Version (2)

May 4, 1971

TO: Evelyn Hess, M.D.
Chairman, Committee on Research, Cincinnati General Hospital

FROM: Edward B. Silberstein, M.D.
Principal Investigator
Eugene L. Saenger, M.D.
Department Head

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

PURPOSES:

1. To investigate the use of whole and partial body irradiation in the palliation and prophylaxis of certain forms of cancer as compared to other methods of treatment or to no treatment.
2. To develop biological indicators to determine effects of radiation so as to predict consequences of high dose and dose rate and large volume therapy.
3. To develop and evaluate therapeutic methods to increase margins of safety from the radiation effects.

Introduction:

Most forms of widespread metastatic cancer in man are not amenable to conventional chemotherapy with a few notable exceptions such as carcinoma of the breast and prostate. Curative radiotherapy requires doses of radiation generally in excess of 3500 rads, and such large amounts of irradiation cannot be given over wide areas of the body. [illegible] radiotherapy has been employed by several groups (1-5) as palli[illegible]static carcinoma and lymphoma. Table 1 summarizes the number of patients and types of irradiation employed to date [illegible] our belief in the

efficacy of this mode of treatment is the fact that the mammalian D_0 (the dose of radiation required to kill 63% of a mammalian cell population) lies between 100-180 rads for all cell types studied (6). Thus, even though larg masses of tumor in vivo may not always be comparable to these in vitro dose-response assays, some proportion of tumor cells irradiated should always be killed. This in turn should frequently relieve symptoms secondary to tumor pressure within or on an organ and, by lowering the number of tumor cells present, possibly prolong life.

Preliminary results published by E.L. Saenger, M.D. (Table 2) suggest an effect on prolonging life (7).

Results of these studies will be analyzed by means of the life table technique, i.e. percent surviving each year of those at risk for that year as compared to the survival figures of several papers which have examined the natural history of various carcinomas with liver or osseous metastases (8-9) and data from the Tumor Registry at the Cincinnati General Hospital. To further evaluate the efficacy of low dose-wide field radiotherapy, a protocol is under consideration randomizing patients with adenocarcinoma of the colon metastatic to the liver into groups receiving 5-fluorouracil 15 mg. per kg. (10) or 300 rads cobalt gamma-irradiation to a wide field from xiphoid to feet. Quality of survival will be measured by criteria revised by T.L. Wright, M.D. and appended to this protocol. We are cooperating with Frank Hendrickson of Presbyterian-St. Luke's Hospital, Chicago, and William Rider, M.D. of Princess Margaret Hospital, Toronto in sharing data on survival of whole body irradiated children with Ewing's tumor. The 3 children whom we have treated are alive and well 1 to 2 1/2 years post-diagnosis.

For patients receiving radiation doses in excess of [illegible] protection is required to avoid life-threatening leukopenia and thrombocytopenia. Provision of "sterile" environments (11,12), platelet (13) and

leukocyte (14) transfusions, and radioprotective chemicals (15) have been employed by others; we are using marrow autotransplantation to avoid these complications.

Proposed Procedure:

The patient has marrow removed from 0800 to 1000 hours in the operating room, is irradiated at about 1300-1400 hours and receives his marrow (stored at 4° C.) intravenously from 1430 to 1600 hours. The method of radiation dose calculation can be found elsewhere (16). The marrow transplantation technique follows:

Materials Required:

1 lavender top Vacutainer tube, labeled with patient's name and number, plus a form for CBC

1 red top Vacutainer tube, labeled with patient's name and number for the blood bank, plus a blood bank form

6 Kurnick needles

4 Vim-Silverman needles

10 20-gauge needles

3 Bierman needles

200 cc. heparin solution containing 50 units heparin per cc. This is made by putting one cc. of preservative-free heparin, 10,000 units per cc., in 199 cc. of normal saline. An extra vial of this heparin is brought to the operating room.

TC-199 culture medium, 100 cc. buffered with sodium bicarbonate to pH 7.3-7.4 and added to a Fenwal pack.

150 12 cc. sterile syringes to be obtained by the operating room.

