Antibody Pretargeted Radiotherapy: A New Approach and a Second Chance

Alan R. Fritzberg, PhD

As discussed in an article in *Newsline* in October 1997 on antibody-mediated targeted radiotherapy or radioimmunotherapy (*J Nucl Med* 1997;38 (10):19N), the promise of using monoclonal antibodies to efficiently and selectively deliver radiation to treat solid tumors has not yet been realized. This has been the case when antibodies have the radioactivity directly attached to them and are allowed to accumulate at the tumor following systemic administration. Over the last several years, pretargeting, in which the radiation is delivered separately from the antibody, has been explored by several groups, and results are promising for improved efficacy potential for solid tumors.

Conventional radioimmunotherapy (radioactivity directly attached to antibody) has achieved success in the treatment of leukemias and lymphomas, in which the tumors are radiosensitive and the tumor cells are relatively accessible. In these cases, targeting of tumor cells occurs rapidly, and the amount of radiation required for significant tumor response is lower than for solid tumors. However, the slow accretion of IgG antibodies of 150 kD molecular weight to peak levels at solid tumors over a 24- to 48-hr period has generally resulted in dose-limiting toxicity to radiosensitive bone marrow before sufficient dose for tumor response results. Although tumor responses measurable by CT or MRI imaging have resulted, they remain anecdotal for systemic delivery.

Attempts to improve the efficiency of radiotherapy delivery by antibodies have been made by modifying the protein form of the antibody. Thus, nonbinding portions of the antibody have been removed, resulting in smaller forms: ΔCH2 deletion (removal of a portion of the IgG constant region) of 120 kD, $F(ab')_2$ fragment of 100 kD, Fab or Fab′ fragments of 50 kD and engineered Fv forms of 25 kD. Studies in animal models have shown that the resulting smaller forms have more rapid tumor uptake and disappearance from the blood, thus improving the therapeutic index (Fritzberg AR, Beaumier PL, Bottino BJ, Reno JM. *J Controlled Release* 1994;28:167-173). Peak tumor uptake and time of peak uptake as well as area under the curve (AUC) relative values, which are proportional to the radiation therapy dose, from results in a colon xenograft model with the various forms of radioiodinated antibody NR-LU-10 are shown in Table 1. However, this selectivity advantage has come at the cost of lower tumor retention, thus substantially reducing how much radiation is actually delivered to the tumor. As only a small fraction of the dose is actually taken up in the tumor, losses in tumor uptake and retention further reduce the potential for radiotherapy efficacy.

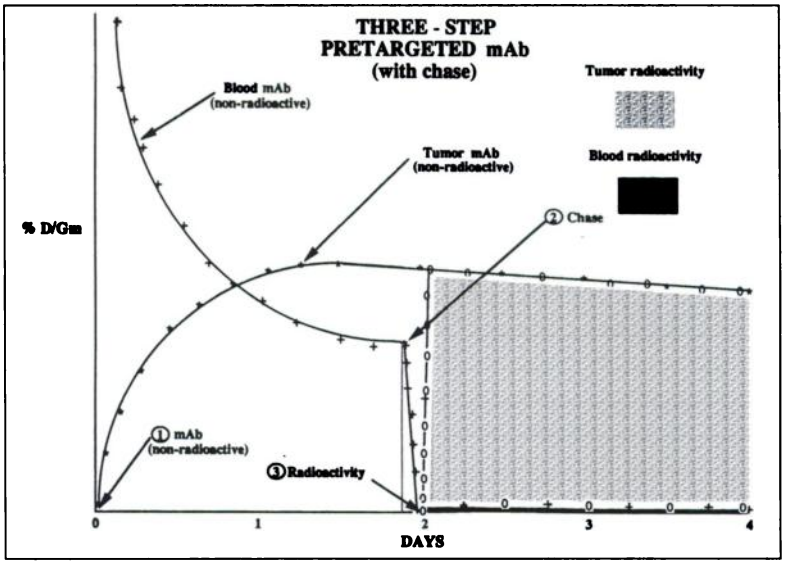

FIGURE 1. Pharmacokinetics of three-step pretargeting. Rapid uptake at 3 hr and slow release of hapten from the tumor is shown over 4 days with pretargeted MAb. Note the large difference between the rates of diffusion into and out of the tumor: very rapid uptake (hours) compared to very slow loss (days) from the tumor. Blood levels are low at all times. (Reprinted from Goodwin DA. *J Nucl Med* 1995;36:876-879.)

