Acta Oncologica 40, *No.* 6, *pp.* 779–783

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.*

Dietary prevention of gastrointestinal cancer—Epidemiologic studies of fruit, vegetables and cereals

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The widely accepted inverse associations between fruit, vegetables, dietary fiber and colorectal cancer risk, have recently been called into question. In addition, dietary studies of certain cancers, such as adenocarcinomas of the esophagus and gastric cardia, and prospective cohort studies of stomach cancer, remain scarce.

To study cancers of the esophagus, gastroesophageal junction (cardia), stomach, colon and rectum in relation to diet, we analyzed data from three large studies in Sweden: a nation-wide case-control study of cancers of the esophagus and gastric cardia, a nation-wide cohort of Swedish twins, and a population-based prospective cohort study of women living in two counties in Central Sweden.

We found fruit, vegetables and antioxidants to be inversely related with all of the studied cancers except gastric cardia cancer. For esophageal and cardia cancers, the absolute risk is so low that tens of thousands of people in the highest risk strata of age and sex would need to increase their consumption in order to prevent one case per year. In relation to stomach cancer, our data hint that individuals with very low consumption of fruit and vegetables were at especially high risk. In relation to colorectal cancer, the inverse association was limited to those who consumed very low amounts of fruit and vegetables (less than 2 servings per day).

Cereal fiber intake showed an inverse association only with cancer risk only in the gastric cardia. Intake of fiber from fruit or vegetables, on the other hand, was unrelated to cardia cancer risk. We can speculate that nitrosamine-scavenging mechanisms of cereal fiber may be more important in the gastric cardia than elsewhere in the gastrointestinal tract, in turn due to a suggested higher production of nitrosamines in the proximal stomach.

While intervention efforts to prevent certain cancer through dietary changes may not be cost effective (such as in the prevention of the rare esophageal cancers), the overall benefit to health, including the prevention of digestive tract cancers, warrants continued efforts to inform and influence the public regarding adequate consumption of these foods.

January 2001

Renal cell cancer—The role of physical activity and body size

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The aim of this thesis was to explore how physical activity, obesity, weight change, and birth weight influence the risk of renal cell cancer.

The relation between occupational physical activity and risk of renal cell cancer was studied in a cohort of Swedish men and women identified in the nationwide censuses in 1960 and 1970, and followed for the occurrence of cancer by linkages to the Swedish Cancer Registry 1971–1989. We identified 2704 male and 587 female cases with the same level of occupational physical activity in 1960 and 1970 (n = 674 025 men and 253 336 women). In multivariate models, men with long-term sedentary jobs had a 25% increased risk compared to men with physically demanding occupations. In contrast, we found no clear evidence of an association between occupational physical activity and renal cell cancer risk among women.

The association between occupational and leisure time physical activity and renal cell cancer risk was further studied in a prospective cohort of 17241 Swedish twins. Exposure information was obtained through a mailed questionnaire. During follow-up from 1967 through 1997 we identified 102 renal cell cancer cases. We found no evidence of a significant association between either occupational or leisure time physical activity and risk of renal cell cancer in this cohort.

To evaluate the existing evidence that obesity increases the risk of renal cell cancer among both men and women, we conducted a quantitative summary analysis of published studies. Fourteen studies on each sex assessed obesity as body mass index (BMI, kg/m^2), or equivalent, and were included in our analysis. In contrast to previous qualitative reviews, our quantitative summary showed that increased BMI is equally strongly associated with renal cell cancer risk among both men and women. The risk increased by 7% per one unit of increase in BMI (1 kg/m^2, corresponding to about 3 kg body weight increase for a subject of average height).

