resistance including MDR1, MRP1 and tubulin mutation. Recently, two additional resistance mechanisms had emerged: overexpression of class III β tubulin (TUBB3) and breast cancer resistance protein (BCRP), a member of the ABC transporter family. We tested whether ixabepilone retains activity in tumor cell lines that overexpress these two resistance proteins.

Methods: Cancer cell lines overexpressing TUBB3 were evaluated in vivo in mice for sensitivity to ixabepilone, docetaxel and vinorelbine. These include DU4475 and PAT-21 breast (MDR1 negative), as well as H1155 and LX-1 lung cancer lines. BCRP overexpressing HEK293 cell line was studied in vitro for sensitivity to ixabepilone, paclitaxel and mitoxantrone.

Results: Efficacy evaluation in nude mice demonstrated that the 4 xenografts overexpressing TUBB3 were resistant to docetaxel and vinorelbine, yielding antitumor efficacy ranging 0.2−0.9 and 0.1−0.3 log cell kill (LCK), respectively. In contrast, ixabepilone was active in all 4 tumors, yielding 1.6−4.2 LCK (Table 1) when tested at their maximum tolerated doses (MTD). The BCRP overexpressing HEK293/BCRP cell line demonstrated resistance to paclitaxel and mitoxantrone by 9.8-fold (IC50 = 26.4 nM) and 4.1-fold (IC50 = 8.7 nM), respectively, in comparison with the vector-transfected control line. This resistance can be reversed by fumitremorgin C, a selective inhibitor of BCRP. In contrast, ixabepilone was far less susceptible to the BCRP-mediated resistance, resulting in a resistance factor of only 1.9-fold (IC50 = 4.1 nM).

Conclusion: Ixabepilone demonstrated reduced susceptibility to multiple resistance mechanisms affecting agents commonly used in breast cancer. These include overexpression of TUBB3, BCRP, MDR1 and MRP1, and β-tubulin mutations. Together, these results suggest ixabepilone may offer breast cancer patients a potentially valuable treatment option.

Table 1. Comparison of the antitumor efficacy of ixabepilone, docetaxel and vinorelbine in 4 human tumor xenografts overexpressing TUBB3

	Antitumor efficacy (Log Cell Kill)		
Tumors	Ixabepilone	Docetaxel	Vinorelbine
H1155	4.2	0.2	0.1
DU4475	2.6	0.9	0.2
Pat-21	1.6	0.3	0.3
LX-1	2.6	0.5	0.1

576 Poster

Possible targets for dasatinib sensitivity in triple negative breast cancer

D. Tryfonopoulos[1], B. Corkery[1], N. O'Donovan[2], M. Clynes[2], J. Crown[1]. [1]*St Vincents University Hospital and National Institute of Cellular Biotechnology, Dublin City University, Dublin, Ireland;* [2]*National Institute of Cellular Biotechnology, Dublin City University, Dublin, Ireland*

Background: Triple-negative breast cancers (TNBCs) lack expression of oestrogen, progesterone, and HER-2 receptors. At present there is no specific targeted therapy for this sub-type of breast cancer. The multi-target kinase inhibitor, dasatinib, has shown promising results in inhibiting growth of triple negative breast cancer cells in vitro. To identify the specific target or targets which are responsible for sensitivity to dasatinib we have compared sensitivity to imatinib, sunitinib and dasatinib in triple negative breast cancer cell lines.

Materials and Methods: Imatinib, sunitinib and dasatinib were tested in TNBC cell lines (MDA-MB-231, BT20, HCC1937) using the acid phosphatase proliferation assay. IC50 values were determined using CalcuSyn software.

Results: The TNBC cell lines displayed the greatest resistance to imatinib, which targets Bcr-Abl, PDGFR and c-Kit (Table). The TNBC cell lines showed greater sensitivity to sunitinib, although still in the 6−10 μM range. Sunitinib targets PDGFR, VEGFR, c-Kit, FLT3, CSF-1R, and RET. As previously reported, the TNBC cell lines display significant sensitivity to the multi-target kinase inhibitor dasatinib, which targets Src, Abl, PDGFR, Kit, and EphA receptors.

IC50 values for multi-target kinase inhibitors in TNBC

	MDA-MB-231	BT-20	HCC-1937
Imatinib (μM)	23.6±2.0	32.6±3.6	27.3±2.0
Sunitinib (μM)	6.7±1.4	9.3±2.5	9.1±1.8
Dasatinib (μM)	0.04±0.01	2.5±0.6	0.13±0.07

Conclusions: TNBC cells are sensitive to dasatinib and based on response to other multi-target kinase inhibitors with overlapping target specificities, our results suggest that sensitivity to dasatinib in triple negative breast cancer is due to inhibition of Src kinase and/or EphA receptors.

