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Current status of cancer therapy with radiolabeled monoclonal antibody

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Molecular targeting therapy has become a relevant therapeutic strategy for cancer. There are several monoclonal antibodies used for the treatment of malignant tumors. Radioimmunoconjugate is composed of antibody and radionuclide showing a synergistic effect of radiation and immune-mediated cellular toxicity and thereby enabling increased efficacy and minimizing toxicity. Radioimmunotherapy using ^{131}I- and ^{90}Y-labeled anti-CD20 monoclonal antibodies is now indicated for the treatment of patients with CD20 antigen-expressing relapsed or refractory, low-grade or transformed non-Hodgkin's lymphoma (NHL), including patients who are refractory to anti-CD20 monoclonal antibody (rituximab) therapy in the United States. It has been exhibiting favorable anti-tumor efficacy in patients with NHL as compared with rituximab. Myelosuppression is the main side effect associated with the radioimmunotherapy but is usually reversible, and nonhematologic adverse reactions are mild to moderate.

Following the impressive results of therapy using radiolabeled monoclonal antibodies for NHL, radioimmunotherapy for solid tumors has been examined; however, the results were unfavorable and did warrant further clinical trials as a single agent. Future studies on radioimmunotherapy for solid tumors should focus on the new strategies of targeting such as locoregional administration for intraperitoneal dissemination, and combination therapy with chemotherapy or cytostatic therapy.

Although radioimmunotherapy for NHL has shown excellent results comparable to aggressive chemotherapy without severe adverse effects, additional clinical trials should be performed to define the proper role of radioimmunoconjugates as a relevant strategy for cure of NHL.

Key words: monoclonal antibody, radioimmunotherapy, radioimmunoconjugate, malignant lymphoma

INTRODUCTION

MOLECULAR TARGETING THERAPY has become a relevant therapeutic strategy for the clinical management of cancer. Immunotherapy using monoclonal antibody targeted to the antigen is one of the molecular targeting therapies in a broad sense. Although the concept of targeted antibody therapy was introduced by Paul Ehrlich in 1906 as the term "magic bullet," anti-sera to the causative molecules of disease lacked efficacy and produced serious side effects. The development of hybridoma technology by Kohler and Milstein made it possible to use monoclonal antibody highly specific to the target molecule,[1] and it took another two decades for that monoclonal antibody to become clinically available as an approved therapeutic tool following advances in protein engineering that could produce a variety of antibodies and cumulative knowledge of preclinical studies, as well as subsequent clinical trials that were conducted to show that the targeted therapy using monoclonal antibodies was effective and safe. Since monoclonal antibodies bind to cell-surface antigens with high affinity and specificity, targeted antibody therapy is theoretically effective for cancer.

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Antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity are the two major mechanisms of action,[2,3] but the direct anti-tumor effects via induction of apoptosis and suppression of cell proliferation by the block of signal pathways are probably important mechanisms.[4–6] It is totally different from chemotherapy in terms of specificity to cancer cells, so that it results in low toxicity to normal organs such as bone marrow. Thus, high specificity and low toxicity are the characteristics of this method.

In 1997, anti-CD20 chimeric monoclonal antibody (rituximab, Rituxan®) was approved by the Food and Drug Administration (FDA) in the United States for use in the treatment of NHL. There are several monoclonal antibodies used for the treatment of malignant tumors. Humanized anti-erbB-2 antibody for breast cancer, humanized anti-VEGF antibody, and chimeric anti-EGFR antibody for colon cancer are also accepted therapeutic agents at present.

Radioimmunotherapy uses immunoconjugate composed of antibody and radionuclide that shows combined effects of radiation-induced cell death and immune-mediated cellular toxicity. It used to be an experimental treatment procedure, but has become an important therapeutic modality as a novel anti-cancer therapy in clinical oncology.

The properties of radioimmunotherapy are the specificity of antibodies to the cellular target, enabling increased efficacy, while minimizing toxicity to normal tissues. Multiple metastatic disease and dissemination as well as hematologic malignancy are the ideal indications of this modality. Immunoconjugates containing an antibody to B-cell surface antigen and a beta-emitting radionuclide exhibited excellent results in clinical trials in patients with B-cell NHL. The first radiolabeled monoclonal antibody approved for the treatment of malignant tumor was ^{90}Y-labeled anti-CD20 monoclonal antibody (ibritumomab tiuxetan, Zevalin®) in 2002. This is a conjugate of the murine parent of rituximab and pure beta-emitter ^{90}Y, that shows additive anticancer efficacy and limited toxicity.

Following the impressive results of therapy using radiolabeled monoclonal antibodies for NHL, radioimmunotherapy for various malignancies including solid tumors has been extensively examined. This article reviews the development and present status of radioimmunotherapy for malignant tumors.

