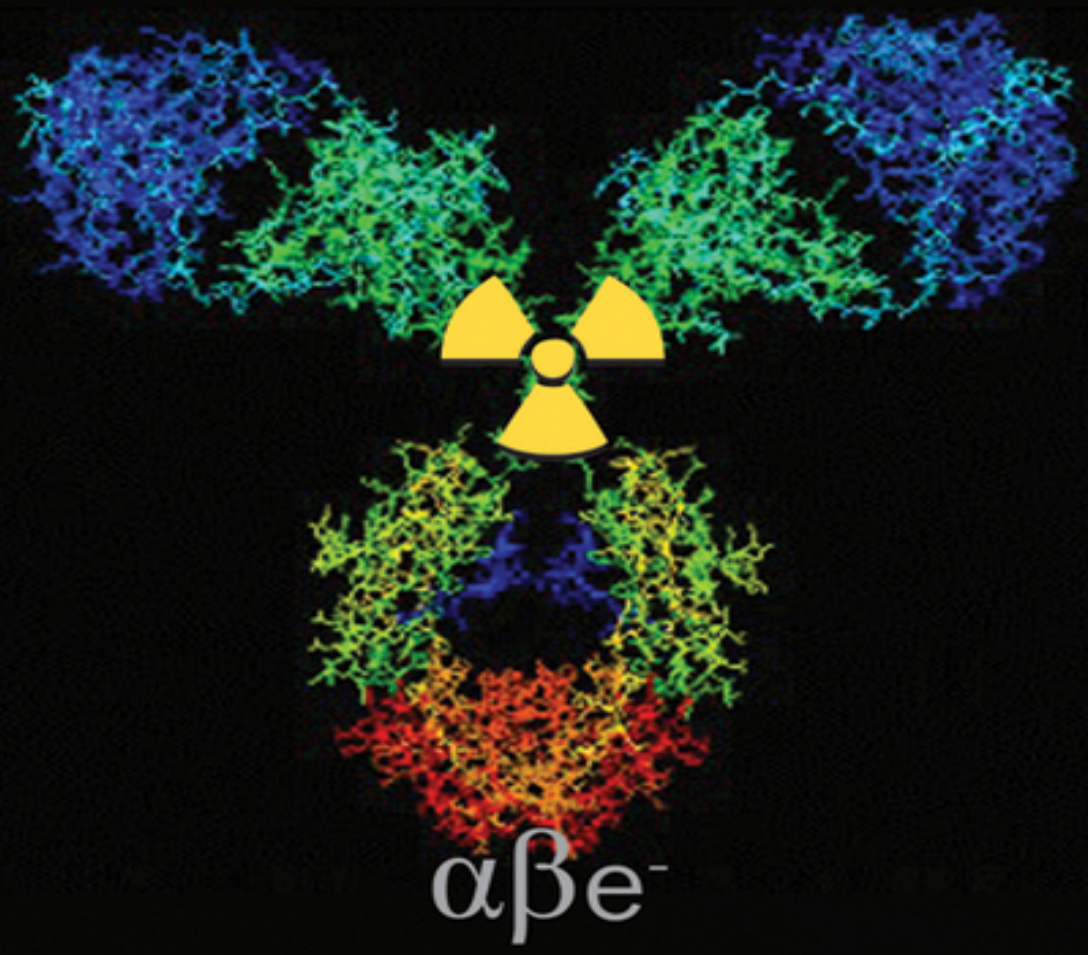
Monoclonal Antibody and Peptide-Targeted Radiotherapy of Cancer



