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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 tanles 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.*

Development of a general framework for optimization of radiation therapy

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A general framework for optimization of radiation therapy has been developed. The framework has been implemented in a new object-oriented code called Orbit and some of the principal capabilities of this code are presented. It is shown that simultaneous optimization of beam directions and intensity modulation can considerably improve the treatment outcome, especially for few field techniques. The flexibility in formulating and solving clinically relevant optimization problems is also demonstrated. In this context, a new optimization strategy P_{++} is introduced, which makes it possible to minimize complications with only a marginal reduction in complication free cure, P_{+}.

Orbit has been closely integrated with a clinical radiation treatment planning system, which thus combines Orbit's advanced ability to optimize treatment plans with the clinical versatility and accuracy of forward dose calculation algorithms.

By viewing a fractionated treatment as a dynamical system, the time structure of radiation therapy optimization can be utilized to organize the calculations in a recursive manner. For this purpose, a new mathematical framework for calculating the probability of complication free tumor control, and its expectation value, has been constructed. All the main clinical parameters and events that affect P_{+} are gathered into four sequences of data describing the delivered energy fluence distributions, patient geometry, radiobiological response parameters and time-dose fractionation schedule. Because of the difficulty in measuring all aspects of the intra- and interfractional variations in the patient geometry, such as internal organ displacements and deformations, as well as inter-patient variations in radiation sensitivity, such uncertainties are accounted for by the method of stochastic optimization.

The dynamic optimization approach to radiotherapy planning allows for information feedback so that patient specific data which are generated as the treatment proceeds, e.g. by in vivo dosimetry, portal imaging, radiotherapeutic computed tomography, PET- and MR-imaging, can be used to refine the beam configurations and beam shapes in the subsequent treatment sessions. It is shown that organ motion can be accurately accounted for in the treatment plan by intensity modulation using stochastic optimization. Since setup uncertainties are detectable during treatment delivery with present portal imaging techniques, they are corrected for during dose delivery. Optimal corrections both to the beam-patient alignment and the delivered fluence profiles, are determined by applying optimal control theory. It is demonstrated that by using such techniques the effects of both random and systematic setup errors can practically be eliminated.

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Nuclear medicine imaging of breast cancer and regional lymph nodes

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The aim of this thesis was to explore the role of nuclear medicine in diagnosis of primary breast carcinoma and regional lymph node involvement. The introduction includes a review over nuclear medicine techniques currently under investigation.

Patients were preoperatively investigated by scintimammography with 99m-Tc-Sestamibi. Ninety-six patients were imaged in prone position as a standard. All patients were routinely assessed by triple diagnosis (TD), i.e., physical investigation, mammography and fine-needle aspiration cytology. The results of TD had lead to the decision that the patient was to be operated upon. Twenty-six patients were additionally imaged with single photon emission tomography (SPECT). In some papers Sestamibi uptake in breasts and uptake in axillary lymph nodes were studied. In one paper detection of sentinel node (SN) in patients with breast carcinoma was studied. Lymphoscintigraphy with 99m-Tc-Nanocolloid, preoperative injection of Patent blue dye and peroperative use of gamma probe were applied on 75 patients.

Scintimammography with 99m-Tc-Sestamibi showed a sensitivity of 84% and specificity of 74%, which was not improved by the additional use of SPECT. The sensitivity of scintimammography in the detection of primary breast lesion depends on tumour size, site and histological features. Furthermore, also benign lesions showed increased Sestamibi uptake, which lead to false-positive findings. The method had unsatisfactory diagnostic accuracy in the detection of axillary lymph node metastases. Complementary use of scintimammography to TD improved the sensitivity in diagnosing cancers, and was specially valuable in patients with mammographically dense breast parenchyma.

The combined use of preoperative lymphoscintigraphy, injection of blue dye and the peroperatively use of a gamma probe resulted in a detection rate of SN of 92% in all patients. SN correctly predicted the axillary status in 96% of the cases and might therefore be a potential concurrent to axillary lymph node dissection.

Scintimammography can be recommended as a complementary method to TD, especially in patients with mammographically dense breasts. Biopsy of SN can be used instead of axillary lymph node dissection in selected patients.

