712341

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Oak Ridge Associated Universities
Post Office Box 117
Oak Ridge, Tennessee 37830

June 20, 1984

Dr. William R. Bibb, Director
Energy Programs and Support Division
Department of Energy
Oak Ridge, TN 37830

Subject: GRANT APPLICATION TO NIH FOR NEW PROPOSAL ENTITLED "RADIOLABELED MONOCLONAL ANTIBODIES FOR CANCER THERAPY", DOE NO. 20-104-84.

Dear Dr. Bibb:

Enclosed are three copies of a new proposal to NIH for a project entitled "Radiolabeled Monoclonal Antibodies for Cancer Therapy". Draft copies of this proposal were forwarded to your office on May 18, 1984 and verbal approval for formal transmittal was given by Robin Spradlin on May 30, 1984.

The proposed research will be supervised by Dr. Lee C. Washburn and will be carried out under policies and procedures previously established between ORAU and DOE.

We will keep you advised concerning the status of the grant.

Sincerely yours,

William E. Felling

William E. Felling
Executive Director

RYAN:sm

Enclosures

REPOSITORY Oak Ridge Operations Records Holding Area
COLLECTION Documents 1944-1994
BOX No. B-53-16 Bldg. 2714-H
FOLDER 20-104-84 1984

Cy Sent to D. H. Moneyhun 7/12

20-104-84

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Form approved
OMB No. 0925-0001

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

GRANT APPLICATION

FOLLOW INSTRUCTIONS CAREFULLY

LEAVE BLANK		
TYPE	ACTIVITY	NUMBER
REVIEW GROUP		FORMERLY
COUNCIL/BOARD *(Month, year)*		DATE RECEIVED

1. TITLE OF APPLICATION *(Do not exceed 56 typewriter spaces)*
RADIOLABELED MONOCLONAL ANTIBODIES FOR CANCER THERAPY

2. RESPONSE TO SPECIFIC PROGRAM ANNOUNCEMENT ☒ NO ☐ YES *(If "YES," state RFA number and/or announcement title)*

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

3a. NAME *(Last, first, middle)*
WASHBURN, Lee C.

3b. SOCIAL SECURITY NUMBER
[redacted]

3c. POSITION TITLE
Scientist

3d. MAILING ADDRESS *(Street, city, state, zip code)*
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, Tennessee 37831

3e. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
Medical & Health Sciences Division, Pre-clinical Radiopharmaceutical Development Group

3f. MAJOR SUBDIVISION
Medical & Health Sciences Division

3g. TELEPHONE *(Area code, number and extension)*
(615) 576-3099

4. HUMAN SUBJECTS
☒ NO ☐ YES ☐ Exemption # _____ OR ☐ Form HHS 596 enclosed

5. RECOMBINANT DNA
☒ NO ☐ YES

6. DATES OF ENTIRE PROPOSED PROJECT PERIOD
From: 4/01/85 Through: 3/31/88

7. DIRECT COSTS REQUESTED FOR FIRST 12-MONTH BUDGET PERIOD *(from page 4)*
$ 88,160

8. DIRECT COSTS REQUESTED FOR ENTIRE PROPOSED PROJECT PERIOD *(from page 5)*
$ 284,980

9. PERFORMANCE SITES *(Organizations and addresses)*
Medical & Health Sciences Division
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, Tennessee 37831

10. INVENTIONS *(Competing continuation application only)*
☒ NO ☐ YES ☐ Previously reported OR ☐ Not previously reported

11. APPLICANT ORGANIZATION *(Name, address, and congressional district)*
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, Tennessee 37831

Third Congressional District

12. TYPE OF ORGANIZATION
☐ Public, Specify ☐ Federal ☐ State ☐ Local
☒ Private Nonprofit
☐ For Profit *(General)*
☐ For Profit *(Small Business)*

13. ENTITY IDENTIFICATION NUMBER
1620476816A

14. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR BIOMEDICAL RESEARCH SUPPORT GRANT
Code 610 Description

15. OFFICIAL IN BUSINESS OFFICE TO BE NOTIFIED IF AN AWARD IS MADE *(Name, title, address and telephone number.)*
Mr. W. F. Countiss
Manager of Finance
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, TN 37831 (615) 576-3304

16. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Name, title, address and telephone number)*
Dr. William E. Felling
Executive Director
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, TN 37831 (615) 576-3300

17. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense *(U.S. Code, Title 18, Section 1001)*.

SIGNATURE OF PERSON NAMED IN 3a *(In ink. "Per" signature not acceptable)*: Lee C. Washburn
DATE: 6/20/84

18. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense *(U.S. Code, Title 18, Section 1001)*.

SIGNATURE OF PERSON NAMED IN 16 *(In ink. "Per" signature not acceptable)*: William E. Felling
DATE: 6/27/84

PHS 398 (Rev. 5/82)

1077389

ABSTRACT OF RESEARCH PLAN

KEY PROFESSIONAL PERSONNEL ENGAGED ON PROJECT

NAME	POSITION TITLE	DEPARTMENT AND ORGANIZATION
Lee C. Washburn	Scientist	Medical & Health Sciences Div. Oak Ridge Associated Universities
Karl F. Hübner	Assistant Chairman, and Chief of Clinical Nuclear Medicine	Medical & Health Sciences Div. Oak Ridge Associated Universities
James E. Crook	Director, Radiopharmaceutical Development and Preclinical Nuclear Medicine and Assistant Director, Clinical Nuclear Medicine	Medical & Health Sciences Div. Oak Ridge Associated Universities
Zenon S. Steplewski	Professor	The Wistar Institute Philadelphia, PA

ABSTRACT OF RESEARCH PLAN: State the application's long-term objectives and specific aims, making reference to the health relatedness of the project, and describe concisely the methodology for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. The abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. **DO NOT EXCEED THE SPACE PROVIDED.**

Monoclonal antibodies and monoclonal antibody fragments with high specificities for human malignancies will be covalently coupled to DTPA via the cyclic DTPA anhydride technique to provide a means for labeling such agents with polyvalent metal radionuclides. The potential of radioimmunotherapy using monoclonal antibodies labeled with the alpha-emitting radionuclide fermium-255, ($T_{1/2}$ = 20.1 h) and beta-emitting yttrium-90, ($T_{1/2}$ = 64.2 h) will be studied preclinically. Two gastrointestinal monoclonal antibodies with specificity for both colorectal and pancreatic carcinomas, 1083-17-1A (17-1A) and 1116-NS-19-9 (19-9), and a monoclonal antimelanoma antibody, 691-19-19 (19-19), will be investigated. Antibodies derivatized via the bifunctional chelate technique will be tested by a radioimmunoassay technique to verify their retention of immunological activity. Nude mice bearing the appropriate tumor type for each monoclonal antibody will be used to test the specificity of tumor uptake. Antibodies labeled with the gamma-emitting radionuclides indium-111, ytterbium-169, and yttrium-88 will be used for scintigraphic and tissue distribution studies to determine the time course of the tumor uptake of radiolabeled monoclonal antitumor antibodies. A radiolabeled monoclonal anti-influenza antibody will be used to control for nonspecific binding. The antibodies will then be labeled with Fm-255 and Y-90 as well as einsteinium-253 (a longer lived alpha emitter to be used as a model for Fm-255), and further tissue distribution studies will be carried out. These tissue distribution studies will be used for radiation dosimetry calculations. In vivo tumor growth studies in nude mice will be carried out to aid in assessment of the therapeutic potential of ^{255}Fm- and ^{90}Y-labeled monoclonal antibody derivatives. This multidisciplinary research approach, which combines the fields of bio-organic chemistry, immunochemistry, and radiopharmacology, is aimed at improving radioimmunotherapy methods for malignant processes in man.

VERTEBRATE ANIMALS INVOLVED ☐ NO ☒ YES If "YES," identify by common names and underline primates.

Nude mice bearing transplanted tumors.

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Number pages consecutively at the bottom throughout the application. Do not use suffixes such as 5a, 5b. Type the name of the Principal Investigator/Program Director at the top of each printed page and each continuation page.

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C. Progress Report/Preliminary Studies *(Not to exceed eight pages)*	26
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SECTION 3. Appendix *(Six sets) (No page numbering necessary for Appendix)*

Number of publications: 0 Number of manuscripts: 0

Other items *(list)*:

Letters from Drs. Z. Steplewski, R. L. Hayes, J. E. Bigelow, and R. Bigler. 44-47

☒ Application Receipt Record, Form PHS 3830

☐ Form HHS 596 if Item 4, page 1, is checked "YES" and no exemptions are designated.

1017391

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Lee C. Washburn

DETAILED BUDGET FOR FIRST 12 MONTH BUDGET PERIOD
DIRECT COSTS ONLY

FROM 4/01/85 THROUGH 3/31/86

DOLLAR AMOUNT REQUESTED (Omit cents)

PERSONNEL (Applicant organization only)			TIME/EFFORT				
NAME		POSITION TITLE	%	Hours per Week	SALARY	FRINGE BENEFITS	TOTALS
Washburn, Lee C.	Ph.D.	Principal Investigator	20	8	8,832	2,030	10,862
Hubner, Karl F.	M.D.	Co-Investigator	5	2	3,270	750	4,020
Crook, James E., Ph.D., M.D.		Co-Investigator	10	4	5,448	1,253	6,701
Coffey, Jack L.	M.S.	Physicist	10	4	3,820	880	4,700
Cabot, Myles C.	Ph.D.	Biochemist	5	2	1,888	434	2,322
Sun, Tan Tan	M.S.	Research Associate	30	12	9,150	2,105	11,255
Carlton, James E.	B.S.	Research Associate	20	8	7,358	1,692	9,050
To Be Named	Ph.D.	Post-doctoral	100	40	19,000	2,850	21,850
		Research Associate					
		SUBTOTALS →			58,766	11,994	70,760

CONSULTANT COSTS Raymond L. Hayes, Ph.D. (retired Head of Preclinical Radiopharmaceutical Development for ORAU's Medical and Health Sciences Division) four consulting visits per year @ $200.00 each — 800

EQUIPMENT (Itemize)

Corning model MP-6A Mega-Pure Still (water purification system) with automatic collection bottle and demineralizer. — 4,000

SUPPLIES (Itemize by category)

Animals (200 nude mice @ $11,50 ea; animal care costs are included in indirect costs.)	$2,300	
Chemicals	1,000	
Glassware and Tissue Culture Supplies	2,000	
Radioisotopes	3,000	8,300

TRAVEL	DOMESTIC Attendance at scientific meetings	2,000
	FOREIGN	
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	

ALTERATIONS AND RENOVATIONS (Itemize by category)

CONSORTIUM/CONTRACTUAL COSTS

Zenon S. Steplewski, M.D., D.M.S., D.S., The Wistar Institute, Philadelphia, will serve as a Co-Investigator at 5% effort. The only expense involved will be for his travel for three trips to Oak Ridge per year @ $600.00 ea. — 1,800

OTHER EXPENSES (Itemize by category)

Publication costs — 500

TOTAL DIRECT COSTS (Also enter on page 1, item 7) → $88,160

10771392

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		1st BUDGET PERIOD *(from page 4)*	ADDITIONAL YEARS SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL *(Salary and fringe benefits.) (Applicant organization only)*		70,760	80,685	86,335		
CONSULTANT COSTS		800	800	800		
EQUIPMENT		4,000	-0-	-0-		
SUPPLIES		8,300	8,600	9,000		
TRAVEL	DOMESTIC	2,000	2,200	2,400		
	FOREIGN					
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
CONSORTIUM/ CONTRACTUAL COSTS		1,800	2,100	2,400		
OTHER EXPENSES		500	700	800		
TOTAL DIRECT COSTS		88,160	95,085	101,735		

TOTAL FOR ENTIRE PROPOSED PROJECT PERIOD *(Also enter on page 1, item 8)* → $ 284,980

JUSTIFICATION (Use continuation pages if necessary): Describe the specific functions of the personnel and consultants. If a recurring annual increase in personnel costs is anticipated, give the percentage. For *all* years, justify any costs for which the need may not be obvious, such as equipment, foreign travel, alterations and renovations, and consortium/contractual costs. For any additional years of support requested, justify any significant increases in any category over the first 12 month budget period. In addition, for COMPETING CONTINUATION applications, justify any significant increases over the current level of support.

Recurring annual increase in personnel costs of 7% anticipated. Increased effort of physicist in years 2 and 3 included.

Travel costs are for three investigators to each attend one domestic scientific meeting per year, such as the Annual Congress for Hybridoma Research and national and regional meetings of the Society of Nuclear Medicine.

The water purification system requested will provide the ultrapure water which is required for the chromatographic separations to be done in this project.

Justification of Personnel:

1. Dr. Washburn, the Principal Investigator, is a radiopharmaceutical chemist who will provide the overall guidance for this research.
2. Dr. Hubner, a Co-Investigator, is a physician with strong backgrounds in both immunology and nuclear medicine. He will provide on-site leadership in these areas.
3. Dr. Crook, a Co-Investigator, is a clinical pharmacologist who will provide expertis in the animal biodistribution work.

(continued on next page)

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Justification of Personnel: (continued)

4. Dr. Steplewski of The Wistar Institute, a Co-Investigator, is one of the foremost authorities in the area of monoclonal antibody research. In addition to providing the monoclonal antibodies and tumor lines required for the project, Dr. Steplewski will also work with ORAU investigators to set up at ORAU the radioimmunoassay procedure necessary to verify the retention of immunological activity of the antibodies after derivatization.

5. Mr. Coffey, a physicist, will perform the radiation dosimetry calculations.

6. Dr. Cabot, a biochemist with a strong background in tissue culture techniques, will maintain in tissue culture the tumor lines obtained from The Wistar Institute.

7. Mrs. Sun, a bioorganic chemist with expertise in radiopharmaceutical synthesis, will carry out syntheses such as attachment of chelating groups to antibodies.

8. Mr. Carlton, a chemist with many years' work in radiopharmaceutical development, is experienced in chromatographic separations of proteins, radiolabeling techniques, and tissue distribution studies.

9. We will attempt to fill the opening for a post-doctoral research associate with an immunochemist or protein chemist who can provide expertise in the techniques for radiolabeling antibodies on a micro scale.

10. Dr. Hayes, the retired Head of ORAU's Radiopharmaceutical Development program, has over 30 years experience in this area. He has expertise in tumor-localizing radiopharmaceuticals, physical characteristics of radionuclides, and radiolabeling techniques. He will be a consultant in these areas (four visits per year).

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director: Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Lee Cross Washburn	Scientist (Chemist)	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1972	Research Associate, Chemistry Department, Vanderbilt University, Nashville, TN
1972 - 1973	Presidential Intern, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN
1973 - present	Scientist (Chemist), Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN

Honors: Alpha Chi; Shell Oil Foundation Predoctoral Fellowship (1971-1972)

Publications:

Washburn, L.C., Blair, L.D., Byrd, B.L., and Sun, T.T. Comparison of Ga-68 EDTA, [1-^{11}C]α-aminoisobutyric acid, and [^{99m}Tc]sodium pertechnetate in an experimental blood-brain barrier lesion. J. Nucl. Med. (submitted)

Washburn, L.C., Sun, T.T., Byrd, B.L., and Callahan, A.P. Resolution of [^{11}C]DL-leucine and [^{11}C]DL-tryptophan by high-performance liquid chromatography. J. Labelled Comp. Radiopharm. (submitted)

Blank, M.L., Byrd, B.L., Cress, E.A., Washburn, L.C., and Snyder, F. Liposomal preparations of calcium- or zinc-DTPA have a high efficacy for removing colloidal ytterbium-169 from rat tissues. Toxicology (in press)

Littlefield, L.G., Joiner, E.E., Colyer, S.P., DuFrain, R.J., and Washburn, L.C. Evaluations of cellular proliferation and chromosome breakages after in vitro exposure of human lymphocytes to calcium or zinc DTPA. Radiat. Res. (in press)

Washburn, L.C., Sun, T.T., Byrd, B.L., and Kabalka, G.W. Effect of structural alterations on the uptake and clearance of primary aliphatic amines in rat lung. J. Labelled Compds. 19(Nos. 11-12):1282-1283, 1982.

Washburn, L.C., Sun, T.T., Byrd, B.L., Rafter, J.J., Hayes, R.L., Frey, K.A., and Agranoff, B.W. ^{11}C-ACHC, a potential agent for positron tomographic measurement of brain amino acid transport. In Nuclear Medicine and Biology, Vol. I, C. Raynaud, ed., Pergamon Press, Paris, France, 1982, pp. 642-645.

Kabalka, G.W., Gooch, E.E., Hsu, H.C., Washburn, L.C., Sun, T.T., and Hayes, R.L. Rapid and mild syntheses of radioiodinated estrogen derivatives via organoborane technology. In Applications of Nuclear and Radiochemistry, Chapter 17, R.M. Lambrecht and N. Morcos, eds., Pergamon Press, New York, 1982, pp. 197-203.

Publications: (Lee C. Washburn)

Hübner, K.F., King, P., Gibbs, W.D., Partain, C.L., Washburn, L.C., Hayes, R.L. and Holloway, E. Clinical investigations with ^{11}C-labeled amino acids using positron emission computerized tomography in patients with neoplastic diseases. In "Medical Radionuclide Imaging 1980", Vol II, IAEA, Vienna, 1981, pp. 515-529.

Hübner, K.F., King, P., Gibbs, W.D., Washburn, L.C., and Hayes, R.L. Positron emission computerized tomography: A potential tool for in vivo quantitation of the distribution of radiopharmaceuticals. In Third International Radiopharmaceutical Dosimetry Symposium, E. E. Watson, A. T. Schlafke-Stelson, J. L. Coffey, and R. J. Cloutier, eds., Bureau of Radiological Health, Rockville, MD, 1981, pp. 77-86.

Hübner, K.F. Krauss, S., Washburn, L.C., Gibbs, W.D., and Holloway, E.C. Tumor detection with 1-aminocyclopentane and 1-aminocyclobutane [^{11}C] carboxylic acid using positron emission computerized tomography. Clin. Nucl. Med. 6:249-252, 1981.

Casey, D.L., Digenis, G.A., Wesner, D.A., Washburn, L.C., Chaney, J.E., Hayes, R.L., and Callahan, A.P. Preparation and preliminary tissue studies of optically active C-11-D- and L-phenylalanine. Int. J. Appl. Radiat. Isot. 32:325-330, 1981.

Kabalka, G.W., Gooch, E.E., Sun, T.T., and Washburn, L.C. A rapid and mild method for labeling functionally substituted molecules with iodine radionuclides. J. Labelled Compd. Radiopharm. 18:117-118, 1981.

Washburn, L.C., Ringenberg, R.E., Sun, T.T., and Hayes, R.L. ^{11}C-Labeled 1-aminocyclohexanecarboxylic acid (^{11}C-ACHC), a potential agent for studies of amino acid transport in the brain. J. Labelled Compd. Radiopharm. 18 (Nos. 1-2):13-14, 1981.

Blank, M.L., Cress, E.A., Byrd, B.L., Washburn, L.C., and Snyder, F. Liposomal encapsulated Zn-DTPA for removing intracellular ^{169}Yb. Health Physics 39:913-920, 1980.

Hübner, K.F., Andrews, G.A., Buonocore, E., Hayes, R.L., Washburn, L.C., Collmann, I.R., and Gibbs, W.D. Carbon-11-labeled amino acids for the rectilinear and positron tomographic imaging of the human pancreas. J. Nucl. Med. 20:507-513, 1979.

Washburn, L.C., Sun, T.T., Byrd, B.L., Hayes, R.L., and Butler, T.A. 1-Aminocyclobutane[^{11}C]carboxylic acid, a potential agent for tumor localization. J. Nucl. Med. 20:1055-1061, 1979.

Washburn, L.C., Sun, T.T., Byrd, B.L., Hayes, R.L., and Butler, T.A. DL-[Carboxyl-^{11}C]tryptophan, a potential agent for pancreatic imaging: production and preclinical investigations. J. Nucl. Med. 20:857-864, 1979.

Washburn, L.C., Sun, T.T., Byrd, B.L., Hayes, R.L., Butler, T.A., and Callahan, A.P. High-level production of C-11-carboxyl-labeled amino acids. In Radiopharmaceuticals II, Soc. Nucl. Med., New York, 1979, pp. 767-777.

Washburn, L.C., Sun, T.T., Anon, J.B., and Hayes, R.L. Effect of structure on tumor specificity of alicyclic α-amino acids. Cancer Res. 38:2271-2273, 1978.

Washburn, L.C., Wieland, B.W., Sun, T.T., Hayes, R.L., and Butler, T.A. [1-^{11}C]DL-Valine, a potential pancreas-imaging agent. J. Nucl. Med. 19:77-83, 1978.

Hayes, R.L., Washburn, L.C., Wieland, B.W., Sun, T.T., Anon, J.B., Butler, T.A. and Callahan, A.P. Synthesis and purification of ^{11}C-carboxyl-labeled amino acids. Int. J. Appl. Radiat. Isotopes 29:186-187, 1978.

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1017396

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
James Edward Crook	Clinical Pharmacologist	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological [redacted] ence, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1964 - 1966	Laboratory Scientist (clinical chemistry and coagulation) University of Maryland Shock-Trauma Research Center, Baltimore, MD
1967 - 1974	Physical Science Technician, United States Public Health Service Hospital, Baltimore, MD
1979 - 1980	Assistant Director of Clinical Pharmacology, USV Laboratories, Tuckahoe, NY
1980 - 1983	Assistant Professor of Medicine, Assistant Professor of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI
1980 - 1983	Staff Physician, VA Medical Center, Wood (Milwaukee), WI
1983 - present	Director of Radiopharmaceutical Development and Preclinical Nuclear Medicine, Assistant Director of Clinical Nuclear Medicine, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN
Honors:	University of Maryland Graduate School Fellowship

Publications:

Crook, J.E. and Mroczkowski, P.J. Red Cell Na-K Cotransport: Dose-Response Effects of Furosemide Inhibition in Black and White Hypertensives. In Proceedings The First International Conference on Diuretics, Elsevier Science Publishing Co., N.Y. 1984 (in press)

Crook, J.E. and Mroczkowski, P.J. Dose Response Effects of Furosemide Inhibition on Erythrocyte Sodium-Potassium Co-Transport in White and Black Normotensives and Hypertensives. Clin. Res. 32:204A, 1984

Crook, J.E. and Mroczkowski, P.J. Increased Red Cell Na-K ATPase Activity Following Exposure to a New Calcium Entry Blocker - Nitrendipine. Presented, International Symposium on Calcium Entry Blockers and Tissue Protection, Rome, Italy, 1984.

1071397

Crook, J.E. and Mroczkowski, P.J. Red Cell Na-K Co-Transport: Dose-Response Effects on Furosemide Inhibition in Black and White Hypertensives. Presented, First International Conference on Diuretics, Miami, Florida, 1984.

Crook, J.E., Mroczkowski, P.J. and Gill, J. Decreased Furosemide Sensitive Sodium-Potassium Co-Transport in Sickle Trait Red Cells from Hypertensive Subjects. Clin. Res. 31:730, 1983.

Crook, J.E., Feix, J. and Mroczkowski, P.J. Red Cell Membrane Fluidity in Essential Hypertension. Clin. Res. 31:730A, 1983.

Crook, J.E. and Mroczkowski, P.J. Erythrocyte Co-Transport in White and Black Hypertensive Subjects. Clin. Res. 30:733A, 1982.

Crook, J.E. and Robertson, D. and Wharton, R. Prostaglandin Suppression: Inability to Correct Severe Idiopathic Orthostatic Hypotension. South. Med. J. 74:318-320, 1981.

Shevitz, S.A., Jamieson, R.C., Petrie, W.M. and Crook, J.E. Compulsive Water Drinking Treated with High Dose Propranolol. J. Nerv. Ment. Dis. 168:246, 1980.

Davis, B.A., Crook, J.E., Vestal, R.E. and Oates, J.A. The Prevalence of Renovascular Hypertension in Patients with Grade III and IV Hypertensive Retinopathy. N. Engl. J. Med. 303:1273, 1979.

Crook, J.E., Woosley, R.L., Leftwich, R.B. and Natelson, E.A. Agranulocytosis During Combined Antiarrhythmic Therapy with Procainamide and Phenytoin. South. Med. J. 72: 1599, 1979.

Crook, J.E. Idiopathic Pulmonary Ossification. J. Tenn. Med. Assoc. 72:668, September, 1979.

Crook, J.E. and Nies, A.S. Drug Interations with Antihypertensive Drugs. Drugs 15: 72, 1978. Reprinted in New Ethicals and Medical Progress, February, 1978. Selected for abstraction in the Digests, 1978.

Davis, B.A., Crook, J.E., Vestal, R.E. and Oates, J.A. Occurrence of Renovascular Hypertension in Patients with Malignant Hypertension and Accelerated Hypertension. Presented before the American Heart Association. 51st Annual Scientific Session, November, 1978.

Crook, J.E. and Dietz, A.J., Jr. Neurochemical Changes in Response to a Structurally Related Stimulant and Depressant II. Fed. Proc. 30:495, 1971. Presented at FASEB, 55th Annual Meeting.

Crook, J.E. and Dietz, A.J., Jr. Neurochemical Changes in Response to a Structurally Related Stimulant and Depressant. Fed. Proc. 29:485, 1970. Presented at FASEB, 54th Annual Meeting.

Crook, J.E. and McLaughlin, J.S. Methyl Alcohol Poisoning. J. Occup. Med. 12:467, 1966.