PRODUCTION OF MONOCLONAL ANTIBODIES

Genetic engineering has enabled the production of a variety of monoclonal antibodies of various constructs and immunological characteristics. Traditionally, monoclonal antibodies of murine origin were the most commonly used monoclonal antibodies for radioimmunotherapy. One of the problems in targeted therapy was the development of human anti-mouse antibodies (HAMA) to monoclonal antibodies of murine origin. A HAMA response occurs usually 2 to 3 weeks after monoclonal antibody infusion. Once HAMA response occurs, the clearance of monoclonal antibody on subsequent administration is accelerated and the targeting is impaired. To overcome the limitation of immunogenicity of murine monoclonal antibodies, several approaches have been proposed. Fragments of monoclonal antibody have been produced by classical protein engineering. Development of HAMA response has been reduced by using these fragments. However, they showed fast blood clearance and decreased amount of uptake in the tumor. Chimeric antibodies consist of constant region of human origin and variable region of murine origin, and decrease the production of HAMA significantly. Moreover, biodistribution of chimeric monoclonal antibodies is different from that of murine monoclonal antibodies. Chimeric monoclonal antibodies show slower blood clearance than murine monoclonal antibodies that show equivalent tumor uptake in general. According to recent advances in protein engineering, humanized antibodies have been created to reduce the possibility of immune responses that limit the efficacy of immunotherapy using monoclonal antibodies. Although human anti-chimera antibody (HACA) or human anti-humanized antibody (HAHA) is seen occasionally, increased toxicity to normal organs or impaired targeting is unusual.[7] Human antibody is produced by a transgenic mouse induced with gene encoding human antibody or produced by means of phage-displaying single chain Fv (scFv) library system.[8–11] The transgenic mouse is capable of producing various antibodies following immunization with various human antigens and various hybridoma clones expressing fully human antibodies. The phage library enables production of antibodies to a variety of antigens such as autoantigens, toxins, and small molecules that are not used to produce antibodies by the traditional method of immunization. Specificity to the antigen and variability of producing antibody are the characteristics of this method; however, low affinity and time-consuming production step are limitations of the method. Antibody structures now can be readily manipulated to facilitate selective interaction with host immune effectors. Development of monoclonal antibodies of these types enabled the selection of treatment regimens that require multiple administrations to achieve optimal response.

Other structural manipulations that improve the selective targeting and rapid clearance from the blood and normal organs of immunoconjugates should lead to the design of effective new treatments, particularly for solid tumors. Although monoclonal antibodies have anti-tumor efficacy described above, they have been conjugated to toxins, cytokines, chemotherapeutic agents, and radionuclides. Radioimmunotherapy is a specific targeted therapy. In particular, radiolabeled monoclonal antibody does not

Table 1 Characteristics of radionuclides used in radioimmunotherapy

Radionuclide	Particle emitted	Half-life	Particle energy (MeV)		Path length (mm)
			α	β	
Iodine (^{131}I)	β^-	8.1 d		0.61	0.8
Yttrium (^{90}Y)	β^-	2.7 d		2.27	2.7
Renium (^{186}Re)	β^-	3.8 d		1.07	1.8
Renium (^{188}Re)	β^-	17 h		2.12	2.4
Lutetium (^{177}Lu)	β^-	6.7 d		0.50	1.5
Astatine (^{211}At)	α	7.2 h	6.8		0.05–0.08
Bismuth (^{212}Bi)	α	0.8 h	8.4		0.05–0.08

need to be internalized, or radionuclide does not need to be dissociated from the monoclonal antibodies as is needed for toxins or chemotherapeutic agents. These characteristics help reduce toxicity to non-target tissues and induce killing of adjacent cells even when the radiolabeled monoclonal antibodies are not bound to the target cells due to heterogeneous expression of target antigen or poor vascularity.

RADIONUCLIDES USED FOR RADIOIMMUNOTHERAPY

Beta-emitters and alpha-emitters are used mainly for radioimmunotherapy. Characteristics of representative radionuclides used in radioimmunotherapy are summarized in Table 1. Each radionuclide has its proper half-life, and the particle emitted from the radionuclide has its proper path length and linear energy transfer (LET). The selection of radionuclide depends on many factors including radiation characteristics and disease that is being treated by radioimmunotherapy. Since a high accumulation and homogeneous distribution at the target site and rapid elimination from non-target tissues are desirable, the half-life of the radionuclide should be long enough to allow the radiolabeled monoclonal antibody to reach the tumor, and short enough to limit radiation exposure to normal tissues. For these purposes, radiolabeled monoclonal antibodies should be specific to the target and easy to be eliminated from the excretory organs. From the view point of efficacy, a longer half-life and a longer path length are advantageous for bulky and poorly perfused tumors, whereas a shorter half-life and a shorter path length are more suited for hematologic malignancies.

Alpha particles are better suited to target hematologic malignancies due to their short path length and high LET. Although alpha-particles travel only a few diameters of tumor cells, their high LET limits cells from repairing DNA damage from radiation, which accounts for their high relative biological effectiveness and cytotoxicity.[12] The short path length and short half-life result in less radiation exposure to non-target and less toxicity to normal tissues.[13]

Auger-electron emitters such as ^{125}I and ^{111}In are also used for radioimmunotherapy. Since the path length of Auger electron is shorter than the diameter of tumor cells, the monoclonal antibodies labeled with these radionuclides must be internalized to be effective.[14]

Most monoclonal antibodies for radioimmunotherapy to date are labeled with either ^{131}I or ^{90}Y. Both radionuclides have an anti-tumor effect due to significant radiation doses as they undergo beta decay. Beta particles have a relatively long path length which is dependent on their energy and a low LET.[15] The path lengths of beta-particles are significantly greater than the diameter of cancer cells and the radiation effect of beta particles from the site of antibody binding on the neighboring cells is the so-called 'crossfire effect,' which allows for a therapeutic radiation doses to cells in bulky, poorly vascularized tumors, or even in tumors with heterogeneous antigen expression. The low LET necessitates a high dose and longer exposure of the cancer cells to induce cell killing.

