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IMPROVING CANCER TREATMENT WITH CYCLOTRON PRODUCED RADIONUCLIDES

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Introduction:

OVERALL OBJECTIVE/RELATIONSHIP TO DOE'S PROGRAM/GENERAL PLAN AND APPROACH: This renewal application pursues our continuing long term goal of promoting nuclear medicine applications by improving the scientific basis for tumor diagnosis, treatment and treatment follow-up based on the use of cyclotron produced radiotracers in oncology. This program fits into the nuclear medicine component of the DOE mission, which is aimed at enhancing the beneficial applications of radiation, radionuclides, and stable isotopes in the diagnosis, study and treatment of human diseases. This program is administered within the Medical Applications and Biophysical Component of the Office of Health and Environmental Research, Office of Energy Research, DOE.

The grant includes 3 interactive components: Radiochemistry /Cyclotron; Pharmacology; and Immunology. An essential strategy is as follows: novel radionuclides and radiotracers developed in the Radiochemistry/Cyclotron section under the DOE grant during the 1989-1992 grant period, will be employed in the Pharmacology and Immunology sections of the DOE grant during the 1992-1995 grant period. The development of novel radionuclides and tracers is of course useful in and of itself, but their utility is greatly enhanced by the interaction with the immunology and pharmacology components of the program. In addition to its primary research mission, this project also provides a basis for training of research scientists in radiochemistry, immunology and bioengineering.

A DOE review, coordinated by the Office of Program Analysis, Office of Energy Research of this MSKCC DOE grant took place in Los Angeles in April 21-22, 1992. In this review, we were asked to emphasize "the scientific methods, recent progress, current project activities, and near-term research directions on your current contract or grant. Deviations, if any, from your original project task statement should be explained". The result of the review was

strongly positive (see attachment 1 to this report).

Project 1. Radiochemistry/Cyclotron: Innovations in target design and radiochemistry (R. Finn, PI).

A. Original Objectives- Development of novel I-124, and Ga-66 targets, as well as methods of radiolabeling to antibodies, and production on "baby" cyclotrons; development of novel targets for radiolabeled precursors; radiolabeling of anti-tumor drugs, including FUDR, methotrexate, and the cross-resistant drug colchicine, for studies of multi-drug resistance in animal and human tumors. Enzymatic methods of labeling of amino acids and thymidine, which were included in the original grant have been set aside for the time being, in favor development of IUDR as a substitute for thymidine, in assessing proliferative rates in vivo.

2. Synthesis of radiotracers for drugs important in tumor pharmacology and multidrug resistance. (C-11 methotrexate, colchicine; F-18 FUDR)

Production methods of FUDR, along with characterization of chemical composition of the precursors has been accomplished, in support of drug retention studies which are now completed in a demonstration project in patients. The C11 target system is being installed, and the basic organic chemistry for production of C-11 methotrexate, C-11 Colchicine, has been accomplished. These drugs will be utilized in a series of experiments that will permit improved correlation of clinical resistance of tumors with the pharmacology and biodistribution of these clinically important drugs.

3. Production of novel radionuclides for immunology research (Iodine-124, Gallium-66)

Physical parameters necessary for production of these radionuclides measured, and a novel solid target designed. Availability of these radioisotopes will lead to improved production methods of these interesting radionuclides with potential as labels for antibody research and immunotherapy.

4. Synthesis of radioiodinated IUDR (I125, I131), and the development of unique methods of production that greatly enhance shelf life of the radiolabeled reagent.

Production of IUDR for animal studies, and clinical demonstration projects (see below under "Pharmacology").

5. Radiolabeling of monoclonal antibodies A33,M195,3F8 with Iodine 124 for animal and human demonstration studies.

Technical features of radiolabeling with this radioisotope have been worked out which gives good yields and immunoreactivity of final products.

6. Radiolabeling of antibody 3F8 with Tc99m using a modification of

the Schwarz method.

Reduction of disulfide bonds and the development of rapid approaches to Tc99m labeling of an important antibody with potential for use in neuroblastoma and small cell lung cancer.

Project 2. Pharmacology (S.M. Larson, PI; B. Mehta, Co-PI; J. Biedler, R. Blasberg and J. Bertino, Consultants).

a. A novel method for assessing multi-drug resistance in vivo

b. Assessing anti-tumor to chemotherapy agents based on assessment of DNA metabolism with radiotracers

c. Retention of antimetabolite in tissue in relationship to anti-tumor effects.

