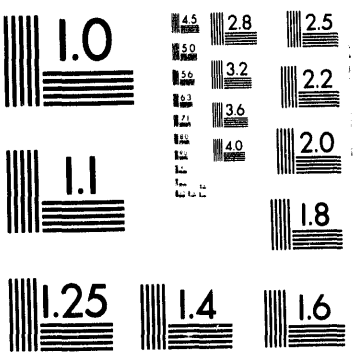
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IMPROVING CANCER TREATMENT WITH CYCLOTRON PRODUCED RADIONUCLIDES

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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 preceding grant periods, will be employed in the Pharmacology and Immunology sections of the DOE grant during the next year of the grant period, 1994. 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.

1. MAIN RESEARCH ACCOMPLISHMENTS OF THE OVERALL GRANT PERIOD AND INCLUDING THE LAST GRANT PERIOD January 1993 to present

During the grant period to date (November 1, 1993), there have been 28 published papers,(9 in the last year) 3 Master's theses (1 in the last year) and 1 PhD thesis; 1 patent application; 4 papers in press; 2 papers submitted and 22 abstracts (13 in the last year) based directly on work supported by this grant. (See Bibliography)

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

A. Original Objectives - Development of unique iodine-124 and gallium-66 targetry; development of targetry for production of rhenium-186; development of targetry for PET radiolabeled precursors; synthesis of novel radiolabeled anti-tumor drugs including methotrexate and the cross resistant drug, colchicine for studies of multi-drug resistance in animals and human tumors; development of IUdR as a potential measure of proliferating tissue; preparation of oxygen-14 labeled water as an alternative to oxygen-15 water for blood flow measurements "in vivo."

B. Radiochemistry/Cyclotron Research Accomplishments

1. Refurbishment of CS-15 cyclotron and upgrading of "routine" radiopharmaceutical preparations.

The entire water cooling monitoring system and interlock system has been replaced during this period of the grant. Experiments and modifications to the ion source cathode filaments are extending the useful lifetime of the source. An automated commercial "black box" synthetic unit has been procured to minimize the exposure to staff involved in "routine" FDG preparations. Subtle modification are necessary to integrate the unit with the MSKCC targetry system.

2. Development of a novel target and holder for "in-line" irradiations of solid and/or powder targets.

The targetry system developed for the CS-15 cyclotron operates with or without the foil cooling and cyclotron vacuum isolation windows to achieve maximum beam energy upon the target surface. The target is further positioned at an incline with the beam to overcome the need for thick target materials and excessive chemistry processing steps.

3. 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.

4. Production of novel radionuclides for immunology research (iodine-124, gallium-66,

rhenium-186)

The verification of the production capability of the CS-15 cyclotron alpha beam and the determination of radionuclidic purity of gallium-66 radionuclide has been concluded. Extensive efforts for the production of Gallium-66 were made during this period of the grant to optimize the operational characteristics of the novel target holder. The target required a minimum of 50 mg of natural copper electroplated onto the reusable silver backing plate. Several electroplating baths are currently being evaluated for application in the production of iodine-124 on the MSKCC cyclotron.

5. Synthesis of radioiodinated IUdR (I-125, I-131, I-123) and the unique development of novel methods of production to greatly enhance the shelf life of the radiolabeled reagent.

Formulation of the IUdR radiolabeled with the various radionuclides for both animal and clinical investigations have successful completed. Emphasis is on the optimization of yields and an appreciation of methods for the stabilization of the radiopharmaceutical.

6. Radiolabeling of monoclonal antibodies A33, M195 and 3F8 with radiometals for animal studies.

The radiolabeling of several monoclonal antibodies using CHX-DTPA coordination has begun using both indium-111 and gallium-67. Good yields and consistent immunoreactivity of the final formulations have been achieved. Currently bismuth-213 is being evaluated as a radiotracer to label huM195.

