Teaching Editorial

Radiolabeled Monoclonal Antibodies: A "Decisive" Technology

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***"The genuinely decisive technology of modern medicine... comes as a result of a genuine understanding of disease mechanisms, and when it becomes available, it is relatively inexpensive, relatively simple, and relatively easy to deliver."*—Lewis Thomas, *The Lives of a Cell*, Viking Press, p. 35.**

The public and Congress are greatly concerned about the high cost of medical care in the United States today. This cost is approaching 10% of the Gross National Product, and much of the cost is alleged to be related to excessive use of technologies that prolong life but that are not "decisive" in curing the basic disease process. Thus, we are confronted with the mental image of a comatose patient, sustained on a ventilator in an intensive care unit, with no hope of cure, facing a painful existence prolonged by artificial means at high cost. Diagnostic approaches have also been severely criticized (*1*) and have become the focus for the government's initial attempts to reduce medical costs. This effort was probably triggered in part by the proliferation of CT scanning, which was associated, probably unfairly (*2*), with a significant increase in health care costs. The resulting Diagnosis-Related Groups (DRGs) have already had a dramatic effect on the psyche of the hospital-based health care worker and have markedly altered practice patterns, for instance, in New Jersey, a pilot program state (*3*).

It is in this climate that proposed advances in diagnostic imaging will be introduced into clinical nuclear medicine. These advances will dictate high standards for new tests that are likely to be "decisive" in the sense that Lewis Thomas has described. The use of radiolabeled antibodies in diagnosis is an example of such a "decisive" technology for nuclear medicine imaging. [Antibody molecules, or immunoglobulins, are produced by plasma cells in higher animals in response to the introduction of foreign substances (antigens).] At least conceptually, the proper antibody preparation can be used to target radioactivity selectively to any tissue type in the body and, as such, represent the ultimate in "selective" radiopharmaceuticals for nuclear medicine. This at least is the promise of radiolabeled monoclonal antibodies for nuclear medicine.

One consequence of this promise is that in nuclear medicine it is important to learn some immunologic terms in order to be conversant with others in the field. A brief glossary of relevant immunologic terms is listed in Table 1.

The principles on which the technique is based are disarmingly simple—and are summarized in Fig. 1, using specific anti-tumor antibodies as an example. The radiolabeled antibody recognizes specific attachment sites on the tumor, and after intravenous injection the antibody is carried by the blood throughout the body but attaches only at the tumor site where antigen combining sites serve as anchoring points. Thus, the radiolabel is carried through the "normal" organs but lodges in the tumor. Over time, there is a buildup of the radioactivity in the tumor to a level sufficient either for diagnostic applications, at tracer level, or for therapy when larger amounts of radioactivity are used.

In fact, the concept presented in Fig. 1 is one of the oldest in modern medicine and was first expressed by Ehrlich as a pharmacologic principle—the "magic bullet" (*4*). The idea that antibodies could be used to target radioactivity to tumors was developed very soon after the era of nuclear medicine began in 1945. In 1953, Pressman and Korngold (*5*) reported specific targeting to kidney and later to rat osteogenic sarcoma. Subsequently, Bale (*6*) extended this approach to targeting fibrinogen in tumors with anti-fibrinogen antibodies. However, this approach was not broadly applied because the state of knowledge of immunologic methods including antibody production was still too primitive to produce large amounts of specific antibodies.

In the 1950s and 1960s, immunology made great strides toward a better understanding of "specific" tumor-associated antigens [e.g., carcinoembryonic antigen (CEA) and radio-

