In order to investigate the potential effect of genistein we have tested different steps in the mitochondrial apoptotic pathway that may be affected by genistein in B-CLL.
Genistein was found to have pro-apoptotic and antiproliferative efects in many cells types and in this particular cell line (EHEB) our results indicated that genistein, down regulate the expression of bcl-2 anti-apoptotic protein, upregulate the expression of bax proapoptotic protein and induce dissipation of the mitochondrial transmembrane potential.
All this data suggest that genistein is implicated in reestablished of a normal apoptotic process in leukemic cells and also that this agent may be used in chemoprevention or for new strategies of combined therapy for this type of leukemia.

790 Scribble deficiency: a novel model of prostate cancer

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Prostate cancer is a heterogeneous and multifocal disease, which is currently the most commonly diagnosed male cancer in Australia. The adult prostate gland is a highly organised network of ducts composed of polarised layers of epithelial cells. Loss of polarity is a hallmark of epithelial cancer progression, suggesting that cell polarity mediators may play a crucial role in prostate tumourigenesis. The polarity regulator Scribble (Scrib) regulates several events that have been shown to be deregulated in epithelial cancers, including apical-basal cell polarity, proliferation, migration, apoptosis and stem cell maintenance [1]. Scribble mislocalisation and deregulated expression have been observed in both human colon adenocarcinoma [2] and mammary tumours [3]. This suggests that Scribble may be crucial for the homeostatic maintenance of other epithelial tissues by coordinating multiple biological processes and signalling pathways that underlie its tumour suppressive function. To address the role of Scribble within prostatic epithelium we have generated a cohort of Scribble heterozygous (Scrib+/−) transgenic mice, as Scribble null mice are neonatal lethal. Histological analysis of Scrib+/− male mice revealed a predisposition to prostate hyperplasia. These lesions display a marked increase in proliferation, androgen receptor expression and activated MAPK signalling. Taken together, this data indicates that Scribble plays a tumour suppressive role within the prostate and presents a direct mechanism for tumourigenesis, whereby Scribble loss instigates deregulation of both the androgen and MAPK signalling networks. By crossing Scribble floxed (Scribfl) mice to the PBCre transgenic line we have been able to specifically deplete Scribble within prostate epithelial cells. PBCre Scrib+/fl mice also displayed prostate hyperplasia indicating that the observed phenotype is cell intrinsic. Immunohistochemical analysis of a human prostate tissue microarray has validated this novel murine prostate cancer model, revealing a correlation between Scribble mislocalisation and advanced stages of prostate cancer. It is hoped further dissection of the molecular mechanisms underlying the development of prostate cancer in the context of Scribble loss will divulge innovative therapeutic routes of intervention in the clinic.

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Tuesday 29 June 2010 09:45−17:30

Poster Session
Oncogenomics

791 An oligo microarray design for detection of known and putative oncogenic fusion transcripts

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Background: In a pilot study, we have validated a novel approach for fusion gene detection, using a custom made oligo microarray combining direct measurements of chimeric transcript junctions with shifted expression levels between sequences up-and downstream of the fusion break-points (Skotheim *et al*., Mol Cancer, 2009). We have now further developed this universal fusion gene detection tool to cover all known fusion genes and the design also include novel fusion transcripts identified from deep-sequencing studies (*e.g*. Maher *et al*., Nature, 2009) to serve as a high-throughput validation strategy.

Material and Method: A database including 556 fusion genes has been compiled by a combined literature survey and database integration. A Python script, using the exon sequences from all known fusion partners as one of the inputs, generates 599,000 oligos covering all theoretically possible exon-exon junctions between known fusion gene partners as well as all exons in the different genes. NimbleGen HD2 3-plex microarrays (max. 3 × 720 k oligos per slide) are used as platform for custom production of the fusion gene microarray. Also, a prototype automated scoring of all potential fusion transcripts has been developed. We are now utilising this universal assay to investigate the presence of fusion genes in a series of 67 cell lines from 15 different cancer types.
Results: In five out of ten leukaemia cell lines with known fusion gene status, the correct fusion transcript score as the number one hit among the 1,180,103 theoretical combinations per sample. These include *BCR-ABL1* (cell lines KU812 and K562), *TCF3-PBX1* (RCH-ACV and 697), and *MLL-MLLT3* (THP-1). Among the remaining 57 cell lines, we have found promising hits in several cancer types, including colorectal. An RT-PCR-based approach has been initiated to experimentally validate the presence of fusion genes in cancer types without previous fusion gene record.
Conclusions: We present here the 2nd generation of a universal microarray based assay for detection of oncogenic fusion transcripts. With this new and improved assay we are able to identify the correct fusion genes in several cell lines with known fusion gene status. Furthermore, promising hits are found in cancer types not previously known to carry fusion genes.

