Nimotuzumab is a humanized monoclonal antibody (MAb) directed against the extracellular domain of human Epidermal Growth Factor Receptor (EGFR). Clinical experience in more than 2000 patients indicates that it does not exhibit the typical skin and gastro-intestinal (GI) toxicity seen with other agents within the same class. A phase II trial was designed to assess the feasibility of administering Nimotuzumab in conjunction with whole brain radiation therapy (WBRT) in patients with advanced NSCLC brain metastases. Here we outline the preliminary results of this trial of Nimotuzumab given concurrently with palliative WBRT.
It was an open, controlled trial in which 30 patients with unresectable brain metastases were randomized to receive Nimotuzumab plus irradiation or irradiation alone.
Nimotuzumab (200 mg) was administered as weekly IV infusions over weeks 1–6 while irradiation in both groups consisted in 40 Gy in 4 weeks. If response or disease stabilization was observed, Nimotuzumab was continued until disease progression or unmanageable toxicity. Primary endpoint was disease control rate (DCR = CR+PR+SD) and secondary endpoints were overall survival (OS) and safety.
Here we present the data from the first 21 patients. DCR was 91.6% (11 pts SD and1 pt PD) for the Nimotuzumab and radiation arm, compared to 44.4% (4 pts SD and 5 pts PD) for the control group. The analysis of overall survival (OS) showed that patients treated with the combination had a mean and median survival of 7.32 and 7.00 months respectively (5 patients still alive), compared with the control group for whom the mean and median survival was of 3.03 and 2.47 months, respectively (1 patient still alive). This difference reached statistical significance ($p = 0.0039$, Log Rank test).
Nimotuzumab administered concurrently with whole brain radiation therapy was well tolerated in NSCLC patients with unresectable brain metastases. The antibody did not provoke skin rash or GI adverse events. Substantial radiological responses and meaningful clinical responses have been seen in patients treated to date.

506 POSTER

The apogenic anti-CD9 antibody, AR40A746.2.3, inhibits tumor growth in breast and pancreatic cancer and targets cancer stem cells in acute myeloid leukemia

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Background: CD9 is a 24 kDa member of the tetraspanin family. It interacts with assorted receptors and is involved in a variety of cellular functions. Its surprising role in cancer biology and its function on cancer stem cells is only now being delineated. Using the ARIUS FunctionFIRST™ platform, AR40A746.2.3, an apogenic monoclonal antibody targeting CD9, was discovered.
Methods: Immunohistochemical (IHC) and FACS were performed to determine the distribution of the AR40A746.2.3 epitope on normal and tumor cells. The cytotoxicity of AR40A746.2.3 was confirmed on a variety of cancer cell lines in vitro. Sub-cutaneous human pancreatic and breast cancer, as well as orthotopic established primary human AML xenograft models were used to define in vivo efficacy. Phosphoproteome profiler arrays were used to monitor cell signaling molecules. FACS was used to measure apoptosis in cells stained with Annexin-V.
Results: IHC revealed moderate to strong staining in 38% of assorted solid tumor samples and 60% of human pancreatic cancer samples. 16 of 20 AML samples contained a CD34+38− cancer stem cell population that was CD9 positive (42.7±10.1%). Stem cells from 8 normal lineage depleted cord blood samples expressed low levels of CD9 (9.9±2.8%). Similar results were found in bone marrow. AR40A746.2.3 induced cytotoxicity in a variety of solid tumor cell lines in vitro. AR40A746.2.3 treatment showed significant tumor growth inhibition in human pancreatic (99.56%TGI, $p < 0.0001$) and breast cancer (80.6%TGI, $p < 0.0001$) xenografts. AR40A746.2.3 treatment also inhibited established patient derived AML outgrowth of CD34+CD38−CD9+ AML stem cells in primary and secondary transplanted NOD/SCID mice. AR40A746.2.3 treatment led to a decrease in phosphorylation of receptor tyrosine kinases (ex. ErbB3 and RON). A reduction in phosphorylation of Akt and GSK-3 was also observed, indicating that AR40A746.2.3 affects important survival pathways in cancer. AR40A746.2.3 treatment potently induced apoptosis in BxPC-3 pancreatic cancer cells.
Conclusions: CD9 is a differentially expressed on AML CD34+CD38− cancer stem cells compared to normal cord blood and bone marrow stem cells. It is also broadly distributed in solid tumors. As a naked MAb, AR40A746.2.3 has significant anti-tumor efficacy in solid and liquid tumors and produces apoptosis through well established signaling pathways. These findings highlight the potential novel therapeutic value of targeting CD9 with a MAb in cancer.