A. Original Objectives - Kinetic studies with long lived radiotracers (H-3 and C-14) in multi-resistant and sensitive animal tumors of a variety of types will be pursued to develop an appropriate "cross-resistant" drug that can serve as a marker substance for the demonstration of the multi-drug resistant phenotype in vivo. When the appropriate marker drug has been successfully radiolabeled with a positron emitter (our initial candidate is the drug colchicine), studies will be pursued in animals with PET imaging and if successful a demonstration project in humans is anticipated. Results of the radioactivity from the marker drug will be correlated with the quantitative expression of P-glycoprotein, using an unique antibody. Since many antitumor drugs act by interfering with DNA metabolism, a component of the original grant was directed toward the use of metabolic tracers in assessing tumor proliferation. Initially, a project was planned to evaluate the use of C-11 thymidine obtained by enzymatic labeling, but because other groups already had a sizeable program in this area, our direction was changed to pursue the use of IUDR, as a radiotracer for DNA synthesis. In addition, FUDR, an antimetabolite drug which is used extensively in cancer treatment, particularly for colorectal cancer of the liver was proposed for studies in a limited series of patients with colorectal cancer using a specially designed gamma camera system, to assess retention of the drug in tumor versus liver.

B. Pharmacology Research Accomplishments

1. Demonstration of multidrug resistance based on less retention of radiolabeled colchicine in resistant in comparison to sensitive tumors.

Initial "proof of principle" studies have been completed and published, using H-3 Colchicine, and the studies repeated with C-14 colchicine using C-14 colchicine labeled in the same position as the C-11 compound, showing identical results. An extensive metabolite analysis, in plasma and tumor has been completed, with the C-14 compound that will serve as a basis for developing a

modeling approach based on PET. Multidrug resistance is thought to be a major mechanism of clinical tumor resistance in patients. Techniques which could detect MDR in tumors, and monitor the effects of strategies that will reverse this, could have a major impact on treatment strategies.

2. Preliminary animal and human studies with radioiodinated IUDR demonstrate the possibility that PET and SPECT imaging in humans may be used as a basis for monitoring DNA synthesis.

We have demonstrated that IUDR is taken up and retained within DNA in an animal tumor model system. Although the uptake is less than thymidine labeled with C14 at the 3 position in the purine ring, the possibility of labeling with longer lived radioiodine isotopes that could be used for either SPECT or PET imaging led us to pursue this. Animal studies performed in Dr. Blasberg's laboratory at MSKCC demonstrated that uptake was greatest in the most rapidly proliferating tumors, and also, that more than 95% of total tumor radioactivity at 24 hours was retained in DNA, with the only other metabolite being free Iodide. This could simplify modeling kinetics for metabolic imaging methods based on PET or quantitative SPECT. Another important potential advantage, especially for brain tumors, is that since IUDR has increased lipophilicity, it has better penetration (3 to 4 fold) into brain than thymidine itself. This will be an advantage, particularly for brain tumors. Initial SPECT studies in humans using Iodine-131 IUDR, performed at MSKCC show the feasibility of early (4 hr) and delayed (24 hr) imaging of brain tumors.

3. Pharmacokinetics studies in humans with metastatic colorectal cancer of [F-18]FUDR tumor uptake after intra-arterial injection, suggest strategies for improving tumor uptake and retention of the drug.

The uptake and transport of FUDR, an antimetabolite anticancer drug used in colorectal cancer, was rate limiting in the majority of patients with colorectal cancer metastatic to the liver. Five patients, 4 with colorectal cancer received injections of high specific activity FUDR (Xcuries/mg). The FUDR that was taken up into the tumors, was retained, but the amount taken up varied and was limited by the initial transport step from blood to tumor. Four of the 5 patients responded, 2 PR, 2 MR and the fifth had stable disease. The number of patients is too small to draw meaningful conclusions about the correlation between levels of tumor uptake and response, since all of the patients had at least some apparent drug effect, but the new finding that transport may be rate limiting in the final concentration of FUDR in tumor is important. This suggests certain strategies to increase uptake, based on actually slowing the flow of blood through the tumor, may permit more uptake and retention. Increasing blood flow to the tumor is unlikely to increase FUDR localization substantially.

Project 3. Immunology: Quantitative immunokinetics and dosimetry of anti-tumor antibodies. (Keith Pentlow, Martin Graham, Farhad

Daghighian and Gene DiResta, Project Leaders).

