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Efficacy of EGFR and IGF-1R antibody therapy is independent of PTEN status in a selection of tumor models

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Mutational inactivation or deletion of the phosphatase and tensin homologue on chromosome10 (PTEN)/MMAC1/TEP gene in human cancer cells leads to a constitutive activation of the PI3 kinase/Akt pathway in cancer cells. This constitutive activation may underlie resistance to therapies targeting receptors such as epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 receptor (IGF-1R). We have evaluated whether loss of PTEN can alter the therapeutic response towards Cetuximab and IMC-A12, monoclonal antibodies targeting EGFR and IGF1R, respectively. The present study was aimed at evaluating the in vitro and in vivo responses to Cetuximab and IMC-A12 on PTEN null cancer cells (PC3 and U87MG) transfected with a functional PTEN gene. In addition we evaluated the effects of siRNA based abrogation of PTEN expression in PTEN wild type cells (BxPC3). The presence or absence of PTEN was demonstrated by western blotting or ELISA and functional PTEN was demonstrated by pAKT western blotting. In vitro proliferation assay showed no difference in growth pattern of either phenotypes of BxPC3. PTEN transfected PC3 and U87 cells showed 10–15% less tumor growth in vivo compared to mock transfected cells. However, presence or absence of PTEN has no significant impact on the efficacy of either cetuximab or IMC-A12 on these established xenograft models. In a resistant PC3 xenograft model response towards cetuximab or IMC-A12 remained the same in either phenotypes with %T/C values of cetuximab: 60% on PC3-Mock and 63% on PC3-PTEN; IMC-A12: 73% on PC3-Mock and 69% on PC3-PTEN. Similarly response towards cetuximab and IMC-A12 on U87MG remained the same. %T/C of cetuximab: 60% and 77% on mock transfected and PTEN cells respectively; IMC-A12: 50% and 57% respectively.
While some results are pending, the existing results suggest that cetuximab and IMC-A12 efficacy are not significantly related to PTEN status in the selected models.

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A monoclonal antibody (AR36A36.11.1) with potent in vivo efficacy in multiple human cancer models targets CD59

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Complement (C) activation leads to cell lysis resulting from the formation of membrane attack complexes (MAC). MACs are blocked by CD59 a C regulatory protein that is over expressed in tumor cells such as breast, colon and prostate carcinomas to evade C lyses. Targeting CD59 with antibodies to prevent this evasion is a potentially effective therapeutic approach. AR36A36.11.1, a functional monoclonal antibody targeting CD59, discovered using Arius' FunctionFIRST™ antibody generation platform, has been studied for its potential as a cancer therapeutic.
Tissue expression and distribution of CD59 detected with AR36A36.11.1 were determined by immunohistochemistry. Cytotoxicity was determined in vitro. In vivo efficacy was tested in xenografts of human cancers. PEPSCAN technology was used to determine the epitope for AR36A36.11.1 and affinity was determined by Biacore. Cytotoxic mechanism of action was assessed using in vitro C dependent cell (CDC) lysis and antigen-dependent cell-mediated cytotoxicity (ADCC).
The epitope for AR36A36.11.1 was present in various normal human tissues and was over expressed in several cancer types. AR36A36.11.1 induced in vitro cytotoxicity in the absence of effector cells and C in prostate cancer cell lines. AR36A36.11.1 exhibited potent in vivo efficacy resulting in tumor growth inhibition of 100% ($p < 0.0023$), 86% ($p < 0.0009$), 58% ($p < 0.031$) and 48% ($p < 0.0216$) in prophylactic xenografts of breast, prostate, lung and colon cancers, respectively. In an established model of breast cancer, AR36A36.11.1 exhibited highly potent dose-dependent efficacy and extended survival of the animals. In an established prostate cancer model, AR36A36.11.1 compared favorably with Taxotere. Humanized AR36A36.11.1 has similar affinity and efficacy to its murine form. The epitope for AR36A36.11.1 resides within the CD59 MAC inhibitory site consistent with the ability of AR36A36.11.1 to enhance CDC. This antibody also enhanced ADCC.
AR36A36.11.1 has significant in vivo anti-tumor activity towards a broad range of high incidence cancers. The effectiveness of AR36A36.11.1 may be due to enhanced CDC through its CD59 blocking functions. However, the potency of the antibody is maintained at low dose levels that do not activate CDC in vitro. Therefore, C-independent pathways such as ADCC or intracellular signaling may also play a role in the efficacy of AR36A36.11.1. Antibody-mediated blockade of CD59 represents a novel approach to cancer treatment.

