1st Protocol

Mar 1966

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Petty
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To: Clinical Research Committee

From: Ben I. Friedman — Principal Investigator

Ben I Friedman

Eugene L. Saenger — Department Head

Eugene L. Saenger

Subject: Protection of Humans with Stored Autologous Marrow

Purpose of Study: Protocol is attached

Proposed Procedure: Protocol is attached

Potential Hazards:

a. With any irradiation therapy there is the danger of radiation injury. As stated in the protocol (page 4) we have observed consistent depression of hematologic values.

The use of autologous marrow in humans has been investigated by others (Ref. 5, 6, 7, 8) without significant complications. There is thought to be some hazard but this is preventable by proper handling of the marrow and controlling the rate of infusion.

b. All patients will be followed carefully by medical personnel cognizant of the radiation syndrome and course of cancer. Hematologic data are accumulated at frequent intervals (page 3) and all measures to control infection will be pursued such as plastic barrier if indicated.

Previous Work Done in this Area: Refer to protocol

Method to be Used in Procuring Consent of Subjects (Volunteers) above:

Consent forms, as attached, have been used since May 1965. These forms are signed only after the patient has been advised of the research and study aspect of the procedures to be used. They are told that therapeutic effects are hoped for, but not assured.

General Remarks:

The experimental design of this program will permit statistical evaluation of the data. It is a study that does carry a hazard of radiation injury. However, the benefits of possible proof and improvement of marrow storage methods seem to justify this approach on patients who have given informed consent.

SECTION 1

NOT FOR PUBLICATION OR PUBLICATION REFERENCE

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

RESEARCH OBJECTIVES

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SRS PROJECT NUMBER

ABBREVIATED TITLE OF PROJECT

Protection of Humans with Stored Autologous Marrow

NAME, SOCIAL SECURITY NUMBER, OFFICIAL TITLE AND DEPARTMENT OF ALL PROFESSIONAL PERSONNEL ENGAGED ON PROJECT

Ben I. Friedman, M.D. 285-20-2206	Associate Professor of Medicine Assistant Professor of Radiology	Radioisotope Lab.
James G. Kereiakes, Ph.D. 406-20-4626	Associate Professor of Radiology	Radioisotope Lab.
Harry Horwitz, M.D. 095-32-0197	Associate Professor of Radiology	Radioisotope Lab.

NAME AND ADDRESS OF APPLICANT ORGANIZATION

University of Cincinnati College of Medicine
Eden and Bethesda Avenues
Cincinnati, Ohio 45219

USE THIS SPACE TO MAKE A BROAD STATEMENT OF YOUR RESEARCH OBJECTIVES

The initial objective is to prove the effectiveness of stored autologous human bone marrow in repopulating the marrow space. Ultimately the experimental design will be used to study various methods of storage and infusion.

The protective effect of autologous marrow will be studied in patients who are given 150-200 rad total body irradiation as a single dose. Changes in peripheral blood hematologic values together with observation of aspirated marrow specimens from the sternum and anterior ilium will be investigated.

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PHS-398 (REV. 5-61)

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Protection of Humans with Stored Autologous Marrow

1.

A. Although the effectiveness of stored bone marrow has been well verified in animal work (1, 2, 3, 4), the value of autologous stored marrow administered following total body irradiation has not been established by an experimental program in human beings (5, 6, 7, 8). In patients receiving single doses of 150-200 rad total body irradiation we have observed severe consistent fall in circulating leucocytes and platelets. It is proposed to study the protective effects of autologous stored marrow on these changes.

The initial goal is to prove the effectiveness of stored marrow. Ultimately the experimental design proposed will be used to study various methods of marrow storage and determine optimum times for storage and infusion. If autologous human bone marrow is useful in repopulating the marrow space, it has an invaluable role in the management of patients receiving large doses of ionizing radiation or cytotoxic agents.

B. Two groups of patients will be investigated. Both groups of patients will receive or will have received 150-200 rad total body irradiation. The first or control group will include patients who have already been irradiated at the 150-200 rad dose level. Additional patients will be added to this group who will be given autologous bone marrow two to three weeks after irradiation, the exact time of administration depending on the severity of leucocyte depression. The second or experimental group of patients will be given 150-200 rad total body radiation, followed by infusion of their previously stored marrow on the second day after irradiation.

All patients studied will have metastatic malignancy from various primary sites. They will have stabile hematologic values. Many will have received local irradiation previously.

Complete blood counts including hemoglobin, hematocrit, erythrocyte count, leucocyte count, platelet count, reticulocyte count, differential smears, and cell indices will be obtained three to five times in the five to fourteen day pre-irradiation period. The same hematologic studies will be obtained on the day of irradiation and 1, 2, 3, 6, 9, 12, etc. days after irradiation according to the standard test days of Thoma and Wald (9). Additional counts will be obtained as indicated. Bone marrow specimens from the sternum and anterior ilium will be obtained prior to irradiation for cytologic and pathologic examination of the clotted specimens, two days after irradiation (prior to infusion), and at weekly intervals after infusion.

