(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.