Edited by
RAYMOND M. REILLY

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Contents

Chapter 12: Radiation Biology of Targeted Radiotherapy

MONOCLONAL ANTIBODY AND PEPTIDE-TARGETED RADIOTHERAPY OF CANCER

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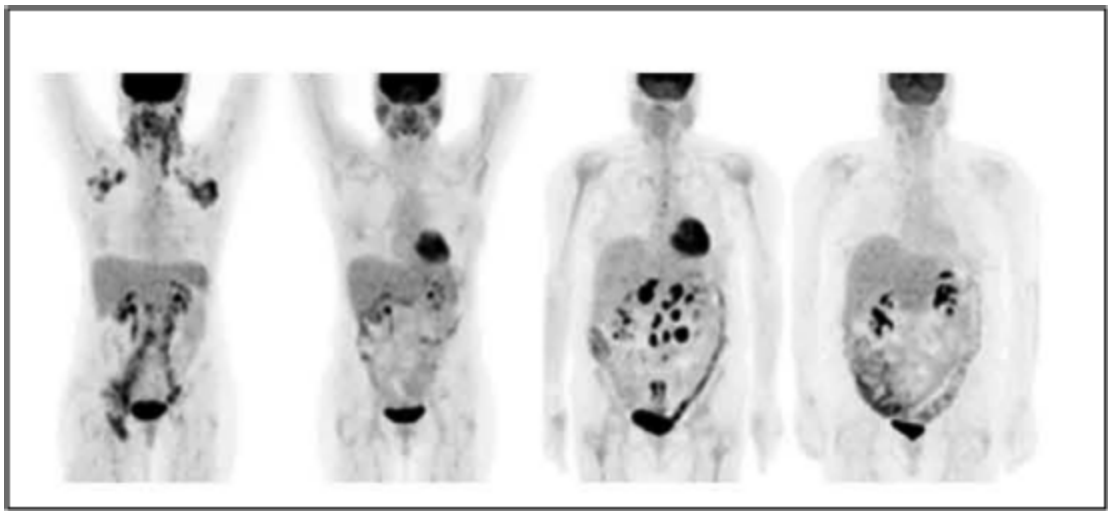
Dedication

To my mother, who always used to ask me, “What is a monoclonal antibody?” and, in another life would have been a wonderful scientist with her inborn fascination with medical discovery and knowledge.

Preface

In June 2009 at the 56th annual meeting of the Society of Nuclear Medicine in Toronto, the “Image of the Year” was selected by Dr. Henry N. Wagner Jr. from Johns Hopkins University [Figure 1 (1)]. This image illustrated the high sensitivity of positron emission tomography (PET) with ^{18}F-2-fluorodeoxyglucose (^{18}F-FDG) to reveal complete responses as early as 3 months post-treatment with ^{90}Y-ibritumomab tiuxetan (Zevalin) or ^{131}I-tositumomab (Bexxar) in patients with non-Hodgkin's lymphoma (NHL) (2). These two radioimmunotherapeutics are the first to be approved by regulatory authorities for treating cancer. By highlighting this image, Dr. Wagner not only recognized the great advances that have been made over the past three decades in radioimmunotherapy (RIT) of NHL (3) but also pointed the way toward how this approach could be combined with achievements in imaging (4) to help further advance the field of molecularly targeted radiotherapy.

Figure 1 Whole-body PET scans using ^{18}F-2-fluoro-deoxyglucose demonstrating complete response in two patients receiving ^{131}I-tositumomab (Bexxar; left two images showing pre- and post-treatment) or ^{90}Y-ibritumomab tiuxetan (Zevalin; right two images showing pre- and post-treatment). (Reprinted with permission from Reference (1).)



There remain many challenges to be overcome, however, particularly to extend the impressive results seen in NHL to RIT of the more prevalent solid tumors (3). RIT and peptide-directed radiotherapy (PDRT) of solid tumors have been restricted by low tumor uptake, dose-limiting toxicity to normal tissues including the bone marrow, and an intrinsically greater radioresistance (3). Nonetheless, the success of RIT of NHL has proven that this approach is scientifically sound, translatable to clinical practice, and feasible. Moreover, there has recently been progress in the treatment of solid tumors with targeted radiotherapeutics, particularly using innovative pretargeting techniques and in the setting of minimal residual disease (3).

My goal in assembling this book was to provide a single resource that would constitute an expert discussion of the diverse aspects of the field of monoclonal antibody and peptide-targeted radiotherapy of cancer. The chapters cover a wide range of topics including the optimization of design of biomolecules and their radiochemistry, cell and animal models for preclinical evaluation, important discoveries from key clinical trials of their effectiveness for the treatment of malignancies, an understanding of their radiation biology and dosimetry, considerations in

their regulatory approval, and health economics issues that need to be appreciated to ultimately see their widespread use in clinical oncology. New emerging areas such as the role of molecular imaging in evaluating the response and resistance to targeted radiotherapy, a discussion of the bystander effect that may enhance its effectiveness, and the potential of combining cytolytic virus therapy with targeted radiotherapy have also been included.

Many of the chapters were authored by internationally renowned experts who have made seminal discoveries in the field and by others who are leaders in areas that will be important to its future. I am grateful to all authors for their excellent contributions and thank them all for their patience as this book emerged. I am also indebted to my wife, Anita who tolerated the workload and spared some of the precious time that we have to spend together to accomplish this task. I believe that the book not only celebrates the substantial achievements of mAb and peptide-targeted radiotherapy of cancer but also acknowledges its limitations and failures—as Henry Ford said, “Failure is simply an opportunity to begin again, this time more intelligently.” A great deal has certainly been learned, approaches are now more informed and elegant, and it is expected that this new knowledge will build on the pioneering discoveries in targeted radiotherapy of NHL that have proven so successful as aptly presented in Dr. Wagner's selection of the Image of the Year. I hope that this book will provide the impetus for discussion, encourage continued contributions to the advancement of the field, and stimulate the imagination of those who would aspire to set its future.