A. Original Objectives- Positron emitting radionuclides with complex decay schemes, such as I-124 and Ga-66, have not figured prominently in applications to anti-tumor antibody dosimetry despite the obvious advantages of positron emission tomography for quantification. The reasons relate to concern about the ability to quantitatively image such radionuclides because of potential interference from time coordinated gamma rays that could fall in the time coincident window at an energy which would allow them to be detected as a coincident event. In a series of phantom studies, we propose to demonstrate that quantitative imaging of I-124 and Ga-66 is possible under conditions likely to pertain in vivo, for "hot-spot" imaging of tumors after parenteral injection. The purpose of this series of projects is to thoroughly evaluate the imaging physics of detection and quantification of I-124 and Ga-66 like radionuclides with modern PET scanners, and to extend the application to animal studies using a unique antibody system, radiolabeled 3F8, an antibody against neuroblastoma tumor. These observations in animals would lay the foundation for quantitative dosimetry of radiolabeled anti-tumor antibodies. In addition, it is likely that animal studies would be pursued with other antibodies, such as A-33, an anti-colon carcinoma antibody, and MX35, an anti-ovarian antibody. In addition, limited patient studies may be done (imaging and immunokinetic studies only). Also, some studies on the physiology of antibody localization will be undertaken.

B. Immunology Research Accomplishments

1. Physics of Positron Emission Tomography Imaging of Iodine-124

Iodine-124, despite a complicated decay scheme that includes only 25% positron decay, can be reliably imaged and quantitated with PET cameras of a variety of designs, even in the presence of large quantities of Iodine-131.

2. Development of mathematical models to analyze the physiology of penetration of anti-tumor antibody into tumor deposits in vivo.

3. Development of analytical techniques to experimentally verify for the first time the essential features of the Baxter-Jain hypothesis, which describes a variety of physical factors that can impede the transport of radiolabeled antibody into the center of tumor masses.

4. An artificial lymphatic system (ALS) has been developed and its function described in a mathematical model, an extension of the Baxter-Jain hypothesis, which is able to predict experimentally determined effects of mechanically changing interstitial fluid pressure in experimental animal tumors.

The experimental studies have been performed with a nude rat tumor model that is suitable for PET imaging studies. These systems have the potential for practical application in settings such as brain

tumors, and possibly liver tumors, where reducing interstitial pressure in the center of tumors could substantially increase penetration and uptake of cytotoxic radiolabeled antibodies.

5. Development of pharmacokinetics models for antibody localizing to human tumor in vivo.

Optimizing dose and timing of dose to improve response of human tumors may result. An important feature of this model, was to incorporate the effects of the vastly different volumes of distribution of antibody and antigen into computations regarding the pharmacology of the distribution of radiolabeled antibodies in vivo.

6. Demonstration of feasibility of Iodine-124 labeled antibody as a method for improved estimates of radiation absorbed dose during radioimmunotherapy.

A child was studied with Iodine-124 labeled 3F8 antibody and the radiation absorbed dose computed from direct measurements using PET. This was the initial application of PET to an individual estimate of radiation absorbed dose for the purpose of radioimmunotherapy.

7. A mathematical model for immunokinetics of anti-tumor antibody in human brain tumors with emphasis on implications for microdosimetry of radioimmunotherapy.

Improvements in knowledge about basic immunology of penetration of antibody into tumors, as well as improved radioimmunotherapy.

Bibliography -August 1, 1991 to date

In the last year there have been 7 published papers, 2 in press, 2 submitted; 3 abstracts; 1 Ph.D. thesis; and 1 patent; that is derived from this work

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3. DiResta GR, Lee J, Arbit E. Measurement of brain tissue specific gravity using pycnometry. J Neuroscience Methods 1991;39:245-251.

4. Mehta BM, Rosa E, Fissekis JD, Bading JR, Biedler JL, Larson SM. In vivo identification of multidrug resistance with radiolabeled colchicine, a potential agent for positron

emission tomography. J Nucl Med 1992;33:1373-1377.

5. Mulshine JL, Shuke N, Carrasquillo J, Daghighian F, Ghosh B, Walsh T, Avis I, Reynolds JC, Cuttitta F, Larson SM. The correct dose: Pharmacologically guided end point for anti-growth factor therapy. Cancer Research (Suppl) 1992; 52:2743s-2746s.

