chromatography and characterized by a variety of techniques, including SE-HPLC, SDS-PAGE, in vitro transcription translation (IVTT) assay using luciferase reporter system, and competition ELISA to measure the binding affinity for CD74. The in vitro cytotoxicity was determined in a B-cell lymphoma cell line (Daudi) and a multiple myeloma cell line (MC/CAR), using the MTS tetrazolium dye reduction assay or a BrdU colorimetric assay.

Results: The purified protein was shown to be a single peak by SE-HPLC and its MW determined by MALDI-TOF to be 177,150, which is in agreement with the MW of one IgG (150,000) plus two rpRNase molecules (24,000). Reducing-SDS-PAGE revealed the presence of 3 bands, one corresponding to the heavy chain and the other two appearing to be derived from the rpRNAse-fused light chains (38,526 and 36,700 by MS). Occurrence of the 2 light chains was due to uneven glycosylation of rpRNase, since the two light chain bands converged to a single band after treatment with N-glycosidase. The EC_{50} of RNAse activity, as measured by the IVTT assay, was 300 pM for rpRNAse-hLL1 and 30 pM for free rpRNAse. The binding affinity of rpRNAse-hLL1 for CD74 was indistinguishable from that of hLL1. rpRNAse-hLL1 was significantly more cytotoxic to Daudi (EC_{50} 280pM (than MC/CAR (EC_{50} 50nM). Free rpRNAse did not demonstrate significant cytotoxicity at the concentrations tested.

Conclusion: A novel immunotoxin was expressed in a mammalian system. About 60% of the rpRNAse was found to be glycosylated. The fusion protein retained activity of rpRNAse and the binding affinity of hLL1 antibody, and demonstrated potent toxicity to CD74+ cells.

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Therapeutic implications of an antibody to the human macrophage-stimulating protein receptor tyrosine kinase (RON)

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The Macrophage-Stimulating Protein receptor aka. MSP-R or RON belongs to the c-MET family of receptor tyrosine kinases. The ligand for c-MET – Hepatocyte Growth Factor (HGF) as well as RON's ligand, MSP are members of the kringle-domain plasminogen-related protein family. As its name implies, MSP was originally found to stimulate macrophages by a variety of means. For example, addition of MSP to certain RON-expressing macrophages induced shape changes, chemotaxis, macropinocytosis and phagocytosis. RON was also found to be expressed in epithelial cells such as keratinocytes where MSP was shown to phosphorylate RON and activate a number of signaling pathways that elicited cell adhesion/motility, anti-apoptotic and proliferative responses. Within the last few years, however, over-expression of RON has been observed in several epithelial tumors and cell lines (ex. colon, breast and lung). In addition, the oncogenic potential of RON was recently demonstrated when lung tumors developed in transgenic mice engineered to over-express RON in their lungs. Although these data suggest a link between RON expression and cancer, studies to address whether inhibition of RON could abrogate tumor or cancer cell line growth have not been reported. Through the screening of a Fab phage display library, we have developed IMC-41A10, a monoclonal antibody that binds to human RON with an affinity of ~1.5 nM and inhibits MSP binding to RON with an IC_{50} of ~2 nM. IMC-41A10 demonstrated significant inhibition of the proliferation of RON-expressing HT-29 colon cancer cells grown adherently or as colonies in soft agar. Moreover, IMC-41A10 showed a 50–60% inhibition of tumor volumes when HT-29 cells were grown subcutaneously in nude mice. To our knowledge, this is the first demonstration that inhibition of the RON receptor tyrosine kinase negatively influences the proliferation of colon cancer cells *in vitro* and *in vivo*. In addition, it underscores the potential therapeutic utility of inhibiting RON in colon and possibly other cancers.