577 Poster

Combination of nab®-paclitaxel and bevacizumab eradicates large orthotopic breast tumors and metastasis to lymph nodes and lungs

S. Ran[1], L. Volk[1], C. Bivens[1], V. Trieu[2], N. Desai[2]. [1]*Southern Illinois University School of Medicine, School of Medicine, Springfield IL, USA;* [2]*Abraxis BioScience LLC, R&D, Los Angeles CA, USA*

Background: *Nab*-paclitaxel (Abraxane®, *nab*-pac) is an albumin-bound 130-nm particle form of paclitaxel that demonstrated greater efficacy and was well tolerated compared to solvent-based paclitaxel (Taxol®) and docetaxel (Taxotere®) in preclinical and clinical studies. We have previously shown that reactionary angiogenesis induced by chemotherapy correlated with increased VEGF production in tumors, and the combination of *nab*-pac and anti-VEGF-A antibody (bevacizumab, bev) has superior efficacy against both primary tumors and metastasis than monotherapies in medium-sized MDA-MB-231 tumors (~230 mm^3) (Ran et al., AACR 2007, #2201). Herein, we studied the combination of *nab*-pac and bev on the growth and metastasis of large-sized (450–600 mm^3) breast tumors.

Materials and Methods: Luciferase-tagged MDA-MB-231-Luc$^+$ human breast carcinoma cells were implanted into mammary fat pads of *nu/nu* mice and allowed to reach an size of 450–600 mm^3, before treatment with *nab*-pac at 10 or 30 mg/kg on the qd×5 schedule for 1, 2 or 3 cycles separated by one week. Bev (4 mg/kg) was administered either concurrently with or after *nab*-pac treatment; and either continued for the duration of the experiment or discontinued after cessation of *nab*-pac therapy. Primary tumor growth was monitored and metastases to lymph nodes and lungs analyzed by monitoring luciferase activity.

Results: Complete regressions and total elimination of metastasis were achieved in 100% of mice bearing large orthotopic MDA-MB-231-Luc$^+$ tumors after treatment with 2 cycles of concurrent 30 mg/kg *nab*-pac and 4 mg/kg bev. Three cycles of combined therapy with 10 mg/kg *nab*-pac resulted in 80% regressions and 98% reduction in metastatic burden. Bev effect was optimal when administered concurrently with *nab*-pac and continued for the duration of the experiment. Bev administered sequentially after *nab*-pac delivered no benefits of the combined therapy.

Conclusions: Repetitive cycles of *nab*-paclitaxel given concurrently with bevacizumab at 4 mg/kg can eradicate both large primary tumors (450–600 mm^3) and lymphatic and pulmonary metastases in an aggressive breast cancer xenograft model. The suppression of paclitaxel-induced reactionary angiogenesis by bevacizumab can significantly enhance the antitumor and antimetastatic efficacy of Abraxane (*nab*-paclitaxel).

578 Poster

First-line trastuzumab (H), oral vinorelbine (NVBo) and capecitabine (X) combination therapy for HER2-positive metastatic breast cancer (MBC): efficacy and safety in a multinational phase II study

A. Chan[1], V. Ganju[2], D. Becquart[3], P.F. Conte[4], L. Petruzelka[5], L. Goedhals[6], M. Gil Gil[7], G. Bernardo[8], G. Villanova[9], N. Tubiana-Mathieu[10]. [1]*Mount Hospital, Medical Oncology, Perth, Australia;* [2]*Frankston Hospital, Medical Oncology, Frankston, Australia;* [3]*AZ Middelheim, Medical Oncology, Antwerp, Belgium;* [4]*Policlinico di Modena, Medical Oncology, Modena, Italy;* [5]*General Teaching Hospital, Medical Oncology, Prague, Czech Republic;* [6]*National Hospital, Medical Oncology, Bloemfontein, South Africa;* [7]*ICO – Hospital Duran i Reynals, Medical Oncology, L'Hospitalet de Llobregat, Spain;* [8]*Fondazione Maugeri, Medical Oncology, Pavia, Italy;* [9]*Institut de Recherche Pierre Fabre, Clinical Research, Boulogne-Billancourt, France;* [10]*CHU Dupuytren, Medical Oncology, Limoges, France*

Background: In HER2-positive MBC, H combined with chemotherapy has shown high efficacy. In HER2-negative MBC, doublet combinations of NVBo and X are active and well tolerated. Therefore we evaluated a triple combination (NVBo+X+H) as first-line therapy for HER2-positive MBC.

