THE PHARMACOKINETICS AND TOXICITY OF INTRAPERITONEALLY ADMINISTERED RADIOLABELLED MONOCLONAL ANTIBODIES IN OVARIAN CANCER PATIENTS

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ABSTRACT

The pharmacokinetics and toxicity of intraperitoneally administered radiolabelled monoclonal antibody (MAb) were studied in 47 cancer patients. Twenty-three patients received ^{131}Iodine-labelled MAb and 24 patients ^{90}Yttrium-labelled monoclonal antibody. The pharmacokinetics, biodistribution and normal tissue dosimetry were different for these two isotopes.

Patients receiving ^{131}Iodine-labelled MAb were divided into three groups. The 13 patients in the first group had no previous exposure to murine immunoglobulin, three of these patients had ascites. The five patients in the second group had human antimouse-immunoglobulin antibodies (HAMA) following previous exposure to murine antibody. The five patients in the third group were HAMA negative but received affinity purified HAMA intravenously. In the first group ^{131}Iodine-labelled MAb was efficiently absorbed from the peritoneal cavity into the circulation producing myelosuppression. The degree of myelosuppression could be predicted by the radiation dose the marrow received from ^{131}Iodine in the vascular compartment. ^{131}Iodine-labelled MAb was not efficiently absorbed from the peritoneal cavity in patients with ascites, radiation enteritis was a more significant toxicity than myelosuppression. The HAMA positive patients in the second group had different pharmacokinetics, ^{131}Iodine-labelled MAb was rapidly cleared from the circulation, with a nine fold reduction in the radiation dose to bone marrow. Similar pharmacokinetics could be induced in HAMA negative patients by the intravenous administration of

affinity purified HAMA. Although the liver was responsible for clearing immune ^{131}Iodine-labelled complexes, the increased radioactivity in this organ was only transient.

Although the blood pharmacokinetics of ^{131}Iodine and ^{90}Yttrium-labelled MAb were similar, ^{90}Yttrium accumulated in various organs such as liver, spleen, bone and bone marrow rather than being excreted in the urine (like ^{131}Iodine). The concentration of ^{90}Yttrium in the bone and marrow made these more important sources of radiation to the marrow than the vascular compartment. Eight of these patients received intravenous infusions of EDTA in an attempt to chelate ^{90}Yttrium released by the MAb and to increase the excretion of ^{90}Yttrium in the urine. The urinary excretion of ^{90}Yttrium was increased four-fold by EDTA with less myelosuppression.

Myelsuppression is the toxicity that limits the amount of ^{131}Iodine and ^{90}Yttrium labelled antibody that can be administered. Different methods must be adopted to decrease the toxicity from these radiolabeles. Anti-antibodies can increased the clearance of ^{131}Iodine-labelled MAb as this isotope is easily excreted. ^{90}Yttrium needs to be adequately chelated before reaching its full potential as a radiolabel in radioimmunotherapy.

I would like to dedicate this thesis to my wife, Cathy
and my children, Hamish and Anna.

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TABLE OF CONTENTS

	Pages
Title	1
Abstract	2
Acknowledgments	5
List of Figures	12
List of Tables	16
Abbreviations	19

CHAPTER 1
Introduction

Radioimmunotherapy.	21
Historical.	21
Systemic RIT.	25
Animal Studies.	25
Studies in Man.	29
Limitations.	32
Regional RIT.	35
Ovarian Carcinoma and RIT.	38
Natural History.	38
Surgery.	39
Chemotherapy.	39
Radiotherapy.	40
Rationale for RIT in Ovarian Cancer.	44
Radiolabelled Antibodies for RIT.	44
Antibody of antibody fragments in RIT?	46
Antibody Quantity.	46
MAb Preparations in these Studies.	47
Which isotope in RIT?	47
Radioimmunoscintigraphy.	50
Radioimmunotherapy.	50
Radio-isotopes for intraperitoneal RIT.	54
The Objectives of this thesis.	55

CHAPTER 2:
Intraperitoneal ^{131}I-MAb in non-immunized patients

Introduction. 57

Patients, Materials & Methods.

- Patients. 59
- ^{131}I-labelled MAb Preparations. 59
- Intraperitoneal Administration of ^{131}I-labelled MAb. 61
- Measurement of ^{131}I Activity in the Blood. 63
- Measurement of ^{131}I Activity in the Urine. 64
- Measurement of the Radiation Dose to the Peritoneal Serosa. 64
- Bone Marrow Radiation Dosimetry. 67
- Myelosuppression. 69

Results.

- Pharmacokinetics of ^{131}Iodine in the Blood. 70
- Urine Excretion of ^{131}Iodine. 74
- The Radiation Dose to the Peritoneal Serosa. 74
- The Radiation Dose to the Bone Marrow. 75
- Toxicity. 80

Discussion

- Patients without ascites. 85
- Patients with ascites. 87

Summary 89

CHAPTER 3
Intraperitoneal ^{131}I-MAb to Patients Immunized to Murine Antibody

Introduction. 91

Patients, Materials & Methods.

89

- Patients. 93
- ^{131}I-labelled MAb Preparations. 93
- Intraperitoneal Administration of ^{131}I-labelled MAb. 95
- Pharmacokinetics & Dosimetry. 95

Results. 97

- Pharmacokinetics. 97
- Bone Marrow Dosimetry. 101

Radiation Dose to the Peritoneal Serosa. 101
Toxicity. 107
Discussion. 109
Summary. 112

CHAPTER 4
A Study of Intravenously Administered Human Antimouse Immunoglobulin Antibody to Patients Receiving ^{131}I-labelled MAb

Introduction. 114
Patients, Materials and Methods. 118
Patients. 118
^{131}I-labelled MAb. 118
Human Antimouse Antibody. 120
Intraperitoneal Administration of ^{131}I-labelled MAb. 121
Pharmacokinetics of MAb & HAMA. 122
Radiation Dosimetry to the Bone Marrow. 123
Radiation Dosimetry to the Liver. 124
Results. 125
Pharmacokinetics of ^{131}I-labelled MAb. 125
Hepatic Clearance of ^{131}I-labelled MAb. 132
Pharmacokinetics of HAMA. 135
Urinary Excretion of ^{131}Iodine. 136
Radiation Dose to Bone Marrow. 149
Radiation Dose to Liver. 141
Toxicity. 141
Discussion. 146
Summary. 149

CHAPTER 5
Intraperitoneal Radioimmunotherapy with ^{90}Yttrium labelled MAb

Introduction. 151
Patients, Materials and Methods. 154
Patients. 154
^{90}Yttrium-labelled MAb. 154

Intraperitoneal Administration of ^{90}Y-labelled MAb. 157
Pharmacokinetics of ^{90}Y-labelled MAb. 157
Radiation Dose to Marrow. 158
Radiation Dose to the Peritoneal Serosa. 150
Toxicity. 160
Results
Pharmacokinetics. 162
Radiation Dose to the Peritoneal Serosa. 168
Radiation Dose to the Bone Marrow. 172
Toxicity. 175
Discussion. 178
Summary. 181

CHAPTER 6
The Biodistribtuion of Intraperitoneally Administered ^{90}Y-labelled MAb in Rabbits

Introduction. 183
Methods and Materials. 185
Results.
Non-immunized Rabbits. 189
Immunized Rabbits. 191
Discussion. 193
Summary. 197

CHAPTER 7
Intravenous EDTA to chelate ^{90}Yttrium Released from ^{90}Y-labelled MAb during RIT

Introduction. 199
Patients, Materials and Methods. 202
Patients. 202
Intraperitoneal Administration of ^{90}Y-labelled MAb. 202
Intravenous Administration of EDTA. 204
Pharmacokinetics of ^{90}Y-labelled MAb. 204
Radiation Dosimetry of Bone Marrow. 204
Toxicity. 205
Results. 206

Pharmacokinetics. 206
Radiation Dose to Bone Marrow. 213
Toxicity. 215
Discussion. 218
Summary. 223

CHAPTER 8
Discussion

Dose Effect Curves in Radiotherapy. 225
Local Toxicity of Radioimmunotherapy. 227
Without Ascites. 227
With Ascites. 228
Systemic Toxicity of Radioimmunotherapy. 228
^{131}I-labelled MAb's in HAMA negative patients. 229
^{131}I-labelled MAb's in HAMA positive patients. 230
Intravenous HAMA in patients receiving RIT. 231
^{90}Y-labelled MAb's in HAMA negative patients. 232
Further Developments of Intraperitoneal RIT. 233

APPENDIXES

Appendix A: Measurement of blood ^{131}I activity. 236
Appendix B: Measurement of urine ^{131}I activity. 239
Appendix C: Measurement of the radiation dose to the peritoneal serosa by TLD. 240
Appendix D: Measurement of the % of isotope bound to protein by dialysis. 245
Appendix E: Measurement of the % of isotope bound to proten by Gel Filtration. 246
Appendix F: Measurement of blood ^{90}Yttrium activity. 248
Appendix G: Measurement of urine ^{90}Yttrium activity. 250
Appendix H: Calculation of the radiation dose to bone marrow from radiolabelled MAbin the vascular compartment. 253
Appendix I: Mann Whitney U Test for the comparison of non-parametric data. 257
Appendix J: Separation of HAMA from serum using affinity column chromatography. 258

References 260

LIST OF FIGURES

CHAPTER 1
Introduction

Figure 1.1: An IgG immunoglobulin and its F(ab')2 and Fab fragments. 45

CHAPTER 2:
Intraperitoneal ^{131}I-MAb in non-immunized patients

Figure 2.1: The intraperitoneal administration of ^{131}I-labelled MAb. 62
Figure 2.2: Intraperitoneal Lithium Fluoride Catheter. 66
Figure 2.3: Blood ^{131}I activity following the i.p. infusion of ^{131}I-labelled MAb. 71
Figure 2.4: Blood ^{131}I activity in mCi following the i.p. administration of ^{131}I-MAb in eight patients. 73
Figure 2.5: Cumulative urine excretion of ^{131}I following the i.p. infusion of ^{131}I-labelled MAb. 76
Figure 2.6: Myelosuppression according to the radiation dose the bone received from ^{131}I in the vascular compartment. 83
Figure 2.7: Platelet count in patients receiving 145 - 157 mCi of ^{131}I-labelled MAb. 84

CHAPTER 3
Intraperitoneal ^{131}I-MAb to Patients Immunized to Murine Antibody

Figure 3.1: ^{131}I activity in HAMA positive and negative patients following the i.p. administration of ^{131}I-MAb. 99
Figure 3.2: Biological Half life of ^{131}I-MAb in HAMA positive patients. 100
Figure 3.3a: Free ^{131}I in the blood of a HAMA positive patient. 103
Figure 3.3b: Sepharose S-200 FPLC of serum of a HAMA positive patient. 103
Figure 3.4: Urinary ^{131}I excretion in HAMA positive patients. 104
Figure 3.5: Radiation Dose to the bone marrow in HAMA positive and negative patients. 105

Figure 3.6: Radiation dose to the peritoneal serosa in HAMA positive and HAMA negative patients. 106
Figure 3.7: Myelosuppression in HAMA positive and negative patients according to the radiation dose the bone marrow. 108

CHAPTER 4
A Study of Intravenously Administered Human Antimouse Immunoglobulin Antibody to Patients Receiving ^{131}I-labelled MAb

Figure 4.1: Blood ^{131}I activity following the administration of i.p. ^{131}I-MAb and i.v. HAMA in patients with ovarian cancer. 126
Figure 4.2: Total ^{131}I-activity and fraction of free ^{131}I in a patient. 126
Figure 4.3: Total ^{131}I-activity and fraction of free ^{131}I in two patients. 129
Figure 4.4: Total ^{131}I-activity and fraction of free ^{131}I in two patients. 130
Figure 4.5: ^{131}I activity in the liver following the clearance of ^{131}I-MAb. 133
Figure 4.6: Blood pharmacokinetics of intravenously injected HAMA. 134
Figure 4.7: Urine excretion of ^{131}I following the clearance of ^{131}I-MAb by HAMA. 137
Figure 4.8: Comparison of ^{131}I excretion in HAMA negative patients, HAMA positive patients, and patients receiving i.v. HAMA. 138
Figure 4.9: Comparison radiation dose to marrow in HAMA negative patients, HAMA positive patients, and patients receiving i.v. HAMA. 140
Figure 4.10: Comparison radiation dose to liver in HAMA negative patients, HAMA positive patients, and patients receiving i.v. HAMA. 140
Figure 4.11: Platelet and leucocyte count following the clearance of ^{131}I-MAb. with intravenous HAMA. 144
Figure 4.12: Comparison of the degree of myelosuppression according to the radiation dose the bone marrow received in HAMA negative patients,HAMA positive patients and patients receiving i.v. HAMA. 145

CHAPTER 5
Intraperitoneal RIT with ^{90}Yttrium labelled MAb

Figure 5.1: Mean blood ^{90}Y activity in patients receiving ^{90}Y-MAb. 165
Figure 5.2: Fraction of blood ^{90}Y not bound to protein in patients receiving

^{90}Y-MAb. 166
Figure 5.3: Rabbit Antimouse immunoglobulin affinity chromatography of the serum of a patient receiving ^{90}Y-MAb. 166
Figure 5.4: Biological half life of ^{90}Y-MAb in the blood. 169
Figure 5.5: Urine excretion of ^{90}Y in patients receiving ^{90}Y-MAb. 171
Figure 5.6: Radiation dose to the peritoneal serosa in patients receiving i.p. ^{90}Y-silicate colloid and ^{90}Y-MAb. 174
Figure 5.7: Myelosuppression according to tbe radiation dose to bone marrow in patients receiving ^{90}Y-MAb. 176
Figure 5.8: Platelet count in patients after the administration of ^{90}Y-MAb. 177

CHAPTER 6

The Biodistribution of Intraperitoneally Administered ^{90}Y-labelled MAb in Rabbits

Figure 6.1: The tissuebiodistribution of ^{90}Y-MAb in rabbits following the i.p. injection of ^{90}Y-MAb. 195
Figure 6.2: The tissuebiodistribution of ^{90}Y in rabbits (immunised to the MAb following the i.p. injection of ^{90}Y-MAb. 196

CHAPTER 7

Intravenous EDTA to chelate ^{90}Yttrium Released from ^{90}Y-labelled MAb during RIT

Figure 7.1: Mean blood ^{90}Y activity in patients receiving i.p. ^{90}Y-MAb and i.v. EDTA. 207
Figure 7.2: Blood ^{90}Y activity in a patient with ascites receiving i.p. ^{90}Y-MAb and i.v. EDTA. 208
Figure 7.3: Biological hlal life of ^{90}Y-MAb in the blood in patients receivingi.p.^{90}Y-MAb and i.v. ETDA. 209
Figure 7.4: Percent of blood ^{90}Y not bound to protein. 211
Figure 7.5: Comparison of the urinary excretion of ^{90}Y in patients receiving i.p. ^{90}Y-MAb with and without i.v. EDTA. 212

Figure 7.6: A comparison of thrombocytopenia in patients who received i.p. infusions of ^{90}Y-MAb with or without i.v. EDTA. 216
Figure 7.7: Myelosuppression according to tbe radiation dose to bone marrow in patients receiving ^{90}Y-MAb with or without i.v. EDTA. 217
Figure 7.8: The effect of EDTA on the stability of ^{90}Y on MAB. 221
Figure 7.9: The effect of i.v. EDTA on the stability of ^{90}Y on MAb in a patient. 221

CHAPTER 8
Discussion

Figure 8.1: The sigmoid dose-effect curve for tumour cure. 226
Figure 8.1: The sigmoid dose-effect curve for tumour cure and fatal complications. 226

APPENDIXES

Figure A.1: Volume Correction Factors for Scintillation Counts. 237
Figure C.1: The TLD catheter. 242
Figure C2: The Calibration of LiF TLD micro-rods. 243
Figure E1: The protein concentration (absorbance at 280 Å) in the eluent of a Sephadex G-50 column. 246
Figure F.1: Volume Correction Factors for Scintillation Counts. 249

LIST OF TABLES

CHAPTER 1
Introduction

Table 1.1: Biodistribution data of the amount of intravenously injected radiolabelled MAb in human tumours. 33

Table 1.2: A table of the physical data for the isotopes most commonly used in RIT or RIS. 47

Table 1.3: The maximum b-particle energy Emax (MeV), maximum range in tissue D_{max}, and the half life of suitable isotopes for RIT. 50

CHAPTER 2:
Intraperitoneal ^{131}I-MAb in non-immuized patients

Table 2.1: ^{131}I-labelled MAb preparations. 58

Table 2.2: Blood ^{131}I activity. 68

Table 2.3: Cumulative ^{131}I excretion. 72

Table 2.4: Radiation dose to the peritoneal serosa. 74

Table 2.5: Integrated blood ^{131}I mCihrs. in HAMA negative patients. 75

Table 2.6: Myelosuppression (WHO scale) according to the radiation dose the bone receives from ^{131}I-MAb in the blood. 78

CHAPTER 3
Intraperitoneal ^{131}I-MAb to Patients Immunized to Murine Antibody

Table 3.1: ^{131}I-MAb preparations and previous RIT details. 90

Table 3.2: Blood ^{131}I activity follwoing the i.p. administration of ^{131}I-MAb in five HAMA positive patients. 94

Table 3.3: ^{131}I excretion in the urine in patients receiving i.p. ^{131}I-MAb. 98

Table 3.4: Integrated blood ^{131}I activity in mCi.hrs in HAMA positive patients. 99

Table 3.5: Radiation dose to the peritoneal serosa. 101

Table 3.6: Myelosuppression (WHO scale) in HAMA positive patients. 103

CHAPTER 4
A Study of Intravenously Administered Human Antimouse Immunoglobulin Antibody to Patients Receiving ^{131}I-labelled MAb

Table 4.1: ^{131}I-MAb preparations in patients receiving i.v. HAMA. 113
Table 4.2: Myelosuppression according to the WHO scale in patients receivingi.v HAMA. 136

CHAPTER 5
Intraperitoneal Radioimmunotherapy with ^{90}Yttrium labelled MAb

Table 5.1: ^{90}Y-MAb preparations.
146
Table 5.2: Blood ^{90}Y activity in patients receiving ^{90}Y-MAb. 153
Table 5.3: Cumulative urinary excretion of ^{90}Y in patients receiving ^{90}Y-MAb. 159
Table 5.4: Integrated ^{90}Y activity in the blood and the radiation dose to bone marrow in patients receiving ^{90}Y-MAb.
161

CHAPTER 6
The Biodistribution of Intraperitoneally Administered ^{90}Y-labelled MAb in Rabbits

Table 6.1: The tissuebiodistribution of ^{90}Y-MAb in rabbits following the i.p. injection of ^{90}Y-MAb. 178
Table 6.2: The tissuebiodistribution of ^{90}Y in rabbits (immunised to the MAb) following the i.p. injection of ^{90}Y-MAb. 180

CHAPTER 7
Intravenous EDTA to chelate ^{90}Yttrium Released from ^{90}Y-labelled MAb during RIT

Table 7.1: ^{90}Y-MAb preparation in patients receiving i.v. EDTA. 191

Table 7.2: Blood ^{90}Y activity in patients receiving EDTA. 195

Table 7.3: Cumulative urinary excretion of ^{90}Y in patients receiving i.p. ^{90}Y-MAb and i.v. EDTA. 200

Table 7.4: Integrated ^{90}Y activity in the blood and the radiation dose the bone marrow receives from this in patients receiving i.v. EDTA. 202

ABBREVIATIONS

The following abbreviations have been used in this thesis:

AUC	Area Under the Curve,
ELISA	Enzyme Linked Immunosorbant Assay,
FPLC	Fast Liquid Protein Chromatography,
GBq	Gigabecquerel (10^9 Bq or 27 mCi)
HAMA	Human Anti-Murine Immunoglobulin Antibody,
HMFG1	Human Milk Fat Globule 1,
^{125}I	^{125}Iodine,
^{131}I	^{131}Iodine,
i.p.	Intraperitoneal,
i.v.	Intravenous,
LiF	Lithium Fluoride,
MAb	Monoclonal antibody,
MBq	Megabecquerel (10^6Bq or 27μCi),
μCi	Microcurie (10^{-6} Ci or 37 kBq),
mCi	Millicurie (10^{-3} Ci or37 MBq),
RES	Reticuloendothelial System
RIS	Radioimmunoscintigraphy,
RIT	Radioimmunotherapy,
TLD	Thermoluminescent Dosimetry,
^{90}Y	^{90}Yttrium.

INTRODUCTION

1. Radioimmunotherapy

If we could selectively irradiate tumour cells then disseminated cancer would be a curable disease; unfortunately this is rarely possible. Tumours do not concentrate radio-isotopes, and the inclusion of normal tissue in a treatment volume limits the effectivness of extrenal beam radiotherapy. Well differentiated follicular thyroid carcinomas which concentrate iodine in their colloid are an exception: if these tumours concentrate iodine sufficiently then they can be cured by the administration of ^{131}I even when widely disseminated. If radioactive isotopes could be targeted to other more common malignant tumours then these would be equally curable. Radioimmunotherapy (RIT) is a therapeutic strategy which aims to achieve this by using anti-tumour antibodies to deliver radioactive isotopes to tumour cells.

1.1 Historical

1.1.1 Polyclonal Antibodies

Using radiolabelled antibodies to target radiation to tumours is an old idea. Antibodies have been used to deliver radio-isotopes to organs since 1948 when Pressman and Keighley were able to demonstrate specific targeting of renal tissue in rats by injecting ^{131}I-labelled anti-kidney antibodies. For many years tumour targeting by radiolabelled

antibodies was hampered by the lack of any known tumour associated antigens. In the 1950's transplanted tumours in animals were found to have a high concentration of fibrin [Bale et al., 1960]. Tumours were localized in animals and man by radioimmunoscinitigraphy (RIS) using ^{131}I labelled anti-fibrin antibodies. Seventy-five percent of tumours from 175 consecutive patients with a range of malignancies from different sites (breast, bronchus, melanoma, and head & neck) were detected by RIS [Spar et al., 1967]. This successful imaging study was followed by a RIT study in 12 patients with advanced carcinoma receiving 50 - 150 mCi (1.85 - 5.55 GBq) of ^{131}I labelled anti-fibrin antibody. The estimated radiation dose to tumours in this study was up to 2000 cGy and there was a single documented response in a patients with squamous carcinoma of the tongue. Fibrin however was found to be a consequence of the inflammatory reaction surrounding experimental transplanted tumours in animals and was not truly tumour associated [Day et al., 1959]. Much of the tumour imaging from anti-fibrin antibodies was probably non-specific.

With the discovery by Gould & Freidman of the tumour associated antigen carcinoembryonic antigen (CEA), the beta subunit of human chorionic gonadotropin (βHCG) and alpha fetoprotein (αFP) it became possible to develop antibodies that were more specific to tumour cells. These antigens are not tumour specific as they can be expressed by foetal tissue (βHCG, αFP), by normal tissue (CEA) or non malignant diseased tissue (CEA, αFP). They are however expressed in high concentrations by some tumours than normal tissue. In 1974 Mach et al., described how xenografts tumours (from a colon cancer cell line) in nude mice could be imaged scintigraphically by ^{131}I-labelled goat anti-CEA antibody; 25% of the injected ^{131}I activity was targeted to

each gram of tumour tissue. Antibody specificity was demonstrated by tumours not concentrating ^{131}I-labelled non-specific antibody to the same degree. In the same year Goldenberg et al., were able to scintigraphically image tumours as small as 70 mgs by injecting ^{125}I labelled goat anti-CEA against a colon cancer cell line called GW-39 grown in hamster cheek pouches. This group also demonstrated that antibody specificity was important with 6.6% and 1.9% of the injected activity in each gram of tumour from specific and non-specific antibody respectively. Tumours did however take up the non-specific immunoglobulin and tumours of more than 200 μm could be imaged scintigraphically with the non-specific antibody. These animal studies were quickly followed clinical studies of ^{131}I labelled anti-CEA in cancer patients; using affinity purified goat anti-CEA [Goldenberg et al., 1978] and sheep anti-CEA [Hine et al., 1980]. Both groups demonstrated positive tumour immunoscintigraphy with ^{131}I-labelled anti-CEA: 10 of 11 primary tumours and 24 of 29 secondary tumour deposits were detected by Goldenberg's group, and 4 of 5 primary tumours and 8 of 11 secondary deposits identified by Hine's group. Mach et al [1980] had less success in imaging colorectal tumours with ^{131}I goat anti-CEA antibody and were only able to correctly identify 11 tumours in 27 patients. They were able to demonstrate that antibody specificity was important by injecting dual labelled specific (^{131}I-labelled anti-CEA) and non-specific (^{125}I labelled goat IgG): the ratio of specific to non-specific antibody in tumours deposits taken from four patients ranged from 4:1 to 5:1. Similar imaging studies were also reported with an ^{131}I-labelled anti-αFP antibody in patients with hepatoma and germ cell tumours [Kim et al., 1980]. All these studies used polyclonal antibody preparations; these were obtained by passing the serum taken from an animal immunized to the antigen down an

affinity column containing the selected antigen. Polyclonal antibody preparations contain antibodies with a wide range of affinities and avidities to several epitopes on the selected antigen, and different polyclonal antibody preparations can behave differently.

1.1.2 Monoclonal Antibodies

In 1976 Kohler and Milstein developed the technology to produce monoclonal antibodies to selected antigens by fusing murine myeloma cells with antigen stimulated splenic B cells [Kohler et al., 1976; and Kohler & Milstein, 1976]. It became possible to maintain immortal cell lines that produced an unlimited supply of monoclonal antibody to a single epitope. Monoclonal antibodies can now be produced to any desired antigen and there are a whole range of MAb's to antigens associated with tumour cells.

Radiolabelled MAbs have been investigated in patients since 1980. In 1981 Mach et al., repeated their RIS study in 28 patients with CEA positive carcinomas (26 colorectal and 2 pancreatic tumours. They were able to positively identify tumours in 14 patients with i.v. ^{131}I-labelled anti-CEA MAb, 6 patients had ambiguous scans and the remaining 8 patients had negative scans; ^{99m}Tc blood pool subtraction was used to increase the sensitivity. The sensitivity was increased by using SPECT[1] in a further 14 patients; they were able to identify 13 tumours with one false negative scan. Since then there have been numerous RIS studies using either whole the whole antibody, F(ab')$_2$ or Fab fragments to antigens expressed by melanoma [Larson et al., 1983a]; Murray et al., 1985 & 1987; Taylor et al., 1988; Early et al.,

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1 Single positron emission tomography

1989], colorectal carcinoma [Carrasquillo et al., 1988b], T-cell lymphoma [Carrasquillo et al., 1987], prostatic carcinoma [Dillman et al., 1987], ovarian carcinoma [Epenetos et al., 1986a; Burzon et al., 1989; Chatal et al., 1987; Pateisky et al., 1985], and testicular teratoma [Epenetos et al., 1986a]. Tumours have been detected by RIS when they were undetectable by conventional radiology including CT scanning [Early et al., 1989, Epenetos et al., 1986a; Chatal et al., 1987; Bomanji et al., 1988]. The success in targeting isotopes to tumour cells in these RIS studies have given impetus to using radiolabelled MAb for radioimmunotherapy

1.2. Systemic RIT

Before the application of hybridoma technology RIT research was restricted to studies of polyclonal antibodies to the few known tumour associated antigens such as CEA, βHCG, αFP, and ferritin. The development of MAb's with specificity to antigens expressed by the majority of tumour cells has made a huge impact. Studies of RIT in animals and man will be discussed separately.

1.2.1 Studies in animals

Goldenberg and coworkers decribed a RIT animal study of radiolabelled antibody to a tumour associated antigen in 1981. Tumours that expressed CEA (GW-39) were grown in hamster cheek pouches: the inhibition of tumour growth following the administration of polyclonal ^{131}I-labelled goat anti-CEA and non-specific goat immunoglobulin were compared. They found that although both radiolabelled antibodies were able to inhibit tumour growth, the anti-

CEA antibody was twice as effective. This was particularly evident in animals with a low tumour burden. The ^{131}I labelled anti-CEA actually prolonged survival by decreasing tumour related deaths: unlabelled anti-CEA antibody was not effective. The following year Scheinberg and Strand studied the effect of an ^{131}I labelled MAb (103A) on Rauscher erythroleukemia in mice. They were unable to demonstrate that the ^{131}I labelled antibody was any more effective than unlabelled 103A in killing erythroleukemic cells. At the time they suggested that the radiation emitted by ^{131}I was too penetrating. A year later however, Scheinberg & Strand [1983] found that although the radiolabelled specific antibody targeted Rauscher erythroleukemia cells to a much greater extent than non-specific MAb, the iodine radiolabel was rapidly removed from tumour cells in the spleen and this may have been responsible for the low poor therapeutic response.

Zalcberg et al., [1984] were able to demonstrate tumour regression in animal RIT studies. This group did a series of experiments using two xenograft tumours ITT-1-7SN and COLO 205 growing in nude mice. COLO 205 tumour nodules of up 0.8 cm in size regressed following the administration of 1.1 mCi (41 MBq) ^{131}I-labelled specific MAb (250-30.6) but not when ^{131}I-labelled non-specific or unlabelled 250-30.6 were injected. The estimated radiation dose to the tumour nodules was 700 cGy. This group also found that the administration of propyl-thiouracil (a drug that inhibits dehalogenase enzymes) increased the therapeutic effect of the ^{131}I-labelled MAb. Badger et al., [1985] undertook a dose escalation study of ^{131}I-labelled anti-Thy 1.1 in nude mice bearing AKR1 SL2 xenograft lymphomas. The ^{131}I activity was increased from 0 to 2000 μCi (74 MBq): there was a 6% partial response with unlabelled MAb, an 11% complete response rate with

500 μCi (18.5 MBq) and a 26% complete response rate with 1000 μCi (37 MBq) of ^{131}I-labelled MAb. The radiation dose to tumour was 1600 and 380 cGy following the injection of 500 μCi (18.5 MBq) of ^{131}I-labelled anti-Thy 1.1 and non-specific MAb respectively. Although the response rate increased with increasing ^{131}I activity, the death rate from myelosuppression also increased, the LD_{50} occurred after 1500 μCi (55 MBq) of ^{131}I-labelled MAb. Tumour relapses when they occurred were from Thy 1.1 negative cells. Goldenbergs group have also undertaken a RIT study using an ^{131}I-labelled MAb anti-CEA instead of the goat anti-CEA [Sharkey et al., 1987]. Tumour expressing CEA (GW-39) were grown in hamster cheek pouches while the animals received either ^{131}I-labelled NP-4 (an anti-CEA MAb) or non-specific murine MAb. They found that ^{131}I-labelled specific MAb was more effective in suppressing tumour growth that non-specific MAb, and that there was an dose response between ^{131}I activities of 500 and 1000 μCi (18.5 - 17 MBq). Tumour burden also appeared to be important as ^{131}I labelled NP-4 was less effective if its administration was delayed by four to seven days. Tumours were received radiation doses of up to 2400 cGy.

Lee and coworkers have reported RIT studies of ^{131}I-labelled MAb (81C6) to human glioma xenografts growing in nude mice (Lee et al.,1988a) and rats (Lee et al., 1988b). This group were able to demonstrate a four-fold advantage of the ^{131}I-81C6 over a ^{131}I-labelled non-specific MAb. Only one of ten mice receiving one mCi (37 MBq) of ^{131}I-labelled non-specific MAb showed any tumour regression compared to 15 of 28 mice receiving ^{131}I-labelled 81C6. They were able to repeat this study in nude rats using intracranial rather than subcutaneous xenograft tumours. They were able to demonstrate that

1.25 - 2.5 mCi (46 - 92 MBq) of ^{131}I-labelled 81C6 produced tumour regression and increased survival which was not seen with ^{131}I-labelled non-specific MAb. The radiation dose delivered to intracranial xenograft tumours was 1565 cGy, nine times higher than that delivered by the non-specific antibody. The radiation dose to the normal brain in this study was only 31 cGy; the bone marrow however received a radiation dose of over 700 cGy.

Although ^{131}I has been the traditional radiolabel, there has been an increasing interest in alternative isotopes for reasons that will be discussed later. Anderson-Berg et al., [1987] have investigated the efficacy of a ^{90}Y-labelled MAb (103A) in eradicating erythroleukemia from the spleens of mice. These investigators found that ^{90}Y-labelled 103A was more successful than ^{90}Y-labelled non-specific MAb in eradicating erythroleukemia. Although myelosuppression was observed in these mice, this was not considered to be a dose limiting factor. Goldenbergs group have also investigated ^{90}Y-labelled MAb in a nude mouse xenograft model [Sharkey et al., 1989]. They investigated the ^{90}Y-labelled NP-2 MAb in nude mice carrying colon xenograft tumours (GW-39). They were able to demonstrate tumour regression following the injection of 50 μCi (1.85 MBq) ^{90}Y-labelled NP-2 , this was not observed with an ^{90}Y-labelled non-specific MAb. However, ^{90}Y activities of 75 μCi (2.78 MBq) were associated with severe myelosuppression and ^{90}Y activities greater than this produced fatal marrow hypoplasia. There has also been some work on the an α-emitter astatine-211 labelled MAb for RIT. Harrison and Royle found that an ^{211}As labelled anti-thy MAb (OX7) increased the survival of nude mice bearing A120 tumours.

1.2.2 Studies in man

There have been several RIT studies in man using radiolabelled polyclonal and monoclonal antibodies. The first study, after Pressmans work with ^{131}I-labelled antifibrin, was from Order's group at the Johns Hopkin Medical School. This group pioneered the RIT of hepatocellular carcinoma and conducted a series of studies using radiolabelled polyclonal anti-ferritin antibodies to irradiate hepatocellular carcinoma [Order et al.,1980; Ettinger et al.,1982; Order et al.,1985]. The clinical efficacy of RIT in these studies is difficult to interpret for several reasons. The RIT was combined with external beam irradiation and chemotherapy as these were thought to be synergistic. External beam irradiation has been found to increase the permeability of the tumour to radiolabelled antibody [Leichner et al., 1988] and adriamycin and 5FU may have a radiation-sensitizing effect as well being directly cytotoxic. The antibody used in the RIT protocols varied with ^{131}I-labelled polyclonal anti-ferritin from rabbits, goats, monkeys, pig, baboon, cow and mouse given in either one or more treatments. Fifteen to 150 mCi (0.55 - 5.55 GBq) of ^{131}I-labelled anti-ferritin was administered intravenously in either single or sequential injections. When sequential RIT treatments were given the animal species of antiferritin was changed so that patients did not develop anti-antibodies to the injected antiferritin. The effects of RIT could only be assessed in 56 patients: 4 (7%) patients had a complete response, 22 (39%) patients had a partial response, no change in tumour volume was seen in 18 (32%) patients, and tumour progression occurred in the remaining 12 (21%) patients. Myelosuppression appeared to be the dose limiting toxicity. They did not correlate of the degree of myelosuppression with the ^{131}I activity administered, with the

radiation dose to bone marrow, or with the type of antibody used. Myelosuppression appeared to be more severe when more that 100 mCi (3.7 GBq) of ^{131}I-labelled anti-ferritin was given or when adriamycin and 5FU were administered concurrently with RIT [Order et al., 1985]. The radiation dose to the whole body was estimated to be 1 to 1.5 cGy per mCi of ^{131}I labelled antibody. More recently this group have investigate the use of ^{90}Y labelled anti-ferritin for hepatocellular carcinoma [Leichner et al., 1988]: pre-irradiation and chemotherapy are still used however which makes the efficacy and toxicity of RIT difficult to assess.

^{131}Iodine labelled anti-ferritin antibodies have also been used to treat patients with recurrent Hodgkins Disease following combination chemotherapy [Lenhard et al., 1985]. Thirty-seven patients received 30 mCi (1.11 GBq) of ^{131}I-labelled polyclonal anti-ferritin antibody with a further 20 mCi (0.74 GBq) eight weeks later if the tumour had not increased in size. Fifteen (40%) patients had a partial response and 77% of patients had relief of type B symptoms. There was however considerable myelosuppression with 16 patients developing thrombocytopenia (< 100 x 10^9 platelets per litre) and 4 patients severe thrombocytopenia (<20 x 10^9 platelets per litre). The nadir blood counts were observed to occur about three to five weeks after treatment.

Other groups have treated fewer patients. Larson et al., [1983b] reported a small study of ^{131}I-labelled Fab MAb (anti-p97) in seven patients with metastatic melanoma. The ^{131}I activity was increased from 132 mCi to 529 mCi (4.9 - 19.6 GBq) given in several injections about five weeks apart. The radiation doses per 100 mCi (3.7 GBq) of ^{131}I to the tumour, liver, and bone marrow were calculated to have

been 1040, 325, and 30 cGy respectively: two minor tumour responses were later reported by Carrasquillo et al., in 1984. Although myelosuppression was mild and thrombocytopenia only occurred in two patients, this is difficult to interpret as the patients who received the highest ^{131}I activity developed their own antibodies to the murine MAb. This may have increased clearance of ^{131}I-labelled antibody from the blood with a lower radiation dose to the bone marrow. Rosen et al., [1987] reported treating five patients with cutaneous T-cell lyphoma with 100 to 150 mCi (3.7 - 5.55 GBq) of ^{131}I-labelled T101. Although the radiation dose to the lymphomatous deposits was only calculated to have been 40 - 510 cGy, all patients had some response following treatment with a decrease in lymph-adenopathy, in the extent of the skin lesions and in pruritis. This treatment was also associated with significant myelosuppression and this was the dose limiting toxicity. Again the pharmacokinetics and toxicity of ^{131}I-labelled MAb may have been altered by the presence of anti-murine antibodies (all patients had been previously exposed to murine immunoglobulin in radio-immunoscintigraphy studies). Lashford et al., reported a small study in 1987 of an ^{131}I-labelled MAb to neuroectodermal tissue (UJ13) in five children with chemoresistant neuroblastoma. The administered ^{131}I activity ranged from 35 to 55 mCi (1.3 - 2.03 MBq) and the maximum radiation dose the tumours received was 2800 cGy. Although one patient achieved a worthwhile response lasting eight months, two patients developed severe marrow aplasia. Patients who received a second treatment (may have developed anti-UJ13 antibodies) cleared the ^{131}I-labelled MAb from their blood rapidly. A pilot study of RIT for patients with B-cell lyphomas was recently reported by DeNardo et al., [1988]. Three patients with a B-cell lymphoma and two patients with chronic lymphocytic leukemia

received ^{131}I -labelled LYM-1 (a MAb produced against B cell lymphoma): a total of 20 doses[1] of 17 - 66 mCi (0.63 - 2.44 GBq) of ^{131}I-labelled LYM-1 were given at various intervals over a 16 month period. Three of the five patients responded to treatment with decreased lymphadenopathy. Toxicity appeared to be limited to mild myelosuppression although a patient did develop a fistula at the site of previous disease.

1.2.3 Limitations of Sytemic RIT

One of the major obstacles of systemic RIT is the poor concentration radiolabelled isotope by tumour deposits. Antibodies of greater affinity and avidity would increase tumour localization and improvements over the next few years can be expected. The problem of tumour cell antigen heterogeneity may be overcome by using MAb cocktails. Large tumours in particular have a low concentration of antibody: Hagan and coworkers have suggested that the antibody concentration in tumours is inversely related to tumour size. Macroscopic tumours are poorly vascularized with a hypoxic and often necrotic centre; Pervez et al., have shown in animal studies that injected radiolabelled MAb is concentrated to the perivascular areas with little localization in the tumour centre. It has also been suggested [Jain & Baxter, 1989] that tumours contain a higher interstitial pressure than the surrounding normal tissue and that this also decreases the concentration of MAb in tumour deposits. Even if the tumour deposit was as well vascularized as the surrounding normal tissue, and had the same

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1 One patients received 10 doses, 2 patients 5 doses, 2 patients 3 doses , and one patient a single dose.

interstitial pressure, a MAb would only pass through a one gram tumour deposit once every 49 days [Weintein et al., 1988].

The biodistribution studies of radiolabelled antibody in animals were promising with 2 to 24 % of the injected isotope activity in each gram of tumour tissue [Mach et al., 1974, Goldenberg et al., 1974; Goldenberg et al., 1981; Badger et al., 1985, Rowlinson et al., 1987; Ward et al., 1987; Lee et al., 1988a, Lee et al., 1988b; Sharkey et al., 1988]. Unfortunately the mouse was the most frequently used animal in many of these studies; the concentration of antibody in tumours grown in these animals is particularly high even when the antibody was not of murine origin. Mach and coworkers [1974] found 24% of the injected isotope activity per gram of tumour using goat anti-CEA in xenograft tumours in mice, and Ward using ^{131}I labelled MAb, 31% per gram of the injected isotope activity in tumour deposits.

The biodistribution data of intravenously administered radiolabelled MAb in humans are less promising. A summary of the biodistribution data in patients is given in Table 1.1 below.

Table 1.1: Biodistribution data of the amount of intravenously injected radiolabelled MAb in human tumours. The activities are given as the % injected activity per gram of tumour. (Data from selected references).

References	IgG/F(ab')$_2$	Isotope	Tumour % inject/g
Moldovsky et al., 1985	IgG	^{131}I	colorectal 0.0047
Murray et al., 1987	IgG	^{111}In	melanoma 0.000028
Taylor et al., 1988	IgG	^{111}In	melanoma <0.052
Early et al., 1989	F(ab')$_2$	^{99m}Tc	melanoma 0.03
	Fab	^{99m}Tc	melanoma 0.0041
Carrasquillo et al., 1988c	IgG	^{111}In	melanoma <0.007
Carrasquillo et al., 1987	IgG	^{111}In	lymphoma 0.02 (nodes) 0.0044 (skin)
Epenetos et al., 1986b	IgG	^{131}I /^{125}I	adenoCa <0.11

A radiation dose in the region of 60 Gy delivered over one week is required to eradicate macroscopic tumour. The minimum concentration of radiolabelled antibody in tumour deposits must be 0.05% if they are to deliver a radiation dose of 60 Gy to the tumour without exceeding whole body tolerance [Vaughan et al., 1987a; Epenetos et al., 1986]. Unfortunately this is rarely achieved when radiolabelled antibodies are injected intravenously.

1.3. Regional RIT

Some tumours do not spread throughout the whole body but remain confined to a body cavity; ovarian carcinoma and mesothelioma for example are often regional diseases, being confined to the peritoneal and pleural cavities. Tumours that produce ascites, pleural and pericardial effusions may be systemic malignancies but produce symptoms by their invasion of a single body cavity. These tumour deposits may concentrate more isotope if the radiolabelled MAb is injected directly into the body cavity rather than intravenously. This approach may be particularly useful in treating patients with ovarian cancer where intraperitoneal chemotherapy has been suggested to be advantageous [Brenner, 1986; Dedrick et al., 1978]. There is some evidence that intraperitoneal RIT may increase the tumour localization of intraperitoneal deposits.

Rowlinson and coworkers have conducted a biodistribution study using dual labelled MAb (AUAI) given intravenously (i.v.) and intra-peritoneally (i.p.) to nude mice bearing an intraperitoneal tumour cell line called LoVo. The tumour to normal tissue ratio for the i.p. administered isotope was 50 times that of the i.v. administered isotope: this i.p./i.v. advantage lasted 24 hours after which the i.p. to i.v ratio of isotopes became one. A similar study was described by Ward & Wallace [1987a]. They were only able to show a concentration advantage of intraperitoneally administered radiolabelled antibody in ascites cells where the ascites to normal tissue isotope ratio was 69.3, and the ratio of specific to non-specific antibodies on ascitic cells was 30.8. When the radiolabelled MAb was given intravenously the ascites

to normal tissue isotope ratio was only 5 and the ratio of specific to non-specific MAb on ascitic cells 7.5. Wahl and coworkers also found enhanced RIT of intraperitoneal human colonic cancer xenografts when radiolabelled MAb was given intraperitoneally (only an abstract of this work has been published [Wahl et al., 1987b]).

There are data from patients receiving intraperitoneally administered radiolabelled antibody that suggests that there is an advantage in regional delivery. Colcher and coworkers in 1987 described the biodistribution of intraperitoneally administered specific (B72.3) and non-specific MAb to colonic carcinoma in ten patients with colorectal tumours. They also compared the biodistribution of intraperitoneally administered ^{131}I-B27.3 with intravenously administered ^{125}I-B27.3 in intraperitoneal and extraperitoneal tumour deposits in four patients. They found that there was at least a two fold advantage (range 2 - 7.6) in the i.p. route of administration in 35 out of 55 tumour biopsies. The advantage of the i.p. route was particularly marked in tumour deposits that were true peritoneal implants without a blood supply. The i.p. route of administration was superior to the i.v. route in 35 of 40 such tumour deposits, the majority having a five fold advantage. Conversely there was in i.v. advantage in all the ten haematogenous deposits: five deposits could not be classified. There is limited biodistribution data [Malamitsi et al., 1988] from seven patients with suspected ovarian tumours who had laparotomies following the i.p. administration of radiolabelled MAbs HMFG2 and H17E2. Up to 0.1% of the injected isotope activity was found per gram of tumour tissue. Ward et al., [1987b] however, were unable find any advantage in the i.p. route of administration in patients with solid i.p. deposits of ovarian cancer. They did however demonstrate an advantage in the i.p. route of

administration when targeting malignant ascitic cells with as much as 0.68% of the injected dose dose per gram of malignant cells. If 100 mCi (3.7 MBq) of ^{131}I-labelled Mab were given then these ascites cells would receive a radiation dose in excess of 10,000 cGy.

The early phase I/II studies of regional RIT would appear to support the impression that small volume disease may respond to regional RIT. Epenetos et al., [1986c] reported controlling malignant ascites by injecting 46 mCi of an ^{131}I-labelled MAb (AUA1) intraperitoneally in a patient with chemoresistant ovarian carcinoma. This decreased the rate of ascites accumulation: when this did occur the cells were negative for AUA1. A later report describing the results of i.p. RIT in 24 patients with chemoresistant ovarian cancer found that all the responses were limited to patients with minimal residual disease [Epenetos et al., 1987]. Pectasides et al., used intracavitary RIT to treat 10 pleural and 3 pericardial effusions in 11 patients. Ten of the 13 effusions responded to regional RIT with a mean follow-up time of nine months (range 2 - 16 months).

There is evidence that other regional routes of RIT administration may be advantageous. Keenan et al., [1987] have investigated the possibility of using intradermal ^{111}In-labelled T-101 (an anti pan T-cell antibody) to image lymphomatous deposits in lymph nodes (lymphangio-immuno-scintigraphy). There appeared to be a good regional advantage with this route of administration with up to 2% of the injected isotope activity per gram of lymph node. There has also been a pilot study of intrathecal RIT in patients with carcinomatous meningitis [Lashford et al., 1988]. Five patients with carinomatous meningitis received between 11 and 40 mCi of ^{131}I-labelled a MAb selected to the tumour cell. Four

of these patients responded to treatment for seven months to two years with minimal toxicity.

Systemic RIT has not yet fulfilled it's early promise due to the poor tumour : normal tissue ratios of radiolabelled antibody. However regional RIT may have a role particularly for small volume disease. The I.C.R.F. group at the Hammersmith Hospital have investigated the feasibility of using intraperitoneal RIT to treat patients with ovarian cancer. The reasons for choosing this malignancy are discussed in the following section.

2. Ovarian Carcinoma & Radioimmunotherapy

2.1. Natural History

Carcinoma of the ovary is common in well developed countries. In the USA, United Kingdom, West Germany and most European countries it is the gynaecological malignancy with the greatest mortality [Becker et al., 1984; D.H.S.S., 1987; Silverberg & Luberg, 1987]. In 1983 there were 3,732 deaths from ovarian cancer in England and Wales and there is evidence that the incidence of ovarian cancer has been increasing steadily over the last 50 years [Beral, 1987].

Carcinoma of the ovary occurs most commonly in women of 45 to 75 years of age. There are few symptoms associated with the early stages of malignancy so 75% of women present with disease spread throughout the peritoneal cavity. Despite the widespread involvement of the peritoneal cavity the tumour is not invasive and tends to coat the

serosal surface of the bowel, liver, diaphragm, and peritoneum rather than invading deeply through these structures. There is also a tendency for ovarian cancer to remain confined to the peritoneal cavity rather than spreading to distant organs. Intra-abdominal disease is responsible for most of the morbidity and mortality in these patients. Surgery, chemotherapy and radiotherapy each have a role in the management of patients with ovarian cancer.

2.1.1. Surgery

As about 75% of patients with ovarian cancer have advanced disease with diffuse intraperitoneal involvement, initial surgery usually involves cytoreduction rather than cure. The aim of surgery in these circumstances is to decrease the bulk of tumour so that modalities such as chemotherapy and radiotherapy have a greater chance of cure. Although there had been no randomized controlled trial between full cytoreductive surgery and lesser procedures, it is the impression of most gynaecology oncologists that radical cytoreductive surgery increases the response to postoperative chemotherapy and long term survival [Wharton & Herson, 1981; Young et al., 1978; Hacker et al., 1983].

2.1.2. Chemotherapy

As the majority of patients will have residual disease following cytoreductive surgery further treatment is clearly indicated. Cisplatinum and its analogue carboplatin are regarded as the most active single agents with response rates of 55 - 65% [Wiltshaw et al., 1985, 1986]. Combination chemotherapy is regarded as having a greater response rate than alkylating agents alone [Young et al., 1978]

but the effects on long term survival are less certain [Kaye et al., 1987]. There is also only a slight difference between single agent cisplatinum and chemotherapy combinations containing platinum: there are two randomized trials that suggest that there is a better response rate in the latter although survival was not altered [Conte et al., 1986; G.I.C.I.O.G., 1987]. With platinum containing chemotherapy one may expect a response rate of 70 - 80% [Conte et al., 1986; Neijt et al., 1984; G.I.C.I.O.G., 1987]; long term survival however is poor with only 30% of patients surviving five years.

2.1.3 Radiotherapy

After cytoreductive surgery most patients have small tumour deposits left scattered throughout the peritoneal cavity from which relapse will eventually occur. Ovarian cancer is usually a regional disease: if these could be eradicated, thereby consolidating the effect of the previous surgery and chemotherapy, long term cure could be possible. Radiotherapy is successfully used to eradicate small volume disease, left after surgery, in many other sites in the body. Lymph node metastasis and microscopic spread from carcinomas of the upper respiratory digestive tract, breast, cervix, bladder, prostate, can be eradicating by external beam irradiation. To achieve this a radiation dose of 50 Gy in 25 fractions delivered over 5 weeks or its biological equivalence is required, with and additional 10 Gy to areas of bulk disease. Unfortunately it is not possible to irradiate the entire abdominal cavity to a radiation dose of 50 Gy as this would exceed the radiation tolerance

of the kidneys, liver, and small bowel[1]. Despite this some radiotherapy centres have had some success in eradicating small volume residual ovarian cancer.

Dembo and Bush [Dembo, 1985; Dembo et al., 1979] pioneered the use of external beam radiotherapy in ovarian cancer, irradiating the entire abdominal cavity to a radiation dose of 24 - 30 Gy in 20 fractions over 4 weeks. The pelvis could receive an additional 10 - 15 Gy but the kidneys required shielding so that the radiation dose did not exceed 20 Gy. Patients treated at the Princess Margaret hospital received whole abdominal radiotherapy rather than chemotherapy after surgery. There were 109 patients with stage III disease: after surgery 48 had no residual tumour, 43 had tumour deposits less than 2 cms in diameter, and 18 had deposits greater than 2 cms in diameter; the five year relapse free survival in these three groups were 40%, 28%, and 7%. Martinez et al., 1985; Fuller et al., 1987; Weiser et al., 1988; Goldberg & Peschel., 1988; and Goldhirsh et al., 1988; have also found whole abdominal radiotherapy beneficial in selected groups of patients.

Many radiotherapy centres have however had less success with radiotherapy for residual ovarian carcinoma. A randomized trial of whole abdominal radiotherapy versus melphalan at the M.D. Anderson Hospital showed no significant difference in diseased free survival [Smith et al., 1975]. Blacklege et al., [1987] have found that in whole abdominal radiotherapy is no better than chlorambucil in consolidating the initial response after cisplatinum chemotherapy. They concluded

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1 The radiation tolerance of the entire kidney, liver, and small bowel are 20, 26, 30 Gy delivered in 20 fractions over 4 weeks (Dembo, 1985).

that neither long term alkylating agents or chemotherapy were useful agents in prolonging survival as the two year survival in this group of patients was only 30%. It is difficult to comprehend how a radiation dose of 30 Gy delivered in 20 fractions over four weeks could sterilize tumour deposits when much greater radiation doses are required to sterilize adenocarcinoma deposits at other sites.

It is possible to deliver a much greater radiation dose to the peritoneal serosa by instilling a radioactive isotope in the form of a non-absorbable colloid into the peritoneal cavity. Radiocolloids containing ^{198}Au, ^{32}P, and lately ^{90}Y have all been used with some success; all deliver a high radiation dose to the peritoneal serosa , which may be as high as 10,000 cGy [Currie 1981]. The radiation dose at depth falls off rapidly, according to the inverse square law, so radiocolloids are only likely to be successful if the diseased volume is small. The radiation dose at a depth of 2 mms is a approximately 1/100th the surface dose [Currie et al., 1981]. There is limited data on the effectiveness of radiocolloids and there have been no large randomized trials. ^{32}Phosphorous has been used to salvage patients who had residual ovarian carcinoma after cytoreductive surgery and cisplatinum chemotherapy: with a 27% disease free survival at four years [Soper et al., 1987]. Kosltad et al., [1977] found that the five year survival in patients with Stage II disease who received intraperitoneal ^{198}Au radiocolloid was greater than those who received pelvic irradiation (54% versus 40%). Varia et al., [1988] described the results form a group of 118 patients who either received or did not receive ^{32}P radiocolloid after a second look laparoscopy following cytoreductive surgery and chemotherapy. ^{32}Phosphorous increased the four year survival rate in patients with minimal residual disease from 22% to 59%; in patients who were "disease free" at

second look laparotomy the four year survival was increased from 67% to 89% . Combining external beam irradiation and radiocolloids in an attempt to achieve a high radiation dose to the peritoneal serosa and a greater radiation dose in tissue deep to the serosa has been associated with unacceptable local toxicity [Klassen et al., 1985, Bakri et al., 1984; Heath et al., 1988].

2.2. Rationale for RIT in Ovarian Cancer

Long term survival in patients with ovarian cancer has remained the same for the last 50 years [Silverberg et al., 1987] despite the advances in chemotherapy surgery and radiotherapy outlined earlier. A new treatment modality is required that will destroy the regional micrometastasis left by cytoreductive surgery and chemotherapy. Epenetos et al., [1984 &1987] have suggested that as RIT is best given by a regional route it may be a useful modality in these patients, whose disease is confined to the peritoneal cavity. By administering the radiolabelled MAb directly into the peritoneal cavity one can increase the amount of MAb targeted to tumour deposits. As antibodies are large molecules one would not expect them to readily cross the peritoneal cavity: local radiation toxicity rather than systemic toxicity would be the dose limiting factor. This thesis describes the pharmacokinetics and toxicity of intraperitoneally administered radiolabelled MAb in patients with ovarian cancer.

3. Radiolabelled Antibodies in RIT

For any radiolabelled MAb prepreparation in RIT there are three major variables: The type of antibody (whole antibody or fragments), the amount of antibody injected, and the isotope used as a radiolabel. Immunoglobulin IgG molecules (the majority of MAbs are IgG) consist of two heavy and two light chains bound together by disulphide bridges as shown in Figure 1.1. The antibody binds to the epitope at the variable regions which are made from portions of both light and heavy chains. The antibody can be digested by enzymes to an Fc (constant region) and either $F(ab')_2$ or Fab fragments; the type of fragment

depending on the enzyme. As the $F(ab')_2$ and Fab fragments retain the variable part of the antibody molecule they can still bind to the epitope: these fragments can be radiolabelled and used in RIT instead of the whole MAb. The Fc part of the molecule is used for various functions such as activating complement of binding onto T-cell or macrophage receptors and is not involved in the binding to an epitope. The advantages and disadvantages of radiolabelled IgG and it's fragments for RIS and RIT are discussed below.