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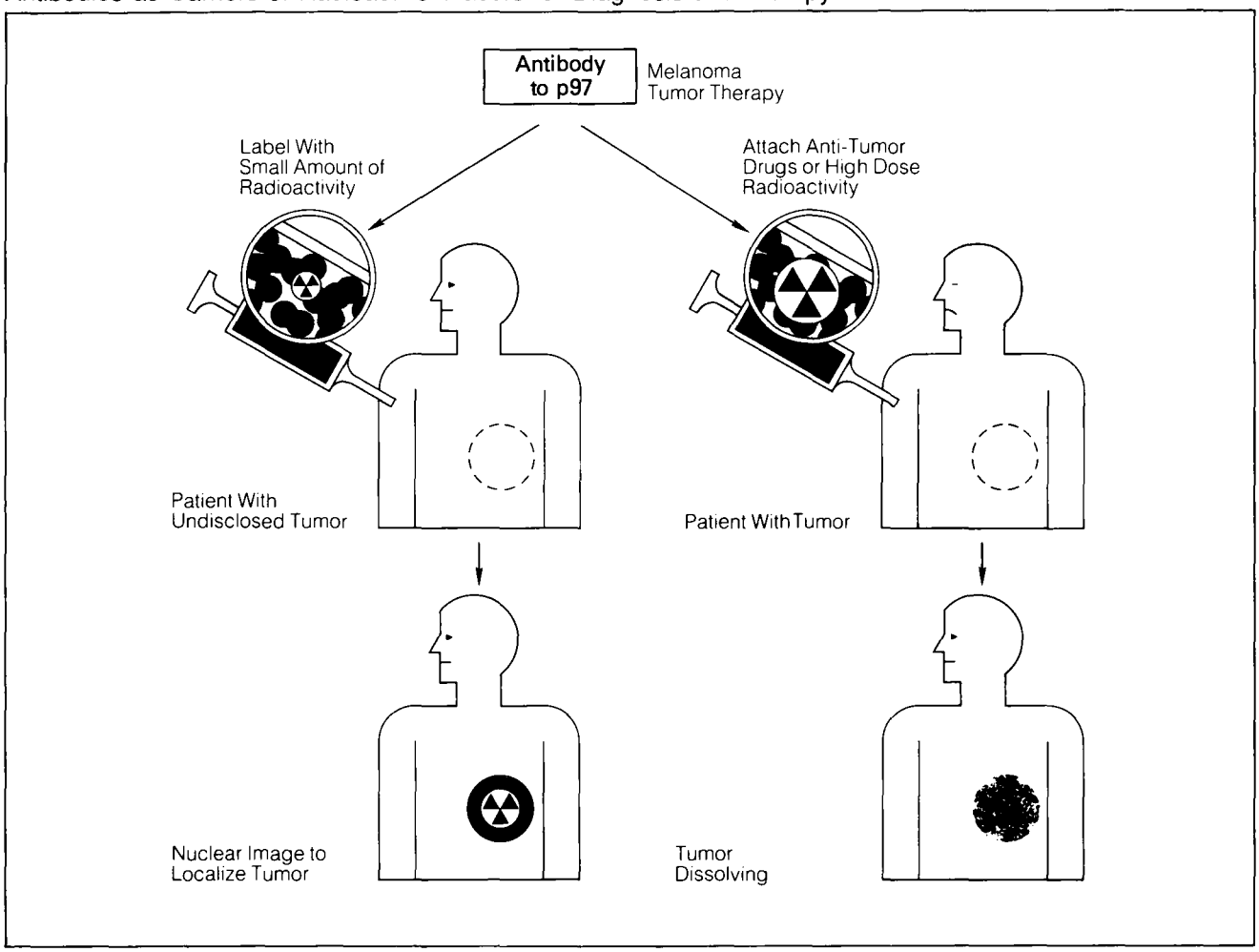

FIG. 1. Basic principle of using anti-tumor antibodies as carriers for radioactivity for diagnosis and therapy of human tumors.

immunoassay methods, as described by Berson and Yalow (*7*)]. It was a combination of these more basic advances that made further studies possible with radiolabeled antibodies against some of the newly discovered antigens: CEA, α-fetoprotein, and human chorionic gonadotropin. DeLand and Goldenberg (*8*) have pursued these studies aggressively. In taking advantage of this step-wise evolution over a period of 20 years, their work is characterized by the application of classic immunologic techniques such as the development of processes for raising antisera in animals and the application of improved purification procedures to "clean up" and purify complex mixtures of antibodies.

After the classic era of development of Pressman and Korngold (*5*), DeLand and Goldenberg (*8*) generated, if not fundamental changes, evolutionary and gradual increments of improved techniques that made imaging studies possible. Nonetheless, these applications were still limited by the relatively small amount of antibody available, and the fact that the heterogeneous mixture of antibodies are difficult to characterize precisely in terms of their reactivity with tumor and its nonreaction with nontumor tissues.

