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Oral presentations

O.01
THE HUMAN GENOME AND GENOMICS: WHERE DO WE STAND?

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The Human Genome Project, the mapping of our 30.000–50.000 genes and the sequencing of all of our DNA, will have major impact on biomedical research and the whole of therapeutic and preventive health care. The tracing of genetic diseases to their molecular causes is rapidly expanding diagnostic and preventive options. The increased insights into molecular pathways, gained from high throughput 'functional genomics', using DNA- and protein-chip approaches and specially-designed animal model systems, will open great perspective for pharmacological and genetic therapies. Powerful bioinformatics and biostatistics will further improve our pattern recognition and accelerate progress. A rapidly expanding area of high expectations is that of 'pharmacogenomics': The design of more effective drugs with lower toxicity, through tailoring of drug treatment to individual, genetically-determined differences in drug metabolism. Not only will this reduce the cost of health care through decrease of adverse drug reactions, but a better stratification of populations will also provide more statistical power more upstream in drug trials. However, the optimal benefits from the current explosion of 'data mining' will only be realised when the basic data are made and kept publicly accessible, while at the same time safeguarding the protection of intellectual property arising from downstream inventions. This is one of the goals of HUGO, the international Human Genome Organisation, established 15 years ago to assist coordinating data acquisition and exchange and societal implementation of the genome project. Additional points of attention in this historic endeavour are the prevention of stigmatisation and discrimination and the safeguarding of a worldwide balance in the contribution by – and benefits to – different populations, while respecting the diversity in cultures and traditions.

O.02
THE GENOMIC REVOLUTION IN CELLULAR PATHOLOGY

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(No abstract available.)

O.03
GENOMICS IN MOLECULAR PATHOLOGY

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Genetic instability is a hallmark of cancer cells and the resulting chromosomal aberrations and gene mutations are key events in the initiation and progression of cancer and can be detected in virtually all tumors. This has been firmly established by the complementary analysis of solid tumor genomes using spectral karyotyping (SKY) and comparative genomic hybridization (CGH). For instance, invasive cervical carcinomas almost invariably carry extra copies of chromosome 3q. To visualize genomic alterations in diagnostics samples, we have developed multicolor fluorescent probe sets that allow for the detection of gene amplification and chromosomal imbalances that are specific for cervical carcinomas. The probe cocktail simultaneously highlights the centromere of chromosomes 3 and 7, and the RNA component of human telomerase gene, which maps to chromosome 3q26. Applied to conventional or monolayer PAP smears, we could show that specific genomic imbalances are rarely detected in CIN1 lesions (6% of samples were positive), but begin to appear in CIN2 lesions (57%), and are frequently present in lesions diagnosed as CIN3 (76%). In order to explore the predictive potential of our diagnostic approach, we hybridized the probe cocktail to previously stained, normal PAP-smears from women who developed invasive cancer after only 2–3 years. In four of 12 cases we unambiguously detected extra copy numbers of the TERC gene on chromosome 3q26 already in the precancerous PAP-smears, therefore predicting the emergence of invasive disease. Our results suggest that the visualization of specific genetic aberrations in directly in

PAP-smears could reduce the disturbingly high rate of false negative diagnoses in cervical cytology. In addition to improved diagnosis and prognosis, the analysis of complex changes of tumor transcriptomes might assist in the prediction of therapy response: we have therefore explored whether parallel analysis of gene expression profiles would predict the response of rectal carcinomas to radiochemotherapy. Our results suggest that pretherapeutic prediction of radiochemosensitivity is indeed possible.

O.04
FRAGILE SITES, EVOLUTIONARY BREAKPOINTS, ENZYMATIC DNA CLEAVAGE AND DNA REPAIR – WHAT WE KNOW ABOUT THE MECHANISMS OF CHROMOSOMAL TRANSLOCATIONS

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Chromosomal aberrations have been identified in virtually all tumors and reflect genomic changes that contribute to the transformation of normal cells into tumor cells. Using screening techniques, such as chromosome banding analysis, comparative genomic hybridization (CGH) and spectral karyotyping (SKY), chromosomal aberrations specific to many different tumors and their respective developmental stages have been detected. The data support the notion that epithelial cancers predominantly show gains and losses of distinct chromosomal regions reflecting copy number changes of oncogenes and tumor suppressor genes. In contrast, recurrent chromosomal translocations are the primary aberrations found in leukemias, lymphomas and sarcomas. The principal biological mechanisms causing either reciprocal translocations or chromosomal gains and losses have not been identified, yet.

The highly recurrent nature of tumor-(tissue/lineage)-specific chromosomal translocations in leukemias, lymphomas and sarcomas supports our hypothesis, that the occurrence of DNA breaks at specific sites followed by non-homologous break repair reflects a targeted process rather than a random event. We suggest that the formation of specific chromosomal translocations might be initiated or influenced by:

- Increased expression of Fragile Sites based on DNA replication errors that might result in DNA double-strand breaks and chromosomal translocations.
- Integration of viral sequences may trigger DNA double-strand breaks at specific sites followed by non-homologous recombination.
- Specific motifs in the DNA sequence, e.g., cleavage sites for topoisomerase II, hypersensitive sites for DNase I or interstitial telomeric repeat sequences (TTAGGG)n, might give rise to breakage during tissue/lineage specific DNA-transcription and during DNA replication.
- Intragenomic heterogeneity in chromatin structure involving histones, i.e. histone acetylation, other associated protein binding sites, the attachment of DNA loops to the nuclear matrix and DNA-folding mechanisms provide links to targeted chromosome fragility in conjunction with tissue specific gene expression and origins of replication.
- Repair mechanisms of double strand breaks via DNA end-joining between DNA ends that have non-homologous sequences or very short regions of homology may fail when the repair occurs between different DNA molecules.

O.05
UNCOUPLING OF MISMATCH CORRECTION AND DNA DAMAGE RESPONSE FUNCTIONS OF THE DNA MISMATCH REPAIR MACHINERY

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A substantial portion of tumors in humans shows microsatellite instability (MSI), i.e., alterations of the length of mono- or dinucleotide repeat sequences in DNA. MSI was discovered in hereditary non-polyposis colon cancer (HNPCC) and found to result from defects in the cells' DNA mismatch-repair (MMR) system. Loss of MMR leaves DNA replication errors unnoticed, strongly accelerating mutagenesis and hence development of cancer. MMR activity is also crucial for the toxicity of several DNA damaging drugs. E.g., cells lacking the central MMR protein MSH2 were found to tolerate high doses of methylating drugs like N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), Streptozocin and Temozolomide (the active metabolite of Dacarbazin), and 6-thioguanine (used in treatment of leukemias). In addition, some studies reported MMR-deficient cells to be moderately tolerant to busulfan, etoposide, doxorubicin and cisplatin. Thus, the role of MMR in the cellular response to DNA

damaging agents may have significance for both the etiology and treatment of cancer. However, by generating a conditionally MMR-deficient mouse embryonic stem (ES) cell line in which the Msh2 gene could be *de novo* inactivated and reactivated, we found no effect of MSH2 activity on response to cisplatin. Previous suggestions on the clinical significance of MMR for the outcome of chemotherapy should therefore be taken with caution.

Nevertheless, MMR was unambiguously shown to mediate the cellular response to methylating agents. We found that the threshold level of MSH2 protein to execute this function is higher than for correction of replication errors. Thus, ES cells expressing MSH2 at only ten percent of wild-type level, were almost fully capable to suppress spontaneous mutagenesis, however they had, similar to fully MSH2-deficient cells, lost the sensitivity to methylation-damage-induced cell death and were highly susceptible to mutation induction by methylating and ethylating agents.

By generating mice expressing a low level of MSH2 in all tissues we are investigating to which extent the enhanced spontaneous mutation rate and the altered response to environmental agents contribute to oncogenic transformation of MMR-deficient cells.

O.06
EXPRESSION AND GENOMIC PROFILING OF HEREDITARY COLORECTAL CANCER

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The annual worldwide incidence of colorectal cancer (CRC) is approximately 950,000, 10–15% of which is estimated to be hereditary. The two main hereditary CRC syndromes, familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC), have been extensively characterized. However, a large proportion of the hereditary CRC cases have not been elucidated yet and new molecular approaches are needed to pinpoint the responsible genes in these families. In order to improve the classification of familial CRC, we are analyzing hereditary colorectal tumors by expression and genomic profiling to define syndrome-specific signatures. To this aim, large numbers of tumors from known and yet uncharacterized CRC familial syndromes are first processed by Laser Capture Microdissection to isolate the nucleic acids from parenchymal cells. Subsequently, the isolated and amplified RNA and DNA samples are analyzed by cDNA and BAC-CGH microarrays, respectively. The use of multiple polyps from individual FAP patients allows the powerful analysis of large number of tumors arising from identical APC germline mutations in the same genetic background and exposed to the same environmental modifiers. This large collection of data sets is stratified by size, histology and anatomical location. The same approach is also being applied to HNPCC tumors and to others from additional patients where mutations in the canonical FAP and HNPCC genes could not be identified or where novel candidate genes (e.g., MYH) have been found to be mutated. Unsupervised hierarchical clustering of the expression data indicate that polyps from FAP patients show APC-specific profiles, different, for example, from those observed in polyps from MYH patients. Also, preliminary BAC-CGH analysis of the same polyps seems to suggest the existence of patient-specific genomic profiles.

The comparison of the expression and the genomic data sets will allow us to pinpoint new genes responsible for the yet uncharacterised familial CRC syndromes and provide further insights into the molecular mechanisms underlying CRC initiation and progression.

O.07
PRB2/P130 TARGET GENES IN HUMAN LUNG CANCER CELLS IDENTIFIED BY MICROARRAY ANALYSIS

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The final deciphering of the complete human genome, together with the improvement of high throughput technologies, is causing a fundamental transformation in cancer research. DNA microarray is a new powerful tool for studying the molecular basis of interactions on a scale that is impossible using conventional analysis. Here we describe a study using oligonucleotide microarrays to identify potential pRb2/p130 target genes in a human lung cancer cell line (H23) overexpressing the human RB2/p130 gene.

Lung cancer is the leading cause of death by cancer worldwide and one of the most common malignancies diagnosed in the United States. During the past 6 years the involvement of pRb2/p130 in lung cancer has

been studied. Previous data has demonstrated an independent role of the reduction or loss of pRb2/p130 expression in the formation and/or progression of lung carcinoma. In this the present study we infected H23 cells with adenovirus carrying the RB2/p130 gene (Ad-CMV-RB2/p130) or adenovirus alone (Ad-CMV). The efficiency of transduction was validated by FACS, Northern and Western blot analyses. pRb2/p130 overexpression resulted in 81% of the cells accumulating in the G0/G1 phase of the cell cycle when compared to the empty adenovirus (54%). The microarray experiments were performed comparing H23 vs. H23-Ad-CMV; H23-Ad-CMV vs. H23-Ad-CMV-RB2/p130; and H23 vs. H23-Ad-CMV-RB2/p130 cells. We identified 40 genes that were down-regulated more than 2.0-fold by pRb2/p130 adenovirus-mediated overexpression. These genes are primarily involved in the following: cell division, signaling and communication, cell structure and motility, gene expression, metabolism, and disease. The modulation of 11 randomly selected genes was confirmed by semi-quantitative RT-PCR and Western blot analyses.

In conclusion, this study identifies a cluster of genes that are modulated by pRb2/p130 expression. Although some of these genes could not be the target of pRb2/p130 at its physiological level, most of them could potentially mediate new therapeutic effects of RB2/p130 in lung cancer.

O.08
SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) AND CANCER

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SEQUENOM has completed whole genome SNP scans in melanoma, breast cancer, prostate cancer and lung cancer. In each study, affected populations and matched Caucasian controls were compared. Each gender-specific cohort numbered about 300 persons. DNA samples from individuals were pooled, and at least 28,000 gene-associated SNPs were compared between cases and controls. The SNPs cover about half of all known human genes. Statistically significant associations were repeated in triplicate. In a typical study about 100 SNPs show statistically significant reproducible association in pools. Half or more of these were still significant at the level of individual genotypes.

The SNPs positively associated in individual genotyping are studied further. Since the studies are performed blind, we can ask, as a positive control, whether genes known, from prior studies to be cancer associated were rediscovered in our SNP scans. A number of such proof of concept genes were found including the B-RAF kinase in malignant melanoma, and a known loss of heterozygosity region in lung cancer. The gold standard, that a SNP is truly associated to cancer, is replication in multiple populations. This has been found for a number of the initial hits. Interestingly, some genes found to be significantly associated in one type of cancer were also associated in other types of cancers. For each candidate cancer gene, an analysis of all nearby SNPs is carried out to map the linkage disequilibrium in the region. In some very gene-dense regions, linkage disequilibirum is too extended to make an unambiguous choice of causal gene, but usually this is not the case.

In breast cancer, RNAi methods were used to explore the functional role of several genes with a certain association to cancer. Some RNAi's strongly inhibit cell proliferation, and in one case, inhibit cell migration in a model for metathesis. At least one of the novel breast cancer genes we have discovered acts as an oncogene in transfection studies.

O.09
METHYLATION CHANGES IN CELL BIOLOGY

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We are in an era where the potential exists for deriving comprehensive profiles of DNA alterations characterizing each form of human disease, specially cancer. DNA methylation is the main epigenetic modification in humans. Tumor cells show aberrant methylation of several CpG islands, but global demethylation versus the counterpart normal cells. Our results show that CpG island promoter hypermethylation has a tumor-type specific pattern.

