particularly, if inhibition of TGF-beta 2 by trabedersen also affects TGF-beta 1 levels.
Material and Methods: The human glioma cell line A-172 was used. Cells were treated with trabedersen or recombinant human TGF-beta 2 (rhTGF-beta 2). Cellular proliferation was assessed via DNA quantification. TGF-beta protein levels in cell culture supernatants were determined by ELISA, mRNA was quantified by qRT-PCR.
Results: While in cell culture medium containing serum, trabedersen potently inhibited TGF-beta 2 expression and barely affected TGF-beta 1 expression, a strong inhibition of both isoforms was observed under serum-free conditions. Serum contains considerable amounts of TGF-beta 1 and 2, which may affect TGF-beta expression by auto- and cross-regulatory loops. Treatment of cells with rhTGF-beta 2 strongly induced expression of TGF-beta 1 on the mRNA as well as on the protein level. The inhibitory effects of trabedersen on TGF-beta 1 expression could be reversed by addition of rhTGF-beta 2. Cellular proliferation of A-172 cells was not affected by trabedersen or rhTGF-beta 2, which might be explained by the fact that growth of A-172 cells is not affected by TGF-beta.
Conclusions: We have demonstrated that by cross-regulatory loops TGF-beta 2 induces the expression of TGF-beta 1 in human glioma cells. Specific inhibition of TGF-beta 2 expression by trabedersen interrupts this cross-regulatory loop and additionally down-regulates TGF-beta 1. This combined inhibition of different TGF-beta isoforms is assumed to contribute to the potency of trabedersen in tumors expressing several TGF-beta isoforms.

360 POSTER

BIO, the GSK3 beta blocker, is a potent inhibitor of cell proliferation and inducer of cell death of cervical carcinoma and rhabdomyosarcoma tumor cells

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Introduction: Since tumor disease is still an very important clinical problem new therapeutic strategies are needed. Cervical carcinoma (CC) is one of the major causes of death among women suffering from tumor. The highest mortality is observed in the group of patients with late diagnosis and with a metastatic disease. Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma among children. Also in this case the mortality is very high in patients with metastatic disease. The 5 year survival years in this group of patients is only about 20%.
Objectives: We have studied the influence of GSK3b inhibitor BIO on cervical carcinoma and rhabdomyosarcoma cell lines proliferation and survival.
Materials and Methods: CC cell lines (HeLa and HTB35) and RMS cell lines (RH30 and SMS) were used as a models. The influence of BIO on cell proliferation was studied using cell count assay and MTT assay. To check the influence of BIO on cell survival we used Hoechst 33342 staining and Annexin V and Caspase 3 staining. We also studied the influence of BIO on normal cells (MSC, HUVEC and myoblasts) and nonmalignant satellite cell lines (C2C12). The expression and activation of GSK3b was studied using western blot.
Results: We used three doses of BIO − 1, 5 and 10 uM. The lowest concentration was not able to inhibit proliferation of tumor cells or to induce apoptosis. Moreover, at this concentration in some instances proliferation was even increased. At higher doses the strong inhibitory effect on cell proliferation and survival was observed. We used two different rhabdomyosarcoma cell lines reflecting two RMS subtype. Interestingly, the alveolar subtype which is recognized as more malignant and highly metastatic was significantly more sensitive to the BIO. This phenomenon could be related to the different expression of GSK3b in this subtype in comparison to the embrional one (SMS). The cervical carcinoma cells were highly sensitive to the BIO inhibition as well. After 96 hours of incubation at the highest concentration of BIO more than 95% was inhibited in proliferation assay. At the same time profound cell death was observed. 5−10 uM BIO exerts massive cell death in HUVECs. On the other hand, mesenchymal stem cells and muscle cells were much more resistant to the action of BIO.
Summary: In the war with tumor new therapeutic strategies are still needed. In this study we showed for the first time that blocking of GSK3 function by specific small molecule inhibitors is able to block proliferation of cervical carcinoma and rhabdomyosarcoma cells and decrease their survival.