Raymond M. Reilly

Toronto, Ontario, Canada

January 2010

References to the preface

1. Anonymous. International interest focuses on SNM annual meeting. *J Nucl Med.* 2009; **50**: 16N–18N.

2. Iagaru A, Mittra E, Goris M. ^{131}I-tositumomab (Bexxar) vs. ^{90}Y-ibritumomab (Zevalin) therapy of low grade refractory/relapsed non-Hodgkin lymphoma. *J Nucl Med.* 2009; **50** (Suppl. 2): 12P (abstract no. 47).

3. Goldenberg DM, Sharkey RM. Advances in cancer therapy with radiolabeled monoclonal antibodies. *Quarterly J Nucl Med.* 2006; **50**: 248–264.

4. McLarty K, Reilly RM. Molecular imaging as a tool for targeted and personalized cancer therapy. *Clin Pharmacol Ther.* 2007; **81**: 420–424.

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Chapter 1

Antibody Engineering: Optimizing the Delivery Vehicle

Diane E. Milenic

1.1 Introduction

The progression of monoclonal antibodies (MAbs) for radioimmunotherapy (RIT) has been driven by the need to solve a series of problems. As variants of antibodies have been developed and evaluated in preclinical studies, opportunities and limitations have become evident. Recent advances in DNA technology have led to the ability to tailor and manipulate the immunoglobulin (Ig) molecule for specific functions and *in vivo* properties. This chapter discusses the use of monoclonal antibodies for radiotherapy with an emphasis on the problems that have been encountered and the subsequent solutions.

The exploration of monoclonal antibodies as vehicles for the delivery of radionuclides for therapy has been ongoing for almost 50 years [1]. In 1948, Pressman and Keighley reported the first *in vivo* use of a radiolabeled antibody for imaging [2]. Ten years later, the first report of radiolabeled tumor-specific antibodies was utilized for radioimmunodiagnosis, and in 1960, radiolabeled antibodies were used to selectively deliver a therapeutic dose of radiation to tumor tissue [1, 3]. Even at these

early stages, investigators were quick to realize the obstacles associated with utilizing antibodies for radioimmunotherapy. Radiation doses delivered to tumors in patients were too low to have significant effects on tumor growth, and the prolonged retention of the radiolabeled antibodies in the blood led to toxicity complications [4]. The inherent heterogeneity in specificity and affinity of polyclonal antibodies resulted in *in vivo* variability. The advent of hybridoma technology and the ability to generate monospecific, monoclonal antibodies produced a resurgence in the use of antibodies as “magic bullets” [5, 6]. In the 1980s, the literature exploded with reports of radiolabeled MAbs being evaluated in the clinical setting, initially in radioimmunodiagnostic applications, confirming that MAbs against tumor-associated antigens could target tumors in patients. Subsequently, RIT clinical trials were initiated to deliver systemically administered radiation to tumors with a specificity that would spare normal tissues from damage [7]. This optimistic viewpoint was quickly tempered by the realization of the obstacles inherent to the use of a biological reagent, especially one of xenogeneic origin.

The preclinical and clinical RIT trials exposed the major constraints to the successful clinical use of radiolabeled MAbs: (i) development of human anti-murine immunoglobulin antibodies (HAMA); (ii) inadequate (low) therapeutic levels of radiation doses delivered to tumor lesions; (iii) slow clearance of the radiolabeled MAbs (radioimmunoconjugates) from the blood compartment; (iv) low MAb affinity and avidity; (v) trafficking to, or targeting of, the radioimmunoconjugates to normal organs; (vi) and insufficient penetration of tumor tissue [8, 9]. In addition, there were toxicities associated with conjugated radionuclides when the

