Timing for Evaluating "Specific" Binding of ^{99m}Tc-Sulesomab in Peripheral Bone Infection

TO THE EDITOR: I congratulate Skehan et al. (*1*) on their recent study on the mechanism by which ^{99m}Tc-sulesomab (LeukoScan; Immunomedics, Inc.) accumulates in inflammation and infection. ^{99m}Tc-Sulesomab is a Fab′ monoclonal antibody directed against the nonspecific cross-reacting antigen of granulocytes (NCA-90), which is overexpressed by activated granulocytes. I waited some months before writing this letter because I wished to discuss the ^{99m}Tc-sulesomab kinetic data of Skehan et al. in light of clinical data that emerged from a recent study at our center (*2*). Interestingly, Skehan et al. compared 2 proteins characterized by a similar molecular weight—^{99m}Tc-sulesomab and ^{99m}Tc-labeled human serum albumin (HSA)—to investigate ^{99m}Tc-sulesomab clearance and uptake by the infectious site. As is known, capillary permeability increases in inflamed tissues. Both HSA and ^{99m}Tc-sulesomab are therefore expected to accumulate, at least in part, in the inflammation region by a nonspecific mechanism. The accumulation is expected to be bidirectional in the case of a "neutral" protein, as happens for HSA, whereas the accumulation should be monodirectional in the case of a protein that specifically binds to cells or tissues expressing the NCA-90 antigen and located in the extravascular interstitial space, as should happen for ^{99m}Tc-sulesomab.

The study of Skehan et al. (*1*) elucidated some important points: The monoclonal Fab′ antibody of ^{99m}Tc-sulesomab does not significantly bind to circulating quiescent granulocytes (<5% binding). Instead, clearance of ^{99m}Tc-sulesomab, as evaluated by Patlak–Rutland analysis, is about 3 times greater than that of HSA. These data agree with an in vitro study conducted by the same authors (*1*), in which, unlike HSA, primed and activated granulocytes showed a 3–4 times higher affinity for ^{99m}Tc-sulesomab than for quiescent granulocytes. Despite these observations, the authors found that the target-to-background ratios of HSA and ^{99m}Tc-sulesomab were similar at 60, 180, 240, and 360 min after radiotracer injection. I think that the authors decided to stop their study 6 h after ^{99m}Tc-sulesomab administration because some large clinical trials have found that ^{99m}Tc-sulesomab makes possible imaging and diagnosis of infection very soon, that is, within a few hours, after radiotracer injection (*3,4*).

However, on the basis of the study of Skehan et al. (*1*), the nuclear medicine reader might realize that despite specific binding of ^{99m}Tc-sulesomab to primed and activated granulocytes in the infectious site, the prevalent mechanism of ^{99m}Tc-sulesomab accumulation is related simply to the nonspecific increase of capillary permeability exactly as for HSA, at least within 6 h after injection. Thus, the question remains open of what, if any, timing should be adequate for evaluating a specific ^{99m}Tc-sulesomab binding prevailing on the nonspecific binding.

In a recent prospective study by Rubello et al. (*2*), the results of 253 consecutive ^{99m}Tc-sulesomab examinations of 220 patients with proven or suspected peripheral bone infection were evaluated. The protocol used in our study included both early (4 h) and, at variance with previous protocols, delayed (24 h) acquisition of ^{99m}Tc-sulesomab images. Moreover, for interpreting ^{99m}Tc-sulesomab findings, we evaluated the early (4 h) uptake pattern versus the delayed (24 h) uptake pattern. Specifically, a pattern of increasing uptake was judged as infection (true-positive result), whereas a pattern of decreasing uptake was judged as nonspecific early accumulation (false-positive result). By adopting these interpretation criteria, we obtained a significantly improved specificity for the ^{99m}Tc-sulesomab examination. In details comparing early and delayed imaging, specificity was 75% versus 87.5% in patients with diabetic foot infection and 76.2% versus 85.7% in patients with other peripheral bone infections or prosthetic joints (*2*). This increase in specificity was related strictly to the identification of some false-positive findings due to nonspecific ^{99m}Tc-sulesomab uptake on early images alone, for example, when blood-pool activity was significantly high. Thus, in our experience, delayed 24-h ^{99m}Tc-sulesomab imaging was useful in detecting nonspecific early ^{99m}Tc-sulesomab uptake and, as a consequence, in identifying cases of specific uptake to granulocytes at the site of infection. Of note, 24 h after ^{99m}Tc-sulesomab injection, background activity is nearly negligible in evaluations of peripheral bone, especially considering the blood-pool activity present (*2*).

