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Abstracts of Theses from the Nordic Countries

Short abstracts of theses on oncologic subjects are published under this heading. The abstract should contain background, problems, results and conclusions and be an independent informative unit that can be read without access to the thesis. It should not contain references to literature, figures or tables in the thesis. A suitable size is about 500 *words. The abstract can be sent to Acta Oncologica together with information about department, faculty and university and date of dissertation.*

Genetics changes in childhood acute lymphoblastic leukemia and other lymphoid malignancies

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Malignant transformation of normal cells is the result of defects in cell growth control, differentiation and programmed cell death. It has been convincingly shown that malignant cells carry mutations in the genes controlling these cellular processes.

Acute lymphoblastic leukaemia (ALL) is the most frequent malignant disease of childhood, constituting approximately 25% of all childhood malignancies. Despite the great improvement in the outcome of these children, approximately 20% of the patients still die from their disease, due to recurrence, to side effects of the treatment or, rarely, to resistant disease. Clonal chromosomal abnormalities can be shown by karyotyping in 80% of the ALL cases, the genetic alterations in the remaining 20% can only be detected by molecular techniques.

Deletions in 6q, 9p, 13q and the translocation t(12;21) frequently resulting in loss of heterozygosity (LOH) in 12p, are examples of mutations found in ALL. Our screening of primary ALL-clones for genetic alterations showed LOH in 6q in 24% of the cases, deletion in chromosome 9 in 31%, LOH or deletion in chromosome 13 in 10% and LOH in 12p in 12% of the cases. An inverse correlation between LOH in 12p and deletions in the Ink4-locus on 9p was found. This may suggest that the mutations on the respective chromosomes are affecting the same molecular pathway, alternatively it reflects the immunophenotypic groups commonly affected by these mutations.

Deletions in the long arm of chromosome 6 are frequently found in lymphoid malignancies. Expanded LOH studies revealed that LOH in 6q occurred in 32% of the ALL cases studied: a higher rate than previously reported. A minimally deleted region of less than 1 centiMorgan (cM) around the marker D6S283 was identified. Our results suggest that this region may harbour a tumour suppressor gene relevant for the development of ALL. Our results also indicate a second less well defined region on 6q telomeric of the region lost in ALL, in which LOH can be detected in non-Hodgkin's lymphoma (NHL) of adulthood.

The Ink4-locus in 9p21 contains three genes involved in the regulation of the G1 phase of the cell cycle (*p16ink4a*, *p15ink4b* and *p14ARF*). Malignant cells from children with ALL were analysed for mutations within the Ink4-locus, using Southern blot hybridisation, single-strand conformation polymorphism (SSCP) analysis and nucleotide sequencing. The results showed that inactivation of the *p16ink4a* and *p14ARF* genes within the Ink4-locus is associated with an inferior prognosis. Although this genetic marker co-varied with other high-risk features such as high white blood cell count and T-cell phenotype, it was identified as an independent prognostic factor by multivariate analysis.

Hyperdiploid karyotype has been associated with a subgroup of childhood ALL with favourable clinical features and a low probability of relapse. DNA-hyperdiploidy, measured as an increased DNA-index by image-DNA-cytometry (ICM), and bi-allelic deletions within the Ink4-locus in ALL, showed a significant inverse correlation. Ink4-locus deletion had a stronger impact on outcome than DNA-hyperdiploidy.

H9 is a malignant T-cell line that has bi-allelic deletions of the Ink4-locus as well as mutated *Rb* and *p53*. H9 is deficient in P21 protein expression although *p22Waf1/Cip1* mRNA was detected. A stretch of approximately 400 bp missing from the coding exon 2 was found in the cDNA, possibly explaining the lack of P21 protein expression in H9 cells.

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Clinical and immunopathological studies in Hodgkin's disease with special reference to prognosis

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Hodgkin's disease (HD) and non-Hodgkin lymphoma (NHL) have certain features in common, but also distinct clinical and pathological differences. Thus, HD has many features of both an infectious (fever, leukocytosis, eosinophilia, night sweats, lymphadenopathy, splenomegaly) and a malignant process with few tumour cells, T-cell accumulation and sclerosis. Today, current therapies fail to cure about 1/3 of patients with advanced HD. The main purpose of the present study has been to improve the understanding of certain biological and clinical features of HD and look for their clinical applications with regard to diagnosis, treatment and prognosis.

