325 POSTER

Decline in serum HER-2/neu predicts response to trastuzumab-based therapy

K. Leitzel[1], F. Esteva[2], M. Fornier[3], J. Gligorov[4], L. Harris[5], W. Kostler[6], D. Luftner[7], M. Pichon[8], C. Tse[4], A. Lipton[1]. *Serum HER-2 Study Group. [1]Penn State University/Hershey Medical Center, Hematology–Oncology, Hershey, PA, USA; [2]M.D. Anderson Cancer Center, Houston, TX, USA; [3]Memorial Sloan Kettering Cancer Center, New York, NY, USA; [4]Tenon Hospital, Paris, France; [5]Yale University, New Haven, CT, USA; [6]University Hospital of Vienna, Vienna, Austria; [7]Humboldt University, Berlin, Germany; [8]René Huguenin Cancer Center, Paris, France*

Background: Trastuzumab (Herceptin) monotherapy has a 34% objective response rate (ORR) in patients with HER-2/neu IHC 3+ or FISH-positive first-line metastatic breast cancer (C. Vogel et al., JCO 20:719–726, 2002). Predicting response and survival to trastuzumab-based therapy is an unsolved problem. The HER-2/neu extracellular domain (ECD) is released after cleavage by the ADAM metalloproteinases, and the remaining membrane-bound internal domain is constitutively activated. Trastuzumab inhibits cleavage of the HER-2/neu ECD.
Materials and Methods: A pooled analysis of 7 trials of first-line trastuzumab therapy (with or without chemotherapy) with serial serum HER-2/neu levels were included. The FDA-approved HER-2/neu ELISA (Oncogene Science/Bayer HealthCare) was used to determine serum HER-2/neu levels. A pretreatment and post-treatment serum (16–120 days) from 235 patients with HER-2/neu IHC 3+/FISH+ primary tumors were available. Kaplan Meier Life table analysis was performed to compare duration of response (DRP), time to progression (TTP), and overall survival (OS).
Results: The median decrease in serum HER-2/neu levels for all patients was 31.0% (Range: 98% decrease to 239% increase). Patients with > 20% decrease in HER-2/neu levels had a significantly higher objective response rate (ORR, complete + partial response) and longer DRP, TTP and OS. The results were similar regardless of the timing of the second serum draw (⩽30 days vs. >30 days) after the start of trastazumab.

HER-2/neu levels (Baseline to follow up)	ORR %	DRP (days), Median	TTP (days), Median	OS (days), Median
>20% decrease	58.3	403	334	1023
⩽20% decrease	25.0	245	173	519
p-value	<0.001	0.075	<0.001	0.004

Conclusion: Patients with HER-2/neu IHC 3+/FISH+ primary tumors and < 20% decrease in serum HER-2/neu levels have decreased benefit from trastuzumab therapy. Patients who do not have a significant decrease in serum HER-2/neu levels should be considered for additional HER-2/neu-targeted therapies.

326 POSTER

Prognostic significance of human kallikrein 7 protein expression levels in ovarian cancer by using automated quantitative analysis

P. Kountourakis[1], A. Psyrri[1], A. Scorilas[5], D. Kowalski[2], R. Camp[2], S. Markakis[3], M. Dimopoulos[4]. *[1]Yale University, School of Medicine, Medical Oncology, New Haven, USA; [2]Yale University, School of Medicine, Pathology, New Haven, USA; [3]Athens University, School of Medicine, Pathology, Athens, Hellas; [4]Athens University, School of Medicine, Clinical Therapeutics, Athens, Hellas; [5]University of Athens, Department of Biology, Hellas, Greece*

