showed synergism with LTBI when mice were treated on day +7 has failed to show a therapeutic effect when mice were treated on day +10. High dose IL-2 (600.000 CU) on the other hand, led to a significant reduction in metastatic burden compared to control group. Combining high dose IL-2 with LTBI led to further significant reduction in tumour burden. Moreover, this combination was associated with less vascular leakage syndrome (VLS) compared to IL-2 alone. IL-2 and combination treatment was associated with an increase in the number of tumour infiltrating immune cells, but only the number of tumour infiltrating NK-cells reflected therapeutic efficacy.

Conclusion: We conclude that tumour burden at the time of treatment and IL-2 dose are 2 crucial factors affecting the synergism between LTBI and IL-2. The combination may not only be more effective than IL-2 alone but also less toxic.

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Dolichol as tumour marker in pancreatic cancer control

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Background: Urinary Dolichol (Dol) have been reported to be 5 to 40 times the normal values, suggesting a metabolic abnormality of N-glycoprotein synthesis in patients with cancer. Pancreas contains a highest level of Dol concentration in human tissues. With focus on a tumour marker, the present study was carried out to estimate blood and urinary levels of Dol in patients with pancreatic cancer (PCA) and chronic pancreatitis (CHP).

Materials and Methods: The samples obtained from 28 patients with PCA (male, 32-69 years old) and 40 patients with CHP (male, 36-65 yeras old). Dol in blood and urine was assayed by HPLC method (Turpeinen, 1986).

Results: Dol in healthy men's blood and urine are 125,9 + 7,8 ng/ml and 6,8 + 0,7 mg/mmol creatinine respectively. In CHP Dol content in urine was much the same, but Dol content in blood showed an increase of 18-22% Blood Dol concentration in patients with PCA increased at stage I up to 25%,at stage II up to 45%, at stage III up to 55%, making up 204,5 + 14,9 ng/ml at stage IV. There was a significant difference between urinary Dol content in patients with CHP and that of cancer patients. Urinary Dol concentration increased at stage I up to 75-90%, making up 44,9 + 6,9 mg/mmol at stage II. At stage III the level of urinary Dol was 7-10 -fold increased.

Conclusions: These findings suggest that Dol appeared in urinary excretion is one of the first manifestations of carcinogenesis in pancreas. In this way CHP therapy should be carried out under Dol excreation control. The interest drown to the employment of Dol as marker is explained by the fact that known PCA markers are glycoproteins (CA-50 and CA-19-9). Monitoring CHP patients with monthly urinary Dol determination is a reliable method to diagnose a PCA.

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EBV-DNA in unfractionated whole blood specimens of patients with EBV-related diseases and seropositive healthy individuals

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Epstein-Barr virus is related with various benign and malignant diseases. Circulating EBV-DNA has been detected in virus-related malignancies and has been shown to be valuable in the diagnosis, prognosis and monitoring of such patients. However EBV-DNA can be found in healthy individuals so caution must be used in interpreting such results. We performed a study to detect EBV-DNA in unfractionated freshly obtained whole blood in Greek patients with EBV-related diseases and healthy volunteers. Peripheral whole blood (PWB) from 95 patients with nasopharyngeal carcinoma (NPC), 34 patients with Hodgkin lymphoma (HL), 13 patients with EBV-related non Hodgkin lymphoma (NHL), 10 patients with active infectious mononucle-osis (IM) and 83 seropositive healthy volunteers were collected between December 1999 and February 2003. Total genomic DNA was extracted using kit according to manufacture's protocol. Qualification and quantifi-cation of DNA were performed in all specimens using spectrophotometry. Additionally, randomly selected samples were analyzed using restriction enzymes and agarose gel electrophoresis. The extracted DNA was used

as a template in PCR with specific primers to amplify region of EBNA-1 gene. The protocol used was for 45 cycles. The products of PCRs were analyzed by electrophoresis on 1.5% agarose and visualized after ethidium bromide staining. The detection rate for EBNA-1 was 83% in NPC (79/95 pts), 82% in HL (28/34 pts), 69% in (NHL) (9/13 pts), 80% in IM (8/10 pts) whereas EBV genome was found only in 11 of the 83 healthy volunteers (13%). Differences in the detection rate of EBV-DNA between patients with EBV-related diseases and healthy volunteers were statistically significant (p<0.005) in all cases, whereas odds ratio was 32,31 (95% CI 14.07 to 74.22) for NPC, 30.54 (95% CI 10.30 to 90.51) for HL, 14.72 (95% CI 3.86 to 56.12) for NHL and 26.18 (95% CI 4.90 to 139.69). Our results showed that detection rate was significantly higher in patients with EBV-related malignancies than in healthy controls which suggests a causative role of Epstein-Barr in human carcinogenesis. The results are similar with those reported in literature where peripheral blood cells were used to detect EBV-DNA instead of free-cell plasma or serum. It seems that whole blood could serve as the preferred clinical sample type since it is simple and reflects the total viral load in the circulation.

