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

Human Transcription Factors RT2 Profiler PCR Array (PAHS-075A − SABiosciences − Qiagen) was used to evaluate the profile of the expression of 84 transcription factors using real time PCR. The PCR array was validated through analysis of the expression levels of 8 genes by quantitative real time PCR using EVA Green dye.
Results: We analyzed transcription factors downstream of signaling from cytokines, chemokines and growth factors, signaling from androgen, B-cell, G-protein, T-cell and Toll-like receptors, and of signal transduction pathways like JAK/STAT, JNK, MAPK, NFkB, Notch and WNT. The SET profile showed 74 down-regulated and 7 up-regulated genes. Eight genes were validated (6 down-regulated and 2 up-regulated) in HEK293 cells overexpressing SET and confirmed in HN13 cells after SET knockdown.
Conclusions: SET accumulation in OSCC (previously validated by us) may control the expression of many transcription factors fundamental in the cell signaling and this could have implications in cancer progression and treatment.

544 POSTER

DNA methylation: an epigenetic pathway to cancer and a promising target for anticancer therapy in breast cancer

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Objective: To determine whether Estrogen Receptor 1(ESR1) (+) and ESR1(−) status relates to epigenetic changes in breast cancer-related genes and to correlate with molecular breast cancer subtypes.
Methods: Since January/02 to June/05,we quantified methylation levels ERS1 gene in serum of 92 pts breast cancer.A PCR quantitative technique was used to analyze levels of methylation gene.We also examined and correlationed the expression of ESR1 in tumors by immunohistochemistry with molecular phenotype.
Results: Median age was 58 years (32−88); 69% were postmenopausal women. Nodal involvement (N0; 63%, N1; 30%, N2; 7%),tumor size (T1; 58%, T2; 35%, T3; 4%,T4; 4%) and grade (G1;20%,G2;37%,G3;30%).Of the cases, 37pts (40%) were Luminal A (LA), 32 pts (33%) Luminal B (LB), 14 pts (15%) Triple-negative (TN) and 9 pts (10%) HER2+. The methylated ESR1 in serum was significantly associated with ESR1(−) in breast tumors >80%($p = 0.0179$). Methylation ESR1 was preferably associated with TN(80%) and HER2+(60%) subtype. Nevertheless unmethylation ESR1 was found more frequently in LA(71%) and LB(59%) phenotype. With a median follow up of 5 years, we found worse overall survival (OS) with more frequent ESR1 methylation gene($p > 0.05$), Luminal A;ESR1 Methylation OS at 5 years 81% vs 93% when was ESR1 Unmethylation. Luminal B;ESR1 Methylation 86% SG at 5 years vs 92% in Unmethylation ESR1. Triple negative; ESR1 Methylation SG at 5 years 75% vs 80% in unmethylation ESR1. HER2; ESR1 Methylation SG at 5 years was 66.7% vs 75% in unmethylation ESR1.
Conclusions: Gene promoter region hypermethylation is a significant event in primary breast cancer. However, its impact on tumor progression and potential predictive implications remain relatively unknown. Our study identifies the presence of variations in global levels of methylation promoters ESR1 genes in breast cancer with different phenotype classes and shows that these differences have clinical significance. Although numerous issues remain to be resolved,quantitative measurement of circulating methylated DNA may be of significance in the assessment and search of targeted therapy resistance related to ESR1 and HER2 status by epigenetic or transcriptional cancer therapy.

545 POSTER

Expression of methylthioadenosine phosphorylase (MTAP) in malignant pleural mesothelioma (MPM) and its implication for pemetrexed-based chemotherapy

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Introduction: The *MTAP* gene encodes for a key enzyme in the methionine salvage pathway. This gene is located at chromosomal locus 9p21, 100kb telomeric to *p16*, which is frequently deleted in malignant pleural mesothelioma (MPM). MTAP-deficient tumors are dependent on the *de novo* purine synthesis pathway, which is inhibited by drugs such as pemetrexed. This study investigates the MTAP expression in MPM and its relationship to pemetrexed-based therapy.
Material & Methods: DNA was extracted from tissue sections of paraffin-embedded tumor samples from MPM patients treated with pemetrexed-based therapy. Gene copy variation (GCV) of MTAP was determined using multiplex ligated PCR assay (MLPA), which included target probes for *MTAP* and *p16*. In addition, immunohisto- chemistry (IHC) was used to detect MTAP protein, using a validated monoclonal antibody. IHC was graded by two independent assessors using a composite score system which consisted of percentage score for positively stained areas multiplied by the intensity of staining, resulting in a score of 0–300. These findings were then correlated to tumor histopathology and clinical outcome, including disease control rates (DCR), median time to treatment failure (TTF) and overall survival (OS).
Results: Data for 52 MLPA and 59 IHC specimens were available for analysis. MTAP GCV was noted in 55% of samples. In all samples with MTAP deletion, there was co-deletion of *p16*. IHC score of 0–100 (null or minimal staining) and 101–300 (moderate to strong staining) were found in 55% and 45% of samples respectively. An association was observed between loss/minimal expression of MTAP protein and early disease stage ($p = 0.04$). The loss of MTAP gene or protein however, showed no significant association with DCR, TTF or OS.
Conclusion: Loss of MTAP protein expression was associated with early stage disease, but neither its gene copy status nor protein expression were predictive for clinical outcome in MPM patients treated with pemetrexed-based therapy.

	MTAP MLPA			MTAP IHC		
	+	−	p	+	−	p
DCR (%)	51	57	0.2	60	71	0.4
TTF (mths)	5.3	7.4	0.7	5.1	7.4	0.5
OS (mths)	6.2	10.3	0.9	10.0	10.1	0.7

546 POSTER

Promoter methylation of the RGC32 gene in non-small cell lung cancer and its clinical implications

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Background: Lung cancer is the leading cause of cancer-related deaths worldwide. Epigenetic inactivation of certain genes by aberrant promoter methylation is recognized as a crucial component in the initiation and progression of lung cancer. Response gene to complement 32 (*RGC32*) is formerly identified as a cell cycle regulator induced by activation of complements; however, its role in carcinogenesis is still controversial.
Methods: We have examined the methylation status in the promoter region of *RGC32* gene in non-small cell lung cancers (NSCLCs) using a methylation-specific PCR, and correlated the results with clinicopathological features.
Results: *RGC32* methylation was found in 45 of 173 NSCLCs (26.0%) and was related to the gene expression. *RGC32* methylation was more frequent in females than in males ($P < 0.05$). *RGC32* methylation was not significantly associated with the prognosis of patients; however, when the patients were categorized by *TP53* mutational status, the effect of *RGC32* methylation on prognosis was significantly different between those with and without *TP53* mutations ($P = 0.005$ [test for homogeneity]); specifically, *RGC32* methylation was associated with a significantly worse survival in the cases with wild-type *TP53*, whereas it exhibited a better survival outcome in the cases with *TP53* mutations.
Conclusion: The current findings suggest that methylation-associated down-regulation of *RGC32* plays an important role in the pathogenesis of NSCLC, particularly in females. However, further studies with a large number of cases are needed to confirm our findings.

547 POSTER

Immunomodulatory activity of SGI-110, a 5-aza-2′-deoxycytidine-containing demethylating dinucleotide

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Background: We have recently reported that aberrant DNA hypermethylation down-regulates the expression of components of the "tumor recognition