BIOLOGICAL RESEARCH REPORTS FROM THE UNIVERSITY OF JYVÄSKYLÄ

67

Tuomo Nikula

Development of Radiolabeled Monoclonal Antibody Constructs: Capable of Transporting High Radiation Dose into Cancer Cells

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 1998

BIOLOGICAL RESEARCH REPORTS FROM THE UNIVERSITY OF JYVÄSKYLÄ 67

Tuomo Nikula

Development of Radiolabeled Monoclonal Antibody Constructs: Capable of Transporting High Radiation Dose into Cancer Cells

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Villa Ranan Blomstedt-salissa
toukokuun 30. päivänä 1998 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Natural Sciences of the University of Jyväskylä,
in the Building Villa Rana, Blomstedt Hall, on May 30, 1998 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 1998

Development of Radiolabeled Monoclonal Antibody Constructs: Capable of Transporting High Radiation Dose into Cancer Cells

BIOLOGICAL RESEARCH REPORTS FROM THE UNIVERSITY OF JYVÄSKYLÄ 67

Tuomo Nikula

Development of Radiolabeled Monoclonal Antibody Constructs: Capable of Transporting High Radiation Dose into Cancer Cells

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 1998

Editors
Jukka Särkkä
Department of Biological and Environmental Science, University of Jyväskylä
Kaarina Nieminen
Publishing Unit, University Library of Jyväskylä

URN:ISBN:978-951-39-9026-8
ISBN 978-951-39-9026-8 (PDF)
ISSN 0356-1062

Jyväskylän yliopisto, 2022

ISBN 951-39-0120-3
ISSN 0356-1062

Jyväskylä University Printing House,
Jyväskylä and ER-Paino, Lievestuore 1998

This study is dedicated with love to my wife
Leena
and my children
Jubo, Simo and Ottilia

ABSTRACT

Nikula, Tuomo
Development of radiolabeled monoclonal antibody constructs: capable of transporting high radiation dose into cancer cells
Jyväskylä: University of Jyväskylä, 1998, 45 p.
(Biological Research Reports from the University of Jyväskylä,
ISSN 0356-1062; 67) ISBN 951-39-0120-3
Yhteenveto: Radioleimattujen monoklonaalisten vasta-ainerakenteiden kehittely
Diss.

The successful application of radioimmunotherapy for cancer has been limited by the availability of the small number of suitable isotopes, mostly emitting β-particle. Iodine-131 is the most prevalent radionuclide for labeling monoclonal antibodies to high specific activity for therapeutic applications. Also yttrium-90 is a promising radiolabel because of its higher energy, longer ranged β-particles and lack of γ-rays. The α-particle emitting radionuclides have a number of physical characteristics that make them attractive candidates for radioimmunotherapy: (a) high linear energy transfer; (b) short path lengths (50-80 μm); (c) limited ability of cells to repair damage to DNA; (d) cell kill via apoptotic mechanisms.

The present study is focused on developing a radiolabeled monoclonal antibody construct that is capable of transporting a high radiation dose into cancer cells. Also the effects of iodination on the tyrosine residues and coupling a chelate on lysine residues to the immunoreactivity fraction of mAb have been studied.

Direct iodine-131 radiolabeling methods yield decreased immunoreactivity of the antibody as a function of increased iodine incorporation. The amino acid sequences of a therapeutic IgG (HuM195), and in particular its complementary determining regions (CDR) were studied, in order to correlate the iodination of tyrosine residues in the antigen binding site with changes in immunoreactivity. The CDR contained an overabundance of tyrosines relative to an expected random distribution of amino acids. The immunoreactivity measured after varying levels of iodination fit to a theoretical curve generated based on the assumption that a single iodine incorporation anywhere on a tyrosine residue in a CDR destroys the immunoreactivity of the antibody. Using enzyme digestion, the distribution of iodine on different parts of the antibody was also studied. The iodinated residues were distributed non-uniformly throughout the IgG, with the heavy chain variable region tyrosines having a higher propensity for iodine incorporation than tyrosines in the other regions of the IgG. The data also suggest that high tyrosine fraction in the CDRs is a limiting factor for using direct radioiodination at the level required for the radioimmunotherapy.

Conjugation of HuM195 to CHX-A-DTPA resulted in the attachment of up to 10 ligand molecules per antibody, and labeling efficiency with Bismuth-213 was typically over 90%. After injection into mice, there was no uptake or loss of bismuth to mouse tissues, that do not express antigen or to kidney, which has avidity for free, unbound bismuth. Toxicity of ^{213}Bi-CHX-A-DTPA was evaluated in normal mice with doses from 0.5 to 20 mCi/kg showing no toxicity, but at 70 mCi/kg significant toxicity was detected. Cell killing experiments with different specific activities of ^{212}Bi or ^{213}Bi labeled conjugate showed dose and specific activity dependent killing of HL60 cells.

The results of this thesis indicate that bismuth-213 labeled HuM195 has high potency to specifically kill the target cells without remarkable toxicity to other tissues.

Key words: Alpha-particles; bismuth-213; CHX-A-DTPA; HuM195; monoclonal antibodies; radioimmunotheraphy

Tuomo Nikula, MAP Medical Technologies Oy, Elementtitie 27, FIN-41160 Tikkakoski, Finland, and Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FIN-40351 Jyväskylä, Finland

Contents

List of original publications

This thesis is based on the following original articles, that will be referred to by their Roman numerals I - V. I have performed most of the work in the papers III - V and a significant proportion of it in the works I and II.

I. Penttilä, P.I., Hiltunen, J.V., Nikula, T.K. & Kairemo, K.J.A. 1990. Labeling and purification of DTPA-coupled monoclonal antibodies with ^{57}Co, ^{90}Y and ^{165}Dy. In: Maddalena, D.J., Snowdon, G.M. & Bonigace, G.R. (eds), Advances in Radiopharmacology, Proceedings of the sixth international symposium on radiopharmacology: 227-231. International Association of Radiopharmalogy.

II. Kairemo, K.J.A., Hiltunen, J.V., Penttilä, P.I., Nikula, T.K. & Laine, A. 1990. Radioimmunotherapy using ^{90}Y-labelled monoclonal antifibrin antibody in nude mice. In: Maddalena, D.J., Snowdon, G.M. & Bonigace, G.R. (eds), Advances in Radiopharmacology, Proceedings of the sixth international symposium on radiopharmacology: 232-237. International Association of Radiopharmalogy.

III. Nikula, T.K., Curcio, M.J., Brechbiel, M.W., Gansow, O.A., Finn, R.D. & Scheinberg, D.A. 1995. A rapid single vessel method for preparation of clinical grade ligand conjugated monoclonal antibodies. Nucl. Med. Biol. 22: 387-390.

IV. Nikula, T.K., McDevitt, M.R., Wu, C., Kozak, R.W., Garmestani, K., Brechbiel, M.W., Curcio, M.J., Pippin, C.G., Tiffany-Jones, L., Finn, R.D., Geerlings, M.W., Sr., Apostolidis, C., Molinet, R., Gansow, O.A. & Scheinberg D.A. 1997. Alpha particle emitting bismuth cyclohexylbenzyl DTPA constructs of recombinant humanized anti-CD33 antibodies for therapy of myeloid leukemias. (manuscript submitted to J. Nucl. Med.)

V. Nikula, T.K., Bocchia, M., Curcio, M.J., Sgouros, G., Ma, Y., Finn, R.D. & Scheinberg D.A. 1995. Impact of the high tyrosine fraction in complementarity determining regions: Measured and predicted effects of radioiodination on IgG immunoreactivity. Molecular Immunol. 32: 865-872.

Abbreviations

B_{max}	maximal binding
CA-DTPA	cyclic dianhydride of DTPA
CDR	complement determining region
CHX-A-DTPA	2-(p-SCN-Bz)-cyclohexyl-DTPA
DNA	deoxyribonucleic acid
DOTA	1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetra-acetic acid
2B-DOTA	2-(p-isothiocyanatobenzyl)-DOTA
DTPA	diethylenetriaminepenta-acetic acid
1B-DTPA	1-isothiocyanatobenzyl-DTPA
1B4M-DTPA	1-isothiocyanatobenzyl-4-methyl-DTPA
1B3M-DTPA	1-isothiocyanatobenzyl-3-methyl-DTPA
1M3B-DTPA	1-methyl-3- isothiocyanatobenzyl-DTPA
2B-DTPA	2-isothiocyanatobenzyl-DTPA
EDTA	ethylenediaminetetra-acetic acid
F_{ab}	antigen binding fragment of immunoglobulin
F_c	complement binding fragment of immunoglobulin
F_v	light chain fragment, containing antigen binding aminoacid sequence
HPLC	high-performance liquid chromatography
HuM195	Humanised mouse antibody M195
IgG	immunoglobulin G
i.p.	intraperitioneal
i.v.	intravenous
ITLC-SG	instant thin layer chromatography, siliga gel
K_a	association constant
LC	liquid chromatography
LET	linear energy transfer
mAb	monoclonal antibody
MAG_3	mercaptoacetyltriglycine
MTD	most tolerated dose
NOTA	1,4,7-triazacyclononane-1,4,7-triacetic acid;
C-NOTA	2-(p-isothiocyanatobenzyl)-NOTA
pI	isoelectric point
^{xxx}Me	a metal with mass number

1 INTRODUCTION

Although monoclonal antibodies (mAbs) can selectively target malignant cells in humans, most mAbs lack the ability to destroy cells by immunologic mechanisms. Therefore, the use of mAb to deliver radioisotopes specifically to tumor cells has become an appealing approach. There are several factors to be overcome before radioimmunoconjugates can be effectively used clinically (Juric & Scheinberg 1994).

The successful application of radioimmunotherapy for cancer has been limited by the availability of a small number of suitable isotopes, mostly emitting β-particles (Table 1). Despite recent interest in a variety of radionuclides for radioimmunodiagnosis and therapy, iodine-131 remains the most prevalent radionuclide for labeling monoclonal antibodies to high specific activity for applications *in vitro* and *in vivo* (DeNardo et al. 1988, Caron et al. 1994, Behr et al. 1997). Yttrium-90 is also a promising radiolabel because of its higher energy, longer-ranged β-particles and lack of γ-rays (Hird et al. 1993, Stemmer et al. 1994, Paganelli et al. 1997). Because of considerable bystander cell killing, the use of β^-- and β^+-emitters with long range (1-10 mm) particles is generally restricted to settings of large tumor burden or cases where a bone marrow transplant is possible for rescue. The similarity of rhenium and technetium chemistry has initiated high interest in the use of rhenium isotopes ^{186}Re and ^{188}Re in radioimmunotherapy. Both isotopes have also suitable gamma quantums to imaging (Jacobs et al. 1993, Rhodes et al. 1996).

Auger and conversion electron emitters are effective only when internalized into proximity of the nucleus (Kassis et al. 1980). Because they act only at subcellular ranges (<1 μm), they will yield single cell killing without damaging the surrounding tissue. Effective Auger radioimmunotherapy remains to be demonstrated in humans.

TABLE 1 The most potent radionuclides for radioimmunotherapy.

Isotope	Half-life	Energy	Source	references
α-emitters				
Bismuth-212	60.5 min	7.8 MeV	generator	Zucchini and Friedman 1982
Bismuth-213	47 min	8.4 MeV	generator	Geerlings et al. 1993
Astatine-211	7.2 h	6.7 MeV	accelerator	Humm 1987
electron capture				
Iodine-123	13.3 h	27 keV	accelerator	
Iodine-125	60 d	27 keV	accelerator	
Platinum-193m	4.3 d	~10 keV	accelerator	Willins and Sqouros 1995
β-emitters				
Yttrium-90	64 hr	2.27 MeV	generator	Chinol et al. 1987
Rhenium-188	17 hr	2.1 MeV	generator	Kamioki et al. 1994
Rhenium-186	3.8 d	1.1 MeV	nuclear reactor	Schroff et al. 1990
Iodine-131	8 d	0.81 MeV	fission product	
Copper-67	2.4 d	0.57 MeV	accelerator / nuclear reactor	Hughes et al. 1997
Lutetium-177	6.7 d	0.50 MeV	nuclear reactor	Schlom et al. 1991

Alpha-particle emitters have several advantages over β^--particle emitters. Because of their high linear energy transfer and the short path lengths, they are up to 100 times as potent as beta particles, and the nonspecific irradiation of normal tissue around the target cell is greatly reduced or even absent. Furthermore, cells have a limited ability to repair the damage to DNA from α-particle irradiation, which makes alpha-particles extremely cytotoxic (Gansow et al. 1984). That makes them attractive to cancer therapy, particularly for applications where focusing cytotoxic effects in a volume equivalent to a few cell diameters is desirable.