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Karl F. Hübner, M.D.	Assistant Chairman, Medical & Health Sciences Div. Chief of Clinical Nuclear Med.	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1960 - 1961	Internship (rotating) at 2nd General Hospital of the U.S. Army and at the 86th Tactical Hospital of the U.S. Air Force, Germany.
1962 - 1964	Resident in Clinical Investigation, Medical Division, Oak Ridge Institute of Nuclear Studies (now Oak Ridge Associated Universities), Oak Ridge, TN
1964 - 1967	Resident in Pediatrics, University of Tübingen, Medical School, Germany
1967 - 1970	Research Associate in Experimental Immunology, Medical Division, Oak Ridge Associated Universities, Oak Ridge, TN
1971 - 10/73	Senior Staff Member with Clinical Staff, Medical Division, Oak Ridge Associated Universities, Oak Ridge, TN
1973 - 1974	Senior Research Scientist, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN
1975 - present	Director, Outpatient Nuclear Medicine, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN
1977 - 6/82	Director, Radiation Emergency Assistance Center/Training Site, Oak Ridge Associated Universities, Oak Ridge, TN
1982 - present	Assistant Chairman, Medical & Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN

Honors: 1954 - "Scheffel Preis" for History and German (High School); 1959 - M.D. Degree, Summa cum laude, Heidelberg University; 1979 - American Men and Women in Sciences; listed in Guide to Energy Specialists, Intl. Environment Information

Publications:

Hübner, K.F. and Lushbaugh, C.C. Radiation injuries. In Critical Decisions in Trauma, eds, E. E. Moore, B. Eiseman, and C. W. Van Way III, St. Louis, The C. V. Mosby Co., 1984, pp. 452-457.

Lushbaugh, C.C., Hübner, K.F., Ricks, R.C., Saenger, E.L., and Wald, N. Panel discussion: Session IV Care of the radiation-exposed and injured. Bull. N.Y. Acad. Med. 59:1141-1149, 1983.

Hübner, K.F. Radiation injury, In Pharmacologic Approach to the Critically Ill Patient, (B. Chernow and C. R. Lake, eds.) Williams and Wilkins, Baltimore, 1983, pp. 707-714.

Hübner, K.F., Fry, S.A., and Lushbaugh, C.C. Biological effects of radiation in man. In Funk & Wagnalls New Encyclopedia, ed. R. S. Phillips, New York: Funk & Wagnalls, 1983, pp. 69-70.

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Publications: (Karl F. Hübner, M.D.)

Hayes, R.L. and Hübner, K.F. Basis for the clinical use of gallium and indium radionuclides. In Metal Ions and Biological Systems, (H. Sigel, ed.). New York, Marcel Dekker, Inc., 16:279-315, 1983.

Berger, M.E. and Hübner, K.F. Excessive exposure to radiation in the health environment: diagnostic testing. Am. J. Nursing 83:1155-1159, 1983.

Hübner, K.F. and Buonocore, E. Positron emission tomography (P.E.T.). In Alimentary Tract Radiology, 3rd Edition, Harshberger, S. E., ed., The C. V. Mosby Company, St. Louis, MO, 1983, pp. 2486-2494.

Fry, S.A., Lushbaugh, C.C., and Hübner, K.F. An overview of radiation accidents and injuries. Int. J. Nucl. Med. and Biol. 9:152-153, 1982.

DuFrain, R.J., Batson, J.S., Joiner, E.E., Colyer, S.P., Littlefield, L.G., and Hübner, K.F. Cytogenetic assessment of radiation sensitivity in lymphocytes from individuals with Huntington's chorea. Radiat. Res. 91:301-303, 1982.

Partain, C.L., Hübner, K.F., Price, R.R., Patton, J.A., Erickson, J.J., Pickens, D.R., Hayes, R.L., and James, A.E., Jr. Functional mapping of the brain: Positron emission tomography and nuclear magnetic resonance imaging. In Functional Mapping of Organ Systems, 11th Annual Symposium on the Sharing of Computer Programs and Technology in Nuclear Medicine, P.D. Esser, ed., New York, Society of Nuclear Medicine, 1981, pp. 13-19.

Lushbaugh, C.C., Hübner, K.F., and Fry, S.A. The impact of estimates of human radiation tolerance upon radiation emergency management. In The Control of Exposure of the Public to Ionizing Radiation in the Event of Accident or Attack; Proceedings of a Symposium Reston, Virginia, 1981. National Council on Radiation Protection, Bethesda, MD, 1982, pp. 46-57.

Hübner, K.F., Purvis, J.T., Mahaley, S.M., Jr., Robertson, J.T., Rogers, S., Partain, C.L., Gibbs, W.D., and King, P. Brain tumor imaging by positron emission computerized tomography (ECT) using C-11 labeled amino acids. J. Comput. Asst. Tomogr. 6:544-550, 1982.

Partain, C.L., Price, R.R., James, A.E., Jr. Hübner, K.F., Mahaley, M.S., Scatliff, J.H., and Robertson, J.T. Positron emission tomography: brain tumor imaging using carbon-11 amino acids. In Medical Readionuclide Imaging 1980, Proceedings of an International Symposium, Heidelberg, Germany, International Atomic Energy Agency, Vienna I:397-398, 1981.

Hübner, K.F., Krauss, S., Washburn, L.C., Gibbs, W.D., and Holloway, E.C. Tumor detection with 1-aminocyclopentane and 1-aminocyclobutane [^{11}C]carboxylic acid using positron emission computerized tomography. Clin. Nucl. Med. 6:249-252, 1981.

Hübner, K.F., King, P., Gibbs, W.D., Washburn, L.C., and Hayes, R.L. Positron emission computerized tomography: a potential tool for in vivo quantitation of the distribution of radiopharmaceuticals. In Proceedings of the Third International Radiopharmaceutical Dosimetry Symposium, Oak Ridge, Tennessee, 1980, E.E. Watson, A. T. Schlafke-Stelson, J. L. Coffey, and R. J. Cloutier, eds., Rockville, MD. HSS Publication FDA 81-8166, U.S. Bureau of Radiological Health, Food and Drug Administration, 1981, pp. 77-87.

Hübner, K.F., King, P., Gibbs, W.D., Partain, C.L., Washburn, L.C., Hayes, R.L., and Holloway, E. Clinical investigations with ^{11}C-labeled amino acids using positron emission computerized tomography in patients with neoplastic diseases. In Medical Radionuclide Imaging 1980, Vol II, IAEA, Vienna, 1981, pp. 515-529.

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Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Jack L. Coffey	Scientist (Health Physicist)	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
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1971 - 1973 Laboratory Assistant, Cumberland College, Williamsburg, KY
1973 - 1974 Industrial Engineer, Henry I. Siegel Co., Inc., Bruceton, TN
1974 - 1982 Health Physicist/Internal Dosimetrist, Manpower Education, Research and Training Division, Oak Ridge Associated Universities, Oak Ridge, TN
1982 - 1984 Medical Physicist, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN
1984 - present Health Physicist, Manpower Education, Research and Training Division, Oak Ridge Associated Universities, Oak Ridge, TN

Publications (selected):

Coffey, J.L., Hayes, R.L., Rafter, J.J., Watson, E.E., and Carlton, J.E. Radiation Dosimetry and Chemical Toxicity Considerations for Tc-99. Health Physics 46:2: 418-422, 1984.

Wirth, N., Swanson, D., Shapiro, B., Nakajo, M., Coffey, J.L., Eckhauser, F., and Owyang, C. A Conveniently Prepared Tc-99m Resin for Semi-Solid Gastric Emptying Studies. J. Nucl. Med. 24:6: 511-514, 1983.

Stathis, V.J., Miller, C.M., Doerr, G.F., Coffey, J.L., and Hladik, Wm. B. III Effect of Technetium Tc-99m Pertechnetate on Bacterial Survival in Solution. Am.J. Hosp. Pharm. 40: 634-637, 1983.

Coffey, J.L., Holloway, E.C., and Hübner, K.F. Advantages and Disadvantages of Three Different Ring Source Designs for Use in Positron Tomography. J. Nucl. Med. 24:P103, 1983.

Washburn, L.C., Hübner, K.F., Sun, T.T., Byrd, B.L., Coffey, J.L., and Callahan, A.P. C-11-Tryptophan, a Potential Agent for Diagnosis of Pancreatic Disease Using PET. J. Nucl. Med. 24:P121, 1983.

Som, P., Oster, Z.H., Srivastava, S.C., Yamamoto, K., Meinken, G.E., Yonekura, Y., Sacker, D.F., Richards, P., Brill, A.B., Coffey, J., Carlton, E., and Hübner, K.F. The Effect of Carrier Amphetamine and KI on the Biodistribution of Iodoamphetamine. J. Nucl. Med. 24:P118, 1983.

Coffey, J.L., Knapp, F.F., Jr. and Goodman, M.M.: Radiation Absorbed Dose Estimates for a New Myocardial Agent I-123-15-(p-Iodophenyl)-6-Tellura-pentadecanoic Acid. J. Nucl. Med. 23:P68, 1982.

Publications: (Jack L. Coffey)

Coffey, J.L., Rafter, J.J., Watson, E.E., and Hayes, R.L. Radiation Dosimetry and Chemical Toxicity Considerations for Tc-99. Health Physics 43:148 (p.232) 1982.

Coffey, J.L. and Watson, E.E. S Values for Selected Radionuclides and Organs with the Heart Wall and Heart Contents as Source Organs. In Proceedings of Third International Radiopharmaceutical Dosimetry Symposium. HHS Publication FDA-81-8166, pp. 563-594, June 1981.

Coffey, J.L., Cristy, M., and Warner, G.G. Specific Absorbed Fractions for Photon Sources Uniformly Distributed in the Heart Chambers and Heart Wall of a Heterogeneous Phantom. MIRD Pamphlet No. 13. J. Nucl. Med. 22:65-71, 1981.

Elmaleh, D.L., Knapp, F.F., Jr., Yasuda, T., Coffey, J.L., Kopiwoda, S., and Strauss, H.W. Myocardial Imaging with Te-123m-9-telluraheptadecanoic Acid. J. Nucl. Med. 22:994-999, 1981.

Coffey, J.L. and Watson, E.E. Radiation Dose to the Injection Site from Intradermally Administered Xe-133. J. Nucl. Med. 22:P64, 1981.

Coffey, J.L. and Watson, E.E. Radiation Dose from Three Short-Lived Gamma-Emitting Radionuclides. Clin. Nucl. Med. 6:162, 1981.

Watson, E.E., Schlafke-Stelson, A.T., Coffey, J.L., and Cloutier, R.J., eds. Third International Radiopharmaceutical Dosimetry Symposium. Proceedings of Symposium held in Oak Ridge, TN, October 7-10, 1980, HHS Publication, FDA 81-8166, 1981.

Hayes, R.L., Byrd, B.B., Rafter, J.J., Carlton, J.E., and Coffey, J.L. Yb-169 for Detection of Inflammatory Lesions. In Proceedings of IAEA International Symposium on Medical Radionuclide Imaging. Heidelberg, Germany, September 1-5, 1980.

Woo, D.V., Knapp, F.F., Ambrose, K.R., Callahan, A.P., and Coffey, J.L. Radiation Dosimetry of Two New Tellurium-123m-Labeled Adrenal-Imaging Agents. J. Nucl. Med. 21:454-458, 1980.

Coffey, J.L. and Knapp, F.F., Jr. Radiation Dosimetry of Sn-117m Labeled 23-(Trimethyl-stanna)-24-nor-5 -cholan-3 -ol (Sn-117m-23-TSC): A Potential Adrenal Imaging Agent. J. Nucl. Med. 21:P86, 1980.

Coffey, J.L. and Watson, E.E. Radiation Dosimetry of Two Ga-68 Radiopharmaceuticals. Presented by J.L. Coffey at the Annual Meeting of the American Pharmaceutical Association, Washington, DC, April 1980.

Coffey, J.L., Cristy, M., and Warner, G.G. Estimates of Specific Absorbed Fractions for Photon Sources Uniformly Distributed in the Heart Contents and Heart Wall of a Heterogeneous Phantom. J. Nucl. Med. 20:607, 1979.

Watson, E.E. and Coffey, J.L. Contribution of Contaminants to the Radiation Dose from Thallium-201. J. Nucl. Med. 20:607, 1979.

Watson, E.E., Coffey, J.L., Schlafke, A.T. and Cloutier, R.J. A Computer Data Base of Bibliographic Entries on Radiopharmaceuticals. J. Nucl. Med. 20:607, 1979.

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Myles C. Cabot	Scientist (Biochemist)	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
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1981 - 1984 Senior Staff Scientist, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN

1978 - 1981 Scientist I, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN (Biochemist, April 1979)

1976 - 1978 Damon Runyon-Walter Winchell Postdoc. Fellow in Cancer Research, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN

1975 - 1976 Instructor; 1974-1976: Assistantship; 1972-1974, Fellowship—The Hebrew University Jerusalem, Israel.

1970 - 1972 Graduate Assistantship; 1969-1972, Laboratory Assistant—Western Carolina University, Cullowhee, NC

Honors: Member American Society of Biological Chemists, Sigma Xi Research Award (1972, Masters Thesis); Distinguished Alumni Award, Western Carolina University.

Publications:

Cabot, M.C., Faulkner, L.A., Lackey, R.J., and Snyder, F. Vertebrate class distribution of 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine acetylhydrolase in serum. Comp. Biochem. Physiol. B. (in press)

Lackey, R.J. and Cabot, M.C. Acetate utilization and fatty acid metabolism in phorbol ester and dimethyl sulfoxide-differentiated human leukemia cells. Cancer Lett. 20:291-297, 1983.

Snyder, F., Lee, T-c, Blank, M.L., Cabot, M.C., Malone, B. and Albert, D.H. Enzymatic pathways for platelet activating factor (Proceedings of the 1st International Symposium on Platelet-Activating Factor, 1983, Paris, Elsevier Biomedical Press, Amsterdam), INSERM (Inst. Nat. Sante Rech. Med.) Symp. (in press)

Cabot, M.C. Effect of cellular phospholipid modification on phorbol diester binding. Cancer Res. 43:4233-4238, 1983.

107403

Publications: (Myles C. Cabot)

Arneson, R.M., Wander, J.D., Cabot, M.C., Tan, E.L., Shenley, R.L., and Hsie, A.W. Resistance to lipid peroxidation by cultured neoplastic cells. In: Protective Agents in Cancer, (McBrien, D.H.C. and Slater, T.F., eds.) (N.F.C.R. Cancer Association Symposia No. 2), 1982, Uxbridge, England, pp. 101-124, Academic Press, New York, 1983.

Lackey, R.J. and Cabot, M.C. Serum lipase active in the hydrolysis of the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate. Cancer Lett. 19:165-172, 1983.

Cabot, M.C., Blank, M.L., Welsh, C.J., Horan, M.J., Cress, E.A., and Snyder, F. Metabolism of 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine by cell cultures. Life Sci. 31:2891-2898, 1982.

Cabot, M.C., Welsh, C.J., and Snyder, F. Increasing the levels of ether-linked lipids in L-M cells by glyceryl ether supplementation depresses growth and choline utilization. Biochem. Biophys. Acta 713:16-22, 1982.

Welsh, C.J., Sayer, A.M., Littlefield, L.G., and Cabot, M.C. Modification of lipid acyl groups by serum deprivation does not affect phorbol ester-induced differentiation of human leukemia cells. Cancer Lett. 65:145-154, 1982.

Cabot, M.C. and Welsh, C.J. Fatty acid metabolism in phorbol ester-differentiating human leukemia cells. Cancer Res. 41:4910-4915, 1981.

Cabot, M.C. and Welsh, C.J. Ether lipid studies in mouse C3H/10T1/2 cells and a 3-methylcholanthrene-transformed clone. Arch. Biochem. Biophys. 211:240-244, 1981.

Cabot, M.C. and Lumb, R.H. The activity of a low temperature lipase in the larvae of Sarcophaga bullata (Diptera:Sarcophagidae) Comp. Biochem. Physiol. 68B:325-328, 1981.

Cabot, M.C. and Goucher, C.R. Chaulmoogric acid: Assimilation into the complex lipids of mycobacteria. Lipids 16:146-148, 1981.

Cabot, M.C., Welsh, C.J., Callaham, M.F., and Huberman, E. Alterations in lipid metabolism induced by 12-O-tetradecanoylphorbol-13-acetate in differentiating human myeloid leukemia cells. Cancer Res. 40:3674-3679, 1980.

Cabot, M.C. and Snyder, F. Manipulation of alkylglycerolipid levels in cultured cells: Fatty alcohol versus alkylglycerol supplements. Biochem Biophys. Acta 617:410-418, 1980,

Cabot, M.C. and Gatt, S. The hydrolysis of triacylglycerol and diacylglycerol by rat brain microsomal lipase with an acidic pH optimum. Biochim. Biophys. Acta 530:508-512, 1978

Cabot, M.C. and Snyder, F. The manipulation of fatty acid composition in L-M cell monolayer supplemented with chaulmoogric acid. Arch. Biochem. Biophys. 190:838-846, 1978.

Cabot, M.C. and Gatt, S. Rat brain microsomal lipase activity. Adv. Exp. Med. Biol. 101: 101-111, 1978.

Cabot, M.C. and Gatt, S. Hydrolysis of endogenous diacylglycerol and monoacylglycerol by lipases in rat brain microsomes. Biochem. 16:2330-2334, 1977.

Cabot, M.C. and Gatt, S. Hydrolysis of neutral glycerides by lipases of rat brain microsomes Biochem. Biophys. Acta 431:105-115, 1976.

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Zenon S. Steplewski, M.D., D.M.S., D.S.	Professor	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
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1953 - 1963	Research Assistant and Senior Assistant, Dept. of Histology and Embryology, Silesian Medical School, Zabrze, Poland.
1963 - 1966	Adjunct, Dept. of Tumor Biology, Institute of Oncology, Gliwice, Poland.
1966 - 1968	Visiting Scientist, The Wistar Institute, Philadelphia, PA.
1969 - 1973	Head, Tumor Virus Laboratory, Dept. of Tumor Biology, Institute of Oncology, Gliwice.
1973 - 1977	Associate Scientist, The Wistar Institute, Philadelphia.
1977 - 1984	Associate Professor, The Wistar Institute, Philadelphia.
1984 - present	Professor, The Wistar Institute, Philadelphia.

HONORS:

1970 - 1973	Several awards from the Polish Ministry of Health and Polish Scientific Societies.
1970 - 1973	Committee of Cell Biology and Tumor Biology, Polish Academy of Sciences
1980	Editor, Hybridoma; Editor, Monoclonal Antibody News.

PUBLICATIONS: (selected)

Adams, D.O., Hall, T., Steplewski, Z., and Koprowski, H. Tumors undergoing rejection induced by monoclonal antibodies of the IgG2a isotype contain increased numbers of macrophages activated for a distinctive form of antibody-dependent cytolysis. Proc. Natl. Acad. Sci, USA, 81, 1984 (June).

Powe, J., Pak, K.Y., Paik, C.H., Steplewski, Z., Ebbert, M.A., Herlyn, D., Ernst, C., Alavi, A., Eckelman, W.C., Reba, R.C., and Koprowski, H. Labeling Monoclonal Antibodies and $F(ab')_2$ Fragments with (^{111}In) Indium Using Cyclic DTPA Anhydride and Their In Vivo Behavior in Mice Bearing Human Tumor Xenografts. Cancer Drug Delivery, Vol. 1, No. 2, 125-135, 1984.

Blaszczyk, M., Pak, K.Y., Herlyn, M., Lindgren, J., Pessano, S., Steplewski, Z., and Koprowski, H. Characterization of Gastrointestinal Tumor-associated Carcinoembryonic Antigen-related Antigens Defined by Monoclonal Antibodies. Cancer Research, 44, 245-253, 1984.

Pak, K.Y., Randerson, D.H., Blaszczyk, M., Sears, H.F., Steplewski, Z. and Koprowski, H. Extraction of Circulating Gastrointestinal Cancer Antigen Using Solid-Phase Immunoadsorption System of Monoclonal Antibody-Coupled Membrane. Journal of Immunological Methods, 66 (1984) 51-58.

Pak, K.Y., Blaszczyk, M., Herlyn, M., Steplewski, Z. and Koprowski, H. Identification and Isolation of Lewis Blood Group Antigens from Human Saliva Using Monoclonal Antibodies. Hybridoma, Vol. 3, 1-10, 1984.

1077405

Publications: (Zenon S. Steplewski)

Mach, J.,Chatal, J., Lumbroso, J., Buchegger, F., Forni, M., Ritschard, J., Berche, C., Douillard, J., Carrel, S., Herlyn, M., Steplewski, Z., and Koprowski, H. Tumor Localization in Patients by Radiolabeled Monoclonal Antibodies against Colon Carcinoma. Cancer Research 43, 5593-5600, 1983.

Moldofsky, P.J., Powe, J., Mulhern, C.B., Jr., Hammond, N., Sears, H.F., Gatenby, R.A., Steplewski, Z., Koprowski, H. Metastatic Colon Carcinoma Detected with Radiolabeled $F(ab')_2$ Monoclonal Antibody Fragments . Radiology 1983; 149:549-555.

Steplewski, Z., and Koprowski, H. Use of Monoclonal Antibodies to Recognize Tumor Antigens. Oncodevelopmental Markers. Academic Press, Inc., pp. 155-166, 1983.

Paik, C.H., Ebbert, M.A., Murphy, P.R., Lassman, C.R., Reba, R.C., Eckelman, W.C., Pak, K.Y., Powe, J. Steplewski, Z. and Koprowski, H. Factors Influencing DTPA Conjugation with Antibodies by Cyclic DTPA Anhydride. Journal of Nuclear Medicine, December, 1983, Volume 24, Number 12.

Herlyn, M., Blaszczyk, M., Sears, H.F., Verrill, H., Lindgren, J., Colcher, D. Steplewski, Z., Schlom, J. and Koprowski, H. Detection of Carcinoembryonic Antigen and Related Antigens in Sera of Patients with Gastrointestinal Tumors Using Monoclonal Antibodies in Double-Determinant Radioimmunoassays. Hybridoma, Volume 2, Number 3, 1983.

Magnani, J.L., Steplewski, Z., Koprowski, H. and Ginsburg, V. Identification of the Gastrointestinal and Pancreatic Cancer-associated Antigen Detected by Monoclonal Antibody 19-9 in the Sera of Patients as a Mucin. Cancer Research 43, 5489-5492, November, 1983.

Pak, K.Y., Blaszczyk, M., Steplewski, Z. and Koprowski, H. Identification and Isolation of a Common Tumor-Associated Molecule Using Monoclonal Antibody. Molecular Immunology, Vol. 20, No. 12, pp. 1369-1377, 1983.

Steplewski, Z. Monoclonal Antibodies: Selection Approaches for Human Solid Tumors. Radioimmunoimaging and Radioimmunotherapy, pp. 105-107, 1983. Ed. Scott W. Burchiel and Buck A. Rhodes, 1983, Elsevier, USA.

Paik, C.H., Ebbert, M.A., Lassman, C.R., Reba, R.C., Eckelman, W.C., Pak, K.Y., Powe, J., Steplewski, Z., and Koprowski, H. pH Effect on DTPA conjugation to antibodies via cyclic DTPA anhydride. Hybridoma 2(1):126, 1983.

Steplewski, Z., Mitchell, K.F., and Koprowski, H. Biological functions of anti-melanoma monoclonal antibodies. Melanoma Antigens and Antibodies, Reisfeld, R., and Ferrone, S., eds., Plenum Press, New York, 1982, pp. 365-380.

Steplewski, Z. and Koprowski, H. Anti-colorectal carcinoma monoclonal antibodies. Hybridomas in Cancer Diagnosis and Treatment, Mitchell, M.S. and Oettgen, H.R., eds., Raven Press, New York, 1982, pp. 207-211.

Steplewski, Z. and Koprowski, H. Monoclonal antibody development in the study of colorectal carcinoma-associated antigens. Methods in Cancer Research, Busch, H., ed., 20:285-316, 1982.

Mazauric, T., Mitchell, K.F., Letchworth, G.T., III., Koprowski, H., and Steplewski, Z. Monoclonal antibody-defined human lung cell surface antigens. Cancer Res. 42:150-154, 1982.

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Raymond L. Hayes	Chief Scientist (retired)	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

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1944 Senior Scientist, U.S. Rubber Co., Charlotte, North Carolina
1950 - 1983 Chief Scientist, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee

HONORS:
Sigma Xi; AEC (NSF) Predoctoral Fellowship 1948-1950.

Publications: (selected)

Hayes, R.L. Detection of malignancies with radionuclides. In Recent Advances in Nuclear Medicine, Vol. 6, J.H. Lawrence and H.S. Winchell, eds., Grune & Stratton, Inc., New York, pp. 177-197, 1983.

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OTHER SUPPORT

(Use continuation pages if necessary)

For each of the professionals named on page 2, list, in three separate groups: (1) active support; (2) applications and proposals pending review or funding; (3) applications and proposals planned or being prepared for submission. Include *all* Federal, non-Federal, and institutional grant and contract support. If none, state "none." For each item give the source of support, identifying number, project title, name of principal investigator/program director, time or percent of effort on the project by professional named, annual direct costs, and entire period of support. (If part of a larger project, provide the titles of both the parent project and the subproject and give the annual direct costs for each.) Describe the contents of each item listed. If any of these overlap, duplicate, or are being replaced or supplemented by the present application, delineate and justify the nature and extent of the scientific and budgetary overlaps or boundaries.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:

(1) ACTIVE SUPPORT:

Lee C. Washburn	90% Salary and Benefit Support - DOE Contract No. DE-AC05-76OR00033
	10% Salary and Benefit Support - NCI Contract No. CA29490-03
Karl F. Hübner	60% Salary and Benefit Support - DOE Contract No. DE-AC05-76OR00033
	40% Salary and Benefit Support - NCI Contract No. CA29490-03
James E. Crook	100% Salary and Benefit Support - DOE Contract No. DE-AC05-76OR00033
Myles C. Cabot	100% Salary and Benefit Support - DOE Contract No. DE-AC05-76OR00033

(2) Grant Application: Pending Review
"Labeled Amines as Markers of Pulmonary Injury and Repair,"
James E. Crook, Principal Investigator, submitted to NIH, April 27, 1984.

