Acta Oncologica Vol. 35, No. 2, pp. 249–255, 1996

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.

Ovarian epithelial tumours—Studies on steroid hormones and growth factors

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Ovarian carcinoma is one of the most common types of cancer in women and is associated with the highest mortality rate among genital tract malignancies. Normally, in the premenopausal stage, the ovarian stroma is the major source of sex steroid hormones. Women suffering from ovarian carcinomas have elevated plasma levels of steroid hormones as compared with healthy controls. There exists a need to clarify the relationships between steroid hormones, growth factors and proliferation in order to increase the possibilities of optimizing endocrine therapy in this disease. The aim of this investigation was to study the in vitro production of steroid hormones and epidermal growth factor/transforming growth factor-alpha (EGF/TGF-α) in malignant and benign ovarian tumours using an incubation model. The production was studied in relation to DNA content and to immunohistochemically determined expression of receptors and proliferation markers. Malignant tumours released more progesterone and androstenedione than benign tumours or normal postmenopausal ovaries. Tissue samples containing more epithelial tumour cells than stromal cells released more progesterone than those with predominantly stromal cells. All postmenopausal tumour-free ovaries contained both estrogen (ER) and progesterone receptors (PR), while tumourous ovaries to a varying extent had lost receptor expression. Among the malignant tumours, all the high producers of progesterone expressing PR were low-proliferating, diploid and p53 negative. Malignant tumours released significantly more EGF/TGF-α than did benign tissues. Aneuploid carcinomas released more than diploid. Addition of progesterone and estradiol increased EGF/TGF-α release from benign tumours. EGF/TGF-α and progesterone were measured in the urine of 74 ovarian carcinoma patients. Radically operated women excreted significantly less into urine than women with residual tumour mass. The patients who died from ovarian carcinoma had significantly higher concentrations of growth factor in urine than patients who were alive and disease-free at follow-up. A significant correlation between concentrations of progesterone and EGF/TGF-α in urine was not noted.

April 1995

cAMP-dependent protein kinase—Its regulation and its role in apoptosis of a promyelocytic leukemia cell line

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The following is taken from a rectica called 'Conclusion and Perspectives':

– Autodephosphorylation of cAKII is favored under conditions with sufficient substrate and adequate ATP supply, using the phosphate acceptor ADP present in the ATP-site of C. Autodephosphorylation is seen at the intracellular range of kinase-, substrate- and ATP/ADP-concentrations which makes it likely that autodephosphorylation is a physiological process.

C-MgADP has increased affinity for phospho-RII compared to RI, suggesting that phospho-RII is a better buffer than RI against C subunit which is continuously phosphorylating substrate. On the other hand, at low substrate availability, RI may be the best buffer of C. Determination of the C-MgADP effect in intact cells requires discriminating measurement of cAKI or cAKII activity in cells in which C has normal access to its substrates and in cells where substrate becomes limited e.g. by blocking of phosphatases. An indirect determination of isozyme activation is possible, based on high salt fixation of cAMP to its binding sites, immunoisolation of the isozyme and determination of its free cAMP sites (IV). A more direct determination of this effect in intact cells using cytoimmunochemistry would be more elegant. Imaging of a living cell with tagged C which relocates from RI to RII at high cAMP stimulation with high substrate availability would be a nice demonstration of this hypothesis. Preparative steps are being taken to produce the required antibodies.

– The antagonistic cAMP-analog (*R*p)-8-Cl-cAMPS is an instrument for inhibition of cAK isozymes in intact cells. However, the (*R*p)-cAMPS analogs have to be used with care, illustrated by (*R*p)-N^6-phenyl-cAMPS, an analog with a high cAK activating power. This implicates that modifications in the adenine moiety can dominate the antagonistic effect of the equatorial diastereoisomer.

Further characterization of (*R*p)-cAMPS analogs might reveal not only compounds with enhanced inhibitory potential, but even compounds activating one isozyme and inhibiting the other isozyme of cAK. Thses possibilities are actively pursued in collaboration with German groups.

– cAMP-binding to RI is closely correlated with induction of apoptosis in the murine leukemia cell line IPC-81 (IV). Additionally, microinjection of C induced apoptosis with the same morphological changes as following cAMP increase (V). Further, subcellular localization of cAK subunits in preapoptotic cells may shed further light of the connections between C and regulation of apoptosis.

A point mutation in the cAMP binding site B of one RI allele decreased the affinity for cAMP, and resulted in a dominant negative function which inhibited, but did not completely prevent, cAMP-induced apoptosis (IV). An even more potent quencher of cAMP action would be a transferable RI where both site A and B were similarly mutagenized. Such a construction is now being made and will presumably be a useful adjunct in our efforts to counteract cAMP in intact cells.

