

CHAPTER VI

Clinical Applications of Bone Marrow Transplantation and Related Experiments

The identification of haemopoietic chimaerism following the transplantation of bone marrow to lethally irradiated animals offered a variety of theoretical possibilities not only for the restoration of an atrophic haemopoietic system but also for the replacement of abnormal blood forming tissue. In addition, this development seemed to be the beginning of a real breakthrough in the field of organ transplantation because it had been demonstrated in experimental animals that the homograft rejection could be completely avoided by the replacement of the host's immunological system by cells of the future organ donor.

Interest has been very much concentrated on the application of bone marrow transplantation in the treatment of two conditions: haemopoietic failure—radiation-induced or from other causes—and leukaemia. In neither of these conditions, however, has any consistent success been achieved, although a considerable number of clinical trials have been made in the past 5 years. In addition, a few unsuccessful trials have been made with high doses of whole body irradiation and bone marrow replacement as a preliminary to kidney transplantation.

Apart from many disappointing experiences, it seems, however, that some limited but real progress had been made, which justifies a careful continuation of clinical work concerned with bone marrow transplantation. Many of the clinical trials were destined to fail from the outset, however, because some if not all conditions known from animal experiments to be necessary for graft acceptance were ignored. In other instances the results have made it quite clear that extrapolation from rodents to man is a very unreliable approach in transplantation biology.

One of the fundamental advances of recent years seems to be the realisation that the reactions of monkeys to both irradiation and bone

marrow transplantation resemble, in many respects, those of humans. It is reasonable to expect, therefore, that continued experimentation with monkeys will eventually contribute substantially to a more precise evaluation of the possibilities and the limitations of bone marrow transplantation in human patients. In one or two centres the same problem is being thoroughly studied in dogs but it is not certain, as yet, whether this species exhibits a graft versus host reaction of comparable severity and pathology following homologous bone marrow transplantation as is seen in primates.

The problems associated with graft rejection and with a graft versus host reaction are absent when isologous bone marrow is available. A number of leukaemic partners of identical twins have been treated with a high dose of whole body irradiation followed by transfusion of fresh isologous bone marrow. These trials will be discussed in more detail later: let it be sufficient to note here that it is now generally accepted that the leukaemia cannot be eradicated by doses of radiation after which bone marrow transplantation could be expected to be beneficial.

The only other application of isologous bone marrow which may be justified is its use for the restoration of haemopoietic function in cases of bone marrow failure. The opportunity for this kind of therapy arises, of course, only rarely, but the results so far reported are encouraging.

Autologous bone marrow, while having the same immunological advantages as isologous cells, is obviously not available for the treatment of spontaneous bone marrow failure. Its application in the treatment of malignant diseases in which the bone marrow is not affected would seem, however, to be a logical development. If a sufficient quantity of autologous bone marrow is collected and stored before the administration of large doses of radiation or of cytotoxic drugs, a subsequent haemopoietic failure can be treated effectively. In most cases this approach requires adequate freezing and storage facilities but, unfortunately, the effectiveness of storage methods cannot be evaluated accurately because of the absence of markers to demonstrate the proliferation of the infused cells.

It has been pointed out previously (Chapter II) that the best approach to the problem of preservation of bone marrow cells at low temperatures is provided by the experiments with monkey bone marrow. It seems worth while to consider the collection and storage of autologous bone marrow from patients who are undergoing kidney

transplantation followed by prolonged treatment with immunosuppressive agents. Bone marrow aplasia is a not infrequent complication in such cases and the reinfusion of stored autologous bone marrow might be tried to restore the patient, although this measure obviously entails the risk of a rejection of the kidney graft.

When autologous cells are not available the question arises, whether or not homologous bone marrow is of any use in the treatment of bone marrow aplasia caused by cytotoxic drugs. These problems have received extensive, but by no means exhaustive, study in experimental animals.

Treatment of haemopoietic failure following irradiation

HOMOLOGOUS BONE MARROW TRANSPLANTATION

The restoration of lethally irradiated subjects with bone marrow transplants has without any doubt a most impressive experimental basis. Much of this basic work with animals was initiated with the aim of finding a method to treat accidentally irradiated people and victims of nuclear warfare. Thus far, clinical bone marrow transplantation has been performed only once for this purpose, namely, in the treatment of the people involved in the now famous Vinca accident. Six laboratory workers, five men and a woman, were heavily irradiated with neutron and gamma rays in the course of a critical nuclear excursion of a Zero energy reactor at the Boris Kidrich Institute of Nuclear Sciences, Belgrade, Yugoslavia. The victims were flown to Paris and treated by a team of French and Yugoslav physicians headed by Jammet and Mathé^{197, 198, 265}.

The doses of radiation to which the victims were exposed have been estimated in a number of ways by several groups of investigators, who arrived at widely differing results (Table VI: 1). The clinical findings were in accord with the view that patient B. had received a sublethal dose of irradiation and that V. had probably been the most heavily exposed. Although the other patients had all been exposed to irradiation within the lethal dose range, it was not possible to decide whether in fact the irradiation would have proved lethal without bone-marrow transplantation. It has to be taken into account that an inhomogenous dose distribution is very likely to occur under the conditions of such accidents which tends to lower the biologically effective total dose.

The patients were nursed under conditions of strict aseptic isola-

TABLE VI: I. Estimated neutron and gamma doses received by the persons involved in the Vinca accident

Patient	Jammet <i>et al.</i> (1959) ¹⁹⁸		Hurst and Ritchie (1959) ¹⁸²		Auxier (1961) ¹²		
	neutron (rem)	gamma (rem)	neutron (rem)	gamma (rem)	charged particle dose (rad)	H(n, γ)D gamma dose (rad)	total radiation dose (rad)
V	210	630	320	320	89	133	214
M	214	642	290	290	87	130	209
G	230	690	300	300	90	135	189
D	256	768	250	250	91	136	192
H	174	522	210	210	66	99	158
B	102	306	175	175	45	67	95

tion to prevent the introduction of pathogenic micro-organisms (so-called barrier nursing), and treated with antibiotics, blood and platelet transfusions and other supportive measures.

Fourteen days after the exposure, patient V. received 4×10^9 spleen and liver cells from a 5 month old human foetus, which failed to result in any clinical or haematological improvement. During the 4th week the patient developed peritonitis and symptoms of intestinal invagination, followed by anuria and jaundice. On the 27th day this patient received 8.5×10^9 homologous bone marrow cells, but died a few days later from a massive haemorrhage. The other 4 patients received between 8.5 and 14×10^9 nucleated homologous bone marrow cells between days 27–32 after the exposure. This was followed in some cases by a rapid and in others by a more gradual recovery of the peripheral blood elements. In 3 patients a slight increase of donor type erythrocytes was seen for a short time; however, the proportion of donor type erythrocytes decreased to insignificant values within a month or two. Satisfactory clinical and haematological recovery was obtained 4 months after the exposure and a follow-up study over 2 years revealed persistent low lymphocyte values but no other haematological abnormalities.

Although the clinical course and the treatment of these patients has been reported extensively, there is no general agreement over the contribution made by bone marrow transplantation to the recovery of the patients. Cronkite and Bond¹⁰⁶ in particular have favoured the possibility that the increase of the peripheral blood cell counts which occurred soon after the bone marrow transfusions may have been spontaneous, and that the presence of donor type red cells for a few weeks cannot be considered as convincing evidence for the take of the bone marrow graft.

The fate of these patients without bone marrow treatment is, of course, open to speculation but in view of the other radiation sequelae, for instance the prolonged azoospermia³⁴², it seems likely that in at least 2 cases, the bone marrow was life-saving. Even a limited and temporary proliferation of the graft could well be of great benefit, since it might carry the patient over an extremely dangerous period. Although the patients survived for as long as four weeks prior to bone marrow transplantation it does not follow that the dose of irradiation was, in each case, sublethal because extensive symptomatic treatment was given during that period.

Regeneration of the host's haemopoietic tissues with concomitant

disappearance of the grafted cells prevented the occurrence of secondary disease in all patients. The cause of this rather early reversal is not clear, but it may have been related to the type of radiation exposure which undoubtedly resulted in an inhomogeneous dose distribution. The experience with the Yugoslav patients also indicates that the risk of an MLD effect in man is probably less than would be expected from the experiments with certain mouse strains (see Chapter II).

The feasibility of homologous bone marrow transplantation was thoroughly discussed at a Symposium of the World Health Organization on the Diagnosis and Treatment of Radiation Injury, held in 1960. Loutit²³², in his appraisal of the reports, thought that the MLD effect could be largely discounted in man and that if this were so, marrow could be prescribed with much more confidence than was formerly justified. The only remaining concern would be the development of a graft versus host reaction after higher dose levels of irradiation, when a permanent take of the foreign bone marrow is more likely to occur.

It is evident that at these dose levels the alternative to the possibility of the development of secondary disease is the risk of the patient succumbing to bone marrow failure.