This thesis is focused on developing a radiolabeled monoclonal antibody construct capable of transporting a high radiation dose into cancer cells.

2 REVIEW OF LITERATURE

2.1 Metal chelates

2.1.1 First chelate agents

In the first chelated immunoglobulins, chemically modified derivatives of EDTA and DTPA were used (Figure 1) (Meares et al. 1976, Krejcareck and Tucker 1977, Leung et al. 1978, Yeh et al. 1979). The bifunctional chelate was saturated with ferric ion and coupled to immunoglobulins. The chelated immunoglobulin was purified using a column consisting of a mixture of ion exchange and size-exclusion resins. Afterwards, the ferric ion was removed from the chelate by dialysis (Scheinberg et al. 1982).

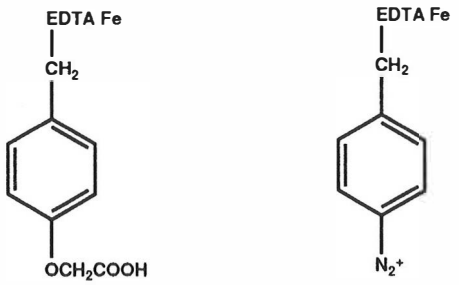
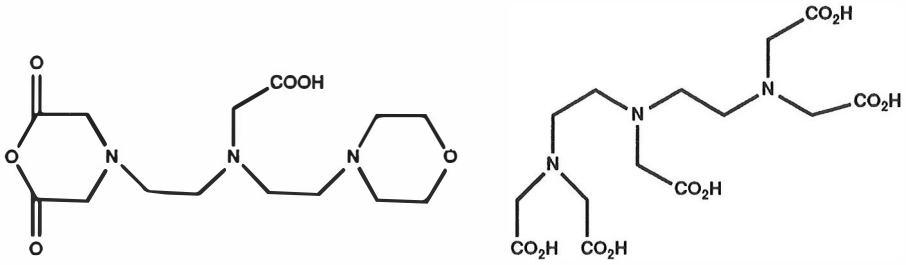


FIGURE 1 Molecular structures of ferri salts of 1-(p-carboxymethoxybenzyl)-EDTA and p-(diatzonium)benzyl-EDTA.

2.1.2 CA-DTPA

In the early 1980's, cyclic dianhydride of DTPA (CA-DTPA) method (Figure 2) was developed (Hnatowich et al. 1983, Paik et al. 1983a, Paik et al. 1983b) and it is widely used with some modifications. The advantages of the method are that it is relatively simple, and all chemicals are commercially available. However, the coupling of DTPA to proteins via cyclic dianhydride DTPA is accompanied by side reactions (Maisano et al. 1992): 1) Inter- and intramolecular cross-linking, which occur to a considerable extent even at low anhydride to protein molar ratio; 2) Tyrosine residue acylation is detected on a rather high anhydride protein molar ratio (200:1). These side reactions reduce immunoreactivity of mAbs even at small final DTPA/mAb ratio (Paik et al. 1983b).



CA-DTPA DTPA

FIGURE 2 Molecular structures of CA-DTPA and DTPA chelates.

2.1.3 Carbon backbone substituted DTPA chelating agents

Even though DTPA has excellent *in vitro* stability over large amount of trivalent metal ions, it lacks high *in vivo* stability with many metals. The reduced stability is price from the fact that at least one "arm" of the DTPA molecule is used to connect it to the antibody. When the antibody is conjugated to the chelate through an isothiocyanate linker (Meares and Wensel 1984, Brechbiel et al. 1986), all five arms of the DTPA are capable of binding to the metal ions. When 1B-DTPA (Figure 3) is conjugated with B72.3 mAb, the body clearance from non-targeted ^{111}In after 168 h was remarkably better when it was compared to conjugates described in Figure 2 (Brechbiel et al. 1986).

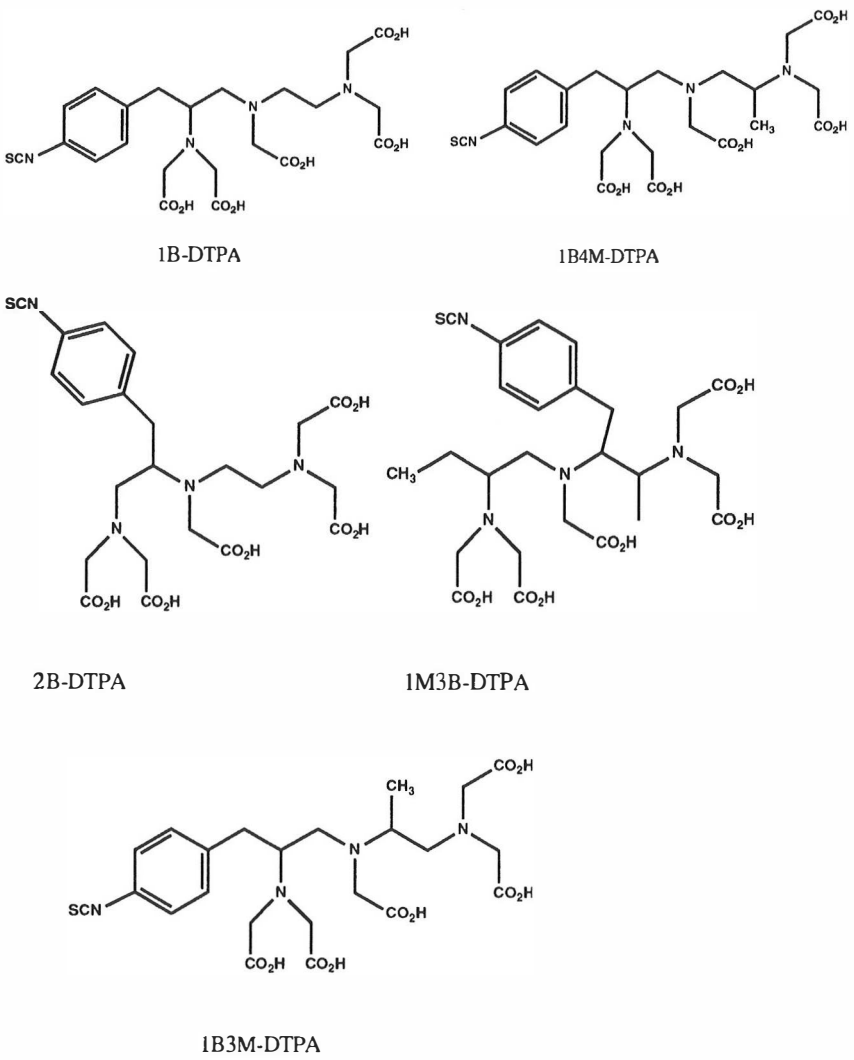
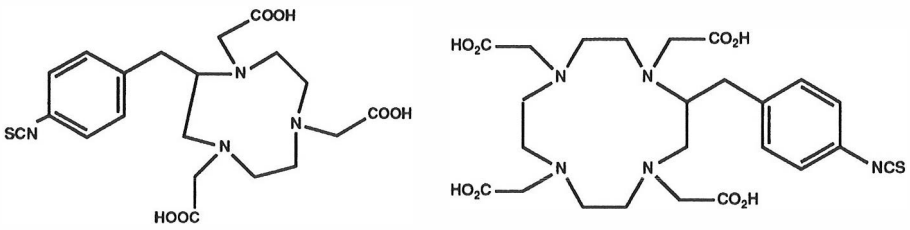


FIGURE 3 Molecular structures of some carbon backbone substituted DTPA analoque chelating agents (Sharkey et al. 1990).

2.1.4 Specific chelators

Ionic radius of the metallic ions appears to be an important parameter for the stability of metal complexes. The differences on the ionic radius of Ga^{3+} (76 pm) and In^{3} (93 pm) effect the stability of ^{67}Ga-C-NOTA (Figure 4) and ^{111}In-IB4M-DTPA (Figure 3) complexes: if Ga^{3+} is chelated with IB4M-DTPA, it forms a less stable complex, and similarly In^{3+} with NOTA so that they have higher stability constants than ^{67}Ga-IB4M-DTPA and ^{111}In-C-NOTA (Lee et al. 1997). Also stereochemical forms of chelator have influence on *in*

vivo stability (Camera et al. 1994, Wu et al. 1997). Large number of "specific" chelators have been developed to the technetium, rhenium (Rusckowski et al. 1996), copper (Blower et al. 1996) and lanthanides (Hemmilä & Mikkola 1990). The chelators for Tc and Re are mainly based on SN, SN_2 and SN_3 formulations such as hydrazino nicotinamide and MAG_3 derivatives (Schwartz et al. 1991, Lei et al. 1996).



C-NOTA 2B-DOTA

FIGURE 4 Molecular structures of some macrocyclic ligands (Deshpande et al. 1990, Lee et al. 1997).

2.2 Number of chelates per mAb

One of the critical issues in the use of metal-chelate antibodies in radioimmunotherapy is the number of chelates per mAb molecule. Several analytical methods have been developed to quantify the number of metal binding sites in ligand-mAb conjugates.

When excess In^{3+} or Co^{3+} doped with ^{111}In or ^{57}Co, respectively, is added to the protein conjugate solution, both free and protein conjugated ligand will be saturated with metal. There are three metal species: 1) Free Me^{3+}, 2) free ligand complexed metal and 3) metal complexed protein-ligand conjugate. The amount of each component can be analyzed by using paper chromatography. Some methods require addition of extra EDTA into the labeling solution (Paik et al. 1983b, Meares et al. 1984b, Mathias et al. 1990, Capillon et al. 1992). When ^{111}In or ^{57}Co is used without carrier, there is a risk that the amount of protein conjugated chelate is underestimated because the free ligand is more efficient in coordinating Me^{3+} than the protein conjugated one (Meares et al. 1984b).

A spectrophotometric method can be used to measure the reaction between a DTPA-ligand mAb conjugate and Y^{3+} complex of arsenazo III (Pippin et al. 1992). An electrophoretic method, based on the measurement of pI changes of chelated-mAb compared with plain mAb (Pham et al. 1995) is also available. In the fluorescence method, the number of chelate per mAb is measured by fluorescence emission of lanthanide metals such as Tb^{3+} and Eu^{3+} (Brandt et al. 1991). A method using ^{14}C labeled ligands is the only one measuring the total number of ligands, not the number of free chelate (Brechbiel et al. 1986, Mirzadeh et al. 1990).

2.3 Radiolabeling antibodies with iodine

Several useful and effective methods have been developed to label antibodies with radioisotopes of iodine. Chloramine-T (Hunter and Greenwood 1962), Iodogen (Fracker and Speck 1978) and lactoperoxidase (Marchalonis 1969) all oxidize iodine to a cationic species capable of replacing a hydrogen atom on the phenyl group of tyrosine. Other specific radioiodinating agents, such as Bolton-Hunter reagent (Bolton and Hunter 1973), succinimidyl para-iodobenzoate (Zalutsky and Narula 1987) and N-succinimidyl 3-hydroxy-4-iodobenzoate (Vaidganathan et al. 1997), directly conjugate to protein-NH_2 residues. These indirect methods are relatively expensive and typically result in lower specific activities than direct radioiodination of the tyrosine residues. Despite some advantages of these newer methods, chloramine-T and iodogen are still most commonly used.

The immunoreactivity of an IgG is its most important biological feature when IgG is used as a therapeutic agent for delivery of a radionuclide (Larson 1986). All labeling procedures pose a risk of decreasing the biological activity of the target protein. There are differences between Fab's in their sensitivity to iodine incorporation. However, the immunoreactivity of therapeutic IgG-Fab fragments is decreased exponentially as the iodine-to-Fab ratio is increased (Ferens et al. 1984, Larson et al. 1984 Matzku et al. 1987). The higher immunoreactivity fraction of mAb was obtained when its antigen-binding site is occupied by its antigen during the labeling procedure (Van del Abbeele et al. 1991).

2.4 Yttrium-90

Monoclonal antibodies labeled with yttrium-90 were first described by Hnatowich et al. (1985) and since then several other groups have reported its use (Roselli et al. 1989, Stemmer et al. 1994, Paganelli et al. 1997).

2.4.1 The source of yttrium-90

Antibody labeling requires carrier free ^{90}Y, which can be obtained from $^{90}Sr/^{90}Y$ -generators. Numerous systems have been described where a cation exchange resin has been used to retain the ^{90}Sr while daughter ^{90}Y activity is eluted with various eluants such as EDTA (Chinol et al. 1987), citrate (Doering et al. 1963), lactate (Rane et al. 1966), oxalate (Suzuki 1964), methanol and acetate (Kawashima 1966), or acetate alone (Chinol et al. 1997). The alternative method to produce yttrium-90, which has received less attention, is solvent extraction (Wike et al. 1990, Lee et al. 1991). The method

has advantages especially with high radioactivities, because the radiation damage to organic cation resins used in the generators is a limiting factor in the use of several qiqabequerels of radioactivity (Gangwer et al. 1977).

