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.