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