Bone marrow from the posterior ilium will be stored during the week prior to irradiation by the method of Kurnick (5, 6). In vitro viability of the marrow will be estimated by trypan blue exclusion initially, but tissue culture and DNA synthesis methods may be used later. The marrow will be infused intravenously without filtration two days after irradiation in the experimental group. In the control group infusion will be two to three weeks post therapy.

Patients will be treated with 150-200 rad (tissue dose at trunk midline) of total body irradiation at an exposure dose rate of 4-6 r/min from a Cobalt 60 source. Trunk midline is 282 cm from the source. One half of the dose will be given laterally to one side and then one half to the opposite side. The dose falls off approximately 15% at the head and feet. The total dose is given within one hour.

Peripheral blood data will be compared to that which we have already obtained in sixteen patients who have been irradiated at the same dose level but have not been infused at two days. The least squares optimized curve fit analysis on ratio data has been used to determine the best estimate of transition and equation characteristics for degradation and recovery periods. The data are based on the mean of the pre-irradiation values being one. Total leucocyte count and platelet count have been most revealing.

The multiple aspirated specimens of bone marrow will be studied for morphologic evidence of recovery.

C. This is a functional experimental design which permits interpretation of the effectiveness of autologous human bone marrow storage and infusion. The problem of not being able to justify giving an LD 50 of total body radiation to humans with other than primary hematologic disease or severe renal disease has made it difficult to determine the efficacy of autologous marrow infusion. Our experience with patients who have received 150-200 rad total body radiation is unique in that we have been able to estimate the dose which was given by the fall in leucocyte count. Though an LD 50 is not used, still a consistent response of the bone marrow to radiation injury is available for comparison with the experimental group.

Even if the present method of storage does not provide satisfactory protection, still the approach offers a test system for other methods of storage and other times for infusion. The ultimate goal of any such research program is to develop the best possible ways of handling human bone marrow in vitro, maintaining adequate viability for repopulation of the marrow space.

Hematologic failure limits the use of radiation and cytologic agents for the management of cancer. It is the goal of this program to establish a method of protecting patients against this problem.

D. The facilities of the University of Cincinnati Medical Center are to be used for this study. Patients for this program will be from the wards and clinics of the Cincinnati General Hospital. All patients will be informed of the research and study nature of these techniques. Consent forms have been utilized by our group since 1964.

A Canalco programmed reduction freezer and a Harris freezer at -80° Centigrade are available. A controlled rate Sigmamotor pump is presently used for infusion of marrow intravenously. Analysis of data will be undertaken by the University of Cincinnati Medical Computer Center staff and equipment.

2.

A. During the past five years an ongoing study of the metabolic effects of total body radiation has been in progress. To date, thirty-seven patients have received from 25 to 200 rad total body radiation. Sixteen of the patients were given 150-200 rad. Bone marrow from twenty-two patients has been stored; it has been infused intravenously in only four.

Severe hematologic depression occurred in all sixteen patients who received more than 125 rad total body radiation. This was manifested by total leucocyte count depression below 2000 and platelet count depression 25-40 days after irradiation. Data from this group has been used for computer analysis.

The mean minimum leucocyte count of the patients who received 150 rad total body radiation was 1264 ± 1140 without previous radiation and 1140 ± 816 when there had been previous therapy. Patients who received 200 rad had leucocyte counts of 983 ± 369. When the leucocyte data have been analyzed by computer techniques, the slope of the degradation curve separates the groups of patients who received more than 125 rad from those who received less than 125 rad. Satisfactory statistical evaluation of these data will establish the presence or absence of a protective effect from marrow infusion.

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2.

B. Personal Publications.

Friedman, B.I., Will, J.J., and Kilgore, J.J. A four day in vivo iron-59 citrate test of bone marrow erythropoiesis in humans. Clinical Research 10:282, 1962. *

Berry, H.K., Saenger, E.L., Perry, H., Friedman, B.I., Kerciakes, J.G., and Scheel, C. Deoxycytidine in urine of humans after whole-body irradiation. Science 142:396, 1963.

Friedman, B.I., Saenger, E.L., and Kreindler, M.S. Endoreduplication in leucocyte chromosomes. Preliminary report of its relation to cancer and whole body irradiation. The Lancet 2:494, 1964.

Saenger, E.L., Friedman, B.I., Kerciakes, J.G. and Perry, H. Metabolic Changes in Humans Following Total Body Irradiation. Report for May 1, 1963 to February 29, 1964, DASA Contract No. DA-49-146-XZ-029, Defense Atomic Support Agency, Washington, D.C.