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

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 lower retention of radiolabeled colchicine in resistant tumors as compared 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. In the last grant period, we have developed quantitative autoradiography techniques for which permit fusion imaging of the histologic slices and the radioactivity content slices for the determination of the uptake of the C-14 Colchicine distribution throughout tumored animals. This permits site by site assessment of local concentration of radiolabeled drugs, and helps us further assess the possibilities of using these markers in non-invasive imaging studies in man.

2. Demonstration that MRK-16, a monoclonal antibody, can be used to detect MDR phenotype based on localization in neuroblastoma tumors growing as heterografts in nude mice.

The monoclonal antibody MRK-16, which targets the P-glycoprotein receptor, has been made available to us by one of our collaborators (Professor Tsuruo, NCI of Japan, Tokyo), and when labeled with Iodine-125, targets effectively to neuroblastoma tumors in vivo. Targeting is most effective for the resistant xenograft tumors, and there is a statistically significant increase (about twice) for resistant tumors in comparison to sensitive tumors.

3. 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 approach. 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. 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. In the current grant period, we performed demonstration project in patients with glioma tumors, using I-131 IUDR and SPECT. At 24 hours, there was an increased uptake of the IUDR, at the very perimeter of the tumor- in some cases, well beyond the area of contrast enhancement on the MRI or CT scan. These findings if confirmed, would be the first imaging demonstration in man that there is frequently extension of tumor into regions beyond the area of blood brain barrier break-down, and could be very important in establishing the optimal zones for treatment planning of radiation therapy and surgical extirpation.

4. 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. 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 partial response, 2 mixed response, 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, such as actually slowing the flow of blood through the tumor. Increasing blood flow to the tumor is unlikely to increase FUDR localization substantially.

Project 3. Immunology: **Quantitative immunokinetics and dosimetry of anti-tumor antibodies. (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.

ALS studies are proceeding well. By the year's end we expect to submit two papers for publication. The first will introduce the ALS concept, its associated mathematical model and animal data with non-specific MoAb. The second paper will present the recent ALS probe design and 3F8 Neuroblastoma uptake studies in dual tumor animals. These results indicate that ALS significantly enhances MoAb uptake and that our mathematical model is appropriate.

Current research involves refining the ALS methodology and its application to chemotherapy. The application studies are currently being performed with three rat tumor models.

i. We have completed a pilot study to determine if reducing IFP increases uptake of smaller molecular weight therapeutic agents. Working with Dr. M. Burt's rat sarcoma model, we determined that reducing this tumor's IFP enhances uptake of Adriamycin. Experiments are underway to determine the mechanism of effect. The improved uptake is believed to be related to the increased blood flow observed in the tumor when IFP was reduced.

ii. New ALS probes with internalized vacuum battery have been developed and are undergoing animal evaluation. This system is implantable and the animals can ambulate freely. First studies have been performed with Sprague Dawley rats with dual Walker 256 flank tumors. Following instrumentation, the animals received an injection of Evan's Blue Dye. Post mortem examination 24 hours following injection revealed that the dye was present in greater amounts in ALS tumor over control.

iii. Preliminary studies conducted with athymic rats with dual neuroblastoma tumors have shown that 3F8 is significantly enhanced in tumor with ALS probe. Current studies are underway to determine impact of new ALS probes and internalized vacuum battery on MoAb uptake. These studies will be performed using CC49 MoAb with LS174T tumor line as well as 3F8 with neuroblastoma.

