Parallel Sessions (Proffered Papers)

Parallel session 1: Cancer genomics (1)

PP01
RECURRENT GENETIC CHANGES IN INTESTINAL-TYPE SINONASAL ADENOCARCINOMA

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Background: Intestinal-type adenocarcinomas of the nasal cavity (ITAC) represent a small percentage of all head and neck cancers and are histopathologically similar to colorectal adenocarcinoma. Professional exposure to wood dust is a strong etiological factor, although the mechanism is not understood. In addition, little is known about the genetic changes in sinonasal adenocarcinomas. Our goal was to identify genome-wide recurrent DNA copy number changes and to evaluate their relation to clinico-pathological characteristics.
Material and methods: Forty-four ITAC, 36 of wich related to professional exposure to wood dust, were studied by microarray CGH analysis using a 30,000 oligonucleotide set. In addition, MLPA was applied to identify copy number changes of 60 different tumor related genes.
Results: The most frequent chromosome gains were, in descending order: 5p15, 20q13, 8q24, 20q11 and 8q21. Losses occurred most frequently at: 4q31-qter, 18q12-22, 8p12-pter, 5q11-qter, 10q22-23 and 17p13. Recurrent high level amplifications were detected at 7p11, 8p11 and 8q24. MLPA confirmed the overall pattern of gains and losses found by array CGH. Frequent gains were found of genes in 8q (PTP4A3, RECQL4, MYC) and 20q (BCL2L1, PTPN1) and deletions of genes in 18q (BCL2, CDH2), 8p (CTSB, N33, MFHAS1) and 17p+q (CRK, TP53, TIMP2). Gains of MYC and PTPN1 were more frequent in the more aggressive solid and mucinous type ITACs. Loss of TIMP2 was associated with advanced tumor stage and worse survival.

Conclusion: This study confirmed a pattern of chromosomal aberrations unique to ITAC and not related to the pattern known in colorectal adenocarcinoma. The results suggest a role for TIMP2, MYC and PTPN1 in ITAC tumor progression.

PP02
CANCER-PREDICTIVE METHYLATION PATTERNS IN HEREDITARY BREAST CANCER

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Background: Promoter methylation is a common epigenetic mechanism to silence tumor suppressor genes during breast cancer development. Methylation of a selected set of genes was detected in ductal fluids of sporadic breast cancer patients. We investigated whether BRCA-associated breast tumors show cancer-predictive methylation patterns similar to sporadic tumors.
Material and methods: Quantitative Multiplex Methylation-Specific PCR (QM-MSP) of eleven genes involved in breast carcinogenesis (RARB, RASSF1, TWIST1, CCND2, ESR1, SCGB3A1, BRCA1, BRCA2, CDKN2A, APC, CDH1) was performed on 40 BRCA1/2-associated and 46 sporadic breast carcinomas, and on normal breast tissue from 9 BRCA1/2 mutation carriers and 13 non-carriers.
Results: The median cumulative methylation index (CMI) of all studied genes was significantly higher in tumors (87.8) than in normal tissue (9.7) ($p<0.001$). The median CMI was significantly lower in hereditary (59.5) than in sporadic breast tumors (121.7) ($p=0.001$), in estrogen receptor negative tumors (77.0) than estrogen receptor positive tumors (122.1, $p=0.001$) and in lymph node negative (76.6) compared to lymph node positive tumors (127.1) ($p=0.01$). The extent of cumulative methylation increased with age ($p<0.001$). The panel of genes predicted malignancy with an accuracy of 92% in sporadic cases and with an accuracy of 86% in hereditary cases.

Discussion: BRCA1/2-associated breast cancers show a lower extent of promoter methylation compared to sporadic breast carcinomas. Importantly, the methylation status of our panel of breast cancer genes is highly associated with hereditary breast cancer, and can be useful for early detection of cancer in nipple fluids of genetically predisposed patients.

PP03
TWIST1 EXPRESSION AND METHYLATION IN BREAST CANCER

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TWIST1 is an anti-apoptotic and pro-metastatic transcription factor involved in regulation of epithelial to mesenchymal transition (EMT). It is expressed in a number of epithelial cancers. In breast cancer, TWIST1 expression has been found to relate to metastases formation. Interestingly, methylation of the TWIST1 promoter that generally silences gene expression frequently occurs in breast cancer. This does not fit with the paradigm of an oncogene. We therefore studied TWIST1 methylation in relation to its expression in normal breast tissue and invasive breast cancers. We found that TWIST1 promoter methylation is significantly enhanced in malignant compared to healthy breast tissue. Furthermore, TWIST1 protein expression was significantly higher in breast malignancy compared to matched healthy tissue from the same patients. There was no correlation between TWIST1 promoter methylation and protein or RNA expression. This might point to a biphasic role of TWIST1 in breast cancer development.

PP04
DISTINCT CHROMOSOMAL ALTERATIONS DURING LUNG METASTASES OF COLORECTAL CARCINOMAS ASSOCIATED WITH TISSUE SPECIFIC METASTASIS

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Comparative genomic hybridization was used to screen colorectal carcinomas for chromosomal aberrations that are associated with the metastatic phenotype of the lung. Thirteen lung metastases, six primary tumors, one lymph node metastasis, one liver metastasis, and one ovarian metastasis were investigated and added to our CGH colon cancer tumor collective, comprising 85 tumor specimens from 56 patients (see CGH online tumor database at http://amba.charite.de/cgh). Lung metastases showed more alterations than liver metastases, particularly more deletions at 1p, 3p, 9q, 12q, 17q, 19p and 22q and gains at 2q, 5p, and chromosome 6. Comparing lung metastases with their corresponding primary tumors, particularly more deletions at 3p, 8p, 12q, 17q, and 21q21 and gains at 5p were observed. Based on our results, we wish to suggest a metastatic progression model. Specific subpopulations of metastatic cells have a distinct metastatic potential which is reflected by a non-random accumulation of chromosomal alterations. Distinct alterations already exist within the primary tumor and this "ready to go package"gives the cells the metastatic potential to achieve the complex series of events needed for metastasis.

PP05
GENE PROFILING HIGHLIGHTS SENSITIVITY TO AS WELL AS PROTECTION FROM RADIO-INDUCED RECTAL TOXICITY IN PROSTATE CANCER PATIENTS

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Purpose: Despite optimal 3D conformal radiotherapy (CRT), 5-10% patients (pts) treated for prostate cancer can still present significant morbidity thus suggesting, at least in part, a potential genetic component of normal tissue radio-sensitivity. The present study was designed to the aim of identifying genetic markers predicting late rectal bleeding (lrb), analysing 35 genes involved in DNA-repair or pathways known as targets for radiation. Pts were selected within the AIROPROS0101 trial,

designed to study the correlation between lrb and dosimetric parameters. Accurate dose-volume information (DVH) helps minimizing the potential bias due to inaccurate dose delivery.

<u>Materials/Methods</u>: 30 pts undergoing >74 Gy CRT (minimum follow-up: 24 mos) selected: 10 pts in the low-risk group (rectal DVH with V70Gy<15% and V50Gy<45%) showing G2-G3 lrb ("radio-sensitive" pts); 10 pts in the high-risk group (V70Gy>25% and V50Gy>60%) with G2-G3 lrb; 10 pts in the high-risk group showing no toxicity ("radio-resistant" pts). 10 healthy donors were also considered. Quantitative RT-PCR was performed using Taqman Assays-on-Demand on RNA from LCLs obtained from EBV-immortalized PBMCs. LCLs from each pt were partly irradiated (5Gy) and partly left untreated. A similar analysis on PBMCs is now on progress. Inter- and intra-group expression levels before/after irradiation were compared using the Kruskall-Wallis test.

<u>Results</u>: Inter-group comparison highlighted many differences before irradiation, mostly among the "radio-resistant" and the "radio-sensitive" groups: 9 genes were significantly down-regulated in "sensitive" pts. It was possible to define a cut-off level allowing the identification of the "sensitive" pts for 4/9 genes. In addition 4 genes were significantly up-regulated in "resistant" pts and in all cases it was possible to define a cut-off level for the identification of the "resistant" pts. Results on genes suggesting a protection from adverse reactions were previously presented (Valdagni et al. Int J Rad Oncol Biol Phys, Abs 11, 2007).

<u>Conclusions:</u> This study provided the identification of genes which might be proposed as putative genetic markers for the prediction of late toxicity sensitivity/resistance. The knowledge of the individual gene profile might help in the planning/optimization of radiation treatment.

Parallel session 2: Cancer cytometry (1)

PP06
HIGH PERFORMANCE FRAMEWORK FOR THE RAPID ANALYSIS OF TMAS

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Virtual microscopy is changing pathology by producing diagnostic quality digital images of entire tissue sections. This provides enormous opportunities for automated image analysis, particularly for tissue microarray (TMA) immuno-biomarker analysis, which is conventionally carried out by visual inspection and scoring which is slow and subjective. However the size and format of virtual slide images makes image analysis a challenge. In order to best utilise virtual slides for objective analysis, there is a need to build high performance cluster (HPC) systems that allow rapid analysis and understanding of these large images.

This study aims to produce a framework which overcomes these problems by using a HPC to rapidly analyse virtual slides regardless of image format. A HP server consisting of 32 Processors and 8 terabytes of memory running Microsoft Windows Compute Cluster Server (WCCS) was used for the study. The framework was written in C++ using the Message Passing Interface (MPI) parallel library and a layer of abstraction was built between the image access and image processing to ensure the algorithms run independent of the image format. The framework takes a TMA and a XML map containing positional information for each of the cores as inputs. A core is farmed out to each of the worker processors which runs the desired algorithm, the results are then collected by a manager processor for presentation to the end user.

A number of algorithms (which measure densitometric, morphological and texture features) have been written in C++ to run inside the framework. Preliminary tests show a significant speedup when using a HPC, making the high throughput automated analysis of TMAs possible for the first time. Work is currently underway in collaboration with Hewlett Packard to trial the framework on a much larger system in the USA and to further optimise the framework.

