874 PUBLICATION

Weekly paclitaxel/carboplatin and radiotherapy in inoperable squamous cell carcinoma of head & neck and lung

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Purpose: Treatment result in Patients with unresectable Head&Neck Cancer (H&NC) as well as Non Small Cell Lung Cancer (NSCLC) is not satisfying. We conducted a phase II trial to asses the efficacy and feasibility of weekly Paclitaxel (P) and Carboplatin (CP) in an outpatient setting.

Methods: From 02/96 to 11/97 35 patients with H&NC (25) and NCSLC (10) were treated with P: 90 mg/m" and CP: 150 mg/m" weekly × 6 with 2 weeks rest. Induction therapy with two cycles was followed by one cycle with simultaneous radiotherapy 2 0 Gy daily 5 times weekly for a total dose of 62–65 Gy. In this cycle the dose of P and CP was adapted to the mucositis, normally with >50% dose-reduction of both: P and CP.

Results: In Feb. 97 19/35 pts had completed therapy and were evaluated for toxicity and response.

We observed complete response in 3, partial response in 11, no change in 3 and progressive disease in 2 pts, overall response 14/19 (74%).

Toxicity: (Only grade 3 + 4) Alopecia: 19/19, haematotoxicity 3/19, mucositis 5/19, infections 3/19, diarrhoea 1 pt; during first cycle 1/19, second cycle 3/19 and third cycle (with radiotherapy) 7/19.

Conclusion: By preliminary evaluation treatment results and toxicity seem to be similar to other regimens with Cisplatin and 5 FU – by dose reduction to 33% in the third cycle toxicity become low and acceptable.

875 PUBLICATION

Effects of radiation in radiotherapy treatments of laryngeal carcinoma: Clinical manifestations

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Purpose: The aim of our study is to evaluate the normal tissue reactions at irradiation and to establish the spectrum of radiotherapy results in laryngeal carcinoma.

Methods: We have analyzed the early and late irradiation reactions at 119 patients (T2-4No-1Mo) with laryngeal carcinoma, in distinct clinical dosimetric situation, in order to establish: the incidence of late oedema and fistula as a function of the field size, the incidence of pharyngo-cutaneus fistulae, the influence of age in the incidence of late injury, influence of the administration of prophylactic metronidazol, the reduction of normal tissue damage by increasing complexity of radiotherapy treatments.

Results: After a quantitative analyse of resulted data from the study we have made a description of the laryngeal carcinoma treatment morbidity and we have bilt a system for classification of normal tissue injury.

Conclusion: Because in clinical practice to obtain a therapeutic gain is necessary to reduce the risk of complication by taking into account the biological impact of the radiotherapy treatment we use with success the data gived by this system of clinical manifestation of normal tissue damages after irradiation.

Breast cancer pathology and predictive factors

876 ORAL

Prognostic Relevance of Microvessel Density (MVD) in primary human breast cancer and lymph node metastases

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The analysis of prognostic factors in breast cancer is of great importance for subsequent therapeutic approaches. Tumor angiogenesis is of high relevance in this context. We analyzed the MVD in 70 primary tumors (PT) (patient age: 33–85 yrs, mean 59.8 yrs) and 27 derived lymph node (LN) metastases using an immunohistological evaluation of CD31 expression. The results were correlated with other tumor/patient characteristics of potential prognostic interest and clinical outcome (median follow-up: 34 months).

Compared to PT, LN metastases showed a much stronger expression of CD31 (median 11.1 vs. 5.3 MV/high power field (HPF), $p < 0.0001$) in general. However, MVD in LN and PT within one individual did not seem to be correlated with each other. In 8 of 10 T4 tumors, 5.3 MV/HPF were found. No other association between stage and CD31 expression was observed. A higher MVD was associated with higher serum levels of CA15-3 ($p = 0.028$). No correlation was found with age, alcohol intake, histology, grade, ploidity, LN involvement, serum CEA, and tumor expression of cathepsin D, c-erbB2, CD44s, CD44v6 and E-cadherin. After 36 months disease free survival (DFS) and overall survival (OS) of patients with <5.3 MV/HPF in PT were 90% and 93% resp. compared to 63% (DFS, $p = 0.015$) and 77% (OS, $p = 0.055$) for higher MVD. The CD31 expression in LN showed no correlation to OS or DFS

In conclusion, assessment of MVD by histological determination of CD31 in PT has prognostic implications in breast cancer. In contrast, analysis of MVD in LN metastases does not provide prognosis related information.

