analysis of genomic and cDNA PCR products on an ALF DNA sequencer allowed the identification of APC mutations.

Results: 125 FAP families were enrolled in our genetic analysis program. We detected APC germline mutations in 68% (85/125) of these families. These 85 families represented 123 FAP-patients and 82 individuals at risk. More than 90% (75/82) of the at-risk individuals were excluded as mutant allel carriers, causative APC germline mutations were detected in about 9% of at risk individuals. In one case mutation detection led the patient to undergo endoscopy.

Conclusions: This study demonstrates the potential of combinatorial molecular and clinical approach for genetic screening of FAP families and could serve as a general strategy for routine molecular diagnostics in a clinical laboratory. New genotype-phenotype correlations might further improve the efficacy of the molecular diagnosis of affected FAP patients.

33 ORAL

Cancer phenotype in 19 families with TP53 mutations

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Purpose: The main components of classical Li-Fraumeni cancer family syndrome (LFS) are sarcomas, breast cancer, brain tumours, adrenocortical carcinoma (ACC) and leukaemia Germline TP53 mutations are detected in more than 50% of LFS families and in some families not strictly conforming to LFS criteria. We have investigated 40 LFS and LFS-like families to define the characteristics of cancers in carriers of TP53 mutations.

Methods: TP53 mutation status was analysed by direct sequencing of constitutional samples. In families with mutations, the distribution of cancers by age and morphology in mutation carriers was analysed.

Results: Mutations were detected in 19 families. First primary cancers in carriers included 20 sarcomas, 14 breast cancers, 9 brain tumours, 5 ACC, 2 leukaemias and 1 each of 4 other types. All except 3 cancers were diagnosed under 40 years of age. There were 7 unaffected carriers aged 9 to 50 years. Multiple primary cancers developed in 18 patients.

Conclusion: TP53 mutation phenotype closely resembles classical LFS. The age distribution of cancers is much younger than in other cancer family syndromes. Very high penetrance with greatest risk under 30 years.

34 ORAL

Inhibition of tumor growth by an antibody against a cell subtrate adhesion molecule

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Purpose: The cell substrate adhesion molecule (defined by the MAb14C5) is typically present in high amounts on the tumour surface of in-situ and invasive breast cancer tissue. Furthermore the cell substrate adhesion receptor is involved in the metastatic process. We introduced the mAb 14C5 in an in-vivo model to prove his capacity for inhibition of invasion and metastasis.

Methods: The model uses a tumor cell line (HH16cl. 1/2, rat adenocarcinoma/fibrosarcoma), which is overexpressing the human tumor-associated antigen CA 14C5. 6 day old Sprague-Dawley rats (n = 2 × 12) received tumor cells,; (2 × 10^6) subcutaneously. After one week series Ab1 received mAb 14C5 intraperitoneally at a dosage of 100–250–500 μg weekly (n = 4 per group, total group n = 12). A control group received polyvalent mouse IgG at the same dosage. The tumor incidence in the used model was >90%. The tumor growth was evaluated over a period of 60 days. 8 applications were administered in total.

Results: The results showed a dose dependent highly significant difference in the tumor growth as the 14C5 treated group developed a mean tumor size of 15.3 ± 18.1 mm and the control showed a mean diameter 37.2 ± 14.9 mm ($p < 0.005$ t-test).

Conclusion: In summary, the monoclonal antibody 14C5 against a human cell substrate adhesion molecule is able to inhibit tumor growth and invasion of CA14C5 overexpressing tumors in-vivo and therefore serves as a target for passive cytotoxic immunotherapy.

35 ORAL

Germline mutations in the CDKN2 and CDK4 genes are rare in melanoma families in the UK

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Purpose: Germline mutations in genes having a role in controlling phase G1 of the cell cycle (CDKN2 and CDK4) have been reported worldwide in melanoma families. A limited number of mutations in the CDKN2 gene have been reported in Europe (In Swedish and Italian families). We collected melanoma families in the UK with the purpose of establishing the frequency of germline mutations in the UK

Methods: Families with a history of melanoma were interviewed and examined and blood was collected for DNA extraction. All coding sequences of the CDKN2 and CDK4 genes were sequenced.

Results: 100 families were recruited to the study. From these, 14 were selected with at least 3 cases of melanoma, or 2 cases one of which had the Atypical Mole Syndrome (AMS) phenotype or multiple primaries. 5 families with 1 case of melanoma but multiple cases with the AMS were also selected. In these 19 families only one mutation in the CDKN2 gene was identified: a 24 base pair insertion in exon 1. No CDK4 mutations were identified.

Conclusion: Although there is no doubt now that germline mutations of the CDKN2 and CDK4 underlie susceptibility to melanoma in some families, the percentage of such families is low. It seems likely that other melanoma susceptibility genes remain to be identified.