This situation has been fundamentally altered by the development of the monoclonal antibody technique. Now for the first time the nuclear medicine clinic has available large quantities of radiolabeled antibodies against tumors that have a single antigenic reactivity and for which the quality of the antibody preparation can be reliably assessed and reproduced. The production scheme for monoclonal antibodies is shown in Fig. 2.

In the typical preparation of milligram quantities of monoclonal antibodies, a colony of mice are immunized with a specific antigen. Subsequently, the mouse spleens are removed, white blood cells (B-lymphocyte cells) are extracted, and are chemically fused with certain cancer cells (myelomas) to create new cells called hybridomas (from hybrid myelomas). Each of these hybridomas will replicate endlessly, producing a specific antibody. Quantities of these monoclonal antibodies can be obtained by growing the hybridomas in conventional tissue cultures and collecting the antibodies from the media. Alternatively, samples of a hybridoma may be injected into the abdomen of mice, leading to the formation of ascites tumors. Several weeks later the mice are sacrificed and the monoclonal antibody is separated from the collected ascites fluid.

The above processes are not only time consuming, but the

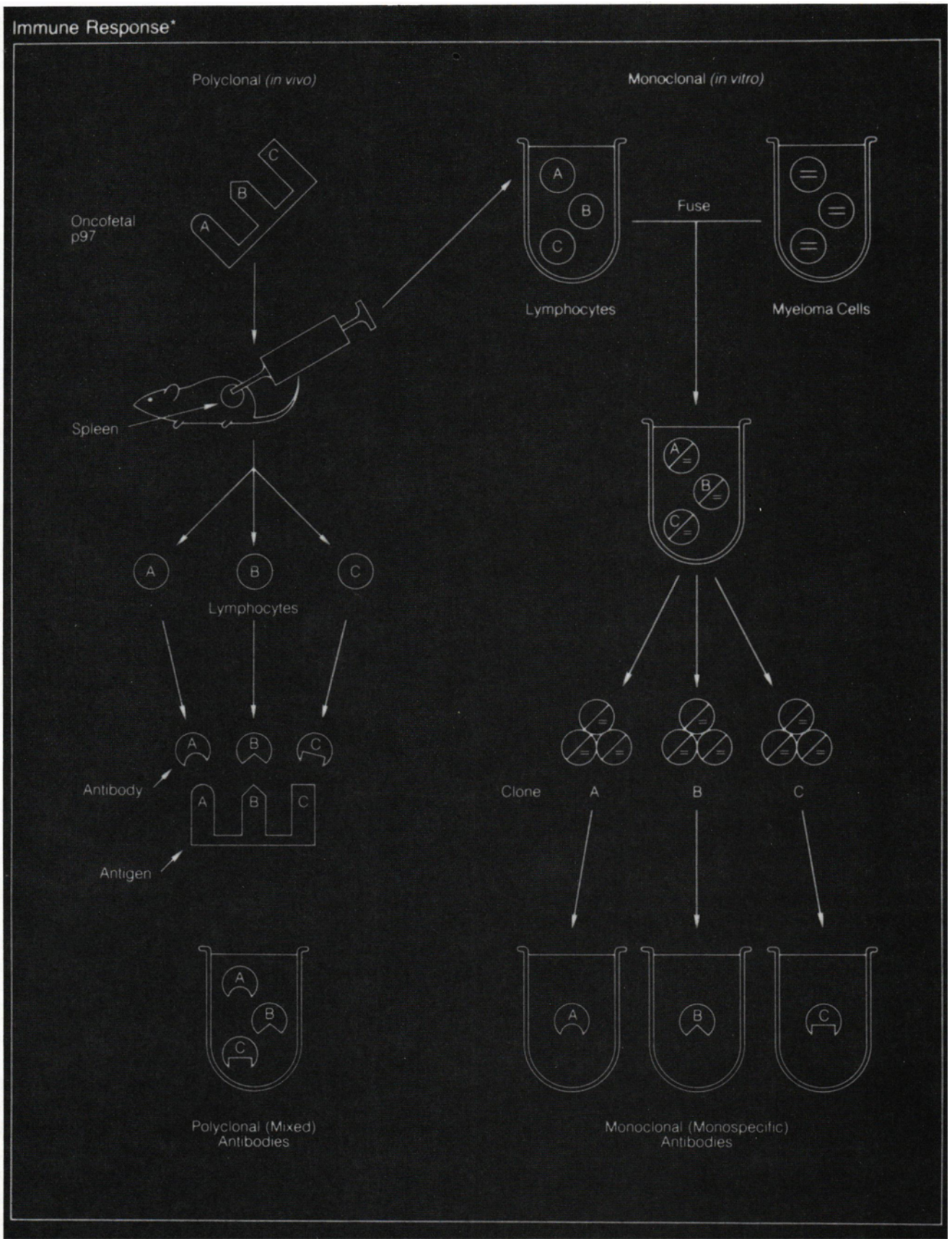

FIG. 2. Monoclonal antibody production by hybridoma techniques.

