markers, including Beclin 1 and ATG5, as well as a relocalization of LC3B protein, was observed, suggesting an enhanced propensity of the cells to undergo autophagic cell death as a determinant of their higher sensitivity to cisplatin. When we assessed the susceptibility of PCa cells to other chemotherapeutic agents, we observed an increased resistance of DU145/miR-205 cells to the mTOR inhibitor RAD001, whereas a comparable sensitivity to paclitaxel was observed for the two cell lines. Overall, these findings suggest that modulation of EMT in PCa cells may result in a different response as a function of the tested drug and that, only for selected agents, combination treatments including EMT-modulators, such as miR-205, can be envisaged to improve cell response.

536 POSTER

Newcastle disease virus Iraqi local isolate as a therapy for murine mammary adenocarcinoma: In vitro and in vivo study

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The avian paramyxovirus, Newcastle disease virus (NDV), is considered to be very promising. Since cytolytic strains of NDV possess a number of desirable properties in this regard. To evaluate the effectiveness of NDV Iraqi isolate (NDV-Iraqi Ahmed Nahi − IAN) as a tumor cytolytic agent, we have performed in vitro and in vivo experiments. In vitro tests studied oncolytic activity on different tumor cell lines by light and electron microscope. In vivo experiment using murine mammary adenocarcinoma allograft grown in mice. We compared antitumor activity of intratumoral injection of NDV-IAN to systemic intraperitonial treatment. In vitro results revealed necrosis and apoptosis induction. While in vivo results showed intratumoral treatment caused average of 92% growth inhibition ($p < 0.0001$), while intraperitonial treatment show 79% growth inhibition at the end of the experiment ($p < 0.0001$) compared to control group. Furthermore treatment groups showed prolong surviving. Histopathological pictures showed massive area of necrosis with infiltration of inflammatory cells mainly lymphocyte. Ultrastructural study showed budding of the virus from the treated tumor cells. Our results suggest that NDV Iraqi isolate (NDV-IAN) as a promising antitumor agent.

Genetics and epigenetics

537 POSTER

Potent in vitro and in vivo anti-tumor activity of ITF2357 by modulation of c-myc related miRNA signature in human Burkitt's lymphoma

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Background and Objectives: Recent studies support the existence of a c-*myc*–microRNA (miRNA) interaction within the genesis and the maintenance of Burkitt's lymphoma (BL). Myc oncoproteins have been found to inhibit the transcription of tumor suppressor genes by recruiting histone deacetylase (HDAC) proteins to target genes. We studied the in vitro and in vivo anti-tumor activity of a novel hydroxamate HDAC pan-inhibitor ITF2357 (Givinostat®, Italfarmaco S.p.A.) on BL cell lines with respect to its ability to modulate BL miRNA expression profile and c-myc target genes.
Methods: Standard MTT assay was used to define the half maximal cell-growth inhibitory concentration (IC_{50}) of ITF2357. Apoptosis and cell cycle phase distribution of treated and untreated cells were analysed by flow cytometry. MiRNA modulation was investigated by array analysis and real time PCR. Cell signalling proteins affected by ITF2357 treatment were analyzed by western blot, immunohistochemistry and confocal microscopy. In vivo anti-tumor activity of ITF2357 alone or in combination of cyclophosphamide (CTX) was studied in subcutaneous Raji xenografted SCID mice.
Results: Namalwa and Raji cell lines treated with 200 nM ITF2357 (48h IC_{50}) showed late and early apoptosis, with subG1 peak formation and G1 arrest respectively. To identify the molecular pathways affected by ITF2357, we investigated c-myc expression and NF-kB activation before and after treatment. Noteworthy, c-myc protein expression was reduced in treated BL cells while its mRNA levels did not change or even increased. As a possible mechanism impairing c-myc translation, we investigated the modulation of miRNA expression profile after treatment with ITF2357. Interestingly, in treated BL cell lines, let-7a and miR-26a that can negatively affect c-myc translation were up-regulated. According to recent evidences about the pro-apoptotic effects of NF-kB activation in human BL, we found that ITF2357 increased the acetylation of NF-kB subunit RelA and NF-kB nuclear localization in BL treated cell lines. The administration of 50 mg/kg ITF2357 to Raji xenografted SCID mice significantly reduced tumor growth compared to untreated control mice. Results from molecular analyses of the in vivo treated tumors were consistent with those obtained in in-vitro experiments. Finally, the combination of ITF2357 and CTX resulted more effective compared to CTX alone in completely eradicating the tumor in vivo.
Conclusion: The in vitro and in vivo anti-tumor effects of ITF2357 against BL cell lines were found associated with the reversion of crucial events in the *c-myc* driven lymphomagenesis, including the restoration of NF-kB activity and let-7a and miR-26a expression. The potent in vivo anti-tumor effects provided by the combined administration of ITF2357 and CTX might be translated in a novel and more effective therapeutic option for BL patients.

