357 POSTER

Antibody-mediated cytotoxic therapy eradicates chemoresistant breast cancer

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Breast cancer is one of the most fatal cancers due to its extremely high chemoresistance. Highly metastatic breast cancer cell line extracted by the collagenase method from human malignant tissue has exhibited aberrations of the p53 tumour suppressor gene by PCR-SSCP analysis. Furthermore, PCR has exhibited high expression of MRP gene, low expression of bax oncogene and overexpression of the tyrosine kinase receptor erbB2 (HER-2/neu), which is a functional membrane protein, playing an important role in the pathogenesis of breast cancer. Monoclonal antibodies directed against c-erbB2 have been conjugated on the surface of liposomes. In the lipophilic bilayer of these immunoliposomes we entrap paclitaxel molecules and we incubate with the tumour cells. As controls we incubate tumour cells with empty liposomes. After incubation for the test samples we observe by PCR upregulation of bax oncogene. Furthermore, analysis by TEM exhibits morphological evidence of liposomal binding onto the cell surface, and subsequent endocytosis leading to lysosomes, where disintegration of liposomes occurs, releasing paclitaxel molecules into the cytoplasm, exerting their oncolytic action.

Furthermore, morphological apoptotic signs are observed, such as pyknotic nuclei, condensed and marginated chromatin of the nucleus forming distinct crescents, segregated nucleus, cell shrinkage, denser nucleus and cytoplasm, cytoplasmic megavacuolization and budding of cell surface, resulting in the formation of apoptotic bodies. Also, these results are confirmed biochemically, where metabolic activity is reduced according to MTT analysis and DNA synthesis is proportionally reduced according the BrdU analysis, compared to control samples.

Conclusion: We achieved to eradicate chemoresistant breast cancer cells by targeting specifically immunoliposomes, circumventing drug efflux pumps such as MRP, and protecting chemotherapeutic molecules from biological milieu interactions, such as HDL attach or albumin binding. Most importantly, this apoptotic induction is independent of p53 mutation, probably due to upregulation of bax-gene caused by paclitaxel.

358 POSTER

Frequency domain laser scanning mammography of the breast – First clinical evaluation study

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We recently demonstrated feasibilty and contrast features of frequency-domain laser scanning mammography (FLM). We now report the first clinical evaluation of this new diagnostic method.

The FLM instrument was equipped with two laser diodes, emitting at 690 nm and at 810 nm, modulated at 110,080 MHz and 110,100 MHz respectively. Breast scans were performed in a reproducible slab geometry in two perpendicular views. Amplitude and phase signals were measured by lock-in amplification and heterodyne detection for each pixel. Edge-effect corrected images (N-algorithm) were calculated based on both amplitude and phase signals. Positivity of FLM was judged by two independent reviewers, if correlating images were discernible on both views.

133 symptomatic patients were investigated, 92 of those (59 malignant 20 benign tumours, 13 mastopathy only) are evaluable for correlation between FLM and histological results. FLM revealed 2/9 non-invasive cancers, 37/50 invasive cancers, 3/20 benign tumours and 0/13 mastopathy lesions. 2/3 mammographically occult cancers were visualized. There was no correlation between FLM imaging contrast and radiological contrast, tumour grade, or age of the patient.

FLM provides independent and complementary information to conventional imaging. The reproducible geometry of the system will allow for further technological improvements as to sensitivity, i.e. by 3-dimensional reconstruction.

359 POSTER

Sequential adriamycin (A), docetaxel (D) and CMF in the adjuvant treatment (AT) of breast cancer (BC)

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In preparation for a phase III trial evaluating D and dose-intensity in the AT of BC, we studied the feasibility of these regimens: 1) non-accelerated (NA): A 75 mg/m^2 q 3 wks × 3 → D 100 mg/m^2 q 3 wks × 3 → CMF days 1, 8 q 4 wks × 3; 2) accelerated (ACC): A 75 mg/m^2 q 2 wks + lenograstim (G) × 3 → D 100 mg/m^2 q 2 wks + G × 3 → CMF (as NA). Radiotherapy was given during/after CMF. 48 pts are evaluable: 19 treated with NA and 29 with ACC. Pt characteristics are: median age 48 (29–66), stage II/III untreated BC 43/5. 164 and 165 cycles were delivered so far in NA and ACC, respectively. Median relative dose-intensity is 100% in both arms. Skin toxicity occurred in 62% of pts receiving ACC (17% G3–G4) and consisted of erythematous plaques in different body areas. Skin lesions appeared during treatment with A in 24% of cases.