Sigmamotor pump with tubing attachments.

4 2-way stopcock adapters

• Sterilized double filter system with two mesh filter openings of 297, and 149 micra diameter respectively.

o 2 Fenwal Blood Pack Units No. TA-10, 1000 cc.

1 thioglycolate culture tube

1 box sterile microscope slides

1 box cover slips

+ Abbott Venopak adapter

Normal saline for injection, 1- 30 cc. vial

+ 3 Abbott blood administration sets with filter opening measuring 150 by 200 micra.

10 alcohol sponges

1 hemostat

2 50 cc. syringes with 18 gauge needles

2 10 cc. syringes with 18 gauge needles

Formalin tube for Pathology Department study of marrow aspirate.

Blood bank request form

Scissors

Procedure:

The patient is premedicated with 0.6 milligrams atropine and 100 mg. Seconal intramuscularly, at 6 a.m. The patient goes to the operating room at approximately 7 a.m. where, after induction with Pentothal, endotracheal intubation is performed, and the patient is given general anesthesia with

---

:h Chicago, Illinois (Panheprin)

Buckeye Supply Co., 7775 Montgomery Rd., Cincinnati, Ohio 45236

oFenwal Laboratories, Div. of Travenol Laboratories, Inc., Morton Grove, Ill.60053

Halothane and placed in the prone position. After appropriate antiseptic preparation of the patient's iliac crest area, bone marrow aspiration may begin.

At this time, prior to aspiration, blood for peripheral white cell count and differential is obtained and 5 cc. of blood are sent to the blood bank for typing and crossmatching of 2 units of whole blood. As an alternat 500 cc. of blood may be obtained 1 week before the transplant and reinfused during the marrow aspirations, so as to eliminate artefactual results from heterogeneous cell populations.

Bone marrow aspirations are performed using the Bierman needles for the sternum, and Kurnick needles for the iliac crests. No more than 3 cc. are obtained with the needle bevel in one position. The bevel is rotated through four quadrants for each aspiration. Aspiration is performed with a syringe which has been filled with one cc. of the heparin solution containing 50 units per cc. An Abbott Venopak adapter connects a sterile bottle containing 200 cc. of heparin solution, 50 units per cc., to a two-way stopcock adapter in which each syringe is placed to fill it with one cc. of heparin. This stopcock is also turned so that the heparin solution is a closed system between aspirations.

The syringe with aspirated marrow is inverted several times to distribute the heparin and handed to an assistant. She injects it through a two-way stopcock adapter into the Fenwal pack which contains TC 199. The emptied syringe is left in place but the stopcock handle is turned each time so as to close off the Fenwal pack system. After each aspiration another assistant

applies pressure to the area from which marrow has been obtained to decrease hematoma formation. The areas to be aspirated will be posterior and anterior iliac crests and the sternum. After 500 cc. of marrow are collected, the bag is inverted 5-10 times and then a sample is obtained for nucleated cell count and differential.

Infusion:

For infusion we employ the Sigmamotor pump and infusion set, alcohol sponges, hemostat, 10 cc. syringe fitted with a 20-gauge needle, a sterile bottle of 30 cc. of 0.9% saline for injection, scissors and the double filter system noted above. Three Abbott blood infusion sets are present for emergency use.

After the marrow has been collected and stored in the blood bank at 4° C. for several hours while the patient receives whole body irradiation, it is then taken to the patient's ward where the Fenwal pack holding the marrow is inverted several times to insure homogeneous distribution of cells and then fitted to the Sigmamotor infusion system. The Sigmamotor pump is turned on and the marrow passes through the double filter system downstream from the pump at a rate of 60 to 90 drops (4-6 cc.) per minute Aliquots are collected immediately after the pump is turned on for the determination of cell viability, nucleated cell count and differential, as well as blood culture.

If there is evidence of malfunction of the filter system, as shown by bubbles appearing in the infusion tubing after the marrow is filtered, the pump is turned off and the intravenous drip of normal saline through which the marrow has been infused "piggyback" is restarted. The Fenwal

pack containing the marrow is then connected to a standard Abbott blood infusion set which is attached to the bottle containing this saline solution and the infusion begun again. After the infusion has been completed, sterile normal saline is flushed through the filter set which has been disconnected from the pump.

