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

samples. The specific expression profile reflects activated processes related to re-organisation of the microenvironment.
Conclusions: The DCIS lesions within the subgroup are diverse in their classic histopathological subtypes and intrinsic molecular subtypes, suggesting that the signature inherent in these lesions is common across breast cancer subtypes. This raises interesting possibilities for identification of DCIS lesions both with and without invasive characteristics, which potentially could be used in clinical assessment of a woman's risk of progression, and lead to improved management that could avoid the current over- and under-treatment of patients.

797 Modeling BRCA2 associated breast cancer progression through genomic profiling

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Background: During the development and progression of cancers, their genomes undergo different types of modifications, including DNA copy number changes by which gene expression can be affected. In our previous report, we showed that genomic alterations occur in non-random patterns throughout breast cancer genomes which, furthermore, relate to *BRCA* abnormalities and molecular phenotypes (Stefansson et al 2009). The study presented here was carried out to follow-up on results described in our previous report which suggested progression for *BRCA2* tumours involving degree of genomic complexities and histological grade.
Materials and Methods: Copy number changes in 34 breast tumours derived from *999del5 BRCA2* germline mutation carriers were analyzed by high-resolution (~7kbp) array comparative genomic hybridization (385K aCGH; NimbleGen Systems). Tumour phenotypes were established by analysis of expression using immunohistochemistry (IHC) on tissue arrays for selected biomarkers (ER, PR, HER2, EGFR, CK5/6, Ki-67, RB and p16) and histologic grade was determined by the modified Bloom-Richardson system.
Results: Molecular characteristics and patterns of copy number changes differed substantially between *BRCA2* tumours displaying luminal- and triple-negative phenotypes. The observed differences include deletions at the *BRCA2* gene locus which were strongly associated with increased growth advantages in *BRCA2* tumours displaying luminal characteristics reflected in expression of Ki-67 proteins. The same was not found for triple-negative *BRCA2* wherein the event of *BRCA2* deletion appears to be stochastic. Network analysis for copy number changes identified several candidate genes that may cooperate with loss at the *BRCA2* gene locus.
Conclusions: The differences identified between *BRCA2* tumours displaying luminal- and triple-negative phenotype suggests that they have developed in different ways and we show here that this involves the *BRCA2* gene locus. These results have potential implications regarding therapeutic choice for future patients with *BRCA2* germline mutations.

798 Transcriptional modules predicting response of colorectal cancer to EGFR-targeted therapy

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Background: Only a fraction of colorectal cancer (CRC) patients respond to antibodies targeting Epidermal Growth Factor Receptor (EGFR), such as cetuximab. It is known that oncogenic mutations in KRAS or BRAF, downstream effectors of EGFR, impair such response. Such cases however account for only about 70% of the non-responder cases. We considered that gene expression profiling could provide new response predictors for CRC cases with wild-type KRAS or BRAF, and developed two complementary molecular signatures respectively linked to "untractable" and "tractable" EGFR pathway activation, and therefore associated with resistance and sensitivity to EGFR-blocking therapy.
Material and Methods: We collected tissue samples from 93 liver metastases of CRC and carried out global gene expression profiling and mutational profiling for KRAS and BRAF. Using mutational information, we derived a transcriptional signature of genes associated to KRAS mutation, whose summarized expression was defined as the "KRAS signature". We also carried out transcriptional profiling of the response to targeted therapy of various cancer cell lines addicted to EGFR or BRAF oncogenic signaling, and defined a common *in vitro* "Addiction signature", whose genes were mapped and further analyzed in CRC expression datasets.
Results: In our CRC dataset, the KRAS signature sharply distinguished a high-score group, formed not only by samples with mutated KRAS or BRAF but also by some non-mutated samples, and a low-score group, formed by the remaining non-mutated samples. Interestingly, also the Addiction signature partitioned the samples in well-distinguished subgroups, but the partition was only partially overlapping with that of the KRAS signature. We then analyzed the behavior of the two signatures in an independent dataset of CRC-liver metastases, annotated with the mutational status and with the response to cetuximab, administered after the biopsy. In samples with wild-type KRAS or BRAF, both the KRAS and the Addiction signatures were correlated to responsiveness in an opposite manner: drug resistance was associated to either very high KRAS signature or very low Addiction signature. In one case, therefore, the RAS pathway was "untractable", similar that of mutated KRAS-driven cases, in the other case the pathway was not active at all, and therefore not responsive to inhibition. According to this hypothesis, the combination of the two signatures (Addiction signature minus KRAS signature) yielded a much more robust response predictor, confirming that responders must have an active EGFR pathway (high Addiction signature), but still a "tractable" one (low KRAS signature).
Conclusions: These data show that gene expression profiling can be successfully used to dissect the molecular alterations that take place in colorectal cancer and to define how they determine response to targeted therapy also in cases without KRAS or BRAF oncogenic activation.

799 Molecular subgroups of breast cancer show distinct genomic profiles and different clinical courses: a novel definition of disease subclasses

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Background: Breast cancer is notoriously heterogeneous and molecular studies have fostered great expectations in the prospect of defining a renewed classification of breast cancer. However the definition of breast cancer molecular subgroups has been debated, pointing at their instability and elevated dependence on the original set of samples or genes. Three broad classes of breast tumours, commonly used in the clinic, were drawn along their ER, PR and HER2 status but this simple classification lacks precision.
Materials and Methods: We believe that breast cancer can be broken down in smaller and more homogeneous subsets based on their genetic characteristics. To reach such a goal we worked on a large dataset (comprising 712 breast tumours 537 analyzed for expression Affy U133A chips and 655 by BAC-array CGH) in order to enhance statistical power and build a robust molecular classification.
Results: Using a combination of supervised and unsupervised analysis of expression profiling data we defined 6 well-delineated molecular subgroups. Array-CGH analysis revealed that each of the 6 molecular subgroups showed distinct profiles of copy number and associated gene expression changes that will be presented. Of particular notice were the findings of chromosomal regions showing inverse patterns (gain in one subgroup/loss in another). We could associate to each molecular subgroup a specific set of signaling pathways and interaction networks. These differences at the molecular level were consonant with significant differences in tumour grade, metastatic sites, relapse free survival among molecular subgroups. Furthermore, we determined the existence of important differences in response to chemotherapy among subgroups and showed that our classification bore independent prognostic power.
Conclusion: Owing the strong prognostic significance of this classification we propose that it could be the keystone of future investigations aiming at identifying novel prognostic factors or therapeutical targets in breast cancer.

800 Genome copy number variation and gene mutation profiling show different somatic development of colorectal cancers in young and elderly patients

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Background: Colorectal cancer (CRC) is one of the most common cancers in the Western world, and has an average age of diagnosis around 70 years. The majority of patients have tumours with sporadic origin, and less than five percent of all CRC cases have a known hereditary defect causing the disease. However, young age at diagnosis, and/or familial clustering of cancers without known hereditary cancer syndromes, indicate a genetic increased risk and the