Grant Application: Pending Review
"Lipid Role in Phorbol Ester Binding," Myles C. Cabot, Principal Investigator, submitted to NIH, October 24, 1983.

(3) None

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RESOURCES AND ENVIRONMENT

FACILITIES: Mark the facilities to be used at the applicant organization and briefly indicate their capacities, pertinent capabilities, relative proximity and extent of availability to the project. Use "other" to describe the facilities at any other performance sites listed in Item 9, page 1, and at sites for field studies. Using continuation pages if necessary, include an explanation of any consortium arrangements with other organizations.

[XX] Laboratory: Three well-equipped, air-conditioned laboratories with a total of approximately 2,000 sq. ft. are available in the Vance Road building of Oak Ridge Associated Universities (ORAU) Medical and Health Sciences Division (M&HSD).

[ ] Clinical:

[XX] Animal: At ORAU M&HSD modern accredited animal facilities with adequate floor space to house cages for all animals in this project are located in the same building as the office and laboratories of the Principal Investigator. Shared specimen collecting and work laboratories are also located directly within the animal facility. Adequate caging, which complies with minimum size and construction requirements as given in the DHEW Guide, is available.

[XX] Computer: ORAU has a Digital Equipment Corporation PDP 11/44 computer with 768 kilobytes of memory. The minitab data analysis software package (Minitab Project, University Park, PA) is available for statistical testing of experimental data.

[XX] Office: Each investigator has a well-equipped, air-conditioned office.

[ ] Other (_____):

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each. Laboratory Data Control (LDC) gradient high performance liquid chromatograph with LDC refractive index detector, LDC variable wavelength UV detector, Packard custom liquid-column single-channel analyzer monitor (for detecting the elution of gamma-emitting radionuclides), and Radiomatic Flo-One Model HP radioactivity detector (for beta emitters) with Model ES electronic stream-splitting device; Beckman Model LS 2800 liquid scintillation counter with external standardization by H#, DPM capability, chemiluminescence monitor, and multi-user package; Packard Model 5220 Auto-Gamma scintillation spectrometer; Searle Pho-Gamma V scintillation camera; and Ohio-Nuclear dual head rectilinear scanner are located at ORAU M&HSD.

ADDITIONAL INFORMATION: Provide any other information describing the environment for the project. Identify support services such as consultants, secretarial, machine shop, and electronics shop, and the extent to which they will be available to the project.

RESOURCES AND ENVIRONMENT

Dr. Zenon Steplewski of The Wistar Institute, who has an excellent record in monoclonal antibody research, will be a Co-Investigator on this project. Dr. Steplewski's laboratory will provide the monoclonal antibodies and monoclonal antibody fragments as well as the tumor lines which are required for this project. He will also work with ORAU investigators to set up at ORAU the radioimmunoassay procedure necessary to verify the retention of immunological activity of the antibodies after derivatization. Dr. R.L. Hayes, former head of ORAU's M&HSD preclinical radiopharmaceutical development program, will serve as a consultant on this project. There is also a wide variety of scientific expertise available both

 (continued on next page)

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RESOURCES AND ENVIRONMENT (continued)

within ORAU and in the Oak Ridge community at large, especially the Oak Ridge National Laboratory. Additionally, the University of Tennessee is nearby and has resources available to us within the graduate, medical, and veterinary divisions. Full-time secretarial services with access to word processor will be available to the project as required, and ORAU's well-equipped machine shop and the Division's electronic shop will also be available as needed.

'USE OF DOE FACILITIES AND DOE CONTRACT REQUIREMENTS'

This research grant application includes a segment of activity that would be performed in facilities of the U. S. Department of Energy and governed by an existing contract between Oak Ridge Associated Universities (ORAU) and the DOE. The DOE has reviewed this proposal and has concurred in ORAU conducting the described work in the DOE facilities made available for biomedical research, subject to payment to the DOE by ORAU from NIH funds of the applicable direct and indirect cost of the work (not including any charge for the use of DOE facilities) as determined by the provisions of the DOE's contract with ORAU.

By way of general information, ORAU's contract with the DOE is a cost-type contract financed under a Government-fund account. The specific contract work is formulated in cooperation with the DOE and authorized within general guidelines in the contract. Ownership of all equipment and facilities acquired by ORAU with DOE funds is vested in the U. S. Government at the time of acquisition. The contract also contains all the terms generally common to Government contracts of the type under which ORAU conducts research operations in Government-owned facilities.

In particular, ORAU's contract with DOE includes standard articles on the subjects of small business, contracting with labor surplus firms, contracting with women-owned concerns, disabled veterans, employment of the handicapped, clean air and water, cost accounting standards, cost or pricing data, equal opportunity and safety and health. We believe that in these areas, the contract conditions parallel conditions which NIH ordinarily applies to its grants. In the event of differences between NIH grant terms and these DOE contract terms, ORAU is agreeable to meeting both to the extent that they are not in conflict, and to applying those most favorable to the United States Government where this is involved. If NIH is aware of problems that such an approach would produce or suggest, ORAU upon receipt of such advice would refer the matter to the DOE for direct resolution with NIH.

The contract also includes DOE standard patent rights and technical data articles for nonprofit organizations. We propose applying these or those which NIH applies to grants, whichever provides ORAU with the greater rights in these areas. We believe this approach is consistent with Public Law 96-517.

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RESEARCH PLAN

A. Specific Aims

The specific aim of this research is preclinical development and evaluation of tumor-specific ^{255}Fm- and ^{90}Y-labeled monoclonal antibodies or monoclonal antibody fragments for potential application in radioimmunotherapy of cancer. A bifunctional chelate technique involving coupling of the chelating agent, diethylenetriamine-pentaacetic acid (DTPA), to monoclonal antibodies via the cyclic DTPA anhydride will be used. This will permit radiolabeling monoclonal antibodies with a variety of metal ion radionuclides. A radioimmunoassay technique will be used to verify retention of immunological activity after radiolabeling. Monoclonal antibodies labeled with In-111, Yb-169, and Y-88 will be used for preliminary scintigraphic and tissue distribution studies in nude mice bearing the appropriate tumor type for each antibody to determine the time course of tumor localization. A radiolabeled monoclonal anti-influenza antibody will be used as a control to assess the importance of nonspecific binding. Tissue distribution studies using monoclonal antibodies labeled with Fm-255, Y-90, and Es-253 (to be used as a longer-lived model for Fm-255) will then be carried out, and this data will be used for radiation dosimetry calculations. In vivo tumor growth studies will be carried out in tumor-bearing nude mice to assess the potential of ^{255}Fm- and ^{90}Y-labeled monoclonal antitumor antibody derivatives for future clinical radioimmunotherapy trials.

B. Significance

Research on tumor localization of radiolabeled antibodies dates back some 30 years to the work of Pressman and Korngold (1), who showed that labeled antibodies against Wagner osteosarcoma cells were concentrated in vivo by the solid tumor. Antibodies to tumor-associated antigens have been considered as leading candidates for the long-sought "magic bullet" (2). Antibodies having a high enough specificity for tumor tissue could in theory be used, in a radiolabeled form, for diagnosis of cancer and also as carriers of radionuclides and cytotoxic drugs for cancer therapy.

The conventional production of antibodies in animals has serious limitations, however. Even when highly purified antigen preparations are used, a whole spectrum of antibodies with diverse specificities is still obtained, and separation of the desired antibody from such mixtures is very difficult. Also, considerable batch-to-batch variability in the quality of the antibody obtained is quite common. Finally, even when large animals are used, there are practical limitations to the quantity of antibody which can be produced.

The discovery of a technique for production of monoclonal antibodies by Kohler and Milstein in 1975 (3) provides a solution to these problems. Monoclonal antibodies, produced in tissue culture by lymphocyte-myeloma hybridomas (4), are homogeneous, require little effort for purification, and can be reproducibly prepared in large quantities. Since their discovery, the potential of monoclonal antibodies for a variety of clinical, basic research, and even industrial applications has been recognized (2). One of the most important of these is their possible use in diagnosis and therapy of cancer.

Table 1 lists the radionuclides which have been used for labeling monoclonal antibodies for diagnostic and therapeutic investigations. Most of these studies to date have used I-131 as a radiolabel. Studies of the diagnostic (5-24) and therapeutic (23-26) potential of ^{131}I-labeled monoclonal antibodies have been carried out in both

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TABLE 1

RADIONUCLIDES USED AS LABELS FOR MONOCLONAL ANTIBODIES

Radionuclide	Half-Life	Decay Mode(s)	Energy of Principal Photon(s) (KeV)	References
Iodine-131	8.05 d	β^-	364	5-26
Iodine-123	13 h	EC	160	27,28
Iodine-125	60 d	EC	35	19-22,29-31
Technetium-99m	6.0 h	IT	140	36,51
Indium-111	2.81 d	EC	173, 247	33,43-51
Gallium-68	68 m	β^+, EC	510	51
Bismuth-212	60.6 m	β^-, α	None	52
Yttrium-90	64.2 h	β^-	None	53

animals and man. Short-lived I-123 has been used as a label for imaging studies in animals (27,28) and man (28), and I-125, although unsuitable for imaging, has been used as a radiotracer in animal studies (19,22,29-31). Subtraction of background activity (32) has been used in many of these investigations to enhance tumor imaging (6,11,14-16,18,22). Another way of improving the tumor images obtained with radiolabeled monoclonal antibodies involves use of single photon computerized emission tomography (SPECT) (13,22). For ^{131}I-labeled monoclonal antibody against carcinoembryonic antigen, SPECT was shown to be clearly superior to rectilinear scanning, permitting correct visualization of 16 out of 17 tumor sites studied (13). One can often actually increase the tumor-to-nontumor ratios by enzymatically removing non-binding portions of the antibody molecule before radiolabeling is carried out. This results in labeled fragments that maintain their specificity but eliminate non-specific binding (9,16-20,22,23,30,33).

Studies using radioiodinated derivatives clearly show the potential of radiolabeled monoclonal antibodies for cancer diagnosis and therapy. A major drawback to using radionuclides of iodine as labels for monoclonal antibodies is, however, the problem of in vivo deiodination, which obscures the images by producing a high general background as well as abnormally high concentrations in the stomach, bladder, and kidney (34,35). In addition, I-131 is far from ideal for both diagnostic and therapeutic applications because of its long half-life and medium energy gamma emission which results in high radiation doses to normal tissues.

Kits have been developed for labeling $F(ab')_2$ fragments of monoclonal antimelanoma antibodies with Tc-99m (36). Although promising results were obtained in animals, the initial clinical results failed to demonstrate uptake in metastatic lesions (36).

The bifunctional chelate technique involves covalent attachment of a chelating group to proteins, permitting strong binding of polyvalent metal ions. This concept was first introduced by Sundberg and co-workers (37), who used a diazonium coupling reaction to attach a phenylethylenediaminetetraacetic acid (phenyl-EDTA) moiety, permitting labeling of proteins with In-111. Krejcarek and Tucker (38) used a mixed carboxycarbonic anhydride to attach diethylenetriaminepentaacetic acid (DTPA) to proteins via an amide linkage. Yokoyama and co-workers (39) used glutaraldehyde as a coupling agent to link desferrioxamine, an excellent chelating agent for radionuclides of gallium and indium, to proteins. Perhaps the most attractive method, however, involves use of a cyclic anhydride to couple DTPA to proteins, again via an amide linkage. The cyclic DTPA anhydride method was first developed by Eckelman and co-workers (40) to make chelating derivatives of fatty acids and was adapted by Hnatowich and co-workers (41,42) to radiolabeling of proteins. Several papers have recently been

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published describing methods for radiolabeling monoclonal antibodies with indium-111 via the cyclic DTPA anhydride (33,43-47). Advantages of the cyclic DTPA anhydride procedure are the simplicity of the syntheses, mild coupling conditions, and easy purification of the protein after coupling.

Paik and co-workers (45,46) have investigated the effect of pH on DTPA conjugation to antibodies via the cyclic DTPA anhydride technique. Out of four buffer solutions studied for the coupling reaction, 0.1 M bicarbonate buffer at pH 8.2 gave the optimal results. For a monoclonal antibody against colorectal carcinoma, a cyclic DTPA anhydride to antibody molar concentration ratio of 50:1 allowed chelation of 1.3 indium atoms per molecule of antibody with only 7% deactivation of the antibody. Higher concentration ratios gave disproportionately greater antibody deactivation.

The bifunctional chelate technique has been investigated for labeling monoclonal antibodies with metal ions. Hnatowich and co-workers found that ^{111}In-labeled monoclonal antibody to carcinoembryonic antigen labeled by the cyclic DTPA anhydride approach had a higher tumor uptake than the same antibody labeled with radioiodine (44). Likewise Wang and co-workers (47) found better binding of ^{111}In-labeled monoclonal antibody against high-molecular weight melanoma-associated antigen than ^{125}I-labeled antibody when incubated in vitro with melanoma cells. However, Powe and co-workers (33) found that ^{111}In-labeled $F(ab')_2$ fragments of monoclonal antibodies to human colorectal carcinoma and melanoma-associated antigens were inferior to the analogous ^{131}I-labeled fragments for imaging tumor xenografts in nude mice, possibly because of increased catabolism of the ^{131}I-labeled antibody. Chelate-conjugated monoclonal antibodies have also been labeled with In-111 by the mixed anhydride technique (29,48-50). A DTPA-conjugate of Fab fragments of antibody to canine cardiac myosin was chelated with In-111 and used to image myocardial infarctions in dogs (51). Preliminary results with this same antibody conjugate were also reported using Tc-99m and Ga-68 as radiolabels (51).

Bismuth-212 has also been used to label monoclonal antibodies through a bifunctional chelate derivative, and a study of the therapeutic efficacy of these agents is in progress (52). However, the short half-life (60.6 min) may not be adequate for proper blood clearance. Preliminary studies of monoclonal antibodies labeled with the pure beta emitter yttrium-90 ($T_{1/2}$ = 64.2 h) have also been carried out (53).

As seen from the above description of previous research with radiolabeled monoclonal antitumor antibodies, the bulk of this has emphasized the diagnostic rather than the therapeutic potential of these agents. This proposed research involves labeling highly specific monoclonal antibodies or monoclonal antibody fragments with the alpha emitter fermium-255 (Fm-255) and the beta emitter yttrium-90 (Y-90) for studies of radiotherapeutic efficacy. Alpha emitters are ideal for therapy because their entire radiation energy is confined to a very small range (54). Fermium-255 appears to be an excellent radionuclide for therapy because it decays by alpha emission with a half-life (20.1 h) which is long enough to allow good tumor uptake and blood clearance, yet short enough to prevent an excessive radiation dose to normal tissues. It decays to Cf-251, which is so long-lived ($T_{1/2}$ = 900 y) that its contribution to the radiation dose would be negligible. Yttrium-90 will also be evaluated in detail as a label for monoclonal antibodies for radioimmunotherapy. Monoclonal antibodies labeled with Fm-255 or Y-90 may be very promising new agents for cancer therapy.

C. Preliminary Studies

As was pointed out in section B., of the methods that have been developed to date for the covalent coupling of polyaminocarboxylates to proteins, such as monoclonal antibodies, the cyclic DTPA anhydride technique appears to be the method of choice for labeling antibodies with polyvalent cationic radionuclides. It is rapid, proceeds at

physiologic pH, and yields chelating products that retain their immunological activity (29).

In preliminary studies directed toward establishing a general technique for labeling monoclonal antibodies, we have used a modification of the methods of Hnatowich et. al. (44) and Paik et. al. (45) for coupling DTPA to antibodies. The coupling procedure for a 1:1 ratio of cyclic DTPA anhydride to monoclonal antibody is as follows: Ten µl of a 1.5 mg/ml solution of the cyclic DTPA anhydride in chloroform was evaporated to dryness under nitrogen gas in a glass tube. Purified colorectal monoclonal antibody 17-1A IgG2a (5.8 mg) in 100 µl of 0.05 M $NaHCO_3$/1M NaCl buffer was added to the dry anhydride and the solution agitated for 30 minutes at room temperature.

Ten µl of this stock solution of DTPA-coupled monoclonal antibody was combined with 10 µl of In-111 in 0.5 M acetate buffer (pH 6). After standing for 30 min at room temperature, 5 ml of 0.01 M citrate buffer was added and the mixture centrifuged in an ultrafiltration membrane cone (Amicon Corporation, Danvers, MA). From the percentages of radioactivity associated with the filtrate and residue fractions, it was found that the coupling efficiency was 63% (an average of 0.63 DTPA group per antibody molecule).

The ^{111}In-labeled DTPA-coupled antibody was separated from ^{111}In-DTPA using a Sephadex G50 gel filtration column (1.5 x 9 cm) eluted with 0.02 M Na_2HPO_4/0.5 M NaCl buffer (pH 7). Elution of radioactivity from the column was monitored using a custom liquid-column single-channel analyzer monitor system (Packard Instrument Company, Downers Grove, IL). At a flow rate of 0.2 ml/min, the retention time of the ^{111}In-labeled DTPA-coupled antibody was 20-25 min and that of ^{111}In-DTPA was 50-60 min (Fig. 1). We have also found that the separation can be carried out prior to labeling, if desired, by using UV absorption at 280 nm to monitor elution of the DTPA-coupled antibody.

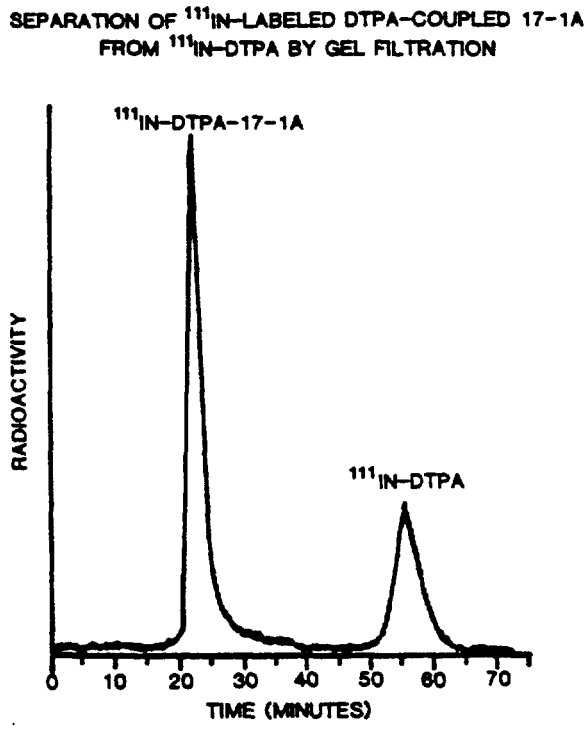

Fig. 1. Separation of ^{111}In-labeled DTPA-coupled 17-1A from ^{111}In-DTPA by gel filtration.

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Five preparations were sent to Dr. Z. Steplewski of The Wistar Institute for radioimmunological testing (see method in Section D.4.) to determine the retention of immunological activity of the derivatized antibodies: (1) Purified monoclonal anticolorectal antibody 17-1A IgG2a (as a standard); (2) a solution of 17-1A which was lyophilized and resuspended in water to a concentration of 5.8 mg/100 µl (because the original 17-1A solution was too dilute for the coupling reaction); (3) DTPA-coupled 17-1A prepared from the solution in (2) as described above (1:1 ratio of anhydride to antibody) and purified by gel filtration; (4) DTPA-coupled 17-1A which was complexed with a molar amount of stable In^{+3} equivalent to the DTPA present (to completely saturate the chelating sites) and then purified by gel filtration; (5) DTPA-coupled 17-1A prepared from the solution in (2) as described above except that the anhydride-to-antibody concentration ratio was 20:1 (which gave an average of 4.5 DTPA groups per antibody molecule). The immune reactivity of samples 2-5 were unchanged relative to the purified 17-1A preparation (sample 1), when tested using the colon carcinoma cell line SW1116, which expresses about 5 x 10^5 binding sites per cell for this antibody (Fig. 2). They were also tested using human melanoma cell line WM-9, which should not bind the antibody (Fig. 3). There was some apparent binding of the conjugates at higher concentrations, but this was probably due to aggregation rather than specific binding.

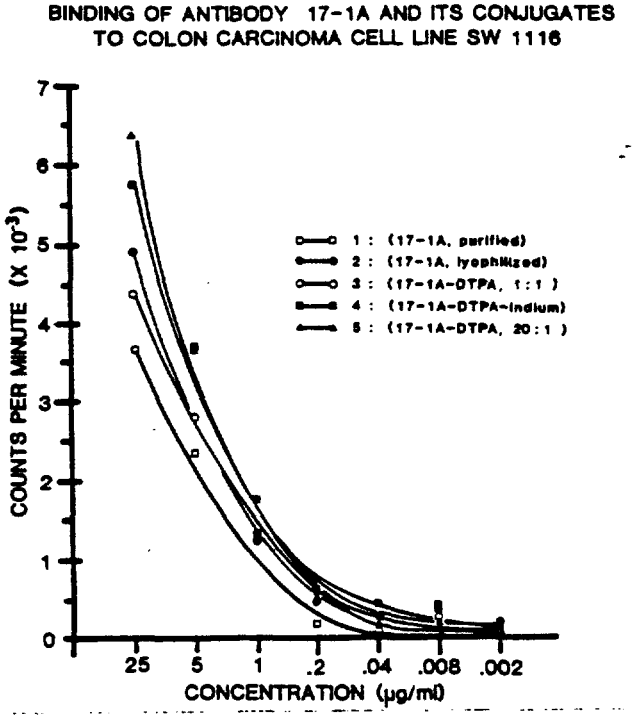

Fig. 2. Binding of antibody 17-1A and its conjugates to colon carcinoma cell line SW 1116.

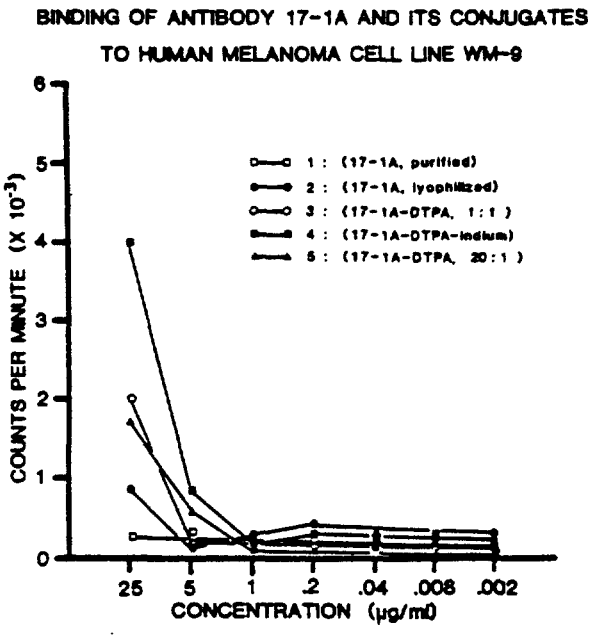

Fig. 3. Binding of antibody 17-1A and its conjugates to human melanoma cell line WM-9.

Preliminary biodistribution experiments were carried out in nude mice bearing human tumor implants using ^{111}In-labeled, DTPA-coupled monoclonal antibody 17-1A against colorectal carcinoma. The purified ^{111}In-labeled 17-1A was prepared by reaction of 17-1A with cyclic DTPA anhydride as described above, addition of an acetate-buffered solution of In-111 (pH 5) to radiolabel the chelate-conjugated monoclonal antibody by ligand exchange, and finally purification of the ^{111}In-labeled 17-1A from ^{111}In-DTPA by gel filtration. Nude mice were inoculated with 10^7 human tumor cells and used for the biodistribution studies when the tumors were 5-10 mm in diameter. Human colon carcinoma cell line SW 948 was used to test for specific tumor uptake of the ^{111}In-labeled 17-1A, and human melanoma cell line WM 164 was used to assess the importance of nonspecific uptake. Each mouse received 6.7 µg of antibody. The results

are shown in Table 2. At 48 hours after injection of the radiolabeled antibody, a tenfold greater uptake was found in the colon carcinoma than in the melanoma. A 5:1 tumor-to-blood concentration ratio was observed.

TABLE 2

48-HOUR TISSUE DISTRIBUTION OF ^{111}IN-LABELED 17-1A IN TUMOR-BEARING NUDE MICE

	% Administered Dose/g/25g	
Tissue	Human Colon Carcinoma Cell Line SW 948	Human Melanoma Cell Line WM 164
Tumor	27.9	2.8
Blood	5.6	2.1
Liver	10.1	15.3
Spleen	11.1	41.4
Kidney	10.5	6.0
Lung	4.5	4.4
Muscle	1.0	0.6
Heart	2.3	2.0
S. Intestine	5.8	10.3

These encouraging preliminary results lead us to feel that the above approach to chelate coupling, labeling, and purification should be quite generally effective for preparation of monoclonal antibodies labeled with polyvalent cationic radionuclides such as the alpha emitter, fermium-225, and the beta emitter, yttrium-90.

D. Experimental Design and Methods

1. Radionuclides to be used as labels

The radionuclides that we propose to study as labels for monoclonal antibodies are listed in Table 3. The advantages of alpha-emitting radionuclides for internal radiotherapy have already been discussed in Section B. The properties of Fm-255 ($T_{1/2}$ = 20.1 h) make it nearly ideal for use in radioimmunotherapy. This radionuclide is unique in that it is available from parent Es-255 ($T_{1/2}$ = 38 d) and in turn decays solely by alpha emission to Cf-251 ($T_{1/2}$ = ~900 y). Tracer quantities of Fm-255 and Es-253 ($T_{1/2}$ = 20.5 d) (to be used as a longer-lived model for Fm-255) can be obtained one or two times a year from the Transuranium Processing Plant at Oak Ridge National Laboratory (see letter from Dr. J. E. Bigelow).

