82% (9/11) cell lines, including 4 primary and 7 commercial cell lines, after 4 days treatment ($p < 0.001$). The cell death resulted from suppression of PAX8 was profound in GBM harboring mutant p53 (mtp53), whilst it was delayed in U87MG, which has wtp53.
Conclusion: The current study represents the first extensive analysis of the expression of PAX2, 5, and 8 and their phosphorylated proteins in gliomas. Our results showed that it is possible to induce a potent cytocidal effect by silencing PAX8 expression in a significant proportion of GBMs. PAX8 serves a pro-survival function in GBM, and therefore a potential therapeutic target in GBM.

336 POSTER

Combination of proapoptotic gene and cisplatin for the treatment of resistant SCLC xenografts in nude mice

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Background: Small cell lung cancer (SCLC) metastasizes at an early stage, and most cases relapse and become resistant to current therapies. A systemic therapeutic approach with higher specificity and efficacy is needed.
Materials and Methods: Lipids: DPEPC, DPPC, and DSPE-PEG2000 were used to make a stable liposome formulation to encapsulate the plasmid. Plasmids: an activity-enhanced human telomerase reverse transcriptase promoter (hTRpG) was used to drive bik (Bcl-2 interacting killer) or luciferase gene. CMV promoter was used as comparison in the similar constructions. Cells: human SCLC cell lines H69, H82, H466, H345 (ATCC) and human normal bronchial epithelium cells were used in vitro and in vivo studies. Nude mice were used in the efficacy studies. The main method was to use liposome delivered tumor-specific proapoptotic gene combined with chemotherapy for systemic treatment of human SCLC xenografts in nude mice.
Results: The transfection efficiency of the liposome formulation in human SCLC cell lines was equivalent to Fu-gene 6. The hTRp-driven luc specifically expressed in human SCLC cell lines but not in the normal cells, the RLU in the cancer cells was 5- to 20-fold higher than that in normal cells ($p < 0.002$). The liposome delivered hTRp-bik could significantly sensitize the chemo-resistant human SCLC cell lines for cisplatin treatment (by increasing the response [%killing] by >5-fold, $p < 0.004$). In nude mice bearing orthotopic human SCLC, the reporter gene expression after IV injections of the liposome delivered hTRp-luc was significantly higher in tumor but not in other organs compared with the same formulation delivering CMV-luc. The combination therapy of liposome delivered hTRp-bik and cisplatin in human SCLC xenografts in nude mice was 2- to 4-fold higher than the chemotherapy alone ($P < 0.01$).
Conclusions: The nonviral gene delivery system is capable for the systemic gene delivery in animal. The combination strategy presented above is significantly more effective than the optimal chemotherapy alone in the resistant human SCLC xenografts models. The survival genes of Bcl-2 family may be used as targets for sensitizing the resistant SCLC.

337 POSTER

Long-term suppression of tumor growth by intermittent administration of oblimersen sodium in combination therapy with taxanes and kinase inhibitors