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Novel antibody-maytansinoid conjugates with efficacy against multidrug resistant tumors

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Chemotherapy often leads to a multidrug resistant (MDR) phenotype characterized by resistance of tumor cells to a broad spectrum of anticancer drugs. One of the best studied mediators of MDR is P-glycoprotein (Pgp), also known as MDR1 or ABCB1. Pgp blocks the uptake of drugs by cells from extracellular space, and pumps various neutral or positively charged compounds out of the cells. Maytansine and its derivatives are substrates of Pgp. In this study, we used antibodies to deliver our proprietary maytansine derivatives, DM1 and DM4, inside Pgp-positive cells. These maytansinoids were conjugated to an EpCAM-binding antibody, B38.1, via either a SPDB disulfide linker, a SMCC non-reducible thioether linker, or a PEG4-containing non-reducible linker. After the conjugates bound to and entered EpCAM-expressing cells, the disulfide-linked conjugate was metabolized to neutral maytansinoid derivatives (DM4 and S-methyl-DM4), the thioether-linked conjugate to a charged product (Lysine-SMCC-DM1), and the PEG4-linked conjugate to a polar, charged compound (Lysine-PEG4-DM1). The cytotoxic potencies of these conjugates in vitro were tested toward three cell lines, the human colon carcinoma COLO 205 (EpCAM+/Pgp−), a COLO 205 clone (COLO205-MDR (EpCAM+/Pgp+) engineered to overexpress Pgp, and the naturally evolved MDR human colon carcinoma HCT15 (EpCAM+/Pgp+). The three conjugates had similar potencies toward COLO 205 cells, but differed in their activities toward COLO205-MDR and HCT15 cells. The PEG4-linked conjugate demonstrated the greatest potency, while the disulfide-linked conjugate was the least active one. The activity of the disulfide- and SMCC-linked conjugates toward COLO205-MDR and HCT15 cells was enhanced in the presence of the Pgp inhibitor cyclosporin A, suggesting that Pgp is the likely cause for the different activities of the conjugates. The activity of these conjugates against these cell lines in vivo were evaluated in subcutaneous xenograft models in SCID mice. The B38.1-PEG4-DM1 conjugate showed greater anti-tumor activity against HCT15 and COLO205-MDR tumors than either the B38.1-SMCC-DM1, or B38.1-SPDB-DM4 conjugates. The advantage of the PEG4 linker was also demonstrated with maytansinoid conjugates of an anti-CanAg antibody, huC242. Thus, antibody-maytansinoid conjugates bearing the novel PEG linker represent a promising approach for the treatment of multidrug resistant tumors.

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A phase 2, single-arm study of volociximab (an anti-a5β1 integrin antibody) monotherapy in patients with platinum-resistant advanced epithelial ovarian cancer or primary peritoneal cancer

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Background: A critical survival step for proliferating vascular endothelial cells is the ligation of fibronectin in the extracellular matrix to $\alpha5\beta1$ integrin. Volociximab, a chimeric monoclonal antibody, blocks fibronectin binding to $\alpha5\beta1$ and induces apoptosis in proliferating endothelial cells.
Materials and Methods: Volociximab was studied in a phase 2, single-arm, multicenter, 2-stage study in platinum-resistant epithelial ovarian or primary peritoneal cancer patients (pts) who had measurable disease with progression after topotecan or pegylated liposomal doxorubicin. Volociximab was administered at 15 mg/kg IV weekly until progression of disease