TABLE 3

RADIONUCLIDES PROPOSED AS LABELS FOR MONOCLONAL ANTIBODIES

Radionuclide	Half-Life	Decay Mode(s)	Proposed Application
Fermium-255	20.1 h	α	Radioimmunotherapy
Yttrium-90	64.0 h	β-	Radioimmunotherapy
Einsteinium-253	20.5 d	α	Model for Fm-255
Yttrium-88	106.6 d	EC	Model for Y-90
Indium-111	2.8 d	EC	Scintigraphic Studies
Ytterbium-169	31.0 d	EC	Scintigraphic Studies

However, the supply of Fm-255 in this country at the present time is limited to that available from the Oak Ridge National Laboratory. Although it is not as suitable as Fm-255 for internal radiotherapy, the pure beta emitter Y-90 ($T_{1/2}$ = 64 h) is readily available from its parent Sr-90 ($T_{1/2}$ = 28 y), and it therefore could act as a substitute for Fm-255. For this reason, together with possible unexpected difficulties with the use of Fm-255, we will also develop monoclonal antibody derivatives labeled with Y-90. Longer-lived, accelerator-produced Y-88 ($T_{1/2}$ = 106.6 d) will be used as a model for Y-90.

Initial scintigraphic and tissue distribution studies for each monoclonal antibody derivative will be carried out with gamma-emitting radionuclides of intermediate half-life, such as commercially available Yb-169 ($T_{1/2}$ = 31 d) and Y-88 ($T_{1/2}$ = 106.6 d) in order to study the tumor uptake and background clearance of the agent over a period of a few hours to several days after administration. Indium-111 ($T_{1/2}$ = 2.8 d) will also be used as an antibody label in order to facilitate comparison of the results obtained in this study with other published work on ^{111}In-labeled DTPA-coupled monoclonal antibodies. These radionuclides all have high stability constants with both DTPA and EDTA (see Table 4, ref. 55), and therefore polyaminocarboxylate ligands coupled to monoclonal antibodies should bind them very strongly. The metal ion used would not be expected to have a significant effect on the biodistribution of a particular antibody derivative. Tissue distributions that vary with isotopic label could be explained only by major differences in their stability constants or by the influence of in vivo exchange of the isotope with plasma proteins.

Einsteinium-253, an alpha-emitting radionuclide of intermediate half-life that is chemically very similar to Fm-255, will also be used as a label for monoclonal antibodies for tissue distribution studies. Assay by liquid scintillation counting techniques will be required for Es-253, as described in our previous study of Es-253 citrate uptake by tumors (56).

TABLE 4

STABILITY CONSTANTS FOR DTPA AND EDTA WITH METAL IONS PROPOSED AS RADIOLABELS

	Log of Equilibrium Constant (K_1)	
Metal Ion	DTPA	EDTA
Fm^{+3}	22.7	na
Y^{+3}	22.1	18.1
Yb^{+3}	22.7	19.5
Es^{+3}	22.6	na
In^{+3}	28.4	24.9

If good potential is seen in these preliminary investigations, the antibody derivative will then be labeled with Fm-255 and Y-90, the nuclides of interest for radioimmunotherapy, for further tissue distribution studies as well as tumor regression and radiation dosimetry studies.

2. Methods for radiolabeling monoclonal antibodies

Covalent coupling of the chelating agent DTPA to monoclonal antibodies and monoclonal antibody fragments will be accomplished using the cyclic anhydride of DTPA (44,45). This will permit labeling these antibody derivatives with selected metal ion radionuclides for investigation of their radioimmunotherapeutic potential. The cyclic anhydride technique, illustrated in Fig. 4, appears to be the method of choice for the reasons discussed in Section C. Hnatowich and co-workers (57-58) have found that DTPA forms stable chelates despite the utilization of one carboxyl group in the formation of the amide bond. Since four carboxyl groups still remain, attachment of DTPA onto a protein would produce a net negative charge, even after complexation with a trivalent metal ion. However, this apparently does not affect the biological activity of an

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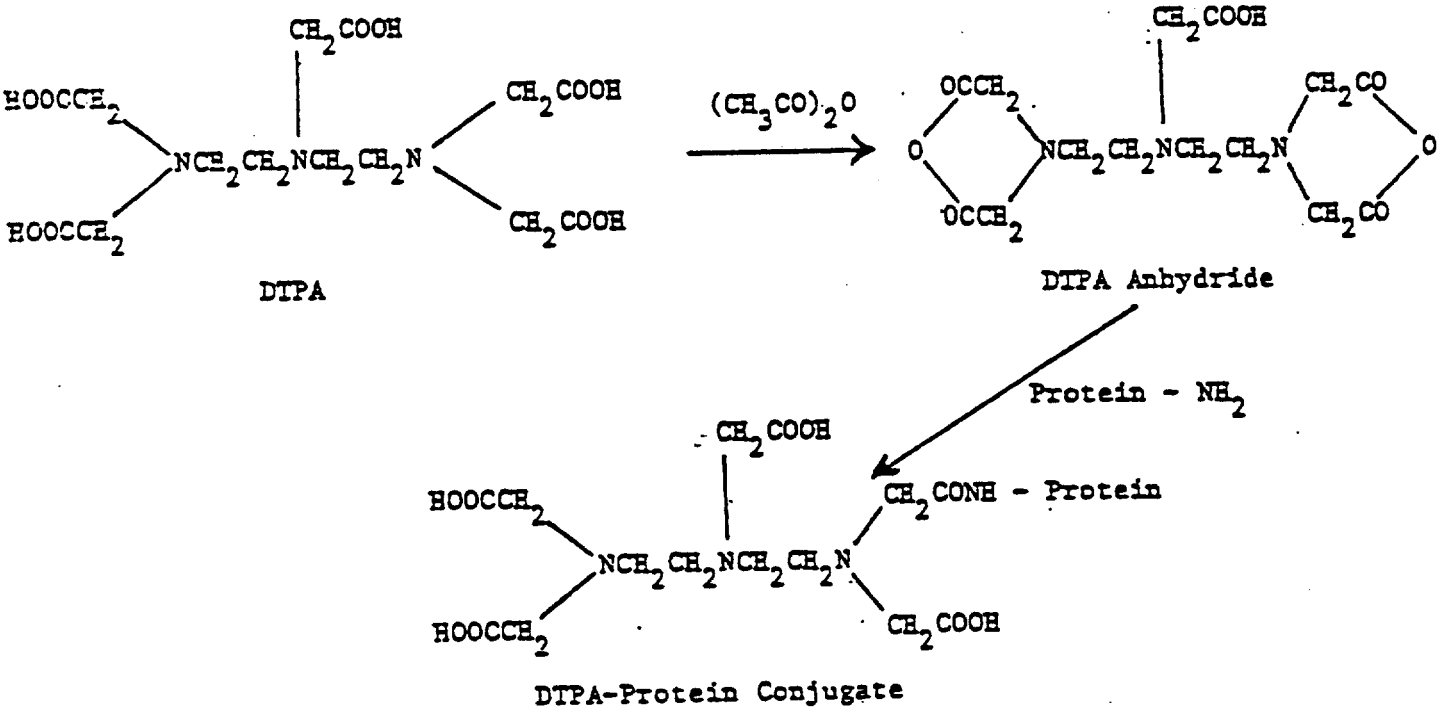

Fig. 4. Covalent attachment of DTPA to proteins via the cyclic DTPA anhydride.

antibody or other protein. For instance, fibrinogen has been modified in this way with no loss of activity in the clotting process or change in biodistribution (42), and a monoclonal antibody to colorectal carcinoma has been conjugated with 1.3 DTPA groups per antibody molecule with only 7% deactivation (45). In our preliminary studies (see Section C.), monoclonal anticolorectal antibody 17-1A was conjugated with up to 4.5 DTPA groups per antibody molecule with no loss of immunological activity.

The experimental conditions described in Section C. for synthesis and purification of the DTPA-coupled monoclonal antibodies are expected to be quite general for the monoclonal antibodies and monoclonal antibody fragments to be investigated. Radiolabeling will be accomplished by ligand exchange, by adding a buffered solution of the appropriate radioactive metal ion to the reaction solution either before or after purification from free DTPA. If necessary, a weak chelate such as citrate will be added to avoid cation hydrolysis.

Since Hnatowich has reported great variability in the coupling efficiency [as much as 70% for albumin (41) to as little as 13% for fibrinogen (42)], we will determine the coupling efficiency at various anhydride-to-antibody molar ratios for each monoclonal antibody to be studied. This will allow us to determine an optimal anhydride-to-

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antibody molar ratio which will give adequate radionuclide binding and maximum retention of immunological activity. The molar ratio used for a particular monoclonal antibody will depend upon the coupling efficiency for that antibody and the specific activity of the radionuclide to be used as a label. The number of DTPA groups per antibody molecule will, of course, be kept as small as possible in order to minimize perturbations in the structure and immunological activity of the DTPA-coupled monoclonal antibodies.

If for any reason the cyclic DTPA anhydride technique should be unsuccessful, we will couple DTPA to monoclonal antibodies and monoclonal antibody fragments using the mixed carboxycarbonic anhydride technique developed by Krejcarek and co-workers (38) and modified by Paik and co-workers (59) to work on the micro scale necessary for labeling antibodies.

3. Monoclonal antibodies to be radiolabeled

Colon carcinoma is the second leading cause of cancer deaths in the United States (60). Demographic data show that the age group primarily affected by carcinoma of the colon is increasing in size. Thus, one major area of focus of our investigation of radiolabeled monoclonal antibodies for cancer therapy will be on colon carcinoma.

Radiolabeling of two monoclonal antibodies that have exhibited great specificity for gastrointestinal carcinomas will be performed. The individual characteristics of these two antibodies are described in Table 5 (61). The monoclonal antibodies and monoclonal antibody fragments which we propose to study will be obtained from The Wistar Institute in Philadelphia, Pennsylvania. Researchers at this institute, including Dr. Zenon Steplewski, a co-investigator on this project, have been at the very forefront of monoclonal antibody research almost from the beginning of this work (see Dr. Steplewski's Biographical Sketch).

TABLE 5

CHARACTERISTICS OF THE MONOCLONAL ANTIBODIES TO BE STUDIED

MONOCLONAL ANTIBODY	17-1A	19-9	19-19
Isotype	IgG2a	IgG1	IgG1
CDC	no	no	no
ADCC	yes	yes	no
Tumor inhibition	yes	no	no
Antigen:	protein	sialylated lacto-N-/fuco-pentaose II	proteo-glycan
Spent Medium	no	yes	yes*
Serum	no	yes	yes*
Immunoperoxidase Assay:			
Frozen Section	yes	yes	yes
Paraffin embedded, fixed tissues	no	yes	no
Specificity:	colorectal & pancreatic carcinoma	colon, pancreas, gastric, and liver carcinoma	melanoma & astrocytoma

*Only in very advanced cases.

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The Wistar Institute has available a panel of tumor cell lines which are able to form tumors in nu/nu mice. These include:

Colon carcinoma	11 cell lines
Gastric carcinoma	1 cell lines
Pancreatic carcinoma	3 cell lines
Melanoma	20 cell lines
Astrocytoma	5 cell lines
Lung adenocarcinoma	3 cell lines
Breast adenocarcinoma	5 cell lines

Our investigations with monoclonal antibodies to colorectal carcinoma will begin with 1083-17-1A (17-1A). Utilizing radioimmunoassay to detect binding of mouse monoclonal antibody to live cells by using rabbit anti-mouse $F(ab')_2$ radioiodinated IgG, 17-1A was found (62) to bind selectively to all nine colorectal carcinoma cell lines studied. Specificity of 17-1A for pancreatic carcinoma has also been demonstrated. However, three lung carcinomas, ten melanomas, two astrocytomas, one breast carcinoma, and a panel of other tumor and normal cell lines did not express the antigen against which the antibody is directed.

The monoclonal antibody 17-1A has been studied fairly extensively. Labeling of the monoclonal antibody 17-1A with I-131 has been accomplished (14). Although I-131 is not a good choice of a radiolabel because of in vivo deiodination, specific uptake of the labeled antibody by human colorectal carcinomas was easily observed (11,16,19,22).

The second gastrointestinal monoclonal antibody to be used is 1116-NS-19-9 (19-9). Unlike the 17-1A antibody, it belongs to the IgG1 isotype. Studies in an immunoperoxidase system have shown binding of the antibody to gastric, liver, pancreatic and colorectal carcinomas (63). The finding of a high specific binding of both 17-1A and 19-9 to pancreatic carcinoma tissue is of great interest. Our group has had extensive experience in the detection of pancreatic tumors utilizing positron-emitting radiopharmaceuticals such as ^{11}C-labeled 1-aminocyclobutanecarboxylic acid and the resolved stereoisomers of ^{11}C-labeled valine and tryptophan in conjunction with the ECAT positron tomographic imaging system. Although the frequency of occurrence of pancreatic adenocarcinoma is, fortunately, much less than that of colon and rectal carcinomas, it is a devastating malignancy in terms of five-year survival rates (1-3%), which have shown little improvement over the past years. This figure reflects the difficulty in both early detection and efficacy of therapy once detected.

As seen in Table 5, of the two anticolorectal antibodies chosen for study only one (17-1A) is tumoricidal. This trait is more generally associated with antibodies of the IgG2a isotype. Monoclonal antibody 17-1A has undergone Phase I trials in four patients with gastric carcinoma (64). It appeared generally well tolerated but, with the injection of therapeutic amounts of the murine immunoglobulin, the possibility of an untoward response exists. Utilization of radiolabeled monoclonal antibodies may be able to further decrease potential problems by enabling use of much smaller quantities of the immunoglobulin.

The third and final monoclonal antibody to be studied is the monoclonal antimelanoma antibody 691-19-19 (19-19), isotype IgG1. This antibody is directed against human cutaneous malignant melanoma. It reacts with the majority of melanoma cells tested and with astrocytomas. The radioimmunoassay results against all other tumors, including colorectal carcinoma, and normal cells were clearly negative (65).

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When a radiolabeled monoclonal antibody is found which has the desired tumor specificity, Fab and $F(ab')_2$ fragments of that antibody, isolated by enzymatic cleavage with papain and pepsin, respectively, will be tested to determine whether the rate of blood clearance of the agent is increased without loss of tumor specificity.

4. In vitro testing of radiolabeled antibodies

The purified chelate-conjugated monoclonal antibodies and monclonal antibody fragments will be subjected to an indirect radioimmunoassay (RIA) procedure to determine the specific binding of the antibody preparations to target cells (33,45). Dr. Z. Steplewski of The Wistar Insitutue in Philadelphia, a co-investigator on this application, has agreed to spend two weeks in Oak Ridge assisting ORAU investigators in setting up the RIA method.

In the RIA procedure, varying concentrations of the antibody or antibody fragment preparation to be tested are incubated in microtiter plates for 1 hour at room temperature with 2.5×10^5 tumor cells of the appropriate cell line in 100 µl of phosphate-buffered saline containing 5% agammaglobulinemic horse serum and 0.02% sodium azide. The cells are then washed three times with ice cold buffer to remove unbound antibody, and a second antibody against mouse immunoglobulin $F(ab')_2$ labeled with I-125 (50,000 cpm in 50 µl) is added to each well. After incubation for 1 hour at room temperature, the cells are washed three times and radioactivity associated with the cell pellet is determined. If the activity of I-125 bound to the cells is plotted against the logarithm of the antibody concentration, the fractional antibody binding activity can be determined by dividing the concentration of the control antibody giving 50% bound radioactivity by the concentration of the conjugated antibody giving the same percentage I-125 bound activity. This fractional antibody binding activity will provide a good measure of the immunological activity of the derivatized antibody. For each assay, several tumor cell lines that express the antigen against that particular antibody will be used, as well as several that do not (negative binding).

Initially the RIA procedure will be carried out on the purified conjugates both before and after radiolabeling. However, our preliminary studies (see Section C.) have indicated that the presence of the metal ion does not affect the relative immunoreactivity of the antibody preparation. If this continues to hold true, we will carry out the RIA procedure only on the final radiolabeled products.

The relative immunoreactivity of the radiolabeled DTPA-conjugated antibody preparations will be assessed by a direct RIA procedure in which 2.5×10^5 target cells are incubated with varying quantities of radiolabeled DTPA-conjugated antibody in a total volume of 100 µl. After 1 hour at room temperature and three washes with cold buffer, the radioactivity associated with the cell pellet is counted, and the fraction of added activity bound is determined for each dilution and compared with the equivalent fractions of ^{131}I-labeled antibody preparation.

5. Tumor localization studies

Preliminary in vivo testing will consist of studies to assess the localization of radiolabeled monoclonal antitumor antibodies or their fragments in transplanted tumors by scintigraphy and subsequent radioisotopic tissue distribution analysis. Solid tumors of the appropriate type for each antibody will be produced by inoculation of nude mice with 10^7 cells of the appropriate tumor type (maintained in tissue culture at ORAU). For each radiolabeled monoclonal antitumor antibody tested, control studies to assess the importance of nonspecific binding will be done using monoclonal anti-influenza antibody labeled with the same radionuclide by the same procedure. The radiolabeled antibodies will be dissolved in Hepes-buffered normal saline containing 2% bovine serum albumin and injected into the mice via the tail vein. Tumor-bearing nude mice will be used for these studies when the tumors are 5-10 mm in diameter.

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Serial scintigraphic images of the ^{111}In-, ^{169}Yb-, and ^{88}Y-labeled monoclonal antibodies will be obtained using our Searle Pho-Gamma V gamma camera. The serial image data will provide the basis for choosing an appropriate sacrifice time for further tissue distribution studies with that particular antibody derivative. Gallium-67 citrate will be studied analogously in each tumor type to provide a basis for comparing the tumor uptake of the radiolabeled monoclonal antibodies with that of this widely-used tumor-imaging agent. At the end of this study the animals will be sacrificed, and the tissue distribution of the radiolabel will be measured by standard gamma counting techniques.

The monoclonal antibody derivatives will then be radiolabeled with Fm-255, Es-253, and Y-90, and tissue distribution studies will be carried out at the optimal sacrifice time found in the studies with gamma-emitting radionuclides. For these radionuclides, alpha (Fm-255 and Es-253) and beta (Y-90) counting techniques utilizing liquid scintillation counting must be used. Radiolabeled anti-influenza antibody will be studied as a control, as described above. Each experimental group will consist of five animals. The tissue distribution data obtained will be used in calculations of the radiation dosimetry of these radiolabeled monoclonal antitumor antibodies (see Section D.7.).

6. <u>In vivo tumor growth studies</u>

It has been shown by other investigators that hybridoma-derived monoclonal antibodies against colorectal carcinoma suppress the growth of adenocarcinomas (66). The studies were carried out in nude mice. The parameters measured are the ones which we intend to follow as evidence of efficacy of the radiolabeled monoclonal antibodies, viz., tumor volume, time for appearance of tumor (latency), and incidence of tumor.

Thymus-deficient 6- to 8-week-old nude mice (nu/nu, BALB/C background) will be used as experimental animals. Approximately 10^7 tumor cells, in appropriate media will be inoculated under the skin of the neck. When the tumors are 5-10 mm in diameter, the animals will concurrently be injected intravenously with monoclonal antibodies or their fragments labeled with Fm-255 and Y-90. Control groups will be inoculated in the same manner and will receive the same amount of nonradiolabeled antibody or antibody fragment. Tumor sizes will be monitored weekly by measuring the three major diameters using a caliper. The efficacy of the radiolabeled monoclonal antibody will be determined also by changes in the latency period between the control group (nonradiolabled antibody) and the group receiving the radiolabeled antibody. Results from this portion of the study will be used to design the experiments to determine an approximate amount of radiolabeled monoclonal antibody or its fragments to be administered concomitantly and at daily intervals following tumor implantation. The control group will receive the same amount of nonlabeled antibody or fragments at the same time interval. As well, the total number of nude mice with palpable masses or nodules in the experimental and control groups will be recorded as additional quantitative evidence that radiolabeled monoclonal antibodies or their fragments are efficacious in affecting tumor appearance and progression. The statistical significance of differences between groups will be tested using ORAU's Digital Equipment Corporation PDP 11/44 computer (768 kilobytes of memory) equipped with the Minitab data analysis software package.

7. <u>Radiation dosimetry estimates</u>

Radiation dosimetry is an essential element in planning a treatment protocol for radiolabeled antibodies so that the physician can determine how much activity is required to achieve the desired effect. Although the radiation dose for an individual patient cannot be accurately calculated from the results of animal studies, initial estimates can be made that will help in analyzing the usefulness of the proposed antibody and in comparing the effectiveness of the different radionuclidic labels.

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The staff of the DOE-FDA Radiopharmaceutical Internal Dose Information Center will participate in this study by advising on data that should be collected to facilitate the dose estimates and by calculating radiation doses for different radionuclidic labels and different types and sizes of tumors, as well as doses in normal tissues from activity in the tumors.

During the first year of the study, preliminary work on the radiation dosimetry will be performed. Mathematical models of mouse tumors of various shapes and volumes will be developed. Computer programs will be developed that will facilitate the estimation of the dose distribution in the tumor and in the normal tissue around the tumor.

As data are collected on the tissue distribution, the radiation dose within the tumor volume from the alphas emitted during decay of Fm-255 and from the betas emitted during decay of Y-90 will be calculated to assist in correlating response to the radioactivity (regression of tumor) with the radiation dose. In addition, calculations of the dose to normal tissues from the betas, conversion electrons, and photons will also be performed.

In the final phase of study, if these agents prove to have potential value for treatment of human carcinomas, preliminary estimates of the radiation dose in human tumors will be made. This will require development of mathematical models of human tumors and calucations of dose distributions in and around the tumors. Because of the nonuniformity of the activity distribution, estimates of the dose at multiple sites within the tumor volume will be made to give estimates of maximum and minimum doses in the tumor.

Because traditional methods of expressing radiation doses such as the MIRD technique are not adequate for characterizing the energy deposition in tissue at the microscopic level, another method must be utilized. The National Cancer Institute (NCI) is funding a three-year program at Memorial Sloan-Kettering Cancer Center in New York to develop methods for calculating the dose from radioactively labeled antibodies. Rodney Bigler, principal investigator for the NCI program, has agreed to serve as an unpaid consultant on this proposed project (see attached letter from Dr. Bigler) to eliminate any duplication of effort and to ensure that the methods used for expressing energy deposition are in harmony with those developed in the NCI program. This is extremely important in the comparison of different agents and protocols. Dr. Bigler has also agreed to make measurements of the fermium-255 and yttrium-90 in the anthropomorphic phantom being developed as part of the NCI program. This will verify calculations of the radiation doses to normal tissues around the target tissues.

8. Projected clinical trials

Although no funds are requested in this proposal for clinical studies, it is obvious that any radiolabeled antibodies and/or antibody fragments found in these preclinical studies to have sufficient potential will be submitted to clinical evaluation. It is expected that eventual clinical testing of the ^{255}Fm- and/or ^{90}Y-labeled monoclonal antibodies for radioimmunotherapy of cancer will be carried out at the American Oncological Hospital of the Fox Chase Cancer Institute in Philadelphia, PA. The clinical protocols will be developed for NCI approval when appropriate.

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9. Timetable for the investigation

a. First year

During the first year of the grant award period, our efforts will be primarily directed toward optimization of the conditions for coupling DTPA to monoclonal antibodies and monoclonal antibody fragments and for radiolabeling the antibody conjugates. The radioimmunoassay procedure will be developed at ORAU and used to insure retention of immunological specificity of the radiolabeled antibodies. Scintigraphic and tissue distribution studies of the antibodies labeled with gamma-emitting radionuclides will begin.

b. Second year

During this period the basic scintigraphic and tissue distribution studies will be completed, including tissue distribution studies of monoclonal antibodies labeled with Fm-255, Y-90, and Es-253. Calculations of radiation dosimetry using the tissue distribution data will begin. We will also begin to test antibodies labeled with Fm-255 and Y-90 to determine the appropriate dose for best tumor regression.

c. Third year

The radiation dosimetry calculations and in vivo tumor growth studies will be completed. The radiolabeled antibodies that show the most promise will undergo further extensive animal testing as required for future clinical investigation.

E. Human Subjects, Derived Materials or Data

Does not apply.

F. Vertebrate Animals

Nude mice bearing transplanted tumors of the appropriate type for each radiolabeled monoclonal antibody to be tested will be used for the biodistribution experiments. Approximately 200 of these animals per year are expected to be required. Sacrifice of these animals will be necessary in order to accomplish the objectives of this research. Procedures to be used for animal care are in full accordance with the DHEW Guide as indicated in the Assurance Statement.

G. Consultants

A letter from Dr. R.L. Hayes, the retired Head of Preclinical Radiopharmaceutical Development for ORAU's Medical and Health Sciences Division, who will be a consultant on this project, is attached.

H. Consortium Arrangements or Formalized Collaborative Agreements

The major collaboration involved in this project is between ORAU and The Wistar Institute (see attached letter from Dr. Zenon Steplewski, a co-investigator on this project). This collaborator will provide the monoclonal antibodies and monoclonal antibody fragments, as well as the tumor lines which are required for this project. He will also work with ORAU investigators to set up at ORAU the radioimmunoassay procedure necessary to verify the retention of antibody specificity after radiolabeling. The only costs to the program will be for Dr. Steplewski to make three visits per year to Oak Ridge.

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In addition, letters from Dr. John Bigelow of Oak Ridge National Laboratory and Dr. Rodney Bigler of Memorial Sloan-Kettering Cancer Center (New York) are also attached. Dr. Bigelow has agreed to provide the fermium-255 and einsteinium-253 needed for this project, and Dr. Bigler has agreed to grant us access to the model he is developing for the National Cancer Institute for estimating the absorbed radiation dose from radiolabeled monoclonal antibodies.