RADIOIMMUNOTHERAPY OF SOLID TUMORS

Present status of radioimmunotherapy in solid tumors

While encouraging results have been obtained using radioimmunotherapy to treat patients with hematologic malignancies, the results in solid tumors are less favorable. Radioimmunotherapy for solid tumors has been studied in various types of tumor, including colon, breast, ovary, prostate and brain; however, the results have not been sufficiently promising to warrant further clinical trials. Carcinoembryonic antigen (CEA) has been studied as a target of radioimmunotherapy.[16–18] In a recent phase II trial with ^{131}I-labeled humanized anti-CEA monoclonal antibody (hMN-14, Immunomedics Inc.), 21 patients with small volume metastasis refractory to treatment received a single dose of 2220 MBq/m^2 (60 mCi/m^2) and showed an overall response rate (ORR) of 58% with a mean duration of response of 9 months.[19] Although these results appear promising, the study was limited to small-volume disease rather than bulky disease.

Subsequent radioimmunotherapy studies were performed using other radionuclides such as ^{90}Y and ^{64}Cu, and other antigen-binding constructs such as antibody fragments and scFv.[20–23] A recent phase I trial of

^{90}Y-labeled chimeric anti-CEA monoclonal antibody with 5-fluorouracil (5-FU) in heavily pretreated 21 patients with metastatic colorectal cancer revealed 11 patients with stable disease (SD) of 3–8 months and 1 patient with a mixed response, although no objective responses were observed.[24] Radioimmunotherapy did not appear to increase non-hematologic toxicities associated with 5-FU, and 5-FU did not appear to enhance the hematologic toxicity of ^{90}Y-labeled monoclonal antibody. These results support the possibility of radioimmunotherapy combined with established 5-FU therapy.

TAG-72 is another secreted antigen that is used for the target of adenocarcinoma. Monoclonal antibody CC49 labeled with ^{131}I was used for colon cancer,[25] and then a non-immunogenic construct was used for a variety of solid tumors,[26–28] although the anti-tumor efficacy was not favorable. Antibodies against cell surface antigens including 17-1A and A33 have a promising property in terms of internalizing into a cell after binding to the antigen. These antibodies could be labeled with ^{125}I, which emits low-energy electrons to damage the nucleus.[29–31] A phase I/II study of ^{125}I-labeled monoclonal antibody A33 demonstrated one patient with a mixed response and 2 patients with stable disease out of 21 patients, and there was no significant toxicity or requirement of radiation hazard control.[31]

Strategy for future radioimmunotherapy in solid tumors

Although most studies showed acceptable tumor targeting, no significant clinical response was observed in solid tumors. To overcome the limited clinical response as well as normal tissue toxicity and immunogenicity, intraperitoneal administration for peritoneal dissemination was performed.[32–34] Trials with scFv have been performed to increase tumor penetration and accelerate clearance from the blood.[35–38] These small molecules are potentially applied as the vehicle of a drug delivery system. However, the clearance of scFv is too rapid to allow for sufficient targeting and retention in the tumor. Increased affinity is required to enhance the efficacy of radioimmunotherapy by minimizing the radiation to normal tissue.

Pre-targeting and multi-step targeting are examples of a strategy that increases the efficacy of radioimmunotherapy for solid tumors. In the pre-target system, the effecter molecule (e.g., radionuclides or drugs linked to a carrier) is given some time after the targeting agent such as antibody. This allows for the targeting agent to localize in the tumor and to clear from the body. The radionuclide is usually bound to a low molecular-weight "effecter" such as chelate or peptide, and administered to be localized at the target site, while it is rapidly cleared from the non-target.

Bispecific antibodies have one binding site for the target and the other antibody arm for the effecter molecule.[39,40] In the avidin/streptavidin-biotin system, antibodies coupled with streptavidin or biotin, which is used as the primary targeting agent are administered followed some time later by the effecter molecule, which is conjugated with biotin or with avidin/streptavidine, respectively.[41–43] Another configuration relies on a three-step approach: biotin-conjugated antibody is administered as a targeting molecule followed a bridging with streptavidine/avidine, after which the biotin-conjugated effecter molecule is given.[44]

There are several problems relating to the clinical use of antibodies for solid tumors. Many factors are supposed to be responsible for the unfavorable anti-tumor efficacy of radioimmunotherapy for solid tumors. First of all, solid tumors are inherently radioresistant and histologically heterogeneous. Heterogeneity of antigen expression and vascularity is another characteristic of solid tumors. Therefore the choice of monoclonal antibody and radionuclide as well as the method of administration as described above is considerably important for designing therapy of solid tumors. Because the cost of producing new antibodies is high, it is cost-effective to develop a hybridoma cell line that produces a high-affinity antibody with high yield.

RADIOIMMUNOTHERAPY OF B-CELL LYMPHOMA WITH ANTI-CD20 MONOCLONAL ANTIBODIES

Radioimmunotherapy of NHL has advanced significantly over the past decade, and several radiolabeled monoclonal antibodies against CD20, CD22 (^{90}Y-epratuzumab) or human leukocyte antigen DR (^{131}I-Lym-1, ^{90}Y-Lym-1) have been tested in clinical trials.[45–48] Among them two radiolabeled monoclonal antibodies against CD20, ^{90}Y-labeled ibritumomab tiuxetan and ^{131}I-labeled tositumomab (Bexxar®) have been used for treating recurrent follicular lymphoma. Although CD20 is not specific to malignant lymphoma, it is the most commonly used antigen for the radioimmunotherapy of lymphoma.

