No serious renal disorders or ONJ cases were reported during this time frame. Overall, the incidence of adverse events was not different between the two arms.
Conclusion: This geographically diverse study confirms the effectiveness of the zoledronic acid to prevent AIBL as documented in the North American Z-FAST study and the rest of world study, ZO-FAST.

2009 POSTER

Safety of the combination of lapatinib (L) plus trastuzumab (T) in patients (pts) with HER2-Positive (+) metastatic breast cancer (MBC)

A. Storniolo[1], M. Koehler[2], A. Preston[2], E. Rappold[2], J. Byrne[2], S. Stein[2], M. Ewer[3]. *[1]Indiana University Medical Center, Oncology, Indianapolis IN, USA; [2]GlaxoSmithKline, Oncology Medicine Development Center, Collegeville, USA; [3]MD Anderson Cancer Center, Oncology, Houston, USA*

Background: L, an oral, dual EGFR/HER2 tyrosine kinase inhibitor, and T, a humanized anti-HER2 antibody, are approved in HER2+ MBC. Based on preclinical synergy and different mechanisms of action, L+T was studied in HER2+ MBC. Data from 4 trials were analyzed to assess safety of L+T.
Methods: From July 2003 to Mar 2007, 393 women of median age 51 years (range: 22−81 years) with HER2+ MBC received L±T (n = 351) or L+T+ paclitaxel or docetaxel (n = 42). L dose range: 500–1500 mg/day; 297 pts received ⩾1000 mg. T dose: 2 mg/kg/week. Drug-related adverse events (AE) graded by NCI CTCAE were analyzed. Cardiac function (LVEF) was assessed at screening, 8 weekly after starting L+T, and at withdrawal via MUGA or echocardiogram. Rate of symptomatic cardiac events (CE; CTCAE Grade 3/4 LV systolic dysfunction) or asymptomatic LVEF decreases (⩾20% relative to baseline and below institution's lower limit of normal) were assessed.
Results: Common drug-related AEs were diarrhea (53%), rash (25%), nausea (24%), fatigue (19%), and vomiting (13%). Maximum grade (G) reported by most was G1 or G2. G3 AE rate was ⩽3% except diarrhea in 12% (including G4 in <1%). Eight pts had single asymptomatic LVEF decreases, 2 had 2 asymptomatic decreases, and 2 (0.5%) had symptomatic CEs, totaling 14 decreases in 12 (3.1%) pts. Pts received prior T± anthracyclines (A; n = 8), A (n = 2), or unknown therapy (n = 2). For asymptomatic events, mean baseline and nadir LVEFs were 65.3% (range: 58−74%) and 46.1% (range: 42.5−51%), respectively. Mean absolute decrease was 19.4% points (range: 13−29%). Median time to onset and duration of LVEF decrease was 55 (range: 18–282) and 9 days (range: 4–113), respectively. L+T was interrupted in 4 pts and continued in 4 despite LVEF decrease. Two events occurred after L was discontinued. Asymptomatic LVEF decrease resolved without sequelae in 7 pts, unresolved in 2, ongoing at death (disease progression) in 1. Two pts had symptomatic CEs (LVEF 58% to 25% and 69% to 25%) after 365 and 42 days of L+T, respectively. L+T was discontinued in both; 1 recovered after 17 days and 1 died (cardiac insufficiency/pulmonary thromboembolism).
Conclusion: Preliminary data indicate L+T was well tolerated in pts with HER2+ MBC. Rates of drug-related AEs were consistent with those reported for L and T alone. Combined HER2 inhibition with L+T does not unexpectedly increase the risk of CE. L+T is currently being studied in neoadjuvant and adjuvant trials in HER2+ BC.

2010 POSTER

Comparative analysis of circulating tumor cells (CTCs) in peripheral blood and disseminated tumor cells in the bone marrow (DTC-BM) of breast cancer patients

C. Schindlbeck, B. Rack, J. Jückstock, W. Janni, H. Sommer, K. Friese. *Ludwig-Maximilians-University, 1st Dept. Obst. Gynecol., Munich, Germany*