– Different modulators of serine/threonine phosphorylation (cAMP, okadic acid, calyculin A) induce different apoptotic phenotypes in a single cell line (VI). Phosphorylation seems to be involved in regulation of cell death, presumably on several steps in the apoptotic signal cascade (VII). The different apoptotic phenotypes suggest multiple cell death pathways regulated by phosphorylation.

Two-dimensional gel electrophoresis could be a useful approach to demonstrate the correlation between the morphological phenotypes and phosphorylated substrates. This method allows identification of phosphorylated target proteins and opens possibilities for microsequencing, cloning, and production of antibodies. Presumably, by combining this approach and screening for cell death modulating genes by differential hybridization of cDNA libraries (underway in cAMP-treated IPC-81 cells) it may become possible to tell the complete story of how cAMP-dependent protein kinase triggers apoptosis.

April 1995

Studies of MHC class I molecules and their peptide ligands in T cell responses to malignant and virally infected cells

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The molecular details of T cell responses against *ex vivo* human tumor cells or virus infected cells were studied. Expression of MHC class I molecules on the surface of human lung and ovary carcinoma cells was found to be required for their *in vitro* interactions with autologous lymphocytes. *In vitro* induction of MHC expression on autologous human tumor cells potentiated their stimulatory capacity and generated CTL which also lysed the MHC negative tumor cells. The products of several HLA class I alleles were found to serve as restriction elements of the CTL response against autologous, EBV-immortalized lymphoblasts (LCL) of a given individual. As binding of a peptide to the presenting MHC molecule is a prerequisite of its antigenic function, assessment of the binding capacity of peptides can be a practical method for identification of potential CTL epitopes. A screening assay was therefore established for detection of peptide-MHC class I binding, based on the finding that peptides which bind to a given class I product can stabilize it and elevate its cell surface expression. HLA-A2 binding peptides of the wild type and mutated tumor suppressor protein, p53, were identified by screening peptides selected on the basis of the A2 binding motif. Some of the peptides did not bind although they contained the motif. Analysis of HLA-A2 and H-2D^d binding Ala substitution analogs of a 12-mer HIV gp 120 peptide revealed several peptide residues to be important for binding to these class I molecules. Molecular modelling suggested that the second and eleventh peptide residues serve as anchor amino acids when binding to A2 and D^d. Thus peptides that are longer than a prototypic nonamer may use other amino acids than the C-terminal residue for binding to the F pocket of the class I antigen binding groove. Peptides with binding capacities for different class I allele products were identified using overlapping peptide series of the HIV-1 gag and the EBV-encoded EBNA-2 proteins, respectively. These data may serve as a basis for epitope identification. Again, several peptides carrying the relevant motif did not bind to the respective class I product. On the other hand, binding peptides without motifs were also identified. This suggested that the motifs had to be refined. Peptides of an overlapping peptide series encompassing the entire sequence of the EBV lytic cycle protein, BZLF-1 were screened for binding capacity to HLA-A2, HLA-B7 and HLA-B8. An HLA-B8 binding octamer peptide was identified as an epitope for HLA-B8 restricted CTL. Another BZLF-1 peptide, overlapping with the HLA-B8 restricted epitope, was demonstrated to serve as an epitope for HLA-Cw6 restricted CTL. The EBV-encoded EBNA-1 protein is mandatory for EBV latency and is expressed in all EBV carrying cells. In contrast to other EBV-encoded proteins expressed in EBV-transformed cells, EBNA-1 does not induce *in vitro* CTL response. We have identified several HLA-A2 and HLA-B7 binding EBNA-1 peptides. Thus, the inability of EBNA-1 to provoke a CTL response is not due to lack of class I binding peptides in its sequence. However, none of these peptides could sensitize target cells to lymphocytes stimulated with autologous LCLs of donors with relevant HLA-type, nor did lymphocytes of these donors, stimulated with the peptides, lyse cells of the autologus LCL. Thus, class I binding peptides may not be presented, or may not be recognized by CD8$^+$ cells due to tolerance of the relevant T cells or due to lack of peptide residues recognizable by the TCR.