36 ORAL

Allelotype of Barrett's adenocarcinoma: A search for novel tumour suppressor genes

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The loss of function of a tumour suppressor gene can be identified by loss of heterozygosity (LOH) studies, which were performed on 22 cases of Barrett's adenocarcinoma. All chrmosomal arms were studied using 135 polymorphic markers, and eight arms demonstrated LOH in more than 40% of tumours: 3p, 5q, 9p, 11p, 13q, 17p, 17q and 18q. Further refinement of these areas of high LOH indicated that LOH on chromosomes 3p, 9p, 13q, 17p and 18q occurred at the sites of the VHL, MTS1, Rb, p53 and DCC tumour suppressor genes respectively. The refined sites on 5q, 11p and 17q are thus putative sites of novel tumour suppressor genes associated with the development of adenocarcinoma in Barrett's oesophagus. It is of note that 70% of tumours displayed LOH at the 17q11.2–q12, and we propose that this is the site of the main tumour suppressor gene associated with the development of Barrett's adenocarcinoma. These tumour suppressor genes may be useful as markers of future carcinogenesis in patients undergoing endoscopic surveillance for Barrett's oesophagus.

37 ORAL

Breast & ovarian cancer risk in BRCA mutation carriers: Implications for prevention

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Purpose: To assess the risk for breast and ovarian cancer in BRCA 1/2 mutation carriers and to suggest prevention policies.

Methods: Ashkenazi Jewish patients were analyzed for mutations common in this population: BRCA1-185delAG, 5382insC and BRCA2-6174delT.

Results: For breast cancer, mean age of onset was 38 yrs. (median 45 yrs., range 26–50, n = 11) and 48 yrs. (median 45 yrs., range 31–67, n = 7) for BRCA1 and BRCA2 respectively ($p = 0.04$). For ovarian cancer mean age of onset was 50 yrs. (median 48 yrs., range 34–72, n = 12) and 62 yrs. (median 59 yrs. range 42–81, n = 9) for BRCA1 and BRCA2 respectively ($p = 0.02$). In 6 patients with both breast and ovarian cancer (4 BRCA1 carriers, 2 BRCA2 carriers) breast cancer always preceded ovarian cancer, with onset at means of 45 yrs. and 58 yrs. respectively. Analysis of patients' families revealed a significant, twofold higher risk in BRCA1 vs. BRCA2 carriers ($p = 0.01$).

Conclusions and Suggestions: Breast cancer occurred at an earlier age than ovarian cancer in both BRCA 1 and BRCA2 carriers, suggesting that breast cancer surveillance should start early in this group. Bilateral oophorectomy should be considered at the time of breast cancer diagnosis or at completion of childbearing.

Timing of prophylactic measures should take into account the lower risk and later age of onset for the BRCA2 6174delT mutation.

38 ORAL

A common Scottish *BRCA1* mutation

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Aims: To assess which mutations are present in breast cancer families in Scotland.

Methods: Families identified as "at risk" by the Dept. Of Medical Genetics had lymphocyte DNA from index cases in breast and ovarian cancer families screened for germline *BRCA1* and *BRCA2* mutations. The screening was carried out by Protein Truncation Testing (PTT) and Single Stranded Conformation Polymorphism (SSCP) on 40 families. Any mutations found were also screened for in a total of 276 patients.

Results: Individual families were found to have the 185 AG deletion and a C insertion at position 5382 in *BRCA1* a 5445 del 7, a 5574 del AA, and an 8525 del C mutations in *BRCA2*. All resulting in truncated protein products Six separate families however showed the same *BRCA1* mutation, an AA deletion in exon 11 of *BRCA1* at position 2800 (2800 del AA). One individual was shown to be homozygous for this mutation, thereby giving rise to a naturally occurring human "knockout" for this gene. All six families share a common haplotype around the *BRCA1* gene.

Conclusions: There appears to be a common mutation which has a prevalence rate of 2%. We have now cloned portions of wild-type and mutant *BRCA1* genes into bacterial expression plasmids, and isolated recombinant proteins. Using these antigens, we have developed antibodies to the carboxy terminal of the 2800 del AA *BRCA1*, and to 3 portions of the wild-type protein. These are currently being tested to see if they are of use in identifying mutation carriers and in identifying interacting proteins.

39 POSTER

Effects of hypusinylatin of eIF-5a on cell cycle progression and specific mRNA translation in human tumor cells

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Purpose: eIF-5a is very likely involved in the translation of specific mRNA, similar to its function in HIV infected cells (transporting late mRNAs out of the nucleus as a cofactor of HIV-REV). Hypusinylation of eIF-5a is essential for cell division. Inhibition of hypusinylation by mimosine leads to a reversible cell cycle arrest at the G1/S border. We have analysed cell cycle progression and specific mRNA translation in human mammary tumor cells after withdrawal of mimosine.