Traditional analysis of TMAs is subjective and slow and desktop computer systems do not yet have the capability to rapidly analyse the high content images presented by

virtual microscopy. The HPC framework presented here can solve these problems and can also help to rapidly advance the efficiency of algorithm development in digital pathology.

PP07
MORPHOMETRY OF ISOLATED TUMOR CELLS IN BREAST CANCER SENTINEL LYMPH NODES: METASTASES OR DISPLACEMENT?

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Background: The nature and significance of isolated tumor cells (ITC) in breast cancer sentinel nodes (SN) continues to be debated. Iatrogenic displacement and mechanical transport of epithelial cells to the SN has been suggested to result in false SN positivity. Numerous studies have indicated that malignant epithelial cells show large nuclei compared to normal breast epithelium. Nuclear morphometry of SN ITC could provide relevant information related to the malignant origin-or-not of breast epithelial cells in the SN.
Objective: To find clues by nuclear morphometry as to the malignant potential of SN ITC in breast cancer patients.
Methods: In patients with primary invasive breast cancer and histologically visible SN ITC with (N=7) or without (N=18) non-SN involvement, nuclear morphometry was performed on the primary tumor as well as on the ITC in the SN. Nuclear size in the primary tumor was compared to that in the corresponding ITC. Patients with SN micro- (N=30) and SN macrometastases (N=30) served as controls.
Results: Nuclei of SN ITC in patients without non-SN involvement were significantly smaller compared to the corresponding primary tumor (P=0.03). In contrast, there were no differences in nuclear size features between the SN ITC of patients with non-SN involvement, SN micro- and macrometastases on the one hand and their corresponding primary tumors on the other. In addition,

nuclei of SN ITC without non-SN metastases after ALND were smaller compared to SN ITC with non-SN metastases (p=0.04). None of the patients with SN ITC and a nuclear area < 40.0 μm² had non-SN involvement after ALND.
Conclusion: Nuclei of SN ITC without non-SN involvement are significantly smaller compared to nuclei in the corresponding primary tumor which supports the hypothesis that some of these deposits could represent benign epithelium. Alternatively, these small cell clusters may represent degenerated malignant cells, either true metastatic or mechanically displaced, which might indicate lack of outgrowth potential. Nuclear morphometry of SN ITC could therefore contribute in identifying patients that do not have non-SN involvement and might therefore be spared from an axillary lymph node dissection.

PP08
ACCURATE DNA IMAGE CYTOMETRY FOR AUTOMATED SCREENING SCENARIOS

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Background: DNA image cytometry is a widely accepted method for the detection of both single cell and stemline aneuploidy in early cancer diagnosis. Its working procedure was standardised by the ESACP. Based on chromosomal aneuploidy, the detection of DNA aneuploidy allows a reasonable sensitive and highly specific early detection of and screening for different carcinomas on cells that can be obtained non-invasively. Yet some practical shortcomings in recent implementations hamper this application: Firstly, corrections of some errors induced by the imaging process, particularly for glare, have not been flexible enough to prevent erroneous results, which must be carefully assessed by the expert but cannot be manually adjusted. Secondly, selection and segmentation of cell nuclei is so far only viable if user-guided, in order to prevent histograms from being contaminated by either inaccurately segmented nuclei or debris, which result in broader peaks or overlapping nuclei grouped to clusters which generate artificial peaks.
Methods: We examined all image processing steps from segmentation to analysis and visualisation of results. An imaging strategy without narrow-band filter and a nucleus segmentation technique which exploits multi-

channel information from a colour camera are proposed. Overlapping nuclei are also detected by a segmentation method. We found the glare effect to vary strongly between specimens and developed an automated and robust technique for its adaptive correction. Traditional binned histogram visualisation does not meet the continuous nature of the data and was replaced by curves based on kernel density estimation. Simulated data sets with given multiple cell populations, measurement precision, and glare are used to test error correction and histogram peak detection techniques.

Results: Precision of non-filtered and filtered imaging are shown to be equal. The developed cytometry system is compared with a commercial product using nine measurements on Pararosaniline Feulgen-stained rat liver specimens and shown to be clearly superior regarding coefficients of variation and correlation coefficients of all peaks, accuracy of stemline positions, 4c/2c ratio, and reproducibility.

Conclusion: The proposed improvements to DNA image cytometry increase its accuracy and applicability and diagnostic significance in manual and in automated workflows, preparing DNA aneuploidy-based cancer screening.

PP09
GENOMIC INSTABILITY AS MEASURED BY DNA PLOIDY IN A TOTAL POPULATION OF UTERINE SARCOMAS

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Uterine sarcomas are rare tumors, with an incidence rate of 1.7 per 100 000 in Norway. The most common histological subtypes are leiomyosarcomas (LMS) and endometrial stromal sarcomas (ESS) followed by undifferentiated uterine sarcomas (UUS) and adenosarcomas. Uterine sarcomas are associated with poor outcome of disease, which is dependent on stage and histological subtype. The 5-year survival is approximately 50% for the stages I-II, and decreases to 15% or lower in advanced stages. There are conflicting results on the prognostic value of DNA ploidy in uterine sarcomas, and previous studies include few cases. To clarify the prognostic role of DNA ploidy analyses by image cytometry, we studied all the uterine sarcomas registered in Norway from 1970-2000. Of 419 histopathological confirmed uterine sarcomas, 354 were eligible for DNA ploidy classification. Of these 222 were LMSs, 78 ESSs, 21 adenosarcomas, 16 UES, 10

sarcomas not otherwise specified and 7 rare tumors. Univariate analyses showed that patients with diploid (n=110) tumors had significantly better outcome than the 244 non-diploid tumors (63 tetraploid, 31 polyploid and 150 aneuploid, Figure 1, P<0,000), as based on both crude and disease specific survival. Separate univariate analyses on the histological subtypes, showed significant differences in disease specific survival between the DNA ploidy groups in ESS (P<0,000) and adenosarcomas (P=0,029), and close to significant in LMSs (P=0,060). However, this observation did only reach significance for adenosarcomas and UUS in Cox multivariate analyses. The independent prognostic markers for ESS were stage of disease, mitotic index and tumor cell necrosis, and for LMS mitotic index, tumor size and stage of disease.

DNA ploidy analyses have prognostic value in uterine sarcomas, but not independent prognostic value in the largest groups: ESS and LMS.

PP10
GENOME-WIDE ALLELIC STATE ANALYSIS OF FORMALIN-FIXED ARCHIVAL TUMOR SAMPLES

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SNP micro-arrays allow detection of relative copy number abnormalities (CNA) and of loss of heterozygosity (LOH) in human cancers. Exact copy numbers might provide a more detailed knowledge on genomic alterations during tumour progression and human cancers in general. Furthermore, varying concentrations of normal cells present in tumour samples hinder the detection of these genomic alterations.

Using a modified version of a recently developed algorithm and integration of the DNA index during analysis we achieved to determine CNAs and LOH in terms of their allelic state, e.g. [AB] (normal genotype), [A] (LOH), [AA] (copy neutral LOH) or [AAB] (allelic imbalance) etc. in flow-sorted keratin-positive tumour cell populations from formalin-fixed, paraffin-embedded cervical and colon cancer samples. For several loci CNAs found were confirmed by FISH analysis on flow-sorted tumour cells. Sorted stromal cells served as a reference.

We conclude that flow-sorting together with integrative analysis significantly improves the identification and determination of genomic alterations in heterogeneous tumour samples allowing defined allelic profiles from tumour cell subpopulations using SNP micro-arrays.

Furthermore, this combined approach allows a detailed mechanistic view of genomic alterations in human carcinomas and can be implemented for large-scale retrospective studies using archival samples.

Parallel session 3: Cancer genomics (2)

PP11
FUNCTIONAL EVALUATION OF CANDIDATE ONCOGENES MAPPING TO NARROW AMPLICONS IN ORAL SQUAMOUS CELL CARCINOMA

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Genomes of solid tumors are characterized by gains and losses of regions, which may contribute to tumorigenesis by altering gene expression. Application of genome-wide array comparative genomic hybridization (CGH) in oral squamous cell carcinoma (SCC) has revealed the presence of narrow amplicons. The amplicons are rare, occurring in less than 5% of tumors and they are narrow (< 3Mb), which facilitates identification of candidate driver oncogenes. The narrow amplicons also are present in oral pre-malignant lesions. Genes mapping to the amplicons implicate aberrations in integrin signaling, apoptosis, adhesion and migration, as well as deregulation of networks specifying cell fate, including the hedgehog and notch pathways as contributing to tumor development. In contrast to genome-wide scanning of tumors, which rapidly highlights aberrant genomic regions, identifying the important cancer genes in these regions and determining how they contribute to tumor development remains a difficult task. We have begun the analysis of candidate oral SCC oncogenes by expressing them in keratinocytes and assessing the functional consequences of oncogene expression by co-culturing the keratinocytes and fibroblasts in three dimensional organotypic cultures. These cultures allow communication between cell types and differentiation of the epithelium. They offer a relatively high throughput model system for evaluating candidate tumor genes identified by genome-wide profiling. These studies show that overexpression of an oncogene may induce a number of tumor phenotypes, including enhancing genomic instability, blocking differentiation, and promoting modification of stromal cells to create an environment permissive for invasion. The oncogene induced features observed in organotypic cultures in vitro both recapitulate the histology of tumors with oncogene amplification and predict previously unappreciated molecular alterations in the in vivo tumors.

