infusion time of gemcitabine. M is as effective as doxorubicin, but provides a lower risk of cardiotoxicity. Prolonged infusion of G can result in higher levels of active metabolites compared to shorter administration. The primary objectives were to determine the optimal doses and the efficacy for G, M and T as primary chemotherapy for locally advanced breast cancer.

Patients and Methods: Patients with histologically confirmed, locally advanced BC were treated with M and T on d1 and G as 4h infusion on d4 every 3 weeks. Patients with an objective response received a maximum of 6 cycles. Treatment was discontinued in case of stable disease or progression after the 2nd cycle. All patients received G-CSF. For the phase I part doses were assigned at registration. Starting dose was G 350mg/m^2, M 50mg/m^2 and T 60mg/m^2. The maximum tolerated dose (MTD) was used for all patients within the phase II part.

Results: 39 pts have been enrolled (Stage: T2 24pts, T3 4pts, T4 11pts, inflammatory BC 7pts. Pretreatment Nodal Status: N0 13 pts, N1/2 26 pts). Currently, 37 pts and 32 patients are evaluable for toxicity and efficacy, respectively. Dose-limiting toxicities were observed in 3/3 pts at G 400mg/m^2, M 60mg/m^2 and T 75mg/m^2 consisting of grade 3 diarrhea and infection in 2 pts and grade 3 stomatitis in 1 pt. The MTD was G 350mg/m^2, M 60mg/m^2 and T 75mg/m^2. Preliminary clinical response data: 2/32 pts with CR, 21/32 pts with PR, 8/32 pts with NC and 1 pt with PD accounting for an overall response rate of 71%. Pathologic response data are pending. Hematological toxicity was moderate (grade 3/4 leucopenia in 23% of courses, thrombocytopenia in 2%, anemia in 2%). Predominant non-hematological toxicities were stomatitis (grade 2 in 26% of courses, grade 3 in 7%), asthenia (grade 2 12%) and nausea (grade 2/3 14%). Other non-hematological toxicities were mild (<10% grade 2/3, except for alopecia). No grade 4 non-hematological toxicity was observed.

Conclusions: GMT is a well tolerated and effective combination. The MTD is G 350 mg/m^2, M 60 mg/m^2 and T 75 mg/m^2. The phase II part of the trial is still ongoing.

377 POSTER

A double marker RT-PCR approach for the detection of disseminating breast cancer cells in peripheral blood

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The detection of disseminated cancer cells in peripheral blood of in breast cancer patients may help to predict disease recurrence and potential metastasis. Cytokeratin 19 (CK19) is commonly used marker for the detection of occult epithelial cells in blood. Mammaglobin 1 (MG1) expression is limited to mammary epithelium and is over-expressed in 95% of primary breast tumours. Our aim was to determine whether the use of two markers (Cytokeratin19 and Mammaglobin I) for the detection of metastatic breast cancer cell using quantitative RT-PCR improves specificity as well as sensitivity of the RT-PCR assay. In several spiking experiments, breast cancer cells (ZR-75) were added to 5ml of human peripheral blood of a healthy donor. After epithelial immunomagnetic enrichment, total RNA was extracted using a silica-based extraction kit. RNA specific primers for MGI and CK19 were designed to amplify these genes in a single-enzyme RT-PCR. cDNA amplicons were visualised in 2% agarose gel. All samples were analysed using Real-time quantitative TaqMan RT-PCR for both CK19 and MG1 primers. We demonstrated similar sensitivity levels for CK19 and MG1 using solution phase RT-PCR. We also showed improved specificity using MG1 as a second breast specific marker. Using quantitative RT-PCR we demonstrated increased sensitivity compared to solution phase RT-PCR. Real-time quantitative RT-PCR is faster, less laborious and sensitive method for detecting occult metastatic breast cancer cell in peripheral blood. Combined markers are the way in the future for the detection of tissue-specific tumour cells.

378 POSTER

Serum levels of matrix metalloprotinase 2 and 9 in patients with breast cancer.