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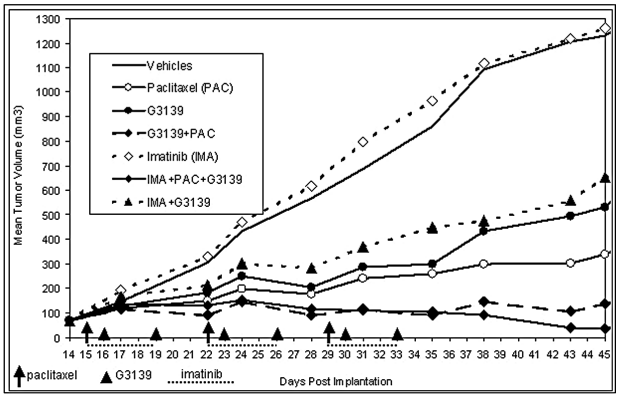
Background: Periodic dosing of the Bcl-2-targeted antisense oligonucleotide oblimersen sodium (G3139, Genasense®) has been shown to be as efficacious as or more efficacious than daily dosing against xenograft tumor models both as monotherapy and in combination with other agents. These results are highly consistent with the analysis of tumor sections taken from animals treated with fluorescently labeled oligonucleotide (FAM-G3139 or G4243). In these studies, intermittent dosing caused greater oligonucleotide uptake into xenograft tumor tissues, even when the total oligonucleotide dose was held constant or reduced by a periodic administration schedule. Finally, G3139 has been shown to increase the efficacy of other antitumor agents with distinct mechanisms of action, such as paclitaxel, DTIC, and kinase inhibitors gefitinib and erlotinib.
Materials and Methods: Xenograft tumors (A375 melanoma, A549 NSCLC, H460 NSCLC, HT29 colon carcinoma) grown in C.B-17/SCID mice were used to evaluate antitumor efficacy. G3139 was administered intravenously (i.v.) via bolus injections at levels ranging from 2.5 mg/kg/day daily to 20 mg/kg twice a week. Kinase inhibitors (erlotinib, imatinib, sunitinib, sorafenib) or temozolomide were administered orally (p.o.) daily. Taxanes (paclitaxel and paclitaxel albumin nanoparticles) were administered once a week i.v. Weight loss was used as a marker of overall toxicity.
Results: Intravenous administration of G3139 as infrequently as twice a week, in triplet combinations with a taxane and a kinase inhibitor was more efficacious than any doublet combination therapy. This result was most striking for imatinib in the A549 model, where single agent imatinib alone has no detectable activity at doses as high as 100 mg/kg/day. Weight loss was dependent on dosing schedules and drug sequencing. Highly effective therapeutic regimens were identified that were well tolerated and that could be administered repeatedly to completely suppress xenograft tumor growth. Growth delay and life span results will be presented.



A549 NSCLC treated with imatinib + paclitaxel + G3139.

Conclusions: G3139 does not require continuous administration to significantly inhibit tumor growth. On the basis of the preclinical efficacy of G3139 administered periodically via either subcutaneous bolus injections or short intravenous infusions, periodic dosing regimens will be incorporated into clinical trials combining oblimersen sodium with other agents such as alkylators, taxanes and kinase inhibitors.

338 POSTER

Carcinoma of the Ampulla of Vater with CIMP+ after treatment with pegylated liposomal based formulation of Ras siRNA combined with vinorelbine-tartrate exhibited inhibition of Raf/MEK/ERK, PI3K/AKT, DNA methylation and re-expression of tumor suppressor genes inducing type I, II, III PCD

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Background: Activated Ras signaling pathway due to mutations is common in carcinoma of the ampulla of Vater(CAV) causing upregulation of DNA and DNMT1 which leads to inactivation of tumor suppressor genes with subsequent chemoresistance due to inhibition of programmed cell death.
Materials and Methods: We treated chemoresistant CAV characterised by Ras codon 12 point mutations which activated the Ras/DNMT1/DNA methylation pathway with pegylated liposomal double stranded short interfering RNAs (siRNA) of synthetic 21 nucleotides specific to Ras combined with vinorelbine.
Results: Post-treatment, we observed Ras gene silencing after recognition of cognate mRNA through hydrogen bonding of the complementary short interfering RNA sequence leading to inhibition of DNA MeTase, DNMT1, Raf/MEK/ERK, PI3K/AKT, Wnt, p44/42 MAPK, cyclin D1, FGF-8, FGFR3, TGF-b, cyclin E-CDK2, RFC1, IL-8 and VEGF-R3 leading to reduced vessel density as assessed by CD31. Angiogenesis and lymphangiogenesis was inhibited. The complete degradation of Ras mRNA after the siRNA mediated RNA interference (RNAi) led to upregulation and re-expression due to epigenetic inactivation of PTEN, RB1, small GTPase RhoB, Par-4, GADD153, p16INK4a/p19Arf, p27Kip1, Skp2, thrombospondin-1, PKC-ä, CKIp21, HIC-1, p15, RARB, p53-MDM2, CHK2, RASSF2A, pcdc-25c and ATM. Vinorelbine-tartrate depolymerized microtubules at G2/M of tumor and endothelial cells and inhibited by phosphorylation the expression of anti-apoptotic and metastatic oncogenes bcl-2, bcl-xL, bcl-w and bcl-G, while it downregulated mcl-1, cIAP1, CAP2, XIAP, bfl-1/A1. Furthermore, vinorelbine upregulated 15-PGDH, ARHI, ICAD, Omi, Diablo, cyt-c, procaspase 7, bax, bak, bok, bad, bid, bcl-xs, bin, krk, Mtd, Nip3,

