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NATO ADVANCED STUDY INSTITUTE ON "Radiolabeled Monoclonal Antibodies for Imaging and Therapy - Potential, Problems, and Prospects"

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Scientific Highlights

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A two-week NATO Advanced Study Institute (ASI) was held at Il Ciocco International Center, Barga, Italy, during July 20 - August 1, 1986, on "Radiolabeled Monoclonal Antibodies for Imaging and Therapy - Potential, Problems, and Prospects". In addition to the primary sponsorship by NATO, this ASI was co-sponsored by the United States Department of Energy/Office of Health and Environmental Research. Additional financial support was provided by industrial companies from the U.S. and Europe, including Abbott Laboratories, USA; Bristol Meyers Co., USA; Capintec, Inc., USA; Compagnie Oris Industrie, France; Cytogen Corporation, USA; Dow Chemical Company, USA;

Medi-Physics, Inc., USA; Organon Teknika, NV, Belgium; Siemens Gammasonics, Inc., USA; and Sorin Biomedica, Italy. The ASI was organized and directed by Suresh C. Srivastava. He was assisted by two co-Directors, R.C. Reba (Washington, USA) and J.F. Chatal (Nantes, France), as well as by an International Advisory Committee consisting of D.A. Goodwin (Palo Alto, USA), A.A. Epenetos (London, U.K.), J.P. Mach (Lausanne, Switzerland), and P. Paras (Silver Spring, USA). In addition, the tutorial faculty included S.J. Adelstein (Boston, USA), R.P. Baum (Frankfurt, FRG), W.H. Beierwaltes (Ann Arbor, USA), K.E. Britton (London, U.K.), G.L. Buraggi (Milan, Italy), G.L. DeNardo (Sacramento, USA), S.J. DeNardo (Sacramento, USA), A. Gottschalk (New Haven, USA), S.M. Larson (Bethesda, USA), J.C. Liehn (Reims, France), J.D. Lumbroso (Villejuif, France), J.G. McAfee (Syracuse, USA), A.A. Noujaim (Edmonton, Canada), P. Riva (Cesena, Italy) and M.J. Welch (St. Louis, USA). Approximately 110 participants from 15 countries were selected to attend the Institute.

The NATO Advanced Study Institutes program aims at the dissemination of advanced knowledge and fostering of contacts among scientists from different countries. The ASI's are primarily high-level teaching activities at which a selected team of international experts provides in-depth lectures and presentations in a carefully defined subject area of recent research interest. Considerable benefit is derived from the fact that the ASI's are small-sized meetings and consist of a systematic and coherent program involving extensive interaction through discussions and workshops. The audience is largely post-doctoral and selected primarily from within the countries of the NATO Alliance.

The objective of this ASI was to present a comprehensive program in the

area of research on radiolabeled monoclonal antibodies. The following topics were covered in depth:

1. Production, purification, and fragmentation of monoclonal antibodies and immunochemistry of hybridomas;
2. Production and the chemistry of radionuclides;
3. Radiohalogenation and radiometal labeling techniques;
4. In-vivo pharmacokinetics of radiolabeled antibodies;
5. Considerations of immunoreactivity (including affinity) of radiolabeled preparations;
6. Instrumentation and imaging techniques, particularly as applied to radioimmunodetection;
7. Radiation dosimetry in diagnostic and therapeutic use of labeled antibodies;
8. Radioimmunoscintigraphy and radioimmunotherapy studies;
9. Clinical results and experience, especially resulting from multicenter trials; and
10. Perspectives and directions for future research.

Tutorial as well as scientific lectures describing the latest research data on the above topics were presented. In addition, three workshop panels were convened on "Methods for Determining Immunoreactivity of Radiolabeled Monoclonal Antibodies - Problems and Pitfalls", "Radiobiological and Dosimetric Considerations for Immunotherapy with Labeled Antibodies", and "The Human Anti-Mouse Antibody Response in Patients". A session for oral presentations by the participants and a poster session were also included. Each presentation was followed by an extensive discussion period. Ample opportunities were provided for informal discussions and interaction during meals,

social events, and organized sight-seeing trips. Development of new contacts, renewing old ones, and establishment of international collaborative research projects were fostered and promoted.