The opposing view-point has been expressed with great perspicuity by Lajtha²¹³ who concluded that any dose of single whole-body radiation which would allow survival with marrow grafting, would also allow recovery of the remaining host marrow elements without the need for marrow grafting, if careful symptomatic therapy were applied. In his opinion "there appears to be no *prima facie* case for marrow grafting either following irradiation or following treatment with tumour chemotherapeutic agents". His thesis is based mainly on the use of dose-survival curves for proliferating cells of various types following *in vitro* and *in vivo* irradiation, to predict haemopoietic recovery in humans. According to this reasoning, the number of haemopoietic cells which survive an LD₅₀ of whole body irradiation would be equal to the minimum number of haemopoietic cells required for successful autologous therapy after lethal irradiation. The former cell number (10⁹ cells/kg body weight using the survival curve from Lajtha's paper and 500 rads as an LD₅₀ for man) appears to be greater than those which have usually been employed in clinical trials (10⁸ isologous cells/kg body weight). Pegg³⁰² has calculated that the number of bone marrow cells which would be

expected to survive a 100 per cent lethal dose of whole body irradiation in various species is 3–16 times higher than the number of isologous cells known to provide complete protection. One explanation of this discrepancy would be provided by the demonstration of the greater radiosensitivity of bone marrow cells *in vivo* than the hitherto accepted value of $D_0=140$ r.* Recently, McCulloch and Till²⁶⁸ have indeed demonstrated a D_0 of 95 rads for mouse bone marrow irradiated *in vivo*. This would leave less than 10^6 cells intact out of the total number of 10^9 bone marrow cells of a 25 gram mouse following 800 rads of whole body irradiation ($\sim LD_{100}$). For effective isologous marrow therapy in mice most authors have found 10^5 – 10^6 cells necessary.

These considerations tend to invalidate many of Lajtha's objections to marrow therapy. If the same radio-sensitivity applies to human bone marrow, a dose of 500 rads would leave 7×10^7 surviving cells per kg body weight which makes the therapeutic effects of 10^8 isologous cells/kg much more acceptable. Lajtha also refutes the idea of tiding the patient over the crucial days with a temporary *homograft* because daily platelet transfusions would be much more effective. Theoretically this may be true, but it is general clinical experience that the prolonged maintenance of adequate thrombocyte levels in thrombopenic patients is extremely difficult. In addition, a bone marrow graft which is functioning only temporarily may also produce granulocytes which may be more effective in the prevention of dangerous infections than any substitution therapy or antibiotic treatment. The difficulties in the collection of a sufficiently large number of cells, as pointed out by Lajtha, have been largely overcome, and Mathé's group has shown that even a single living donor can supply enough cells to repopulate an irradiated recipient to a significant extent.

* For an exponential survival curve, D_0 (mean lethal dose or inactivation dose) is defined as the dose required to reduce the surviving fraction of cells to 37 per cent of the original (approximately to e^{-1}). For other survival curves the D_0 may be described as a measure of the rate at which surviving cells are killed by a given increment in dose. D_0 instead of a LD_{50} has been used most often because of its convenience in the application of the target theory of the action of ionizing radiations on individual cells. Lajtha used a D_0 value of 160 rads for his calculations: after a whole body dose of 500 rads, 10 per cent of the bone marrow cells would survive; that is, if adult marrow contains 5×10^{11} bone marrow cells, 5×10^{10} cells are expected to survive. This value is to be compared with 10^8 isologous cells/kg body weight which was found to bring about regeneration in patients following even higher doses of whole body irradiation.

In conclusion, it seems fair to say that in Lajtha's categorical rejection of bone marrow transplantation, theoretical considerations have been allowed to outweigh an impressive body of experimental data obtained from experiments both with a variety of animal species and also the few proven takes of homologous bone marrow in leukaemic patients; the latter will be discussed in a later section.

AUTOLOGOUS BONE MARROW REINFUSION FOLLOWING IRRADIATION

The study of autologous bone marrow therapy is preferable in larger animals because of the difficulty in obtaining a sufficient quantity of material in smaller ones. Bone marrow can be stored at room temperature for several hours without appreciable loss of viability so that there is ample time for irradiation of the animal if fresh bone marrow has to be reinfused. In the treatment of human patients, whole body irradiation at lethal doses is usually spread over one to two days and in some cases over much longer periods of time so that the preservation of the marrow at low temperatures becomes imperative.

Alpen and Baum² have shown that dogs can be protected from a lethal dose of 600 r (midline dose in air) by a minimal number of 1.5×10^8 fresh autologous marrow cells per kg body weight. The Cooperstown group has studied the therapeutic value of autologous marrow after storage at 4° C for periods of up to 96 hours and at -79° C for 25-120 hours²⁴⁹, and has reported on more than 30 dogs which survived lethal whole body irradiation as a result of this treatment¹⁶⁶. The midline X- and γ -ray doses varied between 600 and 1800 r (dose rates as low as 2 r/min. were used in some cases). The number of bone marrow cells which were administered varied between 2×10^8 and 1×10^9 nucleated cells/kg but the minimal number required for protection was not reported.

Similarly in monkeys, fresh autologous bone marrow was effective following whole body X-ray exposures of 850-925 r¹⁰⁸, the minimum number of cells required for consistent recovery being about $10^8/\text{kg}$ ³³⁶.

In view of these data it would be expected that adequate treatment of adult human patients requires at least 5×10^9 autologous bone marrow cells or 10^8 cells per kg.

The use of frozen autologous bone marrow for the treatment of haemopoietic depression in patients with malignant tumours following partial body irradiation has been studied most extensively by

TABLE VI: 2. Amount of isologous bone marrow administered to irradiated leukaemic patients

Authors	Age	Midline dose of radiation	No. of isologous bone marrow cells ($\times 10^9$)	Effect on haemopoiesis
Atkinson <i>et al.</i> (1959) ¹¹	1 y./6 m.	255 r	2.65	Regeneration
Thomas <i>et al.</i> (1959) ¹⁰⁶	2 y./11 m.	1000 r*	2.5	Regeneration
	4 y./9 m.	750 r	3.9	Late regeneration
Thomas <i>et al.</i> (1961) ¹⁰⁴	25 y.	840 r	17.0	Regeneration
	38 y.	1595 r	9.6	Defective regeneration

* This patient had received 200 r whole-body irradiation 3 months previously

Kurnick and his collaborators^{209, 210, 212}. His data reveal no clear relationship between therapeutic success in terms of haemopoietic recovery and the number of reinfused cells; successes were recorded with as few as 4×10^8 cells and failures with 10 times as many cells. This may be related to the fact that many patients still had areas of active bone marrow although the total production of cells was inadequate. Kurnick considers autologous bone marrow of definite value in the treatment of radiation induced bone marrow aplasia even in cases of long duration. In his opinion, however, haemopoietic depression following chemotherapy does not generally require treatment with bone marrow because of the strong tendency for spontaneous recovery.

Following whole body irradiation of three children in the terminal stage of leukaemia with doses between 470 and 550 r, McGovern *et al.*²⁷¹, administered autologous bone marrow, which had been stored for 5 months at -70° C. In the two patients who received 5.4 and 3.0×10^9 nucleated cells no repopulation of the marrow was seen, while in the third patient recovery of haemopoiesis occurred following the administration of 2.3×10^9 cells. The effectiveness of the preservation in all these studies with frozen autologous marrow remains completely unknown and, moreover, no proof has been obtained that a take of transplanted cells was responsible for the recovery of the blood forming system.

The number of cells used in the therapy of whole body irradiation with fresh isologous marrow is somewhat higher than 10^8 /kg body weight (Table VI: 2), but it remains possible that an overdose of cells was administered to these patients. An evaluation of the results in terms of numbers of bone marrow cells administered is difficult, in view of the wide variations in radiation exposure and because the subjects were suffering to a varying degree from a variety of diseases.

Autologous bone marrow after chemotherapy

EXPERIMENTS WITH ANIMALS

Studies with experimental animals on the feasibility of bone marrow grafts as a way to combat the lethal effects of high doses of cytotoxic drugs have been in progress since 1957. As would be expected with those drugs that cause death from haemopoietic failure, the results leave no doubt that this method can be effective in the absence of immunogenetic differences between host and donor.

Inspection of Table VI: 3 reveals, however, that the available data leave many questions unanswered. The number of bone marrow cells administered has been uniformly rather high, so that no systematic information has emerged concerning the minimal quantity of bone marrow required for protection. Since in many experiments only partial protection was obtained, it must be concluded that bone marrow therapy is possibly less effective in these cases than with cases of whole body irradiation. At least in mice, this could be due to the relatively more pronounced damage to the intestinal epithelium produced by many of these drugs.

Since the majority of the experiments have been performed with a single dose of the toxic agent at a particular level, the toxic range in which bone marrow grafting may be useful still remains unknown. The work published to date does not represent much more than "screening" of the efficacy of bone marrow, and a more detailed and refined evaluation of the possibilities must involve far more time and larger numbers of animals.

It must also be realised that it is still uncertain whether the results thus obtained could be extrapolated with safety to humans.

CLINICAL TRIALS

Clinicians, nevertheless, have been quick to use this new addition to their therapeutic arsenal, particularly since reinfusion of autologous bone marrow entails no undue risk to the patient. As with its application in heavily irradiated patients, the availability of autologous bone marrow would seem to allow the administration of higher doses of chemotherapeutic agents, which might otherwise induce irreversible damage to the haemopoietic system. It must be pointed out at once that clinical experience with this technique is far too limited to allow an evaluation of its possibilities in the treatment of malignant tumours. So far it has been used predominantly in very advanced cases, in which the response to any form of treatment is difficult to assess.

The minimum conditions necessary for a sensible application of autologous bone marrow in combination with massive doses of cytotoxic drugs can, however, be listed.