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HGF regulates the activity of GSK3 in rhabdomyosarcoma cells

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Introduction: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma among children. Two major subtypes are recognized based on morphological and molecular features: alveolar (ARMS) and embryonal (ERMS). There are major differences between AMRS and EMRS e.g. presence of fusion proteins and higher rate of metastasis into distant organs like lungs and bone marrow in case of ARMS. Epithelial–mesenchymal-transition (EMT), is a phenomenon whereby epithelial cells temporarily or permanently acquire mesenchymal phenotype. EMT has been shown to play a key role during embryogenesis and wound healing. Latest reports have shown that EMT plays a crucial role in the development and progression of various tumors.
Aim: Dissecting the role of HGF in regulation of GSK3 activity and EMT related genes.
Materials and Methods: Cell lines used in experiments: RH30 (ARMS), SMS-CTR (ERMS). Real time RT-PCR and western blotting to evaluate gene expression and activation of various intracellular signaling pathways, respectively. Evaluation of protein activation in RMS cell lines was performed after stimulation with HGF.
Results: We observed the phosphorylation of GSK3b on serine 9 after HGF stimulation. To dissect the intracellular pathways responsible for GSK3b phosphorylation several inhibitors such as PI3K inhibitor − Ly294002, MEK inhibitor − U0126 and MET phosphorylation inhibitor were used. Use of both MET and PI3K inhibitors completely attenuated phosphorylation of GSK3b in HGF stimulated RMS cells. We observed also the accumulation of beta-catenin and Snail1 in the nucleus of RMS cells stimulated with HGF. This effect was father augmented when HGF was used together with BIO, small molecular inhibitor of GSK3b. When we study the expression of genes regulated by GSK3b, Snail1 and beta-catenin we noticed downregulation of E-cadherine expression and upregulation of cyclin D1 in cells stimulated with HGF.
Conclusion and future directions: Based on our data we can postulate that stimulation of GSK3b phosphorylation by HGF leads to stabilization and nuclear translocation of EMT activating proteins Snail1 and beta-catenin. This action subsequently causes the downregulation of E-cadherin and upregulation of cyclin D1. Finally, we think that GSK3b could be used as the new therapeutic target to block invasion and metastasis of RMS.
Future experiments will include (i) silencing of *GSK3b* (by viral transduction with shRNA), (ii) establishing the level of GSK3b expression at protein level in different RMS cell lines (by Western Blot) and RMS patients (by immunohistochemistry).

Molecular-targeted therapies − clinical trials

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First in human trial of a poly(ADP)-ribose polymerase (PARP) inhibitor MK-4827 in advanced cancer patients (p) with antitumor activity in BRCA-deficient and sporadic ovarian cancers

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Background: MK-4827 is a potent, selective, PARP1/2 inhibitor with IC_{50} of 3.8 nM. It induces selective synthetic lethality in homologous recombination (HR) repair deficient tumors with BRCA1/2 loss and in tumor cell lines with non-BRCA-related HR defects, supporting clinical utility in sporadic tumors.
Methods: MK4827 was administered orally once daily in cohorts of 3−6 p, enriched for BRCA-deficient and sporadic cancers associated with HR repair defects. Dose escalation was guided by toxicity, pharmacokinetic (PK) and pharmacodynamic (PD) data. Permission was obtained from the appropriate regulatory authorities and properly informed consent given.
Results: 59 p (M13, F46; median age 56 years; 23 BRCA-mutation carriers) were treated at 10 dose levels [30 mg (n = 6), 40 mg (n = 3), 60 mg (n = 7), 80 mg (n = 6), 110 mg (n = 5), 150 mg (n = 6), 210 mg (n = 6), 290 mg (n = 5), 300 mg (n = 9), 400 mg (n = 6)] on days 1−21 of a 28 day cycle (C) in C1, followed by continuous dosing. Prior systemic treatments were 1−2 (n = 5p), ⩾3 (n = 11 p), and ⩾4 (n = 40 p). Overall, dose-limiting toxicity was observed in 4 p: grade (G) 3 fatigue in 1/6 p at 30 mg, reversible G3