On the opening day, welcome addresses were provided by the Director, S.C. Srivastava, and by L. Donato (University of Pisa, Italy) and F. Fazio (University of Milan, Italy). The latter two speakers gave a general overview of the status of biomedical research in Italy, especially the recent work on monoclonal antibodies supported by the Italian National Research Council (CNR). Both these speakers are associated with the governing body of the CNR. Under the university - industry collaboration that the CNR has fostered and supported during the last five years in this area of research, an impressive quantity of work has been accomplished. In particular, the concept of "multi-center clinical trials" with industrial support and participation has progressed into a very successful experiment, and support for this kind of effort is being renewed for the next several years. Spearheaded by the research efforts of G.L. Buraggi and others, the Italian investigators have evaluated two highly specific anti-tumor monoclonal antibodies in over 500 patients so far and the results look quite promising for the use of these reagents in radioimmunoscintigraphy.

The morning session concluded with the Keynote Address by W.H. Beierwaltes of the University of Michigan. The subject was: "Radioimmunotherapy of Cancer - Historical Perspectives and Prospects for the Future". Beierwaltes has spent over 35 years carrying out research in nuclear medicine and has made highly important contributions in the area of radiotherapy of cancer. He voiced a note of pessimism with regard to the therapeutic potential of radiolabeled antibodies. The limited promise that these

reagents have displayed, according to Beierwaltes, was real but perhaps due to a complex set of immune reactions involving cancer cell peptides, and other effects, rather than due to the radiation dose to the tumor itself. He also noted that only regression of the disease, not cure, has been experienced so far. In subsequent discussions throughout the meeting, these issues were addressed and the consensus that finally evolved was more optimistic regarding the therapeutic promise of radiolabeled monoclonal antibodies.

The afternoon session on the opening day was devoted to a detailed introductory lecture by Srivastava entitled "State-of-the-Art in Radiolabeled Monoclonal Antibody Research - What this Institute Hopes to Accomplish". This lecture briefly summarized the scientific and administrative planning of this ASI and then proceeded to an in-depth evaluation of the field as it stands today. Recent research accomplishments in the area of radiolabeled monoclonal antibodies at Brookhaven National Laboratory were described. A perspective of achievements, problems and pitfalls, and of future directions in research that are needed for this modality to fulfill its potential, was also provided. These opening day sessions thus set the stage for the program to follow.

The following sessions were arranged such that they allowed a logical progression into the various aspects of labeled monoclonal antibody research. The tutorial lectures provided an in-depth treatment of the basics. Focused scientific presentations, interspersed throughout the program were made by experts in their respective fields, and dealt with a discussion of the latest research data. The immunochemistry of hybridomas, the selection of monoclonal antibodies and fragments for immunodiagnosis and

immunotherapy, and the design, purification, and evaluation of labeled preparations were discussed by Mach (Switzerland), Gorevic (Stony Brook, USA), S. DeNardo (Sacramento, USA), Noujaim (Canada), and Ferrone (New York, USA). The existing techniques and their complexity were defined and the possibility of improvements were suggested. Emphasis was placed on careful analysis of the fragments, determination of the specificity and the affinity of whole antibodies or fragments using appropriate assay systems, and the development of human-human hybridomas to circumvent the human anti-mouse antibody response in patients. Synthetic schemes were presented for the modification of effector groups as well as for the production of hybrid antibodies employing recombinant DNA technology. These lectures provided an insight into the complexity of the techniques and pointed out various strategies to avoid the pitfalls and carefully develop ideas for future research.

The clinical experience at the U.S. National Institutes of Health in the diagnosis and treatment of lymphoma, melanoma, and colon cancer using labeled monoclonal antibodies was summarized by Larson (Bethesda, USA). This group has had better success with the use of Fab fragments, particularly in diagnostic applications. In many patients, the technique detected disease which was not evident with other methods such as CT scanning.

