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

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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.

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

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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.

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

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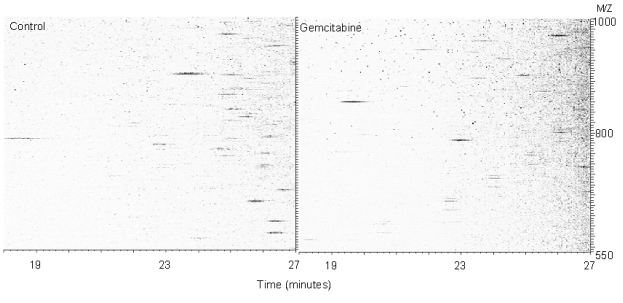
Background: Formalin-fixed paraffin-embedded (FFPE) specimens represent potentially valuable resources for analysis of proteomic changes in cancer and/or response to therapies. Recently published protocols for FFPE tissue preparation for mass spectrometric (MS) analysis rely on deparaffinization through 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.

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Rapid, efficient and reproducible SELDI chip robotic preparation method

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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

(H4/H50, SAX, IMAC-Cu). All samples were processed using a Beckman Coulter Biomek 2000 robotic workstation and examined in duplicate spots in each array type. The MS analyses were performed using a Ciphergen Biomarker System-IIc SELDI-TOF MS. Statistical analysis of MS data was performed with CiphergenExpress. In addition, spectra were preprocessed using the R/Bioconductor PROcess package, corrected for baseline drift and smoothed using a k-nearest neighbor algorithm. Peaks were identified, aligned and normalized. Consistency and reproducibility were assessed by inspection of the mean, standard deviation and coefficient of variation across ProteinChip® arrays within each chip type.
Results: We have modified default robot operating parameters to optimize the sample loading and processing steps so that the same aliquot of sample could be used for the preparation of multiple types of chips in all SELDI array types. Using 3 types of chip arrays in a sequence, we have achieved >80% time savings over sample arrays prepared by hand, along with a significant sample conservation resulting in a 75% reduction of specimen consumption. We continue to explore the ways to reduce variability of instrumental response through implementation of alternative sample and matrix application techniques. Statistical analyses of the mass spectra have revealed that the robotic preparation technique results in more uniform sample intensity results.
Conclusions: We have developed an efficient robotic method for the preparation of samples for SELDI-TOF MS analysis. The uniformity of sample preparations allows for semi-quantitative comparisons of resulting MS spectra.

Gene therapy and antisense approaches

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A phase IIb study in patients with recurrent malignant glioma with the TGF-beta2 inhibitor AP 12009

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Background: In 3 preceding phase I/II dose escalation studies the TGF-beta2 specific antisense compound AP 12009 was administered to patients with recurrent malignant glioma by convection-enhanced delivery (CED). AP 12009 proved to be well tolerated and revealed a good safety profile. Moreover, long lasting and complete tumor remissions were observed. Here we report on a subsequent phase IIb trial G004.
Methods: A total of 145 adult patients with histopathologically confirmed recurrent anaplastic astrocytoma (AA, WHO grade III) or glioblastoma (GBM, WHO grade IV) were enrolled. Objective of the study is to compare the efficacy and safety of two doses of AP 12009 (10 μM or 80 μM) to standard chemotherapy (TMZ or PCV). AP 12009 was administered intratumorally by CED for up to 11 cycles during a 6-month period with 7-day-on, 7-day-off treatment cycles. Endpoints include both survival and tumor response parameters. Post-study MRI for survival and tumor progress will be continuously collected during follow-up.
Results: Active treatment of 134 patients (96 GBM, 38 AA) is completed. Out of all patients 89 patients received AP 12009, 45 patients standard chemotherapy. Median age and Karnofsky performance status (KPS, data not shown) differed between treatment groups, particularly in GBM (GBM: 57.0, 45.0 and 52.0 years for 10 μM, 80 μM and control group, respectively; AA: 39.0, 40.5 and 35.0 years). Adverse events were evaluated by an independent Data and Safety Monitoring Board. Dose finding was achieved as efficacy and safety parameters for the AP 12009 10 μM group are superior to the 80 μM group. Up to now, in GBM patients treated with AP 12009 only 9 SAEs related or possibly related to the study drug were observed, none in AA patients. As in the previous studies, long-lasting responses were observed in AA and GBM patients. Median overall survival (mOS) in AA patients was 84.3 weeks in the control group, mOS in both AP 12009 groups has not been reached since more than 50% are still alive in these groups as of May 2006. In AA patients AP 12009 is superior to gold standard chemotherapy despite the fact that patients in the control group were younger and had better KPS at the time of inclusion. Evaluation of tumor response rates by central MRI reading is currently ongoing. Updated results will be presented.
Conclusion: These results show AP 12009 mediated TGF-beta2 suppression to be a highly promising therapeutic approach for TGF-beta2 overexpressing tumors.