5. MoAb Transport Phenomena

Studies to characterize the parameters affecting MoAb transport to tumor were expanded this year. Experiments were performed in both rat and mouse. The findings from each study indicated that tumor IFP was significantly higher than normal tissue.

i. We completed and published the tumor growth, interstitial fluid pressure(IFP) and velocity profile(IVF) study for the neuroblastoma (NMB-7) rat xenograft model. This model's pH profile and glucose metabolic rate has recently been measured; Its pO_2 profile measurements are currently in progress.

ii. A series of experiments were conducted with Dr. M. Choti to measure IFP in Dr. E. Sigurdson's (Colorectal Cancer Service) rat colorectal-hepatic tumor model. Measurements were performed pre and post infusion of Angiotensin II. The findings indicate that IFP increases following infusion of the drug.

iii. IFP measurements were also performed with a new tiny wick-in-needle pressure probe on LS174T tumor propagated in mouse. IFP, measured in the tumor's center, was directly correlated with tumor size.

iv. Elevated IFV and reduced blood flow was measured in Dr. Burt's (Thoracic Surgery Service), rat sarcoma model. This work was conducted in anticipation of ALS studies.

v. In collaboration with Dr. Arbit,(Neurosurgery Service) IFP has been measured in human brain tumors. As anticipated, IFP is elevated in these tumors but to a smaller extent than observed in rat flank models. Further using a rat brain tumor model, we observed that the measured tumor IFP is influenced by ICP. A manuscript is currently in preparation.

6. 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.

7. 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.

8. 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.

9. PET Imaging Study: ^{66}Ga citrate

The first non-invasive PET imaging study performed with gallium-66 in a living animal was conducted on our PC4600 scanner to measure tissue distribution of ^{66}Ga citrate in a Sprague Dawly rat with a single Walker 256 tumor implanted over its hind flank. The animal was imaged for 1 hour during infusion of ^{66}Ga and for fifteen minutes 19 hours post injection. The first session was performed in quantitative fashion to obtain uptake data, i.e. thirteen frames were acquired during the measurement interval along with arterial blood samples. The surgical and anesthetic procedure followed was identical to that which we developed to perform quantitative ^{18}F deoxyglucose measurements with our xenograft model. The second imaging session was performed in qualitative fashion, i.e. a single fifteen minute frame was acquired along with one venous blood sample. The animal was sacrificed following the second imaging session and tissue samples were taken for counting in our LKB Gamma counter. Both the PET imaging and tissue distribution data indicate that the ^{66}Ga citrate was avidly taken up by the tumor. Modeling of its pharmacokinetics is currently underway.

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.

Previous Human Subject Research Activity

Approved protocol 91-136: We studied patients with brain tumors for IUDR incorporation into tumor DNA. A total of 8 patients were studied and there was increased uptake in

rapidly proliferating tumors of the CNS. No adverse reactions were encountered and this project was also covered under the auspices of the Radioactive Drug Research Committee and the MSKCC Radiation Safety Committee. No other human studies were undertaken during this grant period.

2. PLANS FOR CONTINUATION OF PRESENT OBJECTIVES AND NEW OBJECTIVES FOR GRANT PERIOD NOVEMBER 1, 1993 TO JANUARY 31, 1994

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

Original Objectives: Development of unique iodine-124 and gallium-66 targetry; development of targetry for production of rhenium-186; development of targetry for PET radiolabeled precursors; synthesis of novel radiolabeled anti-tumor drugs including methotrexate and the cross resistant drug, colchicine for studies of multi-drug resistance in animals and human tumors; development of IUdR as a potential measure of proliferating tissue; preparation of oxygen-14 labeled water as an alternative to oxygen-15 water for blood flow measurements "in vivo."

Planned Continuation of Objectives: Iodine-124 production via the irradiation of enriched tellurium-124 with energetic deuterons in collaboration with Brookhaven National Laboratory and the alpha irradiation of natural antimony metallic targets with continue. The completion of target electroplating bath procedures for both gallium-66 production and iodine-124 should be a significant priority during this period of funding. The confirmation of cross sectional data which become a basis for the realized potential to produce iodine-124 in amounts suitable for animal studies on the MSKCC cyclotron. The separation of iodine-124 from the antimony target via a dry distillation technique using an induction heater shall be evaluated.