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The Matrix Metalloprotinases MMPs are a large family of proteolytic enzymes, which are involved in the degradation of many different components of the extracellular matrix. MMPs have been reported to be associated with invasive and metastatic behaviours of human malignant tumours. Enhanced expression of matrix metalloprotinases-2 (MMP-2) has been reported to be associated with increased metastatic potential in various tumours and aggressiveness in breast cancer. This study was designed with the aim to elucidate the possible relationship between the preoperative circulating MMP-2, MMP-9 and breast cancer. Eighty consecutive patients with invasive breast cancer undergoing surgery were prospectively included and evaluated. Venous blood samples were collected before surgery. Sera were obtained by centrifugation, and stored at -80° C until assayed. The control group consisted of 20 patients with benign breast disease (10 with fibrocystic disease and 10 with fibroadenoma). Serum concentrations of MMP-2 and MMP-9 were measured by the ELISA technique. The data on the primary tumour, age, grade and TNM staging were reviewed and recorded. The mean value of serum MMP-2 and MMP-9 in patients with breast cancer were 241.65± 65.668 ng/ml and 1185.00± 773.37 ng/ml respectively and the difference was significant from the control group. Furthermore, there were significantly higher serum levels of MMP–9 in the patients with more advanced primary tumour staging and in the patients with more advanced lymph node status. In multivariate analysis, TNM staging appeared as independent factor and patients with more advanced TNM staging were shown to have higher serum MMP-2 and MMP-9 Levels. Thus preoperative serum metalloprotinases level might reflect the severity of invasive breast cancer and deserve further evaluation with postoperative serum levels.

379 POSTER

Male breast cancer - review of 93 patients

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Background: Male breast cancer (MBC) is a rare disease with similarities to breast cancer in women. The clinical and pathologic features of MBC, the prevalence of known risk factors, prognostic factors, treatments, and survival, were studied.

Material and methods: Retrospective analysis of 93 patients with MBC admitted and treated at the Portuguese Institute of Oncology, Oporto, Portugal, in a 28-year period (1974 and 2002). Clinical data were obtained from each patient's record. Information regarding macroscopic and microscopic characteristics of the lesion were obtained from the pathologist original report. Patients who died of non-cancer-related causes were censored at last follow-up. Overall and disease-specific survival, were calculated using the Kaplan-Meyer method, and univariate comparisons of survival were performed using the log-rank test. A p value of 0.05 or less was considered significant.

Results: The mean follow-up time was 58 months (±47), 10 patients (11%) abandoned follow-up. Median age at diagnosis was 65 years (34-94). Most patients came from rural areas, working in the primary sector, and were in contact with known risk products for cancer. Family history of oncology disease was positive in 23 patients (25%), and 11 (12%) have family breast cancer. In our population 78 patients (85%) present a breast nodule, 39 in a subareolar location (42%), and 63 with firm consistency (69%). The mean interval time between first symptom and diagnosis was 19 months (±25). The most frequent histopathologic type of MBC was invasive ductal carcinoma, representing 65% of the cases. Lobular carcinoma, rarely described, was present in 2 of our patients. Node dissection was performed only in 59 patients (64%), metastization involvement in 39 of them (66%). Histologic grade, according the WHO classification modified by Bloom and Richardson, was mainly grade II (26 patients, 28%). Hormonal receptors were analyzed in 68 patients, 38 (56%) were simultaneously positive for estrogen and progesterone receptors. Primary treatment was surgery in 72 patients (78%). Adjuvant treatment was performed in 63 patients (68%), being radiotherapy (RT) in 52 patients (57%), chemotherapy (CTX) in 12 patients (13%), and hormonal (HT) in 38 patients (41%). Five and ten years overall survival (OS) was 69% and 51%, respectively. Twenty patients (22%), recurred, predominantly with bone and lung metastasis. The mean time for the first recurrence was 42 months (±38). Five and ten years disease-free survival (DFS) was 80% and 53%, respectively. The OS and DFS were not statistically influenced by survival comparisons regarding age, tumour size, lymph node metastization, stage at diagnosis, grade of differentiation, and hormonal receptors.

Conclusions: Our results are similar to the available literature. We observed a long mean time to diagnosis, but it did not influence OS. Treatment changes, with emphasis in the adjuvant setting to RT, CTX and HT, probably contribute to the better outcome achieved.