in 11 pts and 1 in 1 pt. A total of 147 cycles of ND was administered. 24 pts were evaluable for efficacy with 6 CRs and 11 PRs leading to an overall response rate of 71%. All patients were treated with C after the completion of 6 cycles ND. A total of 130 cycles of C were delivered. 13 CRs and 7 PRs were observed for an overall response rate of 83%. Median time to progression and median survival were 28 and 33 months respectively. No Grade IV toxicity was observed during treatment with ND. Grade III neutropenia was observed in 3 pts (12%) with febrile neutropenia in 1 pt. Grade II-III anemia was seen in 3 & 1 pts. Most frequent non hematological toxicities were: nausea/vomiting, Grade I in 20 pts (80%) and Grade II in 5 pts (20%), Gr III alopecia in 23 pts (92%), nail disorder in 5 pts (20%), cutaneous erythema in 3 pts (12%) and oedema in 2 pts (8%). While on capecitabine, the toxicities were: Gr II HFS in 1 pt, Gr II anemia in 2 pts & Gr I neutropenia in 3 pts.

Conclusion: VD followed by C is an effective regimen as first line treatment of MBC with a favorable toxicity profile and very encouraging response rates.

414 Poster

Preliminary results of a Phase II study of low dose weekly paclitaxel (TXL) plus high dose tremifene (TOR) in patients with metastatic breast cancer (MBC)

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Background: No synergistic effect has been reported for the combination of chemotherapy and endocrine therapy. However, a recent *in vitro* report demonstrated that tremifene (TOR) inhibits the excretion of intracellular paclitaxel (TXL) due to P-glycoprotein (P-gp) expression in an anthracycline resistant cell line treated with TOR and TXL simultaneously. In this study, we report the results of a phase II study of low dose weekly TXL and high dose TOR as treatment for metastatic breast cancer (MBC) patients.

Patient and Methods: Eighteen patients were included in the study and treated as described. Eligibility criteria for inclusion in the study included ECOG PS 0–2 and adequate hematological, renal and hepatic function. The primary endpoint was the response rate (RR), and the secondary endpoint was toxicity. TXL (80 mg/m^2) was administrated by intravenous infusion over 60 minutes on days 1, 8, and 15 of a 28 day treatment cycle. Patients were subjected to at least 6 cycles. At the same time, TOR (120 mg/body) was administrated orally, once a day without a break. Prior chemotherapy regimens were as follows: oral 5-FU agents; 3 cases, anthracycline; 2 cases, taxanes; 1 case, and hormonal therapy; 1 case. The metastatic sites: bone 2, lung 5, brain 1, liver 3, lymph node 2 and others 5 cases. Hormone receptor (HR) status was positive in 8 cases, negative in 7 cases, and unknown in 3 cases. The average administration cycle was 6.2 cycles.

Results: Nine responders were observed (4 CR, 5 PR), so the response rate was 50.0% (95% CI 26.9–73.1%). Time to progression was 8.8 months. Only one case experienced grade 3 neutropenia. No cases showed withdrawal.

Discussion: Despite the small number of patients, our results show that weekly TXL administration in conjunction with high dose TOR may be an effective treatment for MBC patients. The recruitment of patients is ongoing and an updated report of response and analysis of P-gp expression as predictive factor will be presented.

415 Poster

In vitro models of breast cancer lymph node metastasis

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Introduction: Lymph node metastasis is a common feature of many cancers. It is associated with considerable morbidity and is often linked to poor prognosis. As part as the European Framework 6 Consortium METABRE, we used the parental cell line MDA-MB-435 and two metastatic sublines, LN1 and LV1, derived from lymph node and liver metastases respectively, as a model to study organ-specific metastasis and to compare the mechanisms of lymphatic and hematogenous metastasis. When injected into mammary fat pads of athymic mice, LN1 (but not LV1) produced spontaneous lymph nodes metastases, whereas when injected intravenously all three lines generated experimental lymph node metastases as well as lung metastases. These distinct patterns of spread – due respectively to direct (intralymphatic) and indirect (hematogenous) colonisation of nodes – will enable us to explore determinants of both mechanisms independently.