Epigenetic silencing affects all cellular pathways: from DNA repair (hMLH1, MGMT, BRCA1) to cell cycle (p16INK4a, p14ARF, p15INK4b, p73). Promoter hypermethylation of particular genes have important consequences for the biology of that particular tumor. This is, for example, the case of the DNA repair gene MGMT which methylation-mediated silencing leads to transition mutations, but, at the same time, 'marks' chemosensitivity. Those genes inactivated by epigenetic mechanisms are not only silenced and hypermethylated in sporadic cases, but also hypermethy-

lation can be observed in hereditary tumors as a 'second hit'. We have also developed massive genomic screenings to find new hypermethylated genes in cancer cell. From these assays we have identified new candidate tumor suppressor genes with important potential roles in the pathogenesis of human cancer.

It is also widely accepted that methyl-CpG binding proteins (MBDs) couple DNA methylation to gene silencing through the recruitment of histone deacetylase and chromatin remodelling activities that modify chromatin structure. Chromatin Immunoprecipitation (ChIP) assays and restriction nuclease accessibility analysis demonstrate how the vast majority of tumor suppressor genes with CpG island promoter hypermethylation-associated inactivation also present histone hypoacetylation and histone methylation. Furthermore, an exquisite specific profile of different methyl-binding proteins (MBD1, MBD2, MBD3, MeCP2) exists in function of the gene studied.

Overall, our data demonstrates that human tumors suffer a profound, but specific, disturbance in their DNA methylation and chromatin patterns. Further research is required to understand the epigenetics of cancer.

O.10
MICROARRAYS: PROFILERS OF INSTABILITY AND SELECTION

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The development of solid tumors is associated with the acquisition of genetic and epigenetic alterations and corresponding changes in gene expression that modify normal growth control and survival pathways. It is now generally agreed that in order for a sufficient number of alterations to accumulate to cause a malignancy, one or more of the mechanisms that work to maintain genetic integrity in cells and/or to regulate cell cycle progression must be compromised, presumably through mutations that occur early in tumorigenesis. Genomic DNA copy number aberrations are frequent in solid tumors and are one of the mechanisms expected to contribute to tumor evolution by copy number induced alterations in gene expression. Microarray-based comparative genomic hybridization (array CGH) is revealing a great variation in the spectra of copy number changes in tumors, which are likely to reflect a composite of selection acting on the variation that is permitted to arise by the particular failures in genomic surveillance mechanism(s) present in the tumor. We are investigating the interplay between selection and genetic instability in shaping tumor genomes by analysis of the types of copy number aberrations arising in tumors and in cells following challenge with methotrexate. These analyses distinguish tumor subtypes and indicate that the genetic backgrounds of cells alter the propensity for development of different types of copy number changes (e.g., low level gains or amplifications) in methotrexate resistant cells. The descriptions of aberration spectra in model systems will facilitate interpretation of the more complex copy number profiles of tumors.

O.11
IMAGES OF THE GENOMIC EVOLUTION OF MELANOMA

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Array CGH has provided significant information on the different paths of genomic evolution of benign and malignant melanocytic tumors. The arrays used for this work consist of ∼2500 BAC clones spanning the genome, including clones containing many known cancer genes and unique sequences near telomeres. Arrays are printed using a custom-built printer employing capillary tube printing pins, and imaged with a custom-built CCD system. The imaging system has several advantages compared to the more common laser scanning systems, including the ability to use a wide range of fluorochromes and to properly quantify signals over a wide range of intensities. Genomic analysis of melanocytic tumors shows that genomic aberrations are detectable in essentially all malignant tumors and some benign tumors. However, the locations of the aberrations allow discrimination of the two classes, providing a potential diagnostic for histologically ambiguous lesions. Malignant melanomas occurring on different anatomic sites, which are subject to different amounts and patterns of sun exposure, show significantly different paths of genomic evolution. For example, high-level amplifications are present in all Acral melanomas, which occur on the palms of hands, soles of feet and under the fingernails, loca-

tions that are shielded from the sun. The amplifications are present before the tumors are histologically visible, and thus indicate substantial genomic evolution in cells that appear phenotypically normal by conventional criteria. Conversely, amplifications are rare and occur late in the most common melanomas of people of European decent, which occur on skin subject to intermittent sun exposure. In addition, analysis of BRAF finds that mutations occur with significantly different frequency in different melanoma types. Approximately 50% of the most common melanomas in Europeans contain mutations, but mutations are very rare in Acral melanoms and in Mucosal melanomas, which are on sites subject to low solar exposure, and in melanomas that occur on highly sun exposed skin that has visible sun damage.

O.12
MICRO-ARRAY TECHNOLOGY: WHICH LIBRARIES, ARRAYERS, SLIDES, AND CONDITIONS?

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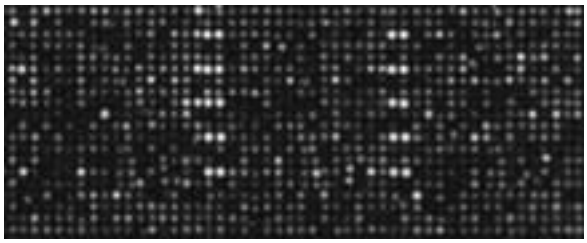
In recent years, the array technology has matured to a stage where data interpretation has become the main bottleneck in microarray throughput. One could thus argue that our focus should shift away from the array technology hardware, hence the Saturday afternoon Bioinformatics and Microarray data analysis sessions. Truth of matter is that even after 10 years of microarrays, there is still a lot to gain in the quality of the arrays. Fine tuning of the array technology hardware and technique yield significant improvements in the ease of data interpretation and throughput. Lowess smoothing and image flattening are examples of bioinformatics 'tricks' that should theoretically not be necessary. Such data treatment to correct artifacts concurrently introduces noise. We therefore argue that all artifacts should be solved within the technique and not by the biostatistician. We will show that miscalled spots, cross-hybridizing spots and missing spots can be effectively dealt with by selecting the right hardware and hybridization conditions. Our final quest is to move from high quality to perfection, and happy biostatisticians, biologist, researchers and clinicians. During this lecture we will present trial and error based ideas, which we believe will lead us to our goal.



Non-contact printed oligo expression array (detail).

O.13
LABELLING AND AMPLIFICATION STRATEGIES IN GENE AND GENE EXPRESSION ANALYSIS

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Labelling of nucleic acids is essential to highly multiplexed genome and transcriptome analysis afforded by micro-arrays. In the great majority of transcriptome analyses, enzymatic cDNA labeling by oligo-dT primed reverse transcription of polyadenylated mRNA is applied. Due to limited processivity of the reverse transcriptase and secondary RNA structure, many of the mRNA species are represented in the labelled target only with their 3′ ends, a bias also reflected in amplified cRNA. Chemical labelling of mRNA based on fluorescent cis-platin derivatives overcomes this 5′-end representation problem as shown by efficient detection of 5′-ends of mRNAs in a micro-array format and opening up a possibility of studying alternative splice variants using oligonucleotide probes that span splice junctions. In array comparative genomic hybridisations cis-platin labelling of genomic DNA performed at least as good as enzymatic labelling.

The multiplexing capacity of micro-arrays is superior to classical FISH. Yet it is important to maximize FISH color multiplicity for detection of structural chromosomal aberrations, particularly those that are not associated with DNA copy number changes as well as for analysis of complex karyotypes. We have developed Combined Binary Ratio (COBRA) FISH to allow discrimination of all human chromosome arms with complex arm-specific paints as well as with PAC/BAC probes for all sub-telomeric regions. In combination with a chemical premature chromosome condensation technique, COBRA-FISH allows karyotyping of G1 and G2 chromosomes with minimal culture artefacts.

Single nucleotide polymorphism detection *in situ* is hampered by low efficiency. In view of their association with a range of diseases mitochondrial DNA mu-

tations and the high copy number, mtDNA constitutes a convenient and pathologically interesting target for *in situ* genotyping. We have applied padlock probes to analyze single-nucleotide variants of mitochondrial genomes (mtDNA) *in situ*. The methodology will enable more direct studiesof heteroplasmy drift of normal and pathogenic mtDNA variants by permitting analysis of the organization, kinetics and mode of segregation of mtDNA variants during mitosis in different cell types.

O.14
VISUALISING MOLECULAR INTERACTIONS IN LIVING CELLS

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Immunocytochemistry and (FISH) are of limited value when applied to living cells. Fortunately, there is the GFP labelling technology that allows for visualisation of proteins in live cells. Specific DNA sequences however, are difficult to detect in live cells, since denaturation is not an option. Single stranded RNA, can be demonstrated using 2′-O-methyl modified oligonucleotides, PNA (peptide nucleic acid) probes or molecular beacons. We obtained optimal hybridisation results by using 2′-O-methyl-oligoribonucleotide probes that are labelled at their 5′ end with a fluorophore. Molecular beacon type of probes revealed specific hybridisation signals for the different target sequences though hybridisation signals were less intense while a considerable amount of 'background' signal was present in cell nuclei. Apparently, the molecular beacon probes, as constructed, opened up in the cell nucleus before reaching their target sequences. PNA probes proved to be very suitable for detecting telomere sequences in living cells under non denaturing conditions. This is an important finding because it will allow studying the dynamics of telomere positioning in the cell nucleus under various experimental conditions and during the cell cycle.

The labelling technology for RNA was combined with GFP constructs allowing the study the dynamics of proteins (labelled by GFP) involved in transcription, and in processing and transport of RNA. To demonstrate functional association of labelled proteins with RNA, spatial (co)localization studies are insufficient. More reliable information is obtained when functional association is assessed by measurements of fluorescence resonance energy transfer (FRET), either on the basis of spectral analysis or by life-time measurements.

As an example, we studied the role of heterochromatin protein 1 (HP 1), considered as a key component of constitutive heterochromatin and a player in gene silencing. GFP-HP-1 protein appeared to accumulate in a number of nuclear subdomains, such as PML bodies. We demonstrated that HP-1 proteins form complexes with SP 100 in PML bodies using CFP and YFP as tags, by measuring their interaction by FRET-FLIM.

O.15
PROGNOSTIC STRATIFICATION IN FOLLICULAR LYMPHOMA BY GENE EXPRESSION PROFILING TO GUIDE THE CHOICE OF TREATMENT

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Follicular lymphoma (FL) is the second most frequent lymphoma in adults. In the Netherlands, approximately 400 new cases of FL are seen yearly. Generally FL has an indolent disease course, but transformation to more aggressive disease is a common event. Diagnosis of the phase of the disease (indolent or aggressive) is imperative to guide the choice of optimal therapy. Currently histological grade is one of the most important parameters that guide the decision to employ aggressive therapy. However, histological grading is rather subjective, poorly reproducible and leaves a large proportion of FL cases as undecided.

To improve the prognostic stratification of FL, gene expression profiling was used for the development of a molecular diagnostic tool to guide treatment choice. In this study 106 biopsy samples of patients with indolent and transformed FL, including the full morphological spectrum of grade 1 to 3 and diffuse large B-cell lymphoma were examined using gene expression profiling. These included a set of paired samples from the indo-

lent and aggressive phases of the disease in 12 patients based on morphology and clinical behavior, an independent validation series of 58 patient samples, as well as 18 patients with ambiguous histological grades.

Using supervised classification, a gene expression profile of 81 genes was established that could, with an accuracy of 100%, distinguish clinically and morphologically indolent from aggressive disease in the training series. This profile accurately classified 93% in the independent validation set, and could accurately predict clinical behavior in 17 of the 18 cases with ambiguous morphological features.

Conclusions: The FL-stratification profile is a more reliable predictor of clinical behavior than the currently used histological grading and IPI scoring systems. It may provide an important aid to guide the choice of therapy in FL patients at presentation and at relapse. The implementation in patient care will direct the use of aggressive therapy at the time it is most effective.

O.16
THE IDENTIFICATION OF COMMON GENETIC ALTERATIONS USING ARRAY CGH TO PREDICT A RESPONSE TO RADIOTHERAPY IN LARYNGEAL CARCINOMAS

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Early stage laryngeal cancer are preferably treated with radiation therapy. Therapeutic options for the more advanced (T3-T4) carcinomas are primary surgery with severe morbidity, or primary radiotherapy with a 50% failure rate. The consequential secondary surgery after failure of radiation therapy is associated with severe morbidity in 15% of cases. Loss of the vocal cords after surgery results in impaired communication and disruption of normal social interaction and respiratory disease in a majority of patients. Radiotherapy is the treatment of choice for advanced laryngeal carcinoma.

Up till now the search for relevant biological markers that predict a response to radiotherapy in laryngeal carcinomas has revealed some candidate genes, but none are of prognostic relevance. Molecular markers that enable accurate prediction of radiotherapeutic effects are yet to be identified.

We are developing a whole genome array CGH, consisting of $\pm$7000 BACs with an average clone interval of $\pm 1/700$ kb. Whole genome array CGH is used to identify amplified genes/markers that are commonly altered in laryngeal carcinomas to radiation therapy.

We will screen for common altered regions with DNA obtained from 30 laryngeal carcinomas. These data will be used to screen for differences between a group of approximately 120 patients that have developed a recurrent tumor after radiotherapy and a group of patients that have no recurrent disease (matched for age tumor stage and smoking habits). For this purpose we use Quantitative Microsatellite Analysis (QuMA) and quantitative RT-PCR (qRT-PCR). We will determine the prognostic value of our results by comparing them with the conventionally used indicators (i.e. staining for cyclin D1, ki-67 and P53).