The relation between body size and renal cell cancer was evaluated in more detail in a population-based case-control study with 877 patients with newly diagnosed renal cell cancer and 1508 control subjects, frequency-matched by age. Exposure information was obtained through a mailed questionnaire. General and abdominal obesity (measured as BMI and waist-to-hip ratio, respectively) were independently associated with increased risk of renal cell cancer among both men and women. Furthermore, tall height was associated with an increased risk among both sexes. Weight gain and repeated weight changes in adult life were associated with an increased risk, especially among those with a high BMI already at age 20.

The relation between birth weight, a marker of fetal nutrition and growth, and renal cell cancer was evaluated in the case-control study described above. A total of 648 cases and 900 control subjects reported their birth weight and were included in the analyses. An increased risk of renal cell cancer was suggested among men with a high ($\geq 3\,500$ g) birth weight, compared to men with a birth weight between 3000 and 3499 g. We found no clear association among men with a low ($< 3\,000$ g) birth weight, or among women. Our study shows that conditions in utero, reflected by birth weight, might affect the risk of renal cell cancer in adulthood.

February 2001

Hepatic and peritoneal colorectal metastases—Aspects of prognosis and treatment

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Although two-thirds of colorectal cancer patients are cured by surgery, approximately 50% of the patients with this disease develop locally recurrent or distant metastases during the course of their illness. The aim of this study was to identify metastatic sites associated with poor prognosis in rectal cancer and then to investigate methods that can prevent the development and growth of metastases and optimise uptake of drugs at these sites in animal models.

In a defined population, 151 patients with irresectable metastatic or local rectal cancer were identified. Bilateral liver involvement, abnormal liver function tests, peritoneal growth or abdominal lymph node metastases implied a poor prognosis.

In a study on Wistar rats with liver metastases from colorectal cancer, blocking of hyaluronan uptake and elimination by the liver enhanced the hyaluronan uptake in liver metastases. Hyaluronan may thus be used to promote uptake of drugs in specific hyaluronan receptor-positive tumour sites.

Adjuvant intravenous radioimmunotherapy delivered as a specific or unspecific monoclonal antibody prevented human colonic cancer cells inoculated into the portal vein of nude rats from developing into liver metastases. Furthermore, intraperitoneally administered radioimmunotherapy inhibited the growth of peritoneal metastases.

Blocking of 5-FU absorption with a vasoconstrictive agent enhanced the uptake of 5-FU in peritoneal metastases. In addition, the uptake of 5-FU in peritoneal metastases could be improved when these tumours were mechanically disintegrated by surgical tumour reduction and the drug was given intraperitoneally.

February 2001

Quality of life in patients with endocrine gastrointestinal tumours

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The overall aim of this thesis is to investigate health-related quality of life (HRQoL), anxiety and depression in patients with endocrine gastrointestinal (GI) tumours. Patient as well as staff perceptions were assessed. HRQoL was studied with the EORTC QLQ-C30, and anxiety and depression with the Hospital Anxiety and Depression Scale. In addition, patient perceptions of the importance of and satisfaction with selected HRQoL aspects were investigated. Semi-structured interviews with open-ended questions were conducted to identify disease- and treatment-related distress, what constitutes a good quality of life and strategies to 'keep a good mood' among these patients. Patients reported a relatively good HRQoL and low levels of anxiety and depression. However, they reported a lower HRQoL than could be expected for healthy people of similar age and gender. Staff gave a more pessimistic view of patient satisfaction with HRQoL aspects than did patients, and staff did not accurately judge individual patients' levels of anxiety and depression.

Importance > satisfaction discrepancies for HRQoL aspects may identify patients with a low quality of life. HRQoL, anxiety and depression did not change substantially during the first year of treatment. Categories identified through content analysis of interview data concerning distress and quality of life were referred to physical, emotional or social dimensions. Identified strategies to 'keep a good mood' were classified as Internal or External. Most categories of distress that were identified are covered by the EORTC QLQ-C30 and/or the HADS, but some additional emotional and social aspects of distress emerged from the interview data. Receiving good care was identified as a strategy to 'keep a good mood'. This result indicates a possible and potentially important relation between the quality of care and patient HRQoL.