PP12
GENOMIC PROFILING IDENTIFIES A SUBSET OF HIGH-GRADE PREMALIGNANT CERVICAL LESIONS CLOSELY RESEMBLING INVASIVE CANCER

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Cervical cancer is caused by a persistent infection with high-risk human papillomavirus types (hrHPV). However, hrHPV positive high-grade premalignant cervical lesions (HGCIN) represent a very heterogeneous disease as they can spontaneously regress, persist, or, in a small subset of cases, progress to invasive cancer. A better understanding of the recurrent (epi)genetic alterations that occur during cervical carcinogenesis may enable better risk stratification of hrHPV positive women for the development of cervical cancer. We performed array-based comparative genomic hybridisation (array CGH) of 46 HGCINs, to determine whether the clinical heterogeneity of this disease is reflected in the genomic signature. The obtained genomic profiles were related to hrHPV type, genomic profiles of invasive cervical squamous cell carcinomas (SCCs) and methylation of the CADM1 (formerly known as TSLC1) promoter. The latter was previously found to be a marker for ×HGCIN.

On average HGCINs showed 7.2 alterations, 2.8 gains and 4.4 losses. Frequent alterations included gains located at chromosome 1, 3, 7 and 20 and losses located at 4, 11, 16, 17 and 19. Losses of 17q22-24.3 were significantly less frequent in HPV16-positive HGCINs compared to HGCINs with other hrHPV types (FDR<0.10). Unsupervised hierarchical clustering identified a subset of 13 HGCINs whose genomic profiles were similar to those of invasive carcinomas. As expected, HGCINs resembling carcinomas showed on average higher numbers of alterations compared to the other HGCINs (12.6 and 5.3 respectively). Subsequent analysis revealed that gains of 1, 3q and 20 were most

discriminative between these two groups of HGCINs. In addition, dense promoter methylation of the CADM1 gene was significantly more frequent in HGCINs resembling carcinomas compared to the other HGCINs (p=0.0004).

In conclusion, genomic profiling identified two distinct subclasses within a group of histologically similar HGCIN lesions. Genomic profiles of one of the subclasses closely resembled those of invasive carcinomas, which was predominantly due to frequent gains at chromosome 1, 3q and 20. The current data suggest that these gains are associated with malignant progression and therefore provide promising starting points in the search for novel biomarkers for cervical cancer.

PP13
PATTERNS OF CHROMOSOMAL INSTABILITY AND GENE MUTATION RELATED TO COLON CANCER PROGRESSION

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We investigated the relationship between chromosomal alterations and specific gene mutations in colon cancer progression in a consecutive series of colon cancer patients.

Fifty two sporadic colon cancers from patients from our Institute were analyzed for somatic mutations in APC, KRAS and TP53 genes and for loss-of-heterozygosity (LOH) of nine microsatellite loci spanning the long arm of chromosome 18. DNAs from the same cases were hybridized to Affymetrix GeneChip(tm) Human Mapping 250K NspI Arrays. The SNP microarray data presence call was estimated using GTYPE 4.1 software and copy number and LOH analysis was performed using the CNAG 2.0 software.

When the gain and loss data were analyzed over all patients, most results were consistent with those reported in the literature for colon cancer (Diep CB et al., Genes Chromosomes Cancer. 2006,45:31-41), thus highlighting the robustness of the platform for detecting gains and losses using SNP data. In addition, new regions of interest were detected such as gains on 5p (5.5%), 12q (20%), 7q (24%) and losses on 1p36.33-p32.3 (46.3%), 19 (26% 19p and 20% 19q).

Next we investigated the relationship between gene mutation data and chromosomal aberration with respect to tumor progression using different staging definitions. A clearer increase of both mutation and copy number data was observed when patients having chromosome 18q LOH were reallocated to their next higher stage compared to standard staging. When we analyzed patterns of gain and loss around the genomic loci harboring APC, KRAS and TP53 for each patient we noticed that APC did not have any particular pattern of aberration whereas the regions around KRAS and TP53 were consistently amplified and lost respectively. This information provides us evidence that these two key players are driving genes involved in two distinct crucial pathways leading to the progression of colon cancer.

These finer resolution mappings of gain and loss coupled with the mutational status of the two key genes (KRAS and TP53) could help in the search for candidate tumor suppressors or activated genes related to colon tumorigenesis.

PP14
ARRAY CGH PROFILE AS A MARKER TO PREDICT NEO-ADJUVANT RADIO-CHEMOTHERAPY RESPONSE OF RECTAL CARCINOMA

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Locally advanced rectal tumors, subjected to standard neo-adjuvant radio-chemotherapy, show a wide range of clinical responses from a complete disease regression to no response whatsoever. However, such responses cannot be deduced from conventional clinical-pathological characteristics.

This study proposed to define the genomic profile of locally advanced rectal tumors, and to identify alterations predictive of a response to neo-adjuvant radio-chemotherapy, with the final aim to stratify patients, at the point of diagnosis, as "responsive"or "non-responsive", for better treatment and clinical management.

Pre-therapy biopsies were collected from 39 patients, 9 females and 30 males, with a median age of 66 years

(range 37-82). Six tumors (15%) were classified as stage T2, 31 (80%) were T3, and 2 (5%) were T4.

Tumor DNA was analysed by array CGH using whole genome glass slides to obtain an exhaustive representation of the genomic alteration profile. This approach allowed an extremely detailed molecular karyotype to be obtained, with a highly sensitive and specific identification of DNA copy number variations, as well as sub-microscopic changes present within the entire genome.

All patients were subjected to a radiotherapeutic regimen consisting of 50 Gy/week for 5 weeks with conventional fractionation, and central catheter infusion of 400 mg/m2 5-fluorouracil during radiotherapy. A clinical response, evaluated as tumor regression grade (TRG) status according to Dworak criteria, was obtained in 41% of cases.

Array CGH profiles, analysed in relation to clinical response, were able to identify a subgroup of non-responsive tumors characterized by a high number of genomic alterations. The most frequent DNA copy number changes, observed in more than 30% of cases, consisted of DNA amplifications, mainly involving chromosomes arms 1p, 1q, 2q, 5p, 5q, 8q, 10p, 11q, 15q and 20p. Indeed, a number of these "hot spot"regions are currently under investigation to identify the principal genes implicated in the resistance to radio-chemotherapy.

These data, together with a more exhaustive evaluation of single gene alterations, should allow a tailored therapeutic intervention for individual patients, improving the clinical results in terms of cost-benefits.

PP15
CHARACTERIZATION OF MYOEPITHELIAL SALIVARY GLAND TUMORS BY PROTEIN EXPRESSION AND DNA PROFILING

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Background: Salivary gland tumors arising from myoepithelial cells have an unpredictable clinical course. A better understanding of the molecular alterations underlying their development and progression could significantly improve their prognostication. Polycomb group (PcG) genes contribute to the regulation of normal cellular development and the regulation of the cell cycle. These proteins interact with several negative (p16, p53, pRb, p14) and positive cell cycle regulators (E2F1, Cyclin D1). Abnormal PcG expression is believed to result in the development of various human epithelial and hematological malignancies.

Methods: We investigated protein expression (PcG proteins BMI-1, MEL-18, EED, and EZH2 together with p53, p16, Cyclin D1, KI-67, and E2F1) by immunohistochemistry (IHC) in myoepitheliomas (benign and malignant) and adenoid cystic carcinomas (ACCs) in relation to tumor behavior. Furthermore, we investigated the genomic profiles of these tumors by microarray-based comparative genomic hybridization (array CGH).

Results IHC: Our data indicate that expression of the two PcG complexes is deregulated in the malignant tumors. Also, high expression of EZH2 in ACC is predictive of a short survival (p=0.04). Expression of p16, Ki-67, Cyclin D1, and E2F1 proteins is increased in all tumors compared to normal tissue. Deregulation of the p53 apoptosis pathway seems to be confined to the malignant tumors.

Results CGH: In all three tumors, the most frequent gains harbor numerous growth factor (FGFs and PDGF) and growth factors receptor (FGFRs and PDGFR) genes. Gains at the FGF(R) regions occur significantly more frequently in the recurred/metastasized ACCs compared to indolent ACCs. Furthermore, ACC patients with ×17 chromosomal aberrations have a significantly less favorable outcome than patients with less chromosomal aberrations (p=0.02).

Conclusions: Deregulations of the p16 and p53 pathways seem to be major events in myoepithelial tumor development. Benign tumors are characterized by p16 pathway deregulation and p53 overexpression is evident in the malignant lesions. PcG proteins are highly disturbed in ACC, but do not seem to have a major role in myoepitheliomas. EZH2 seems to have prognostic relevance in ACC. Frequent DNA copy number gains at loci of growth factors and their receptors suggest their involvement in myoepithelial tumor initiation and progression.

PP16

DENSE CADM1 PROMOTER METHYLATION ASSOCIATED WITH REDUCED IMMUNO-EXPRESSION IN HIGH-GRADE CIN AND CERVICAL SCC

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We previously showed that silencing of TSLC1, recently renamed CADM1, is functionally involved in high-risk HPV-mediated cervical carcinogenesis. CADM1 silencing often results from promoter methylation. Examination of a 93bp promoter region revealed frequent promoter methylation in high-grade squamous premalignant lesions and squamous cell carcinomas (SCC; ×CIN3). Here, we determined the extent of promoter methylation and its relation to gene silencing to select a CADM1-based methylation marker for identification of women at risk of cervical cancer.

Methylation specific PCRs targeting three regions within CADM1 promoter were performed on PBMCs, normal cervical smears and (pre)malignant lesions. CADM1 immuno-expression in cervical tissues was analysed by immunohistochemistry. All statistical tests were two-sided.

Methylation frequency and density increased with severity of cervical squamous lesions. Dense methylation (×2 methylated regions) increased from 5% in normal cervical samples to 30% in CIN3 lesions and 83% in SCCs. Dense methylation rather than occasional methylation was significantly associated with decreased CADM1 immuno-detection in both CIN3 and SCC (p<0.00005). Age-adjusted logistic regression analysis showed that frequency of dense methylation was significantly higher in ×CIN3 compared with ÖCIN1 (Odd's ratio (OR)= 5.6, 95% confidence interval [CI]= 1.7-18.5, p=0.005). In adenocarcinomas, dense CADM1 methylation was significantly less common compared with SCC (23% versus 83%; OR= 17.2, 95% CI= 2.9-101.1, p=0.002). Currently, we are exploring a quantitative MSP approach to determine methylation patterns of CADM1 in cervical smears representing the various stages of cervical disease.

