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Letter to the Editor

Correspondence re: M. I. Bernhard, K. M. Hwang, K. A. Foon, A. M. Keenan, R. M. Kessler, J. M. Frincke, D. J. Tallam, M. G. Hanna, Jr., L. Peters, and R. K. Oldham. Localization of ^{111}In- and ^{125}I-labeled Monoclonal Antibody in Guinea Pigs Bearing Line 10 Hepatocarcinoma Tumors. Cancer Res., *43:* 4429–4433, 1983

The Importance of Traditional Methods of Measuring Tracer Biodistributions

The recent monoclonal antibody report by Bernhard *et al.* (1) has introduced an unfortunate methodology into the conventional techniques of measuring biodistributions of radioactive tracers. This is a strategy of mincing and washing each organ prior to assaying its tracer content. The authors, in fact, declined to show either the percentage of injected dose or percentage of injected dose per g in any intact tissue. Instead, they tabulated residual activity per g remaining after mincing, washing, and triple centrifugation of excised organs. There appear to be at least 2 problems associated with this approach.

A question of clinical relevance seems the more important. Scintigrams of tumor-bearing patients will be made in conditions consistent with intact organs and no internal lavage. Both tightly and loosely bound radionuclides would contribute to the imaging process. In these cases, the amount in the tumor and other tissues is conventionally given in percentage of injected dose or percentage of injected dose per g (3, 5). Such explicit measurements permit comparisons of different tumor-targeting mechanisms in a quantitative fashion. Clinical success in imaging depends on the variation of these values between target and nontarget tissues.

It should also be pointed out that calculations of internal emitter radiation dosimetry, done according to the Medical Internal Radiation Dose formulation (2, 4), are directly proportional to the percentage of injected dose in the various intact source organs. Clinical dosimetrists must use these values to estimate the dose delivered to a given tissue and to compare various possible diagnostic and therapeutic protocols involving radiolabeled monoclonal antibodies. Again, tightly and loosely bound radionuclides contribute to the radiation damage and must be considered jointly.

Processing of biological samples without prior conventional assays leads to a second fundamental uncertainty. Logically, these rather arbitrary manipulations could cause various effects in different organs and disparate species. We were not given either the fraction of deposited radionuclide removed by each step of the process or by the total procedure. Thus, the reader has little understanding of the scientific significance of this particular type of tissue assay. It simply appears as an arbitrary methodology presumably invoked to improve tumor/background ratios. In this regard, the authors may even have been unfair to themselves.

Many researchers in the field of monoclonal antibodies will appreciate the possible improvement in target/background ratios obtainable using tissue processing prior to γ-counting. Removal of loosely bound tracer can help to indicate the specificity of the antibody targeting. But this must not be done to the exclusion of other more important and more conventional measurements. Investigators need to know the actual uptake in the standard format.

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Reply

As stated in the introduction to our paper, most imaging studies using murine monoclonal antibodies have been in a murine host, thus providing data in an allogeneic or syngeneic system. However, the use of murine monoclonal antibodies in humans (xenogeneic host) may present additional complications not addressed by these studies. Our purpose was to determine the fate of a murine monoclonal antibody raised to a tumor-associated antigen in a xenogeneic tumor-bearing host with an intact immune system analogous to that of humans. We believe

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we have accomplished this goal.

Dr. William's letter raises 2 problems presumably associated with our method of tissue analysis. First, it is pointed out that one cannot relate the tissue data as measured by our *in vitro* method to the results of imaging. No attempt was made to do so. Rather, the imaging data were collected and analyzed independently of the *in vitro* data, and were measured quantitatively from the raw computer data according to established region of interest techniques. The images and derived values speak for themselves, irrespective of the tissue biodistribution studies. They indicate the target to background ratio in true context, *i.e.*, *in vivo*, as measured with a γ-camera, using the same scintigraphic procedures that would be used to study tumor-bearing patients.

In vitro measurements of tissue-bound radioactivity were for the purpose of quantitatively analyzing specifically bound radiolabel and, thus, the mince/wash procedure was necessary. Quantitation on the basis of residual radionuclide in intact organs would not have provided these data.

Although concern about clinical relevance is raised, the D3 antibody is specific for an antigen associated almost solely with the guinea pig L10 hepatocarcinoma, and therefore, is not intended for human clinical studies. The letter correctly points out that dosimetry measurements are conventionally based on the percentage of injected dose according to the Medical Internal Radiation Dose formulation, which is designed for measurements in humans, not guinea pigs. No projections of patient dosimetry or toxicity were considered.

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