538 POSTER

Id1 enhances RING1b E3 ubiquitin ligase activity through the Mel-18/Bmi-1 polycomb group complex

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The helix–loop–helix inhibitor of differentiation and DNA binding (Id1) is well-known as an oncogene in various tumors. Although it has been reported that Id-1 promotes several oncogenic processes, it is still unclear whether Id1 functions through epigenetic transcriptional regulation. In this study, we examined the effect of Id1 on polycomb group (PcG) proteins, which are crucial epigenetic gene silencers, and found that Id1 regulated the expression of Mel-18 and Bmi-1, both of which belong to PRC1. We also confirmed that Id1 induced Mel-18 downregulation, which was mediated by the Akt pathway, and consequently upregulated the transcription of its target gene, c-Myc. Using a promoter-reporter, we demonstrated that Id1 regulated Bmi-1 transcription through c-Myc binding to its E-box in the promoter. Finally, we examined the activity of E3 ligase RING1b whose catalytic activity is increased by binding with the RING finger protein Bmi-1, and found that Id1 over-expression enhanced RING1b E3 ligase activity leading to accumulation of H2A ubiquitination and ubiquitin/proteasome-mediated degradation of geminin. Taken together, our study provided a novel link between Id1 and PcG proteins and suggested that Id1 may contribute to tumor development through PcG-mediated epigenetic regulation.

539 POSTER

Hydroxamate-tethered short chain fatty acid designer cancer prevention molecule

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Among various classes of histone deacetylase (HDAC) inhibitors, short-chain fatty acids exhibit the least potency, with IC(50) in the millimolar range. We rationalized that this weak potency was, in part, attributable to their inability to access the zinc cation in the HDAC active-site pocket, which is pivotal to the deacetylation catalysis. Based on the knowledge that the acetylation status of core histones plays a pivotal role in regulating gene transcription through the modulation of nucleosomal packaging of DNA. In a hypoacetylated state, nucleosomes are tightly compacted, resulting in transcriptional repression due to restricted access of transcriptional factors to their targeted DNA. Conversely, histone acetylation leads to relaxed nucleosomal structures, giving rise to a transcriptionally permissive chromatin state. The level of this posttranslational modification is maintained by a dynamic balance between the activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs), both of which are recruited to target genes in complexes with sequence-specific transcription activators. Aberrant regulation of this epigenetic marking system has been shown to cause inappropriate gene expression, a key event in the pathogenesis of many forms of cancer. For cancer prevention safety and low toxicity are of high importance for drug development. Hence, starting from butyric acid (present in dietary sources) however, concentrations are in milimolar range for this to be meaningful. Here we report an Hydroxamate-tethering approach wheer we explored the structural optimization of valproate, butyrate, phenylacetate, and phenylbutyrate by coupling them with Zn(2+)-chelating motifs (hydroxamic acid and o-phenylene diamine) through aromatic omega-amino acid linkers. This strategy has led to a novel class of Zn(2+)-chelating, motif-tethered, short-chain fatty acids that exhibited varying degrees of HDAC inhibitory potency. One hydroxamate-tethered

phenylbutyrate compound, N-hydroxy-4-(4-phenylbutyrylamino)benzamide (HTPB), displayed nanomolar potency in inhibiting HDAC activity. Exposure of several cancer cell lines to HTPB at the submicromolar level showed reduced cell proliferation accompanied by histone hyperacetylation and elevated p21(WAF/CIP1) expression, which are hallmark features associated with intracellular HDAC inhibition.