In conclusion, dense methylation of the CADM1 promoter associated with decreased immuno-expression of CADM1 provides a valuable diagnostic marker for the triage of high-risk HPV-positive women at risk of ×CIN3.

Parallel session 4: Cancer cytometry (2)

PP17

THE PROGNOSTIC VALUE OF DNA PLOIDY IN GASTRIC CANCER ASSESSED BY FLOW AND IMAGE CYTOMETRY

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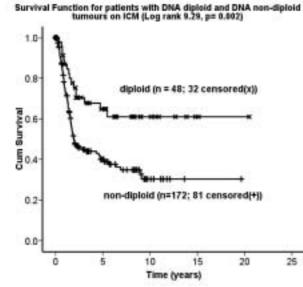[1]*VU University medical center, Amsterdam, The Netherlands*
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<u>Background:</u> DNA aneuploidy reflects gross genomic changes and can be measured either by flow cytometry (FCM) or image cytometry (ICM). The prevalence of DNA aneuploidy in gastric cancer has been reported to range from 27% to 100%. This variability and lack of consistency in its association with clinicopathological factors may be due to methods of cytometry used. We aimed to compare DNA ploidy in gastric cancer measured by both ICM and FCM and evaluate its association with patient survival.

<u>Material and methods:</u> Nuclei were isolated from formalin fixed paraffin embedded tissue samples. FCM was performed with a Partec PAS system and ICM with the PIPE system. Data were obtained from 220 gastric adenocarcinomas with long-term follow up and histograms analyzed with MultiCycle. Ploidy status of both methods were compared to each other and correlated to survival.

<u>Results:</u> With FCM 166 (75.5%) aneuploid cases were found and with ICM 172 (78.2%). Results from both methods were concordant in 170 cases. 28 FCM diploid cases were classified as aneuploid by ICM and vice versa 22 cases classified diploid by ICM were aneuploid by FCM (kappa 0.36). Patients with diploid tumours based on ICM survived significantly longer than those with aneuploid tumours as shown by ICM (Log rank 9.29, p= 0.002). Ploidy determined by FCM was not associated with survival differences (p = 0.118).

Survival Function for patients with DNA diploid and DNA non-diploid tumours on ICM (Log rank 9.29, p= 0.002)

diploid (n = 48; 32 censored(x))

non-diploid (n=172; 81 censored[+])

Conclusion: ICM based on ploidy status predicts clinical outcome in gastric cancer, in contrast to FCM based ploidy status.

PP18
TMPRSS2: ERG REARRANGEMENT IS AN INDEPENDENT MARKER OF GOOD PROGNOSIS IN PROSTATE CANCER

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The frequent fusion of the androgen inducible promoter of TMPRSS2 to ERG in prostate tumours renders the expression of ERG androgen-dependent. Nearly all prostate cancers express the androgen receptor (AR), which mediates androgen action. We assessed the frequency of ERG overexpression and TMPRSS2:ERG rearrangement in prostate cancer and their association with clinicopathological variables and prognosis.
We studied the expression of the TMPRSS2:ERG transcript and the rearrangement status in seven prostate cancer cell lines and 19 xenografts by RT-PCR and fluorescence in situ hybridisation (FISH). ERG expression was studied in 49 freshly frozen clinical prostate samples including 9 BPH, 28 untreated and 12 locally recurrent hormone-refractory prostate cancers (HR-CaP). Fusion frequency was studied by three-

colour FISH on tissue microarrays (TMA) including 150 untreated prostate cancers and 75 HR-CaP.
Of the cell lines studied, only VCaP and DuCaP carry the TMPRSS2:ERG rearrangement, and 7/19 of the xenografts overexpress ERG and contain the rearrangement. Two other xenografts also contain the rearrangement, but they do not express ERG as they are AR negative. 17/28 (61%) untreated clinical prostate carcinomas and 6/12 (50%) HR-CaP overexpressed ERG. In the samples where the TMPRSS2:ERG rearrangement could be evaluated by FISH, the overexpres-sion was associated with it (n=16, p=0.0019). FISH showed that 50/150 (33%) untreated prostate cancers and 28/76 (37%) HR-CaP contained the rearrangement. In the untreated samples, the re-arrangement was not associated with Gleason score, pT-stage, diagnostic PSA, or proliferation rate. It was associated with longer progression free time (p=0.019) and according to multivariate analysis, it was an independent predictor of favorable outcome (relative risk 0.54, 95% confidence interval 0.30-0.98).
In conclusion, the TMPRSS2:ERG rearrangement almost invariably leads to overexpression of ERG, is found in approximately one third of prostate cancers, and may identify a subgroup of prostate cancers with a favourable prognosis.

PP19
THEORETICAL AND EXPERIMENTAL CELLULAR SOCIOLOGY SYSTEMS BIOLOGY: CANCER AS COMPETING CLONES

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The pre-invasive neoplastic development process is one of competing normal and molecularly altered clonal populations. The altered internal molecular dynamics of these normal and abnormal cell populations interact at the physical level where they compete on the basis of cell proliferation, apoptosis rates, resource request signalling and usage, as well as interacting with the host immune system and surrounding stroma. An in depth understanding of all these interactions is key to understanding and altering the neoplastic process for therapeutic (chemoprevention for pre-neoplastic tissue and chemotherapy for invasive cancer) purposes.
Our approach to understanding this process is two fold; 1) a computational 3D static and dynamic model of tissue epithelium and stroma (based on quantitative measures of physical and molecular interactions) and 2) an automated system for multi-colour (FISH and

immunohistochemistry) imaging of tissue sections for the identification of genetically related clones in excised tissue samples.

Our current mathematical model is based on quantitative data on the nuclear phenotypes of the cells, molecular characteristics from quantitative IHC and quantitative tissue architectural information (spatial arrangement of cells in tissue) from more than 10,000 of lung, cervix and oral sections.

We have built an automated system for multi-colour FISH and immunohistochemisrty which is designed to scan up to 6 colours (FISH labels) automatically across an entire tissue section and tabulate the results cell by cell for use in an architectural analysis of clonal neighbourhoods. Several definitions of clonal neighbourhood have been tested. These are derived from the Voronoi tessellation of the imaged tissue based upon the spatial arrangement of the individual cells and their FISH attributes. Initial testing of this system showed that populations defined by genetic amplifications and by genetic deletions can be used to recognise clonal cell clusters. It is also able to recognise of cells resistant to lung cancer chemotherapy based up on a specific gene copy number alteration profile, derived form array CGH data. The intent is to use the novel finds/predictions of the neoplastic process of one approach to validate/understand the results of the other approach.

PP20
CORRELATION BETWEEN TEXTURAL ANALYSES OF CELL NUCLEI AND GENE-COPY NUMBER VARIATIONS

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Chromosomal instability (CIN) has been associated with cellular transformation in multiple cancer types. The most commonly used methods to study changes in overall genome structure and integrity either rely on imaging-based technologies or molecular methods. Based on analysis of monolayer preparations of cell nuclei purified from tumors and high resolution imaging techniques, we have developed a method for high throughput scoring of a large number of nuclear textural parameters ('nucleotyping'). In addition to ploidy, nucleotyping can give information about organization of heterochromatin vs. euchromatin, nucleolus placement and several other layers of information regarding transcriptional activity and structure of the genome. We have attempted to correlate these observations with high resolution microarray-based comparative genomic hybridization (CGH) data. CGH gives a detailed map of regions that are either over or under represented (amplified or deleted) in the tumor samples, and we have tried to link these molecular changes with nucleotyping profiles as well as clinical data. The molecular causes of CIN are poorly understood, but we believe that our integrative approach might enable us to understand the interplay between CIN and tumorigenesis.

PP21
DEVELOPMENT OF DENSITOMETRIC AND TEXTURE ALGORITHMS WITHIN THE APERIO ALGORITHM FRAMEWORK (AAF) FOR VIRTUAL SLIDE ANALYSIS

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Digitisation of an entire glass slide at high power produces a high resolution, diagnostic quality, digital image known as a virtual slide. Virtual microscopy is an exciting, emerging technology that is revolutionising pathology, opening up new opportunities for e-learning, image analysis and machine vision. Despite the rapid advantages in scanning technology, progression in the area of algorithm development for virtual microscopy has been relatively slow, mainly due to image size and the propriety image formats used by many of the scanning manufactures.

This study set out to produce a suite of low-level quantitative features (including densitometric, morphological and Markovian texture features) which can be combined to produce high-level algorithms to run on Virtual slide images. The features were implemented inside the AAF which is a Software Development Kit (SDK) that allows users to run C-code from within Aperio ImageScope (a standalone application for viewing and annotating virtual slides). The code can be run directly on the entire image or by defining a region of interest.

The algorithms produced consistent results on a variety of virtual slides and their pathological relevance was demonstrated in three main applications: (1) the assessment of tumour differentiation in tissue microarrays (TMAs) using texture computation, (2) the densitometric evaluation and scoring of TMA immunohistochemistry and (3) the analysis of disorganised nuclear chromatin signatures in cancer using texture analysis.

Virtual microscopy and whole slide image analysis has enormous potential but the application of complex algorithms applied to entire slides is computationally intensive. This will require novel high performance computing (HPC) approaches to deliver accuracy and speed in the quantitative evaluation of tissue histology and this group have been working in tandem to deliver an HPC framework for virtual slide imaging. High performance computing together with advanced algorithm development on virtual slides could potentially revolutionise the tools available to diagnostic pathologists, support emerging fields such as automated tissue microarray analysis and establish digital pathology as a relevant discipline over the next few years.

