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Preclinical efficacy evaluation of MLN2704: A chemotherapeutic-monoclonal antibody conjugate targeting prostate-specific membrane antigen (PSMA)

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Targeted delivery of chemotherapeutic agents coupled to tumor-specific monoclonal antibodies may enhance anti-tumor efficacy and reduce systemic toxicity. We are developing MLN2704, a novel therapeutic combining DM1, a microtubule-inhibiting maytansanoid, with MLN591, a de-immunized Tumor targeted Monoclonal Antibody Vehicle (T-MAV) specific for PSMA. *In vitro* analysis of MLN2704 demonstrated rapid intracellular uptake and potent nanomolar, antigen-dependent cytotoxicity against LNCaP cells. Furthermore, *in vitro* pharmacodynamic analysis of MLN2704 indicates that exposure time is a relatively more important variable than concentration for cytotoxic effect on PSMA-positive LNCaP cells. We examined MLN2704 efficacy in the CWR22 and 22Rv1 prostate cancer xenograft models. By probing sections of CWR22 xenografts for anti-human IgG, we found that intravenously delivered MLN2704 distributed into the tumor through the vasculature, achieved maximal staining intensity by 24 hours and maximal distribution by 72 hours and human IgG was still detectable up to 21 days post-injection. In the CWR22 model, MLN2704 exhibited dose- and schedule-dependent efficacy that was distinct from either its antibody or DM1 constituents when administered individually. When delivered on a q3dX5 schedule, treatment of CWR22 xenografts with MLN2704 delayed tumor growth to 1000mm3 by 46.4 days relative to PBS controls. On this regimen, MLN2704 treatment produced no overt toxicity as judged by body weight loss or mortality. By comparison, molar equivalent doses of DM1 and the MLN591 antibody delivered on the q3dX5 schedule produced tumor delays of 9.5 and 1.9 days, respectively. After the initial course of treatment with MLN2704, CWR22 xenografts eventually resumed growth, but tumors averaging ~1000mm3 were sensitive to a second course of MLN2704 treatment, exhibiting 75-80% of the growth delays obtained in the initial treatment. Additionally, MLN2704 was effective against the growth of 22Rv1 xenograft models; however, these models exhibited a different dose interval dependency compared to the CWR22 model. Response of the CWR22 xenograft to MLN2704 was correlated with serum PSA levels. In summary, MLN2704 demonstrated efficacy against the growth of PSMA-expressing prostate cancer cells and xenograft tumors. Length of exposure to MLN2704 was a critical determinant of efficacy both *in vitro* and *in vivo*.

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CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of non-hodgkin's lymphoma

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Antibody-targeted chemotherapeutic strategy relies on antibody-mediated preferential delivery of the cytotoxic agent to the tumor and reduces the exposure of normal tissues to the same cytotoxic agent. This has now been clinically validated in the form of Mylotarg (CMA-676) which remains the only US FDA-approved antibody-targeted chemotherapeutic to date and is indicated for the treatment of relapsed CD33+ AML. It is an immunoconjugate in which a derivative of Calicheamicin gamma (Calich.) is conjugated to a humanized IgG4 anti-CD33 mAb. Calicheamicin gamma binds DNA in its minor groove and causes double strand breaks leading to cellular apoptosis. Employing the same conjugation technology used in Mylotarg, we have developed a new immunoconjugate, CMC-544, targeted for B lymphoid malignancies. CMC-544 is an immunoconjugate in which Calich. is linked to a humanized IgG4 anti-CD22 antibody (G544). CD22, expressed by most B lymphoid malignancies, is internalized efficiently when bound by the antibody and thus, is ideally suited for the intracellular delivery of cytotoxic agents. CMC-544 binds CD22 with high affinity (KD = 120 pM) and is cytotoxic *in vitro* to a series of human CD22+ B lymphoma lines (IC_{50} = 10 - 500 pM) being 20 fold more potent than a control conjugate CMA-676. In RL and RAMOS B lymphoma xenograft models in nude mice, CMC-544, when administered ip q4dx3 post-tumor-staging at 20 mcg of conjugated calich./kg, causes strong inhibition of B lymphoma growth (Therapeutic index = 12). CMC-544 also causes complete regression of both small and large established tumors at doses that are less than half of the LD10 of CMC-544, which is 256 mcg of conjugated calich./kg. CMC-544 produces cures (tumor-free for >100 days) at 120 mcg of conjugated calich./kg in both these models (Curative index of >2). In contrast, the CD33-targeted conjugate, CMA-676, used as a negative control, fails to impact growth of CD22+ CD33- B lymphomas. Neither unconjugated calich. nor unconjugated anti-CD22 mAb G544 has any effect on the growth of B lymphoma xenografts in these models. In addition, in a model of systemically disseminated B lymphoma, CMC-544, but not the control conjugate CMA-676, significantly prolongs survival (>80 days) of mice with systemically disseminated CD22+ CD33- B lymphoma. Taken together, this demonstration of preclinical antitumor efficacy of CMC-544 strongly supports its clinical evaluation as a targeted chemotherapeutic for B lymphomas.

