preliminary data suggest that lowering TAM dose by 1/2 or even by 1/4 still allows maintenance of the full effect on total cholesterol.

28 POSTER

ALL-*trans*-Retinoic (ATRA) acid induces cell cycle perturbations and apoptosis in human breast cancer

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Purpose: The antiproliferative effect of ATRA on MCF-7 human breast cancer cells was correlated with the specific effects on the cell cycle progression and apoptosis.

Methods: These effects of ATRA were investigated using DNA content evaluation and dual parameter flow cytometry (FCM) of bromodeoxyuridine incorporation and of the expression of cell cycle-related proteins (Ki-67 as proliferation marker and Statin as quiescence marker) vs DNA content. Apoptosis was studied by FCM of both DNA content and labelling of phosphatidilserine residues by Annexin V.

Results: After 4-days of ATRA treatment, the % of S-phase cells decreased significantly and cells accumulated in the G0/G1 range of DNA content. FCM analysis showed a decrease in the % of Ki-67+ve cells with a simultaneous increase of the 57% of Statin +ve cells. From 5 days of treatments onwards, apoptosis was found to occur.

Conclusions: ATRA-induced inhibition of MCF-7 cell growth is related with the block of cell proliferation mostly in a pre-DNA synthetic phase and the induction of apoptosis. This should be taken into account in the attempt to associate ATRA with other antiproliferative drugs.

29 PUBLICATION

Presence of germline BRCA2 mutations in sporadic breast cancer: Clinical correlation

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Purpose: Breast cancer is the most common malignancy in women. It occurs in hereditary and sporadic forms. At present, the sporadic breast cancer accounts for 90% of all breast cancers. Germline BRCA2 gene mutations have been identified in families prone to breast cancer. We designed the present study, now under way, to detect the presence of germline mutations at BRCA2 gene in sporadic breast cancer.

Methods: Our series consists of 93 patients diagnosed of breast cancer, without family history of breast and ovarian cancer. The mean age was 55 years, and the median age 53. The following clinical parameters were analyzing: Birth and diagnosis date, family history, menopausal status, histology of tumors, pathological stage, hormonal receptors, survival and vital status. The mutational study was performed by PCR-SSCP in peripheral blood lymphocytes DNA of the patients.

Results: We observed in 4 patients (4.3%) presence of aberrant fragment migration (now under sequencing process), 3 of them located at the same fragment (exon 11.19). The comparison of the 9 clinical parameters, between the two subgroups of patients, with and without mutation, did not show any significant difference.

Conclusion: The prevalence rate of mutations in sporadic breast cancer, considering their age, is higher than expected. It is possible that we detected a founder mutation. No implication of germline BRCA2 mutations as prognostic factor.

30 PUBLICATION

Analysis of bcl-2 and p53 genes in patients with breast cancer

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Methods: 46 primary nonmetastatic breast carcinomas. Immunohistochemical expression of bcl-2 and p53 proteins was analyzed in formalin-fixed paraffin-embedded sections. Exons 5 to 9 of the p53 gene were analyzed by PCR-SSCP in 40 of the 46 tumors. Genomic organization of bcl-2 was analyzed by southern blot from tumor tissues and from the peripheral blood of 11 patients (pt).

Results: There was a significant direct correlation between Estrogen and Progesterone receptor expression and bcl-2 protein expression ($p = 0.01$ and $p < 0.0001$). Significant correlation with tumor size was obtained ($p < 0.0001$) but not with lymph node invasion. We did not detect any molecular abnormality in the genomic organization of bcl-2; None of the 11 pt in whom peripheral blood was analyzed showed loss of heterozygosity at bcl-2 locus. The immunohistochemical expression of bcl-2 was inversely related to p53 protein expression, ($p = 0.05$), however the analysis of the correlation between p53 gene mutations and bcl-2 protein expression did not show significant correlation ($p = 0.79$). p53 gene mutations were present in 12 tumors (30% of the cases). (We detected p53 protein expression in 8 of the 12 tumors carrying p53 mutation. p53 protein staining was also detected in 14 cases without mutations at exons 5 to 9).

Conclusion: We have not found any correlation between bcl-2 immunohistochemical expression and p53 gene mutations. Bcl-2 is expressed in tumors with wild-type p53 as well as in those carrying a p53 mutation. This lack of correlation may reflect that regulation of bcl-2 expression is independent of p53.

Cancer genetics

31 ORAL

DNA mismatch repair deficient tumors exhibit length variability of repetitive DNA sequences in diverse promoter regions

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Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is a cancer predisposing trait characterized by germline mutations in DNA mismatch repair (MMR) genes. Loss of MMR function in tumors of HNPCC patients results in length variability (LV) of DNA repeats within microsatellites, of coding and non-coding sequences. DNA repeats are also present within promoter regions, but have not been analyzed in HNPCC-associated cancer so far. Therefore, we analyzed HNPCC tumors for genetic instability of DNA repeats present in different human promoter regions.

Promoter repeats of the human Interleukin-10 (IL10, $[CA]_{21}$), Retinoblastoma (Rb, $[A]_{20}$), WAF1/CIP/p2l $[A_{12}]$, CyclinB1 ($[A]_{11}$), PhospholipaseA_2 (PLA2, $[CA]_{13}$), Glucokinase (GLK, $[GT]_{15}$), and Collagenase type IV (COL4, $[CA]_{21}$) genes were amplified and analyzed on an A.L.F. DNA sequencer.

11/14 HNPCC tumors showed LV within the IL10, 8/12 in the Rb, 8/14 in the CyclinB1, 9/21 in the WAF1, and 3/14 in the COL4 promoter repeat. In contrast, LV of the PLA2 and GLK promoter repeats was absent in 11 and 3 HNPCC tumors, respectively.

Our data reveal a high frequency of LV of DNA repeats within different promoter regions in tumors with DNA MMR deficiency. Case alterations might affect regulatory properties of the promoter elements and subsequently modify the gene expression profile. This new type of 'promoter instability' (PIN) might represent a common mechanism contributing to the pathogenesis of HNPCC.

32 ORAL

Genetic analysis of familal adenomatous polyposis (FAP) families: Lessons and Implications

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Aim: Germline mutations in the APC gene cause FAP. Correlations between the site of the APC mutation and the manifestation of the disease have been established. The detection of a germline mutation in the APC gene in an affected family member permits the identification of carriers and non-carriers of the mutant APC allele among relatives at risk. Non-carriers may be excluded from further endoscopic screening.

Methods: Clinical parameters were considered before mutation analysis was initiated. The APC coding region was screened for germline mutation using a non-radioactive protein truncation test (PTT). Direct sequence

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 ($p = 0.01$).