bcl-b and Noxa. There was inhibition of cellular viability exhibited by trypan blue assay, while Ki67 and MIB1 markers exhibited inhibition of cell proliferation. BrdU, MTT and XTT assays exhibited inhibition of DNA synthesis and metabolic activity in CAV and endothelial cells. Finally, we observed induction of apoptosis or nuclear PCD type I, paraptosis (necrapoptosis) or cytoplasmic PCD type III and autophagic PCD type II leading to a bystander killing effect (BKE) of CAV and endothelial cells.
Conclusion: We achieved to eradicate chemoresistant CAV and endothelial cells by using a functional genomic tool such as siRNAs in a pegylated liposomal formulation which inhibited DNA methylation re-establishing normal function and expression of vital tumor suppressor genes combined with the cytostatic and apoptotic action of vinorelbine-tartrate leading to the synergistic inhibition of angiogenesis, lymphangiogenesis, metastasis and cellular proliferation after the induction of type I, II, III PCD.

339 POSTER

Implication of tumor suppressor maspin in the eradication of lung cancer

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Our work focuses on identifying factors critical to the progression of lung cancer. Here, we investigated the possible role of maspin in this context. In breast and prostate cancers, maspin acts as a tumor suppressor capable of inhibiting motility, invasion and metastasis; in pancreatic cancer it functions as a malignant factor. Few studies to date have investigated the role of maspin in lung cancer, likely because it is not expressed in normal lung tissue. In the present study, we investigated whether engineered overexpression of maspin in NCI-H157 lung cancer cells cells, which do not express endogenous maspin, would suppress the tumorigenicity of this particular tumor cell type. NCI-H157 cells overexpressing maspin displayed a dramatically reduced growth rate compared to the parental cell line when subcutaneously implanted in athymic (nu/nu) mice. Furthermore, gene transfer of maspin suppressed the growth of established NCI-H157 tumors. The data suggest that maspin gene therapy and/or agents that increase maspin expression could have utility in the treatment of lung cancers.

340 POSTER

Gene therapy with plasmid IL-12 delivered by electroporation in patients with malignant melanoma: results of first human Phase I trial

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Metastatic melanoma is a devastating disease lacking effective treatment. In the B16.F10 melanoma model, electroporation of plasmid encoding IL-12 (pIL-12) into established tumors resulted in an 80% cure rate as well as resistance of the cured mice to subsequent challenge with B16.F10 cells. Furthermore, minimal toxicity was observed when pIL-12 was delivered with electroporation as compared to untreated mice or mice that only received injection of pIL-12. These experiments provided the rationale for a Phase I safety, dose escalation and biological efficacy trial in patients with accessible subcutaneous metastases with melanoma.
Methods and Study Design: The primary objective was to determine the toxicity and maximum tolerated dose (MTD) of intra-tumorally electroporated pIL-12. The secondary objectives were to determine the efficacy of this treatment and to evaluate local and systemic cytokine response. Patients received intra-tumoral injection of pIL-12 followed immediately by 1300 V/cm 100 μs duration electric pulses at the tumor site. Electroporation treatments were performed on days 1, 5 and 8. Each patient had a minimum of 2–4 tumors treated. Dose escalation was performed by varying the plasmid amount, (0.6 mg, 1.5 mg, 3 mg, 6 mg, 12 mg) three patients were treated per cohort.
Results: Three patients were enrolled into each of five cohorts of this Phase I study. No Grade 2, 3 or 4 toxicity was noted. Patients expressed significant but transient pain during the administration of electric pulses (lasting a few seconds). Biopsies were done following treatment and showed significant necrosis of melanoma cells within the tumor in the majority of treated lesions. Significant lymphocytic infiltrate was seen in biopsies from patients in cohorts 3, 4 and 5. In addition, IL-12 expression was documented in the tumor samples biopsied but not in serum.
Conclusion: This first-in-human phase I study demonstrated the feasibility and efficiency of *in vivo* electrogene delivery in humans. Transient pain during electroporation has been the only toxicity seen at this point in the trial. Expression of IL-12 was documented at the tumor site as well as tumor necrosis and lymphocytic infiltrate. Further evaluation of this method in melanoma and other tumors is warranted by the current trial.