The synthetic routes to carbon-11 labeled methotrexate and colchicine should be finalized. Initial experiments utilizing methyl iodide as the precursor shall be confirmed. Moreover, the fluoro-colchicine analogue is being considered as an alternative compound. The approach involves the fluorine-18 fluoride anion Kryptofix 2.2.2 carbonate complex as fluorinating reagent to react with the triflic acid analogue on the C-ring.

The radiolabeling of monoclonal antibodies with fluorine-18 para fluoro benzoyl succinimide linkage and gallium-66 as the chloride is also being evaluated.

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

Hypothesis 1: Tumor retention and anti-tumor effect are highly correlated for certain anti-

cancer drugs; i.e. anti-metabolites and biologically derived drugs affected by the multidrug phenotype.

Hypothesis 2: Uptake and retention of IUDR in human tumor may be used as an indication of DNA synthetic rate. In preliminary studies, uptake will be sufficient for adequate quantitative assessment using PET and SPECT in brain tumors, and at 24 hours, the large majority of tumor contained radioactivity will be in the DNA fraction.

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. Limited patient studies with [F-18]-FUDR will be pursued to assess the role of uptake and retention in the anti-tumor response of colorectal cancers, metastatic to liver. A specially designed gamma camera will be used for these studies.

Planned Continuation of Present Objectives: Having established the basic principal of increased uptake of cross-resistant drugs in the more sensitive cell lines, and decreased uptake in the resistant lines, we will refine the analysis of retention to determine the effect of verapamil and the monoclonal antibody on this retention, and to determine the effect of such action on improving animal survival for certain drugs which are active against the target tumors chosen, including adriamycin and vincristine. Our basic study plan will employ sensitive and resistant neuroblastoma cell lines, and in addition to using ^{14}C-labeled tracers and biodistribution studies, as soon as the ^{11}C-colchicine is available, animal studies will be performed with the PC4600 PET scanner, to determine the parameters of detection of MDR sensitive versus resistant tumors, by quantitative imaging of labeled drug retention in tumors versus normal tissues. Depending on the magnitude of uptake of colchicine uptake in tumors, and the metabolite pattern, we will evaluate the possibility of developing formal PET modeling approaches to quantitate transport into tumors using PET. Recent studies with MRK16 antibody have shown promise for detecting the presence of the P-glycoprotein on the tumor cells, and parallel studies will be performed with I-125 or I-124 MRK as a way of detecting resistant tumors, in comparison to the colchicine methodology.

Using the newly developed antimetabolite tracer drug C-11 methotrexate, we will pursue the correlation of retention of drug in tumors, with anti-tumor results, and the role that a radiotracer could have in predicting response of these tumors to therapy. These radiotracer studies are based on the recent work of one of our consultants, Dr. J. Bertino, whose recent studies with a series of anti-metabolites including methotrexate has shown that the development of tumor resistance occurs when tumors no longer retain high drug concentrations.

IUDR has been developed by the radiochemistry group, and initial studies performed by Dr. R. Blasberg, our consultant indicates that the biology is favorable for use of this tracer to assess DNA synthetic rate, particularly in brain tumors, but possibly in lung tumors as well. Serious questions remain, however, about whether enough IUDR will be taken up to permit statistically accurate quantitative imaging, either with SPECT(I-131,I123) or PET(I124). We will do limited patient demonstration studies to address this question. We plan to extend these studies to limited demonstration projects in humans. Our initial studies in brain tumors using I-131 IUDR SPECT have shown late uptake at 24 hours at the perimeter of the tumor. We will label the IUDR with I-123, in order to improve the statistical certainty of SPECT imaging at these sites. In addition, we plan to assess the biology of localization of I-131 IUDR in colorectal cancer after intra-hepatic injections, to see if it is possible to assess DNA synthetic rates, based on local accumulation in the colorectal tumors. Our plans include the concomitant use of blood flow tracers, to determine the input function in these tumor systems. Recent studies by Mariani and colleagues in Milan, have demonstrated the feasibility of such approaches in colorectal cancer.