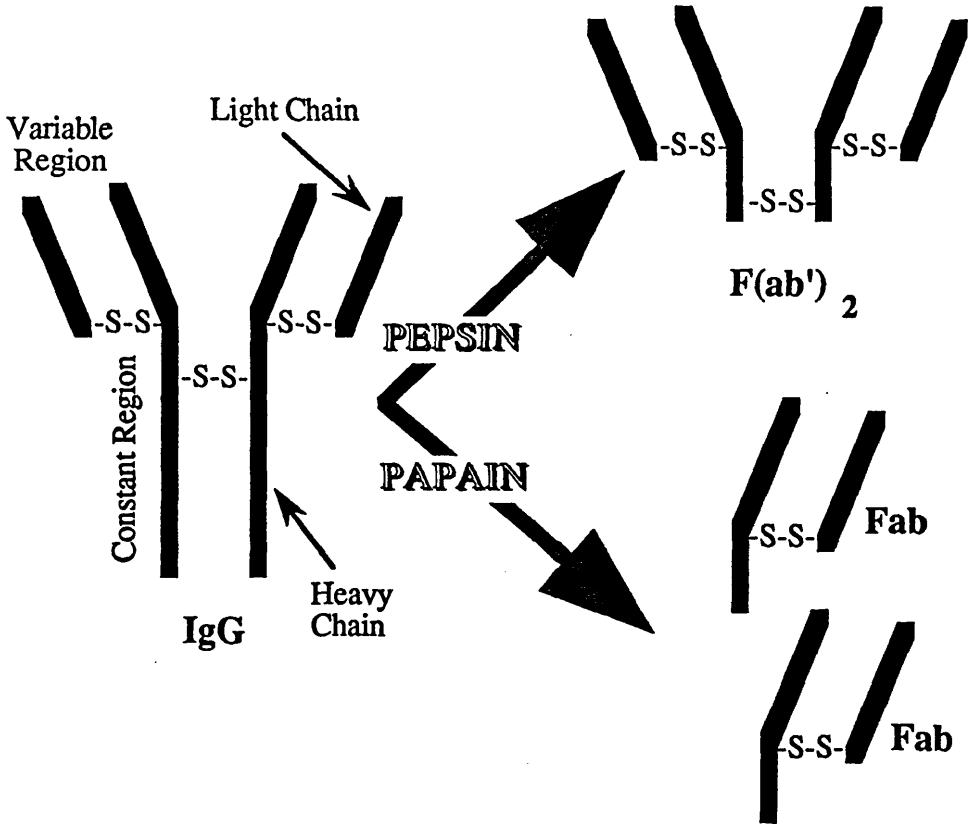


Figure 1.1: An IgG immunoglobulin and its F(ab')2 and Fab fragments

3.1. Antibody or Antibody Fragments in RIT?

Radiolabelled fragments are cleared from the circulation at a faster rate then radiolabelled IgG with less uptake into the liver and spleen [Wahl et al., 1983]. The isotope tumour to normal tissue ratio of isotope is usually greater when $F(ab')_2$ and Fab fragments are used instead of IgG, so that radiolabelled fragments are often used in RIS [Burzon et al., 1989; Larson et al., 1983b]. The absolute amount of MAb targeted to tumour cells is less when radiolabelled fragments are used in place of whole IgG [Wahl et al., 1983], this makes them less advantageous in RIT. Despite this $F(ab')_2$ fragments have been used in RIT with some success [Larson et al., 1983b]. Radiolabelled IgG may be superior to radiolabelled fragments for intracavitary RIT for two reasons. Firstly the absolute amount of isotope on tumour deposits is likely to be higher, and secondly the larger IgG molecule may be absorbed less rapidly into the systemic circulation , decreasing myelosuppression.

3.2 Antibody quantity

Another variable is the amount of antibody injected for RIT. In some antibody preparations all the antibody is radiolabelled while other preparations contain a large amount of unlabelled "cold" antibody. There is evidence administering cold antibody will increased the tumour to normal tissue isotope ratio by decreasing the non-specific uptake of radiolabelled MAb by the liver [Wahl et al., 1983] spleen and bone marrow [Carrasquillo et al., 1988c]. The blocking of non-specific antibody binding sites also appears to decreased the clearance of radiolabelled MAb from the circulation [Early et al., 1989;

Carrasquillo et al., 1988c]. Any increase in therapeutic effect by increased targeting may be accompanied by increasing toxicity from the prolonged retention of MAb in the blood.

3.3 Monoclonal Antibody Preparations in these Studies.

In the studies described in this thesis, three whole IgG murine monoclonal antibodies were used; HMFG1, AUA1, and H17E2. Human Milk Fat Globule 1 (HMFG1) is an IgG1 antibody that binds to large mucin-like molecules that were originally expressed by normal lactating breast tissue. The same antigen is also expressed by 90% of well differentiated breast and ovarian cancers [Arklie et al., 1981; Burchell et al., 1983]. AUA1 is a murine IgG1 monoclonal antibody that binds an antigen expressed by a colon carcinoma cell line called LoVo. It is also however expressed by 75% of ovarian carcinomas particularly the less well differentiated tumours [Epenetos et al., 1982]. The monoclonal antibody H17E2 is a mouse IgG1 antibody that binds to placental alkaline phosphatase expressed by up to 85% of ovarian cancers [Travers & Bodmer, 1984]. Between 10 and 30 mgs of MAb were used for the intraperitoneal RIT studies; all the antibody was radiolabelled, no cold antibody was injected.

3.3. Which Isotope in RIT?

Because of it's wide availability and the well established methods of antibody iodination to a high specific activity ^{131}I is the isotope "gold standard" in RIT. ^{131}Iodine emits gamma rays and β-particles: the energy of the former (see the table below) means that these are too

highly penetrating to be useful in RIT although they can be used for gamma immunoscintigraphy. There had also been widespread experience in using this isotope to treat patients with thyrotoxicosis and thyroid carcinoma. There are however more suitable radiolabels for RIS and RIT.

Table 1.2: A table of the physical data for the isotopes most commonly used in RIT or RIS. The initial EC denotes electron capture: a nuclear transformation where an orbiting electron is captured by the nucleus and energy is emitted as X-rays. IT denotes isomeric transition: where a nucleus emits discrete quanta (gamma rays) returning to the ground state from the metastable state. This data was obtained from The Radiochemical Manual [Wilson et al., 1966].

Isotope	Half Life	Type of Decay & Particle energy MeV		Gamma Energies MeV
^{111}Indium	67 hrs	EC	(100%)	0.173 (87%)
				0.247 (94%)
^{125}Iodine	60 days	EC	(100%)	0.035 (7%)
				0.027 (138%)
^{131}Iodine	193 hrs	β^-	0.25 (2.8%)	0.08 (2.2%)
			0.33 (9.3%)	0.28 (6.3%)
			0.61 (87.2%)	0.36 (79 %)
			0.81 (0.7%)	0.64 (9.3%)
				0.72 (2.8%)
^{32}Phosphorous	14 days	β^-	1.17 (100%)	none
^{99m}Technetium	6hrs	IT	100%	0.140 (90.1%)
				0.142 (8.5%)
^{90}Yttrium	64.2 hrs	β^-	2.27 (100%)	none

3.2.1. Radioimmunoscintigraphy

Iodine is not the most suitable radiolabel for RIS for several reasons. This isotope emits β-particles which are of no use in RIS and the γ-ray energies are greater than necessary. Scintillation counters are less sensitive to higher energy gamma rays, and excessive collimation is required. Several investigators have bound ^{99m}Tc to antibodies [Friztberg et al., 1988; Early et al., 1989] with better imaging results than could be achieved with ^{131}I-labelled antibodies [Endo et al., 1987, Bomanji et al., 1988]. The half life of ^{99m}Tc is rather short for RIS (6 hrs), the tumour to normal tissue ratio increases with time and an isotope with a longer half life may be advantageous. Numerous investigators have used ^{111}In-labelled MAb for RIS: ^{111}In has a longer half life than ^{99m}Tc (67 hours) and is easier to bind to antibodies. Although ^{111}In has several physical advantages over ^{131}I (a pure gamma emitter, gamma rays of a more suitable energy and a more suitable half life), one of the major advantages appears to a greater stability of the isotope bound to tumour deposits [Khaw et al., 1986; Halpern et al., 1983, Anderson & Strand, 1987]. ^{111}Indium is a more suitable radiolabel than ^{131}I for RIS except when trying to define lesions near the liver, which are obscured by the liver concentrating free ^{111}In released from the MAb [Murray et al., 1987; Taylor et al., 1988].

3.2.2. Radioimmunotherapy

The energy emitted from isotopes used in RIT must be absorbed by tissue in the immediate vicinity of the MAb. Highly penetrating γ-rays will not deposit their energy in the targeted tissue, pure β or α emitters are preferred unless RIS is also required. Monoclonal antibodies have

been radiolabelled with ^{67}Cu [Deshpande et al., 1988; Cole et al., 1988], ^{90}Yttrium [Hnatowich et al., 1985a; Vaughan et al., 1987b; Sharkey et al., 1989], ^{109}Palladium [Fawwaz et al., 1984], ^{212}Bismuth [Anderson & Strand, 1987; Kozac et al., 1986] , and ^{211}Astatine [Harrison & Royle, 1987]. The choice of isotope must take into account the heterogeneity of isotope distribution and the size of tumour deposit. If all the cells in a tumour deposit were equally targeted by a radiolabelled MAb then the chosen isotope should have low energy; energetic β-particles would irradiate non-targeted normal tissue. If however one requires an evenly distributed radiation dose in a tumour deposit from heterogeneously distributed radiolabelled MAb, a more penetrating β-emitter is required. The isotope that would be suitable to treat micro-metastasis (small clumps of cells surrounded by normal tissue) would not be suitable for the treatment of large tumour deposits where the MAb has been unable to penetrate the poorly vascularized hypoxic centre. Howell et al., have recently discussed the most suitable isotopes for different MAb distributions in tumour deposits. Using the formula, $E_{max} = 5.9 (D_{max} + 0.007)^{0.505} + (0.00413 D_{max})^{1.33} - 0.307$, the maximum ranges in tissue for the following isotopes were calculated [Howell et al., 1989]; as shown in Table 1.3.

Table 1.3: The maximum β-particle energy Emax (MeV), maximum range in tissue Dmax, and the half life of suitable isotopes for RIT (Howell, 1989).

Isotope	Emax (MeV)	Dmax (mm)	T1/2
^{32}P	1.71	8.7	14.3 days
^{67}Cu	0.576	2.2	61.7 hrs
^{90}Y	2.273	11 .9	64.2 hrs
^{111}Ag	1.035	4.8	7.45 days
^{131}I	0.606	2.4	8.06 days
^{118}Re	2.132	11.1	16.8 hrs
^{193}Pt	0.057, 0.126, 0.010, 0.001.	0.2	4.33 days

If the isotope is evenly distributed in a tumour deposit then ^{193}Pt (^{193}Platinum) would be ideal as more energetic β-particles would irradiate normal tissue. The radiation dose to a one mm tumour deposit from one KBq (27 nCi) of ^{193}Pt and ^{90}Y evenly distributed throughout the tumour would be 2200 & 700 cGy respectively. However most macroscopic tumour deposits have hypoxic and necrotic centres so that the radiolabelled MAb is concentrated around the periphery. In this situation more energetic β-particles are preferable although there may still be disadvantages with very high energy β-particles emitted from isotopes like ^{90}Y. Howell examined the hypothetical problem of a 1.2 cm tumour deposit with a 0.44 cm necrotic centre and radiolabelled MAb concentrated in the vascularized periphery. Although ^{90}Y-labelled MAb would give a better dose distribution across the whole deposit, isotopes with less energetic β-particles such as ^{131}I and ^{67}Cu would

deliver a greater radiation dose to the tumour periphery. The radiation dose to the periphery may be more important as this area contains 90% of the tumour deposit's cells, and the remaining 10% of cells in the hypoxic centre may not be viable. Howell concluded that ^{90}Y-labelled MAb would be best for tumour deposits of greater than one cm, ^{67}Cu-labelled MAb for deposits of a mm, and ^{137}Pt-labelled MAb for micrometastasis less than a mm in size. Several other investigators have concluded that ^{90}Y has the best physical characteristics for the RIS of larger tumour deposits [Wessels, 1984; Humm, 1986]. The isotope half life is also important, Wessels has shown that the ideal half life should be 1.5 to 3 greater than the time (after the MAb injection) of the maximum isotope tumour to normal tissue ratio [Wessels, 1984].

Isotopes that emit α-particles or Auger electrons are an alternative to low energy β-particles emitters. These particles have a minute range in tissue and can only travel a few cell diameters; they can only be used for the treatment of micrometastasis or tumour deposits that are homogeneously targeted. These particles deliver high LET radiation which is many times more destructive than the low LET radiation of β-particles. Only a few disintegrations are required to produce irreparable damage to a cell nucleus. Currently there is interest in two α-emitters, ^{211}Astatine and ^{212}Bismuth . ^{211}Astatine has a half life of 7.2 hours and it's α-particles have a maximum range of 67 μm in tissue; these deliver extremely high LET radiation (120 KeV/μm) so that only 65 disintegrations on a cell surface are required for a 37% cell death rate [Humm, 1987]. ^{212}Bismuth has a similar range in tissue (40 - 80 μm) but has a physical half life of only one hour which limits its usefulness in RIT.

The biological aspects of an isotope are also important, as this determines the biodistribution of the isotope should it become detached from the MAb. This may occur if there is any instability of the antibody/radiolabel complex or when the antibody is catabolized by the tumour or the reticuloendothelial system. Many investigators have found that ^{131}I-labelled antibodies are not stable in vivo [Khaw et al., 1986; Scheinberg & Strand, 1983; Hagan et al., 1985] and that dehalogenation of the MAb decreases the effective half life of ^{131}I in tumour deposits. This does not occur if radiometalic isotopes are used [Despande et al., 1988; Halpern et al., 1983; Anderson & Strand, 1987]. Ideally the radiolabel should be rapidly excreted once it becomes detached from the body. This (is one ignores the thyroid uptake), is the great advantage of iodine labelled antibodies; free iodine is rapidly excreted by the kidneys.

3.2.3. Radio-isotopes for intraperitoneal RIT

In the intraperitoneal RIT studies in this thesis ^{131}I and ^{90}Y-labelled MAb's were used. The former radiolabel was selected as this was the first radiolabel used at the Hammersmith Hospital and was initialy the only isotope available. ^{90}Yttrium labelled MAb's were used as more β-particles of greater energy were required. Monoclonal antibodies do not penetrate tumour spheroids deeply [Sutherland et al., 1987] and it is extremely doubtful if the β-particles of ^{131}I could reach the centre of a one cm tumour nodule if the MAb was confined to the surface of the tumour deposit.

4. This Objectives Of This Thesis

The purpose of this thesis is to study the pharmacokinetic and toxicity of intraperitonealy administered ^{131}I and ^{90}Y-labelled MAb in patients with ovarian cancer. The reasons for the i.p. route of administration for patients with ovarian cancer, and the choice of the two radiolabelled has been discussed. By studying the local and systemic radiation toxicity in these patients one can investigate the following.

1) The local regional toxicity of radiolabelled MAb in the peritoneal cavity. This is only relevant to patients with ovarian cancer or other tumours largely confined to a body cavity.

2) The effects of radiolabelled MAb absorbed from the peritoneal cavity on systemic toxicity. This is relevant to all applications of RIT, particularly if systemic RIT is to have a future role in oncology .

I have been able to study the pharmacokinetics and toxicity of i.p. administered radiolabelled MAb in a series of 47 patients, using ^{131}I and ^{90}Y labelled antibodies. I have also investigated methods of decreasing the degree of systemic toxicity from these two radiolabels, which should allow the administration of greater isotope activity with an increase in the therapeutic effect.

CHAPTER 2

INTRAPERITONEAL ^{131}I-LABELLED MAb IN NON-IMMUNIZED PATIENTS

INTRODUCTION

The purpose of this study was to determine the pharmacokinetics and normal tissue dosimetry of intraperitoneally adminstered ^{131}I-labelled monoclonal antibody. The haemopoietic bone marrow and the peritoneal serosa are organs of special interest as the radiation dose to these organs may limit the isotope activity that can be administered without unacceptable radiation induced myelosuppression (systemic toxicity) or enteritis (local toxicity). If radiolabelled antibody is absorbed from the peritoneal cavity into the general circulation; the radiation dose to the bone marrow will limit the ^{131}I activity that can be given [Vaughan et al., 1987]. If the radiolabelled antibody is not absorbed, the radiolabelled antibody will decay in the peritoneal cavity and the radiation dose to bowel will be the dose limiting factor.

The absorption of monoclonal antibody from the peritoneal cavity into the circulation was monitored by measuring the ^{131}I activity in the blood after the i.p. administration of ^{131}I-MAb. The integrated ^{131}I activity in blood for 100 hours post RIT was determined by measuring the ^{131}I activity in blood samples taken at 12 hourly intervals. This integrated blood ^{131}I activity (μCi hours) was used to estimate the radiation dose to bone marrow (see below). The urinary excretion of ^{131}I was monitored by measuring the cumulative ^{131}I activity in urine for five days after the RIT infusion.

The non-specific (background) radiation dose to the peritoneal serosa from the ^{131}I-labelled MAb in the peritoneal cavity was measured by lithium fluoride (LiF) thermoluminescent dosimetry (TLD). Lithium

fluoride TLD is a well established method of measuring the absorbed radiation dose in radiotherapy departments and has previously been used to measure the radiation dose to peritoneal serosa from radiocolloids [Currie et al., 1981]. By using LiF microrods (each measuring 6 x 1 mm), we were able to measure the radiation dose the peritoneal serosa received over a five day period (appendix C).

PATIENTS , MATERIALS AND METHODS

Patients

Between January and December 1987, eleven patients received intraperitoneally administered ^{131}I-labelled MAb (**Table 2.1**). Nine patients had residual ovarian cancer following cytoreductive surgery and/or chemotherapy, one patient had ascites from metastatic breast cancer, and one patient had positive peritoneal cytology following a total hysterectomy for papillary endometrial cancer. Patients were accepted for RIT if more than 50% of tumour cells were positive for the MAb's HMFG1 and AUA1[1] on an immunoperoxidase examination of the original tumour histology.

^{131}I-Labelled Monoclonal Antibody

In seven patients ^{131}I-labelled HMFG1 was obtained from CIS in France and transported at minus 20 ^{o}C to the Hammersmith Hospital. These antibody preparations contained approximately ten millicuries (0.37 GBq) of ^{131}Iodine per milligram antibody (**Table 2.1**). The remaining six antibody preparations were iodinated at the Hammersmith Hospital[2] with 130 -148 mCi of ^{131}Iodine. Trichloroacetic acid precipitation of samples from all five preparations indicated that more than 70% of ^{131}Iodine was protein bound.

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1 Immunoperoxidase staining was undertaken by Ms Angela Cross, ICRF & Dept of Clinical Oncology, Hammersmith Hospital.

2 Iodinations were performed by Ms Debbie Snook, Me Babu Dhokia, or Mr Greg Sivolapenko.

Table 2.1 I-131 labelled MAb Prepaarations

Patients without ascites	Iodine-131 Activity in mCi	mgs of MAb	% protein bound
1 (Box)	130	20	70
2 (Phi)	151	30	>80
3 (Buc)	146	30	>80
4 (Craw)	157	30	>80
5 (Moo)	147	30	>80
6 (But)	153	30	>80
7 (Gra)	125	10§	>85
8 (Hai)	87	10§	>85
9 (Hut)	105	10§	>85
10 (Ash)*	122	10§	>85
Patients with ascites			
11 (Sut)	102	10§	>85
12 (Vin) ¶	75	10§	>85
13 (Reg)	100	10§	85

* endometrial cancer
¶ breast cancer
§ I-131 labelled HMFG1 obtained from Unipath Ltd. UK.

Intraperitoneal Administration of ^{131}I-Labelled MAb

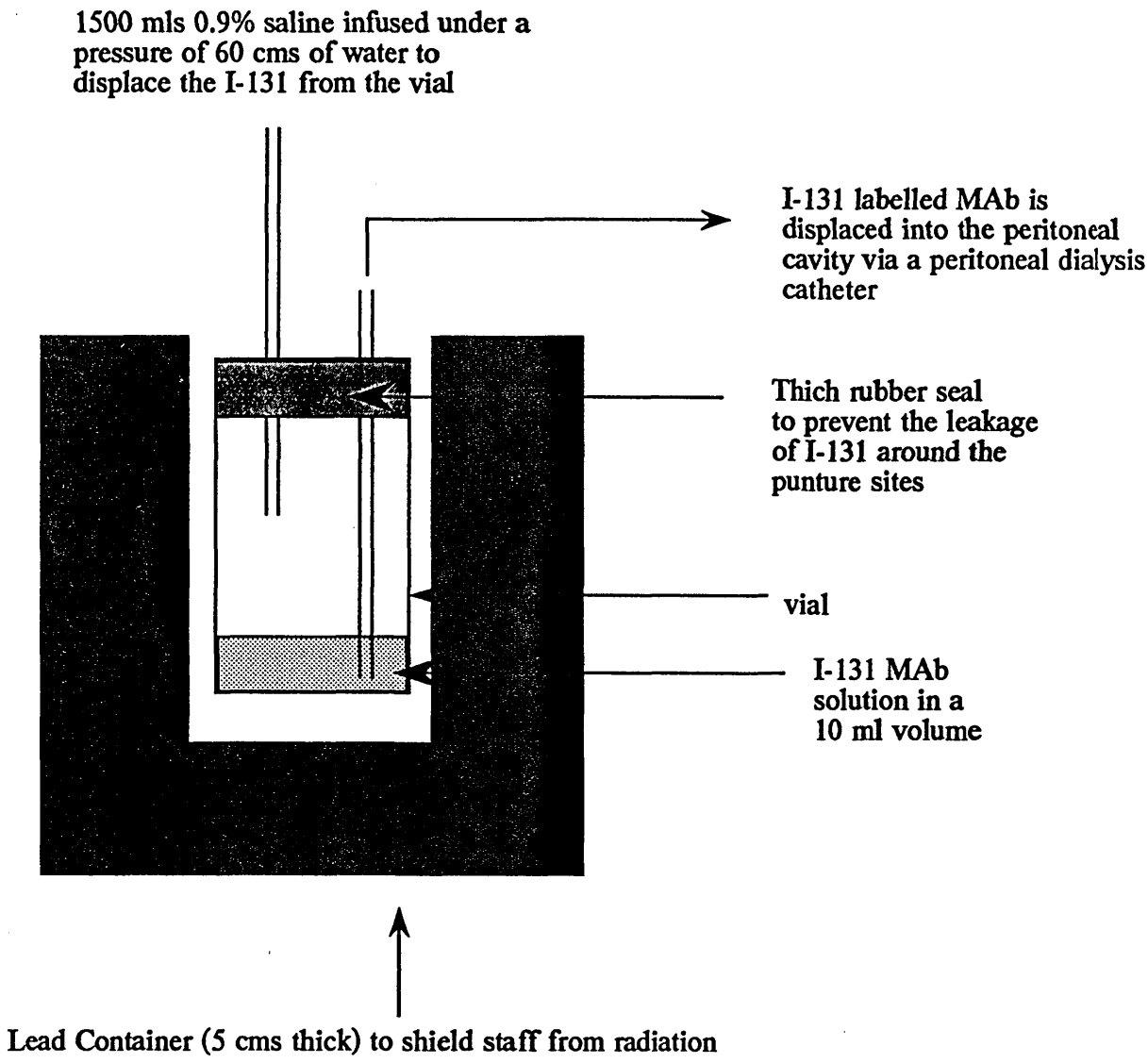
Peritoneal dialysis catheters were inserted into the peritoneal cavity before the infusion of ^{131}I-labelled MAb. This was performed as a "blind procedure" using local anaesthesia in three patients and under direct vision at open laparoscopy[1] in the remaining eight patients. The peritoneal dialysis catheters were inserted into the centre of the peritoneal cavity to ensure even distribution of isotope [Boye et al., 1984]. If free access to the peritoneal cavity was uncertain, as in all cases

of "blind" catheter insertion, the intraperitoneal distribution of one millicurie of ^{99m}Tc in 500 mls of 0.9% sterile saline was imaged by a gamma camera scintigraphy. ^{131}Iodine- labelled monoclonal antibody was infused into the peritoneal cavity using the sterile non-touch technique illustrated in **Figure 2.1** below.

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1 Open laparoscopy was performed by either Mr M. Griffiths, MRCOG., or Ms V. Hird, FRCS., MRCOG.

Figure 2.1: The Administration of I-131 labelled Mab using a sterile non-touch technique. As the I-131 solution is diluted as it is displaced into the peritoneal cavity, over 95% of the I-131 Mab is infused in the first 250 mls of the 1500 ml volume.



The ^{131}I-labelled MAb was stored in sealed in a glass vial (10 mls of PBS) at temperatures between -20 to 4 ^{o}C. The radiation exposure to medical staff was reduced by shielding the vial containing the ^{131}I-labelled MAb in a 5 cm thick lead container. The ^{131}I-labelled MAb was displaced out of the vial into the peritoneal cavity by the introduction of 1500 mls of saline. This technique avoided direct handling of large activities of ^{131}I and reduced the risk of radioactive contamination due to accidental spillage. However using large volumes of saline does not ensure an even distribution of ^{131}I-MAb in the peritoneal cavity as the rate most of the ^{131}I-MAb is introduced into the peritoneal cavity with the first 50 mls of saline.

Dialysis catheters were removed as soon as the ^{131}I-MAb had been completed: leakage of radioactive fluid was prevented by securing two "purse-string" sutures around the puncture site. Attempts were made to improve the intraperitoneal distribution of ^{131}I-MAb by changing the patient's position every 20 minutes for two hours after the infusion. Patients were barrier nursed in a radiation controlled room until their total body ^{131}I activity was less than 30 mCi.

Measurement of ^{131}I Activity in the Blood

The ^{131}I activity in the blood after the i.p. infusion of ^{131}I-MAb was monitored every 12 hours. Five millilitres of blood were stored in pre-weighed glass vials containing EDTA and stored at 4 ^{o}C and the ^{131}I activity in each blood sample was measured in a scintillation well counter as described in appendix A. The ^{131}I activity was corrected for

any radioactive decay after the time of injection when describing the pharmacokinetics of ^{131}I-MAb; absolute (uncorrected) ^{131}I activities were used for dosimetry calculations. The fraction of ^{131}I in the serum that was protein bound was measured by dialysis in four patients (appendix D).

Blood ^{131}I activity against time was plotted for each patient over the first 110 hours after RIT. From each curve, the ^{131}I activity at 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 hours was calculated and the results pooled to express the mean ^{131}I activities at these times. Patients with ascites were studied separately as they were found to have different pharmacokinetics.

Measurement Of ^{131}I Activity in Urine

Urine was collected from each patient for the 110 hours after the infusion of ^{131}I-labelled MAb and stored in graduated 2 litre containers. Each day the container was thoroughly shaken and a two millilitre aliquot taken before discarding the urine sample. The ^{131}I activity in the urine was measured in a scintillation well counter as described in appendix B. Urinary ^{131}I excretion was expressed as the cumulative excreted ^{131}I activity (corrected for radioactive decay since the time of injection). Urine was dialysed against phosphate buffered saline in four patients to estimate the fraction of ^{131}I bound to protein (appendix D).

Measurement of the Radiation Dose to the Peritoneal Serosa

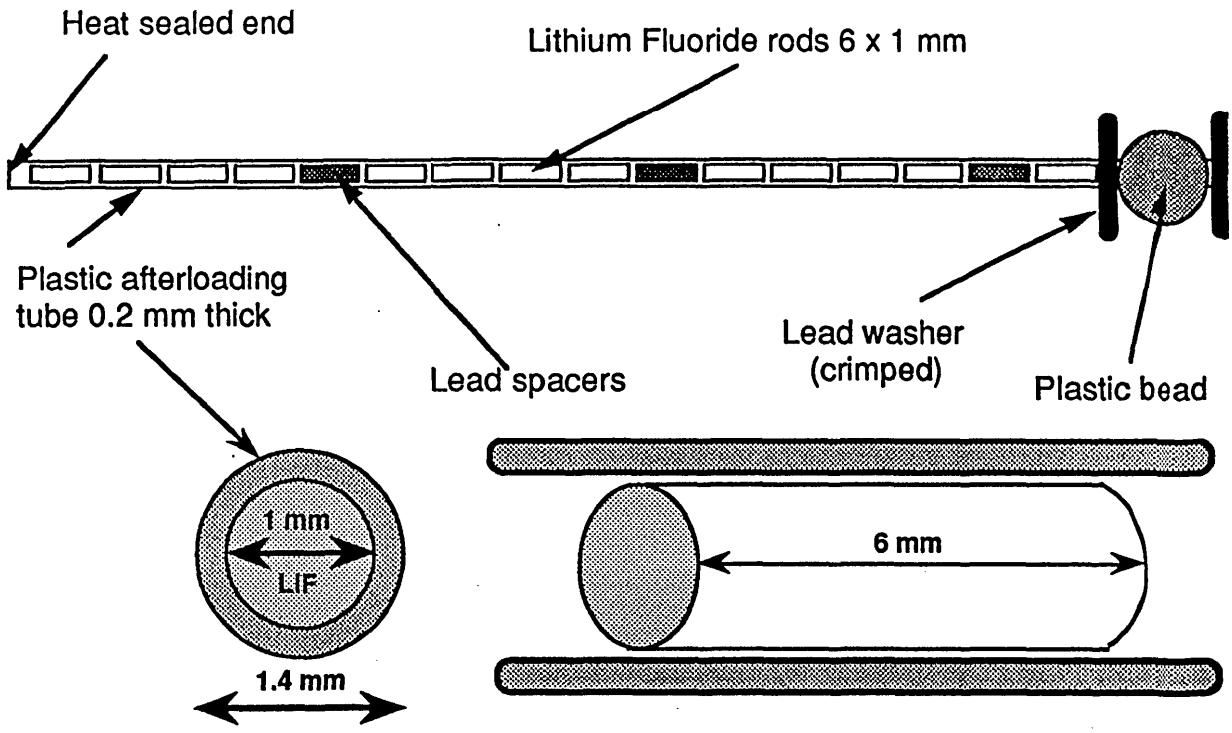
In five patients the radiation dose to peritoneal serosa from ^{131}I-labelled MAb was measured by lithium fluoride (**LiF**) thermoluminescent dosimetry (**TLD**), a well established method of measuring the radiation dose absorbed by tissue [Horowitz, 1981; Meredith & Massey, 1977]. Lithium fluoride micro-rods[1], each of six millimetres long and one millimetre in diameter were chosen as these could be contained in thin hollow plastic tubes[2] which were easily inserted into the peritoneal cavity. The wall thickness of the plasstic tube was only 0.2 mm, and would absorb little of the radiation dose. Between 15 and 53 rods were carried in each tube with metallic radio-opaque markers every five centimetres. The overall dimensions of TLD catheter were 15 to 40 cms in length and 1.4 mm in diameter (see illustration below). The TLD catheters were inserted into the peritoneal cavity through a 14 gauge cannula (at laparoscopy or under local anaesthesia) and the rods position verified by orthogonal radiographs. The rods were calibrated by exposing them to known doses of radiation delivered by radiotherapy treatment machines (appendix C) delivering XRs and electrons from 50 KeV to 4 MeV. As we observed the thermoluminescent response to be dependant on X-ray energy we finally calibrated the LiF rods with an ^{131}I solution of known activity. The rods were immersed in the ^{131}I solution for various time intervals, and the radiation dose to the rods calculated by applying the MIRD formulation (see appendix C).

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1 Obtained from the Harshaw/Filtrol Partnership, Ohio, USA.

2 Iridium wire interstitial afterloading tubes were used (Amersham International Plc. UK)

Figure 2.2: Intraperitoneal Lithium Fluoride TLD Catheter



Bone Marrow Radiation Dosimetry

The radiation dose the haemopoietic bone marrow received from ^{131}I in the marrow circulation was estimated by applying the MIRD[1] formulation to the integrated blood ^{131}I activity for 100 hours after the ^{131}I-MAb infusion (appendix H). The 100 hour period was chosen as 85% of the administered ^{131}I had either decayed or been excreted from the body by this time. I have made a number of assumptions in estimating the radiation dose to bone marrow from the blood ^{131}I activity .

Firstly animal studies have shown that ^{131}I-labelled antibodies are located predominantly in blood rather than bone or other tissue [Rowlinson et al., 1987; Buchsbaum et al., 1985; Badger et al., 1985; Goldenberg et al., 1981]. Secondly marrow is extremely vascular and macromolecules would rapidly reach equilibrium with the blood [Trabowitz et al., 1974], so that the ^{131}I activity in the blood and the marrow circulation are the same. The haemopoietic bone marrow in women constitutes 2.2% of the body weight [I.C.R.P., 1975] i.e. 31% of the weight of the total blood volume, and I have arbitrarily assumed that the bone marrow circulation contains a fifth of the whole blood ^{131}I activity. If one just considers the radiation dose to bone marrow from β-radiation, the only possible radiation source organs are the bone, the bone marrow, and the bone marrow circulation. The estimated bone marrow dose in cGy can be expressed by applying the MIRD

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1 Medical Internal Radiation Dosimetry Formulation (Snyder et al., 1978), see appendix H.

formulation [Snyder et al., 1978] where the radiation dose to the bone marrow, D would equal

$$D = (0.2 \times A_{bl} \times S_{[mar \rightarrow mar]}) + (A_b \times S_{[bone \rightarrow mar]}) + (A_{mar} \times S_{[mar \rightarrow mar]}).$$

Where A_{bl} represents the integrated ^{131}I activity in the whole blood over 100 hours and 0.2 is estimated to be the fraction of A_{bl} in the marrow circulation. The constant $S_{[mar \rightarrow mar]}$ is the S value for marrow as a source and target organ and $S_{[bone \rightarrow mar]}$ the S value for bone as the source organ and marrow as the target organ. The values A_b and A_{mar} refer to the integrated ^{131}I activity in the bone and bone marrow respectively. As I have assumed that the ^{131}I is concentrated in the marrow circulation and that the ^{131}I activity in the bone and marrow is negligible, the expression $\{(A_b \times S_{[bone \rightarrow mar]}) + (A_{mar} \times S_{[mar \rightarrow mar]})\}$ can be ignored. The radiation dose the bone marrow receive from beta radiation is therefore

$$D = (0.2 \times A_{bl} \times S_{[mar \rightarrow mar]}) .$$

The bone marrow does in fact receives an additional gamma ray radiation dose from ^{131}I labelled MAb in the rest of the body. This can be estimated by the MIRD expression $A_{bl} \times S_{[wb \rightarrow mar]}$ where $S_{[wb \rightarrow mar]}$ is the S value for the whole body as a radiation source and the marrow as the target organ. The radiation dose to haemopoietic bone marrow was estimated to be

$$D = (0.2 \times A_{bl} \times S_{[mar \rightarrow mar]}) + (A_{bl} \times S_{[wb \rightarrow mar]}).$$

Myelosuppression

Patients were reviewed at weekly intervals after treatment to assess myelosuppression and other toxicity: this was not always possible if patients were followed up at another centre. Leucopenia and thrombocytopenia were scored by the fall in blood counts expressed as percent of the pretreatment value, and according to the WHO classification [Miller et al., 1981]. In the WHO classification leucopenia and thrombocytopenia are classified as follows.

WHO Grade	Leucoctye Nadir	Platelet Nadir
Grade 0	$> 4 \times 10^6$/L	$> 100 \times 10^9$/L
Grade 1	$3\text{-}3.9 \times 10^6$/L	$75\text{-}99 \times 10^9$/L
Grade 2	$2\text{-}2.9 \times 10^6$/L	$50\text{-}74 \times 10^9$/L
Grade 3	$1\text{-}1.9 \times 10^6$/L	$25\text{-}49 \times 10^9$/L
Grade 4	$< 1 \times 10^6$/L	$< 25 \times 10^9$/L

Combined myelosuppression was scored by adding the leucocyte and platelet grades to produce and arbitrary scale of bone marrow toxicity graded from zero (no toxicity) to eight (life threatening neutropenia and thrombocytopenia.

RESULTS

Pharmacokinetics of ^{131}I in the Blood.

In patients without ascites ^{131}I-labelled MAb appeared to be efficiently absorbed from the peritoneal cavity into the systemic circulation (**Figure 2.3**). Forty hours after the antibody infusion, a mean of 27% (range 18-36%) of the injected ^{131}I activity was in the circulation (**Table 2.2**). After the peak blood radioactivity, the clearance of ^{131}I-labelled antibody from the circulation obeyed single phase exponential clearance (**Figure 2.4**), with a mean half life of 67.5 hours (range 45-95 hours).

Figure 2.3 also illustrates the different pharmacokinetics observed in the three patients with ascites. In these patients the maximum injected ^{131}I activity in the blood was 8% at 100 hours (Table 2.2) with a mean maximum value of 4.8% at 60 hours. As the peak activity in blood was delayed were we unable to study the clearance of antibody from the blood in these patients. The AUC[1] of individual patients with and without ascites were compared using a Mann Whitney U Test . Patients with ascites had significantly lower ^{131}I activity in their blood in the first 100 hours after treatment (Mann Whitney U=0, $P<0.001$). Dialysis of serum 24 hours after RIT in four patients demonstrated a 11% (mean) loss in ^{131}I activity (range 0-19%) indicating that the majority of ^{131}I in serum was bound to protein.

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1 Area Under the Curve

Figure 2.3: Blood I-131 activity following the IP infusion of I-131 labelled Monoclonal Antibody, expressed as mean of the % injected dose.

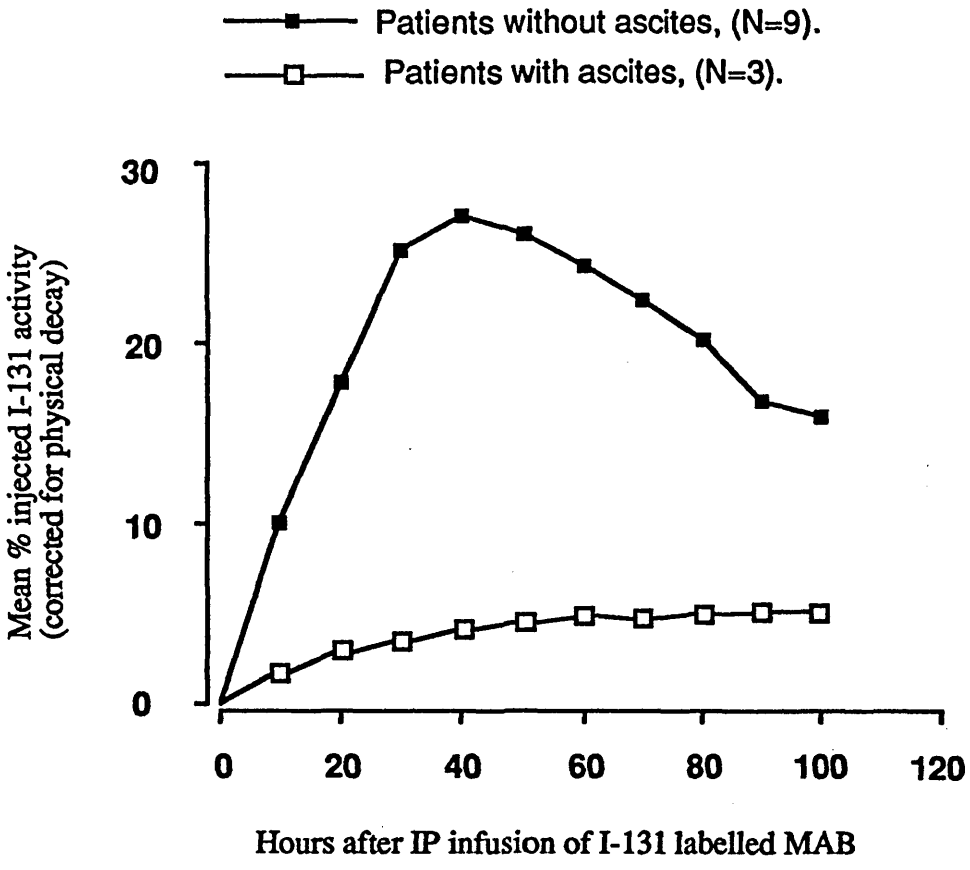


Table 2.2 Blood I-131 activity in patients with and without ascites

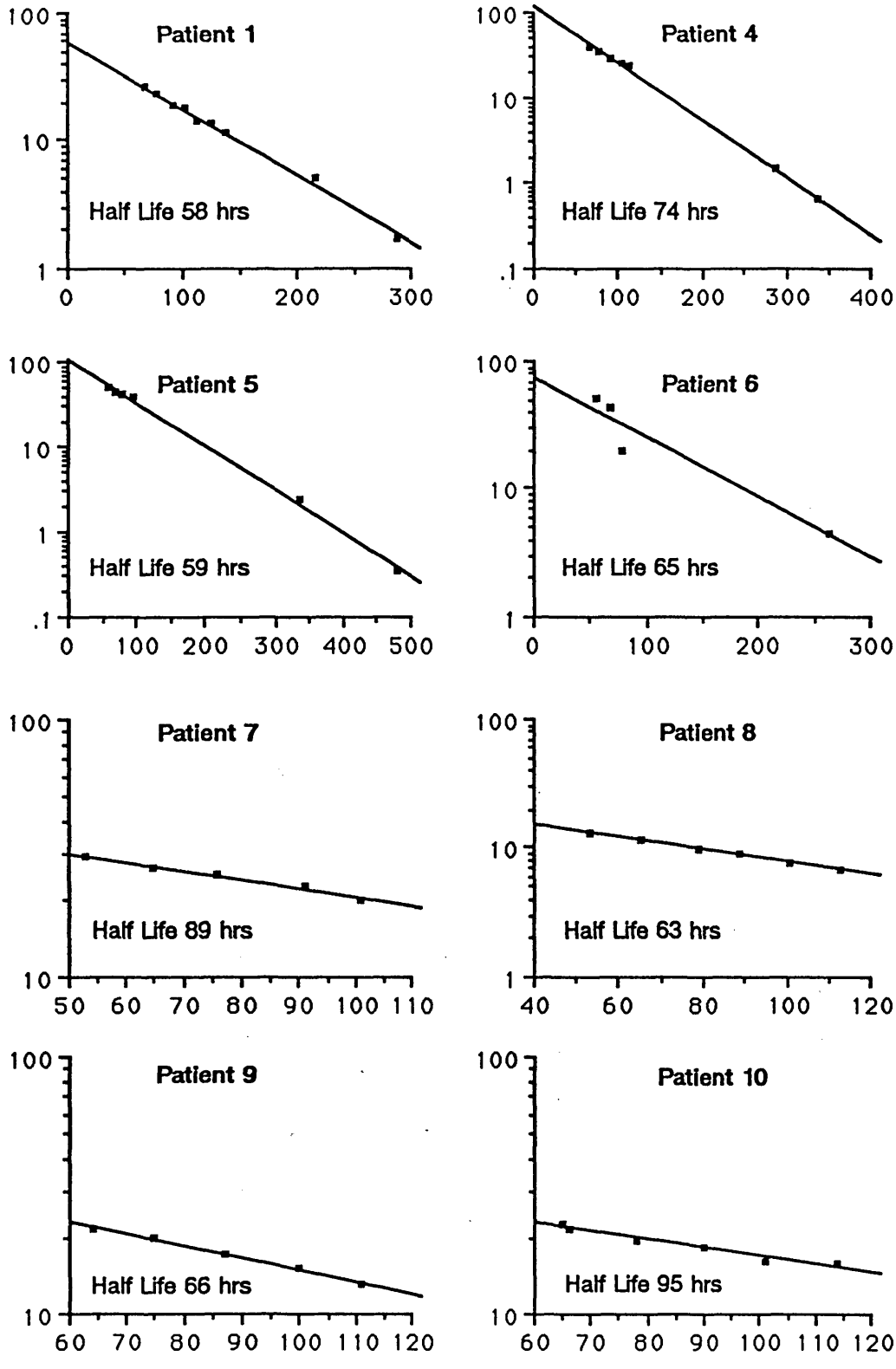
% injected administered I-131 activity in patients without ascites

Hours after IP Infusion	Mean (N=9)	Standard Error	Standard Deviation	Range
10	10	1.3	3.8	6 - 18
20	17.8	1.5	4.3	13 - 28
30	25.1	1.8	5.4	19 - 36
40	27	2.3	6.8	18 - 8
50	26	2.6	7.7	16 - 39
60	24.3	2.6	7.9	14 - 39
70	22.3	2.5	7.6	12 - 37
80	20.1	2.1	6.4	11 - 32
90	16.8	1.5	4.6	10 - 27
100	15.9	1.6	4.7	9 - 24

% injected administered I-131 activity in patients with ascites

Hours after IP Infusion	Mean (N=3)	Range
10	1.8	1.4 - 2.5
20	3	2.0 - 4.2
30	3.6	2.4 - 5.4
40	4.1	3.0 - 6.3
50	4.5	3.1 - 6.8
60	4.8	3.1 - 7.2
70	4.7	2.6-7.4
80	5	2.8 - 7.9
90	5	2.6 - 7.9
100	5	2.5 - 8.0

Figure 2.4: Blood I-131 activity (Log % injected activity on y-axis) following the i.p. injection of I-131 labelled MAb. The clearance half life is given for each patient. The x-axis represents the time in hours following injection of MAb.



Urine Excretion of ^{131}Iodine.

The urinary excretion of ^{131}I in patients receiving radiolabelled monoclonal antibodies is illustrated in **Figure 2.5.** In the eight patients without ascites a mean of 70% of the injected ^{131}I activity was excreted. Only a mean of 40% of the injected activity was excreted in the three patients with ascites **(Table 2.3).** A Mann-Whitney U Test indicated that patients with ascites excreted significantly less ^{131}I (U=0, P=0.005). Dialysis of urine demonstrated a 99% loss in ^{131}I activity, indicating that urinary ^{131}I was not protein bound.

The Radiation Dose to the Peritoneal Serosa

The radiation dose to the peritoneal serosa was measured by thermoluminescent dosimetry in six patients. Lithium fluoride TLD microrods remained in the peritoneal cavity for 110 hours after the intraperitoneal infusion of ^{131}I-labelled MAb. There were 206 measurements in six patients; the mean radiation dose to the serosa per millicurie ^{131}I was 2.85 cGy (range 1.62 to 4.44 cGy). The variation in the radiation dose the peritoneal serosa received in individual patients was quite small, with standard deviations ranging from 6 to 16% of the mean **(table 2.4).**

The Radiation Dose to the Bone Marrow

The integrated ^{131}I activities in blood and estimated radiation doses to bone marrow are tabulated in **Table 2.5.** As the clearance of ^{131}I-labelled antibody from the blood after 60 hours appears to be mono-exponential (Figure 2.4), one can extrapolate the integrated blood activity over a 200 hour period, and estimate the marrow radiation dose over this time.

Figure 2.5 Cumulative urine excretion of I-131 following the IP infusion of I-131 labelled monoclonal antibody.

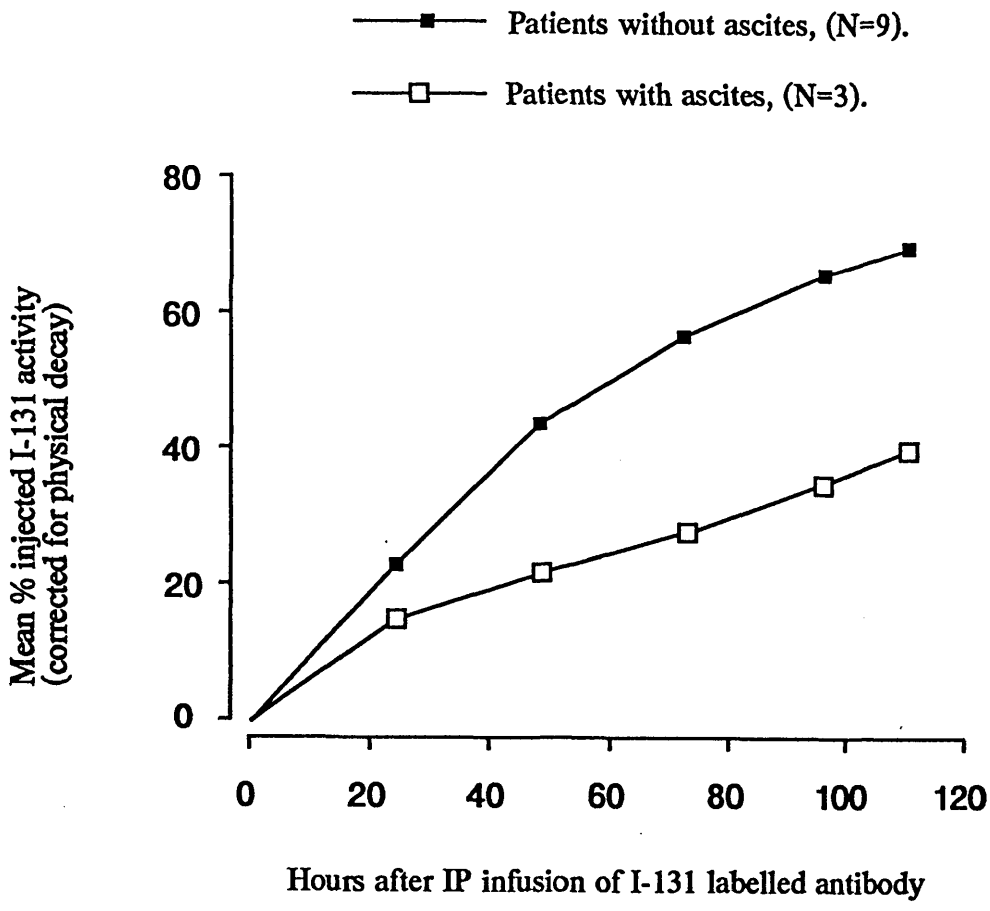


Table 2.3 Cumulative I-131 Excretion

Expressed as % administered activity (corrected for physical decay)

Patients Without Ascites

Hours after IP infusion	Mean (N=8)	Standard Error	Standard Deviation	Range
24	23	2.5	6.6	13-30
48	44	3	8.4	31-53
72	57	2.8	7.4	48-73
96	66	2.1	5.6	58-73
110	70	2	5.1	65-78

Patients With Ascites

Hours after IP infusion	Mean (N=3)	Range
24	15	7-24
48	25	12-34
72	32	17-41
96	35	21-46
110	40	24-55

Table 2.4 Radiation Dose to the Peritoneal Serosa

(measured by Lithium fluoride TLD)

Patients	I-131 Activity in mCi	Radiation Dose to the peritoneal serosa in cGy		
		mean	range	standard deviation
1 (Box)	130	338 (N=30)	322-404	29
4 (Cra)	157	423 (N=35)	255-498	52
5 (Moo)	147	431 (N=15)	343-495	34
7 (Gra)	125	501 (N=53)	423-555	28
9 (Hut)	105	290 (N=38)	217-377	42
10 (Ash)	122	257 (N=35)	266-293	16

Table 2.5 Integrated blood I-131 mCihrs in HAMA negative patients. The radiation dose to bone marrow was estimated by applying the MIRD formulation to the integrated I-131 activity (see text).

Patients without ascites	I-131 Activity in mCi	Integrated Blood I-131 activity in mCi.hrs over 100 hrs & 200 hrs		Radiation Dose to Bone Marrow in cGy 100 hrs & 200 hrs	
1 (Box)	130	1940	2560	109	143
3 (Buc)	146	2320	3200	130	176
4 (Craw)	157	2900	3740	162	210
5 (Moo)	147	2760	3980	155	223
6 (But)	153	2980	4060	167	227
7 (Gra)	125	1780	2380	100	133
8 (Hai)	87	980	1260	55	71
9 (Hut)	105	1580	2100	87	118
10 (Ash)	122	1640	2320	92	130
Patients with ascites					
11 (Sut)	102	174	240	10	13
12 (Vin)	75	94	120	5	7
13 (Reg)	100	188	280	11	16

These calculations are only valid if the clearance of ^{131}I-labelled antibody from blood continues to be mono-exponential, i.e. the effective half life remains unchanged. The radiation dose to bone marrow increased with increasing activity of administered ^{131}I-labelled antibody, from 55 cGy to 167 cGy over 100 hours following 87 and 153 mCi ^{131}I-labelled antibody respectively. A mean of 74% (range 22-30%) of the estimated radiation dose to marrow was delivered in the first 100 hours.

Toxicity

Radiation induced toxicity in these patients appeared to be confined to the bone marrow. There was no evidence of local bowel toxicity; none of these patients had diarrhoea, and vomiting when it did occur, was mild and probably related to the administration of a general anaesthetic. There was however, significant myelosuppression following intraperitoneal RIT. The degree of myelosuppression, shown in **Table 2.6**, appeared to be closely related to the radiation dose the marrow was estimated to have received, and was only observed in patients without ascites. **Figure 2.6** illustrates the relationship between myelosuppression, expressed by the combined WHO grades, and the radiation dose to bone marrow. Although patient numbers are small there does appear to be a steep dose response curve for myelosuppression.

Figure 2.7 illustrates the thrombocytopenia observed in the seven patients who received the highest ^{131}I activities (146-157 mCi) in i.p. RIT between 1983 and 1987. Three of these patients were not included in my study and did not have their blood monitored for ^{131}I activity

(patients B, F, & V). All patients demonstrate a fall in platelet count with nadirs 25-39 days after treatment. In five patients (pts 5, V, 4, B, 6) thrombocytopenia was potentially life threatening and two patients required platelet transfusions. All patients except patient B had complete recovery of myelosuppression. Patient "B" died 29 days after RIT with a platelet count of 19×10^9 /L. A post-mortem examination however found the cause of death to be progressive tumour rather than due to thrombocytopenia. Leucopenia followed an identical pattern in all patients.

Table 2.6: Myelosuppression according to the WHO scale and the radiation dose the bone marrow was calculated to have received from I-131 labelled MAb in the vascular compartment in individual patients. The percentages represent the nadir counts expressed as a percent of the pretreatment count.

Patient No	Radiation Dose to Bone Marrow in cGy	WHO MARROW TOXICITY		
		WBC	Platelets	Total
8	55	0 (88%)	0 (50%)	0
9	87	1 (63%)	0 (76%)	1
10	92	1 (48%)	0 (39%)	1
7	100	1 (57%)	3 (22%)	4
1	109	2 (35%)	0 (53%)	2
3	130	2 (38%)	1 (36%)	3
5	155	4 (23%)	4 (8%)	8
4	162	3 (24%)	4 (11%)	7
6	167	3 (16%)	4 (8%)	7

Figure 2.6: Myelosuppression in an arbitrary scale (combined WHO grade) for the radiation dose the bone marrow was estimated to have received from I-131 in the vascular compartment.