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Improving radioimmunotargetin g

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Radiolabeled monoclonal antibodies offer the potential of highly localized, targeted radiation treatment of cancer. Radioimmuno-

targeting is a promising and developing field, with clinical applicability as a treatment alternative for metastatic, subclinical disease, and in combination with external beam radiotherapy and chemotherapy. There is still a need to further improve radioimmunotargeting, especially in the therapeutic setting, due to the relatively low tumor accumulation of radiolabeled antibodies. In addition, the large amount of redundant, non-targeted radiolabeled antibody circulating in the blood causes damage to normal tissues. In this thesis, investigations have been performed attempting to further increase the knowledge of various parameters influencing targeting: 1) Epitope mapping of 30 anti-cytokeratin monoclonal antibodies which can be used for radioimmunotargeting. 2) Epitope definition of the anti-cytokeratin 8 monoclonal antibody, TS1, on a molecular level. 3) Determination of clearing efficacy and interaction patterns of TS1 and the anti-placental alkaline phosphatase monoclonal antibody, H7, and their respective anti-idiotypes. 4) Iodination effects of ^{125}I on TS1 both in vitro and in vivo, and putative gains in increasing the specific activity of the injected radiolabeled antibody. 5) The potentially positive effects in terms of targeting efficacy by combining external beam radiotherapy with radioimmunotargeting, using the TS1-cytokeratin 8 system.

Cytokeratins are promising antigens for radioimmunotargeting. They are released from epithelial tumor cells and deposited in necrotic tumor regions. The epitope specificities of 30 monoclonal antibodies against the most common human cytokeratins (8, 18 and 19) were determined. At least 10 different, non-competitive epitopes were demonstrated. One of the cytokeratin 8 specific antibodies, TS1, was studied further and the epitope was defined to be contained within the unique sequence of aa 343–357. The specific aa residues involved in antibody binding were determined by alanine scanning.

A parameter that could contribute significantly to improve targeting is the clearance of non-targeted circulating radiolabeled antibodies from the vasculature. Anti-idiotypic monoclonal antibodies have been suggested as clearing agents, and their administration can result in a sparing of normal tissues, without decreasing the tumor:non-tumor dose ratio. In this thesis, the in vitro complex formation between the idiotypic antibody H7 and the three anti-idiotypes, αH7:1. αH7:35 and αH7:38, was studied using native PAGE and gel precipitation. When mixed with H7 in equimolar amounts, the anti-idiotypes displayed three different interaction patterns. Furthermore, the anti-Ids in vivo clearing efficacy of H7, was studied using a nude mouse model. The anti-idiotypes were all capable of significantly reducing the levels of H7 in nude mice. However, αH7:38 was twice as efficient as the other two. The in vitro complex formation and in vivo clearing capabilities and metabolism of TS1, and its anti-idiotype, αTS1, were also studied. Immunoelectron microscopy demonstrated that the two antibodies rarely formed dimers, but instead formed rings of four or six. These results probably reflect a location of epitopes incompatible with tight, dimeric interactions and/or insufficient flexibility of the IgG1 subtype hinge regions. The in vivo study indicated that the anti-idiotype clearing is efficient and that the accumulation and degradation of TS1-αTS1 immune complexes, to a large extent, takes place in the liver.

Radiolabeling and storage can have detrimental effects on antibodies. However, if the specific activity can be increased without affecting the immunoreactivity of the radiolabeled antibodies, a larger dose to the tumor can be achieved without requiring a larger accumulation of antibodies. The immunoreactivity, stability and in vivo kinetics of TS1 were investigated following different degrees of labeling with ^{125}I. Heavily iodinated TS1 lost immunoreactivity rapidly after the labeling procedure, as determined by ELISA. However, the in vivo study demonstrated that the tumor : non-tumor dose ratio was relatively unaffected by the different degrees of iodination.

The potential benefits of combining external beam radiotherapy with radioimmunotargeting, in terms of targeting efficacy, were investigated using TS1. The external beam radiotherapy was given as three fractions of 5 Gy during three consecutive days. Three different timing strategies were employed and evaluated for the combination of the two modalities. External beam radiotherapy preceding radioimmunotargeting, on nude mice xenografted with human HeLa Hep 2-cells, proved to be the most beneficial treatment set-up, in terms of antibody accumulation. These findings may be of clinical importance in the future since they offer the potential of increasing the dose to solid tumors.

In summary, this thesis presents a number of areas in which momentum can be gained in the achievement of more efficacious targeting of tumors.

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Chromosomal instability and genomic amplification in bone and soft tissue tumours

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Acquired genetic abnormalities are found in all types of malignant tumours and may contribute to neoplastic processes by altering protein structure or dosage. Many bone and soft tissue tumours (BSTT) are characterised by complex patterns of chromosome changes, including extensive intratumour heterogeneity and amplification of DNA sequences. The results presented in this thesis demonstrate that the cytogenetic heterogeneity in a number of BSTT may, to a considerable extent, be caused by ring and dicentric chromosomes involved in breakage-fusion-bridge (BFB) events, as shown by the strong correlation between sequences present in mitotically unstable chromosomes and anaphase bridges. In borderline/low-malignant tumours with ring chromosomes carrying amplified sequences from chromosome 12, BFB rearrangements are associated with intratumour variability in the organisation of amplified material and abnormalities in centromeric alpha satellite sequences, including neocentromere formation. After the healing of broken ends by telomeric sequences from other chromosomes, rings may transform into giant marker chromosomes, which may occasionally be dicentric and rearrange further. BFB instability is also present in highly malignant tumours exhibiting a complex, unspecific, and heterogeneous pattern of chromosome aberrations. These neoplasms typically show unstable dicentric chromosomes and a general destabilisation of the chromosome complement. Tumour cells with BFB instability exhibit a decreased elimination rate of mitotically unstable chromosomes, which may be partly related to disruption of the normal TP53-dependent DNA damage response. In both BSTT and epithelial tumours, BFB events are associated with specific abnormalities in nuclear shape, indicating that nuclear atypia may serve as an indicator of ongoing genomic reorganisation in neoplastic cell populations.

