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Abstracts of Theses from the Scandinavian Countries

Abstracts of Scandinavian theses on oncologic subjects are published under this heading. The full theses are as a rule published by the universities or as supplements to different journals. They can usually be obtained after contact with the author.

Stomach cancer in Norway—A prospective study on surgical treatment and prognostic factors

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Based on clinical and epidemiological studies the following conclusions are drawn: Patient selection to different hospitals is documented as is differences in surgery provided. The current organization of hospital care (local, county and university hospitals) seems appropriate in Norway, since no statistically significant differences in complication, mortality or survival rates are documented between comparable subgroups of patients treated. The overall treatment results for stomach cancer in Norway compares favourably with that of the best from single institutions in the West. Individuals affected by gastric carcinoma in Norway are older and the disease diagnosed in a more advanced stage than that reported from Japan. The Laurén classification system of gastric carcinoma is valuable in epidemiological research, but does not influence long term survival. The diffuse tumour type is likely to infiltrate the proximal resection margin more frequent than the intestinal type. When corrected for stage of disease symptom duration and weight loss are not independent risk factors for long term survival. The main risk factors for postoperative death are age and sex of the patient. Proximal gastric resection carries a significantly higher complication rate than any other type of resection and should be abandoned. Total gastrectomy is the treatment of choice for proximal gastric cancer. Palliative resectional surgery is safe and may improve survival for some patients, but data on quality of life are not available. Bypass procedures and intubation are followed by a great number of complications and death. No survival benefit of total gastrectomy for distally located tumours is documented compared to distal resection. Splenectomy does not influence survival but increases the postoperative complication rate. Removal of the spleen is not indicated in antral tumours. The only identifiable prognostic factors in curatively resected gastric carcinoma which independently affected long term survival were stage of disease, age, and macroscopic appearance of tumour.

January 1992

Cytogenetic and molecular genetic analyses of human malignant pleural mesothelioma

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Mesothelioma is an asbestos-related rare neoplasm, the cytogenetic and molecular features of which are not well known. We performed successful cytogenetic analyses on tumor and pleural effusion samples of 34 patients with diffuse malignant pleural mesothelioma, 67% of whom had been exposed to asbestos. In eight of these, cells with normal karyotype and/or cell with nonclonal abnormalities were detected. Clonal chromosomal changes were found in 26 patients, most of them having a near-dipolid stemline. Most of the karyotypes were complex, involving several structural and numerical chromosomal aberrations, and no tumor-specific abnormality was found. Frequently observed polyploidization and heterogeneity of various abberrations in individual cases were indicative of clonal evolution. The most frequent chromosomal losses occurred in chromosomes 1 (overlapping area of losses in 1p11-p36.3), 3 (overlapping area of losses in 3p13-p21), and 22 which present candidate chromosomes carrying tumor suppressor genes. On the other hand, chromosomal gain may be a mechanism elevating the expression of oncogenes or growth factor genes. The most frequent chromosomal gains observed were total or partial polysomy of chromosomes 1 (q-arm), 7, and 11. Deletions and unbalanced translocations were the most frequent types of structural chromosomal aberrations. Clustering of breakpoints was detected in 1p11-p22, 1q12-q25, 3p11-p21, and 7q11.2. Similar patterns of chromosomal aberrations have been detected in 92 mesotheliomas published by other investigators. The general complexity of chromosomal changes in mesothelioma reflect the fact that the tumors analyzed have progressed into late stages in their evolution. Clearly most of the chromosomal aberrations are secondary and related to tumor progression. Aberrations of chromosomes 1, 7 and 22 were further studied at the DNA level by RFLP analysis. No gene amplifications or rearrangements were found in NRAS, EGFR, MET, EPO, PGY3, PLANH1, PDGFA, and PDGFB in 23 cytogenetically characterized mesothelioma tumors. In two of the tumors, numerical aberrations of chromosome 7 were detected that had not been found by cytogenetic analysis. Additional information on aberrations of chromosomes 1 and 7 in mesothelioma was obtained by interphase cytogenetic analysis on paraffin-embedded sections of 13 cytogenetically characterized tumor samples. More heterogeneity in chromosome copy numbers was observed and new clones were detected in comparison to cytogenetic analysis. The modal and mean chromosome numbers determined by cytogenetic analysis were correlated with flow cytometric findings in 31 mesotheliomas. A statistically significant positive correlation was found between the DNA indices and both modal and mean chromosome numbers ($p = 0.04$ and 0.006 respectively). As in other tumors, the cytogenetic aberrations are numerous and thus of no direct diagnostic value in mesothelioma. However, some of the chromosomal findings correlate with prognosis. The copy number of chromosome 7 p-arms was found to be inversely correlated with clinical survival ($p = 0.02$). Moreover, the patients with hyperdiploid mean chromosome number have shorter survival compared to patients with normal karyotype or hypodiploid chromosome number ($p = 0.0007$). Supernumerary copies of chromosomes may lead to evevated expression of genes critical in the pathogenesis of mesothelioma. The lung content of asbestos fibers was determined from 16 patients. Losses of chromosomes 1 and 4, and structural aberrations involving 1p11-p22 were found to correlate with high amounts of asbestos fibers in the lung tissue ($p = 0.0001$, 0.003, and 0.009 respectively). Cells with these aberrations may have a growth advantage in the microenvironment produced by the inflammatory reaction caused by the fibers.

Though the patient material was limited even in this study, it has formed a basis to molecular approaches on the pathogenesis of human malignant mesothelioma. This interesting malignancy deserves further studies that are aimed at the elucidation of human carcinogenesis in general and asbestos-induced malignancy in particular.

April 1992

Malignant mesothelioma—The prognostic significance of biological and clinical characteristics

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From 1960 to 1990, 163 patients with confirmed malignant mesothelioma were registered at the Helsinki University Central Hospital. Studies were performed retrospectively on those registered up to 1980, and prospectively on those registered between 1981 and 1990. The ratio of male to female patients was 2:1 between 1960 and 1980, and almost 4:1 between 1981 and 1990. 46% of patients registered between 1960 and 1980, and 41% of patients registered from 1981 to 1990 were either not known or not likely to have been exposed to asbestos. After mineral fiber analysis, the latter finding was reduced to 30%. The main fibre type was crocidolite/amosite, found in 15/27 lung tissue specimens. It was also found 4 patients with malignant mesothelioma who had been exposed mainly to anthophyllite fibres, indicating that there may be a carcinogenic role for anthophyllite. In both periods, the main symptoms at presentation were dyspnoea, cough and chest pain; the median delay in diagnosis was 3 months. For the retrospective study, good performance status (WHO 0-1) and early clinical stage were found to be independent favourable clinical prognostic factors. For the prospective study, diagnostic delay of more than 6 months, epithelial histology and female gender were also found to have favourable prognostic significance.

Five locally-aggressive hemithorax multi-modality treatment programmes were prospectively evaluated, using high-dose hemithorax irradiation schedules combined with chemotherapy, after debulking surgery. A new system for evaluating tumour responses in pleural mesothelioma was used. None of the combined treatment programmes prevented local invasive growth, or the spread of mesothelioma outside the hemithorax. The median survival time remained unchanged between 1981 and 1990, despite changing treatment policies. However, survival was increased, from 8 to 12 months, for those patients who completed the protocol treatment. This indicates the tumour's resistance to both chemotherapy and radiotherapy, although there is some effect of treatment. The results obtained from the 5 programmes only indicate trends: they cannot be properly compared using statistical methods. The favourable experimental prognostic factors were: low total fibre concentration ($< 1 \times 10^6$ f/g dried lung tissue), anthophyllite as the main fibre type; and normal chromosome 7. In addition to identifying these experimental prognostic factors in univariate analyses, the results suggested that the size of the S-phase fraction (as assessed by DNA flow cytometry) had independent prognostic significance. There was a correlation between high total fibre concentration, and partial or total loss of chromosomes 1, 4, or 9, or chromosomal rearrangement involving a breakpoint at 1p11-p22. There was also a correlation between crocidolite/amosite as the main fibre type, and partial or total loss of chromosomes 1, 3 or 4 or chromosomal rearrangements involving del(3p). Diploid cells predominated in 39 out of 63 tumours analysed.

Despite the recent rapid increase in the number of cases, mesothelioma remains a rare disease. For this reason it is essential that carefully-designed multi-institutional Phase III trials are carried out. However no Phase III trials can be contemplated until an effective treatment is found. Our Phase II studies contribute to the understanding of effective treatment design for mesothelioma. The identification of prognostic factors, both clinical and experimental, is important for stratification in future therapeutic trials, and for directing treatment strategies. However more data are needed to confirm the significance of the experimental factors.