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Synergistic antitumor activity of oncolytic reovirus and chemotherapeutic agents against non-small cell lung cancer (NSCLC)

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Reovirus type 3 Dearing strain (T3D) is a double stranded RNA virus, known to preferentially replicate in and kill cancer cells with an activated Ras pathway. Its in vitro/in vivo oncolytic efficacy has previously been demonstrated against colon, pancreatic, ovarian, breast cancers, malignant gliomas and lymphoid malignancies. The safety, feasibility and potential efficacy of reovirus cancer therapy are currently investigated in phase I/II trials. In this study, we examined the oncolytic activity of T3D in human NSCLC cell lines included in the NCI 60 cell line panel, and explored the therapeutic feasibility of reovirus-chemotherapeutic combination regimens against NSCLC. To determine the susceptibility of each cell line to T3D-induced cell killing, the cells were incubated with serially diluted virus inocula (4.3−8.3 $\log_{10}$ pfu/mL) in a 96-well microplate and examined for cell death by XTT assay at 48 hrs post-infection. The effect of combination of T3D and chemotherapeutic agents was evaluated in selected cell lines with differing levels of T3D- or drug-sensitivity by using the Chou and Talalay's combination index-isobologram method. Progeny virion production was assessed by plaque assay. Seven of 9 NSCLC cell lines from the NCI 60 cell line panel exhibited significant susceptibility to T3D-induced cytopathic effect with ED50 (50% effective dose defined by the initial MOI to achieve 50% cell killing) ranging from 1.46±0.12 to 2.68±0.25 (mean±SD from 3 experiments) $\log_{10}$ pfu/cell. The combination of T3D with cisplatin, gemcitabine, mitomycin or vinblastine was in general synergistic against NSCLC cell lines sensitive to the anticancer drugs when tested as a single agent. However, in cell lines with high-level resistance to the compounds (50% inhibitory concentration $\geqslant$50−100 μM), the T3D-drug combination was found antagonistic regardless of their sensitivity to T3D. Interestingly, the combination of T3D and paclitaxel was uniformly synergistic in all 6 cell lines examined, including in those resistant to paclitaxel or T3D. The plaque assay data indicated that progeny virion production was increased in T3D-infected cells in the presence of paclitaxel. Reovirus has been shown to exploit microtubules for the formation of viral replication complexes. Our data suggest that microtubule-stabilizing agents may enhance reoviral replication, resulting in a more efficient and synergistic oncolytic effect.
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Recombinant adeno-associated virus mediated RNA interference inhibits metastasis of nasopharyngeal cancer cells in vivo and in vitro by suppression of Epstein-Barr virus encoded LMP-1

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Background: In this study, we used a recombinant adeno-associated virus type 2 vector (rAAV-2) to deliver small hairpin RNA (shRNA) targeting EBV LMP-1 into the EBV-positive human NPC C666–1 cells and evaluated the effect of long-term suppression of LMP-1 on NPC growth and metastasis *in vivo* and *in vitro*.
Material and Methods: A NPC metastasis nude mice model with NPC xenograft transplanted in liver was established. The NPC C666–1 cells infected with rAAV-shRNA-LMP-1 or rAAV-EGFP were inoculated in the livers of nude mice. Formation of liver and lung metastasis was evaluated at days 14 after tumor inoculation.
Results: rAAV-shRNA-LMP-1 could effectively infect C666–1 cells and suppress LMP-1 expression. Such suppression, in turns, did not significantly inhibit tumor growth, but prevented NPC metastasis in the liver as well as in the lung. Consistent with *in vivo* data, the *in vitro* studies in NPC C666–1 cell cultures showed that suppression of LMP-1 by rAAV-shRNA-LMP-1could significantly reduce cell mobility and transmembrane invasion ability.
Conclusions: Our results demonstrate for the first time that long-term suppression of EBV encodes LMP-1 *in vivo* is an effective mean for preventing NPC metastasis.