tumor models. G3139 (Genasense, oblimersen sodium; Genta Inc., Berkeley Heights, NJ) is a phosphorothioate oligonucleotide complementary to bcl-2 mRNA. Treatment with G3139 can reduce Bcl-2 protein levels in vivo. Since Bcl-2 overexpression is thought to contribute to chemotherapeutic resistance, Bcl-2 suppression by G3139 may enhance the anti-tumor efficacy of standard cytotoxic chemotherapy. We have previously reported that a combination of G3139 and paclitaxel led to prolonged stable disease in a small number of patients with advanced chemorefractory SCLC. Here we report initial results of a phase I study evaluating the combination of G3139, carboplatin, and etoposide in patients with previously untreated extensive stage SCLC. Eleven patients have been treated to date in 3 dose cohorts. The primary goals of this study are to assess toxicity and to determine a maximally tolerated dose for this combination. Cohort 1 initially received G3139 5 mg/kg/d IVCI days 1-8 on a 21 day cycle, with carboplatin AUC=6 on day 6 and etoposide 80 mg/m^2/d, days 6-8. Of the 4 patients evaluable for toxicity in cohort 1, 2 developed grade 4 neutropenia in cycle 1. Cohort 2 was initiated at identical G3139 and etoposide doses, with carboplatin dose reduced to AUC=5. One patient in each of the first two cohorts elected to discontinue therapy before completing cycle 1. All other patients in both dose cohorts have completed 6 cycles of therapy. In cohort 2, 0 of 4 patients experienced cycle 1 DLT. Several patients did require dose delays in later cycles due to hematologic toxicity. This may be due to the truncation of days from last chemotherapy to next cycle from 19 to 14 because of the 5-day lead-in period of G3139 administration. No non-hematologic toxicities > grade 2 have been attributed to therapy. Enrollment is continuing in cohort 3, with G3139 7 mg/kg/d, carboplatin AUC=5, and etoposide 80 mg/m^2 × 3; 2 patients in this cohort are in cycle 3 and 4, respectively. Overall, in 9 patients evaluable for response, we have documented PR in 7, and SD in 2. Final toxicity and clinical outcome data will be presented. The phase II dose will be tested in a randomized trial within the CALGB.

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G3139 (Genasense; Oblimersen) induces production of reactive oxygen species and hydrogen peroxide in human prostate and bladder carcinoma cells in a backbone and cpg-dependent manner

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PC3 and T24 cells, when treated with 400 nM G3139/Lipofectin or Oligofectin, produce reactive oxygen species (ROS) and H2O2, as measured flow cytometrically by hydrethidium-ethidium and fluorescence and The production of ROS and H2O2 were measured flow cytometrically by hydroethidine to ethidium oxidiation and 2'-7'-dichlorofluorescein fluorescence, respectively. The increase in ROS and H2O2 were is as much as 500% and 250% respectively, vs. untreated cells after 72 h incubation. Maximum generation of ROS and H2O2 was observed after 72 hours, and near maximal generation as long as 5 days after initial incubation, and at a 200 nM oligo concentration, but no lower. A control oligo, 4126, (a two base mismatch of G3139 in which the two CpG motifs are eliminated), produced only a small increase in ROS and H202 production (<50%). However, the mechanism of production was unlike that in immune cells because cells treated with naked oligo (5 microM) did not increase ROS or H2O2 production. Treatment of cells with 2006, a 24mer phosphorothioate triple tandem repeat of an optimized CpG motif (GTCGTT) did not downregulate bcl-2 expression, but did induce ROS and H2O2 production to the same extent as G3139, indicating that bcl-2 downregulation did not cause the increase in production. Confirming this observation, we found that oligo 2009, which is directed to the coding region of the bcl-2 mRNA, downregulated the expression of bcl-2 protein to the same extent and with identical kinetics as G3139, yet did not induce the production of ROS and H2O2. Elimination of the increase in production of ROS and H2O2 could be accomplished by either cytosine C5-methylation, or even more dramatically by C5-propynylation of both of the CpG motifs of G3139. The rate of growth of cells treated with either of these two oligomers (which do not induce the production of ROS and H2O2) was approximately identical to those cells treated with 2009 or 4126 (both of which do not), and much faster than those cells treated with either G3139 or 2006 (both of which do, although the latter does not downregulate the expression of bcl-2). However, 4126 could be transformed into an ROS and H202 producing oligo by modification of the backbone with five LNA (locked nucleic acid) linkages at the 3' and 5' positions. Thus, it appears that there is a CpG directed, non-bcl-2 dependent induction of production of ROS and H2O2 in PC3 and T24 cells that strongly affects the rate of cell growth.