In conclusion, this study assessed the relative importance of various prognostic factors for the course of malignant mesothelioma. The results offer a basis for stratification in the design of treatment trials for this disease.

May 1992

Late-effects after treatment for germ-cell cancer with cisplatin, vinblastine, and bleomycin

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During a 5-year period from 1979 to 1983 all patients in Demark with metastatic non-seminomatous and extragonodal germ cell cancer were treated with 6 cycles of cisplatin, vinblastine and bleomycin (PVB). Thirty-nine patients referred to the Finsen Institute accepted a follow-up examination of side-effects 3.5–9 years after chemotherapy. Renal toxicity consisted of an irreversible decrease in glomerular filtration rate (GFR) in 47% of the patients, while the decrease in GFR was fully reversible in 23%. Significant pulmonary toxicity was observed in smokers and consisted of an irreversible decrease in carbon monoxide diffusion capacity of median 72% of the predicted value. Neurotoxicity was the most pronounced long-term side-effect. Nearly all patients had a peripheral sensory neuropathy probably caused by axonal degeneration. A central conduction defect was observed in 88% of the patients by measuring auditory brain-stem potentials. Irreversible high-frequency hearing loss was induced in 39% of the patients. Parasympathetic nerve dysfunction was found in 36% of the examined patients, including 2 patients with impotence. Half of the patients revealed Raynaud's phenomenon (RP), and the mechanism underlying this side-effect was found to be hyperreactivity of the central sympathetic nervous system. Vascular toxicity was found only in terminal arterioles and was not responsible for RP. PVB treatment caused low sperm counts and a subclinical Leydig cell dysfunction in the majority of patients. Azoospermia was observed in 27% of the patients. Six patients had hypertension and this was related to renal impairment. Objective measurements revealed several long-term complications, which for the most part proved to be without clinical significance at the time of follow-up.

May 1992

Prognostic factors in Hodgkin's disease

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The aim of the study was to find a better method for prognostic assessment in patients with Hodgkin's disease. The hypothesis was that the total tumour burden has decisive influence on the prognosis. A method is described for estimation of the total tumour burden and the relation of this measure to the prognosis is studied. The volume of tumour in each single region is estimated by simple clinical and roentgenological methods, used routinely for staging of Hodgkin's disease. The entire added tumour volume was then used as a measure of the macroscopic

tumour burden. A method for assessment of the total tumour cell burden is also described. This method involves a combination of the macroscopic tumour mass with a measure of tumour cell concentration in the tumour tissue, obtained by counting tumour cells in representative histological sections. The prognostic significance of the tumour burden (for stages I and II also of the tumour cell burden) was studied in relation to previously known prognostic factors by use of multivariate survival analyses according to the Cox's model. The patient material consisted of 506 consecutive patients from the medical department at Finsen Institute (1969–1983) and 300 patients from the whole of Denmark included in the Danish LYGRA-project in 1971–1983. The total tumour burden (and for stages I and III the total tumour cell burden) was found to be the most important prognostic factor. Most previously known important factors were correlated to the tumour burden and lacked independent prognostic significance when tumour burden was included in the multivariate model. It is suggested that the tumour burden should be used as guidance for rational choice of treatment of patients with Hodgkin's disease.

June 1992

Prognosis and prognostic factors in adenocarcinoma of the lung

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Prognosis and prognostic factors in patients with adenocarcinoma of the lung were studied. Staging of pulmonary adenocarcinoma is based on extension of the disease at the time of diagnosis and constitutes the basis for choice of treatment. Patients with disease limited to one lung, possibly with local lymph node metastases in the homolateral lung or hilus, can be operated. However, despite microscopic and macroscopic radical operation, the median survival was only 28 months for the 137 patients in the present investigation. By statistical methods (multivariate regression analysis) it was possible to find parameters associated with the prognosis. Such prognostic factors were performance status, histologic subtype of tumour, size and extension of the primary tumour, and spread to lymph nodes. With use of these factors the patients could be divided into groups with different prognosis and expected 5-year survival varying from 19% to 76%.

Patients with more extensive disease are as a rule inoperable and have a much poorer prognosis. Among the 259 inoperable patients in the present study treated with chemotherapy, the median survival was only 29 weeks. Brain metastases were common and observed in 24% of the patients; the expected cumulative risk of brain metastases for patients who were alive after 2 years was estimated to 40%. Factors associated with survival in the inoperable patients were performance status, stage of the disease, previous non-radical surgical tumour reduction, liver metastases, white cell blood count, and abnormal enzyme values in blood (LDH and ASAT). Histologic subtype was in the inoperable patients not associated with survival time. Previous non-radical surgical tumour reduction could not be regarded as a prognostic factor in itself but rather as an indicator of minimal (but inoperable) extent of the disease.

Treatment of patients with inoperable pulmonary adenocarcinoma is at present experimental which means that there exists no standard treatment with known positive influence on the survival. Knowledge of prognostic factors can be used for choice of experimental therapy and for comparison between treatment results obtained by different groups.

June 1992

Studies on the uptake of L-(methyl-^{11}C)methionine in cancer with positron emission tomography

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Uptake of [^{11}C]methionine in human cancer was studied by PET in order to elucidate the role of [^{11}C]methionine in detecting malignant tumors and assessing the proliferation activity of cancer. Four different analysis methods of the uptake of [^{11}C]methionine in cancer were studied.

[^{11}C]Methionine accumulated avidly in 57/61 (93%) malignant non-brain tumors (lymphoma, breast cancer, and head and neck cancer). The reason why 4 tumors were not visualized in their small size (diameter <20 mm) and small proliferation rate. There was some accumulation of [^{11}C]methionine in an abscess, but no accumulation in an inflammatory lesion or a benign breast tumor. The uptake of [^{11}C]methionine in low grade lymphomas was marked, while the uptake rate of FDG was low in 5/10 low or intermediate grade lymphomas. The uptake rate from the plasma to the lymphoma tumor was lower for FDG than for [^{11}C]methionine.

The uptake rate (K_i) of [^{11}C]methionine from the plasma to the tumor can be measured according to a graphical method of Patlak et al. (182). However, the radioactivity accumulation in tumor adjusted for the injected dose and body surface area (RDA) correlates strongly with the uptake rate K_i ($r = 0.92$, $p < 0.0001$). For clinical purposes, RDA and K_i are comparable ways to analyze the accumulation of [^{11}C]methionine by PET, but RDA is simpler to derive, especially since blood samples are not needed and the dynamic scanning time is reduced from 40 minutes to 5 minutes. The radiation exposure to the personnel is also reduced when frequent blood samples need not to be taken. The capacity of the PET unit is improved.

A high uptake rate K_i of [^{11}C]methionine in the tumors of 22 patients tended to be associated with a large S-phase fraction ($r = 0.40$, $p = 0.07$, $n = 22$), except for one case, where the uptake was clearly higher than predicted by the size of the S-phase fraction ($r = 0.62$, $p = 0.0003$, $n = 21$, outlyer excluded). The correlation between the uptake rate of [^{11}C]methionine in DNA aneuploid tumors and the S-phase fraction was good ($r = 0.73$, $p = 0.05$; for RDA 0.60, $p = 0.03$). The correlation between the uptake rate of [^{11}C]methionine from the plasma to the tumor (K_i) and the S-phase fraction and between the accumulation adjusted for the injected dose and the body surface area (RDA) and S-phase fraction were of the same order of magnitude. The method could be valuable in certain clinical situations, e.g. for the assessment of the proliferation rate of deep-seated tumors and in cases where taking a surgical biopsy is not possible.