Background: The detection of Disseminated Tumor Cells in the Bone Marrow (DTC-BM) of breast cancer patients is an independent prognostic factor in all stages of the disease. As less invasive procedure the analysis of Circulating Tumor Cells (CTCs) in Peripheral Blood (PB) could be an alternative especially for repeated follow up examinations. Automated systems und molecular methods (PCR) could increase sensitivity and offer the possibility of further characterizations of those cells.
Methods: BM aspiration and blood draw is performed simultaneously. Immunocytochemical examination of DTC-BM with the anti-Cytokeratin (CK) antibody A45B/B3 follows a standardized protocol. Analysis of PB (7.5 ml) for the presence of CTCs is performed with the CellTracks Analyzer system (Veridex, NJ, USA). After immunomagnetic enrichment by anti-Epcam antibodies CTCs are stained against CK, CD 45, and, optionally, HER2 by immunofluorescence. Positive events are recognized automatedly and presented on a screen for evaluation.
Results: Up to now, comparison of BM and PB of 44 patients could be performed. DTC-BM and CTCs in PB were detected in 15/44 (34%) cases each. Overall congruence of positive and negative findings was 68% (p = 0.05). 32 pts were examined at primary diagnosis. Of those, 19 (59%) showed both negative BM and PB, 6 (19%) DTC-BM (1−11) with negative PB, 6 (19%) CTCs (2–123) with negative BM, and 1 (3%) both. Patients with presence of CTCs at primary diagnosis tended to have higher tumor stage (T2-T4), Grading 2/3, 4 presented with lymph node metastases. Of 6 patients at recurrence free follow up examination, 3 had both positive BM and PB and 3 both negative status (100% congruence). Of the 6 pts with distant metastases, 5 showed DTC-BM (1–>1000) and 5 CTCs (2−77), all 4 patients with visceral metastases both.
Conclusion: If our results can be confirmed in a larger series, examination of CTCs in PB could add valuable information and allow monitoring of the disease during follow up. Further characterization of CTCs might enable risk stratification and application of targeted therapies. Aim of our ongoing research is the detection and characterization of CTCs by rt-PCR for tumor specific mRNA.

2011 POSTER

Serum BCL-2 and VEGF in women with breast cancer – can they detect the recurrence before CEA and CA15−3?

R. Iosifidou[1], G. Galaktidou[2], F. Patakiouta[3], A. Bousoulegas[4]. *[1]Anticancer Hospital Theageneio, 3rd surgical clinic, Thessaloniki, Greece; [2]Anticancer Hospital Theageneio, clinical research, Thessaloniki, Greece; [3]Anticancer Hospital Theageneio, pathology, Thessaloniki, Greece; [4]Anticancer Hospital Theageneio, 3rd surgical clinic, Thessaloniki, Greece*

Purpose: The purpose of our study is to investigate if serum VEGF and bcl-2 can be used as prognostic factors during the follow-up of the patients with breast cancer and to compare these two factors with CEA and CA15−3.
Patients and Methods: 200 patients with breast cancer stage I and II are enrolled in our study. The mean age of the patients was 59.65±11.65 years. 102 patients had quadrectomy and axillary lymph node dissection and 98 had mastectomy. After the surgical treatment they had supplementary therapy. The size of the tumor was <2 cm in 105 patients and >2 cm in 95 patients. The histological type was ductal carcinoma in 169 patients, lobular in 10 and DCIS in 2 patients. 54 patients had <3 lymph node positive, 46 had >3 positive lymph node and 100 had negative lymph node. 14 patients had recurrence of the disease after the 18 months of the surgical treatment. We measured serum VEGF and bcl-2 before and after the operation and the first and second year of their follow-up with ELISA. CEA and CA15−3 were measured every 4 months after the surgical treatment until the two years. The results have been analysed with curves ROC and Pearson method to find if VEGF and bcl-2 can be used during the follow up of the patients to investigate the recurrence of the disease before the clinical appearance. Also we examine if they can detect the recurrence earlier of CEA and CA15−3.
Results: After the analysis with ROC curves we found that bcl-2 can detect the recurrence of breast cancer preoperative (p = 0.066) and also postoperative (p = 0.037) and at second year (p = 0.029) of the follow-up of the patients. VEGF can detect the recurrence after the operation (p = 0.003). On the other side CEA can detect the recurrence in 20 months after the operation (p = 0.098) and CA15−3 in 8 (p = 0.045). There was no correlation with the size of the tumor, the histological type and the lymph node status.
Conclusion: These results shows that bcl-2 and VEGF in serum can be used in the follow-up of the patients with breast cancer as they can detect the recurrence of the disease much earlier of the clinical appearance. CEA and CA15−3 can also detect the recurrence before the clinical appearance but later of the other two factors. Serum bcl-2 is the most significant factor as it can detect the recurrence in three measurements.