Treatment (n° pts)	N° Pts Withdrawn			% Pts
	radiodermatitis	bone pain	skin toxicity	
NA (19)	1	–	–	5
ACC (29)		1	4	17

This study shows that: 1) ACC is not feasible as adjuvant treatment; 2) NA can be safely given at full doses. The phase III trial will start in June 1997.

360 PUBLICATION

Optimal duration of neoadjuvant chemotherapy: 3 or 4 versus 6 cycles in 3 schemes for operable breast cancer

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Most studies use 3 or 4 cycles of induction chemotherapy, in contrast with adjuvant therapy (most often 6 cycles). From 01/82 to 06/96, 283 patients (pts) (stage II and IIIa) received primary chemotherapy in our center with 3 different regimens (all doses mg/m^2): AVCF/AVCFM, 157 pts (adriamycin 30, vincristine 1 d1, cyclophosphamide 300, fluorouracil 400 d2–d5 and methotrexate 20 d2 and 4, every 28 d); NEM, 83 pts (navelbine 25, epirubicin 35, methotrexate 20 d1 and d8, every 28 d); TNCF, 43 pts (THP-adria 20, d1–d3, navelbine 25 d1 and d4, cyclophosphamide 300, fluorouracil 400 d1–d4, every 21 d). All patients were evaluated after 3 or 4 and 6 cycles by 3 methods: clinical, mammography and ultrasound. The clinical complete (CR) and overall (OR) response rates were:

	AVCF/AVCFM		NEM		TNCF	
Evaluation after	3	6	3/4	6	3/4	6 cycles
CR (%)	6	27^{-}	19	26N	41	58N
OR (%)	39	64^{-}	78	89•	88	90*

N $p < 0.05$, • $p < 0.01$, $^{-}$ $p < 0.001$, * non significative

Similar differences were found with the other 2 evaluations. In conclusion, a significative difference between 3 versus 6 cycles was observed with AVCF/AVCFM and NEM. For TNCF regimen, OR was already important after 3 cycles, but 6 cycles seem necessary to improve CR and therefore pathological complete response (ECCO 8, S13, 53).

361 PUBLICATION

COLLATE: A collaborative authoring tool for the development of clinical oncology guidelines

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ECOLE/GRIP (European Collaboration in Oncology Literature Evaluation/Getting Research Into Practice) is a European project whose goal is to develop a telematics infrastructure to support the development and dissemination of evidence based, quality assured Clinical Practice Guidelines in Oncology. The project involves National Cancer Organisations from

France, Germany, Italy and Netherlands, and is supported by experts in information retrieval and analysis, evaluation and software development.

The consortium is developing a tool (COLLATE), based on emerging information and communication technologies. COLLATE has been designed to achieve two main goals: (a) to support networks of clinicians collaborating on clinical guideline development (involving activities such as the collection, analysis and systematic review of evidence, and consensus forming); (b) to support the dissemination of guidelines and promote their use in clinical practice by making them available to clinicians over the internet.

The impact of COLLATE on guideline development and dissemination is being assessed initially in the diagnosis and treatment of breast cancer. Evaluation is concerning on such aspects as ease of use, the time taken to complete each step of the process, resources used and the quality of guidelines produced. COLLATE is to be demonstrated at the conference, and will be available for comment and discussion by attending clinicians.

Use of COLLATE is expected to produce benefits for clinicians and patients including reducing variations in clinical practice for similar conditions; promoting the appropriate use of medical interventions; speeding up the transfer of state-of-the-art clinical research results into daily practice; improving quality, consistency in clinical care.

362 PUBLICATION

Angiosarcoma of the breast after lumpectomy and radiotherapy for early breast cancer: The key to an early diagnosis

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Secondary angiosarcoma is a rare disease. In recent years, post-treatment angiosarcomas of the breast are described more often. Most reports deal with therapeutic options and bad prognosis. The prognosis is determined by early diagnosis which is often very difficult.

The cases reported in literature and 4 cases in our department are reviewed with special emphasis on diagnosis. The tumor characteristically presents as a painless mass in the breast often accompanied by blueish, reddish, purple or even black skin discoloration. This may be confused with mastitis carcinomatosa in an irradiated breast or with post radiotherapy sequellae. Mammography, ultrasound, fine needle aspiration cytology and MRI have a low diagnostic sensitivity. Incisional biopsy, including discolored skin and underlying tumor is the most accurate way for diagnosis.

Since the only curative treatment seems to be radical surgery, no valuable time should be lost with repeated and confusing diagnostic procedures. When the clinical picture is present, even though imaging does not provide further information, an incisional biopsy provides the fastest way to early diagnosis and treatment.

363 PUBLICATION

The effect of the interval between surgery and radiotherapy on local control and overall survival in patients with breast cancer

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Purpose: We analyzed the effect of the interval between surgery and radiotherapy (RT) on local control and overall survival in patients (pts.) with breast cancer.