It can be concluded that, from a clinical point of view, nuclear medicine physicians should consider reevaluating acquisition protocols and interpretative criteria for ^{99m}Tc-sulesomab imaging, particularly delayed ^{99m}Tc-sulesomab imaging. Lastly, I encourage Skehan et al. to continue their investigation and, in particular, to evaluate the kinetic characteristics of ^{99m}Tc-sulesomab on delayed 20- to 24-h imaging. Further scientific contributions to this field would be extremely useful in better establishing the role of ^{99m}Tc-sulesomab imaging in the diagnosis of peripheral bone infection.

REFERENCES

1. Skehan SJ, White JF, Evans JW, et al. Mechanism of accumulation of ^{99m}Tc-sulesomab in inflammation. *J Nucl Med.* 2003;44:11–18.
2. Rubello D, Casara D, Maran A, Avogaro A, Tiengo A, Muzzio PC. Role of anti-granulocyte antibody scintigraphy (LeukoScan) in evaluating bone infection: acquisition protocol, interpretation criteria and clinical results. *Nucl Med Commun.* 2004;23:39–47.
3. Becker W, Bair J, Behr T, et al. Detection of soft-tissue and osteomyelitis using a technetium-99m-labeled anti-granulocyte monoclonal antibody. *J Nucl Med.* 1994; 35:1436–1443.
4. Hakki S, Harwood SJ, Morrisey MA, Camblin JG, Laven DL, Webster WBJ. Comparative study of monoclonal antibody scan in diagnosing orthopaedic infection. *Clin Orthop.* 1997;335:275–285.

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REPLY: Dr. Rubello makes an interesting point that is reminiscent of claims for other speculative specific, infection/inflammation-targeting agents—Infecton (Draximage Inc.), for instance (*1*)—that imaging at 4 and 24 h yields a higher clinical specificity than imaging at 4 h alone. It would perhaps be helpful, here, to draw a distinction between 2 different definitions of specificity: Clinical specificity is a measure of the number of false-positive results; "radiopharmaceutical" specificity is to do with whether an agent localizes in a lesion through a well-defined physiologic process for which it was designed. There appears to be a widely held view, as also hinted at by Dr. Rubello in his letter, that if accumulation (i.e., target-to-background ratio) continues to increase over 24 h, the agent must be specific by the second of these 2 criteria. Labeled leukocytes are specific on both, but a radiola-

beled protein, such as polyclonal IgG, only on the first. That does not mean to say that IgG would not show increasing localization over time. It clearly does (*2*), and moreover, ^{99m}Tc detached from the protein may be retained in tissue (*3*). Therefore, the fact that an agent, including ^{99m}Tc-sulesomab (LeukoScan; Immunomedics, Inc.), gives better images at 24 h throws no light on its mechanism of accumulation, which could still be "nonspecific."

The purpose of our study was obviously not a clinical comparison between ^{99m}Tc-sulesomab and human serum albumin but an attempt to clarify mechanisms of ^{99m}Tc-sulesomab accumulation in an inflammatory lesion, especially because the concept that ^{99m}Tc-sulesomab binds to circulating granulocytes is clearly erroneous, as shown by negligible cell binding in blood obtained ex vivo (*4*). Perhaps we should have extended our study to 24 h, although by then, among other problems, there would have been significant detachment of ^{99m}Tc from the respective proteins, rendering quantitative studies difficult to conduct or interpret.