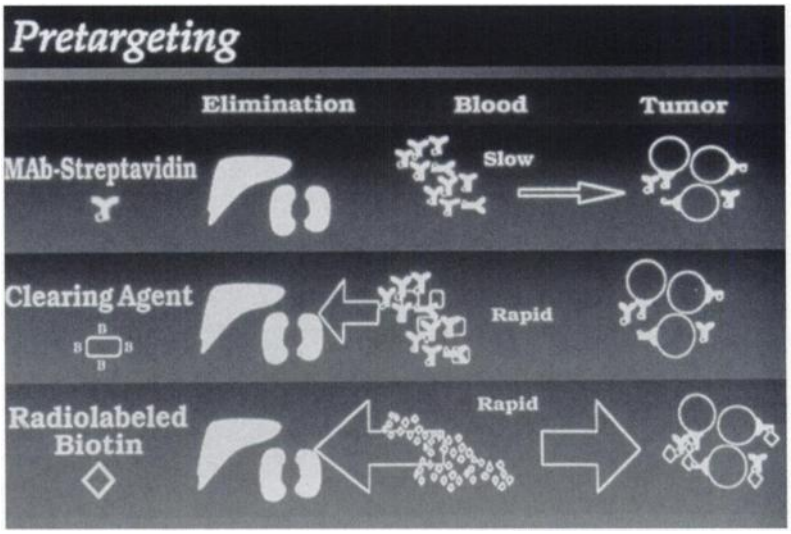

FIGURE 2. Graphic depiction of pretargeting. In the first step, MAb-streptavidin in the blood slowly accumulates on the surface of tumor cells. After 24- to 48-hr accumulation, the clearing agent with biotin for binding to the streptavidin portion and galactose for liver uptake is given and removes the conjugate from the blood. Finally, small-molecule radiolabeled biotin is given and rapidly binds to tumor-targeted MAb-streptavidin or is excreted.

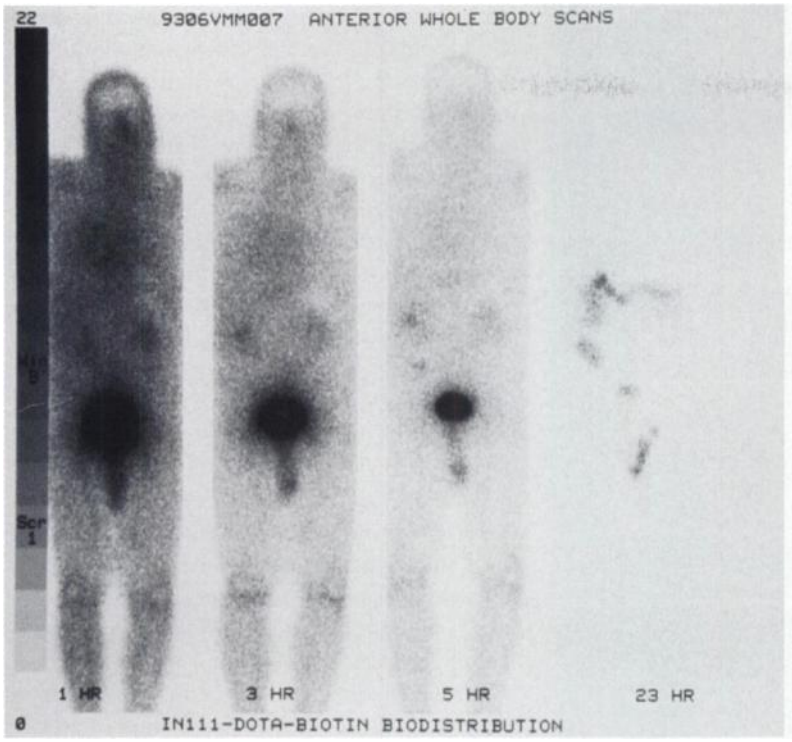

FIGURE 3. Images of ^{111}In-DOTA-biotin in a patient at 1, 3, 5 and 23 hr postinjection in a control study. Rapid extravasation and renal excretion as well as lack of organ retention are seen for the radioactivity delivery agent in the pretargeting system.