June 1992

Cell proliferation rate in prostatic carcinoma—Prognostic value and relation to epidermal growth factor receptor and p53 expression

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This present study investigated the prognostic value of cell proliferation rate in prostatic carcinoma with DNA flow cytometry and PCNA immunohistochemistry. The association of p53, EGFR and ERBB2 expression with cell proliferation and prognosis was evaluated. The use of DNA histogram background subtraction to correct the influence of sliced nuclei improved the prognostic

value of the cell proliferation rate defined as flow cytometric SPF. Good reproducibility of the cell cycle distribution analysis and improved prognostic value of SPF warranted the use of background subtraction in flow cytometric measurement of cell proliferation. Cell proliferation rate determined by flow cytometry was an independent prognostic factor in multivariate analysis of M0-stage patients. A very high cell proliferation rate also predicted a poor response to endocrine therapy for patients who received solely endocrine therapy. DNA aneuploidy, which predicted a poor prognosis in univariate analysis, failed to yield additional prognostic value when cell proliferation rate, histological grade and T-stage were taken into account. Immunohistochemical analysis of PCNA using either PC10 or 19A2 antibody was not associated with the cell proliferation rate determined by FCM. PCNA analysis with 19A2 antibody was marginally associated with prognosis, while analysis with PC10 antibody revealed no prognostic value at all. The prevalence of p53 accumulation was low, indicating that the impact of p53 mutation on the pathogenesis of prostatic carcinoma is marginal. P53 accumulation was associated with poor tumor differentation and a high cell proliferation rate. It also predicted poor prognosis, although it was not an independent prognostic factor in multivariate analysis. A small subgroup of prostatic carcinomas lacking the EGFR expression was found. These tumors are well differentiated and proliferate slowly. The prognosis for patients with EGFR negative tumor was good, although EGFR expression was not an independent prognostic factor in multivariate analysis. Only a small group of prostatic carcinomas showed a very low level of ERBB2 immunostaining. This staining probably did not describe the expression of ERBB2 oncoprotein at all. It seems the ERBB2 overexpression is not important in the pathogenesis of prostatic carcinomas.

The study indicates that flow cytometric analysis of the cell proliferation rate gives independent information on the prognosis for M0-stage prostatic carcinoma. The expressions of EGFR and mutated p53 are both involved in dysregulation of the cell proliferation rate of prostatic carcinomas, but are not as strong prognostic factors as determination of the cell proliferation rate itself. Flow cytometric cell proliferation measurements could help to detect the aggressive prostatic carcinomas and thus probably help in the selection of the most effective therapy for individual prostatic carcinoma patients.

June 1992

Development of methods for mutation detection—Their use in the analysis of human tumor suppressor genes

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We have through the course of this work developed three new methodological approaches for detection and characterization of DNA alterations in tumors. We have used two of these methods to detect such alterations in a variety of tumors in order to gain biological understanding of the tumor suppressor genes RB1 and TP53, and to examine the current limitations of the approaches.

The advent of recombinant DNA technology has dramatically changed both the rate of increase in our biological understanding, and the scope and techniques for biological experiments. This rapid change is illustrated with our examination of the DNA alterations in the tumors from a single patient. It has now become possible to answer questions of tumorigenesis at the base level, and expect precise answers, within a timespan sufficient to be useful for the clinician.

The evolution of methods in tumor DNA analysis, and the consequent increase in the understanding of the mechanisms in tumor biology, will no doubt continue and replace current methods. A scenario with genetic testing for cancer disposing traits, and with more precise prognosis and treatment based on genetic examinations of the relevant genes in tumorigenesis, is now very much in sight.

August 1992

Clinical and histomorphometric assessment of prognostic factors in breast cancer

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A retrospective study was done including 688 patients with an invasive breast cancer. The patients were diagnosed, treated and followed up for over 12 years during 1962–1990 at Kuopio University Hospital, Kuopio, Finland.

Axiallary lymph node status ($p < 0.001$), tubule formation ($p < 0.001$), volume-corrected mitotic index (M/V index) $p = 0.001$), tumour diameter ($p = 0.001$), the age of the patient ($p = 0.020$) and the year of treatment ($p = 0.008$) predicted breast cancer survival independently in a multivariate analysis. In axillary lymph node negative patients, tubule formation ($p = 0.005$), the degree of intraductal growth ($p = 0.015$) and the SD of nuclear perimetry ($p = 0.048$) predicted survival independently. In axillary lymph node positive patients, significant independent predictors were the M/V index ($p < 0.001$), tumour size (0.005), the patient's age ($p = 0.005$), the year of treatment ($p = 0.028$) and tubule formation ($p = 0.024$). In sex steroid receptor positive tumours, the M/V index had a strong correlation to prognosis. The most important predictors of poor outcome after the first recurrence were positive axillary lymph node status ($p < 0.001$), high SD of nuclear area ($p = 0.01$), absence of tubule formation ($p < 0.003$) and postmenopausal age ($p = 0.0023$). A recurrence within two years after primary therapy and a distant recurrence were highly unfavourable prognostic indicators.

In conclusion, axillary lymph node status and the volume corrected mitotic index are accurate predictors in breast cancer. Tumour diameter also provides important prognostic information. Nuclear morphometric variables and histological characteristics may offer additional prognostic information in some cases.

August 1992

Asbestos exposure and risk among asbestos cement workers—Studies of exposure and fibre concentrations, retention patterns and histopathological changes in lung tissue, and cancer risk, mortality and survival

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In a cohort of 3 144 asbestos cement workers the estimated median exposure was 1.2 fibres/ml air. In preserved lung tissue from necropsy, the 96 asbestos cement workers had higher asbestos fibre concentrations (transmission electron microscopy) than matched referents (medians: asbestos cement workers with mesothelioma 189, without mesothelioma 50, and referents 29×10^6 f/g dry weight). This was less pronounced for chrysotile

than for amphibole (crocidolite, amosite, tremolite, anthophyllite) fibres, in spite of the fact that the exposure was mainly to chrysotile. Amphibole concentrations increased with duration of exposure. Asbestos bodies in lung tissue were associated both with duration of exposure and tissue concentrations of amphibole fibres. Further, association were found between amphibole concentrations in lung tissue and fibrosis. Workers dead from mesothelioma had higher amphibole, but not higher chrysotile, concentrations than the others. Thus, the amphibole fibre concentrations and the number of asbestos bodies reflect risk.

The mortality from non-malignant (relative risk (RR) = 2.6), and malignant (RR = 2.5) respiratory disease was higher among the 1 929 male Swedish asbestos cement than in 1 233 referent workers. Dose-response relations with cumulated asbestos dose were found for pleural mesothelioma and colorectal cancer. The proportion of adenocarcinoma was high among asbestos cement workers with lung cancer and a high exposure (45%, referents 15%). Survival, as analysed by use of Cox's proportional hazards regression models, was a couple of years shorter among the asbestos cement than in the other industrial workers. A causal relation with the exposure is supported by the facts that the risk seemed to be confined to the period 20–40 years from start of employment, and was most pronounced among workers starting employment before the age of 30.

August 1992

Radiolabelled monoclonal antibodies and tumour scintigraphy

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Seven monoclonal antibodies directed against human colon adenocarcinoma associated antigens were iodinated with ^{125}I and ^{131}I. One of the antibodies was also internally labelled with ^{75}Se and dual-labelled with ^{75}Se and ^{125}I. Two unspecific monoclonal antibodies were either internally labelled with ^{75}Se or iodinated.

The biokinetics of the monoclonal antibodies were studied in tumour-bearing nude mice. The animals were killed 1 h, 1, 3, 6, 9 or 10 days after injection of the radiolabelled monoclonal antibodies and the radionuclide concentration was measured in various organs. Two cancer patients were injected with iodinated monoclonal antibodies and the biokinetics was studied by gamma camera measurements and measurements of the radionuclide concentration in tumour and blood samples. Chromatography was performed on samples from the injection solutions and animal and human blood.

The radionuclide in plasma was mainly bound to IgG-sized molecules. The normal tissue-to-blood radionuclide concentration ratios for the iodinated monoclonal antibodies were similar for all measured organs. The tumour-to-blood ratios were similar in animals and humans. The biokinetics in the animals were influenced by the choice of radiolabel with longer retention in blood of the internally labelled monoclonal antibody. When the internally labelled monoclonal antibody was iodinated, the blood concentration both radionuclides was more like that of iodinated monoclonal antibodies than internally labelled ones.

A patient study, performed using a $^{99}Tc^{m}$-labelled monoclonal antibody, was supplemented with a tumour phantom study. Artificial tumours with 10 mm diameter and three different activity concentrations, were placed into transverse SPECT images, at ten different sites. Reconstruction was performed using a Hanning filter with three different cut-off frequencies. The sensitivity in tumour detection was determined from the results of two readers. The sensitivity was calculated for other tumour diameters and activity concentrations. The cut-off frequency of 0.5 cm^{-1} had the highest signal-to-noise ratio in the phantom study and had also the highest sensitivity in tumour detection of the simulated patient studies. The results showed that tumours with diameters below 10 mm are difficult to detect using conventional SPECT gamma camera technique.

September 1992

T cell-mediated immune recognition of human tumors regulated by MHC class I and ICAM-1 molecules

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The aim of this thesis was to study the expression of MHC class I and ICAM-1 molecules on ex-vivo tumor cells and the function of these molecules in the recognition of tumor cells by T lymphocytes in vitro.