Project 3. **Quantitative immunokinetics and dosimetry of anti-tumor antibodies. (M. Graham, G. Sgouros, K. Pentlow, Farhad Daghighian and Gene DiResta, Project Leaders).**

Hypothesis 1: Quantitative imaging approaches with PET will lead to non-invasive methods for improved dosimetry and immunokinetics for radioimmunotherapy.

Hypothesis 2: Increased antigen expression by tumors and reduced central pressure in tumor masses will lead to improved localization of antibodies for radioimmunotherapy.

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.

Planned Continuation and New **Objectives**

a) to thoroughly evaluate the imaging physics of detection and quantification of I-124 and Ga-66 like radionuclides with modern PET scanners.

b) **to determine tumor physiology which is important to tumor localization, and if possible provide approaches which will lead to improved uptake of radiolabeled antibody.**

c) **to perform animal studies and limited patient demonstration studies to validate the concept that quantitative imaging with PET can be used to more accurately measure radiation absorbed dose (macro and micro-dosimetry) and immunokinetics of tumor targeting.**

Imaging physics studies performed under DOE to date (November 1, 1993) have conclusively demonstrated for all of the diverse PET systems so far studied (6 distinct designs) , that I-124 can be quantitatively imaged, even in the presence of large quantities, up to 100 times greater, of I-131. In two in-vivo animal tumor systems, namely, nude mice bearing neuroblastoma tumors, and nude rats bearing human ovarian cancer, the ability to measure concentrations of radiolabeled antibody in vivo has been validated. Having established the quantitative nature of imaging with positron emitting radiolabeled antibodies, in the initial grant period, and having developed quantitative models for immunokinetics that permit estimates of microdose from auger emitting forms of Iodine, such as Iodine 125,124,123,we propose studies to validate these concepts of radiation dosimetry in animal model systems, predominantly nude rats bearing NMB7 neuroblastoma tumor. In addition, we have developed a quantitative autoradiographic system, for detailed study of microdistribution in tumors in animals, as a basis for improving the understanding of tissue factors that influence uptake, particularly the relationship of antigen expression to tumor uptake. This system will also make it possible to evaluate microscopic antigen distribution is tumors, and will also be valuable in assessing local microdistribution of dose, and its impact on. We propose further refinement of these methods to assess the role of antigen expression to determining tumor localizations of 3 F8 antibody. IL4 is said to stimulate GD2 antigen expression in neuroblastoma tumors, and we will assess the impact of IL4 in terms of effects on both antigen expression and uptake. The NMB7 tumor system in nude mice, shows a sharp decline in uptake of the antibody with increasing size of tumor, and detailed studies may give insight into the mechanisms of these changes. Recent progress in assessing the impact of tissue pressure an mechanical factors on antibody localization have come from the work of this DOE grant, and further studies are planned to validate a mathematical model which describes the impedance to inflow of antibody into the region of the tumor. Furthermore, a system for reducing the interstitial pressure within tumors has also been developed, to assess the Baxter-Jain hypothesis. These studies will be pursued in parallel, to determine to what extent the tumor localization of 3F8 antibody into animal tumors can be improved. In addition, it is likely that animal studies would be pursued with other antibodies, such as A-33, an anti-colon carcinoma antibody, and antigen binding peptides that react with TAG-72 antigen. In addition, limited patient studies may be done (imaging

and immunokinetic studies only), in patients with brain tumor, colorectal and pediatric tumors, as limited demonstration of the principles developed in the animal model systems.

In addition, the positron emitter Gallium-66 is now available to us, and Dr. Graham has completed physics evaluation of this isotope as well. This can be quantitatively imaged with PET scanners, despite the energetic photon, and we have completed imaging studies in a nude rat, with good imaging quality. This isotope will now be coupled to 3F8, using a chelate designed for Gallium by Dr. Otto Gansow. Preliminary work with Gallium-67 labeled 3F8 has been completed, and there is good targeting to neuroblastoma xenografts. We plan in the next grant period, to assess the utility of Ga-66 labeled 3F8 antibody for tumor targeting in xenografts, and we will compute the dosimetry for potential application to tumor detection and therapy.