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Enhanced antitumor activity of anti-epidermal growth factor receptor monoclonal antibody cetuximab (IMC-C225) in combination with irinotecan (CPT-11), 5-FU, and leucovorin against human colorectal carcinoma xenografts

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Colorectal carcinomas (CRC) frequently express the epidermal growth factor receptor (EGFR) and this expression correlates with more aggressive disease and poor prognosis. Specific inhibitors of EGFR activation such as the anti-EGFR monoclonal antibody ERBITUX™ (cetuximab) have been shown to inhibit the *in vitro* and *in vivo* growth of human CRC tumor cells in pre-clinical models. Cetuximab is currently undergoing clinical testing in a number of EGFR+ human cancers including CRC. In this study, we have evaluated the activity of cetuximab combined with a three-drug chemotherapy regimen of CPT-11/5-FU/leucovorin (LV) in both subcutaneous xenograft and intra-hepatic models of CRC. Athymic mice with established (0.2 cm3) DLD-1 or HT-29 tumors were treated with cetuximab (1 mg; q3d), CPT-11/5-FU/LV (42 mg-, 125 mg-, 6.7 mg/kg; q7dX4) or the combination. Treatment with cetuximab alone resulted in a modest inhibition of DLD-1 (48%) and HT-29 (29%) tumors. Combination therapy with cetuximab and CPT-11/5-FU/LV resulted in the greatest anti-tumor effect inhibiting both DLD-1 and HT-29 tumor growth 76% and 80%, respectively. Enhanced or synergistic activity between cetuximab and the three-drug regimen was seen from day 26 onward. Histological examination of tumors treated with combination therapy showed increased tumor cell apoptosis and extensive tumor necrosis. Cetuximab monotherapy inhibited the growth of intrahepatic DLD-1 and HT-29 tumors by 26% and 13%, respectively. The three-drug regimen of CPT-11/5-FU/LV resulted in >86% inhibition whereas combining cetuximab with the three-drug regimen resulted in >93% inhibition of intrahepatic tumor growth. Moreover, the majority of the animals given combination therapy showed no evidence of disease. No hepatic or metastatic masses were found in 56% of the DLD-1 or 67% of the HT-29 implanted livers. These results are consistent with previous reports showing the potentiation of anti-tumor activity when combining cetuximab with chemotherapeutic drugs. The present study suggests that combinations of cetuximab and CPT-11/5-FU/LV may be an effective therapy in the treatment of advanced colorectal carcinoma.

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A calicheamicin conjugate that targets LewisY selectively destroys LewisY-positive human carcinoma cells and xenografts

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The low therapeutic index of most anticancer drugs prohibits administration of sufficient amounts to obtain a curative effect. Antibody-targeted chemotherapy can provide a solution to this problem. This strategy uses a cytotoxic agent bound to an antibody that recognizes a tumor-associated-antigen. The antibody can deliver the cytotoxic agent specifically to the tumor and reduce systemic toxicity. We asked whether a conjugate of calicheamicin (CM) and an antibody that recognizes the LewisY (LeY) antigen would eliminate carcinomas that express this oligosaccharide antigen. CM is a potent cytotoxic antibiotic that causes double strand breaks of DNA. It has proven therapeutic value against acute myeloid leukemia when conjugated to an anti-CD33 antibody (MylotargTM). Expression of CD33 is limited to myeloid malignancies. In contrast, LeY is highly expressed on