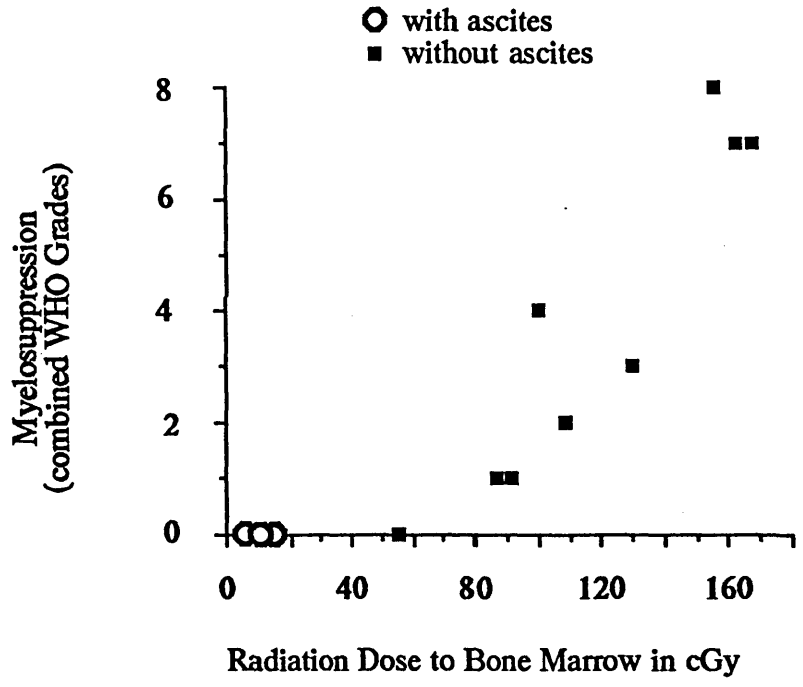
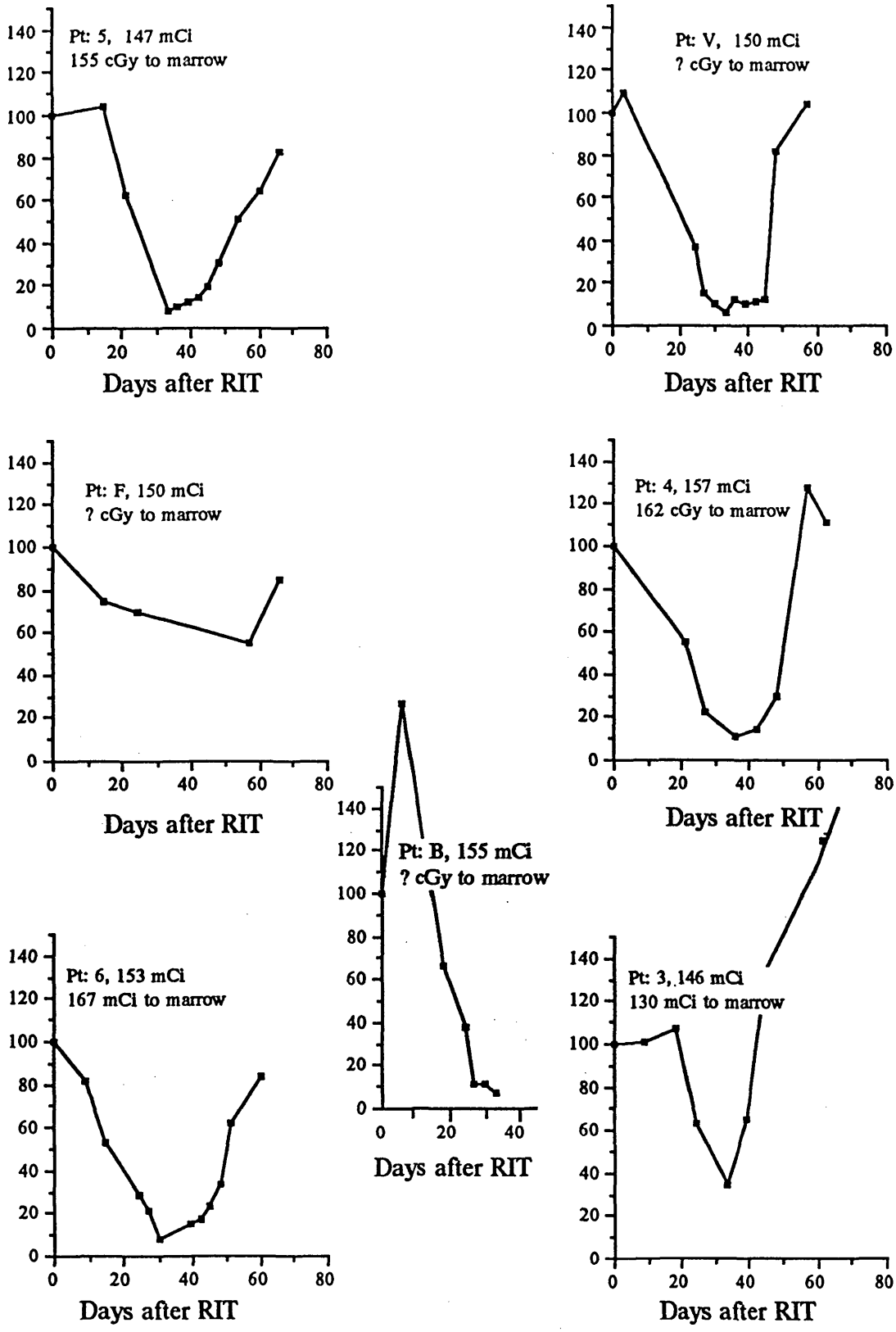


Figure 2.7: Platelet counts in patients receiving 145-157 mCi I-131 labelled monoclonal antibody. Counts are expressed as a % of the pre-treatment value.



DISCUSSION

Patients Without Ascites

In patients without ascites radiolabelled antibody was efficiently transported from the peritoneal cavity into the circulation. This transport may involve the lymphatics as it was not observed in the three patients with ascites. If radiolabelled antibody is efficiently transported into the circulation, the radiation dose to the peritoneal serosa can be expected to be low and local radiation toxicity to bowel should not occur. TLD measurements of the peritoneal serosal radiation dose in six patients without ascites ranged from 217 to 555 cGy delivered over 110 hours. Radiocolloids remain in the peritoneal cavity and deliver a much greater radiation dose to local structures [Currie et al., 1981][1]. As at least 70% of radiolabelled antibody reaches the circulation (65-78% is excreted in the urine), myelosuppression can be expected to limit the isotope activity that can be given without life threatening toxicity [Vaughan et al., 1987].

The bone marrow is irradiated by β-particles and high energy γ-rays from ^{131}I-labelled antibody. Beta-particles contribute 90 to 95% of the radiation dose; as the maximum range in tissue of these β-particles is 2.4 mm, the only possible source organs for β radiation are the marrow, marrow circulation, and bone. The γ-ray contribtuion from ^{131}I in the rest of the body can only accounts for only 5 - 10% of the absorbed radiation dose to bone marrow. I have attempted to calculate the radiation dose to bone marrow, by applying the MIRD formulation to

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1 See Chapter 5 for a comparison of the radiation doses delivered to the peritoneal serosa by Yttrium-90 labelled antibody & Yttrium-90 silicate colloid.

the integrated ^{131}I in the marrow circulation, which was estimated from the integrated ^{131}I in the blood. As macromolecules pass freely between blood and bone marrow [Bigler 1985, Trabowitz et al., 1964], the blood ^{131}I activity may be representative of marrow activity. I was unable to directly measure the volume of either blood or bone marrow, and assumed that the blood volume was related to the individual patients surface area [Hurley, 1975], and that the haemopoietic marrow represented a certain fraction of the blood volume. None of these assumptions are likely to be totally accurate. The greatest error however is likely to be fraction of blood ^{131}I activity that is assumed to be in bone marrow. As active marrow occupies 2.2% of the body weight of women [I.C.R.P., 1975] or 33% the weight of blood, I have arbitrarily assumed that the amount of radiolabelled antibody in marrow to be 20% of whole blood activity. This figure is totally arbitrary and previous estimations have been higher [Stewart et al., 1989]. The actual radiation dose the bone marrow receives may be higher or lower than my estimated value depending on the true fraction of antibody actually in the marrow space.

The radiation dose to marrow increased with the administered activity of ^{131}I-labelled MAb (Table 2.4). In the 100 hours following treatment the bone marrow received radiation doses ranging from 55 cGy (87 mCi ^{131}I) to 167 cGy (153 mCi ^{131}I). Bone marrow radiation doses above 55 cGy were associated with myleosuppression (Figure 2.6) and serious myelosuppression was observed following radiation doses to marrow of 160 cGy. The dose response curve for myelosuppression in Figure 2.6 appears to be steep and is similar to the that observed by Benua and others in 1962 when treating thyroid cancer patients with ^{131}Iodine. In these patients there was little myelosuppression when that

marrow received a radiation dose of less than 200 cGy but there was a sharp escalation in toxicity following greater radiation doses. Administering ^{131}I activities greater than 150 mCi could be hazardous in patients receiving RIT. Much greater ^{131}I activities can be given to patients with thyroid cancer where the iodine is non protein bound and rapidly excreted in the urine [Trunnel et al., 1949; Benua et al., 1962; van Norstrand et al., 1986].

Patients with Ascites

The pharmacokinetics and toxicity of ^{131}I-labelled antibody following intraperitoneal RIT are different in patients with ascites. Radiolabelled antibody is not absorbed efficiently into the circulation, presumably due to lymphatic obstruction. This decreased the radiation dose to bone marrow (Table 2.4), so that myelosuppression was not observed (Figure 2.6). As a greater fraction of the isotope's physical decay takes place in the peritoneal cavity in these patients, the radiation dose to the peritoneal serosa should have been higher than in patients without ascites. We were unable to measure this radiation dose by TLD, as reaccumulating radioactive ascites leaked from the TLD catheter puncture site. This was an unacceptable radiation hazard and the TLD catheters had to be removed.

If radiolabelled monoclonal antibody remains in the peritoneal cavity, behaving like a radiocolloid, small bowel toxicity rather than bone marrow toxicity would limit the isotope activity that can be safely administered. The radiation dose to small bowel may be particularly high when isotopes with relatively long half lives are used such as ^{131}I.

The recommended isotope activity for radiocolloid radiotherapy of the peritoneal cavity is 100 to 150 mCi for ^{90}Yttrium and ^{198}Gold with half lives of about 64 hours, but only 15-20 mCi for ^{32}Phosphorous with a 14 day half life.

SUMMARY

In patients without ascites, ^{131}I-labelled monoclonal antibody was absorbed from the peritoneal cavity into the circulation with myelosuppression from the irradiation of bone marrow. Myelosuppression limits the ^{131}I activity that can be administered.

The degree of myelosuppression increased rapidly with increasing marrow radiation dose; there was also a wide variation in the clearance of ^{131}I-MAb from the blood in individual patients. The combination of these two features will make predictions of myelosuppression in individual patients difficult .

The radiation dose to the peritoneal serosa in patients without ascites was low.

^{131}I-labelled antibody was not absorbed easily from the peritoneal cavity in patients with ascites.

In patients with ascites the radiation dose to the peritoneal serosa and bowel rather than the bone marrow may limit the ^{131}I activity that can be safely given.

CHAPTER 3

INTRAPERITONEAL ^{131}I-LABELLED MAb IN PATIENTS IMMUNIZED TO MURINE ANTIBODY

INTRODUCTION

Myelosuppression due to bone marrow irradiation limits the isotope activity that can be safely administered in RIT. This was clearly seen in Chapter 2 where a steep dose response curve was demonstrated between myelosuppression and the radiation dose the marrow received. The bone marrow is irradiated by ^{131}I-MAb in its circulation which is in equilibrium with the ^{131}I-MAb in the general circulation. The radiation dose is much greater (per mCi ^{131}I) than it would be in patients receiving ^{131}I for thyroid cancer as ^{131}I-MAb has a greater biological half life than free ^{131}I. Increasing the clearance of ^{131}I-labelled MAb from the blood would decrease the amount of ^{131}I-MAb in the marrow circulation and decrease the degree of myelosuppression. This could allow one to administer greater ^{131}I activities with less toxicity resulting in an increase of the therapeutic ratio of regional RIT.

Several investigators have observed a humoral immune response to murine MAb in patients receiving RIT [Miller et al., 1983; Courtenay-Luck et al., 1986; Sears et al., 1986; Zimmer et al., 1988]. These anti-murine immunoglobulin antibodies are called human antimouse antibodies or "HAMA". These anti-antibodies bind to MAb and may clear them from the circulation [Begent et al., 1982; Bradwell et al., 1983; Sharkey et al., 1984; Hyams et al., 1987;]. If this occurred the pharmacokinetics and toxicity of ^{131}I labelled MAb could be very different in HAMA positive patients. If ^{131}I-labelled MAb was cleared from the blood with rapid excretion of the ^{131}I radiolabel there would be less ^{131}I in the circulation to irradiate the marrow. In these

circumstances RIT would be less toxic than single treatments with the same total activity of isotope. However if the anti-antibodies were able to bind to the MAb in the peritoneal cavity the ability of the MAb to bind to tumour cells would be decreased, and this would limit the efficacy of fractionated RIT. I was able to study the pharmacokinetics of ^{131}I-labelled MAb in five HAMA positive patients who had received previous intraperitoneal RIT. The purpose of this study was to determine the pharmacokinetics of intraperitoneally administered ^{131}I-labelled monoclonal antibody in HAMA positive patients and to document the dosimetry and toxicity of fractionated RIT.

PATIENTS, MATERIALS AND METHODS

Patients

Five HAMA positive patients, aged between 39 and 72 years, who had previously received intraperitoneal RIT were given a further i.p. infusion of ^{131}I-labelled MAb. All five patients had ovarian cancer and had previously received RIT to residual tumour following cytoreductive surgery and chemotherapy. The previous RIT had been with ^{90}Yttrium-labelled MAb in four patients and with ^{131}I-labelled MAb in the remaining patient. The time interval between RIT treatments ranged from seven to twenty weeks: an ELISA assay confirmed the presence of HAMA in the serum in all patients[1]. Tumour histology in all five patients demonstrated positive immunohistochemical staining with the MAb's, HMFG1 and HMFG2.

^{131}I-Labelled Monoclonal Antibody

In four patients ^{131}I-labelled HMFG1 was obtained from CIS in France and transported at minus 20 ^{o}C to the Hammersmith Hospital. These antibody preparations contained approximately ten millicuries (0.37 GBq) of ^{131}I per milligram antibody (**Table 3.1**). The remaining antibody preparation consisted of a mixture of 15 mgs HMFG1 and 10 mgs of HMFG2 and was iodinated at the Hammersmith Hospital[2] with 148 mCi of Iodine-131

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1 The serum from patients receiving RIT was routinely monitored for HAMA by an ELISA assay by Dr. N. Courtenay-Luck, ICRF & Dept of Clinical Oncol, Hammersmith Hospital.

2 Iodination by Ms. D. Snooks, BSc.

Table 3.1 I-131 Monoclonal antibody preparations and previousRIT details for patients receiving a second intraperitoneal radio-immunotherapy treatment.

Patient Number	Iodine-131 Acivity in mCi	mgs of HMFG1	Previous RIT
14. Dun	148	25.0*	5 mCi Y-90 HMFG1 7 weeks previously
15. Tho	119	9.2	12.5 mCi Y-90 HMFG1 16 weeks previously
16. Jen	99	12.0	16.9 mCi Y-90 HMFG1 12 weeks previously
17. Edw	106	12.0	15 mCi Y-90 HMFG1 20 weeks previously
18. Gra	100	9.0	125 mCi I-131 HMFG1 18 weeks previously

* the monoclonal antibody in this patient was a mixture of 15 mgs HMFG1 and 10 mgs HMFG2

^{131}I. Trichloroacetic acid precipitation of samples from all five preparations indicated that more than 85% of ^{131}I was protein bound.

Intraperitoneal Administration of ^{131}I-MAb

All five patients underwent a pretreatment laparoscopy, during which peritoneal dialysis and TLD catheters were inserted. Radiolabelled antibody was administered, using the non-touch technique described in Chapter 2. As in chapter 2 patients were barrier nursed in a radiation controlled environment for five days after RIT.

Pharmacokinetics & Dosimetry

Following the intraperitoneal infusion of ^{131}I-labelled MAb, blood was taken at 12 hourly to monitor ^{131}I activity. Urine was also collected for the five days after RIT to monitor the urinary excretion of ^{131}I . In all five patients the radiation dose to the peritoneal serosa was measured by lithium fluoride TLD (see chapter 2 & appendix C). The methods of estimating the radiation dose to the peritoneal serosa have been already outlined in chapter 2 and appendix H.

The proportion of ^{131}I in the blood, not bound to protein (free ^{131}I), was estimated by dialysing serum against phosphate buffered saline (PBS)[1]. The proportion of free ^{131}I in blood 18-24 hours after treatment was measured in three of the five HAMA positive patients (pts. 15, 17 &18). In patient 18, serum was taken on eight occasions

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[1] see Appendix D

after RIT, to determine the proportion of free ^{131}I in blood over a longer time interval.

RESULTS

Pharmacokinetics

The pharmacokinetics of intraperitoneally administered ^{131}I-labelled antibody in the five HAMA positive patients are shown in **Table 3.2.** Peak blood radioactivity occurred 20 hours after the IP infusion with a mean 4.6% (range 3.4 - 6%) of the injected ^{131}I activity in the circulation. These ^{131}I blood activities are compared with those from the HAMA negative patients **Figure 3.1.** HAMA positive patients have greatly reduced whole blood ^{131}I activity (mean peak blood activity: 4.6% versus 28%). If one compares the AUC's between individual HAMA positive and negative patients in Figure 3.1 then HAMA positive patients have significantly lower ^{131}I activities than HAMA negative patients (Mann Whitney $U= 0$, $p < 0.001$).

After the peak ^{131}I activity in the blood at 20 hours the clearance appeared to be bi-exponential. This is shown in **Figure 3.2** where the percentage of the injected ^{131}I activity is plotted (semi-log plot) against time. In all five patients there appeared to be a rapid clearance of ^{131}I from the circulation with biological half lives ranging from 13 to 25 hours (mean 19 hours). Sixty-five hours after the IP infusion the clearance of ^{131}I from the blood decreased with mean biological half life of 68 hours (range 29 - 126 hours).

The majority of blood ^{131}I was not bound to protein during the initial rapid clearance of ^{131}I from the circulation. In three patients (patients 15, 17, & 18) 62%, 78% and 71% of ^{131}I activity could be dialysed from serum 18-24 hours after treatment. In one patient, serum was

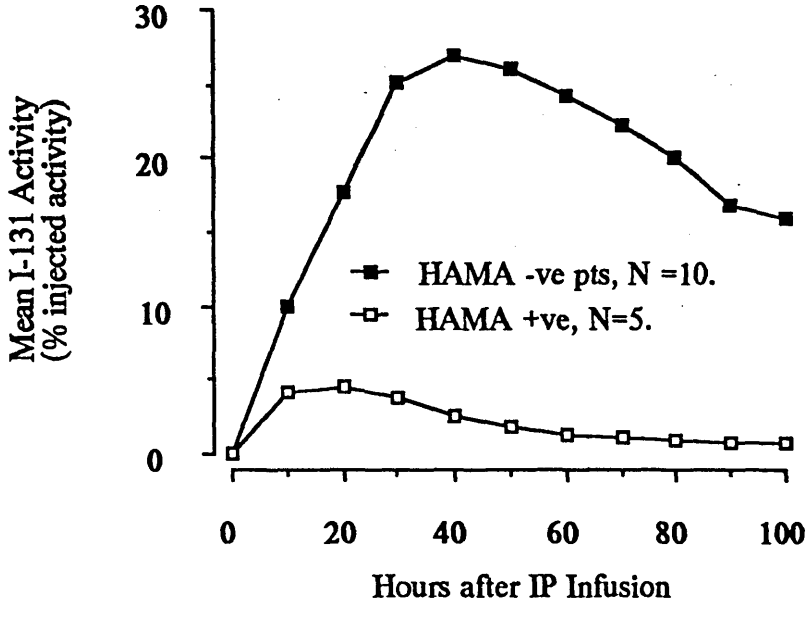
dialysed at 6, 13, 24, 38, 66, 86, 110, and 186 hours after RIT. The results are shown in **Figure 3.3a** where it is evident that the proportion of iodine that is non protein bound falls rapidly after 50 hours. These findings were confirmed by analysing the serum, taken 13 and 86 hours after RIT, through a Sepharose S-200 column. The FPLC trace in **Figure 3.3b** shows that the majority of circulating ^{131}I is protein bound at 86 hours but was "free" 13 hours after the IP infusion.

Table 3.3 shows the cumulative urinary ^{131}I excretion in the five HAMA positive patients. ^{131}Iodine was rapidly excreted with a mean of 80% of the injected ^{131}I activity excreted in the first two days. The urinary ^{131}I excretion in HAMA positive patients is compared to that of HAMA negative patients in **Figure 3.4.** The greater urinary excretion of iodine was significantly higher in HAMA positive patients, Mann Whitney $U = 0$, $p < 0.001$.

Table 3.2: Blood I-131 Activity Following the Intraperitoneal Administration of I-131 Labelled Antibody in Five HAMA Positive Patients.

Hours after IP Infusion	Percentage of the Injected I-131 Activity	
	Mean I-131 Activity	Range
10	4.2	2.3 - 6.6
20	4.6	3.4 - 6.0
30	3.8	2.2 - 5.4
40	2.6	1.7 - 3.8
50	1.8	1.3 - 2.5
60	1.3	0.8 - 1.9
70	1.1	0.5 - 1.6
80	0.9	0.3 - 1.4
90	0.8	0.3 - 1.3
100	0.7	0.2 - 1.2

Figure 3.1: I-131 activity expressed as the mean of the injected activity in patients with and without HAMA following the IP administration of I-131 labelled antibody.



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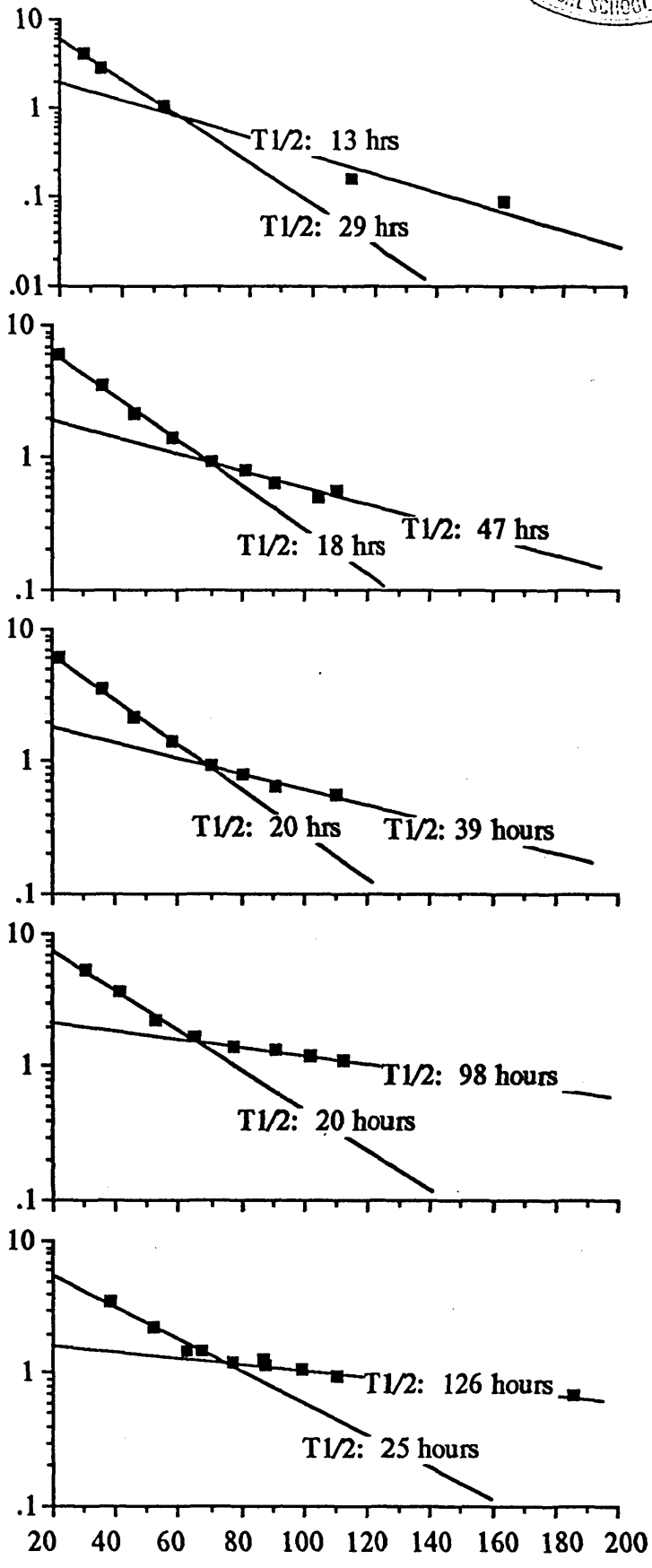


Figure 3.2: Log-linear plot of the % injected blood I-131 activity in HAMA positive patients following IP I-131 labelled MAb. The biological half lives for the two components of exponential clearance are given.

Radiation Dose to Bone Marrow

The radiation dose to bone marrow from the ^{131}I-labelled antibody in the bone marrow circulation was calculated by applying the MIRD formulation [Snyder et al., 1978] to the measured blood ^{131}I activity. This radiation dose is only an estimate as numerous assumptions are made in it's calculation (Chapter 2 & Appendix H). These estimates are given in **Table 3.4** and compared to those in HAMA negative patients in **Figure 3.5**. There is a significant difference in the marrow radiation dose between these groups of patients (expressed as cGy per mCi ^{131}I) as HAMA positive patients have much lower ^{131}I blood activities. The mean radiation dose to bone marrow, for each mCi ^{131}I, in the five HAMA negative patients was 0.098 cGy, (range 0.04 - 0.13 cGy). This was significantly lower that the mean radiation dose to marrow in the nine HAMA negative patients of 0.88 cGy per mCi ^{131}I (Mann Whitney $U = 0$, $p < 0.001$).

Radiation Dose to the Peritoneal Serosa

The peritoneal serosa receives a radiation dose from non-targeted ^{131}I-labelled antibody in the peritoneal cavity. Lithium fluoride TLD was used to calculate this radiation dose in the five HAMA positive patients. Each patient had between 33 and 40 lithium fluoride micro-rods inserted into the peritoneal cavity. These rods were removed 110 hours after the RIT infusion and their light output read on a Toledo TLD reader: the calibration of these rods has already been described (Chapter 2 and Appendix C). The radiation dose the peritoneal serosa received in the five HAMA positive patients is shown in **Table 3.4**. The serosa in HAMA positive patients received smaller radiation doses than the serosa in HAMA negative patients. The peritoneal serosa radiation

dose per mCi of ^{131}I (cGy serosa/mCi I-131) was significantly lower in HAMA positive patients. This comparison is shown in **Figure 3.6**: the difference between HAMA positive and negative patients was significant (Mann Whitney $U = 0$, $p < 0.001$).

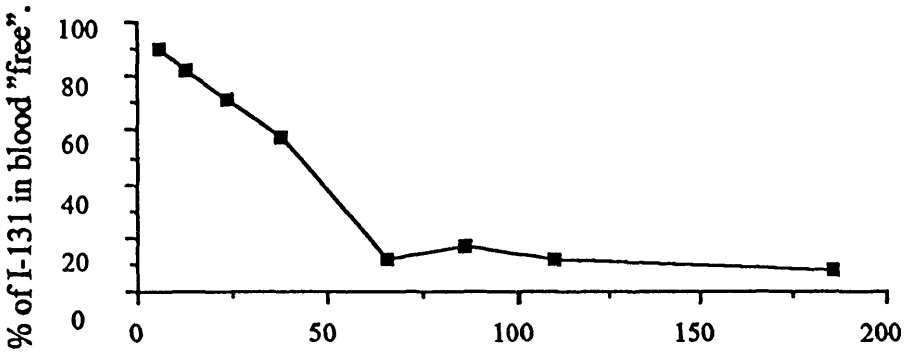


Figure 3.3b: % of the I-131 in blood that is able to be dialysed (free I-131) following the IP administration of I-131 MAB in a HAMA positive patient.

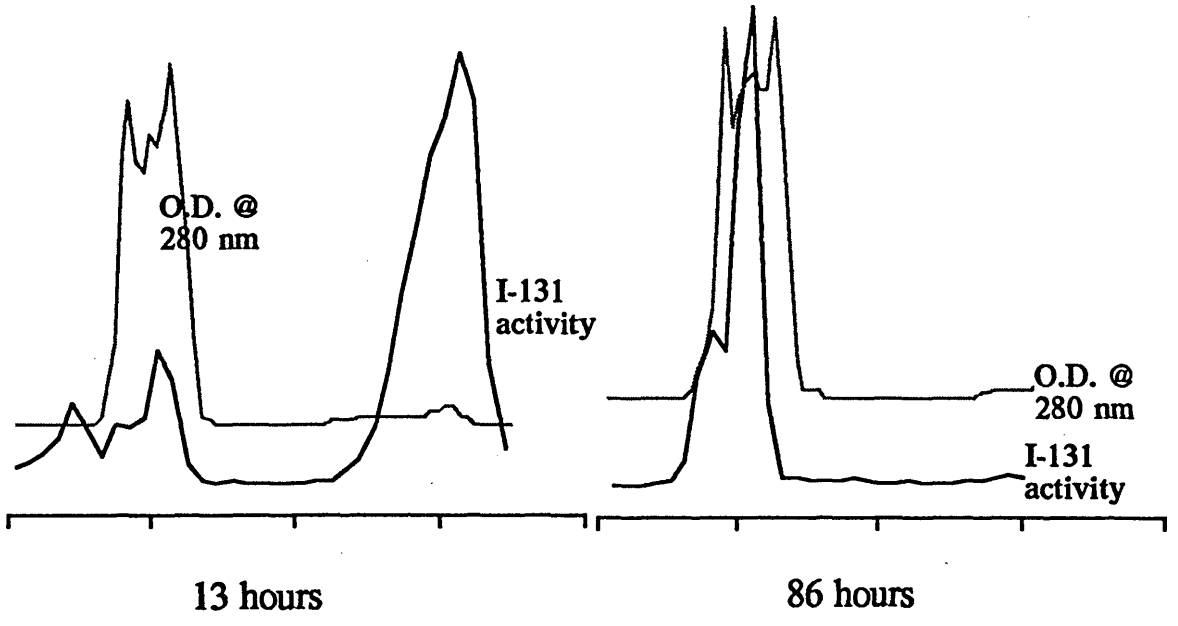


Figure 3.3b: Sepharose S-200 FPLC of the serum taken 13 hours and 86 hours after RIT in a HAMA positive patient (Pt. 18).

Table 3.3: I-131 excretion in urine following the IP administration of I-131 labelled monoclonal antibody in five HAMA positive patients.

Hours after IP infusion	% injected I-131 activity (decay corrected) Mean Cumulative I-131 excretion	Range
24	42.6	32 - 58
48	80	77 - 84
72	88.4	83 - 91
96	90	84 - 93
110	92.8	91 - 94

Figure 3.4: Urinary I-131 excretion in HAMA positive and HAMA negative patients following the IP administration of I-131 labelled monoclonal antibody.

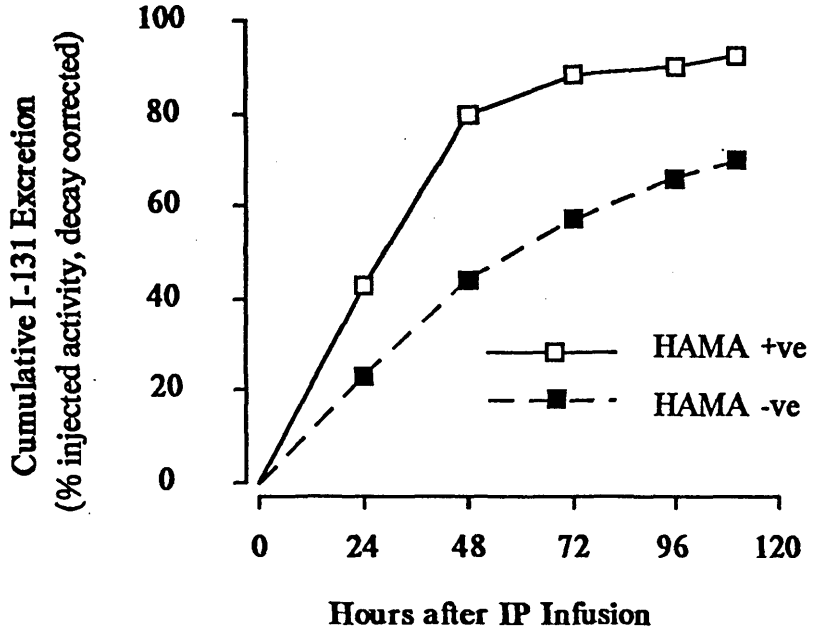


Table 3.4: Integrated blood I-131 activity in mCi.hrs in HAMA positive patients. The radiation dose to bone marrow was estimated by applying the MIRD formulation to the integrated I-131 activity (see text).

HAMA +ve Patients	I-131 Activity in mCi	Integrated I-131 Activity in Blood over 100 hours	Radiation Dose to Marrow over 100 hrs
14. Dun	148	188 mCi.hrs	11 cGy
15. Tho	119	281 mCi.hrs	16 cGy
16. Jen	70	72 mCi.hrs	4 cGy
17. Edw	106	251 mCi.hrs	14 cGy
18. Gra	100	187 mCi.hrs	11 cGy

Fig. 3.5: Radiation dose to bone marrow, expressed as cGy to marrow per mCi I-131 labelled monoclonal antibody, in patients with and without HAMA.

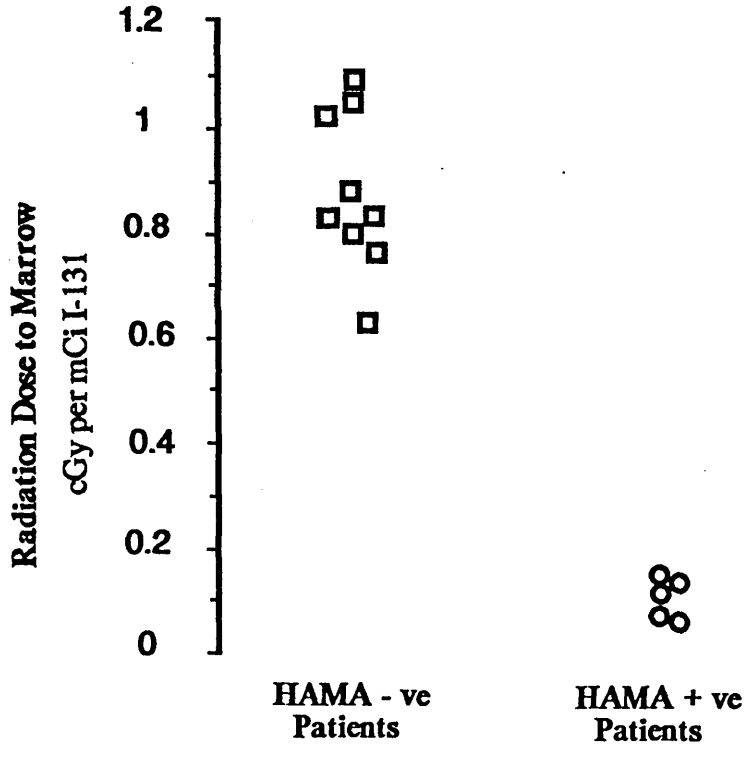
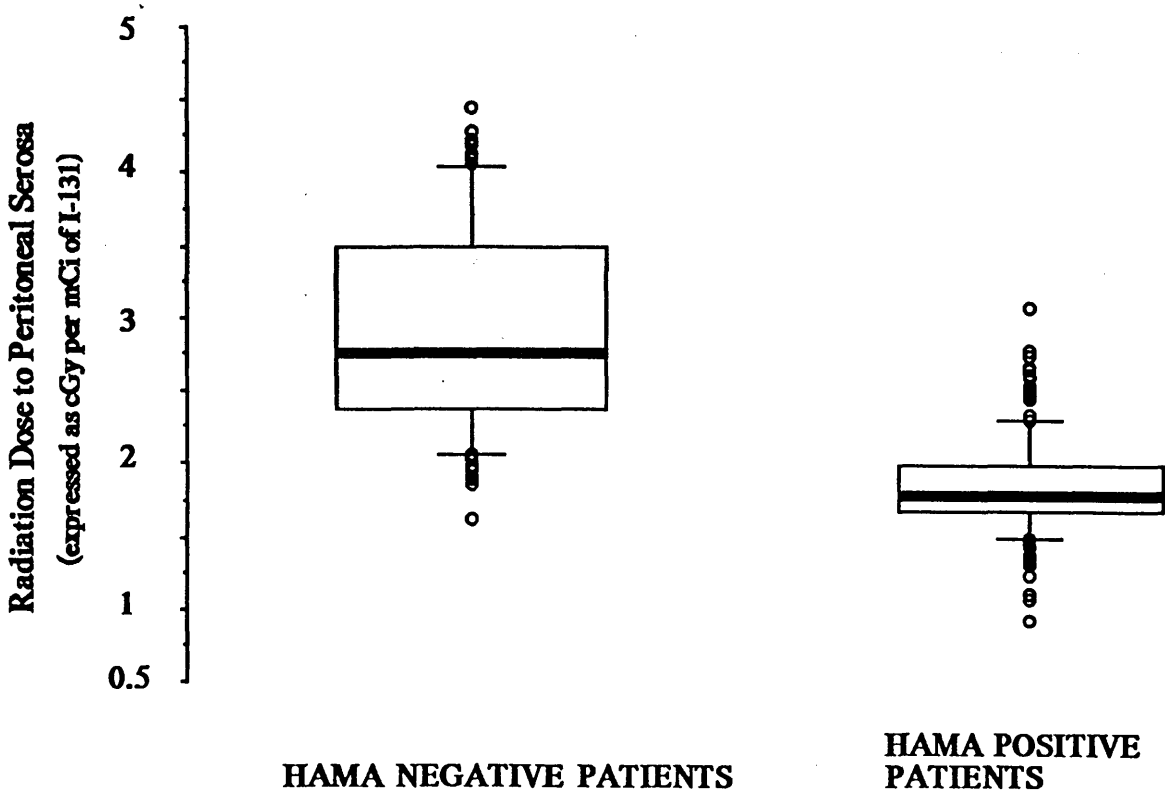


Table 3.5: Radiation dose to the peritoneal serosa as measured by lithium fluoride TLD, in HAMA positive patients.

Patients	I-131 Activity in mCi	Radiation Dose to the Peritoneal Serosa in cGy		Standard Deviation
		Mean	Range	
14. Dun	148	269 (N=35)	201 - 332	38
15. Tho	119	118 (N=40)	158 - 265	27
16. Jen	99	147 (N=33)	115 - 213	28
17. Edw	106	197 (N=40)	118 - 282	38
18. Gra	100	185 (N=39)	92 - 211	25

Fig 3.6: Box Plot of the radiation dose to the peritoneal serosa from I-131 labelled antibody in HAMA positive and HAMA negative patients. The thick line represents the median value, the other lines the 10%, 25%, 75% & 90% percentiles. Individual data points are represented beyond these values.



Toxicity

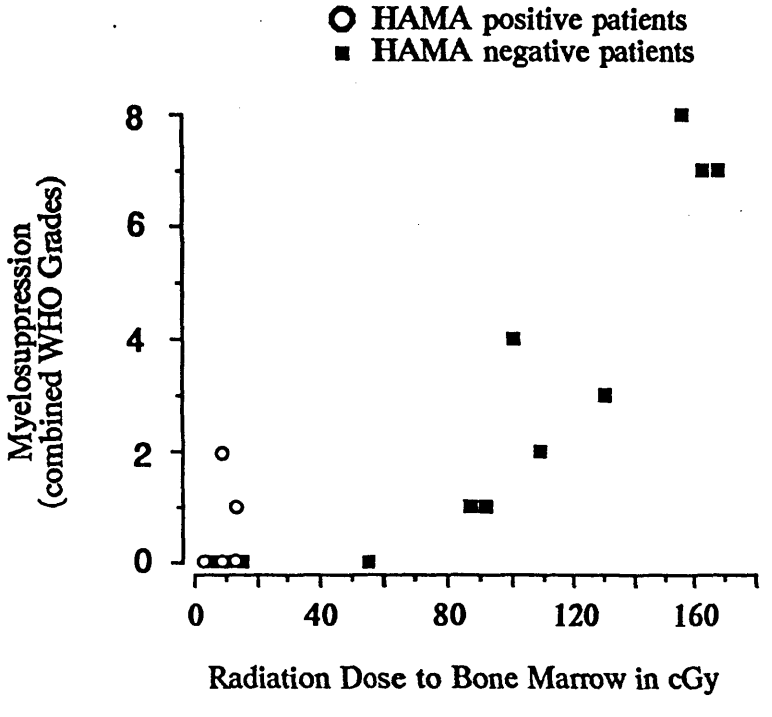
Patient No.15 had severe abdominal pain with nausea and vomiting following the i.p. administration of ^{131}I-labelled antibody with the clinical signs of peritonitis (rebound tenderness, guarding, and absent bowel sounds). This occurred 30 minutes after the end of the infusion and lasted two hours: the pain was severe enough to require narcotic analgesia. It is possible that a local type I hypersensitivity reaction was responsible for this reaction, as we have observed "peritonism" previously in a HAMA positive patient. The other four RIT infusions in HAMA positive patients were not associated with any abdominal pain, nausea or vomiting.

Leucopenia and thrombocytopenia were each scored out of four : 0 (none) to four (severe and life threatening) according to the WHO classification described in the previous Chapter. For each patient the two scores can be added to given and arbitrary scale of myelosuppression from 0 to 8. The leucopenia and thrombocytopenia are shown in **Table 3.6**, the degree of myelosuppression was mild compared to that observed in HAMA negative patients (**Figure 3.7**).

Table 3.6: Myelosuppression (WHO classification) in HAMA positive patients according to the radiation dose the marrow receives in 100 hours after the IP infusion of I-131 labelled monoclonal antibody.

Patients	Radiation Dose to Marrow in cGy	WHO MARROW TOXICITY		
		WBC	Platelets	Combined
14. Dun	11	2	0	2
15. Tho	16	0	0	0
16. Jen	4	0	0	0
17. Edw	14	1	0	1
18. Gra	11	0	0	0

Fig 3.7: Myelosuppression following RIT in patients with and without HAMA. The myelosoppresion is the sum of the WHO leucopenia and thrombocytoenia scores.



DISCUSSION

The presence of HAMA altered the pharmacokinetics of intra-peritoneally administered ^{131}I-labelled antibody. The peak blood ^{131}I activity occurred at 20 hours rather than 40 hours after injection with a six fold decrease in the peak ^{131}I activity (mean of 28% decreased to 4.5% of the injected ^{131}I activity). These results are consistent with the data in animals and man where anti-antibodies are known to clear MAb from the circulation [Begent et al., 1987, Klein et al., 1988, Zimmer et al., 1988;]. The clearance of MAb by anti-antibody is thought to be via the reticuloendothelial system, particularly the liver and spleen [Sharkey et al., 1984; Begent et al., 1982; Goldenberg et al., 1987]. In these HAMA positive patients the clearance of ^{131}I from the blood was biphasic with particularly rapid clearance in the first 60 hours. After 60 hours the mean biological half life of ^{131}I in blood increased from 19 to 68 hours. The final half life is similar to the 71 hour biological half life observed in the HAMA negative patients in chapter 2 . During the first 48 hours most of the ^{131}I was non protein bound, but during the second slower clearance phase the majority of ^{131}I was bound to immunoglobulin (Figures 3.3 a & b). The rapid initial clearance of ^{131}I from the circulation was presumably due to the reticuloendothelial system clearing HAMA-MAb immune complexes from the blood. Dehalogenases could rapidly liberate free ^{131}I into the circulation and this would account for the high proportion of ^{131}I observed in the first 60 hours. After 60 hours most of the ^{131}I appeared to be bound to immunoglobulin with the same half life as that observed in HAMA negative patients. The most obvious explanation for the second much

slower phase of clearance would be the depletion of HAMA. However we have monitored the serum HAMA levels[1] in two patients following intraperitoneal RIT (results not given): only a slight decrease in the serum HAMA titres were observed following treatment. It would be difficult to deplete HAMA, as several grams are present in the blood and only 10 to 30 mgs of MAb were injected. There are two alternative explanations for the slow terminal clearance of MAb in HAMA positive patients. If HAMA had a low affinity for a small proportion of the ^{131}I-labelled antibody, this portion of the MAb would not be cleared by HAMA and this would be left with a long biological half life. Alternatively HAMA may complex the remaining MAb but the mechanism for clearing these immune complexes may have become saturated.

The rapid clearance of MAb from the circulation combined with the efficient excretion of ^{131}I in the urine meant that the radiation dose to bone marrow from circulating ^{131}I was decreased (Figure 3.5). Even though the estimated radiation dose to marrow from circulating ^{131}I was low, mild myelosuppression was observed in two of the five HAMA positive patients (Figure 3.7). The reticuloendothelial system in the marrow may be partially responsible for clearing the HAMA-MAb complexes from the circulation. If this occurred then the radiation dose to marrow would be greater than that calculated from my MIRD formulation calculation which only took into account the ^{131}I activity in the circulation. The radiation dose to bone marrow would then depend on the effective half life of ^{131}I in the marrow reticuloendothelial

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1 By ELISA assay carried out by Dr N. Courtenay-Luck ICRF & Dept of Clin Oncol, Royal Postgraduate Medical School, DuCane Road, London W12 0HS.

system. However, antibody deiodination in these patients appeared to be extremely efficient, with 80% of the injected ^{131}I activity excreted in the urine by 48 hours after the IP infusion. If this degree of dehalogenation occured in the marrow RES then the additional radiation dose to marrow from cleared ^{131}I-labelled immune complexes would be low.

It appears that HAMA decreases the radiation dose to bone marrow from ^{131}I-labelled antibody. As myelotoxicity limited the ^{131}I activity that could be given in HAMA negative patients, repeated RIT may allow one to administer greater isotope activities more safely. HAMA may however decrease the therapeutic effect of ^{131}I-labelled MAb if it binds to MAb in the peritoneal cavity and prevents it from binding to tumour cells. There is unfortunately some evidence that this may occur in these patients. The non-specific radiation dose in the peritoneal cavity in HAMA positive patients (Figure 3.6) was reduced, and this may indicate increased intraperitoneal clearance of complexed MAb. We have also detected HAMA in the free peritoneal fluid from one of these patients. The presence of HAMA in the peritoneal cavity would seriously decrease the efficacy of intraperitoneal RIT, even if the toxicity from myelosuppression was decreased.

SUMMARY

The following conclusions about intraperitoneal RIT with ^{131}I-labelled antibodies were made from this study of HAMA positive patients.

The presence of HAMA alters the pharmacokinetics of ^{131}I-labelled antibody, with rapid clearance of MAb from the blood and excretion of ^{131}I in the urine.

Radioimmunotherapy in HAMA positive patients was less myelotoxic due to the decreased radiation dose to bone marrow from circulating ^{131}I-labelled MAb.

The increased clearance of ^{131}I-labelled MAb from the peritoneal cavity in HAMA positive patients (decreased radiation dose to the peritoneal serosa) may indicate that HAMA is able to bind to MAb in the abdomen and prevent it from targeting tumour cells . This may decrease the effectiveness of repeated RIT in HAMA positive patients.

CHAPTER 4

A STUDY OF INTRAVENOUSLY ADMINISTERED HUMAN ANTIMOUSE-IMMUNOGLOBULIN ANTIBODY TO PATIENTS RECEIVING ^{131}I-LABELLED MAb

INTRODUCTION

Myelosuppression is the most significant toxicity in patients receiving RIT with ^{131}I-labelled MAb's and is caused by the irradiation of bone marrow from ^{131}I in the vascular compartment. As the ^{131}I bound to immunoglobulin it's biological half life is greater than that of free ^{131}I used to treat patients with thyroid carcinoma. This means that patients receiving RIT have a greater radiation dose to bone marrow for a given activity of ^{131}Iodine. The radiation dose to bone marrow from the vascular compartment is reduced in HAMA positive patients as ^{131}I-labelled MAb is rapidly cleared from tbe blood and the free ^{131}I excreted in the urine: myelosuppression is also appears to be reduced. Unfortunately HAMA has been found in the peritoneal cavity and may prevent MAb binding to tumour cells. In our HAMA positive patients the ^{131}I-labelled MAb appeared to have an increased clearance from the peritoneal cavity as the non-specific radiation dose to the serosa was significantly lower in these patients. This means that although repeated RIT treatments may be less toxic, they would also be less effective, and the therapeutic ratio may not be improved.

If HAMA was confined to the blood and did not enter the peritoneal cavity, then ^{131}I-labelled MAb would be able to bind to intraperitoneal tumour cells. Any ^{131}I-labelled MAb absorbed via the lymphatics into the circulation would be rapidly cleared from the blood and the ^{131}I excreted in the urine. Greater activities of ^{131}I could be delivered with less myelosuppression and the therapeutic ratio would be increased. Administering anti-antibodies intravenously to HAMA negative patients

receiving i.p. RIT would be a logical solution to the problems outlined in Chapters 2 and 3. The clearance of antibody from the blood to the peritoneal cavity in humans is very slow [Young et al., 1987] so HAMA injected intravenously would only be slowly absorbed into the peritoneal cavity and would not interfere with MAb binding to tumour cells. Wahl & Fisher [1987a] have confirmed this in animals studies using i.v. anti-antibody to clear intraperitoneally administered MAb from the circulation. In these studies the amount of MAb on intraperitoneal tumour deposits was not decreased by intravenous injection of anti-antibody. The MAb was cleared from the circulation with a 70 to 100% enhancement in the regional delivery advantage.

Anti-antibodies have been used to clear MAb from the circulation in several studies in animals [Wahl & Fisher, 1987; Sharkey et al., 1984; Hyams et al., 1987: Bradwell et al., 1982] and man [Begent et al., 1982 & 1987]. Most of these studies have concentrated on increasing the tumour to non-tumour ratio for intravenously administered radiolabelled MAb. The anti-antibody is given at a time interval after the radiolabelled antibody to clear unbound radiolabelled MAb from the circulation. All these studies have demonstrated that radiolabelled MAb can be rapidly cleared from the blood. There may be some loss of isotope activity from the tumour deposits [Sharkey et al., 1984; Klibanov et al., 1988] but the tumour to blood isotope ratio was increased in all above studies.

The clearance of radioactive MAb/anti-antibody immune complexes by the reticuloendothelial system may lead to an increased radiation dose to the, spleen, liver and bone marrow. If the radiation tolerance of the latter two organs were exceeded, serious toxicity would occur. Several

studies have shown an increased isotope activity in the liver and spleen [Hyams et al., 1987; Sharkey et al., 1984; Wahl et al., 1987] when anti-antibodies are used to clear MAb's from tbe blood. Begent [1982 & 1987], Sharkey [1984] and Wahl [1987a] however had found that the increased hepatic isotope retention was transient. The hepatic radiation dose may even be decreased as the liver receives less radiation from the blood. Not all the reticuloendothelial system may behave in the same way as the retention of ^{131}I (from cleared immune complexes) in the spleen appears to be greater than in the liver [Sharkey et al., 1984; Wahl & Fisher 1987]. Little is known about the clearance of ^{131}I from the bone marrow reticuloendtothelial system although Hyams found that the bone marrow received a lower radiation dose if anti-antibodies were used to clear MAb from the circulation.

We have undertaken a pilot study using anti-antibody to clear ^{131}I-labelled MAb from the circulation of patients receiving intraperitoneal RIT. Human rather than animal antimouse immunoglobulin antibody was chosen as this was thought to be more physiological and less immunogenic. Human antibodies have been used extensively in clinical practice to provide passive immunity (hepatitis A, anti-blood Group D, zoster immune globulin). The amount of HAMA required to clear antibody from the blood is not known although the ratio of anti-antibody to MAb in the literature has ranged from 400:1 [Goldenberg et al., 1987] to 3:1 [Begent et al., 1987]. We chose the latter ratio as this would keep the amount of immune complexes in the circulation to a minimum.

We obtained permission from the Hammersmith Hospital Ethics Committee to isolate HAMA from the blood of patients who had

received RIT, and to give this purifed HAMA to five HAMA negative patients receiving intraperitoneal RIT.

PATIENTS, MATERIALS AND METHODS

Patients

Patients were selected for treatment if their tumour histology demonstrated positive immunohistochemical staining for HMFG1. All five patients had residual ovarian cancer following cytoreductive surgery and postoperative chemotherapy. Patient's age ranged from 42 years to 69 years. Assessment of tumour bulk and the insertion of a peritoneal catheter was performed at a pre-treatment laparoscopy. None of the five patients had been previously exposed to murine immunoglobulin and had no detectable serum HAMA by ELISA assay[1].

^{131}I- labelled Monoclonal Antibody

The monoclonal antibody HMFG1 was iodinated with ^{131}I by C.I.S. in France. Antibody preparations were radiolabelled two days before use: stored and transported at -40 ^{o}C. These preparations had approximately ten millicuries of ^{131}I per milligram antibody (**Table 4.1**). Trichloroacetic acid precipitation of samples taken from the ^{131}I-labelled antibody preparations were done immediately before the administration of antibody to patients[2]. No ^{131}I labelled HMFG1 preparation contained more than 10% free iodine.

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1 ELISA assay for HAMA was performed by Greg Sivolapenko.

2 The I-131 activity of I-131 labelled antibody preparations and the TCA precipitation were performed by Ms. D. Snooks BSc, ICRF & Dept Clin Oncol, Hammersmith Hospital.

Table 4.1: Iodine-131 monoclonal antibody preparations given to the HAMA negative patients who received intravenous HAMA.

Patient No.	Iodine-131 Activity in mCi	mgs HMFG1	Time of iv injection of HAMA	mgs HAMA
19. Lang	117	12	17 hrs	30
20. Whit	113	10	18 & 30 hrs	15 + 18
21. Adam	115	12	16 & 39 hrs	18 + 18
22. Pars	113	10	0 & 30 hrs	15 + 15
23. Howe	131	15	0 + 36 hrs	18 + 18

Human Antimouse Antibody

Human antimurine immunoglobulin antibody was purified with the collaboration of Greg Sivolapenko from the Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital. Donors for human anti-mouse antibodies (HAMA) were chosen from patients who had received i.p. radiolabeled MAB in other studies and had developed high HAMA titres. Consent was obtained to venesect a 200 ml blood specimen; a sample was previously tested for the presence of Hepatitis B antigen and antibodies to human immunodeficiency virus, by the department of Virology at Hammersmith Hospital. The venesected blood was left to clot overnight, and the serum removed and stored at -20 ^{o}C until use. Anti-HMFG1 antibodies were isolated from the serum samples using an affinity chromatography column (appendix J), where HMFG1 was coupled on CNBr-activated Sepharose 4B beads (Pharmacia, Sweden). An appropriate amount of dry beads were washed with 1mM HCL, and then mixed with the HMFG1 in coupling buffer (0.1 M NaHCO3, 0.5 M NaCl, PH 8.5) for 2 hrs at room temperature. The mixture was then transferred to the blocking buffer (1 M Ethanolamine, PH 8.0), and incubated for a further 2 hrs at room temperature. Finally the gel was washed with coupling buffer and 1 M NH3 followed by Phosphate buffered Saline (PBS, PH 7.4). The patient's serum was applied and the column washed with PBS. The anti-HMFG1 antibodies were eluted off using molar solution of ammonia. Fractions were collected, and the ones containing the antibodies (determined by absorption at 280 nm) were pooled together, and dialysed against PBS as soon as possible. Finally the anti-HMFG1 antibodies were filtered with a sterile millipore

filter (Millipore, France), and kept at 4 ^{0}C until use. All samples were tested for sterility and pyrogenicity[1]. Fast Protein Liquid Chromatography (FPLC) analysis, using Superose 6 column (Pharmacia, Sweden), of the purified HAMA revealed that over 90% of the antibody was IgG. This was also biologically confirmed, when the immunoreactivity of the antibodies was tested by an enzyme linked immunosorbant assay (ELISA). Purified HAMA recognized HMFG1 almost as well as its F(ab)'2 fragment. The small portion of HAMA that recognized the Fc part of the MAB (< 10%) did not seem to cross-react with the Fc part of human IgG[2].

In three patients the HAMA was labelled with a tracer activity of ^{125}Iodine. Approximately 2 mg of HAMA was labelled with 0.5 mCi of ^{125}I using the N-Bromosuccinamide method. The kinetics of the exogenously administered HAMA were assessed in patients No. 20, 21 & 22.

Intraperitoneal Administration of ^{131}I-labelled MAb

All five patients underwent a pretreatment laparoscopy, during which peritoneal dialysis were inserted. Radiolabelled antibody was administered using the non-touch technique described in Chapter 2.

Intravenous Administration of HAMA

HAMA was administered at different times after the infusion of ^{131}I-labelled MAb, so that the effects of HAMA could be studied. A ratio of

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1 By Blood Products Laboratory, Elstree, England.