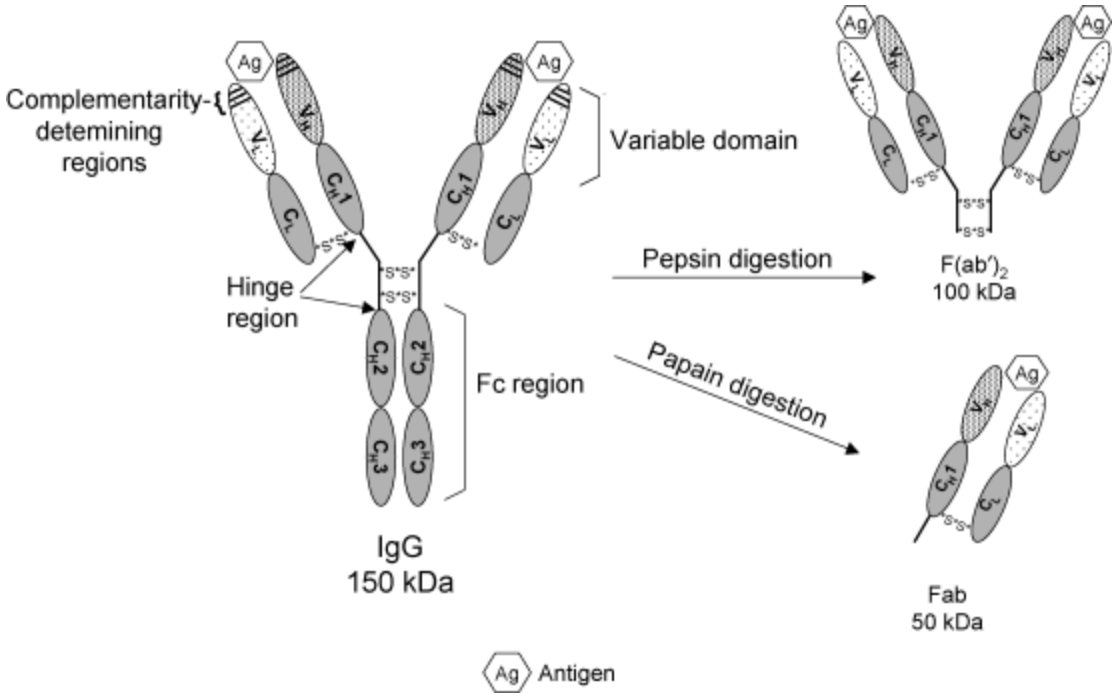
radioimmunoconjugates were metabolized or when the radionuclide dissociated from the immunoconjugate [9]. With these problems in mind, a primary focus has been to optimize RIT by manipulating the MAb molecule. As technology permitted, this was initially accomplished with chemical or biochemical techniques to generate a variety of immunoglobulin forms but is now predominated by genetic engineering.

1.2 Intact Murine Monoclonal Antibodies

In May 2008, a perspective on MAbs by Reichert and Valge-Archer [10] reported that in the periods 1980–1989, 1990–1999, and 2000–2005, 37, 25, and 8 murine MAbs, respectively, were evaluated in the clinic as cancer therapeutics. During this entire 25-year period, radiolabeled MAbs comprised 33% of the murine MAbs [10]. To date, only two radiolabeled murine (mu) MAbs, both targeting CD20, have received FDA approval. Zevalin, ^{90}Y-rituxan (ibritumomab-tiuxetan), was approved in 2002 and is indicated for relapsed or refractory low-grade follicular transformed non-Hodgkin's lymphoma (NHL). The overall response rate of patients is reported to be 80%; 46% for those with rituximab refractory disease [11]. Bexxar (^{131}I-tositumomab) was approved in 2003 for the treatment of non-Hodgkin's B-cell lymphoma in rituximab refractory patients (see Chapter 6). Objective responses following ^{131}I-tositumomab therapy have ranged from 54% to 71% in patients who have undergone previous therapies while for newly diagnosed patients the response rates are 97% with 63% of those experiencing a complete response [12].

In clinical trials using muMAbs for RIT of solid tumors, approximately 73% (ranging from 16% to 100%) of the patients developed HAMA following a single infusion of MAb [13]. In contrast, only about 42% of the patients in RIT trials for treatment of hematologic malignancies develop HAMA. When multiple doses of a radioimmunoconjugate have been administered, the amount of MAb that effectively targets tumor tissue is usually compromised after the second administration [13]. In general, the human antibody response, especially at earlier time points, is directed against the Fc portion of the MAb molecule (Fig. 1.1). With the passage of time and particularly after repeated infusions, the specificity of the human antibody response matures and becomes increasingly specific for the variable region of the MAb [13]. In some instances, anti-variable region antibodies develop after a single infusion of the MAb [13, 14]. This response has the potential of directly inhibiting the ability of the injected MAb from interacting with the targeted tumor [14]. As with any therapeutic regimen, for RIT to be effective, multiple treatment cycles will be necessary. Immunomodulatory drugs such as deoxyspergualin, cyclosporin A, or cyclophosphamide have been evaluated as a means of minimizing or suppressing a patient's immune response during RIT [15].