If new tracers for inflammation require imaging beyond 4 h, as the evidence seems to support, then perhaps we should be looking for radionuclides more appropriate than ^{99m}Tc with which to label them. Alternatively, perhaps we should be imaging at a single time point, 7–8 h, instead of the 4- and 24-h time points that seem to be ingrained in our imaging protocols, and not just those for imaging inflammation.

REFERENCES

1. Larikka MJ, Ahonen AK, Niemela O, Junila JA, Hämäläinen MM, Syrjälä HP. Specificity of ^{99m}Tc-ciprofloxacin imaging [letter]. *J Nucl Med.* 2003;44:1368.
2. Nijhof MW, Oyen WJ, van Kampen A, Claessens RA, van der Meer JW, Corstens FH. Evaluation of infections of the locomotor system with indium-111-labeled human IgG scintigraphy. *J Nucl Med.* 1997;38:1300–1305.
3. Claessens RA, Koenders EB, Oyen WJ, Corstens FH. Retention of technetium-99m in infectious foci in rats after release from technetium-99m labelled human non-specific polyclonal immunoglobulin G: a dual-label study with hydrazinonicotinamido and iminothiolano immunoglobulin. *Eur J Nucl Med.* 1996;23:1536–1539.
4. Skehan SJ, White JF, Evans JW, et al. Mechanism of accumulation of ^{99m}Tc-sulesomab in inflammation. *J Nucl Med.* 2003;44:11–18.

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Radioimmunotherapy of Non-Hodgkin's Lymphoma Revisited

TO THE EDITOR: We appreciate the views communicated by Britton (*1*) in his comments on the papers by Koral et al. (*2*) and Wiseman et al. (*3*) pertaining to therapy of non-Hodgkin's lymphoma (NHL) with ^{131}I-tositumomab and ^{90}Y-ibritumomab tiuxetan, respectively, and desire to offer some other thoughts. Because the carriers of radioactivity for therapy of differentiated thyroid cancer and neuroendocrine tumors are not active against these neoplasms absent the radioactivity delivered, it is not appropriate to use these as examples of a similar paradigm in NHL radioimmunotherapy, because the antibody carriers have in fact been shown to be active on their own, especially rituximab. Predosing with rituximab and adding high amounts when giving the murine radiolabeled antibody certainly differentiate this from any other non–antibody-based therapy. Indeed, experimental evidence has been presented that some biologic effects exerted by the naked antibody can enhance the effects of radiation (*4*).

We would be gratified if there were indeed a direct relationship between targeting and estimated tumor dose delivered with therapeutic response in radioimmunotherapy. However, evidence is accumulating that tumors can have significant responses despite receiving lower estimated doses than others that receive a higher dose. Even tumors that are not visualized (i.e., tumor dosimetry cannot be determined) can undergo major responses (*5,6*).

Although Koral et al. (*2*) defined a trend in previously untreated patients whereby those receiving higher radiation doses to the tumor were more likely to have a complete response, even they acknowledged other factors that can contribute to a response and did not advocate, as Britton does, the use of tumor targeting and dosimetry as a means of selecting which NHL patients should receive radiolabeled antibody treatment. Although taking this course might enhance the likelihood of a response, there is as yet no body of data that proves the contrary; namely, that patients do not respond if their tumors are not targeted with the radioactive antibody. Indeed, Koral et al. did emphasize the limitations of dosimetry methods for tumors and the effects of unlabeled antibody and also found that some tumors responded at lower radiation doses and others failed to respond at higher doses.