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Principal Investigator
Lee C. Washburn

Soc. Sec. No.
[redacted]

May 4, 1984

Dr. Lee C. Washburn
Radiopharmaceutical Development
Medical and Health Sciences Division
Oak Ridge Associated Universities
P.O. Box 117
Oak Ridge, TN 37831

Dear Lee:

I am, as you are aware, very much interested in the progress of the work with chelating derivatives of tumor-specific monoclonal antibodies for possible use in radiotherapy of cancer. Should your grant proposal entitled "Radiolabeled Monoclonal Antibodies for Cancer Therapy" be funded, I would consequently be delighted to act in a consultant capacity to the project. My best wishes.

Sincerely yours,

Ray Hayes

R. L. Hayes, Ph.D.

RLH:bb

1077432

Principal Investig r
Lee C. Washburn

Soc. Sec. No.
[redacted]

THE WISTAR INSTITUTE

THIRTY-SIXTH STREET AT SPRUCE
PHILADELPHIA, PA. 19104

HILARY KOPROWSKI, M.D.
DIRECTOR

PHONE: (215) 898-3700
CABLE ADDRESS: WISTARINST

May 4, 1984

Dr. Lee C. Washburn
Radiopharmaceutical Development
Oak Ridge Associated Universities
Post Office Box 117
Oak Ridge, Tennessee 37830

Dear Dr. Washburn:

This is to confirm my willingness and enthusiasm for our collaboration in radiolabeling of monoclonal antibodies. I will be happy to serve as a Co-Investigator with 5% effort, no salary. We will continuously supply you with purified monoclonal antibodies and I will participate in all experiments, as designed in the grant proposal.

Very truly yours,

Z Steplewski

Zenon Steplewski, MD, D.Sc.

ZS:ets

1077433

Principal Investig r
Lee C. Washburn

Soc. Sec. No.
[redacted]

OAK RIDGE NATIONAL LABORATORY
OPERATED BY MARTIN MARIETTA ENERGY SYSTEMS, INC.

POST OFFICE BOX X
OAK RIDGE, TENNESSEE 37831

May 8, 1984

Dr. Lee C. Washburn
Radiopharmaceutical Development
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, TN 37831

Dear Dr. Washburn:

I would be pleased to work with you on the development of radiolabeled monoclonal antibodies for cancer therapy. The isotopes to which you referred, ^{253}Es and ^{255}Fm, are produced at ORNL along with other isotopes of heavy elements under the National Transplutonium Element Production Program administered by the Office of Energy Research of the U.S. Department of Energy. These nuclides become available one or two times a year when groups of targets irradiated in the High Flux Isotope Reactor are processed at the Transuranium Processing Plant. The products of these operations are made available to interested researchers in the United States upon approval of the Director of the Division of Chemical Sciences, based on allocations recommended by the Transplutonium Program Committee. I cannot commit these groups to supply materials in the future, but it is their policy to make materials available to qualified researchers if at all possible. As a result of your verbal request made in October, 1982, you have been allocated tracer amounts of ^{253}Es and ^{255}Fm from our next campaign, now scheduled for the fall of 1984.

The quantities available in a typical campaign are 0.6 TBq of ^{253}Es and 2.5 GBq of ^{255}Fm. The einsteinium is far in excess of your needs. The fermium is present as the daughter of 40-day ^{255}Es and could be milked repeatedly from the mixture of einsteinium isotopes. However, processing losses and decay during purification, packaging and shipment would limit the amount of ^{255}Fm delivered to your laboratory to the order of 0.4 GBq (~10 mCi).

We can furnish modest amounts of these isotopes for research and development purposes without charge (except for Isotopes Distribution Office handling charges). Should your requests increase to the point of consuming a significant portion of our resources, we would have to reevaluate the supply and cost picture.

Yours very truly,

J. E. Bigelow

J. E. Bigelow, Sc.D.

JEB:amsp

1077434

Principal Investigator
Lee C. Washburn

Soc. Sec. No. [redacted]

MEMORIAL SLOAN-KETTERING CANCER CENTER
ESTABLISHED 1884

(212) 794-7404

May 14, 1984

Lee C. Washburn, Ph.D.
Scientist
Oak Ridge Associated Universities
Radiopharmaceutical Development Group
Medical & Health Sciences Group
P. O. Box 117
Oak Ridge, Tennessee 37831-0117

Dear Dr. Washburn:

The purpose of this letter is to confirm our willingness to collaborate with you and your associates, including Evelyn Watson and Jack Coffey, in your proposal related to antibody therapy. The possibility of using fermium-255 attached to antibodies looks very promising to me.

As you know I am the Principal Investigator for a National Cancer Institute contract (N01-CM-37565) titled, "Dose calculations for cancer therapy using radioactively labeled antibodies directed to tumor associated and/or tumor specific antigens". Within the scope of this contract we will be happy to assist you in preparing treatment plans based upon the use of data to be made available by your proposal. In addition we will be glad to give you the results of our cellular level dose estimates for ^{255}Fm and to make radiation dose measurements where appropriate with our dose measuring instrumentation.

If I can be of further assistance during this proposal phase of this research, please don't hesitate to ask.

Sincerely,

Rodney E. Bigler, Ph.D.
Biophysics Laboratory
Sloan-Kettering Institute for
Cancer Research

1077435

Memorial Sloan-Kettering Cancer Center
1275 York Avenue, New York, New York 10021

Oak Ridge
Associated Universities
Post Office Box 117
Oak Ridge, Tennessee 37831-0117

Executive Office
Office 576-3300
May 18, 1984

Dr. William R. Bibb, Director
Energy Programs and Support Division
AMERD/Federal Building
Department of Energy
Oak Ridge, TN 37830

Subject: DRAFT PROPOSAL TO NIH FOR NEW PROJECT ENTITLED "RADIOLABELED MONOCLONAL ANTIBODIES FOR CANCER THERAPY"

Dear Dr. Bibb:

Enclosed are three draft copies of a new proposal to NIH for a project entitled "Radiolabeled Monoclonal Antibodies for Cancer Therapy". The principal investigator for the proposed research is Dr. Lee C. Washburn. Should the proposal be funded, the work would be carried out under policies and procedures already established between ORAU and the DOE. A statement concerning use of DOE facilities for such work is included on page 21 of the proposal.

We would appreciate your review and approval of this proposal. Final copies should be forwarded to NIH no later than June 26 in order to meet the July 1 deadline. Should any questions arise during your review, please do not hesitate to call Dr. Washburn at 6-3099.

Sincerely yours,

William E. Felling
Executive Director

RYAN:sm

Enclosure

1071436

Cy sent to Dr. H. Moreyhum 6/14 [illegible]
Call Billie Ryan + gave her approval based on her conversation w/Bibb that she change Hubner's time on final. RQS 5/30

20-104-84

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEAL SERVICE

GRANT APPLICATION

FOLLOW INSTRUCTIONS CAREFULLY

LEAVE BLANK		
TYPE	ACTIVITY	NUMBER
REVIEW GROUP		FORMERLY
COUNCIL/BOARD (Month, year)		DATE RECEIVED

DRAFT COPY

1. TITLE OF APPLICATION (Do not exceed 56 typewriter spaces)
RADIOLABELED MONOCLONAL ANTIBODIES FOR CANCER THERAPY

2. RESPONSE TO SPECIFIC PROGRAM ANNOUNCEMENT ☒ NO ☐ YES (If "YES," state RFA number and/or announcement title)

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

3a. NAME (Last, first, middle)
WASHBURN, Lee C.

3b. SOCIAL SECURITY NUMBER
[redacted]

3c. POSITION TITLE
Scientist

3d. MAILING ADDRESS (Street, city, state, zip code)
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, Tennessee 37830

3e. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
Medical & Health Sciences Division, Pre-clinical Radiopharmaceutical Development Group

3f. MAJOR SUBDIVISION
Medical & Health Sciences Division

3g. TELEPHONE (Area code, number and extension)
(615) 576-3099

4. HUMAN SUBJECTS
☒ NO ☐ YES ☐ Exemption # _____ OR ☐ Form HHS 596 enclosed

5. RECOMBINANT DNA
☒ NO ☐ YES

6. DATES OF ENTIRE PROPOSED PROJECT PERIOD
From: 4/01/85 Through: 3/31/88

7. DIRECT COSTS REQUESTED FOR FIRST 12-MONTH BUDGET PERIOD (from page 4)
$ 84,140

8. DIRECT COSTS REQUESTED FOR ENTIRE PROPOSED PROJECT PERIOD (from page 5)
$ 272,055

9. PERFORMANCE SITES (Organizations and addresses)
Medical & Health Sciences Division
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, Tennessee 37830

10. INVENTIONS (Competing continuation application only)
☒ NO ☐ YES ☐ Previously reported OR ☐ Not previously reported

11. APPLICANT ORGANIZATION (Name, address, and congressional district)
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, Tennessee 37830

Third Congressional District

12. TYPE OF ORGANIZATION
☐ Public. Specify ☐ Federal ☐ State ☐ Local
☒ Private Nonprofit
☐ For Profit (General)
☐ For Profit (Small Business)

13. ENTITY IDENTIFICATION NUMBER
1620476816A

14. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR BIOMEDICAL RESEARCH SUPPORT GRANT
Code 6 0 Description

15. OFFICIAL IN BUSINESS OFFICE TO BE NOTIFIED IF AN AWARD IS MADE (Name, title, address and telephone number.)
Mr. W. F. Countiss
Manager of Finance
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, TN 37830 (615) 576-3304

16. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Name, title, address and telephone number)
Dr. William E. Felling
Executive Director
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, TN 37830 (615) 576-3300

17. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense (U.S. Code, Title 18, Section 1001).

SIGNATURE OF PERSON NAMED IN 3a (In ink. "Per" signature not acceptable)
Lee C. Washburn

DATE

18. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense (U.S. Code, Title 18, Section 1001).

SIGNATURE OF PERSON NAMED IN 16 (In ink. "Per" signature not acceptable)

DATE

PHS 398 (Rev. 5/82)

1077431

ABSTRACT OF RESEARCH P' N

KEY PROFESSIONAL PERSONNEL ENGAGED ON PROJECT

NAME	POSITION TITLE	DEPARTMENT AND ORGANIZATION
Lee C. Washburn	Scientist	Medical & Health Sciences Div. Oak Ridge Associated Universities.
Karl F. Hübner	Assistant Chairman, and Chief of Clinical Nuclear Medicine	Medical & Health Sciences Div. Oak Ridge Associated Universities.
James E. Crook	Director, Radiopharmaceutical Development and Preclinical Nuclear Medicine and Assistant Director, Clinical Nuclear Medicine	Medical & Health Sciences Div. Oak Ridge Associated Universities.
Zenon S. Steplewski	Associate Professor	The Wistar Institute Philadelphia, PA.

ABSTRACT OF RESEARCH PLAN: State the application's long-term objectives and specific aims, making reference to the health relatedness of the project, and describe concisely the methodology for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. The abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. DO NOT EXCEED THE SPACE PROVIDED.

Monoclonal antibodies and monoclonal antibody fragments with high specificities for human malignancies will be covalently coupled to DTPA via the cyclic DTPA anhydride technique to provide a means for labeling such agents with polyvalent metal radionuclides. The potential of radioimmunotherapy using monoclonal antibodies labeled with the alpha-emitting radionuclide Fm-255 ($T_{1/2}$ = 20.1 h) and beta-emitting Y-90 ($T_{1/2}$ = 64.0 h) will be studied preclinically. Antibodies derivatized via the bifunctional chelate technique will be tested by a radioimmunoassay technique to verify their retention of immunological activity. Following radiolabeling nude mice bearing the appropriate tumor type for each monoclonal antibody will be used to test the specificity of tumor uptake. Antibodies labeled with the gamma-emitting radionuclides In-111, Yb-169, and Y-88 will be used for scintigraphic and tissue distribution studies to determine the time course of the tumor uptake of radiolabeled antitumor monoclonal antibodies. A radiolabeled anti-influenza monoclonal antibody will be used to control for nonspecific binding. The antibodies will then be labeled with Fm-255, Es-253 (a longer-lived alpha emitter to be used as a model for Fm-255), and Y-90, and further tissue distribution studies will be carried out. This tissue distribution data will be used as the basis for radiation dosimetry calculations using the anthropomorphic phantom being developed elsewhere. Tumor regression studies will be carried out to aid in the assessment of the therapeutic potential of ^{255}Fm- and ^{90}Y-labeled monoclonal antibody derivatives. This multidisciplinary research program, combining the fields of bio-organic chemistry, immunochemistry, and radiopharmacology, could lead to improved methods for radioimmunotherapy of malignant processes in man.

VERTEBRATE ANIMALS INVOLVED ☐ NO ☒ YES If "YES," identify by common names and underline primates.

Nude mice bearing transplanted tumors.

REMOVE AND USE FOR DRAFT COPY

10114 38

51

TABLE OF CONTENTS

Number pages consecutively at the bottom throughout the application. Do not use suffixes such as 5a, 5b. Type the name of the Principal Investigator/Program Director at the top of each printed page and each continuation page.

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SECTION 2. Research Plan

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SECTION 3. Appendix *(Six sets) (No page numbering necessary for Appendix)*

Number of publications: 0 Number of manuscripts: 0

Other items *(list)*:

Letters from Drs. Z. Steplewski, R. L. Hayes, J. E. Bigelow, and R. Bigler.

REMOVE AND USE FOR DRAFT COPY

10714 39

☒ Application Receipt Record, Form PHS 3830

☐ Form HHS 596 if Item 4, page 1, is checked "YES" and no exemptions are designated.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Lee C. Washburn

DETAILED BUDGET FOR RST 12 MONTH BUDGET PERIOD
DIRECT COSTS ONLY

FROM 4/01/85 THROUGH 3/31/86

DOLLAR AMOUNT REQUESTED (Omit cents)

PERSONNEL (Applicant organization only)

NAME		POSITION TITLE	TIME/EFFORT %	TIME/EFFORT Hours per Week	SALARY	FRINGE BENEFITS	TOTALS
Washburn, Lee C.	Ph.D.	Principal Investigator	20	8	8,832	2,030	10,862
Hübner, Karl F.	M.D.	Co-Investigator	5	2	NC	NC	NC
Crook, James E., Ph.D., M.D.		Co-Investigator	10	4	5,448	1,253	6,701
Coffey, Jack L.	M.S.	Physicist	10	4	3,820	880	4,700
Cabot, Myles C.	Ph.D.	Biochemist	5	2	1,888	434	2,322
Sun, Tan Tan	M.S.	Research Associate	30	12	9,150	2,105	11,255
Carlton, James E.	B.S.	Research Associate	20	8	7,358	1,692	9,050
To Be Named	Ph.D.	Post-doctoral Research Associate	100	40	19,000	2,850	21,850
		SUBTOTALS →			55,496	11,244	66,740

CONSULTANT COSTS Raymond L. Hayes, Ph.D. (retired Head of Preclinical Radiopharmaceutical Development for ORAU's Medical and Health Sciences Division) four consulting visits per year @ $200.00 each — 800

EQUIPMENT (Itemize)

Corning model MP-6A Mega-Pure Still (water purification system) with automatic collection bottle and dimineralizer. — 4,000

SUPPLIES (Itemize by category)

Animals (200 nude mice @ $11.50 ea; animal care costs are included in indirect costs.)	$2,300	
Chemicals	1,000	
Glassware and Tissue Culture Supplies	2,000	
Radioisotopes	3,000	8,300

TRAVEL	DOMESTIC Attendance at scientific meetings	2,000
	FOREIGN	
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	

ALTERATIONS AND RENOVATIONS (Itemize by category)

CONSORTIUM/CONTRACTUAL COSTS

Zenon S. Steplewski, M.D., D.M.S., D.S., The Wistar Institute, Philadelphia, will serve as a Co-Investigator at 5% effort. The only expense involved will be for his travel for three trips to Oak Ridge per year @ $600.00 ea. — 1,800

OTHER EXPENSES (Itemize by category)

Publication costs — 500

TOTAL DIRECT COSTS (Also enter on page 1, item 7) — 1077440 → $84,140

B' GET FOR ENTIRE PROPOSED PRC CT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		1st BUDGET PERIOD (from page 4)	ADDITIONAL YEARS SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL (Salary and fringe benefits.) (Applicant organization only)		66,740	76,385	81,730		
CONSULTANT COSTS		800	800	800		
EQUIPMENT		4,000	-0-	-0-		
SUPPLIES		8,300	8,600	9,000		
TRAVEL	DOMESTIC	2,000	2,200	2,400		
	FOREIGN					
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
CONSORTIUM/ CONTRACTUAL COSTS		1,800	2,100	2,400		
OTHER EXPENSES		500	700	800		
TOTAL DIRECT COSTS		84,140	90,785	97,130		

TOTAL FOR ENTIRE PROPOSED PROJECT PERIOD *(Also enter on page 1, item 8)* → $ 272,055

JUSTIFICATION (Use continuation pages if necessary): Describe the specific functions of the personnel and consultants. If a recurring annual increase in personnel costs is anticipated, give the percentage. For *all* years, justify any costs for which the need may not be obvious, such as equipment, foreign travel, alterations and renovations, and consortium/contractual costs. For any additional years of support requested, justify any significant increases in any category over the first 12 month budget period. In addition, for COMPETING CONTINUATION applications, justify any significant increases over the current level of support.

Recurring annual increase in personnel costs of 7% anticipated. Increased effort of physicist in years 2 and 3 included.

Travel costs are for three investigators to each attend one domestic scientific meeting per year, such as the Annual Congress for Hybridoma Research and national and regional meetings of the Society of Nuclear Medicine.

The water purification system requested will provide the ultrapure water which is required for the chromatographic separations to be done in this project.

Justification of Personnel:

1. Dr. Washburn, the Principal Investigator, is a radiopharmaceutical chemist who will provide the overall guidance for this research.
2. Dr. Hübner, a Co-Investigator, is a physician with strong backgrounds in both immunology and nuclear medicine. He will provide on-site leadership in these areas.
3. Dr. Crook, a Co-Investigator, is a clinical pharmacologist who will provide expertise in the animal biodistribution work.

(continued on next page)

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Justification of Personnel: (continued)

4. Dr. Steplewski of The Wistar Institute, a Co-Investigator, is one of the foremost authorities in the area of monoclonal antibody research. In addition to providing the monoclonal antibodies and tumor lines required for the project, Dr. Steplewski will also work with ORAU investigators to set up at ORAU the radioimmunoassay procedure necessary to verify the retention of immunological activity of the antibodies after derivatization.

5. Mr. Coffey, a physicist, will perform the radiation dosimetry calculations.

6. Dr. Cabot, a biochemist with a strong background in tissue culture techniques, will maintain in tissue culture the tumor lines obtained from The Wistar Institute.

7. Mrs. Sun, a bioorganic chemist with expertise in radiopharmaceutical synthesis, will carry out syntheses such as attachment of chelating groups to antibodies.

8. Mr. Carlton, a chemist with many years' work in radiopharmaceutical development, is experienced in chromatographic separations of proteins, radiolabeling techniques, and tissue distribution studies.

9. We will attempt to fill the opening for a post-doctoral research associate with an immunochemist or protein chemist who can provide expertise in the techniques for radiolabeling antibodies on a micro scale.

10. Dr. Hayes, the retired Head of ORAU's Radiopharmaceutical Development program, has over 30 years experience in this area. He has expertise in tumor-localizing radiopharmaceuticals, physical characteristics of radionuclides, and radiolabeling techniques. He will be a consultant in these areas (four visits per year).

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Lee Cross Washburn	Scientist (Chemist)	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1972	Research Associate, Chemistry Department, Vanderbilt University, Nashville, TN
1972 - 1973	Presidential Intern, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN
1973 - present	Scientist (Chemist), Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN

Honors: Alpha Chi; Shell Oil Foundation Predoctoral Fellowship (1971-1972)

Publications:

Washburn, L.C., Blair, L.D., Byrd, B.L., and Sun, T.T. Comparison of Ga-68 EDTA, [1-^{11}C]α-aminoisobutyric acid, and [^{99m}Tc]sodium pertechnetate in an experimental blood-brain barrier lesion. J. Nucl. Med. (submitted)

Washburn, L.C., Sun, T.T., Byrd, B.L., and Callahan, A.P. Resolution of [^{11}C]DL-leucine and [^{11}C]DL-tryptophan by high-performance liquid chromatography. Eur. J. Nucl. Med. (submitted)

Blank, M.L., Byrd, B.L., Cress, E.A., Washburn, L.C., and Snyder, F. Liposomal preparations of calcium- or zinc-DTPA have a high efficacy for removing colloidal ytterbium-169 from rat tissues. Toxicology (in press)

Littlefield, L.G., Joiner, E.E., Colyer, S.P., DuFrain, R.J., and Washburn, L.C. Evaluations of cellular proliferation and chromosome breakages after in vitro exposure of human lymphocytes to calcium or zinc DTPA. Radiat. Res. (in press)

Washburn, L.C., Sun, T.T., Byrd, B.L., and Kabalka, G.W. Effect of structural alterations on the uptake and clearance of primary aliphatic amines in rat lung. J. Labelled Compds. 19(Nos. 11-12):1282-1283, 1982.

Washburn, L.C., Sun, T.T., Byrd, B.L., Rafter, J.J., Hayes, R.L., Frey, K.A., and Agranoff, B.W. ^{11}C-ACHC, a potential agent for positron tomographic measurement of brain amino acid transport. In Nuclear Medicine and Biology, Vol. I, E. Raynaud, ed., Pergamon Press, Paris, France, 1982, pp. 642-645.

Kabalka, G.W., Gooch, E.E., Hsu, H.C., Washburn, L.C., Sun, T.T., and Hayes, R.L. Rapid and mild syntheses of radioiodinated estrogen derivatives via organoborane technology. In Applications of Nuclear and Radiochemistry, Chapter 17, R.M. Lambrecht and N. Morcos, eds., Pergamon Press, New York, 1982, pp. 197-203.

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Publications: (Lee C. Washburn)

Hübner, K.F., King, P., Gibbs, W.D., Partain, C.L., Washburn, L.C., Hayes, R.L. and Holloway, E. Clinical investigations with ^{11}C-labeled amino acids using positron emission computerized tomography in patients with neoplastic diseases. In "Medical Radionuclide Imaging 1980", Vol II, IAEA, Vienna, 1981, pp. 515-529.

Hübner, K.F., King, P., Gibbs, W.D., Washburn, L.C., and Hayes, R.L. Positron emission computerized tomography: A potential tool for in vivo quantitation of the distribution of radiopharmaceuticals. In Third International Radiopharmaceutical Dosimetry Symposium, E. E. Watson, A. T. Schlafke-Stelson, J. L. Coffey, and R. J. Cloutier, eds., Bureau of Radiological Health, Rockville, MD, 1981, pp. 77-86.

Hübner, K.F., Krauss, S., Washburn, L.C., Gibbs, W.D., and Holloway, E.C. Tumor detection with 1-aminocyclopentane and 1-aminocyclobutane [^{11}C] carboxylic acid using positron emission computerized tomography, Clin. Nucl. Med. 6:249-252, 1981.

Casey, D.L., Digenis, G.A., Wesner, D.A., Washburn, L.C., Chaney, J.E., Hayes, R.L., and Callahan, A.P. Preparation and preliminary tissue studies of optically active C-11-D- and L-phenylalanine. Int. J. Appl. Radiat. Isot. 32:325-330, 1981.

Kabalka, G. W., Gooch, E.E., Sun, T.T., and Washburn, L.C. A rapid and mild method for labeling functionally substituted molecules with iodine radionuclides. J. Labelled Compd. Radiopharm. 18:117-118, 1981.

Washburn, L.C., Ringenberg, R.E., Sun, T.T., and Hayes, R.L. ^{11}C-Labeled 1-aminocyclohexanecarboxylic acid (^{11}C-ACHC), a potential agent for studies of amino acid transport in the brain. J. Labelled Compd. Radiopharm. 18 (Nos. 1-2):13-14, 1981.

Blank, M.L., Cress, E.A., Byrd, B.L., Washburn, L.C., and Snyder, F. Liposomal encapsulated Zn-DTPA for removing intracellular ^{169}Yb. Health Physics 39:913-920, 1980.

Hübner, K.F., Andrews, G.A., Buonocore, E., Hayes, R.L., Washburn, L.C., Collmann, I.R., and Gibbs, W.D. Carbon-11-labeled amino acids for the rectilinear and positron tomographic imaging of the human pancreas. J. Nucl. Med. 20:507-513, 1979.

Washburn, L.C., Sun, T.T., Byrd, B.L., Hayes, R.L., and Butler, T.A. 1-Aminocyclobutane[^{11}C]carboxylic acid, a potential agent for tumor localization. J. Nucl. Med. 20:1055-1061, 1979.

Washburn, L.C., Sun, T.T., Byrd, B.L., Hayes, R.L., and Butler, T.A. DL-[Carboxyl-^{11}C]tryptophan, a potential agent for pancreatic imaging: production and preclinical investigations. J. Nucl. Med. 20:857-864, 1979.

Washburn, L.C., Sun, T.T., Byrd, B.L., Hayes, R.L., Butler, T.A., and Callahan, A.P. High-level production of C-11-carboxyl-labeled amino acids. In Radiopharmaceuticals II, Soc. Nucl. Med., New York, 1979, pp. 767-777.

Washburn, L.C., Sun, T.T., Anon, J.B., and Hayes, R.L. Effect of structure on tumor specificity of alicyclic α-amino acids. Cancer Res. 38:2271-2273, 1978.

Washburn, L.C., Wieland, B.W., Sun, T.T. Hayes, R.L., and Butler, T.A. [1-^{11}C]DL-Valine, a potential pancreas-imaging agent. J. Nucl. Med. 19:77-83, 1978.