Tumor cells express relatively low level of MHC class I molecules. By isoelectric focusing analysis selective loss of certain class I allele products was detected in 23 of 50 carcinomas. Selective defects randomly occurred within the HLA A, B and C locus. This selective downregulation of class I molecules may have functional consequences, because the MHC class I molecules present antigen epitopes to cytotoxic T cells. In one ovarian carcinoma the assembly of heavy and light chains was defective. One HLA A2 binding peptide and IFNγ could correct this defect and induce the expression of class I molecules.

The function of MHC class I molecules in immune response against tumor cells was investigated by generation and characteristics of CTLs in MLTCs. Clonal expansion of autologous tumor specific $CD8^{+}$ CTLs in 1/21 MLTCs was induced by repeated stimulation with autologous tumor cells. The lytic function of these CTLs was restricted by HLA B5. These results demonstrated that MHC class I molecules are essential, but not sufficient, for generation of immune response to tumors. By clonal analysis of MLTC responders we found that both CD4 and CD8 clones could express auto-tumor specific and class I restricted lytic function. In addition to earlier findings that CD4 T cells function as helper or suppressor, the class I restricted CD4+ CTLs reflected the heterogeneity of CD4+ T cells in response to class I positive and class II negative tumor cells.

It has been described that TCR has low affinity interaction with MHC-antigen. Therefore, adhesion molecules are required for generation of cellular immune response. The induction of ICAM-1 expression on tumor cells, which carried class I antigens, could elevate the auto-tumor recognition. The regulatory function of ICAM-1 on the lymphocytes was also studied. The results of the existence of lytic function in ICAM-1 positive lymphocytes and reduction of lytic function by pretreatment of lymphocytes with anti-ICAM-1 mAb indicated that ICAM-1 on lymphocytes could promote and regulate the functional activation of lymphocytes when it interacted with LFA-1.

These results demonstrated that MHC class I molecules, which are frequently altered in solid tumors, were important for generation of auto-tumor response in vitro. Expression of ICAM-1 molecule on both tumor and lymphocytes could increase and/or regulate the immune response against tumor.

October 1992

Ultrastructural evaluation of some aspects of the endocrine activity of pituitary adenomas

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Some ultrastructural aspects of human pituitary adenomas that remain to be elucidated are presented. The importance of considering the morphology of neoplastic cells in concert with the features of the extracellular matrix is emphasized. A review of the literature on the ultrastructural aspects of the synthesis and secretion of pituitary hormones is presented. As will be focused on later, some BM components seem to play a role in the secretory process. For this reason, a survey of the morphology of BM characterizing the anterior lobe of the pituitary gland is included. Based on the available literature, the salient morphological features of human pituitary adenomas are reviewed. The outline of the current studies including details on the human material and methods is presented.

Based on electron microscopic studies, the secretory granules (SG) in human pituitary adenomas are characterized qualitatively and quantitatively. The average SG-size of endocrine active adenomas significantly exceeds that of the silent adenomas. A novel subclass of SG, labelled SIG, with a mottled core, placed eccentrically in a vesicle is described. This structure occurs exclusively in hormonally inactive, silent adenomas. The light body is a 300–1 500 nm electron-lucent granule with a filamentous substructure, confined by a double membrane. Hitherto, this structure has been recorded in rodent pituitaries only. It is here documented that light bodies also occur in human pituitary adenomas and furthermore, that the presence of this structure correlates positively with endocrine activity of the tumors.

Thirty silent pituitary adenomas are subclassified according to the extent of RER, Golgi complex, and mitochondria. When correlating these ultrastructural features with clinical data, it appears that typical oncocytomas and organelle-poor adenomas procedure pituitary insufficiency more commonly than do moderately oncocytic adenomas and non-oncocytic, organelle-rich adenomas. It is further shown that the latter two tumor types tend to expand suprasellarly more often than do the former two.

The distribution pattern of BM-material is examined immunohistochemically and ultrastructurally in 38 human pituitary adenomas. This material was seen intercellulary in 10 of 24 endocrine active adenomas, but in none of the 14 silent adenomas. It is hypothesized that the intercellular BM-material, identified in some of the adenomas, is involved in the extracellular transport of hormones, thereby compensating for the diminished vascularity characterizing the lesions.

Intracellular laminin is confined to gonadotropic cells of normal rat pituitaries. By electron microscopic immunohistochemistry, laminin as well as tubulin are seen to concentrate to light bodies. Upon estrogen administration, which depresses the gonadotropic cells, laminin-positive cells virtually disappear from rat pituitaries and the number of light bodies diminishes. Castration which stimulates the gonadotropic cells, increases the number of laminin-positive cells and light body-bearing cells. Based on these results, it is concluded that the presence of cytoplasmic laminin and the number of light bodies reflect the hormonal activity of the gonadotropic cells of the rat pituitary gland.

It is concluded that a combined morphological assessment of intra-og extracellular details of pituitary adenomas adds to the understanding of events concerned with hormone secretion. An evaluation from that angle of the enigmatic silent adenomas, that may elaborate/secrete hormones, may be informative. This approach opens new avenues in the research of both the pituitary and other endocrine glands.

November 1992

Studies on biologically rhythmic natural modifiers of cancer treatment in mice and man—Chronotherapeutic concepts and applications

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The following conclusions are drawn:

—Each cytostatic drug tested chronobiologically has been shown to exhibit a circadian rhythm in toxicity (Ellen-effect; Chronesthesy). This is a general principle which applies to compounds used to treat other diseases and illnesses, as well.

—Each drug may exhibit a different circadian timing in chronotolerance even when its molecules differ only slightly from another compound. Therefore, each drug needs to be tested for its own unique chronotoxicity rhythm.

—Circadian 'stage', and not 'time of day', is the dominant factor in determining phasing of the chronotolerance observed for a drug. For example, 6 a.m. will be the 'best' time for Adriamycin administration to someone sleeping at night, but the 'worst' time for a night worker finishing a work-shift at that time. A stable synchronizer schedule prior to treatment and measurement of a marker rhythm, such as body temperature, cortisol in the blood or potassium in the urine, will help ensure that treatment is administered at the desired stage of rhythm.

—By proper timing of a drug, toxicities can be reduced and efficacy increased, resulting in cost-effective treatment. Dose may also be increased by proper timing.

—Proper timing of each drug used in combination chemotherapy of cancer can result in better tumor control, more cures and reduced toxicities. The finding in rats that Adriamycin near awakening (late light) and DDP late in activity (dark) resulted in more cures has since been extrapolated to humans treated on a similar schedule (Adriamycin at 6 a.m., DDP at 6 p.m.) with similar results.

—The stage of the fertility cycle is likely to play an important role in treatment outcome due to a complex interaction and cycling of sex and stress hormones. While this has documented for cancer, it may well be true for other diseases as well.

—The physician can no longer ignore the endogenous network of interrelated biological rhythms with frequencies along the scale of the day, the week, the month and the year which influence the outcome of treatment. In addition to the skill of the health-care giver, 'when' something is done must become as critical as 'what' and 'how much' for treatment of every illness, including cancer and AIDs.

November 1992

Psychoneurooncology—Psychological dynamics in glioma patients

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Patients with brain tumors, above all high- and low grade gliomas, were studied by psychometric tests, neuropsychological methods, including behavioral observations of frontal functions,

personality assessments, CT, 2-d regional cerebral blood flow (rCBF), and single photon emission tomography (SPECT) measurements.

The aim was to investigate the effects of the brain tumor on various mental functions and to compare patients with high grade gliomas with those with low grade gliomas. The main findings are that supratentorial high grade, even nonfrontal, astrocytomas influence the frontal lobe functions as shown by qualitative neuropsychological methods and functional brain imaging techniques. These patients showed impairments of energy level, attention, motor executive control, speech initiative, goal directed behavior, sequencing, simultaneous/synthetic ability, verbal fluency and of abstract conceptualization. Cerebral blood flow recordings revealed a selective reduction of blood flow in the frontal lobes, which correlated with the grade of malignancy of the posterior tumor, expressed as intensity of focal thallium-201 uptake. No correlation between tumor volume and frontal lobe blood flow reduction was found.

Uninformed of final diagnosis, patients with high grade gliomas showed primary anxiety, and an inability to master and handle it, implying a regression deep enough to block the ego functions. This finding may, in parts, explain the frontal lobe dysfunctions in the patients with high grade gliomas. The results are discussed in relation to current theories of brain function and the biochemistry of the fronto-striatal loops.