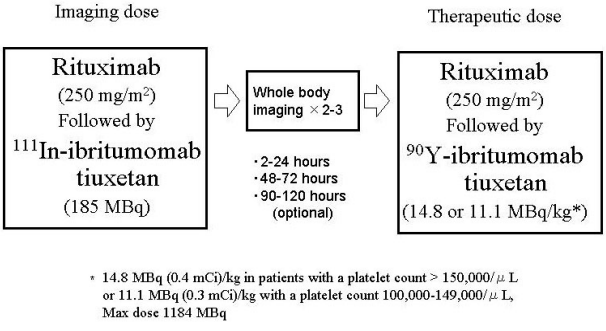
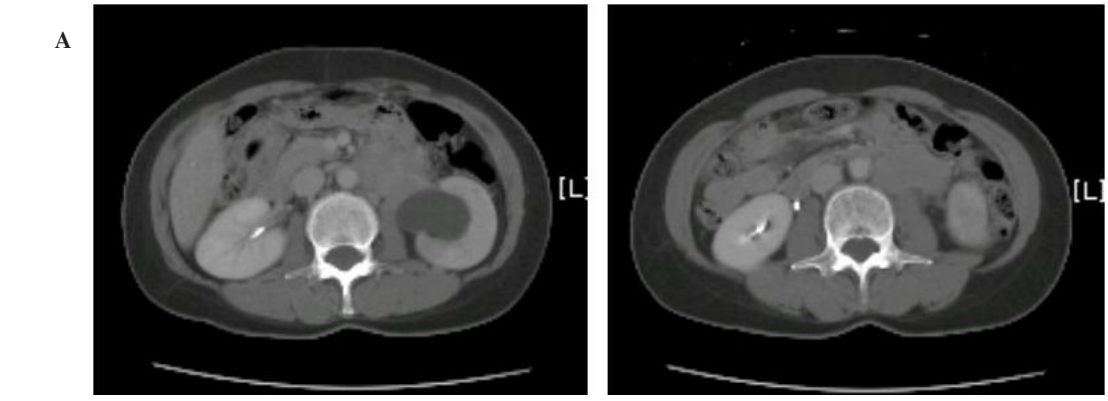


Fig. 1 Anti-CD20 human/mouse monoclonal antibody (rituximab) and ^{111}In-labeled anti-CD20 murine monoclonal antibody (^{111}In-ibritumomab tiuxetan) are administered for imaging to determine the biodistribution of the radioimmunoconjugate, followed by the therapeutic dose with rituximab and ^{90}Y-labeled anti-CD20 murine monoclonal antibody (^{90}Y-ibritumomab tiuxetan).



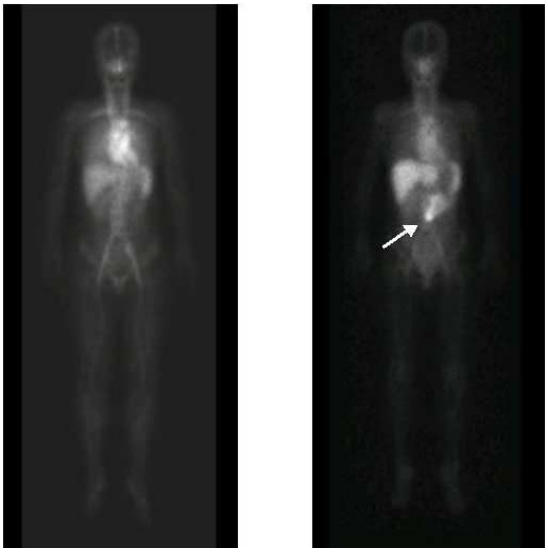


Fig. 2 (A) Contrast-enhanced CT of the abdomen in a patient with non-Hodgkin's lymphoma shows a mass lesion in the paraaortic area and hydronephrosis of the left kidney. (B) Serial gamma camera imaging at 3 hours (*left*) and 72 hours (*right*) after administration of ^{111}In-labeled anti-CD20 murine monoclonal antibody (^{111}In-ibritumomab tiuxetan). On 72-hour image, uptake of ^{111}In-ibritumomab tiuxetan is seen in the tumor (*arrow*) without abnormal visualization of the bone marrow. Hepatic uptake is within normal limits.

^{90}Y-ibritumomab tiuxetan is the first radioimmunoconjugate to be approved by the FDA for the treatment of malignant tumor, and ^{131}I-tositumomab was then approved in June 2003. These agents have shown impressive response rates and an acceptable toxicity profile. Myelosuppression is the main side effect but is usually reversible. Radioimmunotherapy with ^{90}Y- and ^{131}I-labeled anti-CD20 monoclonal antibodies have produced overall response rates of approximately 80% in patients with low-grade lymphomas, and 25% to 30% of them have achieved a complete remission. Patients with bone marrow involvement by lymphoma (>25%) should not be treated with radiolabeled monoclonal antibody in order to minimize potential damage to hematopoietic stem cells.