6. Cheung N-KV, Pentlow KS, Graham MC, Yeh SJ, Finn RD, Larson S. Radiation absorbed dose and tumor response during therapy with 3F8 Iodine-131 conjugated monoclonal antibody. In: Watson EE, Schlafke-Stelson AT, eds. Fifth International Radiopharmaceutical Dosimetry Symposium (CONF-910529). Proceedings of a conference held at Oak Ridge, Tennessee; Oak Ridge Associated Universities, 1992: 95-112.

7. Daghighian F, Pentlow KS, Larson SM, Graham MC, DiResta GR, Yeh SDJ, Macapinlac H, Finn RD, Arbit E, Cheung N-K V. Application of PET and compartmental modeling in estimation of cellular level microdosimetry: PET studies of I-124 labeled 3F8 MAb in human glioma. In: Watson EE, Schlafke-Stelson AT, eds: Fifth International Radiopharmaceutical Dosimetry Symposium (CONF-910529). Proceedings of a conference held at Oak Ridge, Tennessee: Oak Ridge Associated Universities, 1992: 396-413.

Abstracts Published:

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2 Daghighian F, Pentlow KS, Larson SM, DiResta GR, Graham MC, Yeh SDJ, Macapinlac H, Finn RD, Arbit E, Cheung NKV. In vivo kinetics of radiolabeled antibody: PET studies of I-124 labeled 3F8 MAB in human glioma. J Nucl Med 1991; 32:1021.

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Articles in Press

1. Larson SM, Pentlow KS, Volkow ND, Wolf AP, Finn RD, Lambrecht RM, Graham MC, DiResta G, Augenson F, Malawi O, Bendriem B, Daghighian F, Yeh SDJ, Wang GJ, Kalaigian H, Cheung NKV. PET

scanning of ^{124}I-3F8 as a novel method of tumor dosimetry during treatment planning for radioimmunotherapy in a child with neuroblastoma. (J Nucl Med 1992).

2. Conlon KC, Bading JR, McDermott EWM, Corbally MT, Talvo AJ, Brennan MF. Extremity metabolism in the cachectic, VX-2 carcinoma-bearing rabbit. (J of Surgical Research 1992)

Submitted:

1. Bading JR. A method for analytic estimation of solute transport and distribution volume parameters. (Submitted to Am J Physiol, Modeling Methodology Forum, 10/1/91).

2. Daghighian F, Pentlow KS, Larson SM, Graham MC, DiResta GR, Yeh SDJ, Macapinlac H, Finn RD, Arbit E, Cheung NK. Development of a method to measure kinetics of radiolabeled monoclonal antibody in human tumor and applications to microdosimetry: PET studies of I-124 labeled 3F8 MAb in glioma. (Submitted to Eur J Nucl Med 7/92)

Thesis

1. Lee J. (Ph.D.) Characterization of parameters affecting macromolecular transport in neuroblastoma xenograft. Polytechnic University, Bioengineering 1992.

Patents

DiResta G, Lee J, Arbit E. **United States Letters Patent**
Process and device to reduce interstitial fluid pressure in tissue.
Submitted April 1992 (patent pending)

6. Descriptive Summary

Our goal is to **improve the scientific basis for tumor diagnosis, treatment and treatment follow-up based on the use of cyclotron produced radiotracers in oncology.** The grant includes 3 interactive components: Radiochemistry /Cyclotron; Pharmacology; and Immunology. The radiochemistry group seeks to develop innovative cyclotron targetry, radiopharmaceuticals, and radiolabeled antibodies, which are then used to assess important unanswered questions in tumor pharmacology and immunology. Examples include selected positron emitting radionuclides, such as Iodine-124, and Ga-66; I-124, I-123, I-131 labeled iododeoxyuridine, C-11 colchicine, and antimetabolites, like C-11 methotrexate; and radiolabeled antibodies, 3F8, M195, A33, and MRK16 for application in the pharmacology and immunology projects. The pharmacology program studies tumor resistance to chemotherapy, particularly the phenomenon of multidrug resistance and the relationship between tumor uptake and retention and the tumor response for anti-metabolite drugs. The immunology program studies the physiology of antibody localization at the tissue level as the basis for novel approaches to improving tumor localization, such as through the use of an "artificial lymphatic system" which mechanically reduces intratumoral pressures in tumors in vivo. Quantitative imaging approaches based on PET and SPECT in radioimmunotherapy are studied to give greater insight into the physiology of tumor localization and dosimetry.

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