UNIVERSITY OF CINCINNATI

(INTERDEPARTMENTAL CORRESPONDENCE SHEET)

Revised Version of Mar 1967

To E. A. Gall, M.D.

From Ben I. Friedman, M.D. Date April 13, 1967

UCM1 951211 030

Dear Dr. Gall:

Enclosed is the revised proposal covering "The Therapeutic Effect of Total Body Irradiation Followed by Infusion of Stored Autologous Marrow in Humans." I think that this revision answers all of the questions which have been presented by the Faculty Committee on Research.

Should there by any additional questions, please contact me. I would appreciate hearing about this as soon as possible.

Sincerely yours,

Ben I. Friedman, M.D.

BIF/rla

Enclosure

Dr. Goffy, Radford, Kaylor

TO: Faculty Committee on Research

FROM: Ben I. Friedman, M.D. Principal Investigator

Eugene L. Saenger, M.D. Department Head

SUBJECT: The Therapeutic Effect of Total Body Irradiation Followed by Infusion of Stored Autologous Marrow in Humans

Purpose of Study:

The goal of this study is to test the hypothesis that total body irradiation followed by infusion of stored autologous marrow is effective palliative therapy for metastic malignancy in human beings. Total body irradiation has been used for palliation by others (1,4,5,6) but the dose used has been limited to less than 200 R because of the bone marrow depression which follows large doses of radiation.

A combination of total body radiation and infusion of autologous bone marrow may make it possible to use larger doses of total body radiation. Effective total body radiation therapy without the hematologic morbidity would be the most innocuous and least disturbing form of therapy available for the palliation of patients who have extensive neoplasm. Patients given this type of treatment have less morbidity from their therapy than comparable patients given 5-flurouracil or many other chemotherapeutic agents.

It is also planned to investigate techniques of marrow storage and to evaluate the optimum times for storage and infusion. Since we have already successfully separated viable peripheral blood lymphocytes from polymorphonuclears, in the future we expect to be able to separate bone marrow cells.

Ultimately, by tissue culture techniques, we intend to grow relatively pure cultures of "stem cells" from the fractionated marrow. Intravenous infusion of a few milliliters of multipotential "stem cells" should repopulate the marrow space as well as 600 ml. of the crude marrow cell suspension used at this time.

According to Pegg[1] "Autologous marrow aspiration and storage may be considered a wise precaution when new drugs and treatment schedules are being explored; it may then be possible to treat the occasional unexpectly severe hemopoietic depression more effectively." Mathe[2] has demonstrated in one patient that after 800 rad total body irradiation, preceded by 4 days administration of methyl-nitro-imidazolyimercaptopurine, a successful take of homologous marrow occurred.

It has already been proved that intravenous administration of bone marrow cells will repopulate the marrow space in mice[19], guinea pigs[19], rabbits[20], and dogs[21]. In mice[22], rabbits[23] and dogs[21] intravenous administration of stored marrow cells prevented death after an otherwise lethal dose of radiation. It is not possible to apply this information directly to human beings because of the variable response of different species. We, and others[24] have established <u>in vitro</u> viability of bone marrow cells stored at -80°C for as

long as one year. However, almost all *in vivo* studies in human beings where marrow transplant has been attempted did not involve total body radiation or adequate doses of radiomimetic chemotherapy. Therefore, it is necessary to test *in humans* the effectiveness of marrow in repopulating the marrow space following total body radiation and to proceed with developmental techniques and refinements of marrow infusion. In the management of patients with malignancy who are to receive myelotoxic agents or of patients who incur marrow injury in other ways (e.g., warfare, nuclear accident or exposure in space) the availability of stored autologous marrow may well be life saving.

Proposed procedures:

Five groups of patients will be investigated. Three groups of patients will receive or will have received 150 -200 rad total body irradiation as treatment for metastatic malignancy. The first group (Group I) will include patients who have *already been* irradiated at the 150-200 rad dose level and did not receive bone marrow infusion. A second group (Group II) will be given filtered* autologous bone marrow two weeks after irradiation. The third group

of patients (Group III) will be treated with 150-200 rad total body irradiation, followed by infusion through filters* of their previously stored marrow on the second day after irradiation.