Methods: Cell cycle progression of mimosine-treated (300 μM/24 h) and released MDA231 cells was monitored by FACS analysis. mRNA translation was analysed by differential display RT-PCR (DD-RT-PCR) of polysomal RNA.

Results: After withdrawal of mimosine, DNA synthesis becomes visible after 3 h and is completed after 8 h. Mitosis occurs after 12 to 15 h. Readministering mimosine 15 minutes after release does not inhibit S-phase entry. However, S-phase is decelerated and mitosis does not occur. DD-RT-PCR analysis indicates that mRNAs can be identified that are bound to ribosomes and thus translated in cycling cells, but not in cells blocked by mimosine and are bound again and translated shortly after mimosine withdrawl (hypusine-dependent mRNAs).

Conclusion: Hypusinylation of eIF-5a influences the generation of structures necessary for S-phase entry and progression. Hypusine-dependent mRNAs that presumably code for proteins involved in S-phase entry and transit can be identified in the beginning of the S-phase. Interference with tumor-specific hypusine-dependent mRNAs may lead to novel strategies to interfere with tumor cell growth. This work was supported by the Wilhelm Sander Stiftung, Grant 96.022.1

40 POSTER

High resolution HLA-DRB1 genotyping in patients with RCC

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Purpose: A variety of malignancies have been linked to MHC complex genes, including the *DRB1* alleles. The association of certain DRB1 antigens with renal cell carcinoma (RCC) has been both claimed and disclaimed. To determine whether HLA-DRB1 genotypes are associated with RCC, we for the first time performed HLA-DRB1 genotyping in RCC patients.

Methods: we used the modified PCR-RFLP method for the high-resolution HLA-DRB1 genotyping of 96 Japanese RCC patients.

Results: There were no significantly frequent *HLA-DRB1* alleles, whereas the *DRB1*0101* and **0405* alleles had significantly lower frequencies (P = 0.004, RR = 0.2 and P = 0.002, RR = 0.4) in the RCC patients than in the healthy Japanese controls (n = 1216). Moreover, patients with the *HLA-DRB1*0101* or **0405* allele tended to be in earlier stages and to have less aggressive tumors than patients with neither of these alleles. The corresponding serotyping subclassification, however, showed a significantly lower frequency only for DRB1-DR1 (P = 0.01, RR = 0.3).

Conclusion: High-resolution genotyping is essential because the polymorphism of the peptide-binding domain of MHC class II molecules is more precisely determined by genotypes than serotypes. In addition, inherent technical difficulties and potential typing errors render serotyping inefficient. Our data suggest that *HLA-DRB1*0101* and **0405* are protective alleles for both RCC development and tumor progression.

41 POSTER

Differential transcriptional regulation of the human gene for the M1 subunit of ribonucleotide reductase by p53

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Purpose: Wild-type p53 is implicated in the regulation of genes involved in cellular proliferation, differentiation and DNA repair. Ribonucleotide reductase is a potential target for p53 dependent regulation as it is an essential enzyme in the production of deoxyribonucleotides required for DNA synthesis and repair. Using transfection studies we have analysed the effects of p53 on the human gene for the M1 subunit of ribonucleotide reductase *(RRM1)*.

Methods: pSGp53, a vector which drives expression of human wt-p53 via the SV40 promoter or pCMVp53, a vector which drives expression of p53 via the CMV promoter was transfected into p53-null K562 cells together with a reporter plasmid containing the *RRM1* promoter sequence.

Results: Expression of p53 by pSGp53 resulted in 3–11 fold transactivation of the *RRM1* promoter. Using stepwise deletions of the *RRM1* promoter, we have identified a region close to the transcription start site which confers p53 responsiveness on the *RRM1* promoter. Expression of p53 by pCMVp53 resulted in repression rather than transactivation of *RRM1* transcription. Quantitation of p53 suggests that this differential effect of p53 on *RRM1* transcription may be related to the amount of p53 protein expressed in the transfected cells.

Conclusion: p53 differentially regulates *RRM1* transcription. This has important implication regarding the role of p53 in DNA repair, growth arrest and apoptosis.

42 POSTER

Unusual distribution of HLA-DRB alleles in tumour patients

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Purpose: Distribution of alleles of DRB locus of HLA class II genes was compared in 212 tumour patients (44 breast, 17 ovarian, 53 colorectal, 23 lung, 10 thyroid, 3 melanoma, 3 soft tissue neoplastic processes and 59 haematological malignancies) and 120 healthy donors.

Methods: Restriction fragment length polymorphism (RFLP) of DRB locus was analyzed by Southern-blot procedure.

Results: The frequency of DRB homozygous patients in both solid tumour group (42 of 153; 27.4%) and leukemia cohort (10 of 59; 16.9%) was significantly higher than in control (9 of 120; 7.5%) (p = 0.000001 and