507 POSTER

Potent antitumor activity of the anti-CD19 auristatin antibody-drug conjugate hBU12-vcMMAE in rituximab sensitive and resistant lymphomas

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The cell surface antigen CD19 is expressed on most cancers of lymphoid origin, including the vast majority of Non-Hodgkin Lymphoma (NHL), Chronic Lymphocytic Leukemia (CLL) and Acute Lymphoblastic Leukemia (ALL). Despite major advances in treatment options for NHL patients, including the use of cytotoxic compounds and the anti-CD20 antibody rituximab, a significant fraction of NHL patients will eventually relapse and salvage treatments with non-cross resistant compounds are needed to further improve patient survival. Here, we evaluated the antitumor effects of the microtubule destabilizing agent monomethyl auristatin E (MMAE) conjugated to the humanized anti-CD19 antibody hBU12 via a protease sensitive valine-citrulline (vc) dipeptide linker. Potent tumor cell killing by hBU12-vcMMAE was identified in rituximab resistant and sensitive CD19 /20 double-positive NHL cell lines. CD21 is a cell surface receptor with the potential to form heterodimers with CD19 and high levels of CD21 expression were reported to interfere with internalization and cell killing of some antibody-drug conjugates (ADCs) targeting CD19. We found similar frequencies of durable tumor regressions in mice xenografted with CD21 high and low expressing NHL tumors, suggesting that CD21 levels do not affect potency of the hBU12-vcMMAE conjugate. In support of this notion, comparable ADC internalization, intracellular trafficking and release of free drug was observed in CD21 low and high expressing tumor cell lines treated with hBU12-vcMMAE. Combined, our data support further studies with hBU12-vcMMAE as a novel therapeutic option for the treatment of untreated and rituximab refractory NHL as well as other hematologic malignancies, including CLL and ALL.

508 POSTER

PDL192, a novel, humanized antibody to TWEAK receptor, shows potent anti-tumor activity in preclinical models

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Background: TWEAK receptor (TweakR) is a member of the tumor necrosis factor receptor superfamily, a group of receptors that has garnered significant interest as therapeutic targets in cancer and autoimmune disease. TweakR expression has been reported in some solid tumors. In addition, signaling via TweakR can induce apoptosis in certain cancer cell lines.
Methods: Expression of TweakR in primary tumors was assessed by immunohistochemistry (IHC). PDL192, a novel, humanized, IgG1 monoclonal antibody to TweakR, was assessed for its ability to inhibit the growth of cancer cell lines both *in vitro* (soft agar and anchorage-dependent proliferation assays) and *in vivo* (xenograft models in SCID mice). In addition, antibody-dependent cellular cytotoxicity (ADCC) assays were performed with PDL192 on tumor cell lines using peripheral blood mononuclear cells from normal human donors in a ^{51}Cr release assay.
Results: TweakR was found to be expressed by IHC in a variety of solid tumors, including pancreatic, breast, lung, and renal cancer. In *in vitro* assays, the anti-TweakR antibody, PDL192, inhibited the growth of approximately 30% of TweakR-expressing cancer cell lines tested. PDL192 also potently induced ADCC against TweakR-expressing cancer cell lines *in vitro*. PDL192 significantly inhibited tumor growth in several xenograft models representing a variety of tumor types. In an orthotopic model of breast cancer, PDL192 inhibited both primary tumor growth as well as the growth of lung metastases. In a pancreatic model, PDL192 or gemcitabine significantly slowed tumor growth; however, the two agents in combination caused complete tumor regression.
Conclusions: The humanized anti-TweakR antibody, PDL192, has been found to have anti-tumor effects against multiple TweakR-expressing tumor cell lines both *in vitro* and in xenograft models. In addition, PDL192 has been found to enhance the anti-tumor activity of some chemotherapeutic agents in xenograft models. These data, together with the histological data showing that TweakR is expressed on a variety of tumor types, suggest that PDL192 has the potential to be a therapy for patients with solid tumors. This data is the basis for an upcoming Phase I safety study of PDL192 in patients with solid tumors.