TABLE 1. Glossary of Immunologic Terms

Antiserum:	The serum obtained from an animal that has been immunized with a particular antigen.
Antibody:	The immune protein produced by immune lymphocytes in response to a particular antigenic stimulation. Antibodies are classified into several types: IgG, the predominant immune protein circulating in blood (>80% of the antibodies used in nuclear medicine are in this class); IgA, immunoglobulins secreted in glandular products; and IgM, a large immunoglobulin produced usually in early reaction against the antigen. IgD and IgE are other "classes" of immunoglobulins.
Antigen:	The "foreign" material, usually a protein but may also be carbohydrate, lipid, cerebroganglioside or other macromolecule, that induces an immune response.
Antibody fragment:	Using enzyme treatments, the IgG molecule may be broken into several parts: Fc fragment (contains complement typing site, hence the "c"); Fab fragment, the portion containing the antibody binding site; and the $(Fab')_2$ fragment, which contains two binding sites but no Fc portion.
Epitope:	The subpart of the antigen that actually provokes an immune response. Many antigens have more than one "epitope," and, so, when antigens are used to immunize, multiple anti-epitopic antibodies may be produced.
Idiotype:	The portion of the antibody (antibody 1) that contains the antigen binding site. This part of the antibody can itself evoke an immune response that is sometimes considered an "anti-idiotypic" antibody and will appear chemically as somewhat like the original antigen used to create the antibody.
Subclass:	Immunoglobulins vary in specific ways within each broad classification. The IgG's, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, are the more commonly described subclasses. These vary in terms of their biologic properties. For example, IgG_{2a} will fix complement, whereas IgG_1 does not. From the standpoint of nuclear medicine, one important factor is that there appears to be some subclass directed immunity.

yields are small and the purification processes are expensive. Commercial production of kilogram quantities of monoclonal antibodies requires the significant development of a biochemical process design (*16*). One such "novel bioreactor" system* successfully adapted is the ENCAPCEL™ process in which human hybridomas are cultured inside semipermeable microcapsules from which antibodies are extracted through chemical processing.

A listing of the advantages and disadvantages of immune sera and monoclonal antibodies is shown in Table 2. The availability of unique monoclonal antibodies against a number of the most common human tumors has now been described. A partial list of these are shown in Table 3. It would appear that most, if not all, human tumors will have antigens (and in some cases more than one antigen) which will provoke an

TABLE 3. Monoclonal Antibodies Against Human Tumors

Tumor Type	Antigen	Source
Melanoma	p97 HMWA proteoglyco-cerebroganglioside	Mouse
Colon	Glycoprotein B72.3 CEA 17.1 (undefined)	Mouse
Breast	6.2 Undefined	Mouse Human
Lung	Undefined Ab 4.1, Coulter Bone lesion	Mouse
Hodgkins (Reed-Sternberg cell)	Undefined	Human
Osteosarcoma	Undefined	Mouse
Thymocytes	65,000 Proteoglycan (T65) Thymocyte activating factor (anti-TAC)	Mouse

TABLE 2. Comparison of Immune Sera and Monoclonal Antibodies

Item	Immune Heterosera	Monoclonal Antibodies
Source	Immunized animal sera	Hybridoma cells, usually mouse lymphocyte/myeloma
Quantity	Relatively limited unless large domestic animals are employed, usually in the mg range	Can be produced in gram quantities
Composition reproducibility	Contains mixture of antibodies of varying affinity and correlation. Varies from animal to animal/bleeding to bleeding	Homogeneous monospecific protein
Advantages	Relatively simple to create immune sera	High degree of reproducibility and reliability over a hybridoma is created
Applicability	RIA (small amounts of reagents) Decreasing	Immunotherapy trials Immunohistochemistry Immunodetection and immunotherapy