O.17
INCIDENCE AND CLINICAL RELEVANCE OF RECEPTOR TYROSINE KINASE AND RAS MUTATIONS AS POTENTIAL TREATMENT TARGETS IN PEDIATRIC ACUTE MYELOID LEUKEMIA (AML)

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AML arises from two types of mutations. Type-I mutations mainly induce proliferation, and include mutations in receptor tyrosine kinases (RTK) and the ras oncogene. Type-II mutations (e.g., translocations) result in chimeric transcription factors that cause maturation arrest and increase self-renewal capacity. We retrospectively studied de novo pediatric AML samples for FLT3/internal tandem duplications (FLT3/ITD, $n = 234$), c-kit exon 8 and 17 mutations ($n = 150$) and N-ras exon 1 or 2 mutations ($n = 147$). Patients were treated with intensive chemotherapy. The incidence of FLT3/ITD was 11.5%, of c-kit mutations

11.3% (4.0% exon 8, 7.3% exon 17), and of N-ras mutations 15% (10.9% exon 1, 4.1% exon 2). One patient had both N-ras exon 2 and c-kit exon 8 mutations, therefore we conclude that most mutations are mutually exclusive. The c-kit mutations clustered with specific type-II abnormalities: 69% had a t(8;21) or inv(16), versus 16% in the non-mutated patients ($p < 0.0001$). For FLT3/ITD positive patients the opposite relation was found: 54.5% had a normal karyotype versus 22.3% in ITD negative patients ($p = 0.003$). Only FLT3/ITD was age-dependent: no mutations in infants, 5.1% for 1–10 years and 19.4% for 10–18 years of age. Patients with FLT3/ITD showed a worse prognosis as compared to patients without this mutation: 5-year probability for event free survival (pEFS) 29% vs. 46% ($p = 0.005$). C-Kit mutations did not have prognostic significance. N-ras mutated patients seemed to have a better prognosis than patients without ras mutations: pEFS 68% vs. 41% ($p = 0.06$), with a significantly lower relapse rate of 9% vs. 42% ($p = 0.02$). Multivariate analysis including AML-BFM risk group, white blood cell count and bone marrow transplantation in the first complete remission, showed that FLT3/ITD was the strongest independent unfavorable prognostic variable for EFS ($p = 0.01$, relative risk 1.92, 95% CI: 1.16–3.17). N-ras mutations independently predicted a 5-fold (relative risk 0.2, 95% CI: 0.05–0.81) lower risk of relapse ($p = 0.025$). In conclusion, type-I mutations occur in almost 40% of pediatric AML patients who are therefore candidates for treatment with specifically targeted drugs.

O.18
CHROMATIN PATTERNS AND CANCER PROGNOSIS

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(No abstract available.)

O.19
MICROMIRROR DEVICES IN CELLULAR MICROSCOPY

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With the advent of MEMs such as the Digital Micromirror Device (DMD, Texas Instruments Inc.) precise digital control of light flow within microscopy setups has become possible. This technology enables several forms of microscopy which previously was either very expensive or very difficult to perform practically. One form is a novel optical computed tomography transmission microscope which employs the DMD in the back pupil plane of the objective where it enables digital control over the angles of illumination of the sample. This in combination with a modified 3D Radon transform reconstruction algorithm was used to generate 3D volumetric reconstructions of absorption stained thick sections with a resolution similar to early confocal efforts.

Another form allows one to take a DMD based confocal microscope using precise illumination mapping of the individual fibres of a coherent bundle to visualize epithelial tissue *in vivo*.

O.20
3D NUCLEAR ORGANIZATION OF TELOMERES IN NORMAL AND CANCEROUS MAMMALIAN CELLS

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We present the high resolution 3D imaging and analyses of all the FISH-stained telomeres in mammalian nuclei. By using either confocal microscopy or 3D high-resolution microscopy measurements followed by an adequate 3D analysis that we have developed, we analyzed the space-filling properties of all the telomeres in the nucleus.

The method was applied to normal, cancerous and c-myc disrupted cells. In normal cells we have found that telomeres occupy distinct and non-overlapping telomere territories (TTs), they localize in the middle of the nucleus during G0/G1 and S phases and assemble into a central telomeric disk (TD) during G2 phase. In tumors we have found that telomeres form aggregates and do not align in a telomere disk. 3D telomere order therefore forms an important tool for cancer research and possibly diagnostics.

To analyze the data, a 3D image analysis program was developed. It segments the nucleus volume, counts the spots that are found and for each spot various parameters are calculated (volume, intensity and center of gravity). Then, the 3D distribution is determined by analyzing the shape of the volume occupying the telomeres. We have found that this volume can be described as a spheroid (i.e. an ellipsoid having two axes of equal length, $a = b$ and a third different one c) and it's varia-

tion from a perfect sphere is described by the ratio a/c. This parameter provides objective criteria for analyzing the telomeres distribution and forms the basis for the study and the conclusions mentioned above.

Typical distribution of telomeres in a mouse Pre-B lymphocyte cell line is shown in the figures. Figure 1 shows the distribution of telomeres in a normal cell and figure 2 shows the distribution in a cell upon a transient activation of MycER with 4-hydroxy-tamoxifen and exhibit alterations of its telomere organization. A large aggregate is clearly observed.

We will describe the experimental and analysis methods and discuss about some of our results.

O.21
TEXTURAL ANALYSIS OF CHROMATIN PHENOTYPE IN THE GRADING OF CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN)

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The classification and grading of cervical intraepithelial neoplasia (CIN) is subjective and poorly reproducible. The quantitative analysis of chromatin phenotype by computation of nuclear texture may provide an innovative approach for the objective classification of CIN. A series of cervical colposcopy cases was selected as showing normal stratified epithelium (18), koilocytosis (25), CIN 1 (27), CIN 2 (22) and CIN 3 (28). Multimegapixel images of full thickness epithelium stained with H&E were recorded in full colour at $\times 60$ objective magnification using automated image merging algorithms. A machine vision system was developed for the automated segmentation of nuclei from the images which were stored for morphometric and chromatin texture analysis. High resolution digital texture analysis was carried out on each of the three colour channels (R/G/B) and a total of 96 features computed for each channel on each individual nucleus (5,000 nuclei in each diagnostic group). There was high degree of correlation between colour channels and individual texture features. Analysis of correlation matrices allowed a reduction to 37 independent features. Highly significant differences were observed between chromatin phenotype in nuclei from normal and CIN grades ($p < 0.05$). A discriminant function gave 100% correct classification between normal epithelium and CIN 3, however, it was difficult to define a function which could accurately classify all nuclei into the five predefined groups. This indicates the pathologic continuum that exists and a chromatin phenotype index was defined to map progression in cervical neoplasia. Compartmental analysis of chromatin phenotype within upper, middle and lower regions of the epithelium also revealed differences in chromatin phenotype between the compartments and provided added diagnostic value for mapping progression. Chromatin texture provides a useful means of describing phenotypic progression in CIN. Further work is needed to define morphological/molecular signatures which can be used in routine diagnostic pathology and also for precise phenotypic characterisation in studies on the molecular biology of CIN.

O.22
LOW RESOLUTION IMAGE MATCHING STRATEGY TO ANALYSE SERIAL IMMUNOSTAINED CANCER TISSUE SECTIONS*

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Acquisition of whole histochemically or immunohistochemically stained cancer tissue sections can be achieved using a slide scanner equipped with a medical slide holder [1,2]. Resulting numerical images can then be analysed thanks to fully automatic image analysis routines. This technique eases and speeds up the quantification of tumour tissue, stromal (or sclerosis) compartment, blood vessels as well as any nuclear or cytoplasmic markers. Matching of serial binary images of automatically detected structures is of particular interest for further analysis of cell sociology or vascular/cancer cell interactions. In that perspective, a specific mathematical approach was developed. This fully automatic iterative method allows matching couple of images, but does not require any landmark. First, a list of paired points is computed from outlines of the two images ('reference' and 'test' images) in order to evaluate an initial cost function. The rotation and translation, which must be applied to the test image, result from the minimisation of the cost function.

The method accuracy was tested on a topographic analysis of vascularisation in a series of 230 Hodgkin's lymphoma sections of patients enrolled onto a European Organisation for Research and Treatment of Cancer controlled clinical trial. In this series, the sharing

out of blood vessels between sclerosis and lymphoid compartments was found significantly different, with respects to histological subtypes.

We conclude that the approach developed may be helpful in analysing morphological parameter relationships from serial sections of well-identified tumours.

References

[1] N.T. Kim, N. Elie, B. Plancoulaine, P. Herlin and M. Coster, *Anal. Cell. Pathol.* **25**(2) (2003), 63–75.

[2] C.F. Chantrain, Y.A. DeClerck, S. Groshen and G.J. McNamara, *Histochem. Cytochem.* **51**(2) (2003), 151–158.

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O.23
EPIGENETIC EVENTS AND THEIR RELATIONSHIP TO CHROMATIN ORGANISATION IN PROSTATIC INTRAEPITHELIAL NEOPLASIA AND PROSTATIC ADENOCARCINOMA

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Introduction: DNA methylation and histone acetylation are epigenetic events that attribute to changes in gene expression. Alterations in epigenetics are thought to be associated with higher order chromatin organization. The aim of this study is to detect changes in epigenetic events and nuclear chromatin texture in relation to disease progression in prostatic adenocarcinoma.

Materials and methods: A tissue microarray (TMA) was constructed from paraffin embedded prostatectomy specimens of Benign Prostatic Hyperplasia (BPH) (58), low grade prostatic intraepithelial neoplasia (LGPIN) (12), high gradePIN (HGPIN) (10), and prostatic adenocancenoma (PCa) (41) defined by an experienced uropathologis (RM). Three sequential sections from the TMA were stained with H&E and with anti-acetylated histone lysine 9(AcH3K9) and anti-5′methylcytosine (5Mec). High resolution digital texture analysis was used to measure chromatin phenotype and the density and distribution of 5Mec and AcH3K9 density and distribution within at least 30 nuclei from each TMA core.

Results: Numerous textural measurements for chromatin, 5Mec and AcH3K9 were significantly different between BPH, PIN and PCa. The sum of nuclear chromatin density showed a significant increase in chromatin content in PIN and PCa, compared to BPH, although HGPIN had the highest chromatin density values. Increase in global nuclear density was however, associated with an increase in light density area and moderate/high density objects in cancer nuclei. Evaluation of methylation density showed distinct hypomethylation in PCa. Interestingly, PIN nuclei are hypermethylated and form a unique epigenetic subtype. Conversely, the acetylation of H3K9 shows a monotonic trend, increasing from BPH, through LGPIN, HGPIN to PCa. Discriminant analysis indicated that measurement of methylation and acetylation by quantitative immunocytochemistry may provide stronger biomarkers of malignancy than chromatin phenotype.

Conclusions: Epigenetic markers are strongly associated with changes in chromatin phenotype in prostatic neoplasia and PIN and may be useful biomarkers for diagnosis and prognosis. PIN however forms a distinct epigenetic phenotype which shows highly disorganized chromatin and hypermethylation.

O.24
INTEGRATING MICRO-ARRAY ANALYSES IN CLINICAL MEDICINE

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(No abstract available.)

O.25
EXPRESSION PROFILING CHANGES TREATMENT IN BREAST CANCER

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Microarray gene expression profiling combined with advanced bio-informatics is beginning to show its power in delineating disease entities that are otherwise indistinguishable. This refinement in tumor classification allows a more accurate prediction of outcome of disease for patients that present with the same stage of disease based on conventional clinical and histopathological criteria. Gene activities determining the biolog-

ical behaviour of the tumor may indeed be more likely to reflect the aggressiveness of the tumor than general parameters like tumor size, age of the patient, or even tumor grade. Therefore, the immediate clinical consequences are that treatment schemes can be tailored based on the gene activity patterns of the primary tumor.

We used gene expression profiling with DNA microarrays harboring 25 000 genes on 78 primary breast cancers of young lymph node negative patients to establish a signature, predictive for a short interval to distant metastases. This 'poor prognosis' signature consists of genes involved in cell cycle, invasion and angiogenesis. The prognosis signature is superior to currently available clinical and histo-pathological prognostic factors in predicting outcome of disease (OR $=$ 18 (95%CI 3.3–94), $p < 0.001$, multivariate analysis). At present we have validated our findings of this poor prognosis profile on a large independent series of LN0 as well as LN+ (lymph node positive) young breast cancer patients ($n = 187$). Analysis confirms that the profile is a strong factor in predicting outcome of disease for LN0 patients (OR $=$ 17). Furthermore, the profile is as powerful for LN+ patients (OR $= 12$).

Nowadays, consensus guidelines in the management of breast cancer select up to 90% of lymph node negative young breast cancer patients for adjuvant systemic therapy (e.g., St Gallen). As 70–80% of these patients would have remained disease-free without this adjuvant treatment, these patients are 'overtreated'. Our 'poor prognosis' signature provides a novel strategy to accurately select patients who would benefit from adjuvant systemic therapy and can greatly reduce the number of patients that receive unnecessary treatment.

O.26
CONSTRUCTION OF A SUB-MEGABASE RESOLUTION CGH ARRAY WITH COMPLETE COVERAGE OF THE HUMAN GENOME

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Background: Genomic DNA copy number changes are common in many human diseases including cancer. Detection and mapping of these genetic alterations allows for the rapid identification of candidate genes involved in disease. Array comparative genomic hybridization (aCGH) is a new method for detecting genomic alterations in human cancers. Our approach represents a >410-fold increase in resolution over conventional CGH allowing for genome-wide identification of genetic alterations at a resolution of 100 kbp.

Objectives: This array was designed to provide complete coverage of the human genome at sub-megabase resolution. This will allow the rapid identification of genes important to disease progression, by detecting aberrations that would be missed by conventional technologies.