November 1992

DNA and large bowel carcinomas—DNA ploidy pattern and genetic alterations in relation to clinicopathological variables

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A detailed description is given of a total material comprising 406 patients with colorectal carcinomas. Sixty-one per cent of the tumours were DNA aneuploid. Based on the data obtained from DNA flow cytometric analysis in control tissue (internal controls), we could establish that the first peak in the DNA histograms represented DNA diploid cell populations. We defined DNA aneuploidy as a cell population with DNA content deviating by 10% or more from the diploid peak (DNA index $\geqslant 1.1$), limited by the resolution of the flow cytometric methods. This definition of DNA near diploid and DNA aneuploid tumours was used throughout the study. We found a significant association between DNA aneuploidy and both increase in tumour spread (Dukes' stage), as well as increasing degree of cellular atypia. There was no association between DNA ploidy pattern and histological grade. The evaluation of the degree of cellular atypia and tumour differentiation are both subjective and empirical. In the present study, inter- and intraobserver variability analysis showed a relatively great scatter, but no systematical bias.

Flat colonic mucosa, polypoid lesions, carcinomas, and metastatic carcinomas from ten patients with longstanding ulcerative colitis. Of ten carcinomas found in six patients, six showed DNA aneuploidy. Three patients developed metastatic carcinomas, and these lesions had similar DNA indices as found in the main tumour. Aneuploid cell populations with similar DNA indices often occurred, both in different samples from flat mucosa in the individual patient, as well as in mucosa--and carcinoma samples from the same patient. DNA aneuploidy was found in flat mucosa in five of the six patients with carcinoma, and in one of the four patients without carcinoma ($p > 0.1$). High grade dysplasia was found in only four of the six cases with carcinoma, demonstrating that high grade dysplasia is insufficient as the only marker for malignant development. DNA aneuploidy was not confined to dysplastic epithelium, but could be demonstrated almost as often in non-dysplastic mucosa. This strongly suggests an incipient malignant transformation of these cells, either prior to or bypassing the stage of dysplasia. DNA aneuploidy in flat mucosa may therefore constitute an additional marker for the identification of patients at increased cancer risk who might benefit from a closer surveillance.

Carcino-embryonic antigen (CEA) levels in relation to survival, DNA ploidy pattern, Dukes' stage, and recurrent disease were studied in the 406 patients with colorectal carcinomas. Elevated preoperative CEA levels ($> 5\ \mu g/l$) was found in 42% of the patients. The survival was significantly longer for patients that had normal preoperative CEA levels compared with those with elevated levels. This was seen in the total patient group ($p = 0.0001$), and in patients with tumours in Dukes' stage B ($p = 0.001$). A weak but significant correlation was found between preoperative CEA level and Dukes' stage (Kendall's $\tau = 0.05$m $p < 0.01$). Twenty-eight (56%) of the patients with postoperative normal or normalized CEA values and subsequent clinical recurrence, had a rise in CEA before or at the time of the recurrence. A significantly higher proportion of patients with DNA aneuploid (AN) carcinomas than DNA near diploid (ND) carcinomas developed recurrent disease. A rise in CEA prior to or at the time of recurrence occurred in a significantly higher proportion of the patients with carcinoma in the colon compared with those with carcinoma in the rectum. Rise in CEA levels was found significantly more frequent in patients with liver recurrences and abdominal recurrences, compared with those who had local pelvic recurrence. The sensitivity of the CEA test for primary as well as for recurrent disease was not significantly different in the AN and ND tumour groups.

Alterations within the Rb1 gene on chromosome 13q were studied in 255 of the colorectal carcinomas. Thirty-five per cent of the tumours had alternations within this gene. The high frequency of alterations suggests that the Rb1 gene is involved in colorectal carcinogenesis. The most frequent alteration was amplification, suggesting that the Rb1 gene has an oncogene-like function in colorectal carcinogenesis. A significant association was demonstrated between amplification within the Rb1 gene and DNA aneuploidy ($p < 0.01$). Heterozygous loss on chromosome 1p was found in 22.2% of the tumours, indicating that this chromosome may also be involved in the carcinogenesis in a subset of colorectal carcinomas. There was no association between loss on 1p and amplification of the Rb1 gene. Significantly more tumours with heterozygous loss on chromosomes 1 and 2 were aneuploid (85.0% and 83.8%) compared with those without alterations on these chromosomes (55.8% and 57.7%). No association was found between amplification within the Rb1 gene and the clinicopathological characteristics of the tumours.

The relationship between chromosome 17 alterations, especially of the TP53 tumour suppressor gene located on 17p, and DNA ploidy pattern and clinicopathological variables was studied in 231 of the patients with colorectal carcinomas. Loss of the TP53 gene was found in 68% of the carcinomas. TP53 gene loss was strongly associated with DNA aneuploidy ($p < 0.0001$), but only when the whole chromosome was lost. These results indicate that the loss of the TP53 gene may have significance in the aneuploidization process. A further subdivision of the DNA aneuploid carcinomas into 'moderately' and 'grossly' DNA aneuploid tumours (DNA index $\geqslant 1.1$ and < 1.3, and DNA index $\geqslant 1.3$, respectively) showed that TP53 gene loss was significantly more frequent in the grossly DNA aneuploid tumours compared with the rest of the tumours ($p < 0.0001$), as well as compared with the moderately aneuploid tumours ($p < 0.0001$). Allelic amplification

on 17q was observed in 15%. The amplification on 17q was neither associated with loss of the TP53 gene, nor with loss on the other 17q arm. There was a significant association between loss of the TP53 gene and histological grade ($p < 0.01$), and between loss of the TP53 gene and degree of cellular atypia ($p < 0.05$), with TP53 gene loss being most frequent in moderately differentiated carcinomas, and in carcinomas with severe cellular atypia, respectively. A significant association was found between loss of the TP53 gene and tumour site, the frequency of TP53 gene loss increasing from the right colon to the rectum. Loss on 17q was found in 28% of the carcinomas, of which only 6% had loss only on this chromosome arm. We found no evidence of additional tumour suppressor genes on chromosome 17.

November 1992

Colorectal carcinoma—Nuclear DNA heterogeneity and bacterial translocation

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In patients undergoing elective surgery of colorectal carcinoma, the nuclear DNA content of primary and metastatic carcinomas was studied. Microbial colonization of the colonic mucosa, primary tumours and regional metastatic and non-metastatic lymph nodes was also investigated. For comparison, a group of patients with non-malignant colorectal disease was included in the investigation. Patients were prospectively followed for 28 to 73 (mean 50) months.

Multiple samples analysis showed that the primary colonic carcinoma had DNA heterogeneity in nearly half of the cases. Multiple (3–4) samples are therefore necessary for proper evaluation of the tumour's DNA pattern.

Multiple metastases from the same primary tumour may have different DNA patterns. Aneuploidy was present more often in metastases from aneuploid than in those from non-aneuploid primary tumours. Patients with homogeneously aneuploid tumours had a significantly shorter disease-free survival than those with homogeneously non-aneuploid tumours. Tumours with aneuploid DNA-patterns in the various Dukes' stages tended to be associated with a worse outcome. The prognostic significance of the DNA pattern may be affected by the classification system employed and the thresholds of aneuploidy selected. An aneuploidy limit set at > 15% of the cells exceeding the 5c ploidy level (5c exceeding rate) had the best prognostic value. Regardless of the aneuploidy thresholds selected, patients with heterogeneous tumours were found to occupy an intermediate position between those with a more favourable outcome associated with homogeneously non-aneuploid tumours and those with a less favourable outcome associated with homogeneously aneuploid tumours. The DNA pattern can thus serve as a useful complement to traditional histopathological parameters in establishing the patient's prognosis.

Imprints (IMP) of tissue biopsies were found to be comparable to fine needle aspiration smears and adequate for single cell DNA cytometry. The DNA pattern can accordingly be satisfactorily determined on IMP of tissue biopsies, such as those obtained endoscopically before surgery.

'Bacterial translocation' which is the passage of viable gut microflora into the lymphatic system, blood and other deep organs was present in 38% of the mesenteric lymph nodes (MLN) with metastases and in 25% of MLN without metastases in patients with colorectal carcinoma but in only 12% of MLN in patients with non-malignant colorectal disease. Anaerobic microorganisms were more frequently detected in MLN with metastases than in those without. Clostridial species were detected only in nodes with metastatic growth. The primary carcinomas were the most heavily colonized and showed significantly lower concentrations of the prophylactic antimicrobial agent given during surgery than did the adjacent colonic mucosa. MLN, even in the same patient, showed variable antimicrobial tissue concentrations which were not related to the microbiological findings or to the presence of metastatic tumour growth. Colonic carcinoma and the associated pathophysiological changes may promote bacterial translocation.