792 The cancer cell line project – systematic resequencing of known cancer genes in over 750 cancer cell lines

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Cancer cell lines are used extensively as model systems in many areas of cancer research. An understanding of their genetic background allows for a more informed choice of cell lines for biological experiments and drug screening, and helps with the interpretation of the results.
At the Cancer Genome Project, the Cancer Cell Line Project is a large project set up to characterise a set of over 750 cancer cell lines. The cell lines in the set are derived from a wide variety of different cancers, with examples from all the major types. We have included many of the most commonly used cell lines, including the NCI60 set. As part of this project we have systematically screened the cell lines for mutations in a set of known cancer genes. Mutation screening was performed by capillary resequencing of PCR products covering all the coding exons of the genes. In addition, homozygous deletions of six tumour suppressor genes (CDKN2A, PTEN, RB1, STK11, MAP2K4 and SMAD4) were investigated by multiplex PCR and agarose gel analysis. Copy number data from SNP6 Genome Wide Affymetrix arrays is also available for the majority of the cell lines.
To date we have screened 58 known cancer genes for mutations. The results are released regularly on our COSMIC (catalogue of somatic mutations in cancer) web site (http://www.sanger.ac.uk/genetics/CGP/CellLines/). Over 1700 mutations have been released so far on the cancer cell line web pages. These mutations, classified as likely to be oncogenic, are sequence changes which have previously been shown as somatic mutations in cancer or are consistent with the position and type of mutations for a given cancer gene. An additional 2100 variants, also identified in the screen, are available to down load from the web site. The role in oncogenesis of these additional variants is considered tentative or unknown. Matched normal controls are not available for the vast majority of the cell lines. Therefore, the additional set of variants will include rare SNPs as well as passenger somatic mutations.
This ongoing project provides an extensive resource of genetic information on a large set of publically available cancer cell lines. The data can be utilised not only for own in house research projects but is freely available for public use. The data set increases the value of these cell lines as reagents for drug discovery and the evaluation of new therapies.

793 Integration of gene expression and DNA copy number changes in progressive vs. complete response ovarian cancer samples improves survival prediction

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The Cancer Genome Atlas (TCGA) project has generated a very significant quantity of genomic data for different cancer types. The availability of such large amount of high quality data can be viewed as a goldmine for establishing better understanding of the biological processes in cancer, similarities and differences between cancer types and the complex interaction of various genetic and epigenetic changes in cancer. Here, we will describe our analysis of DNA copy number changes and mRNA expression changes in the Ovarian Cancer dataset. We obtained from the TCGA web site raw data for a total of 489 samples hybridized on 2-color oligo-arrays with 244 K probes per array. Raw log-ratio data representing DNA copy number values relative to a DNA pool of normal samples were used in the analysis. The raw data

was processed using Nexus Copy Number version 5 (BioDiscovery, Inc., El Segundo, CA) and regions of copy number change were identified using the built-in FASST segmentation algorithm with significance threshold of 1.0E-5. Our sample set contained 237 Primary Tumour samples having therapeutic outcome classified as either Complete Response (n = 130), Partial Response (n = 33), Stable Disease (n = 2), Progressive Disease (n = 15), and Unknown (n = 57). Using a Fisher's Exact test and a maximum p-value of 0.05 we identified significant regions of copy number change between the Complete Response (CR) group and the Progressive Disease (PD) group. These areas included greater loss of 4q13.3−4q35.2, increase in copy number gain for the short arm of chromosome 5 and 6p21.33−6p12.1, in CR vs. PD among other changes. Collectively these regions include 1938 genes and 59 miRNAs. Performing enrichment analysis on these genes, Complement activation, positive regulation of cell-substrate adhesion, and positive regulation of blood vessel endothelial cell migration, are of the highest statistical significance. Taking advantage of mRNA expression data on the same samples, we identified 20 genes that were significantly differentially expressed between the PD and the CR groups from this subset of genes. These 20 genes were then used to cluster the samples into 5 different groups and generate Kaplan-Meir survival analysis showing different degrees of survival for each group with log-rank significance as low as 0.002. This work has allowed us to create a 20 gene signature that is able to predict survival better than just response to therapy.

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794 Allele-specific copy number analysis of breast carcinomas