The marrow infusion is regularly followed by chills and fever unless 10 grains of aspirin are given orally or rectally during the procedure. Blood cultures are routinely obtained and have always been sterile.

To calculate the actual number of marrow cells administered, we count a filtered specimen using Kurnick's technique (17) and obtain the nucleated cell count per single hemocytometer square by counting eight squares and dividing the total by eight. Then we multiply this number by (10,-to get count per mm^3) x (40, dilution factor in counting) x (1000, number of mm^3 in 1 cm^3) x (fraction of viable cells) x (500 cc. volume of marrow) x (1.4, the dilution correction from 100 cc. TC 199 and 100 cc. of heparin in the Fenwal pack) x (fraction of marrow made up of erythroid and myeloid precursors, including stab cells). This simplifies to (cell count per hemocytometer square) x (2.8) x (10^8) x (fraction of viable cells) x (fraction of marrow cells).

Intravenous infusion of bone marrow has been known to repopulate the marrow space (18-22) of animals and there is now ample experience with human subjects as well (23). The marrow transplant technique developed in our laboratory involves a fully closed system, a significant advance over the technique published recently by the Seattle group (24).

At present we are continuing the immediate retransfusion of autologous marrow. Our earlier studies on marrow transfused after storage at -83° C indicated (by vital staining) viability of 20-50% compared to 95-99% if infused within 6 hours of aspiration; this storage technique will require improvement. It will then be possible to consider evaluation of transfusion at intervals between the onset of irradiation and 21 days especially if therapy is fractionated. We wish to return unirradiated marrow to its necessary microenvironment (25) as soon as possible to avoid any delay in stem cell proliferation and maturation in vivo. If one delays giving marrow until the cell counts have fallen to clinically dangerous levels in the fourth week, one must still allow 5 to 10 days for the precursors of circulating leukocytes (26) and probably the same time for platelets (27) to differentiate. By this time the patient is either seriously ill or recovering spontaneously. With infusion of marrow at this late time one cannot then tell if the marrow transplant has been efficacious, whereas if the marrow is given on the day of radiotherapy the severe cytopenia during the expected nadir of peripheral blood cell counts does not occur, indicating a successful transplant.

Our patients receive daily white blood cell counts and differential white counts, platelet and reticulocyte counts, and a check of the hematocrit and hemoglobin levels. They are sent home two days post-irradiation and the degree of cytopenia observed post-irradiation in our patients has not warranted rehospitalization. Thus the total time consumed for the procedure is 3 1/2 hospital days with or without marrow transplantation, divided as follows:

Related Studies:

Assessment of the effects of large field low dose irradiation is under way for the following parameters:

1. Psychologic performance decrement.
2. Chromosome dose-response.
3. Biochemical indicators of radiation damage.
4. Radiation-induced alterations of the function of circulating peripheral blood cells.
5. Radiation-induced alteration in immunologic response to a set of phages, covered under a separate protocol of Hess, Litwin, et al.

Method to be Used in Procuring Consent:

These forms, containing in lay language an explanation of the procedures described above have been forwarded to your Committee.

The patient is told that the radiotherapy to be given is not a cure but that it may relieve pain (sham radiation does not have this placebo effect in our cancer patients) and that its efficacy in prolonging life is under study.

Table 1

TOTAL CASES RECEIVING LARGE FIELD IRRADIATION
IN RADIOISOTOPE LABORATORY
88 CASES

TBR	8	20	17	14	-
UBR	-	1	1	2	3
LBR	1	2	2	6	9
TRUNK	-	-	-	2	-

Table 2

ELAPSED TIME TO DEATH FOLLOWING
TOTAL AND PARTIAL BODY RADIATION

Midline dose (rads)	N	Days from irradiation to death: Mean	Median
Total-body			
50	6	242	96
100	12	257	198
150	12	197	64
200	6	348	246
	36 patients		
Partial-body			
100	3	72	38
150	2	101	--
200	6	138	133
300	2	212	--
	13 patients		

Ref. 7. From "Effects of Total and Partial Body Therapeutic Irradiation in Man", E.L. Saenger, M.D. in Proc. 1st International Symposium in the Biological Interpretation of Dose from Accelerator Produced Radiation, Lawrence Radiation Laboratory, Berkeley, Calif., March 13-16, 1967. USAEC/Div Technical Information Conf. - 670305

MODIFICATION OF THE KARNOFSKY (1951) PERFORMANCE STATUS RATINGS

Thomas L. Wright, M.D.