Methods: Key eligibility criteria for this multicentre, single-arm trial were: IHC 3+ or FISH+ disease, documented measurable MBC with no previous chemotherapy exposure, relapse >6 months after completing (neo)adjuvant chemotherapy, Karnofsky PS greater than or equal to 70, age greater than or equal to18 years. Each 3-week cycle consisted of NVBo 80 mg/m^2 (first cycle at 60 mg/m^2 in the absence of G3/4 neutropenia) days 1 and 8;

X 1000 mg/m^2 bid (750 if greater than or equal to 65 years) days 1−14; H 4 mg/kg day 1 as a loading dose then 2 mg/kg i.v. weekly starting on day 8. Treatment was continued until progression or unacceptable toxicity.

Results: Fifty patients (pts) have been treated. At baseline median age was 53.5 years (18% greater than or equal to 65); 54% had received chemotherapy for early breast cancer, including anthracycline + taxane in 16%, anthracycline without taxane in 30%, and taxane without anthracycline in 2%. The majority of pts (82%) had visceral involvement. Median number of cycles given was 9. The median relative dose intensities were: H 96%, NVBo 75%, X 77%. NVBo dose was escalated to 80 mg/m^2 in 87% of pts. The objective response rate (RR) in 46 evaluable pts was 74%, including complete response in 13%. Disease was stabilised in a further 20%. Subpopulation analysis showed an 85% RR in 20 chemonaive pts, a 68% RR in 38 pts with visceral metastases, and a 65% RR in 26 pts with liver metastases. After median follow-up of 17.6 months, median progression-free and overall survival have not been reached. Treatment is ongoing in 12 pts. The regimen was well tolerated. NCI CTC v2 G3/4 adverse events were: neutropenia 69% of pts, hand-foot syndrome (G3 only) 18%, diarrhoea 16%, vomiting 12%, febrile neutropenia 8%, fatigue 8%, infection with G3/4 neutropenia 4%, LVEF decline 4%, stomatitis 4%.

Conclusion: The combination of NVBo + X + H is a very effective and well-tolerated first-line treatment for HER2-positive MBC.

579 Poster

Inhibition of angiogenesis and breast cancer progression in vivo by an N-terminal 80 kDa recombinant fragment of human thrombospondin-2

T. Hawighorst[1], M. Koch[2], F. Hussein[1], A. Woeste[2], G. Emons[1].
[1]*Georg-August University Göttingen, Obstetrics and Gynecology, Göttingen, Germany;* [2]*Medical School Cologne, Institute for Biochemistry, Cologne, Germany*

Background: Thrombospondin-2 (TSP-2) is an endogenous angiogenesis inhibitor. During multistage carcinogenesis tumor development and angiogenesis is enhanced in TSP-2 deficient mice. In addition, stable overexpression of the TSP-2 gene inhibited the tumor growth and angiogenesis of human squamous cell carcinoma xenotransplants. Here we investigated the potential antitumoral efficacy of systemic TSP-2 therapy in breast cancer.

Method: We expressed a recombinant 80 kDa fragment of human TSP-2 (NTF-TSP-2), encompassing the N-terminal globular region through the three type 1 repeats, in human kidney 293 EBNA cells, using a modified pCEP4 expression vector. Breast cancer growth was investigated using an established xenotransplantation model of MDA-MB-453 breast cancer cells. Lymph node and lung metastasis were analyzed by quantification of human Alu sequences by real-time PCR.

Results: Daily intraperitoneal injections of NTF-TSP-2 resulted in a significant inhibition of the growth of human MDA-MB-435 breast carcinoma cells in vivo and tumor angiogenesis was significantly reduced. In mice systemically treated with NTF-TSP-2 both lymph node as well as lung metastasis were significantly reduced. NTF-TSP-2 inhibited vascular endothelial growth factor induced tube formation of human dermal microvascular endothelial cells on Matrigel in vitro. Moreover, NTF-TSP-2 potently induced human dermal microvascular endothelial cell apoptosis in vitro.

Conclusions: These data identify NTF-TSP-2 as a potent systemic inhibitor of tumor growth and metastasis, acting by inhibition of angiogenesis.

580 Poster

Continued trastuzumab therapy for patients with HER2-positive breast cancer

F. Haslbauer[1]. [1]*Federal Hospital Vöcklabruck, Department of Internal Medicine Oncology, Vöcklabruck, Austria*

Background: Patients (pts) with HER2-positive breast cancer (BC) are at increased risk of brain metastases (BM). This may be a biological characteristic of HER2-positive BC and/or may result from significantly longer survival in these pts as use of trastuzumab (H) has become more widespread. To explore the possible benefits of continued H in pts with BM, we reviewed cases from our clinic.

Patients and Methods: We identified 12 pts with HER2-positive BC with BM who received H for early or metastatic BC.

