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REVIEW

Novel radiolabeled antibody conjugates

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This article reviews the development of radioimmunoconjugates as a new class of cancer therapeutics. Numerous conjugates involving different antigen targets, antibody forms, radionuclides and methods of radiochemistry have been studied in the half-century since radioactive antibodies were first used in model systems to selectively target radiation to tumors. Whereas directly conjugated antibodies, fragments and subfragments have shown promise preclinically, the same approaches have not gained success in patients except in radiosensitive hematological neoplasms, or in settings involving minimal or locoregional disease. The separation of tumor targeting from the delivery of the therapeutic radionuclide in a multistep process called *pretargeting* has the potential to overcome many of the limitations of conventional, or one-step, radioimmunotherapy, with initial preclinical and clinical data showing increased sensitivity, specificity and higher radiation doses delivered. Our particular focus in pretargeting is the use of bispecific, trimeric (three Fab′s) constructs made by a new antibody engineering method termed 'dock-and-lock.'
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Challenges and prior studies

The use of antibodies to target and treat cancers predates hybridoma-generated monoclonal antibodies (MAbs), starting with the pioneering studies in the 1950s and 1960s that showed radiolabeled immunoglobulins to normal tissue and to undefined tumor antigens could specifically localize in their respective target tissue as compared to other tissues (Pressman and Korngold, 1953; Korngold and Pressman, 1954; Bale and Spar, 1957; Bale *et al*. 1960; McCardle *et al*., 1966). Immune sera raised in various animal hosts against defined human tumor-associated antigens, such as α-fetoprotein and carcinoembryonic antigen (CEA), were then used in the mid-1970s to illustrate the potential for cancer detection by external scintigraphy, a technique termed immunoscintigraphy or radioimmunodetection, first in animal–human tumor xenograft models and then clinically (Goldenberg *et al*., 1974, 1978). The first clinical studies used affinity-purified ^{131}I-labeled goat anti-CEA IgG. Although animal studies had upheld the principle of selective tumor targeting by a specific antitumor antibody, tumor visualization by external imaging was not apparent for several days after injection because of an excessive amount of antibody remaining in the blood pool. The first clinical studies tried to overcome this by co-administering ^{99m}Tc-albumin and ^{99m}Tc-pertechnetate with the radiolabeled IgG. A computer-assisted subtraction method was used to account for radioactivity in the blood and extravascular space based on the ^{99m}Tc-distribution, with the remaining activity representing an enriched uptake of the radiolabeled antibody in the tumor (Goldenberg *et al*., 1978). Although the technique was technically challenging and often difficult to interpret (Mach *et al*., 1980), it was an effective method for localizing a number of different cancers (Goldenberg *et al*., 1980; Kim *et al*., 1980; van Nagell *et al*., 1980). With the development of MAb technology in the early 1980s, it soon became possible to evaluate radioiodinated antibody fragments (Mach *et al*., 1983; Goldenberg *et al*., 1990a, 1993). These agents cleared more quickly from the blood and allowed earlier detection without the need for computer manipulation. Radiochemistry advances brought new capabilities to examine conjugates prepared with ^{111}In and ^{99m}Tc, and clinical studies showed improved disease disclosure or upstaging with these new agents, leading to several product approvals (Goldenberg and Larson, 1992; Goldenberg *et al*., 1997; Sharkey and Goldenberg, 2006). As imaging agents, they had excellent specificity, but relatively poor sensitivity and image resolution, because the target/noise ratios did not compare well with ^{18}F-fluorodeoxyglucose (FDG), which replaced most of these first-generation products. Nevertheless, with the demonstration that murine MAbs could target cancers very specifically, interest in therapy ensued. It soon became apparent that repeated administrations with radiolabeled murine MAbs were restricted because of human anti-mouse antibodies (HAMA) (Larson, 1990; Murray *et al*., 1994; De Nardo *et al*., 1995, 1997a). Thus, chimeric or humanized antibodies needed to be introduced for antibodies conjugated with radionuclides, as also was the case for naked MAb therapies (Goldenberg *et al*., 1990b, 1992; Goldenberg and Larson, 1992).

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The adoption of more human constructs for cancer therapy or imaging with radiolabeled antibodies did not solve the problems of improved imaging or radiation dose delivered for therapy, except for allowing repeated dosing. The fundamental issues for imaging were related to signal enhancement over adjacent normal tissue radioactivity, translating to image resolution, whereas in therapy, it was delivering a tumoricidal dose at radioactivity levels tolerated by normal tissues.