November 1992

Chronic lympocytic leukaemia—Newer prognostic approaches in relation to clinical state

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The present work has dealt with descriptive analysis of bone marrow histology and a number of cellular characteristics in relation to clinical stage and their possible clinical implications, Existing clinical staging systems are reviewed and it is concluded that a combination of the systems of Binet et al. (1981) and Rai et al. (1973) offers a simple and robust tool for identifying patients that should be treated. We have found that a heavily lymphocyte infiltrated bone marrow and some characteristics of the malignant B cell (number of chromosome abnormalities, amount of surface IgM, expression of CD23) are important for the prognosis whereas this could not be demonstrated for the numbers and ratios of T-cell subsets or SmIg class. Our findings may supplement clinical staging, but we did not succeed in identifying any biological marker that can explain the prognostic heterogeneity of CLL or replace clinical staging.

Others have shown that the CLL cell is clonogenic and able to differentiate in vitro, but in vivo it is the seat of leukaemic maturation arrest, an arrest that has taken place just at the time of activation of the mature B cell. The clonogenic cells are slowly dividing, and the main mechanism for clonal expansion is that they are long living. The expansion clone interacts with T cells, NK cells, and monocytes which apparently via unknown growth factors can influence the growth rate of the clonogenic cells, but is also influenced by known or putative growth factors: IL2, IL4, IL7, soluble CD23, TNF. The hypogammaglobulinaemia in B CLL is not explained, but in our and other reports it is found directly correlated to the number of leukaemic B cells, probably secondary to the maturation arrest in vivo.

In a disease, in which the tumor cell can be stimulated to divide and differentiate in vitro but in vivo is a long living but inert, maturation arrested, non-invasive lymphocyte that mainly circulates in the blood, a search for a regulatory defect e.g. an inappropriate lymphokine simulation or inhibition, or inappropriate cell-cell contact between leukamic B cells and regulatory non-B cells, is presently an important field of research.

November 1992

Experimental studies on estramustine and radiation in the treatment of prostate cancer

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Estracyt (estramustine phosphate) has been used in the treatment of advanced prostate cancer for many years. In man estramustine phosphate is rapidly dephosphorylated to estamustine.

the main active cytostatic metabolite and its oxidized analogue estromustine. Estramustine is accumulated in prostate cancer cells, partially due to the presence of estramustine binding protein (EMBP).

The hormone independent cell line DU 145 was heterotransplanted in male and female nude mice and the expression of EMBP was evaluated within the tumours. The expression did not differ between tumours grown in male and female mice, indicating an androgen independent expression of the protein.

The expression of EMBP is higher in cancer cells than in benign epithelial cells, preferentially in moderately and poorly diffrentiated cancer. A high expression of EMBP is also observed in prostatic intraepithelial neoplasia (PIN), which is probably the main precursor of prostate cancer. PIN and prostate cancer have the highest prevalence in the peripheral zone of the prostate, which suggest that EMBP is mainly expressed in the peripheral zone.

In prostate tumour transplants in nude mice treated with estramustine a 7 to 8 fold increase in the number of abnormal metaphase was produced, i.e., highly contracted and unaligned chromosomes. This was not observed in the mice treated with estrogens, showing that estramustine has a non-estrogenic cytostatic effect in vivo. This raised the question whether estramustine might have a radiosensitizing effect.

The human prostate cancer cell line DU 145 was studied in in vitro, irradiation was delivered with doses ranging from 2–8 Gy after incubation with estramustine in doses which are in the same range as used in the clinical situation. A radiosensitizing effect was observed in the clonogenic survival assay showing a 23% sensitization at the dose level of 5 mg/ml and a 30% sensitization of the dose level 20 mg/ml.

The effect of estramustine in combination with radiation was also studied in vivo, in nude mice heterotransplanted with the prostate cancer cell line DU 145. The combination resulted in a significant growth retardation, a larger proportion of necrosis and a decreased proliferative activity in the tumour. The data indicate that estramustine acts as a radiosensitizing agent in vivo and encourages further studies with respect to clinical application.

November 1992

Functional and biochemical studies of blood myeloid cells in polycythemia vera, with emphasis on the defective stimulus-response coupling for oxidative reactions in granulocytes

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Polycythemia vera (PV) is a malignant myeloproliferative disorder characterized by an increased red cell mass, and most often accompanied by leukocytosis and thrombocytosis. The pathogenetic mechanisms behind PV are still largely unknown. In PV, circulating polymorphonuclear granulocytes (PMN) are derived from the malignant clone of stem cells, implying that PMN could serve as a model system for the study of functional and biochemical changes that occur in this disease.

No evidence for an altered function of unstimulated PV PMN was found. Furthermore, fMLP induced PMN adhesion, chemotaxis and enzyme release was normal in PV PMN. However, a stimulus-specific defect was discovered in oxidative metabolism, measured as chemiluminescence (CL), after stimulation with the surface receptor dependent stimuli fMLP, leukotriene B_4 and platelet activating factor, whereas the calcium ionophore A23187 and phorbol myristate acetate (PMA) elicited a normal response. This defect was specific for PV since CL was normal in chronic myelogenous leukemia (CML), essential thrombocythemia (ET) and secondary erythrocytosis. The biochemical background behind the impaired CL response was shown to be a reduced production of superoxide anion. Studies of intracellular hydrogen peroxide (H_2O_2) production in single PV cells, using flow cytometry technique, revealed that the defective response to fMLP was present both in single PMN and single monocytes, and no subpopulation of less responsive cells was found.

Studies of the stimulus-response coupling for the oxidative burst showed that fMLP induced oxidative metabolism was reduced despite a normal fMLP receptor function and a normal activation of phospholipase C, since both the production of inositol-1,4,5-trisphosphate, as well as the increase of cytosolic calcium, was normal in PV PMN. The formation of diacylglycerol, in normal PMN largely generated by phospholipase D (PLD) acting on phosphatidylcholine, was significantly reduced after fMLP stimulation, but normal when induced by PMA. In accordance with this, the activation of PLD, measured as the formation of phosphatidylethanol, was also significantly reduced after fMLP stimulation, but, once again, the response to PMA was normal. Since two different cell lineages exhibited the same defective response to fMLP, it is possible that the proposed impairment of PLD activation could be a consequence of the evolution of the malignant stem cell clone, and might conceivably be involved in the pathogenesis behind PV.

The production of leukotrienes (LT) and lipoxins (LX), metabolites derived from arachidonic acid thought to be involved in the regulation of normal myeloid progenitor growth, was studied in PV, CML and ET. The production by PMN of LTB_4 and LTC_4 was normal in PV, whereas in CML a significantly increased production of LTC_4 was discovered. Furthermore, platelets from patients with CML, PV and ET demonstrated a significantly reduced capacity to form LXA_4, and this defect was particularly noted in CML patients in blastic crisis. These abnormalities of lipoxin and leukotriene production could be of relevance for the uncontrolled myelopoiesis characteristic for myeloproliferative disorders in general and blastic crisis of CML in particular.

November 1992

The epidermal growth factor receptor system in rat liver growth and carcinogenesis

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The epidermal growth factor receptor (EGF-R) system includes the EGF-R and its ligands EGF and transforming growth factor α (TGF-α). Both EGF and TGF-α are potent mitogens for epithelial cells, including hepatocytes. In rat liver, EGF-R is highly expressed, TGF-α is induced in different growth situations, whereas EGF is not expressed in the liver but is present in the afferent blood.

TGF-α is a comparatively more potent stimulator of hepatocyte proliferation than EGF, however, TGF-α was found to bind EGF-R with a lower affinity than EGF in isolated rat liver membranes. No other differences between EGF and TGF-α could be observed in the interaction with EGF-R or in the subcellular distribution following administration of ^{125}I-labeled EGF/TGF-α.

The pre-replicative regulation of the EGF-R system was studied during liver regeneration following partial hepatectomy in the rat, both in the initial priming phase and in the subsequent progres-

sion phase. The EGF binding capacity was found to undergo two modes of regulation. The EGF-binding levels increased during the priming phase, followed by a decrease below the initial level in the progression phase. Neither changes in properties of the EGF-R, such as the size of EGF-EGF-R or TGF-α-EGF-R complexes or binding affinity for EGF and TGF-α, nor intracellular relocalization was found to be contributing explanations for the decreased EGF binding. Instead, both the initial induction and the subsequent decrease in EGF binding were correlated to changes in the rate of EGF-R gene transcription, and parallel changes were also seen in steady-state levels of EGF-R mRNA. EGF administered as a single dose into the portal vein of intact rats was found to induce hepatic EGF-R mRNA and EGF-binding capacity in a dose-dependent fashion, peaking 2–4 h after injection.