A disadvantage common nearly to all of the above systems is that the radioactivity is not eluted in chemical form suitable for direct labeling. In addition, most methods require an extra purification step to remove trace metals (Chinol et al. 1987).

2.4.2 Specific chelators to yttrium

Chelators, which hold radiometals with high stability under physiological conditions, are considered to be important in avoiding radiation damage to nontarget tissue due to radiometal dissociation. Initially, the cyclic anhydride of DTPA was used to attach yttrium-90 to mAb (Hnatowich et al. 1983). ^{90}Y-DTPA-mAb has been found to be unstable *in vivo* (Mather et al. 1989) and free ^{90}Y has a bone marrow seeking behavior. The critical organ in human studies has been the bone marrow (Hnatowich et al. 1988). Several other DTPA analogues have been used to conjugate ^{90}Y to mAb, but all of them have lacked stability *in vivo* (Motta-Hennessy et al. 1991).

In vivo stability of the macrocyclic benzyl-DOTA 2B-DOTA (Figure 4) has been demonstrated (Deshpande et al. 1990), but unfortunately the patients developed anti-chelate antibody responses to it (Kosmas et al. 1992). Also a nonmacrocyclic compound (S)-4-{2,3-bis[bis(carboxymethyl)-aminopropyl]isothyocyanate} of DTPA (CITC-DTPA), an analogue of benzyl-DTPA, is found to develop a humoral immune response. These findings strengthen the hypothesis that an immunogenic hapten (chelate) enhances the immunogenicity of the carrier (mAb) (Kosmas et al. 1995).

Recently, a new DTPA-analogue CHX-A-DTPA (Brechbiel and Gansow 1992) has been found to be stable *in vitro* and *in vivo* (Camera et al. 1994, Wu et al. 1997).

The evaluation of pharmacokinetics with yttrium-90 is difficult because it decays by beta emission, with no associated photons to permit easy external monitoring. Yttrium isotopes ^{86}Y and ^{88}Y have been used to evaluate the pharmacokinetics of yttrium labeled antibodies (Deshpande et al. 1990, McDevitt et al. 1996).

2.5 Alpha-emitting nuclides

Since their proposal in 1982 (Scheinberg et al. 1982), the development of feasible alpha emitting radionuclide-conjugated monoclonal antibodies for radioimmunotherapy has been complicated by two major problems: (A) lack of a suitable source of alpha emitting radionuclides and (B) inadequate methods to stably attach the radionuclide to the IgG. Investigations of α-

particle emitting radionuclides have to date focused primarily on two sources: astatine-211 (Vaughan et al. 1982, Zalutsky et al. 1989) and bismuth-212 (Black et al. 1988, Macklis et al. 1988, Simonson et al. 1990). Recently, a new radionuclide, bismuth-213, has been proposed for alpha-therapy (Geerlings et al. 1993). The use of lead-212 (Jones et al. 1996) and actinium-225 (Geerlings et al. 1993) for alpha therapy has also been discussed.

2.5.1 Astatine-211

Astatine-211 is produced by cyclotron in small quantities (Lambrecht and Mirzadeh 1985) and it has a half-life of 7.2 h. Astatine is a halogen with an even weaker bond to carbon than iodine, and would be unstable with respect to dehalogenation *in vivo*, if linked directly to the tyrosine residues. The first attempt to produce *in vivo* stable astatine-211 conjugates has been done with p-astatobenzoic acid (Friedman et al. 1977). Similar succinimidyl benzoate derivatives have succesfully been made to astatine, and the have also been used with iodine (Zalutsky et al. 1989, and Zalutsky et al. 1997). Because half life of ^{211}At does not give time for mAb's to penetrate into solid tumors, biotinyl-3-[^{211}At]astatoanilide has been developed.That can be produced with one-step synthesis from tributylstannyl precursor (Foulon et al. 1997).

2.5.2 Bismuth-212 and bismuth-213

Bismuth isotopes 212 and 213 have half-lives of approximately one hour. The sources of the bismuth radionuclides are isotope generators (Zucchinie and Friedman 1982, Atcher et al. 1988, Geerlings et al. 1993). The decay cascades of these generator systems are presented in Figure 5.

The advantages of ^{213}Bi over ^{212}Bi are:

1) The parent radionuclide actinium-225 for bismuth-213 has a half-life of 10 days compared to radium-224 half-life of 3.6 days.

2) The ^{213}Bi decay cascade includes much less γ-emission, and emitted γ-rays have energies far lower than those in the ^{212}Bi decay cascade.

For the bismuth radionuclides ^{212}Bi and ^{213}Bi, which have half lives of about one hour, target diseases are limited to those where cancer cells reside in the blood vessels, such as leukemias or micrometastatic carcinomas.

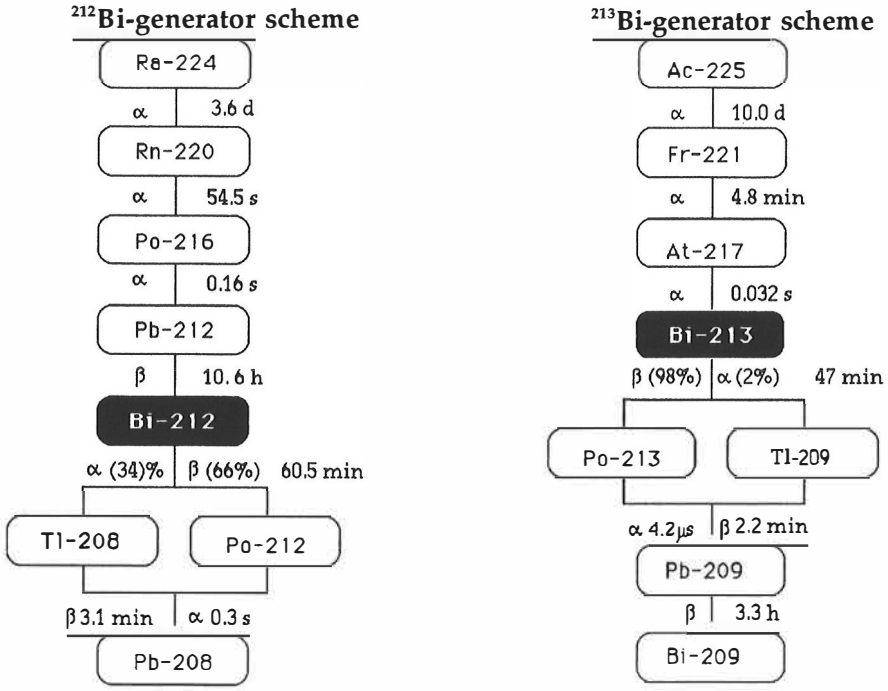


FIGURE 5 The decay cascades of bismuth-212 and bismuth-213 generators.

2.5.3 Chelators to bismuth

One major problem in the development of alpha-therapy has been the instability of chelated bismuth-radionuclides *in vivo*. Several chelates have been investigated to conjugate bismuth radionuclides to mAbs, and variable stabilities have been found. Using Ca-DTPA as a chelating agent has turned out to be as bad as free bismuth, due to rapid losses of bismuth to the kidney (Ruegg et al. 1990, Gansow 1991). Several other DTPA-derivatives are introduced to bismuth chelates (Ruegg et al. 1990, Langmuir et al. 1990, Simonson et al. 1990). Extensive kidney localization of $^{205/206}$Bi, the bismuth isotope with 6.2d half-life, was not observed when the bifunctional macrocyclic ligand DOTA was employed for bismuth (Gansow 1991). Unfortunately, the labeling efficiency with bismuth-212 was very poor.

Recently, a novel isothiocyanatobenzyl derivative of CHX-A-DTPA (Figure 6) was introduced, which both rapidly sequesters bismuth and is stable *in vivo* (Brechbiel et al. 1991, Brechbiel and Gansow 1992 and Huneke et al. 1992).

The specific killing of α-emitter-conjugated mAbs is about 10-100 times higher compared to non-specific α-irradiation and over 1000 times more effective than γ-irradiation (Kozak et al. 1986 and Kurtzman et al. 1988).

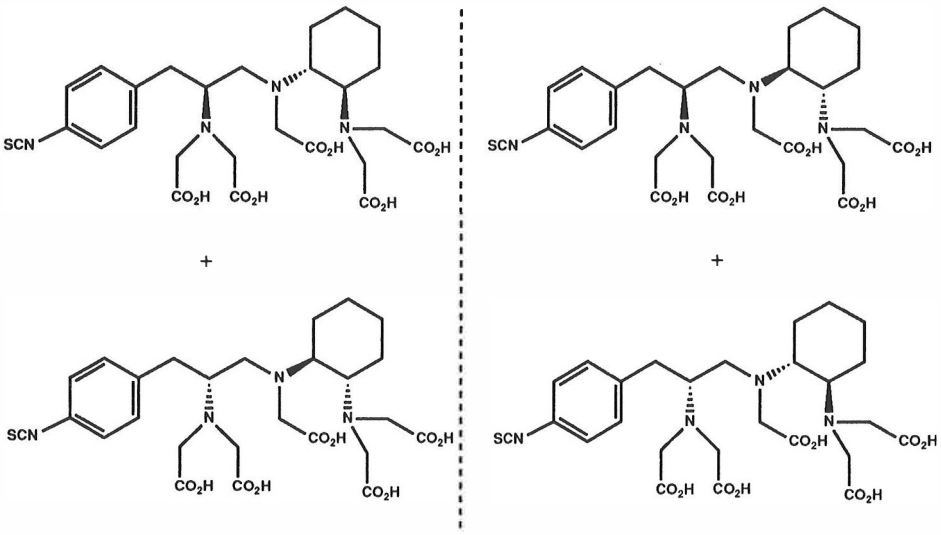


Figure 6 The different isomers of 2-(p-SCN-Bz)-cyclohexyl-DTPA.

2.6 Cell killing by alpha particles

Programmed cell death (apoptosis) is a normal process by which cells are eliminated during normal embryonic development and in adult life. A significant portion of the radioactivity pattern observed after radioimmunoconjugate therapy may be attributable to the induction of apoptotic signals (Sachs and Lotem 1993, Palayoor et al. 1993).

Low doses of high-LET radiation induce double stranded DNA breaks, chromosomal aberrations, cell killing and mutations more efficiently than low-LET radiation. The killing of cells by α-particle irradiation is one to two log more efficient than killing caused by the same dose exposed to γ-irradiation. It has been proposed that this effect is related to an inability to repair alpha-particle induced DNA damage (Nagosawa et al. 1991).

Using α-particle sources, the number of α-particles, which must pass through a cell nucleus in order to kill the cell is estimated to be 2 to 7 depending on the cell line (Raju et al. 1991). Similar results are obtained *in vitro* experiments with radiolabeled antibodies (Macklis et al. 1992).

3 AIM OF THE STUDY

The main purpose of the present study was to develop a radiolabeled monoclonal antibody construct capable of transporting a high radiation dose into the tumor.

Aims of the study:
1. To develop yttrium-90 labeled antibodies and evaluate their *in vivo* properties (I and II).

2. To develop a method for preparation of ligand conjugated monoclonal antibodies of clinical grade (III).

3. To evaluate alpha particle emitting bismuth constructs of anti-CD33 antibodies for therapy of myeloid leukemia (IV).

4. To study effects of radioiodination on IgG immunoreactivity with respect to chelate chemistry (V).

4 SUMMARY OF MATERIALS AND METHODS

The materials and methods are described in detail in the original publications (I-V).

4.1 Antibodies

HuM195 (Protein Design Labs Inc., Mountain View, CA) is a well-characterized genetically engineered CDR grafted humanized monoclonal antibody (human IgG1). Both HuM195 and original murine M195 bind with high affinity to the CD33 antigen (Tanimoto et al. 1989, Scheinberg et al. 1989, Caron et al. 1992, Co et al. 1992 and 1993, Schwartz et al. 1993). HuM195 is under investigation as a carrier of radioisotopes in the treatment of acute leukemia (Caron et al. 1994).

59D8-Fab fragment (Centocor Europe, Leiden, Netherlands) recognizes a synthetic heptapeptide, that is characteristic of fibrins but not of fibrinogens (Hui et al. 1983). OC-125-F(ab´)$_2$-fragment (CIS, Gif-Sur-Yvette, France) reacts specifically with the tumor marker present also in epithelial ovarian carcinoma CA-12-5 (Bast et al. 1983).