2 Personal Communication from Greg Sivolapenko.

HAMA : MAb of approximately 3 : 1 was chosen as ratios of this order have been effective [Begent et al., 1987]. Table 4.1 shows the quantity of HAMA and the times after the i.p. MAb infusion they were injected. One intravenous HAMA injection was given to the first patient , the remaining four patients received two i.v. injections. In the first three patients (No's. 19, 20, & 21) the HAMA was given after the i.p. infusion of MAb, so that the effect of HAMA on ^{131}I-labelled HMFG1 already in the blood could be observed. In the last two patients (No. 22 & 23) the first i.v. HAMA injection was given just prior to the i.p. infusion of ^{131}I-labelled HMFG1, to see if we could decrease the I^{131} activity in blood still further, simulating the pharmacokinetics of ^{131}I-labelled MAb in HAMA positive patients more closely. HAMA was injected intravenously over a 20 minute period through a 21 gauge "butterfly" canula in 50 mls of 0.9% saline.

Pharmacokinetics of MAb & HAMA

Patients were barrier nursed in a radiation controlled environment for five days after the i.p. infusion of ^{131}I-labelled HMFG1. During this time blood samples were taken every twelve hours to monitor the ^{131}I activity in the blood (Appendix A), and to measure the fraction bound to protein using Sephadex G-50 column chromatography (Appendix E). Blood was taken more frequently after the i.v. injection of HAMA so that the immediate effect of i.v. HAMA on circulating ^{131}I-labelled MAb could be observed. In three patients (No. 20, 21 & 22) the administered HAMA was labelled with tracer quantities of ^{125}Iodine. In these patients ^{131}I and ^{125}I activities were measured by counting blood samples in a gamma counter using dual I-131 and I-125 channels. Counts in each

channel were corrected for the 'false' counts due to the presence of the other isotope. The 'true' counts in the ^{131}I channel ($C_{T[131]}$) were,

$$C_{T(131)} = \{C_{O[131]} - (K_{125}.C_{0[125]})\} / \{1-(K_{131}.K_{125})\},$$

where $C_{O[131]}$ and $C_{O[125]}$ were the observed counts in the ^{131}I and ^{125}I channels respectively. K_{125} and K_{131} were constants calculated by counting pure ^{125}Iodine and ^{131}Iodine respectively. The constant K_{131} = the number of counts in ^{125}I channel divided by the number of counts in ^{131}I channel when pure ^{131}Iodine was counted; the constant K_{125} is equal to the number of counts in ^{131}I channel divided by the number of counts in ^{125}I channel when pure ^{125}Iodine was counted. Likewise the 'true' counts in the ^{125}I channel were,

$$C_{T[125]} = \{C_{O[125]} - (K_{131}.C_{0[131]})\} / \{1-(K_{131}.K_{125})\}.$$

Blood isotope activity was expressed as total activity. The fraction of ^{131}I or ^{125}I bound to protein was measured by passing 50 µl serum samples through a 20 ml Sephadex G-50 column.

Radiation Dosimetry to the Bone Marrow

As in chapters 2 and 3 the radiation dose to the bone marrow from ^{131}I in the vascular compartment was estimated by the applying the MIRD formulation to the integrated ^{131}I activity in blood for 100 hours after RIT (Appendix H). This calculation did not take into account any additional radiation dose the bone marrow may have received from the reticuloendothelial system clearing HAMA/MAb complexes from the blood.

Radiation Dosimetry to the Liver

The radiation dose to the liver was also estimated by applying the MIRD formulation to the integrated ^{131}I activity for the first 100 hours after RIT. Unlike the bone marrow, the extracellular space of the liver is not functionally part of the circulation. The liver is thought to contain only 6% of the blood volume [I.C.R.P., 1975]. If one ignores any specific uptake of immune complexes by the liver's reticuloendothelial system, hepatic irradiation would mostly be due to radiolabeled antibody in the liver circulation (mostly β-particles). In addition there would be a small contribution from the rest of the body (γ-rays). The radiation dose the liver received in the first 100 hours $D_{[liver]}$ was estimated by

$$D_{[liver]} = [\, 0.06 \,.\, A \,.\, S_{[liver\ to\ liver]} \,] + [\, A \,.\, S_{[whole\ body\ to\ liver]} \,],$$

where A was the integrated ^{131}I activity over 100 hours following the i.p. infusion of ^{131}I-labelled antibody. The factors $S_{[liver\ to\ liver]}$ and $S_{[whole\ body\ to\ liver]}$ are the S factors for the liver and the whole body as radiation sources. This formula does not take into account the additional radiation dose the liver received from the increased uptake of immune complexes. The increased hepatic uptake of ^{131}I after the i.v. injection of HAMA was assessed in three patients by counting the radiation activity over the liver by a specially shielded scintillation counter. These counts were compared to counts taken at the same time over the heart and left subclavian vessels. Measurements taken from these areas were taken every 20 minutes after injection of HAMA until the measured counts had fallen to pre-HAMA levels. Counts over the liver, heart and left subclavian vessels were then taken every six hours.

RESULTS

Pharmacokinetics of ^{131}I-labelled MAb

Figure 4.1 compares the blood pharmacokinetics of ^{131}I-labelled MAb in the five HAMA negative patients receiving i.v. HAMA, with those of the nine HAMA negative patients in Chapter 2 who did not receive HAMA, and with the five HAMA positive patients from Chapter 3. In HAMA negative patients the administration of HAMA appeared to decrease the blood ^{131}I activity but not to the levels observed in the five HAMA positive patients. One can compare the individual AUC's for the three groups of patients in figure 4.1 by using a Mann Whitney U test. Patients given i.v. HAMA have significantly lower ^{131}I activities in their blood when compared to HAMA negative patients without HAMA ($U=0$, $p < 0.001$), but have significantly greater activities than HAMA positive patients ($U=0$, $p < 0.005$).

The pharmacokinetics of MAb in individual patients receiving i.v. HAMA are studied in more detail in Figures 4.2 to 4.4. The pharmacokinetics following a single injection of 30 mgs of HAMA 17 hours after the i.p. MAb infusion are illustrated in **Figure 4.2.** Until the injection of HAMA the ^{131}I activity in the blood was increasing as one might expect from the data in Chapter 2, with 82% of the serum ^{131}I activity bound to immunoglobulin. Following the i.v. injection of HAMA there was a rapid decrease in blood ^{131}I activity from 6.8% to 2.7% of the injected activity. In fact 83% of the MAb had been cleared from the blood as only 32% of the remaining ^{131}I activity was bound to immunoglobulin. Twenty hours after the i.v. injection of HAMA the

Figure 4.1: The blood I-131 activity following i.p. radioimmunotherapy in five patients receiving i.v. HAMA —— . These curves are compared to the mean I-131 activities observed in 9 HAMA negative patients — — and 5 HAMA positive patients ▬▬ .

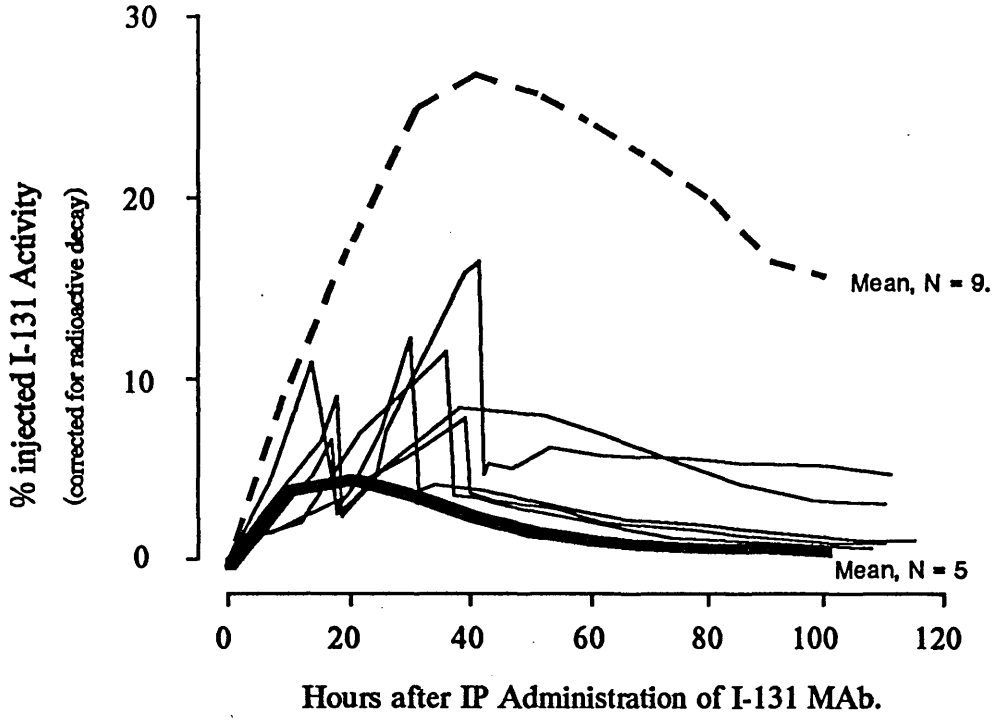
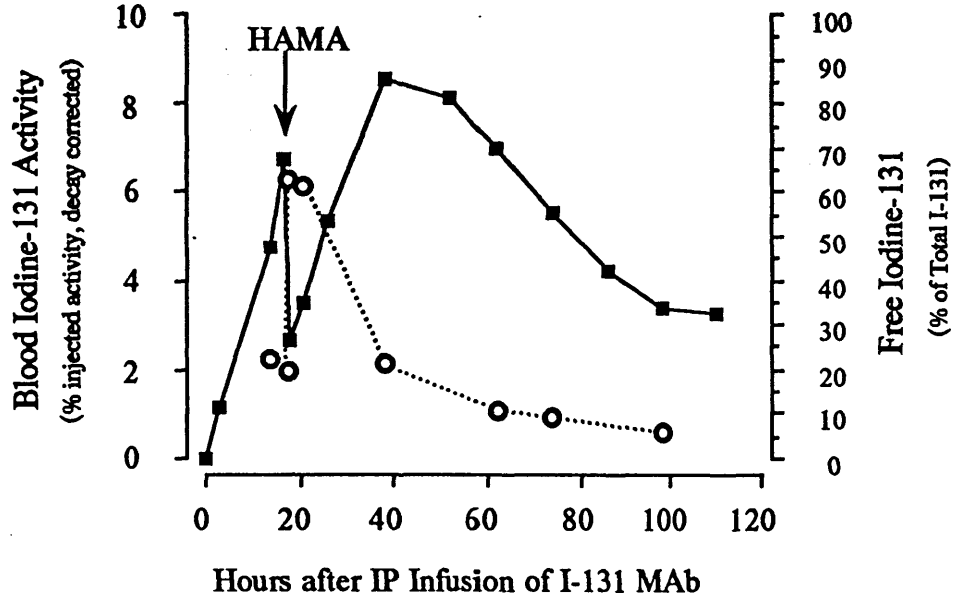


Figure 4.2: Total blood I-131 activity —■— and the percentage of this I-131 that is not protein bound ····o···· in patient 19. The arrow indicates the iv infusion of HAMA.

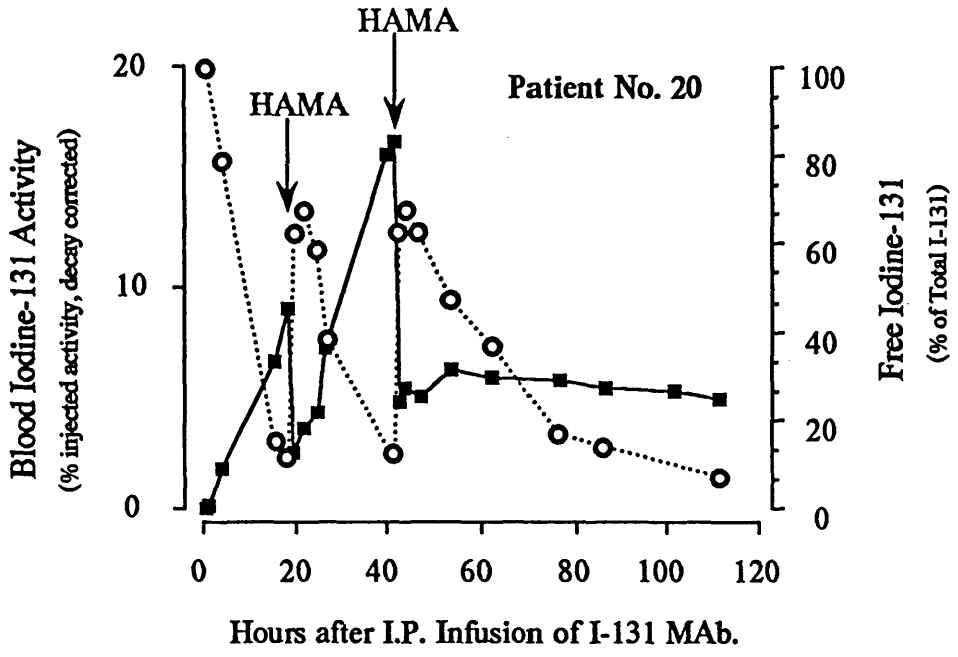


percentage of free ^{131}I fell to 21% and the amount of MAb rose rapidly.

After treating the first patient with i.v. HAMA it was decided that we should give approximately the same quantity of HAMA but inject it in two bolus infusions. By injecting all the HAMA in one infusion one may produce immune complexes with an excess of HAMA to MAb, thereby using up more HAMA than might really be necessary. By monitoring the serum ^{131}I activity and in particular the fraction of free ^{131}I we felt we could determine when further HAMA injections were necessary.

The first HAMA injections in the next two patients (No. 20 & 21) were given 15 and 18 hours after the i.p. infusion of ^{131}I-labelled MAb, the pharmacokinetics of the ^{131}I in the serum are illustrated in **Figure 4.3.** Immediately after the i.p. infusion of ^{131}I-labelled MAb the serum ^{131}I activity was low and mostly consisted of free ^{131}Iodine. This is presumably due to the more rapid diffusion of free ^{131}I out of the peritoneal cavity compared to the slower lymphatic transport of ^{131}I-labelled MAb. Immediately before the first i.v. injection of HAMA, the serum ^{131}I activity had risen to 9.1% and 11% of the injected ^{131}I activity with 89% of the ^{131}I bound to immunoglobulin. One hour after the i.v. injection of HAMA 88% and 90% of the MAb had been cleared from the blood with only 2.5% and 3% of the injected ^{131}I activity remaining. Only 33% and 25% of the remaining ^{131}I activity was protein bound. In the 20 hours after the HAMA injection the blood ^{131}I activity began to rise, with an increasing fraction of the ^{131}I activity bound to protein. Immediately before the second HAMA injection at 30 and 39 hours, the blood ^{131}I activity had increased to 16.6% and 8% of the injected activity with 89% and 74% of this activity bound to protein.

Figure 4.3: Whole blood iodine-131 activity —■— in patients 20 & 21 following the IP infusion of I-131 labelled MAB with intravenous HAMA (arrows). The percentage of blood I-131 not bound to protein is represented by ···o··· .



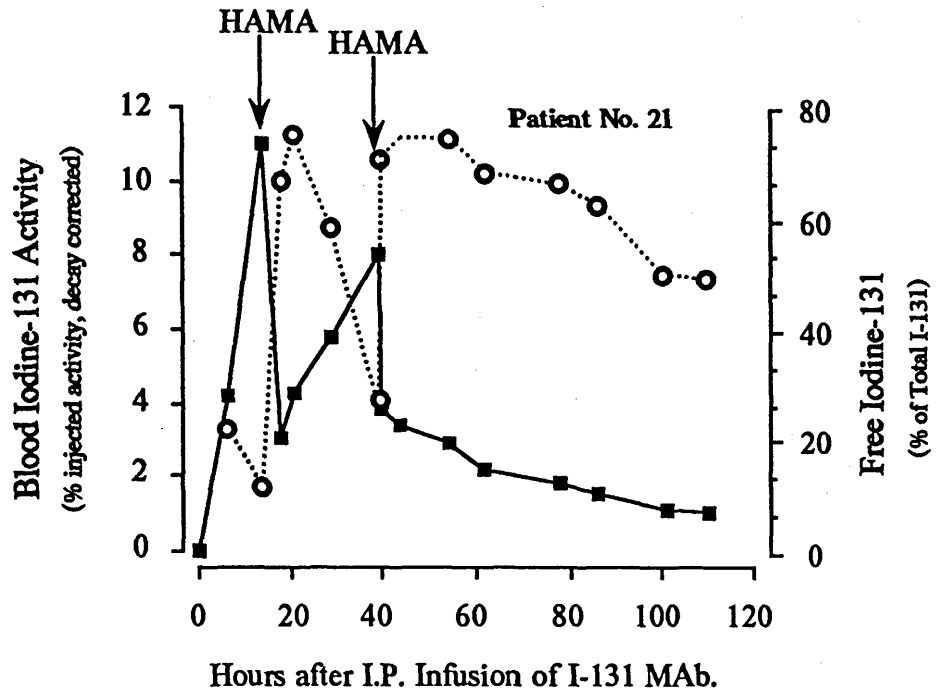
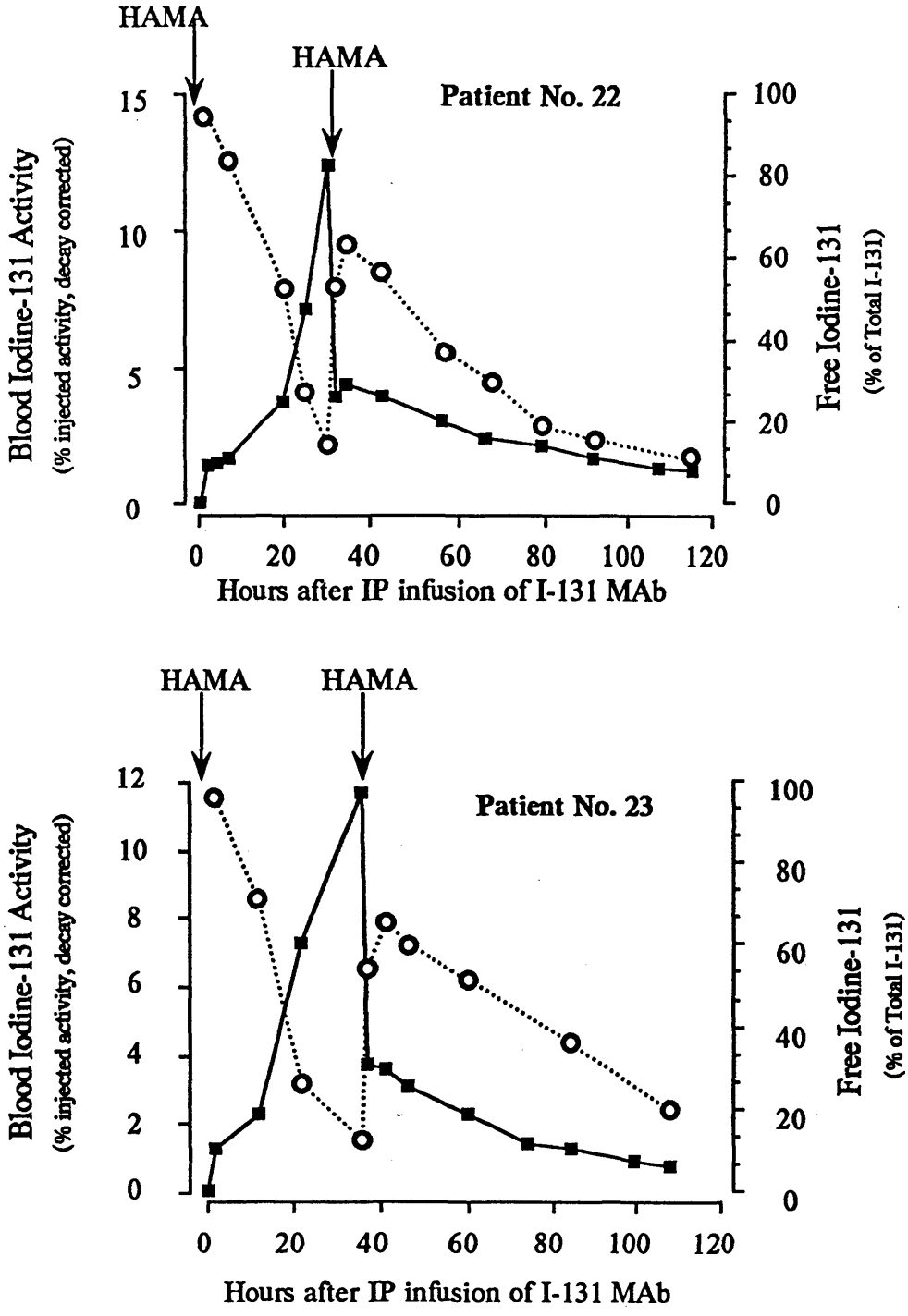


Figure 4.4: Whole blood iodine-131 activity —■— in patients 22 & 23 following the IP infusion of I-131 labelled MAB with intravenous HAMA (arrows). The percentage of blood I-131 not bound to protein is represented by ....o.... .



After the second injection of HAMA, the blood ^{131}I activity fell to 4.8% and 3.4% of the injected activity as 88% and 86% of the circulating MAb was cleared from the blood. Sixty-seven percent and 74% of the serum ^{131}I remaining was free and not bound to protein.

In the last two patients the first i.v. HAMA injection was given immediately prior to the i.p. infusion of ^{131}I-labelled MAb, to imitate the pharmacokinetics of ^{131}I-labelled MAb in HAMA positive patients more closely. The pharmacokinetics of ^{131}I in the blood of these patients are illustrated in **Figure 4.4.** In these patients there was a delay in the initial accumulation of ^{131}I in the blood, and it took 30 and 36 hours before 12.4 and 11.6% of the injected ^{131}I activity was detected in the circulation. By this time 89% of the ^{131}I activity was bound to protein. The second injection of HAMA cleared 83% of the MAb from the circulation with only 3.9 and 3.7% of the injected ^{131}I activity remaining of which only 47% was protein bound.

Hepatic Clearance of ^{131}I-labelled MAb

In patients No. 20, 21, and 22, the increased hepatic uptake of ^{131}I after the i.v. injection of HAMA was assessed by counting the radiation activity over the liver with a specially collimated sodium iodide crystal scintillation counter attached via a multichannel analyser. The counts over the liver were compared with counts taken simultaneously over the left subclavian vessels (representing the blood pool): the results are shown in **Figure 4.5.** Fifteen minutes after the i.v. injection of HAMA there was a rapid increase in hepatic ^{131}I activity, accompanied by a fall in the counts recorded over the subclavian vessels. Peak hepatic ^{131}I activity occurred

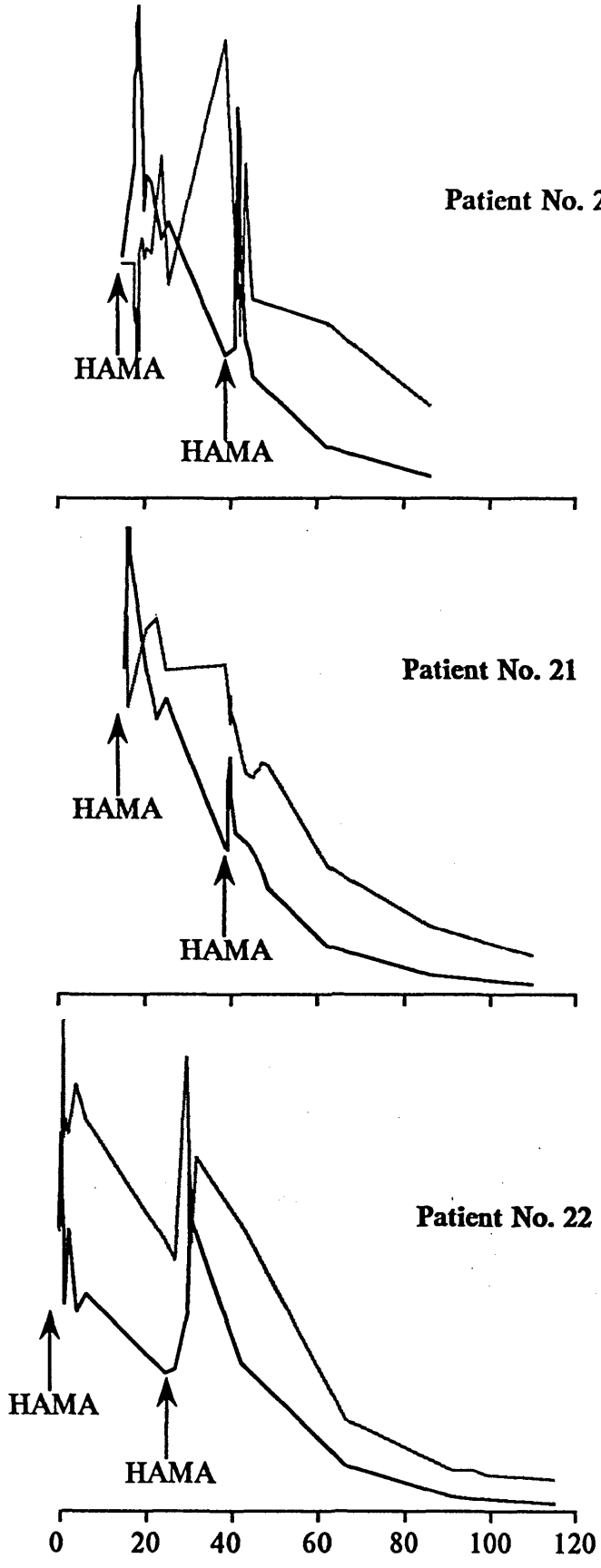
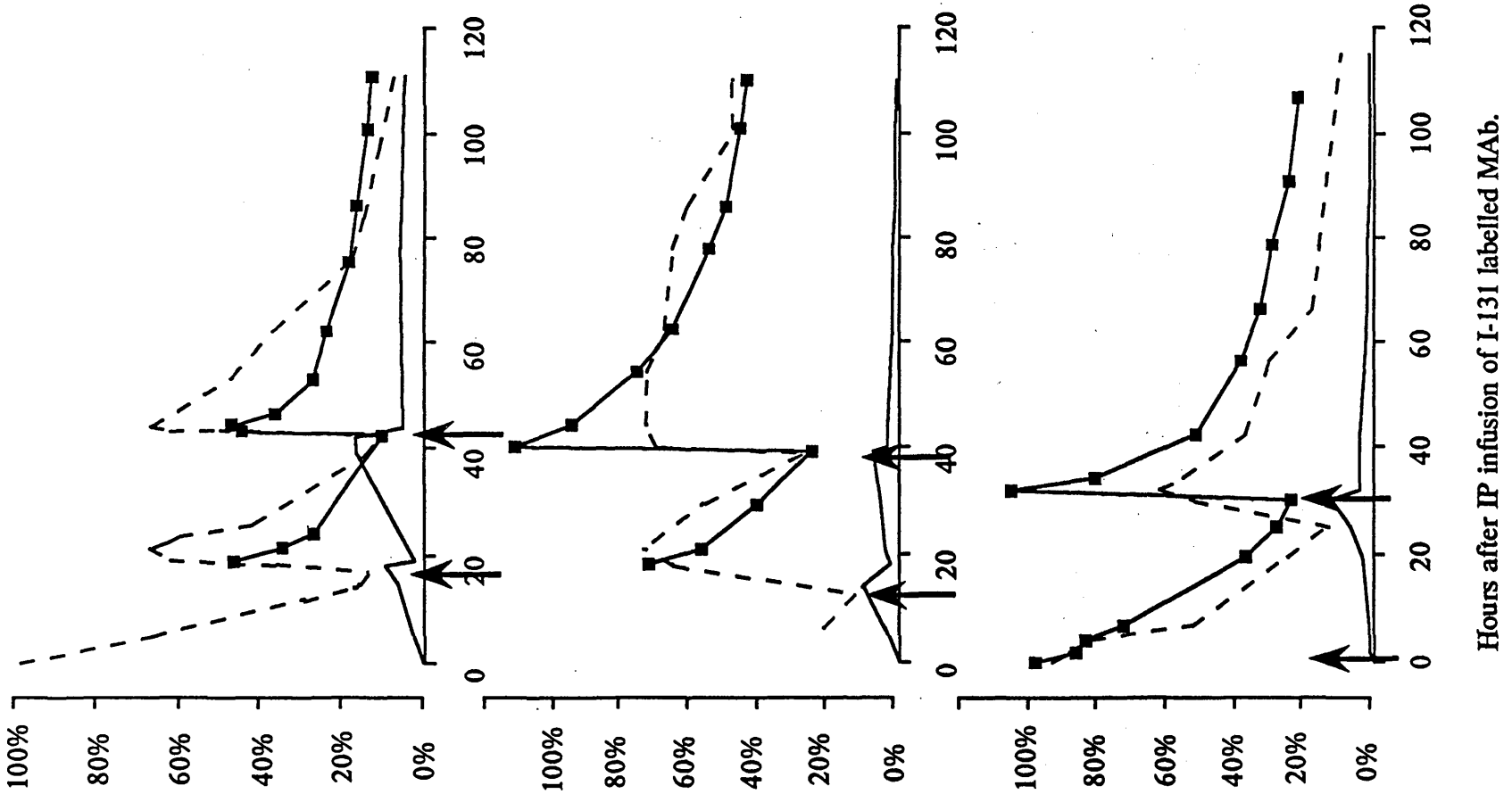


Figure 4.5: I-131 activity over the liver —— and the subclavain vessels —— (measured by a shielded scintillation counter) after the i.v. injection of HAMA in three patients.

Figure 4.6: Serum HAMA levels expressed as a % of the total injected activity in three patients (20, 21, & 22) who received iv I-125 labelled HAMA after the ip infusion of I-131 labelled MAb—■—. The times of iv HAMA administration are given by the arrows. The total I-131 content in patients blood (% of injected activity) is given by ——, and the % of serum iodine-131 that is free by the line– – –



one hour after the injection of HAMA, following which the activity fell to pre-HAMA levels 4 to 8 hours later. There was a small peak in serum ^{131}I activity immediately after the serum trough associated with the increased hepatic uptake.

Pharmacokinetics of HAMA

The pharmacokinetics of HAMA were studied in three patients by labelling HAMA with a trace activity of ^{125}Iodine, as is illustrated in **Figure 4.6.** According to gel chromatography using a Sephadex G-50 column, more than 95% of serum ^{125}I activity was bound to protein. HAMA concentrations decreased (non-exponential kinetics) in the 20 hours after their injection. The rate of clearance may have been dependent on the amount of MAb in the circulation, as clearance was slower following second HAMA injection. The decline in HAMA in the blood was accomanied by a fall in the percentage of ^{131}I that was free and not bound to immunoglobulin. The percentage of ^{131}I that was non- protein bound appeared to be a good indicator of serum HAMA levels. When more than 80% of the serum ^{131}I activity was bound to protein HAMA levels were low and a second HAMA injection was required.

Urinary Excretion of ^{131}Iodine

The cumulative excretion of ^{131}I in these five patients is illustrated in Figure 4.7, and compared to the excretion of ^{131}I observed in HAMA positive patients and HAMA negative patients who did not receive i.v. HAMA in Figure 4.8. In the first three patients there was a rapid increase in the urinary excretion of ^{131}I after the i.v. injection of HAMA (Figure 4.7). This was not seen in the two last two patients as they had received HAMA prior to the i.p. infusion of MAb. Patients who received i.v. HAMA had an increased ^{131}I excretion compared to those HAMA negative patients who did not receive i.v. HAMA (Figure 4.8). The increased ^{131}I excretion only occurred after 24 hours. This delay in excretion may be due to two factors: HAMA was not administered until 16 hours after the MAb in three patients, and when it was administered prior to the i.p. MAb infusion, there was some delay before the ^{131}I-labelled MAb reached the circulation.

Figure 4.7: Cumulative urine excretion of iodine-131 following the i.p. administration of I-131 labelled MAb in HAMA negative patients receiving iv injections of HAMA (arrows).

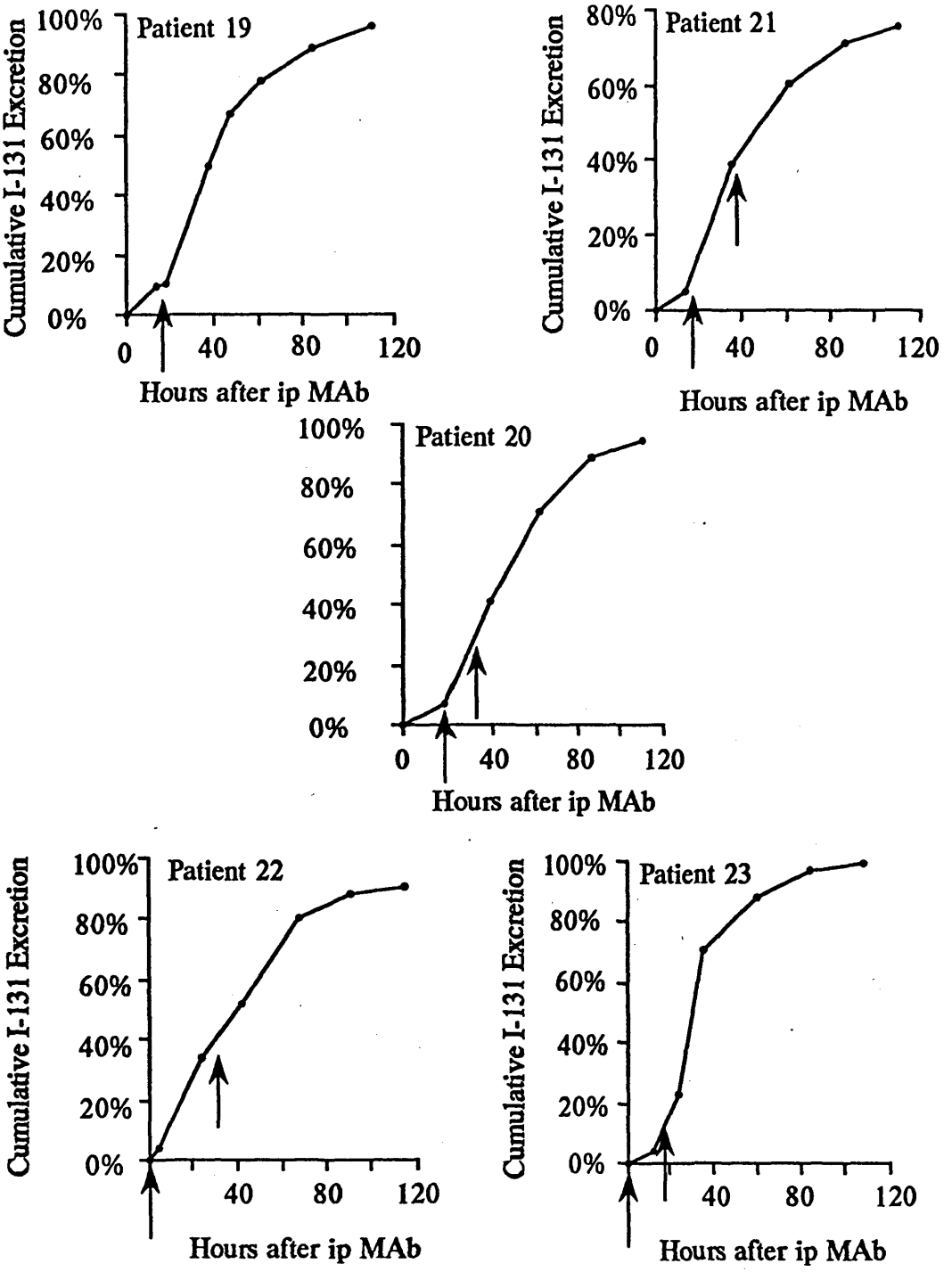
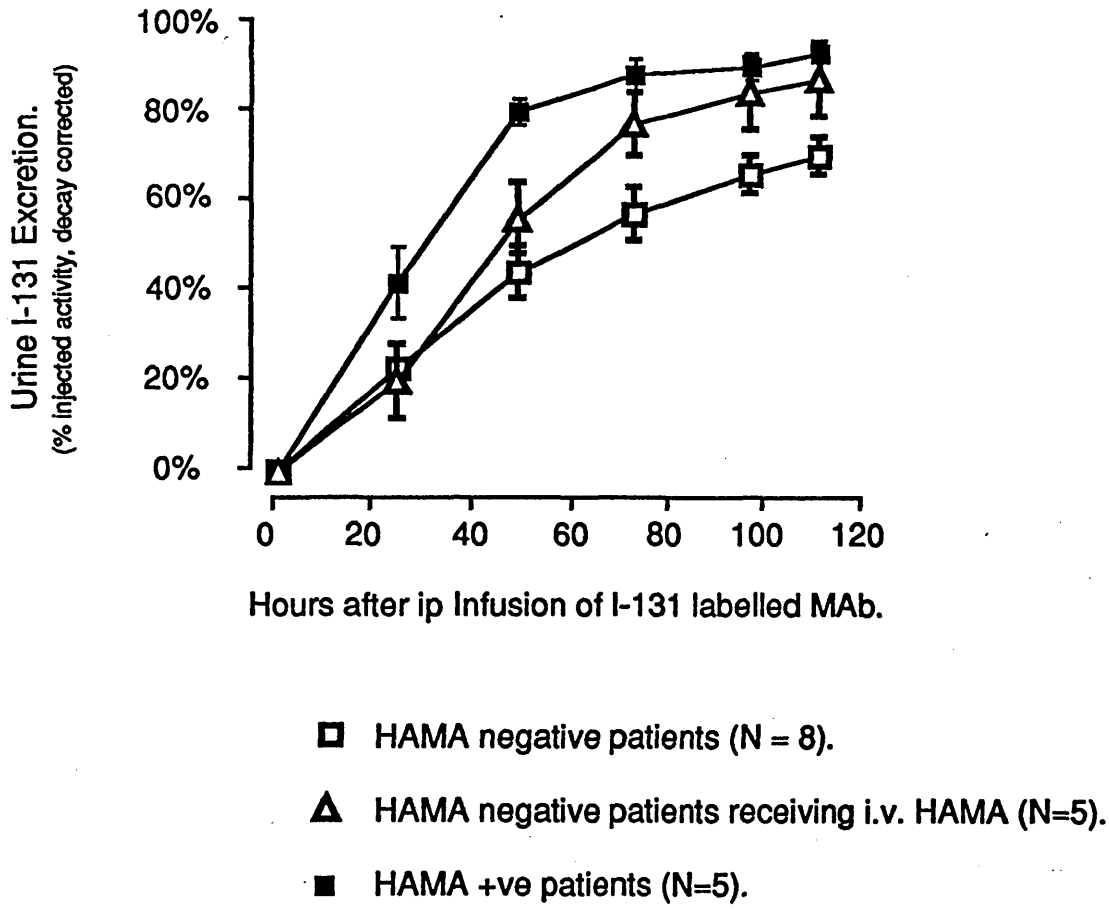


Figure 4.8: Mean I-131 excretion in patients with no HAMA, endogenous HAMA, or given i.v. "exogenous" HAMA. The bars represent =/- two standard errors of the mean.



Radiation Dose to Bone Marrow

The radiation dose to the bone marrow from ^{131}I in the marrow was calculated by applying the MIRD formulation to a fraction (20%) of the integrated blood ^{131}I activity for the first 100 hours after the i.p. infusion of MAb (appendix H). It must be emphasized that this calculation does not take into account any additional radiation dose the marrow may have received from the reticuloendothelial system[1]. The radiation dose in **Figure 4.9** is expressed in cGy per mCi of administered ^{131}I-labelled MAb. Comparisons have also been made with the radiation doses the bone marrow received from ^{131}I-labelled MAb in HAMA negative and HAMA positive patients. The mean radiation dose per mCi ^{131}I in HAMA negative patients receiving i.v. HAMA was 0.205 cGy (range 0.14 - 0.28 cGy). This was significantly lower than the mean radiation dose of 0.855 cGy (range 0.57 - 1.01 cGy) in HAMA negative patients who did not receive i.v. HAMA (Mann Whitney $U = 0$, $p < 0.002$). The mean radiation dose to

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1 The reticuloendothelial system in the marrow may clear the MAb/HAMA immune complexes from the circulation.

Figure 4.9: Scattergram of the estimated radiation dose to bone marrow in patients receiving ip I-131 labelled monoclonal antibody.

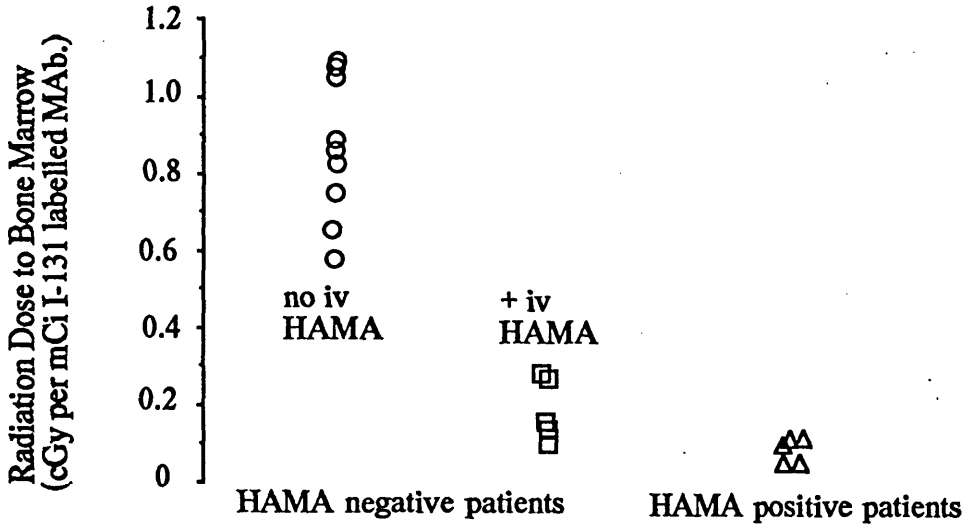
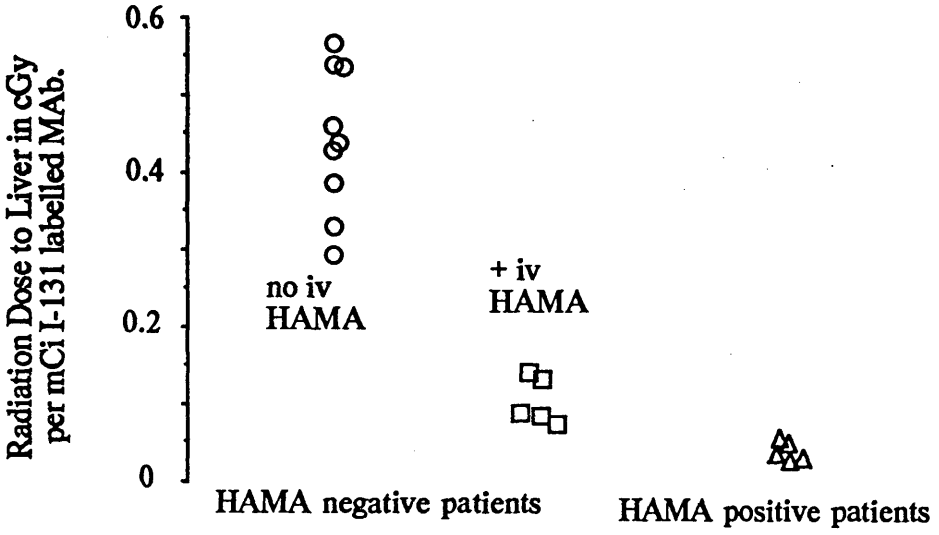


Figure 4.10: Scattergram of the estimated radiation dose to liver in patients receiving ip I-131 labelled monoclonal antibody.



bone marrow in the five HAMA positive patients was only 0.098 cGy/mCi (range 0.06 - 0.13 cGy) which is a significantly lower radiation dose than that seen in patients receiving i.v HAMA (Mann Whitney U=0, $p< 0.005$).

Radiation Dose to Liver

The radiation dose to the liver from ^{131}I in the liver circulation was calculated by applying the MIRD formulation to a fraction (6%) of the integrated blood ^{131}I activity for the first 100 hours after the i.p. infusion of MAb (see methods). No allowance has been made for any additional radiation dose the liver received by clearing immune complexes from the circulation as this is a small fraction of the area under the curve of ^{131}I activity (Figure 4.5). The mean radiation dose per mCi ^{131}I in HAMA negative patients receiving i.v. HAMA was 0.166 cGy (range 0.07 - 0.15 cGy) which was significantly lower than the mean 0.443 cGy (range 0.30 - 0.57 cGy) in HAMA negative patients who did not receive i.v. HAMA (Mann Whitney U = 0, $p< 0.002$) as illustrated in **Figure 4.10.** The mean radiation dose to bone marrow in the five HAMA positive patients was only 0.051 cGy/mCi (range 0.03 - 0.07 cGy) which is a significantly lower dose than that in patients receiving i.v HAMA (Mann Whitney U=0, $p< 0.005$).

Toxicity

Acute toxicity could be associated with both the i.p. administration of ^{131}I-labelled MAb and with the i.v. administration of HAMA. Only one patient (No. 21, see below) developed acute toxicity associated with the

i.p. administration of ^{131}I-labelled MAb: the other observed toxicities appeared to be related to the i.v. administration of HAMA.

Patient No. 19 complained of a warm feeling after the i.v. injection of HAMA. This lasted 30 minutes and was accompanied by a sinus tachycardia[1] of 100 beats per minute. There was no accompanying skin rash, urticara, respiratory wheeze, bronchospasm, or hypotension. One hour after the first HAMA injection, Patient No. 20 complained of feeling cold and had a 'shivering' episode. Examination revealed peripheral vasoconstriction, a slight tachycardia but no hypotension or pyrexia; the second injection of HAMA in this patient was uneventful. Patient No. 21 had a moderate degree of nausea and vomiting in the 24 hours after the i.p. administration of ^{131}I-MAb, this was controlled by anti-emetics and there was no adverse reaction following the i.v. injection of HAMA. Patient No.22 complained of feeling warm and developed a mild tachycardia following the first HAMA injection lasting 20 minutes: an hour after the second HAMA injection she felt cold and had a short rigor, accompanied by a sinus tachycardia of 110/minute but no pyrexia or hypotension. There were no other symptoms or signs and the patient returned to normal after 60 minutes. Patient No. 23 experienced no side effects following the i.v. injection of HAMA.

Myelosuppression was the most serious toxicity encountered in these patients. The degree of myelosuppression according to the WHO classification is shown in **Table 4.2**: all but one patient developed a degree of myelosuppression in the six weeks following treatment. The nadir blood counts for leucocytes and platelets are illustrated in

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1 Confirmed by electrocardiograph.

Figure 4.11. All patients had demonstrated a fall in platelet count after RIT and all but one patient (No. 21) a fall in leucocyte count. The fall in blood counts were prolonged and up to 150 days following treatment.

Figure 4.11: The platelet ■ and leucocyte count □ (expressed as the % of the day 7 count) in five patients who received i.p. I-131 labelled MAb and intravenously administered HAMA.

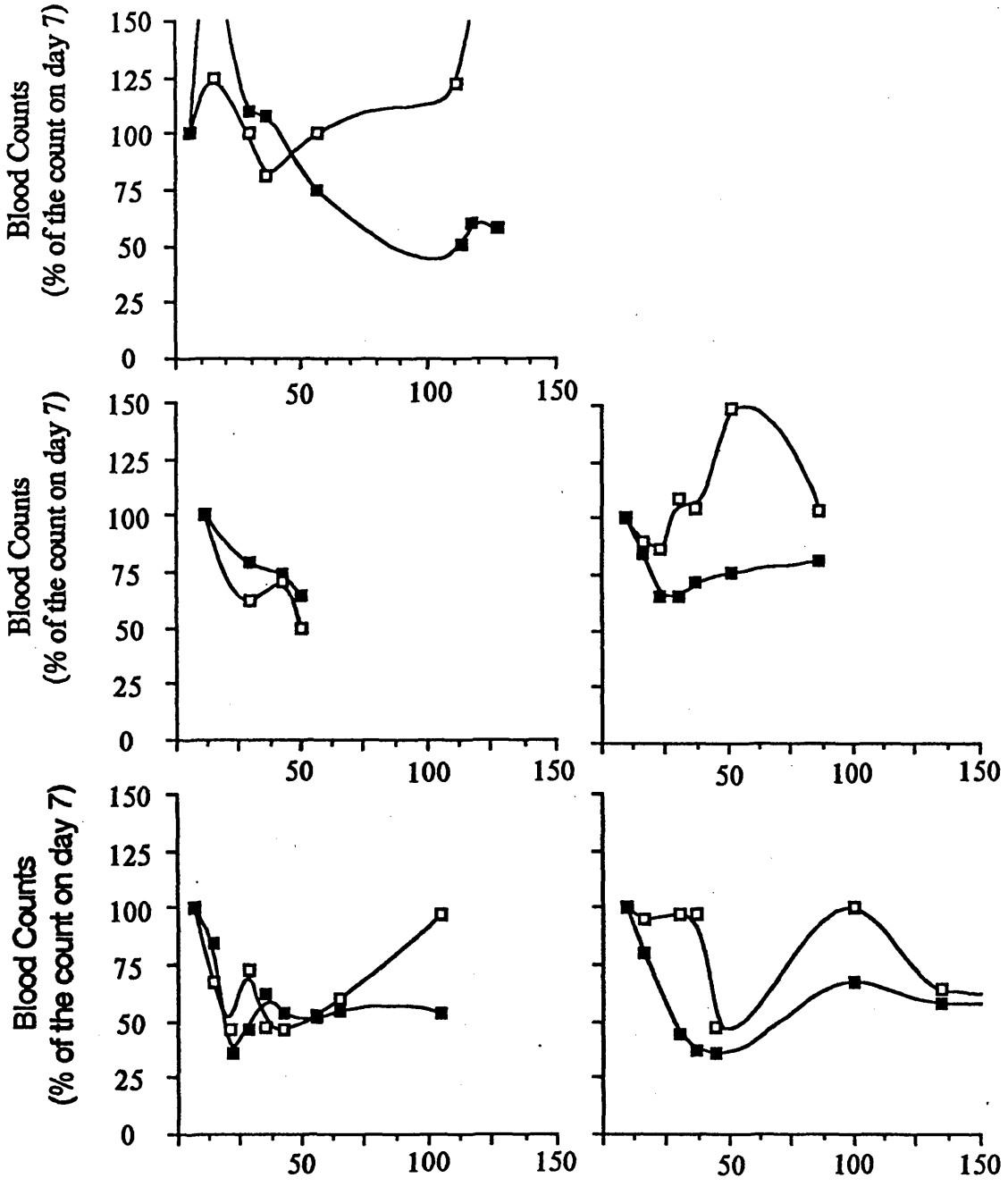
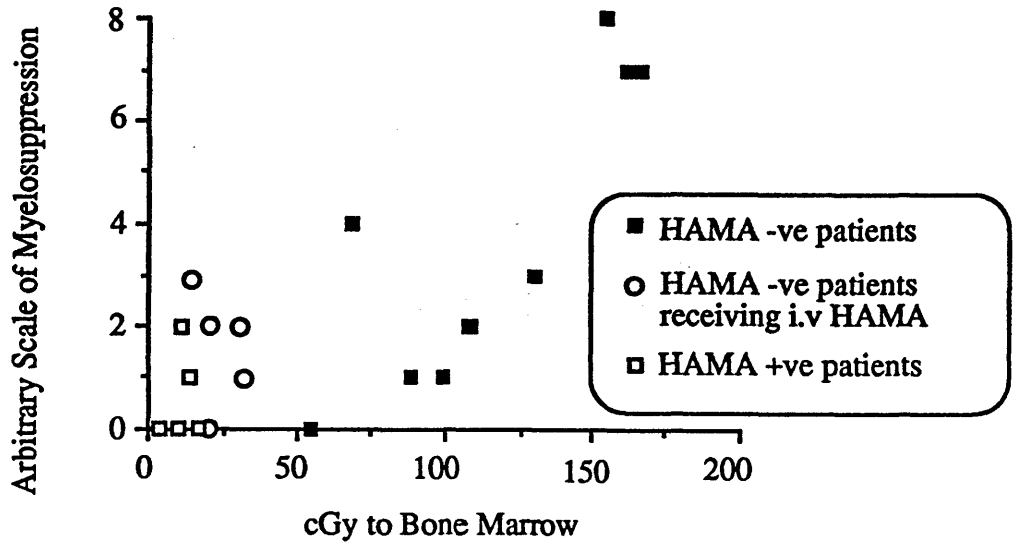


Table 4.2: Myelosuppression (WHO) scale in patients who received i.p. I-131 labelled MAb with i.v. HAMA. The radiation dose to bone marrow was calculated by applying the MIRD formulation to the integrated blood I-131 activity, assuming that the vascular compartment was the only radiation source.

Patient No	Radiation Dose to Bone Marrow in cGy	WHO MARROW TOXICITY		
		WBC	Platelets	Combined
19	31	0	2	2
20	32	1	0	1
21	20	0	0	0
22	16	3	0	3
23	21	2	0	2

Figure 4.12: Myelosuppression according to the radiation dose the bone marrow received from I-131 in the vascular compartment in patients receiving intraperitoneally administered I-131 labelled MAb. Patients have been divided into three groups; HAMA negative, HAMA positive and HAMA negative patients receiving i.v. HAMA. Myelosuppression is scored in an arbitrary scale by combining the WHO scale for thrombocytopenia and neutropenia



DISCUSSION

We were able to demonstrate that intravenously administered HAMA produced a four-fold reduction in the circulating ^{131}I activity, due to the rapid clearance of HAMA/MAb immune complexes from the blood by the liver and other parts of the reticuloendothelial system (Fig 4.5). The radiolabelled MAb was rapidly dehalogenated, the 'free' ^{131}I released into the circulation (Figs 4.2 to 4.4) and excreted in the urine (Fig 4.7). As the ^{131}I activity in the blood was decreased to a quarter of its predicted value, there was a four-fold reduction in the radiation dose the bone marrow received from the ^{131}I in the marrow circulation (from 0.86 cGy per mCi to 0.21 cGy per mCi of ^{131}I-labelled MAb). Patients who were HAMA positive (had endogenous HAMA from previous exposure to MAb) had even lower ^{131}I activities in their blood following RIT: In these patients the mean radiation dose to marrow was only 0.098 cGy per mCi of adminstered ^{131}I-labelled MAb.

One of the concerns about using anti-antibodies to clear radiolabelled MAb from the blood in RIT is that one may increase the radiation dose to the organs containing the reticuloendothelial system. The radiation dose to the liver is of paramount importance, as hepatic radiation tolerance is known to be quite low [Schray et al., 1989]. Although the spleen is even more radiosensitive it is less important as a functioning spleen is not essential for life. The liver receives a radiation dose from three major sources: ^{131}I in the hepatic circulation, ^{131}I in the hepatic RES, and ^{131}I from the rest of the body (gamma radiation). The radiation dose to the liver from the hepatic circulation has been

estimated by applying the MIRD formulation to the integrated ^{131}I activity in the blood and this is the radiation dose quoted in Fig 4.10. The radiation dose from the second source (the hepatic RES) may be very important in patients who either receive i.v. HAMA or have endogenous HAMA as the RES appears to clear 80% of the whole blood ^{131}I activity. To measure the radiation dose the liver receives from this second source one would need to measure the additional ^{131}I activity in the liver, either by serial liver biopsies or by gamma camera scintigraphy. Unfortunately the former is not practical and we do not have a gamma camera that can image more than five millicuries of ^{131}I. I was able however, by using a heavily shielded scintillation counter, to monitor the increased uptake and clearance of ^{131}I by the hepatic RES following the i.v. injection of HAMA. As the increased ^{131}I activity in the liver following the i.v. injection of HAMA was transient (Fig 4.5), the additional radiation dose from this second source was likely to be small. The hepatic dehalogenation of MAb in our patients appears to be similar to that observed by Begent [1982 & 1987] and Shareky and coworkers in 1984. The radiation dose the liver received from ^{131}I from the rest of the body (gamma irradiation) would be reduced by the administration of i.v. HAMA as the total body ^{131}I burden is reduced due to the increased ^{131}I excretion in the urine.