That a slow tumor-targeting protein was poorly matched for the delivery of radiation was noted by Goodwin and his colleagues in the late 1980s. They and several other groups worked on the pretargeting concept, in which the slow antibody-targeting step was dissociated from the delivery of the radioactive moiety. Thus, antibody with "receptor"-binding potential was administered as a first step. After tumor targeting and disappearance of the antibody from circulation, the radioactivity was administered as a small molecule attached (i.e., a ligand). The small molecule could rapidly perfuse the tumor containing the pretargeted antibody and bind to it while the nontargeted small molecule would be rapidly excreted through the kidneys, thus reducing the radiation exposure of the bone marrow (Goodwin DA. *J Nucl Med* 1995;36:876-879). As shown in Figure 1, the addition of a clearing step significantly improved the tumor-to-blood AUC ratio.

Early work by Goodwin and Meares (*J Nucl Med* 1988;29:226-234) and work done in collaboration with the Hybritech group (*Cancer Res* 1991;51:6670-6675) focused on bifunctional antibodies combining antitumor and antichelate binding affinities, allowing the radiometal chelate to be captured by the pretargeted bifunctional antibody. Further improvements on small-molecule radioactivity capture were made by exploring the high affinity, 10^{15}, of avidin or streptavidin (SA) for biotin (Hnatowich DJ, Virzi F, Rusckowski, et al. *J Nucl Med* 1987;28:1294-1302). This system has been studied extensively for imaging and radiotherapy in patients by Paganelli et al. (*Cancer Res* 1991;51:5960-5966).

The use of antibody-SA, clearing agent and DOTA-biotin for pretargeting has been studied in animals and patients by the NeoRx (Seattle, WA) group. A graphic depiction of the reagents and steps of the pretargeting protocol is shown in Figure 2. In the first step, the antibody-SA is administered and allowed to circulate for tumor uptake and penetration. In the second step, the clearing agent is given and circulating conjugate is removed by the liver. Finally, in the last step, the radiolabeled biotin is given, which rapidly binds to pretargeted antibody-SA at tumor, and what is not bound is rapidly excreted through the kidneys into the urine. Conjugation of SA to pancarcinoma antibody NR-LU-10 (Breitz HB, Weiden PL, Vanderheyden J-L, et al. *J Nucl Med* 1992;33:1099-1112) did not compromise tumor uptake or retention. Clearing agents based on biotin for binding to the conjugate and galactose for removal by the Ashwell receptors of the liver have been effective in clearing circulating conjugate. The DOTA macroacrylic chelator is very stable for a wide variety of +2 and +3 metals with the slow, chelate formation kinetics overcome easily by heating the radiometal and the small, heat-stable DOTA-biotin molecule. Thus ^{111}In for imaging and ^{90}Y and ^{177}Lu for beta particle radiotherapy are all useful in this chelate-biotin system. The rapid perfusion of the small-molecule DOTA-biotin with ^{111}In into extravascular tissues and lack of retention in organs is clear from the images in Figure 3, in which ^{111}In-DOTA-biotin was given without the antibody-SA and clearing agent components of pretargeting.

There have been extensive studies of pretargeting with NR-LU-10-SA in tumor xenografts. Optimal tumor uptake and retention have been achieved with high doses of the antibody-SA conjugate to load the tumor with the SA receptor and to maximize tumor homogeneity. After determining appropriate ratios of clearing agent to amount of circulating antibody-SA and stoichiometry of DOTA-biotin to tumor-targeted antibody-SA, the tumor

TABLE 1
Radiation Exposure Comparison for Tumor and Blood for Different Carriers

Carrier	Peak tumor uptake	Time of peak uptake	AUC-infinity		Tumor-to-blood ratio
			Blood	Tumor	
MAb	24% ID/g	24 hr	1697	4819	2.89
ΔCH2	23% ID/g	24 hr	441	815	1.85
$F(ab')_2$	12% ID/g	8 hr	238	559	2.35
Fab	8% ID/g	4 hr	34	319	9.38

AUC = area under the curve; ID = injected dose.