4.2 Radionuclides

Yttrium-90 was eluted with 0,03 M EDTA from the $^{90}Sr/^{90}Y$ generator and purified with AG 50W x 4 column (Chinol et al. 1987). After evaporation to

the dryness, yttrium-90 was dissolved into 0.5 M sodium acetate pH 6 solution.

Bismuth-206 was used in place of ^{213}Bi or ^{212}Bi in certain assays because of the short half-lives of ^{213}Bi and ^{212}Bi. ^{206}Bi was obtained as a 3:2 molar ratio of ^{206}Bi:^{205}Bi (carrier free) in 1.0 M HNO_3 (Crocker Nuclear Laboratory, Davis, CA). The radionuclide solution was diluted with H_2O to 0.1 M HNO_3 and then applied to 5-mm x 2-cm column of the cation exchange resin AGMP50 (200 - 400 mesh; Bio-Rad Laboratories, Inc., Hercules, CA) pre-equilibrated with 0.1 M HNO_3. The column was rinsed with 1000 ml of 0.1 M HNO_3, followed by 1000 ml of metal free H_2O. The ^{206}Bi was then eluted from the column with 600 µl 0.1 M HCl, NaI (Ruegg et al. 1990).

^{212}Bi and ^{213}Bi were eluted from $^{224}Ra/^{212}Bi$ generator (National Cancer Institute, Bethesda, MD) (Atcher et al. 1988) and $^{225}Ac/^{213}Bi$ (Institute for Transuranium Elements, Karlsruhe, Germany) (Geerlings et al. 1993) respectively. 200-500 µl of 0.2 M NaI solution was added to the eluant.

Other radionuclides were obtained from commercial sources and purified if necessary.

4.3 Conjugation of chelates with mAbs

Two different conjugates were used. CA-DTPA, which was conjugated to the mAbs using the method of Paik et al. 1983. Shortly: Cyclic DTPA, solid or dissolved in dry dimethyl sulfoxide, was added into antibody solution of 0.1 M bicarbonate buffer pH 8.2. The ratio DTPA/mAb varied between 0.7 and 5.

CHX-A-DTPA [2-(p-SCN-Bz)-cyclohexyl-DTPA) (Brechbiel and Gansow, 1992)] was dissolved into solution of mAb. The reaction was allowed to proceed for 19 h at room temperature. The reaction mixture was next exchanged into an acetate buffer by continuous dilution with 750 ml of buffer using Amicon concentrator with reservoir (Amicon Inc., Beverly, MA) over approximately two hours. The final product was concentrated to appropriate volume as described in (III). The average number of chelates per antibody ranged from 2 to 10.

4.4 Radiolabeling with metal ions

The metal ions were in the acetate or sodiumiodine-acetate buffer. An appropriate amount of isotope was incubated with antibody for 20-30 min with continuous stirring. The reaction was quenched by adding 20 ml of 10 mM EDTA. The reaction mixture was purified by size exclusion chromatography HPLC or LC (I-V).

4.5 Iodination (with stable (^{127}I) and radioactive iodine (^{131}I))

^{111}In-CHX-A-DTPA-HuM195 in 750 ml of 0.1 M Na-phosphate buffer at pH 7.4 and freshly prepared ^{127}I solution (1 ml 0.5 M Na-phosphate buffer pH 7.4) were added to a glass tube that contained 1 mg of iodogen (Pierce Chemical Co., Rockford, IL) and allowed to react for 15 min. ^{127}I was used at 1 to 10 molar ratios to mAb. The ^{111}In-CHX-A-DTPA-HuM195-I was used in immunoreactivity assays without further purification as described in (V).

4.6 The quality control

The quality control was carried out by using ITLC-SG strip plates (Gelman Science Inc., Ann Abbor, MI) with 0.9% NaCl and 0.1 M EDTA as eluant. In the case of iodine-131 the quality control was carried out by trichloroasetic-acid precipitation (III-V).

Biochemical Methods

4. 7 Total immunoreactivity (Immunoreactive fraction)

The immunoreactivity was determined by incubating 2 ng of radiolabeled mAb with a 20 to 30 fold excess of antigen (10×10^6 or 15×10^6 CD33 positive HL60 cells). After incubation, the cells were collected and unbound IgG was removed to a second set of the cells and reincubated with the same amount of excess antigen as in the first incubation for 90 min at 0°C. The mmunoreactivity percentage was calculated as equal to (bound ^{xxx}Me-IgG to cells#1 plus cells#2)/(total bound plus unbound ^{xxx}Me-IgG) times 100 as described in (IV).

4.8 Determination of relative affinity and competition radioimmunoassay

In the publication (III), the chelated HuM195 and natural HuM195 were compared. The relative affinity was determined using Scatchard analysis. The least squares' method was used to fit the plot of bound/free vs. bound antibody, and apparent relative K_a and B_{max} were read from the slope of the

line and the intercept (Co et al. 1992). Comparisons of antigen-antibody avidity between the starting material (HuM195) and the product (CHX-A-DTPA-HuM195) were carried out by competition with ^{111}In-CHX-A-DTPA-HuM195. The relative avidity of binding was estimated by comparison of mAb concentrations where unlabeled competitor yields 50% inhibition of binding (Caron et al. 1992) as described in (III).

4.9 Antibody fragments

Fab and Fc fragments were prepared by using standard methods (Harlow and Lane 1988). The intact and reduced HuM195 IgG_1 molecules were fractionated on a 8% SDS-polyacrylamide gel. Bands corresponding to the heavy and light chains were identified, cut from the gel, and the radioactivity in the proteins was determined as described in (IV).

4.10 In vitro stability

Approximately 3 mg of ^{206}Bi-CHX-A-DTPA-HuM195 was added into 1 ml of human serum and incubated over time at 37°C in 5% CO_2. At various time points, samples of 10 µl were taken from solutions and added to 100 µl of a protein A bead slurry in phosphate buffered saline at pH 7.4 and mixed well. Samples were incubated at 4°C for 1 h. After incubation, the beads were collected by centrifugation, and unbound IgG was removed to a second set of the protein A beads and reincubated for 60 min. After incubation, the beads were washed twice with cold PBS. Mab bound to beads and free mAb were counted in a gamma-counter.

An *in vitro* stability in percent was calculated as equal to (bound ^{206}Bi-IgG on beads#1 plus beads#2)/(total bound plus unbound ^{206}Bi) times 100. This method was considered more accurate than other traditional chromatographic methods used to separate radiometal labeled protein from other metal compounds because it can separate radiometal labeled IgG from other metal binding proteins such as transferrin that comigrate with IgG, and will also separate the labeled IgG from free metal ions and free metal chelates (IV).

4.11 Targeting and biodistribution of radiolabeled antibody

Stability of the Bi- or Y-labeled radioimmunoconjugate *in vivo* was assessed by analyzing the biodistribution of ^{206}Bi-HuM195 in normal BALB/C mice or

^{90}Y in nude mice with ovarian carcinoma tumor. ^{206}Bi and ^{90}Y allowed the sampling of tissues for a prolonged time period. Mice were given injections of 2 μg (^{206}Bi) or 22 μg (^{90}Y) mAbs i.p. or i.v. in the tail vein. The biodistribution of the radiolabeled preparation was determined by removing blood, bone, brain, colon, heart, kidney, liver, lung, muscle, spleen and stomach and counting the tissue samples directly in the gamma counter at various time points after injection as described in (II and IV).

4.12 Cell killing

The potency of ^{212}Bi- and ^{213}Bi-CHX-A-DTPA-HuM195 for killing of leukemia cells was measured by using different specific activities of the Bi-antibody (3 to 20 mCi/mg). Serial dilutions of bismuth labeled antibody were added over HL60 cells (CD33 +) or RAJI cells (CD33-), the final activity ranging from 0.02 to 20 mCi/ml. The cells were incubated 24 h at 37°C in 5% CO_2. After incubation, cell viability was determined by ^{3}H-thymidine incorporation as described in (IV).

4.13 Toxicity of ^{212}Bi and ^{213}Bi in normal BALB/C mice

The toxicity of ^{212}Bi-labeled mAb has been described in (Huneke et al. 1992). Toxicity of the ^{213}Bi-labeled radioimmunoconjugate *in vivo* was assessed by analyzing viability, activity, weights, white blood cell counts and red blood cell counts in normal BALB/C mice over a three week period after injection. Mice received injections of 10, 30 or 80 μCi 213Bi-CHX-A-DTPA-HuM195 ip (0.5 to 4 mCi/kg body weight). Each dose group contained three mice. A control group included three mice without any treatment.

5 REVIEW OF THE RESULTS AND DISCUSSION

5.1 Chelate conjugation

Two moderate scale conjugations of CHX-A-DTPA to HuM195 were performed using Amicon concentrator with reservoir as a reaction vessel, concentrator and buffer chancing apparatus. Reactions yielded chelate/protein ratios of 2.3 to 5.0. The chelate/protein ratios were similar to those achieved using conventional methods. No aggregate formation was detected. HuM195-CHX-A-DTPA was labeled with ^{111}In at specific activities up to 30 mCi/mg protein. Labeling efficiency was 70-95% (III).

OC-125 was conjugated with cyclic dianhydride (CA-DTPA-OC-125) at final ratio 0.7 to 5 and labeled with yttrium-90. Specific activities of 4.5 mCi/mg protein were obtained (I).

The immunoreactivity of ^{111}In labeled HuM195-CHX-A-DTPA was remarkably high (75-95%), and it was independent of the chelate/protein ratio (up to 10 chelates per mAb). Immunoreactivity of the iodinated HuM195 construct was 50-70% (III). The immunoreactivity of CA-DTPA constructs has not been measured, but the antibody accumulation on the cancer cells was confirmed by immunohistochemical staining (II).

The affinity of the chelated construct was determined by Scatchard analysis, and the conjugate was found to have an apparent K_a of 1.1×10^{-9} M^{-1} (Figure 2A) (III) similar to affinity of unconjugated HuM195. Also no difference between HuM195 and HuM195-CHX-A-DTPA was found when a competive radioimmunoassay comparing starting material and product was used (Figure 2B) (III).

The use of only one vessel decreases the risks of a bacterial and a metal contamination. The process was run rapidly in a single vessel resulting in a product with radiolabeling efficiency, radiochemical purity, total

immunoreactivity and affinity equal to or better than in products achieved with other methods. It required only 24 hours run-time and the actual time needed to clean and prepare the equipment for the process was also reduced. In addition, the CA-DTPA -method (Hnatowich et al. 1983) results in a loss of immunoreactivity largely due to side reactions, such as cross-linking and tyrosine residue alkylation (Maisano et al. 1992), which are not problems for thiocyanated backbone derivatives of EDTA or DTPA.

5.2 Tyrosine and lysine distribution in CDRs

The amino acid sequences of the different regions of HuM195 were examined to determine the numbers of tyrosines (for iodination) and lysines (for chelate conjugation). The tyrosine and the lysine contents in HuM195 for a single and light chain are reported in Table 1 (V). 46% of the tyrosines of HuM195 are in the variable regions as a whole, and 23% of tyrosines are in the CDR. Variable regions and CDRs represent approximately 33 and 8% of the entire IgG sequence, respectively. The lysines are distributed more uniformly: 6% of lysines are found in the CDR, and 27% in the variable regions as a whole.

The relatively high number of tyrosines on the CDRs appears to be characteristic of CDR's. The typical numbers of tyrosines on the framework and CDR of numerous monoclonal antibodies (Kabat et al. 1991) are similar to HuM195. The CDR's typically include 20% to 30% of the tyrosines. Table 3 (V) presents the contents of lysine and tyrosine on the variable chains of several humanized antibodies (Co et al. 1992, 1991 and 1994, Queen et al. 1989, Riechmann et al. 1988).

To get a better definition of the sites of labeling, HuM195 was labeled using iodine-131. The radiolabeled IgG was digested with papain to F_{ab} and F_c fragments. IgG was also reduced to heavy and light chains. The data are summarized in Table 2 (V). The distribution of radioactivity on the fragments and heavy and light chains indicated that iodine was not randomly conjugated to the IgG molecule. The radioactivity was 10% to 15% higher on the heavy chain and F_c fragment than expected, based on the amounts of tyrosine in each region.

Tyrosines in the variable region appeared to be preferentially labeled relative to other tyrosines (Table 2) (V). The results of the iodine-131 distribution on the different fragments of IgG indicated that the iodine incorporation onto the tyrosines of the variable heavy chain is greater than on those of the rest of the IgG. One reason for the higher specific activity of iodination onto the variable heavy chain may be the external presentation of CDR tyrosines compared to tyrosines elsewhere.

Because cold iodine was used, all loses of immunoreactivity could be directly attributed to the attachment of the iodine atom to tyrosine and not to radiation damage or oxidation.