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Monoclonal antibodies and cytokines for therapy of patients with advanced colorectal carcinoma—A clinical and immunological study

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There is a great need for developing and improving treatment alternatives in colorectal carcinoma (CRC). The tumour associated antigen (TAA) GA733/CO17-lA is expressed by more than 90% of all CRCs, on the majority of both primary and metastatic CRC cells. This antigen can be utilised as a target structure for passive as well as active immunotherapy.

Mouse MAbl7-1A has been produced against this antigen and shown to induce clinical responses in patients. The clinical effect might be improved by adding cytokines, which might augment immune effector functions utilised by MAb. In this study we have analysed clinical and immunological in vivo functions of patients with advanced CRC treated with a combination of the MAb17-1A, GM-CSF and IL-2.

The first analysis concerned the clinical effect of IL-2 alone, without MAb. Low doses of IL-2 were given in combination with IFN-α to 15 patients with metastatic CRC. No patient showed a major response. Six patients had a stable disease. In the subsequent study the clinical response and immune effector functions were analysed when IL-2 was added to a combination of MAb/GM-CSF, the so far best treatment regimen. 20 patients with metastatic CRC were included in this study. One patient obtained a partial remission and 2 patients stable disease. The therapeutic effect did not seem to be improved when IL-2 was added to MAb/GM-CSF therapy, which was in contrast to expectations from preclinical data. There might be a tendency to a lower response rate and impaired survival for MAb/GM-CSF/IL-2 treated patients as compared to patients treated with MAb/GM-CSF.

When analysing different in vivo effects a suppressed human anti-mouse (HAMA) and anti-idiotypic antibody (Ab_2) response as well as an impaired antibody dependent cellular cytotoxicity (ADCC) was observed. There was also a tendency to reduction of the frequency and severity of allergic reactions in MAb/GM-CSF/IL-2 treated patients as compared to MAb/GM-CSF treated patients. A highly significant increase in neopterin and sIL-2R serum concentration was noted in the MAb/GM-CSF/IL-2 treatment group as compared to the MAb/GM-CSF group. These data may indicate induction of immune suppression when IL-2 was added to MAb/GM-CSF. Increases in neopterin and sIL-2R concentrations may reflect activation of cellular immune responses mainly involving macrophages and lymphocytes. High concentrations of these serum markers might indicate generation of oxidative stress.

Induction of cytokine antibodies has earlier been shown to hamper the clinical responses of biotherapeutic agents. This study revealed differences in immunogenicity between different preparations of GM-CSF and that neutralising anti-GM-CSF antibodies had biological implications. Furthermore, it was obvious that non-neutralising IL-2 antibodies had a profound impact on IL-2 pharmacokinetics. In IL-2 antibody positive patients a decreased cytokine induced increment of leukocytes was observed. As the use of therapeutic cytokines is increasing it seems to be of particular importance to establish the optimal biological doses of different cytokines as well as to carefully evaluate the induction of cytokine antibodies to be able to use cytokines rationally in the clinic.

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Activity of tumor-antigens in polyomavirus replication

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Polyomavirus replication needs an active cell environment, and the viral DNA replication is cell cycle regulated. Tumor antigens (T-antigens) of mouse polyomavirus, especially large T-antigen, are necessary for the replication. Large T-antigen is a multifunctional protein, and like other T-antigens, its N-terminal region functions as a DnaJ-domain. This thesis focuses on identification of the factors regulating polyomavirus DNA replication.

Large T-antigen is a phosphoprotein which is the only essential viral protein in the initiation viral DNA replication. We found that the host cell cycle is critical for viral DNA replication and that threonine 278 of large T-antigen is a G2/M phase cyclin-dependent kinase phosphorylation site, regulating viral DNA replication.

In the DnaJ-domain, the first alpha-helix is important for T-antigen function, especially for its ability to regulate viral DNA replication. Mutant Ll3V large T-antigen is defective in the regulation of DNA replication, whereas the trans-activating function of mutant large T-antigens does not change. When all results are considered, we conclude that the functional defect of LI3V large T-antigen is not related to the known activity of J-domain, it is possibly due to underphosphorylation of the mutant protein.