pneumonitis in 1/6 p at 60 mg, and two reversible G4 thrombocytopenias in 2/6 p treated at 400 mg. The MTD was established at 300 mg. Other MK-4827 related G1−2 reversible adverse events included fatigue, anorexia, nausea and myelosuppression. Dose proportional PK was observed with a mean terminal $t_{1/2}$ of 40 hours (range 37−42 hours). PD studies confirmed PARP inhibition in peripheral blood mononuclear cells at doses of ⩾80 mg. Antitumor responses were observed in both sporadic and BRCA-mutation associated (BRCA-MA) cancers. There have been 9 p with partial responses (PR) (8 confirmed, 7 ovarian, 2 breast, 8/9 BRCA-MA cancers, 8/9 with ongoing treatment), and 4 p with stable disease (SD) (2 ovarian, 2 NSCLC, 2/4 BRCA mutation carriers, 1/4 with ongoing treatment) ⩾120 days. PRs have ranged from 46–357 days and SD from 136–354 days.
Conclusions: MK-4827 was well tolerated, had linear PKs, evidence of target modulation, and promising antitumor activity. Specific cohort expansions are ongoing. Evidence of both PARP blockade and antitumor activity in both BRCA-MA and sporadic cancer has been observed.

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Dose of the molecularly targeted agents (MTA) in Phase 1 trials correlates with clinical benefit

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Introduction: The primary objective of phase 1 trials to date has been to safely select the maximum tolerated dose (MTD) of a drug or drug combination for utilization in subsequent trials. Conventional cytotoxic chemotherapy is generally more effective at the MTD. Recent single-institution data suggests that the novel MTAs may not require a MTD for efficacy. We analyzed patient (Pt)outcome results in MTA Phase 1 trials at multiple institutions throughout North America sponsored by the National Cancer Institute's (NCI) Cancer Therapy Evaluation Program (CTEP).
Methods: Data on Pts treated on monotherapy phase I trials investigating novel MTAs with a defined MTD, from 2001–2009, were collected and analyzed retrospectively. Pts were grouped into 6 cohorts depending upon the dose of best response [(complete response (CR), partial response (PR) or stable disease (SD)] as a percentage of the final identified MTD for the drug (<20%, 21−40%, 41−60%, 61−80%, 81–100%,>100%). Outcomes including response rates, overall survival and toxicity were compared. Logistic regression analysis was used to test whether there was an increase in the probability of a response as dose increased. A Cox proportional hazards model was used to determine if survival increased with increasing dose.
Results: A total of 1908 Pts treated on 53 eligible clinical trials were analyzed. Median Pt age was 61 (range: 16−93), with 59% males and 41% females and median number of prior treatments was 3 (range: 1−16). Distribution of Pts according to dose levels was as follows: <20% MTD = 93 pts, 21−40% MTD = 213 pts, 41−60% MTD = 263 pts, 61−80% MTD = 310 pts, 81–100% MTD = 508 pts and >100% MTD = 344 pts. Non-progression rates (NPR) defined as CR, PR or SD at first assessment, 3 months and 6 months was 44%, 26% and 11% respectively. The probability of both overall response (CR+PR) or NPR increases with increasing dose, p = 0.10 and p = 0.24 respectively after controlling for study influences. Overall survival also increased with increasing dose, p = 0.041.
Conclusions: Pts treated in the context of phase 1 trials with MTAs continue to derive reasonable clinical benefit. Contrary to other single institution data, our results suggest that the potential clinical benefit in terms of overall response, non-progression rate and overall survival significantly correlates with the administered dose level, with increasing benefit for patients treated at doses at or near the MTD.

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The first-in-human, first-in-class study of CUDC-101, a multi-targeted inhibitor of HDAC, EGFR, and HER2: A Phase I study in patients with advanced cancer