PP22
MULTIMODAL CELL ANALYSIS (MMCA) FOR EARLY CANCER DETECTION

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We describe a system for computer-assisted microscopical identification and classification of cancer cells that allows repeated semiautomatic measurements of various cellular markers after multiple restainings and relocations of cells, fixed on glass slides. For that purpose individual cells are repeatedly identified by digital image analysis after application of different stainings or marker-demonstrations. Relocations are performed by a combination of scanning stage-movements and digital image alignments. The system is based on a Leica DMLA computerized microscope, a CCD color camera and a PC. We report on the performance of the system and on results of first diagnostic applications.

Cell loss after Feulgen- and Silvernitrate-restaining was lowest in primarily air dried smears (0%), medium in alcohol-fixed smears (8.4%) and highest in liquid-based monolayer preparations (20.3%). Rough mechanical relocation of cells and pixel wise, precise overlay of their images worked well, even after three different staining procedures. The CV of DNA-measurements of rat liver lymphocytes could be lowered from 3.25% (AutoCyte QUIC) to 1.72% by improved software-correction of glare- and diffraction-errors.

In 30 oral smears (12 from cancers) staining sequence was: Papanicolaou, Feulgen and silver-nitrate. Image-cytometry correctly identified 93.1% of cancer samples by detection of DNA-aneuploidy alone. Adding AgNOR-analysis, all smears that contained cancer cells were correctly detected. 33 reference- and 261 abnormal epithelial cells were measured on an average per slide.

In 35 smears from serous effusions (13 due to metastatic carcinomas, 11 due to malignant mesotheliomas and 11 due to non neoplastic causes) DNA-aneuploidy alone identified 62.5% of specimens containing tumor cells. Addition of AgNOR-counting increased the rate of tumor cell-detection to 92%.

In 30 fine-needle-aspiration biopsies from thyroid nodules (14 from follicular adenomas, 6 from - carcinomas and 10 from colloid goiters) the prevalence of DNA-aneuploidy was only 55% in neoplasias. Adding AgNOR-analysis, 92% of neoplasias were correctly identified. The coefficient of correlation of modal DNA-values between MMCA- and AutoCyte QUIC measurements was r=0.988.

Thus we could prove that multimodal, digital cell analysis is technologically feasible and may increase diagnostic accuracy on few cells only by creation of feature-vectors for few individual cells.

Parallel session 5: Cancer cell biology/ Cancer stem cells

PP23
EPHRIN B3 RECEPTOR EXPRESSION IN NORMAL ESOPHAGUS AND ESOPHAGEAL ADENOCARCINOMA: A CLINICOPATHOLOGICAL STUDY OF A NEW CANCER-ASSOCIATED BIOMARKER

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<u>Introduction:</u> Esophageal adenocarcinoma shows a heterogeneous behaviour in progression and response to neoadjuvant chemotherapy. In order to understand the diverse aspects involved, differential expression analyses have been performed. Ephrin receptor tyrosine

kinases and their ligands, the ephrins, have been primarily described for their roles in controlling angiogenesis and cell intermingling, which are both important issues in esophageal adenocarcinomas.

Material and Methods: Biopsy specimens were obtained in 30 patients with an esophageal adenocarcinoma (AEG type I) during primary staging. Ten patients with a healthy esophagus served as the control group. All patients with AEG I were treated with a neoadjuvant chemotherapy followed by resection. For a gross screening microarray analysis was performed and compared to the normal tissue. One of the most predominant differentially expressed genes Ephrin B3 receptor was confirmed by immunohistochemistry and correlated with the histophathological postoperative staging and the response status.

Results: Ephrin B3 receptor was located close to the basal membrane in the control group and around the tumor area in the case of an invasive carcinoma. However there was a pronounced difference in the expression pattern of responders and non-responders showing a higher concentration of the receptor in the group of responders. This was true for the microarray analysis as well as for the protein expression.

Conclusion: The identification of this new biomarker in esophageal adenocarcinoma provides first evidence that the diverse behaviour in response and progression might be determined very early during the development of the stem cells.

PP24
PEUTZ-JEGHERS SYNDROME POLYPS ARE POLYCLONAL WITH EXPANDED PROGENITOR CELL COMPARTMENT

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Background & Aims: The nature of polyp formation in Peutz-Jeghers Syndrome (PJS) has remained elusive. Consequently, whether PJS polyps harbor an inherent risk of transformation as proposed by the hamartoma-carcinoma sequence is unclear. We previously postulated that PJS polyp formation results from benign mucosal prolapse due to a germline defect in asymmetric stem cell division. Here we determine the clonality status of PJS polyps and investigate cell proliferation in normal and hamartomatous PJS intestinal epithelium.

Methods: The clonality of PJS polyps was studied using an X-chromosome inactivation assay and length of the progenitor zone of epithelial crypts was assessed by Ki67 labeling. Furthermore, LKB1 mutation and LOH analyses were performed.

Results: Clonality assay and loss of heterozygosity analysis on PJS polyps from two PJS patients revealed that all polyps were polyclonal. LKB1 mutation analysis identified new germline mutations leading to a premature stop codon in both patients. Ki67 staining showed that the progenitor zone of normal intestinal crypts of PJS patients was expanded compared to crypts in normal controls ($p<0.001$).

Conclusions: PJS polyps are polyclonal and by strict definition not pre-neoplastic. Normal mucosa from PJS patients exhibits an expanded progenitor zone possibly from a disturbance in asymmetric stem cell division, leading to mucosal prolapse and polyp formation. The hamartoma-carcinoma sequence in PJS remains unproven and risk of neoplastic transformation in PJS polyps should not direct surveillance strategies in these patients.

PP25
PROTEOMICS OF STEM CELL LINES OBTAINED FROM LOW- AND HIGH-GRADE HUMAN ASTROCYTOMAS

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In a previous study, the protein expression profiles of low- and high-grade human brain astrocytoma tissues was compared by two-dimensional gel electrophoresis (Odreman et al., J. Proteome Res. 2005). In this way, a number of proteins differentially expressed between the low- and high-grade tumors were identified. These proteins could be potentially used as novel moleculars biomarkers for an accurate classification of the grade of astrocytomas. In this study, we extended the same analysis to cell lines obtained from the low- and high-grade tumor samples.

For this reason, main objectives of our study were: 1. Optimize a method to isolate and expand in vitro cancer stem cell (CSC) population from low-grade and high-grade human brain astrocytomas (named, respectively, l-CSCs and h-CSCs); 2. Demonstrate in vitro l-CSC and h-CSC stemness and tumorigenicity; 3. Compare the protein expression profiles of l-CSCs and hCSCs.

Low- (n=17) and high- (n=19) grade astrocytomas were obtained by surgical resection at the Neurosurgical unit of the Azienda Ospedaliero-Universitaria of Udine. Neoplastic tissues were mechanically-enzimatically dissociated and the single cell suspension cultured in a medium selective for the growth of Multipotent Adult Stem Cells. Cultured cell lines showed a mesenchymal immunophenotype, being usually CD13high CD59high CD49bhigh CD90high CD73int CD44int HLA-ABCint CD29int CD105int KDRint CD49aint CD49dint CD14neg CD45neg CD38neg HLA-DRneg CD133neg CD117neg CD34neg, and expressed Oct-4, REX1 and NANOG, pluripotent state-specific transcription factors. Tumorigenicity of expanded cell lines was confirmed by the demonstration of anchorage independence growth (soft agar assay) and loss of contact inhibition (sphere formation assay, transformation foci assay, and growth curve analysis).

The proteomic studies performed to compare h-CSCs and l-CSCs allowed us to identify a number of proteins that are more abundant either in the low- or in the high-grade tumors and were not detected in the previous study using the brain tissues.

PP26
A NEW METHOD TO ISOLATE AND IN VIRO EXPAND CANCER STEM CELLS FROM HUMAN GLIOMAS

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Background: The possibility to isolate and expand in vitro cancer cell with stem cell properties from human gliomas could open new perspectives in "patient tailored"therapies aimed at targeting specifically this rare population of cells, possibly responsible for recurrences and drug resistance.

Aims: To develop a methodology alternative to in vitro neurosphere formation or CD133 selection, capable of isolating and rapidly expanding a population of cells with stem cell features, both from low- (I, II) and high-grade (glioblastoma multiforme, GBM) human glial tumors.

Methods and Results: 0,2-2 g in weight, non necrotic fragments of low- (n=20) and high- (n=21) grade lesions were obtained by surgical resection at the Neurosurgical Unit of the hospital of Udine (Italy). Neoplasms were mechanically-enzimatically dissociated and cells were cultured in a medium optimized in our laboratory for the growth of Multipotent Adult Stem Cells. Cell lines were tested for growth kinetics, stemness and in vitro tumorigenicity.

Results: Cell lines were obtained from all the samples with high efficiency and reproducibility. Cell population doubling time of glioma cell lines (calculated at passage 3) was 25 hours, allowing obtaining 106 cells in less than 20 days. About stemness, CSCs expressed a typical mesenchymal immunophenotype, being usually CD13high/CD59high/CD49bhigh/CD90high/CD73low/ CD44low/HLA-ABClow/CD29low/CD105low/KDRlow/CD49alow/CD 49dlow/CD14neg/CD45neg/CD38neg/HLA-DRneg/CD133neg/CD117neg/CD34neg. Further, the average percentages of Oct-4 and Nanog, pluripotent state-specific transcription factors, were 86% and 91%, respectively, and cell lines displayed in vitro multipotency.

Regarding tumorigenicity, the majority of CSCs displayed aberrant growth features such as loss of contact inhibition (transformation foci assay) and adhesion independent growth (soft agar assay).

Importantly, CSCs at passage 3 demonstrated a 160 fold enrichment in soft agar colony formation capability with respect to uncultured cells.

Conclusions: We developed a methodology, alternative to in vitro neurosphere formation or CD133 selection, for the rapid expansion, from low- and high-grade gliomas, of cells that retain stem cell features and aberrant growth properties. The high reproducibility and yield of the method would allow to obtain a sufficient number of cells, even for high throughput approaches and drug screening in a short period of time.