May 1995

Kaposi's sarcoma in humans and malignant lymphomas in simian AIDS

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Kaposi's sarcoma (KS) and malignant lymphoma (ML) are frequently seen in patients with acquired immunodeficiency syndrome (AIDS). To elucidate factors involved in the pathogenesis of these tumors we have studied endemic African (EKS), AIDS-associated (AKS) and sporadic (SKS) Kaposi's sarcoma biopsies and a cynomolgus monkey model of AIDS lymphomagenesis. These studies show that EKS, AKS and SKS have very similar histopathological and immunophenotypic features. The spindle cell (SC) compartment in all KS forms consisted of two major cell populations, one fibroendothelial cell population corresponding to 65% of the SC and immunoreactive for Mabs TE7, EN4, CD31, CD34, CD36 and variably to PAL-E and vWf. The second distinct SC population corresponding to 35% of the SC, immunoreacted with Mabs to CD45 and the CD68 monocytic markers. In vitro propagated KS-derived cells expressed TE 7, vimentin, collagens I and III and to a less extent (5%) α actin but not CD45 indicating similarity with the TE7+ (fibroendothelial) SC in the lesions. Furthermore AKS and EKS spindle cells expressed α5, α6 and αv integrin subunits which are usually induced by inflammatory cytokines and also αx integrin shown to be expressed by monocytoid cells. Expression of E-selectin was only seen on vascular endothelial cells but not in SC. A strong tenascin expression and lack of thrombospondin expression was noted in the SC. This integrin profile in all KS forms suggests similar pathogenic mechanisms and an important role of paracrine and autocrine cytokines in SC growth of KS. These observations also suggest a low tumorigenicity of the KS lesions. Proliferation and ploidy studies showed that EKS, AKS and SKS are low to intermediate rate proliferating lesions with euploid DNA content suggesting that most KS lesions do not develop clonal sarcomatous-like tumors. No HIV antigens or proviral DNA were demonstrated by immunohistochemistry or polymerase chain reaction (PCR) respectively in the KS SC indicating that the role of HIV in the pathogenesis of AKS is indirect. Recently DNA-sequences of a putative, novel herpes virus (HHV8) were detected by PCR in AKS (Chang et al., 1994). Using the same PCR assay we demonstrated HHV8 DNA in EKS, SKS as well as AKS suggesting an etiopathogenic role for HHV8 in all KS forms. Preliminary results also showed the HHV8 sequence in some malignant lymphomas. Cynomolgus monkeys infected with SIVsm developed clinical manifestations similar to human AIDS, including lymphadenopathy with initial follicular hyperplasia and later involution and destruction of the follicular dendritic cell (FDC) network accompanied by disappearance of demonstrable follicular trapping of SIV antigen. Other histological changes included systemic multinucleated giant cells (MGC) formation of monocytic origin and a high frequency of non-Hodgkin's lymphoma (approx. 40%). No KS developed in these monkeys. The lymphomas were anatomically disseminated, of B cell origin had high grade malignant morphology and were associated with an EBV-like cynomolgus herpes virus (HVMF-1). 6/21 (29%) were oligoclonal, developing in animals with a rapid CD4 cell decline and 15/21 (71%) were monoclonal with longer induction time and slower CD4 cell decline. A predominant B-cell clone could in some cases be detected in lymphnodes before clinical lymphoma presentation, suggesting an early induction of the final malignant clone. No c-myc rearrangement or p53 overexpression could be demonstrated in a few tested cases. However 6/6 lymphomas expressed

IL-6 and IL-10 in the tumor cells. These findings are markedly similar to features of AIDS-related lymphomas (ARL). These studies indicate that during its development KS is a tumor-like, predominantly reactive lesion possibly with an infectious etiology and dependent on dysregulated cytokine production, secondary to HIV and/or other viruses (HHV8). This observation is important for the development of new treatments for KS. The high incidence of simian ARL in SIVsm infected cynomolgus monkeys indicate the importance of this SIV-cynomolgus model for studies of EBV-associated lymphomagenesis and possibly, therapeutic trials.

May 1995

Studies on podophyllotoxin derivatives—Etoposide pharmacokinetics and liposomal formulation of teniposide