Results of clinical trials using ^{90}Y-labeled anti-CD20 monoclonal antibody

Of the numerous radioimmunoconjugates that have been tested for efficacy in animal models and in clinical trials, ^{90}Y-ibritumomab tiuxetan is the agent that has been most used in routine clinical practice. Ibritumomab is a murine immunoglobulin G_1 kappa monoclonal antibody that targets the CD20, a B-lymphocyte antigen, which is present on more than 90% of B-cell lymphomas and is not shed or internalized.[49] ^{90}Y-ibritumomab tiuxetan is composed of the anti-CD20 monoclonal antibody ibritumomab, which is covalently bound to the chelator 1-(2)-methyl-4-isothiocyanatobenzyl (MX)-DTPA (tiuxetan). Ibritumomab tiuxetan is radiolabeled with either ^{111}In or ^{90}Y, and used for imaging or therapy, respectively. Biodistribution of the antibody is assessed by using ^{111}In-labeled anti-CD20 monoclonal antibodies to confirm the indication of radioimmunotherapy using ^{90}Y-labeled anti-CD20 monoclonal antibodies.

The therapeutic regimen of ^{90}Y-ibritumomab tiuxetan is as follows (Fig. 1). Human/mouse chimeric monoclonal antibody to CD20 (rituximab, 250 mg/m^2) and ^{111}In-ibritumomab tiuxetan (185 MBq [5 mCi]) are infused initially for evaluating biodistribution of ^{111}In-ibritumomab tiuxetan, followed 1 week later with rituximab (250 mg/m^2) and ^{90}Y-ibritumomab tiuxetan (14.8 MBq/kg [0.4 mCi/kg]). Whole-body imaging is performed serially at 2–24 hours and 48–72 hours after the administration of ^{111}In-ibritumomab tiuxetan (Fig. 2). Abnormally increased accumulation of ^{111}In-ibritumomab tiuxetan in the normal organs such as bone marrow on 48–72 hour image makes it impossible for the patient to be treated with ^{90}Y-ibritumomab, because it indicates the possibility of myelosuppression and poor therapeutic efficacy due to the inappropriate ^{90}Y-ibritumomab tiuxetan uptake. A phase I/II ^{90}Y-ibritumomab tiuxetan study was performed to evaluate dosimetry in patients with relapsed or refractory NHL to estimate the radiation absorbed dose to normal organs and bone marrow from ^{90}Y-labeled anti-CD20

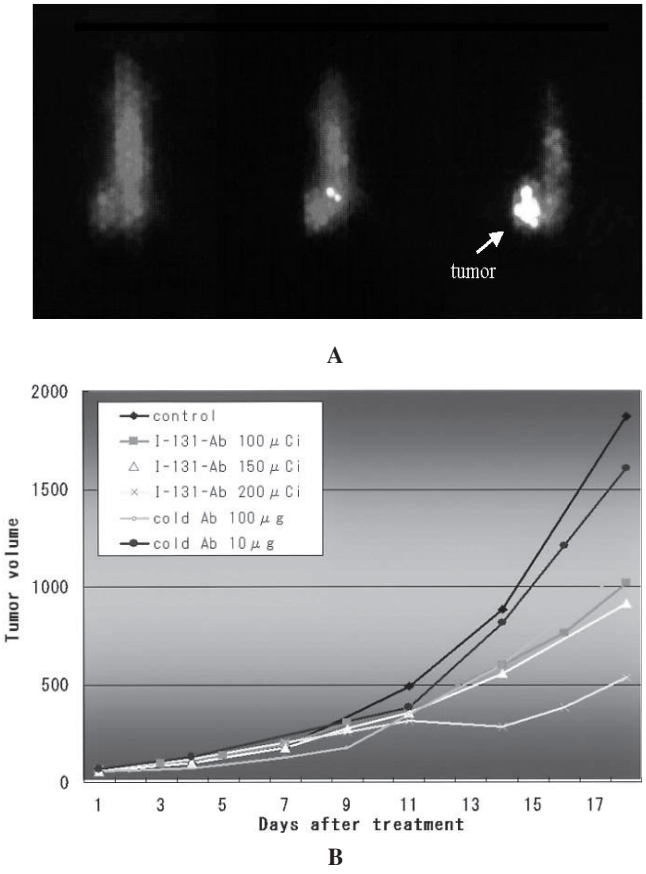


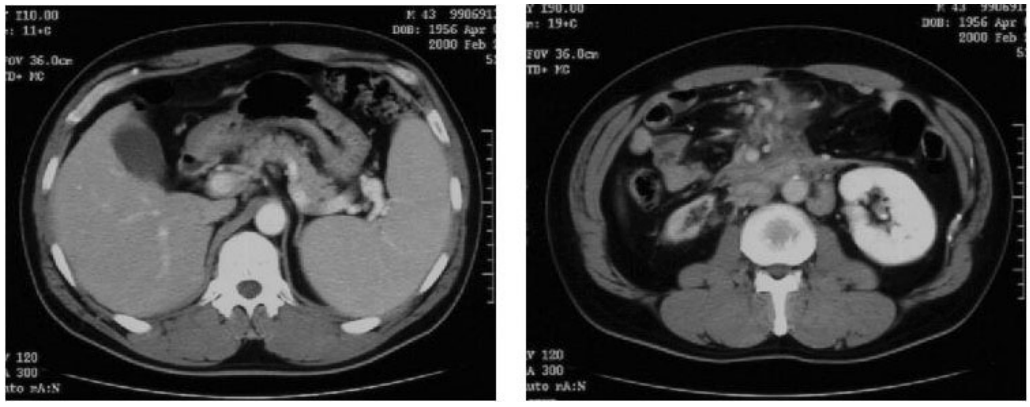
Fig. 3 (A) Gamma camera imaging of ^{131}I-labeled anti-CD20 murine monoclonal antibody NuB2 (^{131}I-NuB2) in an SCID mouse bearing human B-cell lymphoma xenograft. (B) Serial changes in the size of tumors i.v. injected with ^{131}I-NuB2 and NuB2 alone. Anti-tumor efficacy of ^{131}I-NuB2 is dose dependent and superior to that of NuB2 alone. I-131-Ab: ^{131}I-NuB2, cold Ab: NuB2 alone, control: seline, tumor volume (mm^3)