540 POSTER

Clinical phase II development of resminostat, a novel HDAC inhibitor

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Resminostat (4SC-201) is a promising, oral pan-HDAC inhibitor being under clinical phase II development. It has shown excellent anti-tumour activity in a wide panel of preclinical models.
A First-in-Man study yielded a favourable safety profile, together with superior pharmacokinetic characteristics and a consistent modulation of the HDAC target. After 4 treatment cycles (2 months), a stabilization of tumour diseases was observed in the majority of patients with various late-stage solid tumours.
Currently, a clinical phase II programme with resminostat explores its therapeutic activity in a spectrum of indications as follows:
(I) Hepatocellular carcinoma (HCC). A phase II trial (SHELTER study) in patients with advanced HCC investigates the therapeutic efficacy in an exploratory second-line setting after treatment failure of first-line standard therapy with sorafenib. In the study, patients are treated with the combination of resminostat and sorafenib (including MTD determination) or with resminostat as monotherapy. Study endpoints are the estimation of the progression-free survival, overall survival, response rate, and the analyses of safety, PK and biomarkers.
(II) Hodgkin's lymphoma (HL). A phase II trial (SAPHIRE study) evaluates the therapeutic activity of resminostat in relapsed or refractory HL patients. Resminostat is applied as monotherapy in an open-label, single-arm, Simon-two stage design. Primary endpoint is the overall response rate (ORR), the secondary endpoints are similar to the SHELTER study.
(III) Colorectal carcinoma (CRC). A phase II study in patients with advanced k-ras mutant CRC is to be commenced shortly. Resminostat will be administered in combination with the standard FOLFIRI chemotherapy regimen to patients being refractory to a 5-FU-comprising first-line therapy. Following a dose escalation part of the combination treatment, patients will be randomized into two study arms, receiving either the resminostat/FOLFIRI combination or FOLFIRI alone. Primary end point is the estimation of progression-free survival (PFS), the secondary endpoints correspond to the SHELTER study.
Clinical data from this development program of resminostat will be presented at the conference.

541 POSTER

ERBB3 promoter polymorphisms and mRNA expression associated with lung cancer risk in Korean lung cancer patients

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Lung cancer has been the leading cause of cancer-related deaths in Korea, and its incidence continues to rise. Recent therapeutic strategies have focused on the development of "targeted therapies" that aims to specifically disrupt critical oncogenic mechanisms. The EGFR is one such target, because it is known to promote growth of cells and function as an oncogene, expressing in up to 80−90% of NSCLC.
The ERBB3 is unique among the EGFR families in that it has been shown to have weak or no tyrosine kinase activity. The correlation between ERBB3 protein expression and distant metastasis in lung cancer was reported.
To evaluate the role of ERBB3 gene in lung cancer risk, genotypes of the ERBB3 promoter region (−536 A/G and −276 C/T) were determined in 430 lung cancer patients and 429 normal subjects by TaqMan assay. Furthermore, to examine potential effects of the common haplotypes (A-T and A-C haplotypes) on ERBB3 transcriptional activity, luciferase reporter assays were performed in H2009 and H358 cells. The ERBB3 mRNA expressions were quantified by real-time PCR using immortal lymphocytes originated from lung cancer patients. The genotypes of ERBB3 polymorphisms showed no association with susceptibility to the lung cancer risk. However, in the analysis stratified by smoking status, the effect of −276 C/T on the lung cancer risk was found in non-smokers (OR: 0.11, 95% CI: 0.02–0.87) in the recessive model. And, the subsequent analysis revealed that A-C haplotype was associated with susceptibility to the lung cancer risk in codominant model (OR: 1.31, 95% CI: 1.01–1.70). In particular, the A-C haplotype showed an increased risk of lung cancer in non-smokers (dominant OR: 8.82, 95% CI: 1.14–68.36) and the A-T haplotype showed a decreased risk of lung cancer in non-smokers (codominant OR: 0.63, 95% CI: 0.41–0.98). Interestingly, A-C haplotype induced transcriptional activity by 30% compared with A-T haplotype. And, the ERBB3 mRNA levels were higher in A-C haplotype (0.51 ± 0.09) than in A-T haplotype (0.25 ± 0.05). These results suggest that ERBB3 promoter polymorphisms affect ERBB3 mRNA expression, further contributing to the genetic susceptibility to lung cancer.
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542 POSTER