These new performance status ratings are suggested by the fact that in the group of patients in Phase I of the study, the performance ratings according to the system of Karnofsky (1951) seemed to bunch around 70 and then fall precipitously when clinical metastatic disease supervened. These modifications, it is hoped, will provide a greater range in the upper performance ratings and somewhat more objective criteria for any given rating. This new system ignores completely objective signs of disease and is concerned entirely with performance.

Out-Patient Only	Full Activity	100%	No symptoms
		90%	Minor symptoms (e.g. cough, sputum) which do not interfere with patient's activities.
		80%	Minor symptoms but normal activity is only minimally impaired. Capable of full-time work.
	Impaired Activity	70%	Not capable of full-time work but still able to perform part-time work.
		60%	Not capable of part-time work but able to care completely for self at home.
	Minor Care Problem	50%	Some assistance required but this is minor (e.g., cooking, washing clothes for women; men no longer doing minor household tasks). Spends less than 4 hours in bed at home per day. Capable of leaving home unassisted.
Generally Hospitalized	Major Care Problem	40%	Disabled. Spends more than 4 hours in bed at home per day. Hospitalization may be required if no assistance available at home. Hospitalized for acute course palliative therapy.
		30%	Severely disabled. Major care problem in the home. May be hospitalized although patient and family may elect to remain at home.
		10%	Hospitalization necessary for terminal care or for active supportive treatment. Requires full and complete nursing care.

Reference

Karnofsky, D. A., et al., Arch. Int. Med. 87, 477 (1951)

REFERENCES

1. Saenger, E.L., Silberstein, E.B., Aron, B., Horwitz, H., Kereiakes, J.G., Chen, I., Winget, C. and Gleser, G.C. Radiation Effects in Man: Manifestations and Therapeutic Efforts, Defense Atomic Support Agency Report No. 2599, 30 April, 1970, pp. 92.

2. Johnson, R.E. Total Body Irradiation of Chronic Lymphocytic Leukemia: Incidence and Duration of Remission, Cancer 25:523-530, 1969.

3. Johnson, R.E. Total Body Irradiation with Fractionated Dosage Schedules, Front. Radiation Ther. Onc. 3:152-162, 1968.

4. Collins, V.P., Loeffler, R.K. and Hyman, G.A. Comparative Effects of Total Body Radiation, Nitrogen Mustard and Triethylene Melamine on the Hematopoietic System of Terminal Cancer Patients, Science 118:161-163, 1963.

5. Miller, L.S., Fletcher, G.H. and Gerstner, H.G. Systemic and Clinical Effects Induced in 263 Cancer Patients by Whole Body X-irradiation with Nominal Air Doses of 15 to 200 R. School of Aviation Medicine, U.S.A.F., Report No. 57-92, 1957.

6. Little, J.B., Cellular Effects of Ionizing Radiation, New Eng. J. Med. 278:308-315, 1968.

7. Saenger, E.L. Effects of Total- and Partial-Body Therapeutic Irradiation in Man, in Proc. 1st International Symposium on the Biological Interpretation of Dose from Accelerator Produced Radiation, Lawrence Radiation Laboratory, Berkeley, California, March 13-16, 1967; USAEC/Div. of Technical Information, CONF-670305.

8. Rapoport, A.H. and Burleson, R.L. Survival of Patients Treated with Systemic Fluorouracil, Surg., Gyne. and Obstet. 130:773-777, 1970.

