigation as a therapeutic target in renal cancer.

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Human ependymoma tumor-initiating cells (TICs) as a model for preclinical studies on EGFR-kinase inhibitors (EGFR-KIs)

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Background: Ependymomas represent 10% of pediatric brain tumors, with a dismal prognosis in 50% of patients. Increasing evidence shows that ependymomas arise from TICs, which are believed to be responsible for tumor development, progression, and recurrence. Given the high dependence of TICs on EGF, we explored the effects of the EGFR-KIs gefitinib and AEE788 on ependymoma TICs.
Material and Methods: We used neural stem cell permissive conditions to isolate TICs from pediatric ependymomas. Cells were characterized for the expression of stemness markers (CD133, nestin, and brain lipid binding protein), neurosphere-renewal ability, multipotency and tumorigenicity. The effects of gefitinib (which targets EGFR), and AEE788 (which simultaneously targets EGFR, HER2, and VEGFR1/2), were evaluated on cell proliferation and EGF-induced signaling *in vitro* and in TIC-driven xenografts.
Results: We established two TIC-lines that fulfilled all TIC criteria. When orthotopically implanted into nude mice, cells gave rise within 2–3 months to tumors that phenocopied parental tumors. Both lines exhibited a high pEGFR/EGFR ratio. EGFR-KIs reduced dose-dependently the proliferation and survival of TIC-lines, and effectively blocked EGF-induced and basal activation of EGFR, HER2, Akt and ERK. Treatment reduced CD133 expression dose- and time-dependently, suggesting selective effects of EGFR-KIs on the TIC subpopulation. On removal of growth factors, lines showed morphological changes towards neuronal- and astrocytic-like cells, and strongly up-regulated GFAP, while down-regulating the expression of CD133 and activated HER2. Differentiated TICs were less sensitive

to EGFR-KIs because of reduced cell death. AEE788 (50 mg/Kg twice a week for 8 weeks) administered to orthotopic TIC-xenografts did not significantly affect the survival. Experiments are ongoing to see whether AEE788 pretreatment reduces tumorigenicity of TIC-cells *in vivo*.
Conclusions: (1) These established cell lines and xenografts represent valuable models for both basic and preclinical research on ependymoma, for which the availability of tumor models is extremely limited. (2) Human ependymoma TICs are sensitive to EGFR-KIs *in vitro,* but not *in vivo*, prompting preclinical evaluation of combination treatment strategies.
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Anti-tumoral effects of the multi-targeted kinase inhibitor AEE788 in BRAF mutated colorectal cancer cells

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Background: Advanced colorectal cancer patients with tumours harboring a mutation in the KRAS or BRAF genes do not derive benefit from the administration of epidermal growth factor receptor (EGFR)-directed monoclonal antibodies, such as cetuximab or panitumumab. Therefore, other targeted therapies are needed. AEE788 is a novel synthesized oral small-molecule multitargeted kinase inhibitor with potent inhibitory activity against both EGFR and vascular endothelial growth factor receptor (VEGFR). The aim of this study was to determine the efficacy of AEE788 to inhibit cell proliferation in colorectal cancer cells with different RAS/BRAF mutational status, and to explore the involved mechanisms.
Materials and Methods: The human colorectal cancer cell lines SW48 (KRAS/BRAF non-mutated), Caco-2 (BRAF V600E) and HCT-116 (KRAS G13D) were treated with different doses of AEE788, in the presence or the absence of EGF or VEGF. Cell proliferation was measured using an XTT assay. Apoptosis was determined using both cell death detection ELISA and annexin flow cytometry assays. The expression and phosphorylation levels of EGFR, VEGFR, Akt and Erk1/2, and COX-2 expression were determined by western-blot using the corresponding specific antibodies.
Results: In all the three cell lines AEE788 effectively inhibited the phosphorylation of EGFR induced by EGF. In addition, AEE788 was capable to reduce the EGF-driven cell proliferation of SW48 and Caco-2 cells, but not of HCT-116 cells. Significantly, AEE788 reduced the VEGF-dependent cell proliferation of Caco-2 cells, that efficiently expresses cyclooxygenase-2 (COX-2), but not of SW48 or HCT-116 cells with low or undetectable expression of this enzyme, respectively. The antiproliferative effects of AEE788 in Caco-2 cells were associated to reduced activation of the EGFR/VEGFR downstream kinases Akt and ERK1/2 and enhanced apoptosis.
Conclusions: AEE788 exerts anti-proliferative and apoptotic effects in BRAF mutated colorectal cancer cells, by inhibiting both EGF- and VEGF-dependent intracellular signaling. Our results support that AEE788 may be effective in the management of colorectal cancer in a non-mutated KRAS setting, independently of BRAF mutational status.

