MEDAREX

Single, low dose treatment of lymphoma and renal cancer xenografts with human anti-CD70 antibody-toxin conjugates, results in long term cures

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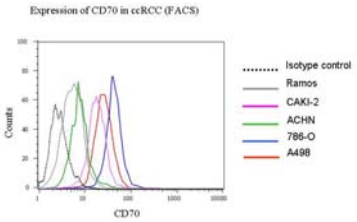
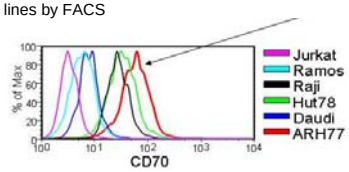
Introduction

CD70 is highly expressed in multiple tumor types including many lymphomas and clear cell renal carcinomas. The expression in lymphomas is not surprising given the role of CD70 in B and T cell activation. However, expression of CD70 at high levels and very high prevalence in renal cancer was initially unexpected. The tightly controlled and limited expression in normal tissues, together with this high level tumor expression makes CD70 an extremely attractive target for antibody-directed therapy. In order to exploit this specificity we have isolated and characterized a human anti-CD70 antibody of high affinity and specificity from transgenic mice. This antibody has been conjugated to highly potent DNA alkylating agents via hydrazone and amino acid based linkers. The resulting CD70 targeted antibody-drug conjugates show potent and curative anti-tumor activity at low doses; highly specific antigen targeting activity (as shown by comparing CD70 antibody conjugates with isotype control conjugates *in vivo*), and an extremely wide therapeutic window in both lymphoma and renal cancer models. Furthermore, these CD70 antibody drug conjugates are demonstrating favorable toxicity profiles in larger animals at levels far in excess of the xenograft model determined efficacious doses.

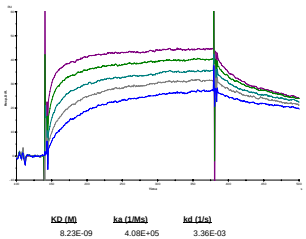
CD70 antibody binding



CD70 HuMAb binding to lymphoma cell lines by FACS

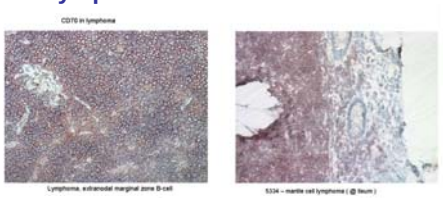


CD70 HuMAb binding to CD70_CD8 fusion protein



KD (M)	ka (1/Ms)	kd (1/s)
8.23E-09	4.08E+05	3.36E-03

Expression of CD70 in lymphoma

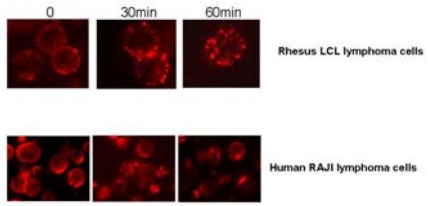


CD70 prevalence in lymphoma by IHC

Disease Type	Prevalence
Marginal Zone B Cell	7 / 11
DLBCL	14 / 15
Follicular Lymphoma	5 / 13
Peripheral T cell lymphoma	3 / 3
Hodgkins	4 / 4 (inflammatory cells)
Mantle Cell lymphoma	2 / 4

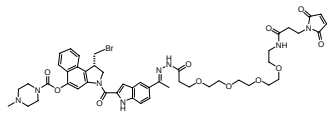
For such a specific anti-cancer target to function to its maximum with a toxin conjugate system, it is desirable that the target antigen is internalized upon antibody binding. The images **below** show rapid internalization of CD70.1 in lymphoma cell lines from humans and monkeys. Thus CD70 appears to be a suitable target for an antibody drug conjugate and that Rhesus is a suitable tox species for this anti CD70 HuMAb

Internalisation on Ab binding (lymphoma)



Development of toxins

MED2220 is a synthetic analogue of Duocarmycin which is conjugated to CD70.1 via an acid labile hydrazone linker) For internalizing antigens such as CD70, once the hydrazone linker is cleaved in the acid environment of the lysosome or endosome, the active toxin is released by carboxylesterase cleavage. The active toxin is a DNA alkylating agent. **MED2220 is shown below**.