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ProteinChip® proteomics for early detection of cancer

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The emergence of new sophisticated proteomic tools has opened up a new approach to perform diagnosis, prognosis and therapy monitoring. Where previous protein based methods involving proteins have been based on single protein markers, such as PSA and CA125 with their inherent limitations, these new methods now allow for the monitoring of many proteins simultaneously, thus allowing for the use of patterns and neural networks in the evaluation of data.

Analysis of complex body fluids like serum, plasma, urine or CSF with Surface Enhanced Laser Desorption/Ionization (SELDI) (Ciphergen Biosystems Inc.) allows for direct monitoring of the proteome. This has been exploited when investigating several different cancers. When combining the information of signal intensity and mass of several hundred proteins it is possible to discover patterns of proteins that are unique for certain diseases and thereby perform diagnoses with very high specificity and sensitivity.

This approach has been applied to a range of cancers using serum samples from affected and age matched control individuals. Using as little as 20 μL of serum automated fractionation and ProteinChip® analysis on various surfaces can be performed. Including data analysis to compare profiles between groups hundreds of samples can be run in a matter of days. By submitting SELDI data to a classification software it is possible to build decision trees that yield much higher sensitivity and specificity compared to single marker assays. The same approach can be used to perform prognoses, stage determination and therapy monitoring.

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Effects of p53 status and wortmannin treatment on potentially lethal damage repair in vivo, with emphasis on the response of intratumor quiescent cell populations

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Purpose: To examine the effects of p53 status and wortmannin treatment on potentially lethal damage repair in vivo, referring to the response of intratumor quiescent cells.

Methods: Human head and neck squamous cell carcinoma cells trans-fected with mutant TP53 (SAS/mp53) or with neo vector as a control (SAS/neo) were injected subcutaneously into both the hind legs of Balb/cA nude mice. Mice bearing the tumors received 5-bromo-2'-deoxyuridine (BrdU) continuously to label all proliferating (P) cells in the tumors. The mice then received gamma-rays with or without subsequent wortmannin administration. Right after or 24 h after gamma-ray irradiation alone or 24 h after wortmannin administration following irradiation, the tumors were excised, minced and trypsinized. The tumor cell suspensions thus obtained were incubated with a cytokinesis blocker (= cytochalasin-B), and the mi-cronucleus (MN) frequency in cells without BrdU labeling (= quiescent (Q) cells) was determined using immunofluorescence staining for BrdU. The MN frequency in total (P + Q) tumor cells was determined from the tumors that were not pretreated with BrdU.

Results: On the whole, larger values of MN frequency and surviving fraction were observed in SAS/mp53 cells than in SAS/neo cells, and Q cells showed lower MN frequencies than total cells. Without wortmannin, SAS/neo tumor cells, especially Q cells within SAS/neo tumors, showed large potentially lethal damage repair (PLDR) capacities, compared with total or Q tumor cells within SAS/mp53 tumors that showed little PLDR capacity. Wortmannin treatment inhibited the PLDR in SAS/neo tumors very effectively, but showed no apparent effect on either total or Q tumor cells within SAS/mp53 tumors.

Conclusion: PLDR in vivo was thought to be a p53-dependent event whether in total or Q tumor cell populations and might reflect the nonhomologous end-joining process for DNA double-strand break repair.

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Increased sensitivity in the detection of isolated tumor cells (ITC) in bone marrow by automated screening analysis

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The presence of isolated metastatic cells in bone marrow (BM) of breast cancer patients at the time of diagnosis indicates occult hematogenous tumor cell dissemination and predicts an increased risk for subsequent distant disease. However, manual microscopic evaluation of an adequately high number of BM cells leads to a substantial risk of decreasing read-out, and might, therefore, be unreliable. Purpose of the present study was to evaluate, whether the sensitivity of this method can be improved by using digital microscopy. In a retrospective series of 244 breast cancer patients with stage I-III disease, we analyzed BM aspirates, which were previously stained with monoclonal anti-cytokeratin antibody A45-B/B3. Only well preserved slides were included. All samples, screened manually between 1995 and 1999, were now reevaluated by two independent observers, after pre-screening with the MDS digital microscope (Applied Imaging Inc, USA), without knowledge of the initial results. By manual screening, ITC were detected in 40 patients (16%), compared to 88 patients (36%) using digital microscopy (P

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Correlation between sensitivity to capecitabine in xenograft models and mRNA expression levels of pyrimidine-metabolizing enzymes in tumor tissues

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Background: Analysis of factors predicting sensitivity to anticancer drugs is important and useful not only for selecting potential responders but also for developing new combinations. The oral fluoropyrimidine capecitabine (Xeloda®) is sequentially activated by three enzymes, carboxylesterase, cytidine deaminase (CD) and thymidine phosphorylase (TP), and generates 5-FU selectively within tumor tissues. We have demonstrated previously that the sensitivity of human tumor xenografts to capecitabine correlates significantly with the ratio of TP to dihydropyrimidine dehydrogenase (DPD) in tumor tissues. On the other hand, thymidylate synthase (TS) and other pyrimidine-metabolizing enzymes are also reported as predictive factors for 5-FU sensitivity.

