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## PUBLICATION

**Differential loss E-cadherin in breast carcinomas**

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**Purpose:** Invasion, eventually leading to metastasis, is presented as a result of a balance between the activation of two sets of genes, invasion promotor and invasion suppressor genes. E-cadherin is an invasion suppressor gene product. Our study try to analyse the E-cadherin expression in breast tumors, linked to histological type.

**Methods:** Tissue material from 35 breast lesions of untreated patients was included in this study. There were made parallel samples for paraffin-embedding and staining with H.E. For immunohistochemistry 5  $\mu$ m thick cryostat sections were cut from the frozen tissue blocks. We used the MoAb L-cam Boehringer Mannheim, for the E-cadherin staining. Bound antibody was detected using SBC (Boehringer Mannheim).

**Results:** Histologically normal tissue structures present in breast tumors were studied. The antibody anti-L-cam stained the intercellular borders of the luminal cells of the interlobular ducts. E-cadherin was expressed in all cases of benign and malignant lesions with different grades of intensity. Our results show that the most intensive reactions were remarked at the benign level, and to low grade of malignancy (well differentiated tumors).

**Conclusion:** E-cadherin is expressed in the majority of breast tumors at different levels. We observed a good correlation between the loss of expression of E-cadherin and high grade of malignancy. A future extension of the study and larger correlation with histological grade, ER status and other oncogenes products can converg to a real test for malignancy evaluation.

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## PUBLICATION

**The TNF level in plasma and in primary tumor conditional medium in gastric cancer patients**

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**Purpose:** The TNF is responsible for paraneoplastic symptoms in gastric cancer patients. We have investigated the TNF level in plasma and in primary tumor conditional medium in 21 gastric cancer patients and in 10 donors plasma.

**Methods:** The bioassay was performed. 16 patients with G4-G3 and in 5 patients with G1-G2 tumor grades. According to Bormann types: I type – 4 patients, I – 3, II – 4, IV – 10. Tumor location: upper portion of stomach – 4, middle portion – 6, lower portion – 11. Staging of process: II – 1 patient, III – 20 patients.

**Results:** The plasma TNF level in G4-G3 tumors was  $1.8 \pm 0.24$  ng/ml, in G1-G2 –  $0.514 \pm 0.15$  ng/ml, in donors plasma –  $0.088 \pm 0.014$  ng/ml. The supernatant TNF level of G4-G3 tumors was  $0.12 \pm 0.008$  ng/ml, and G1-G2 –  $0.15 \pm 0.02$  ng/ml. Thus, we have found the corelation between the tumor grade, agressiveness of tumor process and TNF level in plasma.

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## PUBLICATION

**Apoptotic effects of taxol and radiation on the intestinal kript cells of swiss-albino mice**

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**Purpose:** Taxol, a natural product isolated from the bark of the yew tree *taxus brevifolia*, exhibits significant antitumor activity against various experimental tumors of animals and humans. The drug induces nuclear fragmentation in cultured cells, a feature characteristic of apoptotic cell death. In this experiment, we investigated apoptotic effects of taxol and ionizing radiation on the intestinal kript cells of swiss-albino mice.

**Materials & Methods:** 170 Swiss-albino mice obtained from Test Animals Brooding Center of Uluda University were used for this experiment. They were 3–4 months old and weighing 20–25 gr. Each group consisted of 5 mice. Taxol was administered bolus IV through the tail vein. Radiation was given with a special device using linear accelerator (6 MV photon). Dose was calculated manually as Dmax dose. There were four groups in the study. First group (taxol alone, n = 15) received IV bolus taxol in three different doses (20, 30 and 40 mg/m<sup>2</sup>). Radiation was administered in three different doses (0.25, 0.5 and 1 Gy) to mice in the second group (radiation alone, n = 15). Third group (radiation and taxol, n = 135) was taxol and radiation group. Mice were administered taxol intravenously (20, 30 and

40 mg/m<sup>2</sup>) initially. They were irradiated after 12, 24 and 48 hours after taxol administration (0.25, 0.5 and 1 Gy). Fourth group was control group which did not receive either taxol or radiation. Mice were sacrificed 24 hours after taxol or radiation administration using ether anesthesia. Using light microscope, apoptotic and mitotic index were counted on jejunal kript cells of mice that were stained with hemotoxilein-eozin. Differences between groups were evaluated with student t-test statistically.