monoclonal antibodies.[45] The study determined the dose of rituximab which are given prior to the radioconjugate to bind to peripheral blood B-cells and optimize biodistribution, and then to determine the maximum tolerated dose of ^{90}Y-ibritumomab tiuxetan (7.4, 11.1, or 14.8 MBq/kg [0.2, 0.3, or 0.4 mCi/kg]). Following administration of ^{111}In-ibritumomab tiuxetan, serial anterior/posterior whole-body scans were acquired and major-organ radioactivity versus time estimates was calculated using regions of interest. Estimated radiation absorbed dose using the MIRDOSE3 revealed that normal organs and red marrow radiation absorbed doses were estimated to be under the upper limit of 20 Gy and 3 Gy, respectively with these values being acceptable. The only toxicity was hematologic and was not correlated to the radiation absorbed dose estimates to the red marrow or $T_{1/2}$, reflecting that hematologic toxicity was dependent on bone marrow infiltration of lymphoma cells in pretreated patients.

The results of an early clinical trial were reported by Witzig et al.[50] They conducted a phase I/II multicenter trial for treatment of relapsed or refractory B-cell NHL to determine the maximum-tolerated single dose of ^{90}Y-ibritumomab tiuxetan that could be administered without stem-cell support, and to evaluate safety and efficacy. They showed that the maximum-tolerated dose was 14.8 MBq/kg and 11.1 MBq/kg for patients with baseline platelet counts >150,000/μl and 100,000 to 149,000/μl, respectively. The ORR in 51 patients was 67% (26% complete response [CR]; 41% partial response [PR]; for low-grade disease (n = 34), 82% (26% CR; 56% PR); for intermediate-grade disease (n = 14), 43%; and for mantle-cell disease (n = 3), 0%. Kaplan-Meier estimate revealed that time to disease progression in responders and duration of response was 12.9+ months and 11.7+ months, respectively. Adverse events were primarily hematologic and correlated with the baseline extent of marrow involvement of lymphoma cells and baseline platelet count. They concluded that radioimmu-notherapy with ^{90}Y-labeled anti-CD20 monoclonal anti-bodies was a safe and effective therapy for relapsed or refractory NHL on an outpatient basis.

Randomized controlled trial of ^{90}Y-ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell NHL was performed.[51] The radioimmunotherapy group (n = 73) was pretreated with two rituximab doses (250 mg/m^2), and the control group received rituximab 375 mg/m^2 weekly for four doses (n = 70). OR and CR rates were significantly higher with ^{90}Y-ibritumomab tiuxetan, compared with rituximab (OR rate 80% vs. 56%, p = 0.002; CR rate 30% vs. 16%, p = 0.004). Time to progression (TTP) was 11.2 vs. 10.1 months (p = 0.173). Radioimmunotherapy with ^{90}Y-ibritumomab tiuxetan was well tolerated and produced significantly higher ORR and CR compared with rituximab alone.

Based on the previous study,[50] two patients with baseline platelet counts of 100 to 120,000/μl developed grade 4 thrombocytopenia (<25,000/μl), whereas none of 8 patients with normal baseline platelet counts developed thrombocytopenia at the same dose level of ^{90}Y. A statistically significant correlation was observed between percentage of bone marrow involvement with lymphoma and hematologic toxicity following ^{90}Y-ibritumomab tiuxetan therapy. Then the safety and efficacy of radioimmunotherapy with a reduced dose of ^{90}Y-ibritumomab tiuxetan (11.1 MBq/kg [0.3 mCi/kg]; maximum 1,184 MBq [32 mCi]) were evaluated in 30 patients with mild thrombocytopenia (100,000–149,000/μl) who had advanced, relapsed or refractory, low-grade, follicular, or transformed B-cell NHL.[52] The overall response rate was 83% (37% CR, and 40% PR), and Kaplan-Meier estimated median TTP was 9.4 months (range, 1.7–24.6) for all patients and 12.6 months (range, 4.9–24.6) for responders. The primary toxicity was hematologic: the incidence of grade 4 neutropenia, thrombocytopenia and anemia



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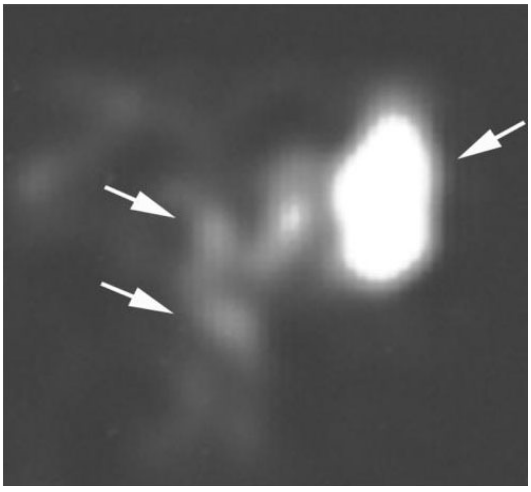

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Fig. 4 (A) Contrast-enhanced CT of the abdomen in a patient with non-Hodgkin's lymphoma shows splenomegaly and enlargement of the paraaortic lymph nodes. (B) Gamma camera imaging of ^{131}I-labeled anti-CD20 murine monoclonal antibody NuB2. Anterior view of the abdomen shows an increased uptake of ^{131}I-NuB2 in the spleen and paraaortic lymph nodes (*arrow*).

was 33%, 13% and 3%, respectively, although they were transient and reversible. The study confirmed that reduced-dose ^{90}Y-ibritumomab tiuxetan was safe and well tolerated and has significant clinical efficacy in patients with mild thrombocytopenia.