- (1) *A sufficiently large number of bone marrow cells should be available for reinfusion.* The procedure employed for instance by Black *et al.*⁶⁸ in cancer cases, in which 20 ml. of sternal marrow (by our estimate $0.3-1 \times 10^9$ nucleated cells) were reinfused

TABLE VI: 3. Effects of treatment with isologous and autologous bone marrow after lethal doses of cytotoxic drugs: animal experiments

Chemotherapeutic agent	Animals	Bone marrow	Results	Authors
Thioguanine 2 doses of 85 mg/kg i.p.	C ₃ H mice carrying Ehrlich ascitis carcinoma	2 × 10 ⁷ isologous cells i.p.	Prolongation of survival time	Sartorelli and Le Page (1957) ³⁶⁰
T.E.M. 5 mg/kg (LD ₁₀₀)	C ₃ H and C57BL mice	Over 10 ⁷ isologous cells i.v.	7% 30-day survival	Rudovic <i>et al.</i> (1958) ³⁵⁷
T.E.M. 0.3 mg/kg i.v. (LD ₂₀₀)	Dogs	10-15 ml. autologous cells 18 hr later	5 out of 6 dogs survived	Costakel <i>et al.</i> (1960) ¹⁰³
N mustard two doses: 3 and 4 mg/kg (LD ₉₀)	Swiss mice	10 ⁷ isologous cells i.v.	30% survival	Tran BaLoc <i>et al.</i> (1958) ⁴¹²
Myleran 21 mg/kg per os (LD ₁₀₀)	Inbred rats	32-128 × 10 ⁶ isologous cells i.v.	40-60% 30-day survival	Dunjic and Maisin (1960) ¹³²
Dimethyl-Myleran (CB 2348) 7.5 mg/kg i.p. (LD ₁₀₀)	Inbred August rats	3-7 × 10 ⁷ isologous cells i.v. or i.p.	Up to 100% survival when injected within hours	Talbot and Elson (1958) ³⁹³

Dimethyl-Myleran 16 mg/kg	CBA mice	3×10^7 isologous cells	Complete protection	Floersheim and Elson (1961) ^{141a}
6-mercaptopurine 100 mg/kg + Myleran 16 mg/kg	CBA mice	3×10^7 isologous cells	Complete protection	Floersheim and Elson (1961) ^{141a}
1, 6-bis-chloro-ethyl- amino-1,6 deoxy-D- mannitol 120 mg/kg s.c. (LD ₅₀) and 140 mg/kg s.c. (LD ₂₀₀)	C57BL mice	5×10^6 isologous cells i.v.	Reduction of mor- tality at LD ₅₀ to 16%, at LD ₁₀₀ to 55%	Rudvic (1962) ³⁵⁶
Thio-T.E.P.A. 28-40 mg/kg i.v. (LD ₂₀₋₁₀₀)	BDF1 mice	10^7 isologous cells i.v.	Slight decrease of mortality at lowest dose of drug	Lochte <i>et al.</i> (1963) ²²⁴
5-6 mg/kg i.v.	Dogs	Stored autologous marrow i.v.	Decreased mortality, no effect at 8 mg/kg of drug	Lochte <i>et al.</i> (1963) ²²⁴

i.p., intraperitoneal i.v., intravenous

after treatment with 0.4–0.65 mg/kg nitrogen mustard, seems bound to result in complete failure because of the small number of cells used. Black *et al.*⁶⁸ were nevertheless of the opinion that the recovery of the haemopoietic system was more rapid than in comparable cases without marrow reinfusion. It is, however, always questionable whether small numbers of patients with advanced disease provide sufficient basis for comparison.

(2) *The effect of marrow reinfusion cannot be evaluated when doses of chemotherapeutic agents have been administered which themselves allow a rapid and spontaneous recovery of haemopoiesis.* A typical example of this is shown in the report of Smiley *et al.*³⁷⁷. These authors reinfused between 1.3 and 3.0×10^9 autologous bone marrow cells into five patients after treatment with 0.4 mg/kg of nitrogen mustard. The rate of recovery of the haemopoietic systems of these patients was equalled by a further five patients who received only the nitrogen mustard; in all cases complete or nearly complete recovery had occurred by the 26th day after treatment.

The recent results of Meyer *et al.*²⁷⁶ also seem to be in this category. Their treatment consisted of four daily doses of 0.2–0.3 mg of nitrogen mustard, after which spontaneous recovery of the haemopoietic system was seen in all seven patients. The rise in the blood counts started as early as 14 days after treatment. In 9 other patients bone marrow preserved by freezing was reinfused 6–12 days after the last injection of nitrogen mustard; this failed to result in a more rapid return of the haemopoietic system to normal.

(3) *The bone marrow should be administered at a time when the chemotherapeutic agent is no longer active.* Nitrogen mustard seems to be inactivated very rapidly so that reinfusion can be performed shortly after an injection or course of injections^{68, 289}. For many other agents information on this point is inadequate. When the toxic action is expected to persist for more than 24 hours, low temperature preservation of the bone marrow becomes necessary, since storage at 4° C for more than 1 day may lead to an appreciable loss of viable cells.

(4) *When low temperature storage of the marrow is employed, a considerable loss of its restorative capacity may be expected.* Methods which allow very good preservation of mouse bone marrow have been found to be inadequate with monkey bone

marrow and, since direct information on this aspect of preservation cannot be obtained with human marrow, even the best methods should be regarded as leaving no more than 50 per cent of the cells viable. The absence of any beneficial effect from autologous marrow in the patients reported on by Meyer *et al.*²⁷⁶ may also be related to the use of rather low numbers of frozen cells: $4-9 \times 10^9$ per patient. An efficiency of 50 per cent in the preservation (the best so far obtained with monkey bone marrow) would leave $2-4 \times 10^9$ cells available which is slightly below the estimated minimum number.

(5) *Autologous bone marrow can serve as an adjunct to "super-dosage" chemotherapy only when the use of larger doses of the drug is prohibited by its depressive action on the bone marrow.* The margin which separates marrow depression from lethal toxic effects on other tissues should be relatively large. In one of the patients described by McFarland *et al.*²⁷⁰, extensive "maceration" of the mucosa of the oesophagus and the stomach was found at autopsy, 19 days after treatment with 1.4 mg/kg of nitrogen mustard. It should also be remembered that "super-dosage" of chemotherapeutics, even when supported by bone marrow, may not be tolerated by patients who are in a terminal stage of the disease.

(6) *"Super-dosage" chemotherapy is likely to cause a severe decrease of immunological defences so that "barrier nursing" as well as supportive therapy with antibiotics and blood or platelet transfusions are required.*

In most of the clinical work reported, the degree of effectiveness of autologous bone marrow reinfusion is limited to the general impression that favourable effects—in terms of a more rapid haemopoietic recovery—are obtained with many patients.^{200, 209, 303} The majority of these studies were carried out after treatment with nitrogen mustard. McFarland *et al.*²⁷⁰ observed rapid recovery of the haemopoietic system in 3 patients who received almost 3 times the recommended dose of nitrogen mustard (1.1 mg/kg versus 0.4 mg/kg) followed by autologous bone marrow. Clifford *et al.*⁷⁶ have investigated the use of large doses of nitrogen mustard administered over a period of 3 days for the palliation of patients in East Africa with advanced malignant tumours, where radiotherapy was not available. They report on 3 patients who survived a dose of 2 mg/kg with autologous bone marrow therapy, while 3 other patients not

treated with bone marrow died after the same dose of nitrogen mustard.

Pegg *et al.*³⁰³ have reported on autologous bone marrow replants not only after administration with nitrogen mustard but also after treatment with mannomustine, phenylalanine mustard and cyclophosphamide. In the patients treated with the latter two drugs the authors failed to observe any benefit from the bone marrow treatment.

In 38 cases reported by Hill and Loeb¹⁷⁸, massive chemotherapy with a variety of agents (actinomycin D, vincalucoblastine, amethopterin and 6-mercaptopurine) and therapy with radioactive isotopes were supported by autologous bone marrow reinfusion.¹⁷⁸ This was followed by a satisfactory response in 21 cases. In agreement with several other authors, they consider the use of autologous bone marrow of value, because it provides a degree of "insurance" against irreversible haemopoietic complications.

Haematological responses similar to those in patients used as controls were seen in 3 patients by Kretchmar *et al.*²⁰⁷ and in 4 patients by Dunnigan and Brown¹³³, after treatment with autologous marrow following large single doses of nitrogen mustard (0.6–1.1 mg/kg). Kretchmar *et al.* stress the fact that an early, quite rapid and *spontaneous* recovery of the haemopoietic system was seen in a number of patients who had received 1 mg/kg of nitrogen mustard in a single dose. Both groups reinfused the marrow shortly after its collection without recourse to freezing. Technical reasons, as Dunnigan and Brown have themselves pointed out, may have been responsible for the failure to demonstrate any effect of the autologous marrow.

Any discussion of the effects of autologous bone marrow would be incomplete without mention of the work of Conrad and Crosby¹⁰⁰. In their studies 8 patients with advanced Hodgkin's disease were treated with massive single doses of nitrogen mustard (0.95–1.5 mg/kg) while orthopaedic tourniquets were applied to one or more extremities during the infusion in an attempt to protect the bone marrow. Radioactive iron uptake studies afterwards provided evidence of a more pronounced erythropoietic activity in the protected areas, but no proof was obtained that the aplastic marrow spaces were seeded with cells from the protected marrow.