**Results:** Mean mitotic index was 2.80 in control group while it was 6.81 in taxol group, 8.79 in radiation group and 3.37 in radiation and taxol group. Although there was no significant difference between taxol alone and radiation alone groups and between control and taxol and radiation groups (p > 0.05), control and radiation and taxol groups were significantly different from either taxol alone or radiation alone groups (p < 0.000). Mean apoptotic index was 1.00 in control group while it was 19.44 in taxol group, 14.56 in radiation group and 5.78 in taxol and radiation group. Differences in apoptotic index between all groups reached significance (p < 0.01). Mean mitotic index was 4.60 in mice irradiated in 12 hours while it was 2.84 in 24 hours and 2.69 in 48 hours. Although there was no difference between 24 and 48 hours (p > 0.05), differences between 12 hours and 24 and 48 hours were statistically significant (p < 0.05). Apoptotic index was 3.43% in 12 hours, 9.96% in 24 hours and 3.92% in 48 hours. Differences between 24 hours and 12 and 48 hours were statistically significant (p < 0.05). There was no difference between 12 and 48 hours (p > 0.05).

**Conclusion:** Addition of taxol to radiation did not seem to be enhancing apoptotic effect of radiation in normal jejunal kript cells.

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## PUBLICATION

**Expression on tumors and its metatases of platelet endothelial cell adhesion (PECAM-1), a molecule involvement in transendothelial cell migration and angiogenesis**

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**Introduccion:** Platelet endothelial cell adhesion molecule-1 (PECAM-1) is a cell-cell adhesion molecule that is expressed on circulating platelets, on leukocytes, at the intercellular junctions of vascular endothelial cells. This is important molecule that mediate in process of transendothelial cell migrations in the inflammatory response and in formation of new vessels. Some investigators shows expression of PECAM-1, in some cellular tumoral lines, in vitro and they have hypothesized about the importance in the metastatic process.

**Aim:** To know if the human tumours and its metastases express PECAM-1.

**Material and Methods:** The tumour biopsies was collected of primary localisation and its metastases in 12 patients and it fixed in formalin and paraffin-embedded. We used the antibody anti CD31, H1/6, in the immunohistochemical staining.

**Results:** We found expression of PECAM-1 in more of 50% of the cells and +++/+++ or superior in colon, breast, and bladder urinary cancer, also in its metastases. We found expression nor in melanoma, ovarian, kidney and non small cell lung cancer, neither its metastases.

**Conclusion:** Some tumours and its metastases express PECAM-1. We believe that it will be very important to know which is the effect of regulation of this molecule in process of dissemination and tumoral angiogenesis, actually we are studying this.

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## PUBLICATION

**Fibronectin in cell adhesion. It's expression in breast tumors**

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**Purpose:** A major cause of morbidity and death due to the cancer is the metastasis of cells from the primary tumor to distant sites where secondary tumors become established. Tumor cell adhesion to component of extracellular matrix and basement membranes is mediated by specific cell surface receptors that bind to extracellular adhesive proteins. The fibronectin-integrin system has provided a valuable model system for the study of the cellular and molecular mechanisms involved in cell adhesive steps in metastasis. We studied the fibronectin (FN) expression in 35 breast tumors (10 benign and 25 malignant) with different histological types.

**Methods:** We analysed tissue material from 35 breast tumors of untreated patients. We made parallel detection for histological type on paraffin-embed-

ded tissues stained with HE, and for FN expression on cryostat sections. We used the MoAb antiFN from Boehringer-Mannheim. SBC (Boehringer-Mannheim) was used for immunohistochemical staining. Hematoxylin was used for nuclear staining.

**Results:** The expression of fibronectin is heterogenous at both benign and malignant lesions. We evaluated as negative, positive and strong positive the staining for fibronectin.

**Conclusion:** These results bring the fibronectin expression at a new level—that of malignancy marker. It also have the quality to reveal the perivascular metastatic capacity. We consider that the fibronectin through its receptor property in cell adhesion can become an useful indicator for metastatic potential of the tumor and also for the tumoral evolution.

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PUBLICATION

### Dominant properties for the suppression of development of preneoplastic foci in the liver of carcinogen resistant inbred DRH strain rats

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**Purpose:** The post-initiation stage of hepato-carcinogenesis was investigated in carcinogen resistant inbred DRH rats and the parental Donryu rats.

**Methods:** Male rats (5 W) were treated with DEN followed by 3'-methyl-DAB and partial hepatectomy. At 8 weeks after the start of treatment, the number and size of glutathione S-transferase (GST-P) positive lesions were determined.

**Results:** The mean area occupied by GST-P positive lesions was 30% in Donryu rats but less than 4% in DRH rats despite of the presence of comparable numbers of foci in livers of both strains. This genetic property was dominantly inherited in the F1 rats by crosses of DRH and carcinogen sensitive inbred F344 rats.