February 2001

Diagnosis and therapy evaluation of bone metastases

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The aim of this study was to investigate different aspects of different imaging modalities in the diagnosis of bone metastases and in the assessment of their response to therapy.

The role of CT, with and without clinical information, was investigated as compared to CT-guided bone biopsy in the evaluation of suspected bone metastases. The diagnostic accuracy of CT alone (44%) increased to 82% when clinical information was taken into account, especially for the lesions diagnosed histopathologically as benign. In most cases, CT in combination with clinical information gave enough information about the nature (malignant or benign) of a bone lesion. In uncertain cases the diagnostic accuracy could be improved by means of CT-guided bone biopsy.

Possible misinterpretation of new sclerotic lesions when judged according to the WHO criteria during treatment was studied. One hundred and thirty-nine breast cancer patients with bone metastases, who participated in a clinical trial of clodronate therapy, were studied retrospectively. In 8 of the 24 patients considered at conventional radiography to have progressive disease according to WHO criteria, 17 of 52 apparently new sclerotic lesions (33%) were detected on previous bone scintigraphy. WHO criteria may give rise to misinterpretations in patients with new sclerotic lesions. For better assessment more sensitive techniques, e.g. bone scintigraphy, can be used as a complement to conventional radiography.

Eighteen breast cancer patients with known bone metastases were studied prospectively regarding evaluation of therapy response. T1-weighted spin echo (SE) and fat-suppressed long echo time inversion recovery turbo spin echo (long TE IR-TSE) MR sequences, conventional radiography, bone scintigraphy and CT-guided bone biopsy were performed before and during systemic chemotherapy. T1-weighted sequences and long TE IR-TSE sequences were compared regarding evaluation of early response of breast cancer bone metastases to chemotherapy, using a combination of clinical, radiographic and scintigraphic examinations as a reference. Therapeutic response evaluation with MR imaging was based on change in tumor size assessed quantitatively by measuring all focal metastases, and on change in pattern and signal intensity (SI) of the metastases, assessed visually. The long TE IR-TSE

sequence demonstrated partial response of breast cancer bone metastases to chemotherapy more accurately than the T1-weighted sequence (58% vs. 17%).

The effect of granulocyte colony-stimulating factor (G-CSF)-supported chemotherapy on MR images of normal red bone marrow was investigated. A diffuse, homogeneous SI increase was observed visually and quantitatively in initially normal bone marrow during G-CSF therapy, obscuring some focal lesions. No such SI change was visible after G-CSF therapy or in patients not receiving G-CSF. We concluded that G-CSF-supported chemotherapy might induce diffuse SI changes in normal red bone marrow on MRI, and that this might lead to misinterpretations in the evaluation of response of bone metastases.

Early response of bone metastases to therapy was assessed in targeted metastatic lesions in breast cancer patients with T1-weighted and long TE IR-TSE MR sequences and CT compared with histopathological findings. The results indicated that the SI increase in the metastatic lesions following therapy on long TE IR-TSE images might be useful in indicating an early response. T1-weighted images are of limited value in assessing alterations in the amount of tumor cells. An increase in electron density on CT can be seen in both responding and progressing lesions.

February 2001

Hypothermic modulation of chemo- and radiotoxicity in vivo and in vitro

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The therapeutic ratio of cytotoxic treatment with ionising radiation and chemotherapy is small, often not permitting curative treatment and causing severe side effects. The goal is to broaden this interval either by sensitising tumour tissue or protecting normal tissue. Studies were undertaken to investigate the influence of clinically feasible hypothermia on the cytotoxic effects and pharmcokinetics in vivo in mice, with or without tumour, and in vitro in tumour cells.