The EGF-R system appears to be involved in both the priming and progression phases of liver regeneration in the rat. The priming phase includes an induction of EGF-R, which can be mimiced by EGF simulation. The progression phase include an induction of TGF-α mRNA, coinciding in time with the decrease in EGF-R levels.

The possible role of growth hormone (GH) as a regulator of hepatic EGF-R was examined in GH-deficient rats and mice, before and after GH administration. GH deficiency was associated with low hepatic levels of EGF-R, and GH was found to increase hepatic EGF-R mRNA level in GH-deficient animals. However, when GH was administred to intact rats, GH was found to reduce hepatic EGF-R levels. Furthermore, aberrant regulation of hepatic EGF-R in hypophysectomized rats after partial hepatectomy may be linked to a retardation of the liver regenerative response.

The EGF-R system was also studied during 2-acetylaminofluorene (2-AAF) induced liver carcinogenesis in the rat. In preneoplastic hepatic nodules and in liver cancer, EGF-R mRNA and EGF-binding levels were reduced in a stepwise fashion, parallelled by a corresponding increase of TGF-α mRNA levels. Hepatic EGF-R mRNA and EGF binding was also found to be reduced by 2-AAF treatment in control rats, whereas hepatic nodules were less sensitive to 2-AAF treatment.

Similarities with respect to the EGF-R receptor system are found during liver regeneration, hepatic development, and liver carcinogenesis in the rat. In all these situations, TGF-α levels are increased in combination with lowered levels of EGF-R, compared with adult control livers. Elevated TGF-α expression might constitute a general mechanism for autocrine hepatocyte growth stimulation. Suppression of EGF-R levels might have been evolved to protect the organism from hepatic cancer development in situations where liver growth is induced, such as after toxic or mechanical insult.

November 1992

Intra-arterial and intra-venous chemotherapy combined with radiation in the treatment of brain tumours

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Based on clinical studies the following conclusions are drawn:

The combination of combined intra-arterial chemotherapy followed by radiation leads to an increased median survival with more long term survivers in patients with anaplastic astrocytomas and in patients older than 40 years with astrocytomas. In patients with glioblastoma multiforme, this modality of treatment do not improve median survival but an increased number of long-term survivors may be seen. *Patients younger than 40 years with* astrocytomas do not benefit from this modality of treatment. A parallelism exists between sensitivity to chemotherapy and response to radiotherapy. Patients who will benefit from the treatment may be selected early, normally two months after treatment start. Combining intra-arterial chemotherapy and radiation does not lead to an increased incidence of adverse CNS reactions. Specific transient abnormalities in the brain may occur during the first year after treatment and may be misinterpreted as tumour recurrence.

EEG may be valuable in predicting adverse CNS reactions following treatment. Nuclear brain scan may be valuable in selecting the patients who are in danger of developing adverse CNS reactions. Intra-arterial chemotherapy does have an effect in patients with brain tumours who have recurrent tumour after radiation. The most important prognostic factors are age, corticosteroid dependency at treatment start, performance status, histology and frontal lobe location.

November 1992

Perioperative predictors of tumor stage and prognosis in colorectal adenocarcinoma

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Overall survival in colorectal adenocarcinoma is approximately 50%. Survival is related to the depth of the tumor and the presence of tumor positive lymph nodes, characteristics that form the basis of the current staging system, Dukes' classification system. Curvative treatment is based on radical surgery. Additional therapy is currently evaluated in patients with more advanced Dukes' stages. As more sophisticated additional programs are developed, there is a need to identify patients that will benefit from their use, i.e. to improve the perioperative prediction of survival for the individual patient. The purpose of the present study was to evaluate the value of different potential perioperative predictors for the survival in colorectal adenocarcinoma.

Two hundred and twelve unselected patients operated on for colorectal adenocarcinoma, of which 177 (83%) were potentially cured, were examined regarding various clinical, histopathological, cellular and serological tumor characteristics. The aim was to improve 1. preoperative staging, and 2. postoperative staging beyond that achieved by Dukes' system.

The number of small-sized blood vessels was inversely correlated to survival. The DNA content, considering both ploidy and S-phase fraction, and the expression of α_3 and PDGF β-receptor were not correlated to the clinical outcome.

Endosonography turned out to be reliable when considering tumor growth in relation to the muscle layers suggesting that growth beyond muscularis propria indicates the need for preoperative irradiation.

All six serum tumor markers had prognostic value in univariate analysis. In multivariate analyses, taking Dukes' stage into account, only TPA remained as an independent prognostic marker. The predictive value of TPA preoperatively was almost the same as that of the Dukes' stage postoperatively and Dukes' stage and TPA together was the best predictor of patient survival. Tumor stage, TPA and age turned out to be the only factors with an independent prognostic value. This was the case both in the group of patients considered as potentially curable and in that considered as potentially cured. An increased TPA level might indicate subclinical metastatic disease even if the preoperative investigation is negative and the patient considered as potentially cured by surgery.

It is also important to evaluate newer prognostic markers properly against previously known, often easily available and/or inexpensive tests, before the newer ones are incorporated into clinical routine.

November 1992

Tumour blood flow—Methodology and determining factors—A study in rats

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Malignant tumours cannot grow without a sufficient blood flow. The tumour vascular network is different from normal vasculature. This difference can be exploited in cancer therapy by manipulating the tumour blood flow.

The aims of this study were to determine the conditions for blood flow measurements with the microstructure method, emphasising nonentrapment, and to investigate the influence of TNF-α on the tumour vascular bed as a basis for treatment of cancer.

Two syngenic tumours, an adenocarcinoma (NGW) and a hepatoma, were implanted in the dorsum of the right hind foot in rats. The microsphere method, the ^{86}Rb-fractional uptake technique and the ^{133}Xe-washout technique were used for blood flow measurements. The tendency for the microspheres to aggregate was evaluated in vitro and in vivo. A method for measurement of the passage of microspheres through the tumour vascular bed—nonentrapment—was developed. Adrenalin, noradrenalin, verapamil and TNF-α were given for intervention of blood flow and nonentrapment.

Injection of 400×10^3 but not 800×10^3 16 μm microspheres could be repeated with 10 minutes' interval without major haemodynamic effects. Ultrasonification increased aggregation of microspheres both in vitro and in vivo. There was significant nonentrapment of microspheres through the tumour vascular bed and the magnitude depended on the type of tumour, microsphere size and vasoactive influence. During a 96-hour observation period TNF-α reduced tumour blood flow by 50% and enhanced tumour growth. Thrombosis and extensive necrosis were also seen in these tumours. Microsphere nonentrapment through the tumour vascular bed was not reduced during the observation period which may indicate that TNF-α stimulated formation of abnormal tumour vessels.

In conclusion, the microsphere method is inappropriate for tumour blood flow measurements as nonentrapment was high, with large variations, and could be influenced by vasoactive drugs. Pharmacological doses of TNF-α reduced tumour blood flow but increased tumour growth rate.

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The role of calcium in cancer cell proliferation and cell death—with reference to mechanisms of action of calcium antagonists

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Calcium regulating mechanisms and the effects of calcium antagonists were studied in tumour cells. The dependency on extracellular Ca^{2+} showed that there was a significant difference in cell growth response between the cell lines. Whereas DU 145 prostatic cancer cells grew well in low Ca^{2+} concentrations, 1 BR fibroblasts and OVCAR-3 ovarian cancer cells grew poorly. Depolarization with high extracellular K^+ showed that voltage operated calcium channels probably exist in DU 145 cancer cells and 1 BR fibroblasts but not in OVCAR-3 cancer cells on Chinese hamster ovary cells. Verapamil did not block Ca^{2+}-influx in DU 145 or OVCAR-3 cancer cells but caused a paradoxical increase in cellular Ca^{2+} as a result of a decreased Ca^{2+}-efflux. Prenylamine in low concentrations reduced Ca^{2+} uptake, but in high concentrations increased cytosolic free Ca^{2+}. Verapamil and prenylamine also caused a significant inhibition of cell proliferation in a dose dependent manner in DU 145 and OVCAR-3 cells. ATP, carbacholine, and histamine increased $[Ca^{2+}]_i$ significantly in OVCAR-3 cells with a biphasic response due to both influx of extracellular Ca^{2+} and release of Ca^{2+} from intracellular stores. The effects of ATP and carbacholine on Ca^{2+} transients were partially inhibited by pertussis toxin and the phorbol ester PMA suggesting involvement of both a GTP-binding protein and protein kinase C. Furthermore, ATP, carbacholine, and histamine also stimulated cell growth in these cells. From these results it was concluded that an increase in $[Ca^{2+}]_i$ is an important factor in mediating both mitogenic action and antiproliferative effects in cancer cells. Muscarinic cholinergic receptors of the M_3 type were found in normal ovarian tissue, well differentiated ovarian tumours and the well differentiated cancer cell line, OVCAR-3, but not poorly differentiated tumours and the poorly differentiated cell line SKOV-3. It is suggested that these receptors are present in ovarian tissues and in ovarian tumours depending on the degree of differentiation.