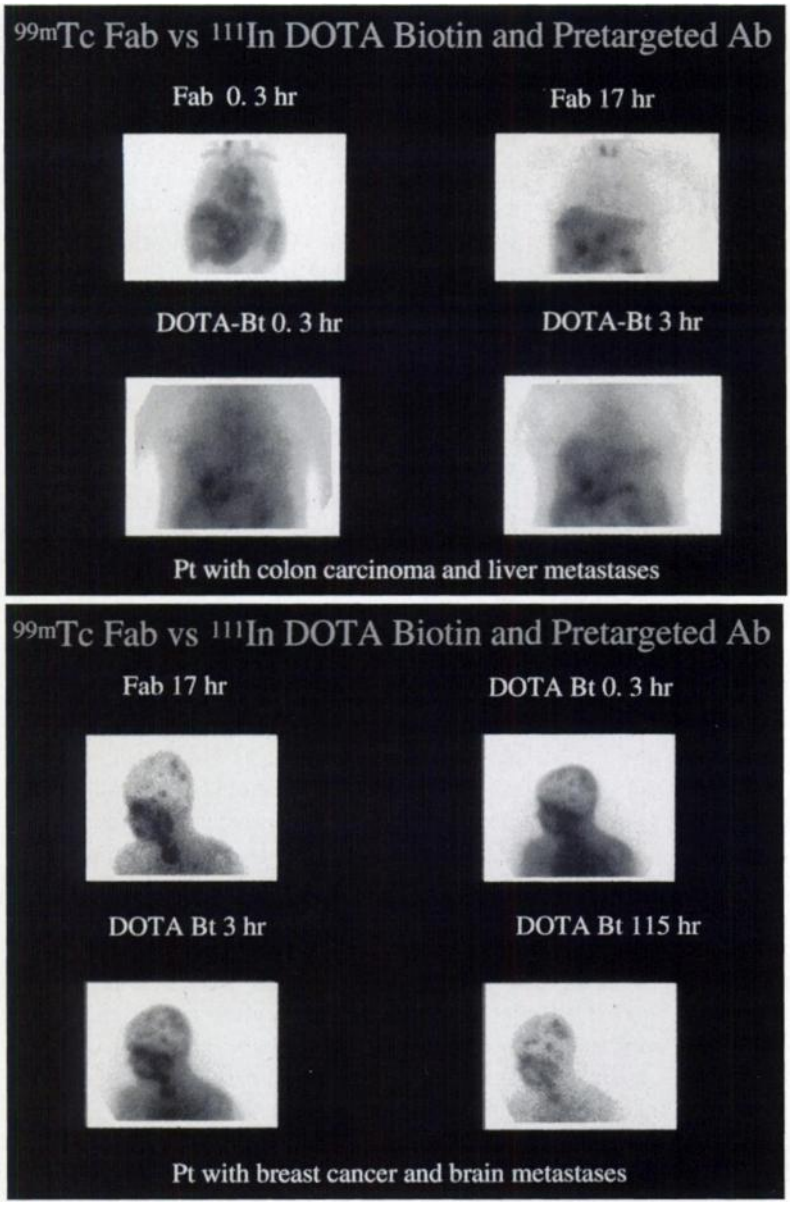

FIGURE 4. (Top) Images comparing ^{99m}Tc NR-LU-10 Fab (Verluma) and pretargeted ^{111}In-DOTA-biotin from a patient with colon cancer metastatic to liver. The radiolabeled Fab fragment shows blood pool at 0.3 hr and clear tumor images at 17 hr while pretargeted ^{111}In-DOTA-biotin shows liver metastases clearly at 0.3 hr as well as at 3 hr. (Bottom) Images comparing ^{99m}Tc-Fab with pretargeted ^{111}In-DOTA-biotin in a breast cancer patient with brain metastases. Tumor uptake is seen at 0.3 hr and is retained through the 115-hr image. (Images courtesy of Hazel Breitz, Virginia Mason Medical Center, Seattle.)

concentration with time gave AUC values of 3288 for tumor and 77 for blood, as determined for the antibody forms in Table 1. These values result in a ratio of 42, about 15 times higher than the whole antibody. Further, the selectivity was achieved without major losses in tumor uptake.