Monoclonal antibodies and immunoconjugates

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A new Tc-99m labelled somatostatin analogue (Tc-99m EDDA-TRYCINE-HYNIC-TOC) for receptor imaging: first clinical results before and during radioreceptor therapy with Y-90 DOTATOC

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Aim: Radioreceptor therapy using radiolabelled peptides is a promising new approach for the treatment of neuroendocrine tumors. We evaluated Tc-99m EDDA-TRYCINE-HYNIC-TOC (TET-H-TOC) in patients with somatostatin receptor (SSTR)-positive tumours (staging, dosimetry and follow-up).
Methods: The Tc-99m labelled somatostatin analogue was synthesized in our pharmaceutical lab using lyophilized kits (radiochemical purity by HPLC, TLC >95%, product stability *in vitro* 4 to 6h). So far, 46 patients (53 examinations) were studied after injection of 580-890 MBq (median 673 MBq) TET-H-TOC. The histologically proven tumours were endocrine neoplasias, renal carcinomas, bronchial carcinoma, mesothelioma and malignant fibrous histiocytoma. The imaging protocol consisted of whole-body scans and planar images of the tumor region (15 min, 1h, 2h, 4h, 8h, 24h p.i.) and additionally SPECT-images (1h und 4h p.i.). For semi-quantitative assessment, individual regions of interest (ROI) were drawn in order to generate time-activity curves and to calculate tumour-to-tissue/background ratios. Pharmacokinetic analysis was carried out (radioactivity kinetics in plasma and urine). In some selected patients, image fusion of the whole-body scans was performed with CT and/or MRT and/or PET using a HERMES computer.
Results: 7 out of 46 patients showed an intense tracer accumulation in the SSTR-positive tumours (visual 3+, tumour / background ratio >2.5). In these patients, radioreceptor therapy was carried out using Y-90 DOTATOC (simultanous injection von 150 MBq In-111 DOTATOC). All pretherapeutic scans with the Tc-99m labelled ligand (4h p.i.) showed a similar overall pattern of biodistribution and tumour uptake in comparison to the therapy scans with In-111/Y-90 DOTATOC (24h p.i.). The Tc-99m EDDA-HYNIC-TOC scans (incl. SPECT) offered superior imaging properties with earlier tumour visualisation (all lesions were detected 1h p.i.) as compared to the In-111 labelled analogue. Hence, the receptor scintigraphy with Tc-99m EDDA-TRYCINE-HYNIC-TOC enables to select patients suitable for radioreceptor therapy with Y-90 DOTATOC.
Conclusion: Our results demonstrate the ability of Tc-99m EDDA-TRYCINE-HYNIC-TOC for receptor scintigraphy of SSTR-positive tumours with superior image performance as compared to In-111 labelled SST-analogues (staging), for an individual selection of patients suitable for a radioreceptor therapy with Y-90 DOTATOC (dosimetry) and for post-therapeutic control.

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Cetuximab (C225, Erbitux) in combination with irinotecan, infusional 5-fluorouracil (5-FU) and folinic acid (FA) is safe and active in patients (pts) with metastatic colorectal cancer (CRC) expressing epidermal growth factor-receptor (EGFR). Results of a phase I study