A morphological review with immunostainings of 238 biopsies diagnosed as HD showed reclassifications to other diagnoses in 11%, with T-cell-rich B-cell lymphoma and anaplastic large cell lymphoma as the most frequent misdiagnosed entities. Thus, immunohistochemistry makes it possible to identify NHL, that are morphologically difficult to distinguish from HD.

The proportion of Epstein–Barr virus (EBV)-positive HD tumours was 40%, detected by latent membrane protein 1 (LMP1; immunohistochemistry) and EBV-encoded RNA (EBER; in situ hybridisation). Apart from EBV-positive tumours being more common in patients with the subtype mixed cellularity (MC), clinical features, blood lymphocyte function and outcome were not related to tumour EBV status. Patients with EBV-positive tumours had elevated soluble (s)CD54 serum levels, higher antibody titres to EBV early antigen restricted and decreased total white blood cell counts. A potential causal relationship between EBV tumour status and these findings needs to be further explored.

A high eosinophil infiltration in HD tumour tissue was seen in 1/3 of 259 untreated patients, most pronounced in younger patients, nodular sclerosis (NS) type 2 and bulky disease. The degree of eosinophilia was not associated with the number of Hodgkin–Reed–Sternberg cells, the phenotype distribution of small lymphocytes, EBV tumour status or clinical outcome. Thus, deter-

mination of tissue eosinophilia is of limited diagnostic and prognostic value in HD. Mixed cellularity HD tumours had significantly higher numbers of lymphocytes positive for CD8, T-cell intracellular antigen-1 and granzyme B, respectively, than NS. This T and NK cell infiltration in MC might be explained by the high levels (mRNA and protein level) of the chemokines IP-10 and Mig previously observed in MC.

Tumour infiltrating lymphocytes had a decreased expression of the CD3-ζ-chain in 66% of biopsies. Among a number of biological markers under study, high serum levels of interleukin-10 and sCD30 were found to be associated with a poor prognosis, and may add prognostic information to that achieved by the International Prognostic Score.

In a randomised study, fixed vs response adapted chemotherapy was evaluated in responding patients with advanced HD. The policy to give 6–8 courses of chemotherapy to this patient group seems valid. The price, however, for such an approach is the over-treatment of a subset of already cured patients.

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Oral health and experience of oral care among cancer patients during radio- or chemotherapy

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Oral complications and symptoms are common among patients with cancer. The aim of this thesis is to study several aspects of oral status, oral health and its relation to quality of life, and oral care among patients treated with radiotherapy or chemotherapy. Descriptive, comparative and correlational designs were used.

A series of consecutive patients admitted to a university hospital or a regional hospital to receive radiotherapy for head and neck cancer or chemotherapy for haematological malignancies, were studied prospectively with regard to oral symptoms and their relation to health-related quality of life using interviews and questionnaires, examination of the oral cavity and saliva tests. All nurses and enrolled nurses who worked with these patients or with patients with lung cancer were interviewed about their education and knowledge in oral care and performed oral care. The medical and nursing records on patients with these cancer diseases at the two hospitals were reviewed.

The results indicate that patients receiving radiotherapy experienced increasing oral symptoms, which remained to a large extent one month after treatment. Patients receiving chemotherapy did not experience oral symptoms to the same extent. The oral symptoms were significantly related to patients' health-related quality of life, particularly among those receiving radiotherapy. Data also indicate that there is a lack of adequate education and continuing education in oral care among nursing staff. All patients were not examined orally before or during treatment, nor did they receive sufficient information or instruction related to oral hygiene. Patient compliance with oral hygiene procedures was acceptable, although some patients reported difficulties. Oral status and oral care were insufficiently documented, particularly in nursing records. The attitudes to oral examination and discussion on oral hygiene differed between nursing staff and patients. Nursing staff objected to examining the oral cavity referring to patient integrity. This was not considered as a hindrance among patients. In conclusion, oral health is related to health-related quality of life, which motivates a multi-disciplinary approach to oral care.