Design: Utilizing the whole-genome human 32,850 BAC re-array clone set (http://www.bcgsc.ca/lab/mapping/bacrearray/human/index_html/view) we have created a CGH array covering the entire human genome at 0.1 megabase resolution. Fingerprinting of each BAC clone post DNA preparation was employed to verify clone identity and quality of preparation. We utilized a linker-mediated PCR approach to create high concentration representations of our BAC clone set suitable for spotting on slides. This approach begins with restriction digestion of an individual BAC, followed by ligation of PCR primers to digestion products. Two subsequent rounds of PCR yielded DNA at 1 μg/μl concentration. Amplified DNA was precipitated and re-dissolved then spotted in triplicate. A subset of the PCR products were verified by sequence analysis using BAC vector based primers for quality control prior to array printing.

Results: Profiling of cancer cell lines has resulted in the identification of breakpoints to within 100 kbp in single experiments. Additionally we have identified novel micro amplifications which have been missed by conventional screening technologies and represent genes potentially relevant to disease progression.

Conclusions: High resolution whole genome aCGH profiling will allow the rapid identification of aberrations important to disease progression from minimal amount of sample by reporting quantitative copy number information for 32,580 genomic loci in a single experiment.

O.27
KIT MUTATIONAL STATUS IN PREDICTING CLINICAL RESPONSE TO IMATINIB MESYLATE IN ADVANCED GASTROINTESTINAL TUMORS

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Activating mutations of KIT tyrosine kinases are critical in the pathogenesis of gastrointestinal stromal

tumors (GISTs). Imatinib mesylate (former STI571; Glivec) is a potent inhibitor of KIT kinase activity and has been proven highly active in patients with unresectable or metastatic GISTs expressing immunohistochemically detectable KIT protein. Preliminary clinical observations linked the response to the presence of KIT mutations in the tumor with the risk of progression during imatinib treatment being higher in patients without detectable KIT mutations. KIT mutations in GISTs differ in their form and in the involved protein domains. The results from the multicentre trials in GIST on the efficacy and safety of imatinib confirmed that the likelihood of a clinical response to the drug correlated with KIT mutational status. Patients with a GIST harbouring an exon 11 KIT mutation had a significantly higher partial response rate than patients whose tumor had an exon 9 or no detectable KIT mutation. In addition, patients with GISTs expressing exon 11 KIT isoforms had longer median survival time and were less likely to progress than other patients. These findings indicate that mutational status of KIT oncoprotein can be useful for prediction of clinical response to imatinib.

O.28
DIAGNOSTIC ASPECTS OF CD117/KIT

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(No abstract available.)

O.29
IMATINIB IN THE TREATMENT OF GASTROINTESTINAL STROMAL TUMOUR

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New drug development in cancer is now focused on molecular targeted therapies. The pathogenesis of common solid tumours is believed to involve multiple genetic events occurring over a period of years that confer a progressively more malignant phenotype. In contrast, certain haematological and mesenchymal malignancies appear largely to be driven by single genetic events. Gastrointestinal stromal tumours (GIST) were characterised in 1998 as arising from a progenitor cell common to the interstitial cells of Cajal cells that coordinate peristalsis in the gut. It was also shown that they were usually associated with activating mutations in the gene coding for the receptor tyrosine kinase KIT (CD117). Chronic myeloid leukaemia (CML) has been known for a number of years to be driven by the t(9;22) translocation that results in the characteristic Philadelphia chromosome and creates the BCR-ABL fusion protein, thus activating the ABL tyrosine kinase. KIT and ABL are both inhibited by imatinib (Glivec), and the drug is an extremely effective treatment of these diseases. In GIST, Phase II studies have reported response rates of 55–71%, with most patients experiencing clinical benefit. Prior to the advent of imatinib there was no effective treatment for patients with unresectable or metastatic disease. In a randomised trial conducted by EORTC comparing 400 mg v 800 mg daily the response rates were identical but progression free survival was superior for 800 mg. Activating KIT mutations are a feature of very early disease and yet remain important even when the disease is advanced and metastatic. Where KIT is wild type, activating mutations have been reported in PDGFR-A. Resistance can occur and the mechanisms for this need to be elucidated. Imatinib represents a major advance in the treatment of GIST and has become a paradigm for the effective targeting of a disease-specific molecular abnormality in cancer therapy.

O.30
NEUROGENOMICS: FROM GENE TO PROTEIN

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Over the last years considerable progress has been made in understanding neuronal function using genomics approaches in the brain of humans and animals using gene expression profiling as well as proteomic approaches. I will exemplify some of the progress that has been made in the field using microarray technology and real-time PCR which addresses the molecular basis of synaptic function, neuronal outgrowth and brain diseases such as addiction and schizophrenia. In particular, I will focus on the progress made in the field of proteomics where the rapid methodological innovation has opened new avenues to identify proteins at high throughput, to characterize their posttranslational modifications and to visualize the dynamics of protein assemblies. I will show most recent data on the identification of the full complement of synaptic proteins involved synaptic transmission and signal transduction and briefly highlight some of the future perspectives.

O.31
SYNAPTIC CONNECTIVITY AND NEURODEGENERATION: A FUNCTIONAL GENOMICS APPROACH

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A final common pathway of neurodegenerative diseases is the loss of synaptic connectivity, preceding the loss of the neurons themselves. We tested the role of use-depedent changes in synaptic conenctivity in relation to neurodegeneration using a number of genetic models that alter the natural communication among neurons.

By *in vivo* gene inactivation using homologous recombination in murine stem cells, we identified several presynaptic genes as essential components of the secretion machinery. The most drastic phenotype was observed upon deletion of the expression of *munc18-1* (see Verhage et al., *Science* 287: 864–869). Null mutantion of this gene leads to a complete abolishment of synaptic communication, also of spontaneous activity (mini's). The null mutants are not viable and die shortly after birth. Histological and ultrastructural analysis revealed massive neurodegeneration, especially in those brain areas known to generate synapses first. To identify the molecular cascades of pathogenesis, we screened for changes in gene expression at timepoints where neurodegeneration was not yet apparent using DNA-array hybridization. We detected expression of 5500 genes at embryonic day (E) 14 neocortex of wildtype and mutant mice and 6000 at E18. We observed significant changes in expression for a small number of these genes ($<$10%), which could be grouped into a small number of categories. Most notably was a reduced expression of most neuropeptide genes and the neurotrophic factor like BDNF, but not their receptors and other components of synaptic transmission. We tested these factors in cultured neurons from the munc18-1 null mutants, but observed only a mild protective effect of insulin and BDNF. Increased trophic support in organotypic cultures of two brain slices, one from wildtype and one from the null mutant delayed neurodegeneration further, but did not prevent it. Finally, we generated a conditional allelle for the munc18-1 gene and deleted expression *in vivo* using Cre-recombinase in specific populations of neurons. We found that the neurons degenerated, without affecting their targets and upstream connections. We conclude that neurodegeneration in the absence of synaptic communication in the munc18-1 null mutant is mainly a cell-autonomous process that can be delayed but not prevented by neurotrofic support and that reduced neuropeptide expression is probably a downstream phenomenon related to the reduced activity in the network.

O.32
GENE EXPRESSION PROFILING OF FIBROBLAST-LIKE SYNOVIOCYTES FROM RHEUMATOID ARTHRITIS PATIENTS

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Rheumatoid arthritis is diverse in its clinical presentation and in the responsiveness to treatment. Previously we observed variation in transcriptional programs between synovial tissues derived from distinct patients that accounts for much of the biological diversity. Here we characterized variation in gene expression patterns in a set of 19 fibroblast-like synoviocytes derived from affected synovial tissue from different RA patients using cDNA microarrays representing approximately 24,000 human genes. The FLS cell lines could be classified into subtypes distinguished bydifferences in their gene expression patterns. Sets from co-expressed genes were identified for which variation in transcript levels could be related to specific features in biological variation. From 10 FLS cell lines we had cDNA microarray gene expression data available from whole synovial tissue from which the cell lines were derived. Most interestingly, the FLS clustering patterns were similar to the subclassification that was obtained by sampling the whole synovial tissue from which they were derived. These data support the notion that heterogeneity between synovial tissue is reflected in the FLS as a stable trait and suggest distinct pathogenic capabilities of human synoviocytes in disease.

O.33
HUMAN PAPILLOMAVIRUS DETECTION BY MICROARRAY AS ADJUNCT TRIAGE IN LIQUID BASED CYTOLOGY OF CERVICAL SCREENING

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In the prevention of cervical cancer, cytologic examination is the basis for selecting women with a chance on preneoplasia. In cervical screening the category of cytological classification with minor abnormalities is the largest group. Human papilloma virus (HPV) has been shown to be a key factor in the development of cervical cancer. The major question of this study is whether HPV typing may be a useful additional triage point in cervical screening when cytologic abnormal cells are present. To this end we retrospectively examined the prospectively collected cases of 1997–1999 with cytological abnormalities. For HPV genotyping we used the in-house developed HPV microarray method. Residual sample vials of liquid-based Pap-test specimens (357) were selected based on their presence of cytological abnormalities (PAP $\star$ 2). The specimen vials were used for DNA isolation, amplification and digoxigenin (DIG)-labeling of HPV E1 genomic regions using PCR. The prevalence of HPV infection was 28% in this study. Percentage of HPV positive samples increases with cytological classification: 16% (PAP 2), 56% (PAP 3a), 89% (PAP 3b), 100% (PAP 4). In the PAP 3b group, HPV types of an unknown risk and high risk were exclusively detected; in PAP 4 only high risk types ($n = 10$) have been detected. HPV screening of the PAP 2/3a group results in $\sim$80% low risk HPV or HPV negative individuals. Results suggest the combination of cervical screening cytology and HPV testing.

O.34
OLIGO HYBRIDIZATION BASED HUMAN PAPILLOMA VIRUS TYPING USING THE "ARRAY OF ARRAYS" TECHNOLOGY

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Human papilloma virus (HPV) DNA can be identified in $>$90% of cervical cancers. Based on their association with high-grade lesions and invasive cancer HPVs are categorized into 'high risk' (HPVs 16, 18, 45, and 56), 'intermediate risk' (HPVs 31, 33, 35, 51, 52, and 58), and 'low risk' (HPVs 6, 11, 42, 43, and 44). In developing countries with ineffective screening due to a lack of coverage, accessibility, and effectiveness, HPV DNA testing should be considered. Although various screening methods have been described there is still a need for a quick easy and high-throughput assay. Here we describe the development of Several cell lines (HeLa, SiHa, pHPV11, CC10) were enriched for HPV DNA using CP general PCR primers. The obtained PCR products were treated with exonuclease and alkaline phosphatase to remove PCR primers and unincorporated dNTPs. A cocktail of HPV specific oligo's (HPV 6-11-16-18-31-33-35-39-45-51-52-53-56-58-59) containing $5'$generic tags was added. Single-base extension (SBE) was performed using thermosequenase and Cy3-labelled ddATP. The complementary anti tags were spotted onto a glassslide in an 'array of arrays' manner. Hybridization was performed for 3 h at 42°C. Afterwards the array was scanned and analyzed. For the 'array of arrays' based HPV typing, mini-arrays can be spotted onto one glass slide allowing the simultaneous typing of up to 80 patient samples. In our pilot study using cell lines the array performed as expected. A dilution experiment to determine the sensitivity of the array confirmed that as little as 10 fg of HPV DNA could be detected. In the future clinical samples will be tested. This study suggests that the SBE- based HPV oligonucleotide microarray is equivalent to previously used techniques for the detection of HPV in cervical specimens. However, the SBE-PCR combined with hybridization to an 'array of arrays' will allow the multiplex, fast, and sensitive typing of clinical samples.

O.35
GENE EXPRESSION PROFILING PREDICTS DISEASE-FREE SURVIVAL IN PROSTATE CANCER

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One of the major problems in management of prostate cancer (PC) is lack of reliable genetic markers predicting clinical course of the disease. We analyzed expression profiles of 12,625 transcripts in prostate tumors from patients with distinct clinical outcome after therapy as well as human PC xenografts exhibiting metastasis-promoting phenotype in nude mice. We identified small clusters of genes discriminating recurrent versus non-recurrent disease with 90% and 75%

accuracy in two independent cohorts of patients comprising discovery (21 tumors) and validation (79 tumors) sets of clinical samples. Kaplan–Meier analysis demonstrated that recurrence predictor signatures are highly informative ($p < 0.0001$) in stratification of patients into sub-groups with distinct relapse-free survival after therapy. Gene expression-based recurrence predictor algorithm was informative in predicting the outcome in patients with early stage disease; either high or low preoperative PSA levels, and provided additional value to the outcome prediction based on Gleason sum. Overall, 88% of patients with recurrence within one year after therapy were correctly classified into poor prognosis group. Identified algorithm provides additional predictive value over conventional markers of outcome and appears suitable for stratification of PC patients at the time of diagnosis into subgroups with distinct disease-free survival probability after therapy.

O.36
EGFR AND CANCER PROGNOSIS

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The epidermal growth factor receptor (EGFR), a member of the erbB receptor family, which also includes erbB2, erbB3 and erbB4, is expressed in many tumour types. Stimulation of EGFR by binding of ligands such as epidermal growth factor (EGF) and transforming growth factor alpha (TGFalpha), results in activation of multiple downstream signalling cascades, including the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinse (PI3K) pathways. Such signalling can promote proliferation, angiogenesis and invasion and inhibit apoptosis. Not surprisingly, therefore, increased EGFR expression has been linked to tumour progression and development of resistance to standard therapies. Indeed, EGFR overexpression strongly associates with reduced over-all survival rates of patients in several tumour types (notably head and neck, ovarian, cervical, bladder and oesophageal cancers). In total, these data provide a strong rationale for targeting EGFR in cancer, and anti-EGFR therapies have proved effective in many preclinical studies. For example, our own experience with the anti-EGFR agent Iressa has revealed a marked inhibition of antihormone-resistant growth and invasion in breast cancer *in vitro*, while emerging investigator-driven trials in this disease show promise for such treatments. However, recent clinical trial data has also demonstrated that a proportion of cancer patients are resistant *de novo* to anti-EGFR therapies, while others can acquire resistance during treatment. Moreover, the prognostic role for EGFR is not clearly established across all cancer types. For example, in gastric, breast, endometrial, colorectal and non-small cell lung cancers, EGFR provides only modest or poor prognostic information. These data suggest that measurement of EGFR expression alone may be too simplistic. Since the EGFR is only one component of a highly complex and interacting signalling network, a more detailed analysis, encompassing its activation status and interplay with other receptors and downstream signalling elements, may prove more informative. Such data would also aid identification of patient populations that may benefit from anti-EGFR therapies and allow accurate monitoring of treatment efficacy.