The following day was devoted to a discussion of the radiochemical labeling techniques. Zalutsky (Durham, USA) and Reynolds (Bethesda, USA) provided an extensive treatment of the radiohalogenation methods and mechanisms. Chemical and radiolytic effects on the immunoreactivity of halogenated antibodies were discussed. The possible use of ^{211}At label for immunotherapy was mentioned. Iodine-131, despite its less than ideal nuclear properties, was advocated as the most practical therapeutic nuclide

for use with antibodies. This point was stressed later on during the meeting as well. Meares (Davis, USA) summarized the status of investigations in the area of radiometal labeling using the bifunctional chelating agent approach. He stressed the importance of kinetic factors in the evaluation of physiological stability of radiometal labeled antibodies. As an example, he showed that ^{67}Cu complexes with nitrogen macrocycles stayed intact in serum for several days despite the fact that their thermodynamic stability constants were several orders of magnitude lower than those of ^{67}Cu aminopolycarboxylate complexes. Paik (Washington, USA) summarized the work by the George Washington University group on ^{111}In and ^{99m}Tc labeling of monoclonal antibody-DTPA conjugates. They have shown two binding sites for ^{99m}Tc on antibodies - a low affinity site and a high affinity site. This group advocates the use of excess free DTPA in the labeling medium since the side reactions of ^{99m}Tc can be avoided by doing so even though the antibody labeling yields are somewhat lower. Welch (St. Louis, USA) presented his thoughts and experience on the labeling of antibodies with positron-emitting radionuclides. His group has developed labeling techniques using ^{18}F and ^{68}Ga. He showed that even though the $t_{1/2}$ of these tracers is short, their application to PET imaging (with its concomitant advantages) with antibodies is feasible in certain situations. Saccavini (Gif sur Yvette, France) described the specific and non-specific attachment of DTPA to antibodies for labeling with ^{111}In and gadolinium (for magnetic resonance imaging). Modification of carbohydrate side chains on the Fc portion was utilized for attaching DTPA and subsequently the radiometal. This approach showed greater immunospecificity retention following labeling, although the propensity of non-Fc carbohydrate residues resulted in incomplete specifi-

city of DTPA attachment to the Fc portion. Eckelman (New Brunswick, USA) and Welch (St. Louis, USA) in a joint presentation expressed their doubts as to the possibility of developing Gd-labeled antibodies suitable for magnetic resonance imaging. From theoretical considerations and based on some published data, they showed that the number of Gd atoms per antibody molecule will have to be >1000 for effective magnetic resonance imaging and that such ratios are impossible to achieve without completely altering the antibodies' in-vivo behavior.

The following two days covered lectures on preclinical and clinical evaluation of labeled antibody preparations. Animal models and in-vivo pharmacokinetics were also discussed. The experience of various investigators was generally favorable to warrant continued work on development of better antibodies, animal models, and radiolabels. Welch (St. Louis, USA) described a rat model based on the dinitrophenyl (DNP)-coupled agarose beads that localize in the lungs. Monoclonal antibodies specific for DNP are then evaluated for miscellaneous in-vivo biodistribution mechanisms in order to provide a general insight into the specific and non-specific uptakes of monoclonal antibodies. Granowska (London, U.K.) described a new imaging approach involving kinetic analysis with probability mapping. This technique utilized temporal changes in biodistribution and provided a method for identifying sub-clinical and sub-radiological recurrences before second-look laparotomy. G. DeNardo (Sacramento, USA) advocated the use of quantitative imaging techniques to provide estimates of radiation dose distributions associated with particular radionuclides and targeting molecules. He presented the results of studies in patients using SPECT to define the pharmacokinetics of labeled antibody distribution. Halpern (San Diego, USA)

summarized his results in patients with the ^{111}In-labeled anti-CEA antibodies and suggested that even though tumor imaging was efficacious in certain situations, problems of background remain and must be addressed before improvements can be expected. McCabe (Bethesda, USA) provided a description of results with the anticolon carcinoma human IgM monoclonal antibodies 16-88 and 28A32 and suggested that clinical diagnostic and therapeutic trials are warranted based on the promising results in-vitro and in mice.

A summary of the pilot and prospective studies using antimelanoma (225.28s) and anti-CEA (F023C5) monoclonal antibodies was presented by Buraggi (Milan, Italy). Results using whole IgG as well as $F(ab')_2$ and Fab fragments labeled with ^{131}I, ^{123}I, ^{111}In, and ^{99m}Tc were described. A specificity of close to 100% was found for both antigen-antibody systems. Sensitivity was over 85% for primary lesions (about 70% for metastases) and both accuracy and predictive values of the positive test were found to be over 90%. These studies have led to the multi-center clinical trials in Italy that were described by Siccardi (Milan, Italy). The progress of patient studies by the Italian groups appears very impressive and further and widespread validation of the data, especially by other investigators, is presently being undertaken. Italian investigators have studied over 250 melanoma patients in ten nuclear medicine centers and studies with colon cancer patients are well underway.