The acute toxicity of various cytotoxic drugs or x-irradiation and tumour specific survival were studied in syngenic mice, with a 'micrometastatic' tumour model, at normal body temperature and at hypothermia, 28°C, induced by chlorpromazine (10–15 mg/kg i.p.). C57/Bl-mice, males and females, with or without, i.v. tumour (MCG101-AA) inoculation, exposed to either whole body irradiation (8 Gy), doxorubicin (15 or 17.5 mg/kg i.p.), cisplatin (20 mg/kg i.p.), nitrogen mustard (6 mg/kg i.v.), vinblastine (12 mg/kg i.p. or 30 mg/kg i.v.) or 5-fluorouracil (350 mg/kg i.p.) were studied at different temperatures by following the time from exposure to imminent death. Blood concentrations of 14-^{14}C-labeled doxo- or epirubicin were studied in mice after i.v. or i.p. administration at 28°C or 37°C. A human glioma cell line (251MG), in early exponential growth, was exposed to doxorubicin (0.05–0.5 μg/ml), cisplatin (1–10 μg/ml), or x-irradiation (2–4 Gy) at 28°C or 37°C with/without chlorpromazine (1 μg/ml).

Hypothermia protected the non-tumour-inoculated mice from radiation-induced toxicity and protected the tumour-inoculated mice from acute doxorubicin toxicity, males gaining more than females. The effects seemed dependent on temperature and not on chlorpromazine. The anti-tumour efficacy was increased among males. Hypothermia protected also mice from acute cisplatin toxicity and increased anti-tumour efficacy in both genders. At hypothermia the acute toxicity of nitrogen mustard was decreased but enhanced for vinblastine and 5-FU. Chlorpromazine-induced hypothermia itself neither caused acute toxicity nor influenced tumour specific survival or distribution of tumour deposits. Blood concentrations and the AUCs were highest under hypothermic conditions and the ratio calculated for the blood concentration under hypothermic/normothermic conditions over time was substantially increased after i.p. administration, most pronounced for epirubicin. Hypothermia reduced the cytotoxic effect of doxorubicin, cisplatin or irradiation in glioma cells. Exposure to chlorpromazine neither influenced cellular growth nor interacted with the drugs or radiation. Hypothermia induced by chlorpromazine profoundly reduces acute radiation- doxorubicin-, cisplatin- or nitrogen mustard-induced toxicity in mice. For doxorubicin and cisplatin the anti-neoplastic activity was not compromised, rather enhanced, indicating an increased therapeutic ratio. It seems to be a difference among genders, males being more salvaged than females upon doxorubicin or radiation exposure. Moderate hypothermia in vitro results in a protection of the cellular insult of ionising radiation, doxorubicin or cisplatin. In summary there may be a gain in the therapeutic index for several clinically used cytotoxic injuries when used in conjunction with hypothermia.

March 2001

Thymidylate synthase expression in colorectal cancer—Its role as a prognostic factor and a predictive factor in adjuvant 5-fluorouracil-based chemotherapy

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The enzyme thymidylate synthase (TS) plays a central role in DNA synthesis and is therefore an important target for chemotherapeutic treatment. We have studied the prognostic value of immunohistochemically detected TS on slices of formalin-fixed paraffin embedded stored colorectal tumors using the monoclonal antibody TS 106.

The level of TS staining in 25 primary colorectal cancers, arbitrarily scored in grades of 0, 1, 2, and 3, correlated with enzyme activity, as measured by a tritium release method.

TS was homogenously expressed in two thirds of 48 studied primary colorectal tumors.

TS expression, assessed in 70 primary colorectal cancers Dukes' stage A-D, was an independent prognostic factor for time to death in colorectal cancer ($p = 0.04$). Low TS expression (TS grades 0 or 1) correlated with better outcome and high TS expression (TS grades 2 or 3) with worse outcome.

243 patients with rectal cancer Dukes' stage A-C were retrospectively studied. Multivariate analysis showed that TS expression was an independent marker for loco-regional recurrence ($p = 0.04$), distant metastasis ($p = 0.0l$) and overall survival ($p = 0.02$).