Clinical trials of the pretargeting system at NeoRx have resulted in an optimized amount and schedule of component administration. In the studies, Verluma, the ^{99m}Tc-Fab fragment of NR-LU-10, was used to qualify patients for the pretargeting study. Thus, comparison of the Fab fragment images with ^{111}In-DOTA-biotin in the pretargeting approach provides a sense of the rapidity of tumor uptake in tumors and also disappearance of radioactivity from the blood. Figure 4 (top) shows a patient with colon cancer and liver metastases and (bottom) a patient with breast cancer and metastases to the brain. At 0.3 hr (18 min) postinjection, tumors are clearly imaged with pretargeting, whereas the Fab fragment image of the colon cancer patient shows only vascular blood pool. In the breast cancer image, tumor retention to 115 hr is shown for the brain metastases.

A ^{90}Y dose-escalation trial has resulted in non-marrow-limiting doses above 200 mCi compared to typical maximum tolerable dose values of 25 mCi to 30 mCi with conventionally labeled ^{90}Y antibodies (Abrams P, et al. *Proc Am Soc Clin Oncol* 1995;14:423). Estimates of tumor uptake (% ID/g) in patients are comparable to conventionally radiolabeled antibodies, and tumor responses have been seen in patients who had failed standard therapies.

The encouraging results in animals and patients has led to consideration of application of pretargeting to radiotherapy with short-lived alpha emitters. Alpha emitters (He atoms) with a mass about 8000 times greater than beta particle electrons exhibit high linear energy transfer (LET) over a short path length of several cell diameters, and thus require very few disintegrations to kill a cell. The numbers of atoms needed have been estimated at 6 or 7 for internalized alphas to 25 for surface-bound atoms (Vaidyanathan G, Zalutsky M. *Phys Med Biol* 1996;41:1915-1931). Further, the high LET results in lack of repair potential and oxygen effects on cytotoxicity. Although several alpha emitters are under consideration, those readily available include ^{211}At (7.2 hr $T_{1/2}$), produced by accelerators, ^{212}Bi (1 hr $T_{1/2}$) from ^{212}Pb (10.6 hr $T_{1/2}$), separated following decay of ^{224}RA (3.6 d $T_{1/2}$) and ^{213}Bi (47 min $T_{1/2}$) from decay of ^{225}Ac (10 d $T_{1/2}$). While the ^{212}Bi and ^{213}Bi emitters are so short lived as to limit application to very rapid targeting processes, the ^{212}Pb decay provides an in vivo generator of ^{212}Bi, thus allowing uses that involves lower targeting processes.

Work by Gansow et al. demonstrated that DOTA forms inert chelates with lead and bismuth (Kumar K, Magerstadt M, Gansow OA. *Chem Comm* 1989;3:145-146). However, application requires the chelate to contain the product ^{212}Bi from ^{212}Pb decay. Mizardeh, Kumar and Gansow showed that about 36% of the ^{212}Bi was released out of the DOTA and in vivo localized in kidney (*Radiochim Acta* 1993;60:1-10). As the DOTA-biotin used in pretargeting in the antibody-SA system contains the DOTA chelate moiety, and understanding the chelation properties of lead and bismuth as mentioned above, studies were

(Continued on page 36N)

ings of government. This 5-day forum, to be held in April 1998, is being coordinated by the SNM-TS as part of HPN's commitment to this project.