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Background: CUDC-101 is a synthetic small-molecule, first-in-class, multi-targeted inhibitor of both receptor tyrosine kinases (RTK), EGFR and HER2, and class I/II HDACs. Along with these direct effects, CUDC-101 also indirectly attenuates the survival signaling pathways Akt, HER3, and MET. Through this inhibition of multiple signaling networks, CUDC-101 effectively suppresses the growth of a broad range of tumor types both *in vitro* and *in vivo*,, including RTK-resistant cell lines.
Material and Methods: This phase I dose-escalation study assessed the safety and tolerability of CUDC-101 to determine the maximum tolerated dose (MTD). The pharmacokinetics (PK), pharmacodynamic (PD) biomarkers and preliminary efficacy were also investigated. Dosing was IV infusion over 1 hr on Days 1−5 of each 14 day treatment cycle. PD measurements included histone acetylation in PBMCs and EGFR, HER2 inhibition in paired skin and tumor biopsies. Tumor response was evaluated by RECIST.
Results: 25 pts (11M/14F, median age 60 [range 37−79], median prior systemic regimens: 3 [range 2−11]) with advanced solid tumors received CUDC-101 at 1of 5 dose levels (75–300 mg/m^2). Frequent tumor types included breast (24%), lung (16%), and head and neck cancers (16%). Dose-limiting-toxicities in cycles 1−2 occurred in 3 pts at 300 mg (elevated creatinine, n = 2; pericarditis, n = 1) both were transient and reversible. MTD was determined to be 275 mg/m^2. The most frequent adverse events were nausea (24%), fatigue (20%), vomiting (20%), dyspnea (20%), pyrexia (16%), and dry skin (16%), being Grade 1/2 in severity. CUDC-101 exposure increased linearly in the range of 75–300 mg/m^2, with a half life of ~2.5 hrs and AUC of 10368 hr*ng/mL at the 275 mg/m^2 dose. PD changes are currently being investigated. One confirmed partial response was achieved in a gastric cancer pt (at 275 mg/m^2) and stable disease of >3 months was seen in one pt with refractory breast cancer (150 mg/m^2). Two additional subjects (salivary gland adenocarcinoma and tongue squamous cell carcinoma) exhibited anti-tumor activity with a decrease of >20% in target lesions.
Conclusions: CUDC-101 exhibited a favorable safety and PK profile up to doses of 275 mg/m^2. Continued clinical development of CUDC-101 is supported by the early evidence of anti-tumor activity observed in this trial. An expansion phase at the MTD in specific tumor types is proposed to seek additional signals of activity and to explore alternative dosing schedules.

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First-in-human, safety, pharmacodynamic (PD) and pharmacokinetic (PK) trial of a first-in-class dual RAF/MEK inhibitor, RO5126766, in patients with advanced or metastatic solid tumour

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Background: Among the signaling pathways most frequently deregulated in human cancer is the Ras-Raf-MEK-extra-cellular signal-regulated kinase 1 and 2 (ERK1/2) pathway. RO5126766, a first-in-class dual Raf/MEK inhibitor, is being tested in a phase I (P1) study. Objectives were determination of maximum tolerated dose (MTD), dose limiting toxicities (DLTs), safety/tolerability, pharmacokinetics (PK)/pharmacodynamics (PD) and preliminary clinical activity (RECIST criteria).
Patients and Methods: Patients (pts) with advanced or metastatic solid tumors received oral RO5126766 administered on a continuous daily dosing schedule (QD) in 28 days cycles. PK and blood PD samples (PBMCs) were collected after a single dose (run-in) and cycle 1 day 15 (C1D15). Paired skin and tumor biopsies (baseline, C1D15) and sequential FDG-PET scans (baseline, C1D15, and C3D1) were taken. To increase activity window, 3 intermittent regimens, 4 days on/3 days off (4/3), 7 days on/7 days off (7/7) and once a week (QW), are currently being tested.
Results: 38 pts (25 QD, 7 4/3 and 6 7/7) in 12 cohorts (QD from 0.1 to 2.7 mg, 4/3 and 7/7 both at 2.7 and 4.0 mg) have been included. Mean age 51y, ECOG 0−1, previous chemotherapy lines median 3 (0−14). Common tumors were melanoma (15), ovarian (5) and CRC (6). Four reversible DLTs were observed on QD: grade (G) 3 blurred vision (2.7 mg), 2 G3 CK elevations (2.7 and 2.25 mg) and G3 transaminitis (1.8 mg). QD MTD was defined as 2.25 mg. QD most common related adverse events include skin (89%), GI (74%), eye (42%) and metabolic (26%) disorders. PK profiles suggest dose-linearity, a half-life ($t_{1/2}$) of 40 to 60 hrs and drug accumulation 3−7 fold in the QD regimen at steady-state. In tumor and skin biopsies modification of target related molecules (e.g. pERK, pMEK) was detected. Target inhibition close to 100% (pERK/pMEK) was observed in stimulated PBMCs. To date, of 25 evaluable pts, 1 melanoma pt has a partial response and 7 pts experienced stable disease for at least 16 wks (median, 23.5; range 16−49) associated with a reduction in SUV-max (mean, −35%; range, −81−+10; n = 6) measured by C1D15 FDG-PET.