Although there was a four-fold decrease in the radiation dose the bone marrow received from ^{131}I in the vascular compartment , myelo-suppression still occurred. The myelosuppression in these patients has been compared to that observed in HAMA negative and HAMA positive patients (chapters 2 & 3) in **Figure 4.12.** In this figure myelosuppression along the y-axis (quantified by adding the WHO grade for neutropenia and thrombocytopenia) is plotted against the radiation

dose the marrow was calculated to have received from the ^{131}I in the marrow circulation. The degree of myelosuppression appears to follow a predictable curve in HAMA negative patients: one could almost draw a sigmoid curve through the points, with no marrow suppression with radiation doses below 50 cGy and life threatening myelosuppression when the bone marrow received a radiation dose in excess of 200 cGy. The pattern in patients who were either HAMA positive or who received i.v. HAMA is less clear. Although no serious myelosuppression was encountered, neutropenia and thrombocytopenia were observed after the marrow was calculated to have received only 20 to 40 cGy. It is possible that the RES in the bone marrow clears ^{131}I at a slower rate than the RES in the liver and retains ^{131}I-labelled proteins for longer periods. A similar finding was observed by Appelbaum et al. [1987] in administering ^{131}I-labelled MAb to dogs. This would increase the radiation dose the bone marrow would receive from the marrow RES above the level calcuclated from measuring only ^{131}I clearance from the circulation.

SUMMARY

- The administration of i.v. HAMA rapidly cleared ^{131}I-labelled MAb from the circulation.

- The liver appeared to be responsible for the clearance of immune complexes as increased ^{131}I activity was detected in the liver within 10 minutes of the i.v.injection of HAMA. However the increased ^{131}I activity in the liver lasted less than four hours.

- Free ^{131}I was released from the immune complexes cleared by the reticuloendothelial system, and rapidly excreted in the urine.

- There was a four-fold decrease in the radiation dose organs received from ^{131}I in the vascular compartment. Organs containing the reticuloendothelial system however may receive an additional radiation dose from the radioactive immune complexes.

CHAPTER 5

INTRAPERITONEAL RADIOIMMUNOTHERAPY WITH ^{90}YTTRIUM-LABELLED MAb.

INTRODUCTION

The first radiolabel to be used in radioimmunotherapy was ^{131}Iodine [Spar, 1965]. This isotope was chosen because it was readily available; there were well established techniques for protein iodination and its β-particles were moderately energetic (E_{max} 0.6 MeV). There was also extensive clinical experience in the administration, dosimetry and toxicity of large activities of ^{131}Iodine in patients with follicular thyroid carcinoma. It was primarily for these reasons that ^{131}Iodine was used in our first three studies. ^{131}Iodine however has several disadvantages, particularly when used for intracavitary RIT. Its beta particles have a maximum energy of 0.6 MeV with a maximum range in tissue of 2.4 mms and a mean range of only 0.5 mm. Intraperitoneally administered macromolecules have a limited penetration into tumour nodules [Dedrick et al., 1978; Myers & Collins, 1983], and radiolabelled MAbs are likely to target cells near the nodule surface [Sutherland et al., 1987]. If one ignores the systemic absorption of MAb from the peritoneal cavity, intraperitoneal tumour nodules will receive a radiation dose primarily from radiolabelled antibody bound to tumour cells on the nodule periphery. This means that ^{131}I-labelled MAbs could not sterilize any tumour nodule greater than a 5 mm in diameter. Another problem with ^{131}Iodine is the emission of γ-rays as well as β-particles.. Although gamma rays are useful for imaging purposes they do not usefully contribute to radiation dose of targeted tumour cells as they are too penetrating. Gamma rays increase the patient's systemic radiation dose, and that of the nursing and medical staff caring for the patient. It has also been suggested that many tissues including tumours contain dehalogenase enzymes, which are able to cleave the bound

^{131}Iodine from the antibody. Although this may decrease the radiation dose to many normal tissue such as the liver and spleen, the tumour radiation dose may also be decreased. This may explain why metalic radiolabels have a longer biological half life on tumours [Sakhara et al., 1987; Carrasquillo et al., 1987; Scheinberg & Strand, 1983; Khaw et al., 1986; Halpern et al., 1983]. The physical half life of ^{131}Iodine (193 hours) is probably too long for intraperitoneal RIT. An isotope with a shorter half life would allow a greater fraction of the isotope's total decay too occur in the peritoneal cavity before being absorbed into the rest of the body. Finally there are special problems in environmental radiation protection associated with iodine isotopes as iodine is excreted in urine, sweat, saliva and vomit. The environment can easily become contaminated and the hazard to medical staff compounded by the fact that free ^{131}Iodine is volatile and could be inhaled or ingested by the nursing/medical staff if precautions are not taken. Nursing patients receiving i.p. RIT with iodine isotopes can be difficult and labour intensive.

Recently several authors have suggested that ^{90}Yttrium may be a more suitable isotope [Humm, 1986; Hnatowhich et al., 1988; Wessels & Rogus, 1988; Leichner et al., 1988] for radioimmunotherapy, and several investigators have coupled ^{90}Yttrium to MAbs using antibody-DPTA conjugates (Hnatowich et al., 1985a; Klein et al., 1988; Sharkely et al., 1988). ^{90}Yttrium is a pure β-emmiter and has no penetrating gamma radiation. The maximum energy of these β-particles is 2.3 MeV with a maximum range in tissue of 11 millimetres. This would allow much larger tumour nodules to be irradiated, even if the radiolabelled antibody was concentrated on the nodule periphery. ^{90}Yttrium has a physical half life of only 64.2 hours which would allow

a greater fraction of its physical decay to occur in the peritoneal cavity. Tumour dehalogenases would have no effect on the biological half life of ^{90}Y-labelled MAb on tumour cells. ^{90}Yttrium unlike the iodine isotopes, is not excreted in sweat, saliva or vomit, which makes the radiation protection precautions are easier. There are however potential problems associated with the use of ^{90}Y-labelled MAb in RIT. ^{90}Yttrium is known to be a bone seeking isotope [Vaughan & Tull, 1953]; if free yttrium was absorbed by bone the radiation dose to the bone marrow would be increased. There is animal data that suggests that bone absorption of free yttrium may be a problem [Sharkely et al., 1988; Klein et al., 1988]. The reticuloendothelial system (liver and spleen in particular) also absorb ^{90}Yttrium and the radiation dose to these organs may be greater that the radiation dose they receive using ^{131}I-labelled MAb.

In March 1988 we started a phase I study to investigate the pharmaco-kinetics and dosimetry of ^{90}Y-labelled MAb infused into the peritoneal cavity in patients with persistent ovarian cancer following surgery and chemotherapy. The object of this study was to determine the maximum safe activity of ^{90}Y-labelled MAb that could be administered by the intraperitoneal route.

PATIENTS, METHODS AND MATERIALS

Patients

Sixteen patients with persistent ovarian cancer following surgery and chemotherapy received i.p. radioimmunotherapy with ^{90}Y-labelled HMFG1. The tumour histology from all patients demonstrated positive immunoperoxidase staining for HMFG1[1]. This study was approved by the Ethics Committee of the Royal Postgraduate Medical School, and informed consent was obtained from all the subjects. Patients were treated at either the Hammersmith Hospital or St. Mary's Hospital, Praed Street.

^{90}Yttrium-labelled MAb.

^{90}Yttrium was obtained from a ^{90}Strontium generator at Harwell, Berkshire, U.K., ^{90}Strontium contamination accounted for less than 1 part per 10^5 to 10^7. HMFG1-DPTA conjugates were made by either Ms. D. Snook or Mr. B. Dhokia at the ICRF laboratory , Royal Postgraduate Medical School & Hammersmith Hospital, DuCane Road. The immunoreactivity of each batch of HMFG1/DPTA conjugate was tested by a radioimmunoassay and an ELISA assay using ^{111}In-labelled HMFG1/DPTA. The purity and fraction of free ^{90}Yttrium in each sample was analysed by fast protein liquid chromatography and a Sephadex G-50 column respectively. At least 90% of ^{90}Yttrium in each sample was bound to monomeric HMFG1, and less than 3% of

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1 Immunoperoxidase staining was performed by Ms Angela Cross, ICRF & Dept. Clinical Oncology, Hammersmith Hospital.

^{90}Yttrium was not protein bound. The immunoreactivity of the radiolabelled antibody was always in the region of 60% or above compared to that of unlabelled antibody. **Table 5.1** lists the activity of ^{90}Y-HMFG1 and the quantity of HMFG1 administered to each patient.

Table 5.1: The yttrium-90 activity and the quantity of HMFG1 in Y-90 labelled antibody preparations used for i.p. radioimmunotherapy.

Patient No.	Yttrium-90 Activity in mCi	mgs HMFG1
24. Dun	5	30.0
25. Col	16.35	16.8
26. Tho	12.48	20
27. Pet	17	20
28. Edw	15	20
29. Ber	15.04	20
30. May	15.1	20
31. Jen	16.9	20
32. Lyn	20	18.14
33. Ale	6.8	28
34. Bur	15.0	20
35. Els	17.6	25
36. Gad	17.7	24
37. Pan	16.8	20
38. Isa	18.2	18
39. Sim	18.4	24

Intraperitoneal Administration of ^{90}Y-labelled MAb

Intraperitoneal catheters were inserted into the peritoneal cavity at open laparoscopy. This procedure allowed us to document the amount of residual disease and to insert intraperitoneal TLD catheters in seven patients (see below). Following recovery from the anaesthetic, the intraperitoneal catheter was connected to an i.v. blood giving set and 1.5 litres of 0.9% saline infused into the peritoneal cavity over 20-30 minutes. The ^{90}Y-labelled MAb was contained in a syringe surrounded by a 2 cm thick perspex syringe shield. This provided almost total shielding and allowed the ^{90}Y-labelled HMFG1 to be slowly injected into the infusing fluid. ^{90}Y-labelled MAb was more evenly distributed in the peritoneal cavity than ^{131}I-labelled MAb as the latter required a non-touch technique that relied on serial dilution of the ^{131}Iodine preparation. The peritoneal catheter was removed immediately after the infusion had ceased and the puncture site secured by two "purse-string" sutures. The patient's position was changed every 20 minutes for the following two hours to encourage an even intraperitoneal distribution of antibody. Patients were nursed in a single room for at least 24 hours after the infusion of ^{90}Y-labelled antibody.

Pharmacokinetics of ^{90}Y-labelled MAb

Patients were venesected at 12 hourly intervals for 110 hours after the i.p. infusion of ^{90}Y-labelled MAb and the blood ^{90}Yttrium activity measured as described in Appendix F. Briefly, five millilitres of blood were stored in pre-weighed glass vials containing EDTA and stored at 4 ^{o}C, and the ^{90}Yttrium activity measured in a scintillation well counter. ^{90}Yttrium activities in blood were corrected for radioactive decay when describing the pharmacokinetics of radiolabelled antibody but not for

calculating the radiation dose to organs by the MIRD formulation. The fraction of ^{90}Yttrium in the blood that was not protein bound (free ^{90}Y) was assessed by dialysing serum taken 24 hours after the i.p. infusion against phosphate buffered saline (appendix D). A curve of whole blood ^{90}Yttrium activity for 100 hours after the i.p. infusion was constructed in each patient. From each curve, the ^{90}Yttrium activities at ten hourly intervals hours were calculated and the results pooled to express mean ^{90}Yttrium activities for these times.

Urine was collected for the first five days after the i.p. infusion of ^{90}Y-labelled MAb. The ^{90}Yttrium activity of representative aliquots taken from the urine each day were measured in a scintillation well counter as described in appendix G. Urine activity was expressed as the cumulative percentage of the injected ^{90}Yttrium activity, corrected for radioactive decay.

Radiation Dose to Bone Marrow

^{90}Yttrium emits non-penetrating radiation with a maximum range in soft tissue of 11 millimetres. The bone marrow can receive a radiation dose from four possible sources, the blood within the marrow (the vascular compartment), the bone marrow itself (including the RES), the bone, and distant organs (bremsstrahlung irradiation). Although bone absorption of radio-isotope was negligible with ^{131}I-labelled MAb, studies in animals and man have indicated that that bone may be an important source organ when ^{90}Yttrium is used as a radiolabel. Estimating the ^{90}Yttrium content in bone would require multiple bone biopsies which was not feasible in our patients. As I was unable to estimate the ^{90}Yttrium activity in bone, I decided to ignored the bone as

a source organ, and assumed that the vascular compartment was the only important source organ. This has been calculated by applying the MIRD formulation to 20% of the integrated blood ^{90}Yttrium activity for the first 100 hours after the i.p. infusion of ^{90}Y-labelled MAb. The radiation dose to the bone marrow was calculated by the formula,

$$D = (0.2 \times A_{bl} \times S_{[mar \rightarrow mar]}) + (A_{bl} \times S_{[w.b \rightarrow mar]}) . \quad (1)$$

A_{bl} is the integrated ^{90}Yttrium activity in blood (μCi. hrs) for 100 hours after the i.p. infusion of ^{90}Y-labelled MAb. I have assumed that the marrow contains 20% of the ^{90}Yttrium activity found in the whole blood volume (appendix H). $S_{[mar \rightarrow mar]}$ is the S factor for marrow as a source organ and a target organ, and $S_{[w.b \rightarrow mar]}$ is the S factor for the whole body as a source organ and the marrow as a target organ. For ^{90}Yttrium, $S_{[mar \rightarrow mar]}$ is 0.00086 and $S_{[w.b \rightarrow mar]}$ would be close to zero. In fact the formula (2) would represent a more accurate estimation of the radiation dose to bone marrow in these patients.

$$D = (0.2 \times A_{bl} \times S_{[mar \rightarrow mar]}) + (A_b \times S_{[b \rightarrow b.m]}) + (A_{mar} \times S_{[mar \rightarrow mar]}) \quad (2)$$

where A_b and A_{mar} are the integrated Y-90 activities in bone and bone marrow for 100 hours after the i.p. infusion of antibody and $S_{[b \rightarrow b.m]}$ is the S factor for bone as a source organ (0.00011). Unfortunately there is no satisfactory method for measuring A_{mar} and A_b in patients. By studying the degree of myelosuppression associated with the radiation dose to bone marrow calculated by equation (1) one may be able to estimate the combined effect of A_{mar} and A_b.

Radiation Dose to the Peritoneal Serosa

In nine patients the radiation dose to peritoneal serosa from ^{90}Y-labelled antibody was measured by lithium fluoride (LiF) thermoluminescent dosimetry (TLD), using the same technique described in Chapter 2 and Appendix C. Hollow heat-sealed plastic tubes containing 31 to 45 lithium fluoride rods (each 6 x 1 millimetre) were inserted into the peritoneal cavity during the pre-treatment laparoscopy. The rods were calibrated by exposing them to known doses of radiation delivered by radiotherapy treatment machines delivering XRs and electrons with energies between 50 KeV and 4 MeV. As we observed the thermoluminescent response to be dependant on X-ray energy we finally calibrated the LiF rods with an ^{90}Yttrium solution of known activity. The rods were immersed in the ^{90}Y solution for different time intervals, and the radiation dose to the rods calculated by applying the MIRD formulation. I have also used lithium fluoride TLD to measured the radiation dose to the peritoneal serosa in two patients who received intraperitoneal Y-90 silicate colloid for ovarian cancer. As radiocolloids are not absorbed from the peritoneal cavity, the radiation dose in these patients represents the radiation dose the peritoneal serosa would receive if the ^{90}Y-MAb was not absorbed.

Toxicity

Patients were seen at weekly intervals after treatment to assess toxicity. During each visit, a full clinical examination was undertaken and blood venesected for analysis of plasma electrolytes, liver function, and blood counts. Leuopenia and thrombocytopenia were expressed as a

percentage fall in leucocyte and platelet count[1]. Myelosuppression was also classified according to the WHO classification [Miller et al., 1981]. In the this classification leucopenia and thrombocytopenia are classified as follows,

WHO Grade	Leucocyte Nadir	Platelet Nadir
Grade 0	$> 4 \times 10^9$/L	$> 100 \times 10^{12}$/L
Grade 1	$3 - 3.9 \times 10^9$/L	$75 - 99 \times 10^{12}$/L
Grade 2	$2 - 2.9 \times 10^9$/L	$50 - 74 \times 10^{12}$/L
Grade 3	$1 - 1.9 \times 10^9$/L	$25 - 49 \times 10^{12}$/L
Grade 4	$< 1 \times 10^9$/L	$< 25 \times 10^{12}$/L

For each patient these scores were combined to produce an arbitrary scale of myelosuppression ranging from 0 where no leucopenia or thrombocytopenia was found, to 8 where life threatening leucopenia and thrombocytopenia occurred.

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1 The counts on the 8th post-treatment day were chosen as the denominator as the pre-treatment blood counts were taken any day in the two weeks prior to RIT.

RESULTS

Pharmacokinetics

I was able to obtain blood pharmacokinetic data from 14 of the 16 patients who received intraperitoneal RIT with ^{90}Y-labelled MAb. The mean blood ^{90}Yttrium activities following treatment are given in **Table 5.2** and illustrated in **Figure 5.1.** The mean peak blood ^{90}Yttrium activity occurred 40 hours after the i.p. infusion with 22.2% (range 14.2% - 26.4%) of the injected ^{90}Yttrium activity in the circulation. The serum from seven of these patients, taken 24 hours after the i.p. infusion, was dialysed against PBS to determine the fraction of ^{90}Yttrium that was free[1]. Seven to sixteen percent (mean 12%) of the ^{90}Yttrium activity was able to be dialysed from serum and is therefore not attached to protein (free ^{90}Y), these results are illustrated in **Figure 5.2.** As the dialysate (PBS) does not contain protein, the post-dialysis serum may be diluted due to the oncotic pressure exerted by the serum protein during dialysis. I have attempted to estimate the dilution effect by weighing serum filled dialysis tubing before and after dialysis. There was about a 5 to 10 % increase in the weight of the dialysed serum which is probably due to this dilution effect. I have also used affinity chromatography of serum before and after dialysis to estimate amount of dilution. By putting serum down an affinity column containing species specific rabbit anti-mouse immunoglobulin antibody one can extract the ^{90}Y-labelled MAb from the serum (appendix J).

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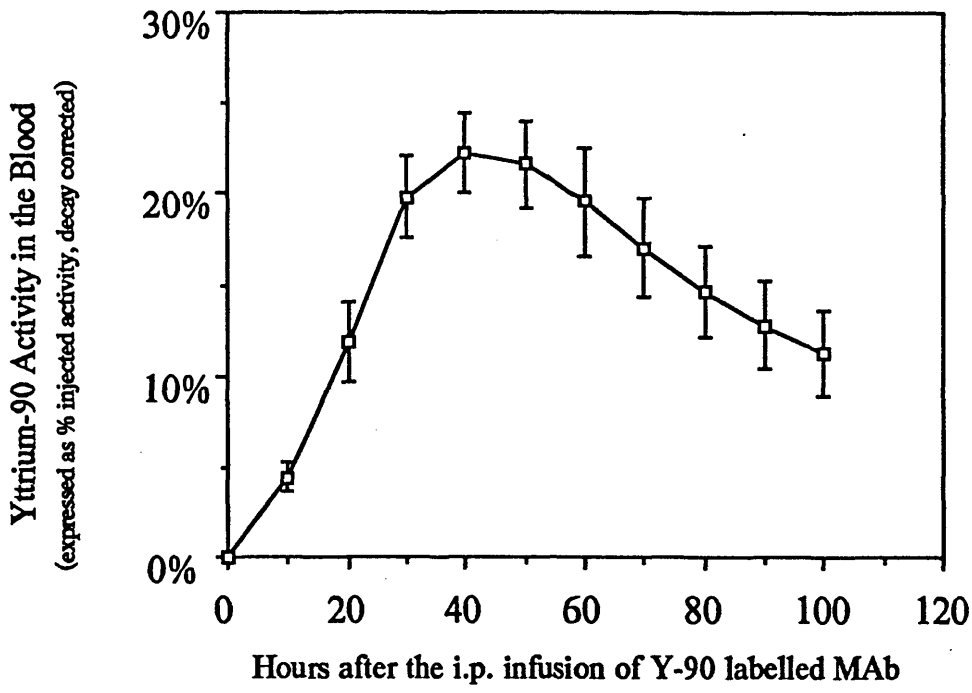
1 not protein bound (or on a molecule of MW of less than 14, 000 daltons).

This is illustrated in **Figure 5.3,** where 200 µl of serum taken 14 hours after the i.p. infusion of Y-90 labelled HMFG1 was passed

Table 5.2: Blood Y-90 activity following the i.p. infusion of Y-90 labelled MAB.

	% injected Yttrium-90 activity in the blood			
Hours after the i.p. infusion.	Mean (N=14)	Standard Error	Standard Deviation	Range
10	4.4	0.42	1.6	1.6 - 7.4
20	11.8	1.1	4.0	6.0 - 20.5
30	19.8	1.2	4.4	12.4 - 25.4
40	22.2	1.1	4.1	14.2 - 26.4
50	21.6	1.2	4.5	14.1 - 26.4
60	19.6	1.4	5.3	12.0 - 28.6
70	17.0	1.4	5.1	10.0 - 27.0
80	14.6	1.3	4.7	8.0 - 23.0
90	12.8	1.2	4.5	6.0 - 20.2
100	11.3	1.2	4.3	5.4 - 18.4

Figure 5.1: Mean blood yttrium-90 activity following the i.p. infusion of Y-90 labelled MAb. The error bars represent +/- two standard errors of the mean value.



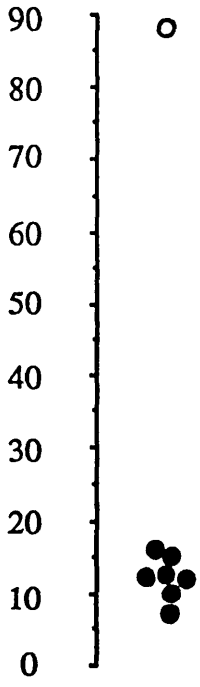
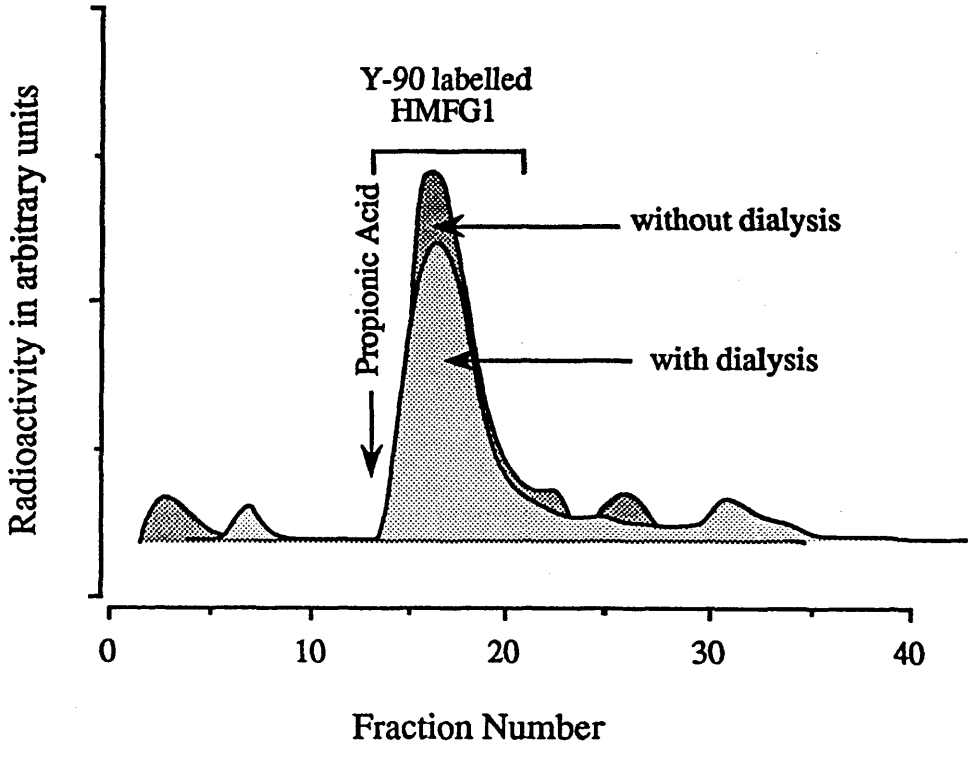


Figure 5.2: A scattergram of the % of yttrium in patients serum that was able to be dialysed (i.e. yttrium that was not bound to protein). The symbol ● denotes patients who received i.p. Y-90 labelled MAb, and the symbol o a single patient who received Yttrium Silicate Colloid.

Figure 5.3: Serum from Patient 37; venesected 14 hrs after the i.p. infusion of Y-90 labelled MAb, has been passed through an affinity column containing rabbit antimouse immunoglobulin. The two curves are for dialyses and non dialysed samples: yttrium-90 activity has been corrected for radioactivity decay during the dialysis period.



through an affinity column containing rabbit antimouse-immunoglobulin antibody. The same volume of serum was then put down the column after being dialysed against PBS for 24 hours and the counts corrected for the radioactive decay that occurred over that time. As the ^{90}Y-labelled MAb cannot pass through the dialysis membrane the 12% difference between the two AUC's in figure 5.3 is due to dilution. It would appear that the majority of ^{90}Yttrium in the serum of patients receiving RIT is bound to protein. This is in contrast with the serum ^{90}Yttrium activity in a single patient who received ^{90}Y-silicate colloid where 88% was free (Figure 5.3). The clearance of ^{90}Y-MAb from the circulation after the peak ^{90}Yttrium activity obeyed mono-exponential clearance kinetics. This is shown in **Figures 5.4.1 & 5.4.2** where the log of the ^{90}Yttrium activities from 14 patients are plotted against time. The mean biological half life of ^{90}Y-MAb in the circulation was 52 hours (range 30 to 84 hours).

Urine was collected for five days from 13 of the 16 patients. The renal excretion of Y-90 are shown in **table 5.3** and **Figure 5.5**: By five days a mean of 11.5% of the injected ^{90}Yttrium activity had been excreted (range 6 - 19%).

Radiation Dose to the Peritoneal Serosa

The radiation dose to the peritoneal serosa was measured by lithium fluoride TLD in seven patients. I was also able to measure the radiation dose to peritoneal serosa in two patients who received i.p. Y-90 silicate colloid. As radiocolloids are not absorbed from the peritoneal cavity the radiation dose to the serosa is much higher than the serosal radiation dose in patients who receive ^{90}Y-MAb. If the ^{90}Y-MAb was not absorbed from the peritoneal cavity, as may be the case in patients with ascites, then the

Figure 5.4.1: The Y-90 activity in blood in individual patients after the i.p. infusion of Y-90 labelled MAb. The Y-90 activity is expressed as % of injected activity (log scale on y-axis), and the time after injection in hours on the x-axis. The clearance half life is given for each patient.

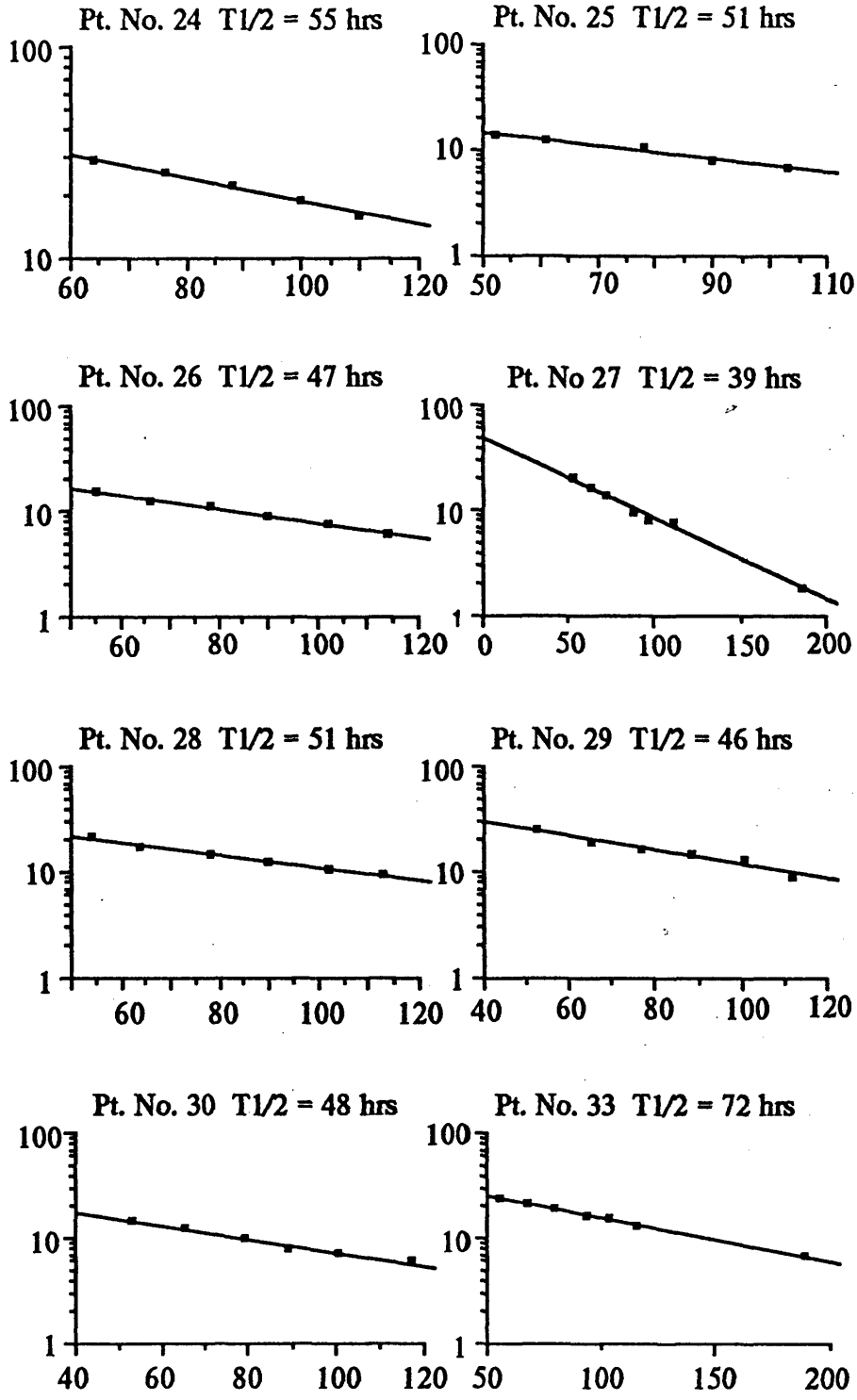


Figure 5.4.2: The Y-90 activity in blood in individual patients after the i.p. infusion of Y-90 labelled MAb. The Y-90 activity is expressed as % of injected activity (log scale on y-axis), and the time after injection in hours on the x-axis. The clearance half life is given for each patient.

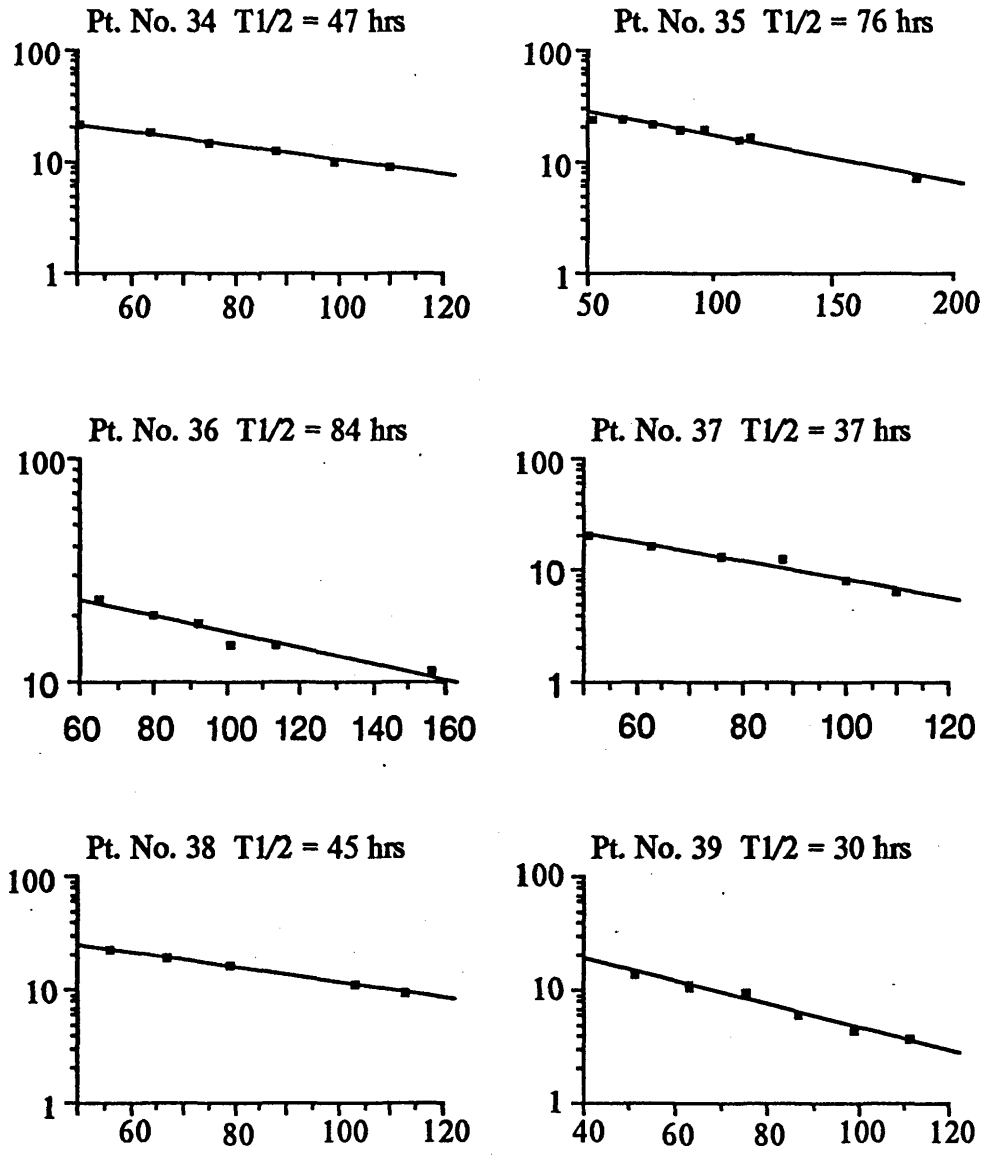
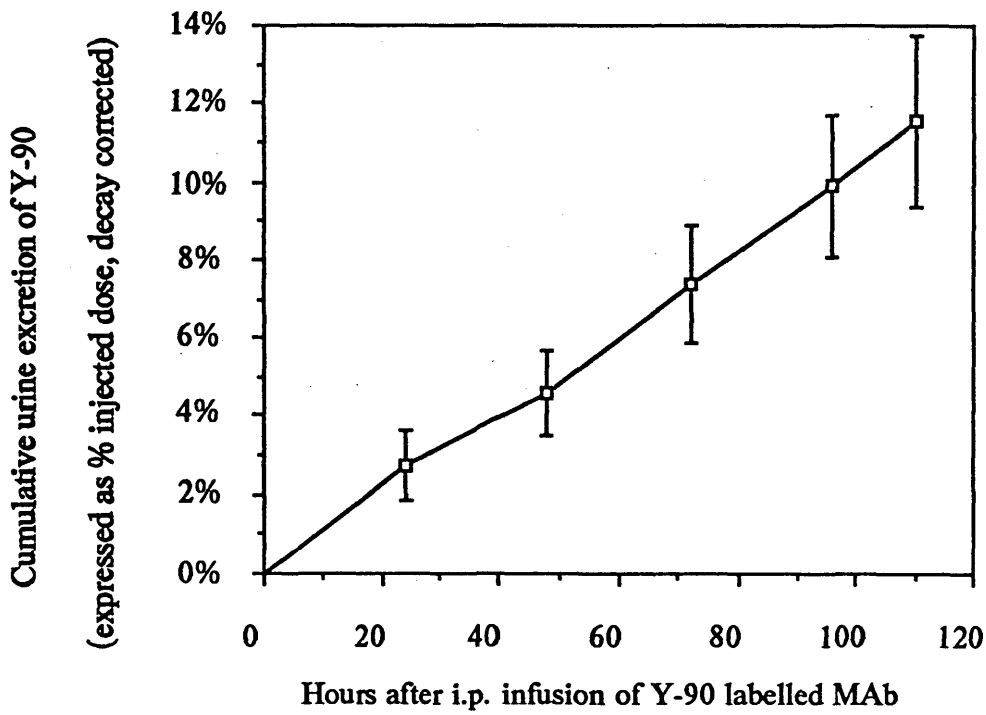


Table 5.3: Cumulative excretion of Y-90 in the urine following the i.p. infusion of Y-90 labelled MAb.

Hours after i.p. infusion of MAb	Cumlative % injected Y-90 activity in urine			
	Mean (N = 13)	Standard Error	Standard Deviation	Range
24	2.7	0.4	1.5	0.5 - 5.8
48	4.6	0.6	2.1	1.9 - 9.0
72	7.4	0.8	2.7	3.7 - 12.5
96	9.9	1.0	2.6	4.7 - 16.7
110	11.5	1.1	4.1	6.01 - 19.4

Figure 5.5: Mean cumulative excretion of Y-90 in the urine in 13 patients who received i.p. Y-90 labelled MAb.



radiation dose to the peritoneal serosa would be similar. One can compare the radiation dose the peritoneal serosa receives from these two modalities by comparing the radiation dose per millicurie of administered ^{90}Yttrium. This is shown in the Box plot in **Figure 5.6** where the distribution of 379 TLD readings from seven patients who received i.p. Y-90 labelled MAb are compared with that of 69 TLD measurements taken form two patients who received ^{90}Y-radiocolloid. The mean radiation dose of 15.7 cGy/mCi ^{90}Y in patients receiving RIT is significantly lower that the mean radiation dose of 96.6 cGy in the two patients receiving ^{90}Y-radiocolloid. There appeared to be a wider variation in the radiation dose the peritoneal serosa received from ^{90}Y-radiocolloid with radiation doses of 200 to 450 cGy per mCi ^{90}Y occasionally recorded.

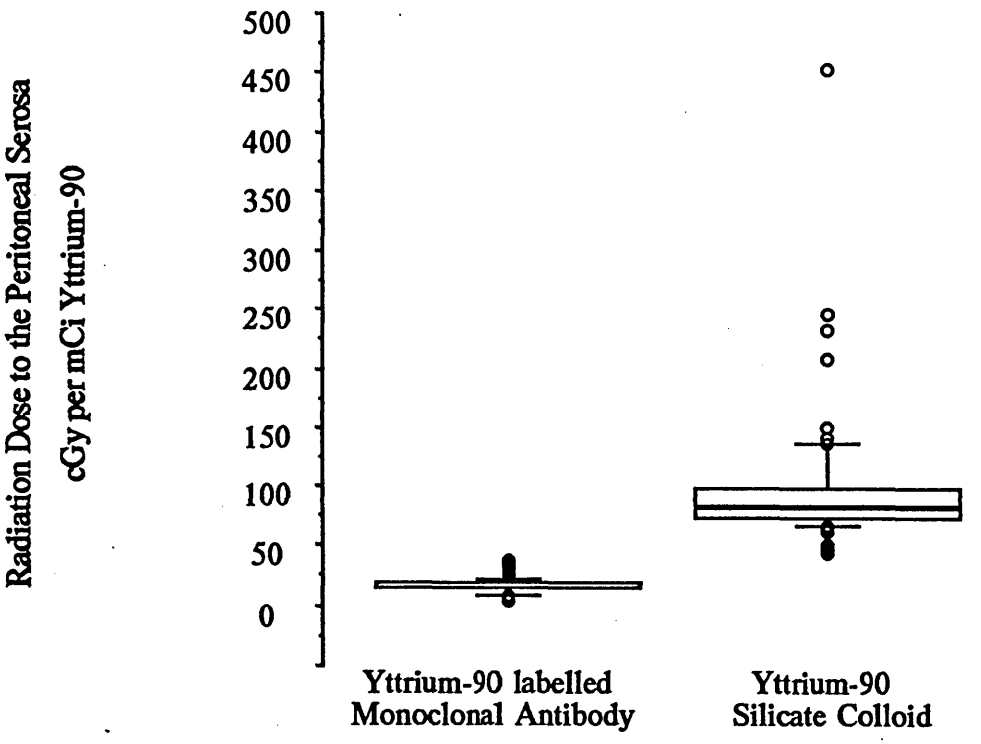
Radiation Dose to Bone Marrow

The Area Under the Curves (AUC's) for whole blood ^{90}Yttrium activity against time in individual patients are given in **Table 5.4.** As the clearance of ^{90}Y-MAb from the circulation is monoexponential I have used the effective half life of the ^{90}Y-MAb to predict the AUC for the second 100 hours after treatment. The radiation doses the bone marrow was estimated to have received from ^{90}Y-MAb in the marrow's vascular compartment (MIRD formulation) are also shown in Table 5.4. The radiation dose the bone marrow received over 100 hours from ^{90}Y-MAb in the circulation was estimated to range from 10.3 cGy following 5 mCi (0.185 MBq) ^{90}Y-MAb to 34.4 cGy following 17.7 mCi's (0.655 MBq) ^{90}Y-MAb. The radiation dose in the second 100 hours after the i.p. infusion only accounted for a mean of 15.4% of the total radiation dose over the 200 hour period (range 7.1% - 23.7%).

Table 5.4: The integrated Y-90 activity in the blood for 100 & 200 hours after intraperitoneal RIT with Y-90 labelled MAb. The radiation dose to the bone marrow from Y-90 in the vascular compartment is also given.

PATIENTS	YTTRIUM-90 ACTIVITY IN mCi	INTEGRATED Y-90 ACTIVITY IN mCi.hrs		RADIATION DOSE TO MARROW (cGy)	
		100 hrs	200 hrs	100 hrs	200 hrs
24	5	48	63	10.3	13.5
25	16.4	95	115	20.4	24.7
26	12.5	96	105	20.6	22.6
27	15	126	144	27.1	31.0
28	15	126	146	27	31.4
29	15	140	165	30	35.5
30	15.1	81	95	17.4	20.4
31	16.9				
32	20				
33	6	64	78	13.8	16.8
34	15	141	165	30.3	35.5
35	17.6	160	203	34.4	43.6
36	17.7	160	200	34.4	43.0
37	16.8	121	137	26.0	29.5
38	18.2	159	194	34.1	41.7
39	18.4	121	130	26.0	28.0

Fig 5.6: Box Plot of the radiation dose to the peritoneal serosa (cGy / mCi Y-90) in patients receiving i.p. Y-90 labelled antibody and Y-90 silicate colloid. The thick line represents the median value, the other lines the 10%, 25%, 75% & 90% percentiles. Individual data points are represented beyond these values.



Mean radiation dose was 15.7 cGy per mCi of Y-90 labelled MAb. There were 379 TLD readings from 7 patients.

Mean radiation dose was 96.6 cGy per mCi Y-90 silicate colloid. There were 69 TLD readings from 2 patients.

Toxicity

Myelosuppression was the most important toxicity, and was observed three to six weeks after the ^{90}Y-MAb infusion. **Figure 5.7** illustrates the thrombocytopenia observed in patients with increasing administered ^{90}Yttrium activity. Significant myelosuppression was observed in patients who received more than 12 mCi (0.44 MBq) of ^{90}Y-MAb, with severe thrombocytopenia in patients receiving 17-18 mCi (0.63 MBq). A similar pattern was observed with post-treatment neutropenia (data not shown). **Figure 5.8** illustrates the relationship between myelotoxicity, expressed in an arbitrary scale 0 to 8 (see methods), and administered ^{90}Yttrium activity.

There did not appear to be any toxicity other than myelosuppression. All patients reported mild abdominal discomfort following the laparoscopy and antibody infusion. Referred pain in the shoulder was also common. These symptoms were mild and were controlled by simple analgesics. Pyrexia lasting less than 48 hours was also occasionally observed following treatment, but was self limiting in all cases. There was no evidence of renal or liver impairment from blood taken for analysis of serum electrolytes, serum transaminases, bilirubin or serum alkaline phosphatase. All patients developed an immune response the murine MAb with increasing titres of human anti-mouse antibody (HAMA) as previously described (Courtenay Luck et al., 1986; 1988).

Figure 5.7: Myelosuppression according to the radiation dose the bone marrow received from Y-90 labelled MAb. Myelosuppression was expressed on an arbitrary scale 0 - 8, according to the sum of the WHO grades for leucopenia and thombocytopenia.

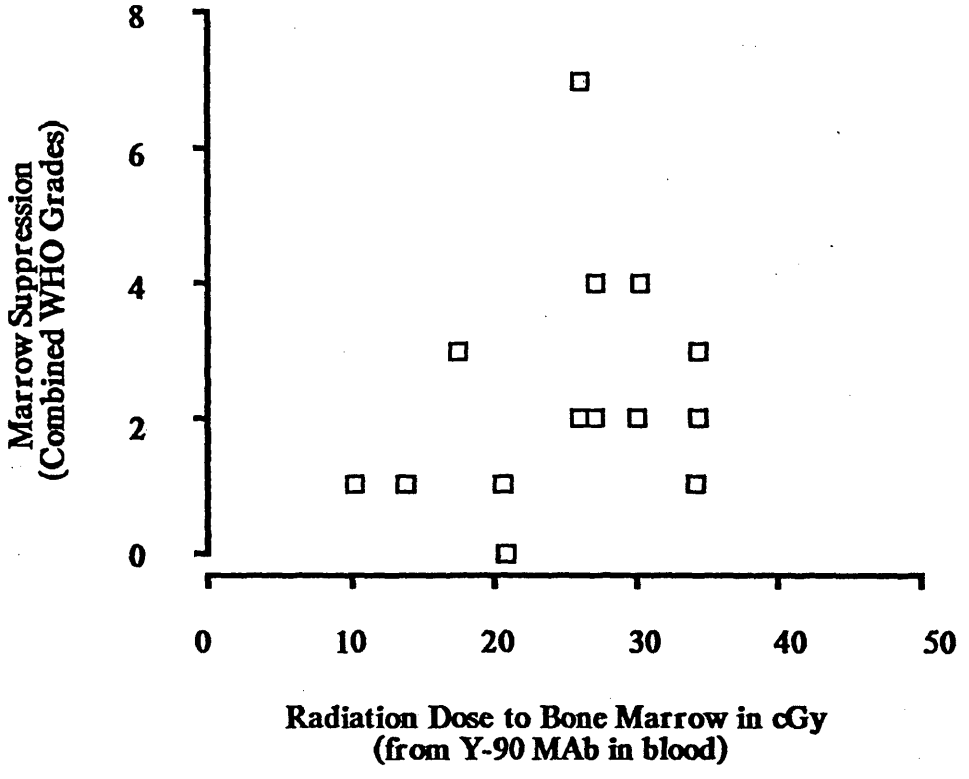
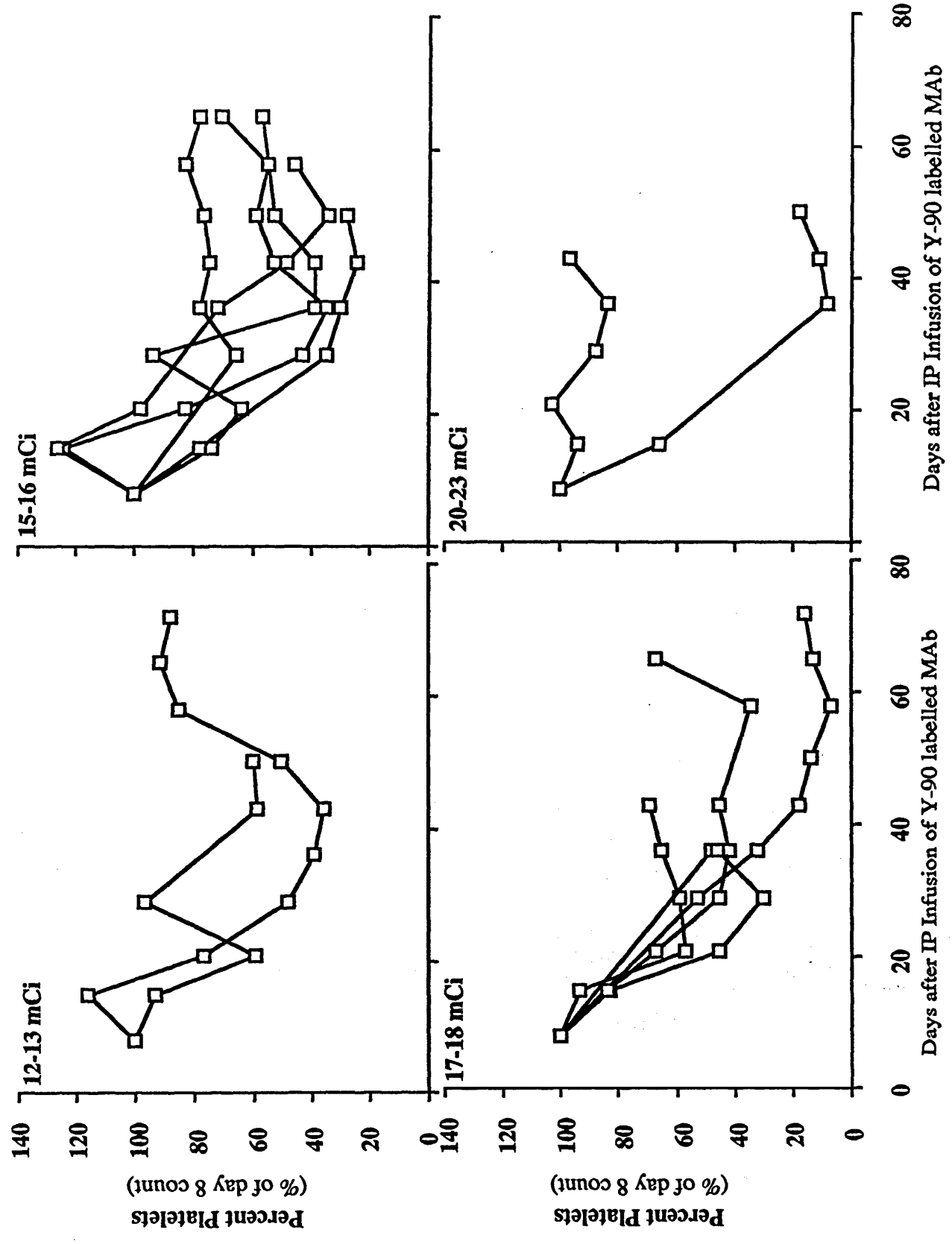


Figure 5.8: Platelet count following the i.p. RIT with yttrium-90 labelled MAb.



DISCUSSION

The pharmacokinetics of ^{90}Y-labelled MAb in the blood appeared to be similar to that of ^{131}I-labelled MAb. There appeared to be efficient absorption of ^{90}Y-labelled MAb from the peritoneal cavity into the circulation. The non-specific radiation dose to the peritoneal serosa was much lower than the radiation dose delivered by ^{90}Y-silicate radiocolloid where there was no absorption. Peak blood ^{90}Yttrium concentrations occurred 40 hours after the i.p. infusion, the clearance of MAb from the blood after this peak activity was mono-exponential. The AUC's of the % injected isotope versus time appeared to be significantly lower in the 14 patients receiving ^{90}Y-labelled MAb than in the 9 patients receiving ^{131}I-labelled MAb in Chapter 2 (Mann Whitney $U = 20.5$, $p < 0.005, > 0.001$). The shorter half life of ^{90}Yttrium does not account for this difference as the blood isotope activity was corrected for radioactive decay. The most significant difference between the pharmacokinetics of ^{90}Yttrium and ^{131}I-labelled MAb's was in the urinary excretion of isotope. Only a mean of 11.5 % of the ^{90}Yttrium was excreted in the urine over a five day period (range 6 - 19%) compared to a mean of 70% (range 65 - 78%) of ^{131}Iodine. The lower renal excretion of ^{90}Yttrium in patients is a disadvantage as more untargeted ^{90}Yttrium decays in the body with possible radiation toxicity to normal organs such as bone and liver. Yttrium chelated to DPTA is stable in the body and rapidly excreted in the urine (Winchell et al., 1964). As this did not occur in these patients, one may assume that ^{90}Yttrium is released free following antibody catabolism.

For the observed degree of myelosuppression, the radiation dose to bone marrow from the ^{90}Y-labelled MAb in the marrow compartment was low (10.3 to 34.4 cGy over 100 hours) compared to that delivered by ^{131}I-labelled antibody (55 - 167 cGy). Although the estimated radiation dose to marrow from the marrow compartment was able to predict the degree of myelosuppression in patients who received ^{131}I-labelled MAb (Figure 2.6), this correlation was not observed in the patients who received ^{90}Y-labelled MAb (Figure 5.8). A possible explanation for the discrepancy between the estimated radiation dose and the degree of myelosuppression in patients receiving ^{90}Y-labelled MAb is that the marrow receives an additional radiation dose from another source organ. Two additional radiation source organs are the reticulo-endothelial system (RES) and bone. The RES clears immune complexes from the circulation and may have been responsible for the excess myelosuppression in Chapter 4. The hepatic and splenic RES are known to concentrate ^{90}Yttrium released from ^{90}Y-labelled MAb [Vaughan et al., 1987], and the RES in the bone marrow may have a similar role. Yttrium is a bone seeking isotope and ^{90}Yttrium concentration in bone has been observed in both animal [Klein et al., 1988 & Sharkely et al., 1988] and human [Hnatowich et al., 1989] RIT studies using ^{90}Y-labelled MAb.

The vascular compartment of the marrow appears to contribute only a fraction of the radiation dose to the marrow: the bone and perhaps the RES are probably more important radiation sources. This means that methods of increasing the clearance of ^{90}Y-labelled MAb from the circulation, with the liberation of unchelated ^{90}Yttrium, would increase rather than decrease the radiation dose to bone marrow. Giving ^{90}Y-labelled MAb to HAMA positive patients, or using HAMA to clear ^{90}Y-

labelled MAb from the circulation could increase the toxicity considerably. One could only decrease the radiation dose to bone marrow if ^{90}Yttrium was released from the MAb still complexed to its chelate. If ^{90}Yttrium remained chelated, it would be immediately excreted in the urine rather like ^{131}Iodine: under these circumstances the toxicity from ^{90}Y-labelled MAb would be greatly reduced and totally predictable.

SUMMARY

Although the pharmacokinetics of ^{90}Y-labelled MAb in the blood were similar to those of ^{131}I-labelled MAb, ^{90}Yttrium was not excreted to the same extent as ^{131}Iodine in the urine.

The radiation dose to the bone marrow from ^{90}Y-labelled MAb in the marrow compartment did not account for the degree of observed myelosuppression.

The absorption of free ^{90}Yttrium by bone could account for the discrepancy between the radiation dose to bone marrow from ^{90}Yttrium in the marrow compartment and the degree of observed myelo-suppression.

The non-specific radiation dose to the peritoneal serosa from ^{90}Y-labelled MAb is much lower than that delivered by the same activity of ^{90}Y-silicate radiocolloid. The absorption of ^{90}Y-labelled MAb from the peritoneal cavity accounts for this difference.

Increasing the clearance of ^{90}Y-labelled MAb from the blood will not decrease the radiation dose to the bone marrow unless the ^{90}Yttrium is released from the antibody in a chelated and rapidly excreted form.

CHAPTER 6

THE BIODISTRIBUTION OF INTRAPERITONEALLY ADMINISTERED ^{90}Y-LABELLED MAb IN RABBITS

INTRODUCTION

When ^{131}I-labelled antibodies are used in RIT, any ^{131}Iodine not targeted to the tumour or taken up by the thyroid gland is largely confined to the circulation or excreted in the urine [Rowlinson et al., 1987; Ward & Wallace, 1987]. Even if ^{131}I-labelled antibody is taken up by the liver, the antibody is rapidly dehalogenated and the ^{131}Iodine returned to the circulation and excreted in the urine (Chapters 3 & 4). One can estimate the radiation dose to an organ by measuring the integrated ^{131}Iodine activity in the organ's circulation. I was able to do this in patients receiving i.p. ^{131}I-labelled antibodies: the estimated radiation doses to bone marrow produced the expected degree of myelosuppression.

The situation is however quite different when ^{90}Y-labelled antibodies are used. The biodistribution data in animals and man indicate that ^{90}Yttrium is not excreted in the urine as ^{131}Iodine would be [Sharkely et al., 1988 & Hnatowich et al., 1988]. These and other studies have also shown the ^{90}Yttrium from ^{90}Y-labelled MAb can accumulate in tissues such as the liver, spleen, and bone [Vaughan et al., 1987; Klein et al., 1988]. This means that organs receive a radiation dose from additional sources to the vascular compartment of the marrow, the additional radiation source organs would be bone and possibly the reticulendothelial system in the bone marrow itself.