O.37
DIAGNOSTIC ASPECTS OF EGFR ASSAYS

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Epidermal growth factor receptor (EGFR) is one of several targets for next-generation therapy for cancer. It is a transmembrane receptor that plays a key role in the maintenance of normal cellular function and survival. Stimulation of the receptor initiates a signaling transduction cascade, which regulates a variety of cellular processes [1].

EGFR is highly expressed in a number of solid tumor types (e.g., colorectal, non-small cell lung, and head and neck carcinomas), and its presence has been shown to be associated with aggressive disease and overall poor clinical prognosis. This fact, together with the role of EGFR in cell division, cell survival, angiogenesis and cell migration, gives a clear rationale for targeting the receptor as a strategy for anticancer therapy [1]. In order to select patients who would derive the most benefit from EGFR-targeted therapy, it is important to be able to detect the presence of the receptor within the target tumor(s), and a number of methods are available to do this [2]. DAKO EGFR pharmDx^{TM} is an immunohistochemical test that was originally developed to establish patient eligibility for one of these therapies, cetuximab, in clinical trials, where the need for a standard, reproducible test method was crucial. Alternative visualization methods, which are perhaps less useful due to their technical complexity, include *in situ* hybridization, RT-PCR, and Western or Northern blotting [1]. Contrary to expectation, several clini-

cal trials did not find any relationship between the level of EGFR expression detected within colorectal tumors and the response to treatment with cetuximab, either as a single agent or in combination with irinotecan; patients with any detectable level of EGFR expression had an equal chance of a response [3–5]. In contrast, there did seem to be a correlation between the severity of cetuximab-induced acneiform rash and the response rate and survival time following cetuximab treatment (monotherapy or combination therapy). This relationship between rash and treatment outcome appears to be important [5,6].

Additional molecular markers for the activity of the EGFR signaling pathway are being investigated, such as MAPK and pAkt. These studies may find ways to better predict who may respond to EGFR-targeted therapy [7].

In summary, next-generation EGFR-targeting therapies offer new options in anticancer therapy. The challenge remains to understand how the patient population can be enriched.

References

[1] R.S. Herbst and C.J. Langer, *Sem. Oncol.* **29** (2002), 27–36.
[2] R.S. Herbst and D.M. Shin, *Cancer* **94** (2002), 1593–1611.
[3] L.B. Saltz et al., *Proc. Am. Soc. Clin. Oncol.* (2001), abstract #7.
[4] L.B. Saltz et al., *Proc. Am. Soc. Clin. Oncol.* (2002), abstract #504.
[5] D. Cunningham et al., *Proc. Am. Soc. Clin. Oncol.* (2003), abstract #1012.
[6] L.B. Saltz et al., *Proc. Am. Soc. Clin. Oncol.* (2003), abstract #817.
[7] J. Mendelson and J. Baselga, *J. Clin. Oncol.* **21** (2003), 2787–2799.

O.38
EGFR INHIBITORS – THERAPEUTIC ASPECTS

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The 2 most advanced EGFR inhibitors in development are C225 (Cetuximab^{TM}) and ZD1839 (Iressa^{TM}). C225 is an antibody directed against the ligand binding domain of human EGFR, which competes for receptor binding with EGF and other ligands. *In vitro*, Cetuximab^{TM} inhibits EGFR tyrosine kinase activity and proliferation of EGFR-overexpressing squamous cell carcinoma cell lines. Synergy was observed with doxorubicin, cisplatin and radiation. In phase I trials, major toxicity has been dermatological (rash and acneic skin reactions); allergic reactions have also been observed in about 3% of cases. This agent, administered i.v. weekly, is presently in phase III trials in HNSCC and colon cancer. Iressa^{TM}, a synthetic molecule which targets the EGFR ATP binding site, is a very specific inhibitor of EGFR TK activity. Synergy has been observed with paclitaxel and cisplatin. In phase I trials, responses were seen in advanced NSCLC, and cutaneous toxicity and diarrhea were the most important side effects. Oral chronic administration daily is feasible. Two large randomized trials have been completed in advanced NSCLC in combination with chemotherapy. A large phase II study in second and third line has demonstrated a single agent activity of 18.5%. Another large phase II study in patients who received prior platinum and docetaxel obtained a response rate of 11%. There was no difference in response rate between the 250 and the 500 mg/day doses, but side effects were higher in patients who received the 500 mg dose. A similar small molecule, OSI-774 (Tarceva), has also shown activity in this setting. Two large randomized phase III studies of Iressa have recently been completed and analyzed in which 2 doses of Iressa (250 or 500 mg/day) or placebo were given in combination with 2 different chemotherapy regimens (carboplatin–paclitaxel or carboplatin–gemcitabine). These studies failed to demonstrate an increase in survival by adding Iressa together with chemotherapy in patients with advanced NSCLC.

O.39
TISSUE ARRAYS FOR PROTEIN STUDIES

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(No abstract available.)

O.40
AUTOMATING TISSUE BASED PROTEOMICS WITH IMAGE ANALYSIS

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An important aim of proteomics is to find molecular targets (proteins) that specifically relate to a particular disorder. Antibodies are then prepared against these targets for diagnostic evaluation. Tissue microarrays (TMAs) play a crucial role in this evaluation process. Limiting factors in the use of TMAs for high throughput analysis relate to acquisition of many images, their

visual evaluation and the subsequent input of the results into a data base. Such a data base is essential to correlate the data with tumor type and outcome, and to evaluate the performance against other markers. So far, these steps are mostly performed by hand, are time-consuming and potentially prone to bias and errors. We have applied image analysis to support the varies steps of TMA evaluation.

An image analysis system Ariol SL-50 (Applied Imaging Corp) consisting of an automated microscope and slide loader was used to process up to fifty bar-coded slides containing tissue micro-arrays unattended. In a first pass using a $1.25\times$ objective the slide is scanned for the presence of tissue cores. The orientation of the array, the indices and the positions of the cores are thereby determined automatically. During a second pass using a $20\times$ objective images of the individual cores are acquired and some global features of the tissue cores are measured. This information is stored in a SQL database. The database allows the visual evaluation of the staining patterns of each of the individual cores and also against other markers achieved in separate experiments. Such a data base is essential to correlate the data with the different tumor types.

Finally, we investigated the possibility of automated classification of TMAs in a set of arrays containing colorectal tumors stained with different markers. Results will be presented.

O.41
MOLECULAR GENETICS OF SMALL BOWEL ADENOCARCINOMAS – AN ANALYSIS USING TISSUE MICROARRAY TECHNOLOGY

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Introduction: Primary small bowel adenocarcinoma is rare (1% of all gastrointestinal malignancies). The reason for its rarity is unknown and the mechanisms of carcinogenesis remain unclear. While no consistent changes have been identified to date, the few small studies performed suggest that the underlying molecular genetics are different from those in colorectal cancer. A molecular study on a larger series is therefore indicated.

Method: Suitable cases of primary small bowel adenocarcinoma were identified from the BSG (British Society of Gastroenterologists) National Survey (June 1998–May 2000) and the histopathology records of Leeds NHS Trust (1980–2002).

Clinical information and survival data was provided by case note review and questionnaires retrieved from the BSG Survey. Based on this information and stringent histological review only primary small bowel adenocarcinomas were included. All specimens were graded and staged according to the UICC (Union-Internationale Contre le Cancer) TNM Classification (1997). TMA (tissue microarray) technology was employed to allow high-throughput immunohistochemistry. Formalin fixed paraffin embedded tumour and control tissues were collected and each case was represented by 3 tissue cores. Monoclonal antibodies for tumour suppressor genes p53, p16, and SMAD4, mismatch repair genes hMLH-1, hMSH-2 and the APC/β-Catenin pathway were used.

Results: 100 cases of primary small bowel adenocarcinoma from the BSG survey and 64 cases identified from histopathology records with the Leeds NHS Trust were collected. Tumour anatomic distribution comprised 10 periampullary, 47 duodenal, 33 jejunal, 38 ileum and 36 indeterminate. The frequency and pattern of expression of each gene will be defined in all tumours from the duodenum, mid small bowel and ileum. All findings will be correlated with clinicopathological parameters and survival.

Conclusion: This is the largest study of the molecular genetics of primary small bowel adenocarcinoma. Knowledge of the underlying molecular mechanisms of this rare tumour may elucidate as to whether pathogenesis is identical throughout the small bowel or whether different aetiopathogenetic mechanisms are operative in the different regions of the intestine.

O.42
CYTOLOGY MICROARRAYS

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The result of the genomic era is the growing need for high throughput methods of marker evaluation and validation. Frequently tissue microarray's are used for this purpose. A very similar somewhat complementary method our group has been developing is cytology microarrays. These are arrays of cytologic material which have been deposited on to slides as distinct spots. Each spot can be controlled to consist of up to

several 1000 cells or only a few cells. These cells may be deposited as fixed or unfixed. Each spot can come from a unique source. Currently we have created hundreds of cytology microarrays consisting of a 5×12 array of spots using both a manual and a computer controlled spotting device. Through the correct optimisation of the spotting suspension and deposition method one can tightly control the number of cells deposited and the size of the spots deposited. We have tested the arrays created to date with a variety of conventional stains, immunohistochemical molecular markers, and with a selection of fish probes. Currently created arrays consist of cytologic material from $\sim$24 cell lines (lung, cervix, breast) cervical scraping samples, sputum samples, and bronchial lavage samples. As an example application the results from cytology microarrays consisting of Normal and Abnormal lung and cervical material, lung cancer cell lines and normal human bronchial cells to tested against a panel of possible lung cancer detection markers will be presented.

O.43
UPREGULATION OF THE BHLH-LZ TRANSCRIPTION FACTOR TFEB IN T(6;11)(P21;Q13)-POSITIVE RENAL CELL CARCINOMAS DUE TO PROMOTER SUBSTITUTION

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The TFE/MITF subfamily of basic helix-loop-helix-Leucine-zipper (bHLH-LZ) transcription factors consists of four closely related members named TFE3, TFEB, TFEC, and MITF, which can mutually form both homo- and heterodimers. We previously positionally cloned the renal cell carcinoma-associated t(X;1)(p11;q21) breakpoint, which fuses the TFE3 gene on the X chromosome to the PRCC gene on chromosome 1, resulting in a malignant phenotype of the affected kidney cells. We now characterized a renal cell carcinoma of the clear cell type with a balanced t(6;11)(p21;q13), and found that this translocation results in a fusion between the anonymous non-coding Alpha gene on 11q13, and the TFEB gene on 6p21. Both fusion transcripts were found to be expressed. However, the AlphaTFEB fusion gene appears to contain all coding exons of the TFEB gene linked to $5'$ upstream regulatory sequences of the Alpha gene. Quantitative RT-PCR analysis revealed that AlphaTFEB mRNA levels are up to 60-fold upregulated in primary tumor cells as compared to wild-type TFEB mRNA levels in normal kidney samples. In complete accordance with this observation we found that also TFEB protein levels are dramatically up-regulated in the tumor cells. Based on these results we conclude that the common RCC-associated t(6;11)(p21;q13) translocation leads to a dramatic transcriptional and translational upregulation of TFEB due to promotersubstitution, thereby severely unbalancing the nuclear ratios of the MITF/TFE subfamily members. We speculate that this imbalance may lead to changes in the expression of downstream target genes, ultimately resulting in the development of RCC. Moreover, since this is the second MITF/TFE transcription factor that is involved in RCC development, our findings point towards a concept in which this bHLH-LZ subfamily may play a critical role in the regulation of (aberrant) renal cellular growth.

O.44
CENTROMERIC CHROMOSOMAL TRANSLOCATIONS SHOW TISSUE-SPECIFIC DIFFERENCES IN COLORECTAL ADENOCARCINOMAS VERSUS ORAL SQUAMOUS CELL CARCINOMAS

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In both adenocarcinomas and squamous cell carcinomas, chromosomal instability leading to imbalances and structural rearrangements is a major mechanism in carcinogenesis and tumor progression. The aim of this study was to define the different patterns of chromosomal instability in two epithelial tumor types, adenocarcinomas and squamous cell carcinomas, and to explore possible differences in the underlying mechanisms. Seven adenocarcinoma cell lines and nine squamous cell carcinoma cell lines were studied using spectral karyotyping (SKY), conventional comparative genomic hybridization (CGH), array CGH and fluores-

cence *in situ* hybridization (FISH) with centromere specific DNA-clones. One fresh, uncultured squamous cell carcinoma was studied. SKY analysis of the adenocarcinomas revealed that 56% of translocations occurred within chromosome arms, while 23% of rearrangements occurred between the centromeres of two different chromosomes. In contrast, in the squamous cell carcinomas these percentages were 33% and 50%, respectively. Squamous cell carcinomas on average showed significantly more whole arm translocations than adenocarcinomas (112 in 9 cases versus 13 in 7 cases, respectively, $p = 0.008$), the majority of which lead to whole chromosome arm loss or gain. Array CGH demonstrated that in all squamous cell carcinomas and in most adenocarcinomas, the breakpoints of imbalanced whole arm translocations occurred between the two clones on the array flanking the centromeres. FISH with centromere specific probes, however, revealed in the majority of cases a remarkable difference. In squamous cell carcinomas, whole chromosome arm translocations in all instances resulted in a marker chromosome containing centromere material of both participating chromosomes, while in adenocarcinomas only centromere material of one of the two chromosomes involved was present. The fresh squamous cell carcinoma case confirmed the results in the cell line counterparts, indicating that our findings are not caused by a tissue culture artefact. This is the firsdt report of a tissue type-specific form of chromosomal instability differences in centromeric breakage and illegitimate recombination in human epithelial malignancies.