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Regulation of tissue factor expression in myeloid and monocytic leukaemia cells

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Tissue factor (TF) is a transmembrane glycoprotein that initiates the blood coagulation cascade and is also involved in cell migration, tumour metastasis and angiogenesis. Pathologic expression of tissue factor by monocytes contributes to several thrombotic complications like acute coronary artery disease and disseminated intravascular coagulation. The aim of this thesis was to investigate the clinically important pathologic expression of TF in myelomonocytic leukaemia cells and reveal the cellular signals leading to the suppression of TF expression.

The studies in this thesis indicate that TF is a marker of immature myelo-monocytic cells. Markedly higher levels of TF were expressed in immature myelo-monocytic cell lines compared to mature monocyte-like cells. Induction of terminal differentiation in immature cells resulted in down-regulation of TF expression, irrespective of the specific phenotypes induced by retinoic acid (RA) or vitamin D_3 in monoblastic U-937 cells. TF suppression was also found independent of differentiation pathways, i.e. monocytic or granulocytic. The nuclear receptor activation requirements for transcriptional suppression of TF by retinoic acid (RA) were shown to differ between acute promyelocytic leukaemia (APL) NB4 and U-937 cells. In NB4 cells the binding of the agonist to the RA receptor (RAR)α alone is needed for down-regulation of TF, whereas ligand binding to both RARα and retinoic X receptor was necessary for efficient suppression of TF expression in U-937 cells.

To analyse the transcriptional regulation of TF, stable NB4 and U-937 clones expressing the luciferase gene under the control of various 5′ flanking regions of the TF gene were selected. Different promoter regions were found to control the basal TF transcriptional activity. Analysis of protein binding to the 140 bp promoter region, responsible for basal TF activity in NB4 cells, revealed binding of RFX-1. RA suppressed the promoter activity in NB4, but not in U-937 cells. The ecotopic expression of the APL fusion proteins PML/RARα or PLZF/RARα in U-937 receptor clones were shown to confer sensitivity to RA-induced suppression of TF promoter activity.

These results provide a more detailed picture of TF regulation in leukaemic and haematopoietic cells and may have a bearing on new clinical treatment strategies in APL and other leukaemias.

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Assessment of UV exposure in a young metropolitan population

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Overexposure to UVR causes $\geq 80\%$ of skin cancers, and $\geq 30\%$ of all diagnosed cancers in western societies, Sweden included, are skin cancers. Overexposure in terms of UV-induced erythema during childhood and adolescence is considered particularly harmful. The widespread use of sunbeds among adolescents and young adults contribute considerably to UV exposure.

The objectives of this thesis were to i) analyze the relation of sunbed use to phenotype, erythema, sunscreen use and skin disease in adolescents, and ii) to analyze adolescent's sunbed use and its relation to sex, age, attitudes, and smoking, iii) to estimate the proportion of artificial/natural UV exposure in terms of population doses, considering exposed skin area, iv) to assess UV exposure and erythema in the population aged 13–50 years, and v) to compare two cross sectional studies (1993 and 1999) of adolescents' sunbed use, including the relation of sunbed use to age, phenotype, sunscreen use and smoking.

Two cross-sectional surveys were applied, with participants (ages 15–19 years) being selected by stratified random sampling of classrooms (1993), and stratified random sampling by age (13–19 years and 20–50 years respectively) from the national census registry.

During 12 months preceding the survey in 1993, 70% of female, and 44% of male adolescents had used sunbeds, 44% of them had reported sunbed erythema. Sunbeds were used in equal proportions, regardless of skin reaction upon sunbed use. Sunbed use was positively related to phenotype, sunscreen use intentional outdoor tanning, and smoking. Population doses of natural UVR in Sweden, and estimates of exposure to artificial UVR suggested that erythemally effective UV-doses from sunbeds may be equivalent to those from the sun, considering exposed skin area. During 12 months preceding the survey in 1999, almost half of responders aged 13–50 years had been on vacation to a sunny seaside resort abroad, 33% of females, and 17% of males tanned outdoors, almost every second female and one of four males used sunbeds. One adult of four had given up sunbeds. Females used sunbeds and tanned outdoors twice as often as males, particularly adolescents aged 17–19 years and adults aged 20–29 years. Sunburn was reported by 55% after tanning outdoors in Sweden, abroad, or both, and one third of those reported sunbed erythema. Outdoor tanning, sunbed use and related erythema was particularly frequent in young females. Levels of sunbed use and sunbed erythema among adolescents aged 15–19 years was 50% lower in 1999 than in 1993, but outdoor tanning had increased. In both 1993 and 1999, the same dominance of females was observed in sunbed use, and its association with phenotype, outdoor tanning, sunscreen use, and smoking.