Centralised analysis of phase I ECG dataset of resminostat, a new oral histone deacetylase inhibitor (HDACi)

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Background: Resminostat (4SC-201) is a newly developed, specific, potent, pan-HDAC inhibitor with broad anti-tumour activity in preclinical models and promising clinical characteristics. The compound is in phase II clinical development in various oncological indications.
Methods: 19 Patients with advanced solid tumours were treated in a first-in-human trial at increasing oral daily dose levels from 100 mg to 800 mg in repeated 14-day cycles consisting of 5 consecutive treatment days followed by a 9-day rest period. Cardiac function was monitored by pulse rate, blood pressure, troponin levels and continuous ECG telemetry. In addition, standard 12-lead rest ECGs were conducted frequently to aid in the determination of potential effects on QT interval prolongation. An intensive profile consisting of 18 single ECGs was performed from Day 1 to 5 during Cycle 1, and a reduced number of ECGs in the following cycles, if there were no clinically relevant findings. Subsequent to the on-site clinical assessment, ECGs were sent to a core lab for further analysis by a trained cardiologist. PR, QRS and QT intervals and heart rate (HR) were measured in lead II across 3 consecutive beats using markers for the respective ECG intervals.
Results: No signal for drug-induced prolongation of QTc was observed. A maximum mean HR increase of up to 22 beats per minute and a corresponding shortening of the PR and QT interval was found in a dose-dependent manner. HR correction with Fridericia's formula did not reveal consistent changes in QTc. The incidence of QTcF outliers was very small. The results suggest that resminostat does not affect the duration of myocardial repolarisation. At doses $\geqslant$ 400 mg, unspecific flattening of the T-wave and slight depression of the ST-segment were observed frequently. However, in some patients such findings were already observed at baseline. No dose-limiting toxicities were seen with regard to cardiac safety in all dose cohorts.
Conclusions: Centralised analysis of phase I ECG data did not reveal a drug-induced prolongation of the QTc interval. Drug administration was frequently associated with moderate increases in HR. At doses levels $\geqslant$ 400 mg, unspecific changes in T-wave morphology and slight ST-segment depression were observed.

543 POSTER

Human transcription factors regulated by SET protein

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Background: Transcription factors exhibit three essential functions: binding to specific DNA sequence, transcription control and response to regulatory signals. The SET/TAF-1β protein, also termed I2PP2A, interacts with several proteins involved in the regulation of cell cycle and apoptosis. In addition, SET is a member of INHAT complex, responsible for inhibiting the activity of the histone acetyltransferase (HAT) proteins by binding to histones and blocking the association between HATs and histones. Consequently, SET influences the state of histone acetylation and affects chromatin structure, promoting epigenetic alterations and gene transcriptional silencing. We previously identified SET protein accumulated in oral scamous cell carcinoma (OSCC). Here, we addressed the impact of SET accumulation on transcription factors expression. Material and Methods: HEK293 cells were transfected with pCMV vector containing SET full length cDNA or empty vector. Small interference RNA (siRNA) was performed in OSCC-HN13 (origin: tongue) cells using oligos against SET or a negative control. After transfection, to promote *SET* overexpression or knockdown, the mRNA was extracted by TriZol reagent and the