IgG and its fragments have different characteristics that determine their targeting properties, such as how quickly they reach the target antigen and clear from the blood, which organ clears the antibody from the blood, penetration into the tumor and amount of the injected product binding to the target. Once antibodies target their respective antigens, they generally bind with high avidity, which in turn determines their tumor residence time, whereas the unbound antibody is processed by various organs in the body and eventually degraded and excreted. IgG, which is the principal antibody form used, clears very slowly from the blood, requiring several days before a sufficient amount leaves the circulation to allow the specific concentration taken into the tumor to be distinguished from blood and adjacent tissue radioactivity. Its slow clearance is in part owing to its large size, ~150 000 Da, that impedes its extravasation, resulting in a slow tumor accretion. Because the concentration of an IgG remains high in the vascular compartment and a tumor's vasculature is leakier than that of most normal tissues (Folkman, 1971; Jain, 1991), the IgG is slowly (i.e., 1–2 days) able to become localized at a relatively high concentration in tumors. In mouse xenograft models, tumor uptake is typically between 10 and 30% of the injected activity per gram tumor, but in humans, with a larger vascular and extravascular volume of distribution, this accretion is reduced to <0.1% per gram (Siegel *et al*., 1990; Buchsbaum, 1995). As the molecular size of an antibody is reduced from a divalent $F(ab')_2$ fragment (~100 000 Da) to the monovalent binding Fab fragment (~50 000 Da), there is a progressively faster clearance from the blood. Higher tumor/blood ratios are achieved with the smaller antibody forms, but generally at the cost of having proportionally less of the injected product reaching the tumor, a commensurately shorter residence time, but perhaps better tumor penetration (Sharkey *et al*., 1990; Behr *et al*., 1995). Molecular engineering has enabled the formation of even smaller antibody structures, such as scFv (~25 000 Da), which are cleared even more rapidly from the blood, and with even a lower uptake in tumors (Colcher *et al*., 1998). However, the rapid clearance of these molecules from the blood and adjacent, antigen-negative tissues, can result in early, high tumor to background ratios, achieving relatively strong signals compared to background (Batra *et al*., 2002; Wittel *et al*., 2005).

Although scFv's exceedingly rapid clearance properties with low tumor accretion are generally considered pharmacokinetically unfavorable for targeting radionuclides, a number of innovative strategies have been undertaken to restore in part the multivalency of an antibody, so as to overcome this deficiency and enhance tumor retention. For example, by deleting the C_H2 sequence of an IgG, the resulting construct, whereas still divalent and nearly 100 000 Da in size, clears extraordinarily fast from the blood, resulting in high-localization ratios within a short period of time (Slavin-Chiorini *et al*., 1993). This resulted in a higher molecular size, allowing the construct to clear from the blood slower than an scFv, but more quickly than an IgG. A number of new constructs, ranging from divalent diabodies, minibodies, $(scFv)_2$-Fc and other assorted constructs, are essentially composed of multiple scFvs tethered in different ways, and illustrated in Figure 1 (Colcher *et al*., 1998; Wu, 2004; Binz *et al*., 2005; Kenanova and Wu, 2006).

Although the tumor to background ratios for imaging and the therapeutic index for therapy were not optimal, they still provided certain prospects for these directly labeled antibodies. For example, in radioimmunodetection, these radiolabeled antibodies could differentiate tumor from nonspecific proliferation, such as in inflammation, when compared to FDG, or when FDG was not suitable (such as in prostate cancer) (Kelloff *et al*., 2005a, b; Jana and Blaufox, 2006; Zalutsky, 2006a). In radioimmunotherapy, evidence for efficacy could be shown in locoregional applications (Alvarez *et al*., 1997, 2002; Mahe *et al*., 1999; Riva *et al*., 1999; Meredith *et al*., 2001; Goetz *et al*., 2003; Paganelli *et al*., 2006; Reardon *et al*., 2006), in an adjuvant setting (Liersch *et al*., 2005; Zalutsky, 2006b) or in patients with