Methods: In an attempt to study the mechanism of lymph node metastasis *in vitro*, we initially compared the migration and invasion potential of these cell lines in Transwell® assays under different conditions. The filter inserts were either uncoated or coated with collagen IV, and the lower chamber contained either standard culture medium supplemented with 5% FCS, lymphatic endothelial cells (LECs) or LEC-conditioned medium.

Results: MDA-MB-435 parental cells and LV1 in all cases migrated more readily than LN1, suggesting that under these conditions, we were unable to detect any organotropism due to the presence of LECs or secreted products.

Future work: We are now exploring 3D models in which fluorescently tagged tumour cells are co-cultured with Matrigel® supplemented with fibroblasts above a layer of LECs, in order better to mimic the *in vivo* environment. Additionally, future studies will compare spontaneous and experimental lymph node metastasis from MDA-MB-435 cell lines and a second model (GI 101 and sublines) using gene expression microarrays, in order to explore the potential different mechanisms involved in these two routes of dissemination.

416 Poster

Pilot study of gemcitabine (G) plus trastuzumab (H) in metastatic breast cancer patients with erb-2 overexpression previously treated with anthracyclines (A) and taxanes (T)

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Trastuzumab and Gemcitabine have demonstrated a survival benefit in combination with other drugs in metastatic breast cancer (MBC) patients (pts). Preclinical data suggests a synergism between both agents. We performed this pilot study to evaluate the clinical benefit (CR+PR+SD) and safety of the GH combination in MBC pts.

Patients with histological confirmation of MBC previously treated with anthracyclines and taxanes, erb-2/neu overexpression (IHQ +++ or ++ and positive FISH), measurable disease (RECIST), age >18 years old, ECOG performance status ⩾ 2, left ventricular ejection fraction > 50%, and adequate bone marrow, renal and hepatic function were included in the study. Treatment consisted of gemcitabine 1200 mg/m^2 days 1, 8 every three weeks up to eight cycles. Trastuzumab was administered weekly at a dose of 2 mg/kg, with a loading dose of 4 mg/kg.

Seventeen pts were recruited.

The median age was 57.3 years old (range 35–72); ECOG PS 0–1, 82%; PS 2, 18%. Histology included ductal carcinoma (88%) and lobular carcinoma (6%). All patients received previously A and T; 15 pts received neo/adjuvant treatment, and 6 first line (4 of them received both). 59% of patients had visceral disease (47% in the liver and 23.5% in lung). Total number of cycles received were 86, with a median number of 5 cycles per patient (range 1–8). Median relative dose intensity for G was 95%. In terms of haematological toxicity per patient (N = 17): neutropenia G 3–4 (47%) without any febrile neutropenia, and anemia grade 3 (6%). Non-hematologic toxicity was generally mild with grade 3–4 fatigue and transaminase elevation in 17% and 13% of pts respectively. The clinical benefit rate (N = 15) was 59% (95% confidence interval (CI) 33–81.5), with 4 PR and 6 SD; six pts progressed during treatment.

These results reveal an encouraging activity and toxicity in a group of patients with an unfavorable prognosis. Further evaluation of this regimen is warranted.

417 Poster

Efficacy and tolerability of taxanes or vinorelbine chemotherapy with trastuzumab as a first combination in Her-2 overexpressing patients with metastatic breast cancer

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Background: Data from clinical trials showed that treatment with trastuzumab and chemotherapy in patients (pts) with Her-2 overexpressing metastatic breast cancer (mbc) significantly increases response rate, time to progression, duration response and reduces mortality in comparison with conventional chemotherapy. Preclinical data suggest synergistic antitumor activity beetwen either taxanes or vinorelbine with trastuzumab.

It is retospective analysis of efficacy and safety of taxanes or vinorelbine in first combination of trasuzumab in metastatic Her-2 overexpressed breast cancer pts.