A theoretical expression for the HuM195 immunoreactivity in relation to iodination was generated using the data above with two assumptions: 1) Labeling of any tyrosine in either of the CDR regions destroys immunoreactivity. Therefore, immunoreactivity is preserved only if all labeling occurs outside the CDR regions. Or, 2) immunoreactivity is destroyed if one iodine atom is bound to the CDR regions of both arms of an antibody. The equations for these assumptions are presented in appendix A (V).

The resulting theoretical immunoreactive curves for iodinated HuM195 for the two different assumptions are shown in Figure 2A (V). The measured data (Figure 2A (V)) fit well to the theoretical curve generated using the assumption that one iodine incorporation into CDR is enough to destroy the immunoreactivity. It is likely that significant losses of avidity will occur in most antibodies with the conversion from bivalent to monovalent binding resulting from distribution of the CDR region.

Curves showing loss of immunoreactivity for the chelate ligand conjugated to IgG on lysines are shown in Figure 2B (V). The data are not sufficient to be compared with the above theoretical curves. When HuM195 was labeled with indium-111 on the F_c fragment, it was found to have 39% of the original radioactivity, which is about same percentage as the lysine content of F_c fragment.

Labeling of HuM195 with up to 7 chelates per IgG resulted in minimal losses of activity, whereas equivalent labeling with iodine reduced binding by more than 80%. Thus radiometal chelates may be more suitable for radiolabeling with high specific activity.

Fragments the the IgG have been proposed as therapeutic agents to gain faster targeting to tumors and faster clearance from the blood pool. The theoretical immunoreactivity of fragments (F_{ab}, F_v) is lost even faster than whole IgG (Figure 3) (V) because a large fraction of the tyrosines is in the CDR (33% and 50% respectively). Because only a labeled fragment can have radioactivity (for diagnosis or therapy), the theoretical immunoreactivity of the radioiodinated Fab and Fv fragments can never be higher than 67% and 50% respectively.

5.3 Radiochemistry of bismuth-213

The sizes of bismuth-213 generators that were used in this work ranged from 0.3 to 15 mCi. Conditions for Bi eluation were optimized. Elution with 0.1 M HCl, 0.1M NaI was suitable in preparation for the next step of directly labeling the mAb with Bi. In addition, elution with HCl/HI permitted the lead isotope daughters to remain on the column and reduced ^{225}Ac-breakthrough as compared to elution with high molarity HCl (Nelson, 1964). Actinium-225 breakthrough was in the range of 0.0001 - 0.01% when 0.1M HCl, 0.1M NaI was used to elute bismuth from the generator. Typically 75 to 90% Bi-recovery was observed when the generator was eluted with 500

- 1000 µl of eluant with eluting speeds of up to 500 µl/min. Trace amount of unidentified radionuclide in the first eluate from the generator was occasionally found as determined by using a multichannel analyzer.

HuM195-CHX-A-DTPA labeling efficiency with ^{213}Bi was typically over 90% at specific activities of up to 20 mCi/mg, but efficiency decreased with increasing specific activity. With activities of 50 mCi/mg, only 50-70% efficiencies were achieved. This reduction may have been due to the small amounts of antibody used to achieve the higher specific activities. The chelation reaction was nearly completed in 10 min. (over 85%), but was allowed to continue for a full 12-13 min. to optimize labeling. These labeling efficiencies were comparable to those seen with ^{111}In, ^{206}Bi and ^{212}Bi labeled HuM195-CHX-A-DTPA.

5.4 Biochemistry of the bismuth-213 construct

The chelated HuM195 antibody was rapidly internalized into the cells in a time-dependent manner ranging from 50% at 1 hour to 65% at 24 hours (Figure 2) (IV). The high percentage of isotope internalized and retained in the cells, representing approximately two to three times more retention of isotope than that seen with iodine labeled HuM195. The likely reason for the higher internalized fraction in HL60 cells is that catabolized free iodine is able to leak out from cells, whereas released radiometals are likely to bind to transferrin and other metal binding proteins in the cells (Yamauchi et al. 1989).

The labeled constructs were stable for at least two days *in vitro* in the presence of human serum at 37°C (Table 2) (IV). Less than 5% decomposition of the product was seen over two days. As ^{213}Bi and ^{212}Bi have half-lives of 1 hour or less, the constructs need to be stable for only 6 hours. These assays also demonstrated that the radiometal was not transferred to another metal binding serum protein at 37°C during this time period.

5.5 Biodistribution of ^{90}Y and ^{213}Bi labeled antibodies

Tissue to blood ratios of HuM195-CHX-A-DTPA-^{206}Bi in the major organs were less than 0.3 over 72 hours (Figure 3) (IV). There was no uptake or loss of bismuth to the mouse tissues that do not express CD33 or to kidney, which has avidity for bismuth (Durbin 1960). A comparison of the biodistribution of free bismuth-206, 103A-ca-DTPA-^{206}Bi (Ruegg et al. 1990), 103A-CHX-A-DTPA-^{206}Bi (Huneke et al. 1992) and HuM195-CHX-A-DTPA-^{206}Bi in mice 1 hour after i.v. injection shows that the newer generation constructs are stable *in vivo* whereas free bismuth and the earlier

generation chelates resulted in renal accumulation (Figure 4) (IV).

Otherwise, the biodistribution of administered ^{90}Y-antifibrin and ^{90}Y-OC-125 showed that yttrium is leaking out of construct *in vivo* and is accumulated in the bone. However, therapeutic effects were found with both antibodies compared with control groups.

5.6 Specific cytotoxicity

Cell killing experiments with different specific activities of bismuth-212 or bismuth-213 labeled HuM195-CHX-A-DTPA showed dose and specific activity dependent killing of CD33+ HL60 cells. ^{212}Bi-HuM195 was 20-30 times more potent at killing CD33+ HL60 cells than CD33 negative RAJI cells at 24 hours in *in vitro* assays (Figure 5A) (IV). The potency against the specific target HL60 cells depended directly on the specific activity of the labeled antibody with the highest specific activities (30 mCi/mg) showing the highest potency. Potency for killing HL60 cells at a specific activity of 0.2 mCi/mg approached the potency for killing the RAJI control cells.

The dependence of selectivity on specific activity can be explained by examining the number of CD33 target sites on each HL60 cell and the number of bismuth atoms labeled per HuM195 IgG molecule. At 0.2 mCi/mg, it is unlikely that specific cell killing can occur because only one cell out of 10 contains ^{212}Bi. Nonspecific cytotoxicity from alpha radiation in the media, or from antibody constructs nonspecifically bound to the cells, dominate the activity. Conversely, at specific activities of 30 mCi/mg, fourteen ^{212}Bi atoms are delivered to each HL60 cell at saturation. Therefore, at high specific activities, cytotoxicity should depend directly on the binding characteristics of HuM195 to HL60 cells.

Similar specific killing of HL60 cells was observed with ^{213}Bi-HuM195 (Figure 5B) (IV). At a specific activity of 10 mCi/mg, potency against HL60 cells was about 10 times higher than against RAJI cells. ^{212}Bi-HuM195 was slightly more potent than ^{213}Bi-HuM195. At the same specific activities, more ^{212}Bi would be conjugated per HuM195 IgG due to its longer physical half-life. Therefore, for equivalent binding of HuM195 to each cell, more alpha decays would be delivered by ^{212}Bi-constructs.

In order to determine the number of bismuth atoms necessary to specifically kill HL60 cells, the cytotoxicity data were replotted as a function of Bismuth atoms per cell (Figure 6) (IV). These data yield an LD_{50} dose of bismuth-213 and bismuth-212 are a range 2 to 2.5 initial atoms per cell.

5.7 Toxicity in mice

Over a three week period, there were no changes in viability, white blood cell counts or red blood cell counts or weight loss in mice injected with doses of ^{213}Bi up to 70 mCi/kg (Table 3). Mice in the highest dose group showed slightly reduced activity on days 3 and 4. These data suggest that there is little toxicity, with up to a 4 mCi/kg dose of 213Bi-CHX-A-DTPA-HuM195.

Cell killing experiments show specific cell killing over one log which is dependent on dose and specific activity. Both bismuth isotopes show approximately 50% killing when two atoms are bound on the cell surface. The possibility that a particle, whose starting point is outside the cell surface, may hit on a cell is negligible, and even though the bismuth is bound on the cell surface the possibility that the alpha particle absorbed enough energy in the cell to make damage is small. Main radio damage is coming from those decays that are internalized. Because approximately 50% of HuM195-CHX-A-DTPA is internalized into the cell in 60 min., it seems that one alpha inside is capable of killing the cell.

Doses of up to 10 mCi/kg have not shown any toxicity (viability, weight, cell count) in health BALB/C, but 18 mCi/kg is above the MTD and significant toxicity was found at 70 mCi/kg of ^{213}Bi-CHX-A-DTPA-HuM195. Based on these data, Phase I studies in humans with myeloid leukemia have been started, and now nine patients have been treated with ^{213}Bi-CHX-A-DTPA-HuM195 (Scheinberg et al. 1997).

5.8 Future views

The successful radioimmunotherapy requires 1) humanized mAb or at least a chimeric one that could effectively target cancer cells, 2) a way to attach a radioisotope to an antibody without losing immunoreactivity, and 3) a good alpha or beta emitter that is readily available and safe to use. Both yttrium-90 and bismuth-213 are available, and both can be attached to the mAbs using new chelates. The most limiting factor for using them in therapy is the specificity of antibodies. Even though several radiolabeled antibodies are introduced to the diagnostic and therapy, there are only few registered mAbs for diagnostic use. There have not yet been any therapeutic radiolabeled mAbs in Phase III multicenter trials. Recently, simultaneously with development of new chelate agents, there has been increased enthusiasm for a therapeutic approach using radiolabeled mAbs. The therapeutic radiolabeled antibodies seem to fulfill expectations which were set for them more than a decade ago. However, radiolabeled antibodies will not be the "magic bullets" that can alone cure the cancer, but together with conventional therapy, they most likely are going to increase the survival rate of cancer patients.

6 CONCLUSION

The main focus of the present study was to develop a radiolabeled monoclonal antibody construct that is capable of transporting a high radiation dose into a tumor. The main conclusions are

1. A rapid single vessel method for preparation of chelate conjugated monoclonal antibodies of clinical grade has been developed.

2. Alpha emitting constructs are among the most potent cytotoxic agents known, yet they are specific and appear safe to non-hematologic tissues *in vivo*. The physical and biochemical characteristics of the ^{213}Bi isotope and its generation, as well as the biochemistry of ^{213}Bi-CHX-A-DTPA-HuM195, make it possible to use these constructs in human immunotherapy.

3. The CDR of HuM195 contains an overabundance of tyrosine relative to an expected random distribution of amino acids. The immunoreactivity measured after varying levels of iodination of HuM195 fits to a theoretical curve that was generated based on the assumption that a single iodine incorporation anywhere on a tyrosine residue in a CDR destroys the immunoreactivity of the antibody. The results also suggest that suitable radiometal-chelate-mAbs may be more effective in therapeutic applications.

YHTEENVETO

(Résumé in Finnish)

Radioleimattujen monoklonaalisten vasta-ainerakenteiden kehittely

Syövän hoito radioimmunoterapialla on rajoittunut muutamaan sopivaan saatavilla olevaan isotooppiin, jotka lähinnä emittoivat β⁻-hiukkasia. Jodi-131 on eniten käytetty radionuklidi leimattaessa vasta-aineita terapeuttisiin sovellutuksiin. Yttrium-90 on myös lupaava radioleima korkean energian, pitkän β-kantaman ja puuttuvan γ-säteilyn vuoksi. Alfa-hiukkasia emittoivilla radionuklideilla on joukko ominaisuuksia, jotka tekevät niistä houkuttelevia kandidaatteja radioimmunoterapiaan: (a) korkea LET arvo, (b) lyhyt kantama (50-80 µm) kudoksessa, (c) solujen rajoittunut mahdollisuus korjata DNA vaurioita, (d) solukuolema apoptoottisesti.

Tässä työssä on keskitytty kehittämään radioleimattu vasta-aine, joka kykenee kuljettamaan suuren säteilyannoksen syöpäsoluihin. Lisäksi on selvitetty vasta-aineen tyrosiinien radiojodauksen sekä lysiineihin kytkettyjen kelaattien vaikutusta immunoreaktiivisuuteen.