Homologous bone marrow after chemotherapy

EXPERIMENTS WITH ANIMALS

In animal experiments, homologous bone marrow has been much less successful than autologous bone marrow in the treatment of chemotherapeutic drug toxicity as is shown in Table VI: 4. Several investigators have compared the effects of isologous and homologous bone marrow after the same drug dosage. However, in some cases the same number of homologous and isologous bone marrow cells were tested, which makes the comparison of limited value because it is known from radiation studies that many more homologous cells are required to obtain a prolonged proliferation of the donor cells. In very few cases has any attempt been made to identify donor type haemopoietic cells in the survivors. It seems that Cree¹⁰⁴ in his experiments with rabbits is the only author who has obtained proof of permanent takes following treatment with aminochlorambucil. The protective effects with homologous bone marrow in myleran treated rats by both Weston *et al.*⁴⁵⁷ and by Dunjic^{130, 131} are so impressive that it is likely that a temporary proliferation of donor bone marrow occurred. Dunjic found that survival was better following higher doses of myleran and the injection of larger numbers of cells; this points to a take of the graft. It is regrettable that Ambrus *et al.*³ made no attempt to demonstrate the presence of donor cells in monkeys which had survived lethal amounts of nitrogen mustard as a result of bone marrow therapy. The absence of secondary disease in these animals suggests that the grafted cells did not persist.

It becomes evident from these data that the problem of foreign bone marrow transplantation following cytotoxic drugs has remained largely unexplored. Admittedly, the dangers of the development of secondary disease which result from a successful take of the bone marrow are discouraging, but this should be no reason to neglect a field which may still conceal interesting possibilities.

CLINICAL TRIALS

Haemopoietic failure caused by cytotoxic drugs has been treated with homologous bone marrow in a limited number of clinical trials. The results tend to confirm the findings obtained with experimental animals, that takes are temporary at best and that beneficial effects are in most cases doubtful or indeed absent altogether. Pegg *et al.*³⁰³ presented data on 12 patients who had been treated with various

TABLE VI: 4. Effects of treatment with homologous bone marrow transplantation following chemotherapy: animal experiments

Chemotherapeutic agent	Animals	Bone marrow	Results	Authors
Thioguanine 2 doses of 85 mg/kg	C ₃ H mice carrying Ehrlich ascitis carcinoma	2 × 10 ⁷ homologous cells i.p.	Prolongation of survival, no proof of "take"	Sartorelli and Le Page (1957) ³⁶⁰
T.E.M. 5 mg/kg (LD ₁₀₀)	C ₃ H and C57BL mice	Over 10 ⁷ homologous cells i.v.	No protection	Rudovic <i>et al.</i> (1958) ³⁵⁷
N mustard 2 doses 3 and 4 mg/kg (LD ₉₀)	Mice: 3 inbred strains	10 ⁷ homologous cells i.v.	No protection	Tran BaLoc <i>et al.</i> (1958) ⁴¹²
N mustard 5-10 mg/kg i.v. (LD ₁₀₀ : 8 mg)	Swiss mice	10 ⁷ homologous cells i.v.	10-60% protection at LD ₁₀₀ when injected 30 min after drug, no identification of donor cells in survivors	Ambrus <i>et al.</i> (1962) ³
1-2 mg/kg i.v. (LD ₁₀₀)	Rhesus monkeys	1-10 × 10 ⁸ homologous cells i.v.	5/6 monkeys survived, no proof of "take"	Ambrus <i>et al.</i> (1962) ³

Aminochlorambucil 30-40 mg/kg s.c. (LD ₅₀)	Rabbits	Homologous bone marrow or foetal haemopoietic tissue	40-60% survival and evidence of graft "takes"	Cree (1962) ¹⁰⁴
Myleran 23-35 mg/kg per os (LD ₁₀₀)	Rats, non-inbred	32-128 × 10 ⁶ homologous cells i.v.	Nearly complete protection with the higher cell number and the larger dose of Myleran, no proof of "take"	Dunjic (1962) ^{130,131}
Myleran 20 mg/kg i.v. (LD ₁₀₀)	Rats, non-inbred	125 × 10 ⁶ homologous cells i.v. 1 or 3 times	Complete protection, no proof of "takes"	Weston <i>et al.</i> (1957) ⁴⁵⁷
Dimethyl-Myleran 16 mg/kg	CBA mice	3 × 10 ⁷ homologous cells i.v.	No effect	Floersheim and Elson (1961) ^{141a}
6-mercaptopurine 100 mg/kg + Myleran 16 mg/kg	CBA mice	3 × 10 ⁷ homologous cells i.v.	No effect	Floersheim and Elson (1961) ^{141a}

i.p., intraperitoneal i.v., intravenous

chemotherapeutic agents, sometimes in addition to radiotherapy, and after which homologous bone marrow was infused. Only 4 patients received the minimal number of cells theoretically required for a take of the graft. In one of these, some evidence of a temporary proliferation of the donated cells was obtained by a rise, for a brief period, in donor type neutrophils. In none of the cases was the bone marrow reported to have had a beneficial clinical effect. Haurani *et al.*¹⁷¹ described a smaller series of patients treated with homologous bone marrow after high doses of nitrogen mustard or 6-mercaptopurine and in which evidence of the proliferation of the grafted cells was absent.

Miller and Diamond²⁷⁹ reported a temporary take of homologous bone marrow in a patient with Hodgkin's disease who developed pancytopenia after treatment with nitrogen mustard. The patient received 5×10^9 fresh bone marrow cells from a donor with the sickle cell trait. The sickle cell count in the recipient rose 6-fold during the first 3 weeks over the immediate post-infusion value, and then decreased to zero over the next 2 months.

Recently a temporary take of myeloid cells was observed in 3 children who received large numbers of peripheral granulocytes (a total of $2-9 \times 10^{11}$ cells) during intensive therapy with amethopterin for acute lymphoblastic leukaemia²²⁰. The cells were obtained by plasmaphoresis from patients with chronic myeloid leukaemia. Persistent mitoses of the transfused cells containing the Philadelphia chromosome were detected as long as 52 days after transfusion. No symptoms of secondary disease were observed. Similar white blood cell transfusions involving $3 \cdot 10^{10}-10^{12}$ nucleated cells were performed by Mathé *et al.* (L. Schwarzenberg, G. Mathé, J. de Grouchy, C. de Nava, M. J. de Vries, J. L. Amiel, A. Cattan, M. Schneider and J. R. Schlumberger, *Israel Journal of Medical Sciences* 1, (1965) 925-56) in 33 patients with leukaemia, haematosarcoma or epithelioma during an agranulocytic stage of the disease. In 7 of those patients symptoms of secondary disease developed after the transfusion, which suggests a take of the lymphoid elements present in the myeloid leukaemic cell suspensions infused.

Evidence suggestive of temporary takes of homologous bone marrow of type O donors after radiotherapy or treatment with cytotoxic drugs was obtained in a number of patients by the observation of a rise of the level of O-positive erythrocytes for as long as 3 weeks after transplantation²⁸⁴.

Three other partial or temporary takes of a homologous haemopoietic graft have been reported in the literature. Beilby *et al.*³⁷ report the treatment of a patient with Hodgkin's disease who developed severe hypoplasia of the bone marrow after receiving aminochlorambucil. Infusion of bone marrow from the patient's sister resulted in an increase of donor type (D positive) erythrocytes which amounted to 24 per cent 6 months after the transplantation. Curiously enough the donor's skin graft was rejected.

Another patient with Hodgkin's disease whose bone marrow became aplastic after treatment with TEM, was treated with pooled bone marrow from 4 donors. Donor type erythrocytes were present in large numbers (up to 50 per cent) 3 months after the grafting but 2 months later these cells had disappeared¹⁷².

Proliferation of donor type cells was also observed⁷¹ in a patient with pancytopenia who was treated with foetal liver cells following a course of thio-TEPA for the treatment of mammary carcinoma. Immediately after the infusion, the white cell and platelet counts rose sharply and donor type erythrocytes increased until a maximum of 9.5 per cent was reached on day 16. Thereafter, host cell regeneration started and the donor type erythrocytes eventually disappeared. The number of foetal liver cells administered was, unfortunately, not reported.

The total number of reported cases in which some evidence was present of a short period of proliferation of the infused homologous cells is certainly larger than would have been expected from a study of the results of experiments with animals. Perhaps this is related to the fact that in some of the patients suffering from Hodgkin's disease or leukaemia the immunological system is already severely depressed before chemotherapy is initiated. Another possibility seems to be that the immune system is sufficiently damaged by certain cytotoxic drugs as to prevent an immediate rejection of the transplanted bone marrow cells. Compared to the situation after whole body irradiation, however, recovery of the host's lymphatic system occurs quite rapidly and results in a rejection of the foreign cells.

It is of interest that both Thomas *et al.*⁴⁰¹ and Cole and Alpen⁷⁸ observed an improved acceptance of a homologous bone marrow graft in dogs treated with radiation plus amethopterin or 6-mercaptopurine. Whether the immune suppression by the addition of chemicals is greater than that provided by increasing the doses of radiation is one of the many questions that invite further experimentation.

Whole body irradiation and transplantation of haemopoietic cells in the experimental treatment of leukaemia

The treatment of leukaemia by irradiation is seriously hampered by the fact that the malignant cells are usually widely disseminated at the time of diagnosis. The local irradiation of a tumour site, e.g. a lymphomatous gland or a spleen, often has a limited effect because of the influx of non-irradiated tumour cells from outside the radiation field. This is illustrated by the early experiments of Hollcroft and co-workers¹⁸⁰ on the treatment of lymphomas in mice. A whole body dose as small as 50 r administered simultaneously with a local dose of 1000 r to a subcutaneously transplanted lymphoma, was found to be at least as effective as 6000 r administered to the tumour alone. When the local dose and the whole body dose were separated by a 5 minute interval a substantial decrease in the effectiveness of the treatment was already apparent.