Poster presentations (Mon, 24 Sep, 14.00−17.00)
Breast cancer – pre-clinical science

2012 POSTER

Activity of capecitabine (C) and docetaxel (D) doublets with and without trastuzumab (T) in a breast cancer xenograft model

C. Moisa[1], K. Kolinsky[2], Y.E. Zhang[3], U. Dugan[4], K. Packman[2], B. Higgins[2]. *[1]Roche Labs Inc., Medical Affairs Oncology, Nj, USA; [2]Hoffmann-La Roche, Discovery Oncology, Nj, USA; [3]Hoffmann-La Roche, Pharmaceutical & Analytical R& D, Nj, USA; [4]Hoffmann-La Roche, Medical Affairs Oncology, Nj, USA*

Background: In the setting of pretreated metastatic breast cancer, C is highly active, well tolerated, and extends survival when D is added to C. Preclinical data on C+D doublets ± T, a humanized monoclonal antibody

against HER2-neu, using a KPL-4 human breast cancer xenograft model (estrogen receptor-negative, HER2+) are presented.
Materials and Methods: The antitumor activity of optimal dose (OD) and $\frac{1}{2}$ OD C and D monotherapy was evaluated along with $\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D or $\frac{1}{2}$ OD C + OD D. Since the results showed that $\frac{1}{2}$ OD C + OD D was toxic, OD C + OD D was not considered further. Both OD C + $\frac{1}{2}$ OD D and $\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D, the optimal doublet from the initial study, were tested $\pm$ T in this HER2+ model.
Results: The initial investigation found that the tumor response (TR) and increased life span (ILS) were significantly better for $\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D than for $\frac{1}{2}$ OD C, OD C, $\frac{1}{2}$ OD D, or OD D. Subsequent investigation found that TR and ILS were not significantly different for the $\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D and OD C + $\frac{1}{2}$ OD D doublets; they were, however, better with the addition of T to each doublet. In comparing triplicates, TR was not statistically different, but survival was significantly better for the OD C + $\frac{1}{2}$ OD D + T group. At day 253, there were 1/10 complete responders (CRs) in the $\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D + T group (ILS = 267%) vs. 6/10 CRs in the OD C + $\frac{1}{2}$ OD D + T group (ILS > 837%, ongoing).

Treatment vs	Treatment	P (TGI)	P (ILS)
$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D	$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D + T	0.021	0.0124
OD C + $\frac{1}{2}$ OD D	OD C + $\frac{1}{2}$ OD D + T	0.038	<0.0051
$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D	OD C + $\frac{1}{2}$ OD D + T	0.002	<0.0016
OD C + $\frac{1}{2}$ OD D	$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D + T	0.273	0.0645
$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D + T	OD C + $\frac{1}{2}$ OD D + T	0.241	<0.0237
$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D	OD C + $\frac{1}{2}$ OD D	0.064	0.2052

OD C = 400 mg/kg qd $\times$ 14; $\frac{1}{2}$ OD D = 10 mg/kg qweek $\times$ 3; T = 20 mg/kg 2x/week x 6. TGI = tumor growth inhibition.

Conclusions: The addition of T to non-toxic CD doublets increases TR and ILS. Results to date support the use of the most dense dose of C in triplicate combinations for sustaining CRs. Based on these results, the clinical testing of CD doublets with or without T in the neoadjuvant setting in HER2-negative and -positive breast cancer patients, respectively, is ongoing.

2013 POSTER

Bone marrow-derived TNF-a promotes tumour growth in a spontaneous model of mammary carcinogenesis

C. Chiodoni, S. Sangaletti, M. Parenza, C. Ratti, M.P. Colombo. *Istituto Nazionale Tumori, Experimental. Immunotherapy and gene therapy, Milano, Italy*

Most solid tumors are composed by tumor cells sourrounded by infiltrating stromal cells, including immune cells and blood vessel cells, which play a key role in tumor development and progression. Cancer cells and infiltrating inflammatory cells communicate through a complex network of pro-inflammatory molecules, many of them still unknown. Recent evidences have highlighted a critical role for the transcription factor NF-κB and for the inflammatory mediator TNF-a in such multifaceted interaction that leads to cancer progression, in some tumor types.On this line, we are investigating the role of TNF-a in mammary carcinogenesis. Treatment with neutralizing Ab to TNF-a of mice injected s.c. with the mammary carcinoma cell line N2C, greatly reduces tumor growth; tumors grown in depleted mice show a less organized stroma and vasculature, with reduction of collagen type IV. To further study TNF-a role, we used the MMTV-HER-2/neuT transgenic mice, which, because of the expression of the mutated rat neu oncogene under the the MMTV promoter, spontaneously develop mammary carcinomas.Bone-marrow transplantation (BMT) experiments from TNF-a KO mice into NeuT significatively delay the onset and reduce mammary tumor growth, indicating a relevant role of TNF produced by cells of BM origin, likely macrophages. Performing BMT at different time points during tumor progression (8, 15, 20 weeks of age) indicates that TNF-a, differently form other models such as skin carcinogenesis where its role is mainly relevant for tumor initiation/promotion, is critical not only in the early steps of the carcinogenic process, but also at later time points when evident carcinomas in situ are already present. Whole mount analysis of mammary glands confirms the less sever tumor phenotype of mice transplanted with TNF-a KO BM in comparison with animals that have received wild type BM. Experiments with mice KO for TNF receptors are planned to identify the cellular target for TNF-a action and to further elucidate the mechanisms of its tumor-promoter activity in mammary carcinogenesis.