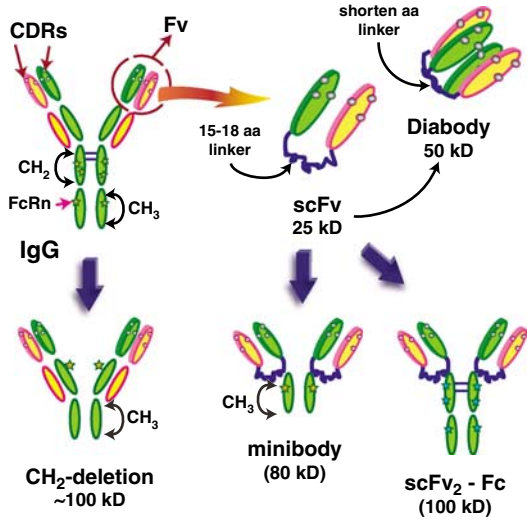

Figure 1 Examples of molecularly re-engineered forms of antibody used for targeting radionuclides. Starting with the IgG (upper left), the separate portions of the Fv can be isolated and tethered together with an amino-acid (aa) linker to form an scFv (single chain) construct. Shortening the length of the aa linker can lead to the formation of homodimeric structures, such as diabodies, but other forms, such as tribodies and tetrabodies have also been described. Larger constructs formed from scFv and various portions of the IgG's C_H3 and $C_H2 + C_H3$ have also been used. The $scFv_2$-Fc site specifically mutates FcR n-binding residues in the heavy chain that control the pharmacokinetic behavior of the IgG. Another construct, the C_H2 deletion, also has accelerated clearance from the blood, but preserves divalent antigen binding.

radiosensitive lymphomas (Goldenberg, 2001; Dreyling *et al*., 2006; Meredith, 2006; Meredith and Knox, 2006; Witzig, 2006). Responses in lymphoma are most certainly related to the radiosensitivity of these tumors, because the measured uptake of radiolabeled antibodies in lymphoma is no higher than that in solid tumors (Sharkey and Goldenberg, 2005). However, it is noteworthy that with both of the radiolabeled anti-CD20 antibodies used commercially, they are combined with a naked CD20 murine (Bexxar) or chimeric (Zevalin) MAbs that have antitumor activity (Witzig *et al*., 2002; Davis *et al*., 2004). Perhaps the responses are augmented to some degree by an underlying synergy between the unconjugated and radioconjugated antibodies (Hernandez and Knox, 2004). Indeed, objective responses have been observed in lymphoma with radiolabeled antibodies having low radioactivity or low doses of antibody given, and in some cases without an ability to visualize targeting to a given lesion (Goldenberg *et al*., 1991; Kaminski *et al*., 1993; Sharkey *et al*., 2003a). Most of the antibodies used as radioconjugates for solid tumors have not been therapeutically active as unconjugated antibodies, and therefore there have been a variety of approaches taken to enhance the therapeutic response (Sharkey and Goldenberg, 2005). An opinion shared by many is that radioconjugates may be best applied clinically in solid tumors if combined with a chemotherapeutic agent (Wong, 2006). Most combinations examined preclinically have sought to add a chemotherapeutic agent to enhance the effect of a full dose of the radioconjugate (DeNardo *et al*., 1997b; Crow *et al*., 2005; Kelly *et al*., 2006; Masters *et al*., 2006), whereas others seek to enhance an effective, yet not curative, chemotherapy regimen with a smaller dose of the radioconjugate (Cardillo *et al*., 2002; Gold *et al*., 2003) A few phase I clinical trials have examined the former approach in patients with advanced solid tumors. Some treatment regimens used myeloablative radioconjugate doses with the aid of bone marrow or peripheral stem cell support, but without evidence of objective responses (Wong *et al*., 2003; Forero *et al*., 2005; Richman *et al*., 2005; Sharkey *et al*., 2005b).

Pretargeting as a new paradigm

The single most perplexing problem with the delivery of antibody-targeting radionuclides is that the efficiency of selective targeting is relatively poor. Maximum radiation absorbed doses measured in solid tumors most often are much <2000 cGy at the maximum tolerated dose (Sharkey and Goldenberg, 2005). The difference between the continuous low-dose rate radiation delivered by a radioconjugate and conventional external beam radiation make it difficult to be certain what threshold might be required to achieve objective responses, but there is little doubt that directly radiolabeled antibodies have not reached this limit, even with hematopoietic support. Thus, alternative strategies that could increase the radiation dose to tumors substantially are necessary.