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This thesis deals with ways to optimize the use of podophyllotoxin derivatives in cancer chemotherapy. The first part includes pharmacokinetic studies of etoposide with the ultimate aim to find a basis for individualized treatment. The second part deals with a liposomal formulation of teniposide developed to reach the mononuclear phagocytic system (MPS) specifically and minimize toxic side effects. The intracellular concentrations of etoposide in leukaemic blast cells during induction treatment of acute myelocytic leukaemia were studied. The intracellular concentration was 13.0 (+/−7.3)% (n = 22) of the plasma concentration, which is low compared to most other cytostatic drugs. The interindividual difference in cellular drug exposure ($AUC_{0\text{-}16h}$) was considerable, with a coefficient of variation of 104% and 54% after 100 and 200 mg etoposide/m^2 respectively. Protein binding of etoposide is extensive and therefore small changes in protein binding capacity may have a great impact on toxicity and the therapeutic effect. The studies showed variable protein binding in adult cancer patients with haematological malignancies (n = 37) or small cell lung cancer (n = 11) with a free etoposide fraction of 5.6 (+/−3.9)% and 5.7 (+/−3.4)% respectively in the two groups. A significantly elevated binding capacity in children (n = 11), the free etoposide fraction being 2.5 (+/−0.6)%, was found. Intracellular drug concentrations and free concentrations of etoposide vary among patients and are of potential value for therapeutic drug monitoring (TDM) of etoposide. Liposomes are man-made phospholipid vesicles. They are taken up by macrophages and could be used for drug targeting to the mononuclear phagocytic system (MPS). Liposomes containing teniposide (2.5 molar %) were produced and the effect of liposomal formulation on drug distribution was studied in mice. The drug uptake in the MPScould be modulated by increasing the size of the vesicles. When vesicles of 200 nm, 1 μm and 3 μm, were administered, the drug levels in the spleen 40 min after injection were increased 2.6, 6.8 and 21.1 times, compared with levels after injection of the commercial teniposide formulation. The organ distribution of teniposide in mice could be modified by administering the drug in liposomal form with the potential of improved treatment of diseases engaging the MPS.

May 1995

Carcinogenic effects of low dose irradiation in early childhood—A dosimetric and epidemiologic study

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The carcinogenic effect after exposure to ionizing radiation during infancy and after low organ absorbed doses was studied in subjects treated for skin hemangioma. During 1920–1959 more than 16,000 infants, less than 18 months old, were admitted to Radiumhemmet for skin hemangioma. Their mean age was 6 months. Most (89%) were irradiated with β, γ and/or x-rays. The treatment modalities changed over the years but radium-226 was the most common one (82%). Absorbed doses to the brain, thyroid, breast anlage, lungs, stomach, intestine, ovaries, testicles and bone marrow were calculated individually. Most of the organs (77%) received absorbed doses $\leq$0.1 Gy. A record-linkage with the Swedish Cancer Register revealed that 300 cancers were diagnosed in the present study during 1958–1986. The standardized incidence ratio (SIR) was 1.11 (95% confidence interval (CI) 0.99–1.24). Most sites did not show any increase in the number of cancers. However, as the median age at end of follow-up was 38 years most subjects had not yet reached ages where cancers are more common. During 1958–1986, 17 thyroid cancers were observed in the cohort (SIR = 2.28; 95% CI 1.33–3.65). The excess relative risk persisted at least 40 years after the irradiation. The mean absorbed dose to the thyroid was 0.26 Gy. A positive dose-response relationship could be observed. No differences in risk estimates between the sexes were seen. Seventy-five breast cancers were observed among the females in the cohort. The SIR was 1.24 which almost reached statistical significance. The excess relative risk increased with increasing time after exposure and did not level off during the current follow-up period. The mean absorbed dose to the breasts was 0.39 Gy. A positive dose-response relationship was observed. No increased incidence of leukemias could be observed neither among children nor adults.

May 1995

The idiotypic network response in cancer patients treated with monoclonal antibodies

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The anti-tumor effector functions of unconjugated monoclonal antibodies (MAb) in cancer therapy are not fully understood. Direct cytotoxic mechanisms such as antibody-dependent cellular cytotoxicity, complement-dependent cytolysis and apoptosis have been suggested. Induction of idiotypic network responses have also been proposed to be of therapeutic significance. Patients with advanced colorectal carcinoma (CRC), treated with mouse or chimeric MAb17-1A, were shown to develop both anti-idotypic (ab_2) and anti-anti-idiotypic (ab_3) antibodies during therapy. Inclusion of granulocyte/macrophage-colony stimulating factor (GM-CSF) into the mouse MAb treatment schedule significantly enhanced the induction of both ab_2 and ab_3 as compared to treatment with mouse MAb alone. Patients exhibiting high ab_2 concentrations posttreatment and a high increase in anti-tumor cell binding antibodies survived significantly longer after institution of MAb therapy than those with low ab_2 concentrations and no or low increase in anti-tumor cell antibodies. In the same patient group induction of a T cell immune response directed against the infused antibody and human anti-idiotypic antibodies was shown. A significant correlation between the induction of ab_2 and nominal antigen (GA733-2E, the antigen mimicked by ab_2) specific T cells and tumor regression was found. Human anti-idiotypic antibodies mimicking a colorectal carcinoma associated antigen were further used for immunization of surgically treated CRC patients without evidence of disease. Induction of both a B and T