Goodwin (Palo Alto, USA) described a novel approach to radiolabeled antibody design with potential for increased control of pharmacokinetics. This involves a multistep but simple protocol producing clear, early images without the need for background subtraction. Use is made of anti-chelate

monoclonal antibodies for the so-called rapid hapten immunoscintigraphy. In collaboration with Meares, his group has prepared and tested a number of bifunctional chelates with haptenic functions. Gottschalk (New Haven, USA) suggested a new concept for data reporting (imaging) that involves the combined application of a sensitivity and efficacy index. He discussed the use of an overall sensitivity index score, and an efficacy index relating the diagnoses made to patient management decisions. He suggested that the use of these concepts could minimize the confusion which results when various investigators report their imaging data in a number of different ways.

The second week started with a full-day session on radiation dosimetry and immunotherapy application of radiolabeled antibodies. Adelstein (Boston, USA) presented his thoughts on the selection of nuclides for therapy with labeled antibodies. He provided an extensive discussion of the advantages and disadvantages of Auger, beta, and alpha-emitters for radiotherapy of tumors. The limitations of each type of decay were critically examined, and possible strategies were presented for the effective use of nuclides for tumor radioimmunotherapy. Bigler (New York, USA) presented an interesting concept for radioimmunotherapy based on the destruction of isolated tumor cells distributed within the hematologic system, as opposed to cells within the solid tumors. He showed what conditions can be expected to control cancer by controlling occult micrometastases when conventional treatment can effectively eliminate primary and large metastatic tumor.

The session that followed included lectures by Mausner (Upton, USA), Fawwaz (New York, USA), and Myers (London, U.K.). Mausner provided an overview of the production and chemistry of therapeutic radionuclides for use with labeled antibody therapy. He discussed the work done at Brookhaven

National Laboratory and by other groups on both accelerator and reactor-produced radionuclides. The concept of the "in-vivo generator" for radioimmunotherapy under development at Brookhaven, was also discussed. Fawwaz presented his ideas on radioimmunotherapy of cancer and proposed that this approach may have only limited effectiveness, and only for isolated and easily accessible tumors. Myers described his views on the properties of low energy particulate radiations distributed in various cell configurations, and how the choice of a particular radionuclidic label influenced the therapeutic radiation dose that could be achieved.

Clinical experience with immunoscintigraphy was the subject of five following lectures. Chatal (Nantes, France) summarized his experience for the comparative prospective detection of carcinoma recurrence with SPECT imaging and a comparison of the results with those obtained by ultrasonography and computed tomography. He concluded that the limitations of each technique are mutually compensated for and that it is important to use them simultaneously when a recurrence is biologically suspected. Britton (London, U.K.) described the results of studies in patients using ^{123}I-labeled antibodies. He emphasized the fact that a very high signal-to-noise ratio was not essential for successful imaging and that the data could be treated appropriately (for example, by using kinetic analysis with probability mapping) to enhance the quality of images despite the usually encountered low tumor-to-background uptake ratios. Iodine-123 was proclaimed to be one of the best nuclides for imaging, although the availability and the cost continue to be a problem. The network for ^{123}I production and distribution has been much more successful in Europe than in the USA.

Riva (Cesena, Italy) described the results of alternative adminis-

tration routes in patients using radiolabeled antibodies against melanoma and colon carcinoma. His presentation emphasized the clinical potential of immunoscintigraphy but noted the need for much additional research for a widespread usefulness of the technique. Baum (Frankfurt, FRG) summarized the clinical experience resulting from a collaborative program in 200 patients for immunoscintigraphy of various tumors and also their initial experience with immunotherapy.

Another session was devoted to the antibody approach for blood cell labeling and for imaging thrombi. McAfee (Syracuse, USA) gave two lectures, one related to leukocyte labeling and the other to an antifibrin antibody. His experience on labeling antibodies against the various leukocyte antigens was moderately successful. Labeling of the antifibrin antibody, however, was more promising. Fresh as well as 5-day old thrombi could be visualized in experimental animal models using this antibody. Thakur (Philadelphia, USA) reviewed his work on platelet labeling using antiplatelet monoclonal antibodies as well as the work by various other investigators, and provided a summary of the results.

