HETEROGENEITY OF FLOW CYTOMETRIC DNA DISTRIBUTIONS AS A PROGNOSTIC VARIABLE IN PRIMARY MALIGNANT MELANOMA.

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Flow cytometric single parameter analysis of 49 patients with primary malignant melanoma of the skin in stage 1 were studied. For most patients, several samples from different regions of the tumour were obtained. The aim of the study was to investigate the prognostic significance of flow cytometric DNA measurements. The median followup time is now greater than 5 years. Maximum likelihood estimates of the DNA distributions showed a marked heterogeneity with respect to the number of subpopulations and the DNA indices. There was a considerable intra- and inter-patient variation. Inter-patient variation was significantly larger than intra-patient variation. Life table analysis of recurrence free survival showed a significantly poorer prognosis for patients with DNA measurements with many subpopulations and high DNA indices ($p < 0.05$). Similar analysis for survival were performed.

HETEROGENEITY OF ESTROGEN RECEPTORS IN HUMAN BREAST CANCER. ITS CLINICAL RELEVANCE?

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It is well recognized that only 50% of patients with estrogen receptor (ER) positive tumors respond on endocrine therapy. Ten years ago it was postulated that this observation could be explained at least in part by tumor heterogeneity viz. tumors featuring both ER positive and ER negative cells. This hypothesis has been evaluated utilizing an immunohistocemical ER analysis recently developed in our laboratory performed on formalin fixed paraffin imbedded sections obtained from breast cancer tissue. The study consisted of A:An evaluation of ER in primary tumors vs. ER content in regional- and distant metastases. B:An evaluation of ER content of primary tumors and patients response to endocrine therapy when they got advanced disease. It was found that: (1) Both primary tumors as well as their corresponding regional and distant metastases showed pronounced ER heterogeneity. However, in 10 to 15% of the cases only a difference in ER content was observed. (2) 60% of patients with ER positive tumors responded on endocrine therapy whereas only 5% ER negative tumors responded. The important feature for succes of treatment appeared to be related to ER positivity rather than to number of ER positive cells. These two observations do not lend support to the hypothesis that ER heterogeneity is of major importance for the relative succes of response to endocrine therapy in patients with breast cancer.(Sponsored by The Danish Cancer Society and The Danish Medical Research Council).

HETEROGENEITY OF HUMAN UTOTHELIAL CELL LINES PROPAGATED IN VITRO.

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The classification and identification of established cell lines are essential questions for all studies of cellular transformation in vitro. We have previously classified a number of human urothelial cell lines according to their grade of transformation based on studies of their morphology, growth pattern, life span, tumorigenicity in nude mice, and ability to invade cocultured normal tissues in vitro. For the identification of the individual cell lines HLA polymorphism and Restriction Fragment Length Polymorphism (RFLP) of various genes (α-globin, Ha-ras-1, HLA-Dr) have proved to be useful. Such studies have revealed the contamination of some cell lines with other cells, and the heterogeneity of other non-contaminated, cloned cell lines.

THE NORDIC CANCER ATLAS - OVERVIEW OF RESULTS.

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The Nordic Cancer Atlas is the result of collaboration between the cancer registries in Denmark, Finland, Iceland, Norway and Sweden, set up to describe the variation in cancer incidence in 72 regions in the five countries. The number of cases of 29 cancers and average population size in the regions and 5-year age classes were collected for the period 1970-79. Standardized morbidity ratios (SMRs) were computed for each cancer site and sex, and used as basis for colouring maps of the five Nordic countries. For international comparability we also computed directly standardized incidence rates (world standard). We found large variations in incidence levels, not only between rural and urbanized areas as expected, but also between countries and within countries across borders. Testis cancer showed a pattern with little variation within countries, but with enormous differences between countries; the incidence rate is 4.5 times higher in Denmark than in Finland. Melanoma of skin is more frequent in southern parts of Scandinavia, and exhibits a patterns that does not follow national borders. Lung

cancer shows the well-known urban-rural variation, except for Finnish males, where the incidence rates are fairly constant throughout the country. All cancers showed a limited variation in incidence, with a ratio of incidence rates between the regions with highest and lowest of 1.7. For both men and women the high incidence area is Copenhagen, but for men the lowest incidences are found in rural Sweden and Norway, and for women in rural Finland.