2014 POSTER

Serum proteome mass spectrometry analyses for identification of novel diagnostic biomarkers in breast cancer patients

M. Pietrowska[1], K. Behrendt[2], E. Nowicka[2], A. Walaszczyk[1], R. Tarnawski[3], J. Polanska[4], A. Polanski[4], L. Marczak[5], M. Stobiecki[5], P. Widlak[1]. *[1]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Experimental and Clinical Radiobiology, Gliwice, Poland; [2]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, II Radiotherapy Clinic/Teaching Hospital, Gliwice, Poland; [3]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Radiotherapy Department, Gliwice, Poland; [4]Silesian University of Technology, Faculty of Automatic Control Electronics and Computer Science, Gliwice, Poland; [5]Institute of Bioorganic Chemistry, Plant Secondary Metabolites Biochemistry Group, Poznan, Poland*

Background: Proteomics is the study of the proteome – a complete protein component of the cell. In contrast to the genome, the proteome is dynamic and its fluctuations depend on combination of numerous internal and external factors. Identifying and understanding changes in the proteome related to a disease development and therapy progression is a subject of clinical proteomics. Here we aimed to identify in the circulating blood a set of polypeptide biomarkers that could be used in diagnostics and monitoring of therapy of breast cancer patients.
Methods: Analysis of the low-molecular-weight region of the blood proteome (using either serum or plasma samples) by mass spectrometry (MS) methods is one of the basic approaches of clinical proteomics. Although no single peptide is expected to be a reliable bio-marker in such analyses, multi-peptide sets of markers selected in numerical tests have been already shown in a few studies to have prognostic and predictive value in cancer diagnostics. In our study we have analyzed low-molecular-weight serum polypeptides (<10 kD) using MALDI-TOF mass spectrometry.
Results: Blood samples were collected in the group of 100 breast cancer patients before the start of therapy, as well as in the group of 400 healthy controls. Specific patterns of low-molecular-weight polypeptides (1−10 kD) were identified due to mathematical analyses and cross-correlated between experimental groups. A multi-component set of polypeptides has been selected as a classifier that differentiate control and cancer samples.
Conclusions: Here we have presented report from the project aimed to identify a set of polypeptide biomarkers that could be used for diagnostics and monitoring of a therapy of breast cancer patients. Preliminary data showed that cancer-specific multi-component polypeptide pattern could be identified in serum of breast cancer patients. However, their importance for cancer diagnostics remained to be verified.

2015 POSTER

Functional analysis of the −2548G/ A leptin gene polymorphism in breast cancer cells

M. Terrasi[1], E. Fiorio[2], A. Russo[3], E. Surmacz[4]. *[1]S.H.R.O., Biology, Philadelphia PA, USA; [2]Department of Oncology University of Verona, Oncology, Verona, Italy; [3]Section of Medical Oncology Department of Surgical and Oncology, Biology, Palermo, Italy; [4]S.H.R.O, Biology, Philadelphia PA, USA*

Background: Leptin, a hormone produced mainly by the adipose tissue, regulates energy balance acting in the brain. In addition, leptin can stimulate mitogenic and angiogenic processes in peripheral organs. Recent data suggested that leptin can be involved in breast cancer progression, as it can induce proliferation, survival and anchorage-independent growth of breast cancer cells and is abundant in breast cancer tissues. The mechanisms of leptin overexpression in breast cancer are not clear.
The G to A substitution at −2548 in the leptin gene (Lep-2548G/A allele) in adipocytes correlated with a two-fold increase of leptin secretion and elevated circulating leptin levels. Furthermore, the occurrence of Lep-2548G/A in leukocytes correlated with increased susceptibility for different neoplasms, including breast cancer. However, molecular bases underlying this association have never been investigated. Here we asked whether occurrence of Lep-2548G/A in breast cancer cells could modulate transcriptional activation of the leptin gene.
Materials and Methods: We evaluated two different breast cancer cell lines, MDA-MB-231 and MCF-7. We used chromatin immunoprecipitation assays, DNA affinity immunoprecipitation, Western blot analysis and real time PCR.
Results: Lep-2548G/A was identified in MDA-MB-231, while it was absent in MCF-7 cells. DNA analysis revealed that Lep-2548G/A mapped near binding site for a transcriptional factor SP-1 and contained a motif for binding a transcriptional repressor nucleolin. Thus, we focused on the impact of Lep-2548G/A on the functional interactions of SP-1 and nucleolin with the leptin gene promoter Chromatin immunoprecipitation assays demonstrated that the existence of Lep-2548G/A improved efficient