PP27
SPECIFIC DETECTION OF ABERRANT AND NORMAL STEM CELLS IN ACUTE MYELOID LEUKAEMIA

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In acute myeloid leukaemia (AML), a fraction of the progenitor population contains tumor initiating cells, referred to as leukemic stem cells (LSCs). LSCs have stem cell characteristics, irrespective of the origin; from normal hematopoietic stem cells (HSC) or from more differentiated cells. In a CD34+ AML, it is believed that

both HSCs and LSCs reside within the CD34+ CD38-compartment. Previous research has shown that frequencies of residual leukemic blasts, including both stem cells and progenitors detected after chemotherapy and known as minimal residual disease, predict survival. Since therapy surviving LSCs initiate relapses, we hypothesized that the LSC frequency after treatment may give more detailed prognostic information. To enable detection of LSCs, these should be discriminated from HSCs. Our recent work shows that LSCs can be differentiated from HSCs using FACS analysis based on aberrant lineage marker expression, including the novel LSC marker CLL-1. However, HSCs and LSCs cannot be distinguished in case of low expression of these aberrant markers.

Therefore, we now investigated additional markers/parameters in order to distinguish LSCs from normal HSCs in all CD34+ AML. In normal bone marrow, HSCs form a distinct clustered population with lower forward scatter (FSC) and side scatter (SSC) properties than CD34+ CD38+ progenitors, indicating smaller size and less granularity. In addition, the HSCs showed a somewhat higher expression of CD45 and CD34. In multiple diagnosis AML cases, a similar HSC-like population was present within the total CD34+ CD38- compartment. These cells were always negative for the antigenic aberrancies found on AML blasts and thus can be hypothesized to be normal CD34+ CD38- cells. In addition to this low FSC/low SSC population, a population was found with slightly higher FSC and SSC often expressing aberrant markers and coinciding in scatter with the total leukemic progenitor population. In part of the cases, this population also showed a different pattern of CD34 and CD45 expression compared to the HSCs.

In AML, LSCs can thus be detected not only based on aberrant marker expression but also using scatter properties and/or CD45 and/or CD34 expression.

PP28
NOVEL PRECLINICAL CELL CULTURE MODEL FOR PREVENTION OF GENETICALLY PREDISPOSED FAMILIAL ADENOMATOUS POLYPOSIS (FAP) SYNDROME

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In the adenoma-carcinoma sequence of colon carcinogenesis, mutations in the adenomatous polyposis coli (APC) gene represent a primary genetic defect for the FAP syndrome. Models exhibiting clinically relevant genetic defects and quantifiable cancer risk may provide valuable leads for efficacious intervention. Cloned colon epithelial cell line from Apc850Min/+ mutant mouse represented the FAP model. Cell cycle progression, apoptosis and anchorage-independent growth represented the biomarkers for carcinogenic risk. Relative to the wild type Apc+/+ cells, the Apc mutant cells exhibited 80.6% decrease in the G0:S+G2/M ratio, 12.7 fold increase in the S+G2/M:SubG0 ratio and 100% increase in anchorage-independent growth, indicating down-regulated homeostatic growth control and enhanced carcinogenic risk. Growth of Apc mutant cells in serum-depleted medium was up-regulated by insulin and epidermal growth factor and down-regulated by transforming growth factor- beta1 and dexamethasone, suggesting receptor-mediated growth modulation. Treatment of Apc mutant cells with low dose combinations of cancer preventive synthetic enzyme inhibitors (CLX, DFMO) and natural phytochemicals (EGCG, CUR, EPA) produced cytostatic growth arrest and inhibited carcinogenic risk. These data validate a novel approach for efficacy of new combinatorial preventive/therapeutic compounds for colon cancer.

Parallel session 6: Cancer cell biology / Tumor microenvironment

PP29
OPPOSITE ROLE AND DIFFERENTIAL SIGNALING OF IL-21 AND IL-15 IN B-CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS

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B-CLL cells require factors, such as cytokines and CD40L presented by Th cells, in the microenvironment of lymphoid tissues for their survival and expansion. IL-15 is expressed by bone marrow stromal cells and in DC present in lymphoid tissues, and may therefore have a role as growth and survival factor for B-CLL cells. IL-15 induces proliferation and partial rescue from anti-IgM-triggered apoptosis of B-CLL cells. IL-15 mitogenic effects are more evident after CD40

triggering, which increases expression of IL-15Ralpha and of IL-2Rbeta chains. The effects of IL-15 are blocked by antibodies reacting to the IL-2Rbeta or IL2Rgamma chains, which are involved in IL-15 signaling in different cell types. We also tested the effects of he structurally related cytokine IL-21, a factor produced by Th and NKT cells. Differently from IL-15, L-21 does not support CLL B cell proliferation and promotes apoptosis. Moreover, IL-21 counteracts the effects of IL-15. To gain information on the mechanisms underlying the opposite effects of these two cytokines, which share the usage of the common-gamma chain, we investigated their signaling cascade. IL-15 predominantly increases tyrosine phosphorylation of STAT5, whereas IL-21 predominantly activates STAT3 and the JAK1/STAT1 pathway in B-CLL cells. The ability of IL-21 to predominantly activate STAT3 may depend on the presence of a STAT3 binding motif in the IL-21R chain. In addition, IL-15, but not IL-21, induced recruitment of Shc and increased ERK1/2 phosphorylation levels. Pharmacological inhibition of JAK3 or of MEK1 (the kinase activating ERK1/2) efficiently blocks IL-15-triggered proliferation and anti-apoptotic effects. In conclusion our data indicate that IL-21 and IL-15 may have opposite roles in the natural history of CLL by differentially regulating the expansion of the neoplastic clone. The usage of IL-21 or of drugs targeting the IL-15 signaling pathways may be further exploited as therapeutic strategies for CLL.

PP30
HYPOXIA SIGNATURE OF NEUROBLASTOMA CELLS: ROLE OF N-MYC OVEREXPRESSION

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High N-myc expression is associated with advanced neuroblastoma stage and poor prognosis, but the relationship between N-myc and immunity has remained obscure. Hypoxia, a local decrease of oxygen tension, is a critical determinant of tumor progression. Transcriptional activation by hypoxia is mediated primarily by the hypoxia-inducible factor-1 (HIF-1). N-myc shares with HIF the ability to bind the E-box and a crosstalk between hypoxia and N-myc has been suggested. Using Affymetrix GeneChip we studied the changes in gene expression caused by hypoxia exposure in 11 neuroblastoma cell lines with or without N-myc overexpression. In addition, we used the SHEP21N cell line with N-myc expression inducible by tetracycline. Cells were exposed to hypoxia (1% oxygen) or normoxia (20% oxygen) for 18 hours. The gene expression data were analyzed using GeneSpring GX 7.3 and new algorithms based on sparsity-based regularization and spectral clustering techniques. We found that the neuroblastoma cell lines responded to hypoxia with modulation of the gene expression profile involving 300 to 750 genes but only 31 genes were concomitantly upregulated in all cell lines. Gene ontology analysis revealed that similar pathways were modulated by hypoxia in the various cell lines (e.g. glycolysis, angiogensis, apoptosis) despite the fact that different genes were represented in the same pathway. Interestingly, we found that N-myc overexpression (whether achieved by cell engineering or by spontaneous mutations) caused a major change in the response of the cells to hypoxia and let us define N-myc dependent and independent hypoxia signatures. Experiments with neuroblastoma spheroids demonstrate that these myc-dependent changes in hypoxic response alter the influx/recruitment of leukocytes to the hypoxic tumor site and inhibit the macrophage influx. We demonstrated the existence of a crosstalk between N-myc and hypoxia in neuroblastoma cells and we speculate that this crosstalk could be instrumental in favoring the expression of tumor promoting genes accounting the poor prognosis of N-myc overexpressing neuroblastoma tumors.

PP31
EPHA2 AND EFNA1: OVEREXPRESSION IN HUMAN OSTEOSARCOMA AND POTENTIAL ROLE IN ONCOGENIC SIGNAL TRANSDUCTION

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<u>Aims:</u> The interaction between the EphA2 receptor and its ligand EFNA1 plays a role in tumor growth and metastasis. Both antigens are found to be upregulated in different carcinomas. Osteosarcomas are a common tumor type in children with a high metastatic potential. We used genome-wide microarray analysis to identify genes differentially expressed between normal bone and

osteosarcomas and found up-regulation of EFNA1 and EphA2 in osteosarcoma tissue.

<u>Methods:</u> Expression of EFNA1 and EphA2 in malignant human osteogenic tissues was analyzed by immunohistochemistry. A tissue microarray of non-tumor bone samples served as control. Signal transduction following EphA2 activation by the ligand was studied in the human osteoblast cell line HOBc and several osteosarcoma cell lines using EFNA1/Fc soluble ligand.

<u>Results:</u> The study verified the upregulation of EFNA1 and EphA2 in human osteosarcoma tissue as compared to normal bone. We observed time- and dose-dependent suppression of EphA2 expression via internalisation and degradation in osteosarcoma cell lines through EFNA1/Fc. EphA2 and EFNA1 are targets of the Mek/Erk pathway and stimulation of EphA2 by EFNA1/Fc leads to an activation of Raf, Mek and Erk. This stimulation resulted in an activation of the transcription factors Elk1 and cJun. MTT-assays showed significantly higher proliferation of high EphA2 expressing MNNG/HOS cells after ligand-treatment.

<u>Conclusion:</u> Our results show that EphA2 and EFNA1 are overexpressed in osteosarcomas suggesting a role of EphA2 dependent signalling during osteogenic tumorigenesis. We suggest that a possible positive feedback loop regulates EphA2-expression and activation through the activation of Raf/Mek/Erk activity.