The predictive value of TS expression was studied in colorectal cancers of Dukes' stage B and C from 862 patients who all were included in Nordic adjuvant trials evaluating the efficiency of adjuvant 5-fluorouracil (5-FU)-based chemotherapy. No benefit of adjuvant chemotherapy was found in this group of patients, which was a subgroup of the 2191 included in randomized Nordic studies. In our study group, TS expression was an independent factor for disease-free survival ($p = 0.05$) and overall survival

($p = 0.05$). In the group of 442 patients treated with surgery only, TS expression was an independent prognostic factor (disease-free survival, $p < 0.001$, overall survival, $p = 0.001$), while in the group of patients treated with surgery and 5-FU-based chemotherapy, TS expression was not of prognostic value. Patients with high TS-expressing tumors had a tendency toward improved clinical outcome (not significant), whereas patients whose tumors expressed a low TS level (28% of the patients) had an impaired clinical outcome following adjuvant therapy (overall survival $p = 0.008$).

A weak but significant association was found between Ki-67 expression and TS expression (low/high) in rectal cancer ($p = 0.02$). There was no significant correlation between TS and Cyclin A expression ($p = 0.1$). The prognostic value of TS expression can be explained only partly by the proliferative activity of the tumor, since, to conclude, TS levels, immunohistochemically assessed in colorectal cancer, are a prognostic factor independent of Dukes' stage. Patients with Dukes' C tumors with low TS expression, as determined by immunohistochemistry, will, according to our findings, have an impaired survival if they are treated with surgery and 5-FU-based adjuvant chemotherapy compared with surgery alone. Further studies in new patient material are needed to see whether the results of the present study can be reproduced. If this is the case, 5-FU-based adjuvant treatment is to be recommended only to high TS expressors, but not to low TS expressors.

March 2001

Bronchial carcinoids

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Bronchial carcinoids are subdivided into typical and atypical. Atypical carcinoids are more malignant, but typical carcinoids may also influence survival. In the present study immunohistochemistry was performed to identify prognostic markers in patients with typical bronchial carcinoids. The diagnostic efficacy of octreoscan was evaluated, in comparison with CT and bone scan, and finally our experience of treating patients with metastatic bronchial carcinoids is reported.

In an unselected material of 43 patients with typical bronchial carcinoids, metastatic disease was found in 12 patients (28%): Five patients (12%) developed distant metastases and died from their disease. High Ki-67 index, as well as positive staining for bcl-2 or p53 was associated with decreased survival time. Positive staining for CD44s, v7-8 and v9, as well as positive nuclear staining for nm23 correlated to decreased mortality. Staining for CD44 and Ki-67 should be performed routinely for prognostic evaluation in these patients.

Octreoscan positive tumors were found in altogether 20/28 patients (71%). The primary tumor was detectable in 81% and intrathoracic metastases in 78% of the patients on octreoscan; the corresponding figures for CT were 94% and 89% respectively. Liver metastases, as shown by CT, were demonstrable by octreoscan in 64% of patients. Octreoscan showed 70% and bone scan 90% sensitivity for identification of bone metastases.

Plasma chromogranin A was elevated in 28/30 patients (94%) with metastatic bronchial carcinoids and was the most sensitive tumor marker. Increased urinary 5′HIAA was found in 68%.

Biotherapy with α-interferon and Octreotide relieved carcinoid syndrome in 7/16 patients. However, only 4/27 patients showed stable disease during median 15 months, while 23 patients progressed. Treatment with cisplatinum + etoposide resulted in an objective response or stable disease for 6–8 months in 3/8 patients with widespread tumors. Doxorubicin combined with streptozotocin or paclitaxel was associated with stable disease for 9 months in 2/2 patients each. All 7 patients treated with streptozotocin + 5-FU progressed.