The studies confirm previous findings that young populations from high-latitude areas actively seek high risk settings of overexposure to UVR. Risk behaviors are positively related, particularly in females, erythema is experienced by a high proportion of the population at an early age, and the accumulated incidence and prevalence of UV-induced erythema is high. From a skin cancer control perspective, initiatives should be taken to promote sun awareness as a conceptual norm from early age, including the implementation of alternative tourism, in combination with restrictive international policies regarding sunbed use, and observation of these policies.

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bHLH transcription factors in differentiating neuroblastoma cells

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Neuroblastoma is a tumor of the sympathetic nervous system, and arises during early childhood. The tumor is highly heterogeneous and evidence, such as expression of genes normally only expressed during embryonic and fetal stages, suggests that the tumor is of embryonic origin and that the tumor arises as a consequence of perturbed differentiation during the development of the sympathetic nervous system. Transcription factors implicated in this differentiation program are therefore of potential interest in order to understand the genesis of neuroblastoma. Based on findings in Drosophila, several transcription factors playing vital roles in the development of the vertebrate peripheral nervous system have been identified, some of them belonging to the basic helix-loop-helix (bHLH) family. Amplification of the protooncogene N-Myc is one of the most important genetic aberrations in neuroblastoma. There is a high positive correlation between N-Myc amplification and malignancy, but the molecular consequences of this phenomenon have only been partly investigated. The work presented here describes the roles of the bHLH transcription factors HASH-1, HES-1, and the bHLHZip transcription factors N-Myc, Mad proteins, Mnt and Max during neuroblastoma cell differentiation. It suggests that a controlled temporal expression patterns of some of these genes are important in order to allow neuronal differentiation to proceed.

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Smoking and breast cancer

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Ex-smokers are exposed to a higher breast cancer risk than are never smokers. This conclusion is based on a follow-up of the 10 902 women in the Malmö Preventive Project. The 31% higher incidence in ex-smokers remained statistically significant when other risk factors were taken into account in the analysis.

In both smokers and ex-smokers there was an increased incidence of oestrogen receptor negative tumours. In ex-smokers, but not in smokers, there was similarly an overrepresentation of poorly differentiated tumours.

Many women who give up smoking gain weight and obesity may be associated with increased lipid levels in the blood. Breast cancer incidence in ex-smokers was higher in women with high serum levels of triglycerides, but not in women with high cholesterol.

Probability of survival following breast cancer is only partly related to stage at diagnosis. In a follow-up of 792 breast cancer patients within the Malmö Mammographic Screening Trial, it was shown that, stage by stage, smokers had a less favourable prognosis than never smokers.

Breast cancer mortality rates differ substantially between residential areas in Malmö. In an analysis of incident cases and deaths between 1986–96, it was shown that stage at diagnosis, rather than incidence, contributes to this pattern. Areas with a high proportion of advanced carcinomas, and a high case-fatality rate, had a higher prevalence of smokers, ex-smokers, foreigners and residents receiving income support.

It is concluded that smoking is a risk factor for breast cancer and that smoking contributes to the pattern of breast cancer mortality.

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Drug resistance in acute myeloid leukaemia—Pharmacokinetic and in vitro studies

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Chemotherapy has improved clinical outcome in adult acute myeloid leukemia (AML) but still the majority of the patients die of their disease. One important explanation is transport mediated drug resistance resulting in refractory or relapsing disease. P-glycoprotein (Pgp) is located in the membrane and can extrude cytostatic drugs. This will cause a reduction of intracellular concentrations of the drug and reduce the effect on the leukemic cells. Down regulation of the enzyme topoisomerase IIα (topo IIα) is another resistance mechanism.