MED-2220

Peptide linkers

we have developed peptide linker versions of MED2220 (n2, and n3)

A key factor in the development of these Ab drug conjugates is the determination of the toxicity, specificity and *in vivo* efficacy profiles. In order to compare the hydrazone and amino acid linker systems of MED2220 and n2/n3 we have used multiple CD70 positive xenograft tumor models. In these systems we have analysed the efficacy of CD70 and isotype control Abs (specificity). The potential toxicity has been assessed in tumor bearing SCID mice and wild type mice

RAJI xenograft : hydrazone linker

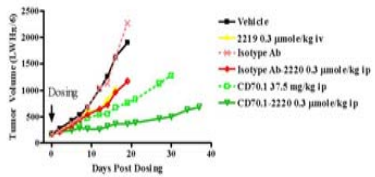


Figure above shows *in vivo* efficacy of MED2220 conjugates in a **RAJI lymphoma** xenograft model. Reasonable tumor control is achieved with a single dose which is more than 3X less than the MTD for MED2220 conjugates. MED2220 conjugated to a non tumor binding antibody shows only minimal efficay (red line). 2219 is the free toxin version of MED2220.

RAJI xenograft : peptide linker

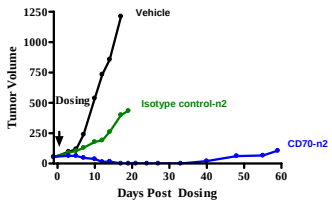


Figure above shows *in vivo* efficacy of CD70-n2 conjugates in a **RAJI lymphoma** xenograft model. These long term regressions were achieved with a single dose of 0.3µmol/kg

Efficacy in ccRCC model

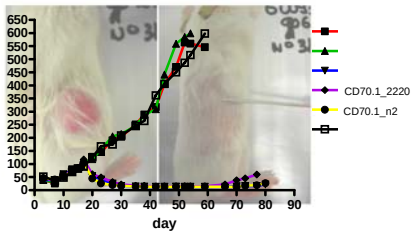


Figure above. Efficacy of MED2220 (2 x 0.3µmol/kg), and CD70-n2 (1 x 0.1µmol/kg) anti CD70 conjugates in a 786-O renal cancer xenograft model.

Single, low dose cures & wide therapeutic window

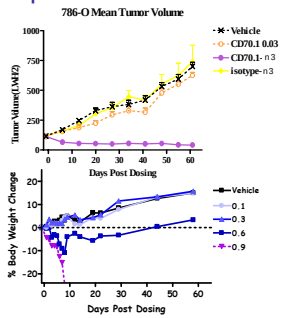
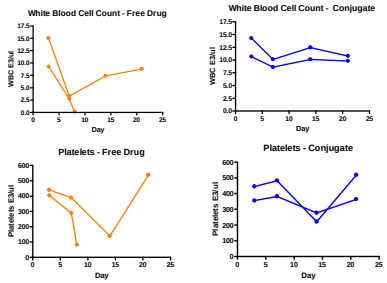


Figure above (top) shows *in vivo* efficacy of CD70-n3 conjugates at a single dose of 0.03µmol/kg in a **786-O xenograft renal cancer** model. Almost all tumors have completely regressed. **Figure above (bottom**) shows the body weight changes observed in balb/c mice treated with CD70.1_n3 at doses between 0.1 and 0.9µmol/kg - showing at least a 20-fold window between curative efficacy and MTD.

Preliminary Safety Study in Dogs

Free drug at 0.15µmol/kg; Conjugate at 0.18µmol/kg - CD70-n2



Conclusions

CD70 is now recognised as a very specific tumor antigen in lymphomas and renal cancer. The challenge is to develop systems to exploit this specificity leading to an efficacious and safe anti cancer therapeutic. We have developed a high affinity fully human anti CD70 antibody as a mechanism to target CD70 positive tumors. With the toxin and linker systems described here we show complete cures in CD70 positive lymphoma and renal cancer models with single, low dose treatments. Cures have been achieved at even lower doses such that the efficacy / toxicity window in mice can be as large as 200 fold. These CD70 antibody drug conjugates have also been tested in larger mammals at doses above the curative doses described here with no evidence of adverse toxicity.