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Preclinical characterization of EMD 1214063 − a selective c-Met kinase inhibitor in clinical phase 1

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The relevance of the oncogenic receptor tyrosine kinase c-Met for tumor progression, metastasis and aggressiveness has been convincingly demonstrated in preclinical and early clinical settings. c-Met can be activated by different mechanisms such as HGF binding and dimerization, over-expression, gene amplification or activating mutations. Several compounds with different selectivity profiles inhibiting c-Met are currently under preclinical/clinical investigation and might emerge as valuable cancer therapeutics in the future.
After the optimization of a hit structure identified during a high throughput screening, the highly selective c-Met kinase inhibitor EMD 1214063 was identified as clinical candidate for further development and is currently being investigated in a phase 1 clinical trial. This compound inhibits enzymatic and cellular c-Met kinase activity with IC_{50} values in the low nanomolar range. The pyridazinone EMD 1214063 displayed an impressive kinase selectivity of at least 300 fold when tested in vitro against a panel of more than 280 kinases at a concentration of 1 μM. The mechanism of action of our clinical candidate, including inhibition of phospho-c-Met, down-regulation of cyclin D1 and up-regulation of p27 in a dose and time dependant manner, has been shown in PK/PD experiments *in vivo*. This compound also demonstrated excellent anti-tumor activity *in vivo* in a variety of xenograft models, e.g. the gastric cancer cell line Hs746T, the lung cancer cell line EBC-1 or the glioblastoma cell line U87MG, either as single agent or in combination. Depending on the sensitivity of the particular model, complete regression and tumor free survival was observed with doses as low as 6 mg/kg/d administered per os. The overall profile of EMD1214063 including the chemical structure, structure–activity relationships, in vitro potency, selectivity profile, pharmacokinetic and *in vivo* data will be discussed.

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Specific TGF-beta receptor-I inhibition using LY364947 impairs signaling, motility, and invasion in parental and multikinase inhibitor-resistant hepatocarcinoma cells

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Background: Hepatocarcinomas (HCC) are highly malignant tumors of unmet medical needs. LY364947, a selective ATP-mimetic inhibitor, specifically inhibits TGF-β receptor (TβR)-I activation at nanomolar concentrations. TβR-I activation induces angiogenesis, cell invasion, and epithelial-to-mesenchymal transition (EMT), offering opportunities for investigating the potential of novel TβR-I inhibitors such as LY364947 in HCC.
Materials and Methods: We investigated the antiproliferative effects of LY364947 in a panel of human HCC and other gastrointestinal cancer cells by MTT assay, baseline and phosphorylated (p-) protein levels by western blot analysis, mRNA expressions by qRT-PCR, motility by wound-healing assay, and invasion by matrigel assay.
Results: LY364947 was tested in SK-HEP1 cells and the derived-counterparts SK-HEP-1R cells selected by stepwise exposure to the multikinase inhibitor sunitinib (cross-resistant to sorafenib). Protein- and mRNA-expressions of TGF-β1, TGF-β2, and TβR-I were detectable in SK-HEP1 and SK-HEP1-R cells, a low expression of mRNA TβR-II (with no protein) signal being observed in these cells. Exogenous stimulation of SK-HEP1 and SK-HEP1-R cells with TGF-β yielded the downstream activations of p-Smad2 and p-Smad3 as well as p-ERK1/2, p-AKT^{ser473}, and p-S6 in SK-HEP1 cells. In TGF-β-stimulated SK-HEP1 and SK-HEP1-R cells, LY364947 inhibit p-Smad3 at μmolar concentrations. LY364947 also inhibits TGF-β-induced downstream p-AKT^{473} and p-ERK1/2 signaling in SK-HEP1 cells. LY364947 displays moderate antiproliferative effects at concentrations up to 20 μM after 72 h exposure in our cell lines without exogenous TGF-β stimulation. Using 5 and 10μM LY364947, a decrease in spontaneous TGF-β-independent cell motility was observed in SK-HEP1 and SK-HEP-1R cells in wound-healing assay. Using 10 μM, LY364947 also decreases TGF-β-independent invasion in both SK-HEP1 and SK-HEP1-R in matrigel assay.
Conclusion: Inhibition of TGF-β/TβR-I activation using LY364947 inhibits TGF-β-dependent cell signaling and reduces cell motility and invasion in parental and multikinase-resistant HCC cells. HCC appears as an interesting tumor model to evaluate and antimetastatic potential of novel TGF-β inhibitors, either as single agents and/or in combination with other anticancer drugs.

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Down-modulation of the androgen receptor (AR) with EZN-4176 inhibits the growth of prostate tumor and potentiates the inhibitory effect of MDV-3100, a novel anti-androgen

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Background: While androgen-deprivation therapies are effective initially for the treatment of prostate cancer (PC), the recurrence of castration-resistant prostate cancer (CRPC) frequently occurs. In such cases, the AR still plays a critical role since new agents that deplete testosterone (Abiraterone; Reid et al., 2010. J. Clin Oncol. *28*: 1447−9) or block ligand binding (MDV-3100; Scher et al., 2010. Lancet. *375*: 1437−46), and are in Phase III evaluation, remain effective. These findings suggest that down-modulation of AR expression may provide an alternative strategy for treating CRPC. Here we describe a novel locked nucleic acid (LNA)-based antisense oligonucleotide (ONs), designated EZN-4176, that down-modulates the AR and inhibits prostate tumor growth in vitro and in vivo.