Results: Median age was 49 yrs (range 33−65). All had HER2 overexpression (IHC 3+ or FISH positive). Tumor stage was 2 in 9 pts and 4 in 3 pts at first diagnosis of BC. All received H + taxane (paclitaxel n = 11); H was also given with vinorelbine (n = 2), carboplatin (n = 3), capecitabine (n = 4), liposomal doxorubicin (n = 4) and letrozole (n = 1) and as monotherapy in 6 pts. First administration of H was for early BC in 3 pts (neoadjuvant 2, adjuvant 1) and for metastatic BC in 9 (1st-line 6, 3rd-line 1, 5th-line 1, 6th-line 1). All pts showed complete or partial response to H-containing therapy. Whole brain radiotherapy (WBRT) was given in 11 pts after development of BM. H is ongoing in 4 pts. Median overall survival (OS) from diagnosis of MBC is 32.5 months. Median OS from diagnosis of BM is 11.5 months.

	OS from diagnosis of BM, median (range)
No. of H-containing regimens	
2 (n = 6)	9.5+ (3–13)
3 (n = 3)	19+ (2–22)
⩾5 (n = 3)	31+ (6–48+)
Setting of first H-containing regimen	
Neoadjuvant (n = 2)	(6+–31+)
Adjuvant (n = 1)	48+
Metastatic (n = 9	11 (2–22)
Treatment after diagnosis of BM	
H alone (n = 3)	8+ (5–12)
H and a single line of chemo (n = 5)	11 (2–19+)
H and multiple lines of chemo (n = 4)	26.5 (6+–48+)

Conclusions: Pts with HER2-positive BC with BM are a heterogeneous population and analysis of such pts is difficult. However, this series suggests that it is appropriate to continue H therapy in pts with evidence of BM. Our data are consistent with those of Bartsch et al. (J Neurooncol 2007), who reported improved OS if H was continued after WBRT for BM compared with historical controls, and Nam et al. (SABCS 2007, #4061), who reported prolonged OS in pts receiving H after the onset of BM.

581 Poster

Continuation of trastuzumab-based therapy beyond disease progression in metastatic breast cancer patients – a retrospective one center analysis

P. Tokajuk[1], B. Czartoryska-Arlukowicz[1], M.Z. Wojtukiewicz[2].
[1]*Comprehensive Cancer Center, Clinical Oncology, Bialystok, Poland;* [2]*Medical University, Clinical Oncology, Bialystok, Poland*

Background: Clinical value of the continuation of trastuzumab-based (TZB) therapy beyond disease progression in HER-2-overexpressing metastatic breast cancer (MBC) patients (pts) still remains uncertain.

Methods: A retrospective analysis was performed to assess the effectiveness of TZB therapy for MBC pts treated in our institution from 2002 to 2007 outside clinical trials.

Results: A total of 46 pts were evaluated. Median age: 48.5 years (range, 33−62). 16 pts (34.7%) were premenopausal. Hormonal receptors status: 18 pts ER(+), 2 pts PgR(+), 2 unknown. HER-2 overexpression was determined by IHC staining (3+ score) in all pts. Metastases location: 31 pts soft tissues/bones, 31 pts visceral disease. Median number of metastatic sites: 2 (range: 1−4). 29 pts (63%) had metastases in ⩾2 locations. 27 pts (58.7%) received neo/adjuvant chemotherapy: 19 pts doxorubicin (FAC or AC), 11 pts CMF, 3 pts docetaxel (AT), 7 pts other protocol. Median previous chemotherapy lines for advanced disease: 1 (range: 0−6). 25 pts received doxorubicin/epirubicin, 19 pts docetaxel, 12 pts vinorelbine as a part of advanced disease chemotherapy. Trastuzumab was administered at standard doses and combined with docetaxel, vinorelbine, cisplatin, capecitabine, etoposide, gemcitabine or administered as monotherapy. Response for the first-line TZB therapy was as follows: CR 7/46 pts (15.2%); PR 20/46 pts (43.5%). Median TTP was 7.0 months (range: 0−46). 33/46 pts (72%) received a second-line TZB therapy beyond disease progression. Response for the second-line therapy: CR 2/33 pts (6%); PR 15/33 pts (45.5%). Median TTP was 4.6 months (range: 0−44). 6/33 pts received a third-line and subsequent lines (up to six lines) of TZB therapy. PR for subsequent lines of therapy was observed in 4 pts. Median survival has not been reached. Pts who received ⩾2 of TZB regimens survived significantly longer than pts who had received only 1 regimen (P = 0.004 logrank). Pts with metastases in 1 location survived significantly longer than pts with metastases in ⩾2 sites (P = 0.02 logrank).

Conclusions: Continuation of trastuzumab-based therapy beyond disease progression seems to be effective in a large proportion of HER-2-overexpressing metastatic breast cancer patients, producing long-lasting responses in some of them.