Among the 43 unselected typical bronchial carcinoid patients, 5-year and 10-year survival was 95% and 91%, respectively. The prognosis in patients with bronchial carcinoids showing distant metastases was poor: 5-year survival was 70% from diagnosis and 22% from treatment start.

March 2001

Molecular endocrinology of target enzymes in androgen metabolism—Implications for prostate cancer

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Genes that are responsible for variations in drug and hormonal responses are sometimes also associated with the pathophysiology of diseases. Understanding the aetiology of a disease is often essential for the design of effective drugs. Androgens are implicated in the development of prostate cancer and benign prostate hyperplasia (BPH). This study aimed at characterising the therapeutic target enzymes steroid 5α-reductase 1 and 2 and the steroid metabolising enzyme cytochrome P450 1B1 in relation to prostate cancer in order to gain a better view of the prostate tumor biology.

The conversion of testosterone to the more potent metabolite dihydrotestosterone by steroid 5α-reductase 2 is a key mechanism in the action of androgens in the prostate. The 5α-reductase 2 specific messenger RNA (mRNA) levels were measured in 50 biopsies obtained from 31 Caucasian outpatients, using a solution hybridization method. Significant differences were observed between cancerous and noncancerous tissues. The median 5α-reductase 2 mRNA level in noncancerous tissue was 3.4 times higher than in cancerous specimens. 5α-Reductase 2-specific gene expression and enzyme activity was further measured in 30 prostatic tissue specimens from 15 Caucasian patients. The enzyme activity at pH 5.5 was significantly correlated to the 5α-reductase 2-specific mRNA expression as measured by reverse-transcription PCR ($R_s = 0.81$). This association makes it possible to predict prostatic 5α-reductase 2 activity using core needle biopsies.

In order to elucidate the role of 5α-reductase 2 polymorphisms, we performed a population based case control study in 176 Caucasian prostate cancer patients and 161 healthy controls of the importance of the V89L and A49T polymorphisms for the risk of prostate cancer, in relation to age and tumor characteristics. Carriers of the LL genotype were at increased risk of bone metastases at the time of diagnosis compared to the combined groups of individuals with VL or VV genotypes, OR 5.67 (95% CI 1.44–22.30), when adjusted for age, differentiation grade, T-stage and PSA. Heterozygous prostate cancer cases carrying the AT genotype were significantly younger than cases harboring the AA genotype (mean age 66 vs 71 years).

In the same patient material, the *CYP1B1* V432L polymorphism was studied in relation to risk of prostate cancer and tumor

characteristics. Carriers of the *CYP1B1* 432 LL genotype had a higher risk of metastases at the time of diagnosis compared to individuals with VL or VV genotypes, (OR 2.46, 95% CI 1.02–5.93) when adjusted for age, differentiation grade, T-stage and PSA. These findings may contribute to further understanding of the etiology of prostate cancer metastases.

March 2001

Development of electron beams for 3D modulated radiation therapy

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This study was initiated to analyse the possibilities with the MM50 racetrack microtron and to achieve similar treatments with more conventional treatment equipment. By using a computer controlled multileaf collimator both for photon and electron beams, automatic and dynamic beam delivery are possible for both electrons and photons. This allows the specific depth modulation characteristics of electrons to be used more easily and more effectively to optimise treatment.

When developing clinical procedures with these beams it is essential to have access to a reliable and well-documented treatment planning system. A detailed evaluation of an electron algorithm was therefore performed. It was shown that this pencil beam algorithm could be used with the same reliability for multileaf collimated beams as for beams with conventional applicator collimation.

The 50 MeV racetrack microtron uses a scanning beam technique where an elementary beam is scanned in arbitrary computer controlled patterns. A standard set of intensity modulated electron beams was designed with theoretical methods and then verified with measurements. These beams have been implemented into a dose planning system and can be used for manual optimisation.