9. Cady, B., Monson, D.O. and Swinton, N.W. Survival of Patients After Colonic Resection for Carcinoma with Simultaneous Liver Metastases, Surg., Gyne., Obst. 131:697-700, 1970.

10. Horton, J., Olson, K.B., Sullivan, J., Reilly, C., Shrider, B. and the Eastern Cooperative Oncology Group, Five-Fluorouracil in Cancer: An Improved Regimen, Ann. Int. Med. 73:897-900, 1970.

11. Bodey, G.P., Hart, J. and Freireich, E.J. Prolonged Survival of a Leukemic Patient in a Protected Environment, Am. J. Med. Sci. 256:112-121, 1968.

12. Penland, W.Z. and Perry, S. [illegible] Isolator, Lancet 1:174-176, 1970

13. [illegible] Gardner, F. Storage of Platelet Concentrates [illegible], Blood 35:549-557, 1970.

14. Eyre, H.J., Goldstein, I.M., Perry, S. and Grew, R.G. Leukocyte Transfusions: Function of Transfused Granulocytes from Donors with Chronic Myelocytic Leukemia, Blood 36:432-442, 1970.

15. Carr, C.J., Huff, J.E., Fisher, K.D. and Huber, T.E., Protective Agents Modifying Biological Effects of Radiation, Arch. Environ. Health 21:88-98, 1970.

16. Saenger, E.L., Friedman, B.I., Kereiakes, J.G., Perry, H., Metabolic Changes in Humans Following Total-Body Irradiation, Defense Atomic Support Agency Report No. 1844, February 1960 through April 30, 1966.

17. Kurnick, N.B. "Autologous Bone Marrow in the Treatment of Severe Iatrogenic Myelosuppression" in Diagnosis and Treatment of Acute Radiation Injury, World Health Organization, Geneva, 1961, pp. 309-327.

18. Lorenz, E., Congdon, C.C. and Uphoff, D. Modification of Acute Irradiation Injury in Mice and Guinea Pigs by Bone Marrow Injections, Radiology 58: 863-877, 1952.

19. Porter, K.A. Effect of Homologous Bone Marrow Injections in X-irradiated Rabbits, Br J. Exper. Path. 38:401-412, 1957.

20. 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 20:730-734, 1962.

21. Ferrebee, J.W., Billen, D., Urso, I.S., Lu, W.C., Thomas, E.D., Congdon, C.C., Preservation of Radiation Recovery Factor in Frozen Marrow, Blood 12:1096-1100, 1957.

22. Porter, K.A. and Murray, J.E., Successful Homotransplantation of Rabbit Bone Marrow After Preservation in Glycerol at -70 Degrees C., Cancer Res. 18:117-119, 1958.

23. Bortin, M., A Compendium of Reported Human Bone Marrow Transplants, Transplantation 9:571-587, 1970.

24. Thomas, E.D. and Storb, R., Technique for Human Marrow Grafting, Blood 36: 507-514, 1970.

25. Knospe, W.H., Blom, J. and Crosby, W.H., Regeneration of Locally Irradiated Bone Marrow. II. Induction of Regeneration in Permanently Aplastic Medullary Cavities, Blood 31:400-405, 1968.

26. Cronkite, E.P. and Vincent, P.C., [illegible] Disorders of Animals and Man, Atomic [illegible] Symposium Series No. 13, A[illegible] Press, 1970.

[illegible] and Finch, C.A., Thrombokinetics in Man, J. Clin. Invest. 48:963-974, 1969.

28. Saenger, E.L., Silberstein, E.B., Horwitz, H., Kereiakes, J.G., Chen, I., Winget, C., Gleser, G. C., Kunkel, R., Radiation Effects in Man: Manifestations and Therapeutic Efforts, Defense Atomic Support Ags Report No. 2428, 30 April 1969. pp. 64.

29. Winitz, M., Adams, R.F., Seedman, D. A., Davis, P.N., Jayko, L. G. Hamilton J.A., Studies in Metabolic Nutrition Employing Chemically Defined Diets, Vivonex Corp. 867 West Dana St., Mountain View, California, 1969, pp. 55.