O.45
HYPERMETHYLATION STATUS OF SERUM-DERIVED GENOMIC DNA FROM IRESSA-TREATED LUNG CANCER PATIENTS

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Epigenetic alterations in the promoter region through methylation of cytosine residues represent an important mechanism to modulate the expression and function of genes. Such modifications can provoke a complete suppression of and alter the biological behavior of the affected cell. We have examined the promoter region of eight candidate genes (APC1A, DAPK, FHIT, MGMT, p14ARF, p16INK4a, RAR beta, RASSF1A) in lung cancer patients for potential hypermethylation. Genomic DNA was purified from serum and subsequently treated with sodium bisulfite to convert unmethylated cytosine bases. The amount of genomic DNA in serum is rather low and, therefore, we have developed a nested PCR approach to increase the sensitivity of our test system. The first PCR amplifies a larger fragment without discrimination between methylated or wildtype status. The second PCR uses nested primers which specifically amplify either transcription. The absence of the affected gene may in turn dramatically influence methylated or unmethylated sequences. We have investigated a group of 23 patients with confirmed diagnosis of lung cancer (mean age 63.1 ± 10.8 years, range 34.1–77.4). All patients were enrolled in a clinical study in which they were treated with Iressa. The following frequencies of hypermethylation were detected: APC1A (26.1%), DAPK (8.7%), FHIT (26.1%), MGMT (4.3%), p14ARF (52.1%), p16INK4a (8.7%), RAR beta (43.5%), RASSF1A (34.8%). More than 60% of the patients showed multiple epigenetic alterations while in 3 patients all tested promoter regions were found to be unmethylated. A possible correlation with clinico-pathological parameters of these patients is presently being investigated. Single parameters or a combination of epigenetic alterations may be useful markers for prognosis and/or therapeutic efficiency.

O.46
GENOMIC INFORMATION AND CANCER

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Global views of the cancer transcriptome are proving to be valuable for the understanding of cancer pathogenesis, for cancer diagnosis, and for the prediction of response to therapeutic intervention. Specifically, we have identified distinct patterns of gene expression that signify a unique subset of acute lymphoblastic leukemia patients that respond poorly to conventional chemotherapy. These patients, harboring MLL gene rearrangement are also characterized by overexpression of the receptor tyrosine kinase FLT3, and xenograft experiments indicate that leukemias induced by human cell lines with FLT3 overexpression (but not activating mutation) regress when the mice are treated with an oral FLT3 kinase inhibitor, suggesting that FLT3 may be an important therapeutic target in MLL-rearranged ALL. We have observed similar pat-

terns of gene expression in lung, prostate, brain, and lymphoid solid tumors. Interestingly, a gene expression of metastatic potential was identified in a broad panel of tumors, suggesting that generic signatures of metastatic potential may exist. In addition, a chemical biology approach to modulating cancer genomes will be presented. We demonstrate that a small molecule screen can be performed based on the measurement of gene expression profiles. Small molecules capable of inducing the gene expression signature of hematopoietic differentiation in leukemia cell lines and primary patient samples have been identified, and these compounds, including the estrogen derivative 16-ketoestradiol, are shown to induce functional evidence of myeloid maturation. These experiments demonstrate the feasibility of chemical genomics-based approaches to small molecule screening.

O.47
HIGH THROUGHPUT LOSS-OF-FUNCTION PHENOTYPIC SCREENS IN MAMMALIAN CELLS USING RNA INTERFERENCE

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One of the major remaining deficits in our understanding of the human genome is that information regarding gene function is available for only some 6,000 of the approximately 30,000 genes. Thus, we need to functionally annotate the tens of thousands of genes for which this information is currently lacking. We have developed functional genetic approaches to obtain information regarding gene function using high-throughput screens. We use both gain-of-function and loss-of-function genetic screens to identify novel components of cancer-relevant pathways. We have designed a mammalian expression vector (pSUPER, suppression of endogenous RNA), which directs the synthesis of short hairpin transcripts that get processed intracellularly into siRNA-like molecules. This vector can mediate persistent inhibition of gene expression in a highly specific fashion. We have used this vector to stably suppress expression of individual members of several gene families, including histone acetyl transferases (HATs), protein phosphatase 2A regulatory "B" subunits, de-ubiquitinating enzymes (DUBs) and all human protein kinases. Using functional screens, we have been able to place several of the members of these gene families in cancer-relevant pathways. Results of these loss-of-function genetic screens will be presented. We are currently using this vector system to generate a large collection of some 25,000 vectors that each targets a single transcript. This collection of siRNA vectors will allow us for the first time to perform genome-wide functional screens for loss-of-function phenotypes. We will use these siRNA libraries to identify novel genes that are involved in control of cell cycle, responses to cytotoxic drugs and replicative senescence. A new technology ("siRNA bar code screens") that will greatly facilitate these large-scale loss-of-function screens will be presented.

References

[1] T.R. Brummelkamp, R. Bernards and R. Agami, A system for stable expression of short interfering RNAs in mammalian cells, *Science* **296** (2002), 550–553.
[2] T.R. Brummelkamp, S.M.B. Nijman, A.M.G. Dirac and R. Bernards, Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-K B, *Nature* **424** (2003), 797–801.

O.48
GENOMICS IN CANCER DRUG DISCOVERY

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Microarray based gene expression analysis is increasingly used to answer a range of questions not only related to Target Discovery but also in biomarker discovery and analysis, signaling pathway analysis and compound characterisation. These applications are particularly valuable in Oncology, where cellular models can be exploited and human disease material is available in sufficient quantity. We have used comprehensive gene expression analysis to characterize several compounds acting either on the same or different signaling pathways.

Genomics technologies can also be applied to characterize disease at the molecular level in accord with molecular approaches to drug/target discovery. Such approaches will hopefully lead to diagnosing disease at the molecular level to allow the correct selection of therapy for a larger percentage of patients.

O.49
GENOMIC-BASED ANTICANCER DRUG DEVELOPMENT

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Gene expression profiling has potential for elucidating the complex cellular effects and mechanisms

of action of novel targeted anticancer agents or existing chemotherapeutics for which the precise molecular mechanism of action may be unclear. Our laboratory has been particularly interesting in using expression profiling by cDNA microarray to enhance the discovery and development of novel anti-cancer agents. Our primary interest is in investigating gene expression patterns induced by cancer therapeutics, both in experimental models and as part of their clinical evaluation. We have used both commercially available and in house arrays (4–30 K cDNA sets) to identify signatures for a number of different drug types. These signatures can be used to confirm of identify the mechanism(s) of drug action, discover genes involved in drug sensitivity or resistance, and develop molecular biomarkers for use as pharmacodynamic and prognostic endpoints. The successful use of gene expression profiling will be exemplified by the following recent studies and work in progress:

- Definition of molecular signatures of HSP90 inhibition following treatment with agents such as 17-allylamino-17-demethoxygeldanamycin, currently undergoing phase I/II clinical studies.
- Identification of genes altered in the tumour tissue of rectal cancer patients during treatment with 5FU, including a cluster of genes regulated by c-Myc (Clarke et al., *Cancer Research*, in press).

The current status and future potential of gene expression profiling in cancer pharmacology and drug development will be discussed.

O.50
GENOME WIDE SEARCH FOR NOVEL GENETIC ALTERATIONS IN PREINVASIVE BRONCHIAL SQUAMOUS CELL CARCINOMA*

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Characterizing genetic alterations in premalignant stages of disease facilitates earlier detection and provides understanding of progression. However, current techniques either require a sizeable amount of DNA or do not provide the resolution needed to define alteration boundaries. Here we describe the identification of novel alterations in bronchial carcinoma *in situ* (CIS) utilizing RAPD PCR and high resolution array CGH.

Objective: Identification of novel genetic alterations in premalignant bronchial lesions and fine mapping these alterations using a microarray of bacterial artificial chromosomes (BACs) spanning these regions.

Design: DNA fingerprints of paired normal and CIS samples were generated using Randomly Amplified Polymorphic DNA (RAPD) PCR to identify amplified or absent bands in premalignant lesions. Using a custom built array of BACs spanning the regions identified by DNA fingerprinting, alteration size and frequency were determined.

Methods: 30 pairs of microdissected CIS and normal DNA specimens were compared. Identifying the same band discrepancy in multiple samples revealed key alterations. For fine mapping, BACs were selected based on the Fingerprint Contig (FPC) map and the UCSC human genome tiling set. These BACs were amplified and arrays constructed. Co-hybridization of differentially labelled CIS and normal reference DNA identified BACs that have been amplified or deleted in the CIS samples.

Results: 17 recurrent changes were observed in the 30 CISs. 10 of the recurrent changes were localized to chromosome 1p, 1q, 7q, 8q, 10q, 13q and 14q. Approximately 300 BACs (>60 Mbp) were selected to span these regions as well as BACs covering the entire 1p, 3p, and 5p arms.

Conclusion: RAPD DNA fingerprinting proves useful in identifying novel genetic alterations in preinvasive lung cancer samples. High density array CGH has identified defined boundaries for these alterations, allowing for the selection of candidate genes. To produce a complete profile of these premalignant lesions, construction of an array containing overlapping clones spanning the entire genome is in progress.

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O.51
HIGH RESOLUTION ANALYSIS OF GENOMIC COPY NUMBER ALTERATIONS IN BLADDER CANCER CELL LINES BY MICROARRAY-BASED COMPARATIVE GENOMIC HYBRIDISATION (CGH)

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Tumour development and progression is often associated with copy number changes. While regions

of DNA amplification commonly harbour oncogenes, regions of deletion have been shown to contain tumour suppressor genes. Although conventional CGH has provided a lot of information on genome wide copy number changes in tumours, its resolution is limited to approximately 3 Mb. This can be overcome by replacing the metaphase chromosomes as the hybridisation targets with mapped sequences arrayed onto glass slides. The resolution is now only limited by the size and spacing of the mapped sequences used.

We have used a genomic DNA microarray consisting of ∼3040 large insert clones spaced at approximately 1 Mb intervals across the human genome and a chromosome 6 tiling path array to examine copy number changes of 23 bladder-derived cell lines, for which some genomic information was available including microsatellite typing and M-FISH analyses.

The comparison of array CGH with existing M-FISH results revealed excellent concordance. However, regions of gain and loss were defined more accurately by array CGH.

The most frequent changes involved complete or partial loss of 4q (83%) and gain of 20q (78%). Other frequent losses were of 18q (65%), 8p (65%), 2q (61%), 6q (61%), 3p (56%), 13q (56%), 4p (52%), 6p (52%), 10p (52%), 10q (52%) and 5p (43%). A region of deletion at 8p21.2–p21.3 previously identified in bladder cancer has also been refined to an interval of approximately 1 Mb. Frequent copy number gains on 8q (61%), 20p (56%), 5p (40%) and 3q (43%), and fifteen high level amplifications have been identified. A previously reported amplification at 6p22.3 was the most frequent amplification detected in all the cell lines studied. Further analysis using a chromosome 6 tiling path array revealed the amplicon structure in more detail and identified several genes of interest which are currently being investigated by real time PCR.

O.52
INNOVATIVE MICROARRAY SCANNING AND ANALYSIS

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Success in microarray technology requires new approaches to microarray reader development. A microarray reader system (Optical Scanning Array reader, or OSA reader) based on high-content automated microscopy has been developed. The reader allows fast capture of high resolution (down to 0.35 mm) microarray images from any fluorescent dyes in the visible range (380–700 nm) and, also, image acquisition of up to 4 simultaneous labels.

Controlled by high performance software, the system is adapted to scanning and quantitative analysis and interpretation of any type of dry microarrays: DNA and protein microarray, cell array, rolling-circle amplification associated microarray, etc.

An innovative feature of the OSA reader allowing a wide range of on-chip chemical and enzymatic reactions including PCR amplification is a microarray-holder with a temperature-controlled hybridization chamber.

Examples will be shown in the fields of biological research and medicine. The system was used for the development of oligonucleotide microarrays for CFTR gene mutation detection. On-chip registration of hybridization kinetics and analysis of duplex stabilities for all spots of the microarray allowed the optimisation the hybridization conditions for maximum match/mismatch discrimination. Registration of on-chip kinetics and melting curves were used to improve the accurate detection and quantification within gene expression microarray. Finally, both capability of capture of high resolution images and advanced algorithms for spots or cell clusters morphology analysis give access to massive parallel functional studies of sub-cellular markers in cell-arrays.

O.53
EXPRESSION PROFILING OF MOUSE MODEL OF GASTRIC TUMOROGENESIS

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Background: Gastric cancer is the second most common cause of cancer mortality worldwide, causing almost 700 000 deaths annually. Establishing an appropriate mouse model and identifying key causative genes is crucial for the progress in this field and may offer novel therapeutic targets for treatment strategies in the pathogenesis of stomach cancer.

Objective: To isolate and identify potential key genes involved in the development of stomach cancer.

Methods: We have previously reported on the generation of a transgenic mouse model, whereby mice develop tumors in the glandular stomach that correlate strongly with increased mortality [1]. Tumor cysts appeared at 3–4 months and increase in severity with age.