Our clinical experience with intraperitoneally administered ^{90}Y-labelled antibody would appear to support this concept. Patients developed marked myelosuppression after their bone marrow received a relatively

low radiation dose from the ^{90}Yttrium in the vascular compartment (Chapter 5). The bone appeared to be a more important radiation source organ than the blood in these patients. To investigate this further, I undertook a biodistribution study of intraperitoneally administered murine MAb in rabbits. Rabbits were chosen rather than mice (used in previous biodistribution studies) as it is easier to obtain adequate samples of bone marrow and bone in these larger animals.

METHODS AND MATERIALS

Half lop-eared rabbits weighing between 2 and 2.5 Kg were used in this experiment. The rabbits were given a normal diet and free supply of water. Nine rabbits were immunized with 1.5 gram of HMFG1 two to three weeks prior to the i.p. injection of ^{90}Y-labelled MAb. These rabbits received a second subcutaneous "booster" immunization five to ten days prior to the i.p. injection of ^{90}Y-labelled MAb. All nine immunized rabbits developed high serum titres of rabbit anti-murine immunoglobulin on an ELISA assay[1].

As before the monoclonal antibody HMFG1 was supplied by Unipath Ltd, UK and purified ^{90}Yttrium from a ^{90}Strontium generator, at Harwell, Berkshire, UK. ^{90}Yttrium was chelated to a DPTA-HMFG1 conjugate by Ms. Debbie Snook at the Imperial Cancer Research Fund laboratory in the Royal Postgraduate Medical School. The immunoreactivity of the DPTA-antibody counjugates were tested by an ELISA assay of ^{111}In-labelled conjugate. Thirty-five to 80 MBq (1 - 2 mCi) of ^{90}Y-labelled to 3.5 mgs of HMFG1 were used in this experiment. The relatively high ^{90}Yttrium activity was necessary to ensure that there were sufficient counts in tissue a week later.

^{90}Y-labelled HMFG1 was injected into the peritoneal cavity with 50 mls of 0.9% saline after the rabbits were anaesthetized by the i.v. injection

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1 The immunization and ELISA assay were carried out by Greg Sivolapenko, Dept of Immunology, Royal Postgraduate Medical School, DuCane Road, London .

of 1.5 to 2 mls of Hypnorm. A small incision was made in the skin of the anterior abdominal wall to facilitate the insertion of a 14 gauge "Venflon" catheter (Vygo) into the peritoneal cavity. Free assess into the peritoneal cavity was first demonstrated by the injection of 10 ml of 0.9% saline: suction was then applied to make sure that faecal material was not aspirated (indicating that the catheter was in the bowel lumen). The ^{90}Y-labelled antibody was diluted in 50 mls of 0.9% saline and injected through the side port of the Venflon catheter: the 50 ml volume was chosen as this was equivalent to two litres of infusate in man. The catheter was then removed and a suture tied around the puncture site to prevent any leakage of ^{90}Y-labelled antibody.

Following the i.p. injection of ^{90}Y-labelled HMFG1, the rabbits were kept in radiation controlled area. The faeces, urine and carcasses were stored for one month to allow the ^{90}Yttrium to decay to 1/1500th of its original injected activity (52 KBq) before being incinerated. The radiation activity of faeces were checked to make sure that there was not excessive activity over the first three days. This would indicate that there had either been leakage of the ^{90}Y-labelled antibody from the rabbit, or that the ^{90}Yttrium had been excreted in the faeces due to the injection of radiolabelled HMFG1 into the bowel lumen (this occurred in one rabbit).

Three rabbits from the non-immunized and immunized groups were killed two, five and seven days after the i.p. injection of ^{90}Y-labelled antibody. Rabbits were first anaesthetized by an intravenous drug cocktail containing one ml of Hypnorm and two ml of pentobarbitone and then killed by the exsanguination of 70 - 110 mls of blood (this decreased the blood pool in organs). After death the rabbits were dissected and the organs listed in Table 6.1 removed. Representative

samples (0.5 - 1 gram) of the organs were blotted dry and put into pre-weighed scintillation tubes. Samples of liver were taken both from the liver edge (near the peritoneal cavity) and from the centre of the liver. The Y-90 activity from these samples were analysed separately in each rabbit, but as the same ^{90}Yttrium activity was found in both samples the mean ^{90}Yttrium activity of the two samples was used. The two most important organs that were investigated in this study were the bone and bone marrow. Two bones were chosen, the femur to represent tubular bone and the rib to represent flat bone. The mid femur was crushed so that a bone marrow sample could be removed and put into a pre-weighed scintillation tube. Small fragments of the midfemur were put into pre-weighed scintillation tubes after being washed, dried, and crushed further into smaller fragments. Bone marrow was extracted from three ribs by the injection of ten millilitres of air into the marrow cavity through a 21 gauge needle. Each marrow sample normally weighing about 30 - 40 μg was placed in a separate scintillation tube and analysed separately. After the marrow had been extracted, the marrow cavity in each rib was washed out by the irrigation of 20 mls of water injected through a 21 gauge needle. The rib was then crushed into small fragments which were dried and put into pre-weighed scintillation vials for separate analysis of the ^{90}Yttrium activity. In each rabbit the ^{90}Yttrium activity in the paired femur samples, the paired femoral marrow samples, the triple rib and rib marrow samples were almost identical. The mean ^{90}Yttrium activities for these organs were used for each rabbit.

The weight of each tissue sample was measured by reweighing the scintillation tubes. Each sample was then dissolved by a 48 hour incubation at 37 ^{o}C in two millilitres of Soluene. After 48 hours all the

samples (except very small translucent chips of bone) had been dissolved. The ^{90}Yttrium activity in each sample was compared with that of "standard ^{90}Yttrium activities" obtained by the serial dilution of aliquots taken the injectate for each rabbit. The ^{90}Yttrium activity in each samples was expressed as the percentage of the injected ^{90}Yttrium activity per gram of tissue.

RESULTS

Non-immunised Rabbits

The biodistribution of ^{90}Yttrium in non-immunized rabbits are shown in **Table 6.1**: the values being the mean taken from three rabbits killed two, five and seven days after the i.p. administration of ^{90}Y-labelled antibody. ^{90}Yttrium accumulated in several organs in the week after the i.p. injection of ^{90}Y-labelled antibody. At two days most organs contained less ^{90}Yttrium than the blood; the exceptions being the ribs, liver and renal cortex, with tissue to blood ratios of 1.1, 1.69 and 1.48 respectively. Three days later the ^{90}Yttrium activity in the blood had fallen to 16% of the day 2 activity while the ^{90}Yttrium activity in the bone, marrow, liver, and spleen had either remained constant or increased. The tissue to blood ratios for these organs five days after the i.p. injection were: liver 9.7, spleen 6.2, rib 7.6, rib marrow 6.2, renal cortex 4.1, femur 4.0, and femur marrow 3.5. These changes were even more evident seven days after the i.p. injection when the blood activity in the blood had fallen to 6% its previous value and the ^{90}Yttrium activity in the bone marrow, bone, liver, spleen and renal medulla had increased still further. Particularly high ^{90}Yttrium activities were found in the liver, renal medulla, bone marrow and bones. The tissue to blood ratios for the following organs were; liver 31.9, renal medulla 27.6, rib 26.0, rib marrow 20.0, femur marrow 11.9 and femur 11.4

Table 6.1: The mean tissue Y-90 content from three rabbits killed 2, 5, & 7 days after the i.p. injection of Y-90 labelled MAb. These rabbits had not been exposed to murine immunoglobulin and had no rabbit antimurine antibody.

ORGANS	% INJECTED Y-90 ACTIVITY I0 -3 / GRAM OF TISSUE (MEAN OF THREE RABBITS)			TISSUE TO BLOOD RATIO (MEAN OF THREE RABBITS)		
	Day 2	Day 5	Day 7	Day 2	Day 5	Day 7
Blood	103	17	6	1	1	1
Marrow (femur)	70	60	74	0.68	3.53	11.94
Marrow (rib)	81	106	124	0.79	6.24	20.00
Bone (femur)	65	68	71	0.63	4.0	11.45
Bone (rib)	113	129	161	1.10	7.59	25.97
Liver	174	164	198	1.69	9.65	31.94
Spleen	71	105	55	0.69	6.18	8.87
Renal Cortex	153	70	34	1.48	4.12	5.48
Renal Medulla	52	29	171	0.51	1.71	27.58
Heart	34	16	15	0.33	0.94	2.42
Lung	40	20	18	0.39	1.18	2.90
Stomach	27	24	17	0.26	1.41	2.74
Colon	38	26	25	0.37	1.53	4.03
Diaphragm	58	46	18	0.56	2.71	2.90
Peritoneum	28	115	39	0.27	6.76	6.29
Omentum	16	31	23	0.16	1.82	3.71
Bile	18	2	5	0.17	0.11	0.81
Muscle	1	2	4	0	0.11	0.64

Immunised Rabbits

The biodistribution data in rabbits previously immunized with HMFG1 are shown in Table 6.2: these values represent the means taken from three rabbits killed two, and seven days after the i.p. administration of ^{90}Y-labelled antibody. Only the results from two of the three rabbits killed five days after the injection were included, as the injection in one of these rabbits was into the bowel lumen rather than into the peritoneal cavity (this rabbit had negligible ^{90}Yttrium activity in its tissue and an extremely high ^{90}Yttrium activity in its faeces).

The major difference in the biodistribution of ^{90}Yttrium between immunized and non-immunized rabbits was the decreased blood activity in immunized rabbits. At two, five and seven days after the i.p. injection, the ^{90}Yttrium activity in the immunized rabbits was 54%, 3.5% and 8.3% the activity of non-immunized rabbits. The ^{90}Yttrium activity in most organs however was essentially either unchanged (liver, spleen and renal cortex) or only slightly decreased (bone and marrow). This meant that the ratio of ^{90}Yttrium activity between these organs and the blood was increased considerably in immunized rabbits. Seven days after the i.p. injection of ^{90}Y-labelled HMFG1 the following tissue to blood ratios of Y-90 were found: Rib 329:1, Liver 239:1, Femur 203:1, Renal Cortex 165: 1, Spleen 165:1, Diaphragm 127:1, Marrow rib 119:1, Peritoneum 110:1 and the omentum 69:1.

Table 6.2: The mean tissue Y-90 content from three rabbits killed 2, 5, & 7 days after the i.p. injection of Y-90 labelled MAb. These rabbits had been previously immunised with murine antibody.

ORGANS	% INJECTED Y-90 ACTIVITY 10 - 3 / GRAM OF TISSUE (MEAN OF THREE RABBITS)			TISSUE TO BLOOD RATIO (MEAN OF THREE RABBITS)		
	Day 2	Day 5	Day 7	Day 2	Day 5	Day 7
Blood	56	0.6	0.5	1	1	1
Marrow (femur)	53	13	39	1	22.4	76.5
Marrow (rib)	66	18	61	1.2	31.0	119.6
Bone (femur)	59	30	105	1.1	51.7	202.9
Bone (rib)	83	49	168	1.5	84.5	329.4
Liver	217	115	122	3.9	198.3	239.2
Spleen	83	74	84	1.5	127.6	164.7
Renal Cortex	117	44	84	2.1	75.8	164.7
Renal Medulla	66	10	33	1.2	17.2	64.7
Heart	29	6	12	0.5	10.3	23.5
Lung	30	5	13	0.5	8.6	25.4
Stomach	35	7	14	0.6	12.1	27.4
Colon	70	32	24	1.3	55.2	47.1
Diaphragm	58	24	65	1.0	41.4	127.4
Peritoneum	132	26	56	2.4	227.6	109.8
Omentum	93	8	35	1.7	160.3	68.6
Bile	3.5	7	17	0	6.0	33.3
Muscle	1.7	0	3	0	0	5.9

DISCUSSION

This biodistribution study in rabbits of intraperitoneally administered ^{90}Y-labelled MAb indicates that ^{90}Yttrium accumulates in normal tissue rather than being excreted in the urine like ^{131}Iodine. The ratio of ^{90}Yttrium between normal tissue and blood is therefore greater than unity in most organs. **Figure 6.1** illustrates the accumulation of ^{90}Yttrium in the normal tissue of non-immunized and immunized rabbits; the tissue to blood ratios of ^{90}Yttrium in these organs are illustrated in **figure 6.2.**

The ^{90}Yttrium activity in the blood was not representative of the ^{90}Yttrium activity in the marrow, bone, kidney, liver or spleen: ^{90}Yttrium accumulated in all these organs. This means that the radiation dose to these organs is more dependant on the ^{90}Yttrium concentration in these organs than the ^{90}Yttrium concentration in the blood. Applying the MIRD formulation to a fraction of the integrated blood ^{90}Yttrium activity will seriously underestimate the radiation dose to these organs. This explains the discrepancy between the estimated radiation dose to bone marrow and the degree of myelosuppression observed in patients receiving ^{90}Y-labelled MAb in chapter 5. This study confirms that the bone marrow and the bone are both major radiation sources for bone marrow (both being more important than the blood). The most likely explanation for the high ^{90}Yttrium activity in the bone marrow is the uptake of ^{90}Y-labelled HMFG1 by the reticuloendothelial system. Although it is impossible to extrapolate the radiation dose to human marrow from the data in rabbits, the bone and marrow are likely to be

more important radiation source organs to marrow than blood by several orders of magnitude.

Rabbits with antibodies to HMFG1 had decreased blood ^{90}Yttrium activity. Presumably this is due to the clearance of HMFG1/anti-antibody complexes from the circulation by the reticuloendothelial system. As less than 20% of ^{90}Yttrium will be excreted in the urine, ^{90}Yttrium will accumulate in tissues such as the liver, spleen and bone marrow (all part of the reticuloendothelial system) and the bone (yttrium is a bone seeking isotope). The ratio of ^{90}Yttrium in these organs compared to blood varied from 150:1 to 300:1. If this occurred in HAMA positive patients then the radiation dose to bone marrow would not be decreased by the HAMA, in fact the radiation dose may even be increased.

A further interesting feature observed in this biodistribution study is the increased ^{90}Yttrium activity in the peritoneum, diaphragm, and omentum in immunized rabbits. This may indicate that anti-HMFG1 combines with ^{90}Y-labelled HMFG1 in the peritoneal cavity with the liberation of ^{90}Yttrium into the peritoneal cavity. If the liberated ^{90}Yttrium formed colloids in the peritoneal cavity rather than being absorbed (like ^{131}Iodine in chapter 3) then the radiation dose to the peritoneal cavity would be increased.

Figure 6.1: Biodistribution of Y-90 following the i.p. injection of -90 labelled MAb in normal rabbits (graphs A & C) and rabbits that have been immunised with murine antibody (graphs B & D). Each point is the mean of three values.

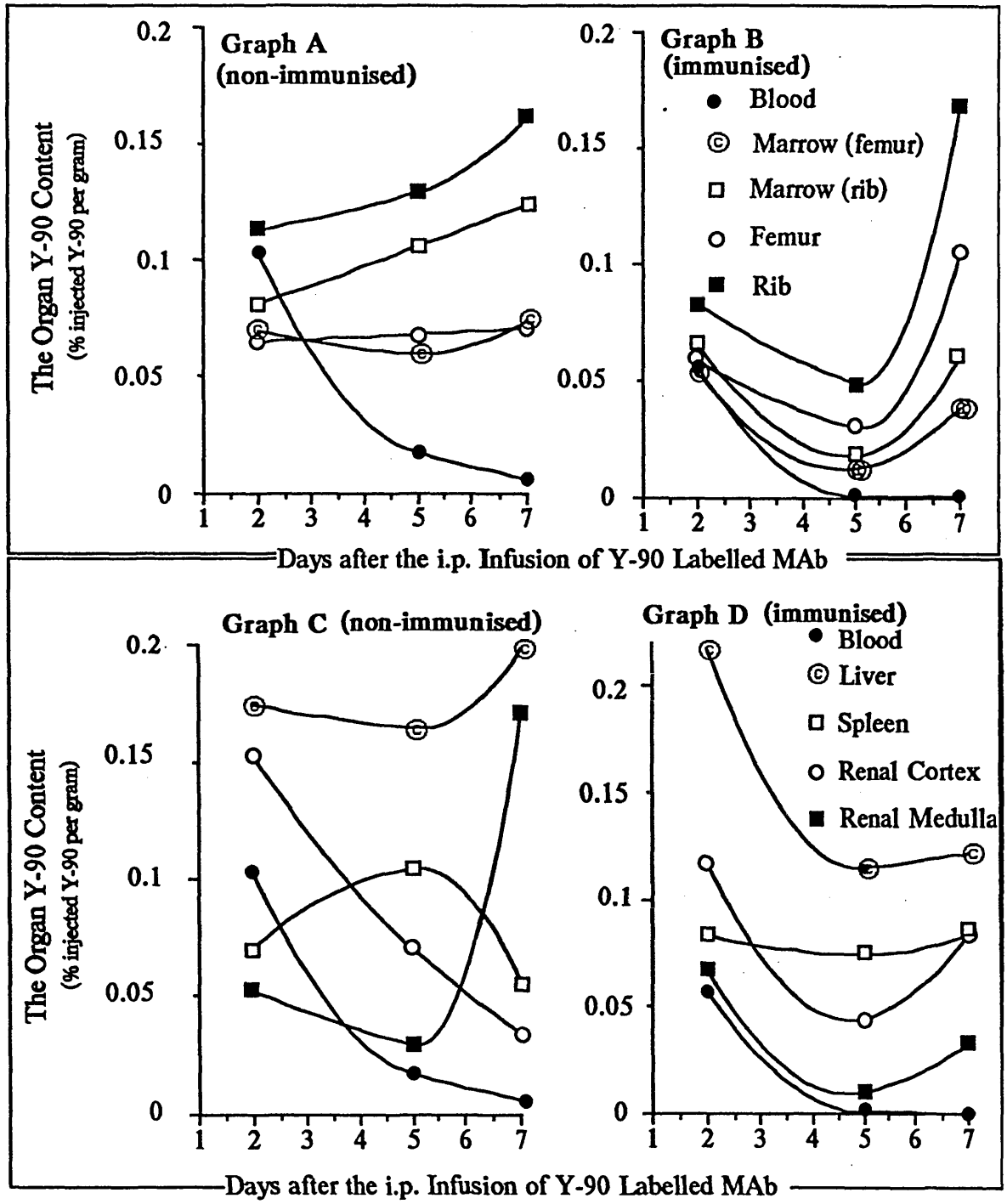
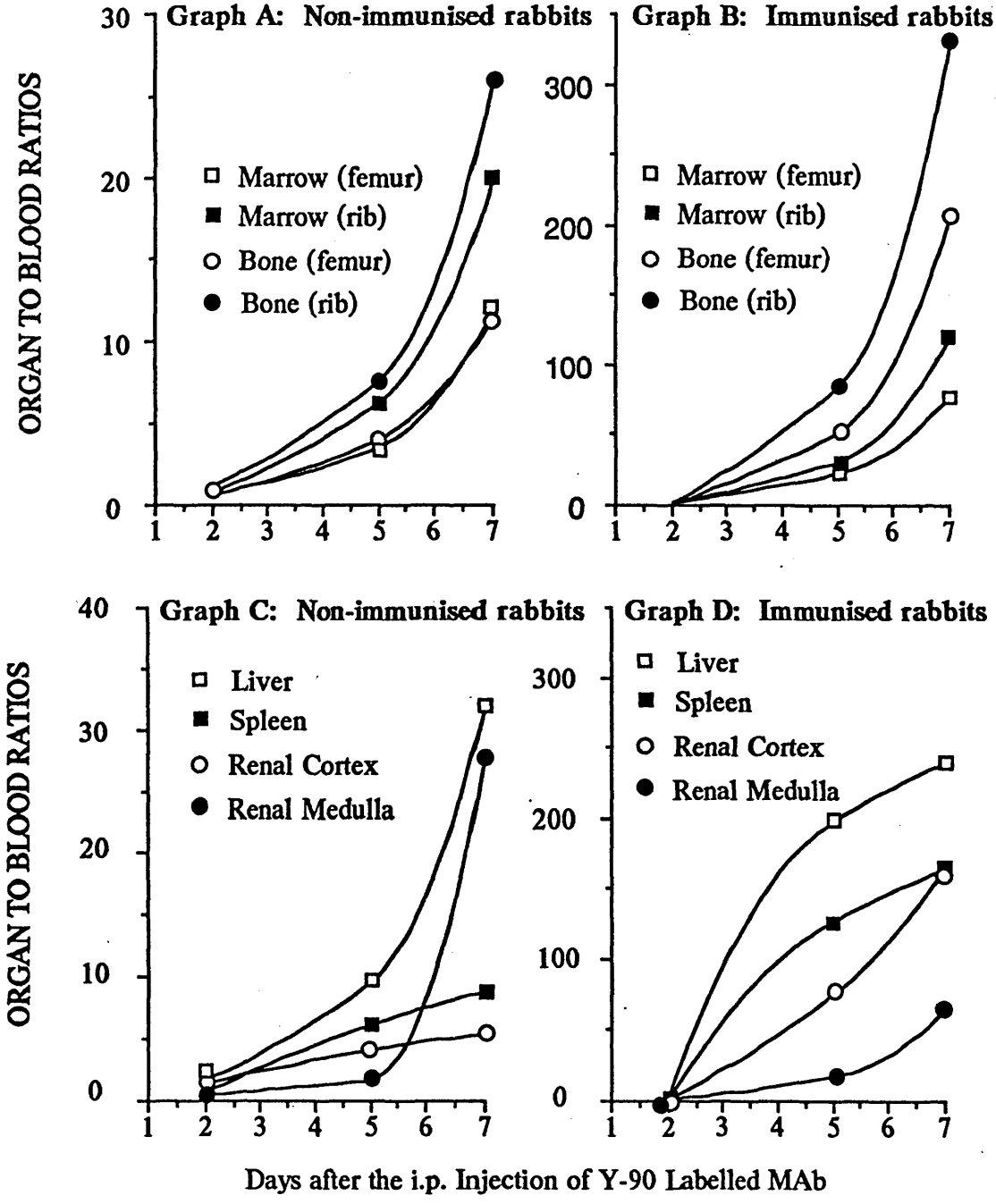


Figure 6.2: The organ to blood ratio of Y-90 activity in rabbits following the i.p. injection of Y-90 labelled MAb. Each point represents the mean from three rabbits. The rabbits in graph B & D had been previously immunised with murine immunoglobulin.



SUMMARY

From this chapter the following conclusions were drawn,

^{90}Yttrium from ^{90}Y-labelled MAb accumulates in normal tissue.

The bone marrow in rabbits receiving ^{90}Y-labelled antibody receives a radiation dose from ^{90}Yttrium in the blood, the marrow and the bone. The latter two organs are the most important radiation source organs.

Anti-antibodies increase the clearance of ^{90}Y-labelled antibody from the blood, but do not decrease the ^{90}Yttrium activity in important organs such as liver, bone, and bone marrow.

The presence of anti-antibodies will not decrease the radiation dose to bone marrow as ^{90}Yttrium concentration in bone and bone marrow will be unchanged or increased.

CHAPTER 7

INTRAVENOUS EDTA TO CHELATE ^{90}YTTRIUM RELEASED FROM ^{90}Y-LABELLED MAb DURING RIT.

INTRODUCTION

Although ^{90}Yttrium may have some advantages over ^{131}Iodine as a radiolabel in RIT, with β-particles of greater energy, a shorter physical half life, and no antibody dehalogenation, there is one major disadvantage. It would appear from the data presented in Chapters 5 and 6 that when ^{90}Yttrium is released from the MAb during antibody catabolism, the ^{90}Yttrium is not excreted in the urine, as ^{131}Iodine would be, but accumulates in various organs. This means that these organs receive a greater radiation dose than they might otherwise: the most important organ being the bone marrow as it had the lowest tolerance to radiation (the LD_{50}[1] in man may be as low as 5 Gy). From the animal data in chapter 6 and the patients data in chapter 5 it would appear that the bone marrow receives most of its radiation dose from ^{90}Yttrium either in the marrow's reticuloendothelial system or adjacent bone.

Chelated ^{90}Yttrium however is much less toxic than unchelated ^{90}Yttrium as it is excreted in the urine. In the early 1960's Whinchell and others did a series of studies using ^{90}Yttrium-DPTA chelates as a means of delivering total body irradiation to animals and man. They were able to inject "Curie" quantities of ^{90}Yttrium chelated to DPTA with entirely predictable radiation doses to body organs including the bone marrow. Chelated ^{90}Yttrium had an extremely short effective half live in man as renal excretion is so efficient; in fact these workers recycled excreted ^{90}Yttrium in urine back into patients by passing the

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1 LD50 is the dose of radiation that may be expected to be fatal in 50% of individuals.

urine through a filter. In these circumstances the radiation dose to organs is from the ^{90}Yttrium in the circulation and extracellular space alone: there was no accumulation in various organs such as the bone or reticuloendothelial system. Delivering total body irradiation by this method proved perfectly feasible and some patients achieved long standing remissions from their lymphoma.

If the ^{90}Yttrium released from the antibody during catabolism was in a chelated form then it would not accumulate in tissue but would be excreted by the kidneys like ^{111}In -DTPA [Fawwaz et al., 1985]. Chelates have been used to chelate metals in the treatment of metal poisoning for many years. A list of chelates and the medical conditions they are used in are given below.

Chelating Agents	Metals	Disease
Desferrioxamine	Fe,	Haemochromatosis Iron poisoning Thalessemia
EDTA[1] (Versene)	Pb, Y, Sr	Lead poisoning Y^{90}/Sr^{90} poisoning
Dimercaprol[2]	Hg, Au, Cd, As, An,	Heavy metal poisoning
Penicillamine[3]	Cu	Wilsons Disease
N-acetyl dl-penicillamine	Hg	Mercury Poisoning

1 Calcium disodium ethylenediaminetetraacetic acid

2 2,3 - dimercaptopropanol or British antilewisite

3 β,β - dimethylcysteine

Versene is commonly used to chelate lead in accidental poisoning but will also chelate other metals including yttrium, strontium, and americum. Radioactive isotopes of the latter three elements may be released into the environment in leaks from nuclear power stations. Vaughan and Tull described the use of versene (EDTA) to remove Y-90 from the skeleton of rabbits in 1953, and EDTA has been used to remove fission products from the skeleton in a man exposed to Americum in radiation leak [Breitenstein, 1983].

As patients receiving ^{90}Y-labelled MAb retain ^{90}Yttrium in their bodies, a study was started to investigate the possibility of using intravenous EDTA to chelate free ^{90}Yttrium and increase its renal excretion. An animal study was set up to see if the intravenous administration of EDTA would alter the ^{90}Yttrium concentration (from intraperitoneally administered ^{90}Y-labelled MAb) in intraperitoneal tumours. Nude mice bearing intraperitoneal tumours from a cell line called LoVo received an i.p. injection of ^{90}Y-labelled AUA1 (a murine MAb that binds to a cell surface antigen expressed by LoVo cells). The administration of intravenous EDTA did not significantly decrease the ^{90}Yttrium targeted to the intraperitoneal tumour cells but did decrease the ^{90}Yttrium concentration in bone[1]. A pilot study was undertaken to investigate the use of intravenous EDTA in patients receiving intraperitoneally administered ^{90}Y-labelled MAb for ovarian cancer. The object of this study was to examine the effect of EDTA on the pharmacokinetics and toxicity of the ^{90}Y-labelled patients using our previous experience of ^{90}Y-labelled MAb as a control.

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1 Data to be published (Rowlinson, G., Stewart, J.S.W., Hird, V., et al.)

PATIENTS, METHODS AND MATERIALS

Patients

Eight patients with persistent ovarian cancer following surgery and chemotherapy received i.p. radioimmunotherapy with ^{90}Y-labelled HMFG1. The tumour histology from all patients demonstrated positive immunoperoxidase staining for HMFG1[1] or AUA1. This study was approved by the Ethics Committee of the Royal Postgraduate Medical School, and informed consent was obtained from all the subjects. Patients were treated at either the Hammersmith Hospital or St. Mary's Hospital, Praed Street. Seven patients had minimal residual tumour (tumour deposits less than two centimetres); the remaining patient had gross residual tumour with ascites.

Intraperitoneal Administration of ^{90}Y-labelled MAb

These patients were treated in exactly the same way as the patients in Chapter 5, although no patient had a TLD catheter inserted. The details of the ^{90}Y-labelled antibody preparations are given in **Table 7.1.** ^{90}Y-labelled AUA1 was given in addition to HMFG1 in two patients (Pts 42 & 45). The EDTA infusion was administered as described below.

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1 Immunoperoxidase staining was performed by Ms Angela Cross, ICRF & Dept. Clinical Oncology, Hammersmith Hospital.

Table 7.1: Details of quantitiy of HMFG1 and Yttrium-90 activities in patients receiving intraperitoneal RIT with an intravenous EDTA infusion to chelate free Y-90.

Patient No.	Yttrium-90 Activity in mCi	mgs HMFG1
40. Hac	20.35	16
41. Hea	19.60	18
42. Car	22.0	18 + 17*
43. Pan	18.2	14.6
44. Cos	20.6	18.2
45. Sla	14.5	19 + 15.6*
46. Ans	20.6	23
47. Lum	25.5	23.1

* denotes additional AUA1

Intravenous Administration of EDTA

Sodium calcium edetate B.P. (Ledclair)[1] was given as a four infusion 14, 26, 38, 50, 62, and 74 hours after the i.p. administration of ^{90}Y-labelled MAb. The dose of Ledclair at each infusion was 30 mgs/Kg body weight in 500 mls of 0.9% saline. During the 78 hours of EDTA infusions the urine volume was maintained at a minimum of 2 litres a day by the administration of intravenous fluids.

Pharmacokinetics of ^{90}Y-labelled MAb

The pharmacokinetics of ^{90}Y-labelled MAb was studied as described in Chapter 5. Every 12 hours the ^{90}Yttrium activity in the blood was measured: the fraction of ^{90}Yttrium activity bound to protein was measured by passing a 50 ml aliquot of serum through a Sephadex G-50 column (Appendix E).

Radiation Dosimetry of Bone Marrow

The bone marrow only receives the a small proportion of its total radiation dose from the vascular compartment. I have decided to ignore the radiation dose to bone marrow from ^{90}Yttrium in the bone and marrow as I was unable to measure the ^{90}Yttrium activity in these organs. One can however use the radiation dose the marrow receives from the blood to make a simple comparison between the patients treated with and without EDTA. As in Chapter 5 the radiation dose to bone marrow would be **D** where,

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1 Sodium calcium ethylenediamine tetraacetic acid (Ledclair) is manufactured by Sinclair Pharmaceuticals Ltd., Godalming, Surrey, UK.

$$D = (0.2 \times A_{bl} \times S_{[mar \rightarrow mar]}) + (A_b \times S_{[b \rightarrow b.m]}) + (A_{mar} \times S_{[mar \rightarrow mar]}).$$

A_{bl} represents the integrated ^{90}Yttrium activity in the whole blood over 100 hours and 0.2 is estimated to be the fraction of A in the marrow circulation. The constant $S_{[mar \rightarrow mar]}$ is the S value for marrow as a source and target organ and $S_{[b \rightarrow b.m]}$ the S value for bone as a source organ and bone marrow as a target organ. The values A_b and A_{mar} refer to the integrated ^{90}Yttrium activity in the bone and bone marrow respectively. As these values are not known I have ignored the more important part of the equation $(A_b \times S_{[b \rightarrow b.m]}) + (A_{mar} \times S_{[mar \rightarrow mar]})$ and just measured the radiation dose to bone marrow from blood.

$$D = (0.2 \times A_{bl} \times S_{[mar \rightarrow mar]}).$$

Toxicity

These patients remained in a radiation controlled room for five days after the intraperitoneal RIT and were then seen weekly as outpatients. Each week patients underwent a full clinical examination, and blood was taken for routine blood counts, liver function tests and serum urea and electrolytes.

RESULTS

Pharmacokinetics

Patients who received EDTA had similar serum pharmacokinetics as patients in Chapter 5 who did not receive EDTA. The mean whole blood ^{90}Yttrium activities in the seven patients without ascites and the standard deviation and range of ^{90}Yttrium activities are given in **Table 7.2.** Peak ^{90}Yttrium activity in the serum occurred 50 hours after the i.p. infusion of ^{90}Y-labelled MAb, with a mean of 19.8% of the injected activity in the blood (range 12.9 - 31%). The mean blood activity in patients receiving EDTA compared to those patients who did not receive EDTA in **Figure 7.1.** Patients who received EDTA had slightly lower ^{90}Yttrium activity in their blood. There is a significant difference between the AUC's of the two groups in Figure 7.1: patients who received EDTA had significantly lower AUC's than patients who did not receive EDTA (Mann Whitney U = 20.5, p = 0.004). Patient No. 47 who had ascites demonstrated quite different pharmacokinetics in the blood as illustrated in **Figure 7.2.** Less than 1.1% of the injected ^{90}Yttrium activity was found in the circulation, with peak ^{90}Yttrium activity 100 hours after the i.p. infusion of ^{90}Y-labelled MAb.

As before the clearance of ^{90}Y-labelled antibody from the blood after peak levels was mono-exponential as illustrated for the seven patients without ascites in **Figure 7.3.** The mean biological half life of ^{90}Y-labelled MAb was 48 hours. This was not significantly different from the biological half life in patients who did not receive EDTA with a mean half life of 52 hours (Mann Whitney U = 37, p = 0.18).

Table 7.2: Blood Y-90 activity following the i.p. infusion of Y-90 labelled MAB.

Hours after the i.p. infusion.	% injected Yttrium-90 activity in the blood			
	Mean (N=7)	Standard Error	Standard Deviation	Range
10	3.7	0.5	1.3	1.8 - 6.0
20	9.1	1.2	3.2	4.2 - 13.8
30	15.9	1.9	4.9	10.1 - 24.0
40	19.5	1.9	5.1	13.9 - 29.0
50	19.8	2.1	5.5	12.9 - 31.0
60	17.8	2.2	5.8	11.5 - 29.1
70	15.4	1.9	5.1	10.0 - 25.2
80	13.2	1.8	4.8	8.0 - 22.0
90	12.6	2.0	5.4	6.0 - 19.9
100	9.5	1.5	3.9	5.4 - 16.6

Figure 7.1: Mean blood yttrium-90 activity following the i.p. infusion of Y-90 labelled MAB. The closed squares ■ represent the mean values from seven patients who received EDTA, and the open square □ the mean from 14 patients who did not recieve EDTA.

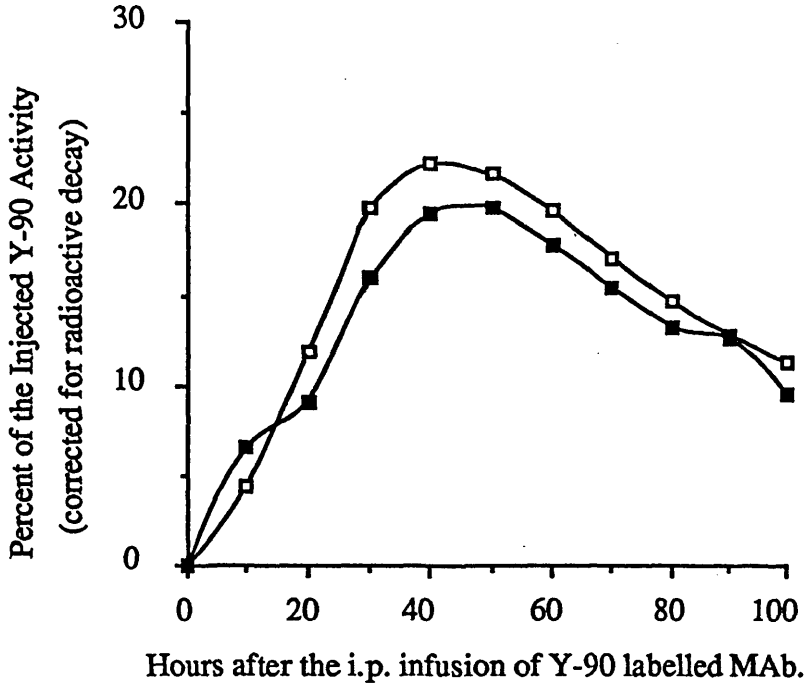


Figure 7.2: Blood Y-90 activity in a patient with ascities who received i.p. Y-90 labelled MAb & i.v. EDTA infusions.

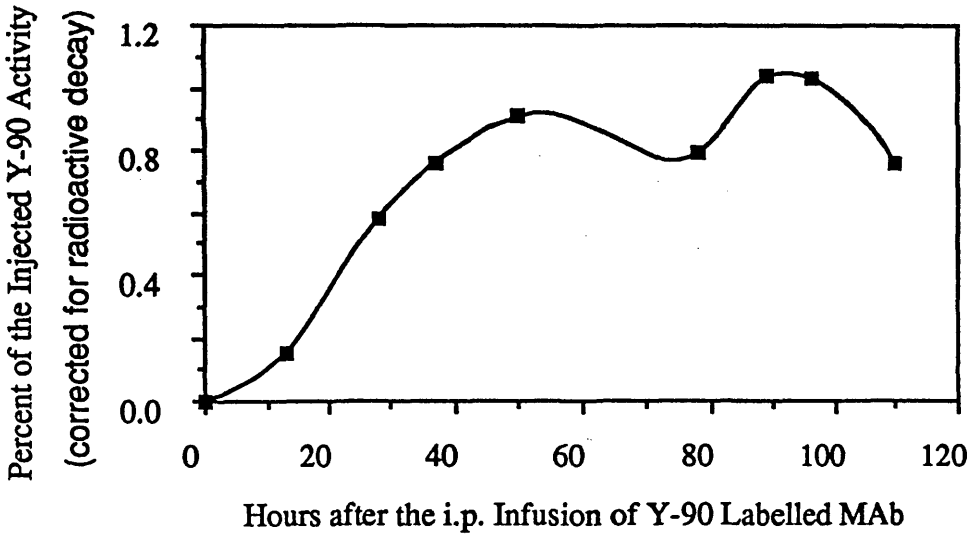
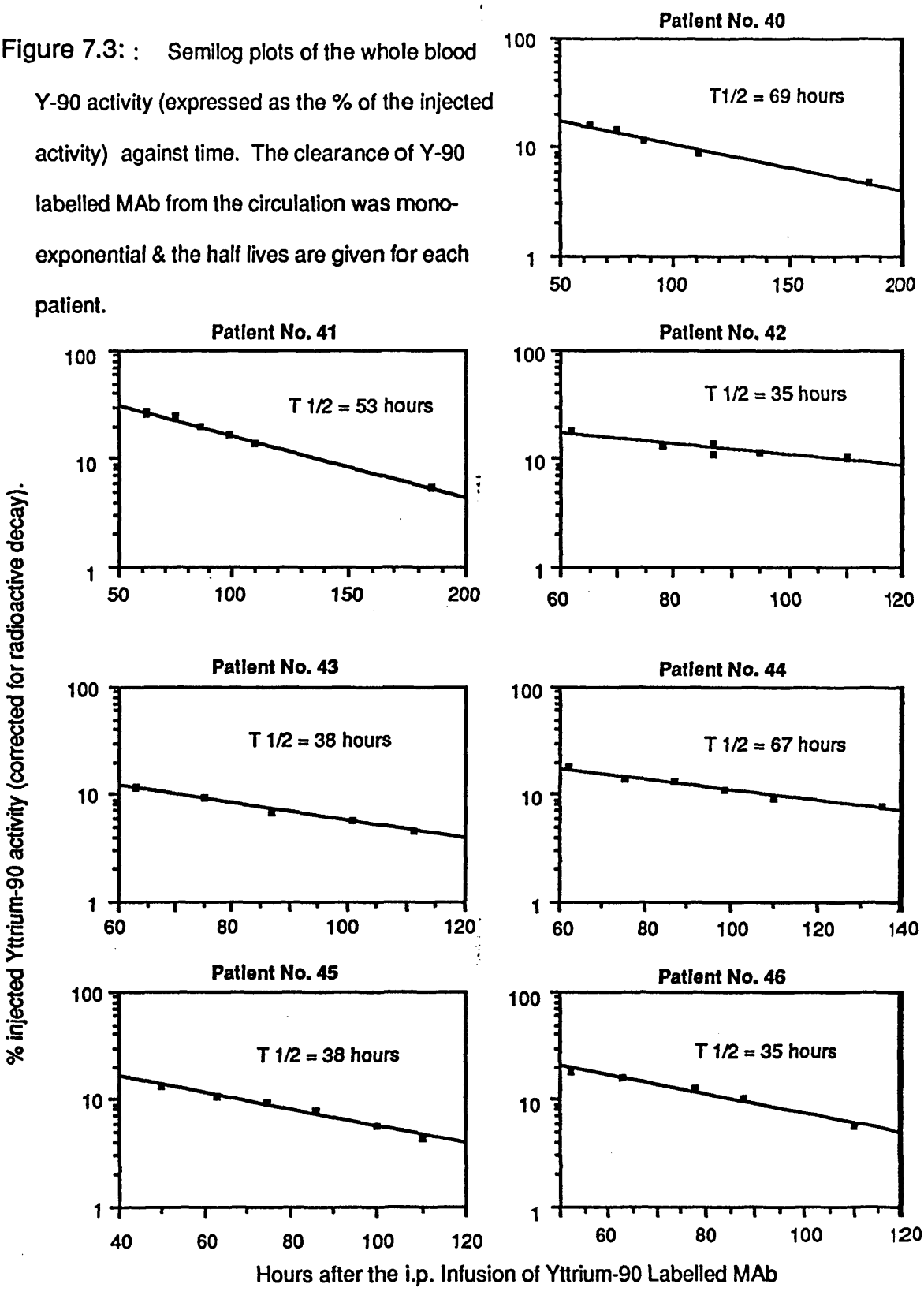


Figure 7.3: : Semilog plots of the whole blood Y-90 activity (expressed as the % of the injected activity) against time. The clearance of Y-90 labelled MAb from the circulation was mono-exponential & the half lives are given for each patient.



In this study the fraction of serum ^{90}Yttrium that was not bound to protein (MAb) was measured by passing a 200 µl aliquot of serum through a 20 ml Sephadex G-50 column. The amount of 'free' ^{90}Yttrium in serum was measured at 12 hourly intervals in five of the seven patients without ascites as illustrated in **Figure 7.4.** Fourteen hours after the i.p. infusion of ^{90}Y-labelled MAb between 2 and 5.5% of ^{90}Yttrium activity in the blood was non- protein bound. Although the fraction of free ^{90}Yttrium appeared to decrease with the EDTA infusion in four of the five patients, this trend was not significant. In this study only a small fraction of the ^{90}Yttrium activity was not bound to protein.

There was a significant increase in the amount of ^{90}Yttrium excreted in the urine in patients receiving EDTA. The mean and the range of the daily cumulative excretion of ^{90}Yttrium is shown in **Table 7.3.** Between 21% and 41% (mean 31.6%) of the injected ^{90}Yttrium activity was excreted in patients receiving EDTA. The renal excretion of ^{90}Yttrium in patients receiving EDTA is compared with that of patients not receiving EDTA in **Figure 7.5.** As soon as the EDTA infusion was commenced there was an increase in the urinary excretion of ^{90}Yttrium. The difference at five days between the cumulative excretion of ^{90}Yttrium was highly significant (Mann Whitney U = p = 0.0002). Although only 1.2% of the injected ^{90}Yttrium activity was found in the blood of the patient with ascites (Pt No. 47) 28% of the injected Y-90 activity was excreted in the urine over the 110 hours period.

Figure 7.4: Free Yttrium-90 in the circulation following the i.p. infusion of Y-90 labelled MAb. Free Y-90 is expressed as a % of the total serum Y-90 (measured by Sephadex G-50 column chromatography). The patient "number" is given with each curve.

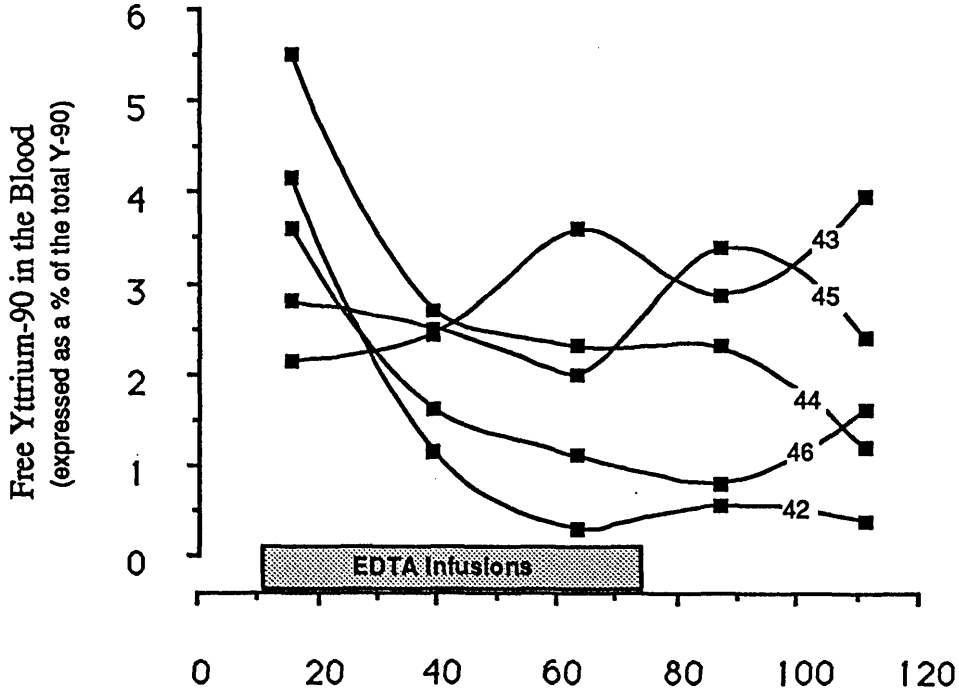
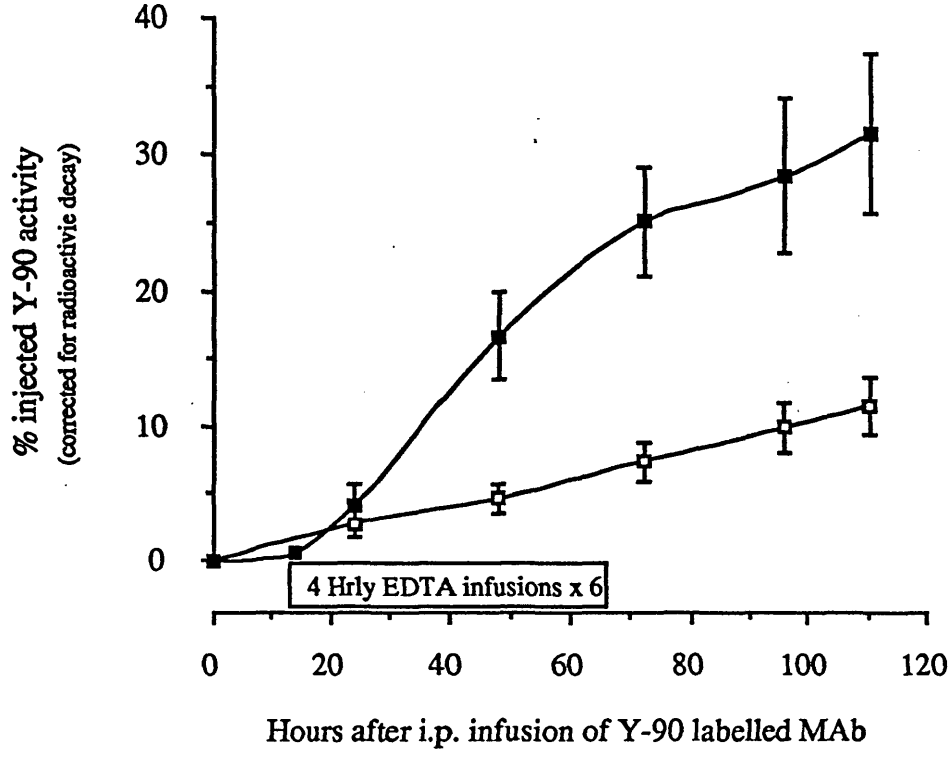


Table 7.3: Cumulative excretion of Y-90 in the urine following the i.p. infusion of Y-90 labelled MAb in patients who received i.v. EDTA.

	Cumlative % injected Y-90 activity in urine			
Hours after i.p. infusion of MAb	Mean (N = 7)	Standard Error	Standard Deviation	Range
14	0.6	0.1	0.2	0.3 - 0.9
24	4.0	0.8	1.6	1.7 - 5.3
48	16.7	1.7	4.1	11.5 - 22.5
72	25.1	2.0	5.0	17.0 - 31.0
96	28.4	2.8	7.5	16.1 - 36.9
110	31.6	2.9	7.6	21.1 - 41.0

Figure 7.5: Mean cumulative urine excretion of Y-90 following the i.p. infusion of Y-90 labelled MAb in 14 patients who did not receive i.v. EDTA and 7 patients who did receive EDTA. The errors bars represent +/- 2 S.E of the mean.



Radiation Dose to Bone Marrow

The integrated ^{90}Yttrium activities in blood (mCihrs) for 100 and 200 hours after the i.p. infusion of ^{90}Y-labelled MAb are given in Table 7.4. Although the ^{90}Yttrium activity in blood was not measured after 110 hours, the integrated ^{90}Yttrium activity for the 110 to 200 hour time period can be estimated by using the calculated clearance half life[1] to extrapolate the ^{90}Yttrium activity beyond the measured values. The estimated radiation doses to bone marrow from ^{90}Y-labelled MAb in the marrow circulation over 100 and 200 hour time periods are also given in Table 7.4, these ranged from 15 to 46 cGy. One can compare the radiation doses to bone marrow in patients receiving and not receiving EDTA (Chapter 5) by expressing the radiation dose as as cGy per mCi injected ^{90}Yttrium. The mean radiation dose in patients receiving EDTA was 1.51 cGy per mCi ^{90}Yttrium (range 1.51 - 2.3 cGy/mCi) which was less than the mean radiation dose of 1.77 cGy per mCi Y-90 (range 1.01 - 2.36 cGy/mCi) for patients not receiving EDTA. This difference however was not significant according to a Mann Whitney U test (U = 28. p = 0.12).

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1 The "effective" half life of Y-90 in the blood was used where 1/T(effective) = 1/T(biological) + 1/T(physical)

Table 7.4 : The integrated yttrium-90 activity in blood for 100 and 200 hours after the i.p. infusion of Y-90 labelled MAb and the radiation dose to bone marrow.

Patients	Yttrium-90 Activity in mCi	Integrated Y-90 Activity in mCi.hrs		Radiation Dose to Bone Marrow	
		(100 hrs)	(200 hrs)	(100 hrs)	(200 hrs)
40	20.3	128	147	27.5	31.6
41	19.6	215	255	46.2	54.8
42	22.0	169	197	36.3	42.4
43	18.2	182	116	21.9	24.9
44	20.6	153	187	32.9	40.2
45	14.5	70	79	15.0	17.0
46	20.6	135	151	29.0	32.5
47	25.5	9	-----	1.6	-----

Patient No.47 had ascites.

Toxicity

There was no toxicity associated with the intravenous infusion of EDTA. Serum electrolytes and liver function tests remained normal in the two months after treatment (no decrease in the serum calcium was expected as the calcium salt of versene was used). A degree of myelosuppression was seen in six of the seven patients[1] who did not have ascites. The fall in platelet count in patients receiving EDTA is illustrated in **Figure 7.6** and compared to that observed in patients from Chapter 5 who did not receive EDTA. The platelet count eight days after treatment is used a a base line as the pre-treatment blood count was not taken on a specific day. It is difficult to compare fall in the platelet count between the two groups of patients as the patients who received EDTA also received a greater activity of ^{90}Y-labelled MAb. It would appear however that patients who received 20 - 23 mCi of ^{90}Y-labelled MAb and EDTA had the same degree of thrombocytopenia as patients receiving 15 - 16 mCi of Y-90 labelled MAb and no EDTA. The difference in myelosuppression between patients receiving and not receiving EDTA is also compared in **Figure 7.7.** This figure plots the degree of myelosuppression (expressed in an arbitrary scale discussed previously) against the radiation dose the bone marrow was calculated to receive from Y-90 labelled MAb in the marrow circulation. Patients who received EDTA appear to have less myelosuppression for a given radiation dose to the bone marrow.

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1 One patient was followed up in Greece, and did not have regular FBC's.

Figure 7.6: Thrombocytopenia in patients who received i.p. Y-90 labelled MAb. The closed circles represent patients who received i.v. EDTA. Platelet counts are expressed as a % of the count 8 days after treatment.

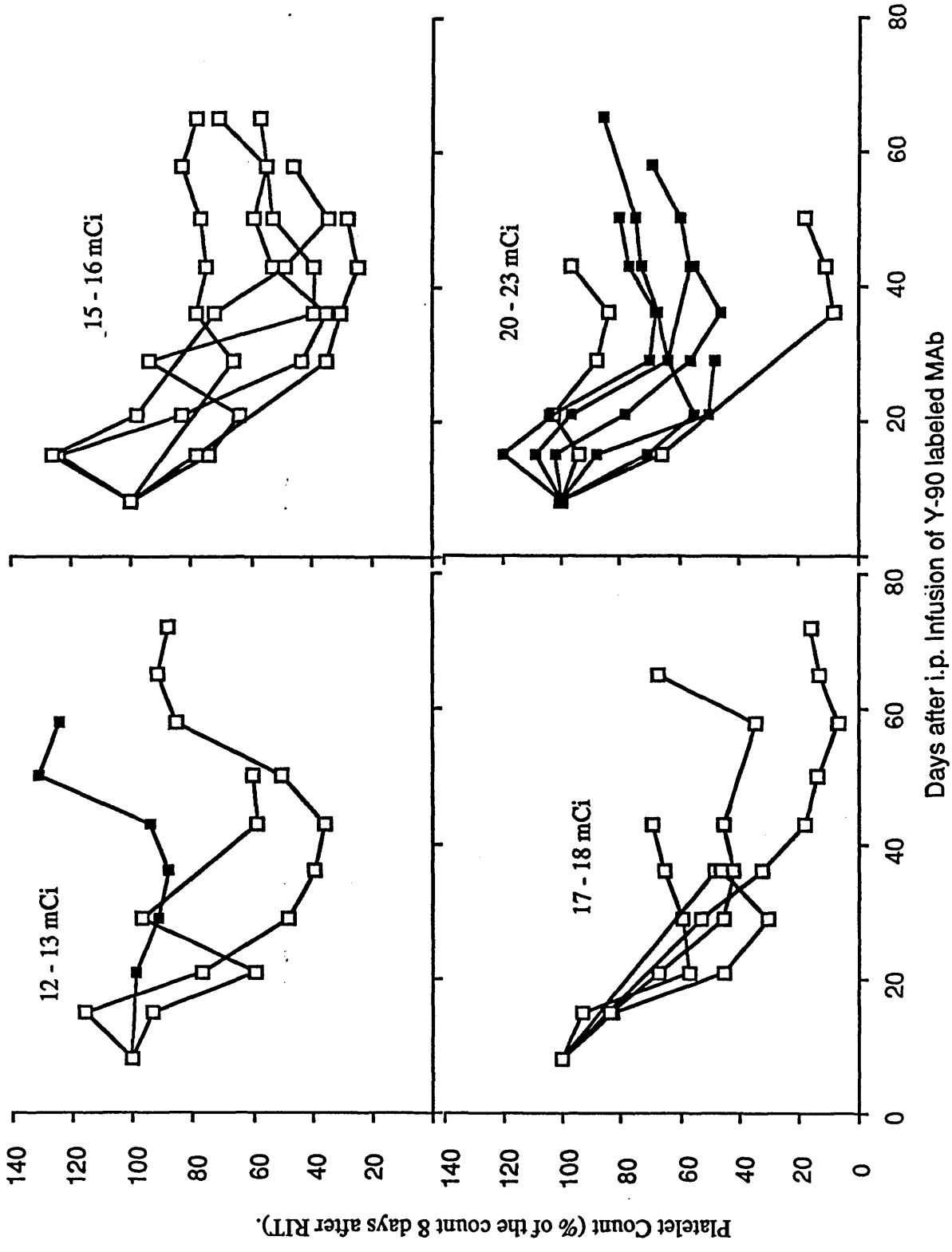
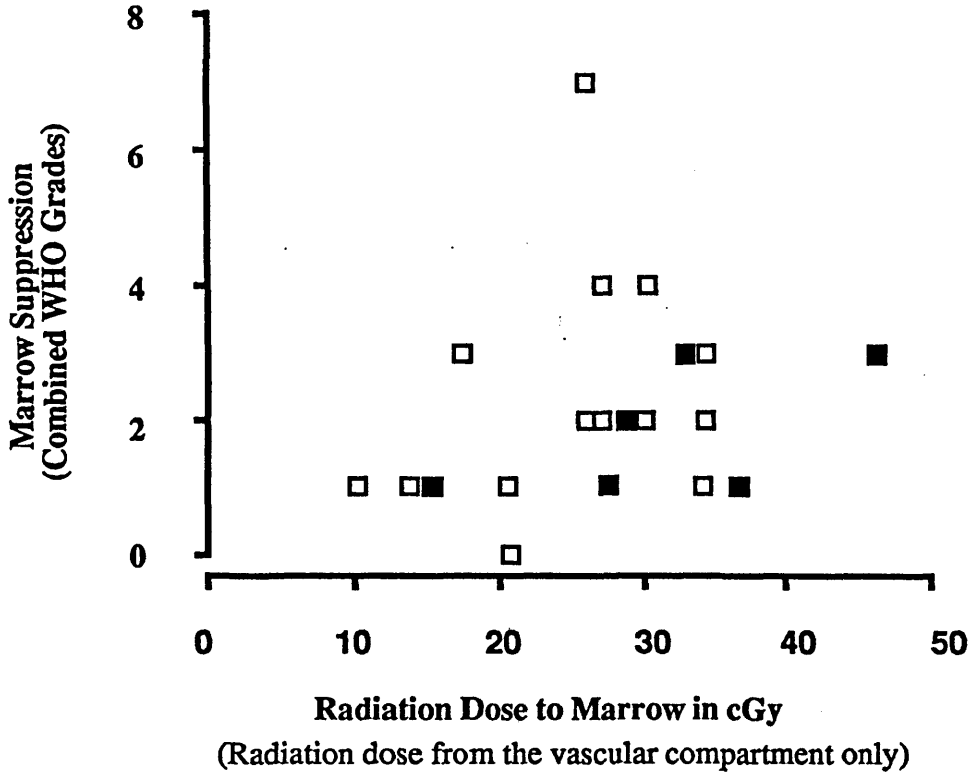


Figure 7.7: Myelosuppression in patients who received i.p. Y-90 labelled MAb. The myelosuppression is scored on an arbitrary scale from 0 to 8 (the sum of the WHO grades for leucopenia & thrombocytopenia. The closed sqaures represent patients who received i.v. EDTA.



