INVITED COMMENTARY

Multivalent Single-Chain Antibodies for Radioimaging of Tumors

Because of their exquisite specificity for their cognate antigens, monoclonal antibodies have long been envisioned as potential clinical reagents for diagnosis of cancer and other diseases. Numerous radiolabeled monoclonal antibodies against tumor-associated antigens have been evaluated in diagnostic clinical trials (*1*). The outcome of these trials highlights the limitations of using IgGs in radioimmunoscintigraphy. The major impediments to their use as reagents for in vivo imaging include their slow clearance from circulation; their poor diffusion from vasculature to the tumor; and, for xenogeneic antibodies, their potential to elicit the immune response in patients. The advent of genetic engineering made it possible to design and generate antibody fragments that could circumvent these problems and target tumors more efficaciously. A single-chain Fv fragment (scFv), consisting of the variable regions of the immunoglobulin light and heavy chains tethered together with a linker peptide, is one such recombinant reagent. After tumor targeting and biodistribution of the radioiodinated scFv derived from an anti-TAG-72 antibody, B6.2, were studied in athymic mice carrying an LS-174T xenograft (*2*), scFv's derived from an array of antitumor antibodies were evaluated for in vivo tumor targeting in xenografted mice and in patients (*3*). By virtue of being smaller molecules (approximately 30 kDa) than intact IgG (approximately 150 kDa), scFv's showed the advantage of higher diffusion capacity and rapid clearance from circulation, resulting in improved tumor-to-blood ratios, albeit within a short time after administration. However, a propensity of scFv's for rapid elimination from the blood pool coupled with their monovalency limited their uptake by the tumor to provide the desired quality of the image. Attempts, therefore, have been made to optimize the size of the tumor-targeting reagents to minimize rapid, first-pass clearance from the circulation. More important, constructs were designed to enhance the functional affinity or avidity of the targeting reagents for improved imaging. Most of the approaches to optimizing the size of the immunoreagents for the desired pharmacokinetics and maximizing tumor uptake to enhance the sensitivity of radioimmunoscintigraphy are based on developing multivalent antibody fragments. In this issue of *The Journal of Nuclear Medicine*, Goel et al. (*4*) report an evaluation of the in vivo tumor-targeting and biodistribution properties of divalent and tetravalent scFv's derived from an extensively studied antitumor antibody, CC49.

Several distinct approaches have been used to impart multivalency to the scFv-based immunologic reagents that have been developed for tumor targeting. One approach is based on connecting the variable regions of the light chain and heavy chain of an antibody through a short peptide linker aimed toward forcing a noncovalent association between the complementary variable domains belonging to 2 different peptide chains. The size of the linker dictates whether a bivalent (diabody), trivalent (triabody), or a tetravalent (tetrabody) molecule is generated (*5*). An alternative approach is based on covalently joining 2 scFv molecules in tandem by way of linker peptide (*6*) or disulfide bonds (*7*). Multimeric scFv's have also been developed by fusing scFv molecules to amphipathic helices (*8*), leucine zippers (*9*), and the k-constant region (*10*). An antibody fragment called minibody has been developed by the fusion of an anti-CEA antibody-derived scFv to the C_H3 domain of the human IgG1 (*11*). Evaluation of the tumor-targeting and biodistribution characteristics of diabodies derived from antibodies against CEA (*12*), c-erbB-2 (*13*), and TAG-72 (*14*) showed significantly higher tumor uptake in comparison with the levels attained by their monovalent counterparts. High tumor-to-blood ratios displayed early after administration made imaging of the target a possibility. Tumor-bearing mice injected with radiolabeled minibody derived from an anti-CEA antibody, T84.66, showed rapid tumor uptake that reached a level of 33% by 6 h, and xenografts could be clearly visualized at 4 and 19 h after administration (*11*).