Figure 1.1 Schematic of an immunoglobulin structure. Enzymatic digestion of the intact IgG molecule yields $F(ab')_2$ and Fab fragments.



To address these challenges of MAb-directed therapy, several strategies have been employed that center around modifying the MAb molecule. These alterations include reduction in the size of the MAb molecule, deglycosylation, or the addition of side groups. Reduction in size of the MAb molecule has been accomplished through methods such as enzymatic cleavage or genetic engineering [16–18]. Digestion of an antibody with pepsin removes the Fc region of the heavy chain on the carboxyl terminus of cysteamine producing F(ab′)$_2$ fragments that retain two antigen binding sites and have a molecular weight of ~100 kDa (Fig. 1.1). Fab fragments are generated by digestion with papain, an enzyme with a specificity for the amino group of cysteines. In this case, the disulfide bridges between the heavy chains are removed with the Fc region, which results in a molecule (M_r ~ 50 kDa) with one antigen binding site. Fab′ fragments are produced through reduction and alkylation of F(ab′)$_2$, which also yields a MAb molecule with a single antigen binding site and an M_r of ~50 kDa [16–18].

Comparisons of intact MAbs and F(ab′)$_2$ fragments (Fig. 1.1) in RIT clinical trials have demonstrated that the F(ab′)$_2$ fragments do have a shorter serum half-life than intact MAbs. Patient antibody responses against F(ab′)$_2$ fragments appear to occur with lower frequency after a single administration of the radioimmunoconjugate. Furthermore, some objective responses to treatment with a radiolabeled F(ab′)$_2$ fragment have been observed [19, 20]. Autoradiographic studies of radiolabeled MAbs administered to athymic mice bearing human tumor xenografts have illustrated the ability of Fab′ and F(ab′)$_2$ fragments to penetrate tumor tissue with greater efficiency than intact MAbs [20, 21]. The pharmacokinetics of Fab or Fab′ fragments is even more rapid than F(ab′)$_2$ fragments ($t_{½}\alpha$ ~ 10 min, $t_{½}\beta$ ~ 1.5 h for Fab′ fragments versus $t_{½}\alpha$ ~ 30 min, $t_{½}\beta$ ~ 12 h for F(ab′)$_2$ fragments) [22]. In general, Fab and Fab′ fragments have proven to be less immunogenic than intact MAbs [23]. Their greatest disadvantage for RIT applications is their high and persistent renal localization, which appears to be a function of molecular size [22], which greatly increases the risk for renal toxicity. The degree to which the radiolabel is retained in the kidneys depends on the radionuclide and the radiolabeling chemistry (see Chapter 2). Radioiodinated MAbs are rapidly dehalogenated and the radioiodine excreted via the kidneys or into the stomach and intestines. Free radioiodine is trapped in the thyroid gland if there is inadequate blocking with stable iodine. Chelated radiometallonuclides, that is, ^{111}In, ^{90}Y, and ^{177}Lu, are not as readily eliminated from normal tissues when the radioimmunoconjugate is metabolized [24]. The retention of radiometals in the kidneys is due to the reabsorption of antibody fragments after their glomerular filtration

followed by degradation of the radioimmunoconjugates with trapping of radioactive metabolites within the renal tubular cells [22, 24, 25]. Although they are readily eliminated from the body, radioiodines may also pose a concern for toxicity to renal tissue, depending on the dose of radioactivity administered. An effective means of enhancing renal excretion of the radioimmunoconjugates is the blocking of its readsorption from the luminal fluid in the proximal tubules by administering basic amino acids such as lysine or arginine, prior to or with the radiolabeled MAb fragment [26, 27].

Fragments of MAb that retain immunoreactivity, however, are often difficult to generate [22]. As mentioned, they are prepared by proteolytic digestion of intact MAb using enzymes, a procedure that must be optimized for each MAb and usually requires threefold or more MAbs to obtain the final desired quantity of the fragment. The process is inefficient and costly when producing the amounts necessitated by a RIT clinical trial.

1.3 Recombinant Immunoglobulin Molecules

Antibodies consist of four polypeptide chains, two heavy and two light chains, connected by disulfide bonds; the heavy chains are glycosylated (Fig. 1.1). Several criteria must be met to generate and produce genetically engineered antibodies. First, a host cell is needed that would produce and secrete a properly assembled functional antibody molecule with the appropriate carbohydrate side chains. Second, the DNA must be introduced into the recipient cell in an efficient manner. Finally, expression vectors must be available that permit the expression of the introduced genes as well as the