Hayes, R.L., Washburn, L.C., Wieland, B.W., Sun, T.T., Anon, J.B., Butler, T.A., and Callahan, A.P. Synthesis and purification of ^{11}C-carboxyl-labeled amino acids. Int. J. Ap Radiat. Isotopes 29:186-187, 1978.

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
James Edward Crook	Principal Investigator Clinical Pharmacologist	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1964 - 1966	Laboratory Scientist (clinical chemistry and coagulation University of Maryland Shock-Trauma Research Center, Baltimore, MD
1967 - 1974	Physical Science Technician, United States Public Health Service Hospital, Baltimore, MD
1979 - 1980	Assistant Director of Clinical Pharmacology, USV Laboratories, Tuckahoe, NY
1980 - 1983	Assistant Professor of Medicine, Assistant Professor of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI
1980 - 1983	Staff Physician, VA Medical Center, Wood (Milwaukee), WI
1983 - present	Director of Radiopharmaceutical Development and Preclinical Nuclear Medicine, Assistant Director of Clinical Nuclear Medicine, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN
Honors:	University of Maryland Graduate School Fellowship

Publications:

Crook, J.E. and Mroczkowski, P.J. Red Cell Na-K Cotransport: Dose-Response Effects of Furosemide Inhibition in Black and White Hypertensives. In Proceedings The First International Conference on Diuretics, Elsevier Science Publishing Co., N.Y. 1984 (in press)

Crook, J.E. and Mroczkowski, P.J. Dose Response Effects of Furosemide Inhibition on Erythrocyte Sodium-Potassium Co-Transport in White and Black Normotensives and Hypertensives. Clin. Res. 32:204A, 1984

Crook, J.E. and Mroczkowski, P.J. Increased Red Cell Na-K ATPase Activity Following Exposure to a New Calcium Entry Blocker - Nitrendipine. Presented, International Symposium on Calcium Entry Blockers and Tissue Protection, Rome, Italy, 1984

Crook, J.E. and Mroczkowski, P.J. Red Cell Na-K Co-Transport: Dose-Response Effects on Furosemide Inhibition in Black and White Hypertensives. Presented, First International Conference on Diuretics, Miami, Florida, 1984.

Crook, J.E., Mroczkowski, P.J. and Gill, J. Decreased Furosemide Sensitive Sodium-Potassium Co-Transport in Sickle Trait Red Cells from Hypertensive Subjects. Clin. Res. 31:730, 1983.

Crook, J.E., Feix, J. and Mroczkowski, P.J. Red Cell Membrane Fluidity in Essential Hypertension. Clin. Res. 31:730A, 1983.

Crook, J.E. and Mroczkowski, P.J. Erythrocyte Co-Transport in White and Black Hypertensive Subjects. Clin. Res. 30:733A, 1982.

Crook, J.E. and Robertson, D. and Wharton, R. Prostaglandin Suppression: Inability to Correct Severe Idiopathic Orthostatic Hypotension. South. Med. J. 74:318-320, 1981.

Shevitz, S.A., Jamieson, R.C., Petrie, W.M. and Crook, J.E. Compulsive Water Drinking Treated with High Dose Propranolol. J. Nerv. Ment. Dis. 168:246, 1980.

Davis, B.A., Crook, J.E., Vestal, R.E. and Oates, J.A. The Prevalence of Renovascular Hypertension in Patients with Grade III and IV Hypertensive Retinopathy. N. Engl. J. Med. 303:1273, 1979.

Crook, J.E., Woosley, R.L., Leftwich, R.B. and Natelson, E.A. Agranulocytosis During Combined Antiarrhythmic Therapy with Procainamide and Phenytoin. South. Med. J. 72: 1599, 1979.

Crook, J.E. Idiopathic Pulmonary Ossification. J. Tenn. Med. Assoc. 72:668, September, 1979.

Crook, J.E. and Nies, A.S. Drug Interactions with Antihypertensive Drugs. Drugs 15: 72, 1978. Reprinted in New Ethicals and Medical Progress, February, 1978. Selected for abstraction in the Digests, 1978.

Davis, B.A., Crook, J.E., Vestal, R.E. and Oates, J.A. Occurrence of Renovascular Hypertension in Patients with Malignant Hypertension and Accelerated Hypertension. Presented before the American Heart Association. 51st Annual Scientific Session, November, 1978.

Crook, J.E. and Dietz, A.J., Jr. Neurochemical Changes in Response to a Structurally Related Stimulant and Depressant II. Fed. Proc. 30:495, 1971. Presented at FASEB, 55th Annual Meeting.

Crook, J.E. and Dietz, A.J., Jr. Neurochemical Changes in Response to a Structurally Related Stimulant and Depressant. Fed. Proc. 29:485, 1970. Presented at FASEB, 54th Annual Meeting.

Crook, J.E. and McLaughlin, J.S. Methyl Alcohol Poisoning. J. Occup. Med. 12:467, 1966.

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel in order on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Karl F. Hübner, M.D.	Assistant Chairman, Medical & Health Sciences Div.; Chief of Clinical Nuclear Med	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1960 - 1961	Internship (rotating) at 2nd General Hospital of the U.S. Army and at the 86th Tactical Hospital of the U.S. Air Force, Germany
1962 - 1964	Resident in Clinical Investigation, Medical Division, Oak Ridge Institute of Nuclear Studies (now Oak Ridge Associated Universities), Oak Ridge, TN
1964 - 1967	Resident in Pediatrics, University of Tübingen, Medical School, Germany
1967 - 1970	Research Associate in Experimental Immunology, Medical Division, Oak Ridge Associated Universities, Oak Ridge, TN
1971 - 10/73	Senior Staff Member with Clinical Staff, Medical Division, Oak Ridge Associated Universities, Oak Ridge, TN
1973 - 1974	Senior Research Scientist, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN
1975-present	Director, Outpatient Nuclear Medicine, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN
1977 - 6/82	Director, Radiation Emergency Assistance Center/Training Site, Oak Ridge Associated Universities, Oak Ridge, TN
1982-present	Assistant Chairman, Medical & Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN

Honors: 1954-"Scheffel Preis" for History and German (High School); 1959-M.D. Degree, Summa cum laude, Heidelberg University; 1979-American Men and Women in Sciences; listed in Guide to Energy Specialists, Intl. Environment Informatio

Publications:

Hübner, K.F. and Lushbaugh, C.C. Radiation injuries. In Critical Decisions in Trauma, eds. E. E. Moore, B. Eiseman, and C. W. Van Way III, St. Louis, The C. V. Mosby Co., 1984, pp. 452-457.

Lushbaugh, C.C., Hübner, K.F., Ricks, R.C., Saenger, E.L., and Wald, N. Panel discussion: Session IV Care of the radiation-exposed and injured. Bull. N.Y. Acad. Med. 59:1141-1149, 1983.

Hübner, K.F. Radiation injury. In Pharmacologic Approach to the Critically Ill Patient, (B. Chernow and C. R. Lake, eds.) Williams and Wilkins, Baltimore, 1983, pp. 707-714.

Hübner, K.F., Fry, S.A., and Lushbaugh, C.C. Biological effects of radiation in man. In Funk & Wagnalls New Encyclopedia, ed. R. S. Phillips, New York: Funk & Wagnalls, 1983, pp. 69-70.

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Publications: (Karl F. Hübner, M.D.)

Hayes, R.L. and Hübner, K.F. Basis for the clinical use of gallium and indium radionuclides. In Metal Ions and Biological Systems, (H. Sigel, ed.), New York, Marcel Dekker, Inc., 16:279-315, 1983.

Berger, M.E. and Hübner, K.F. Excessive exposure to radiation in the health environment: diagnostic testing. Am. J. Nursing 83:1155-1159, 1983.

Hübner, K.F. and Buonocore, E. Positron emission tomography (P.E.T.). In Alimentary Tract Radiology, 3rd Edition, Harshberger, S. E., ed., The C. V. Mosby Company, St. Louis, MO, 1983, pp. 2486-2494.

Fry, S.A., Lushbaugh, C.C., and Hübner, K.F. An overview of radiation accidents and injuries. Int. J. Nucl. Med. and Biol. 9:152-153, 1982.

DuFrain, R.J., Batson, J.S., Joiner, E.E., Colyer, S.P., Littlefield, L.G., and Hübner, K.F. Cytogenetic assessment of radiation sensitivity in lymphocytes from individuals with Huntington's chorea. Radiat. Res. 91:301-303, 1982.

Partain, C.L., Hübner, K.F., Price, R.R., Patton, J.A., Erickson, J.J., Pickens, D.R., Hayes, R.L., and James, A.E., Jr. Functional mapping of the brain: Positron emission tomography and nuclear magnetic resonance imaging. In Functional Mapping of Organ Systems. 11th Annual Symposium on the Sharing of Computer Programs and Technology in Nuclear Medicine, P.D. Esser, ed., New York, Society of Nuclear Medicine, 1981, pp. 13-19.

Lushbaugh, C.C., Hübner, K.F., and Fry, S.A. The impact of estimates of human radiation tolerance upon radiation emergency management. In The Control of Exposure of the Public to Ionizing Radiation in the Event of Accident or Attack; Proceedings of a Symposium Reston, Virginia, 1981. National Council on Radiation Protection, Bethesda, MD, 1982, pp. 46-57.

Hübner, K.F., Purvis, J.T., Mahaley, S.M., Jr., Robertson, J.T., Rogers, S., Partain, C.L. Gibbs, W.D., and King, P. Brain tumor imaging by positron emission computerized tomography (ECT) using C-11 labeled amino acids. J. Comput. Asst. Tomogr. 6:544-550, 1982.

Partain, C.L., Price, R.R., James, A.E., Jr., Hübner, K.F., Mahaley, M.S., Scatliff, J.H., and Robertson, J.T. Positron emission tomography: brain tumor imaging using carbon-11 amino acids. In Medical Readionuclide Imaging 1980, Proceedings of an International Symposium, Heidelberg, Germany, International Atomic Energy Agency, Vienna I:397-398, 1981.

Hübner, K.F., Krauss, S., Washburn, L.C., Gibbs, W.D., and Holloway, E.C. Tumor detection with 1-aminocyclopentane and 1-aminocyclobutane [1-^{11}C]carboxylic acid using positron emission computerized tomography. Clin. Nucl. Med. 6:249-252, 1981.

Hübner, K.F., King. P., Gibbs, W.D., Washburn, L.C., and Hayes, R.L. Positron emission computerized tomography: a potential tool for in vivo quantitation of the distribution of radiopharmaceuticals. In Proceedings of the Third International Radiopharmaceutical Dosimetry Symposium, Oak Ridge, Tennessee, 1980, E.E. Watson, A. T. Schlafke-Stelson, J. L. Coffey, and R. J. Cloutier, eds., Rockville, Md. HSS Publication FDA 81-8166, U. S. Bureau of Radiological Health, Food and Drug Administration, 1981, pp. 77-87.

Hübner, K.F., King, P., Gibbs, W.D., Partain, C.L., Washburn, L.C., Hayes, R.L., and Holloway, E. Clinical investigations with ^{11}C-labeled amino acids using positron emission computerized tomography in patients with neoplastic diseases. In Medical Radionuclide Imaging 1980, Vol II, IAEA, Vienna, 1981, pp. 515-529.

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Myles C. Cabot	Scientist (Biochemist)	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1981 - 1984 — Senior Staff Scientist, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN

1978 - 1981 — Scientist I, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN (Biochemist, April 1979)

1976 - 1978 — Damon Runyon-Walter Winchell Postdoc. Fellow in Cancer Research, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN

1975 - 1976 — Instructor; 1974-1976: Assistantship; 1972-1974, Fellowship--The Hebrew University Jerusalem, Isreal.

1970 - 1972 — Graduate Assistantship; 1969-1972, Laboratory Assistant Western Carolina University, Cullowhee, NC

Honors: — Member American Society of Biological Chemists, Sigma X Research Award (1972, Masters Thesis); Distinguished Alumni Award, Western Carolina University.

Publications:

Cabot, M.C., Faulkner, L.A., Lackey, R.J., and Snyder, F. Vertebrate class distribution of 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine acetylhydrolase in serum. Comp. Biochem. Physiol. B. (in press)

Lackey, R.J. and Cabot, M.C. Acetate utilization and fatty acid metabolism in phorbol este and dimethyl sulfoxide-differentiated human leukemia cells. Cancer Lett. 20:291-297, 1983.

Snyder, F., Lee, T-c, Blank, M.L., Cabot, M.C., Malone, B. and Albert, D.H. Enzymatic pathways for platelet activating factor (Proceedings of the 1st International Symposium on Platelet-Activating Factor, 1983, Paris, Elsevier Biomedical Press, Amsterdam), INSERM (Inst. Nat. Sante Rech. Med.) Symp. (in press)

Cabot, M.C. Effect of cellular phospholipid modification on phorbol diester binding. Cancer Res. 43:4233-4238, 1983.

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ublications: (Myles C. Cab)

rneson, R.M., Wander, J.D., Cabot, M.C., Tan, E.L., Shenley, R.L., and Esie, A.W. esistance to lipid peroxidation by cultured neoplastic cells. In: Protective Agents n Cancer, (McBrien, D.B.C. and Slater, T.F., eds.) (N.F.C.R. Cancer Association Symposia o. 2), 1982, Uxbridge, England, pp. 101-124, Academic Press, New York, 1983.

ackey, R.J. and Cabot, M.C. Serum lipase active in the hydrolysis of the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate. Cancer Lett. 19:165-172, 1983.

Cabot, M.C., Blank, M.L., Welsh, C.J., Horan, M.J., Cress, E.A., and Snyder, F. Metabolism of 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine by cell cultures. Life Sci. 31:2891-2898, 1982.

Cabot, M.C., Welsh, C.J., and Snyder, F. Increasing the levels of ether-linked lipids in L-cells by glyceryl ether supplementation depresses growth and choline utilization. Biochim. Biophys. Acta 713:16-22, 1982.

Welsh, C.J., Sayer, A.M., Littlefield, L.G., and Cabot, M.C. Modification of lipid acyl groups by serum deprivation does not affect phorbol ester-induced differentiation of human leukemia cells. Cancer Lett. 65:145-154, 1982.

Cabot, M.C. and Welsh, C.J. Fatty acid metabolism in phorbol ester-differentiating human leukemia cells. Cancer Res. 41:4910-4915, 1981.

Cabot, M.C. and Welsh, C.J. Ether lipid studies in mouse C3H/10T1/2 cells and a 3-methylcholanthrene-transformed clone. Arch. Biochem. Biophys. 211:240-244, 1981.

Cabot, M.C. and Lumb, R.H. The activity of a low temperature lipase in the larvae of *Sarcophaga bullata* (Diptera:Sarcophagidae) Comp. Biochim. Physiol. 68B:325-328, 1981.

Cabot, M.C. and Goucher, C.R. Chaulmoogric acid: Assimilation into the complex lipids of mycobacteria. Lipids 16:146-148, 1981.

Cabot, M.C., Welsh, C.J., Callaham, M.F., and Huberman, E. Alterations in lipid metabolism induced by 12-O-tetradecanoylphorbol-13-acetate in differentiating human myeloid leukemia cells. Cancer Res. 40:3674-3679, 1980.

Cabot, M.C. and Snyder, F. Manipulation of alkylglycerolipid levels in cultured cells: Fatty alcohol versus alkylglycerol supplements. Biochim. Biophys. Acta 617:410-418, 1980.

Cabot, M.C. and Gatt, S. The hydrolysis of triacylglycerol and diacylglycerol by rat brain microsomal lipase with an acidic pH optimum. Biochim. Biophys. Acta 530:508-512, 19

Cabot, M.C. and Snyder, F. The manipulation of fatty acid composition in L-M cell monolay supplemented with chaulmoogric acid. Arch. Biochem. Biophys. 190:838-846, 1978.

Cabot, M.C. and Gatt, S. Rat brain microsomal lipase activity. Adv. Exp. Med. Biol. 101: 101-111, 1978.

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Zenon S. Steplewski, M.D., D.M.S., D.S.	Associate Professor	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1953 - 1963	Research Assistant and Senior Assistant, Dept. of Histology and Embryology, Silesian Medical School, Zabrze, Poland.
1963 - 1966	Adjunct, Dept. of Tumor Biology, Institute of Oncology, Gliwice, Poland.
1966 - 1968	Visiting Scientist, The Wistar Institute, Philadelphia, PA.
1969 - 1973	Head, Tumor Virus Laboratory, Dept. of Tumor Biology, Institute of Oncology, Gliwice.
1973 - 1977	Associate Scientist, The Wistar Institute, Philadelphia.
1977 - present	Associate Professor, The Wistar Institute, Phliadelphia.

HONORS:

1970 - 1973	Several awards from the Polish Ministry of Health and Polish Scientific Societies.
1970 - 1973	Committee of Cell Biology and Tumor Biology, Polish Academy of Sciences
1980	Editor, Hybridoma; Editor, Monoclonal Antibody News.

PUBLICATIONS: (selected)

Adams, D.O., Hall, T., Steplewski, Z., and Koprowski, H. Tumors undergoing rejection induced by monoclonal antibodies of the IgG2a isotype contain increased numbers of macrophages activated for a distinctive form of antibody-dependent cytolysis. Proc. Natl. Acad. Sci, USA, (In Press).

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Publications: (Zenon S. Steplewski)

Mach, J., Chatal, J., Lumbroso, J., Buchegger, F., Forni, M., Ritschard, J., Berche, C., Douillard, J., Carrel, S., Herlyn, M., Steplewski, Z., and Koprowski, H. Tumor Localization in Patients by Radiolabeled Monoclonal Antibodies against Colon Carcinoma. Cancer Research 43, 5593-5600, November, 1983.

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Herlyn, M., Blaszczyk, M., Sears, H.F., Verrill, H., Lindgren, J., Colcher, D. Steplewski, Z Schlom, J. and Koprowski, H. Detection of Carcinoembryonic Antigen and Related Antigens in Sera of Patients with Gastrointestinal Tumors Using Monoclonal Antibodies in Double-Determinant Radioimmunoassays. Hybridoma, Volume 2, Number 3, 1983.

Magnani, J.L., Steplewski, Z., Koprowski, H. and Ginsburg, V. Identification of the Gastrointestinal and Pancreatic Cancer-associated Antigen Detected by Monoclonal Antibody 19-9 in the Sera of Patients as a Mucin. Cancer Research 43, 5489-5492, November, 1983.

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Raymond L. Hayes	Chief Scientist (retired)	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1944 Senior Scientist, U.S. Rubber Co., Charlotte, North Carolina
1950 - 1983 Chief Scientist, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee

HONORS:
Sigma Xi; AEC (NSF) Predoctoral Fellowship 1948-1950.

Publications: (selected)

Hayes, R.L. Detection of malignancies with radionuclides. In Recent Advances in Nuclear Medicine, Vol. 6, J.H. Lawrence and H.S. Winchell, eds., Grune & Stratton, Inc., New York, pp. 177-197, 1983.

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Hayes, R.L., Rafter, J.J., Carlton, J.E., and Byrd, B.L. Studies of the in vivo uptake of Ga-67 by an experimental abscess: Concise comunication. J. Nucl. Med. 23:8-14, 1982.

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Kabalka, G.W., Gooch, E.E., Hsu, H.C., Washburn, L.C., Sun, T.T., and Hayes, R.L. Rapid and mild syntheses of radioiodinated estrogen derivatives via organoborane technology. In Applications of Nuclear and Radiochemistry, R.M. Lambrecht and N. Morcos, eds., Pergamon Press, New York, 1982, Chapter 17, pp. 197-203.

Washburn, L.C., Sun, T.T., Byrd, B.L., Rafter, J.J., Hayes, R.L., Frey, K.A., and Agranoff, B.W. ^{11}C-ACHC, a potential agent for positron tomographic measurement of brain amino acid transport. In Nuclear Medicine and Biology, Vol. I, C. Raynaud, ed., Pergamon Press, Paris, France, 1982, pp. 642-645.

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Publications: (R. L. Hayes)

Hubner, K.F., King, P., Gibbs, W.D., Partain, C.L., Washburn, L.C., Hayes, R.L., and Holloway, E. Clinical investigations with ^{11}C-labeled amino acids using positron emission computerized tomography in patients with neoplastic diseases. In "Medical Radionuclide Imaging 1980", Vol. II, IAEA, Vienna, 1981, pp. 515-529.

Casey, D.L., Digenis, G.A., Wesner, D.A., Washburn, L.C., Chaney, J.E., Hayes, R.L., and Callahan, A.P. Preparation and preliminary tissue studies of optically active C-11-D- and L-phenylalanine. Int. J. Appl. Radiat. Isot. 32:325-330, 1981.

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Washburn, L.C., Sun, T.T., Byrd, B.L., Hayes, R.L., and Butler, T.A. DL-[Carboxyl-^{11}C]tryptophan, a potential agent for pancreatic imaging: production and preclinical investigations. J. Nucl. Med. 20:857-864, 1979.

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Hayes, R.L. Chemistry and radiochemistry of metal-ion nuclides commonly employed in radiopharmaceuticals. In The Chemistry of Radiopharmaceuticals, N. D. Heindel, H. D. Burns, T. Honda, and L.W. Brady, eds., Masson Publishing, New York, 1978, pp. 155-168.

Washburn, L.C., Wieland, B.W., Sun, T.T., Hayes, R.L., and Butler, T.A. [1-^{11}C]-DL-Valine, a potential pancreas-imaging agent. J. Nucl. Med. 19:77-83, 1978.

Hayes, R.L., Washburn, L.C., Wieland, B.W., Sun, T.T., Anon, J.B., Butler, T.A., and Callahan, A.P. Synthesis and purification of ^{11}C-carboxyl-labeled amino acids. Int. J. Appl. Radiat. Isot. 29:186-187, 1978.

Washburn, L.C., Sun, T.T., Anon, J.B., and Hayes, R.L. Effect of structure on tumor specificity of alicyclic α-amino acids. Cancer Res. 38:2271-2273, 1978.

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Hubner, K.F., Andrews, G.A., Washburn, L.C., Wieland, B.W., Gibbs, W.D., Hayes, R.L., Butler, T.A., and Winebrenner, J.D. Tumor location with 1-aminocyclopentane[^{11}C]-carboxylic acid: preliminary clinical trials with single-photon detection. J. Nucl. Med. 18:1215-1221, 1977.

Hayes, R.L., Rafter, J.J., Washburn, L.C., and Byrd, B.L. Affinity of ^{253}einsteinium for tumor tissue. Nature New Biology 246(No. 149):23-25, 1973.

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OTHER SUPPORT

(Use continuation pages if necessary)

For each of the professionals named on page 2, list, in three separate groups: (1) active support; (2) applications and proposals pending review or funding; (3) applications and proposals planned or being prepared for submission. Include *all* Federal, non-Federal, and institutional grant and contract support. If none, state "none." For each item give the source of support, identifying number, project title, name of principal investigator/program director, time or percent of effort on the project by professional named, annual direct costs, and entire period of support. (If part of a larger project, provide the titles of both the parent project and the subproject and give the annual direct costs for each.) Describe the contents of each item listed. If any of these overlap, duplicate, or are being replaced or supplemented by the present application, delineate and justify the nature and extent of the scientific and budgetary overlaps or boundaries.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:

(1) ACTIVE SUPPORT:

Lee C. Washburn	90%	Salary and Benefit Support - DOE Contract No. DE-AC05-760R00033
	10%	Salary and Benefit Support - NCI Contract No. CA29490-03
James E. Crook	100%	Salary and Benefit Support - DOE Contract No. DE-AC05-760R00033
Myles C. Cabot	100%	Salary and Benefit Support - DOE Contract No. DE-AC05-760R00033

(2) Grant Application: Pending Review
"Labeled Amines as Markers of Pulmonary Injury and Repair", James E. Crook, Principal Investigator, submitted to NIH, April 27, 1984.

Grant Application: Pending Review
"Lipid Role in Phorbol Ester Binding", Myles C. Cabot, Principal Investigator, submitted to NIH, October 24, 1983.

(3) None

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RESOURCES AND ENVIRONMENT

FACILITIES: Mark the facilities to be used at the applicant organization and briefly indicate their capacities, pertinent capabilities, relative proximity and extent of availability to the project. Use "other" to describe the facilities at any other performance sites listed in Item 9, page 1, and at sites for field studies. Using continuation pages if necessary, include an explanation of any consortium arrangements with other organizations.

[XX] Laboratory: Three well-equipped, air-conditioned laboratories with a total of approximately 2,000 sq. ft. are available in the Vance Road building of Oak Ridge Associated Universities (ORAU) Medical and Health Sciences Division (M&HSD).

[ ] Clinical:

[XX] Animal: At ORAU M&HSD modern accredited animal facilities with adequate floor space to house cages for all animals in this project are located in the same building as the office and laboratories of the Principal Investigator. Shared specimen collecting and work laboratories are also located directly withi the animal facility. Adequate caging, which complies with minimum size and construction requirements as given in the DHEW Guide, is available.

[XX] Computer: ORAU has a Digital Equipment Corporation PDP 11/44 computer with 768 kilobytes of memory. The minitab data analysis software package (Minitab Project, University Park, PA) is available for statistical testing of experimental data.

[XX] Office: Each investigator has a well-equipped, air-conditioned office.

[ ] Other (_____):

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each. Laboratory Data Control (LDC) gradient high performance liquid chromatograph with LDC refractive index detector, LDC variable wavelength UV detector, Packard custom liquid-column single-channel analyzer monitor (for detecting the elution of gamma-emitting radionuclides), and Radiomatic Flo-One Model HP radioactivity detector (for beta emitters) with Model ES electronic stream-splitting device; Beckman Model LS 2800 liquid scintillation counter with external standardization by H#, DPM capability, chemiluminescence monitor, and multi-user package; Packard Model 5220 Auto-Gamma scintillation spectrometer; Searle Pho-Gamma V scintillation camera; and Ohio-Nuclear dual head rectilinear scanner are located at ORAU M&HSD.