Solving the problem associated with the deficiencies in targeting directly radiolabeled antibodies requires a separation of the targeting moiety from the effector bearing the radionuclide, so as to permit administration of the radionuclide when optimal targeting has been accomplished. To accomplish this, the targeting was first achieved with a bispecific antibody (bsMAb) that binds both to a target antigen as well as the radiometal–chelate complex of the effector molecule (Reardan *et al*., 1985; Goodwin *et al*., 1986). By separating the targeting steps, the bsMAb does not need to be treated with reagents used for conjugation to a suitable radionuclide, which ensures its immunoreactivity is uncompromised. Other pretargeting methods have been developed and applied, particularly involving biotin–avidin (or streptavidin) binding, with encouraging results shown in certain situations (Goldenberg *et al*., 2006). Two distinct methods for pretargeting using the avidin/streptavidin–biotin method have evolved. Both use biotin as the radiolabeled carrier, and both include a step to remove excess antibody conjugate from the blood, so that radiolabeled biotin can be administered within 1–2 days of the conjugate's injection. Unlike approaches based on avidin/streptavidin that rely on a clearing step, bsMAb pretargeting simply delays giving the radionuclide until the bsMAb has cleared sufficiently from the blood. This is possible because the smaller bsMAb clears more quickly than the larger IgG-based conjugates. In addition, streptavidin's ultra-high affinity would require the concentration of the avidin-based conjugate to be reduced to far lower levels in the blood than that required for a bsMAb, and therefore this step is very important for those methods. In avidin–biotin approaches, radiolabeled biotin is strongly bound to streptavidin localized at the tumor site. BsMAb pretargeting relies on a radiolabeled effector that bears two haptens capable of binding to the anti-hapten binding arm of the bsMAb. This technique, called the affinity enhancement system (AES), improves the uptake and retention of the radiolabeled hapten-peptide (Le Doussal *et al*., 1989; Goodwin *et al*., 1994; Boerman *et al*., 1999). Although there is an affinity advantage for binding biotin to streptavidin in the tumor, as compared to the divalent hapten (i.e., 10^{-15} vs 10^{-9} M, respectively), ultimately these complexes are both held in the tumor by the association of the antitumor antibody to its antigen. The major advantage for a bsMAb approach is the ability to use humanized antibodies that will have a lower immunogenicity than the foreign streptavidin or avidin proteins. Regardless of the pretargeting method used, each has been shown in animal models and in patients to be highly efficient targeting procedures, with improved therapeutic properties as a result of the enhanced tumor/blood ratios (Boerman *et al*., 2003; Sharkey *et al*., 2005c; Goldenberg *et al*., 2006). However, we will restrict our discussion to use of bsMAbs in pretargeted imaging and therapy of cancer.

The first clinical testing of a bsMAb pretargeting method for cancer imaging reported successful localization of colon cancer metastases in the liver using an ^{111}In-chelate complex given 5 days after the

administration of an unlabeled anti-CEA × anti-chelate bsMAb (Stickney *et al*., 1991). In contrast, an ^{111}In-labeled antibody to colorectal cancer had excessive amounts of ^{111}In in the liver that frequently masked tumor targeting (Abdel-Nabi *et al*., 1987, 1990; Lamki *et al*., 1990; Patt *et al*., 1990). In contrast to the monovalent chelate–radiometal complex, Le Doussal *et al*. (1989) found that a bivalent structure would be bound more stably at the tumor site, presumably because the divalent hapten-peptide could be crosslinked by two adjacent bsMAbs (Figure 2). This new affinity enhancement system was applied to an anti-CEA × anti-DTPA(In) bsMAb pretargeting system that was used successfully to target CEA-producing tumors in patients with an ^{111}In- or ^{131}I-labeled di-DTPA-peptide (Le Doussal *et al*., 1993; Peltier *et al*., 1993; Chetanneau *et al*., 1994). However, once ^{99m}Tc-Fab-based imaging agents became available that allowed tumor visualization within a few hours, with minimal uptake in the liver, investigators turned to the simplicity of a single-step, direct targeting method. Although ^{18}F-FDG eventually replaced most antibody-based cancer imaging products, new data with pretargeted PET imaging agents suggest that this technique could be useful for molecular imaging.