(PD) or drug intolerability. Study objectives were to evaluate efficacy, safety, pharmacokinetics (PK), and pharmacodynamics. If ⩾3/23 pts in Stage 1 had a response by RECIST, the study could proceed to Stage 2. Serum samples for PK were obtained on Days 1, 15, 29, and 50. Ascitic fluid obtained when possible was analyzed for drug levels. Archived tumor tissue was analyzed by immunohistochemistry (IHC) for α5 integrin expression.
Results: Sixteen pts were enrolled in Stage 1; interim data are currently available on 14 pts. Median age was 61yrs (54−80 yrs); performance status was 0 (5 pts) or 1 (9 pts); and disease stage was III (7 pts) or IV (6 pts). Fourteen pts received prior chemotherapy (2−4 regimens); no pts had prior anti-angiogenic treatment. All 13 efficacy evaluable pts had investigator assessed PD by Week 8. The majority of pts (11 pts; 85%) had Grade 2 and 3 adverse events (AEs), the most common being headache, abdominal distension, nausea and fatigue in 4 pts (31%). Five SAEs in 4 pts were considered probably/possibly related to volociximab: deep vein thrombosis, hypertensive encephalopathy, facial palsy, pulmonary embolism, and small intestinal obstruction. Peak serum concentrations of volociximab increased approximately 3-fold from Day 1 to Day 50, and trough levels were in excess of 150 mcg/mL, a level which correlated with efficacy in preclinical xenograft models. Volociximab was also measurable in ascitic fluid samples. IHC analysis of archived tumor sections showed low-to-moderate expression (H-score, 145±37) of α5 integrin by malignant epithelial cells.
Conclusions: Weekly volociximab was well tolerated; however, there was insufficient clinical activity to support continued enrollment. Limited clinical activity precluded assessment of the potential relevance of tumor expression of α5 to response.

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A monoclonal antibody targeting Trop-2 exhibits anti-tumor efficacy in human cancer models as a monotherapy and demonstrates efficacy in combination therapy

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AR47A6.4.2, a monoclonal antibody targeting human Trop-2, was generated using the ARIUS FunctionFIRST™ platform. Trop-2 is a signal transduction molecule with widespread expression reported in a number of human carcinomas. Clinically, high levels of Trop-2 expression have been correlated with poor prognosis and decreased survival in colorectal and head and neck carcinomas, and have been implicated breast cancer metastasis. Overexpression studies have validated Trop-2 as an oncogene, as its overexpression alone was sufficient to induce tumorigenesis in mice. Taken together, these findings confirm that targeting Trop-2 in cancer patients may be an effective therapeutic strategy.
Following treatment with AR47A6.4.2 significant tumor growth inhibition was observed in models of human breast, pancreatic, colon, and prostate cancers. There was also an associated increase in the survival. The potency of AR47A6.4.2 was enhanced in a model of pancreatic cancer when administered in combination with Gemcitabine. Combination therapy resulted in tumor regression during the treatment period, and 97% tumor growth inhibition was observed 27 days after treatment ended. In addition, AR47A6.4.2 showed potent anti-tumor activity (96% tumor inhibition) in combination therapy with Irinotecan in a colorectal cancer model.
Mechanism of action studies have revealed that AR47A6.4.2 treatment diminishes MAPK phosphorylation in response to serum stimulation, suggesting that AR47A6.4.2 may exert some of its anti-tumor effects by inhibiting TROP-2 signaling. AR47A6.4.2 was also shown to induce CDC in vitro in pancreatic cancer cell lines where AR47A6.4.2 has exhibited anti-tumor activity.
A humanized version of AR47A6.4.2 was generated with high affinity and potent anti-tumor activity. The Cynomolgus monkey was identified as a relevant pre-clinical toxicology model, and the humanized molecule was assessed in a dose-ranging toxicology study. No dose limiting toxicity was discovered.
AR47A6.4.2 is the first naked therapeutic antibody targeting Trop-2. It demonstrates significant anti-tumor in vivo efficacy in xenograft models of human pancreatic, colon, breast and prostate cancer as a monotherapeutic agent as well as in combination with chemotherapy. It is now undergoing preparation for human clinical studies to confirm its potential therapeutic value in treating solid tumor malignancies.