877 ORAL

Long-term prognostic impact of PAI-1 in primary breast cancer confirmed by six-year median follow-up

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We evaluated the prognostic impact of invasion markers uPA (urokinase-type plasminogen activator) and its inhibitor PAI-1 in primary breast cancer ($n = 316$) after a median follow-up of 77 (41–108) months. In a node-negative subgroup ($n = 100$), their prognostic strength was compared to that of new tumor biological factors S-phase (SPF), MIB1 (Ki-67), HER-2/neu, p53, and cathepsin D.

Levels of uPA and PAI-1 were determined in tumor tissue extracts by ELISA (American Diagnostica, CT), and that of cathepsin D by ELSA. SPF was determined flowcytometrically in paraffin sections. MIB1, p53, and HER-2/neu were assessed immunohistochemically in adjacent paraffin sections using APAAP technique. Optimized cutoff values were determined using isotonic regression.

In all patients, various factors showed significant impact on disease-free survival (DFS) in univariate analysis. However, in multivariate analysis, only lymph node status and PAI-1 remained significant. In the node-negative subset, uPA, PAI-1, and proliferation markers SPF and MIB1 had a significant impact on DFS. In multivariate analysis, only PAI-1 retained its significance. Statistical analysis performed after varying follow-up periods suggested time-dependency with PAI-1 as prognostic factor of delayed relapse, and uPA as indicator of early relapse.

In conclusion, PAI-1 remains a strong prognostic factor in breast cancer after a 6-year median follow-up. Its prognostic impact seems to indicate a time-dependent risk profile, which may help clinicians to individualize follow-up care, as well as provide a more profound insight into the dynamics of breast cancer metastasis

878 ORAL

p53 overexpression in node-negative breast cancer: Prognostic significance in a randomized study?

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Introduction: resistance to chemotherapy of breast cancer with p53 mutations has been reported We have evaluated p53 alterations in 282 consecutive premenopausal patients with node-negative breast cancer who underwent primary surgery and were randomized either to CMF (Endoxan 400 mg/m2), Fluorouracil 400 mg/m2 et Methotrexate 40 mg/m2) or control arm from 1980 to 1989.

Methods: P53 alterations were analyzed by immunohistochemistry using DO7 MoAb, revealed by immunoperoxidase technique and quantitated in terme of percentage of positive cells.

Results: We observed a positive staining in 24% of the tumors. Among them, 10% had a positive staining in more than 75% of the cells. There was a highly significant association between the proportion of positive cells and histologic grade of the infiltrating ductal carcinomas ($p < 0.008$). However there was no association with age, tumor size, histological status, vascular

emboli. There was a non significant trend between positive staining and overall survival either in each arm of the trial and in all population. Interestingly, we observed a higher relative risk of local relapse after conservative therapy in the boosted area in the group of mutated p53 (RR = 1.85).

Conclusions: We conclude that, in this node-negative breast tumor population, the alterations of the p53 cannot predict the response to the chemotherapy. However, it may represent a useful marker of risk of local relapse and of radioresistance.

879 ORAL

Clinical significance of urokinase plasminogen activator and its receptor in breast cancer

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Background: Urokinase plasminogen activator (uPA) is a multifunctional protein involved in tissue proteolysis, cellular migration, cellular proliferation and growth factor activation. Most of these actions are mediated while the protease is bound to a membrane receptor, termed uPAR.

Aim: The aim of this project was to study uPA and uPAR in breast cancer and to relate levels of both to patient outcome.

Methods: uPA and uPAR were measured by ELISAs (American Diagnostica).

Results: Initially, different detergents were evaluated for their ability to extract uPA and uPAR. The most effective detergent for the extraction of uPA was n-dodecylmaltoside (nDM) followed by Nonidet P40, CHAPS and Triton X-100. Neither Tween 20 nor digitonin increased the yield of uPA. nDM and CHAPS were the most effective in extracting uPAR followed by Triton and Tween. As for uPA, digitonin had no effect on the release of uPAR. Median levels of uPAR were 2.2-fold higher in primary carcinomas than in benign samples ($p = 0.043$).

However, levels in primary and metastatic cancers were not significantly different. Median levels of uPA were also significantly higher (7.6-fold) in the primary cancers than in the benign samples ($p = 0.0001$) but as with uPAR, levels of uPA were not significantly different in primary and metastatic samples. Using optimum cut-off points, both uPA and uPAR were significant prognostic markers in breast cancer, including in patients with node-negative disease. However, uPA was a stronger indicator of both disease-free interval ($p = <0.005$) and overall survival ($p = <0.005$) than uPAR (disease-free interval $p = <0.05$; overall survival ($p = <0.025$). Our results are consistent with data from model systems suggesting that both uPA and its receptor are involved in cancer spread. We conclude that both uPA and uPAR are of prognostic value in breast cancer.