Bispecific antibodies were first made by conjugating a Fab fragment of an antitumor antibody to a Fab fragment of the antichelate antibody. Today, the process is more efficiently performed through molecular engineering (Rossi *et al*., 2003, 2005, 2006). Constructs as small as 50 000 Da with monovalent binding to the tumor target and to the hapten have exceptionally fast blood clearance properties, and perform well in pretargeting (Rossi *et al*., 2003), but studies have indicated that a bsMAb with divalent binding to the tumor also has favorable pharmacokinetic properties, and affords higher uptake and retention in tumors (Karacay *et al*., 2002; Rossi *et al*., 2003). More recently, a new, more modular, method for assembling bsMAbs has created highly stable, humanized bsMAb constructs with a molecular weight of ~157 Da, and which have performed well in a pretargeting setting (Rossi *et al*., 2006).

The second critical component of the bsMAb pretargeting system is the radiolabeled hapten-peptide. The hapten portion of this complex is responsible for binding to the antihapten arm of the bsMAb. Initially, the hapten was a radiometal-binding chelate, such as DTPA, which could also be loaded with a radiometal, such as ^{111}In. Two DTPAs could be joined together synthetically by a short (e.g., usually 2–4 amino acids) peptide. By using tyrosine as one of the amino acids, the DTPA-hapten-peptide could also be radioiodinated. Di-DTPA-peptides containing ligands for binding ^{99m}Tc/^{188}Re have also been used (Karacay *et al*., 2000; Gestin *et al*., 2001). The disadvantage of the hapten doubling as the radiolabeled ligand is that not all radionuclides are optimally bound by a single ligand. The antihapten antibody also could be affected by what is bound to the radiolabeled ligand. A more flexible approach would be to separate the hapten from the radiolabeled ligand. In this regard, studies are now focusing on the use of a hapten known as HSG (histamine-succinyl-glycine), showing that hapten-peptides can be prepared with ^{131}I/^{124}I/^{125}I, ^{111}In, ^{90}Y, ^{177}Lu, ^{67}Ga/^{68}Ga and ^{99m}Tc/^{188}Re (Sharkey *et al*., 2003c; Griffiths *et al*., 2004; McBride *et al*., 2006).

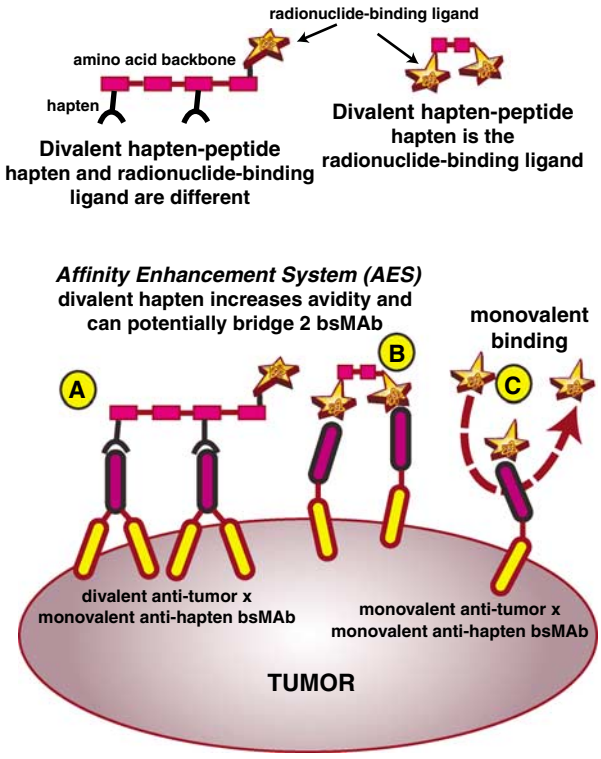

Figure 2 Modifications to the hapten-binding ligand used in bsMAb pretargeting for improved tumor uptake and retention. Initially, bsMAb were formed chemically by linking a Fab of an antitumor antibody to the Fab of an antibody binding to a chelate (i.e., hapten) (**C**). After pretargeting the bsMAb to the tumor, the radiolabeled chelate could bind, but this monovalent binding was not very stable. Linking two chelates together to make a divalent-hapten improved uptake and retention by a process known as the affinity enhancement system (**B**). Additional improvements were made by separating the hapten-binding from the radionuclide-binding component, although maintaining the principles of AES (**A**).