The efficacy of ^{90}Y-ibritumomab tiuxetan radioimmunotherapy was assessed in rituximab-refractory patients with follicular NHL.[53] In this study eligible patients were defined as having no objective response to rituximab (375 mg/m^2 weekly for 4 weeks) or TTP of <6 months. The ORR for the 54 patients with follicular NHL was 74% (15% CR and 59% PR), and the Kaplan-Meier-estimated TTP was 6.8 months (range, 1.1 to >25.9 months) for all patients and 8.7 months for responders. The incidence of grade 4 neutropenia, thrombocytopenia, and anemia was 35%, 9%, and 4%, respectively. The study confirmed that ^{90}Y-ibritumomab tiuxetan therapy is effective in rituximab-refractory patients.

Results of long-term follow-up of a phase I/II study were reported by Gordon LI et al.[54] Based on International Workshop Response Criteria, CR, CR unconfirmed (CRu), and PR rates were 29%, 22%, and 22%, respectively (ORR 73%, 51% in CR/CRu), and mean TTP and duration of response (DR) in responders were 12.6 months and 11.7 months, respectively. TTP and DR in complete responders (CR/CRu) were 28.3 and 27.5 months, respectively, and nine patients (24% of responding patients) had a TTP of more than 3 years. Thus ^{90}Y-ibritumomab tiuxetan radioimmunotherapy produced durable remission in follicular and diffuse large cell lymphoma.

^{131}I-labeled anti-CD20 monoclonal antibody

Since the availability and radiochemical properties of ^{131}I are feasible for use in radioimmunotherapy, ^{131}I-labeled monoclonal antibodies have been shown to be effective in clinical trials as well as in preclinical studies. Some of these radioimmunoconjugates have shown promise as therapeutic agents especially for hematologic malignancies. ^{131}I-labeled anti-CD20 monoclonal antibody NuB2 was an example that showed anti-tumor efficacy for the treatment of B-cell lymphoma xenograft in SCID mice (Fig. 3) and has been tested in a clinical trial (Fig. 4) in our institution.

^{131}I-labeled monoclonal antibody to the CD20 antigen, ^{131}I-tositumomab is now clinically available in the United States and European countries. Tositumomab is a murine immunoglobulin G_{2a} that binds to the CD20 antigen on the surface of normal and malignant human B-cells, and is linked covalently with ^{131}I to produce ^{131}I-tositumomab.[55,56] The ^{131}I-tositumomab regimen was approved by the FDA in June 2003 for the treatment of patients with CD20-positive, follicular NHL, whose disease was refractory to rituximab and had relapsed following chemotherapy[57] (Fig. 5).

The dose-limiting toxicity of ^{131}I-tositumomab was bone marrow suppression, and the whole-body radiation dose varies significantly from patient to patient due to cross-reactivity with normal B-cells and dehalogenation of ^{131}I-tositumomab by normal tissue.[58] Dose of

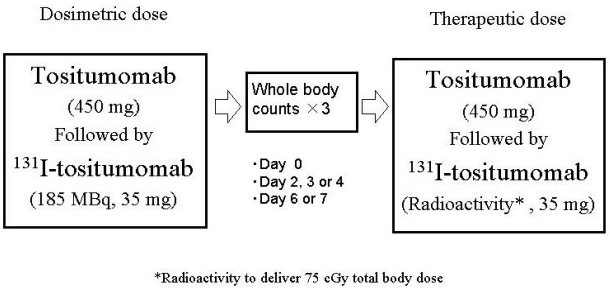

Fig. 5 Anti-CD20 murine monoclonal antibody (tositumomab) and ^{131}I-labeled tositumomab (^{131}I-tositumomab) are administered for imaging to determine the dosimetry, followed by the therapeutic dose with tositumomab and ^{131}I-tositumomab. Dose of ^{131}I-tositumomab is determined by the dosimetry to deliver whole-body radiation of 75 cGy.

^{131}I-tositumomab is therefore determined individually on a patient basis.[57] Following an appropriate prevention of ^{131}I from concentrating in the thyroid gland by potassium chloride or lugol solution, an unlabeled dose of tositumomab is administered over 1 hour to block circulating CD20-positive B-cells, followed immediately by a dosimetric dose (185 MBq) of ^{131}I-tositumomab to determine the whole-body distribution by imaging 3 times for 1 week (Fig. 5). The total counts in each scan are calibrated to total-body activity to calculate whole-body radiation and then a therapeutic dose of ^{131}I-tositumomab is administered to deliver 75 cGy of radiation to the total body to limit hematologic toxicity. Patient-specific dosimetry is actually inevitable, because the ^{131}I activity administered to each patient to achieve the total body dose is variable.[59] A study measuring dose data for hospital staff and family member exposed to patients treated with ^{131}I-tositumomab assured safety, and ^{131}I-tositumomab therapy is now performed on an out-patient basis in the United States.[60,61]