cell immune response with specificity not only for the immunizing agent, the human ab_2, but also for the nominal antigen was shown. The immunity was also analyzed with respect to epitope specificity and structural correlates for the idiotypic mimicry between ab_2 and the nominal antigen. The results provide further support for the notion that an idiotypic network response might be an important indirect effector function at therapy with unconjugated MAb and that MAb based therapy not only should be considered passive immunotherapy but also capable of inducing an active specific immunization. Furthermore, the study indicates that human anti-idiotype antibodies can be used for induction of a humoral and cellular immune response against "self" antigens.

May 1995

Therapeutic options in carcinoid mid-gut disease—Evaluations by clinical and cell culture studies

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The traditional treatment of mid-gut carcinoid patients is based on surgery and embolization of the arteries supplying liver metastases. Additional medical treatment consists of interferon, octreotide and chemotherapy with cytotoxic drugs such as 5-Fluorouracil and Streptocotozin. Although promising in open trials, the appropriate use of interferon and octreotide needed evaluation in controlled trials. We report the results from prospective studies of octreotide and interferon combined with surgery and embolization An in vitro model was established in order to test drug effects and mechanisms of actions as the incidence rate of carcinoid tumours is low.

Our studies showed that interferon either with or without embolization induced response or inhibited progression in 30 out of 42 patients based on the 24-h excretion of 5-hydroxyindole acid 5-HIAA) and in 30 out of 42 patients based on computer tomography (CT) evaluation of the largest liver metastasis. Median survival time in 42 patients with the carcinoid syndrome at inclusion was 39 months. The combined treatment with interferon and embolization provided the best results judged from survival. In this group mean observation time was 61 (39–84) months and eight of nine patients were alive at termination of the study. Survival in a group of patients randomized to stop interferon treatment after one year was significantly shorter than in patients who continued this treatment ($p<0.03$). Octreotide provided improved quality of life even after a short treatment period and that the main symptoms of the carcinoid syndrome, diarrhea and flushing, improved after therapy with relatively low doses.

The heart affection in our patients was studied, since others have shown that this is the single most important predictor of survival. Twenty-six out of 37 patients displayed insufficient valves at echocardiography and tricuspid regurgitation was found in 22. Furthermore, in this study the patients were found to display significantly decreased early (E) through atrial phase (A) transmitral filling velocity ratio (E/A-ratio). This parameter assesses left ventricular myocardial compliance, and indicates a possible left ventricular manifestation of carcinoid disease.

The treatment drugs were tested for their influence on the serotonin (5-HT) metabolism in primary carcinoid cell cultures. Interferon and octreotide both lowered cell medium 5-HT significantly compared with controls. Interferon inhibited intracellular 5-HT as well, indicating an inhibition of synthesis. Octreotide had no effect on intracellular 5-HT. Neither drug reduced total DNA in the culture for the time tested. Additionally, in this model, interferon augmented the effect of irradiation confirming our clinical experience.

Based on our findings the current treatment of midgut carcinoid patients is:

- –initial surgical reduction of tumour load,
- –repeated embolization of liver metastases and
- –subsequent treatment with interferon-a2b when tolerated with additional octreotide for symptom relief.

May 1995

Transformation and replication characteristics of in vitro evolutionary mutant papillomavirus genomes

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Papillomavirus early gene expression is subject to tight regulation in virus infected cells by the operation of incompletely understood transcriptional inhibitory feedback loops. This thesis describes the characterization of in vitro evolutionary mutant bovine papillomavirus type 1 (BPV-1) genomes which were associated with spontaneous focus formation in a latently infected mouse C127 cell line harbouring a weakly transforming mutant BPV-1 derivative. In addition, some novel observations on the mechanisms of latent BPV-1 plasmid DNA replication arising from studies with these spontaneous viral mutants are presented. Four transformation associated spontaneous BPV-1 mutants were analysed. Three had simple deletions within the E1 gene, which was known to encode an essential viral DNA replication factor, and a fourth mutant had an insertion comprising a direct repeat of sequences spanning the 3′ end of E1 and the 5′ half of the transcriptional transactivator gene, E2. Each E1 deletion mutant was both replication-defective and transformation-defective, but 2/3 of these mutants nevertheless stimulated cell transformation to high level when cotransfected with a replication-competent BPV-1 genome. In contrast, the insertion mutant genome was replication-competent and exhibited a high transforming phenotype in the absence of a cotransfected genome. However, a common property of these sponteneous transformation-associated mutants was their ability to overexpress the E2 transactivator gene, and it is suggested that this was achieved by diverting the major control of transactivator gene expression to an upstream viral early gene promoter. These studies support the notion that BPV-1 early gene expression is normally limited due to tight control of E2 transactivator levels, and underline the concept that neoplastic progression associated with papillomavirus infections is likely to occur predominantly by the disruption of viral transcriptional regulatory circuits with a resultant derepression of viral oncogene expression.