To identify differentially expressed genes that could play role in gastric tumorogenesis we used a new protocol [2] to perform subtractive suppression hybridization (SSH) procedure. Briefly, RNA isolated from glandular stomach of three month old transgenic and wild type animals was used as a source for cDNA synthesis followed by SSH. Subtracted cDNA was cloned and used to generate cDNA libraries that are enriched for up- and down-regulated genes, respectively. Colony PCR was performed on approximately 4000 bacterial colonies. PCR products were spotted on nylon membranes using a macroarray robot. The generated filters were hybridized with radiolabeled subtracted cDNA probes, and analyzed with computer software.

Results: 197 clones showed differential expression on processed filters. BLAST analysis revealed 109 individual genes that encode: 23 hypothetical proteins; 27 different factors, regulators and signaling proteins; 26 enzymes; 9 transporters and channels; 7 cytoskeletal and motor proteins; 3 antigens; 14 other proteins. Expression of these candidate genes was confirmed by RT-PCR or Northern blotting. Most interesting candidates are currently the focus of ongoing investigations.

Conclusion: Using this stomach cancer mouse model could prove to be a valuable tool for evaluating the role of key genes in the development of stomach cancer.

References

[1] P. Andersson et al., *Proc. Natl. Acad. Sci. USA* **99** (2002), 9990–9995.

[2] O.D. von Stein, *Methods Mol. Biol.* **175** (2001), 263–278.

O.54
OLIGONUCLEOTIDE MICROARRAYS AS A SCREENING TOOL FOR DETECTION OF GENE REARRANGEMENTS IN CHILDHOOD LEUKEMIA

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Leukemia, the most common childhood malignancy, is associated with chromosomal translocations, allowing the identification of prognostically relevant subgroups of patients with unique biological and clinical features. The risk-stratification of patients is widely used in most therapeutic trials and the precise molecular diagnosis is needed to choose an adequate chemotherapy and to predict clinical outcome.

The multiplex reverse transcription-polymerase chain reaction (RT-PCR)-microarray-based approach have been developed for the detection of twelve most clinically important translocations: t(9;22)p190 and p210; 11q23 rearrangements; t(12;21); t(1;19); t(8;21), t(15;17) and inv16. The method included isolation of RNA, reverse transcription, two-round nested multiplex PCR with translocation-specific primers, labeling of PCR fragments and hybridization with oligonucleotide microarrays. Two different methods were used for labeling of the target: (1) post-PCR fragmentation with DNase I followed by terminal transferase tailing with fluorescent labeled nucleotide and (2) labeling during second round of PCR using fluorescence-labeled nested primers. The microarray represented 25-mers immobilized into gel pads attached to microscopic slide. Fluorescence signals were measured under fluorescent microscope equipped with CCD-camera. The hybridization patterns were very specific and allowed to distinguish not only between different types of translocations, but between breakpoint variants, as well.

More than 200 samples from leukemia patients were analyzed to demonstrate the potential clinical application of the method. The above-mentioned gene rearrangements were found in about 40% of all cases. The parallel study using standard RT-PCR approach was performed. The sensitivity and specificity of the microarray-based assay is comparable with RT-PCR technique, so that it can be used not only to identify translocations in primary samples, but to follow minimal residual disease (MRD) in the course of therapy. A further development of the method, on-chip-multiplex PCR has been applied for the analysis of a common translocation t(9;22). Our data suggest that the microarray-based assay is very effective and reliable tool in clinical screening of gene rearrangements in patients with leukemia.

O.55
IDENTIFICATION OF GENES RELEVANT FOR THE DEVELOPMENT OF MURINE MALIGNANT MESOTHELIOMA USING ARRAY CGH ANALYSIS

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Very little is known about the genetic lesions contributing to the development of malignant mesothelioma. In a subset of tumors in man, inactivation of tumor suppressor genes such as the neurofibromatosis type 2 gene (NF2), p16INK4a and TP53 has been reported.

We have used these observations as a basis to generate a mouse model for mesothelioma using Cre/Lox mediated switching of conditional tumor suppressor genes. Injection of Adeno-Cre virus in the thoracic cavity of conditional knockout mice caused tumor suppressor gene inactivation in the mesothelial cells lining the thoracic cavity. Mesotheliomas developed at high frequency after Adeno-Cre virus administration to Nf2flox/flox p53flox/flox, Nf2flox/flox Ink4aArfflox/flox and Nf2flox/flox Rbflox/flox mice with a latency of 3–5, 6–9 and 6–20 months, respectively.

We are currently analyzing mesotheliomas that developed in our mouse model by comparative genomic hybridization to BAC arrays (array CGH) to identify genes that contribute to tumor progression in this tumor. The CGH arrays used to detect DNA copy number changes consist of approximately 3000 BAC clones providing an average resolution of 1 Mb across the mouse genome.

Initial results for 11 Nf2flox/flox p53flox/flox and 3 Nf2flox/flox Rbflox/flox murine mesotheliomas were obtained by unsupervised hierarchical clustering with the software program Genesis. Cluster analysis revealed that the array CGH profiles segregated into two subgroups that correlated with the genotype of the tumors. We also identified a small region on chromosome 6 that showed high-level amplification in 2 of the 11 Nf2flox/flox p53flox/flox mesotheliomas and confirmed this by Southern blot analysis.

We are currently expanding the number of murine mesotheliomas analyzed by array CGH and are investigating whether genes located in the amplicon on chromosome 6 play a role the development of mesothelioma.

O.56
BOTH MICROARRAY-CGH AND EXPRESSION PROFILES PREDICT SURVIVAL IN GASTRIC CANCER

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Background: Gastric cancer is one of the most frequent and mortal forms of cancer worldwide, and surgery is currently the only curative treatment. Results of gastrectomy largely depend on the stage of the disease including lymph node status. The present study aimed to classify gastric cancer both by microarray based comparative genomic hybridization (microarray CGH) and micro array expression arrays.

Methods: Genomic profiling was performed for 35 gastric tumors by both medium density microarray-CGH (2214 clones) and genome wide microarray based expression profiling (18850, 60-mer oligos). The distribution of genomic profiles in this gastric cancer series was analyzed by hierarchical cluster analysis with Cluster and Treeview software (http://rana.lbl.gov /EisenSoftware.htm).

Results: Unsupervised cluster analysis for 35 array-CGH profiles revealed three clusters of gastric cancers with twelve, eight, and fifteen cancers, respectively. Cluster membership was significantly correlated with lymph node status with $10/12$ (83%) and $7/8$ (88%) positive lymph nodes in clusters 1 and 2, respectively, versus $6/15$ (40%) in cluster 3 ($p = 0.02$). Disease related survival differed significantly between cluster 3 versus cluster 1 and 2 combined ($p = 0.019$). From 25 of the 35 tumors high quality RNA could be isolated suitable for RNA expression microarrays. Unsupervised cluster analysis of the expression data revealed two separate clusters, of fifteen and ten cancers, respectively. Again cluster membership was significantly correlated with survival ($p = 0.03$) and showed a trend of correlating with lymph node status ($p = 0.1$). Cross table analysis showed a high correlation between microarray CGH cluster membership and expression analysis cluster membership.

Conclusion: DNA copy number profiles in gastric cancer correspond to gene expression profiles and both are correlated with clinical outcome.

O.57
TOWARDS IDENTIFICATION OF GENES THAT INFLUENCE THE EFFICACY OF CANCER SELECTIVE CONDITIONALLY REPLICATING ADENOVIRUSES IN LUNG CANCER CELLS USING OLIGONUCLEOTIDE EXPRESSION ARRAYS

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Conditionally replicating adenoviruses (CRAds) provide a novel platform for the treatment of cancer, which are able to eradicate tumor cells during their replicative cycle, a process named oncolysis, that subsequently leads to the spreading of progeny within the tumor mass. Various CRAds have been developed in which the adenovirus genomes have been modified such that replication occurs selectively in tumor cells thereby making use of the affected tumor-suppressor pathways that are often disrupted during tumorigenesis, such as the p53 and Rb pathways. Although effective as a single modality in several preclinical and clinical models, combination with chemotherapy has been shown to enhance their potency. Currently little is known about the mechanism underlying CRAd-induced oncolysis and the influence of host-cell factors/ genes on this process.

Being part of our ongoing interest in studying the mechanisms by which conventional and novel anticancer agents target and kill tumor cells, here we used oligonucleotide expression arrays to identify genes and pathways that may influence the efficacy of CRAds. For this purpose we isolated RNA from NCI-H460 non-small-cell lung cancer (NSCLC) cells that were infected for different periods of time (0, 6, 24 and 48 hrs) with CRAd-D24 RGD, an infectivity enhanced virus targeted to cells with a defective Rb pathway. In addition, treatment was combined with a subtoxic concentration of paclitaxel (8 nM), conditions that we found to act in synergy with the virus. Cy-3-labelled sample RNA was hybridized together with Cy-5-labelled universal human reference RNA (Stratagene) to 19 K human oligonucleotide expression arrays (Compugen/Sigma-Genosys) printed as sixty-mer on Amersham Biosciences CodeLink^{TM} Activated Slides. Results obtained from the time-course experiment with CRAd or paclitaxel alone, or combined CRAd and paclitaxel have been normalized by different methods and are currently analysed in self-organising maps in order to identify gene expression patterns that correlate with the activation of oncolysis and the co-treatment with paclitaxel.

O.58
IDENTIFICATION OF HYPOXIA RESPONSIVE GENES REGULATED BY THE HYPOXIA INDUCIBLE FACTOR 1 (HIF-1)

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When the microenvironment of cells becomes hypoxic, cells undergo a variety of biological responses, including activation of signalling pathways leading to cell survival. The hypoxia inducible factor 1 (HIF-1) plays a critical role in the response of the cell to hypoxia. For the elucidation of the biological pathways induced by hypoxia this study analyzed the RNA profiles of murine fibroblasts at normoxia and hypoxia using micro array (Affymetrix). The regulation of gene expression by hypoxia is further distinguished between regulation mediated by HIF-1 and HIF-1-independent regulation by the use of fibroblasts derived from a HIF-1a knockout mouse. The first results showed that all enzymes involved in the glycolysis were strongly upregulated in a HIF-1 dependent manner as described before, thereby confirming the reliability of our data. A similar increase in the expression of the glucose transporter (GLUT1) was seen. More enzymes involved in metabolic pathways were upregulated by hypoxia, like adenylate kinase 4, galactokinase. Genes already related to hypoxia are also changed under hypoxia in these micro array data. However, new hypoxia regulated genes were identified. Increased expression of genes encoding proteins involved in angiogenesis, apoptosis, and invasion was observed. Genes found to be downregulated by hypoxia included those involved in cytoskeleton, cell growth, and DNA repair. Eight new hypoxia regulated genes of which the function is yet unknown, showed to be upregulated and 20 genes appeared to be downregulated. Surprisingly, the regulation of most upregulated genes appeared to be mediated by HIF-1. The micro array data of 15 genes have been confirmed by quantitative real-time RT-PCR (Lightcycler). A closer identification of the pathways regulated by HIF-1 will contribute to the understanding of the biological consequences of hypoxia.

O.59
THE EFFECT OF ANDROGEN DEPRIVATION ON GENE EXPRESSION IN NORMAL RAT PROSTATE AND PROSTATE TUMORS

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Prostate cancer (PCa) is a very commonly diagnosed malignancy among men in the western world and is the second leading cause of cancer death. Currently, androgen deprivation is the only curative therapy available. Once the cancer has spread locally or distantly androgen deprivation is to be considered palliative and the cancer will inevitably recur as an androgen insensitive malignancy. In order to discriminate a priori between androgen sensitive and insensitive PCa (prognosis) and to identify novel targets for more effective treatment protocols, it is essential to elucidate the molecular mechanisms that are at the basis of androgen (in)sensitivity of normal prostate and prostate cancer cells. Interestingly, the last decade of AR research in hormone refractory PCa (HR PCa) has shown that in HR PCA the AR axis is still active albeit at very low dihydrotestosterone (DHT) levels. We, therefore, reasoned that the AR responsive genes, which are still actively transcribed in tumors of patients that have undergone androgen deprivation, can provide a rational basis for targeting the AR axis. For our analyses we have chosen the Dunning R3327 rat model system in which the progression to androgen insensitivity is mimicked by several androgen-dependent (H) and -independent (HI-s, HI-m, HI-f) prostate-derived tumor cell lines.

We have analyzed gene expression profiles in normal prostates of rats 14 days after castration by using an oligo-based micro array composed of approximately 5000 oligonucleotides, representing over 4800 known genes. These expression profiles were compared with similarly obtained expression profiles of androgen dependent and androgen independent rat prostate tumors cells. Within the panel of AR responsive genes, i.e. after castration induced androgen deprivation, we could identify several clusters that are aberrantly regulated in prostate cancer cells. These gene clusters may serve as novel prognostic identifiers and therapeutic targets.

O.60
COMPARISON OF TRANSCRIPTIONAL RESPONSES TO ACTIVIN AND TGF-B ON A GLOBAL SCALE USING HIGH-DENSITY OLIGONUCLEOTIDE MICROARRAYS

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Activins and TGF-b are members of the TGF-b superfamily, which includes a large number of signaling molecules that regulate cell proliferation, differentiation and apoptosis. These molecules have been demonstrated to suppress tumor formation and TGF-b or activin receptor loss frequently occurs in gastric, colon and pancreatic cancers. Although activins and TGF-b bind to different cognate receptors on the cell surface, they appear to activate common intracellular intermediates. The molecular basis for signaling similarity and specificity between these two ligands is not yet understood. We have investigated Activin A and TGF-b transcriptional responses in immortalized human mammary epithelial cells in gene expression profiling studies using Agilent's Human 1A and Human 1B oligo microarrays. These high-density DNA microarrays, generated using Agilent's SurePrint inkjet technology allow genome-wide scanning of up to 36,000 human genes and transcripts. From these studies, it appears that Activin A activates only a subset of genes normally induced by TGF-b and that activin signaling is relatively transient compared to that of TGF-b. We have identified novel genes that are regulated by both Activin A and TGF-b, as well as genes activated by only one of these ligands. These results suggest that Activin A and TGF-b trigger distinct transcriptomic responses which may be responsible for their different biological activities in human breast epithelial cells.