TABLE 4. Principal Properties Relevant to Radioimmunodetection

Tumor Antigen Binding	Targeting
Affinity of antibody	Type of immune protein used: whole IgG, $(Fab')_2$, Fab
Concentration of antigen	Cross-reactivity with non-tumor tissues (liver, spleen, etc.)
Heterogeneity of expression on tumor cells	Amount of immune fragment employed
In vitro vs. in vivo expression of the tumor antigen	Route of administration
	Radiolabeling characteristics and stability in vivo/in vitro

immune response, and thus result in specific monoclonal antibodies. In the test tube, all of these reagents can be characterized quite precisely in terms of antigen reactivity, and nonreactivity with normal tissues.

The primary characteristics of the antibody (tumor antigen binding) and the secondary characteristics (factors related to in vivo targeting) have been summarized in Table 4. The radiolabels that have been proposed are tailored to the particular purpose for which the antibody is intended. A list of available radionuclides for both imaging and therapy are shown in Table 5.

The specificity of the localization, in terms of uptake in a body tumor, is determined by both sets of factors. Much of the data in support of these factors are, however, still in an anecdotal state. Theoretically, the tighter the binding to antigen, the longer the antibody will be held in the tumor site. This also allows for greater concentration in the tumor site relative to surrounding tissues. Similarly, the variability of antigen expression could very well affect the uptake in tumor sites because low antigen concentration suggests a low uptake of the radiolabeled antibody at this site.

The authors have observed good evidence for the dependence of uptake on antigen concentration in vivo, using anti-p97 Fab fragments, and have documented the absence of antibody binding in some parts of tumor sites in vitro. Antibodies have also been found to vary greatly in terms of their tissue cross-reactivity and that this cross-reactivity can markedly affect the biodistribution of the antibodies in patients. These points are illustrated in Fig. 3. Similarly, the type of immune protein, whether whole IgG, $(Fab')_2$, or Fab, will have a marked effect on the distribution of the radiolabeled material. In general, the whole IgG achieves higher concentration in tumor. A lesser concentration in tumor-to-blood or tumor-to-tissue ratio is achieved using Fab fragments. Fab, however, is excreted much more rapidly via the kidneys.

One intriguing aspect of the work developed so far is that the same antibody which targets for diagnosis can be applied for therapy. The work of Order and associates (*9*) using anti-ferritin antibodies and the initial studies done on melanoma are encouraging in this regard. It is still too soon, however, to say whether these therapies will become a common part of nuclear medicine practice.

What effect will the introduction of monoclonal antibody imaging have on the practice of nuclear medicine technology? Fortunately, the advances in scintillation cameras and emphasis on single photon emission computed tomography (SPECT) tend to favor "hot-spot" imaging. These advances will probably be employed more frequently because of the inherently superior contrast resolution of tomography. For example, a tumor of 3 cm in diameter and a tumor-to-tissue uptake ratio of 5 can be easily seen with SPECT, at 10 cm depth in the body, but less certainly with planar imaging (*10*). This improvement results from SPECT's relative lack of sensitivity

TABLE 5A. Selected Radionuclides for Radioimmunodetection

Nuclide	Half-life	Primary Decay Characteristics	Advantages	Disadvantages
Tc-99m	6 hr	IT (99%); γ = 141 KeV (89%)	Availability Decay energy	Short $T_{1/2}$ Chemistry problem
I-123	13 hr	EC (100%); γ = 159 KeV (83%)	Decay energy Iodine chemistry	Availability Cost ($20/mCi) Short $T_{1/2}$
In-111	68 hr	EC (100%); γ = 171 KeV (88%) γ = 245 KeV (94%)	Decay energy Optimal $T_{1/2}$ Chelation chemistry	In vivo metabolism
I-131*	8.05 d	β^- (100%); γ = 364 KeV	Availability Iodine chemistry Optimal $T_{1/2}$	Decay energy In vivo de-iodination
Ru-97	69 hr	EC (100%); γ = 216 KeV (86%)	Chelation chemistry	In vivo metabolism Availability
Cu-67*	62 hr	β^- (100%); γ = 91 KeV (7%) γ = 93 KeV (17%) γ = 184 KeV (47%)	—	—

*Potentially useful in therapy as well.