The Group III patients will test the hypothesis that bone marrow "take" is best obtained early after radiation when presumably the marrow space is relatively empty and can accept viable cells more readily(3).

Patients selected for Groups II and III will be those who are to receive total body irradiation in the treatment of metastatic malignancy from various primary sites, excluding neoplasms which are treated by internal radiation, e.g., carcinoma of the thyroid. They will have stable hematologic values and may have received local radiation previously. Patients will be treated with 150-200 rad (midline absorbed tissue dose) of total body irradiation at an exposure dose rate of 4-6R/min. from a Cobalt 60 source. This dose of

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* The marrow will be infused intravenously with filtration through successive #50, #100 and #200 mesh filters (corresponding to openings of 297, 149 and 74 microns). Filters and tubing will be sterile and pyrogen free. Initially, all marrow will be infused through the filters. If the filtered marrow does not repopulate the marrow space, then unfiltered marrow will be infused to determine whether filtration was the reason the infusion was not effective.

radiation approximates the therapeutic and hematologic effects of 0.4-0.6 milligrams of nitrogen mustard per kilogram body weight (1, 4, 5, 6).

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 days pre-irradiation period and 1, 2, 3, 6, 9, 12, etc. days after irradiation according to the standard test days of Thoma and Wald[7]. Additional counts will be obtained as clinically indicated.

In Group II, bone marrow specimens for cytologic and pathologic examination will be obtained from the sternum and anterior ilium prior to irradiation, the day of infusion (prior to infusion), and at weekly intervals after infusion. In Group III, bone marrow specimens for cytologic and pathologic examination will be obtained from the sternum and anterior ilium prior to irradiation, 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[8,9]. The bone marrow will be cultured for sterility prior to freezing. In vitro viability of the marrow will be estimated by trypan blue exclusion initially[8], but tests utilizing tissue culture[10], DNA synthesis[11], and acridine orange[12] are being developed.

Peripheral blood data will be compared to those which we have already obtained in the sixteen patients (Group I) who have been irradiated at the same dose level but have not been infused. Total leucocyte count has been the most valuable variable in assessing hematologic injury following irradiation. The specimens of bone marrow will be studied for morphologic evidence of recovery.

All of the studies outlined for Group II and Group III were not performed on patients in Group I. Ideally, all groups should be run concurrently but it would seem unjustified to withhold autologous bone marrow therapy in patients exposed to these doses of radiation when marrow is available.

There will be a continuous review of the results as they are being obtained. If it becomes obvious that the results are statistically significant, the study will be terminated. A maximum of thirty-two additional subjects will be needed for this investigation. Since there are 16 patients in Group I who have already been irradiated, it is advisable to have 16 subjects in Groups II and III respectively.

The data from these three groups of patients will be compared with data obtained from patients with similar neoplasms who have been untreated (Group IV) and from patients who have received chemotherapy (Group V). Patients in Groups IV and V will be paired with patients in Groups I, II, and III according to age, sex, race, type, duration and extent of neoplasm. As a result of this comparison, it will be possible to determine the palliative value of total body irradiation at this dose level with and without bone marrow infusion.

Potential hazards:

(a) With any irradiation therapy there is the danger of radiation injury. We have observed consistent depression of hematologic values in the control group. One might wonder why 150-200 rad of total body radiation is selected as the treatment in this group of patients. As has been noted earlier, this dose range has therapeutic effectiveness equal to 0.4-0.6 mgm/kilo of nitrogen mustard. It is proposed because an observable therapeutic effect would be anticipated. At 150-200 rad, the effect of marrow reinfusion will be detectable. There are important differences between radiation and radiomimetic chemotherapy as well as certain similarities. A particular difference is of importance in this study. The margin of safety separating marrow death and lethal effects on other systems is smaller with chemotherapy than it is with total body irradiation; i.e., the amount of chemotherapy treatment to produce equivalent change in peripheral blood is more hazardous. Marrow infusion has no detectable effect unless very large doses of chemotherapy are used which then result in significant injury to other systems.