With the dock-and-lock modular system for creating humanized bsMAbs and a more universal hapten-peptide-binding system in the HSG-based peptides, pretargeting methods based on bsMAbs have advanced to a point where humanized recombinant bsMAb can be easily produced against other tumor antigens or even other disease markers. The technique can be applied for imaging or therapeutic applications with potentially a wide range of different compounds.

The pretargeting advantage comes principally from the fact that the radiolabeled hapten-peptide is a small molecule capable of exiting the vasculature within minutes of its injection. This allows the hapten–peptide to quickly equilibrate in the extravascular volume, where it can bind to the bsMAb that was localized earlier. Dynamic imaging studies of tumor-bearing nude mice have revealed that the peptide is selectively bound within 10 min of its injection (Sharkey *et al*., 2005a). The amino-acid composition of the hapten-peptide can be modified to give it properties that favor renal excretion

over clearance through the hepatic–biliary pathway (Sharkey *et al*., 2003c). This could have substantial advantages over directly radiolabeled peptide-based compounds, particularly when structural modifications affect a peptide's binding to the target receptor. Unlike many radiometal-labeled peptides or small antibody fragments that in animal models have >50% of the injected dose per gram trapped in the kidneys, the hapten-peptides generally have only 3–4% of the injected dose bound in the kidneys a few hours after injection, with about 40% the remaining product being removed each day afterward. Studies in animals have shown >95% of the radiolabeled di-HSG-peptide is eliminated within 1 day, with the vast majority of this activity removed in a few hours. The end result is an extremely rapid targeting process.

The uptake of the radiolabeled hapten-peptide appears to be highly efficient from a number of perspectives. Often, the hapten-peptide can be radiolabeled at higher specific activities than a directly radiolabeled antibody, which would bring more radionuclide to the tumor per unit mass of hapten-peptide delivered. Most interesting is the finding that pretargeting can often capture almost as much radioactivity in the tumor as a directly radiolabeled IgG (Axworthy *et al*., 2000; Sharkey *et al*., 2003b). Thus, unlike antibody fragments, where their rapid blood clearance results in a net loss of radioactivity in the tumor, when pretargeting is properly adjusted, the percentage of the injected dose bound to the tumor can equal that found with an IgG. In this respect, pretargeting offers the best targeting properties of an IgG, namely high uptake, with the reduced blood and tissue uptake found with an antibody fragment, and normal tissue uptake for pretargeting is reduced even more. Because the hapten-peptide uptake achieves it maximum accretion within minutes, not only is the total radiation dose delivered to the tumor higher, but it is delivered at a much higher dose rate (Karacay *et al*., 2005). For imaging, this has resulted in tumor/blood ratios that are 40-fold higher than a directly radiolabeled Fab fragment (Sharkey *et al*., 2005a). When used in conjunction with microPET, tumor localization with a pretargeting procedure provides a stronger signal in the tumor and less background in the normal tissues, making the images less ambiguous to read than ^{18}F-FDG (McBride *et al*., 2006), and microPET imaging studies have even shown an ability to detect micro-disseminated human tumor xenografts in nude mice as small as 0.2 mm in diameter that were not seen with ^{18}F-FDG (Sharkey *et al*., 2007) (Figure 3).

The only drawback to using a pretargeting system is that the antigen marker being targeted must be accessible (i.e., on the cell surface or in the extracellular space within the target tissue microenvironment), and should stay accessible in sufficient concentration to allow time for the untargeted bsMAb to clear from the blood. A targeted substance that internalizes quickly after being bound by the bsMAb would not be a good candidate for a pretargeting application, and therefore direct targeting would be preferred. However, it is important not to presume that a given marker that could internalize over time would not also benefit from a pretargeting approach. For example, some antigens might require crosslinking before internalization is initiated. In this case, a bsMAb with monovalent binding to the target antigen would be preferred to one that is divalent. A divalent hapten-peptide could still be used in this situation, and may even benefit the targeting system because its binding might trigger the internalization, bringing the imaging or therapeutic payload inside the cell. In situations where the bsMAb might be internalized before it has adequately cleared from the blood, this could potentially benefit from a clearing step or simply a bsMAb construct with more naturally rapid blood clearance properties. As shown in the CEA pretargeting system using a 50 Da bsMAb that cleared more quickly from the blood than a 100 Da Fab × Fab construct, uptake of the ^{111}In-hapten-peptide in the tumor achieved similar levels with the smaller bsMAb construct as with the larger conjugate, but tumor/nontumor ratios were significantly improved because the smaller construct also cleared from the normal tissues more efficiently (Rossi *et al*., 2003). It is also perhaps worth noting that pretargeting systems might be less affected in situations where the antigen is present in the blood. With a directly radiolabeled antibody, complexes formed with the circulating antigen would likely result in higher accretion of radioactivity in the liver or spleen. In pretargeting, as long as the unconjugated bsMAb–antigen complexes are effectively removed before the administration of the radiolabeled hapten-peptide, efficient targeting of the tumor occurs. Problems associated with a large antigen sink in normal tissues are difficult for both directly radiolabeled antibodies and pretargeting systems, but innovative techniques that exploit differences in pharmacokinetics or other properties could further enhance the targeting specificity. Thus, for many targeting situations, a bsMAb pretargeting solution could have an advantage over a directly radiolabeled antibody.