Material and Methods: Forty pts with metastatic HER-2/neu overexpressed breast cancer were treated with weekly trastuzumab (4 mg/kg at initial dose and 2 mg/kg as maintenance dose) and: I – 17 pts with taxanes (docetaxel 100 mg/m^2 or paditaxel 175 mg/m^2) every three weeks or II – 23 pts weekly vinorelbine 25 mg/m^2. It was first combination of trastuzumab and chemotherapy in all pts, in group I – 8 pts were anthracydine pretreated because of mbc, in group II – 9 pts. Median age was I: 52 years (range: 32–64); II: 51 years (range:39–73). Median number of metastatic sites was 2 in both of these groups (r 1–4). All of the patients were evaluable for toxicities and tumor response.

Results: The objective response (OR) in taxanes and trastuzumab group was observed in 15 pts-88% (CR-5 pts, PR-10 pts), stable disease (SD) – in 2 pts 12%. Median TTP in

This group – 44 weeks. In vinorelbine and trastuzumab group: OR-14 pts (61%), SD-8 pts (35%), PD – 1 pt (4%); median TTP – 43 weeks.

There is no severe toxicites (including cardiac) in analyzed groups. Most frequent toxicites were in grade 1/2: fatigue, arthralgia, myalgia, nausea, peripheral neuropathy in group I and neutropenia, fatigue, neurotoxicity in group II.

Conclusion: This analysis showed that both taxanes and vinorelbine are effecive as first combination with trastuzumab in metastatic HER-2 overexpressed breast cancer in first line treatment or anthracycline pretreated patients. Objective response rate was better in group I, TTP are similar but there was no significant difference in these groups. Toxicities are rare and acceptable.

418 Poster

Fulvestrant: a new opportunity in advanced breast cancer (ABC)

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Introduction: Fulvestrant (Ful) is a new oestrogen receptor (ER) antagonist. It downregulates ER without agonistic effects and has established efficacy after tamoxifen (TAM) failure in post-menopausal women with ABC. Preclinical data suggests treatment with Ful may be useful also following failure with an aromatase inibitor (AI), but few clinical data are available.

Methods: We retrospectively evaluated all postmenopausal patients (pts) in 9 Italian hospitals who received Ful 250 mg/monthly, under a compassionate-use programme, from March 2001 until August 2005. The main inclusion criteria were ER+ and/or PgR+; pretreatment with at least two endocrine agents (HT) and measurable disease.

Results: A total of 127 pts were treated. Median age was 67 years (range 39–92). 80 pts (63%) were ER+ PgR+; 27 pts (21.3%) were ER+ PgR-. 79 pts (62.2%) were treated with adjuvant therapy, 31 pts only chemotherapy (CTH), 27 pts with CTH + TAM and 20 pts with only TAM (1 pt unknown); 22 pts were treated with anthracyclines ± taxanes in adjuvant therapy. Prior to commencing Ful for metastatic disease, 125 pts had received ⩾2 lines of HT; 46 pts, 13 pts and 3 pts received 3, 4, and 5 endocrine agents respectively. All pts were treated with AIs and 78 pts (61.5%) with TAM. 83 patients (65.4%) were treated with at least one CTH; 55, 40, 12 and 3 pts respectively with 2, 3, 4 and 5 lines of CTH. 89/127 pts (70%) were exposed to anthracyclines + taxanes for metastatic disease. Our group of women was extremely heavily pretreated in relation to their ABC. The characteristics of metastases were: median site number 2 (range 1–5), 47 pts one site, 41 pts two sites, 29 pts three, 7 pts four and 3 pts five. 63 pts had prevalent visceral metastases, 51 bone metastases and 12 soft tissue metastases (1 pt biochemistry progression). The patients were treated for a median 6.5 months (range 1–34+) with very few side effects (1 pulmonary embolism, 1 deep venous thrombosis, 1 rash, 2 nausea, 1 vomiting, 1 hypertension, 1 muscular pain, 1 gastric pain, 1 asthenia, 1 mucositis, 1 headache and 1 vertigo). We obtained 2 CR, 5 PR (15+, 4+, 22, 15+, 14+ months), 45 SD and 65 PD, giving a clinical benefit (CR+PR+SD) rate of 41% in evaluable pts (median TTP 6.5 months; range 2–34+). 10 pts are not yet evaluable for response.