New drug targets

341 POSTER

The discovery of MP529, a potent and selective aurora kinase inhibitor using CLIMB

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Aurora A kinase is a validated target for a number of human malignancies, including pancreas, breast, prostate, ovarian and colorectal cancers. This serine-threonine kinase is of particular interest as of late, due to its important role in proper spindle formation at mitosis. Overexpression of Aurora A leads to dysregulation of the centrosome cycle resulting in the formation of multipolar mitotic spindles. The resulting abnormal mitotic events lead to genomic instability which is an underlying process in tumorigenesis. Previous studies have shown that inhibition of Aurora A kinase in tumor cell lines effectively disrupts mitosis, leading to monopolar spindles, multinucleate cells, growth arrest and eventually cell death. Through the use of our proprietary CLIMB drug discovery process, we have set out to identify and synthesize a new series of Aurora A kinase inhibitors. In traditional small molecule screening, as many as several million compounds may be tested in order to identify the few that interact selectively with a disease-related protein target. CLIMB can achieve similar results by screening as few as several hundred computationally selected compounds. CLIMB screening is based on the clustering of representative chemical structures and pharmacophores that embody our large virtual library of nearly 50 million compound structures. Using CLIMB, the Aurora A kinase crystal structure was used as a substrate for docking to generate a subset of leads based on calculated binding energies. These leads were then screened and ranked using a number of in silico physicochemical and ADMET prediction algorithms to determine which were most likely to be "drug-like". Biochemical enzyme-based assays with recombinant Aurora A kinase have revealed an array of candidates of the substituted (4-p-tolylsulfamoyl-phenyl) amide class, the MP529 series. These compounds exhibit nanomolar activity or better against the Aurora A kinase enzyme, and have been carried forward into ex vivo and in vivo evaluations. In cell-based assays, mitotic markers of Aurora A kinase inhibition are seen, resulting in a reduction of tumor cell growth. Selected compounds from this series have been appraised in an in vivo xenograft context, and have been shown to be effective, while exhibiting a wide therapeutic window and desirable pharmacokinetic properties. The MP529 series represents a novel scaffold which improves upon the pharmacological activities of known Aurora kinase inhibitors.

342 POSTER

A phase I dose-escalation and pharmacokinetic (PK) study of XL647, a novel spectrum selective kinase inhibitor, administered orally to patients with advanced solid malignancies (ASM)

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Background: XL647 is an orally bioavailable small molecule inhibitor of multiple receptor tyrosine kinases involved in tumorigenesis, angiogenesis, and metastasis, including EGFR/ErbB1, ErbB2/HER2, VEGFR2/KDR, and EphB4.
Methods: ASM patients (pts) were enrolled in successive cohorts to receive XL647 orally as a single dose on Day 1 with PK sampling, then on Day 4, pts received 5 consecutive daily doses with additional PK sampling. Pts continued treatment with XL647 for 5 consecutive days, followed by a 9-day observation period; cycles were repeated every 14 days. Pharmacodynamic plasma samples were collected from all pts and are undergoing analysis for mechanism-of-action related molecules. Pts were allowed to stay on study in the absence of unacceptable toxicity until evidence of disease progression.
Results: A total of 41 pts have been treated across 11 dose levels: 0.06, 0.12, 0.19, 0.28, 0.39, 0.78, 1.56, 3.12, 4.68, and 7.0 mg/kg in liquid formulation, and then at a fixed dose of 350 mg in tablet formulation. One serious adverse event, grade 4 pulmonary embolism, was considered possibly related to study treatment in a pt dosed at 0.28 mg/kg. One pt at 3.12 mg/kg had a dose-limiting toxicity (DLT) of asymptomatic QTc prolongation as assessed by an electrocardiogram, resulting in expansion