Materials and Methods: We have tested a CRC biomarker DNA chip (*RanplexCRC®*, Randox Laboratories) which assays 28 mutations in 4 CRC-related genes (*K-ras, APC, BRAF, TP53*). These represent the most genetic markers ever to be tested simultaneously in a CRC screening study. Our test series include (1) DNA from the matched tumour and normal tissue of German CRC patients (N = 55) and (2) An Irish sample cohort (N = 250) from participants who have undergone colonoscopy following positive iFOBT results, comprising samples of stool and tissue from 100 patients with polyps and advanced adenomas, 50 with CRC, and stool from 100 individuals normal after colonoscopy. We use spin column extraction (using sample specific *Qiagen* kits) for DNA isolation.
Results: From the German CRCs, mutations were observed in 71% of the cancerous tissues (39/55) and 25% of the matched 'normal' tissue (13/55). In a majority of cases mutations were from *Kras* and *p53* (30 of the 46 mutations detected), suggesting a signature for advanced stages as the German CRCs tested were stage 2 and 3. The majority of the mutated tumours had only one mutation (25 out of 39). To test assay utility in screening for colorectal neoplasms we are currently examining in our Irish iFOBT +ve cohort how the mutation profile in the disease and normal matched tissue is reflected in DNA isolated from the stool of our patients. Mutation detection is possible in 1000-fold excess of wild-type DNA. The initial advanced adenoma results (8 mutated out of 16) indicate a maximum detection rate of 50% for these highly screen relevant growths, which if confirmed in the stool screening would be superior to FOBT. None of the matched normal adenoma tissues were mutated.
Conclusions: These data confirm the high detection rate of DNA mutations in CRCs and show that mutations associated with CRC carcinogenesis can be detected from advanced precancerous colorectal lesions using the *Ranplex* method. Genetic assays have great potential to achieve highly specific and sensitive detection of colorectal neoplasias, especially for eminently treatable early stage cancers and advanced adenomas. In addition the types of mutations detected, such as in *Kras*, will also provide important molecular prognostic information separate to detection alone.

129 Molecular diagnosis of pancreatic cancer using a combination of genetic and epigenetic markers

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Background: *KRAS* mutations have proved useful as a diagnostic marker in FNA of pancreatic masses. Methylation markers have shown promise in the early diagnosis of pancreatic carcinoma. The aim of this study was to assess the diagnostic utility of hypermethylation status of candidate genes in combination with *KRAS* mutation detection in the evaluation of pancreatic masses.
Experimental design: As a first step the methylation status of *HRH2*, *EN1*, *SPARC, CDH13, APC, RASSF1A, TMS1, CRBP1, INHBB, CPLX* and *RARB2*, was tested in paired tumoural and non tumoural tissue samples of 11 primary pancreatic adenocarcinoma by melting curve analysis (MCA) optimized to have an analytical sensitivity of 5%. Sixty-one Fine Needle Aspirates (FNA) of pancreatic masses (43 pancreatic adenocarcinomas and 18 chronic pancreatitis) were studied; their methylation status was analyzed using Melting Curve Analysis after DNA bisulfite treatment. *KRAS* mutations were detected by a Restriction Fragment Length Polymorphism/Polymerase Chain Reaction (RFLP/PCR) method.
Results: Five genes where promoter hypermethylation was detected in more than 50% of analyzed tumours were selected: *HRH2*, *EN1*, *SPARC*, *CDH13* and *APC*. The methylation panel had a sensitivity of 73% (27 of 37) and a specificity of 100% whenever two or more promoters were found hypermethylated. *KRAS* mutations showed a sensitivity of 77% (33 of 43) and a specificity of 100%. When combined, genetic and epigenetic analyses, resulted in a sensitivity of 84% maintaining the 100% specificity.
Conclusion: Both genetic and epigenetic molecular analyses offer an excellent diagnostic yield in this setting allowing cancer diagnosis in an overwhelming majority of the cases. The good clinical performance is more relevant at a time where EUS-guided FNA is increasingly used in the preoperative evaluation of small pancreatic masses.

130 GINS proteins as candidate markers for urine-based cancer screening

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Background: A urine-based screening strategy for urogenital tract malignancies would offer an affordable non-invasive alternative to current approaches. The GINS complex (a heterotetramer of Sld5, Psf1, Psf2 and Psf3) is a highly conserved DNA replication factor. GINS interacts with the MCM complex in eukaryotes and archaea. Given the value of MCM proteins as specific and sensitive markers for cancer screening, we investigated whether GINS subunits might also be of potential diagnostic value in applications where the sensitivity and/or specificity of MCM proteins is low.
Materials and Methods: We investigated the expression of GINS proteins in human cell lines by immunoblotting, and in tissue sections by immunohistochemistry. We also tested the stability of GINS proteins by incubating intact tissue culture cells or cell lysates in urine. Finally, we have set up an ongoing clinical study of Sld5 protein as a potential biomarker in urine samples from prostate and bladder cancer patients. In this study, we have screened for Sld5 and Mcm2 by multichannel immunofluorescence microscopy in the cellular fraction and measured Sld5 protein levels in the urine supernatant by ELISA.
Results: Consistent with the published findings for MCM, GINS proteins are expressed in all cycle phases of cultured proliferating cells, but are down-regulated in cells undergoing quiescence. Examination of histological sections indicates that Sld5 and Psf3 expression is restricted to the proliferative compartment in normal tissue, but spreads to the majority of cells in a wide range of dysplastic and malignant tissues, including cervix, colon and skin. Our urine spiking experiments suggest that Sld5 protein is more stable than Mcm2 in harsh extracellular environments. In the pilot clinical study of over 50 prostate and bladder cancer patients, Sld5 was readily and specifically detectable in the cellular fraction of the samples from cancer patients. We also present an evaluation of Sld5 protein levels in the supernatant portion of those same urine samples as an easy-to-screen diagnostic/prognostic marker for male urogenital cancers.
Conclusions: Owing to their stability, GINS proteins hold promise as independent or complementary markers to the MCM proteins for cancer screening in harsh extracellular environments such as urine. Work is ongoing to identify further applications of GINS in cancer screening and prognosis.

131 Relevance of copy number alterations in Ewing Sarcoma: gain of 1q defines a subset of patients with worse outcome, probably caused by DTL/CDT2 overexpression and subsequent cell cycle deregulation

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Background: Despite extensive characterization of the molecular implications of EWS-ETS, little is known about secondary genetic alterations in Ewing Sarcoma (ES), the only ones that could shape molecular subtypes of the disease with prognostic significance. Research in this field is crucial since the prognosis is poor in patients with primary disseminated disease or after relapse, with a survival rate of nearly 20%.
Material and Methods: *Patient and samples*: tumour samples from a total of 105 ES patients were included in the study along with 16 ES cell lines.
BAC-microarrays: Sanger 1Mb BAC collection was amplified and spotted in triplicate onto Codelink slides (GE). Cy5/Cy3-dCTP-labelled DNA from tumour samples and healthy donors were co-hybridized with the BAC-microarrays.
Expression microarrays and SNP-microarrays: Total RNA from *DTL*-silenced cell lines was extracted with Trizol (Invitrogen) and hybridized with the GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix) after quality assessment using an Agilent 2100 Bioanalyzer (Agilent).
Lentiviral shRNA transduction of ES cell lines for functional validations: The MISSION shRNA collection of 5 pLKO.1-shRNA constructions (SIGMA Aldrich) was selected for assays aimed at reducing *DTL* overexpression.
Results: We assessed copy number alterations (CNA) in **67 ES tumours**, finding 1q gain (31% of tumours) markedly associated with relapse and poor