DISCUSSION

^{90}Yttrium appears to be successfully chelated by EDTA in these patients with the chelated ^{90}Yttrium excreted in the urine. The fourfold increase in the renal excretion of ^{90}Yttrium appeared to be accompanied by less myelosuppression. The additional ^{90}Yttrium in urine may either come from ^{90}Yttrium released after the catabolism of ^{90}Y/MAb conjugate, or from the ^{90}Yttrium on the MAb itself. If the ^{90}Yttrium was obtained from the the former source there would be less ^{90}Yttrium available to decay in the bone, liver, spleen and bone marrow: the toxicity would be decreased with no loss in therapeutic potential. If EDTA took ^{90}Yttrium directly from the MAb (i.e. competing with DTPA for ^{90}Y) the amount of ^{90}Y-labelled MAb available to target tumour cells would be decreased and any reduction in toxicity would be accompanied by a reduction in therapeutic effect. The loss in therapeutic effect would be more marked if EDTA was able to take ^{90}Yttrium from MAb in the peritoneal cavity, particularly from MAb already bound to tumour cells.

There is some evidence from this study that at least a proportion of the excreted ^{90}Yttrium comes from MAb in the circulation and/or the peritoneal cavity. Patients treated with EDTA had slightly lower blood ^{90}Ytttrium activities than patients not receiving EDTA (Figure 7.1) and the AUC's (% injected Y-90 activity in the blood versus time) in these patients was significantly reduced. One would not expect this decrease in ^{90}Yttrium activity if EDTA only chelated free ^{90}Yttrium already

released from the MAb. The data obtained from the patient with ascites (Patient No. 47) suggests that some of the excreted ^{90}Yttrium may originate from the peritoneal cavity. This patient, in common with other patients with ascites[1], had a negligible amount of ^{90}Yttrium in the circulation. Although only 1.2% of the injected activity was found in the blood, 28% of the injected activity was excreted in the urine. One obvious explanation for this is that EDTA is able to diffuse into the peritoneal cavity where it chelates ^{90}Yttrium: the soluble chelated ^{90}Yttrium can diffuse back into the circulation from where it is rapidly excreted.

The data from this clinical study suggests that the stability of ^{90}Yttrium on the MAb/DTPA conjugate may be decreased by EDTA. This was investigated further by two invitro studies discussed below. The first study involved an examination of the stability of ^{90}Y-labelled MAb in serum containing different concentrations of EDTA. I was given 30 mgs/Kg of calcium disodium EDTA as a two hour intravenous infusion. A two hour infusion time was used to ensure that the EDTA concentration in my serum was a least equal to that in patients (who received a four hour infusion). Serum was obtained from blood venesected prior to the EDTA infusion and at one, three, five, eight, and thirteen hours afterwards, giving a series of serum samples containing decreasing concentrations of EDTA. ^{90}Y-labelled HMFG1 was incubated in these serum samples at 37 oC for 24 hours. The ^{90}Y-labelled HMFG1 preparations were prepared by Ms D. Snook with the same specific activity used in patients. The concentration of HMFG1 is the serum samples was 1 μg/ml which is similar to the maximum concentration of

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1 See Chapter 2.

MAb in the blood of patients receiving i.p. ^{90}Y-labelled MAb. The amount of free ^{90}Yttrium in the serum samples during the 24 hour incubation was determined by Sephadex G-50 gel filtration and is illustrated in **Figure 7.8.** Even without any EDTA there appears to be some dissociation of ^{90}Yttrium form the HMFG1 in serum, with an increasing amount of the ^{90}Yttrium becoming detached from the MAb with time. After 24 hours incubation 5% of the total ^{90}Yttrium activity was non-protein bound: Hnatowich et al., (1985a) observed a similar dissociation of ^{90}Yttrium from DTPA-IgG. High concentrations of EDTA appeared to increase the dissociation of ^{90}Yttrium from the MAb with 10% to 13% of the total ^{90}Yttrium not bound to protein after 24 hours incubation. This increased dissociation was only observed in the serum samples taken in the five hours following the infusion of EDTA; the degree of dissociation seemed to be independent of the EDTA concentration. The stability of ^{90}Yttrium on the MAb in serum taken eight to 13 hours after the infusion of EDTA appeared to be unchanged, with 5% of the ^{90}Yttrium activity not bound to protein after a 24 hour incubation. This study indicates that the concentrations of EDTA found in the serum in the first few hours after the EDTA infusion may produce a two to three fold increase in the instability of Y-90 on the MAB/DPTA conjugate. This was not observed when the EDTA concentration had fallen to levels found in serum taken more than eight hours after the EDTA infusion.

Figure 7.8: The influence of EDTA on the stability of Y-90 labelled MAb incubated in serum at 37 oC.

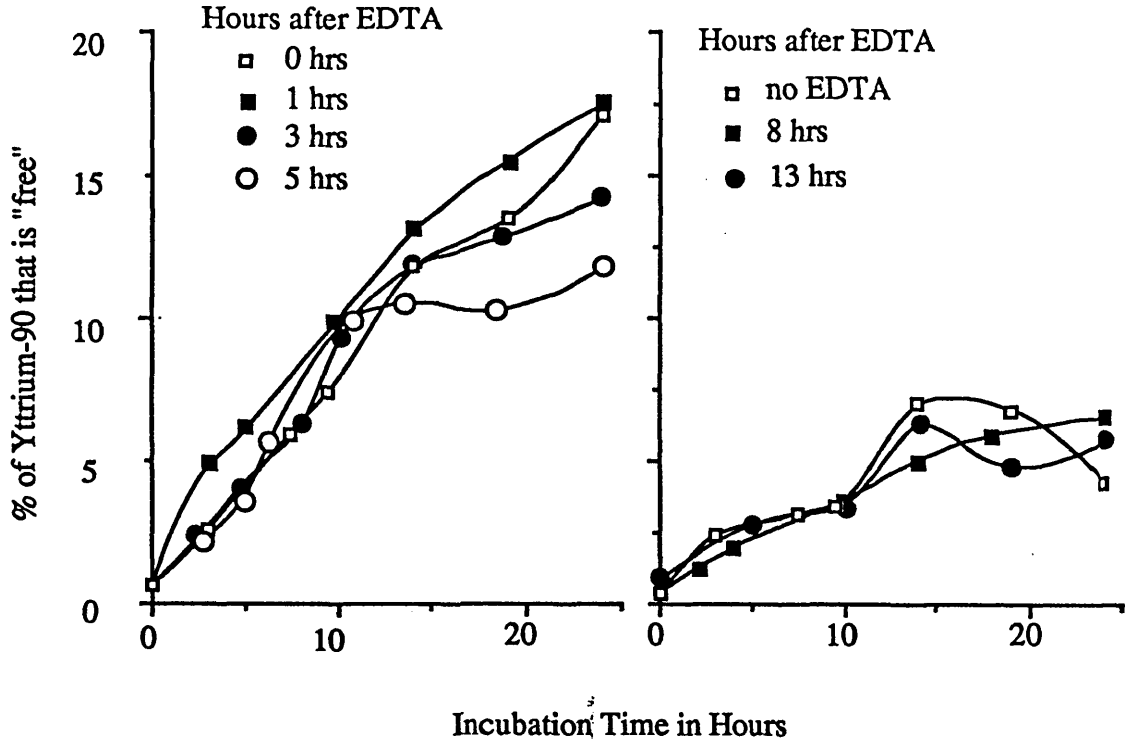
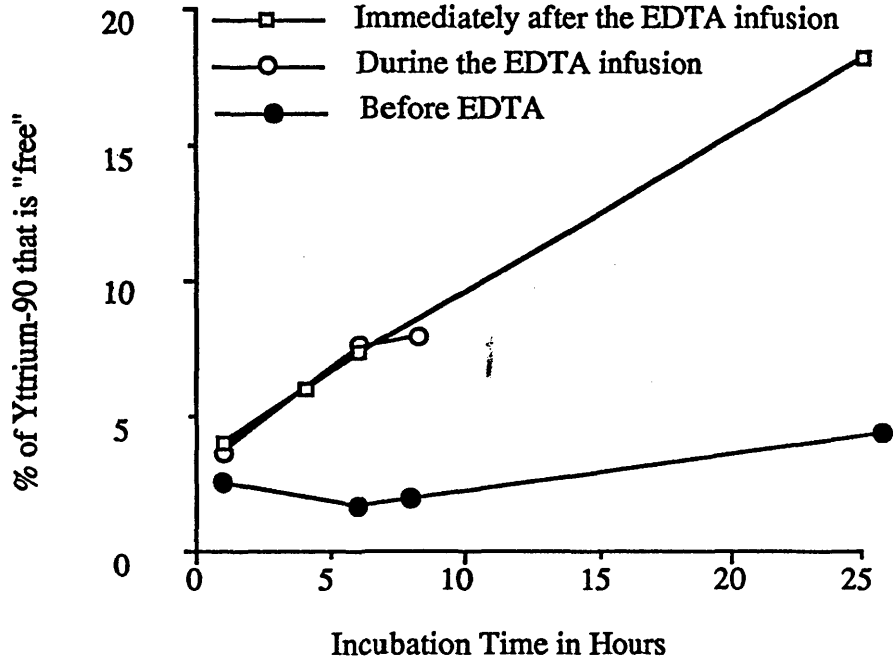


Figure 7.9: The influence of i.v. EDTA on the stability of Y-90 labelled MAb in a patient.



A second in vitro study was undertaken to confirm the instability of ^{90}Y-labelled MAb with EDTA in patients. Serum was taken from a patient who had received a therapeutic activity of ^{90}Y-labelled HMFG1 into the peritoneal cavity. The patient received EDTA (30 mgs/Kg) as a four infusion 14 hours after the i.p. infusion of ^{90}Y-labelled MAb. Serum was taken immediately before, during and immediately after the EDTA infusion. As in the previous study the fraction of Y-90 that was free was measured by gel filtration using a Sephadex G-50 column: the results are illustrated in **Figure 7.9.** In this study the ^{90}Y-labelled MAb appeared to be more stable than in the previous study with only 3.5% of total ^{90}Yttrium not bound to protein after 25 hours incubation. When serum was taken during and immediately after the infusion of EDTA, the stability of ^{90}Yttrium on the MAb decreased; after 25 hours incubation 16.5% of the ^{90}Yttrium activity in the serum was not bound to protein. This in vitro study confirms the increased instability of ^{90}Y-labelled MAb in high concentrations of EDTA.

It would appear that the infusion of EDTA can chelate ^{90}Yttrium in patients receiving ^{90}Y-labelled antibody with decreased toxicity. Some of the chelated ^{90}Yttrium may be from the ^{90}Yttrium bound to MAb in either the circulation or the peritoneal cavity. The competition of EDTA with DTPA for ^{90}Yttrium could probably be decreased by decreasing the EDTA concentration in the patients serum. The best method of delivering EDTA to these patients is unknown, but these results suggest that a continuous infusion may be preferable to four hour infusions. This would avoid the high peak concentrations of EDTA but still provide sufficient EDTA to chelate the free Y-90 released into the tissue after catabolism of MAb.

SUMMARY

The administration of EDTA in patients receiving ^{90}Y-labelled MAb produced successful chelation of ^{90}Yttrium with a fourfold increase in the renal excretion of ^{90}Yttrium.

^{90}Y-labelled MAb is unstable in the presence of high concentrations of EDTA.

A proportion of the chelated ^{90}Yttrium was from ^{90}Yttrium on MAb in the circulation and the peritoneal cavity.

Greater quantities of ^{90}Yttrium would be excreted if a suitable antibody chelate was developed so that the ^{90}Yttrium remained chelated after the MAb had been metabolized.

CHAPTER 8

THE DISCUSSION

The purpose of this thesis is to study the pharmacokinetics and toxicity of RIT. Radiolabelled antibody was given intraperitoneally as the regional route of administration was thought to be advantageous for intraperitoneal tumour localization. Radiolabelled MAb's were absorbed from the peritoneal cavity into the circulation; allowing systemic as well as local toxicity to be investigated.

Dose Effect Curves in Radiotherapy

A typical tumour cure probability curve for increasing doses of radiation is illustrated in **Figure 8.1.** No responses are observed until a threshold radiation dose is reached after which the response rate rises sharply until all the tumours are cured. At the hypothetical radiation doses A, B and C, the probability of cure is 50%, 75%, and 95%. There is also a sigmoidal response curve for fatal toxicity and which may rise equally steeply. The radiocurability of a tumour depends on the relative position of the two curves as illustrated in **Figure 8.2.**

For a radiocurable tumour the dose-effect curve for fatal complications is to the right of that for cure. Both curves rise steeply; increasing the radiation dose from A to B cGy will improve the cure rate by 25% with a 5% increase in fatal complications; increasing the radiation dose further from B to C cGy will increase the cure rate by a further 20% but the fatal complication rate may rise 45%. Any therapeutic manipulation that increased the distance between the curves, whether shifting the fatal complication curve to the right or the cure curve to the left will increase the radiocurability of the tumour.

Figure 8.1: The sigmoid dose-effect curve for tumour cure.

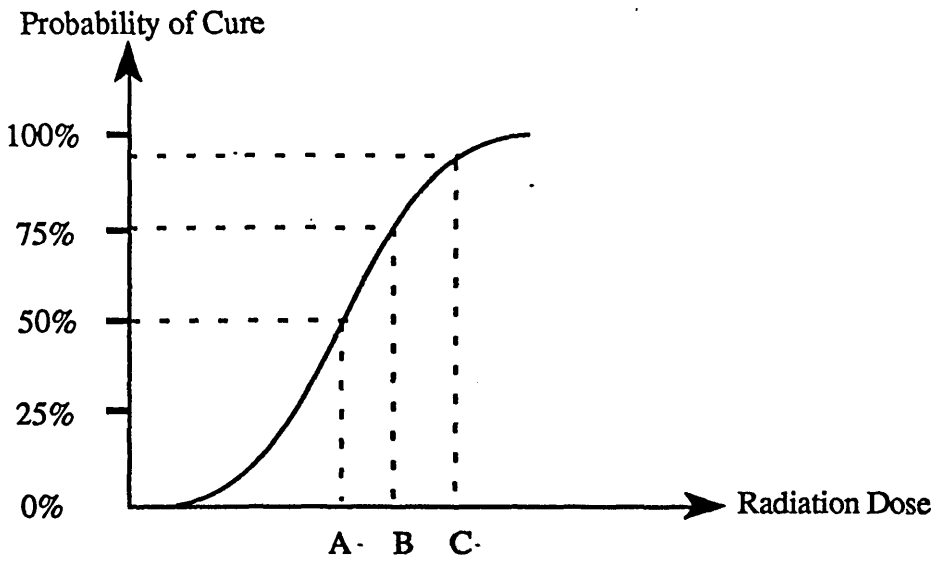
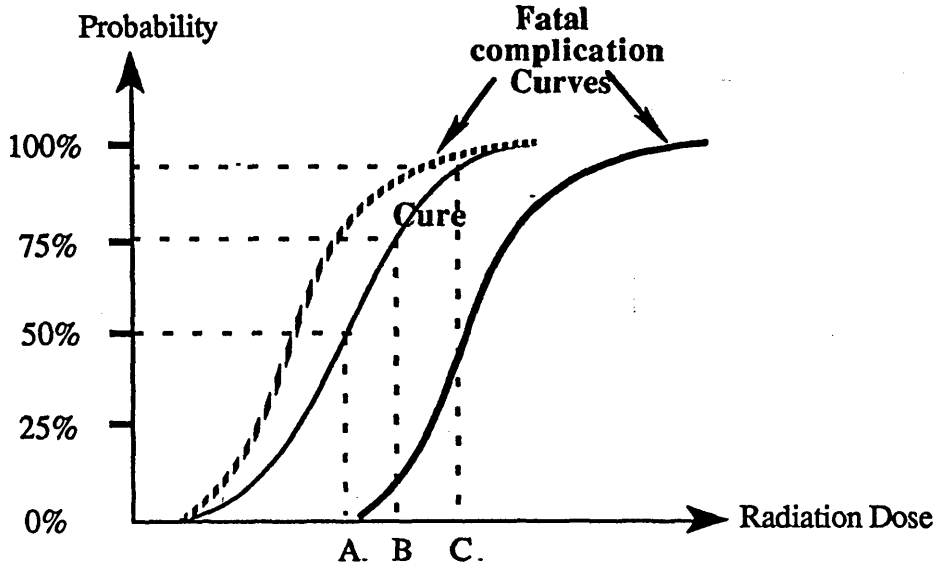


Figure 8.2: Sigmoid dose-effect curves for tumour cure and fatal complications for a radiocurable tumour (thick line) and a radioincurable tumour (dashed line).



Unfortunately the fatal dose effect complication curve for systemic RIT probably lies to the left of the dose-effect curve for tumour cure

[Vaughan et al., 1987; Epenetos et al., 1986]. If one wishes to develop RIT as a therapeutic modality the two curves must be defined, and the dose effect curve for fatal comlications shifted to the right of the curve for tumour cure. In this thesis an attempt has been made to study the dose-effect curve for potentially fatal complications of RIT (myelosuppression). I have also investigated methods of decreasing toxicity (shifting the curve to the right) to increase the therapeutic ratio.

As regional RIT was used in this study the toxicity can be divided into local and systemic toxicity.

L0CAL TOXICITY OF INTRAPERITONEAL RIT

Without Ascites

Radiolabelled MAb is efficiently absorbed from the peritoneal cavity into the sytemic circulation in patients without ascites. This majority of the isotope leaves the peritoneal cavity and is either excreted in the urine (^{131}I) or decays in the rest of the body (^{90}Y). The radiation dose to the peritoneal serosa can expected to be low unless it happenes to be adjacent to tumour deposits that have been localized by radiolabelled MAb. This was confirmed by intraperitoneal TLD measurements in patients receiving ^{131}I and ^{90}Y-labelled MAb. The mean radiation dose to the peritoneal serosa from ^{131}I and ^{90}Y-labelled MAb's were 2.85 cGy and 15.7 cGy per mCi respectively. The radiation dose to peritoneal serosa from ^{90}Y-labelled MAb can be compared to the dose delivered by ^{90}Y-silicate colloid. Radiocolloids are not absorbed from the peritoneal cavity and complete their physical decay there:

^{90}Yttrium-silicate colloid delivered over six times as much radiation to the peritoneal serosa, 96.6 cGy versus 15.7 cGy per mCi of ^{90}Yttrium. As the radiation dose to local organs is low, one would not expect to observe acute or chronic radiation sequelae such as fibrosis, strictures, malabsorption, coloitis, or cystitis; and none of these were seen our patients.

With Ascites

The situation is rather different in patients with ascites as the absorption of radiolabelled MAb from the peritoneal cavity is decreased in these patients. Only 4.8 to 8% of the injected ^{131}I activity was found in the blood and only 40% of the injected activity was excreted in the urine. In these patients radiolabelled MAb behaves more like a radiocolloid with a much greater "non-specific" radiation dose to the serosa. Although we did not observe local toxicity in these patients we have noticed in others studies that most patients with ascites have transient dairrhoea following the administation of radiolabelled MAb. We assume that this is due to the greater non-specific radiation dose to bowel. Local toxicity rather than myelosuppresion limits the isotope activity that can be given in these patients.

SYSTEMIC TOXICITY OF INTRAPERITONEAL RIT

As most of the radiolabelled MAb is absorbed, the toxicity from intraperitoneal and systemic RIT are similar. I have used the isotope activity in the blood to estimate the radiation dose organs such as the

bone marrow receive from radiolabelled MAb in the vascular compartment. Other methods of calculating the radiation dose such as SPECT or tissue biopsies were not appropriate in these patients. One can raise several objections to using the MIRD forumulation and the blood isotope activity to calculate the radiation dose to an organ. There are three major criteria that must be met. Firstly the isotope should be in the blood and not concentrated in the organ of interest or tissue adjacent to that organ. Secondly the isotope must be homogeneously distributed (in the blood) through the organ concerned. Thirdly one must know the porportion of the total blood volume is in the organ. I was not able to satisfy all these criteria but was able to estimate the radiation dose the bone marrow received from ^{131}I-labelled MAb. Even if the calculated radiation dose was not accurate the chosen model is useful for comparing the toxicity of different radiolabelled antibody preparations.

^{131}I-labelled MAb in HAMA Negative Patients

In these patients myelosuppression was the main toxicity. The degree of myelosuppression could be predicted by applying the MIRD model to the ^{131}I activity in the blood, with a steep dose-effect curve. Although myelosuppression could be predicted from the integrated blood ^{131}I activity, it was less easy to predict the degree of myelosuppression form the administered ^{131}I activity as the biological half life of the ^{131}I-labelled MAb was so variable (range 45-95 hours). This unpredicatbility makes phase I/II studies extremely difficult.

^{131}I-labelled MAb in HAMA Positive Patients

The pharmaockinietics of ^{131}I-labelled MAb in HAMA positive patients were totally different. Although HAMA may interfere with ^{131}I-labelled Mab from reaching its target, this study illustrates how the systemic toxicity from RIT can be reduced. The main reason why radiolabelled antibodies are so toxic is their long biological half life; it is possible to give patients with thyroid cancer up to 400 mCi (14.8 GBq) of ^{131}I if the biological half life is sufficiently low [Benua et al., 1982; Dobyns & Maloof, 1951; Trunnel et al., 1949; Van Norstrand, et al., 1986]. If the untragetted ^{131}I-MAb could be converted to free ^{131}I and excreted then the toxicity of RIT would be the same as that of ^{131}I therapy for thyroid cancer; greater ^{131}I-MAb activities could be given with greater therapeutic effect.

This is what happened in the five HAMA positive patients. The peak ^{131}I activity in the blood was only 3.4 - 6% of the injected ^{131}I activity compared to 18-36% in HAMA negative patients. Most of the ^{131}I in the blood of HAMA positive patients was free (62% - 78%) compared to a minority (0 - 19%) in HAMA negative patients. Free ^{131}I was rapidly excreted in the urine with 80% of the injected activity being excreted by 48 hours. Although the bone marrow was calculated to have received a radiation dose of 4 - 16 cGy from ^{131}I in the vascular compartment mild myelosuppression was observed. It is possible that the my application of the MIRD formulation underestimated the radiation dose to bone marrow in these patients for two reasons. Firstly most of the ^{131}I was free and not protein bound. Free ^{131}I may be distributed througout the extracellular space rather than being confined to the blood, as assumed by my MIRD model, and this would be an additional radiation source to bone marrow. Secondly the RES in the marrow may

have a role in removing the ^{131}I -MAb-HAMA immune complexes from the circulation and concentrate ^{131}I. Although hepatic RES (the major organ involved in removing soluble immune complexes) only concentrates ^{131}I for a few hours, others parts of the RES may not be so efficient at dehalogenating antibodies . Sharkey et al., [1984] found that the spleen in hamsters receiving goat anti-CEA and donkey anti-goat immunoglobulin antibody retained ^{131}I for longer than the liver. This group confirmed this finding in patients three years later [Goldenberg et al., 1987]. Hyams and coworkers on the other hand observed that the bone marrow in monkeys who had received anti-antibodies to clear MAb from their blood a much lower bone marrow activity than monkets who did not receive anti-antibody.

Although RIT is less myelosuppressive in HAMA positive patients it is probalbly not very effective. We were able to detect high HAMA levels in the peritoneal fluid from a HAMA positive patient. As there is a huge excess of HAMA compared to MAb, the former would interfere with the latter antibody binding to tumour cells in the peritoneal cavity. It is interesting no see that the non-specific radiation dose to the peritoneal sersosa per mCi of administered ^{131}I was significantly lower in HAMA positive patients. This may indicate that HAMA-MAb complexes are metabolized in the peritoneal cavity and free ^{131}I released is able to diffuse rapidly across the peritoneal cavity.

Intravenous HAMA in Patients Receiving RIT.

A solution to intraperitoneal HAMA preventing MAb reaching its target, is to inject (HAMA negative) patients with HAMA

intravenously. The clearance of immunoglobulin from the blood into the peritoneal cavity is slow [Young et al., 1987], and i.v. HAMA is unlikely to reach the peritoneal cavity unless given in vast excess. Wahl & Fisher were able to demonstrate in rats that the combination of i.p. radiolabelled MAb and i.v. anti-MAb decreased the isotope activity in the blood but not in intraperitoneal tumour deposits. We were able to clear ^{131}I from the blood in HAMA negative patients tby injecting HAMA intravenously; the ratio of HAMA to MAb was about 3 :1. The radiation dose the bone marrow and the liver received from ^{131}I in the vascular compatment was reduced four fold. Hepatic ^{131}I activity was increased immediately after the injection of HAMA but this only this increased activity was only maintained for six hours. We did detect mild myelosuppression which we could not be explained by the radiation dose the marrow received from the vascular compartment. It is possible that the marrow received an additional radiation dose from ^{131}I either in the extracellular or the marrow RES compartments. There is little doubt however that the degree myelosuppression was significantly reduced by the intravenous administration of HAMA.

^{90}Y-labelled MAb in HAMA Negative Patients.

Myelosuppression limits the isotope activity that can be administered for both ^{90}Y and ^{131}I-labelled antibodies. The marrow radiation dosimetry however, is however quite different for the two isotopes. Most of the myelosuppression observed following the administration of ^{131}I-labelled MAb can be explained by the radiation dose the marrow received from the vascular compartment. This was not true with ^{90}Y-labelled MAb where the vascular compartment only accounts for a minority of the marrow's radiation dose . ^{90}Yttrium unlike ^{131}I is not excreted in the

urine after the MAb catabolism, but accumulates in various organs. The biodistribution study in rabbits indicated that the bone and the bone marrow itself were both much more important radiation source organs that blood. The myelosuppresion that accomanied RIT with ^{90}Y-labelled MAb was quite unpredicatable and severely limited the ^{90}Y activity that could be administered.

The administration of HAMA in patients receiving ^{90}Y-labelled MAb may increase rather than decrease myelosuppresion. ^{90}Yttrium from cleared immune comlexes could acculate in the bone and marrow and increase the radiation dose to bone marrow. Although I was unable to demonstrate this in the rabbit biodistribtuion study, the presence of rabbit anti-mouse immunoglobulin antibody did not decrease the ^{90}Y activity in the bone and marrow despite an impressive reduction in the blood ^{90}Y activity.

If ^{90}Y remained chelated to DTPA after ^{90}Y-labelled MAb was catabolised it would be excreted in the urine and behave like ^{131}I. We have attempted to salvage some of the released ^{90}Y by giving patients intravenous infusions of EDTA, and have produced a four-fold increase in the urinary excretion of ^{90}Y with less myelosuppression. In vitro studies indicated however that EDTA increased the instability of ^{90}Y on DTPA-MAb. It is possible that some of the excreted ^{90}Y was taken directly off the antibody rather than salvaged from bone and liver.

Futurer Developments of Intraperitoneal RIT

The administration of intravenous HAMA increased the distance between the two iso-effect curves in Figure 8.2 and will allow increased

activities of ^{131}I-labelled MAb to be given with an increased chance eradicating tumour depoists . There is concern however that ^{131}I may not be the ideal isotope as it's β-particles are of low energy and not very penetrating . ^{90}Yttrium with suitable physical characteristics has an unfavourable biodistribution, being concentrated in important body organs and is not excreted. Ideally ^{90}Y should remain chelated after MAb catabolism and be excreted in the urine. There are a generation of new chelating agents that may allow radiometals to remain chelated with less non-specific uptake by the body and an improved tumour to normal tissue ratio [Esteban et al., 1987; Moi et al., 1989; Bland et al., 1988]. If these are sucessful then the radiation dose to bone marrow would be from the vascular compartment (like ^{131}I-labelled MAb), and HAMA could be used to reduce this toxicity.

Although new chelates and anti-bodies will reduce the toxicity associated with RIT, new techniques in tumour targeting are required as present generation of antibodies and their fragments so not localize tumour deposits sufficiently. For RIT to be a useful therapuetic modality the isoeffect curve for cure must be moved to the left (figure 8.2). Either a new generation of antibodies or methods of amplifying the bound MAb on tumour cells are required.

APPENDIXES

Appendix A
Measurement of blood ^{131}I activity.

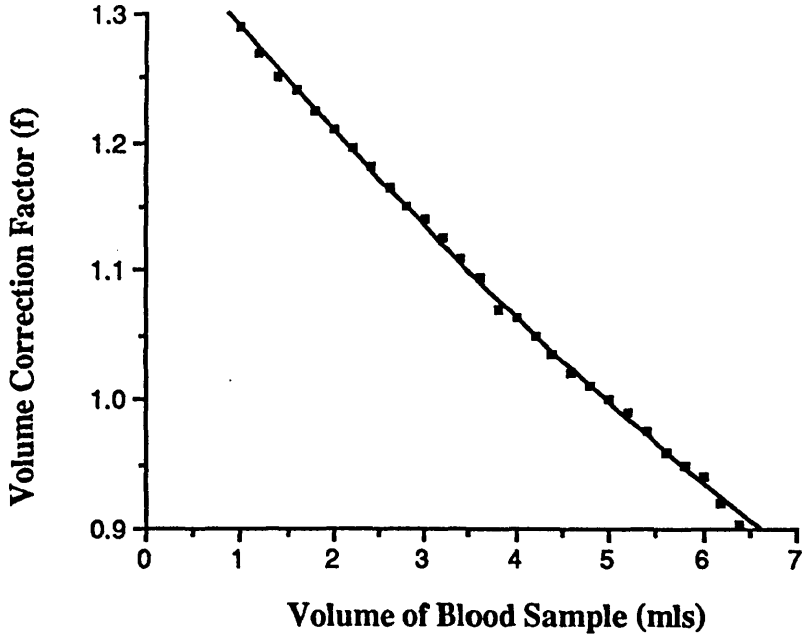
The blood ^{131}I activity in patients receiving ^{131}I labelled MAb was measured in the following way. For five days after intraperitoneal RIT blood samples were taken from each patient at 12 hourly intervals. These were put into pre-weighed tubes containing EDTA and stored at 4 ^{o}C: the weight of each blood sample was calculated by reweighing the filled containers and dividing the weight difference by 1.05 as this is the weight in grams of one ml of blood [I.C.R.U. 1975].

The ^{131}I activity in the blood samples were measured in a scintillation well counter. The number of counts from the blood samples and standard solutions of known ^{131}I activity[1] were recorded. Although the volume of the standard ^{131}I solution was always five mls, the blood sample volume ranged from three to five ml. The number scintillation counts obtained from a given activity of ^{131}I was found to be dependant on the sample volume (V_s). Volume correction factors were determined by recording the number of counts from a standard ^{131}I activity in increasing volumes of saline. The correction factor (called f) was normalized for a correction factor of five ml, as illustrated below.

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1 supplied by Amersham International Plc.

Figure A.1: Volume Correction Factors for the Scintillation Counts.



Using the blood sample to determine the ^{131}I activity in the whole circulation, requires an estimate of the patients blood volume. This was done by applying Hurley's formula [Hurley, 1975] to the each patients surface area. The surface area of a patients was taken to be $Wt^{0.425}$ x ($H^{0.725}$ x 0.007184), where Wt is the patient's weight in Kg and H the height in cm [DuBois & DuBois, 1916].

The ^{131}I activity in a patients circulation at time t after the i.p. infusion of ^{131}I labelled MAb was

$$A' = [(C_s/f)/C_{st}] \times [V_w/V_s] \times A_{st}$$

where A' was the ^{131}I activity in patients whole blood volume at time t, A_{st}[1] and C_{st} were the ^{131}I activity and the number of scintillation counts in the five ml standard

1 I-131 activity at the time of the i.p. infusion of I-131 labelled MAb.

solution, V_s and C_s the volume were the number of scintillation counts from blood sample and V_w was the patients estimated blood volume [Hurley, 1975].

A' is the total ^{131}I activity in the blood assuming that no radioactive decay occur. Expressing decay corrected ^{131}I activity is appropriate if one wishes to describe the biological behaviour of the monoclonal antibody rather than the physical characteristics of the isotope. The true and not the decay corrected ^{131}I activity is required however when the blood ^{131}I activity is used to calculate the radiation dosimetry to organs. The true blood ^{131}I activity can be calculated from the decay corrected activity by

$$A = A' \, 0.693\, t / T$$

where A was the true ^{131}I activity at time t and A' would have been the ^{131}I activity had no radioactive decay occurred since the time of the i.p. injection. The half life of ^{131}I (T) is 193 hours.

Ideally one would wish to determine each patients individual blood volume rather than estimate it from a patients surface area. Hurley [1975] found that a patients blood volume was closely related to their surface area, which is routinely measured in oncological patients. Although a more precise method of determining whole blood ^{131}I activity would be preferable, much greater approximations were made in calculating the radiation dose to individual organs (see below).

Appendix B
Measurement of urine I-131 activity.

The ^{131}I activity in the urine was determined by the following method. Patients were instructed to store all their urine in graduated two litre containers so that the total urine volume (to the nearest 50 mls) could be measured. Each day a five ml aliquot was taken from the previous day's container so that the urine could be discarded[1]. Two ml of urine were taken from the aliquot and placed into sealed scintillation vial. The ^{131}I activity in the urine sample was measured by a scintillation well counter, against two ml 'standard' solutions containing known activities of ^{131}I.[2] The ^{131}I excretion for each day was calculated to be,

$$A' = [(C_s/C_{st}] \times [V_w/2] \times A_{st}$$

where A' is the ^{131}I activity excreted in a whole day (assuming that no radioactive day occurred since the time of the i.p. infusion of ^{131}I labelled MAb) and A_{st} the activity in the standard solution of ^{131}I at the time of the i.p. infusion of ^{131}I labelled MAb. The daily urine volume is represented by V_w; C_s and C_{st} represent the number of scintillation counts from the urine sample and ^{131}I standard. The calculated ^{131}I activity is corrected for radioactive decay. The true ^{131}I excretion (A) is required to determine the residual total body ^{131}I content and is calculated from the decay corrected activity as described in appendix A.

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1 It was necessary to discard large I-131 activities quickly to reduce the radiation exposure to medical and nursing staff.

2 Standard solutions of I-131 were obtained from Amersham International Plc.

Appendix C

Measurement the radiation dose to the peritoneal serosa by Thermoluminescent Dosimetry.

Thermoluminescent Dosimetry (TLD) is used to measure the radiation dose to normal organs in every radiotherapy centre in the United Kingdom. Although it is an indirect method of measuring the radiation dose and requires calibration against standard radiation sources it does several practical advantages. One of these is the ability of being able to measure the radiation dose in discrete anatomical areas. Martin Sullivan[1] and myself have developed a TLD system for measuring the radiation dose to the peritoneal cavity. Lithium fluoride (LiF) microrods were obtained from the Harshaw/Filtrol Partnership in Ohio, USA. The microrods are cylindrical, six mm long and one mm in diameter. The rods were first anealed and exposed to a standard dose of radiation. The light output from the LiF rods was measured in a Toleldo TLD counter and rods whose light output varied more than 10% were discarded.

The LiF microrods were sealed into thin plastic afterloading tubes o(Amersham International Plc.) with an internal diameter of one mm and a wall thickness of 0.2 mm. Each TLD catheter could contain up to 45 LiF micorods with radio-opaque markers between every fifth LiF rod so that the total length of the TLD catheter could be 35 cms. The ends of the TLD catheter were heat sealed and the catheters sterilized for 30 minutes in isopropyl alcohol. TLD catheter were inserted into the peritoneal cavity through a 14 gauge intravenous cannula[2] after the introduction of the intraperitoneal dialysis catheter. When the dialysis catheter was inserted under local anaesthesia (a 'blind insertion') a litre of 0.9% sterile saline was introduced into the peritoneal cavity before the TLD catheter was inserted. This ensured that there was fluid between the anterior abdominal wall and the bowel and decreased the likelihood of perforating the

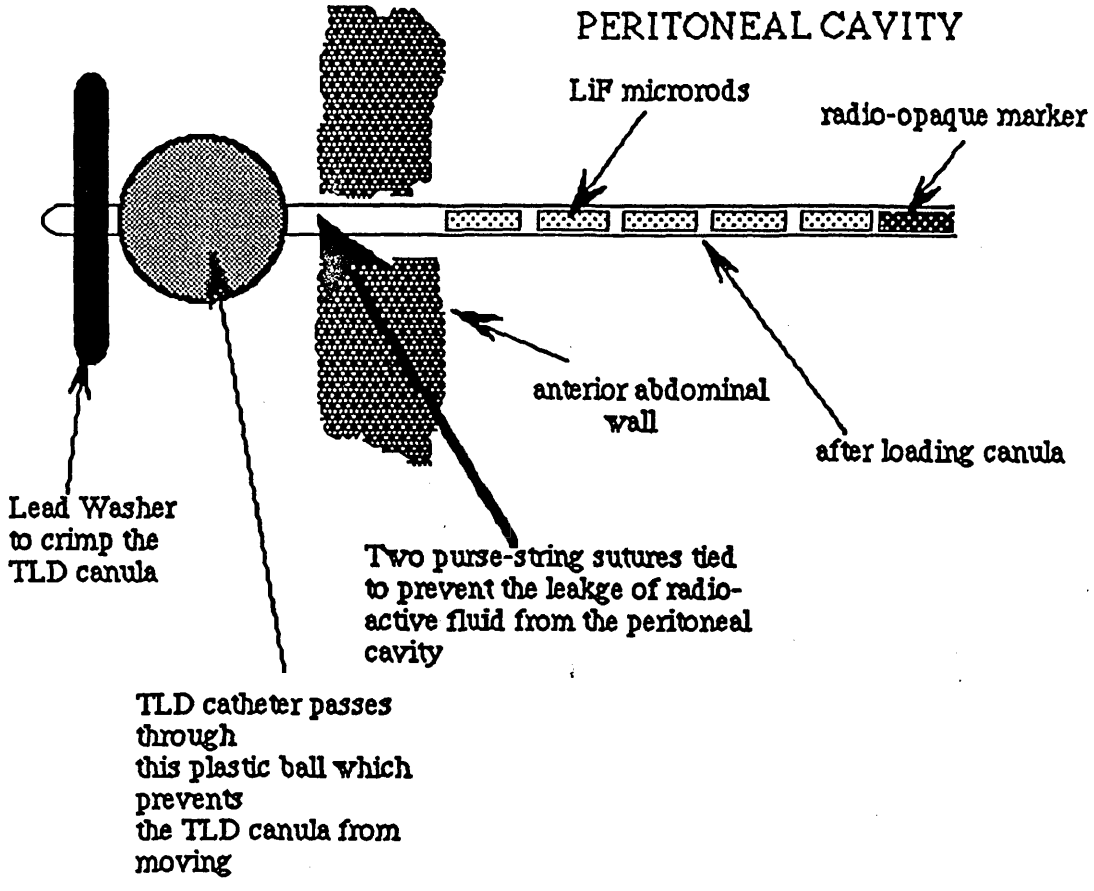
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1 Martin Sullivan is a physicist at the Department of Medical Oncology at the Hammersmith Hospital and was responsible for the calibration of the TLD micor-rods.

2 Normally a Centrasil CVP cannula.

bowel with the TLD catheter (this did not occur). The TLD catheter was secured to the anterior abdominal wall as illustrated below.

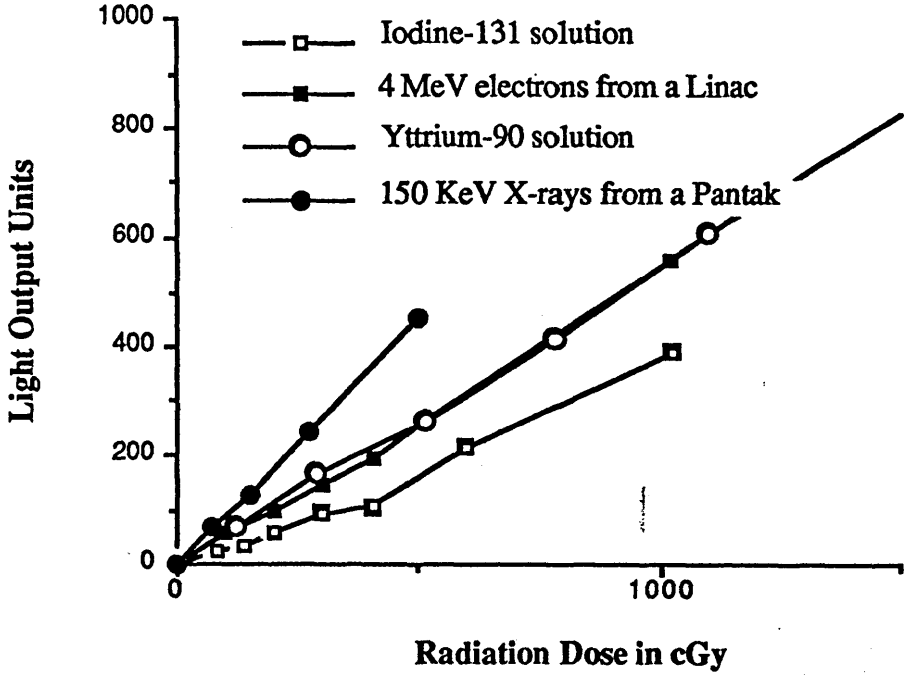
Figure C.1: The TLD catheter



The TLD catheter remained in the peritoneal cavity for 110 hours and was removed before the patients was discharged. Catheters were stored for 24 hours at room temperature before the light output was read in a Toledo TLD reader. The LiF micro-rods were calibrated by exposing them to known doses of radiation of different energies (6 MeV X-rays, 4 MeV electrons and 50 KeV X-rays). As the thermoluminescence response was found to be dependant on the radiation energy (a decreased response observed with 50 KeV X-rays) the rods were finally calibrated by immersing them for different time intervals in a 200 ml solution containing known activities of ^{131}I and ^{90}Y. The radiation dose in cGy to the rods in solution was calculated by multiplying the integrated microcurie hours with the MIRD S factor for spleen (as a radiation source

and target organ) as the container was of similar dimensions to a spleen. The light output from the TLD rods for calibration using different types of radiation are shown below. Each point is the mean light output from five lithium fluoride rods.

Figure C.2: The calibration of LiF TLD micro-rods



The increased thermoluminescent sensitivity of LiF to X-rays (150 KeV) of low energy is a well known phenomena. The decreased thermoluminescent response to the calculated radiation dose from the ^{131}I solution is probably due to the absorption of low energy β particles by the plastic afterloading tube. This would mean that the LiF rods would underestimate the radiation dose from ^{131}I: this is only relevant if the LiF rods are calculated with more energetic source of β particles. When the ^{90}Y solution was

used to calibrate the TLD catheter the more energetic β particles were able to penetrate the TLD catheter and the thermoluminescence increased. There was an excellent correlation between the radiation dose the LiF rods were calculated to have received from the ^{90}Y solution and the dose they received from 4 MeV electrons delivered by a Linac. The calibration curves from the ^{131}I and ^{90}Y solution were used to calculate the radiation dose to the peritoneal serosa from ^{131}I and ^{90}Y labelled MAb respectively. When the TLD catheters were used to measure the peritoneal serosal radiation dose from ^{90}Y radiocolloids (i.e. a radiation dose of several thousand cGy) a fresh calibration was required as the light output per cGy is not linear (supralinearity) at high radiation doses.

Appendix D
Estimation of % isotope bound to protein by dialysis.

Dialysis was used in several studies to determine the fraction of isotope activity that was bound to protein. Dialysis tubing (Visking Co. UK.) was prepared for use by first boiling it for 15 minutes in distiled water immediately before cooling it at 4 °C in phosphate buffered salt (PBS). One ml of serum was dialysed against 2.5 litres of PBS at 4 temperature of 4 °C for 24 hours. The dialysed serum was removed from the dialysis bag and a 500 µl aliquot put into a scintillation vial. The counts in this vial were then compared to those of 500 µl sample of non-dialysed serum. The percentage of non-protein bound isotope was calculated from the following formula.

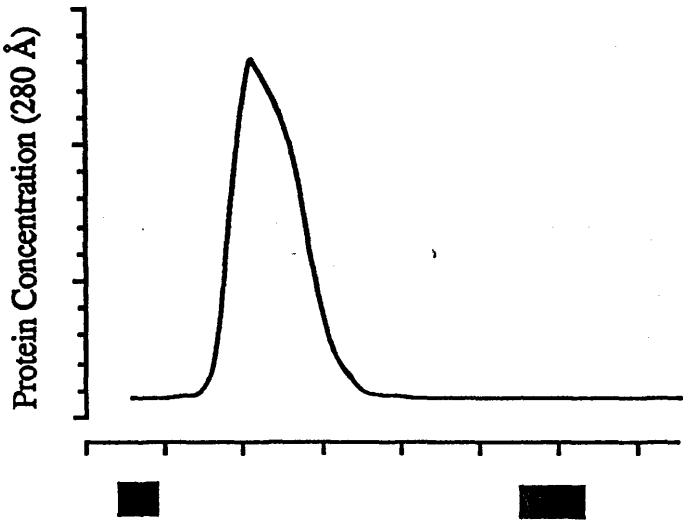
$$\% \text{ free isotope} = (1 - ((C-C_d)/C)) \times 100.$$

Dialysis is not an accurate method of determining the amount of free isotope. Serum contains protein which attract water into the bag from oncotic their pressure. This dilutes the dialysed serum so that the scintillation counts (Cd) are less than they should be. One can estimate the amount of dilution by weighing the serum in the dialysis bag before and after dialysis. The weight-gain was found to range from 5 - 10% of the total weight, so one may assume that there is a 5 - 10 % overestimation in the amount of free isotope determined by this method. A more accurate method of determining the amount of free isotope in serum samples would be by gel filtration (see below).

Appendix E
Estimation of % isotope bound to protein by Gel Filtration.

Gel filtration of serum was the preferred method determining the amount of free isotope in serum samples and was used when a detailed analysis was required. Fresh Sephadex G-50 columns were made each day. Two grams of Sephadex G-50 beads (Pharmacia Fine Chemicals, Uppsala) were mixed with 40 mls of sterile PBS and kept at room temperature for 12 hours. The reconstituted beads expanded to form 20 mls of gel which was poured into a suitable column and washed with PBS. The column was washed with 30 mls of PBS before the (20 - 200 µl)[1] serum sample was applied. The sample was eluted with 30 mls of PBS and collected in 15 fractions. The columns was washed with a further 20 mls of PBS before being used again. The absorbence at 280 Å of fractions taken from a typical Sephadex G-50 column is shown below.

Figure E.1: The protein concentration (absorbence at 280Å) in 15 fractions from a G-50 column. Serum protein was confined to the first seven fractions.



1 depending on the isotope activity of the serum

Fractions were collected in scintillation vials so that the radioactivity could be counted in a gamma counter. The percentage of the total isotope activity that was free (not protein bound) is given by the following expression,

$$\frac{\text{Sum of the Counts in Fractions 8 to 15}}{\text{Sum of the Counts in Fractions 3 to 7.}} \times 100$$

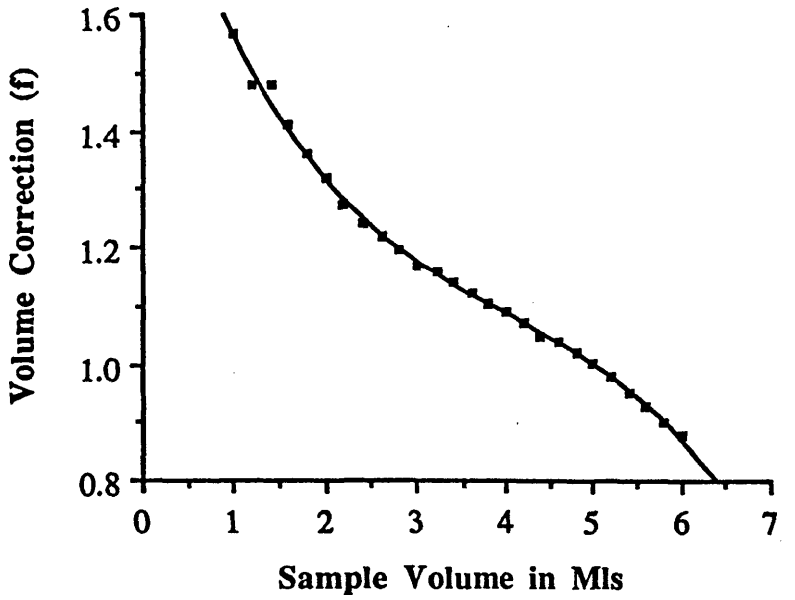
Appendix F

Measurement of blood ^{90}Yttrium activity.

The ^{90}Y activity in the blood of patients receiving ^{90}Y labelled MAb was measured in the following may. Blood samples were collected at 12 hourly intervals for five days after RIT. The samples were put into pre-weighed tubes containing EDTA and stored at 4 ^{o}C. The sample volume was calculated by reweighing the tubes and dividing the weight difference by 1.05, the weight in grams of one ml of blood [I.C.R.U. 1975].

The ^{90}Y activities in the blood samples were measured in a scintillation well counter and compared with those from 5 ml standard solutions of known ^{90}Y activity. Although the volume of the standard ^{90}Y solution was five ml, the volume of the blood samples ranges from three to five ml. The number scintillation counts obtained from a given activity of ^{90}Y was found to be more dependant on the sample volume (V_s) than ^{131}I. Volume correction factors were determined by counting the same activity of ^{90}Y in increasing volumes of fluid. The correction factors (called f) were normalized to a five ml volume and are illustrated below.

Figure F.1: Volume Correction Factors for the Scintillation Counts.



Using the blood sample to determine the ^{90}Y activity in the whole circulation, requires an estimate of the patients blood volume. This was done by applying Hurley's formula [Hurley, 1975] to the each patients surface area. The surface area of a patients was taken to be $Wt^{0.425}.(H^{0.725} \times 0.007184)$, where Wt is the patient's weight in Kg and H the height in cm [DuBois & DuBois, 1916].

The ^{90}Y activity in a patients circulation at time t after the i.p. infusion of ^{90}Y labelled MAb was

$$A' = [(C_s/f)/C_{st}] \times [V_w/V_s] \times A_{st}$$

where A' was the ^{90}Y activity in patients whole blood volume at time t, A_{st}[1] and C_{st} were the ^{90}Y activity and the number of scintillation counts in the five mm standard solution, V_s and C_s the volume were the number of scintillation counts from blood sample and V_w was the patients estimated blood volume [Hurley, 1975].

A' is the total ^{90}Y activity in the blood assuming that no radioactive decay occur. This is a useful assumption if one wishes to study the biological behaviour of the monoclonal antibody rather than the physical characteristics of the isotope. However, when one wishes to use the blood ^{90}Y activity to calculate the radiation dose to organs then the true (not decay corrected) ^{90}Y activity was required. The true blood ^{90}Y activity at t hours after the i.p. infusion of ^{90}Y labelled MAb was

$$A = A' \text{Exp}((t / T) \times 0.693))$$

where A was the true ^{90}Y activity at time t and A' would have been the ^{90}Y activity had no radioactive decay occurred since the time of the i.p. injection. The half life of ^{90}Y (T) is 64.2 hours.

Ideally one would wish to determine each patients individual blood volume rather than estimate it from a patients surface area. Hurley [1975] found that a patients blood volume was closely related to their surface area, which is routinely measured in oncological patients. Although a more precise method of determining whole blood ^{90}Y activity would be preferable, much greater approximations were made in calculating the radiation dose to individual organs (see below).

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1 The was the I-131 activity that the standard solution of I-131 would have had at the time of the i.p. infusion of I-131 labelled MAb.

Appendix G

Measurement of urine ^{90}Yttrium activity.

The ^{90}Y activity in the urine was determined by the following method. Patients were instructed to store all their urine in graduated two litre containers so that the total urine volume (to the nearest 50 mls) could be measured. Each day a five ml aliquot was taken from the previous day's container so that the urine could be discarded[1]. Two ml of urine were taken from the aliquot and placed into sealed scintillation vial. The ^{90}Y activity in the urine sample was measured by a scintillation well counter, against two ml 'standard' solutions containing known activities of ^{90}Y. The ^{90}Y excretion for each day was calculated to be,

$$A' = [(C_s/C_{st}] \times [V_w/2] \times A_{st}$$

A' is the total ^{90}Y activity in the blood assuming that no radioactive decay occur. Expressing decay corrected ^{90}Y activity is appropriate if one wishes to describe the biological behaviour of the monoclonal antibody rather than the physical characteristics of the isotope. The true and not the decay corrected ^{90}Y activity is required however when the blood ^{90}Y activity is used to calculate the radiation dosimetry to organs. The true blood ^{90}Y activity can be calculated from the decay corrected activity by

$$A = A' \; 0.693\, t / T$$

where A was the true ^{90}Y activity at time t and A' would have been the ^{90}Y activity had no radioactive decay occurred since the time of the i.p. injection. The half life of ^{90}Y (T) is 64.2 hours.

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1 It was necessary to discard large Y-90 activities quickly to reduce the radiation exposure to medical and nursing staff.

Ideally one would wish to determine each patients individual blood volume rather than estimate it from a patients surface area. Hurley [1975] found that a patients blood volume was closely related to their surface area, which is routinely measured in oncological patients. Although a more precise method of determining whole blood ^{90}Y activity would be preferable, much greater approximations were made in calculating the radiation dose to individual organs (see below).

Appendix H

Estimation of the radiation dose to bone marrow from radiolabelled MAb in the vascular compartment.