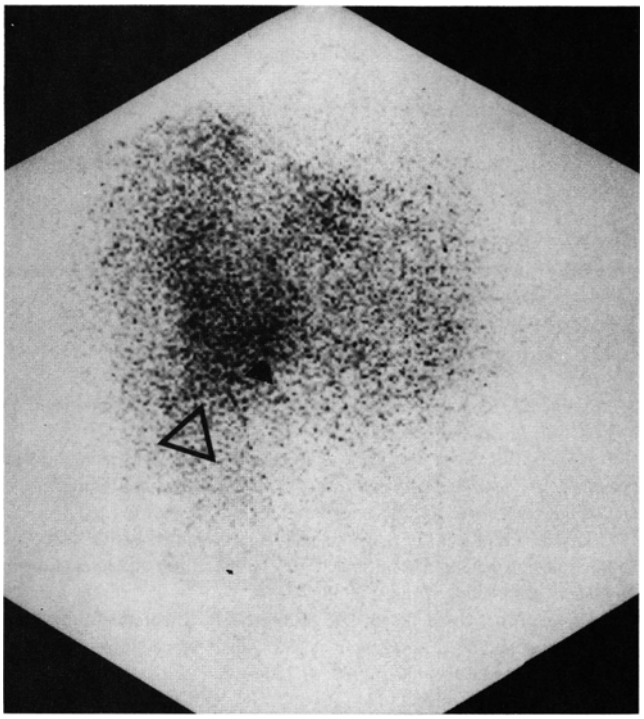

FIG. 3. Anterior posterior liver scans in a 55-yr-old woman with metastatic melanoma. There is uptake of I-131 (anti-p97) Fab by tumor (dark triangle) and also some localization by the normal liver (open triangle).

to the radioactivity in tissues surrounding the hot spot as compared to the usual planar images.

If targeting to tumors becomes selective enough, patients will come to the nuclear medicine laboratory for injection of a therapeutic dose of radiation labeled to antibodies. The authors estimate that maximum tumor-to-tissue ratios of 1,000:1 could be achieved in vivo (*11-14*), although present in vivo uptake ratios are more commonly 10:1. If we could achieve 1,000:1, outpatients could be treated with less than 30 mCi I-131, and receive 30,000 rads to their melanoma tumors. Unfortunately, this is still a dream at present, but many laboratories, in university, government, and industry settings, are working hard to make this dream become a reality.

It was noted at the outset that the utility and decisiveness of a new diagnostic technology would prove to be a function of simplicity of concept, ease of delivery, and competitiveness in the market place. We have briefly documented current efforts to translate concept and simplicity to actual delivery. What about the market place?

With research on applications of radiolabeled monoclonal antibodies to diagnostic imaging and therapy still in embryonic form, it is difficult to be more than vaguely quantitative about the economics (J. Perpich, MD, JD and R. Emyanitoff, PhD, *private communications*).

Estimates of the world-wide market for radiolabeled monoclonal antibodies in diagnostic imaging by 1990 range from 240 to 900 million dollars. This figure is largely dependent upon assumptions about the target population. (This estimate can be tripled if therapeutic applications are considered.)

While some analysts envision the utilization of monoclonals for in vivo mass screening of populations, more conservative estimates suggest that the established sensitivity and specificity of monoclonals will be manifested in earlier, more complete, more specific diagnostic procedures, followed by repeat scans to monitor the effectiveness of existing disease treatment methods.

Assessment of heart damage and identification of colorectal,

TABLE 5B. Selected Radionuclides for Radioimmunotherapy

Nuclide	Half-life	Primary Decay Characteristics	Advantages	Disadvantages
I-131*	8.05 d	β^- (100%); 0.608 MeV (86%) γ = 364 KeV (82%)	Availability Imaging Cost	Long tissue path
Y-90	64 hr	β^- (100%); 2.29 MeV (100%)	^{90}Sr-Generator Pure β^- decay	Chemistry problems In vivo metabolism?
Cu-67*	62 hr	β^- (100%); γ = 91 KeV (7%) γ = 93 KeV (17%) γ = 184 KeV (49%)	Imaging	In vivo metabolism?
Bi-212	1 hr	α (36%) β^- (64%)—^{212}Po (0.3μg•sec $T_{1/2}$, α = 8.78 MeV)	High LET Decay	Short $T_{1/2}$ Unknown chemistry
At-211	7.2 hr	α (41%); 5.9 MeV (41%) EC (59%)	High LET Decay	Short $T_{1/2}$ Unknown chemistry
I-125	60.2 d	EC (100%); γ = 35 KeV x-rays = 27 KeV	High LET Decay	Must be in nucleus to kill tumor