A multicenter, randomized study involving 78 patients with refractory/relapsed NHL revealed that the responses in the ^{131}I-tositumomab and unlabeled tositumomab groups were as follows: ORR 55% and 19% ($p = 0.002$); CR 33% and 8% ($p = 0.012$); median duration of overall response not reached and 28.1 months (95% confidence interval [CI]: 7.6 months, not reached); and median TTP 6.3 and 5.5 months ($p = 0.031$), respectively, for the median follow-up of 42.6 months (range 1.9 to 71.5 months).[62] Thus the study demonstrated that all of the therapeutic outcome measures were significantly enhanced by the conjugation of ^{131}I to tositumomab. The study also indicated that the majority of non-hematologic adverse events associated with ^{131}I-tositumomab were mild to moderate and usually transient.

Based on the recent results of clinical trials, the FDA approved an expanded indication for ^{131}I-tositumomab in January 2005. The new indications excluded the requirement that patients be refractory to rituximab and have relapsed after chemotherapy. The regimen is now indicated for the treatment of a broader population of patients with CD20 antigen-expressing low-grade, follicular or transformed NHL.

The clinical usefulness of ^{131}I-tositumomab was assessed in previously untreated patients.[63] The study included seventy-six patients with stage III or IV follicular lymphoma who underwent a single course of treatment with ^{131}I-tositumomab as an initial therapy. The study revealed that 95% of the patients had some response, and 75% had a complete response. After a median follow-up of 5.1 years, the actuarial 5-year progression-free survival for all patients was 59%, with a median progression-free survival of 6.1 years. Of 57 patients who had a complete response, 40 remained in remission for 4.3 to 7.7 years. Hematologic toxicity was moderate, with no patient requiring transfusions or hematopoietic growth factors. The authors concluded that a single one-week course of ^{131}I-tositumomab therapy as initial treatment could induce prolonged clinical remissions in patients with advanced follicular lymphoma.

The impressive response rate and favorable prognostic profile of this treatment have facilitated prospective randomized trials of ^{131}I-tositumomab alone as compared with chemotherapy or chemotherapy plus rituximab for selected patients.

Future direction of radioimmunotherapy for non-Hodgkin's lymphoma

Current investigations are focusing on the potential role of ^{131}I- and ^{90}Y-labeled anti-CD20 monoclonal antibodies earlier in the treatment algorithm, including as single-agent therapy for relapsed diffuse large B-cell lymphoma patients not eligible for transplantation, and consolidation treatment for low-grade NHL patients after first-line therapy.

Because ^{131}I-tositumomab and ^{90}Y-ibritumomab tiuxetan have been showing comparative efficacy and acceptable toxicity, clinical use would be decided by the different utility of these two radioimmunoconjugates. Because ^{90}Y is a pure beta emitter, patients are treated with ^{90}Y-ibritumomab tiuxetan on an outpatient basis without radiation hazard. Imaging with ^{111}In-ibritumomab tiuxetan is performed prior to the therapy, since it confirms the suitable biodistribution of the antibody. However, the imaging does not seem to be necessary in most cases. On the other hand, radiation exposure to distant organs as well as the public and family members has to be considered for ^{131}I-tositumomab, and the therapy is performed under strict radiation hazard control. The regimen of ^{131}I-tositumomab therapy requires a dosimetric dose of ^{131}I-tositumomab to determine the therapeutic dose, delivering 75 cGy of radiation to the total body.

Pretargeted radioimmunotherapy for B-cell NHL has been investigated.[64,65] A recent phase I trial has been using a tetrameric single-chain anti-CD20-streptavidine

fusion protein as the targeting agent and a synthetic clearing agent at 48 or 72 hours later to remove the unbound former agent in the circulation.[65] ^{90}Y-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-biotin infusion at 24 hours later resulted in rapid tumor localization and urinary excretion. The study demonstrated safety and efficacy with 2 CRs and 1 PR out of 15 patients enrolled. There are several reports showing the favorable clinical results of rituximab and anti-CD33 monoclonal antibody (alemtuzumab) in B-cell chronic lymphocytic leukemia (CLL).[66–68] Radioimmunoconjugate of these monoclonal antibodies in B-cell CLL would be a candidate for clinical trials.

Use of non-radioactive immunoconjugates has been investigated in hematologic malignancies. A clinical trial with humanized anti-CD33 monoclonal antibody joined to a calicheamicin derivative (gemtuzumab ozogamicin) in acute myeloid leukemia and preclinical research with anti-CD22 immunoconjugate of calicheamycin in B-cell lymphoma have been reported.[69,70]

CONCLUSION

Radioimmunoconjugate consisting of a monoclonal antibody against CD20 linked to a beta-emitting radionuclide is effective for the treatment of NHL, because of its selectivity for the lymphoma cells that are markedly radiosensitive. The use of a radiolabeled monoclonal antibody also decreases the toxicity as compared with conventional therapy by limiting radiation to the specific target. However, radioimmunotherapy for solid tumor is not favorable and future studies should focus on the new strategies of targeting, locoregional application, and combination therapy.

More than 20 years has passed since radioimmunotherapy for NHL was initiated. Although radioimmunotherapy for hematologic malignancies has shown promising results, additional clinical trials are still needed to define the proper role of radioimmunoconjugates as a relevant strategy for cure of NHL.

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