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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**215** POSTER

**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.

**216** POSTER

**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.

**217** POSTER

**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.

**218** POSTER

**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.