In this study the prodromal morbidity (anorexia, nausea, vomiting) would approximate 60%, As to mortality figures at the dosages to be evaluated, in human beings bone marrow aplasia occurs at 450 R and "intestinal death" occurs above 1000 R.

Without hospital care it is felt that a human LD 50 may approximate 500 R and with hospital care about 700 R. Therefore total body radiation between 450 R and 1000 R is the dose where death from bone marrow aplasia is expected. The doses proposed are below these levels.

When the mean survival time of patients treated with less than 100 rad, 125 rad, 150 rad, and 200 rad are compared there is no progressive decrease related to dose (Tables I, II). If the patients with survival times of 9 and 10 days are excluded and the patients still living at the present time are included there is still no significant difference in survival time which can be associated with the irradiation as determined by several statistical tests. In fact the data suggests that a therapeutic effect from the radiation therapy has been obtained.

The use of autologous and isogeneic marrow in humans has been investigated by others (8, 9, 13, 14). When marrow has been infused too rapidly, cerebral hemorrhage, hemoglobinemia and hemoglobinuria have been observed. These hazards have not been encountered when the rate of infusion has been maintained below 60 drops/min.

There is disagreement as to the need for filtration. Kurnick does not feel it is necessary at the time of infusion; Thomas, Ferrebee, and Pillow(13, 14, 15) recommend its use to minimize the incidence of pulmonary embolism. A series of filters will be used with the now operational constant rate infusion pump.

To date, we have infused unfiltered marrow intravenously in five patients. One patient had hemoglobinuria. Autopsy material from three of these patients has been studied by Drs. E. A. Gall and B. Yamaguchi. No evidence of pulmonary emboli, pulmonary infarction or other disease as a result of marrow infusion was found on microscopic examination.

b. All patients will be followed carefully clinically. Hematological data are accumulated at frequent intervals and all measures to control infection will be used. They may include plastic barrier and reverse isolation if needed. Prophylactic antibiotic therapy will not be prescribed.

Previous work done in this area:

During the past five years an ongoing study of the metabolic effects of total body irradiation has been in progress. To date, forty patients have been treated with 25 to 200 rad total body irradiation. Eighteen of the patients were given 150-200 rad. The clinical course of these patients has paralleled that of comparable patients treated with other agents. Bone marrow from twenty-three patients has been stored; it has been infused intravenously in five.

Marked hematologic depression occurred in all eighteen patients who received more than 125 rad total body radiation. There was a total leucocyte count depression below 2000 WBC/mm^3

and platelet count depression 25-40 days after irradiation. The mean minimum leucocyte count of previously untreated patients who received 150 rad total body radiation only was 1264 ± 1140, and was 1140 ± 816 when there had been previous therapy. The nadir of the leucocyte counts of patients who received 200 rad was 983 ± 369.

A comparison of the hematologic changes in patients receiving partial body irradiation with those who received total body irradiation revealed a paucity of change in the total WBC, platelets, and hematocrit even when the patient received more than 200 rad partial body radiation. In two of the ten patients who were given partial body irradiation, lymphopenia did not occur until 48 or 72 hours after exposure.

Infusion of stored autologous bone marrow has been completed in five patients. The marrow was infused intravenously without filtration(13). In one patient the response suggested marrow space repopulation.

Chromosome studies have been performed in these patients. In four patients who were studied in detail, endoreduplication has been seen with increased incidence in the peripheral blood chromosome preparations(16). This observation may be associated with malignancy but was increased following total body irradiation. As an outgrowth of the experience with endoreduplication, the question as to whether the malignant cell was the one which might be undergoing endoreduplication was considered. Therefore,

a method of cell separation utilizing a Ficoll gradient and ultracentrifugation was undertaken. Friedman, et.al., have reported adequate separation of lymphocytes from granulocytes. Chromosome culture techniques following this cell separation have revealed metaphases[17].