O.61
TRANSCRIPTIONAL REPROGRAMMING AND ACTIVATION OF ENDOTHELIAL CELLS BY TUMOR CELLS

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One of the most challenging task for oncogenomics approaches are investigations of the impact of genomic

and transcriptomic changes on cellular sociology. Tumor angiogenesis is a drastic example of such complex intercellular crosstalk's with enormous potential for design of the new generation of the anti-tumor agents. To investigate molecular determinants of interactions between tumor and endothelial cells we combined expressional profiling and proteomic approaches with designed by us co-culture system of tumor and endothelial cells. To model tumour/endothelial cell interactions, we co-cultured U87 human glioma cells with human umbilical vein endothelial cells (HUVEC). U87 cells induced an 'activated' phenotype in HUVEC including an increase in proliferation, migration and tube formation. Expressional profiling of tumour-activated endothelial cells revealed global transcriptional changes (reprogramming) including activation of angiogenesis-related markers and the induction of autocrine growth loops. Hierarchical clustering of DNA array data revealed specific temporal patterns of expression of angiogenesis-related genes in endothelial cells, activated by tumor cells, which coincide with phenotypic changes. Suppression of p53 function in tumor cells leads to increased radiation-induced apoptosis in interacting endothelial cells. Parallel expressional profiling of both tumor and endothelial cells revealed coordinated changes of gene expression in both types of cells. In general conclusion data suggest that tumour-induced activation of endothelial cells involves complex molecular changes, which are determined by the genetic background of tumor cells. We believe that combination of co-culture technique with expressional profiling and proteomic approaches provide a powerful tool for understanding of the complex interactions of the tumor and endothelial cells during tumor angiogenesis and discovery of the new molecular targets for anti-tumor vasculature targeted therapy.

O.62
CORRELATIVE STUDIES USING IMMUNOFLUORESCENCE AND IMAGE ANALYSIS OF EGFR INHIBITOR OSI-774 IN METATASTIC COLORECTAL CANCER

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Epidermal Growth Factor Receptor (EGFR) is a trans-membrane glycoprotein, which once activated by a growth stimulus starts an intracellular signal transduction cascade culminating in DNA synthesis and cell division. EGFR over expression is seen in up to 75% of colorectal cancers. Deregulation of EGFR signal transduction pathway is believed to play an important role in tumour formation, growth, metastasis and has been correlated with poor prognosis. OSI-774 (Tarceva), is an orally active, selective inhibitor of the EFGR tyrosine kinase. In a Phase II clinical trial assessing OSI-774 in metastatic colorectal cancer, patients underwent CT-guided biopsy of liver metastases pre-treatment, repeated after 1–2 weeks on treatment. In 8 cases tumour tissue was present in both biopsies. Tissue sections were dual labeled using an immunofluorescence protocol consisting of anti-cytokeratin antibodies conjugated to Cy5, and one of the following conjugated to Cy3: EGFR, tyrosine-1068 phosphorylated EGFR, phospho-ERK1/2, phospho-PKB/Akt, and Ki67/MIB-1. Antibody-stained sections were counterstained with DAPI. A serial HE section was used to identify regions of the core biopsies containing cancer tissue. These areas were then imaged using $10\times$ (EGFR, ERK, PKB) or $20\times$ (Ki67) objectives, and an autostage to create composite, tiled field images for each colour across the entire tumour area. Results were expressed as the mean integrated optical density (IOD) of each molecular marker in cytokeratin-positive tissue at baseline and post-OSI-774 treatment. There was a statistically significant decrease in the mean intensity of both pEGFR and pERK post-treatment. The mean IOD's for all 8 patients were: pEGFR pre-treatment 236, post-treatment 95 ($p < 0.01$) pERK pre-treatment 319, post-treatment 148 ($p < 0.001$). There was no statistical change in PKB/Akt activation while on OSI-774 treatment. Five of the 8 patients showed a decrease in Ki67/MIB-1 after treatment. Although none of the patients treated with OSI-774 had shrinkage of their liver metastases, these data show that OSI-774 can effectively dephosphorylate EGFR and its downstream target ERK in advanced colorectal cancer.

O.63
PROMOTER BINDING AND ACTIVATION PROFILES OF TRANSCRIPTION FACTORS

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The NH_2-terminal Jun Kinases (JNKs) function in diverse roles through "activation" of a number of AP-1 components including ATF2 and c-Jun. These factors play roles in apoptosis, stress, and human carcino-

genesis [1–3]. In order to determine target genes we have developed prototype "promoter arrays" consisting of microarrays of ∼2700 promoters, each ∼1200 nts in length including over 250 AP-1 sites. Treatment of BT474 cells with the DNA damaging agent cisplatin but not the inactive isomer transplatin leads to activation of JNK and phosphorylation of ATF2 and c-Jun [4]. We have used anti-phospho-ATF2 and anti-phospho-cJun in parallel in a chromatin immunoprecipation (ChIP) to isolated ATF2- or c-Jun-bound promoter fragments from living cells. The isolated prmoters were hybridized tot he promoter array to identify the parent genes. Significant hybridization occurred at 35% of AP-1 regulated promoters whereas only 16% of other promoters were hybridized. Anti-phospho-ATF2 captured DNA bound 144 features, anti-phospho-cJun bound 181 features, while only 42 features were bound by both factors suggestive of ATF2/c-Jun heterodimer binding. Twelve promoters were confirmed present or absent using PCR, in complete concordance with the hybridization pattern. ATF2 preferentially bound promoters of DNA repair genes consistent with a known role of ATF2(4). The promoter array is being expanded to 11 K. Promoter arrays may be helpful in understanding the mechanism of JNK action.

References

[1] F. Bost et al., *MCB* **19** (1999), 1938.
[2] O. Potapova et al., *JBC* **275** (2000), 24767.
[3] Y.-M. Yang et al., *Clin. Canc. Res.* **9** (2003), 391.
[4] J. Hayakawa et al., *JBC* **278** (2003), 20582.

O.64
BIOINFORMATICS: PRINCIPLES AND PRACTICAL APPROACHES

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(No abstract available.)

O.65
SUPERVISED DATA ANALYSIS OF MICROARRAYS

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The huge amounts of data produced by microarray experiments, as well as the special features of the data, have induced development of microarray data analysis methodology and software. Two examples of such features are the multiplicity of the number of objects of interest, the genes, as contrasted with the usually small number of replicates per gene, and the dependency between genes. The data analyst has to choose what particular method or software is useful to answer the research questions. This presentation intends to give an overview on the available methods and software together with their pros and cons. Moreover, some statistical concepts are discussed that could improve the usefulness of the results.

Before discussing analysis of data we take a step back: design of the experiment. Statistical design has proven its use in many areas of application. We discuss some of the ideas that may increase the accuracy and precision of the quantities of interest, e.g., differential gene expression.

Methods for the analysis of microarray data can roughly be divided into two groups: first, normalisation together with (multiple) testing and second, model-based approaches.

Biologists usually favour the first approach while statisticians may find more comfort in applying the well-known ANOVA or mixed models. What to use depends on the situation. Some hints will be given to help deciding.

After choosing the method for the analysis, one has to find the software that does it. Besides the capabilities of the software many other criteria such as user-friendliness, compatibility with other software and costs influence the decision on what to use. Three types of software are discussed: (1) Statistical packages; (2) Commercial packages; (3) Freeware sources. A critical note is made on the widely used Significance Analysis of Microarrays Excel add-in.

Finally, the danger of over-interpretation of results is discussed. Some pitfalls and the use of validation as a tool to safeguard against these are presented.

O.66
MULTI-STEP FILTRATION OF DNA ARRAY DATA FOR OPTIMIZED TARGET SELECTION

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In the previous reports (Pelizzari et al., *NAR*, 1999; Khodarev et al., *Genomics*, 2003; Khodarev et al., *PNAS*, 2001) we emphasized some problems, connected with DNA array analysis. Here we represent the step of data filtration as critical step in the DNA array data analysis. Analysis was performed with 3 different type of arrays: Clontech Atlas', Research Genetics GeneFiters' arrays and Affymetrix U95 and

U133A GeneChips'. First rule of the multistep filtration (MSF) is the filtration of genes with statistically significant differences in the levels of expression (or signal intensities). As the second rule of filtration we use ROC analysis, leading to the intensity-based filtration (IBF). ROC analysis identifies false-positive (FP), true positive (TP), false negative (FN) and true negative (TN) data in the any given type of the test. True-positive fraction (TPF) is called sensitivity and true-negative fraction (TNF) is called specificity of the test. 1-specificity is called false positive fraction (FPF). Function of TPF vs FPF is called receiver operating characteristic curve (ROC-curve) and is the basis for the estimation of the optimal cut off levels for discrimination of the false positive and true positive data. In all three types of arrays ROC curves indicates an optimal filtration value, connected with the minimal value of false to true data. As the third rule of filtration we used comparison of the ratios of the features in the same-to-same (control) hybridizations and the same-to-different (control to experimental) hybridizations with estimation of confidence intervals. We will show an example, how such MSF procedure allowed to reduce the number of selected genes from 22,000 presented on the array to 26 genes, where 60% belonged to the single pathway.

O.67
ACGH-SMOOTH: AN AUTOMATIC TOOL FOR BREAKPOINT IDENTIFICATION AND SMOOTHING OF ARRAY-CGH DATA

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We propose a novel tool, called aCGH-Smooth, for the analysis of array Comparative Genomic Hybridization (array-CGH) data.

Array-CGH is nowadays the method of choice for studying DNA copy number changes because of its high sensitivity and resolution. The chromosomal copy numbers of the actual tumor cells are detected relative to a normal reference sample. The signals found in an array-CGH experiment contain a certain level of "noise" from various sources, e.g., due to polymorphic sites in the DNA (sequence variation between individuals), experimental noise as well as compression of the ratios due to admixed normal cells in the tumor sample. Noise reduction can allow for a better interpretation of array-CGH data.

aCGH-Smooth is written in visual C++. It has a user-friendly interface including a visualization of the results, which highlights the obtained smoothing and allows the user to influence the smoothing and number of breakpoints by setting the value of suitable parameters. A-CGH smooth is suitable for CGH data generated by BAC, PAC, cosmid, cDNA and oligo CGH arrays. It facilitates data interpretation and is a superior alternative to smoothing the data using a 'moving average' method.

aCGH-Smooth employs a 'smoothing' algorithm that adjusts the observed array-CGH values such that they represent the copy number of the most prevalent tumor cells. The problem is formalized as model fitting to search for most-likely-fit model given the data. A model describes a number of breakpoints, a position for each, and parameters of the distribution of copy number for each. The algorithm estimates the real parameters of the model from the observed array-CGH values.

O.68
SOUND EQUIVALENT TO CHROMATIN: THE SONIFICATION OF DIGITALIZED FAST-FOURIER TRANSFORMED NUCLEAR IMAGES*

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Texture may be interpreted as a mathematical function. One of the principal tools for image decomposition is the Fast Fourier Transform (FFT), which provides information on the presence of cyclic harmonic structures inherent in the texture of the original image, since each pixel of the FFT image is equivalent to a frequency and an amplitude. Texture analysis by FFT, however, is not very popular among pathologists and cytologists, which may be in part due to its mathematical basis. In order to provide a more intuitive approach to the FFT, and finally to texture analysis, we developed a method for extracting sound from digitalized microscopic images of cell nuclei from cytologic preparations. Gray level transformed images were FFT-transformed and regional maxima extracted. Since music has a time dimension, we defined a vector, like a pointer of a clock, moving in 30 seconds from the zero to the six hour position of this new image. The sound is played when the vector strikes a pixel. Frequency and amplitude of the sound are strictly defined by the position and gray value of the pixels. Thus each microscopic image is represented by a 30-

second sound clip. We compared the sounds generated from cytologic brush preparations from normal bronchial mucosa and adenocarcinomas. Since chromatin condensations in carcinoma cells are equivalent to a spectrum with lower frequencies with high amplitudes in the FFT image, cells from normal bronchial mucosa and adenocarcinoma cells can be differentiated by listening to the 'equivalent sound' of their nuclei. This sonification method for microscopic images gives a more intuitive approach to the FFT image and the principles of the Fourier Transformation. Moreover, it opens a synesthetic way for understanding textures and microscopic structures, since the auditory system can be used for pattern recognition. Finally with this method an interface between science and art is created.

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O.69
THE GLOBAL TEST: TESTING ASSOCIATION OF A PATHWAY WITH A CLINICAL OUTCOME USING A SINGLE TEST

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This paper presents a global test to be used for the analysis of microarray data. Using this test it can be determined whether the global expression pattern of a group of genes is significantly related to some clinical outcome of interest. Groups of genes may be any size from a single gene to all genes on the chip (e.g., known pathways, specific areas of the genome or clusters from a cluster analysis). The test allows groups of genes of different size to be compared, because the test gives one p-value for the group, not a p-value for each gene. Researchers can use the test to investigate hypotheses based on theory or past research or to mine gene ontology databases for interesting pathways. Multiple testing problems do not occur unless many groups are tested. Special attention is given to visualizations of the test result, focussing on the associations between samples and showing the impact of individual genes on the test result. An R-package globaltest is available from http://www.bioconductor.org.