PP32
INSULIN-INDUCED LEPTIN EXPRESSION IN BREAST CANCER CELLS: ROLE OF SP-1 AND HIF-1ALFA

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Pathologic conditions associated with hyperinsulinemia, such as obesity, metabolic syndrome and diabetes, appear to increase the risk of breast cancer. Here we studied molecular mechanisms by which insulin activates the expression of leptin-obesity hormone that has been shown to promote breast cancer progression in an autocrine or paracrine way. Using MDA-MB-231 breast cancer cells, we found that 1) insulin stimulated leptin mRNA and protein expression, which was associated with increased activation of the leptin gene promoter; 2) ins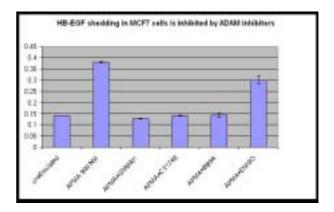ulin increased nuclear accumulation of transcription factors HIF-1 and Sp-1 and their loading on the leptin promoter; 3) siRNA-mediated knockdown of either HIF-1 or Sp-1 significantly downregulated insulin-induced leptin mRNA and protein expression; 4) inhibition of ERK1/2 and PI-3K pathways decreased insulin-dependent leptin expression, which coincided with reduced association of HIF-1 and/or Sp-1 with specific leptin promoter regions. In summary, our data suggest that hyperinsulinemia could induce breast cancer progression through leptin-dependent mechanisms. This process is regulated by the PI-3K and ERK-1/2 pathways and requires Sp-1- and HIF-1 mediated leptin gene transcription.

PP33
THE ROLE OF ADAM PROTEINASES IN SHEDDING OF EGFR LIGANDS

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A Disintegrin and Metalloproteases (ADAMs) are transmembrane proteases able to proteolytically cleave extracellular domains of several growth factors, receptors and cytokines, a process called shedding. Recently several ADAMs are described to play a key role in EGFR signaling by the shedding of EGFR ligands, including HB-EGF, an important EGFR ligand in many malignancies. Since the EGFR pathway plays a major role in development and many diseases including cancer, elucidating the regulatory mechanisms of EGFR ligand shedding is important.

In this study the levels of expression of ADAM 8,9,10 and 17 are measured in several cancer cells in vitro, including LNCaP and PC3 cells. Expression is quantified at the mRNA level by RT-PCR and at the protein level by Western Blotting. In the more advanced cancer cells significantly higher levels of ADAMs expression are detected.

Furthermore, with the use of specific synthetic and physiological ADAM inhibitors the role of ADAMs in HB-EGF shedding is demonstrated in several cancer cell lines. The specific contribution of ADAM10 and 17 on HB-EGF shedding is discussed. SiRNA studies indicate that APMA stimulated HB-EGF shedding is mainly mediated by ADAM17 and ionomycin stimulated HB-EGF shedding by ADAM10.

In conclusion, this study provides insight into mediators of EGFR ligand regulation in vitro: it demonstrates that aberrant EGFR ligand shedding may be caused by the elevated expression of sheddases and discusses activation of different sheddases by distinct stimuli.

Parallel session 7: Translational cancer research (1)

PP35
EXPRESSION OF THE MIRNA CLUSTER MIR-17-92 IS ASSOCIATED WITH DNA COPY NUMBER GAIN OF 13Q DURING COLORECTAL ADENOMA TO CARCINOMA PROGRESSION

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A novel class of non-coding RNAs called microRNAs have shown to regulate central mechanisms of tumorigenesis and contribute to tumour development as tumour suppressors or oncogenes due to altered expression. We have documented that in colorectal chromosomal instable tumours, the combination of gain of 8q and 13q is a major factor associated with colorectal adenoma to carcinoma progression. Functional studies on the mir-17-92 cluster localized on 13q31 have demonstrated that its transcription is activated by the transcription factor c-myc, located on 8q and has antiapoptotic, proliferative and angiogenic activities. These results show an oncogenic function of this miRNA cluster. Therefore, we propose to investigate the contribution of the hsa-mir-17-92 cluster in the tumour biology of colorectal cancer (CRC).

RNA expression levels of the mir-17-92 cluster was determined in 53 colorectal tumours by quantitative RT-PCR. Identification of target genes was accomplished by integrating array comparative genomic hybridization,

mRNA and miRNA expression data from the same tumours and miRNA target prediction programs.

The mir-17-92 cluster shows increased expression in tumours showing 13q gain (p<0.005). Five putative target genes, one of which has known tumour suppressor activity have been identified.

The oncomir mir-17-92 cluster seems to play a role in CRC progression

PP36
FOXP3 EXPRESSION IN HUM CANCER CELLS

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Background and Aims: Transcription factor forkhead box protein 3 (Foxp3) specifically characterizes the thymically derived naturally occurring regulatory T cells (Tregs). Limited evidence indicates that it is also expressed, albeit to a lesser extent, in tissues other than thymus and spleen, while, very recently, it was shown that Foxp3 is expressed by pancreatic carcinoma. This study was scheduled to investigate whether expression of Foxp3 transcripts and mature protein occurs constitutively in various tumor types.

Methods: Twenty five tumor cell lines of different tissue origins (lung cancer, colon cancer, breast cancer, melanoma, erythroid leukemia, acute T-cell leukemia) were studied. Detection of Foxp3 mRNA was performed using both conventional RT-PCR and quantitative real-time PCR while protein expression was assessed by immunocytochemistry and flow cytometry, using different antibody clones.

Results: Foxp3 mRNA as well as Foxp3 protein was detected in all tumor cell lines, albeit in variable levels, not related to the tissue of origin. Irrespective of the level of initial expression, culture in the presence of epigenetic drugs did not alter the levels of Foxp3 transcript. We offer evidence that Foxp3 expression, not epigenetically regulated, characterizes tumor cells of various tissue origin.

Conclusions: The biological significance of these findings warrants further investigation in the context of tumor immune escape, and especially under the light of current anti-cancer efforts interfering with Foxp3 expression.

PP37
GENOMIC ALTERATIONS IN COLEORECTAL CENCER IN RELATIONSHIP TO STAGE AND SURVIVAL ASSESED BY TILING BAC ARRAY CGH

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The aim of this study was to identify early and late DNA alterations significant for colorectal cancer progression by genome-wide array CGH.

Tissue specimens from patients with either short (<1 year, n=13) or long-term survival (>10 years, n=19), as well as from patients with Dukes A, B, C or D tumor stages (n=8 in each group) were analyzed by array CGH with 32K tiling BAC arrays. DNA samples from each patient group were pooled and hybridized to each other and to reference DNA. Mathematical vector analysis was performed on results from short and long-term survivors. Tumor alterations in Dukes (A + B) and Dukes (C + D), were considered related to tumor appearance and progression, respectively.

Tumor DNA contained 600-4500 copy number alterations when related to different patient subgroups. Overall, copy number gains were significantly more common than losses in colorectal tumor tissue from all clinical (short - long survival) or histopathological (Dukes A-D) groups (p<0.05) and major DNA alterations were found on chromosomes 8, 13, 18 and 20. Alterations specifically related to short survival included gain of 8q and loss of 8p. Copy number gains related to tumor progression (Dukes C + D) were most common on chromosomes 7, 8, 19 and 20, while corresponding major losses appeared in chromosome 8. Losses at chromosome 18 were found in all Dukes stages and thus probably related to carcinogenesis. Among the 20 most extreme BAC-clones, identified by vector analysis, were RP11-739G15 at 8q24.21 recently reported by others to be associated to colorectal cancer susceptibility.

The genomic variation in colorectal cancer cells is tremendous and emphasizes that available analytical tools are more powerful than subsequent statistical models for identification of events related to outcome. However, alterations in chromosomes 7, 8, 19 and 20 were evident and are most likely to harbor genetic events contributing to clinical progression of colorectal cancer.

PP38
GATA4 AND GATA5 ACT AS TUMOR SUPPRESSORS AND ARE POTENTIAL BIOMARKERS IN COLORECTAL CANCER

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Background: The transcription factors GATA4 and GATA5 are involved in gastrointestinal development and are inactivated by promoter hypermethylation in colorectal cancer (CRC). Here, we evaluated GATA4/5 promoter methylation as potential biomarkers for non-invasive CRC detection, and investigated the role of GATA4 and GATA5 in CRC.

Methods: Methylation of GATA4/5 was analyzed in colorectal tissue and fecal DNA from CRC patients and healthy controls using methylation-specific PCR (MSP). GATA4/5 functioning was studied by inducing GATA4/5 overexpression in human colorectal cancer cell lines and analyzing colony formation, cell proliferation, migration, invasion and anchorage-independent growth.

Results: GATA4 and GATA5 methylation was observed in 70% (63/90) and 79% (61/77) of CRCs, respectively, and was independent of clinicopathological features. This was confirmed in another independent patient series. Methylation frequencies in normal colon tissues from non-cancerous controls were 6% (5/88, GATA4, p<0.001) and 13% (13/100, GATA5, p<0.001). Examination of GATA4 methylation in fecal DNA from

an independent series of CRC patients (n=21) and controls (n=67) yielded a sensitivity of 67% (95% confidence interval [CI] = 47%-87%) and a specificity of 99% (95% CI = 96%-100%). GATA4 or GATA5 overexpression suppressed colony formation (p<0.005), proliferation (p<0.001), migration (p<0.05), invasion (p<0.05) and anchorage-independent growth (p<0.0001) of CRC cells.

Conclusions: GATA4 and GATA5 exhibit tumor suppressive effects in colorectal cancer cells in vitro. Methylation of GATA4 and GATA5 is a common and specific event in colorectal carcinomas, and GATA4 methylation in fecal DNA as a single methylation marker is a sensitive and specific biomarker for colorectal cancer detection.