The aim of the first part of this thesis was to study effects of interaction with transport mediated resistance. In a phase I–II pharmacokinetic study 22 de novo AML patients were included and received a high single dose of mitoxantrone (30 or 40 mg/m^2) in combination with ara-C. In the leukemic cells we found a high accumulation of mitoxantrone which, in contrast to plasma, remained stable during the 48 hours studied. Compared with previous results with mitoxantrone 12 mg/m^2 the area under the curve (AUC) for intracellular concentrations was increased by 150% (30 mg/m^2) and 260% (40 mg/m^2) respectively. The toxicity was acceptable but a prolonged duration of neutropenia was noted. Several studies have shown that Pgp can be blocked and in the next study 10 AML patients were included and given a continuous infusion of daunorubicin in combination with the Pgp modulator PSC 833. The intracellular in vivo concentrations of daunorubicin in Pgp positive leukemic cells increased as well as the ratio of the AUC for daunorubicin in leukemic cells to the AUC in plasma suggesting that the mechanism was a specific inhibition of Pgp by the modulator. Another aspect of drug resistance and cytostatic treatment is the potential interaction of Pgp and drugs used in the supportive care of AML patients. 21 different drugs were tested in a Pgp expressing leukemia cell line and the change in intracellular rhodamine 123 was measured. However, in this model we found no significant interactions of the tested drugs on Pgp.

In the second part of the thesis we studied two different chemosensitivity assays and markers for drug resistance. In the bioluminiscence ATP chemosensitivity assay cell death is measured as reduced ATP levels. 83 samples from 77 AML patients were included and the in vitro effect of six different cytostatic drugs were tested. We found that in vitro sensitivity to daunorubicin was associated with complete remission and prolonged disease free survival. One problem with in vitro assays is the possible contamination of non malignant cells which can interfere with the results. Therefore we established a new assay where selected myeloid cells were analysed using flow cytometry. 63 samples from 60 AML patients in different stages of their disease were tested. The method was feasible and we found correlations to clinical parameters and Pgp expression. Topoisomerase IIα is a well known target enzyme for many cytostatic drugs. In this final investigation the expression of the enzyme was studied in different phases of the cell cycle in 25 acute leukemia patients. In contrast to normal cells we found that topo IIα was expressed in G0/G1 phase. A low expression of topo IIα in the G0/G1 phase seemed to be associated with resistant disease, which suggests that topo IIα expression in the different cell cycle phases may have a predictive value.

In conclusion a high single dose or the inhibition of Pgp increased the intracellular concentrations in vivo of mitoxantrone and daunorubicin respectively. Drugs used in the supportive care of AML patients did not interact with Pgp in vitro. In vitro sensitivity to daunorubicin was associated with both short and long term outcome using the bioluminiscence ATP assay. In vitro chemosensitivity testing of selected myeloid cells and low expression of topo IIα in the G0/G1 phase, using flow cytometry techniques, correlated to clinical outcome but further studies are needed to evaluate the predictive value.

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Diagnosis, management, quality of life, and long-term survival in prostate cancer patients—A study based on national, regional, and local cancer registry data in Sweden

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Prostate cancer is a common disease with considerable variation in clinical behaviour and therapeutic responsiveness. Uncertainty surrounds almost all aspects of prostate cancer management and it has been difficult to conduct proper randomised controlled trials required for reliable evidence-based decision-making. Although randomised controlled trials are under way and may eventually provide unbiased data on the efficacy of the management of prostate cancer in selected patient groups under optimal circumstances, population-based studies are necessary to evaluate variations in incidence and the effectiveness of management as practised in the community at large.

This study uses three prospectively assembled population-based cohorts and one cross-sectional group of men with prostate cancer:

1. a local register of all men with prostate cancer in the Central District of Östergötland 1974–1986 (n = 813)
2. the South-East Region Prostate Cancer Register 1987–1996 (n = 6 782)
3. the National Prostate Cancer Register 1996–1997 (n = 8 328)
4. a cross-sectional group of all men with prostate cancer residing in Östergötland 1999 comprising patients from cohorts 1 and 2 (n = 1 442).