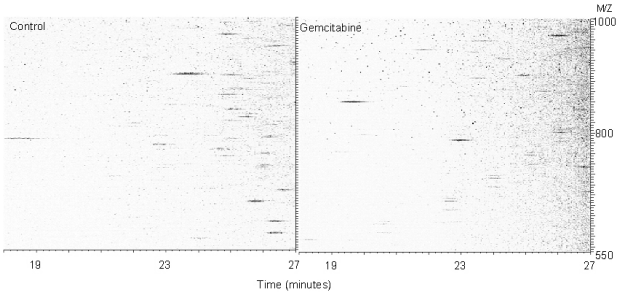
Background: Kallikreins, a subgroup of the serine protease enzyme family, are considered important prognostic biomarkers in cancer. Here, we sought to determine the prognostic value of kallikrein 7 (hkl7) in ovarian cancer using a novel method of compartmentalized in situ protein analysis.
Materials and Methods: A tissue array composed of 150 advanced stage ovarian cancers, uniformly treated with surgical debulking followed by platinum-paclitaxel combination chemotherapy, was constructed. For evaluation of kallikrein 7 protein expression, we used an immunofluorescence-based method of automated in situ quantitative measurement of protein analysis (AQUA).
Results: Mean follow-up time of the cohort was 34.35 months. One hundred twenty eight of 150 cases had sufficient tissue for AQUA analysis. In univariate survival analysis low tumor hkl7 expression was associated with better outcome for overall and disease free survival in 3 years (p values 0.032 and 0.037, respectively). In multivariate survival analysis, adjusting for well-characterized prognostic variables, low tumor hkl7 expression level were the most significant predictor variable for overall survival(95% CI: 0.125–0.729, p = 0.007).
Conclusions: High tumor hkl7 protein expression is associated with inferior patient outcome in ovarian cancer. Hkl7 may represent a promising therapeutic target in ovarian cancer.

327 POSTER

Improved method for preparation of formalin-fixed paraffin-embedded tissue for mass spectrometric analysis

J. Cole, R. Streeper, D. Campos, E. Izbicka. *CTRC Institute For Drug Development, San Antonio, USA*

Background: Formalin-fixed paraffin-embedded (FFPE) specimens represent potentially valuable resources for analysis of proteomic changes in cancer and/or response to therapies. Recently published protocols for FFPE tissue preparation for mass spectrometric (MS) analysis rely on deparaffinization through solvent washes, rehydration through graded alcohols, and tryptic digestion. We examined these methods and found that incomplete removal of paraffin interferes with proteolysis and complicates MS analysis.
Materials and Methods: Human renal carcinoma CAKI-1 cells grown in culture and as mouse xenografts in vehicle- and gemcitabine-treated animals were harvested and preserved as FFPE specimens. Thin (6 to 10 um) sections of FFPE specimens were deparaffinized by sequential washes of non-polar organic solvents, collected into silanized Eppendorf tubes, suspended in digestion buffer, sonicated, and digested with sequencing grade trypsin. Liquid chromatography in an aqueous acetonitrile gradient with 0.03% trifluoroacetic acid in a narrow bore C18 column was performed with a Thermo-Finnigan Surveyor HPLC. The MS analyses were performed using a Thermo-Finnigan LCQ-Duo and a New Objective PicoView 150 nanoelectrospray ionization source. MS data were processed using Mascot database search software.
Results: We have observed numerous quantitative and qualitative differences between the *in vitro* and *in vivo* specimens, along with significant differential up- or down-regulation of multiple molecular species after drug treatment.



Conclusions: Our preparative techniques designed to ensure complete removal of paraffin produces specimens that tend to yield more tryptic peptides for subsequent MS analysis in greater relative quantities than do the published protocols. The insights that we have gained from the preliminary identification of the variant proteins in these specimens continues to drive the investigation of the mechanisms of action of chemotherapeutic agents and proteomic biomarker studies in our laboratory.

328 POSTER

Rapid, efficient and reproducible SELDI chip robotic preparation method

D. Campos[1], R. Streeper[1], J. Cornell[2], E. Izbicka[1]. *[1]CTRC Institute For Drug Development, Biomarkers & Biomedical Proteomics, San Antonio, USA; [2]University of Texas Health Sciences Center, San Antonio, Texas, USA*

Background: Proteomic studies frequently involve the generation of numerous samples for subsequent instrumental analysis. The preparation by hand of large numbers of samples for surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) analysis is a laborious and time-intensive exercise. Robotic sample preparation is compatible with high-throughput analysis, but so far robotic protocols have not addressed issues of reproducibility and sample conservation, highly relevant in clinical studies.
Materials and Methods: Whole cell lysates of 8 human tumor cell lines and control serum specimens were profiled in ProteinChip® arrays