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C225 is a chimeric antibody targeted against EGFR with activity in refractory CRC. Our current phase I trial evaluates the feasibility and safety of C225 combined with irinotecan and weekly infusional 5-FU/FA (modified AIO-regimen) as 1st-line treatment for pts with CRC. 24/27 screened tumors were found positive for EGFR by immunhistochemistry, and 21 positive pts entered the trial. None of them had previous palliative chemotherapy or relevant organ dysfunction. After a loading dose of 400 mg/m^2 i.v., C225 was given weekly at a dose of 250 mg/m^2 i.v. Chemotherapy was administered weekly × 6, followed by 1 week rest, and consisted of irinotecan 80 mg/m^2, FA 500 mg/m^2 and 5-FU 1500 mg/m^2/24h (low dose group) or 2000 mg/m^2/24h (high dose group). Dose limiting toxicity (DLT) was defined as neutropenia or skin toxicity >grade 3, neutropenia/leukopenia with fever;

thrombopenia, diarrhea, mucositis, liver toxicity >grade 2 or other relevant organ toxicity >grade 1, each in the first cycle. Six pts were enrolled in the low dose group, 15 in the high dose group, respectively, with a median age of 61 (36-68) years and a KPS of 100 (70-100). Six pts in the low dose group, and 13 pts in the high dose group are presently evaluable for DLT and response. As of yet, 55 cycles of treatment were administered, ranging from 2-5(+) in the low dose and 1-5(+) in the high dose group. Dose modifications were required in 2/6 pts in the low dose group, and 7/13 pts in the high dose cohort. While no DLTs were observed during the first cycle in the low dose group, three DLTs occurred in the high dose group (diarrhea grade 3: 2 patients; diarrhea grade 4: 1 patient). Characteristic skin toxicity attributable to C225 was seen in all pts, with acneiform rash grade 3/4 limited to one patient in the low dose group, and 2 pts in the high dose cohort during the course of treatment. Response assessment in 19 pts (RECIST): CR 1 (5%), PR 10 (53%), PR (to be confirmed) 1 (5%), SD 6 (32%), PD 1 (5 %), OR 63% (95% confidence interval 41-85%). In summary, the combination of C225 with irinotecan and weekly infusional 5-FU/FA is safe and feasible in pts with EGFR-positive metastatic CRC. Diarrhea is dose-limiting, and reversible acneiform skin rash is the most common toxicity associated with the use of this regimen. The high activity of the combination warrants further investigation, and late phase II studies are in preparation using the recommended low dose schedule established in this trial.

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In vivo activity of recombinant humanized monoclonal antibody 2C4 in xenografts is independent of tumor type and degree of HER2 overexpression

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The proto-oncogene HER2/erbB2 encodes a growth factor receptor, which is overexpressed in about 25 % of breast cancers. Trastuzumab (Herceptin) is a recombinant humanized monoclonal antibody directed against the extracellular domain of the HER2 protein (HER2-ECD) and is approved for the treatment of HER2 positive metastatic breast cancer. Preclinical studies with trastuzumab show inhibition of primary tumor growth of HER2 overexpressing xenografts in athymic nude mice. 2C4 is another HER2-ECD-specific recombinant humanized monoclonal antibody that does not compete with trastuzumab for HER2 binding. *In vitro*, 2C4 binding to HER2 inhibits ligand-dependent heterodimerization of HER2 with other HER family members leading to the inhibition of several downstream signal transduction cascades. We have studied the dose-dependent activity of 2C4 against three human xenografts in immunodeficient mice. The xenografts differed with regard to tumor type (breast cancer and NSCLC) and/or HER2 protein levels (HER2 1+ and HER2 3+). Established tumors of about 100 mm3 were treated by weekly i.p. administration of 2C4. The doses ranged from 0.6 mg/kg to 60 mg/kg with a twofold loading dose given at the first treatment. The determination of 2C4 serum levels revealed a dose-dependent increase of 2C4 serum concentration in the different dose groups. In each of three xenografts (MAXF-449, HER2 1+; NCI-H522, HER2 1+ and Calu-3, HER2 3+) the higher doses (6 mg/kg, 20 mg/kg and 60 mg/kg) of 2C4 resulted in complete tumor stasis. At lower doses (0.6 mg/kg and 2 mg/kg) the HER2 low expressor models MAXF-449 and NCI-H522 still responded with tumor stasis. In contrast, the HER2 high expressor model Calu-3 showed a reduced response with 30 % and 84 % tumor growth inhibition at 0.6 mg/kg and 2 mg/kg, respectively. A possible explanation for the requirement of higher 2C4 doses to achieve the maximum effect in the HER2 high expressor model may be the much higher amount of HER2 target molecules at the surface of HER2 3+ versus HER2 1+ tumors. Together with the previously described activity of 2C4 in prostate cancer and colorectal cancer xenografts our data indicate that 2C4 mediated tumor growth inhibition is not restricted by tumor type or by the degree of HER2 overexpression since an appropriate dose of 2C4 can be as active in low and high HER2 expressing models.