Suora jodi-131-leimaus aiheuttaa immunoreaktiivisuuden laskun vasta-aineeseen liitettyjen jodiatomien funktiona. Terapeuttisen IgG:n (HuM195) sekä erityisesti sen hypervariaabelialueen aminohappojärjestystä tutkimalla selvitettiin antigeenin sitoutumisalueella olevien tyrosiinien jodauksen vaikutusta immunoreaktiivisuuteen. Hypervariaabelialue sisältää huomattavasti enemmän tyrosiineja kuin voisi olettaa aminohappojen satunnaisen jakautuman pohjalta. Havaittu immunoreaktiivisuus eri jodi/vasta-aine suhteilla sopi teoreettisiin käyriin, jotka oli muodostettu olettamalla, että jodin liittyminen mihin tahansa tyrosiinitähteeseen hypervariaabelialueella tuhoaa vasta-aineen immunoreaktiivisuuden. Hajottamalla vasta-aine entsymaattisesti osiin havaittiin, ettei jodi ollut liittynyt tasaisesti eri osissa oleviin tyrosiinitähteisiin. Jodia liittyi suhteellisesti enemmän raskaan ketjun variaabelialueen tyrosiineihin kuin muualle IgG:hen. Tuloksista voidaan myös päätellä, että hypervariaabelialueen korkea tyrosiinipitoisuus on rajoittava tekijä suoran radiojodauksen käytössä sellaisilla aktiivisuustasoilla, joita tarvitaan radioimmunoterapiassa.

Kymmenen CHX-A-DTPA ligandia liitettiin yhtä vasta-ainemolekyylia kohti. Leimausefektiivisyys vismutti-213:lla oli yli 90%. Kun hiiriin injektoitiin Bi-206-CHX-A-DTPA-HuM195:tä ei havaittu vapaata vismutin kertymistä munuaisiin kuten ei myöskään konjugaatin kertymistä muihin elimiin. ^{213}Bi-CHX-A-DTPA-HuM195 toksisuus evaluoitiin normaaleissa hiirissä. Annoksilla 0.5-20 mCi/kg ei havaittu toksisuutta, mutta annos 70 mCi/kg osoitti selvää toksisuutta. Solutappokokeet eri spesifisillä ^{212}Bi- ja ^{213}Bi-konjugaatti aktiivisuuksilla osoittivat annoksesta ja spesifisestä aktiivisuudesta riippuvaa HL60-solujen kuolemaa.

Tämän väitöskirjan tulokset osoittavat, että vismutti-213 leimatulla HuM195:lla on erittäin hyvä kyky tappaa spesifisesti kohdesoluja ilman merkittävää toksisuutta muille kudoksille.

Acknowledgements

This study was carried out in the Memorial Sloan Kettering Cancer Center, New York, during 1993-1995, and it was financially supported by MSKCC, the Finnish Foundation for Cancer Research, MAP Medical Technologies Oy and Pharmactinium, Inc. I wish to express my gratitude to David Scheinberg, M.D., Ph.D., and Ronald Finn, Ph.D., (Memorial Sloan Kettering Cancer Center) for arranging the laboratory facilities for my disposal and for their caring guidance throughout this study. Their encouragement, support and especially their warm friendship made this study possible.

My sincere thanks go to my other supervisor, Antero Salminen, Ph.D., (University of Kuopio) for his advice, excellent cooperation, and friendship.

I wish to thank the official referees of the thesis, Professor Sirpa Jalkanen, M.D., Ph.D., (University of Turku) and Docent Kim Bergström, Ph.D. (Kuopio University Hospital) for their valuable criticism and comments. I also wish to thank Atro Voutilainen, Ph.D., for revising the language of this thesis.

Part of this work is fruit of collaboration with other laboratories in U.S.A. and Europe. I am indebted to late Otto Gansow, Ph.D., and Martin Brechbiel, Ph.D. (National Cancer Institute) for their advice in chelation chemistry. I would also to like to thank Christos Apostolidis Ph.D., and Roger Molinet Ph.D., (Institute for Transuranium Elements) for the collabration in ^{213}Bi-generator.

I wish to express my warmest thanks to Maurits Geerlings, Ph.D., and Neil Bander M.D., Ph.D., for their support and friendship.

I further express my thanks to my collagues Monica Bocchia, M.D., Michael Mc Devitt, Ph.D., Michael Curcio, B.Sc., George Sgouros, Ph.D., and Yan Ma, M.Sc., for their fruitful collabration and friendship.

My deepest gratitude belongs to Jukka Hiltunen, M.Sc., Jaana Kumpulainen, Ph.D., Pirkko Penttilä, M.Sc., and Jarmo Haukka, M.Sc. for their encouragement, collabration and valuable comments.

I owe my sincerest thanks to my parents Össi ja Pentti for their endless love.

There is no way I can express my love and gratitude to my wife Leena and my children, Juho, Simo and Ottilia for their patience and understanding.

REFERENCES

Alvarez, V. L., Lopes, A. D., Lee, C., Coughlin, D. J., Rodwell, J. D. & McKearn, T. J. 1988. Site-specific modification of monoclonal antibodies: studies of indium-labeled antibodies using nude mouse xenograft systems. In: Rodwell J. D. (eds) Antibody-mediated Delivery Systems: 283-315. Marcel Dekker, New York.

Atcher, R. W., Friedman, A. M. & Hines, J. J. 1988. An improved generator for the production of ^{212}Pb and ^{212}Bi from ^{224}Ra. Appl. Radiat. Isot. 39: 283-286.

Bast, R.C., Jr., Klug, T.L., St.John, E., Jenison, E., Niloff, J.M., Lazarus, H., Berkowitz, R.S., Leavitt, T., Griffiths, C.T., Parker, L., Zurawski, V.R., Jr. & Knapp, R.C. 1983. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N. Engl. J. Med. 309: 883-887.

Behr, T.M., Sharkey, R.M., Juweid, M.E., Dunn, R.M., Vagg, R.C., Ying, Z., Zhang, C.-H., Swayne, L.C., Vardi, Y., Siegel, J.A. & Goldenberg, D.M. 1997. Phase I/II clinical radioimmunotherapy with an iodine-131-labeled anti-carcinoembryonic antigen murine monoclonal antibody IgG. J. Nucl. Med. 38: 858-870.

Black, C.D.V., Atcher, R.W., Barbet, J., Brechbiel, M.W., Holton III, O.D., Hines, J.J., Gansow, O.A. & Weinstein, J.N. 1988. Selective ablation of lymphocytes *in vivo* by an alpha emitter, ^{212}bismuth, chelated to a monoclonal antibody. Antibody, Immunoconj. and Rad.Phar. 1: 43-53.

Blower, P.J., Lewis, J.S. & Zweit, J. 1996. Copper radionuclides and radiopharmaceuticals in nuclear medicine. Nucl. Med. Biol. 23: 957-980.

Bolton, A.E. & Hunter, W.M. 1973. The labeling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. Biochem. J. 133: 529-539.

Brandt, K.D., Schnobrich, K.E. & Johnson, D.K. 1991. Characterization of antibody-chelator conjugates: Determination of chelator content by terbium fluorescence titration. Bioconjugate Chem. 2: 67-70.

Brechbiel, M.W., Gansow, O.A., Atcher, R.W., Schlom, J., Esteban, J., Simpson, D. & Colcher, D. 1986. Synthesis of 1-(p-isothiocyanatobenzyl) derivatives of DTPA and EDTA. Antibody labeling and tumor imaging studies. Inorg. Chem 25: 2772-2781.

Brechbiel, M.W., Pippin, C.G., McMurry, T.J., Milenic, D., Roselli, M., Colcher, D. & Gansow, O.A. 1991. An effective chelating agent for labelling of monoclonal antibody with ^{212}Bi for α-particle mediated radioimmunotherapy. J. Chem., Soc. Chem. Commun. 1169-1170.

Brechbiel, M.W. & Gansow, O.A. 1992: Synthesis of C-functionalized trans-cyclohexyldiethylenetriaminepenta-acetic acids for labelling of

monoclonal antibodies with the bismuth-212 α-particle emitter. J. Chem. Soc. Perkin Trans 1: 1173-1178.

Camera, L., Kinuya, S., Garmestani, K., Wu, C., Brechbiel, M.W., Pai, L.H., McMurry, T.J., Gansow, O.A., Pastan, I., Paik, C.H. & Carrasquillo, J.A. 1994. Evaluation of the serum stability and *in vivo* biodistribution of CHX-DTPA and other ligands for yttrium labeling of monoclonal antibodies. J. Nucl. Med. 35: 882-889.

Capillon, Ph., Cuerreau, D., Garron, J.Y. & Pasqualini, R. 1992. A simple chromatographic method to determine by-products in labeling DTPA chelate monoclonal antibodies with (^{111}In) indium. In: IXth international symposium on radiopharmaceutical chemistry. Paris, 6-10 april 1992: 402. Conservatoire National des Arts et Metiers, Paris.

Caron, P.C., Co, M.S., Bull, M.K., Avdalovic, N.M., Queen, C. & Scheinberg, D.A. 1992. Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies. Cancer Res. 52: 6761-6767.

Caron, P.C., Jurcic, J.G., Scott, A.M., Finn, R.D., Divgi, C.R., Graham, M.C., Jureidini, I.M., Sgouros, G., Tyson, D., Old, L.J., Larson, S.M. & Scheinberg, D.A. 1994. A phase 1B trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: Specific targeting without immunogenicity. Blood 83: 1760-1768.

Chinol, M. & Hnatowich, D.J. 1987. Generator-produced yttrium-90 for radioimmunotherapy. J. Nucl. Med. 28: 1465-1470.

Chinol, M., Franceschini, R., Paganelli, G., Pecorale, A. & Paiano, A. 1997. Simple production of yttrium-90 in a chemical form suitable to clinical grade radioconjugates. In: Bergmann, H., Kroiss, A. & Sinzinger, H. (eds), Radioactive Isotopes in Clinical Medicine and Research XXII: 327-332. Birkhäuser Verlag, Basel.

Co, M.S., Avdalovic, N.M., Caron, P.C., Avdalovic, M.V., Scheinberg, D.A. & Queen, C. 1992. Chimeric and humanized antibodies with specificity for the CD33 antigen. J. Immunol. 148: 1149-1154.

Co, M.S., Scheinberg, D.A., Avdalovic, N.M., McGraw, K., Vasquez, M., Caron, P.C. & Queen, C. 1993. Genetically engineered deglycosylation of the variable domain increases the affinity of an anti-CD33 monoclonal antibody. Molecular Immunol. 30: 1361-1367.

Co, M.S., Yano, S., Hsu, R.K., Landolfi, N.F., Vasquez, M., Cole, M., Tso, J.T., Bringman, T., Laird, W., Hudson, D., Kawamura, K., Suzuki, K., Furuichi, K., Queen, C. & Masuho, Y. 1994. A humanized antibody specific for the platelet integrin gpIIb/IIIa. J. Immunol. 152: 2968-2976.

DeNardo, S.J, DeNardo, G.L. & O'Grady, L.F. 1988. Pilot studies of radioimmunotherapy of B cell lymphoma and leukemia using iodine-131 Lym-1 monoclonal antibody. Antibody Immunoconj. Radiopharm 1: 17-33.

Deshpande, S.V., DeNardo, S.J., Kukis, D.L., Moi, M.K., McCall, M.J., DeNardo, G.L. & Meares, C.F. 1990. Yttrium-90-labeled monoclonal antibody for therapy: Labeling by a new macrocyclic bifunctional chelating agent. J. Nucl. Med. 31: 473-479.

Doering, R.F., Tucker, W.D. & Stang, L.G., Jr., 1963. A simple device for milking high-purity yttrium-90 from strontium-90. J. Nucl. Med. 4:54-

59.

Durbin, P. W. 1960. Metabolic characteristics within a chemical family. Hlth. Phys. 2: 225-238.

Fracker, P.J. & Speck, J.C. 1978. Protein and cell membrane iodinations with a sparingly soluble chloramide 1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril. Biochem. Biophys. Res. Commun. 80: 849-857.

Ferens, J.M., Krohn, K.A., Beaumier, P.L., Brown, J.P., Hellström, I., Hellström, K.E., Carrasquillo, J.A. & Larson, S.M. 1984. High-level iodination of monoclonal antibody fragments for radiotherapy. J. Nucl. Med. 25: 367-370.

Foulon, C.F., Schoultz, B.W. & Zalutsky, M.R. 1997. Preparation and biological evaluation of an astatine-211 labeled biotin conjugate: Biotinyl-3-[^{211}At]astatoanilide. Nucl. Med. Biol. 24: 135-143.

Gangwer, T.E., Goldstein, M. & Pillay, K.K.S. 1977. Radiation effects on ion exchange materials, Brookhaven National Laboratory, 32

Gansow, O.A. 1991. Newer approaches to the radiolabeling of monoclonal antibodies by use of metal chelates. Nucl. Med. Biol. 18: 369-381.