THE IMPORTANCE OF TUMOR OXYGENATION STATUS FOR ANTINEOPLASTIC THERAPY.
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The heterogeneity of solid tumors due to differences in intratumoral oxygenation status is believed to influence the effect of several cancer treatment modalities. Based on results from a clamped tumor control assay using a C3H mouse mammary carcinoma in vivo, the effect of various antineoplastic agent on aerobic and hypoxic cells was determined. The agents could be divided into three categories:
(1) preferential activity against aerobic cells: Cisplatinum, Bleomycin, Methotrexate, X-rays.
(2) preferential activity against hypoxic cells: Hyperthermia, Etoposide, Misonidazole.
(3) activity against both cell types: Mitomycin C, Cyclophosphamide, Adriamycin, Vincristine.
The significant killing of hypoxic cells observed for agents in category (2) and (3) correlated with the ability of these agents to cause a significant enhancement in radiation response. The drugs in class (1) did not improve the radiation response. We conclude that tumor hypoxia might be an important factor in the development of drug resistance in solid tumors. The specific toxicity of antineoplastic agents on aerobic and hypoxic cells needs to be considered, especially in the agents are used in combination with radiotherapy.

HETEROGENEITY IN CERVICAL CANCER.
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Heterogeneity can be considered at several levels - the microscopic or the molecular level. Cancers with a possible viral etiology could be considered as one group of cancers with an internal heterogeneity as virusproducts can be demonstrated in certain cells in the tumor but not in all cells. Cervical cancer which at present is considered to be caused by human papilloma virus (HPV) is one example where heterogeneity apparently do exist. Between 60% and 80% of the tumors contain HPV DNA analysed by the Southern Blot test. In situ hybridization on paraffin sections demonstrate HPV-DNA in selected cells within a premalignant lesion. Is this heterogeneity true or is it a result of lack of sensitivity of the tests used? Data from our studies will be discussed in relation to application of the Polymerase Chain Reaction to samples scored negative in the "standard" tests used.

AMPLIFICATION OF myc FAMILY ONCOGENES IN TUMOR BIOPSIES FROM PATIENTS WITH SCLC.
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Activation of oncogenes is found in a variety of human malignancies, and amplification of members of the myc family is often found in small cell lung cancer (SCLC). In this study the level of amplification of selected oncogenes was determined in biopsies of 9 metastatic lesions from patients with SCLC. The tumors CPH 136A and 136B were from the same patient before and after single drug VM-26 chemotherapy. DNA was extracted by standard methods and digested with restriction endonucleases. Ten micrograms per lane was electrophoresed in 0,8% agarose gels and transferred to nylon membranes. Southern blots were probed with oligolabeled oncogene fragments. One tumor (CPH 160) had approx. 8-fold amplification of c-myc. Two tumors (CPH 124 and 136A) had approx. 10-fold amplification of the 10 kb L-myc band. No amplification was seen of N-myc, c-myb, N-ras or H-ras. Thus, amplification of one of the members of the myc oncogene family was found in 3 out of 9 tumor biopsies. This figure is comparable with the high amplification frequency of myc oncogenes reported for SCLC.

ISOLATION AND CHARACTERIZATION OF A HIGH AND LOW TUMORIGENIC SUBLINE OF HUMAN T24 BLADDER CARCINOMA CELLS.
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Two sublines of the T24 human urinary bladder carcinoma cell line, T24A and T24B, which differ in tumorigenicity has been isolated. The sublines differed in morphology and in growth rate in vitro and in vivo. T24A had a 5 times delayed tumor take after subcutaneous injection and at the same time a higher expression of HLA than T24B. Following intravenous injections in the tail vein of nude mice lung metastases were only produced by T24B. No differences were seen with respect to the type of invasion into mouse heart tissue in vitro, but the intercellular adhesion was tighter between the T24A cells than between T24B