880 ORAL

Ductal carcinoma in situ of the breast, an evaluation of a new histopathological classification system

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Purpose: New histopathological classification systems for ductal carcinoma in situ of the breast (DCIS) have recently been suggested. We aimed to evaluate the reproducibility for the system proposed by Holland and co-workers and the correlation to the prognosis. The system is based upon cytonuclear differentiation and cellular polarisation. It divides DCIS into three categories, highly differentiated, intermediately- and low differentiated lesions (R1, R2 and R3 respectively).

Methods: The histopathological specimen from 195 consecutive women diagnosed with a primary DCIS 1986–1994 have been reclassified by two separate observers. The relapse-free survival in relation to the histopathological subgroup was calculated for patients treated with breast conserving surgery (BCS). The material was stratified for postoperative radiotherapy.

Findings: The distribution by histopathological subgroup was 7% (R1), 51% (R2) and 42% (R3) respectively. There was an interobserver agreement in 66% of the first 100 reviewed cases and in 93% for the thereafter reviewed 95 cases. There were 32 local recurrences among 149 patients treated with BCS, with a median follow-up time of 59 months. There were no distant recurrences or deaths in breast cancer. No recurrences occurred in the R1 group. The relapse-free survival did not differ appreciably between R2 and R3. This was true also after stratification for radiotherapy.

Conclusion: Holland's classification had a high reproducibility after a short learning period. One group with an excellent prognosis was easily discerned. Further classification into R2 and R3 groups did not help predict local recurrences.

881 ORAL

Immunoreactive detection of parathormone related protein (PTHrP) in primary breast cancers is a good prognostic factor for subsequent bone metastases

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Purpose: Bone metastases in breast cancer patients are frequent. In a prospective study we evaluated, whether tumor associated production of parathormone related protein (PTHrP) is a valuable prognostic factor for subsequent bone metastases.

Methods: Tumors of 216 patients were stained immunocytochemically with MoAb's against PTHrP. Median time of postoperative care was 5 years (6–134 months) including the most critical interval for metastasis.

Results: PTHrP was positive in 124 (56%) patients. After 5 years distant metastases were found in 63 (28%) women. This subcollective contained 42 (19%) cases of isolated bone metastases. 29 (69%) were positive and 13 (31%) were negative for detection of PTHrP. The calculated risk for metastatic bone disease was twice as high for the former patient group. Significant correlation was found between PTHrP and positive tumor cell detection in bone marrow ($P = 0.031$, Fisher's t-test), and between PTHrP and the chronological sequence of skeletal events ($P = 0.026$, Kaplan-Meier-analysis), too.

Conclusion: We could show that PTHrP is a specific prognostic factor, not for screening metastases in general, but to identify high-risk-patients for subsequent osseous metastases. PTHrP increases bone turnover and thereby chemotaxis on tumor cells. However, prophylactic treatment with bisphosphonates could be useful for these patients to reduce their risk of bone metastases.

882 ORAL

A multivariate analysis of tumour biologic factors predicting response to cytotoxic treatment in advanced breast cancer

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Purpose: To identify factors which could predict response to chemotherapy in breast cancer.

Methods: 173 patients with measurable or evaluable metastatic breast cancer were enrolled in a randomized trial between 11/87 and 1/91. The two groups of patients received the same monthly dose of 5-fluorouracil (500 mg/m^2), epirubicin (60 mg/m^2) and cyclophosphamide (500 mg/m^2) either on a weekly or monthly basis as first line cytotoxic treatment. In 103 evaluable patients we performed a multivariate analysis of the tumour biologic factors (grade, ER, PgR, SPF, ploidy, p 53, c-erbB-2, Bcl-2 and Bax) which showed significance in the univariate analysis according to treatment response, time to progression (TTP) or overall survival (OS).

Results: In the univariate analysis only S-phase fraction (SPF), grade and proapoptotic protein Bax showed statistically significant effect on response to cytotoxic treatment. In the multivariate analysis of these factors S-phase fraction had the strongest effect on response; thereafter grade and Bax.

In the univariate analysis grade, PgR, Bax and Bcl-2 had significant effect on TTP, while in the multivariate analysis only Pgr receptor showed statistically significant effect.

In the univariate analysis Pgr and Bax had effect on OS and both remained significant also in the multivariate analysis.

Conclusion: The factors which had significant effect on response to cytotoxic treatment in the univariate analysis i.e. grade, SPF and Bax seemed to be able to predict independently the response to treatment also in the multivariate analysis. Thus they might be of value for the clinician in the decision making of treatment for metastatic breast cancer. TTP could be predicted partly by the same factors i.e. grade, Pgr, Bax, Bcl-2 and OS by Pgr, Bax although the association was quite weak.