Gansow, O.A., Atcher, R.W., Link, D.C., Friedman, A.M., Seevers, R.H., Anderson, W., Scheinberg, D.A. & Strand, M. 1984. Generator-produced Bi-212 chelated to chemically modified monoclonal antibody for use in radiotherapy. In: Knapp, F.F., Butler, T.A. (eds), Radionuclide generators: New systems for Nuclear Medicine Application: 215-227. Am. Chem. Soc. Symp. Ser. 241, Washington DC.

Geerlings, M.W., Kaspersen, F.M., Apostolidis, C. & Van der Hout, R. 1993. The feasibility of ^{225}Ac as a source of α-particles in radioimmunotherapy. Nucl. Med. Comm. 14: 121-125.

Hemmilä, I.A & Mikola, H.J. 1990. New complexing agents for labeling of proteins with metals. Acta Radiol. Sup. 374: 53-55.

Hird, V., Maraveyas, A., Snook, D., Dhokia, B., Soutter, W.P., Meares, C., Stewart, J.S.W., Mason, P., Lambert, H.E. & Epenetos, A.A. 1993. Adjuvant therapy of ovarian cancer with radioactive monoclonal antibody. Br. J. Cancer 68: 403-406.

Hnatowich, D.J., Layne, W.W., Childs, R.L., Lanteigne, D., Davis, M.A., Griffin, T.W. & Doherty , P.W. 1983. Radioactive labeling of antibody: A simple and efficient method. Science 220: 613-615.

Hnatowich, D.J., Virzi, F. & Doherty, P.W. 1985. DTPA-coupled antibodies labeled with yttrium-90. J. Nucl. Med. 26: 503-509.

Hnatowich, D.J., Chinol, M., Siebecker, D.A., Gionet, M., Griffin, T., Doherty, P.W., Hunter, R. & Kase, K.R. 1988. Patient biodistribution of intraperitioneally administered yttrium-90 labeled antibody. J. Nucl. Med. 29: 1428-1434.

Hughes, O.D.M., Bishop, M.C., Perkins, A.C., Frier, M., Price, M.R., Denton, G., Smith, A., Rutherford, R. & Schubiger, P.A. 1997. Preclinical evaluation of copper-67 labelled anti-MUC1 mucin antibody C595 for therapeutic use in bladder cancer. Eur. J. Nucl. Med. 24: 439-443.

Hui, K.Y., Haber, E. & Matsueda, 1983. Monoclonal antibodies to a synthetic fibrin-like peptide bind to human fibrin but not fibrinogen. Science 222: 1129-1132.

Humm, J.L 1987. A microdosimetric model of As-211-labeled antibodies for radioimmunotherapy. Int. J. Radiat. Oncol. Biol. Phys. 13: 1767-1773.

Huneke, R.B., Pippin, C.G., Squire, R.A., Brechbiel, M.W., Gansow, O.A. & Strand, M. 1992. Effective α-particle-mediated radioimmunotherapy of murine leukemia. Cancer Res. 52: 5818-5820.

Hunter, W.M. & Greenwood, F.C. 1962. Preparation of iodine-131 labelled human growth hormones of high specific activity. Nature 194: 495-496.

Jacobs, A.J., Fer, M., Su, F.M., Breitz, H., Thompson, J., Goodgold, H., Cain, J., Heaps, J. & Weiden, P. 1993. A phase I trial of a rhenium 186-labeld monoclonal antibody administered intraperitoneally in ovarian carcinoma: Toxicity and clinical response. Obstet. Gynecol. 82: 586-593.

Jones, S.B., Tiffany, L.J., Garmestani, K., Gansow, O.A. & Kozak, R.W. 1996. Evaluation of dithiol chelating agents as potential adjuvants for anti-IL-2 receptor lead or bismuth alpha radioimmunotherapy. Nucl. Med. Biol. 23: 105-113.

Juric, J.G. & Scheinberg, D.A. 1994. Recent developments in the radioimmunotherapy of cancer. Cur. Opin. Immun. 6: 715-721.

Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K. S. & Foeller, C. 1991. Sequence of proteins of immunological interest. U.S. Department of Health and Human Services, Puplic Health service National Institute of Health.

Kamioki, H., Mirzadeh, S., Lambrecht, R.M., Knapp, R., Jr., & Dadachova, 1994. ^{188}W->^{188}Re generator for biomedical applications. Radiochimica Acta 65: 39-46.

Kassis, A.I., Adelstein, S.J., Haycock, C. & Sastry, K.R.S. 1980. Radiotoxicity of Se-75 and S-35. Theory and application to a cellular model. Radiat. Res. 84: 407-425.

Kawashima, T. 1966. Separation of carrier-free ^{90}Y from ^{90}Sr by cation exchange in a methanol-ammonium acetate medium. Int. J. Appl. Rad. Isot. 20: 806-808.

Kosmas, C, Maraveyas, A., Gooden, C.S., Snook, D. & Epenetos, A.A. 1995. Anti-chelate antibodies after intraperitoneal yttrium-90-labeled monoclonal antibody immunoconjugates for ovarian cancer therapy. J. Nucl. Med. 36: 746-753.

Kosmas, C, Snook, D., Gooden, C.S., Courtenay-Luck, N.S., McCall, M.J., Meares, C.F. Epenetos, A.A. 1992. Developmentof humoral immune responses against a macrocyclic chelating agent (DOTA) in cancer patients receiving radioimmunoconjugates for imaging and therapy. Cancer Res. 52: 904-911.

Kozak, R.W., Atcher, R.W., Gansow, O.A., Friedman, A.M., Hines, J.J. & Waldmann, T.A. 1986. Bismuth-212-labeled anti-Tac monoclonal antibody: α-particle-emitting radionuclides as modalities for radioimmuinotherapy. Proc. Natl. Acad. Sci. USA 83: 474-478.

Krejcarek, G.E. & Tucker, K.L. 1977. Covalent attachment of chelating groups to mackromolecules. Biochem. Biophys. Res. Commun. 77: 581-585.

Kurtzman, S.H., Russo, A., Mitchell, J.B., DeGraff, W., Sindelear, W.F., Brechbiel, M.W., Gansow, O.A., Friedman, A.M., Hines, J.J., Gamson, J. & Atcher, R.W. 1988. ^{212}Bismuth linked to an antipancreatic carcinoma antibody: model for alpha-particle-emitter radioimmunotherapy. J. Nat. Cancer Inst. 80: 449-452.

Lambrecht, R.M. & Mirzadeh, S. 1985. Cyclotron isotopes and radiopharmaceuticals-XXXV. Astatine-211. Int. J. Appl. Radiat. Isotopes 36: 443-450.

Langmuir, V.K., Atcher, R.A., Hines, J.J. & Brechbiel, M.W. 1990. Iodine-125-NRLU-10 kinetic studies and bismuth-212-NRLU-10 toxicity in LS174T multicell spheroids. J. Nucl. Med. 31: 1527-1533.

Larson, S.M. 1986. A tentative biological model for the localization of radiolabelled antibody in tumor: The importance of immunoreactivity. Nucl. Med. Biol. 13: 393-399.

Larson, S.M., Carrasquillo, J.A. & Reynolds, J.C. 1984. Radioimmunodetection and radioimmunotherapy. Cancer Invest. 2: 363-381.

Lee, J., Garmestani, K., Wu, C., Brechbiel, M.W., Chang, H.K., Choi, C.W., Gansow, O.A., Carrasquillo, J.A. & Paik, C.H. 1997. *In vitro* and *in vivo* evaluation of structure-stability relationship of ^{111}In- and ^{67}Ga-labeled antibody via 1B4M or C-NOTA chelates. Nucl. Med. Biol. 24: 225-230.

Lee, T.-W. & Ting, G. 1991. Study on the separation of carrier free yttrium-90 from strontium-90. Isotopenpraxis 27: 269-273.

Lei, K., Ruskowski, M., Chang, F., Qu, T., Mardrissian, G. & Hnatowich, D.J. 1996. Technetium-99m antibodies labeled with MAG_3 and SHNH: an *in vitro* and animal *in vivo* comparison. Nucl. Med. Biol. 23: 917-922.

Leung, C.S.-H., Meares, C.F. & Goodwin, D.A. 1978. The attachment of metal-chelating groups to proteins: Tagging of albumin by diazonium coupling and use of the products as radiopharmaceuticals. Int. J. Appl. Radiat. Isotopes 29: 687-692.

Macklis, R.M, Kinsey, B.M., Kassis, A.I., Ferrara, J.L.M. Atcher, R.W. Hines, J.J., Coleman, C.N., Adelstein, S.J. & Burakoff, S.J. 1988. Radioimmunotherapy with alpha-particle-emitting immunoconjugates. Science 240: 1024-1026.

Macklis, R.M., Lin, J.Y., Beresford, B., Atcher, R.W., Hines, J.J. & Humm, J.L. 1992. Cellular kinetics, dosimetry, and radiobiology of α-particle radioimmunotherapy: Induction of apoptosis. Radiat. Res. 130: 220-226.

Maisano, F., Gozzini, L. & de Haën, C. 1992. Coupling of DTPA to proteins: A critical analysis of the cyclic dianhydride method in the case of insulin modification. Bioconjugate Chem. 3: 212-217.

Marchalonis, J.J. 1969. An enzymic method for the tracer iodination of immunoglobulins and other proteins. Biochem. J. 113: 299-305.

Mather, S.J., Tolley, D.M. & White, G.W. 1989. Labelling monoclonal antibodies with yttrium-90. Eur. J. Nucl. Med. 15: 307-312.

Mathias, C.J., Sun, Y., Connett, J.M., Philpott, G.W., Welch, M.J. & Martell, A.E. 1990. A new bifunctional chelate, $BrMe_2HBED$: An effective conjugate for radiometals and antibodies. Inorg. Chem. 29: 1475-1480.

Matzku, S., Kirchgeßner, H. & Nissen, M. 1987. Iodination of monoclonal IgG antibodies at a sub-stoichiometric level: Immunoreactivity changes related to the site of iodine incorporation. Nucl. Med. Biol. 14: 451-457.

McDevitt, M.R., Finn, R.D., Scheinberg, D.A., Larson S.M., Nikula, T., & Gansow, O. 1996. The preparation of yttrium-86 labelled monoclonal antibody chelate constructs for evaluation of yttrium-antibody pharmacokinetics. PET and Cancer, European Conference on research and application of positron emission tomography in oncology April 17-19,1996 Groningen, the Netherlands.

Meares, C.F., Goodwin, D.A., Leung, C.S.-H., Girgis, A.Y., Silvester, D.J., Nunn, A.D. & Lavender, P.J. 1976. Covalent attachment of metal chelates to proteins: The stability *in vivo* and *in vitro* of the conjugate of albumin with a chelate of ^{111}indium. Proc. Natl. Acad. Sci. USA. 73: 3803-3806.

Meares, C.F. & Wensel, T.G. 1984. Metal chelates as probes of biological systems. Acc. Chem. Res. 17: 202-209.

Meares, C.F., McCall, M.J., Reardan, D.T., Goodwin, D.A., Diamanti, C.I. & McTigue, M. 1984. Conjugation of antibodies with bifunctional chelating agents: Isothiocyanate and bromoacetamide reagents, methods of analysis and subsequent addition of metal ions. Anal. Biochem 142: 68-78.

Mirzadeh, S., Brechbiel, M.W., Atcher, R.W. & Gansow, O.A. 1990. Radiometal labeling of immunoproteins: Covalent linkage of 2-(4-isothiocyanatobenzyl)diethylenetriaminepentaacetic acid ligands to immunoglobulin. Bioconjugate Chem. 1: 59-65.

Motta-Hennesy, C., Sharkey, R.M. & Goldenberg, D.M. 1991. Labeling of monoclonal antibody conjugates with ^{90}Y. Appl. Radiat. Isot. 42: 421-426.

Nagasawa, H., Little, J.B., Inkret, W.C., Carpenter, S., Raju, M.R., Chen, D.J. & Strniste, G.F. 1991. Response of X-ray-sensitive CHO mutant cells (xrs-6c) to radiation II. Relationship between cell survival and the induction of chromosomal damage with low doses of α particles. Radiat. Res. 126: 280-288.

Nelson, F. 1964. Ion exchange procedures VI. cation exchange of Ac(III) and Fr(I) in HCl and $HClO_4$ solutions: isolation of ^{227}Ac from ^{231}Pa and ^{227}Ac daughters. J. Chromatog. 16: 538-540.

Paganelli, G., Grana, C., Chinol, M., Cremonesi, M., De Cicco, C., De Braud, F., Maggiolo, M., Siccardi, A.G. & Veronesi, U. 1997. Antibody quided three-step radiotherapy with ^{90}Y-biotin in glioma patients. The fourteenth international conference on advances in the application of monoclonal antibodies in clinical oncology. 5-7 May 1997 Thira, Santorini, Greece, 47.