May 1995

Malignant melanoma—Risk factors

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The purpose of the study was to investigate risk factors for malignant melanoma. Two sources of information were used: (i) a case-control study including 127 patients with malignant melanoma and 155 patients with lymphoma or thyroid carcinoma and 794 age-stratified healthy controls randomly selected from the South Swedish Health Care Region (SSHCR); (ii) a population-based, matched case-control stydy of malignant melanoma from

SSHCR including 400 patients with a first histopathological diagnosis of malignant melanoma and 640 controls. The results show that acute intermittent rather than chronic, cumulative sun exposure is associated with increased risk of melanoma. No support was given to the suggestion that sunburns experienced in childhood carry a greater risk of melanoma than exposure in other time periods. Use of sunbeds or sunlamps was found to be a risk factor for malignant melanoma. A higher risk and higher exposure rate was seen in young people. Melanomas of the trunk were strongly associated with use of sunbeds or sunlamps, while lesions of the extremity or head and neck were not. Unexpectedly, the use of sunscreens did not protect against malignant melanoma development, but was instead related to an increase in malanoma risk. When different melanoma presentation sites were considered, lesions of the trunk were associated with sunscreen use in females while lesions of the extremity or head and neck were associated with sunscreen use in males. There was an increased risk of malignant melanoma in individuals with many pigmented naevi, with blond or red hair colour, and/or a tendency of freckling. In addition, family history of malignant melanoma was significantly associated with melanoma risk. The study did not support a relationship between malignant melanoma and reproductive or hormonal factors in females. However, increasing number of live births seemed to be protective. No association was observed between alcohol intake or smoking habits and melanoma risk. Interestingly, some support was lent to the possibility that some specific types of prescribed drugs i.e. beta-blockers, hydralazines and benzodiazepines, may be associated with melanoma development. However, the elevated odds ratios were, at least for benzodiazepines, diminished after adjustments for host factors. The questionnaire used was found to provide information with high test-retest reliability.

May 1995

Radiotherapy of prostatic cancer—with reference to positioning and three-dimensional dose-planning

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Prostatic adenocarcinoma is frequently diagnosed in the Western world. While the latent form of the malignancy is very common throughout the world, the incidence of more aggressive forms varies.

The treatment of prostatic adenocarcinoma is the subject of much debate. In some cases, treatment may not affect survival or quality of life, but there are cases that benefit from aggressive curative treatment. If radical radiotherapy is chosen, several parameters must be taken into consideration for the therapy to be optimal.

Conformal therapy is a term used to denote external beam irradiation based on three-dimensional dose-planning in order to optimise the dose distribution in the target and organs at risk. By altering the number of fields (4–6), margins (10 or 20 mm) and field collimation (multileaf collimator), it was found that field collimation and margin reduction decreased the dose to risk organs. However, an increase in the number of fields did not improve the dose distribution in the rectum or the bladder. On verification films of patients with implanted gold seeds, it was not possible to reduce the margins to 10 mm and a radiobiological model predicted that small displacement errors in the fields would have substantial impact on local control.

In order to improve dose distribution to risk organs, positioning, fixation and verification systems must be developed if margin reductions are to be a feasible therapy option. A system that makes use of implanted magnetic markers was found, in a phantom study, to position the phantom with an accuracy of 0.5 mm.

Artificial intelligence neural networks were trained to evaluate treatment plans presented as dose-volume histograms and found to be able to accept or reject plans according to the assessments of three radiation oncologists.

May 1995

Clinical trials in multiple myeloma—Treatment results and methodological aspects

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The purpose of the present study has been to evaluate new treatment strategies for myeloma, by the use of clinical trials, and to explore some aspects of the methodology of clinical trials, with emphasis on patient accrual.

From October 1983 until December 1986, 162 myeloma stage II-III patients were entered in a randomised study, comparing melphalan-prednisone therapy (MP) with combination chemotherapy. No advantage was found for combination chemotherapy, either for response or survival.