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A monoclonal antibody targeting the breast cancer stem cell marker melanoma-associated chondroitin sulfate proteoglycan improves survival and demonstrates anti-tumor activity in vivo

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Melanoma-associated chondroitin sulfate proteoglycan (MCSP, also known as the high molecular weight-melanoma associated antigen) is a glycoprotein-proteoglycan complex primarily restricted to the surface of melanoma cells. MCSP has been reported to regulate cell proliferation and motility by two distinct Thr phosphorylation events leading to its role in cancer progression. MCSP has been shown recently to be expressed on human CD44+CD24low breast cancer stem cells.
Few antibodies directed against MCSP show any in vivo anticancer activity suggesting the difficulty of drugging this target by conventional means. AR11BD-2E11−2, the first functional monoclonal antibody that targets MCSP was discovered using the FunctionFIRST™ platform. AR11BD-2E11−2 showed cytotoxicity in breast and ovarian cancer cell lines in vitro. When AR11BD-2E11−2 was evaluated in vivo, anti-tumor activity was apparent in breast (MCF-7 and MDA-MB-231) and ovarian (OVCAR-3) tumor models, as well as in a model of human melanoma (A2058). In the MCF-7 xenograft breast cancer model, AR11BD-2E11−2 reduced tumor volume by 79% compared to isotype control treated mice ($p = 0.048$), and conferred a significant survival benefit ($p = 0.03$).
Immunohistochemistry analyses revealed that the staining pattern for the epitope recognized by AR11BD-2E11−2 on frozen human breast cancer and melanoma sections was highly specific for malignant cells. Normal epithelial staining with AR11BD-2E11−2 was generally minimal. Western analysis of tissue lysates showed that the expression profile of AR11BD-2E11−2 was similar across tissues from humans, Chinese hamsters and cynomolgus monkeys indicating that these two species may be relevant models for toxicology and pharmacokinetics evaluations. Different forms of AR11BD-2E11−2 are currently being developed for clinical use.
The anti-MCSP antibody AR11BD-2E11−2 has exhibited significant anti-tumor efficacy in human xenograft tumor models of melanoma, ovarian and breast cancer. Since MCSP was recently identified on breast cancer stem cells, the epitope of AR11BD-2E11−2 may be a particularly important antibody target for the treatment of breast cancer. The fact that this epitope on MCSP was identified using the Arius FunctionFIRST™ platform highlights the importance of this platform in identifying functional epitopes within important cancer targets.

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Discovery of the 6F4 anti-tumor antibody targeting the tight junction molecule JAM-A. 1. Target identification by proteomic approach

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Functional approaches have been used to identify potential new targets for therapeutic application in oncology. After immunisation of mice with tumor cells and fusion, hybridoma supernatants were screened for their anti-proliferative properties. The 6F4 monoclonal antibody (Mab) was selected on the basis of its ability to inhibit in vitro proliferation of MCF-7 breast cancer cells. Its target was further shown to be a 35 kDa membrane glycoprotein, which is highly expressed on various human tumor cell lines. A method based on affinity purification and proteomic analysis was used to identify the target antigen of the 6F4 Mab. Small-scale immunoprecipitation experiments were performed with 6F4 Mab-Sepharose beads to evaluate different conditions of antigen binding, gel washing and protein elution. After binding, washing steps with detergents were introduced to dissociate the target protein from heteromeric protein complexes thereby eliminating strongly associated proteins. Optimal conditions were further applied to develop a large-scale immunopurification process. The 6F4 Mab target was purified from a HT-29 cell membrane extract. Proteins bound to the 6F4 affinity column were eluted by decreasing the pH and subjected to SDS-PAGE and western blot analyses. The 35 kDa band, specifically visualised by western blot with 6F4 Mab, was excised from a 1D gel stained with a MS-compatible Coomassie blue staining method. After in-gel digestion with trypsin, peptides were extracted and analysed by mass spectrometry using MALDI-TOF and ion trap LC-MS/MS mass spectrometers. Protein identification was achieved through database searching with the Mascot software.
The target of the 6F4 Mab was unambiguously identified to be the F11 receptor (F11R) or Junctional Adhesion Molecule A (JAM-A). This identification was further confirmed by immunoprecipitation and