Paik, C.H., Murphy, P.R., Eckelman, W.C., Volkert, W.A. & Reba, R.C. 1983a. Optimazion of the DTPA mixed-anhydride reaction with antibodies at low concentration. J. Nucl. Med. 24: 932-936.

Paik, C.H., Ebbert, M.A., Murphy, P.R. Lassman, C.R., Reba, R.C., Eckelman, W.C., Pak, K.Y., Powe, J., Steplewski, Z. & Koprowski, H. 1983b. Factors influecing DTPA conjugation with antibodies by cyclic DTPA anhydride. J. Nucl. Med. 24: 1158-1163.

Palayoor, S.T., Humm, J.L., Atcher, R.W., Hines, J.J. & Macklis, R.M. 1993. G2M arrest and apoptosis in murine T lymphoma cells following exposure to ^{212}Bi alpha particle irradiation. Nucl. Med. Biol. 20: 795-805.

Pham, D.T., Kaspersen, F.M. & Bos, E.S. 1995. Electrophoretic method for the quantitative determination of a benzyl-DTPA ligand in DTPA monoclonal antibody conjugates. Bioconjugate Chem. 6: 313-315.

Pippin, C.G., Parker, T.A., McMurry, T.J. & Brechbiel, M.W. 1992. Spectrophotometric method for the determination of a bifunctic DTPA ligand in DTPA-monoclonal antibody conjugates. Bioconjugate Chem. 3: 342-345.

Queen, C., Schneider, W. P., Selick, H. E., Payne, P. W., Landolfi, N. F., Duncan, J. F., Avdalovic, N. M., Levitt, M., Junghans, R. P. & Waldmann, T. A. 1989. A humanized antibody that binds to the interleukin 2 receptor. Proc. Natl. Acad. Sci. U.S.A 86: 10029-10033.

Rane, A.T. & Bhatki, K.S. 1966. Rapid radiochemical separations of strontium-90-yttrium-90 and calcium-45-scandium-46 on a cation exchange resin. Anal. Chem. 38: 1598-1601.

Raju, M.R., Eisen, Y., Carpenter, S. & Inkret, W.C. 1991. Radiobiology of α-particles III. Cell inactivation by α-particle traversals of the cell nucleus. Radiat. Res. 128: 204-209.

Rhodes, B.A., Lambert, C.R., Marek, M.J., Knapp, F.F., Jr., & Harvey, E.B. 1996. Re-188 labelled antibodies. Appl. Radiat. Isot. 47: 7-14

Riechmann, L., Clark, M., Waldmann, H. & Winter, G. 1988. Reshaping human antibodies for therapy. Nature 332, 323-327.

Roselli, M., Schlom, J., Gansow, O.A. Raubitschek, A., Mirzadeh, S., Brechbiel, M.W. & Colcher, D. 1989. Comparative biodistributions of yttrium- and indium-labeled monoclonal antibody B72.3 in athymic mice bearing human colon carcinoma xenografts. J. Nucl. Med. 30: 672-682.

Ruegg, C.L., Anderson-Berg, W.T., Brechbiel, M.W., Mirzadeh, S., Gansow, O.A. & Strand, M. 1990. Improved *in vivo* stability and tumor targeting of bismuth-labeled antibody. Cancer Res. 50: 4221-4226.

Rusckowski, K.L.M., Chang, F., Qu, T., Mardirossian, G. & Hnatowich, D.J. 1996. Technetium-99m antibodies labeled with MAG_3 and SHNH: An *in vitro* and animal *in vivo* comparison. Nucl. Med. Biol. 23: 917-922.

Sachs, L. & Lotem, J. 1993. Control of programmed cell death in normal and leukemic cells: New implications for therapy. Blood 82: 15-21.

Scheinberg, D.A., Strand, M. & Gansow, O.A. 1982. Tumor imaging with radioactive metal chelates conjugated to monoclonal antibodies. Science 215: 1511-1513.

Scheinberg, D.A., McDevitt, M.R., Brechbiel, M.W., Finn, R.D., Wu, C., Geerlings, M.W., Sr., Geerlings M., Jr., Nikula T., Bander N., Barendswaard, E., Juric J., Larson S., Sgouros, G., Apostolidis C., Molinet, R. & Gansow, O.A. 1997. Alpha particle emitting bismuth-213 cyclohexylbenzyl DTPA constructs of monoclonal antibodies for therapy of cancer. Presented in α-immuno´97, Karlsruhe 27-28.10.97.

Schlom, J., Siler, K., Milenic, D.E., Eggensperger, D., Colcher, D., Miller, L.S., Houchens, D., Cheng, R., Kaplan, D. & Goeckeler, W. 1991. Monoclonal antibody-based therapy of a human tumor xenograft with a lutenium-177-labeled immunoconjugate. Cancer 51: 2889-2896.

Schwartz, D.A. Abrams, M.J., Hauser, M.M., Gaul, F.E., Larsen, S.K., Rauh, D. & Zubieta, 1991. Preparation of Hydrazino-modified proteins and their use for the synthesis of ^{99m}Tc-protein conjugates. Bioconjugate Chem. 2: 333-336.

Schwartz, M.A. Lovett, D.R., Redner, A., Finn, R.D., Graham, M.C., Divgi, C.R., Dantis, L., Gee, T.S., Andreeff, M., Old, L.J., Larson, S.M. & Scheinberg, D.A. 1993. Dose-escalation trial of M195 labeled with iodine-131 for cytoreduction and marrow ablation in relapsed or refractory myeloid leukemias. J. Clin. Oncol. 11: 294-303.

Schroff, R.W., Weiden, P.L., Appelbaum, J., Fer, M.F., Breitz, H., Vanderheyden, J.-L., Ratliff, B.A., Fisher, D., Foisie, D., Hanelin, L.G., Morgan, A.C., Jr., Fritzberg, A.R. & Abrams, P.G. 1990. Rhenium-186 labeled antibody in patients with cancer: report of a pilot phase I study. Antibody, Immunoconj. and Radiopharm. 3: 99-111.

Sharkey, R.M., Motta-Hennessy, C., Gansow, O.A., Brechbiel, M.W., Fand, I., Griffiths, G.L., Jones, A.L. & Goldenberg, D.M. 1990. Selection of a DTPA chelate conjugate for monoclonal antibody targeting to a human colonic tumor in nude mice. Int. J. Cancer 46: 79-85.

Simonson, R.B., Ultee, M.E., Hauler, J.A. & Alvarez, V.L. 1990. Radioimmunotherapy of peritoneal human colon cancer xenografts with site-specifically modified ^{212}Bi-labeled antibody. Cancer Res. 50: 985s-988s.

Stemmer, S.M., Johnson, T., Kasliwal, R., Lear, J., Matthes, S., Taffs, S., Dufton, C., Glenn, S., Butchko, G. & Jones, R.B. 1994. High dose ^{90}Y-Mx-DTPA-BrE-3 and autologous hematopoietic stem cell support for the treatment of advanced breast cancer. J. Immunother. 16: 161.

Suzuki, Y. 1964. Preparation of carrier-free ^{90}Y from ^{90}Sr with ion exchange. Int. J. Appl. Rad. Isot. 20: 806-808.

Tanimoto, M., Scheinberg, D.A., Cordon-Cardo, C., Huie, D., Clarkson, B.D. & Old, L.J. 1989. Restricted expression of an early myeloid and monocytic cell surface antigen defined by monoclonal antibody M195. Leukemia 3. 339-348.

Vaidyanathan, G., Affleck, D.J. & Zalutsky, M.R. 1997. Method for radioiodination of proteins using N-succinimidyl 3-hydroxy-4-iodobenzoate. Bioconjugate Chem. 8: 724-729.

Van den Abbeele, A.D., Aaronson, R.A., Daher, S., Taube, R.A., Adelstein, S.J. & Kassis, A.I. 1991. Antigen-binding site protection during

radiolabeling leads to a hihger immunoreactive fraction. J. Nucl. Med 32: 116-122.

Van Geel, J., Fuger, J. & Koch, L. 1991. Verfahren zur Erzeugung von ^{229}Th, ^{225}Ac und ^{213}Bi. European Patent Application 91102260.6.

Vaughan, A.T.M., Bateman, W.J., Brown, G. & Cowan, J. 1982. The specific inhibition of cellular clonogenic proliferation using ^{211}At labelled lectins and antibodies-I. Int. J. Nucl. Med. Biol. 9: 167-171.

Wike, J.S., Guyer, C.E., Ramey, D.W. & Phillips, B.P. 1990. Chemistry for commercial scale production of yttrium-90 for medical research. Int. J. Appl. Radiat. Isot. 41: 861-865.

Willins, J.D. & Sqouros, G. 1995. Modeling analysis of platinum-195m for targeting individual blood-borne cells in adjuvant radioimmunotherapy. J. Nucl. Med. 36: 315-319.

Wu, C., Brechbiel, M.W., Gansow, O.A., Kobayashi, H., Carrasquillo, J. & Pastan, I. 1997. Synthesis and evaluation of the four stereoisomers of 2-(p-nitrobenzyl)-trans-CyDTPA for radiolabeling immunoprotein with ^{90}Y and $^{212/213}$Bi. Submitted.

Yamauchi, K., Suzuki, Y., Nagao, T. & Arimori, S. 1989. Kinetics of indium-111 chloride in chronic myelogenous leukemia. Nucl. Med. Comm. 10: 817-826.

Yeh, S.M., Sherman, D.G. & Meares, C.F. 1979. A new route to "bifunctional" chelating agents: Conversion of amino acids to analogs of ethylenedinitrilotetraacetic acid. Anal. Biochem. 100: 152-159.

Zalutsky, M.R. & Narula, A.S. 1987. A method for the radiohalogenation of proteins resulting in decreased thyroid uptake of radioiodine. Appl. Radiat. Isotopes. 38: 1051-1055.

Zalutsky, M.R., Garg, P.K., Friedman, H.S. & Bigner, D.D. 1989. Labeling monoclonal antibodies and $F(ab')_2$ fragments with the α-particle emitting nuclide astatine-211: Preservation of immunoreactivity and *in vivo* localizing capacity. Proc. Natl. Acad. Sci. USA 86: 7149-7153.

Zalutsky, M.R., Stabin, M.G., Larsen, R.H. & Bigner, D.D. 1997. Tissue distribution and radiation dosimetry of astatine-211-labeled chimeric 81C6, an α-particle-emitting immunoconjugate. Nucl. Med. Biol. 24: 255-261.

Zucchini, G.L. & Friedman, A.M. 1982. Isotopic generator for ^{212}Pb and ^{212}Bi. Int. J. Nucl. Med. Biol. 9: 83-84.

I

Labeling and purification of DTPA-coupled monoclonal antibodies with ^{57}Co, ^{90}Y and ^{165}Dy

by

Penttilä P.I., Hiltunen J.V., Nikula T.K. & Kairemo K.J.A.

In: Maddalena, D.J., Snowdon, G.M. & Bonigace, G.R. (eds), Advances in Radiopharmacology, Proceedings of the sixth international symposium on radiopharmacology 1990: 227-231.

II

Radioimmunotherapy using ^{90}Y-labelled monoclonal antifibrin antibody in nude mice

by

Kairemo K.J.A., Hiltunen J.V., Penttilä P.I., Nikula T.K. & Laine A.

In: Maddalena, D.J., Snowdon, G.M. & Bonigace, G.R. (eds), Advances in Radiopharmacology, Proceedings of the sixth international symposium on radiopharmacology 1990: 232-237.

III

A rapid single vessel method for preparation of clinical grade ligand conjugated monoclonal antibodies

by

Tuomo Nikula, Michael Curcio, Martin Brechbiel, Otto Gansow, Ronald Finn & David Scheinberg

Nuclear Medicine and Biology 22: 387-390, 1995

https://doi.org/10.1016/0969-8051(94)00126-5

IV

Alpha particle emitting bismuth cyclohexylbenzyl DTPA constructs of recombinant humanized anti-CD33 antibodies for therapy of myeloid leukemias

by

Tuomo Nikula, Michael McDevitt, Chuanchu Wu, Robert Kozak, Kayhan Garmestani, Martin Brechbiel, Michael Curcio M, Greg Pippin, L. Tiffany-Jones , Ronald Finn, Maurits Geerlings, Sr., Christotos Apostolidis, Roger Molinet, Otto Gansow & David Scheinberg

submitted

https://jnm.snmjournals.org/content/40/1/166

V

Impact of the high tyrosine fraction in complementarity determining regions: Measured and predicted effects of radioiodination on IgG immunoreactivity

by

Tuomo Nikula, Monica Bocchia, Michael Curcio, George Sgouros, Yan Ma, Ronald Finn & David Scheinberg

Molecular Immunology 32: 865-872, 1995

https://doi.org/10.1016/0161-5890(95)00052-G