The possibility of restoring lethally irradiated animals by bone marrow transplantation has greatly stimulated research on the treatment of leukaemia by whole body irradiation. It was hoped that the post-irradiation grafting of bone marrow would allow the use of much higher radiation doses than was formerly regarded as possible. In addition, the total dose could be given in one session, thus further enhancing the lethal effect on the tumour.

It was clear from the beginning, however, that the maximum dose would be limited to about 1000 r in view of the danger of intestinal radiation death, which cannot be prevented by bone marrow transplantation.

The first attempt was made by Hollcroft and co-workers¹⁸⁰ who used a transplantable leukaemia in inbred guinea-pigs. These authors found remissions, as evaluated from the peripheral lymphocyte count, the duration of which varied more or less linearly with the radiation dose. No permanent cure could, however, be obtained. These disappointing results were subsequently confirmed by other workers^{32, 236, 254, 260, 361, 373, 448}, and are in contrast with the initially promising experiments reported by Barnes *et al.*²⁰ These authors obtained a considerable number of cures in mice carrying a transplantable lymphosarcoma following irradiation with a dose of 1500 r given over 25 hours, and treatment with isologous bone marrow. They could not, however, repeat their earlier success³³, nor have others succeeded in obtaining an improved rate of cure by the use of protracted irradiation. Furthermore, the treatment of spontaneous tumours failed to

yield better results than had been obtained in the experiments with transplanted tumours^{254, 260}. Quite satisfactory results have been published by Trentin⁴¹⁵ with the Gardner lymphosarcoma. Of eleven C₃H mice, irradiated and treated with isologous bone marrow on the day of inoculation of the tumour, 10 were alive after 190 days. It might be questioned, however, whether in addition to the irradiation, antigenic differences between tumour and host may have contributed to the tumour regression. Such antigenic differences are not uncommon in tumours which have been frequently transferred over a number of years.

Significant tumour regression has been reported by the use of homologous or heterologous bone marrow and lymphoid cells in animals receiving whole body irradiation^{20, 448}. The prevention of the recurrence of the tumour following radiation doses that by themselves were insufficient to eradicate it, has been attributed to a graft versus host reaction, the tumour being considered as part of the host. The simultaneous occurrence of severe secondary disease, has until recently, however, invalidated this mode of treatment.

In the following paragraphs the factors which determine tumour regression and the survival of experimental animals will be analysed. The experiences so far obtained with this form of treatment in human patients will also be reviewed.

THE EFFECT OF THE IRRADIATION

In clinical literature great emphasis has been placed on the radiosensitivity of tumours. Differences in the results of radiation therapy of different tumours as measured by decrease of tumour volume, remission time, symptom free interval and the rate of cure have been attributed to differences in the radiosensitivity of the cells which form the tumours.

Recent radiobiological research has indicated, however, that the differences in radiosensitivity between many types of cells are quite small when the irradiation is performed *in vitro* under standard conditions of oxidation and if the reproductive capacity of the cells is used as a criterion²¹⁴. It is beyond the scope of this book to discuss all the factors which might possibly modify the radiation response of malignant cells with similar "intrinsic" radiosensitivity, when irradiated *in vivo*. For the present discussion it is relevant to mention only that the important study of Hewitt and Wilson^{173, 174} has shown that the dose-effect relationship for the survival of mouse leukaemic

cells when irradiated in the liver *in vivo* does not differ materially from that obtained with many lines tested *in vitro* (Fig. VI¹).

Hewitt and Wilson reported limited observations on 4 other leukaemic cell lines which indicated that the radiosensitivity of these leukaemias did not differ radically from that found for the CBA leukaemia used in their earlier studies¹⁷⁶. The only factor which significantly influenced the radiosensitivity of the leukaemic cells was the oxygen supply. Tumour cells irradiated under anoxic conditions

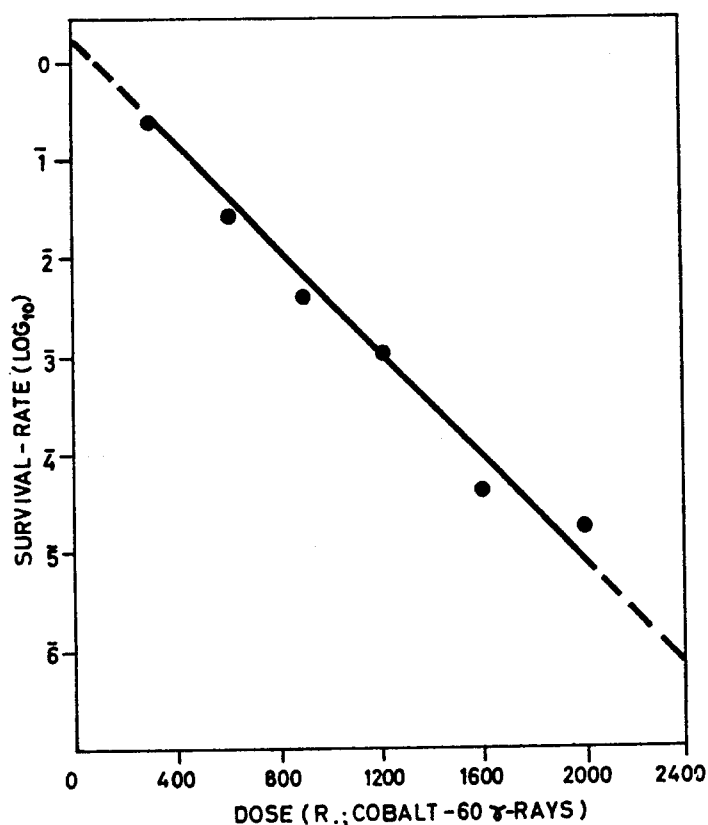


Figure VI¹. Cell-survival curve, obtained by Hewitt and Wilson (1959)¹⁷⁴ for mouse leukaemia cells irradiated *in vivo*

in recently killed mice appeared to be 2.3 times more radioresistant than cells irradiated in living mice¹⁷³.

In the authors' laboratory the assay is carried out with leukaemic spleen instead of liver (Fig. VI²). The D_{010} values* obtained with 3 different strains of leukaemia (2 lymphosarcomas and 1 myeloid leukaemia) were found to be a factor of about 2 higher than those of Hewitt and Wilson¹⁷⁴. This fits in very well with the theory that in contrast to leukaemic cells in the liver, those in the spleen are under anoxic conditions. Hewitt and Wilson also found that leukaemic cells

* The term D_{010} has been explained on page 199.

in the peripheral blood behave as anoxic cells¹⁷⁶. They explained this rather unexpected finding by postulating a rapid turnover of leukaemic cells, so that cells which were at anoxic sites during the irradiation were afterwards found present in the blood. We may extend this hypothesis and assume that these cells entered the circulation from the spleen.

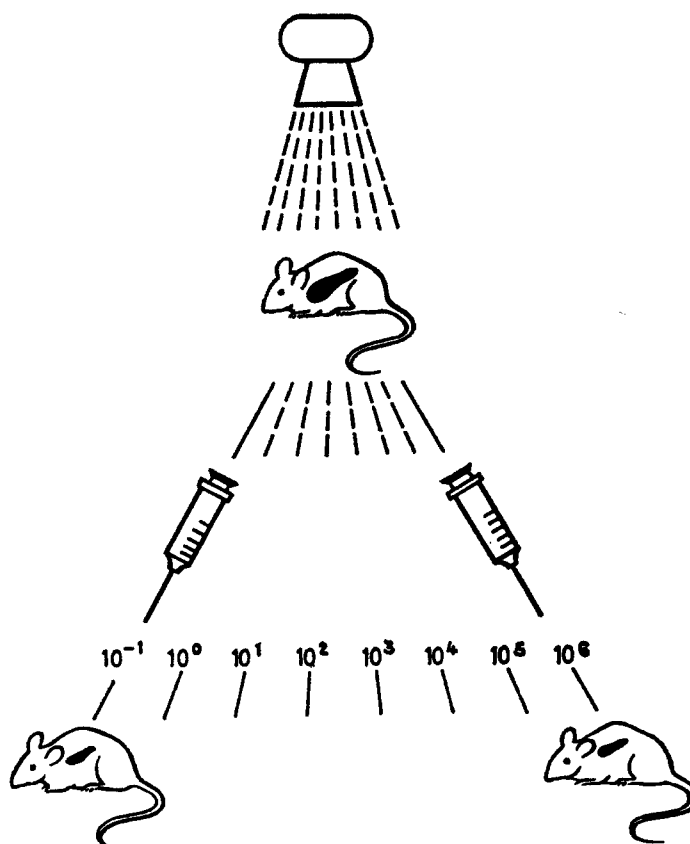


Figure VI². Schematic representation of the *in vivo* assay of the radiosensitivity of leukaemia cells. A tumour-bearing mouse is irradiated. Serial dilutions of a suspension of the leukaemic spleen are prepared after the irradiation and injected into normal mice. The number of cells required to produce leukaemia in 50% of the recipients ED_{50} (Effective Dose 50%) is compared with the ED_{50} for non-irradiated leukaemic cells

Until now, only a restricted number of different leukaemias have been tested, and it might be doubted whether the survival curves of other leukaemias would have a comparable shape. However, for the present discussion we shall assume that this is the case and, furthermore, that tumour cells from all sites are fully oxygenated.