We think that we have made exceptional progress in regard to the development of SPECT and planar imaging methods for assessing radioimmunotherapy dosimetry, based on human imaging and therapy trials supported by clinical NIH grant support. The actual fusion imaging and computer processing has been supported in part by the DOE, however, and we are now in a position to assess estimates of dosimetry made by our SPECT methods, employing quantitative PET imaging with Iodine-124. Accordingly, there will be a big emphasis in the next grant period, to go on with production scale for Iodine-124, to use this isotope as a way to independently validate assumptions made in the dosimetry of radioimmunotherapy by these single photon imaging methods.

Finally, in preliminary studies with the single antigen binding protein in animals (to CC49,an anti-TAG-72 antigen), it has been possible to demonstrate that the single chain significantly improves the therapeutic index (tumor/red marrow) radiation dose, and that a number of positron emitters are good candidates as diagnostic and therapeutic radionuclides, in particular, Iodine-124. We plan to continue these studies, utilizing QAR methods to evaluate the penetrance of antibody into tumor, and whether these calculated improvements actually are seen in animal treatment studies.

Human Studies and Animal Research Activities in the Continuation Period

Human studies will be performed in a limited number (less than 15) of patients. There will be a mixture of studies performed with radiolabeled IUDR and I-124 or Ga-66 labeled monoclonal antibodies. Currently, two protocols are already approved for use of these agents: 91-136 and 87-084A(3). These two protocols involve IUDR and 3F8 I-124 respectively. If any modifications are required based on progress in production of novel radionuclides, all appropriate institutional review boards' approvals will be obtained prior to proceeding with human studies.

There are no significant changes in the use of animals in the continuation period.

3. GRADUATE STUDENTS TRAINED:

Masters Degree Granted

a. **Osama Malawi, M.S.** Bioengineering (Nuclear Medicine Research Laboratory) Maintain PC4600 PET camera and the coincidence detector apparatus; acquire and analyze the H2015 and F18DG studies in animals using the coincidence detector apparatus

b. **Jongbin Lee, M.S.**, Ph.D. Bioengineering. Design, build and implant ALS probes and pumps into tumor bearing animals; fabrication of WIN and CLGH probes; data acquisition and analysis of IFP, IFV and MoAb ALS animal data.

Post-Doctoral Tenure

a. **Vipa Bookitticharon, Ph.D.** (International Atomic Agency Fellow) Technetium-99m labeling of 3F8 monoclonal antibody

b. **Diu-Thu Vo, M.D.**, Post-doctoral fellow QAR methodologies and laboratory research on targeting of radioimmunotherapy
Factors important in the localization of radioiodinated 3F8 to NMB7 neuroblastoma in vivo.

c. **Shangde Cai, Ph.D.** Candidate November 1993, Hunter College. As a post doctoral fellow his efforts concentrate on the synthesis of positron emitting radionuclides incorporated into various monoclonal antibodies.

d. **JiaJu Zhang, M.D.** post-doctoral fellow who is involved in the development of novel computer methods for dosimetry.

Ph.D. Degree Granted

a. **Jongbin Lee, M.S.**, Ph.D. Bioengineering

Ph.D. Candidates

a. **Osama Malawa, M.S.**, Bioengineering at Columbia University (see above)

b. **Sunil Konath, B.S.**, Chemical Engineering; Bioengineering Program, Columbia University (Start date Sept. 1992) Conduct doctoral dissertation research in Nuclear Medicine Research Lab: responsible for developing the mathematical analysis and software necessary for multiple ALS placement.

c. **Ravi Moorthy, M.S.**, Chemical Engineering; Bioengineering Program, Columbia University (Start date July 15, 1992) Conduct doctoral dissertation research in neurosurgical research lab: responsible for tumor propagation; conduct the autoradiographic studies to determine the effects of ALS on MoAb uptake; measure tumor Ph and pO_2 before and after ALS intervention; prepare animals for FDG studies.

