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## EDITORIAL

# Bullets to Magic Bullets—and Miles to Go Before We Sleep

In this issue of the *Journal of Nuclear Medicine*, Klivényi et al. (1) highlight the advantages and limitations in their studies of pretargeting xenografted tumor using a bispecific antitumor/antihapten antibody followed by <sup>68</sup>Ga chelate. They report that the system permits excellent PET imaging; importantly, they delineate the effect of enantiomer selection on tumor uptake and tumor-to-nontumor ratios.

The need to improve relative uptake of radionuclide in tumor using an antibody-based targeting system is urgent. The clearance of intact immunoglobulins is slow, resulting in low target-to-background ratios, especially early after administration, thereby limiting the use of nuclide-based detection and therapy. The considerable advantages of PET have intensified efforts to develop antibody-mediated tumor imaging methods appropriate for use with short-lived positron emitters. Rapid tumor localization is also essential in targeted therapy using short-lived alpha-emitting isotopes.

Seminal studies, using antichelate antibodies followed by a radiolabeled chelate (2) and various avidin–biotin approaches (3), showed the potential of pretargeting a decade ago. Since then, the technique has been refined in many aspects, several of which were used in the present study. Examples include the eval-

uation of various bifunctional antibody constructs with mono- or bivalent binding to tumor antigen (4–6), the application of blood-clearing agents to remove circulating antibodies before injection of effector molecule; and the use of bivalent hapten molecules, which apparently enhance tumor uptake (4,7). A clinical study in colorectal cancer patients demonstrated significantly improved tumor-to-nontumor ratios when comparing bifunctional antibody/<sup>111</sup>In-diethylenetriamine pentaacetic acid (DTPA) with its bivalent <sup>111</sup>In-labeled counterpart (8), underscoring the potential of the method.

The avidin–biotin system has the advantage of the extremely high affinity (10<sup>–15</sup> M) between biotin and the proteins avidin and streptavidin and the possibility of enhancing tumor signal, because both avidin and streptavidin have four binding sites for biotin, the preferred effector molecule. Grana et al. (9) successfully showed the use of their three-step targeting approach; biotinylated antibody followed by avidin/streptavidin and finally radiolabeled biotin, in a variety of solid tumors. The most extensive clinical trial evaluating pretargeting for therapy has been initiated by NeoRx (Seattle, WA). A pretargeted streptavidinylated antibody is followed by a clearing agent, after which biotin, labeled with <sup>90</sup>Y by the metal chelator 1,4,7,10-tetrazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), is administered. Initial clinical trials from both groups have shown that the amount of radioactivity

that can be safely administered is significantly greater with this methodology than with radiolabeled antibody alone (10,11).

An interesting alternative to the antibody/hapten and avidin–biotin systems, presented by Hnatowich et al. (12), is the use of peptide nucleic acid (PNA). The interaction between complementary PNA strands is essentially similar to that of deoxyribonucleic acid, but the peptide backbone of PNA results in greater serum stability, increasing the applicability of single-strand radiolabeled PNA to localize tumor that is pretargeted with an antibody conjugated with the complementary PNA strand. Regardless of the choice of “receptor–ligand” pair, the selection of an appropriate antibody–antigen system is imperative, perhaps more so in pretargeting techniques than in conventional radioimmunotargeting. The complexity of the system necessitates careful optimization.

Pretargeting has also found applications outside the realm of nuclear medicine. Antibody-directed prodrug therapy, in which an antibody is used to direct an enzyme to the tumor site where it can subsequently convert a prodrug to a cytotoxic agent, is in clinical trials (13). In addition, bispecific antibodies that recognize tumor-associated antigens and immune effector antigens have been studied as immunotherapeutic agents. Initial studies used anti-CD3 antibodies (14); later studies have targeted the constant fragment gamma receptor family (15),

Received Jul. 17, 1998; revision accepted Aug. 4, 1998.

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CD28 (targeting cytotoxic T cells) (16) and CD16 (targeting natural killer cells) (17). Early clinical trials demonstrated the safety of bispecific antibodies in Hodgkin's disease (18) and breast cancer (19). Concerns include successful delivery of the putative cytotoxic cell to the tumor site. Another pretargeting strategy relies on the T-cell-activating potency of the superantigen *Staphylococcus* enterotoxin A. A fusion protein consisting of an antitumor-directed antigen-binding fragment and the superantigen were found to be safe in a recently concluded clinical trial (20).

The initial promise of pretargeting to deliver radionuclides selectively to tumor has been tempered by several constraints. Among these are the immunogenicity of compounds such as streptavidin; the presence of endogenous biotin; the rapid tumor clearance of monovalent chelates; the restriction in using antibodies against chelates with limited nuclide applicability; and finally, as shown so elegantly by Klivényi et al., that racemic mixtures of chelates may have variable effects, with enantiomeric nature influencing targeting.

Some of these problems are being addressed successfully. The ability of DOTA to form stable chelates with a number of +2 and +3 metals has permitted DOTA-biotin to be labeled with radionuclides for imaging ( $^{111}\text{In}$ ), beta-particle therapy ( $^{90}\text{Y}$ ) and alpha-particle therapy ( $^{212}\text{Bi}$ ) (21). Bivalent and multivalent chelate constructs have increased targeting efficiency. Current efforts to replace streptavidin with a modified avidin that may be less immunogenic show promise (22).

But will it be good enough? Zhu et al. (23) recently concluded that "pretargeting... was neither sensitive enough for radioimmunodetection nor effective enough for radioimmunotherapy" in their model. These conclusions, by a group that has worked long and hard on the problem, should give pause for thought. That is not, however, a reason to give up the search for an effective pretargeting system. We should examine and seek to obviate limitations through antibodies with more suitable binding characteristics and lower immuno-

genicity, more appropriate antigen systems that are not modulated and more stable effector agents, thus maximizing the immense potential of targeted radionuclide delivery.

The use of radioimmunoscintigraphy has been underscored by U.S. Food and Drug Administration approval of four radiolabeled antibodies for the detection of cancer. Compared with traditional radioimmunoscintigraphy, pretargeting results in improved tumor contrast (8,24), and PET imaging should further increase the sensitivity of this technique. Although we are a long way from an optimal pretargeting system for therapy, there is no reason to be pessimistic. After all, one has only to extrapolate these data to realize that most chemotherapeutic agents would not pass muster under the criteria we set for ourselves. The gloomy predictions of today must be studied and dissected to yield the promise of tomorrow, which will occur as long as careful studies are carried out.

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