The bone marrow may receive a radiation dose from radiolabelled MAb from several source organs. If the radiolabel is a pure β emitter then these source organs must be within the β particle range (i.e 2 mms for ^{131}I and 11 mms for ^{90}Y). As the marrow is surrounded by bone which has a high density the marrow can only be irradiated by β-particles from it's immediate environment. Therefore bone marrow may receive a radiation dose from the two sources.

1 From isotope in the bone.

2 From isotope in the bone marrow

The bone marrow can be divided into yellow and red marrow. The red marrow is the part of the bone marrow that is actively involved in haemopoiesis and consists of a framework of phagocytic cells, connective tissue cells, haemopoietic cells and small nodules of lymphoid tissue in a bed of extremely vascular sinusoids. These sinusoids freely connect with the circulation with an immediate exchange of macromolecules between the two organs [Trabowitz et al., 1964, Bigler, 1975]. Radiolabelled MAb in the systemic circulation will have immediate access to the bone marrow circulation and I have assumed that the concentration of radiolabelled MAb in these two organs is the same. The radiolabel however may not just be confined to the bone marrow circulation. The phagocytic cells in the marrow are part of the reticuloendothelial system, a known known source of MAb catabolism [Rosenblum et al., 1985, Hnatowhich et al., 1985, Halpern et al., 1985, Eger et al., 1987]. If the radiolabel was concentrated in the marrow RES then this may be an important additional radiation source.

If the radiolabel emits gamma rays then the bone marrow will also receive a radiation dose from the rest of the body. Unless the gamma rays are of low energy (not very penetrating) this is a small fraction of the total radiation dose (10% with ^{131}I). Even if the isotope is a pure β emitter (^{90}Y) then the marrow will still receive a small total body radiation dose from bremstrahlung X-rays. The haemopoietic bone marrow will receive a radiation dose from the following sources,

1 From the radiolabel in the marrow circulation

2 From radiolabel in the marrow reticuloendothelial system

3 From radiolabel in bone

4 From radiolabel in the rest of the body (either gamma radiation or bremstrahlung X-rays).

It is very difficult to measure the contribution each of these radiation sources makes to the bone marrow's total radiation dose. Ideally one would take serial bone and bone marrow biopsies from representative areas after RIT. Alternatively one may wish to use dynamic bone and bone marrow gamma camera scintigraphy and calculate the isotope activity in these organs. Unfortunately the first is impractical and the second not possible with the large isotope activities used in RIT. One can however easily measure the isotope activity in the blood and try to estimate the radiation doses from this.

I have chosen to use the MIRD formulation [Synder et al., 1978] to estimate the radiation dose the bone marrow received from the marrow circulation and the rest of the body. The MIRD formulation had been criticized for radiation dose calculations in RIT

[Kwok et al., 1985] because it does not take into account the non-uniform distribution of isotope in soft tissue. Even when the isotope is evenly distributed there may be large errors in the calculated radiation dose that depend on the assumptions made in its calculation [Wooten, 1983]. The complex geometry of the soft tissue-bone intermixture make calculations of absorbed dose to bone marrow extremely difficult [Eckermann, 1985] and the radiation doses calculated by the MIRD formulation would be a gross simplification. Despite these reservations, I have chosen to use the MIRD formulation to estimate the marrow radiation dose, as this is still the standard method of calculating the radiation dose to organs from internal radio-isotopes and there is no better generally accepted method available.

I have assumed that the bone marrow only receives a radiation dose from two source organs, the bone marrow circulation and the rest of the body (the latter is ignored with pure β emitters). I have chosen to ignore the important contribution from the bone and marrow RES as I cannot measure the isotope activity in these tissues. These tissues may however be very important when radiometalic isotopes such as ^{90}Y are used. Haemopoietic marrow accounts for 2.2% of the total body weight of an average women [I.C.R.U., 1975] i.e about 30% of the weight of the blood volume. I have assumed, quite arbitrarily, that the marrow has 20% of the radiolabel isotope activity of the rest of the blood volume. The radiation dose the bone marrow receives from the two radiation sources according to the MIRD formulation would be

$$\{S_{[mar'mar]} \times 0.2\,A\} + \{S_{[wb'mar]} \times A\} \quad cGy$$

$S_{[mar'mar]}$ is the S value for haemopoietic marrow as a radiation source and target organ, $S_{[wb'mar]}$ is the S value for the whole body as a radiation source and the marrow as a target organ, and A is the integrated isotope activity over the time of

radiation (μCi.hrs). Although the contribution from bone $\{S_{[bone'mar]} \times A^*\}^1$ and the marrow reticuloendothelial system $\{S_{[mar'mar]} \times 0.2\ A^{**}\}^2$ are ignored, their importance may be extrapolated by the degree of unexpected myelosuppression associated with the radiation dose calculated from the MIRD formulation.

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1 A^* is the integrated isotope activity in bone

2 A^{**} is the integrated isotope activity in the marrow RES.

Appendix I

Mann Whitney U Test for the comparison of non-parametric data.

A Mann-Whitney U test (Mann & Whitney, 1947) was used to compare the medians of two populations this thesis as there was no evidence that any of the populations were normally distributed. The calculations were assisted by a computer program called Statsview + (distributed by Brainpower Inc, California). Briefly the observations from two populations are combined and ranked in numerical order. The sum of the ranks of one of the populations was taken (which population is arbitrary) and a test statistic calculated from

$$T = S - \frac{n(n+1)}{2}$$

where T was the test statistic, S was the sum of the ranks and n was the number of observations in the population. The significance of T (called U by the statistics program) was determined from standard statistical tables. Where there were more than 20 observations the significance of z was determined from the critical values of a unit normal distribution where

$$z = \frac{T - \frac{mn}{2}}{\sqrt{\frac{nm(n+m+1)}{12}}}$$

The terms n and m are the number of observations in the two populations being compared.

Appendix J
Separation of Human Antimouse Immunoglobulin Antibody (HAMA) from human serum using affinity columns chromatography.

The separation of HAMA from human serum was done in collaboration with Greg Sivolapenko at the Department of Immunology, Royal Postgraduate Medical School, DuCane Road.

Patients who had received previous intraperitoneal RIT and were known to have high titres of HAMA[1] were asked to donate 100 to 200 mls of blood. All blood samples were screened (with informed consent) for antibodies to Hepatitis B and Human Immunodeficiency viruses[2]. Serum was separated from the blood using an aseptic technique and stored at a temperature of - 20 ^{o}C until use. Anti-HMFG1 antibodies were isolated from the serum samples using an affinity chromatography column , where HMFG1 was coupled on CNBr-activated Sepharose 4B beads (Pharmacia, Sweden). Five grams of dry beads (to make 17.5 mls gel) were washed with one litre of 1mM HCL, and then mixed on a rotator with 10 mgs of HMFG1 in coupling buffer (0.1 M NaHCO3, 0.5 M NaCl, PH 8.5) for 2 hrs at room temperature. The mixture was then transferred to the blocking buffer (1 M Ethanolamine, PH 8.0), and incubated for a further 2 hrs at room temperature. Finally the gel was washed with coupling buffer followed by 1 M ammonia and Phosphate buffered Saline (PBS, PH 7.4). The patient's serum was applied and the column washed with PBS. The anti-HMFG1 antibodies were eluted off using a molar solution of ammonia. Fractions were collected, and the ones containing HAMA (determined by absorption at 280 nm) were pooled together, and dialysed against PBS as soon as possible. Finally the anti-HMFG1 antibodies were filtered with a sterile millipore filter (Millipore, France), and kept at 4 ^{0}C until use. All samples were tested for sterility and pyrogenicity[3]. Fast Protein Liquid Chromatography (FPLC) analysis, using Superose 6 column

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1 Routine ELISA assays for HAMA were done routinely on all patients after RIT on weeks 1 to 8 and then 3 monthly.

2 These tests were done by the Department of Virology, Hammersmith Hospital.

3 By Blood Products Laboratory, Elstree, England.

(Pharmacia, Sweden), of the purified HAMA revealed that over 90% of the antibody was IgG. This was also biologically confirmed, when the immunoreactivity of the antibodies was tested by an enzyme linked immunosorbant assay (ELISA). Purified HAMA recognized HMFG1 almost as well as its F(ab)'2 fragment. The small portion of HAMA that recognized the Fc part of the MAB did not seem to cross-react with the Fc part of human IgG.

Anderson, W.T., & Strand, M., (1987). Radiolabeled antibody: Iodine versus radiometal chelates. NCI Monogr. 3: 149-151.

Anderson-Berg, W.T., Squire, R.A., & Strand, M., (1987). Specific radioimmunotherapy using Y-90 labeled monoclonal antibody in erythroleukimic mice. Cancer Res., 47: 1905-1912.

Appelbaum, F.R., Badger, C.C., Deeg, H.J., Nelp, W.B., & Storp R., (1987). Use of Iodine-131-labeled anti-immune response-associated monoclonal antibody as preparative regiman prior to bone marrow transplantation: Initial dosimetry. N.C.I. Monogr. 3: 67 - 71.

Arklie, J., Taylor-Papadimitriou, J., Bodmer, W.F., Egan, M., Millis, R., (1981). Differentiation antigens expressed by epithelial cells in lactating breast are also detectable in breast cancer cells, Int. J. Cancer. 28: 23 - 29.

Badger, C.C., Krohn, K.A., Peterson, A.V., Shulman, H., & Berstein, I.D., (1985). Experimental radiotherapy of murine lymphoma with ^{131}I labeled anti-thy 1.1 monoclonal antibody. Cancer Res. 45: 1536 - 1544.

Bakri, Y.N., Given, F.T., Peeples, W.J., Frazier, A.B., (1984). Complications from intrperitoneal radioactive phosphorous in ovarian malignancies. Gynecol. Oncol. 21: 292 - 299.

Bale, W.F., Spar, I.L., Goodlan, R.L., (1960). Experimental radiation therapy of tumours with ^{131}I carrying antibodies to fibrin. Cancer Res. 20: 1488 - 1494.

Becker, N., Frentzel-Begme, R., Wagner, G., (1984). "In", Ovarian Cancer, Atlas of Cancer Mortality in the Federal Rebublic of Germany. Berlin, Springer-Verlag. 2nd Edition.

Begent, R.H., Bagshawe, K.D., Pedley, R.B., Searle, F., Ledermann, J.A., Green, A.J., Keep, P.A., Chester, K.A., Glaser, M.G., Dale, R.G., (1987). Use of a second antibody in radioimmunotherapy, NCI Monogr. (3): 59-61.

Begent, R.H.J., Green, A.J., Bagshawe, K.D., Jones, B.B., Keep, P.A., Searle, F., Jewkes, R.F., Barret, G.M., & Ryman, B.E., (1982). Liposomally entrapped antibody in cancer radioimmunotherapy detection. Lancet ii: 739 - 742.

Benua, B.S., Cicale, N.R., Sonenberg, M., Rawson, R.W., (1962). The relation of radioiodine dosimetry to results and complications of metastatic thyroid cancer. Am. J. Roentgenol. 87: 171 - 182.

Beral, V., (1987). The epidemiology of ovarian cancer. "In", Ovarian Cancer - The Way Ahead. Published by the Royal College of Obstetricians & Gynaecologists (1987). Editors: Sharp, F., & Souter, W.P., pages 21 - 31.

Bigler, R.E., Zannsonico, P.B., Leonard, R., Cosma, M., Primus, F.J., Alger, E., DeJager, R., Stowe, S., Ford, E., Brennan, K., and Goldenberg, D.M., (1985). Bone marrow dosimetry for monoclonal antibody therapy. In , Fourth International Radiopharmaceutical Dosimetry Symposium, Schlafke-Stelson E.D., Watson, E.E., (Eds.). Oak Ridge Assoc Univ. Publications, pp. 514-534.

Bland, M.J., Greager, J.A., Atcher, R.W., Brown, J.M., Brechbiel, M.W., Gansow, O.A., and Das Gupta, T.K., (1988). Improved sarcoma imaging and

reduced hepatic activity with indium-111-SCN-BZ-DTPA linked to MoAb 19-24. J. Nucl. Med. 29: 1810-1816.

Blackledge, G., Lawton, F., Luesley, D., Redman, C., Spooner, D., Latief, D., Mould, J., Hilton, C., Chan, K.K., (1987). The relative merits of chemotherapy and radiotherapy following debulking chemotherapy. In Ovarian Cancer - The Way Ahead, Ed Sharp, F., Soutter, W.P. (Eds.). London, The Chameleon Press Ltd. , pp. 427-441.

Bomanji, J., Nimmon, C.C., Hungerford, J.L., Gransowska, S.M., & Britton, K.E., (1988). Ocular radioimmunoscintigraphy: sensitivity and practical considerations. J.Nucl.Med 29: 1038 - 1044.

Boye, E., Lindegaard, M.W., Paus, E., Skretting, A., Davy, M., and Jakobsen, E., (1984). Whole-body distribution of radioactivity after intraperitoneal administration of ^{32}P colloids. Birt. J. Radiol. 57: 395 - 402.

Bradwell, A.R., Vaughan, A., Fariweather, D.S., & Dykes, P.W., (1983). Letter to the Edititor: Improved immunodetection of tumours using a second antibody. Lancet i: page 247.

Breitenstein, B.D., (1983). Hanford americum exposure incident: Medical management and chelation therapy. Health Physics 45: 855-866.

Brenner, D.E., (1986). Intraperitoneal chemotherapy: A reveiw. J. Clin. Oncol. 4: 1135 - 1147.

Buchsbaum, D., Randall, B., Hanna, D., Chandler, R., Lohen, M., Johnson, E., (1985). Comparison of the distribution and fate of monoclonal antibodies labeled with 131-iodine or 111-indium, Eur. J. Nucl. Med. 10: 398-402.

Burchell, J., Durbin, H., & Taylor-Papadimitriou, J., (1983). Complexity of expression of antigenic determinants recognised by monoclonal antibodies HMFG1 AND HMFG2 in normal and malignant human mammary epithelial cell. J. Immunol. 131: 508 - 513.

Burzon, G., Mayr, A.C., Langer, M., Becker, R., Cordes, M., Zwicker, C., Koppenhager, K., & Felix, R., (1989). Radioimmunoscintigaraphy of ovarian cancer with I-131 labeled OC-125 antibody fragments. Eur. J. Nucl. Med. 15: 42 - 48.

Carrasquillo, J.A., Sugarbaker, P., Colcher, D., Reynolds, J.C., Estebanm J., Bryant, G., Perentesis, P., Yokoyama, K., Rotman, M., Schlom, J., & Larson, S.M., (1988a). Peritoneal carcinomatosis: Imaging with intrperitoneal injection of 131-I-labeled B27.3 monoclonal antibody. Radiol. 167: 31 - 40.

Carrasquillo, J.A., Sugarbaker, P., Colcher, D., Reynolds, J.C., Estebanm J., Bryant, G., Keenan, A.M., Perentesis, P., Yokoyama, K., Simpson, D.E., Ferroni, P., Farkas, R., Schlom, J., & Larson, S.M., (1988b). Radioimmunoscintiigraphy of colon cancer with iodine-131 -labeled B72.3 monoclonal antibody. J Nucl. med. 29: 1022 - 1030.

Carrasquillo, J.A., Abrams, P.G., Schroff, R.W., Reynolds, J.C., Woodhouse, C.S., Morgan, A.C., Keenean, A.M., Foon, K.A., Perentesis, P., Marshall, S., Horowitz, M., Szmendera, J., Englert, J., Oldham ,R.K., and Larson, S.M., (1988c). Effect of antibody dose on imaging and biodistribution of indium-111 9.2.27 anti-melanoma monoclonal antibody. J.Nucl.Med. 29: 39 - 47.

Carrasquillo, J.A., Mulshine, J.L., Bunn, P.A., Reynolds, J.C., Foon, K.F., Schroff, R.W., Perentesis, P., Steis, R.G., Keenan, A.M., Horowitz, M., & Larson, S.M., (1987). Indium-111 T101 monoclonal antibody is superior to iodine-131 T101 in imaging of cutaneous T-cell lymphoma. J. Nucl. Med. 28: 281 - 287.

Carrasquillo, J.A., Krohn, J.A., Beaumier, P., et al., (1984). Diagnosis of and therapy for solid tumors with radiolabeled antibdoies and immune fragments. Cancer Treat. Rep. 68: 317-328.

Chantal, J.C., Fumoleau, P., Saccavini, J., Thedrez, P., Curtet, C., Bianco-Arco, A., Chetanneau, A., Peltier, P., Kremmer, M., & Guillard, Y., (1987). Immunoscintigraphy of recurrences of gynecologic carcinomas. J. Nucl. Med. 28: 1807 - 1819.

Colcher, D., Esteban, J., Carrasquillo, J.A., Sugarbaker, P., Reynolds, J.C., Bryant, G., Larson, S.M., Schlom, J., (1987). Complementation of intracavitary and intravenous administration of a monoclonal antibody (B72.3) in patients with carcinoma. Cancer Res. 47: 4218 - 4224.

Cole, W., De Nardo, S., Meares, C., McCall, M.J., DeNardo, G.L., Epstein, A.L., O'Brien, H.A., & Moi, M.K., (1987). Comparative serum stability of radiochelates for antibody radiopharmaceuticals. J Nucl Med. 28: 83 - 90.

Conte, P.F., Bruzzone, M., Chiara, S., Sertoli, M.R., Daga, M.G., Rubagotti, A., Conio, A., Ruvulo, M., Rosso, R., Santi, L., Carnino, F., Cottini, M., Mossetti, C., Guercio, E., Gatti, M., Silquini, P.N., Prelato, M.L., Durando, C., Giaconne, G., Calciati, A., Farini, D., Centonze, M., Rugiati, S., Parodi, G., Messineo, M.,

Storace, A., Bernardini, G., Misurali, F., Alessandri, S., Casini, M., Ragni,N., Fogia,G., Bentivoglio, G., and Pescetto, G., (1987). A randomized trial comparing cisplatinum plus cyclophosphamide versus cisplatinum, doxorubicin and cyclophsophamide in advanced ovarian cancer. J.Clin. Oncol. 6: 965-971.

Courtenay - Luck, N.S., Epenetos, A.A., Sivolapenko, G.B., Larche, M., Barkans, J.R., & Ritter, M.A., (1988). Developemnt of anti-idiotype antibodies against tumour antigens and autoantigens in ovarain cancer patients treated intraperitoneally with monoclonal antibodies. Lancet (ii): 894 - 897.

Courtenay - Luck, N., Epenetos, A.A., Larche, M., Moore, M., Pectascides, D., Dhokia, B., Ritter, M.H.: Development of primary and secondary immune responses to mouse monoclonal antibdodies used in the diagnosis and therapy of malignant neoplasms. Cancer Res. 46: 6489-6493, 1986.

Currie, J.L., Bagne, F., Harris, C., Sullivan, D.L., Surwit, E.A., Wilkinson, R.H., Creasman, W.T., (1981). Radioactive chromic phosphate absorption, and effective therapeutic radiation in phantoms, dogs and man. Gynecol. Oncol. 12: 193 - 218.

Day, E.D., Planinsek, J.A., Pressman, D., (1959). Localization of radiolabeled rat fibrinogen in trasplanted rat tumoursJ Natl Cacner Inst 23: 799 - 812.

Dedrick, R.L., Myers, C., Bungay, p., et al., (1978). Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat. Rep. 62: 1-11.

Dembo, A.J., Abdominopelvic radiotherapy in ovarian cancer: A 10 - year experience. Cancer. 55: 2285-2290, 1985.

Dembo, A.J., Bush, R.S., Beale, F.A., Bean, H.A., Pringle, J.F., & Sturgeon, J.F., (1979). The Princess Margaret Hosptial study of ovarian cancer: Stages I, II, and asymptomatic III presentations. Cancer Treat. Rep. 63: 249 - 254.

DeNardo, S.J., DeNardo, G.L., O'Grady, L.F., Levy, N.B., Mills, S.L., Macey, D.L., McGahan, J.P., Miller, C.H., & Epstein, A.L., (1988). Pilot studies of radioimmunotherapy of B cell lymphoma and leukemia using I-131 Lym-1 monclonal antibody. Antibody Immunoclonjugates and Radiopharmaceuticals 1: 17 - 33.

Department of Health and Social Security: InThe State of The Public Health for the year 1986. London, HMSO Publications, 1987.

Deshpande, S.V., DeNardo, S.J., Meares, C.F., McCall, M.J., Adams, G.P., Moi, M.K., & DeNardo, G.L., (1988). Copper-67-labeled monoclonal antibody Lym-1, a potential radiopharmaceutical for cancer therapy: labeling and biodistribution in RAJI tumored mice. J.Nucl. Med. 29: 217 - 225.

Dillman, R.O., Beauregard, J., Ryan, K.P., Hagan, P.L., Clutter, M., Amox, G.S., Burnett, K.G., David, G.S., Carlo, D.J. & Halpern, S.E., (1987). Radioimmunodetection of cancer with the use of indium-111-labeled monoclonal antibodies. NCI Monogr 3: 33 - 36.

Dobyns, B.S., Maloof, F., (1951). The study and treatment of 119 cases of carcinoma of the thyroid with radioactive iodine. J. Clin. Endocrin. 11: 1323 - 1360.

Early, J.F., Schroff, R.W., Abrams, P.G., Fitzberg, A.R., Morgan, A.C., Kasina, S., Reno, J.M., Srinivasan, A., Woodhouse, C.S., Wlibur, D.S., Natale, R.B.,

Collins, C., Stehlin, J.S., Mitchell, M., & Nelp, W.B., (1989). Sucessful imaging of malignant melanoma with technetium-99m-labeled monoclonal antibodies. J. Nucl. Med. 30: 25 - 32.

Eckerman, K. F.: Aspects of the dosimetry of radionuclides within the skeleton with particular emphasis on the active marrow, In , Fourth International Radiopharmaceutical Dosimetry Symposium, Schlafke-Stelson E.D., Watson, E.E., (Eds.). Oak Ridge Assoc Univ. Publications, 1985, pp. 514-534.

Endo, K., Sakahara, H., Nakashima, T., & Koizumi, M., (1987). Preparation and properties of antitumor monoclonal antibodies labeled with metallic radionuclides indium-111, gallium-67, and technetium-99m. NCI Monogr. 3: 135 - 140.

Epenetos, A.A., Munro, A.J., Stewart, J.S.W., Rampling, R., Lambert H.E., McKenzie, C.G., Souter, P., Rahemtulla, A., Hooker, G., Sivolapenko, G.B., Snook, D., Courtenay-Luck, N., Dhokia, B., Krauk, T., Bodmer, W.F., (1987). Antibody-guided irradiation of advanced ovarian cancer with intraperitoneally administered radiolabeled monoclonal antibodies. J.Clin.Oncol. 5: 1890 - 1899.

Epenetos, A.A., Carr, D., Johnson, P.M., Bodmer, W.F., and Lavender, J.P., (1986a). Antibody-guided radiolocalisation of tumours in patients with testicular and ovarian cancer using two radioiodinated monoclonal antibodies to placental alkaline phosphatase. Brit. J. Radiol. 59: 117 - 125.

Epenetos, A.A., Snook, D., Durbin, H., Johnson, P.M., and Taylor-Papadimitriou, J., (1986b). Limitations of radiolabeled monoclonal antibodies for localization of human neoplasms. Cancer Res. 46: 3183 - 3191.

Epenetos, A.A., Hooker, G., Krausz, T., Snook, D., Bodmer, W.F., & Taylor-Papadimitriou, J., (1986c). Antibody-guided irradiation of malignant ascites in ovarian cancer: A new therapeutic method possessing specificity against cancer cells. Obstet. Gynecol. 68: Suppl. 3, 71s-74s.

Epenetos A.A., Hammersmith Hospital Oncology Group and Imperial Cancer Research Fund, (1984). Antibody-guided irradiation of malignant lesions: Three cases illustrating a new method of treatment. Lancet, ii: 1441 - 1443.

Epenetos, A.A., Nimmon, C.C., Arklie, J., Elliott, A.T., Hawkins, L.A., Bodmer, W.F., (1982). Detection of human cancer cells in an animal model using radiolabeled tumor associated monoclonal antibdodies. Br. J. Cancer. 46: 1 - 8.

Esteban, J.M., Schlom, J., Gansow, O.A., Atcher, R.W., Brechbiel, M.W., Simpson, D.E., & Colcher, D., (1987). New method for the chelation of indium-111 to monoclonal antibodies: Biodistribution and imaging of athymic mice bearing human colon carcinoma xenografts. J. Nucl. Med 28: 861 - 870.

Ettinger, D.S., Order, S.E., Wharam, M.D., Parker M.K., Klein, J.L., and Leichner, P.K., (1982). Phase I-II study of isotopic immunoglobulin therapy for primary liver cell cancer. Cancer Treat. Rep. 66: 289 - 297.

Fawwaz, R.A., Wang, T.S.T., Estabrook, A., Rosen, J.M., Hardy, M.A., Alderson, P.O., Srivastava, S.C., Richards, P., and Ferrone, S., (1985). Immunoreactivity and biodistribution of indium-111 labelled monoclonal antibody to a high molecular weight melanoma associated antigen. J. Nucl. Med. 26: 488 - 492.

Fawwaz, R.A., Wang, T.S.T., Srivatava, S.C., Rosen, J.M., Ferrone, S., Hardy, M.A., and Alderson, P.O., (1984). Potential of palladium-109-labeled antimelanoma monoclonal antibody for tumour therapy. J. Nucl. Med. 25: 796 - 799.

Fraker, P.J., Speck, J.C., (1978). Protein and cell membrane iodination with sparingly soluble chloramide. 1, 3, 4, 6 - tetracloro-5, 6-diphenyl glyceril. Biochem. Biophys. Res. Commun. 80: 849 - 854.

Fritzberg, A.R., Abrams, P.G., Beumie, P.L., Kasma, B., Morgan, C., Rao, T.N., & Reno, J.M., Sanderson JA, Srinivasen A, Wilbar DS, Vanderheyden J-L., (1988) Specific and stable labelling of antibodies with technetium-99m with a diamide dithiolate chelating agent. Proc. Natl. Acad. Sci. USA 85: 4025 - 4029.

Fuller, D.B., Sause, W.T., Plenk, H.P., & Menlove, R.L., (1987). Analysis of postoperative radiation therapy in Stage I through III epithelial cancinoma. J. Clin. Oncol. 5: 897 - 905.

Ghose, T., Norvell, S.T., Aquino, J., Belitsky, P., Tai, J., Guclu, A., & Blair, A.H., (1980). Localization of 131I-labeled antibodies in human renal cell carcinomas and in a mouse hepatoma and correlation with tumor decection by photoscanning. Cancer Res. 40: 3018 - 3031.

Goldberg, N., & Peschel, R.E., (1988). Postoperative abdominopelvic radiation therapy for ovarian cancer. Int. J. Radiat. Oncol. Biol. Phys. 14: 425 - 429.

Goldenberg, D.M., Sharkey, R.M., and Ford, E., (1987). Anti-antibody enhancement of iodine-131 anti-CEA radioimmunodetection in experimental and clinical studies. J. Nucl. Med. 28: 1604 - 1610.

Goldenberg, D.M., Gaffar, S.A., Bennett, S.J., & Beach, L.J., (1981). Experimental radioimmunotherapy of a xenografted human colonic tumor (GW-39) producing carinoembryonic antigen. Cancer Res. 41: 4354 - 4360.

Goldenberg, D.M. Deland, F., Kim, E., Bennet, S., Primus, S.J., van Nagell, J.R., Estes, N., DeSimone, P., and Rayburn, P., (1978). Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. New Engl. J. Med., 298: 1384 - 1388.

Goldenberg, D.M., Preston, D.F., Primus, J., and Hansen, H.J., (1974). Photoscan localization of GW-39 tumors in hamsters using radiolabeled anticarcinoembryonic antigen immunoglobulin G. Cancer Res. 34: 1 - 9.

Goldhirsh, A., Greiner, R., Drehor, E., Sessa, C., Kraner, F., Forni, M., (1988). Treatment of advanced ovarian cancer with surgery, chemotherapy, and consolidation of response by whole abdominal radiotherapy. Cancer 62: 40-47.

Gould, P., & Freidman, S.O., (1965). Specific carcinoembryonic antigens of the human digestive system. J. Exp. Med. 122: 467 - 481.

Gruppo Interregionale Cooperativo Oncologica Ginecologica, (1987). Randomised comparison of cisplatinum with cyclophosphamide/cisplatinum and with cisplatinum / cyclophosphamide / adriamycin in advanced ovarian cancer. Lancet. ii: 353 - 359.

Hacker, N.F., Berek, J.S., Lagasse, L.D., Nieberg, R.K., & Elashoff, M., (1983) Primary cytoreductive surgery for epithelial ovarian cancer. Obstet. Gynecol. 61: 413 - 420.

Hagan, P.L., Halpern, S.E., Dillman, R.O., Shawler, D.L., Johnson, D.E., Chen, A., Krisman, L., Frinke, J., Bartholemew, R.M., David, G.S., Carlo, D., (1986). Tumor size: Effect on monoclonal antibody uptake in tumor models. J. Nucl. Med. 27: 422 - 427.

Hainsworth, J.D., Malcolm, A., Johnson, D.H., Burnett, L.S., Jones III, H.W. & Greco, A. (1983) Advanced minimal residual ovarian carcinoma: abdominopelvic irradiation following combination chemotherapy. Obstet. Gynecol. 61: 619 - 624.

Haisma, H.J., Mosely, K.R., Battaile, A., Griffiths, T.C., & Knapp, R.C., (1988). Distribution and pharmacokinetics of radiolabeled monoclonal antibody OC 125 after intravenous and intraperitoneal administration in gynecologic tumors. Am. J. Obstet. Gynecol. 159: 843 - 848.

Hall, E.J. (1978). Acute Effects Of Whole Body Irradiation. In Radiobiology for the Radiologist (2nd Edition), Harper & Row Publishers, Philadephia, USA, page 214.

Halpern, S.E., Hagan, P.L., Garver, P.R., (1983). Stability, characterisation and kinetics of ^{111}In labeled monoclonal anti-tumor antibody in normal animals and nude mouse human xenografts tumors. Cancer Res. 43: 5347 - 5355.

Harrison, A., & Royle, L. (1987). Efficacy of astatine-211-labeled monoclonal antibody in treatment of murine T-cell lymphoma. N.C.I. Monogr. 3: 157-158.

Heath, R., Rosenman, J., Varia, M., & Walton L., (1988). Peritoneal fluid cytology in endometrial cancer: its significance and the role of chromic phophate (^{32}P) therapy. Int. J. Radiat. Oncol. Biol. Phys. 15: 815 - 822.

Hine, K.R., Bradwell, A.R., Reeder, T.A., Drolc, Z., & Dykes, P.W., (1980). Radioimmunodetection of gastrointestinal neoplams with antibodies to carcinembryonic antigen. Cancer Res 40: 2993 - 2997.

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

Hnatowich, D.J., Virzi, F., and Doherty, P.W., (1985a). DPTA-coupled antibodies labeled with yttrium-90. J. Nucl. Med. 26: 503 - 509 .

Horowitz, Y.,S., (1981). The theoretical and micordosimetric basis of thermoluminescence and applications to dosimetry. Phys Med Biol 26: 765 - 824.

Howell, R.W., Rao, D.V., & Sastry, K.S.R., (1989). Macroscopic dosimetry for radioimmunotherapy: Non uniform activity distribution in solid tumours. Med. Phys. 16: 66 - 74.

Humm, J.L.:, (1986). Dosimetric aspects of radiolabeled antibdoies for tumour therapy. J. Nucl. Med. 27: 1490 - 1497.

Hurley, P.J., (1975). Red cell and plasma volumes in normal adults. J.Nucl. Med. 16: 46 - 52.

Hyams, D., Reynolds, J.A., Carrasquillo, J.A., Perentesis, P., Larson, M., Morin, M., Simpon, D., Schlom, J., & Colcher, D. (1987). The effects of circulating anti-murine antibody on the pharmacokinetics and biositribution of injected radiolabeled monoclonal antibodies. J. Nucl. Med. 27: 922-923.

International Commission on Radiological Protection No. 23., (1975). Report of the Task Group on Reference Man. Oxford, Pergamon Press.

Jain, R. K., & Baxter, L.T., (1989). Mechanisms of heterogenous distribution of monocloanal antibodies and other macormolecules in tumors: Significance of elevated interstitial pressure. Cancer Res. 48: 7022 - 7032.

Kaye, S.B., MacClean, A., Cordiner, J., Soukop, M., Kennedy, R., & Duncan, I ., (1987). Chemotherapy for advanced ovarain cancer - sigle agent versus combination chemotherapy. "In", Ovarian Cancer - The Way Ahead. Published by the Royal College of Obstetricians & Gynaecologists (1987). Editors: Sharp, F., & Souter, W.P., pages 323 - 328.

Keenan, A.M., Weinstein, J.N., Carrasquillo, J.A., Bunn, P.A., Reynolds, J.C., Foon, K.A., Smarte, N.C., Ghosh, B., Fekja, R.M., Larson, S.M., and Mulshine, J.L., (1987). Immunolymphoscintigraphy and dose dependence of ^{111}In-labeled T101 monoclonal antibody in patients with cutaneous T-cell lymphoma. Cancer Res. 47: 6093 - 6099.

Khaw, B.A., Cooney, J., Edinton, I., & Strauss, H.E., (1986). Differences in experimental tumor localization of dual labeled monoclonal antibody. J. Nucl. Med. 27: 1293 - 1299.

Kim, E.E., Deland, F.H., Nelson, M.O., Bennet, S., Simmonds, G., Alpert, E., Goldenberg, D.M., (1980). Radioimmunodetection of cancer with radiolabelled antibodies to a-fetalprotein. Cancer Res 40: 3008 - 3012.

Klaassen, D., Starreveld, A., Shelley, W., Miller, A., Boyes, D., Gerulath, A., Levitt, M., (1985). External beam pelvic radiotherapy and intraperitoneal chromic phosphate in early stage ovarian cancer: a toxic combination. Int. J. Radiat. Oncol. Biol. Phys. 11: 1801-1804.

Klein, J.L., Leichner, P.K., Callahan, K.M., Kopher, K.A., and Order, S.E., (1988). Effects of anti-antibodies on radiolabeled antibody therapy. Antibody Immunoconjugates and Radiopharmaceuticals 1: 55 - 64.

Klibanov, A.L., Martynov, A.V., Sliakin, M.A., Sakharov, I.Y., Muzykantov, V.R., Danilov, S.M., & Torchilin, V.P., (1988). Blood clearance of radiolabelled antibody enhancemant by laoctomisation and treatment with biotin-avidin or antimouse IgG antibodies. J. Nucl. Med. 29: 1 951 -1 956.

Kohler, G., Howe, S.C., Milstein, C., (1976). Fusion between immunoglobulin non-secreting myeloma cell lines. Eur J Immunol 6: 292-295.

Kohler, G., Milstein, C., (1976). Derivation of specific antibody producing tissue culture and tumour lines by cell fusion. Eur. J. Immunol. 6: 511-519.

Kolstad, P., Davy, M., & Høeg, K., (1977). Individulaized treatment of ovarian cancer. Am. J. Osbstet. Gynecol. 128: 617 - 625.

Kozak, R.W., Atcher, R.W., Gansow, O.A., Friedman, A.M., Hines, J.J., Waldman, T.A., (1986). Proc. Natl. Acad. Sci. USA 83: 474 - 478.

Larson, S.M., Brown, J.P., Wright, P.W., Carrasquillo, J.A., Hellstrom, I., & Hellstom K.E., (1985a). Imaging of melanoma with I-131 labelled monoclonal antibodies. J. Nucl. Med. 24: 123 - 129.

Larson, S.M., Brown, J.P., Wright, P.W., Carrasquillo, J.A., Hellstom, I., & Hellstrom, K.E., (1983a). Imaging of melanoma with I-131 labeled monoclonal antibodies. J. Nulc. Med 24: 123 - 129.

Larson, S.M., Carrasquillo, J.A., Krohn, J.A., Brown, J.P., McGuffin, R.W., Ferens, J.M., Graham, J.M., Hill, M.M., Beaumier, L.D., Hellström K.E., & Hellström, I., (1983b). Localization of ^{131}I labeled p97 specific Fab fragments in human melanoma as a basis for radiotherapy. J. Clin Invest. 72: 2110 - 2114.

Lashford, L.S., Davies, A.J., Richardson, R.B., Bourne, S.P., Bullimore, J.A., Eckert, H., Kemshead, J.T., & Coakham, H.B., (1988). A pilot study of 131I monoclonal antibodies in the therapy of leptomeningeal tumors. Cancer 61: 857 - 868.

Lashford, L,. Jones, D., Pritchard, J., Gordon, I., Breatnach, F., & Kempstead, J.T., (1987). Therapeutic application of radiolabeled monoclonal antibody UJ13A in children with disseminated neuroblaastoma. N.C.I. Monogr. 3: 53 - 57.

Lee, Y-S., Bullard, D.E., Zalutsky, M.R., Coleman R.E., Wikstrand, C.J., Friedman, H.S., Colapinto, E.V., & Bigner, D.D., (1988a). Therapeutic efficacy of antiglioma mesenchymal extracelluslr matrix ^{131}I-radiolabelled murine monoclonal antibody in a human glioma xenograft model. Cancer Res. 48: 559 - 566.

Lee, Y., Bullard, D.E., Humphrey, P.A., Colapinto, E.V., Friedman, H.S., Zalutsky, M.R., Coleman R.E., & Bigner, D.D., (1988b). Treatment of Intracranial glioma xenografts with ^{131}I-labeled anti-tenascin monoclonal antibody 81C6. Cancer Res. 48: 2904 - 2910.

Lenhard, R.E., Order, S.E., Spunberg, J.J., Aspell, S.O., & Leibel, S.N., (1985). Isotopic immunoglobulin: a new systemic therapy for advanced hodgkins disease. J. Clin. Oncol. 3: 1296 - 1300.

Leichner, P.K., Nai-Chuen, Y., Frenkel, T.L., Loudenslager, B.M., Hawkins, W.G., Klein, J.L., and Order, S.E., (1988). Dosimetry and treatment planning for 90Y-labeled antiferritin in hepatoma. Int. J. Radiat. Oncol. Biol. Phys. 14: 1033 - 1042.

Mach, J.P., Buchegger, F. & Forni, M. (1981) Use of radiolabelled monoclonal anti-CEA antibodies for the detection of human carcinomas by external photoscanning and tomoscintigraphy. Immunol Today 2: 239 - 245.

Mach, J.P., Carrel, S., Forni, M., Ritschard, J., Donath, A., Alberto, P., (1980). Tumor locaization of radiolabelled antibodies against carcino-embryonic antigen in patients with carcinoma. N. Engl. J. Med. 303: 5 - 10.

Mach, J.P., Carrel, S., Merenda, C., Sordat, B., Cerottini, J.C., (1974). In vivo localisation of radiolabelled antibodies to carcinoembryonic antigen in human colon carcinoma grafted into nude mice.Nature 248: 704 - 706.

Malamitzi, J., Skarlos, D., Fotiou, S., Papakostos, P., Aravantinos, G., Vassilarou, D., Taylor-Papadimitriou, J., Koutoulidis, K., Hooker, G., Snook, D., & Epenetos, A.A., (1988). Intracavitary use of two radiolabeled tumor-associated monoclonal antibodies. J. Nucl. Med. 29: 1910 - 1915.

Mann, H.B., and Whitney, D.R., (1947). On a test of whether one or two random variables is stochastically larger than the other. Annals Math. Stats.18: 50 - 60.

Martinez, A., Schray, M.F., Howes, A.E. & Bagshaw, M.A. (1985) Postoperative radiation therapy for epithelial ovarian cancer: The curative role based on a 24-year experience. J Clin Oncol 3: 901 - 910.

Meredith, W.J., & Massey, J.B., (1977). "In" Fundamental Physics of Radiology, 3rd Edition, Year Book Medical Publishers, Chicago, pages 124 - 129.

Miller, A.B., Hoogstraten, B., Staquet, M., and Winkler, A., (1981). Reporting the results of cancer treatment. Cancer 47: 207 - 214.

Miller, R.A., Oseroff, A.R., Stratte, P.T., & Levy, R., (1983). Blood 62: 988-

Moi, M. K., Meares, C.F., and DeNardo, S.J.: The peptide way to macrocycle bifuctional chelating agents: Synthesis of 2-p-nitrobenzyl-1,4,7,10,-tetracyclododecane-N,N',N",N"'-tetraacetic acid, and study of its yttrium (III) complex. J. Amer. Chem. Soc. (In press).

Moldofsky, P.J., Powe, J., Mulhern, C.B., Hammond, N., Sears, H.F., Gatenby, R.A., Stepelwski, Z., & Kolrowski, H., (1985). Metastatic colon carcinoma detected with radiolabeled F(ab')2 monoclonal antibody fragments. Radiol 149: 849 - 858.

Murray, J.L., Rosenblum ,M.G., Lamki, L., Glenn, H.J., Krizan, Z., Hersh, E.M., Plager, C.E., Barhtolomew, R.M., Unger, M.W., Carlo, D.J., (1987). Clinical parameters related to optimal tumor localization of indium-111-labeled mouse antimelanoma monoclonal antibody ZME-018. J. Nucl. Med. 28: 25 - 33.

Murray, J.L., Rosenblum, M.G., Sobol, R.E., Bartholomew, R.M., Plager C.E., Haynie, P., Jahns, M.F., Glenn, H.J., Lamki, L., Benjamin, R.S., Papdopolous, N., Boddie, A.W., Frinke, A.W., David, J.A., Carlo, D.J., &Hersh, E.M.,

(1985). Radioimmunoimaging in malignant melanoma with 111In-labeled monclonal antibody 96.5. Cancer Res. 45: 2376 - 2381.

Myers, C.E., & Collins, J.M., (1983). Pharmacology of intraperitoneal chemotherapy. Cancer Invest. 1: 395 - 407.

Neijt, J.P., Ten Bokkel Huinink, W.W., Van der Burg, M.E.L., van Oosterom, A.T., Vriesendorp, R., Kooiman, C.D., van Lindert, A.C.M., Hammlynk, J.V.T.H., van Lent, M., van Honwelingen, J.C., Pinedo, H.M., (1984). Randomised trial comparing two combination chemotherapy regimes (Hexa-CAF vs CHAP - 5) in advanced ovarian carcinoma. Lancet. ii: 594 - 600.

Order, S.E., Klein, J.L., Leichner, P.K., Frinke, J., Lollo, C. & Carlo, D.J., (1986). ^{90}Yttrium Antiferritin: A new therapeutic radiolabeled antibody. Int. J. Radiat. Biol. Oncol. Phys. 12, 277 - 281.

Order, S.E., Stillwagon, G.B., Klein, J.L., Leichner, P.K., Fishman, E.K., Ettinger, D.S., Haulk, T., Kopler, K., Finney, K., Sordyke, M., Self, S., Leibel, S., (1985). ^{131}Iodine anti-ferritin, a new treatment modality in hepatoma: A radiation therapy oncology group study. J. Clin. Oncol. 3: 1573 - 1582.

Order, S.E., Klein, J.L., Ettinger, D., Alderson, P., Siegelman, S., and Leichner, P., (1980). Use of Isotopic Immunoglobulin in therapy. Cancer Res., 40: 3001-3007.

Paganelli, G., Riva, P., Sorti, G., Tison, V., Cacciaguerra, G., Fiorentini, G., Moscatelli, G., Benini, S., & Agoustini, M., (1986). Intraperitoneal versus intravenous injection of radiolabelled antibody in patients with colorectal carcinoma. Brit. J. Cancer 54: 542 - 543.

Pateisky, N., Philipp, K., Skodler, W.D., Czerwenka, K., Hamilton, G., Burchell, J.: Radioimmunodetection in patients with suspected ovarian cancer. J. Nucl. Med. 26: 1369 -1376, 1985.

Pectasides, D., Stewart, S., Courtenay-Luck, N., Rampling, R., Munro, A., Krausz,T., Dhokia, B., Snook, D., Hooker, G., Durbin, H., Bodmer, W., Epenetos, A.A., (1986). Antibody - guided irradiation of malignant pleural and pericardial effusions. Br. J. Cancer. 53: 727 - 732.

Pervez, S., Epenetos, A.A., Mooi, W.J., Evans, D.J., Rowlinson, G., Dhokia, B., & Krausz, T., (1988). Localization of monoclonal antibody UAA1 and its F(ab')2 fragments in human tumour xenografts: an autoradiographic and immunohistochemical study. Int. J. Cancer Suppl 3: 23 - 29.

Piver, M.S. & Baker, T. (1986) The potential for optimal (<2cm) cytoreductive surgery in advanced ovarian cancer at a tertiary medical centre: A prospective study. Gynecol Oncol 24, 1-8.

Pressman, D., & Keighley, G., (1948). The zone of activity of antibodies as determined by the use of activity of nephrotoxic anti-kidney serum. J. Immunol. 59: 141 - 146.

Rosen, S.T., Zimmer, A.M., & Spies, W.G., (1987). Radioimmunodetection and radioimmunotherapy of cutaneous T-cell lymphomas utilizing a ^{131}I labelled monoclonal antibody. J. Clin. Oncol. 5: 562 - 573.

Rowlinson, G., Snook, D., Busza, A., Epenetos, A.A., (1987). Antibody-guided localistion of intraperitoneal tumors following intraperitoneal or intravenous antibody administration. Cancer Res. 47: 6528 - 6531.

Sakahara, H., Endo K., Nakashima T., Koizumi, M., Kunimatsu, Y., Ohta, H., Nakamura T., Tanaka, H., Kotoura, Y., Yamamuro, T., Hosoi, S., Toyama, S., & Torizuka, K., (1987). Localization of human osteogenic sarcoma xenografts in nude mice by a monoclonal antibody labled with radioiodine and indium-111. J. Nucl. Med. 28: 342 - 348.

Scheinberg, D.A., & Strand, M., (1983). Kinetic and catabolic considerations of monoclonal antibody targeted in erythroleukemic mice. Cancer Res. 43: 265 - 272.

Scheinberg, D.A., & Strand, M., (1982). Leukemic cell targeting and therapy by monoclonal antibody in a mouse model system. Cacner Res. 42: 44 - 49.

Schray, M.F., Martinez, A., Howes A.E., (1989). Toxicity of open-field abdominal irradiation as primary postoperative treatment in gynecologic malignancy. Int. J. Radial. Oncol. Biol. Phys. 16: 397 - 403.

Sears, H.F., Heryln, D., Steplewski, Z., & Koprowski, H., (1986). Hybridoma 5: s109.

Sharkely, R., Kaltovich, F.A., Shih, L.B., Fand, I., Govelitz, G., and Goldenberg, D.M., (1989). Radioimmunotherapy of human colonic cancer xenographs with yttrium-90 labeled monoclonal antibodies to carcinoembryonic antigen. Cancer Res. 48: 3270 - 3275.

Sharkey, R., Pykett, M.J., Siegel, J.A., Alger, E.A., Primus, J., and Goldenberg, D.M., (1988). Radioimmunotherapy of the GW-39 human tumor xemograft with ^{131}I-labeled murine monoclonal antibody to carcinoembryonic antigen. Cancer Res. 47: 5672 - 5677.

Sharkey, R.M., Primus, F.J., & Goldenberg, D.M., (1984). Second antibody clearance of radiolabeled antibody in cancer radioimmunodetection. Proc. Natl. Accad. Sci. 81: 2843 - 2846.

Silverberg, E., Luberg, J.: Cancer Statistics 1987. Ca-A Cancer Journal for Physicians. 37: 2 - 19.

Smith, J.P., Rutledge, F.N., Declos, L., (1975). Postoperative treatment of early carcinoma of the ovary: a randomised trial between postoperative radiotherapy and chemotherapy. NCI Monogr. 42: 149 - 153.

Snyder, W.S., Ford, M.R., Warner, G.G., (1978). Estimates of specific absorbed dose fractions for photon sources uniformly absorbed in various organs of a heterogenous phantom. Medical Internal Radiation Dose (MIRD), New York, Society of Nuclear Medicine, 5: 5 - 67.

Soper, J.T., Wilkinson, R.H., Bandy, L.C., Clarke-Pearson, D.L., & Creasman, T., (1987). Intraperitoneal chromic phosphate P 32 as salvage therapy for persistent carcinoma of the ovary after surgical restaging. Am. J. Obstet. Gynecol. 156: 1153 - 1158.

Spar, I.L., Bale, W.F., Marrack, D., Dewey, W.C., McCardle, R.J., and Harper, P.V., (1967). 131I-Labeled antibodies to hauman fibrinogen. Diagnostic studies and therapeutic trials. Cancer 20: 865 - 870.

Stewart, J.S.W., Hird,V., Snook, D., Sullivan, M., Hooker, G., Courtenay-Luck, N., Sivolapenko, G.B., Griffiths, M., Myers, M.J., Lambert, H.E., Munro, A.J., and Epenetos, A.A., (1989). Intraperitoneal radioimmunotherapy for ovarian cancer.

Pharmacokinetics , toxicity and efficacy of I-131 labeled monoclonal antibodies. Int. J. Radiat. Oncol. Biol. Phys 16: 405 - 413.

Sutherland, C.A., Davis, J.O., & Stirrat, G.M., (1987). Penetration and binding of radiolabeled anti-carcinoembryonic antigen monoclonal antibodies and their antigen binding fragments in human colon multicellular spheroids. Cancer Res. 47: 1627 - 1633.

Taylor, A., Milton, W., Eyre, H., Christian, P., Wu, F., Hagan, P., Alazraki, N., Datz, F., & Unger, M., (1988). Radioimmunodetection of human melanoma with indium-111-labeled monoclonal antibody.J.Nucl.Med. 29: 329 - 337.

Trabowitz, S., Strauss, H., Small, M.J., (1964). Sternal marrow circulation by the radio-isotope clearance technique. J. Nucl. Med. 5: 864 - 870.

Travers, P., Bodmer, M.F., (1984). Preparation and characterisation of monoclonal antibodies against placental alkaline phosphatase and other human trophoblast associated determinants. Int. J. Cancer. 33: 633 - 641.

Trunnell, J.B., Marinelli, L.D., Duffy, B.J., Hill, R., Peacock, W., Rawson, R.U., (1949). The treatment of metastatic thyroid cancer with radioactive iodine: credits and debits. J. Clin Endocrin. 9: 1138 - 1152.

Van Norstrand, D., Neutze, J., and Atkins, F. (1986). Side effects of "rational dose" iodine-131 therapy for metastatic well-differentiated thyroid carcinoma. J. Nucl. Med., 27: 1519 - 1521.

Van Weelde, J.G., Pauels, E.K.J., Jones, B., Van Osterrom, A.Y., (1984). Scintigraphic peritoneooscopy in advanced ovarian malignancies: Its value for chemotherapeutic distribution studies. Clin. Radiol. 35: 465 - 468.

Varia, M., Rosenman, J., Venkatraman, S., Askin, F., Folwer, W., Walton, L., Halle, J., and Currie, J., (1988). Intraperitoneal chromic phosphate therapy after second-look laparotomy for ovarian cancer. Cancer 61: 919 - 927.

Vaughan, A.T.M., Anderson, P., Dykes, P.W., Chapman, C.E., and Bradwell, A.R., (1987a). Limitations to killing of tumors using radiolabelled antibodies. Brit. J. Radiol. 60: 567 - 578.

Vaughan, A.T.M., Yankuba, S.C.S., and Anderson, P., (1987b). Antibodies labeled with metallic radionuclides: Influence of nuclide chemistry on dose distribution. NCI Monogr. 3: 141 - 144.

Vaughan, J.M., and Tull, M.L., (1953). Use of ethylene diamine tetracetic acid (versene) for removing the fission products from the skeleton. Lancet ii: 856 - 859.

Wahl, R.L., & Fisher, S., (1987a). Intraperitoneal delivery of monoclonal antibodies: Enhanced regional delivery advantage using intravenous unlabeled anti-mouse antibody. Nucl. Med. Biol. 14: 611 - 615.

Wahl, R.L., Leibert, M., Fisher, S., & Boland, R., (1987b). Enhanced radioimmunotherapy of intraperitoneal human colon cancer xenografts by intraperitoneal monoclonal antibody delivery. Proc. Amer. Assoc. Cancer Res. 28: 438.

Wahl, R.L., Parker, C.W., & Phillpott, G.W., (1983). Improved radioimaging and tumor localization with monoclonal F(ab')2. J.Nucl.Med. 24: 316 -325.

Waldmann, J.A., (1969). Metabolism of immunoglobulins. Prog. Allergy 13: 1 - 10.

Ward, B.G., Wallace, K., (1987a). Localisation of the monoclonal antibody HMFG2 after intravenous and intraperitoneal injection into nude mice bearing subcutaneous and intraperitoneal human ovarian tumor xenografts. Cancer Res. 47: 4714 - 4718.

Ward, B.G., Mather, S.J., Hawkins, R., Crowther, M.E., Shepherd, J.H., Granowska, M., Britton, K.E., Slevin, M.L., (1987b). Localisation of radioiodine conjugated to the monoclonal antibody HMFG2 in human ovarian carcinoma. Assessment of intraperitoneal and intravenous route of administration. Cancer Res. 47: 4719 - 1727.

Weinstein, J.N., Eger, R.R., Covell, D.G., Black, C.D.V., Mulshine, J., Carrasquillo, J.A., Larson, S.M., and Keenan, A.M., (1988). The pharmacology of monoclonal antibodies. Ann. NY. Accad. Sci. 199 - 210.

Weiser, E.B., Burke, T.W., Heller, P.B., Woodward, J., Hoskins, W.J., Park, R.C., (1988). Determinants of survival in patients with epitheilial ovarian cancer following whole abdominal irradiation. Gynecol. Oncol. 30: 201 - 208.

Wessels, B.W., and Rogus, R.D., (1988). Radionuclide selection and model absorbed dose calculations for radiolabeled tumour associated antibodies. Med. Phys. 11: 638 - 645.

Wharton, J.L., & Herson, J., (1981). Surgery for common epithelial tumours of the ovary. Cancer 48: 582 - 589.

Wilson, B.J., Bayley, R.J., Catch, J.R., Evans, C.C., Gorsuch, T.T., Maynard, J.C., Myerscough, L.C., Newbery, G.R., Sheard, H., & Taylor, C.B.G., (1966). The Radiochemical Manual, 2nd Edition.Published by The Radiochemical Centre, Amersham.

Wiltshaw, E., Evans, B., Rustin, G. Gilbey, E., Baker, J. & Barker, G., (1986). A prospective randomised trial comparing high-dose cisplatin with low-dose cisplatin and chlorambucil in advanced ovarian cancer. J Clin Oncol 4: 722 - 729.

Wiltshaw, E., Evans, B., & Harland, S., (1985). Phase III randomisedtrial of cisplatin versus JM8 (carboplatin) in 112 ovarian cancer patients, Stages III and IV. Proc. Amer. Soc. Clin. Oncol. 4: 121.

Winchell, H.S., Pollycove, M., Longman, W.D., and Lawrence, J.H., (1964). A method for maintenance of Curie quantities of Yttrium-90 DTPA in the human body for defined time periods: Technique and dosimetry. J. Nucl. Med. 5, 16 - 24.

Wooten, W.W., (1983). Radionuclide kinetics in MIRD dose calculations. J. Nucl. Med. 24: 621 - 624.

Young, G.A., Brownjohn, A.M., & Parsons, F.M., (1987). Protein losses in patients receiving continuous ambulatory peritoneal dialysis. Nephron 45: 196 - 201.

Young, R.C., Chabner, B.A., Hubbard, S.P., Fisher, R.I., Bender, R.A., Anderson, T., Simon, R.M., Canellos, G.P., & DeVita, V.T.Jr., (1978). Advanced ovarian adenocarcinoma. New Engl. J. Med. 299: 1261 - 1266.

Zalcberg, J.R., Thompson, C.H., Lichtenstein, M., & McKenzie, I.F.C., (1984). Tumor immunotherapy in the mouse with the use of ^{131}I labelled monoclonal antibodies. J. Natl. Cancer Inst. 72: 697 - 704.

Zimmer, A.M., Rosen, S.T., Spies, S.M., Goldman-Leikin, R., Kazikiecz, J.A., Silverstein, E.A., & Kaplan, E.A., (1988). Radioimmunotherapy of patients with cutaneous T-cell lymphoma using an iodine-131-labeled monoclonal antibody: Analysis of retreatment following plasmaperesis. J. Nucl. Med. 29: 174 - 180.