Ferrone (New York, USA) in his lecture emphasized the selection criteria utilized in radiolabeled monoclonal antibody research. Using their experience with whole and fragmented antibodies (225.28s, 149.53, 763.74, and others) directed against a human high-molecular weight melanoma-associated antigen as examples, he stressed the importance of in-vitro assays, Scatchard plot analysis, and affinity determinations in order to select the best monoclonal antibodies for imaging as well as therapy applications. He also described his latest research results on work with anti-idiotype antimelanoma antibodies.

Instrumentation and imaging techniques were the topics discussed in sessions on the last day of the meeting. Paras (Silver Spring, USA) gave a state-of-the-art lecture on instrumentation for scintigraphic detection of tumors using labeled antibodies. He also summarized the various advantages and limitations of present techniques, including SPECT and PET. Liehn (Reims, France) reviewed the image subtraction techniques for contrast enhancement and described a new algorithm which resolves many problems encountered in this approach. Both planar and tomographic images can be processed using his algorithm which is based on a robust image registration procedure that performs geometric and grey-level registrations at the same time. The process does not require markers and regions where the images are different are automatically excluded. The normalization coefficient is not affected by the target activity. Lumbroso (Villejuif, France) discussed his approach of digital superimposition of SPECT and CT images for successful immunoscintigraphy. This technique was developed to link precise anatomical data from x-ray CT with the radiotracer distribution in SPECT slices. This method (studied in 22 patients with gastrointestinal cancer) increased the clinical specificity of radioimmunoscintigraphy and allowed coupling of functional information with accurate morphological data.

Two sessions of miscellaneous oral presentations, and a poster session, were also included in the program. A variety of topics were covered, including: the detection of lymph node metastases following various routes of labeled antibody administration (Munz et at, Germany); fragmentation techniques and immunological controls in antibody selection (Larue et al, France); immunohistochemical studies of CEA and CA19-9 in patients (Lorenz et al, Germany); SPECT with ^{131}I-MAb's and comparison with CT, in colorectal

cancer follow-up (Scheidhauer, Germany); selection of antibodies for optimal biological functions for clinical use (Powe and Steplewski, Philadelphia, USA); evaluation of site-specific labeled antibodies in neuroblastoma, and renal cell, colon, and breast carcinoma-bearing nude mice (Alvarez et al, Princeton, USA); antiplatelet antibodies for thrombus localization (Meinken and Srivastava, Upton, USA); evaluation in mice of ^{125}I and ^{211}At-labeled antitumor antibodies (Harrison and Royle, U.K.); in-vivo kinetics of ^{131}I-MAb's in patients (Aprile et al, Italy); immunoscintigraphy of human sarcomas (Bruland et al, Norway); a monoclonal antibody reactive with human lung cell carcinoma (Dazord et al, France); improved imaging with antibody fragments conjugated with carboxypeptidase G_2 (Melton, U.K.); and use of second antibody administration for tumor-to-background ratio enhancement (Pedley, U.K.), etc.

The workshops on "Methods for Determining Immunoreactivity of Labeled Monoclonal Antibodies - Problems and Pitfalls" (panel members: Reba, Mach, Powe, and Zalutsky), on "Radiobiological and Dosimetric Considerations for Immunotherapy with Labeled Antibodies" (panel members: Adelstein, G. DeNardo, Gottschalk, and Myers), and on "Human Anti-mouse Antibody Response in Patients" (panel members: Chatal, Ferrone, Reynolds, S. DeNardo, and Noujaim) were very successful. The panelists provided a short presentation first, followed by extensive discussion with audience participation.

The setting for the entire meeting was very informal, and all attendees were provided lodging and board at the same hotel. The participants stayed as a closely-knit group throughout the meeting. This allowed and promoted constant social as well as professional interaction among colleagues on a truly international level.

The proceedings of this ASI will be published in a hard-cover book format by the Plenum Publishing Corporation, New York (Suresh C. Srivastava, Editor) during Spring 1987.

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