This investigation further describes how a conventional treatment head can be modified for use of multileaf collimated electron beams with beam characteristics related to those of the MM50 racetrack microtron. The optimisation was carried out by modifying the treatment head: replacing the air atmosphere in the treatment head with helium, adding a helium bag below the MLC, changing the position of the scattering foils, modifying the monitor chamber, and adjusting the position of the MLC. The results from Monte Carlo calculations show that a standard treatment head using a dual scattering foil system can be redesigned for multileaf collimation with beam characteristics that are clinically acceptable. Furthermore, the results show that the difference between curved leaf ends of the MLC and focused ends is negligible in most practical cases. The results also show the importance of scattering foil optimisation in the optimisation of parameters such as the penumbra and the virtual source position. In addition, scattering foil optimisation reduces the output variation.

Three optimised treatment heads based on commercially available treatment units were designed for multileaf collimation of electron beams. The performance of these three treatment heads was analysed for a number of clinical cases and the results show that they can be used for mixed beam IMRT delivery.

March 2001

Patterns of proliferation in human colorectal cancer

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Colorectal cancer (CRC) is a common cancer diagnosed in Sweden second only to cancer in the breast and prostate. Every year 5 000 new cases are found. Although there has been some progress concerning treatment—refined surgical technique in rectal cancer, adjuvant radiotherapy and chemo- or immunotherapy—no significant improvement of survival has been achieved over the last decades. In the development of better treatment schedules, it has become necessary to search for biological markers that will predict the unique behaviour of individual tumours. This is the only way to identify patients who will benefit from additional treatment to surgery.

In the 1980s a method to calculate the proportion of proliferating cells and to estimate the duration of the DNA-synthesis phase of the cell cycle (S-phase) was developed by A.C. Begg. The non-toxic pyrimidine BrdUrd or IdUrd could be administered to patients by iv infusion. This molecule closely resembles one of the four building blocks of DNA (thymine). Proliferating cells incorporate IdUrd as new DNA is produced in the S-phase of the cell cycle. IdUrd can then be detected by a monoclonal antibody (labelled with a dye or fluorescence) and analysed in tissue sections or by flow cytometry (FCM).

In the present study proliferative patterns in human colorectal cancers have been studied after intravenous infusion of IdUrd before surgery. After immunohistochemical staining the proportion of labelled nuclei was estimated (labelling index of the tumour, LI). We found very high proliferative activity in the tumours which is somewhat surprising since CRCs are considered to be slow growing tumours. There was marked inter- and intratumoural variability of LI. We also found that LI was almost always much higher in the superficial (luminal) part of the tumour than in the deep infiltrating part. Results indicate that low proliferative activity in the deep part of the tumour may be a negative prognostic sign.

FCM analysis of nuclei in suspension was also performed and the advantages and difficulties with this technique are discussed. In FCM analysis of IdUrd labelled cells there is still no technique available that allows exclusion of normal stromal cells from the malignant cells. In one study epithelial tumour cells were double stained for DNA and cytokeratin (cytoskeletal protein in epithelial cells). In this way normal stromal cells could be excluded from the analysis. When epithelial tumour cells only were analysed the S-phase fractions of diploid and aneuploid tumours were equally high. When crude tumour samples were analysed the diploid tumours had false low S-phase fractions due to the admixture of normal stromal cells.

In conclusion, IdUrd administered in vivo provides an excellent opportunity to study proliferation in human tumours. Proliferative activity in human CRC is high in spite of slow overall growth. There is marked variability between tumours and between different areas within a tumour. The proliferative activity is higher in the superficial (luminal) part of the tumour. When biopsies are sampled this must be taken into consideration in order to avoid misleading conclusions from results due to architectural peculiarities specific for CRC. In other words we should not compare apples (superficial LI) with pears (deep LI). Specific architectural patterns in solid tumours should always be evaluated in the microscope. Dynamic information about proliferation kinetics in tumours provided by FCM may predict clinical outcome in patients and thus be of importance for decision making in cancer treatment, but the technique should be handled with care by experienced hands and minds.

March 2001