The incidence of prostate cancer in the South-East Region increased from 613 in 1987 to 780 in 1993 and then slowly declined. The age-adjusted incidence varied from 89/100 000 to 169/100 000 between the different counties included in the National Prostate Cancer Register 1996. In counties where a large percentage of the tumours were detected when still localised, the incidence was higher and the men younger at diagnosis (both $p < 0.05$). In the age interval 50–59 years of age the median PSA was 13 ng/ml, whereas it was 35 ng/ml in those younger than 50. This difference was significant ($p < 0.05$) and is probably explained by larger total tumour volume among men in the youngest

age group. For men with well to moderately differentiated tumours and PSA < 20 ng/ml the risk for regional and distant metastases was below 10%. Between 1987 and 1996 the proportion of men treated with radical prostatectomy in the South-East Region decreased from 11% to 2.5%. During the same period the percentage of patients receiving GnRH-analogues increased from 3.9% to 37.8% while the percentage of patients treated with orchiectomy decreased from 40.0% to 12.8%. For patients treated with radiotherapy the median PSA was 16.7 ng/ml, for those who underwent radical prostatectomy 9.3 ng/ml, for patients receiving GnRH-analogues 61 ng/ml and for those treated with bilateral orchiectomy it was 88 ng/ml. All differences in PSA levels between the treatment groups were significant ($p < 0.05$), indicating that there is a selection process with men having less advanced cancer receiving GnRH-analogues and men with more advanced cancer undergoing bilateral orchiectomy, and similarly a selection of men with smaller tumours being treated with radical prostatectomy rather than radiotherapy. Of the men answering the questionnaire sent to all prostate cancer patients residing in Östergötland 1999 42% had perceived pain during the previous week and 26% stated their quality of life to be 50% or less on a visual analogue scale. A high health-care availability rating and short time since diagnosis were found to significantly predict lower rating of pain on average ($p < 0.05$). Pain on average was found to be a significant predictive factor for decreased quality of life together with high age, low health-care availability rating and palliative treatment ($p < 0.05$). Age > 70 years, advanced stage and poor differentiation were risk factors associated with increased risk for prostate cancer death in Östergötland Central District Cohort ($p < 0.05$). The survival curve followed a continuous exponential course throughout the period of observation.

The geographical as well as temporal variations in incidence are probably explained by differences in diagnostic activity, which also affects the age at diagnosis and distribution of stages. There are also large disparities in management within the country, which reflects the lack of evidence supporting one treatment in favour of another. How diagnostic activity and different local habits in management affect outcome in the long run is still unknown, but the results of our study regarding quality of life and survival may be used as a basis for management decision-making. Patients with localised tumours have a favourable prognosis, even without initial treatment. When deciding on therapy, however, the grade of malignancy should be taken into account as it has a great influence on disease-specific survival. For men with well to moderately differentiated tumours and PSA < 20 ng/ml, further investigation to exclude distant and regional metastases is unnecessary.

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PDGF in cerebellar development and tumorigenesis

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Medulloblastoma is a highly malignant cerebellar childhood tumor. As in many other brain tumors, expression of platelet-derived growth factor (PDGF) and its receptors has been shown in medulloblastoma. To reveal the importance of this growth factor in cerebellar development and tumorigenesis, analyses were performed on human medulloblastoma cell lines and on tissue from normal mouse brain at different stages of development. The in vivo effect of a forced expression of PDGF-B in the cerebellar primordium was examined in transgenic mice.

In the normal mouse embryo, we found PDGF receptor-α-positive cells in the early neuroepithelium and on neuronal precursors. In the postnatal cerebellum, cells in the external germinal layer and Purkinje cells expressed the receptor. In the medulloblastoma cells, expression of all the three PDGF isoforms and PDGF receptors was seen and correlated to neuronal differentiation. Endogenously activated, i.e. tyrosine phosphorylated, PDGF receptors were identified. To reveal the role of PDGF in normal cerebellar development, we established transgenic mice where a PDGF-B cDNA was introduced via homologous recombination into the engrailed-1 gene. Engrailed-1 is specifically expressed at the mid-/hindbrain boundary of the early neural tube, i.e. in an area from which the cerebellar primordium develops. The ectopic expression of PDGF-B caused a disturbance of cerebellar development. Midline fusion of the cerebellar primordium did not occur properly, which resulted in cerebellar dysplasia in the adult mouse.