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Inhibition of FLT3-expressing leukemia cells by a monoclonal antibody-auristatin conjugate

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The receptor tyrosine kinase FLT3 is overexpressed in blasts of ~90% of acute myelogenous leukemia (AML) and the majority of B-lymphoid leukemia patients. Internal tandem duplications (ITDs) in the juxtamembrane region and point mutations in the kinase domain of FLT3 are found in ~37% of AML patients and are associated with a poor prognosis. We have recently developed a fully human monoclonal antibody (IMC-EB10) which binds with high affinity to FLT3 receptor on human leukemia cells. In the present study, a novel auristatin conjugate of the anti-FLT3 antibody (EB10-MMAF) was prepared using a dipeptide linker that allows for drug release inside the lysosomes of antigen-positive cells. The MMAF conjugates were stable in buffers and plasma. EB10-MMAF (drug/antibody raito=7.6) was highly potent, and selectively inhibited the growth of FLT3-expressing leukemia cells with an IC50 of 0.19 nM and 0.08 nM for MV4;11 and BaF3-ITD cells (both positive for FLT3-ITD), 1.11 nM, 6.18 nM and 1.82 nM for REH, EOL-1, EM3 cells (all three positive for wild-type FLT3), and 135 nM for JM1 (negative for FLT3). An MMAF conjugate with a control antibody was not active in these cell lines (IC50s > 5.9 uM). Flow cytometric analysis with annexin V indicated that EB10-MMAF treatment induced apoptosis of leukemia cells *in vitro*. The *in vivo* efficacy of the conjugate is being investigated in several FLT3-positive human xenograft leukemia models in NOD-SCID mice.

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Therapeutic efficacy of the Y-90 labeled antibody 19G9, targeting a novel protein RG-1, expressed in metastatic prostate cancer

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RG-1 (a human mindin homologue) is expressed selectively in prostate tissues in the human male. We have shown by analysis of RG-1 mRNA and protein levels that its expression is high in prostate tumors but low in most other tissues. We have extended this analysis to include prostate tumor metastases to soft tissues and bone and found that RG-1 expression is maintained in greater than 75% of metastatic bone tumors, 70% of lymph node metastases, and 85% of locally recurrent tumors in androgen unresponsive patients. Fully human antibodies, 19G9 and 34E1, have been generated against the RG-1 protein and have been shown to accumulate at high abundance in LNCaP tumor xenografts. This has been observed with hybridoma generated antibodies and antibodies expressed in CHO cells. Conjugates of these antibodies with CHX-A"-DTPA have been generated and radiolabeled with either Y-90 or Y-86. MicroPET imaging with the Y-86 radiolabeled 19G9 antibody demonstrated very specific accumulation of the antibody in LNCaP tumor xenografts with clear tumor delineation apparent at 4 hours and exceptional tumor to background contrast at 72 hours. The therapeutic efficacy of Y-90-CHX-A"-19G9 was evaluated in mice bearing LNCaP xenografts. An MTD study identified a non-toxic therapeutic dose to be 75–100 μCi. Significant anti-tumor efficacy of the Y-90 antibody conjugate was seen with a single administration of radiolabeled antibody to animals bearing 200–400 mm^3 tumors. Inhibition of tumor growth was seen in all treated animals over a 45-day period. At 49 days post treatment, slow tumor growth recurred but this regrowth could be prevented for an additional 40-day period by a second administration of a 75 μCi dose on day 49. We conclude that Y-90-CHX-A"-19G9 is a novel human antibody conjugate that has considerable promise for the effective therapy of metastatic prostate cancer in androgen unresponsive patients.

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Enhanced apoptosis and tumor regression induced by a direct agonist antibody to TRAIL-R2

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Substantial evidence indicates that supraoligomerization of the death receptors for Fas ligand (FasL) and Tumor necrosis factor-related apoptosis-Inducing ligand (TRAIL) is necessary for efficient activation of the apoptotic pathway. Oligomerization of Fas or TRAIL receptors with bivalent IgG antibodies can mimic the natural ligands, but only after these antibodies are further oligomerized by the addition of secondary crosslinking reagents. We report here a novel fully human IgG antibody to TRAIL-R2 (KMTR2, also known as HGS-TR2J) that directly activates tumor cell apoptosis in vitro without the requirement of crosslinking IgG. Size-exclusion chromatography demonstrated the apoptosis activity co-eluted with monomeric IgG and was effective independent of the