Most clinical trials have a regulatory requirement that targeting be confirmed before therapeutic doses are given, but definitive tumor targeting is not required for the approved agents. Trying to select patients on the basis of dosimetry is not unlike the selection of patients for cytotoxic chemotherapy, for which in vitro assays of tumor sensitivity have not been predictive. But when a specific target molecule is recognized before therapy, such as the expression of cluster designation (CD) 20 on lymphoma cells, then use of a radiolabeled anti-CD20 monoclonal antibody is more justified than when the cells have no demonstrable CD20. However, how much CD20 is enough? Is its presence in 10% of cells, 20% of cells, or more cells sufficient, and is weak staining adequate? In vivo imaging of a patient with extensive disease can be problematic and misleading if proper doses and procedures are not followed, including dose titration. Therefore, having both in vitro and in vivo data of antigen expression and antibody accretion is certainly the best situation, but when the naked antibody can have immunotherapeutic activity and when there is still an incomplete understanding of factors that influence a response, targeting the isotope to the tumor may not, by itself, be sufficient for selecting a candidate patient. For these reasons, we do not agree with the implication of Britton's view that even patients who have failed prior therapies should not be given radioimmunotherapy if pretreatment targeting and dosimetry are not convincing of a probable response, especially when therapeutic doses of naked monoclonal antibodies are being given as a part of the therapy. Britton states, "there is clearly a lower limit at which insufficient therapy has no benefit to the patient with a tumor," but there are no data to support this conclusion. In many early phase I trials, responses are observed at the starting therapeutic dose level, and responses have been seen even after the pretherapy imaging dose, but of course the therapeutic dose is always escalated because of the assumption that higher doses will be more beneficial. Certainly the higher complete response rate at myeloablative doses supports the "more is better" dictum, but clearly we must be more cautious in advocating an

expanded use of doses requiring hematopoietic support and instead direct trials toward showing efficacy for radioimmunotherapy with less toxicity in a frontline setting. Indeed, use of ^{131}I-tositumomab as a first therapy in indolent, follicular NHL showed lower toxicity but also a higher response rate than when ^{131}I-tositumomab was given at the same dose after multiple prior drug courses (*7*), but it is not as yet clear whether there is a difference in tumor targeting and accretion between these 2 patient groups.

Also deserving of comment is Britton's example that the dose administered to a patient on the basis of body weight seemingly could have been increased were it adjusted to the dose limit allowed by the estimated bone marrow dose. Although the calculation of red marrow absorbed dose aids in the prediction of hematologic toxicity, there are other factors to consider, particularly in NHL (*8–11*), and therefore it would be irresponsible to suggest that another method be used in place of those that have carefully been evaluated for the 2 approved radioimmunotherapy agents. The simple observation that the majority of patients who receive radioimmunotherapy experience myelotoxicity, sometimes even grade 4, suggests that the current dosing methods (i.e., whole-body clearance and body weight plus blood count) are still imperfect. However, this observation also means that most patients are receiving the highest possible dose without having to rely on more drastic measures to control severe myelosuppression. Nevertheless, between at least 8% and 15% do require hematopoietic growth factors or blood cell transfusions (Bexxar [Corixa Corp.] and Zevalin [Biogen IDEC] product labels). Under the currently approved indication, patients will most likely have undergone several cycles of chemotherapy that can have a profound effect on the marrow reserve. From a practical perspective, reducing the dose of radiolabeled antibody according to bone marrow involvement (with >25% involvement excluding treatment) and baseline platelet counts has been shown to mitigate myelosuppression. Use of red marrow dosimetry in combination with a biologic marker, such as FLT3-L, improved the prediction of myelotoxicity in patients with solid tumors (*12*), but use of this or other markers of hematopoietic status needs further examination to determine utility in lymphoma.