Outreach to Chapter Meetings

The Government Relations Office conducted several chapter visits by Robert Carretta, MD, chairman of the Government Relations Committee, in an effort to expand the visibility of the ACNP and SNM's government relations efforts. Chapter visits included those to the Pacific Northwest, Greater New York, Missouri Valley, and Northern and Southern California meetings. Chapters interested in arranging for a government relations speaker at their upcoming meetings should contact David Nichols, Director of Government Relations, at (703) 708-9773.

Political Action Committee

SNM, through the Government Relations Office, is moving forward with the establishment of a political action committee (PAC) by April 1998. This PAC will allow the Society to become more visible in the Congress and assist those members who are legislative friends of nuclear medicine in their reelection campaigns.

Legislative Network

The SNM-TS continues to operate a very successful legislative network. With more than 50 members in the legislative network, spread out among all the chapters of the SNM, the network enables members to keep informed on legislative issues and contact their members of Congress prior to key votes on Capitol Hill. If you are interested in participating in this legislative network or being included as a key contact in our nuclear medicine database, please contact Amanda Sullivan in the Government Relations Office at (703) 708-9773.

For more information on any of these topics, members are encouraged to routinely check the government relations page on the web at www.snm.org or to contact the Government Relations Office at (703) 708-9773.

—David Nichols is the director of the ACNP/SNM government relations office.

Alpha Particle Therapy

(Continued from page 19N)

parent of ^{229}Th, which is the parent of ^{225}Ac, which is the parent of ^{213}Bi. The DOE is currently working out plans to use U.S. uranium stockpiles for the production of alpha emitters and other medical isotopes. "This is a swords-to-plowshares story about using bomb-grade materials directly toward the treatment of cancer," said Robert E. Schenter, PhD, deputy site manager for the isotope program at PNNL. Scientists at PNNL are currently producing the beta emitter ^{90}Y and the alpha emitters ^{213}Bi, ^{225}Ac and ^{223}Ra from stored nuclear materials.

Still a Long Way to Go

As promising as alpha emitters seem as a potential treatment for cancer, researchers remain reserved in their enthusiasm. They remember the initial excitement over monoclonal antibodies and the resulting disappointment when that treatment failed to work against solid tumors. While acknowledging that they have made tremendous strides in alpha research over the past decade, researchers know they still have a long way to go.

—Deborah Kotz

Antibody Pretargeted Radiography

(Continued from page 22N)

recently initiated to evaluate tumor delivery of ^{212}Pb/^{212}Bi in the pretargeting system.

Initial studies used the gamma-emitting isotopes ^{203}Pb and ^{205}Bi for study of DOTA-biotin stability and pretargeting. The complexes were found to be stable, and pretargeting tumor and normal organ values were similar to those with ^{111}In and ^{90}Y. Then, ^{212}Pb and ^{212}Bi DOTA-biotin were prepared and evaluated. As expected from the results of Mirzadeh et al., about 35% of the biotin binding was lost for the ^{212}Bi from decay of the ^{212}Pb. However, when applied in the pretargeting context with NR-LU-10-SA, both the ^{212}Pb and ^{212}Bi values in tissue resulted in over 20% ID/g in tumor in 15 min, rising to 30% ID/g after 1 hr. Blood values were below 5% ID/g by 15 min, resulting in high tumor-to-blood AUC values. The kidney ^{212}Bi values were increased over the first 3 hr, then diminished, indicating that ^{212}Bi released from circulating forms localized in the kidney, but tumor-targeted radioactivity remained, even following escape from the DOTA chelator.

The preliminary studies of the alpha emitters briefly described establish potential in an efficient targeting system for radionuclides with short half-lives. The pretargeting system provides a means to evaluate the potential of targeted alpha radiotherapy in small and large xenograft tumors as well as metastatic tumor models. Issues for alpha emitters in pretargeting to be addressed in future research include toxicity to normal tissues and efficacy with respect to applicability in micrometastases relevant to adjuvant tumor treatment and the potential for treating established solid tumors.

Note: Pretargeting of ^{212}Pb supported by PHS Grant CA71221.

—Alan R. Fritzberg, PhD, is chief scientist and chairman of the scientific advisory board, NeoRx Corporation, Seattle, WA.