ADDITIONAL INFORMATION: Provide any other information describing the environment for the project. Identify support services such as consultants, secretarial, machine shop, and electronics shop, and the extent to which they will be available to the project.

RESOURCES AND ENVIRONMENT

Dr. Zenon Steplewski of The Wistar Institute, who has an excellent record in monoclonal antibody research, will be a Co-Investigator on this project. Dr. Steplewski's laboratory will provide the monoclonal antibodies and monoclonal antibody fragments as well as the tumor lines which are required for this project. He will also work with ORAU investigators to set up at ORAU the radioimmunoassay procedure necessary to verify the retention of immunological activity of the antibodies after derivatization. Dr. R.L. Hayes, former head of ORAU's M&HSD preclinical radiopharmaceutical development program, will serve as a consultant on this project. There is also a wide variety of scientific expertise available both

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within ORAU and in the Oak Ridge community at large, especially the Oak Ridge National Laboratory. Additionally, the University of Tennessee is nearby and has resources available to us within the graduate, medical, and veterinary divisions. Full-time secretarial services with access to word processor will be available to the project as required, and ORAU's well-equipped machine shop and the Division's electronic shop will also be available as needed.

'USE OF DOE FACILITIES AND DOE CONTRACT REQUIREMENTS'

This research grant application includes a segment of activity that would be performed in facilities of the U. S. Department of Energy and governed by an existing contract between Oak Ridge Associated Universities (ORAU) and the DOE. The DOE has reviewed this proposal and has concurred in ORAU conducting the described work in the DOE facilities made available for biomedical research, subject to payment to the DOE by ORAU from NIH funds of the applicable direct and indirect cost of the work (not including any charge for the use of DOE facilities) as determined by the provisions of the DOE's contract with ORAU.

By way of general information, ORAU's contract with the DOE is a cost-type contract financed under a Government-fund account. The specific contract work is formulated in cooperation with the DOE and authorized within general guidelines in the contract. Ownership of all equipment and facilities acquired by ORAU with DOE funds is vested in the U. S. Government at the time of acquisition. The contract also contains all the terms generally common to Government contracts of the type under which ORAU conducts research operations in Government-owned facilities.

In particular, ORAU's contract with DOE includes standard articles on the subjects of small business, contracting with labor surplus firms, contracting with women-owned concerns, disabled veterans, employment of the handicapped, clean air and water, cost accounting standards, cost or pricing data, equal opportunity and safety and health. We believe that in these areas, the contract conditions parallel conditions which NIH ordinarily applies to its grants. In the event of differences between NIH grant terms and these DOE contract terms, ORAU is agreeable to meeting both to the extent that they are not in conflict, and to applying those most favorable to the United States Government where this is involved. If NIH is aware of problems that such an approach would produce or suggest, ORAU upon receipt of such advice would refer the matter to the DOE for direct resolution with NIH.

The contract also includes DOE standard patent rights and technical data articles for nonprofit organizations. We propose applying these or those which NIH applies to grants, whichever provides ORAU with the greater rights in these areas. We believe this approach is consistent with Public Law 96-517.

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RESEARCH PLAN

A. Specific Aims

The specific aim of this research is preclinical development and evaluation of tumor-specific ^{255}Fm- and ^{90}Y-labeled monoclonal antibodies or monoclonal antibody fragments for potential application in radioimmunotherapy of cancer. A bifunctional chelate technique involving coupling of the chelating agent, diethylenetriaminepentaacetic acid (DTPA), to monoclonal antibodies via the cyclic DTPA anhydride will be used. This will permit radiolabeling monoclonal antibodies with a variety of metal ion radionuclides. A radioimmunoassay technique will be used to verify retention of immunological activity after radiolabeling. Monoclonal antibodies labeled with In-111, Yb-169, and Y-90 will be used for preliminary scintigraphic and tissue distribution studies in nude mice bearing the appropriate tumor type for each antibody to determine the time course of tumor localization. A radiolabeled anti-influenza monoclonal antibody will be used as a control to assess the importance of nonspecific binding. Tissue distribution studies using monoclonal antibodies labeled with Fm-255, Y-90, and Es-253 (to be used as a longer-lived model for Fm-255) will then be carried out, and this data will be used for radiation dosimetry calculations. Tumor regression studies will be carried out in tumor-bearing nude mice to assess the potential of ^{255}Fm- and ^{90}Y-labeled antitumor monoclonal antibody derivatives for future clinical radioimmunotherapy trials.

B. Significance

Research on tumor localization of radiolabeled antibodies dates back some 30 years to the work of Pressman and Korngold (1), who showed that labeled antibodies against Wagner osteosarcoma cells were concentrated in

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vivo by the solid tumor. Antibodies to tumor-associated antigens have been considered as leading candidates for the long-sought "magic bullet" (2). Antibodies having a high enough specificity for tumor tissue could in theory be used, in a radiolabeled form, for diagnosis of cancer and also as carriers of radionuclides and cytotoxic drugs for cancer therapy.

The conventional production of antibodies in animals has serious limitations, however. Even when highly purified antigen preparations are used, a whole spectrum of antibodies with diverse specificities is still obtained, and separation of the desired antibody from such mixtures is very difficult. Also, considerable batch-to-batch variability in the quality of the antibody obtained is quite common. Finally, even when large animals are used, there are practical limitations to the quantity of antibody which can be produced.

The discovery of a technique for production of monoclonal antibodies by Kohler and Milstein in 1975 (3) provides a solution to these problems. Monoclonal antibodies, produced in tissue culture by lymphocyte-myeloma hybridomas (4), are homogeneous, require little effort for purification, and can be reproducibly prepared in large quantities. Since their discovery, the potential of monoclonal antibodies for a variety of clinical, basic research, and even industrial applications has been recognized (2). One of the most important of these is their possible use in diagnosis and therapy of cancer.

Table 1 lists the radionuclides which have been used for labeling monoclonal antibodies for diagnostic and therapeutic investigations. Most of these studies to date have used I-131 as a radiolabel. Studies of the diagnostic (5-24) and therapeutic (23-26) potential of ^{131}I-labeled

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TABLE 1
RADIONUCLIDES USED AS LABELS FOR MONOCLONAL ANTIBODIES

Radionuclide	Half-Life	Decay Mode(s)	Energy of Principal Photon(s) (KeV)	References
Iodine-131	8.05 d	β^-	364	5-26
Iodine-123	13 h	EC	160	27,28
Iodine-125	60 d	EC	35	19-22,29-31
Technetium-99m	6.0 h	IT	140	36,51
Indium-111	2.81 d	EC	173, 247	33,43-51
Gallium-68	68 m	β^+, EC	510	51
Bismuth-212	60.6 m	β^-, α	None	52

monoclonal antibodies have been carried out in both animals and man. Short-lived I-123 has been used as a label for imaging studies in animals (27,28) and man (28), and I-125, although unsuitable for imaging, has been used as a radiotracer in animal studies (19,22,29-31). Subtraction of background activity (32) has been used in many of these investigations to enhance tumor imaging (6,11,14-16,18,22). Another way of improving the tumor images obtained with radiolabeled monoclonal antibodies involves use of single photon computerized emission tomography (SPECT) (13,22). For ^{131}I-labeled monoclonal antibody against carcinoembryonic antigen, SPECT was shown to be clearly superior to rectilinear scanning, permitting correct visualization of 16 out of 17 tumor sites studied (13). One can often actually increase the tumor-to-nontumor ratios by enzymatically removing non-binding portions of the antibody molecule before radiolabeling is carried out. This results in labeled fragments that maintain their specificity but eliminate non-specific binding (9,16-20,22,23,30,33).

Studies using radioiodinated derivatives clearly show the potential of radiolabeled monoclonal antibodies for cancer diagnosis and therapy. A major drawback to using radionuclides of iodine as labels for monoclonal antibodies is, however, the problem of in vivo deiodination, which obscures the images by producing a high general background as well as abnormally

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high concentrations in the stomach, bladder, and kidney (34,35). In addition, I-131 is far from ideal for both diagnostic and therapeutic applications because of its long half-life and medium energy gamma emission which results in high radiation doses to normal tissues.

Kits have been developed for labeling $F(ab')_2$ fragments of monoclonal antibodies with Tc-99m (36). Although promising results were obtained in animals, the initial clinical results were disappointing (36).

The bifunctional chelate technique involves covalent attachment of a chelating group to proteins, permitting strong binding of polyvalent metal ions. This concept was first introduced by Sundberg and co-workers (37), who used a diazonium coupling reaction to attach a phenylethylenediamine-tetraacetic acid (phenyl-EDTA) moiety, permitting labeling of proteins with In-111. Krejcarek and Tucker (38) used a mixed carboxycarbonic anhydride to attach diethylenetriaminepentaacetic acid (DTPA) to proteins via an amide linkage. Yokoyama and co-workers (39) used glutaraldehyde as a coupling agent to link desferrioxamine, an excellent chelating agent for radionuclides of gallium and indium, to proteins. Perhaps the most attractive method, however, involves use of a cyclic anhydride to couple DTPA to proteins, again via an amide linkage. The cyclic DTPA anhydride method was first developed by Eckelman and co-workers (40) to make chelating derivatives of fatty acids and was adapted by Khatowich and co-workers (41,42) to radiolabeling of proteins. Several papers have recently been published describing methods for radiolabeling monoclonal antibodies with indium-111 via the cyclic DTPA anhydride (33,43-47). Advantages of the cyclic DTPA anhydride procedure are the simplicity of the syntheses, mild coupling conditions, and easy purification of the protein after coupling.

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Paik and co-workers (45,46) have investigated the effect of pH on DTPA conjugation to antibodies via the cyclic DTPA anhydride technique. Out of four buffer solutions studied for the coupling reaction, 0.1 M bicarbonate buffer at pH 8.2 gave the optimal results. With anticolorectal carcinoma monoclonal antibody, a cyclic DTPA anhydride to antibody molar concentration ratio of 50:1 allowed chelation of 1.3 indium atoms per molecule of antibody with only 7% deactivation of the antibody. Higher concentration ratios gave disproportionately greater antibody deactivation.

The bifunctional chelate technique has been investigated for labeling monoclonal antibodies with metal ions. Hnatowich and co-workers found that ^{111}In-labeled monoclonal antibody to carcinoembryonic antigen labeled by the cyclic DTPA anhydride approach had a higher tumor uptake than the same antibody labeled with radioiodine (44). Likewise Wang and co-workers (47) found better binding of ^{111}In-labeled monoclonal antibody against high-molecular weight melanoma-associated antigen than ^{125}I-labeled antibody when incubated in vitro with melanoma cells. However, Rowe and co-workers (33) found that ^{111}In-labeled $F(ab')_2$ fragments of monoclonal antibodies to human colorectal carcinoma and melanoma-associated antigens were inferior to the analogous ^{131}I-labeled fragments for imaging tumor xenografts in nude mice, possibly because of increased catabolism of the ^{131}I-labeled antibody. Chelate-conjugated monoclonal antibodies have also been labeled with In-111 by the mixed anhydride technique (29,48-50). A DTPA-conjugate of Fab fragments of antibody to canine cardiac myosin was chelated with In-111 and used to image myocardial infarctions in dogs (51). Preliminary results with this same antibody conjugate were also reported using Tc-99m and Ga-68 as radiolabels (51).

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Bismuth-212 has also been used to label monoclonal antibodies through a bifunctional chelate derivative, and a study of the therapeutic efficacy of these agents is in progress (52). However, the short half-life (60.6 min) may not be adequate for proper blood clearance.

As seen from the above description of previous research with radiolabeled antitumor monoclonal antibodies, the bulk of this has emphasized the diagnostic rather than the therapeutic potential of these agents. This proposed research involves labeling these highly specific monoclonal antibodies or monoclonal antibody fragments with the alpha emitter Fm-255 and the beta emitter yttrium-90 for studies of radiotherapeutic efficacy. Alpha emitters are ideal for therapy because their entire radiation energy is confined to a very small range (53). Fermium-255 appears to be an excellent radionuclide for therapy because it decays by alpha emission with a half-life (20.1 h) which is long enough to allow good tumor uptake and blood clearance, yet short enough to prevent an excessive radiation dose. It decays to Cf-251, which is so long-lived ($T_{1/2}$ = 900 y) that its contribution to the radiation dose would be negligible. The pure beta emitter Y-90 ($T_{1/2}$ = 64.2 h) will also be evaluated as a label for monoclonal antibodies for radioimmunotherapy. Monoclonal antibodies labeled with Fm-255 or Y-90 may be very promising new agents for cancer therapy.

C. Preliminary Studies

As was pointed out in section B., of the methods that have been developed to date for the covalent coupling of polyaminocarboxylates to proteins, such as monoclonal antibodies, the cyclic DTPA anhydride technique appears to be the method of choice for labeling antibodies with polyvalent cationic radionuclides. It is rapid, proceeds at physiologic

pH, and yields chelating products that retain their immunological activity (29).

In preliminary studies directed toward establishing a general technique for labeling monoclonal antibodies, we have used a modification of the methods of Wnatowich et. al. (44) and Paik et. al. (45) for coupling DTPA to antibodies. The coupling procedure for a 1:1 ratio of cyclic DTPA anhydride to monoclonal antibody is as follows: Ten µl of a 1.5 mg/ml solution of the cyclic DTPA anhydride in chloroform was evaporated to dryness under nitrogen gas in a glass tube. Purified colorectal monoclonal antibody 17-1A IgG2a (5.8 mg) in 100 µl of 0.05 M $NaHCO_3$/1M NaCl buffer was added to the dry anhydride and the solution agitated for 30 minutes at room temperature.

Ten µl of this stock solution of DTPA-coupled monoclonal antibody was combined with 10 µl of In-111 in 0.5 M acetate buffer (pH 6). After standing for 30 min at room temperature, 5 ml of 0.01 M citrate buffer was added and the mixture centrifuged in an ultrafiltration membrane cone (Amicon Corporation, Danvers, MA). From the percentages of radioactivity associated with the filtrate and residue fractions, it was found that the coupling efficiency was 63% (an average of 0.63 DTPA group per antibody molecule.

The ^{111}In-labeled DTPA-coupled antibody was separated from ^{111}In-DTPA using a Sephadex G50 gel filtration column (1.5 x 9 cm) eluted with 0.02 M Na_2HPO_4/0.5 M NaCl buffer (pH 7). Elution of radioactivity from the column was monitored using a custom liquid-column single-channel analyzer monitor system (Packard Instrument Company, Downers Grove, IL). At a flow rate of 0.2 ml/min, the retention time of the ^{111}In-labeled DTPA-coupled antibody was 20-25 min and that of ^{111}In-DTPA was 50-60 min

(Fig. 1). We have also found that the separation can be carried out prior to labeling, if desired, by using UV absorption at 280 nm to monitor elution of the DTPA-coupled antibody.

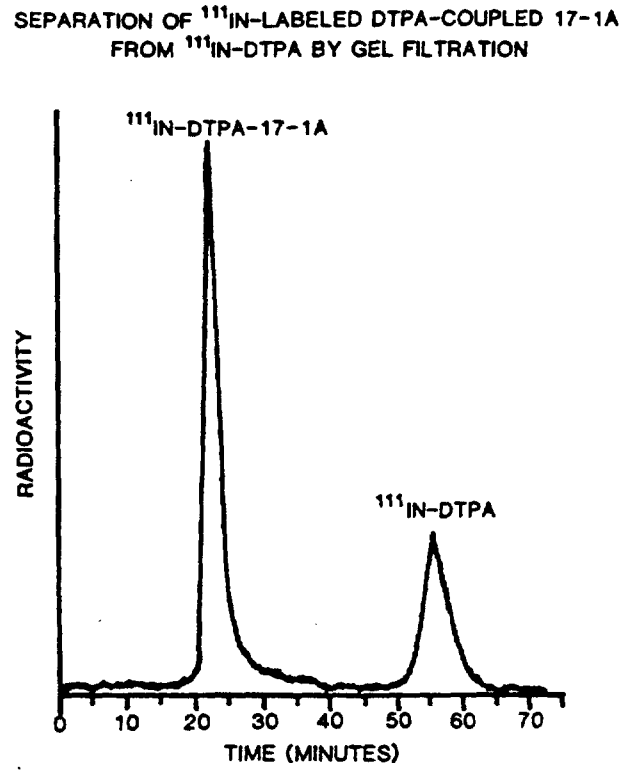

Fig. 1. Separation of ^{111}In-labeled DTPA-coupled 17-1A from ^{111}In-DTPA by gel filtration.

Five preparations were sent to Dr. Z. Steplewski of The Wistar Institute for radioimmunological testing (see method in Section D.4.) to determine the retention of immunological activity of the derivatized antibodies: (1) Purified colorectal monoclonal antibody 17-1A IgG2a (as a standard); (2) a solution of 17-1A which was lyophilized and resuspended in water to a concentration of 5.9 mg/100 µl (because the original 17-1A solution was too dilute for the coupling reaction); (3) DTPA-coupled 17-1A prepared from the solution in (2) as described above (1:1 ratio of anhydride to antibody) and purified by gel filtration; (4) DTPA-coupled 17-1A which was complexed with a molar amount of stable In^{+3} equivalent to

the DTPA present (to completely saturate the chelating sites) and then purified by gel filtration; (5) DTPA-coupled 17-1A prepared from the solution in (2) as described above except that the anhydride-to-antibody concentration ratio was 20:1 (which gave an average of 4.5 DTPA groups per antibody molecule). The immune reactivity of samples 2-5 were unchanged relative to the purified 17-1A preparation (sample 1), when tested using colon carcinoma cell line SW1116, which expresses about 5 x 10^5 binding sites per cell for this antibody (Fig. 2). They were also tested using

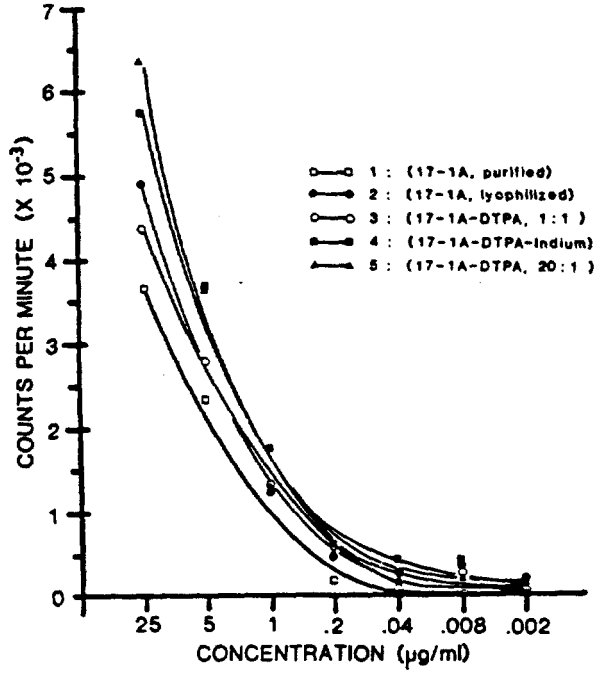

Fig. 2. Binding of antibody 17-1A and its conjugates to colon carcinoma cell line SW 1116.

human melanoma cell line FT-8, which should not bind the antibody (Fig. 3). There was some apparent binding of the conjugates at higher concentrations, but this was probably due to aggregation rather than specific binding.

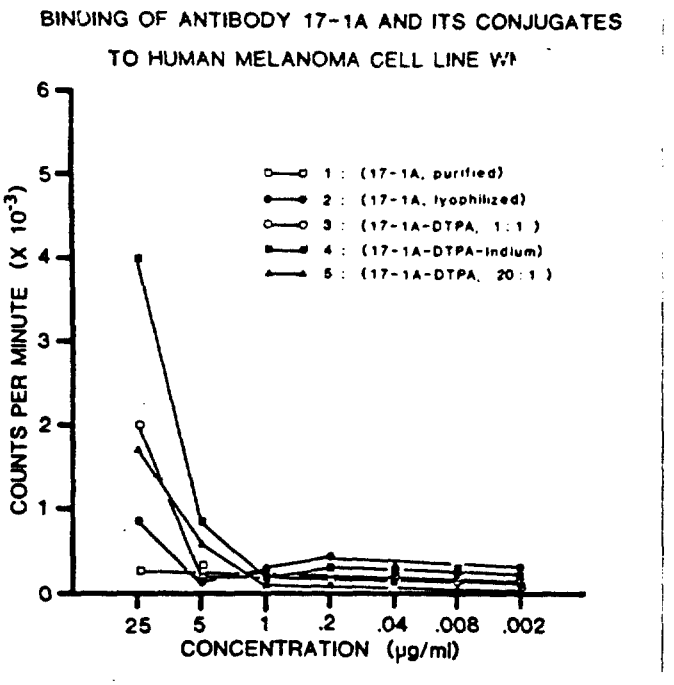

Fig. 4. Binding of antibody 17-1A and its conjugates to human melanoma cell line WM-9.

We feel that the above approach to chelate coupling, labeling, and purification should be quite generally effective for preparation of monoclonal antibodies labeled with polyvalent cationic radionuclides such as the alpha emitter, fermium-225, and the beta emitter, yttrium-90.

D. Experimental Design and Methods

1. Radionuclides to be used as labels

The radionuclides that we propose to study as labels for monoclonal antibodies are listed in Table 2. The advantages of alpha-emitting radionuclides for internal radiotherapy have already been discussed in Section B. The properties of Fm-255 ($T_{1/2}$ = 20.1 h) make it nearly ideal for use in radioimmunotherapy. This radionuclide is unique in that it is available from parent Es-255 ($T_{1/2}$ = 39 d) and in turn decays solely by alpha emission to Cf-251 ($T_{1/2}$ = ~900 y). Tracer quantities of Fm-255 and Es-253 ($T_{1/2}$ = 20.5 d) (to be used as a longer-lived model for Fm-255) can be obtained at intervals from the Transuranium Processing Plant at Oak Ridge National Laboratory (see letter from Dr. J. F. Tigelow in Appendix).

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TABLE 2

RADIONUCLIDES PROPOSED AS LABELS FOR MONOCLONAL ANTIBODIES

Radionuclide	Half-Life	Decay Mode(s)	Proposed Application
Fermium-255	20.1 h	α	Radioimmunotherapy
Yttrium-90	64.0 h	β-	Radioimmunotherapy
Einsteinium-253	20.5 d	α	Model for Fm-255
Yttrium-88	106.6 d	EC	Model for Y-90
Indium-111	2.8 d	EC	Scintigraphic Studies
Ytterbium-169	31.0 d	EC	Scintigraphic Studies

However, the supply of Fm-255 in this country at the present time is limited to that available from the Oak Ridge National Laboratory. Although it is not as suitable as Fm-255 for internal radiotherapy, the pure beta emitter Y-90 ($T_{1/2}$ = 64 h) is readily available from its parent Sr-90 ($T_{1/2}$ = 28 y), and it therefore could act as a substitute for Fm-255. For this reason, together with possible unexpected difficulties with the use of Fm-255, we will also develop monoclonal antibody derivatives labeled with Y-90. Longer-lived, accelerator-produced Y-88 ($T_{1/2}$ = 106.6 d) will be used as a model for Y-90.

Initial scintigraphic and tissue distribution studies for each monoclonal antibody derivative will be carried out with gamma-emitting radionuclides of intermediate half-life, such as commercially available Yb-169 ($T_{1/2}$ = 31 d) and Y-88 ($T_{1/2}$ = 106.6 d) in order to study the tumor uptake and background clearance of the agent over a period of a few hours to several days after administration. Indium-111 ($T_{1/2}$ = 2.8 d) will also be used as an antibody label in order to facilitate comparison of the results obtained in this study with other published work on ^{111}In-labeled DTPA-coupled monoclonal antibodies. These radionuclides all have high stability constants with both DTPA and EDTA (see Table 3, ref. 54), and therefore polyaminocarboxylate ligands coupled to monoclonal antibodies

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should bind them very strongly. The metal ion used would not be expected to have a significant effect on the biodistribution of a particular antibody derivative. Tissue distributions that vary with isotopic label could be explained only by major differences in their stability constants or by the influence of in vivo exchange of the isotope with plasma proteins.

TABLE 3

STABILITY CONSTANTS FOR DTPA AND EDTA WITH METAL IONS PROPOSED AS RADIOLABELS

Metal Ion	Log of Equilibrium Constant (K_1) DTPA	EDTA
Fm^{+3}	22.7	na
Y^{+3}	22.1	18.1
Yb^{+3}	22.7	19.5
Es^{+3}	22.6	na
In^{+3}	28.4	24.9

Einsteinium-253, an alpha-emitting radionuclide of intermediate half-life that is chemically very similar to Fm-255, will also be used as a label for monoclonal antibodies for tissue distribution studies. Assay by liquid scintillation counting techniques will be required for Es-253, as described in our previous study of Es-253 citrate uptake by tumors (55).

If good potential is seen in these preliminary investigations, the antibody derivative will then be labeled with Fm-255 and Y-90, the nuclides of interest for radioimmunotherapy, for tumor regression and radiation dosimetry studies.