Radioimmunotherapy remains complex because of the combination of immunology and radiation medicine, each having its own set of problems and prospects. This complexity was confirmed by the long journey that this modality traveled before the first products reached (only recently) clinical practice. Yet, despite many differences between the first 2 radioimmunotherapy agents approved for use, they are interestingly similar in their ability to achieve higher response rates than either prior chemotherapy or use of the naked antibody by itself (*13*), and they are indeed also gaining interest as potential frontline therapies for NHL (*7*). In this setting, we prefer to advocate that all appropriate patients whose tumors express CD20 should be candidates for CD20 radioimmunotherapy, that those who express CD22 should likewise be treated with radiolabeled CD22 mAbs, and so forth, at least until more reliable in vivo methods of selecting the best responders are confirmed. But standards for determining adequate expression of such markers are not established, and whether these determinations should be made before or after patients fail chemotherapy remains a subject of future investigation and could include study arms with and without patient selection based on prior external imaging.

REFERENCES

1. Britton KE. Radioimmunotherapy of non-Hodgkin's lymphoma. *J Nucl Med.* 2004;45:924–925.
2. Koral KF, Dewaraja Y, Li J, et al. Update on hybrid conjugate-view SPECT tumor dosimetry and response in ^{131}I-tositumomab therapy of previously untreated lymphoma patients. *J Nucl Med.* 2003;44:457–464.
3. Wiseman GA, Kornmehl E, Leigh B, et al. Radiation dosimetry results and safety correlations from ^{90}Y-ibritumomab tiuxetan radioimmunotherapy for relapsed or refractory non-Hodgkin's lymphoma: combined data from 4 clinical trials. *J Nucl Med.* 2003;44:465–474.
4. Hernandez MC, Knox SJ. Radiobiology of radioimmunotherapy with ^{90}Y ibritumomab tiuxetan (Zevalin). *Semin Oncol.* 2003;30(suppl):6–10.
5. Sharkey RM, Brenner A, Burton J, et al. Radioimmunotherapy of non-Hodgkin's lymphoma with ^{90}Y-DOTA humanized anti-CD22 IgG (^{90}Y-epratuzumab): do tumor targeting and dosimetry predict therapeutic response? *J Nucl Med.* 2003; 44:2000–2018.
6. Sgouros G, Squeri S, Ballangrud AM, et al. Patient-specific, 3-dimensional dosimetry in non-Hodgkin's lymphoma patients treated with ^{131}I-anti-B1 antibody: assessment of tumor dose-response. *J Nucl Med.* 2003;44:260–268.
7. Kaminski M, Estes J, Tuck M, et al. Iodine I 131 tositumomab therapy for previously untreated follicular lymphoma [abstract]. *Proc Am Soc Clin Oncol.* 2000;19:5a.
8. Juweid ME, Zhang CH, Blumenthal RD, et al. Factors influencing hematologic toxicity of radioimmunotherapy with ^{131}I-labeled anti-carcinoembryonic antigen antibodies. *Cancer.* 1997;80(suppl):2749–2753.
9. O'Donoghue JA, Baidoo N, Deland D, Welt S, Divgi CR, Sgouros G. Hematologic toxicity in radioimmunotherapy: dose-response relationships for I-131 labeled antibody therapy. *Cancer Biother Radiopharm.* 2002;17:435–443.
10. Shen S, Meredith RF, Duan J, et al. Improved prediction of myelotoxicity using a patient-specific imaging dose estimate for non-marrow-targeting ^{90}Y-antibody therapy. *J Nucl Med.* 2002;43:1245–1253.
11. DeNardo G, Yuan A, Goldstein D, et al. Impact of interpatient pharmacokinetic variability on design considerations for therapy with radiolabeled MAbs. *Cancer Biother Radiopharm.* 2003;18:231–237.
12. Siegel JA, Yeldell D, Goldenberg DM, et al. Red marrow radiation dose adjustment using plasma FLT3-L cytokine levels: improved correlations between hematologic toxicity and bone marrow dose for radioimmunotherapy patients. *J Nucl Med.* 2003;44:67–76.
13. Witzig TE, Gordon LI, Cabanillas F, et al. Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *J Clin Oncol.* 2002;20:2453–2463.

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