Another facet of considerable interest in patients receiving large doses of total body irradiation has been the question of immunologic response. In cooperation with A. J. Luzzio, studies of serum obtained from patients before and after total body irradiation have been performed. These data suggest an alteration in the antigenicity of human serum gamma globulin as a result of in vivo irradiation(18).

Method to be used in procuring consent of subjects above:

Consent forms, as attached, have been used since May 1, 1965. These forms are signed only after the patient has been advised of the research and study aspect of the procedures to be used. The patients are told this is a form of therapy but that benefits cannot be guaranteed. They are advised of the possible complications, including the possibility of pulmonary infarct following marrow infusion. Since total body radiation is another mode of treatment for patients with an ultimately fatal disease, it is only correct to indicate the possibility of therapeutic advantages. All patients are informed that a risk exists, but that all precautions to prevent untoward results will be taken.

General Remarks:

Many studies have been carried out in animals regarding the protective effect of bone marrow following radiation. They are well summarized in these paragraphs from Pegg's book.

"The possibility of protecting mice and guinea pigs with allogenic haemopoietic cells was first demonstrated by Lorenz and Congdon (Lorenz, et.al., 1952; Congdon, et.al., 1952). Similar results have been obtained in rabbits (Kilfinger, et.al., 1953; Porter, 1957b; Porter and Murray, 1958; Kupka, et.al., 1961); rats (Gorizontov, et.al., 1963) and, after some initial failures (Weston. 1958; Rothberg and Akeroyd, 1958), successful protection has also been obtained in the monkey (Crouch and Overman, 1957); (Overman, 1958). Similarly, there have been many failures in irradiated dogs (Porter and Couch, 1959; Stecher and Sullivan, 1959; Alpen and Baum, 1958; Puza, et.al., 1961; Jordan, et.al., 1961) but the Cooperstown group using more closely related dogs have reported more successful allogenic protection (Ferrebee, et.al., 1958; Thomas, et.al., 1958; Thomas, et.al., 1959). Others have since confirmed these findings (Trumbull, et.al., 1963; Winchell, et.al., 1963)."

"The survival of irradiated mice infused with guinea pig marrow was reported by Congdon, et.al., 1952, but others have been unable to confirm this (Barnes and Loutit, 1954a).

There is, however, wide agreement that rat bone marrow will protect the irradiated mouse (Vos, et.al., 1956; Nowell, et.al., 1956; and Gengozian and Makinodan, 1957)."

As was indicated under the "Purpose of this study," it is most important that methods of protecting against the undesirable effects of therapeutic efforts--radiation or other cytotoxic agents--be developed. Since hematologic depression is one of the earliest limiting biologic responses to such treatment, it is the first for which protective methods must be developed. Based on animal work, it would seem that autologous marrow is the safest protective technique to be used initially. This study is proposed as a continuation of the work cited for the following purposes:

1. To evaluate the role of total body radiation and autologous bone marrow in the treatment of extensive neoplasms.
2. To evaluate bone marrow transfusion in the treatment of bone marrow depression from chemotherapy.
3. To determine whether stored autologous bone marrow therapy may play a role in treatment of bone marrow depression following acute radiation exposure in warfare or occupationally induced accidents.

This work is considered to be of vital importance not only for improving the survival of patients with far advanced cancer but for the survival of the citizens of this nation in the event of nuclear warfare or a major peacetime radiation accident.

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TABLE I

Dose	Survival time in days			
	<50 rad	100 rad	150 rad	200 rad
	9	32	10	28
	73	49	25	36
	80	85	30	194
	113	91	32	298
	438	121	33	649
	740	197	54	885
	L*	200	74	
		239	151	
		324	262	
		446	270	
		646	592	
		650	830	
		L		
		L		

*Living

TABLE II

SURVIVAL TIME OF PATIENTS TREATED WITH TOTAL BODY RADIATION*

Dose	50 rad	100 rad	150 rad	200 rad
N	6	12	12	6
Mean	242	257	197	348
Median	96	198	64	246

*Excludes patients living at this time