Material and Method: 672 pts. who were treated with postoperative RT after mastectomy and axillary dissection were analyzed retrospectively. Median age was 48 (21–80) years. Postoperative RT was given to all of the pts.(46–65 Gy, Co60-Linak.) through peripheric lymphatic and chest wall portals. 56% of the pts. received RT and 44% RT+chemotherapy (CT). In 68 pts. FAC and in 230 pts. CMF regimens were used. In 196 pts CT was done after irradiation, and in 102 pts. sandwich method (CT + RT + CT) was used. Median follow up was 60 (36–196) months. RT was started in % 61 of the pts. within 8 weeks (group A), and in 39% of the pts. between 8–20 weeks (group B).

Results: Group A and B were similar according to menopausal status, lymph node metastasis and CT apply. T3 tumors were more in group B ($p < 0.05$). Five and ten year local disease free survivals were 89%, 84% in group A and 84%, 84% in group B. Five and ten year overall survivals were 67%, 51% in group A and 64%, 54% in group B. There were no significant differences in locoregional and distant recurrence incidence, locoregional disease free survival and overall survival between two groups.

Conclusion: The results suggest that administering RT. within 8–20 weeks after mastectomy may not effect local control and overall survival.

364 PUBLICATION

Modulation of tamoxifen adjuvant hormonal treatment according to first generation prognostic factors in 702 pT1–2/pN0–1(≤3)/M0 breast cancer, treated by surgery and external irradiation

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From 1982 to 1992, 702 women under 75 years, without any previous carcinoma, suffering from a unilateral invasive breast cancer classified as pT1–2/pN0–1/M0 were enrolled: 84.3% underwent a breast conservative treatment and 15.7% a modified radical mastectomy. There were 71.7% pT1, 28.3% pT2; 62.4% of the patients were menopaused. Histological axillary lymph node status, Scarff-Bloom and/or cytological grade, ER content were used to set up three groups of patients: 400 were pN0, grade 1–2, ER+ (group 1), 113 were pN0, grade 3, ER+ (group 2), 189 were pN+ ≤ 3, grade 1–2, ER+. Patients from the latter 2 groups received tamoxifen 20 mg/day/2 years, with a pelvic irradiation (12 Gy), should they be not menopaused. In the three groups, the median age is ranging from 51 to 59 (28–75) ($p = 0.07$) and the median participation time from 55 to 60 months (1–178) ($p = 0.47$). 5-year outcomes were assessed with Kaplan Meier method and log-rank test. Overall survival: 97/98/97% ($p = 0.39$), disease free survival: 85/90/91% ($p = 0.08$), local-regional free survival: 93/94/98% ($p = 0.01$), metastatic free survival: 90/93/92 (0.91). As compared to group 1, tamoxifen modifies significantly the outcomes of patients grade 3 or pN1 ≤ 3. Other results are detailed according to age, pT stages, menopausal status, treatment with a Cox multivariate analysis.

Biotherapy–gene therapy–vaccination

366 ORAL

Interleukin 12 supports immune responses towards human breast and ovarian carcinomas following initial CD80 mediated T cell activation

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Introduction/Purpose: One explanation for the poor immunogenicity of tumors is the induction of peripheral tolerance by tumor cells that fail to deliver costimulatory signals. Furthermore, T cells stimulated with wild type tumor cells often fail to secrete cytokines. The present study has been undertaken to identify cytokines that cooperate with CD80 in T cell activation towards human breast (MaCa) and ovarian carcinoma (OvCa) cell lines.

Methods/Results: We developed culture conditions enabling us to modulate immune responses towards CD80 transfected MaCa and OvCa by IL-7 and IL-12. IL-7 amplified the proliferative response towards CD80 transfected MaCa and OvCa but stimulated predominantly $CD4^+$ T lymphocytes. IL-12 represses the proliferative response of naive T cells in primary activations. However, during rechallenge stimulations IL-12 cooperates with CD80 mediated activation. In long-term T cell cultures IL-12 synergizes with CD80 expression in stimulation of $CD8^+$ T cell lines which recognize the parental MaCa line in a HLA-restricted manner.

Conclusion: We showed that CD80 expression is neccessary for tumor cells to function as (allo)antigen presenting cells. Following initial CD80 mediated activation IL-12 supports the propagation of $CD8^+$, HLA-restricted T lymphocytes. Tumor-reactive T cell lines would improve efforts aimed at identifing tumor specific antigens. In clinical approaches immunogenic tumor variants could be directly used for the vaccination of HLA-matched MaCa and OvCa patients.