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2. Methods for radiolabeling monoclonal antibodies

Covalent coupling of the chelating agent DTPA to monoclonal antibodies and monoclonal antibody fragments will be accomplished using the cyclic anhydride of DTPA (44,45). This will permit labeling these antibody derivatives with selected metal ion radionuclides for investigation of their radioimmunotherapeutic potential. The cyclic anhydride technique, illustrated in Fig. 4, appears to be the method of choice for the reasons

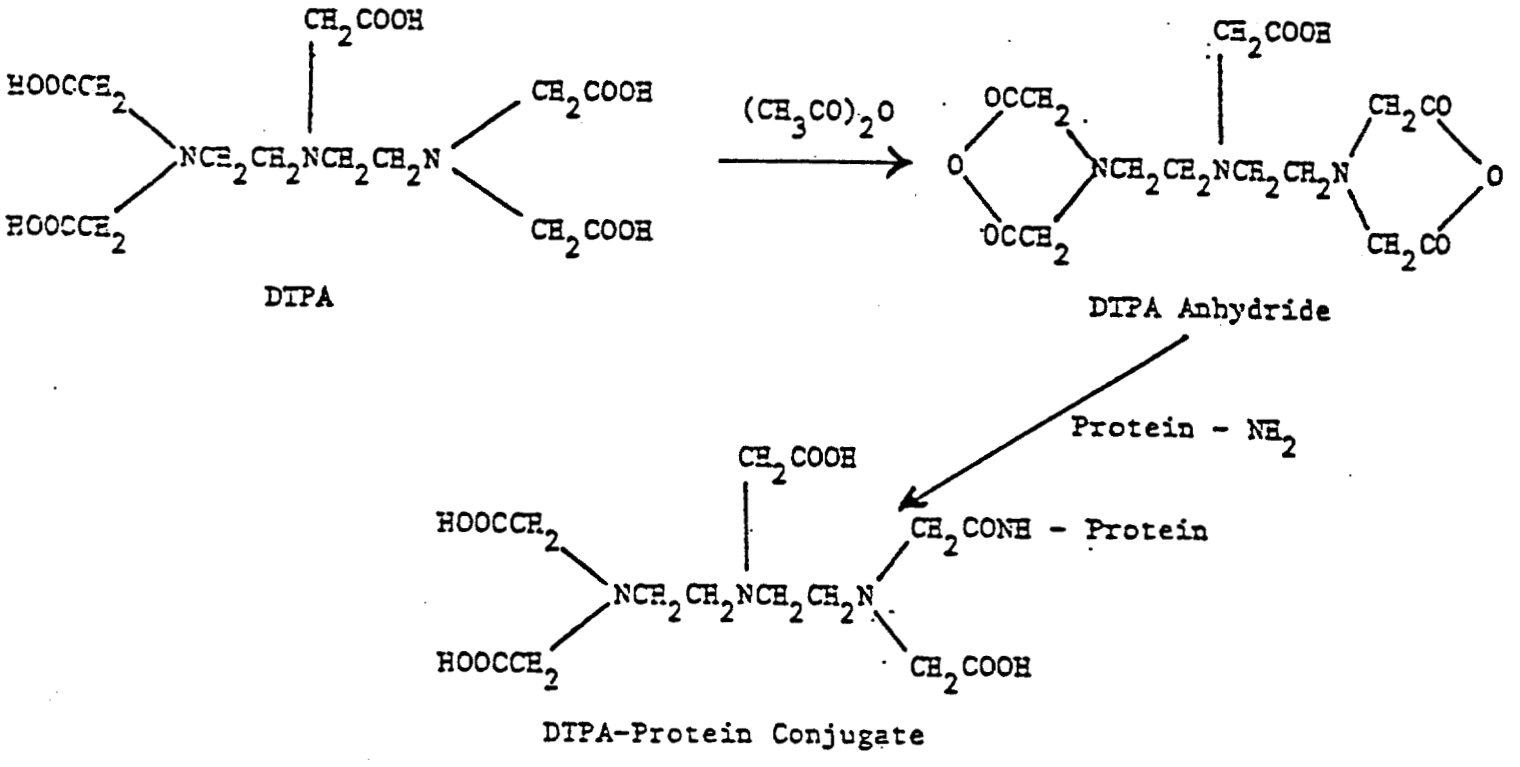

Fig. 4. Covalent attachment of DTPA to proteins via the cyclic DTPA anhydride.

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discussed in Section C. Hnatowich and co-workers (56-57) have found that DTPA forms stable chelates despite the utilization of one carbonyl group in the formation of the amide bond. Since four carbonyl groups still remain, attachment of DTPA onto a protein would produce a net negative charge, even after complexation with a trivalent metal ion. However, this apparently does not affect the biological activity of an antibody or other protein. For instance, fibrinogen has been modified in this way with no loss of activity in the clotting process or change in biodistribution (42), and a monoclonal antibody to colorectal carcinoma has been conjugated with 1.3 DTPA groups per antibody molecule with only 7% deactivation (45). In our preliminary studies (see Section C.), colorectal monoclonal antibody 17-14 was conjugated with up to 4.5 DTPA groups per antibody molecule with no loss of immunological activity.

The experimental conditions described in Section C. for synthesis and purification of the DTPA-coupled monoclonal antibodies are expected to be quite general for the monoclonal antibodies and monoclonal antibody fragments to be investigated. Radiolabeling will be accomplished by ligand exchange, by adding a buffered solution of the appropriate radioactive metal ion to the reaction solution either before or after purification from free DTPA. If necessary, a weak chelate such as citrate will be added to avoid cation hydrolysis.

Since Hnatowich has reported great variability in the coupling efficiency [as much as 70% for albumin (41) to as little as 13% for fibrinogen (42)], we will determine the coupling efficiency at various anhydride-to-antibody molar ratios for each monoclonal antibody to be studied. This will allow us to determine an optimal anhydride-to-antibody molar ratio which will give adequate radionuclide binding and maximum

retention of immunological activity. The molar ratio used for a particular monoclonal antibody will depend upon the coupling efficiency for that antibody and the specific activity of the radionuclide to be used as a label. The number of DTPA groups per antibody molecule will, of course, be kept as small as possible in order to minimize perturbations in the structure and immunological activity of the DTPA-coupled monoclonal antibodies.

If for any reason the cyclic DTPA anhydride technique should be unsuccessful, we will couple DTPA to monoclonal antibodies and monoclonal antibody fragments using the mixed carbomycarbonic anhydride technique developed by Krejcarek and co-workers (33) and modified by Paik and co-workers (59) to work on the micro scale necessary for labeling antibodies.

3. Monoclonal Antibodies to be Radiolabeled

Colon carcinoma is the second leading cause of cancer deaths in the United States (59). Demographic data shows that the age group primarily affected by carcinoma of the colon is increasing in size. Thus one major area of focus of our investigation of radiolabeled monoclonal antibodies for cancer therapy will be on colon carcinoma.

Radiolabeling of two monoclonal antibodies that have exhibited great specificity for the gastrointestinal carcinomas will be performed. The individual characteristics of these two are described in Table 4(59). The monoclonal antibodies and monoclonal antibody fragments which we propose to study will be obtained from The Wistar Institute in Philadelphia, Pennsylvania. Researchers at this institute, including Dr. Zenon Steplewski, a Co-investigator on this project, have been at the very forefront of monoclonal antibody research almost from the beginning of this work (see Dr. Steplewski's Biographical Sketch).

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The Wistar Institute has available a panel of tumor cell lines which are able to form tumors in nu/nu mice. These include:

Colon carcinoma	11 cell lines
Gastric carcinoma	1 cell lines
Pancreatic carcinoma	3 cell lines
Melanoma	20 cell lines
Astrocytoma	5 cell lines
Lung adenocarcinoma	3 cell lines
Breast adenocarcinoma	5 cell lines

Our investigations with anticolorectal carcinoma monoclonal antibodies will begin with 1083-17-1A (17-1A). Utilizing radioimmunoassay to detect binding of mouse monoclonal antibody to live cells by using rabbit anti-mouse $F(ab')_2$ radioiodinated IgG, 17-1A was found (61) to bind selectively to all nine colorectal carcinoma cell lines studied. However, three lung carcinomas, ten melanomas, two astrocytomas, one breast carcinoma, and a panel of other tumor and normal cell lines did not express the antigen against which the antibody is directed. Thus, specificity of this monoclonal antibody for colorectal carcinoma was demonstrated.

The monoclonal antibody 17-1A has been studied fairly extensively. Labeling of the monoclonal antibody 17-1A with I-131 has been accomplished (14). Although I-131 is not a good choice of a radiolabel because of in vivo deiodination, uptake of the labeled antibody by human colorectal carcinomas was observed.

The second gastrointestinal monoclonal antibody to be used is 1116-NS-19-9. Unlike the 17-1A antibody, it belongs to the IgG1 isotype. Studies in a immunoperoxidase system have shown binding of the antibody to gastric,

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pancreatic and colorectal carcinomas (62). The finding of a high specific binding to pancreatic carcinoma tissue is of great interest. Our group has had extensive experience in the detection of pancreatic tumors utilizing positron-emitting radiopharmaceuticals such as ^{11}C-ACPC and the resolved stereoisomers of ^{11}C-valine and tryptophan in conjunction with the ECAT positron tomographic imaging system. Although the frequency of occurrence of pancreatic adenocarcinoma is, fortunately, much less than colon and rectal carcinomas, it is a devastating malignancy in terms of five-year survival rates (1-3%), which have shown little improvement over the past years. This figure reflects the difficulty in both early detection and efficacy of therapy once detected.

As seen in Table 4, one of the two colorectal antibodies chosen for studies is tumoricidal - a trait which is more generally associated with antibodies of the IgG2a isotype. This same monoclonal antibody has undergone Phase I trials in four patients with gastric carcinoma (63). It appeared generally well tolerated but as always with the injection of foreign protein, the question

TABLE 4

CHARACTERISTICS OF THE MONOCLONAL ANTIBODIES TO BE STUDIED

MONOCLONAL ANTIBODY	19-9	17-1A
Isotype	IgG1	IgG2a
CDC	no	no
ADCC	yes	yes
Tumor inhibition	no	yes
Antigen:	sialylated lacto-N	protein
Spent Medium	yes	no
Serum	yes	no
Immunoperoxidase Assay:		
Frozen Section	yes	yes
Paraffin embedded, fixed tissues	yes	no

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of serum sickness remains. Utilization of radiolabeled monoclonal antibodies may be able to surmount the question of serum sickness by being able to use smaller quantities of proteins.

The third and final monoclonal antibody to be studied is the anti-melanoma antibody 691-19-19, isotype IgG1. This antibody is directed against human cutaneous malignant melanoma. It reacts with the majority of melanoma cells tested and minimally with astrocytomas. The radioimmunoassay results against all other tumors, including colorectal carcinoma, and normal cells were clearly negative (64).

When a radiolabeled monoclonal antibody is found which has the desired tumor specificity, Fab and $F(ab')_2$ fragments of that antibody, isolated by enzymatic cleavage with papain and pepsin, respectively, will be tested to determine whether the rate of blood clearance of the agent is increased without loss of tumor specificity.

4. <u>In vitro testing of radiolabeled antibodies</u>

The purified chelate-conjugated monoclonal antibodies and monclonal antibody fragments will be subjected to an indirect radioimmunoassay (RIA) procedure to determine the specific binding of the antibody preparations to target cells (33,45). Dr. Z. Steplewski of The Wistar Insitutue in Philadelphia, a Co-Investigator on this application, has agreed to spend two weeks in Oak Ridge assisting ORNL investigators in setting up the RIA method.

In the RIA procedure, varying concentrations of the antibody or antibody fragment preparation to be tested are incubated in microtiter

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plates for 1 hour at room temperature with 2.5×10^5 tumor cells of the appropriate cell line in 100 μl of phosphate-buffered saline containing 5% agammaglobulinemic horse serum and 0.02% sodium azide. The cells are then washed three times with ice cold buffer to remove unbound antibody, and a second antibody against mouse immunoglobulin $F(ab')_2$ labeled with I-125 (50,000 cpm in 50 μl) is added to each well. After incubation for 1 hour at room temperature, the cells are washed three times and radioactivity associated with the cell pellet is determined. If the activity of I-125 bound to the cells is plotted against the logarithm of the antibody concentration, the fractional antibody binding activity can be determined by dividing the concentration of the control antibody giving 50% bound radioactivity by the concentration of the conjugated antibody giving the same percentage I-125 bound activity. This fractional antibody binding activity will provide a good measure of the immunological activity of the derivatized antibody.

Initially the RIA procedure will be carried out on the purified conjugates both before and after radiolabeling. However, our preliminary studies (see Section C.) have indicated that the presence of the metal ion does not affect the relative immunoreactivity of the antibody preparation. If this continues to hold true, we will carry out the RIA procedure only on the final radiolabeled products.

The relative immunoreactivity of the radiolabeled DTPA-conjugated antibody preparations will be assessed by a direct RIA procedure in which 2.5×10^5 target cells are incubated with varying quantities of radiolabeled DTPA-conjugated antibody in a total volume of 100 μl. After 1 hour at room temperature and three washes with cold buffer, the radioactivity associated with the cell pellet is counted, and the fraction of added

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activity bound is determined for each dilution and compared with the equivalent fractions of ^{131}I-labeled antibody preparation.

5. Tumor localization studies

Preliminary in vivo testing will consist of studies to assess the localization of radiolabeled antitumor monoclonal antibodies in transplanted tumors by scintigraphy and subsequent radioisotopic tissue distribution analysis. Solid tumors of the appropriate type for each antibody will be produced by inoculation of nude mice with 10^7 cells of the appropriate tumor type (maintained in tissue culture at ORAU). For each radiolabeled antitumor monoclonal antibody tested, control studies to assess the importance of nonspecific binding will be done using anti-influenza monoclonal antibody labeled with the same radionuclide by the same procedure. The radiolabeled antibodies will be dissolved in Hepes-buffered normal saline containing 2% bovine serum albumin and injected into the mice via the tail vein. Tumor-bearing nude mice will be used for these studies when the tumors are 5-10 mm in diameter.

Serial scintigraphic images of the ^{111}In-, ^{169}Yb-, and ^{88}Y-labeled monoclonal antibodies will be obtained using our Searle Pho-Gamma V gamma camera. The serial image data will provide the basis for choosing an appropriate sacrifice time for further tissue distribution studies with that particular antibody derivative. Gallium-67 citrate will be studied analogously in each tumor type to provide a basis for comparing the tumor uptake of the radiolabeled monoclonal antibodies with that of this widely-used tumor-imaging agent. At the end of this study the animals will be sacrificed, and the tissue distribution of the radiolabel will be measured by standard gamma counting techniques.

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The monoclonal antibody derivatives will then be radiolabeled with Fm-255, Es-253, and Y-90, and tissue distribution studies will be carried out at the optimal sacrifice time found in the studies with gamma-emitting radionuclides. For these radionuclides, alpha (Fm-255 and Es-253) and beta (Y-90) counting techniques utilizing liquid scintillation counting must be used. Radiolabeled anti-influenza antibody will be studied as a control, as described above. Each experimental group will consist of five animals. The tissue distribution data obtained will be used in calculations of the radiation dosimetry of these radiolabeled antitumor monoclonal antibodies (see Section D.7.).

6. Tumor Regression Studies

It has been shown by other investigators that hybridoma-derived anticolorectal carcinoma monoclonal antibodies suppress the growth of adenocarcinomas (65). The studies were carried out in nude mice. The parameters measured are the ones which we intend to follow as evidence of efficacy of the radiolabeled monoclonal antibodies -- tumor volume, time for appearance of tumor, and incidence of tumor.

Thymus-deficient 6- to 8-week-old nude mice (nu/nu, BALB/C background) will be used as experimental animals. Tumor cells, approximately 10^7, in appropriate media will be inoculated under the skin of the neck and injected intraperitoneally with monoclonal antibodies labeled with Fm-255 and Y-90. Control groups will be inoculated but will not receive the radiolabeled antibody. Tumor sizes will be monitored weekly by measuring the three major diameters using a caliper. The efficacy of the radiolabeled monoclonal antibody will be determined also by changes in the latency period between the control group (no antibody) and the group receiving the radiolabeled antibody. In addition, the lower incidence of

tumors will be measured by comparing the number of tumors developed in the two groups. The statistical significance of differences between groups will be determined using the Student's t test.

7. Radiation dosimetry estimates

Radiation dosimetry is an essential element in planning a treatment protocol for radiolabeled antibodies so that the physician can determine how much activity is required to achieve the desired effect. Although the radiation dose for an individual patient cannot be accurately calculated from the results of animal studies, initial estimates can be made that will help in analyzing the usefulness of the proposed antibody and in comparing the effectiveness of the different radionuclidic labels.

The staff of the DOE-FDA Radiopharmaceutical Internal Dose Information Center will participate in this study by advising on data that should be collected to improve the dose estimates and by calculating radiation doses for different radionuclidic labels and different types and sizes of tumors, as well as doses in normal tissues from activity in the tumors.

Because traditional methods of expressing radiation doses such as the MIRD technique are not adequate for characterizing the energy deposition in tissue at the microscopic level, another method must be utilized. The National Cancer Institute (NCI) is funding a three-year program at Memorial Sloan-Kettering Cancer Center in New York to develop methods for calculating the dose from radioactively labeled antibodies. Rodney Sigler, principal investigator for the NCI program, has agreed to serve as an unpaid consultant on this proposed project (see letter in Appendix) to eliminate any duplication of effort and to ensure that the methods used for expressing energy deposition are in harmony with those developed in the NCI program. This is extremely important in the comparison of different agents

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and protocols. Dr. Bigler has also agreed to make measurements of the fermium-255 and yttrium-90 in the anthropomorphic phantom being developed as part of the MCI program. This will verify calculations of the radiation doses to normal tissues around the target tissues.

D. Projected Clinical Trials

Although no funds are requested in this proposal for clinical studies, it is obvious that any radiolabeled antibodies and/or antibody fragments found in these preclinical studies to have sufficient potential will be submitted to clinical evaluation. It is expected that eventual clinical testing of the ^{255}Fm- and/or ^{90}Y-labeled monoclonal antibodies for radioimmunotherapy of cancer will be carried out at the American Oncological Hospital of the Fox Chase Cancer Institute in Philadelphia, PA.

E. Human Subjects, Derived Materials or Data

Does not apply.

F. Vertebrate Animals

Nude mice bearing transplanted tumors of the appropriate type for each radiolabeled monoclonal antibody to be tested will be used for the biodistribution experiments. Approximately 200 of these animals per year are expected to be required. Sacrifice of these animals will be necessary in order to accomplish the objectives of this research. Procedures to be used for animal care are in full accordance with the DHEW Guide as indicated in the Assurance Statement.

G. Consultants

A letter from Dr. R.L. Hayes, the retired Head of Preclinical Radiopharmaceutical Development for ORAU's Medical and Health Sciences

Division, who will be a consultant on this project, is attached in the Appendix.

H. Consortium Arrangements or Formalized Collaborative Agreements

The major collaboration involved in this project is between ORAU and The Wistar Institute (see attached letter from Dr. Zenon Steplewski, a Co-Investigator on this project). This collaborator will provide the monoclonal antibodies and monoclonal antibody fragments, as well as the tumor lines which are required for this project. He will also work with ORAU investigators to set up at ORAU the radioimmunoassay procedure necessary to verify the retention of antibody specificity after radiolabeling. The only costs to the program will be for Dr. Steplewski to make three visits per year to Oak Ridge.

In addition, letters from Dr. John Bigelow of Oak Ridge National Laboratory and Dr. Rodney Bigler of Memorial Sloan-Kettering Cancer Center (New York) are also attached. Dr. Bigelow has agreed to provide the fermium-255 and einsteinium-253 needed for this project, and Dr. Bigler has agreed to grant us access to the model he is developing for the National Cancer Institute for estimating the absorbed radiation dose from radiolabeled monoclonal antibodies.

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CHECKLIST

This is the required last page of the application.

(Check the appropriate boxes and provide the information requested.)

TYPE OF APPLICATION

[XX] NEW application *(This application is being submitted to the PHS for the first time.)*

[ ] COMPETING CONTINUATION of grant number: _____.
(This application is to extend a funded grant beyond its current project period.)

[ ] SUPPLEMENT to grant number: _____.
(This application is for additional funds to supplement a currently funded grant.)

[ ] REVISION of application number: _____.
(This application replaces a prior unfunded version of a new, competing continuation or supplemental application.)

[ ] Change of Principal Investigator/Program Director.
Name of former Principal Investigator/Program Director: _____.

ASSURANCES *(See GENERAL INFORMATION section of instructions.)*

a. Civil Rights	b. Handicapped Individuals	c. Sex Discrimination	d. Vertebrate Animals *(If applicable)*	e. Human Subjects *(If applicable)*
[X] Filed	[X] Filed	[X] Filed	[X] Filed	[X] Filed
[ ] Not filed	[ ] Not filed	[ ] Not filed	[ ] Not filed	[ ] Not filed

INDIRECT COSTS

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office. If the applicant organization is in the process of initially developing or renegotiating a rate, or has established a rate with another Federal agency, it should, immediately upon notification that an award will be made, develop a tentative indirect cost rate proposal based on its most recently completed fiscal year in accordance with the principles set forth in the pertinent *DHHS Guide for Establishing Indirect Cost Rates*, and submit it to the appropriate DHHS Regional Office. Indirect costs will not be paid on foreign grants, construction grants, and grants to individuals, and usually not on grants in support of conferences.

[X] DHHS Agreement Dated: October 1,1983 (Provisional)

_____ % Salary and Wages *or* 51.9 % Total Direct Costs.

Is this an off-site or other special rate, or is more than one rate involved? [X] NO [ ] YES
(If "YES," explain and provide the basis for the indirect cost calculation.)

[ ] DHHS Agreement being negotiated with _____ Regional Office.

[ ] No DHHS Agreement, but rate established with _____ Date _____

[ ] No Indirect Costs Requested.

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HILARY KOPROWSKI, M.D.
DIRECTOR

PHONE: (215) 898-3700
CABLE ADDRESS WISTARINST

May 4, 1984

Dr. Lee C. Washburn
Radiopharmaceutical Development
Oak Ridge Associated Universities
Post Office Box 117
Oak Ridge, Tennessee 37830

Dear Dr. Washburn:

This is to confirm my willingness and enthusiasm for our collaboration in radiolabeling of monoclonal antibodies. I will be happy to serve as a Co-Investigator with 5% effort, no salary. We will continuously supply you with purified monoclonal antibodies and I will participate in all experiments, as designed in the grant proposal.

Very truly yours,

Z Steplewski

Zenon Steplewski, MD, D.Sc.

ZS:ets

May 4, 1984

Dr. Lee C. Washburn
Radiopharmaceutical Development
Medical and Health Sciences Division
Oak Ridge Associated Universities
P.O. Box 117
Oak Ridge, TN 37831

Dear Lee:

I am, as you are aware, very much interested in the progress of the work with chelating derivatives of tumor-specific monoclonal antibodies for possible use in radiotherapy of cancer. Should your grant proposal entitled "Radiolabeled Monoclonal Antibodies for Cancer Therapy" be funded, I would consequently be delighted to act in a consultant capacity to the project. My best wishes.

Sincerely yours,

Ray Hayes

R. L. Hayes, Ph.D.

RLH:bb

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OAK RIDGE NATIONAL LABORATORY
OPERATED BY MARTIN MARIETTA ENERGY SYSTEMS, INC.

POST OFFICE BOX X
OAK RIDGE, TENNESSEE 37831

May 8, 1984

Dr. Lee C. Washburn
Radiopharmaceutical Development
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, TN 37831

Dear Dr. Washburn:

I would be pleased to work with you on the development of radiolabeled monoclonal antibodies for cancer therapy. The isotopes to which you referred, ^{253}Es and ^{255}Fm, are produced at ORNL along with other isotopes of heavy elements under the National Transplutonium Element Production Program administered by the Office of Energy Research of the U.S. Department of Energy. These nuclides become available one or two times a year when groups of targets irradiated in the High Flux Isotope Reactor are processed at the Transuranium Processing Plant. The products of these operations are made available to interested researchers in the United States upon approval of the Director of the Division of Chemical Sciences, based on allocations recommended by the Transplutonium Program Committee. I cannot commit these groups to supply materials in the future, but it is their policy to make materials available to qualified researchers if at all possible. As a result of your verbal request made in October, 1982, you have been allocated tracer amounts of ^{253}Es and ^{255}Fm from our next campaign, now scheduled for the fall of 1984.

The quantities available in a typical campaign are 0.6 TBq of ^{253}Es and 2.5 GBq of ^{255}Fm. The einsteinium is far in excess of your needs. The fermium is present as the daughter of 40-day ^{255}Es and could be milked repeatedly from the mixture of einsteinium isotopes. However, processing losses and decay during purification, packaging and shipment would limit the amount of ^{255}Fm delivered to your laboratory to the order of 0.4 GBq (~10 mCi).

We can furnish modest amounts of these isotopes for research and development purposes without charge (except for Isotopes Distribution Office handling charges). Should your requests increase to the point of consuming a significant portion of our resources, we would have to reevaluate the supply and cost picture.

Yours very truly,

J. E. Bigelow

J. E. Bigelow, Sc.D.

JEB:amsp

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May 14, 1984

Lee C. Washburn, Ph.D.
Scientist
Oak Ridge Associated Universities
Radiopharmaceutical Development Group
Medical & Health Sciences Group
P. O. Box 117
Oak Ridge, Tennessee 37831-0117

Dear Dr. Washburn:

The purpose of this letter is to confirm our willingness to collaborate with you and your associates, including Evelyn Watson and Jack Coffey, in your proposal related to antibody therapy. The possibility of using fermium-255 attached to antibodies looks very promising to me.

As you know I am the Principal Investigator for a National Cancer Institute contract (N01-CM-37565) titled, "Dose calculations for cancer therapy using radioactively labeled antibodies directed to tumor associated and/or tumor specific antigens". Within the scope of this contract we will be happy to assist you in preparing treatment plans based upon the use of data to be made available by your proposal. In addition we will be glad to give you the results of our cellular level dose estimates for ^{255}Fm and to make radiation dose measurements where appropriate with our dose measuring instrumentation.

If I can be of further assistance during this proposal phase of this research, please don't hesitate to ask.

Sincerely,

Rodney E. Bigler, Ph.D.
Biophysics Laboratory
Sloan-Kettering Institute for
Cancer Research

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