Radioimmunotherapy of Solid Tumors: The Promise of Pretargeting Strategies Using Bispecific Antibodies and Radiolabeled Haptens

Radioimmunotherapy (RIT) of malignancies has been studied for more than 2 decades (*1*). The basic premise of RIT—that monoclonal antibodies (mAbs) directed against a tumor-associated antigen can be used to target radionuclides to cancer cells for in situ radiation therapy—has been proven in tumor-xenografted mouse models and clinically in cancer patients (*2*). Furthermore, the potential for successful treatment of cancer using this approach has been shown for patients with non-Hodgkin's B-cell lymphoma (NHL) using anti–cluster designation 20 antigen (CD20) mAbs labeled with ^{131}I (tositumomab [Bexxar]; Corixa Corp.) or ^{90}Y (ibritumomab tiuxetan [Zevalin]; IDEC Pharmaceuticals Corp.), for which durable response rates of 70%–80% were achieved in clinical trials (*3,4*). Unfortunately, the same cannot be said for solid tumors, for which response rates to RIT are often less than 10% and the doses that can safely be administered are severely restricted by toxicity toward normal tissue, particularly toward the bone marrow (*1,2*). The reasons for inadequate treatment of solid tumors using radiolabeled mAbs are complex and have been the subject of several reviews (*5–7*) and modeling studies (*8,9*), but a critical barrier that has been identified is the poor pharmacokinetic properties of intact IgG antibodies. Intact mAbs are macromolecules (mass, 150 kDa) that exhibit circulation half-lives ranging from 2 to 3 d for murine forms to 4 d for chimeric and humanized forms (*10*). These long residence times in the blood encourage accumulation of radioactivity in tumors by allowing multiple passes at which antibodies may extravasate and interact with tumor cells. However, long residence times are also a major contributor to nonspecific toxicity to hematopoietic stem cells, especially if the antibodies are labeled with long-range β-emitters such as ^{131}I or ^{90}Y (cross-fire effect). Bone marrow toxicity is directly related to the residence time of radioactivity in the blood and is lower for radiolabeled mAb fragments [Fab and F(ab′)$_2$] and peptides (e.g., ^{90}Y-1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid [DOTA]-D-Phe^1-Tyr^3-octreotide [^{90}Y-DOTATOC]) than for intact antibodies, because of their more rapid elimination (*1*). However, antibody fragments and peptides exhibit lower absolute accumulation in tumors than do intact antibodies, a critically important consideration when one is designing a strategy for targeted in situ radiotherapy of malignancies.

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One strategy to preserve the pharmacokinetic benefits of intact mAbs, in terms of providing relatively good tumor localization while minimizing the deleterious effects on the bone marrow, is to separate the delivery of the antibody from that of the radionuclide using pretargeting techniques (*11*). Pretargeting has focused mainly on approaches that use the (strept) avidin-biotin system or those that use bispecific antibodies (BsmAbs), which recognize a tumor-associated antigen and a small-molecule radiolabeled hapten. The essential concept of pretargeted RIT is to deliver the BsmAb to the tumor first, allow sufficient time for clearance of excess circulating antibodies (sometimes combined with a clearing agent "chase" step), and then administer the radiolabeled hapten, which extravasates readily and binds to the tumor-bound BsmAb. Excess radiolabeled hapten is quickly cleared from the blood by the kidneys. The much shorter residence time of radioactivity in the blood for pretargeted RIT than for direct RIT is expected to significantly diminish bone marrow toxicity and allow dose escalation of radioactivity to more therapeutically effective levels.

The paper by Kraeber-Bodéré et al. (*12*) in this issue of *The Journal of Nuclear Medicine* describes a phase I clinical trial of pretargeted RIT in 22 patients with solid tumors (including 9 patients with medullary thyroid carcinoma [MTC]) using a BsmAb (hMN14 × m734) recognizing carcinoembryonic antigen and the dipeptide hapten di-diethylenetriamine pentaacetic acid (DTPA)-indium-tyrosine-lysine labeled with ^{131}I (^{131}I-di-DTPA-In-TL). This group evaluated 2 amounts (40 and 75 mg/m^2) of the BsmAb to identify the optimal dose for pretargeting and then studied increasing doses of ^{131}I-di-DTPA-In-TL (1.8–2.9 GBq/m^2) administered 5 d later to determine the maximum tolerated dose of radioactivity. The study showed excellent localization of ^{131}I-di-DTPA-In-TL to 70% of tumor sites by γ-scintigraphy. Moreover, disease