Wright, T.J., Pahner, P.D., Friedman, B.I. and Will, J.J. The mechanism of formation of a "marker" chromosome in a transplantable spontaneous chloroleukemia in the Wistar rat. Ohio State Medical Journal 61:445, 1965. *

Friedman, B.I. Autologous bone marrow storage and infusion in patients receiving whole body irradiation. Ohio State Medical Journal (to be published in 1966). *

Friedman, B.I. and Toler, S.J. Chromosome studies of peripheral blood leucocytes separated by centrifugation in a Ficoll gradient. Experimental Hematology (to be published in 1966). *

Friedman, B.I., Schaefer, J.W., and Schiff, L. Increased ^{59}iron absorption in patients with hepatic cirrhosis. Journal of Nuclear Medicine (accepted for publication in 1966).

* abstract

2.

C. It is very difficult to determine the best method for storage of human bone marrow. The storage procedure of Kurnick (5, 6) may not be the most effective because of the use of glycerol and storage at only -80° C. However, it has been reported (4) that glycerol is as satisfactory as dimethyl-sulphoxide. The question of storage at -80° C. or -196°C. is a moot one that will warrant further investigation utilizing the experimental design cited here. Cavins (2) used -80° C., as have many other investigators. In the Annals of the New York Academy of Science, Vol. 114, 1964, there is an excellent review of the subject of bone marrow storage. Thomas (8) commented: "a sample of autologous marrow stored in anticipation of the possibility of irradiation accident would be of great value to the individual concerned." In the same publication Meyer (7) found no protective effect from autologous marrow on patients treated with nitrogen mustard. These reports illustrate the continued need for a prospective study as proposed.

References

1. Ashwood-Smith, M.J. Preservation of mouse bone marrow at -79° C. with dimethyl sulphoxide. Nature 190:1204, 1961.

2. Cavins, J.A., Kasakura, S., Thomas, E.D., and Ferrebee, J.W. Recovery of lethally irradiated dogs following infusion of autologous marrow stored at low temperature in dimethyl-sulphoxide. Blood 20:730, 1962.

3. Persidsky, M.D. and Richards, V. Radiation protection of mice with bone marrow and spleen preserved at low temperature using polyvinylpyrrolidone. Blood 23:337, 1964.

4. van Putten, L.M. The effectiveness of different freeze storage techniques for mouse-bone-marrow cell suspensions. Ann. N.Y. Acad. of Sci. 114: 695, 1964.

5. Kurnick, N.B., Feder, B.H., Montano, A., Gerdes, J.C. and Nakamura, R. Some observations on the treatment of postirradiation hematopoietic depression in man by the infusion of stored autogenous bone marrow. Ann. Int. Med. 51:1204, 1959.

6. Kurnick, N.B. Autologous bone marrow in the treatment of severe iatrogenic myelo-suppression. Diagnosis and treatment of acute radiation injury, 1961, Geneva, World Health Organization, p. 309.

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7. Meyer, L.M., Fliedner, T.D., Cronkite, E.P. Autologous bone marrow transfusion following chemotherapy. Ann. N.Y. Acad. of Sci. 114:499, 1964.

8. Thomas, E.D. Observations on supralethal whole-body irradiation and marrow transplantation in man and dog. Ann. N.Y. Acad. of Sci. 114: 393, 1964.

9. Thoma, G.E. and Wald, N. The diagnosis and management of the accidental radiation injury. Journal of Occupational Medicine 1:421, 1959.

3. BIOGRAPHICAL SKETCHES

(Give the following information for EACH key staff member, beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Ben I. Friedman, M.D.	Associate Prof. of Medicine Assistant Prof. of Radiology	Oct. 18, 1926
PLACE OF BIRTH (City, State, Country)	**PRESENT NATIONALITY (If non-U.S. citizen, indicate visa status)**	**SEX**
Cincinnati, Ohio, U.S.A.	American	☒ Male ☐ Female

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED
University of Cincinnati, Cincinnati, Ohio (Pre-Med.)		
University of Cincinnati College of Medicine	M.D.	1948

HONORS

MAJOR RESEARCH INTEREST

Hematology, Radiobiology, Nuclear Medicine

RELATIONSHIP TO PROPOSED PROJECT

Principal Investigator

RESEARCH AND/OR PROFESSIONAL EXPERIENCE (Start with present position; list ALL experience relevant to project)

Associate Professor of Medicine, University of Cincinnati, 1965
Assistant Professor of Radiology, University of Cincinnati, 1964
Assistant Chief Clinician, Hematology Clinic, Cincinnati General Hospital, 1956 - present
Assistant Professor of Medicine, University of Cincinnati, 1962-1965
Assistant Clinical Professor of Medicine, University of Cincinnati, 1959-1962
Instructor in Medicine, University of Cincinnati, 1955-1959
Fellow in Hematology and Nutrition, Cincinnati General Hospital 1953-1955
Senior Resident in Medicine, Duke University Hospital, July, 1950 - Oct. 1950 and Oct. 1952 - June, 1953
Junior Resident in Medicine, Cincinnati General Hospital, 1949-1950

Certified, American Board of Internal Medicine, 1956.
Fellow, American College of Physicians, 1963.