With further reference to Fig. VI¹ it can be seen that a dose of 2000 r will cause a reduction of the tumour cell population by a factor of 10^5 . This means that in a population of mice bearing tumours of a

size of 10^4 cells, 90 per cent would be cured, assuming that each single surviving tumour cell would be able to grow out and cause a recurrence. It will be clear that in overt leukaemia, especially in humans, the population of malignant cells will be much larger than 10^4 . Since the dose of whole body irradiation cannot be increased above approximately 1000 r in view of the danger of death by intestinal damage, radiation alone cannot be expected to cure leukaemia. A logical consequence of these considerations is the recommendation that the irradiation of the patients should be carried out during a remission, when the number of malignant cells is at its lowest and the surviving fraction after a certain fixed dose of radiation is, therefore, as small as possible.

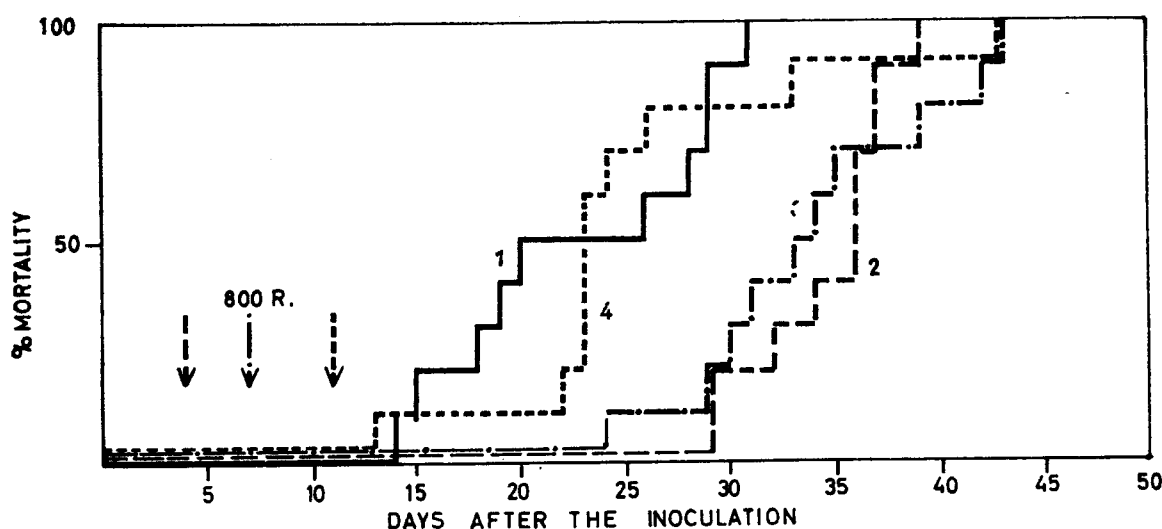


Figure VI³. Survival of C57BL mice, inoculated with 0.5×10^6 lymphosarcoma cells, irradiated with 800 r at 4, 7 or 11 days after the inoculation respectively, followed by injection of 6×10^6 isologous bone marrow cells

- 1 = Non-irradiated control mice
- 2 = Mice irradiated 4 days after tumour inoculation
- 3 = Mice irradiated 7 days after tumour inoculation
- 4 = Mice irradiated 11 days after tumour inoculation

These pessimistic predictions on the efficacy of a lethal dose of radiation as a cure for leukaemia are borne out by the experimental data obtained from mice. In Fig. VI³ the survival of mice treated with isologous bone marrow after irradiation at different intervals following tumour inoculation is shown. Although some prolongation of survival is obtained in mice irradiated within a week of inoculation, the results are not very impressive.

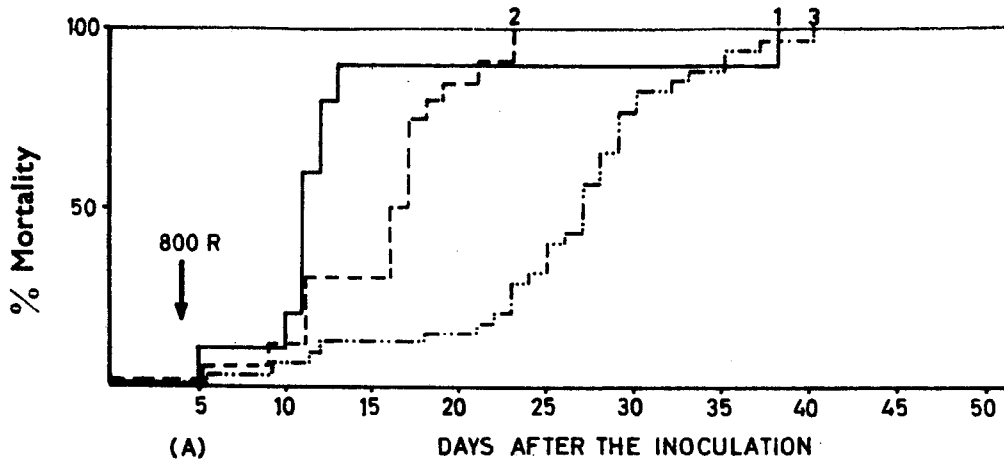


Figure VI⁴(a). Effect of irradiation (800 r) and injection of rat bone marrow and lymph node cells on the survival of mice inoculated with lymphosarcoma (0.8×10^6 cells)

- 1 = Non-treated control mice
- 2 = Mice irradiated and treated with rat bone marrow (36×10^6 cells)
- 3 = Mice irradiated and treated with rat bone marrow (36×10^6 cells) and lymph node suspension (20×10^6 cells)

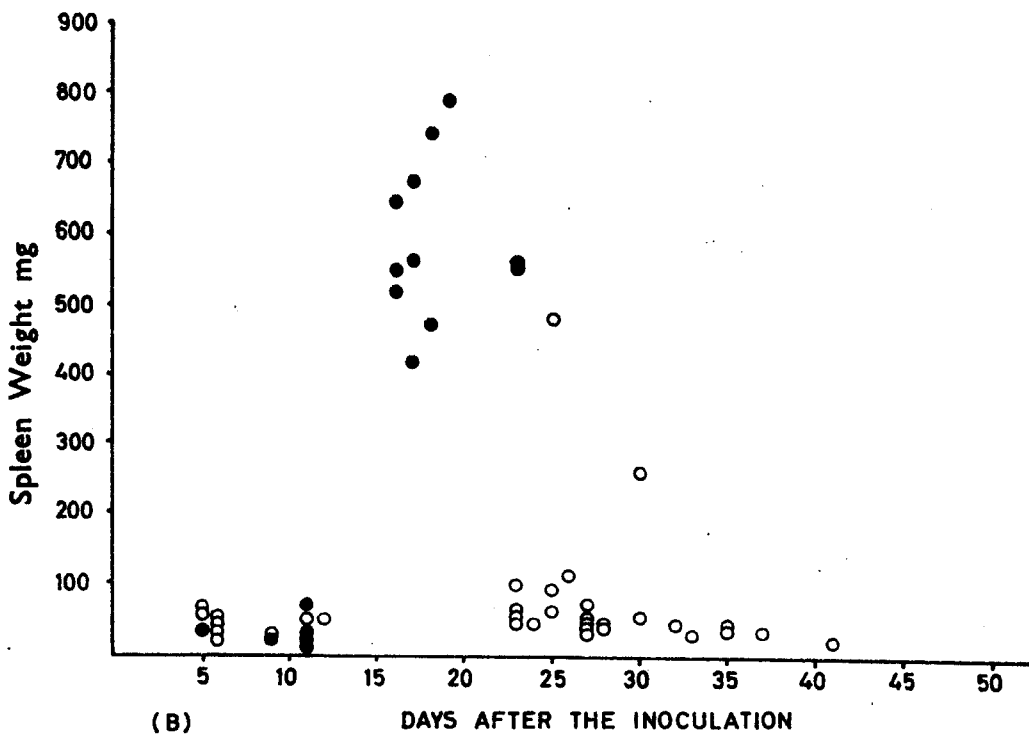


Figure VI⁴(b). Effect of irradiation and injection of rat bone marrow and lymph node cells on the growth of a C57BL lymphosarcoma

Spleen weights of mice of Fig. vi⁴(a)

- Heterologous bone marrow
- Heterologous bone marrow and lymph node suspension

THE HOST-DONOR COMBINATION

If it is accepted that leukaemia cannot be cured by whole body irradiation alone, other means must be sought to supplement the effect of the irradiation. The use of bone marrow transplantation by itself seemed to provide the possibility of a further reduction in the number of malignant cells that survive irradiation. A foreign haemopoietic transplant is able to react immunologically against its host. Since a tumour, even if slightly antigenic, shares most antigens with its host, it might be expected that a graft versus host reaction would also affect the cells of the tumour.

It was indeed found by Barnes and Loutit³², de Vries and Vos⁴⁴⁸ and Mathé and Bernard²⁶¹ that the transplantation of foreign haemopoietic cells could result in a variable prolongation of survival time or even in a permanent cure of a small proportion of animals. As would be expected, the effect of tumour inhibition appeared to be most pronounced when, in addition to bone marrow, lymphoid cells were given to initiate an earlier and more severe graft versus host reactions.