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stabilized for 3 mo to more than 12 mo in 45% of patients receiving a therapeutic dose of the radiolabeled hapten (6 MTC patients and 3 non-MTC patients), although there were no partial remissions or complete responses. Disappointingly, bone marrow toxicity was dose limiting in MTC patients, in whom the incidence of ^{131}I-di-DTPA-In-TL uptake in the pelvic and axial skeleton was 5-fold greater than that in non-MTC patients. A recent study by this same group found that imaging after treatment in 35 MTC patients receiving BsmAbs and ^{131}I-di-DTPA-In-TL revealed bone involvement in 89% of patients, compared with one third of 12 patients with colorectal cancer receiving pretargeted RIT (*13*). Interestingly, the proportion of MTC patients with bone involvement detected by immunoscintigraphy was higher than the proportion with bone involvement detected by bone scanning (57%) or MRI (76%), suggesting that the BsmAb-hapten approach also is a sensitive diagnostic tool. Bone marrow toxicity was greater for the 75 mg/m^2 dose than for the 40 mg/m^2 dose of BsmAb. Using the 40 mg/m^2 dose of BsmAb, the maximum tolerated dose of ^{131}I-di-DTPA-In-TK (defined by grade III or IV hematologic [>14 d] or nonhematologic toxicity) was 1.8 GBq/m^2 for MTC patients but was not reached at 2.9 GBq/m^2 for non-MTC patients. These results suggest that RIT using this BsmAb-hapten approach is promising for treatment of solid tumors but reveal that hematopoietic toxicity remains a challenge—one that appears to be related to tumor type; is dependent on the pretargeting protocol, especially the dose of BsmAb; and, importantly, restricts the dose of radiolabeled hapten that can safely be administered to certain patient populations.

Production of mAbs specific for metal chelators was first described by Reardan et al. in 1985 (*14*). Initially, these antibodies were precomplexed in vitro to radiolabeled metal chelator haptens and then were administered, with reliance on the enhanced permeability of tumor vasculature for selective targeting and on dissociation of the chelator from the antibody complex in vivo and its subsequent renal excretion (i.e., antibodies as reversible carriers) for eliminating circulating radioactivity (*15*). However, for specific pretargeting of tumors, it is necessary to use a BsmAb that can recognize a tumor-associated antigen as well as specifically bind a radiolabeled metal chelator (*16*). In the study by Kraeber-Bodéré et al. (*12*), such a BsmAb was constructed by covalently linking the Fab′ fragment of the chimeric anti–carcinoembryonic antigen mAb hMN-14 to the Fab′ fragment of the murine anti-DTPA-indium mAb m734. Although the use of a murine Fab′ fragment could potentially induce a human antimouse antibody (HAMA) response in patients, in only 1 of 12 patients tested did HAMA develop. Interestingly, there was a higher incidence—4 of 12 patients—of human antihuman antibody responses directed against the chimeric hMN-14 Fab′. Nevertheless, the immunogenicity of this BsmAb was substantially lower than that of immunoconjugates used for pretargeting with the (strept)avidin-biotin system. Streptavidin is a bacterial protein produced by *Streptomyces avidinii* (*17*). In a phase II trial of pretargeted RIT in 25 patients with colorectal cancer receiving streptavidin-conjugated NR-LU-10 antibodies and ^{90}Y-1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA)-biotin (*18*), all patients experienced development of HAMA, human anti-streptavidin antibodies, and antibodies specifically directed against the immunoconjugates. The immunogenicity of hMN-14 × m734 BsmAbs could further be diminished by humanization of the murine anti-DTPA-indium Fab′ and possibly by replacement or elimination of nonessential immunogenic sequences in hMN-14 Fab′ by recombinant antibody–engineering techniques.

Antibodies directed against metal chelators discriminate exquisitely between complexes that contain different metals. For example, by substituting scandium or gallium for indium in an ethylenediaminetetraacetic acid complex, the affinity of binding by an antiindium-ethylenediaminetetraacetic acid antibody was decreased more than 1,000-fold (*14*). Thus, in the study by Kraeber-Bodéré et al. (*12*), ^{131}I-labeled di-DTPA-In-TL that had been precomplexed in vitro with (nonradioactive) indium was used as the hapten for pretargeted RIT. The divalent ^{131}I-di-DTPA-In-TL hapten presents 2 epitopes for recognition by the BsmAb. In tumor-xenografted mouse models, such divalent haptens have been shown to exhibit improved tumor uptake by creating an opportunity for cross-linking of 2 BsmAbs pretargeted to the surface of tumor cells, thereby increasing the avidity of interaction and stabilizing the complexes (*19*). This finding is important, because the affinity of mAbs for metal chelators (affinity constant, $\sim 10^9$ mol/L) is about 1 million times lower than that of avidin or streptavidin for binding biotin (affinity constant, 10^{15} mol/L), thus potentially making BsmAb less attractive for pretargeted RIT strategies (*11*).