Conclusion: The clinical benefit reached in such a heavily-pretreated group of women stresses the ability of Ful as a new and additional hormonotherapy in ABC.

419 Poster

Low incidence of cardiac events in an EORTC phase II study of CMF in combination with Trastuzumab in women with HER-2 positive metastatic breast cancer

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Objectives: Trastuzumab (T) combined with CT has significantly improved the time to progression, overall response rate and survival in metastatic breast cancer patients overexpressing HER-2 but with an increased risk of cardiac toxicity. This trial was designed to assess the incidence of the congestive heart failure and the therapeutic activity of T in combination with CMF.

Patients and Methods: Eligible patients with metastatic breast cancer, 1st or 2nd line, are enrolled in this single arm Phase II study of T IV weekly plus CMF, Bonadonna regimen, for a maximum of 8 cycles, followed by T alone. The cardiac monitoring includes a formal baseline measurement of LVEF before initiating T plus CMF therapy. Patients with abnormal cardiac function, high-doses of prior anthracycline (A) exposure, pre-existing heart disease and prior treatment with T were ineligible. Serial LVEF measurements are performed every 3 months during the study, and a patient experiencing a decline in LVEF of > 15% from baseline, or any decline in LVEF to > 5% below the lower limit of normal for the institution, regardless of symptoms, or who develops CHF was taken off protocol.

Results: The trial remains open but is expected to close to recruitment by March 2006. To date 66 patients have been treated with a median age of 55 (range, 31 to 75). 22 patients had prior exposure to A, 17 patients as adjuvant therapy and 5 for metastatic disease, combined with cyclophosphamide or taxanes. No NYHA grade 3 or 4 clinical CHF was observed. Only one grade 1 and one grade 2 CHF have been reported. The grade 1 occurred after 10 cycles (8 CMF+T, 2 T). The grade 2 occurred after 3 cycles of CMF in a patient who had had prior anthracyclines chemotherapy. The median baseline LVEF was 61% (range, 45% to 85%). In patients previously treated with A, nine have a drop of at least 6% during the combined treatment. The mean drop of LVEF observed was 2.65% (SD:6.22%) at 3 months and 3.15% (SD:6.19%) at 6 months.

Conclusion: There is a very low rate of cardiac events for patients treated with the combination of trastuzumab and CMF. Asymptomatic drops in LVEF occurred in patients irrespective of previous anthracycline exposure.

420 Poster

Redox regulation of Prx II and Prx III in breast cancer metastasis to lung

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Peroxiredoxins are a novel class of antioxidants thought to be of particular importance in H_2O_2-mediated redox signalling and are known to be differentially expressed in various cancers. Previous studies in our group with the MDA-MB-435 parental cell line and various metastatic sublines demonstrated that Prx II and Prx III are specifically over-expressed only in the lung metastatic variants. Since the lung provides an entry for ROS, redox regulation is of particular importance in the lung microenvironment and a higher expression of antioxidant proteins in breast cancer may be a crucial factor in metastasis to lung.

We analyzed expression and redox regulation of Prx II and Prx III in 435 parental cells (435-P) and a lung metastatic variant (435-L3) by western blotting and flow cytometry. Exposing cells to H_2O_2-mediated oxidative stress led to a further induction of Prx II and Prx III in 435-L3, while the expression levels of Prx II and Prx III in 435-P remained almost unchanged. Confocal microscopy furthermore revealed that treatment with H_2O_2 induced the cytosolic Prx II to translocate almost completely to the nucleus while Prx III remained in the mitochondria. Whether Prx II interacts directly with nucleic DNA or simply serves as a scavenger for H_2O_2 has to be further elucidated.

Moreover, 435-L3 cells showed higher resistance and a minor level of cell death when stressed with H_2O_2 compared to 435-P. Depletion of Prx II or Prx III by siRNA methods rendered 435-L3 cells more sensitive to H_2O_2-induced stress. Simultaneous down-regulation of Prx II and Prx III however