In Plate VI: 1 the dramatic effect on tumour growth in one of these experiments is shown. The effect of tumour inhibition shown by the foreign haemopoietic cells seems not to be due to the wasting of the animals which accompanies the secondary disease, as was suggested by Barnes and Loutit³². This was shown by experiments with a C57BL lymphosarcoma which was inoculated into (CBA × C57BL)F₁ hybrids⁴⁴⁸. The growth of the tumour was inhibited following the irradiation and the administration of CBA bone marrow and CBA lymphoid cells, while similar cells of C57BL origin had no effect. Nevertheless, both treatments induced secondary disease and wasting in the F₁ hybrids to a similar extent.

Although the effect of tumour inhibition shown by foreign haemopoietic cells is quite spectacular in some host-donor combinations, the survival time of the animals is, at best, only slightly prolonged when compared to that of non-treated controls or mice treated with isologous marrow (Fig. VI⁴ (a) and (b)). Only exceptionally was an animal found to survive permanently. It seems to be extremely difficult to induce that precise degree of graft versus host reactivity which will kill the leukaemic cells but which is at the same time mild enough to allow survival of the host (Table VI: 5).

ATTEMPTS AT CONTROLLING THE GRAFT VERSUS TUMOUR REACTION

Several attempts have been made to limit the graft versus host

TABLE VI: 5. Effects of the injection of varying numbers of homologous lymphoid cells on tumour recurrence after irradiation and on death from secondary disease*

Treatment	Mortality interval: days after treatment	Mean spleen weight at death (mg)	Main cause of death
Non-irradiated, non-treated	9-10	701	Lymphosarcoma
CBA bm	11-18	510	Lymphosarcoma
CBA bm + 0.8 × 10 ⁶ CBA Ly cells**	12-15	104	Secondary disease
CBA bm + 0.4 × 10 ⁶ CBA Ly cells	12-18	100	Secondary disease
CBA bm + 0.2 × 10 ⁶ CBA Ly cells	18-26	120	Secondary disease
CBA bm + 0.1 × 10 ⁶ CBA Ly cells	15-23	602	Lymphosarcoma

* C57BL mice inoculated with C57BL lymphosarcoma, irradiated 4 days after tumour inoculation with 800 r, treated with 10 × 10⁶ bone marrow cells and varying doses of lymph node cells—10 mice in each group⁴⁴.

** bm, bone marrow Ly, lymph node

reaction in order to prevent death from secondary disease, while preserving the inhibiting effects on the tumour.

(1) *Induced reversion of the chimaeras to host type haemopoiesis shortly before the peak of secondary mortality.* This was accomplished by a second relatively low dose (200 r) of irradiation followed by the injection of isologous haemopoietic cells. The majority of the mice treated in this way died, however, from a recurrence of their lymphosarcoma. This finding indicates that a protracted graft versus host reaction is required to prevent regrowth of the malignant cells.⁴⁴⁴

(2) *Treatment with homologous lymph node cells which are tolerant towards the host.* This approach could only be expected to result in a cure if the tumour contained specific antigens of sufficient strength. Although up to 60 per cent of cures have been obtained in a suitable experimental set-up with this method, these results appear to be of academic interest only. Firstly, it is highly questionable whether human leukaemias possess tumour-specific antigens of sufficient strength to be susceptible to an immunological attack by host-tolerant cells. Secondly, the clinical application of this mode of treatment would require the *in vitro* induction of tolerance of the donor lymphoid cells to the prospective host, the possibilities for which seem to be remote at present.⁴⁴⁴

(3) *The grafting of large numbers of isologous lymphoid cells in addition to isologous bone marrow following lethal irradiation.* One of the present authors has reported a significant prolongation of life and even long term survival of C57BL and (CBA × C57BL)_F₁ mice carrying a transplantable lymphosarcoma, following this form of treatment^{448, 450}. Originally the possibility of a competition between the normal and the malignant lymphoid cells was favoured, but subsequent experiments have made it more likely that the beneficial effect of the treatment has to be ascribed to antigenicity of the tumour cells.

Although the practical significance of these experiments seems to be as remote as that of the preceding ones, treatment with large numbers of *isologous* lymphoid cells might be considered in those rare leukaemic patients who have an identical twin partner available as donor. In the case of tumour specific antigens being present, the likely possibility is that the transplanted cells would start an immunological reaction against the leukaemic cells.

COMPLICATIONS OF THE TREATMENT OTHER THAN SECONDARY DISEASE

Whole body irradiation of leukaemic mice may result in an appreciable rate of mortality in the first week following the irradiation. This is especially true in the cases where the animals have large leukaemic spleens at the time of irradiation. In cases involving such mice, autopsy showed large haemorrhagic areas in both lungs, which on microscopic examination proved to be infarctions, caused by massive blockage of the pulmonary arterioles by large numbers of disintegrated tumour cells and DNA (Plate VI: 2). Such thrombo-emboli have also been described in irradiated leukaemic AKR mice¹⁸¹. The fragments of tumour cells are probably removed from the spleen and the liver after the irradiation and carried to the lung by way of the portal circulation and the hepatic veins.

Another complication, due to massive radiation-induced destruction of tumour cells, is renal insufficiency caused by deposition and subsequent blockage of the renal tubules by urates. This latter complication is well known to occur in human cases of leukaemia following irradiation.

Theoretically, still another seemingly paradoxical complication may result from the irradiation of a tumour-bearing animal, namely, an increase in the rate of the proliferation of tumour cells which survive the irradiation. This may be the case when the tumour has anti-genic properties, which impede its proliferation in a normal animal. The abrogation of the immunological defence by the irradiation would then result in enhanced growth of the tumour. This was actually found to occur in at least one specific host-transplantable tumour combination.

THE CLINICAL APPLICATION OF BONE MARROW TRANSPLANTATION IN THE TREATMENT OF LEUKAEMIA

Until now, about 60 patients suffering from leukaemia have been treated by total body irradiation and the infusion of isologous bone marrow from identical twins^{11, 404, 406}, stored autologous bone marrow²⁷¹ or homologous bone marrow^{8, 171, 258, 262, 263, 271, 404, 407}.

The evaluation of the results, especially with respect to the effect of homologous bone marrow on the leukaemia, is exceedingly difficult, mainly because adequate non-treated control patients are never available, and because in most of the cases proof of a take of the marrow transplant is either lacking, or unconvincing. Careful analysis of the data reveals that evidence for a marrow take has been obtained in only

6 or 7 cases treated with homologous bone marrow. Two takes of homologous bone marrow grafts have been described by Mathé *et al.*²⁶³ in 2 leukaemic children, both of whom died from secondary disease after about one month. In two other patients similarly treated a temporary existence of donor type erythropoiesis was accompanied by symptoms of secondary disease during the second and third month following transplantation^{251, 262}. The symptoms disappeared at the same time as did the donor type erythrocytes, while simultaneously a rise in the lymphocyte count (presumably host type) occurred. These 4 patients were irradiated with a dose of 870 to 950 r and received $11-34 \times 10^9$ bone marrow cells from a single homologous donor each.

Quite recently, a successful take of a bone marrow homograft of more than one year duration was obtained in a 26-year old patient by Mathé and co-workers²⁵⁸. Following whole body irradiation with two divided doses of 400 rads each, this patient was given pooled bone marrow derived from 6 relatives. The bone marrow was administered 5 days after the second irradiation and the patient developed pronounced secondary disease beginning about 10 days after the transplantation and lasting two months. After 8 months the recipient's blood was completely repopulated with erythrocytes of the genotype of one of the donors (a brother). The patient produced γ -globulins of a type characteristic of the donors (a differentiation between the donors could not be made with this technique). A skin graft of the donor, whose erythrocytes were identified in the patient, was retained, while skin from the other donors—simultaneously grafted—was rejected.

It is of great interest that the graft which was retained came from the donor who had been classified as closest to the host according to leucocyte antigen determinations in spite of the fact that the sera used in these determinations were probably not monospecific. This donor was, together with one other donor, also closest to the recipient on the basis of the histocompatibility test* currently employed by the group in Paris²⁵⁶.

The Cooperstown group has reported one temporary and incomplete take of homologous bone marrow in a heavily irradiated

* A subject unrelated to the recipient of the bone marrow and the donor is grafted with the recipient's skin. After this graft has been rejected, the subject receives a skin graft from each of the prospective donors. A second set reaction in any of these grafts is interpreted as an indication that histocompatibility factors between that donor and the recipient are shared.

leukaemic patient. There were no clear-cut symptoms of secondary disease in this case although an active bone marrow was found at autopsy^{398, 404, 407}.

Kurnick²¹⁰ has reported a uniform lack of success in a limited number of homologous bone marrow transplantations. Similarly, disappointing findings were reported by Haurani *et al.*¹⁷¹ in a series of 9 leukaemic patients. Following 300–500 r of whole body irradiation these patients received $4-29 \times 10^9$ fresh homologous bone marrow cells from excised ribs. At no time could donor type cells be demonstrated in the recipient's blood, which is not surprising in view of the low dose of radiation. A single unsuccessful attempt to transplant homologous bone marrow was reported by Meighan and Bean²⁷³ who transfused 4×10^9 cells into a leukaemic patient after 700 r of whole body irradiation.

Andrews *et al.*⁸ described a series of 7 leukaemic patients who had been given homologous bone marrow after whole body irradiation. Four cases received pooled marrow from several donors, in most cases closely related to the patients. The absence of any evidence of "takes" can be explained by the sublethal doses of whole body irradiation employed (270–620 r) in all but one patient. The latter received 6.8×10^9 pooled cells from 5 donors in her "immediate family", following a radiation dose of 940 r. The patient died 17 days later with bone marrow aplasia.