PP39
EXPRESSION PROFILING OF MOUSE COLON REVEALS PLA2G2A TARGET GENES

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Background: Predisposition to colon cancer is influenced by multiple genetic factors. Linkage analysis of susceptibility to ApcMin-induced intestinal cancer revealed the mouse secretory phospholipase A2 group IIA gene Pla2g2a as a candidate gene for susceptibility to cancer of the small and large intestine. Final proof was obtained when the functional AKR-derived Pla2g2a allele was introduced as a transgene onto the B6-ApcMin/+ background (which is a natural Pla2g2a-mutant) and was shown to strongly reduce tumor incidence. Interestingly, unlike most previously identified tumor susceptibility genes, Pla2g2a does not behave like a classical oncogene or tumor suppressor gene.

Aim: We aim to identify the biological function of Pla2g2a in tumor development, expecting that this will shed new light on general molecular mechanisms that modulate colon cancer risk.

Methods: To investigate the genetic pathways underlying Pla2g2a's resistance to tumor development we conducted microarray expression studies (Agilent 4x44K) using gender-matched distal colon tissues obtained from Pla2g2a-transgenic and Pla2g2a-deficient colony littermates. In this way we were able to examine differences in expression profiles in colon that were caused by a single-gene difference that is known to affect cancer risk, i.e. Pla2g2a.

Results: More than 400 transcripts exhibited significant differential expression, including Pla2g2a itself. Our data revealed clusters of significantly altered genes involved in inflammation and microbial defense, cell signaling and cell cycle, transactivation, apoptosis and mitochondrial function, intestinal lipid and energy metabolism, and DNA repair. As such, these data confirm the previously proposed roles of Pla2g2a in anti-bacterial defense, inflammation, eicosanoid generation, clearance of apoptotic cells, and in the Wnt signaling pathway. Moreover, pathway analysis by PLAGE (Pathway Level Analysis of Gene Expression) and GSEA (Gene Set Enrichment Analysis) further substantiated these findings, and revealed a possible link between Wnt-mediated expression of Pla2g2a and the Notch signaling pathway.

Conclusion: Pla2g2a affects several different biological pathways that may influence cancer risk. We propose and discuss three different models: 1. Pla2g2a protects the mucosa from bacterial damage and prevents chronic inflammation; 2.Pla2g2a promotes apoptosis of cancer cells; and 3. Pla2g2a maintains a balance between Wnt, Notch and other key signaling pathways.

Parallel session 8: Translational cancer research (2)

PP40
PRION (PRNP)-BASES ANTISENSE SILENCING INDUCES ASTROCYTOMA CALL DEATH,SUGESSTING A NOVEL THERAPEUTIC APPROACHFOR GLIOMA TREATHMENT

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In this contribution, we have investigated a possible antagonistic mechanism involving two genes of the prion family, prion and doppel, in the context of human astrocytic tumor cells.

The prion gene (PRNP) and its related protein (PrPC) are expressed in the CNS and in almost all other tissues, and it is highly conserved in mammals; although a great deal is known about the role of this protein in the prion diseases, the normal cellular function of PrPC has

remained elusive. The doppel protein Dpl, codified by the PRND gene is a recently discovered paralogue of PRNP, normally expressed only in testis, but ectopically over-expressed in astrocytomas. Additionally, it was reported that in the absence of PrPC, Dpl showed a toxic effect in neuronal and glial cells.

Astrocytomas are fairly common tumors of neuroectodermal origin that typically show a high degree of tumor malignancy. Specific pathological features of astrocytomas comprise a high degree of neoplastic cell proliferation and invasivity within the brain peritumoral tissues.

We have therefore developed a gene-therapy approach through the silencing of PRNP in different astrocytoma cell lines, inducing a significant percentage of cell death. Different transfection and silencing approaches have been compared, by means of siRNA and antisense technologies, using several astrocytoma-derived cell lines (U373-MG, U87-MG, T98G, PRT-HU2, D384-MG and IPDDC-A2) and controls.

Our results suggested that the normal prion protein might have a protective function versus doppel-induced cytotoxicity in glial tumor cells or, alternatively, PrPC per se may function to protect cells from various kinds of internal or environmental stress: consequently, PrPC ablation might activate cell death pathways. Nevertheless, these results suggested a novel gene-interfering approach for astrocytomas, based on the silencing of the PRNP expression in tumor cells.

PP41
A FUZZY-IMMUNE APPROACH USEFUL TO INVESTIGATE CHROMOSOMAL HIGHTROUGHTPUT DATA

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The results of molecular high density data are often differently interpreted depending on the algorithm used. In particular, the elaboration of data coming from Array Comparative Genomic Hybridization (aCGH) platform has been demonstrated to be knotty, mainly because the enormous complexity of the DNA alterations in cancer genomes has been demonstrated to be consistent with a non random patterns of copy number alterations in human cancer.

We verified the possibility to use a new class of algorithm based on Artificial Immune System (AIS) paradigm to elucidate the biological dynamics of cancerous processes using a novel fuzzy rule induction system for data mining (IFRAIS). It was the first time that this algorithm has been used for a biological purpose.

A high-resolution array Comparative Genomic Hybridization based on 2464 BAC clones (HunArray2.0, UCSF Comprehensive Cancer Center Microarray Core), has been carried out in D. Albertson laboratory, as previously described (Fridlyand 2006). We analyze these data with already validated algorithms as J48, Ant Miner and GEP and with IFRAIS with the aim to extrapolate rules classifying breast cancer patients with respect to oestrogen receptor status (ER+ vs ER-) and familiarity (familial vs sporadic). Competitive results have been obtained using IFRAIS with respect to the other 3 methods in terms of accuracy (88,95%) and k-statistic (0,648). Furthermore, a strong correlation of Gene Ontology Terms discovered by the 4 top performing algorithms has been observed. In particular, the attention has to be focused in FER role in ER status determination, while, 3 rules have been extrapolated to define familial breast cancer and 5 to define sporadic. In conclusion, the results showed that AIS based classifier returned results, in terms of accuracy and kappa statistic quite comparable with the ones that characterized best performing tree classifiers. Furthermore, they seem to be consistent from the biological point of view. These results seem to encourage further studies on the employment of such systems in contexts characterized by high dimensional data and complex information distribution.

PP42
APOPTOSIS AND FAS/FASL PATHWAY: ROLE OF FASL FUNCTIONAL POLYMORPHISM IN PROSTATE CANCER DEVELOPMENT

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The Fas/FasL system is one of the major pathways in apoptosis and is important in the regulation of cell proliferation and tumor cell growth. Alterations in Fas ligand expression levels could influence apoptosis induction efficiency. Functional promoter polymorphisms on FASL gene (FASL-844T/C) alter its

transcriptional activity. The role of FASL polymorphisms in prostate cancer has not been studied.

Using the PCR-based restriction fragment-length polymorphism methodology, FASL gene locus -844 genotypes were evaluated in DNA samples from 855 men: 603 prostate cancer patients and 252 healthy controls.

It was found that for FASL-844T/C polymorphism the CC genotype is more frequent in patients with PSA level Öl0 (30.3%), than in PSA>10 (22.5%). Using a recessive model, comparing CC genotype with T carrier genotypes (TT+TC) a statistically significant association was found, with a protection to elevated levels of PSA for patients with CC genotype (OR=0.70; CI: 0.45-0.99).

FASL-844 CC genotype was associated with higher basal expression levels of FasL and this death ligand is expressed in T-cells membrane or in some tumor cells. Assuming that the effect of FASL-844 polymorphism could be associated with a higher expression of FasL in T cell population, it might confer them a more effective apoptosis induction of tumor cells. Consequently, patients with FASL-844 CC genotype, which might have a more effective apoptosis induction by T cell, controlling prostatic cell proliferation, and thus, reducing PSA levels.

According to the results of the present study, FASL-844T/C polymorphism could have influence in PSA levels.

PP43
ANTITUMOR EFFICACY AND MECHANISMS OF ACTION OF NCX 4040 USED AS A SINGLE AGENT OR IN COMBINATION WITH CONVENTIONAL CYTOTOXIC DRUGS

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Objectives: The aim of the present work was to investigate the efficacy and mechanisms of action of the NO-donating aspirin, NCX 4040, in experimental models representative of several tumor histotypes. The potential clinical usefulness of experimental results was further evaluated in in vivo experimental colon cancer models.

Methods: Established tumor cell lines. The activity and molecular mechanisms of NCX 4040 were evaluated on a panel of human colon (LoVo, LoVo Dx, WiDr, LRWZ), bladder (HT1376, MCR), and pancreatic (Capan-2, MIA PaCa-2, T3M4) cancer cell lines. Single and combination drug activity was tested by sulforhodamine B assay. The apoptotic process was investigated by several techniques including TUNEL analysis, fluorescence microscopy, mitochondrial membrane potential ( ) analysis, and western blot. Gene silencing experiments were also performed using siRNA technique.

In vivo studies on xenografted mice. Tumor-bearing mice xenografted with the colon cell lines used for in vitro experiments were treated with NCX 4040 alone or in combination with 5-fluorouracil (5-FU) or oxaliplatin (OXA).

Results: The in vitro studies showed a higher cytotoxic activity of NCX 4040 than that produced by other commercial NO-donors in all cell lines derived from different tumor histotypes. Furthermore, the early onset of 8-hydroxyguanine lesions, indicative of oxidative stress, and the lack of cytotoxic activity of the denitrated NCX 4040 analogue, NCX 4042, underlined the pivotal role of NO moiety in drug activity. Our results also highlighted that NCX 4040 exerts a proapoptotic effect through a mitochondrial-dependent pathway. In particular, in the most sensitive colon cancer cell lines, NCX 4040 induced a cytocidal effect and apoptosis through p53 and NAG-1 expression, early   m collapse followed by cytoplasmatic release of cytochrome c, and caspase -9 and -3 activation. Conversely, in apoptosis-resistant cell lines, our results suggest a determinant role of COX-2 activity in resistance to programmed cell death. The data observed for colon cancer cell lines are similar to those obtained in bladder and pancreatic cell lines.

The in vivo studies on xenografted mice confirmed the antitumor efficacy and limited side-effects of NCX 4040 on colon cancer, and also highlighted its role as a sensitizing agent to OXA cytotoxicity.