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Identification of pan B-cell neoplasm markers using a combination of cDNA PCR subtraction and microarrayanalysis

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Malignant cells express multiple antigens that can be recognized by the immune system and can be targeted by antigen specific immunotherapies including vaccines, antibodies and T cells. To identify genes that are over-expressed in B-cell malignancies PCR subtracted cDNA libraries in conjunction with microarray screening were used. Twelve subtracted libraries (lymphoma n=6, CLL n=2, myeloma n=4) were constructed to enrich for B-cell neoplasm or lymphatic tissue specific cDNA sequences. cDNA pools from lymphomas, B-CLL and multiple myeloma (MM) were subtracted against cDNA pools of related normal hematopoietic tissues or normal non-hematopoietic tissues. Subtracted libraries showed enrichment for both B-cell neoplasm and lymphatic tissue specific genes compared to an unsubtracted library. 14,000 cDNA fragments derived from these B-cell neoplasma specific libraries were analyzed using DNA microarray technology. Genes over-expressed in B-cell malignancies were identified using pairs of fluorescence-labeled cDNA probes synthesized from lymphomas, CLL or MM (n=70) and normal tissue poly A+ RNAs (n=70). Over 1.6 Million hybridization signals were analyzed. Expression patterns of 125 overexpressed genes were confirmed and characterized further by Real Time PCR using a panel of 70 cDNAs comprising of lymphomas, CLL, MM, normal tissues and MACS sorted hematopoietic subpopulations. Expression profiles were compared to known therapeutic antibody targets, CD20, CD52 and CD45. Based on their mRNA expression profile a group of cDNA fragments (Ly1448, Ly1456, Ly1464 and Ly1728) was identified demonstrating expression in normal tissues similar to CD20 but displaying broader coverage in B-cell malignancies. These cDNA fragments are expressed in the majority of B-cell lymphomas, B-CLL and multiple myeloma patient samples and were thus termed Pan B-cell neoplasm markers. So far, potential full length open reading frames of 3 of the 4 cDNA fragments have been identified, encoding for proteins with a predicted transmembrane region. Further characterization of these genes and proteins is in progress.

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Anti-EGFR monoclonal antibody Cetuximab binds the EGFR variant III receptor and internalizes phosphorylated receptor on the cell surface

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The epidermal growth factor receptor (EGFR) plays an important role in the growth and survival of many human tumors of epithelial origin. EGFR variant III (EGFRvIII) is a truncated form of EGFR that does not bind ligand, is constitutively active, and is reported to be co-expressed with EGFR on some human tumors including breast, glioblastoma, lung, and prostate. Here we have tested the anti-EGFR monoclonal antibody ERBITUXTM (cetuximab) for its ability to bind the EGFRvIII. Chinese hamster ovary (CHO) and 32D (non-tumorigenic murine hematopoetic cells) stable transfectants were generated that express the EGFRvIII under the Ecdysone-inducible system. Transfectant cell lines expressed approximately 1.09 $\times$ 106 and 0.6 $\times$ 106 EGFRvIII receptors/cell in CHO and 32D transfectants, respectively. Analysis of receptor phosphorylation showed that the EGFRvIII was constitutively phosphorylated in transfected cells. Flow cytometry and immunoprecipitation analysis of EGFRvIII transfectants showed specific binding of cetuximab to the EGFRvIII. Thus, the binding epitope for cetuximab is outside the region of the EGFRvIII deletion. In internalization studies, binding of cetuximab to the EGFRvIII on the cell surface led to the internalization of the cetuximab-EGFRvIII complex with 50 % of the complex internalized from cell surface after 3 hours. This internalization led to a 80% reduction in phosphorylated EGFRvIII in transfected cells. These data demonstrate that cetuximab binds to and internalizes EGFRvIII and suggests that cetuximab may be a potential candidate for the treatment of tumors that also express EGFRvIII.