Progress towards tumour therapy with ^{90}Y-mAb conjugates

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^{90}Y-cDTPA-mAb conjugates have been examined as potential vehicles for tumour radiotherapy but bone localisation of the radioisotope and the consequences thereof seriously limit their potential. We recently described a series of macrocyclic chelators, functionalised for attachment to mAbs, which form exceptionally stable complexes with Yttrium. We have now performed a series of biodistribution experiments in animals including a detailed bone study which demonstrate that our chelates give significantly lower bone uptake than the acyclic structure.

Order SE *et al. Antibody Immunoconj Radiopharm* 1988, **1**, 163.
Cox JPL *et al. J Chem Soc Chem Commun* 1989, **797**.

Clinical trials with ^{131}I-labelled chimeric antibodies

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We carried out a phase I dosimetry trial of ^{131}I-labelled chimeric 17-1A (IgG-1/K) and a phase I radioimmunotherapy trial with ^{131}I-labelled chimeric B72.3 (IgG-4/K). The pharmacokinetics of ^{131}I-ch-17-1A were identical to our prior studies with non-conjugated antibody (B half-life of 100 h) and the specific activity of the circulating antibody was constant throughout 14 days in the circulation, documenting that dehalogenation does not occur in the circulation. None of the 6 patients developed an antibody response to chimeric 17-1A. This study and the prior non-conjugated chi-1701A study thus had an antibody response to chimeric 17-1A occur in only 1/16 patients.

In the phase I radioimmunotherapy trial (still ongoing), the first 6 patients receiving an initial infusion of 18 mCi/m^2 (n = 3 patients) or 27 mCi/m^2 (n = 3 patients) of ch B72.3 will be reported. The pharmacokinetics of ch B72.3 were similar to ch-17-1A. No evidence for dehalogenation in the circulation was noted. 4 out of 6 patients developed an antibody response to ch-B72.3 after a single exposure to this reagent. The specificity of this immune response will be presented. A comparison of half-life and dosimetry estimates for these two chimeric reagents was carried out.

Antibody structure matters too

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Antibodies are proteins capable of making highly specific interactions with a virtually infinite range of antigens. Since the invention of monoclonal antibodies, numerous applications have been found in biology and medicine. A number of potential future applications have also been described, for example antibodies as biosensors and the generation of proteolytic antibody-enzymes. Such applications rely upon artificial modifications to antibody structure by techniques such as site directed mutagenesis (Roberts S *et al., Nature* 1987, **328**, 731–734). It is possible to modify antibody binding affinity, but without structural information, such modifications are made "blindfold".

Structural information on proteins is gained from X-ray crystallography or nuclear magnetic resonance (NMR) techniques. However, a typical X-ray structure takes some 2 years to solve and, as yet, NMR is unable to solve the structure of protein as large as an antibody F(ab) fragment. Thus modelling of antibody structures is the only practical approach to suggesting possible modifications. The development of modelling protocols allows us to understand something of the relationship between primary aminoacid sequence and tertiary structure. This is important in defining strategies by which to make intelligent modifications to antibody structure. We developed a "combined algorithm" for modelling antibodies which uses elements of both knowledge-based and *ab initio* techniques (Martin ACR *et al., Proc Natl Acad Sci USA*, 86, 9268–9272.

The results of such modelling applied to some structures which have already been solved by X-ray crystallography will be presented. Finally, some preliminary work on an automated procedure for docking antibody and antigen, in the computer, will be described.

Microbial production of anti-tumour antibody fragments

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We have developed yeast and bacterial systems for secretion of genetically engineered antibody fragments. This is accomplished by first fusing mouse light chain variable regions to a human light chain constant region module to form chimeric light chain genes. Similarly, mouse heavy chain variable regions are fused to a genetically engineered human gamma 1 heavy chain constant region module consisting of Ch1 to form chimeric Fd chain genes. For expression in the yeast system, the mature chimeric light and Fd chain genes coding for specificity to tumour cell antigens are fused to a yeast promotor, signal sequence and polyadenylation signal. For expression in the bacterial system, the mature Fd and light chains are fused to a bacterial signal sequence and then to each other to form a dicistronic unit which is placed under the control of a strong, inducible promoter. Simultaneous expression of these genes results in the secretion of antibody fragments into the culture supernatants. These fragments are correctly folded and associated as determined by both direct and competition binding assays with tumour cell lines containing the target antigen.

Tumour localisation and normal human tissue reactivity of a chimeric B72.3 antibody

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The *in vitro* reactivity on frozen normal human tissues and the *in vivo* pharmacokinetics and tumour imaging efficacy in mice of a site-specific GYK-DTPA conjugate of an IgG1 human/mouse chimeric B72.3 antibody (chB72.3) were compared to those of a similar immunoconjugate of murine B72.3 (B72.3-GYK-DTPA). Both antibodies were easily conjugated to GYK-DTPA and both conjugates labelled efficiently with ^{111}In. The normal human tissue reactivity of chB72.3 was consistent with that expected for a TAG-72 reactive antibody. ChB72.3-GYK-DTPA-^{111}In localised to and imaged LS174T human colon adenocarcinoma xenografts grown in nude mice, although the maximum %ID/g tumour observed was less than that observed with murine B72.3-GYK-DTPA-^{111}In. This may be due to the faster whole body and blood clearance of chB72.3-GYK-DTPA-^{111}In in these animals. Thus, the *in vitro* reactivity on frozen normal human tissues and the *in vivo* pharmacokinetics in mice of the chimeric B72.3-GYK-DTPA were similar but not identical to those of murine B72.3-GYK-DTPA. Collectively, these data suggest that clinical studies of the chimeric B72.3 antibody as a site-specific GYK-DTPA immunoconjugate are warranted.