Goel et al. (*4*) have derived a divalent scFv and a tetravalent scFv from an anti-TAG-72 antibody, CC49, and have evaluated them as tumor-imaging reagents. The tetravalent molecule (approximately 120 kDa), potentially the more efficient of the 2 targeting reagents, was generated by spontaneous formation into dimers of the 2 divalent molecules (approximately 60 kDa) that were assembled by covalent linkage between 2 scFv monomers. The larger of the 2 molecules has 4-fold higher affinity than the divalent molecule. Earlier, skepticism had been expressed about the stability of the covalent scFv dimers (*5*), and they were unfavorably compared with diabodies. In an effort to address the question of stability, Goel et al. incubated the radiolabeled

Received Apr. 24, 2001; accepted May 14, 2001.

For correspondence or reprints contact: Syed V.S. Kashmiri, PhD, Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, 9000 Rockville Pike, Bldg. 10, Room 5B38, Bethesda, MD 20892.

protein in vitro with 1% mouse serum at 37°C for 24 h. A 30% loss of product was reported; 20% was recovered with the low-molecular-weight protein, whereas 10% was found with the high-molecular-weight protein. However, the serum concentration tested in the study of Goel et al. is significantly lower than the physiologic concentration. Thus, the in vivo stability of the radioimmunoconjugates remains to be shown. Nevertheless, when administered to xenografted athymic mice, the radiolabeled multivalent scFv's showed an optimum rate of clearance. Although both scFv's showed good tumor targeting, the tetravalent molecule showed 3 times better tumor localization. The level of tetravalent molecule in tumor reached approximately 19 percentage injected dose per gram (%ID/g) in 6 h. At 16 h, the radiolocalization indices (%ID/g of tumor divided by %ID/g of normal tissue) for blood and spleen were impressive, whereas the high liver uptake and a significant association of radioactivity with the pancreas were not so reassuring.

The choice of radionuclide is critical for the effective localization of the tumor target. ScFv's radiolabeled with ^{123}I (*15*) and ^{18}F (*16*) have been used for radioimmunoscintigraphy. Several investigators have used radiometals to label antibody fragments. ^{64}Cu-labeled minibodies provided satisfactory images of LS-174T colon cancer xenografts (*17*). ^{177}Lu was the choice for labeling CC49 scFv (*18*), whereas ^{99m}Tc-labeled scFv derived from anti-CEA antibody MFE-23 provided optimal images of the xenografted tumors (*19*). A comparison of the targeting efficiency of antibody fragments with different rates of clearance (diabody vs. minibody) labeled with radionuclides of different half-lives (^{123}I vs. ^{18}F) led to the conclusion that the highest quality of tumor radioimaging is achieved when the half-life of the radionuclide is compatible with the rate of clearance of the antibody fragment to be radiolabeled. On the basis of this criterion, ^{99m}Tc (half-life, 6 h) was the most appropriate choice for labeling multivalent CC49 scFv's of relatively short biologic half-life. Goel et al. (*4*) used an indirect method to label multivalent scFv's with ^{99m}Tc. This method, based on using a bifunctional chelating agent with a transchelator, circumvents the need for additional steps of genetic engineering to facilitate attachment of the radiolabel to the antibody fragments.

One of the rationales for using scFv's as a diagnostic tool is their reduced immunogenicity, because they do not carry an immunoglobulin constant region. However, the variable region of the xenogeneic antibody has the potential to elicit an antiidiotypic antibody response in patients during multiple imaging. ScFv's derived from humanized antibodies would reduce this risk. Monoclonal antibody CC49 has been humanized, and a minimally immunogenic variant of the humanized CC49 has been characterized (*20*). A radioimaging agent derived from this variant may potentially be more useful. Lastly, one hopes that the preclinical studies will be corroborated by the clinical trials.