Pretargeted RIT using the (strept) avidin-biotin system has yielded impressive preclinical results in human tumor–xenografted mouse models. In a landmark study by Axworthy et al. (*20*), "cures," defined as complete tumor regression with no recurrence for more than 1 y, were obtained in 10 of 10 mice with lung or colon cancer xenografts and in 8 of 10 mice with breast cancer xenografts that received SA-NR-LU-10 immunoconjugates followed by a clearing agent and then by ^{90}Y-DOTA-biotin. Unfortunately, these promising results did not translate into successful outcomes in cancer patients receiving this same pretargeted RIT protocol. In a phase II trial of 25 patients who received SA-NR-LU-10, a clearing agent, and ^{90}Y-DOTA-biotin (4.0 GBq/m^2), there were no complete responses and only 2 partial remissions (*18*). Disease was stabilized in 4 additional patients. Moreover, these SA-NR-LU-10 immunoconjugates cross reacted with

normal bowel, resulting in unacceptable toxicity after the administration of ^{90}Y-DOTA-biotin and preventing further investigation for pretargeted RIT. In the study by Kraeber-Bodéré et al. (*12*), with the exception of the MTC patients who experienced dose-limiting bone marrow toxicity, no major (grade III or IV) nonhematologic toxicities were associated with pretargeted RIT using the hMN-14 × m734 BsmAb and ^{131}I-di-DTPA-In-TL haptens. Grade I or II hepatic toxicity was seen in 5 patients at the higher dose (75 mg/m^2) of the BsmAb and in 1 patient at the lower dose (40 mg/m^2); in 2 patients, this toxicity was attributed to tumor involvement. No other nonhematologic toxicities were noted. These results are encouraging for further dose escalation in non-MTC patients to improve the therapeutic response.

For future research, one direction that may diminish the bone marrow toxicity from pretargeted RIT in MTC patients (and also in non-MTC patients) using BsmAbs could be the use of a hapten labeled with an α-emitter such as ^{211}At or ^{213}Bi. Compared with the 2-mm β-particles emitted by ^{131}I, the much shorter range (50–100 μm) of α-particles would decrease the cross-fire effect on nontargeted bone marrow stem cells from targeted tumor cells in the marrow. Moreover, compared with the low-linear-energy transfer of a β-emitter, such as one labeled with ^{131}I, the high-linear-energy transfer (100 keV/μm) of the α-particles would amplify the DNA-damaging and cell-killing properties of the hapten (*21*). Biotin analogs labeled with ^{213}Bi have recently been studied for pretargeted RIT using the (strept)avidin-biotin system and showed encouraging results in tumor-xenografted mouse models, although dose-limiting renal toxicity was noted (*22*).

A possible explanation for the lower toxicity from the 40 mg/m^2 dose than from the 75 mg/m^2 dose in the study by Kraeber-Bodéré et al. (*12*) may be that less BsmAb was circulating in the blood at the time of ^{131}I-di-DTPA-In-TL administration. This would have diminished the formation of BsmAb-hapten complexes in the blood and, thus, minimized the residence time of circulating radioactivity by allowing renal excretion of the hapten. The incorporation of a clearing agent step may therefore help to further diminish bone marrow toxicity with this approach. The effectiveness of pretargeted RIT using the BsmAb-hapten strategy could also potentially be improved by extension to patient populations that have already been identified as more responsive to direct RIT. These populations include patients with solid tumors who have minimal residual disease and patients with NHL (*1*). For example, in a phase II trial of 30 colorectal cancer patients with minimal residual disease treated with a 2.2 GBq/m^2 dose of ^{131}I-hMN-14, the objective response rate in 19 patients with measurable tumors was 16% (3 partial remissions) but there were also 8 minor responses. Importantly, in the adjuvant setting, 7 of 9 patients remained disease free for up to 3 y (*23*). Despite excellent responses to direct RIT of NHL, bone marrow toxicity remains dose limiting. Pretargeting strategies may allow continued dose escalation to achieve the long complete responses (2–7 y) that were achieved with myeloablative RIT of NHL (*24*). Several groups are investigating pretargeted RIT using the (strept) avidin-biotin system for B-cell lymphomas in mouse tumor models (*25,26*) and clinically in patients with NHL (*27,28*), with excellent tumor response and low hematologic toxicity. Pretargeted RIT using BsmAbs and radiolabeled haptens has not yet been examined in patients with NHL. However, one preclinical study (*29*), on mice with subcutaneously implanted Ramos human B-cell lymphoma xenografts treated with BsmAbs directed against CD20 and an ^{90}Y-labeled histamine-succinyl glycine peptide hapten, revealed that pretargeted RIT was more effective than direct RIT, suggesting that pretargeted RIT is indeed a promising direction for future research.

Nature has provided us with the perfect model for recognition and eradication of human disease: the immune system. This system has allowed humans to survive for millennia despite relentless attack by infectious organisms. The modern challenge is to harness its elegant design to fight cancer, a disease that is diagnosed in almost 10 million people worldwide each year and is responsible for the deaths of more than 7 million individuals annually (*30*). In nuclear medicine, we are still learning how best to apply the model of antigen-antibody recognition in the immune system to the targeting of radionuclides to tumor cells for in situ radiation therapy of malignancies. The study by Kraeber-Bodéré et al. (*12*) represents an important step forward on the path to understanding the potential for treatment of solid tumors using appropriate combinations of antibodies and radionuclides. Each step on this path will bring us closer to achieving our goal of discovering more effective and less harmful therapies for cancer patients.

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