Fifty patients with asymptomatic myeloma stage I were randomised to MP given from the time of diagnosis, or deferred therapy, i.e., MP from the time of disease progression. In the latter group, 50% developed progressive disease and were started on MP before 12 months. Response rate and survival was similar in the two patient groups.

An incidence study in Western Sweden in 1984–1986 showed a crude incidence rate of 6.3 cases of myeloma/100 000 inhabitants/year. Of the 300 new cases, 60% were included in the ongoing clinical trials, 24% were excluded, and 16% were never reported to the study secretariat. The latter two groups had higher ages and inferior survival compared to the included patients. The application of lower age limits and other critera for exclusion of patients with inferior prognosis resulted in longer survival for the remaining patient group.

Interferon-alfa-2b (IFN) for initial therapy in combination with MP, followed by IFN maintenance was evaluated in a multicentre study by the Nordic Myeloma Study Group (NMSG 4/90). From June 1990 until November 1992, 594 patients were randomised to MP or MP + IFN 5 MU three times weekly. In responding patients achieving a plateau phase, MP therapy was interrupted, and then reinstituted at relapse, whereas IFN therapy was continued throughout the plateau phase, until the time of definitive failure on MP. No difference was seen in response rate or survival between the treatment arms. However, response and plateau phase duration was longer for patients treated with IFN as compared to no maintenance.

Patient accrual and quality of participation in the NMSG 4/90 trial was evaluated in a comparison between 13 university hospitals, 25 major county hospitals, and 61 minor county hospitals. About 1/3 of the patient material in the trial was recruited from each of the three hospital categories. No differences in the quality of participation were observed, as judged by adherence to the study protocol and dose intensity of melphalan and IFN.

Physicians' attitudes towards clinical trials were evaluated by a questionnaire that was mailed to 99 investigators in the NMSG 4/90 trial. Several factors were found to influence patient accrual: the scientific aim of the study, ethical aspects, communication

between participators and study organization, and cost and reimbursement awareness. These factors should be taken into account when planning and implementing future trials.

May 1995

Normal skin reactions in radiotherapy—Proliferation, progression and prognostic factors

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Clinical radiotherapy is now in a rapid phase of development with altered fractionation schedules and new techniques. But it is still the reactions of the normal tissue surrounding the tumour that limit the possible dose, which is set according to the reactions of the most sensitive patients. A considerable variation in normal tissue reactions exists. Identification of radiosensitive individuals and extrinsic prognostic factors for this variation would be valuable. All changes in the therapy will also affect the normal tissues. We have therefore studied acute and late skin reactions to radiotherapy in different respects.

A method for assessing the acute skin reactions by determining the epidermal basal cell density (BCD) from skin biopsies during and after irradiation has been evaluated. The reaction in pig skin were investigated for different doses and dose-rates, as well as the reactions in patients with 5 different doses and dose-rates, as well as the reactions in patients with 5 different fractionation schedules. The BCD was found to be a reliable and reproducible measure.

The skin reactions in patients treated with accelerated radiotherapy were compared with those in patients treated with conventional fractionation to the same dose. The interfraction interval was reduced from 24 to 8 h. A decreased level of acute reactions was seen with the accelerated schedule using BCD and skin score as endpoints. The result is interpreted as a relative lack of redistribution of cells between the daily fractions. In contrast, the late reactions were increased with degree of telangiectasia as the endpoint. Incomplete repair of sublethal damage is the most plausible explanation.

In order to identify possible extrinsic factors contributing to the individual variation in normal tissue reactions, 20 patient and treatment-related factors were analysed in 402 patients using a multivariate procedure with acute and late skin reactions as endpoints. Except for dose level and fractionation schedule, the only significant prognostic factors found for acute effects were the systolic blood pressure and the preirradiation skin reflectance value, which is an indicator of the oxygen haemoglobin content in the skin. Significant factors for late effects, additional to the dose level, were the degree of acute reactions and the individual variations in dose.

Differences in cellular radiosensitivity were investigated with clonogenic assay in 10 fibroblast cell lines, derived from skin biopsies of 6 patients treated with radiotherapy ten years earlier. The results were compared with the acute and late skin reactions in these patients. A significant correlation was found between fibroblast radiosensitivity in vitro and the degree of late skin reactions. However, 70–80% of the individual variability in normal tissue reactions could not be explained by extrinsic factors. Genetic cellular differences in radiosensitivity are likely, and a correlation with late tissue reactions was detected. This provides the basis for predictive testing of radiosensitivity with possibilities of individualised treatments and increased tumour control probability.