*Potentially useful in imaging also.

breast, lung, and prostate cancers are often considered the target applications by market estimates. The four cancers account for about 50% of annual cancer incidence in the United States. Considering that diagnostic images have already been obtained with radiolabeled antibodies for most of these conditions, these targets are not unrealistic.

The Biological Response Modifiers Program (BRMP) of the National Cancer Institute (NCI) has worked closely with industry to develop new antibodies for therapy in melanomas and lymphomas. Drs. J. Schlom and D. Colcher, also of NCI, have developed a number of antibodies to target breast, ovary, and colon tumors. The incorporation of alpha-emitting isotopes, potentially very effective radionuclides for therapy, is under study by a group in the radiation oncology program of NCI headed by Dr. Otto Gansow.

The potential market is a strong inducement for continued advances in the technology of monoclonal antibody production. Larger fermenting vessels are being introduced, and continuous flow is replacing batch production. It is estimated that the present cost of $4–10 K/gram of antibody should decrease to $0.5–1 K by 1990. Given dose estimates of 50–500 μg/diagnostic injection, factoring in the cost of isotopes and labeling, it is probable that costs for these new imaging agents will be competitive with that of existing agents, making these procedures cost-competitive with traditional nuclear medicine scans.

FOOTNOTE

*Damon Biotech Corp., U.S.

REFERENCES

1. Goinin PL, Glaser RJ. Misuse of laboratory tests and diagnostic procedures. *NEJM* 1982;307:1336.
2. Evans RG. The economics of computed tomography. Comparison with other health care costs. *Radiology* 1980;136:509.
3. Inglehart, JK. New Jersey's experiment with DRG-based hospital reimbursement. *NEJM* 1982;307:1655–60.
4. Ehrlich P. Collected Studies in Immunity. In *Immunology and Cancer Research*, NY: Pergamon Press, 1957.
5. Pressman D, Korngold L. The in-vivo localization of anti-Wagner osteogenic sarcoma antibodies. *Cancer* 1953;6:7619–23.
6. Bale WF, Spar IL, Goodland RL, et al. In vivo and in vitro studies of labelled antibodies against rat kidney and Walker carcinoma. *Proc Soc Exp Biol Med* 1955;89:564–68.
7. Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. *J Clin Invest* 1960;39:1157.
8. DeLand FH, Goldenberg DM. In vivo cancer diagnosis by radioimmunodetection. In *Radioimmunoimaging and Radioimmunotherapy*, Burchiel SW and Rhodes BA, eds, NY: Elsevier Publ, 1983:329–30.
9. Leicher PK, Klein JL, Siegelman SS, et al. Dosimetry of 131-I labelled antiferritin in hepatoma: Specific activities in the tumor and liver. *Cancer Treatment Reports* July-August 1983;647–58.
10. Rockoff SD, Goodenough DS, McIntire KR. Theoretical limitations in the immunodiagnostic imaging of cancer with computed tomography and nuclear scanning. *Cancer Res* 1980;40:3054–58.
11. Larson SM, Carrasquillo JA, Reynolds JC. Radioimmunodetection and radioimmunotherapy. *Cancer Investigation* 1984;2:363–81.
12. Larson SM, Brown JP, Wright PW, et al. Imaging of melanoma with I-131-labeled antibodies. *J Nucl Med* 1983;24:123–29.
13. Larson SM, Carrasquillo JA, McGuffin RW, et al. Localization of I-131 labeled p-97 specific Fab fragments in human melanoma as a basis for radiotherapy. *J Clin Invest* 1983;72:2101–14.
14. Carrasquillo JA, Krohn KA, Beaumier P, et al. Diagnosis and treatment of solid tumors with radiolabeled Fab. *Cancer Treatment Reports* 1984; 68:317–28.
15. Argyrios M, Wallace JB. *Biotechnology* 1984;5:447–53.