Methods: In the present study, we analyzed the correlation between the sensitivity of xenografts to capecitabine, 5'-deoxy-5-fluorouridine (5'-DFUR, Furtulon® and 5-FU and mRNA levels of the following pyrimidine-metabolizing enzymes: TP, DPD, CD, TS, orotate phosphoribosyl transferase (OPRT), uridine phosphorylase (UP), uridine kinase (UK), UMP kinase (UMPK), ribonucleotide reductase (RNR), thymidine kinase (TK), and TMP kinase (TMPK). mRNA levels in the tumor tissues of 80 xenograft models were determined by real-time RT-PCR.

Results: Significant correlations were demonstrated between mRNA levels of several enzymes: TS, OPRT, UK, UMPK, RNR, TK and TMPK. However, mRNA levels of these enzymes did not correlate significantly with those of TP, DPD, UP and CD. Furthermore, mRNA levels of TP, DPD and CD showed wide variation between xenograft models when compared with levels for other enzymes. Antitumor activity was assessed in 50 xenograft models for capecitabine and 24 models for 5'-DFUR and 5-FU after 3 weeks of treatment at maximum tolerated doses. The antitumor activity

of capecitabine and 5'-DFUR correlated significantly with mRNA levels of TP and with the TP:DPD ratio, whereas the activity of 5-FU correlated significantly with OPRT, TMPK, UMPK and CD. In a multiple regression analysis, only TP and DPD were independent predictive factors for sensitivity to capecitabine and 5'-DFUR and only UMPK was predictive for sensitivity to 5-FU.

Conclusion: The predictive factors for sensitivity to capecitabine and 5'-DFUR in xenograft models are TP and DPD, which are different from those for 5-FU. Therefore, there is a possibility that responders to capecitabine and 5'-DFUR would be different from those responding to 5-FU.

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Expression of hMLH1 and hMSH2 proteins in normal tissues and cancer predisposition in hereditary non-polyposis colon cancer

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Backround: Germline mutations of hMLH1 and hMSH2 genes are associated with hereditary non-polyposis colon cancer (HNPCC), a cancer susceptibility syndrome characterised by increased predisposition to colon cancer, but also endometrial, stomach, small bowel, ovary, hepatobiliary and urinary tract cancer. Skin and brain tumours develop in HNPCC subtypes of Muir-Torre and Turcot's syndrome, respectively. The reasons why a higher incidence of cancer exists in these tissues are not sufficiently explained. We tested a hypothesis that a higher incidence of cancer in the specific tissues is related to their constitutive level of hMLH1/hMSH2 protein expression.

Material and methods: hMLH1 and hMSH2 proteins were studied in paraffin-embedded archival normal samples of various tissues. A total of 89 specimens was analyzed. Indirect immunohistochemical technique was used. The intensity of nuclear staining was graded as 0-3. H-scores were calculated. The results were statistically analyzed.

Results: Tissues with the highest mean H-scores were as follows: A) epithelia of large bowel (hMLH1, 114, hMSH2, 129), stomach (109, 147), small bowel (111, 127); B) endometrium (114, 134); C) ureter (106, 120), ovarian mesothelium (113, 125), liver (93, 112); skin (98, 120), brain astrocytes (92, 118). D) The H-scores for squamous epithelium of eosophagus, uterine cervix, and oral cavity, for bronchioli epithelium, prostate glands, breast tubuli, bone marrow, kidney tubuli, gallbladder and thyreoid gland epithelia ranged from 70 to 100. E) The lowest scores were found in peripheral nerves, brain microglia, lung alveolar epithelium, and kidney glomeruli (below 50). Mean H-score values for group A were hMLH1 112 (SD18.7), hMSH2 133 (SD 25.5), B 114 (SD 29.7), 134 (SD 28.1), C 101 (SD 12.7), 121 (SD 13.4), D 82 (SD 12.2), 93 (SD 13.2) and E 72,9 (SD 22,4), 82 (SD 24,9), respectively. The values for groups A, B and C were higher than for groups D and E. The differences were highly statistically significant (p < 0.0001), both comparing groups and comparing particular tissues between each other.

Conclusions: Tissues predisposed to an increased risk of cancer in hMLH1/hMSH2 mutation carriers express constitutively a higher level of hMLH1/hMSH2 proteins, reflecting their higher need of DNA mismatch-repair. It is possible, that this fact is co-responsible for increased risk of cancer in these tissues along with other biological and environmental factors.

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Apoptotic morphology of adherent cells in vital phase-contrast microscopy compared to scanning electron microscopy images

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Background: Volume regulation is essential for the preservation of cellular functions, but also includes the regulation of cell shrinkage during apoptosis. Our aim was to analyze the geometry and especially the microscopy optical halo around P31 mesothelioma cells for its relevance in the detection and study of morphological changes in cisplatin-induced apoptosis.