What is the potential role of pretargeting for the molecular imaging of cancer? Although pretargeting methods could be considered to be more complicated than a single-agent targeting method, once the optimal conditions are established, the injection sequence is straightforward. Irrespective of the pretargeting method used, patients would be able to receive the injection of the bsMAb or antibody conjugate by their oncologist, and then return one or more days later to receive the radiolabeled product in a nuclear medicine facility. Imaging could be performed within a few hours of this injection. Thus, for a pretargeting procedure, it will take a few days before an image is acquired, whereas it may be possible with some of the smaller, directly radiolabeled antibody constructs to image in a shorter period of time. However, in our experience, preclinical data suggest that image quality with pretargeting is superior to that of a directly radiolabeled antibody fragment (Sharkey *et al*., 2005a; McBride *et al*., 2006).

For therapy, improved antitumor responses, longer survival and higher cure rates have been observed with bsMAb pretargeting in animals bearing solid tumors

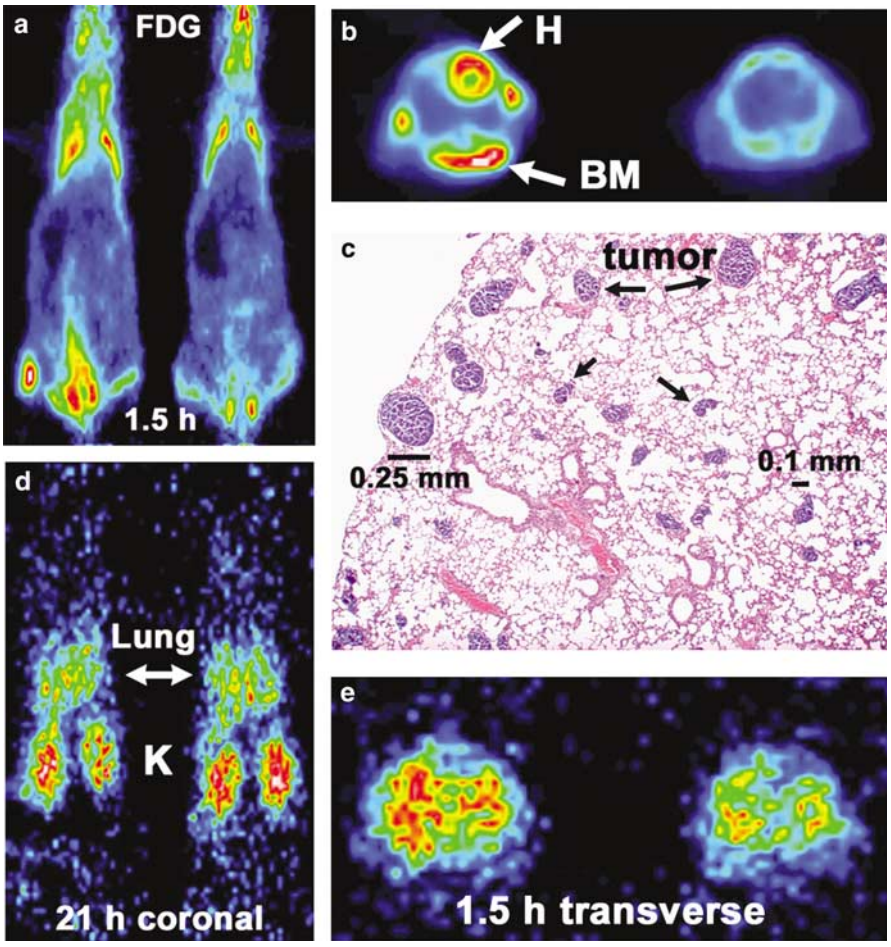

Figure 3 Localization of micrometastatic colon cancer in the lungs of nude mice using microPET. (**a**) Coronal cross-sections of two animals 1.5 h after being given ^{18}F-FDG are shown. No evidence of tumor involvement could be appreciated in the chest. Strong uptake in the bone marrow of the shoulder, ribs, skull and pelvis are seen. In (**b**), transverse sections through the chest of these same animals again fail to reveal any evidence of tumor, but the heart wall was clearly seen. (**c**) Cross-section of the lungs taken from one of these animals at necropsy after this imaging session that illustrates multiple, yet very small, tumor nodules in lungs. (**d** and **e**) Coronal and transverse sections of animals bearing the same sized tumors in the lung, but had received an anti-CEA bsMAb followed 1 day later with an ^{124}I-labeled peptide are shown. Strong uptake in the lungs was clearly evident in all imaging sessions from 1.5 to 21 h after the ^{124}I-peptide injection. Uptake in the kidneys (K) was also seen as a result of the peptide clearing through the kidneys.

and lymphomas (Gautherot *et al*., 1997, 2000; Kraeber-Bodere *et al*., 2002; Karacay *et al*., 2005; Sharkey *et al*., 2005d). As mentioned earlier, there is a growing consensus that radioimmunoconjugates may be best utilized in combination with chemotherapeutic regimens. Because many of these drugs are associated with severe hematologic toxicity, pretargeting might be combined more readily with chemotherapeutic agents. A few reports in animal models have shown improved responses with pretargeting and drugs such as gemcitabine and paclitaxel (Kraeber-Bodere *et al*., 2002; Graves *et al*., 