## Cancer in children

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ORAL

### The standards, options and recommendations (SOR) project for the management of childhood cancer

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**Objectives:** The "Standards, Options and Recommendations" (SOR) project, was started in 1993, and is a collaboration between the Federation of the French Cancer Centres (FNCLCC), the 20 French Cancer Centres and specialists from French Public Universities, General Hospitals and Private Clinics. For pediatric cancer, this project is a collaboration between the FNCLCC and the French Society of Pediatric Oncology (SFOP). The main objective is the development of clinical practice guidelines to improve the quality of health care and outcomes for cancer patients. The methodology is based on literature review and critical appraisal by a multidisciplinary group of experts, with feedback from specialists in cancer care delivery.

**Methods:** Data have been identified by literature search using Medline and personal references lists. The main criteria considered were incidence, risk factors, prognostic factors and efficacy of cancer treatment. Once the guideline, was defined, the documents were submitted for review to national and International Independent reviewers, and to the medical committees of the French Cancer Centres which have expertise in pediatric cancer management, for agreement.

**Results:** To date, SOR documents have been produced for neuroblastoma, rhabdomyosarcoma, medulloblastoma, osteosarcoma and pain management in children. These recommend standard diagnostic and therapeutic approaches on the basis of file strength of published evidence. The SOR guidelines for rhabdomyosarcoma in which imaging, pathological studies, surgery, radiotherapy and chemotherapy strategies are critically reviewed, the details of SOR methodology and the planned methods of dissemination will be presented.

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### Prognostic value of blasts on day 15 in bone marrow and of molecular evaluation of remission in children with acute lymphoblastic leukemia

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Childhood acute lymphoblastic leukemia is curable in approximately 70% of all patients with current treatment protocols. Risk-directed protocol strategies using multiagent chemotherapy have resulted in complete remission rates of 98% after induction therapy. Despite these achievements, still 25 to 30% of these children will subsequently relapse. With conventional prognostic factors at diagnosis and prednisone poor response one third of patients, who will suffer a relapse can be detected. In vivo response to multiple agent therapy appears to be an independent predictor of outcome. In this study we have evaluated the prognostic significance of early response to induction therapy, as measured by the percentage of lymphoblasts in the day 15 bone marrow aspirate and by the detection of MRD by molecular methods using clone-specific immunoglobulin and T cell receptor gene rearrangements in 75 children with ALL treated according to current BFM protocols. All children achieved morphological remission after induction chemotherapy. After a median observation time of 4.5 years (range 1–7 years) 14 children relapsed. Results of blasts on day 15: M1: 47 patients, M2: 18 children, and M3: 10 children. Five, 4, and 5 relapses occurred in the M1, M2, and M3 group, respectively. Results of MRD analysis (combined evaluation after induction and consolidation treatment): MRD low risk group 38 children, no relapse; MRD medium risk group 31 patients with 10 relapses; MRD high risk group 6 patients with 4 relapses. Log rank test showed significant differences for event free survival (RFS) between BFM risk groups ( $p = 0.0039$ ), for M1, M2 and M3 on day 15 ( $p = 0.0014$ ) and for MRD risk groups ( $p = 0.0001$ ). In a stratified log rank test combining BFM risk groups and MRD groups, blasts on day 15 are not predictive for EFS ( $p = 0.28$ ).

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ORAL

### Monitoring and functional characterization of rare tumor cells in the haematopoietic system

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The detection and quantification of rare tumor cells in the bone marrow (BM) or peripheral blood (PB) is becoming of particular significance for the treatment of cancer patients. Moreover, exact genetic and functional data of the infiltrating tumor cells can further help to better understand the biology of the infiltrating cells and thus help to refine the anti-tumor therapy in individual patients. A fully automated scanning system was adapted, to enable a sequential demonstration of tumor specific immunological and molecular cytogenetic investigations at a single tumor cell level. 200 BM samples from 60 pediatric cancer patients were analyzed sequentially using a tumor specific antigen and by demonstrating the tumor typical genetic aberration. The proliferative capacity of tumor cells was assessed by a simultaneous detection of the tumor specific antigen and the proliferation marker Ki67. Automatic analysis of 29 BM samples revealed Ki67 antigen expression in up to 78% of the tumor cells in the BM of newly diagnosed patients. A similar percentage of Ki67 positive cells was found in patients undergoing cytotoxic treatment. Automatic search and sequential genetic and immunological characterization of tumor cells prove to be a powerful diagnostic tool in clinical oncology and allow new insights in the biological nature of rare tumor cells circulating in the haematopoietic system.

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ORAL

### Cutaneous and subcutaneous Ewing's sarcoma – A relatively indolent disease

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To analyze the outcomes of extraosseous Ewing's Sarcoma (ES) in a primary cutaneous or subcutaneous site.

Between July 1985 and March 1997, 14 patients with cutaneous or subcutaneous ES were treated.

13 had definitive surgical resections and 1 had biopsy of the mass at the time of referral. All patients received chemotherapy. Twelve patients on ES-87 and ES-92 protocols received radiation (36 Gy) to the operative bed