In a parallel study, the expression pattern of a glial fibrillary acidic protein (GFAP)-*lacZ* transgene was followed in the embryonic mouse central nervous system. It was shown that the human GFAP promoter was already active by embryonic day 9.5 and as development proceeded, expression occurred in different, independent cell populations. Among these cell populations were the radial glial cells in the neocortex.

April 2001

Familial glioma—Epidemiological and experimental studies

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The aetiology of primary brain tumours is poorly understood. Ionising radiation is the only well established environmental risk factor. Apart from this some rare inherited syndromes are associated with increased risk of glioma. The aim of this thesis was to establish if there is a familial aggregation of glioma and to further characterise the glioma families in order to investigate mode of inheritance and if other cancer sites were associated. The aim was also to investigate possible underlying genetic mechanisms explaining familial glioma.

In a retrospective cohort analysis familial aggregation of glioma in the northern region of Sweden was investigated. A threefold increased risk among first degree relatives (FDR) was observed, and familial glioma was found in about 5% of all cases. Another study used the Swedish Cancer Registry with linkage to the Swedish Family Database. That study also identified an increased risk in glioma families. Novel differences were found, when dividing the cohort into relatives of cases with low-grade glioma (LGG) and high-grade glioma (HGG) separately. Among relatives to LGG patients, there was an increased risk of developing LGG especially among FDR of young probands indicating a genetic origin to the aggregation. The familial risk was even higher, sevenfold, among siblings. Performing a complex segregation analysis based on the cohort of FDR to glioma patients in the northern region of Sweden also lent support for an autosomal recessive gene, where the hallmark is affected siblings without parental disease. Extended pedigrees found an autosomal domi-

nant pattern of inheritance in about 1% of all glioma cases. No increased risks for other cancer sites were found.

An immunohistochemical case-control study revealed differences in p53 and Pgp expression in tumours between familial and sporadic gliomas. P53 is a key regulator in cell cycle control and Pgp is a protein involved in the tumour resistance to drugs. Familial cases had a significantly higher frequency of tumours not expressing p53 and Pgp compared to sporadic gliomas. This might indicate that a subset of familial gliomas has specific mutation pathways not involving p53 overexpression or total p53 truncation. Microsatellite instability (MSI) is a sign of defects in the DNA repair system and the familial gliomas was investigated, and no MSI positive tumours were found. The tumour suppressor genes p53 and PTEN were investigated for germline mutations in 8 samples of DNA in glioma families and no mutations were found.

To summarise, familial aggregation of glioma exists in about 5% of all glioma cases. The families are heterogeneous, autosomal dominant inheritance is rare and a large part of the families are affected siblings. There could be an effect of an autosomal recessive gene, especially in families with multiple LGG. The underlying genetic mechanism involved is unclear, but p53 and PTEN mutation or the mis-match repair system seems not to be involved.

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Chronic leukemic B-cell disorders and trisomy 12—A study of surface markers, protein expression and clinical course

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Chronic lymphocytic leukemia of B-cell type (CLL) is the most common disease entity of chronic leukemic B-cell disorders (CLBD). CLL is the most frequent leukemia in the Western world and the incidence is increasing. The clinical course is very variable and specific therapy is not always needed. Therefore, valid prognostic markers are of great importance managing the individual patient. Disease stage is determined in most cases and has a prognostic value. Chromosomal abnormalities have also been evaluated as prognostic markers in CLBD. The most common numerical chromosomal aberration is trisomy 12. The purpose of this thesis was to use fluorescence in situ hybridization (FISH) to detect trisomy 12 in CLBD and to characterize the patients with trisomy 12 from a biological, clinical, and prognostic perspective.

Totally 156 patients with CLBD were evaluated for the occurrence of trisomy 12. The findings were correlated with the morphology of the leukemic cells and the surface expression of CD25 (IL2-receptor), CD54 (ICAM-1), CD95 (Apo 1) and with the intracellular murine double minute (MDM2) protein. Repeat FISH analyses were performed to study the leukemic clone during the course of the disease.

Trisomy 12 was detected in 2–54% of the blood mononuclear cells (MNC) in 18/118 (15%) of the CLBD patients. The aberration was found in 24% of patients with tumor cells with atypical morphology including lymphoplasmacytoid features, while only 2% of the cases with s typical morphology had trisomy 12. The trisomic patients had higher S-LDH, expressed a monoclonal band more frequently, had shorter median time to treatment and reduced survival compared with the disomic cases.