December 1992

Adjuvant chemotherapy for colorectal cancer—A clinical and experimental study

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Treatment with sequential intravenous 5-fluorouracil 500–600 mg/m^2 and leucovorin 50 mg was tolerable and induced objective tumour regression in 10/34 patients with advanced symptomatic colorectal cancer. A low haemoglobin level, symptoms, a short disease-free interval and a low Karnofsky performance status were predictive of a poor reponse to chemotherapy and a short survival in 340 patients with advanced colorectal cancer. These results indicate an anti-tumour effect of sequential 5-fluorouracil and leucovorin. Intraperitoneal 5-fluorouracil in male Wistar rats after a left colonic resection caused weight loss during the 7-day course and a marked reduction of the breaking strength of the anastomosis and abdominal skin wound. The addition of intravenous leucovorin did not aggravate this effect. Weight loss alone did not impair wound healing to the same extent as 5-fluorouracil. The decrease in anastomotic breaking strength was not affected by an altered concentration of intraperitoneal 5-fluorouracil. Administration of 5-fluorouracil in the early postoperative period might thus increase the risk of wound healing complications, but this risk does not seem to increase further if leucovorin is added. The postoperative course was not more complicated in 50 patients treated with a 6-day course of intraperitoneal 5-fluorouracil and intravenous leucovorin after a curative colorectal cancer resection than in 51 patients treated with placebo. Chemotherapy-related toxicity was rare. Eleven patients who were given a similar course of intraperitoneal chemotherapy had a lower accumulation of hydroxyproline, reflecting collagen deposition, in subcutaneously implanted polytetrafluoroethylene grafts at the end of the treatment compared with 15 treated with placebo, but equal levels were found at two weeks. Experimental signs of altered wound healing thus did not correspond to an increased number of clinical complications in this study.

December 1992

Suppressor genes involved in tumourigenesis of colon and testis—A molecular and cytogenetic study

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In colorectal and testicular tumours, we have described several somatic mutations by using different genetic methods. Comparative RFLP analysis of normal and tumour DNA of the same patient have been used extensively throughout this study. A number of DNA markers have been used. In one study we detected a CA repeat polymorphism, with maximum 10 base pair deviation of alleles, by running the PCR products on a long polyacrylamide gel. Specific molecular techniques to detect base mutations (CDGE, DGGE, sequence analysis), were developed or established in our laboratory during the course of this study, and adapted to this project in a later part of the study. The most significant alterations observed in colorectal tumours were deletions of chromosome arm 17p loci and gain of chromosome arm 13q loci. Inactivation of both alleles of the tumour suppressor gene TP53, mapped to band p13 on chromosome 17, was shown to be an important event in the tumourigenesis of the colon and rectum.

It was previously known that chromosome 13 also harbour a suppressor gene, namely the gene which in an inactivated condition initiates retinoblastoma (RB1). Unexpectedly for a tumour suppressor gene, we found that loci within this gene exhibited increased copy number in colorectal tumours. This gain of DNA may reflect amplification of another yet undescribed gene closely linked to this region. Thus the importance of the RB1 gene itself in development of these tumours remains to be elucidated in future studies.

In 1986, when this work was started, very little was known about the involvement of the RB1 gene in other malignancies. In an adenocarcinoma from a patient with familial adenomatous polyposis, we found loss of heterozygosity at a locus in near proximity to the RB1 gene. This was probably caused by deficiency of chromosome 13, since a whole chromosome 13 was absent in all karyotyped cells. Since lack of chromosome 13 was one of rather few marker chromosomes, we concluded that this event had been involved in the genesis of this particular tumour. Here we observed concordance between DNA and cytogenetic studies of the same tumour. It would be of great interest to study, with a suitable collection of DNA markers, a panel of karyotyped tumours of which frozen samples were available. Such results would shed light on the interpretation of results obtained by both methods.

In 1987, the predisposing gene for familial adenomatous polyposis was shown to be linked to 5q markers. In a selection of patients with this hereditary disease we screened for possible germline mutations at this chromosome arm. Such a constitutional mutation would be of importance in the progress of cloning the gene. However, all were found to exhibit normal high resolution karyotypes without any consistent anomaly. During this work we established a standard for high resolution G-banding in our laboratory. Very little is known about genes possibly predisposing for testicular cancer. Therefore, in order to screen the whole genome for germline mutations, we used high resolution chromosome analysis. The panel of testicular cancer patients was selected on the basis of a possible genetic predisposition to the disease (bilaterality and/or familial occurrence). Normal karyotypes were found in all examined patients. We concluded that his level of resolution is not adequate for the purpose of screening for constitutional mutations, unless a serious phenotypic abnormality is present, in addition to the cancer. A significant frequency of loss of heterozygosity was observed at loci on chromosome arms 3p and 11p in a material of male germ cell tumours. Such losses indicate inactivation of one allele of putative tumour suppressor genes. Several reports had indicated that the 3p and 11p regions contain such genes and our results suggest that these genes are important in the tumourigenesis of the testis. We are now in the process of testing specific regions known to contain tumour suppressor genes, and eventually mutations within sequenced genes of interest, in a larger panel of additional tumours and the corresponding normal samples collected at this hospital.

It is now known that at least two of the genes involved in the development of the pediatric disease Wilms' tumour are located to the short arm of chromosome 11. Our results provide evidence for the influence of at least one of these genes (WT1), during progression of testicular cancer. Furthermore, our data suggest that the deficiency of one gene copy (as represented by a polymorphic locus within the gene) reflects in an early event in the genesis of male germ cell tumours. Explanation of the parental origin of the allele losses detected showed a preferential loss of paternal copies. These results suggest the possibility of inactivation by imprinting of maternally derived 11p gene(s). Thus, inactivation of both alleles of a tumour suppressor gene can be caused by another mechanism besides mutations. The WT1 gene is of great interest in further studies on testicular tumours. Normal and tumour tissue samples have been collected for expression studies. To enable us to follow up the study of imprinting as a possible contributing cause to testicular cancer, collection of additional parental DNA has been initiated.

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Adjuvant treatment of ovarian carcinoma—A clinical and experimental study

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The following conclusions are drawn:

—Adjuvant intraperitoneal ^{32}P therapy is equally effective as adjuvant cisplatin treatment in epithelial ovarian carcinoma.

—Adjuvant ^{32}P therapy is associated with more bowel complications than adjuvant cisplatin treatment. No significant relation existed between the incidence of small bowel complications following intraperitoneal ^{32}P therapy and the following variables: ^{32}P dose, ^{32}P distribution scintigram, type of surgery at instillation, number of prior laparotomies, or whether ^{23}P was given on the same day as surgery or the next day.

—None of 77 patients with well differentiated DNA diploid ovarian carcinoma in FIGO (1986) Stage I relapsed. A high-risk group with graded tumors in FIGO (1986) State I disease was identified. This group consisted of all moderately and poorly differentiated tumors, and highly differentiated DNA non-diploid tumors. The relapse rate in this group was 25%. Tumors with clear cell elements were not graded. The relapse rate was 43% in tumors in FIGO (1986) Stage I with clear cell elements.

—The high energy transfer and the short-range cytotoxicity of the α-emitter ^{211}At might be successfully exploited for intracavitary radiotherapy of intraperitoneal micrometastases.

a. ^{211}At was labeled stably on polymer microspheres.

b. The intraperitoneal distribution of ^{211}At microspheres in mice was favorable compared with ^{32}P and ^{90}Y colloids.

c. The therapeutic efficacy of ^{211}At microspheres in an intraperitoneal hybridoma tumor model was very encouraging compared with ^{32}P and ^{90}Y colloids.

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