One of the biochemical properties of large T-antigen is DNA binding. A mutant large T-antigen (D286N) in which the mutated site is close to the DNA-binding domain was able to rescue viral DNA replication of a mutant with a base substitution in the origin of replication (G4A). BlAcore analysis showed no significant difference in the interaction between the mutant and wild-type large T-antigens with that could explain their different activities in vivo. However, in a cotransfection experiment we found that the D286N mutant large T-antigen showed strongly increased activity in the initiation of DNA replication at both the wild-type and mutant origin of replication.

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Modelling the impact of two forms of hypoxia on novel radiotherapy approaches

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Treatment modelling is now widely used for many purposes. The main interest lies in estimating the possible gain of any new treatment strategy, but recently the theoretical modelling of the biological effect of the treatment has been considered for inclusion into treatment planning algorithms. The interest in treatment modelling of radiotherapy is explained by the fact that it represents a cheap and fast way of estimating the potential benefit of new treatment strategies, before the initiation of lengthy and costly clinical trials. The radiobiological database is now quite large and it allows the modelling of the biological response with constantly improving accuracy. Many models have been devised to describe this response and are already used for analysing large sets of patient data.

The aim of the present thesis was twofold: to compare the predictions in the clinical dose range for the linear quadratic (LQ) model and the linear quadratic model with inducible repair (LQ/IR) and to determine the possible therapeutic gain of different treatment strategies taking into consideration the microenvironmental conditions known to exist in tumours. The main focus was on the availability of oxygen and other nutrients to tumour cells, knowing that tumour vasculature is very poor compared to that in normal tissues. Hypoxia and other nutrient gradients, together with the reduction of cellular energy charge resulting from starvation, were considered in relation to the ability of the cell to resist radiation injury by mounting an adaptive response. The brief interruption of oxygen supply to the acutely hypoxic cells determines an increased radioresistance, while the lack of oxygen and other nutrients in starved chronically hypoxic cells results in a radiosensitisation. It is the first time this latter aspect of tumour sensitivity is considered for a theoretical model.

The modelling performed on the tumour response showed that there are some important differences in the clinical dose range between the predicted responses with the LQ and LQ/IR models. Some of these differences provide an alternative explanation to the success of hyperfractionation and also explain some unusual results reported in the literature with respect to hypoxic protection.

The existence of starved chronically hypoxic cells in tumours and their postulated incapacity to trigger intracellular repair provides a better understanding of the tumour response to radiation treatments. Furthermore, the analysis of the complex relationship between induction of repair and intrinsic radioresistance indicates the possible therapeutic gain that can be expected from more extreme fractionation.

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Cutaneous malignant melanoma—Aspects on prevention

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The general aims of the present thesis were to delineate factors of importance for prevention of cutaneous malignant melanoma (CMM). Three samples of patients with CMM, one sample of patients with dysplastic nevus syndrome (DNS) and a sample from the general population were studied. The patients with CMM, Stage I ($n = 231$), were included in an information program aiming at increased satisfaction with information and knowledge about melanoma. A total of 694 patients were included in a study to investigate factors of importance for presenting with CMM > 2.0 mm and 54 patients were investigated regarding signs and symptoms of CMM $\leqslant 2.0$ mm. Sun-related behaviours and attitudes to sunbathing were studied in a sample of DNS patients ($n = 79$). Reasons for non-attendance in a population based screening program for melanoma were investigated in a sample of the general population ($n = 501$) out of which 201 individuals were identified as non-attenders. The Hospital Anxiety and Depression (HAD) scale, and study specific questionnaires concerning knowledge about melanoma and sun-related behaviours were used in combination with interviews. Data concerning medical status were collected from patient files and from the Regional Melanoma Registry.

The information program increased satisfaction with information and knowledge about melanoma. Women and men participated to the same extent.

Histogenetic type was the only factor distinguishing CMM with tumour thickness > 2.0 mm from thinner lesions. Nodular melanomas $\leqslant 2.0$ mm appeared to be smaller in diameter and were more often described as a new lesion compared to SSM. Family members played an important role in promoting medical attention of suspicious lesions. Accessibility to medical service was important in screening and early detection. Forgetfulness, lack of time and no perceived need for examination were the most frequently reported reasons for non-attendance in skin cancer screening. High and equal levels of knowledge about melanoma were found among attenders and non-attenders. A higher proportion of men was non-attenders. Extensive UV-exposure, although not always expressed in terms of sunbathing, was reported by DNS-patients. The most important reasons for sunbathing were attractiveness and to enjoy the warmth of the sun. The majority estimated their own risk for melanoma as equal or lower compared to the general population.

A number of obstacles in melanoma prevention were delineated, but aspects of improving prevention of CMM were also revealed, such as targeted group information, accessability to medical services, notification that new lesions with small diameter may warrant medical advice and that relatives are important in promoting early detection.

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