Analyses of the occurrence of trisomy 12 in different morphologically defined lymphoid cells by combined conventional cell staining and FISH, (MGG/FISH), showed that the aberration was as frequent in morphologically atypical as in typical cells.

By sequential FISH analyses, we found that acquisition of trisomy 12 during the course of the disease was a rare event (2/60). In all cases with trisomy 12 ($n = 17$), the trisomic clone expanded as the disease progressed. Therapy with alkylators and combination regimens significantly reduced the trisomic clone. In contrast, after treatment with purine analogs a relative increase of the trisomic clone was observed.

Using flow cytometry, CLL patients had a higher frequency of B-cells expressing CD25 than controls. High expression of CD25 ($\geq 30\%$ of the B-cells) was associated with a significantly shorter median time to treatment.

CLL patients had a significantly higher percentage of mononuclear cells (MNC) expressing the MDM2 protein than controls. Patients with trisomy 12 had significantly more MDM2 expressing cells than the disomic cases. This extra copy of chromosome 12 cannot be the only explanation for the difference, since most cases had more MDM2 expressing cells than trisomic MNC.

Even though trisomy 12 was associated with several of the studied biological and clinical factors neither appeared specific for CLBD patients with trisomy 12.

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Applications of high energy photons and mixed beams from an MM50 Racetrack Microtron

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The aim of this thesis was to investigate and illustrate the new possible clinical applications of novel photon and electron beams generated by the MM50 Racetrack Microtron in Umeå. The MM50 can generate photon and electron beams with an energy more than twice as high as the highest available energies for conventional medical accelerators. Further, the design of the treatment head and the beam flattening by computer controlled scanning of the elementary beam makes automatic and dynamic beam delivery feasible for both photons and electrons. These features have been utilised for the development of new possible treatment applications.

An important tool for the design of treatment plans and dose calculations is the treatment planning system (TPS). The accuracy of this system is therefore vital, and since it has been used in parts of this work it was essential to perform a thorough evaluation of the dose calculation algorithm. The results, presented in this work showed that the system was reliable for electron dose calculations in general, but there could exist problems for certain geometries. No significant differences were found between the results for conventional electron beams and those from the MM50.

The importance of motion uncertainties due to set-up variations and internal movements of the tumour in relation to the beams was studied. Standard treatment plans were compared to treatment plans recalculated to incorporate motion uncertainties by applying error distributions on the beams, thus generating a wider effective penumbra. The differences were analysed using

dose-volume evaluation and TCP and NTCP models. The results indicated that optimising the dose to the PTV can be misleading since the effect on the moving CTV and the static PTV (and CTV) is not the same. Models to incorporate motion uncertainties should therefore be included in the TPS.

The feasibility of using the scanning beam technique of the Racetrack to perform intensity modulation of photon beams independent of blocks or MLC was studied. Physical limits to the achievable dose gradients restricted the modulating capabilities, but useful standard modulations like wedge-shaped dose distributions can be performed independent of the MLC and can be included in the TPS.

The choice of treatment energy for lung tumour irradiation has long been debated. The general approach is, however, to use energies in the lower end of the therapy energy scale. Energies as high as 50 MV have not previously been investigated for this purpose. A phantom study and later a study using a real patient geometry was performed to analyse the potential of this beam quality. It was demonstrated that the 50 MV beams could be used to increase the dose to a lung tumour, with the same complication probability to the surrounding healthy lung tissue as for the reference beam of 6 MV. Any general recommendations are difficult to give, however, since it is the complex mechanism of electron equilibrium in the lung, or the lack of it, that is the major reason for the dose escalation possibilities. A combination of high and low energy photons could probably be the optimal choice for dose escalation.

The capabilities of advanced treatment techniques that are unique for the MM50, mixing multileaf collimated electrons together with photons, or electron energy modulation, is not yet available on conventional accelerators. In order to gain a more widespread use of these techniques, an investigation was made to see if conventional treatment heads could be modified to allow for multileaf collimation of electron beams comparable to that of the MM50. The investigation showed that the treatment heads could be modified to obtain that goal, with penumbras narrow enough for isocentric treatments and with a virtual SSD similar to the one for photons.

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