(Edward Leonard, Ph.D., Professor of Chemical Engineering, Chairman, Bioengineering Program, Columbia University.)

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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)

DISCLAIMER

5. OPINION STATEMENT REGARDING STATE OF KNOWLEDGE RELATING TO CYCLOTRONPRODUCED RADIOTRACERSIN ONCOLOGY FOR PHARMACOLOGY AND IMMUNOLOGY; ITS SIGNIFICANCE AND NEEDED FUTURE INVESTIGATIONS.

Cancer research initiatives of the 50's, 60's and 70's have found their way into the everyday oncology practice of the 80' and 90's. The Atomic Energy Agency and its direct descendant, the Department of Energy, have supported nuclear medicine research heavily and we owe much of modern day nuclear medicine practice to this support: the development of Technetium-99m, the modern gamma camera, Single Photon Tomography, Positron Emission Tomography, are examples of such DOE support. Also, when the "War on Cancer" was declared by President Nixon in 1972, and the National Cancer Institute was established, research initiatives were begun which have resulted in major impact on clinical care in the United States and throughout the world. (3). The use of mammography for screening breast cancer patients, combination chemotherapy for many common cancers, the biotechnology revolution, were prompted by NCI/NIH support. Now more than ever, modern nuclear medicine techniques are being widely applied in oncology for diagnosis and therapy of malignant tumors. Diagnostic nuclear medicine techniques contribute to the clinical care of the cancer patient by 1) detecting the presence of occult tumors; 2) staging tumor extent 3) monitoring the response of tumors to therapy. 4) assessing the impact of tumors on normal organ and tissue physiology. Therapeutic nuclear medicine is useful in the curative and palliative therapy of a growing number of tumor types, and in the control of symptoms resulting from cancer, as in the control of cancer pain.
DOE will have an important role in the future in the providing essential support for basic nuclear medicine research that will be translated into clinical practice in the year 2000 and after. This research support will be targeted to growth opportunities in

1) **instrumentation research,** especially computerized imaging modalities like PET and SPECT. The areas of promise here include applications of computers to image analysis, to "fuse" MRI and nuclear medicine images, and for the purposes of dosimetry of radiotracers targeting cancer for the purposes of therapy. This is a major area of need just now, and quantitative imaging approaches such as are discussed in this grant will help establish essential dose response relationships. Also, better hardware is needed and new detector systems for PET, such as Lutetium based detectors, should lead to improved resolution and sensitivity. Cyclotron research and development to improve ease of use of these extremely valuable research instruments must continue to be supported by DOE, in order that novel radiotracers will continue to be developed as a basis for biomedical advances in numerous areas of medical science.

2) **the "new biology"**-especially molecular engineering and genetics research. Novel radiolabeled antibodies and related molecules, receptor binding ligands, improved sites for radiotracer attachment, created by genetic engineering are all feasible projects that if given support could provide major payback in improved health care.

3) **radiochemistry and new radiotracer development**-new ways for radiolabeling of biomolecules, more rapid separation of cyclotron produced radionuclides and their use in radiolabeling both for diagnosis and therapy. Also, there is a tremendous need to improve knowledge about the bioavailability and concentration of anti-cancer drugs and biologic response modifiers in tumors, in order to better understand the phenomenon of dose response relationships and the underlying mechanisms of resistance of cancers to these therapies. The success of **gene therapy**, which seems so promising, may in the end depend on the ability to target adequate quantities of DNA to the right spot within the cell. Radiotracers will aid greatly in this process, and the DOE should support such efforts.

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