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Background: Whole genome SNP genotyping is an expanding technology to measure genomic aberrations in an allele-specific manner. However, to accurately index all genomic aberrations in a cancer sample, both the ploidy of the cancer cells and the infiltration of non-aberrant cells need to be accounted for in the analysis.
Materials and Methods: We performed genotyping of 112 breast carcinoma samples using Illumina 109K SNP arrays and constructed an algorithm (ASCAT, Allele-Specific Copy number Analysis of Tumours) to estimate the fraction of aberrant cells and the tumour ploidy, and to index all genomic aberrations taking both properties into account. ASCAT allows calculation of "Tumour Profiles" (genome-wide allele-specific copy-number profiles) from which gains, losses, copy-number-neutral events and LOH can accurately be determined.
Results: We present the first allele-specific copy number analysis of the *in vivo* breast cancer genome. Using ASCAT, we obtained Tumour Profiles for 91 of the breast carcinomas (81 %). We observe aneuploidy (>2.7n) in 45% of the cases and an average non-aberrant cell admixture of 49%. By aggregation of Tumour Profiles across our cohort, we obtain genomic frequency distributions of gains and losses, as well as first-time genome-wide views of LOH and copy-number-neutral events in breast cancer. In addition, the Tumour Profiles reveal differences in aberrant tumour cell fraction, ploidy, gains, losses, LOH and copy-number-neutral events between the five previously identified molecular breast cancer subtypes. Basal-like breast carcinomas have a significantly higher frequency of LOH compared to other subtypes, and their Tumour Profiles show large-scale loss of genomic material during tumour development, followed by a whole-genome duplication, resulting in near-triploid genomes. Finally, from the Tumour Profiles, we construct a genome-wide map of allelic skewness in breast cancer, indicating loci where one allele is preferentially lost while the other allele is preferentially gained. We hypothesize that these alternative alleles have a different influence on breast carcinoma development.
Conclusions: We infer tumour ploidy, non-aberrant cell admixture and Tumour Profiles (genome-wide allele-specific copy-number profiles) from genome-wide SNP data of breast cancers and identify specific signatures of aberrations in breast carcinoma and breast carcinoma subtypes.

795 Integrative analysis of genome-wide genetic and epigenetic changes in human osteosarcomas

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Background: Genetic and epigenetic alterations are frequently seen in cancer, and are responsible for the deregulation of differentiation and proliferation programs. Using different high-throughput technologies, we have analysed genetic and epigenetic changes genome-wide in the well-characterised EuroBoNeT panel of 19 human osteosarcoma cell lines [1]. By integrating different types of genome-wide information, including DNA copy number changes, mRNA expression and promoter DNA methylation, we can identify important genes and transcriptional networks for osteosarcoma development.
Material and Methods: We have analysed the panel of 19 osteosarcoma cell lines, as well as four normal bone samples and two primary osteoblast cultures. DNA copy number changes have been mapped at high resolution using the Affymetrix Genome-Wide Human SNP Array 6.0, methylation of more than 27,500 CpG islands have been analysed using the Illumina Infinium Methylation27 BeadChip, and global gene expression patterns have been obtained using the Illumina HumanWG-6 Expression BeadChip. Data integration is performed using R scripts, and pathway and network analyses are done using GeneGO.
Results: We have identified a number of recurrent regions of DNA copy number changes in the osteosarcoma cell lines, and a comparison between the cell lines and normal bone revealed a number of genes with altered expression and DNA promoter methylation. Integration of the different types of genome-wide data revealed a number of recurrently altered genes involved in important biological functions. DNA copy number, DNA promoter methylation and mRNA expression was further integrated and compared between osteosarcoma cell lines and osteoblast cultures. Further analysis showed a large overlap between the genes identified for each comparison, suggesting multiple types of aberrations in a limited number of critical pathways. Important alterations identified will be validated and further investigated in the EuroBoNeT panel of osteosarcoma patient samples.
Conclusions: Using an integrative approach, we have identified genes, pathways and transcriptional networks frequently altered in human osteosarcomas. These genes may play an important role for osteosarcoma development and will be further investigated.
The EuroBoNeT osteosarcoma cell line panel will serve as a well-characterized genetic and epigenetic model system for basic and preclinical studies.

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796 Molecular diversity in ductal carcinoma in situ (DCIS) and early invasive breast cancer

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Background: Ductal carcinoma in situ (DCIS) is a non-invasive form of breast cancer where cells restricted to the ducts exhibit an atypical phenotype. Some DCIS lesions are believed to rapidly transit to invasive ductal carcinomas (IDCs), while others remain unchanged. Existing classification systems for DCIS fail to identify those lesions that transit to IDC.
Materials and Methods: We studied gene expression patterns related to progression of breast cancer in 31 pure DCIS, 36 pure invasive cancers and 42 cases of mixed diagnosis (invasive cancer with an in situ component) using Agilent Whole Human Genome Oligo Microarrays 44k. Six normal breast tissue samples were also included. qRT-PCR was used for validation. Various analytical methods were used such as hierarchical clustering, Significant Analysis of Microarrays (SAM), Gene Set Enrichment Analysis (GSEA), Database for Annotation, Visualisation and Integrated Discovery (DAVID), and a logistic regression model for gene identification.
Results: All DCIS and invasive samples could be classified into the "intrinsic" molecular subtypes defined for invasive breast cancer. Hierarchical clustering revealed a trend to group by intrinsic subtypes, and not by diagnosis. We observed heterogeneity in the transcriptomes among the DCIS of high histological grade and identified a distinct subgroup containing seven of 31 DCIS with gene expression characteristics more similar to advanced tumours. A set of genes independent of grade, and both ER and HER2 status was identified by logistic regression that univariately classified a sample as belonging to this distinct DCIS subgroup. qRT-PCR of single markers clearly separated this DCIS subgroup from the other DCIS, which consisted of both high and low grade lesions as well as ER- and HER2-positive and negative