2003). Several clinical studies, mostly phase-I, have also examined the role of pretargeting an ^{131}I-labeled hapten-peptide using anti-CEA bsMAbs (Kraeber-Bodere *et al*., 1999, 2003, 2006; Vuillez *et al*., 1999). Although therapeutic responses were rarely observed in these studies, a recent retrospective assessment of the efficacy of CEA pretargeting and an ^{131}I-hapten-peptide in the therapy of patients with medullary thyroid cancer (MTC) revealed encouraging findings (Chatal *et al*., 2006). These authors observed a statistically significant increase in the survival of a subset of patients who had a calcitonin doubling time of $\leqslant 2$ years and who were treated with pretargeted radioimmunotherapy as contrasted to a group of matched patients who received no therapy. In addition, patients who experienced a 100% increase in their calcitonin doubling time after receiving treatment had a significant survival advantage over nonresponders. Thus, it may be possible in future trials to select patients who might benefit from this treatment. These results are also very encouraging from the perspective that MTC is a very challenging tumor, with few effective chemotherapy and radiation therapy options when it has spread.

Conclusions

Highly specific and very sensitive images are possible with a bsMAb pretargeting, and both experimental and

clinical studies have shown that pretargeting can have encouraging therapeutic effects. Although the studies indicate that PET imaging will likely outperform SPECT-based imaging systems, it is important to note that the strong signal and high tumor/tissue ratios produce excellent image contrast even with conventional gamma scintillation camera, and thus a pretargeting procedure based on a ^{99m}Tc-labeled peptide can be a useful and less expensive alternative to a PET-based system. There are also other positron-emitting radionuclides with better PET imaging properties, such as ^{64}Cu or ^{68}Ga, which might also improve image quality with pretargeting. Thus, humanized bsMAb pretargeting appears to offer new prospects for improved molecular imaging of cancer.

This also appears to be true for radioimmunotherapy, where improved specific targeting and higher tumor radiation doses appear to be feasible. In MTC, initial clinical results with a first-generation pretargeting method have shown evidence of improved survival. Preclinical studies with more advanced pretargeting reagents have also shown superiority over directly labeled, one-step, radioimmunotherapy. Therefore, more clinical studies to evaluate these new procedures are needed. As the reagents for imaging and therapy, except for the final radionuclides chosen, are the same, and integration of both modalities is possible in such trials, we anticipate an eventually broader application of this technology to the management of cancer.

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