Biographical Sketch

Harry Horwitz, M. D. | Associate Prof. of Radiology | March 20, 1927

London, England | American | Male

Education -

	Degree	Year
University of London	M.B., B.S.	1950

Honors

Major Research Interest

Radiotherapy and Chemotherapy of Cancer

Relationship to Proposed Project

Radiotherapist

Research and/or Professional Experience

Associate Professor of Radiology, University of Cincinnati, 1965
Special Consultant, Radiological Health Training, U.S.P.H.S., Taft Sanitary Engineering Center, Cincinnati, Ohio, 1962 - present
Attending Radiation Therapist, Cincinnati General Hospital, 1960-present
Assistant Professor of Radiology, University of Cincinnati, 1960-1965
Senior Registrar, Radiation Therapy, Addenbrookes Hospital, Cambridge, England, 1958-1960
Fellowship in Radiation Therapy, Mt. Sinai Hospital, New York, 1957-1958
Registrar in Radiation Therapy, St. Bartholomew Hospital, London, England, 1954-1958

Biographical Sketch

James G. Kereiakes, Ph.D. Associate Prof. of Radiology August 15, 1924

Columbus, Ohio, U.S.A. American Male

Education	Degree	Year
Western Kentucky State College, Bowling Green, Ky.	B.S.	1945
University of Cincinnati, Cincinnati, Ohio	M.S.	1947
University of Cincinnati, Cincinnati, Ohio		

Honors

Major Research Interest

Radiobiology and Nuclear Physics

Relationship to Proposed Project

Physicist

Research and/or Professional Experience

Associate Professor of Radiology, University of Cincinnati, 1962 - present
Consultant, Radiological Health Research Activities, U.S.P.H.S., Taft Sanitary Engineering Center, 1964 - present
Member, Radiation Medical Advisory Committee, Ohio State Department of Health, 1962 - present
Consultant, Radiation Committee, Ohio State Medical Association, 1961-present
Radiation Safety Officer, University of Cincinnati, 1962 - present
Assistant Professor of Radiology, University of Cincinnati, 1959-1962
Deputy Director, Radiobiology Department, U.S. Army Medical Research Laboratory, Ft. Knox, Kentucky, 1957-1959
Supervisory Physicist, Radiobiology Department, U.S. Army Medical Research Laboratory, Ft. Knox, Kentucky, 1953-1957
Physicist, Environmental Medicine Department, U.S. Army Medical Research Laboratory, Ft. Knox, Kentucky, 1950-1953

CONSENT FOR SPECIAL STUDY AND TREATMENT

I, _____, do hereby give my consent to the members of the professional staff of the Cincinnati General Hospital, University of Cincinnati College of Medicine, to administer to me whole or partial body irradiation on or about _____, 196__.

The nature and purpose of this therapy, possible alternative methods of treatment, the risks involved, the possibility of complications, and prognosis have been fully explained to me. The special study and research nature of this treatment has been discussed with me and is understood by me.

Consent is given for photographs and publication for the advancement of medical education.

_____
Signature

Witnesses to signature:

_____

_____

Relationship _____
(Patient or Guardian)

Date _____ AM PM _____
(Mo. Day Yr.)

Place _____
(Ward-Clinic-Unit)

Chart No. _____

Original: to patient's chart
Copies: to Co-60 file
to TBR file

5/1/65

CONSENT FOR SPECIAL STUDY AND TREATMENT

I, _____, do hereby give my consent to the members of the professional staff of the Cincinnati General Hospital, University of Cincinnati College of Medicine, to perform a bone marrow aspiration and to store my bone marrow on or about _____, 19__.

The nature and purpose of this therapy, the risks involved, the possibility of complications, and prognosis have been fully explained to me. The special study and research nature of this treatment has been discussed with me and is understood by me.

Consent is also given for reinfusion (giving the marrow back to me) when the members of the professional staff recommend it.

Consent is given for photographs and publication for the advancement of medical education.

_____
Signature

Witnesses to signature:

_____

_____

Relationship _____
(Patient or Guardian)

Date _____ AM PM _____
(Mo. Day Yr.)

Place _____
(Ward - Clinic - Unit)

Chart No. _____

Original: to patient's chart
Copies: to Co-60 file
to TBR file

5/1/65