Syed V.S. Kashmiri
National Cancer Institute
Bethesda, Maryland

REFERENCES

1. Goldenberg DM, Larson SM. Radioimmunodetection in cancer identification. *J Nucl Med*. 1992;33: 803–814.
2. Colcher D, Bird R, Roselli M, et al. *In vivo* tumor targeting of a recombinant single-chain antigen-binding protein. *J Natl Cancer Inst.* 1990;82:1191–1197.
3. Huston JS, George AJT, Adams GP, et al. Single-chain Fv radioimmunotargeting. *Q J Nucl Med.* 1996;40:320–323.
4. Goel A, Baranowska-Kortylewicz J, Hinrichs SH, et al. ^{99m}Tc-labeled divalent and tetravalent CC49 single-chain Fv's: novel imaging agents for rapid in vivo localization of human colon carcinoma. *J Nucl Med.* 2001;42:1519–1527.
5. Todorovska A, Roovers RC, Dolezal O, et al. Design and application of diabodies, triabodies and tetrabodies for cancer targeting. *J Immunol Methods.* 2001;248:47–66.
6. Beresford GW, Pavlinkova G, Booth BJ, et al. Binding characteristics and tumor targeting of a covalently linked divalent CC49 single-chain antibody. *Int J Cancer.* 1999;81:911–917.
7. Adams GP, McCartney JE, Tai MS, et al. Highly specific in vivo tumor targeting by monovalent and divalent forms of 741F8 anti-c-erbB-2 single-chain Fv. *Cancer Res.* 1993;53:4026–4034.
8. Pack P, Muller K, Zahn R, et al. Tetravalent minibodies with high avidity assembling in *Escherichia coli. J Mol Biol.* 1995;246:28–34.
9. Kostelny SA, Cole MS, Tso JY. Formation of a bispecific antibody by the use of leucine zippers. *J Immunol.* 1992;148:1547–1553.
10. McGregor DP, Molloy PE, Cunningham C, et al. Spontaneous assembly of bivalent single-chain antibody fragments in *Escherichia coli. Mol Immunol.* 1994;31:219–226.
11. Hu S, Shively L, Raubitschek A, et al. Minibody: a novel engineered anti-carcinoembryonic antigen antibody fragment (single-chain Fv-CH3) which exhibits rapid high-level targeting of xenografts. *Cancer Res.* 1996;56:3055–3061.
12. Wu AM, Chen W, Raubitschek A, et al. Tumor localization of anti-CEA single chain Fvs: improved targeting by non-covalent dimers. *Immunotechnology.* 1996;2:21–36.
13. Adams GP, Schier R, McCall AM, et al. Prolonged *in vitro* tumor retention of a human diabody targeting the extracellular domain of human HER2/neu. *Br J Cancer.* 1998;77:1405–1412.
14. Pavlinkova G, Beresford GW, Booth BJ, et al. Pharmacokinetics and biodistribution of engineered single-chain antibody constructs of MAb CC49 in colon carcinoma xenografts. *J Nucl Med.* 1999;40:1536–1546.
15. Larson SM, El-Shirbini AM, Divgi CR, et al. Single chain antigen binding protein (sFv CC49): first human studies in colorectal carcinoma metastatic to liver. *Cancer.* 1997;80:2458–2468.
16. Wu AM, Williams LE, Zieran L, et al. Anti-carcinoembryonic antigen (CEA) diabody for rapid tumor targeting and imaging. *Tumor Targeting.* 1999;4:47–58.
17. Wu AM, Yazaki PJ, Tsai S, et al. High resolution microPET imaging of carcinoembryonic antigen-positive xenografts by using a copper-64-labeled engineered antibody fragment. *Proc Natl Acad Sci USA.* 2000;97:8495–8500.
18. Schott ME, Milenic DE, Yokota T, et al. Differential metabolic patterns of iodinated *versus* radiometal chelated anti-carcinoma single-chain Fv molecules. *Cancer Res.* 1992;52:6413–6417.
19. Verhaar MJ, Keep PA, Hawkins RE, et al. Technetium-99m radiolabeling using a phage-derived single-chain Fv with a C-terminal cysteine. *J Nucl Med.* 1996;37:868–872.
20. Tamura M, Milenic DE, Iwahashi M, et al. Structural correlates of an anticarcinoma antibody: identification of specificity-determining residues (SDRs) and development of a minimally immunogenic antibody variant by retention of SDRs only. *J Immunol.* 2000;164:1432–1441.