May 1995

Enhancement of chemotherapy and hyperthermia in an experimental brain tumour model by manipulation of tumour microenvironment and drug delivery

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The following conclusions are drawn;

I.p. glucose does not improve the effect of hyperthermia, or chemotherapy with ACNU or BCNU in the BT_4 tumour model, but significantly improves treatment outcome after thermochemotherapy with these drugs. This enhancement is achieved without observed increase of side effects.

The effect of ACNU is substantially increased by local hyperthermia. The drug is therefore suitable for thermochemotherapy.

I.p. glucose did not prevent thermotolerance development. I.p. glucose did not reverse the increased resistance to further thermochemotherapy with BCNU induced by thermotolerance. To optimise the effect of i.p. glucose or treatment with similar circulatory and intratumoral environmental effects, tumours should not be treated in a thermotolerant state.

These studies show timing of i.p. glucose and thermochemotherapy to be a critical factor for treatment outcome. The optimal time interval between glucose i.p. and thermochemotherapy is shorter than the time to develop the lowest intratumoral pH after i.p. glucose, probably due to glucose induced reduction of tumour perfusion and drug uptake.

Administration of glucose i.p. after thermochemotherapy improves tumour response. Because of the suitability of this timing regarding cytotoxic drug uptake and expected systemic toxicity, use of this time interval should be explored further.

In vitro, acute pH reduction increases the effect of thermochemotherapy with BCNU.

I.p. glucose induces hyperglycemia and a substantial haemoconcentration, with a transient and moderate reduction of blood pressure. No indication of a systemic circulatory collapse has been observed. An intratumoral pH reduction was observed after i.p. glucose, and was accentuated by simultaneous hyperthermia. This pH reduction was also seen, but with a lesser magnitude in thermotolerant tumours. The degree of intratumoral pH reduction probably affects the influence of i.p. glucose on the thermochemotherapy effect.

Use of methods that mimic the circulatory and metabolic effects of i.p. glucose should therefore be investigated further, because of the potential benefit in human medicine.

A functioning system and procedure for i.a. drug treatment of intracerebral BT_4An tumours, combined with the use of a system for local brain hyperthermia, have been developed. Local brain hyperthermia increased tumour response after chemotherapy of intracerebral BT_4An tumours with i.a. or i.v. ACNU. Thermochemotherapy of intracerebral tumours was improved when ACNU was administered i.a. instead of i.v.

Thermochemotherapy with i.a. drug administration is a promising method for local enhancement of thermochemotherapy response. Before treatment of humans with a reasonable life expectancy, normal brain tissue tolerance to hyperthermia alone and combined with drugs has to be investigated further in larger animal species.

May 1995

Prostate cancer—Epidemiological studies

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Prostate cancer is a large and increasing medical problem both in Sweden and in the rest of the developed world, with about 300.000 new cases diagnosed world wide annually. Despite the high incidence of this disease, little is known about the aetiology of prostate cancer. The aim of this study was to try to understand more about the natural history and to find possible a etiological risk factors for this tumour. In a population based study of prostate cancer cases in northern Sweden it was found that the large increase in prostate cancer during the last two decades was mainly caused by well (G1) and moderately (G2) differentiated tumours. However, the indicence of poorly differentiated (G3) tumours remained unchanged. The introduction of new diagnostic methods is the most plausible explanation for the increase of these low grade tumours. The relative survival in prostate cancer was found to be independent of patient age at diagnosis, indicating that tumour proliferation and the aggressiveness of this disease is equal in all ages. However, due to the increasing occurrence of concurrent diseases with growing age the number of lost years caused by prostate cancer decreases dramatically in older age groups. The overall cause specific mortality for prostate cancer was found to be around 50%. In accordance with most other cancer tumours, the annual mortality rate decreased with longer survival also for prostate cancer patients. In a study from the Swedish Twin Register it was found that the proband concordance rates for prostate cancer were 4,5 times greater among monozygotic compared to dizygotic twins. In a large nation-wide cohort study of men who had a father with prostate cancer, the overall standardised incidence ratio (SIR) was 1.70 for prostate cancer. Younger age at diagnosis among the fathers were associated with an increased risk among sons. This cohort study and the twin study indicates that both inherited and familial factors are of importance in a subgroup of prostate cancer patients. In a prospective case-control study, both a high mass index (BMI) and a high food intake were found to be independent risk factors for prostate cancer. Both BMI and a high food intake might be indicators of a high fat diet, which so far is the most consistent exogenous risk factor for prostate cancer. The use of tobacco or alcoholic beverages were not associated with prostate cancer risk.

June 1995