A serious difficulty in an evaluation of the results of treatment is that in human leukaemia, remissions lasting for several months may be obtained after sublethal whole body irradiation without the administration of any bone marrow⁸. Remissions of long duration obtained by treatment with autologous bone marrow²⁷¹, isologous bone marrow⁴⁰⁴, or homologous bone marrow^{171, 407} after sublethal radiation doses, can also be ascribed to this unexplained effect of whole body irradiation on human leukaemia. Such remissions may even be obtained in patients unresponsive to treatment with cortisone or chemotherapy. In a case reported by Haurani and co-workers¹⁷¹ whole body irradiation with a dose of only 50 r during a remission apparently sufficed to extend the leukaemia-free period of the patient to 7 months.

Taking all these considerations into account, it appears that the conclusions drawn from the experiments with mice are well borne out by the clinical observations. Radiation only, even with supralethal doses does not eliminate the leukaemia. This disappointing conclusion

is also applicable to the patients who were treated during a remission, when the total number of leukaemic cells would have been minimal. Remission times, obtained after lethal doses of whole body irradiation and treatment with isologous bone marrow (obtained from identical twins) have been two months at the most.

Evidence which suggests that the immunological activity of homologous haemopoietic cells against tumour cells has an additional effect by inhibiting the recurrence has been obtained by Mathé^{258, 262}. Two children with acute lymphoblastic leukaemia were treated with homologous bone marrow. Apart from other evidence of a temporary take of the graft, they both suffered for a short period from secondary disease. Compared with the cases treated with isologous bone marrow, the remissions were of relatively long duration, 5 and 6 months respectively. These two cases, in which spontaneous reversal to host type haemopoiesis occurred, may be compared with the experiments in mice in which reversal was induced early in the period of secondary disease. They tend to confirm the view that in order to completely eradicate the leukaemia the graft versus host activity should not only be severe but also of a protracted nature.

In the patient whom Mathé²⁵⁸ treated with pooled marrow from 6 donors, a remission was obtained, which lasted for 20 months until the time of his death from another cause. The patient suffered from severe secondary disease, recovered and then remained a chimaera. This case is the only one in which a long-lasting suppression of leukaemia in a human chimaera has occurred. The same reservations presented earlier with respect to the possible occurrence of remissions due to radiation only must, however, be applied to this case.

In 2 other patients definite evidence of chimaerism was provided²⁶³. Both died following the irradiation after 29 and 31 days respectively, and the autopsy findings pointed to the presence of severe secondary disease. Histological studies failed to reveal any evidence of leukaemia. Because of the relatively short period of survival of these children and the fact that they were treated during a remission, it is not possible to decide whether the homologous transplant had any additional inhibitory effect on the leukaemic process.

As with mice, several complications other than secondary disease have been observed in human patients. Acute death occurred within 48 hours in 2 patients due to massive destruction of leukaemic cells following the irradiation.⁸ The death of these patients was probably

caused by pulmonary thrombo-emboli of disintegrated cell nuclei and DNA. Such emboli have actually been observed in a patient with a large leukaemic spleen who received irradiation.¹⁸¹

Acute renal failure, due to deposition of urates in the kidney, may occur in the first week following the irradiation¹⁷¹. It is accompanied by a high level of uric acid in both serum and urine. Lethal renal complications have not been reported however.

A complication, not observed in mice, is brought about by the bone marrow transfusion. It appears to occur mainly when haemopoietic cells from multiple sources (more than 1 donor or bone marrow combined with foetal tissues) are given simultaneously or within an interval of a few days. Its clinical manifestations are respiratory distress and sometimes the development of acute cor pulmonale. In the pulmonary arterioles of such patients multiple emboli of fat, bone and bone marrow have been found (Plate VI: 3), accompanied by infarctions and oedema of the surrounding pulmonary tissue^{263, 271, 404}.

Finally, the frequent occurrences of necrotising mycelial infections of the oesophagus and gastro-intestinal tract have to be mentioned^{263, 404}. It might be assumed that leukaemic patients are especially prone to develop such infections. On the other hand it has been found, that monkeys treated with homologous bone marrow display a similar tendency to develop more or less generalised mycelial infections. The same applies to the frequent occurrence of viral diseases in irradiated monkeys and patients treated with homologous bone marrow.

It must be concluded that total body irradiation followed by bone marrow transplantation is an extremely hazardous treatment with, until now, only a few apparent successes. It is in particular the group of Mathé which has made outstanding contributions in this field and which has distinguished itself by a persistent exploration of the possibilities of this method in the treatment of leukaemia. So far, it has not been shown conclusively that such favourable effects as have been reported could not have been obtained with the use of sublethal total body irradiation alone.

Treatment of other blood diseases with bone marrow

Many attempts to treat various kinds of defective haemopoiesis with bone marrow from either animal or human sources can be found throughout the medical literature of the past half century. In a review by Congdon *et al.*⁹⁸ the first report is cited as far back as 1891.

With a few exceptions the bone marrow or bone marrow preparations were always given orally until the 1940's when the intramedullary or intravenous administration of bone marrow became more popular. Few clinicians were thinking in terms of a true replacement of the diseased haemopoietic tissue by proliferating donor cells; the current idea was, in fact, that stimulating factors present in normal bone marrow might induce a recovery of the host's blood-forming system. We shall not discuss the conflicting reports that have appeared on the usefulness of bone marrow in the treatment of pancytopenia and related diseases, but shall limit ourselves to the studies of recent years, when information on the fate of the transplanted cells has been sought.

The only animal experiments in this field were performed by Russell and her collaborators at Bar Harbor, who have published an impressive *oeuvre* on the experimental treatment of congenitally anaemic mice by implantation of normal bone marrow.

They succeeded in curing not only the mild form but also the lethal form of this macrocytic anaemia by the transplantation of marrow from normal isologous donors. This was done initially after the irradiation of the anaemic hosts, but later it was found that irradiation is not required for a successful replacement of the abnormal cells with normal erythropoietic tissue³⁵⁴. It seems that a small implant of normal blood forming tissue can replace the host's erythropoiesis because of the much higher rate of proliferation of the former. This has recently been confirmed by McCulloch *et al.*²⁶⁶ who have shown that marrow from anaemic mice is less capable than bone marrow from normal mice by a factor of 200 in its ability to proliferate and to form visible colonies in the spleen of irradiated animals. It is thus possible to produce (isologous) erythropoietic chimaeras without whole body irradiation but it is as yet unknown whether the chimaeric state extends to the other haemopoietic cell types. In particular, it would be interesting to know if the lymphatic tissue of these animals had been repopulated by donor-type cells as well.

In view of the unpredictability of spontaneous recovery and the fluctuations which occur in human bone marrow hypoplasias, the only reliable criterion for an effective take of the graft in clinical cases is the identification of specific characteristics of the donor cells after haemopoietic regeneration has occurred. As there is usually no reason to expect a critical depression of the immune function in these diseases, it cannot be expected that a graft of homologous bone marrow

will take. In recent years two series of patients with aplastic anaemia or other bone marrow hypoplasias were treated with bone marrow^{171, 303}, and in another series the patients were treated with foetal haemopoietic cells⁶⁹. A few cases responded favourably to the therapy but *in no case* was convincing proof of even a temporary take obtained. Some patients received corticosteroids which were intended to inhibit rejection of the foreign cells and one patient was conditioned with whole body irradiation (200 rads).

A further negative result was reported by Domz¹²⁵ who attempted to restore a case of acquired hypogammaglobinaemia with bone marrow from the patient's husband. Since the homograft reaction in these patients is not always significantly decreased, a take of the foreign cells was not *a priori* to be expected.

Without even entering into all the details such as the number of cells, the source of cells and other crucial factors, it is clear that recent clinical experiences have confirmed previous observations both on human patients and on experimental animals that a take of foreign haemopoietic cells cannot be achieved without a drastic suppression of immune reactivity of the recipient. Even in the anaemic mice used in Russell's experiments, *homologous* bone marrow was not permanently established unless the host animals were pretreated by irradiation. Such pretreatment would hardly seem to be justified even in the most severe cases of bone marrow aplasia in view of the present possibilities of symptomatic treatment.

It should be kept in mind that pancytopenia can be due to toxic factors or unfavourable conditions of internal *milieu* which are not necessarily removed by the introduction of normal bone marrow. This may have been the cause of failure in the case reported by Fernbach and Trentin¹³⁷, who transplanted (isologous) bone marrow from an identical twin partner into a pancytopenic child (Table VI: 6). The anaemia subsided only temporarily and no improvement of the other blood cell types occurred. Four other attempts to treat marrow failure of unknown or toxic origin with bone marrow from an identical twin donor have been reported, three of which were successful (Table VI: 6). The lack of success in the case described by Tocantins and McKenna⁴¹¹ may have been due to the rather small number of cells administered. Although there could obviously be no proof of a take in the patients who responded well to the treatment with isologous bone marrow, their rapid clinical improvement was sound evidence for the proliferation of the injected cells.

TABLE VI: 6. Cases of marrow failure treated with bone marrow from identical twins

Authors	Age (years)	Cause of marrow failure	No. of cells administered ($\times 10^9$)	Recovery after treatment
Fernbach and Trentin (1963) ¹³⁷	3	Chloramphenicol and/or sulfonamides	3.6 (2 months later 2.8)	No
Tocantins and McKenna ⁴¹¹ (cited by ⁴⁰⁸)	15	Unknown	2.0	No
Robins and Noyes (1961) ³⁴⁸	7	Anticonvulsant drugs	5.5	Yes
Mills <i>et al.</i> (1964) ³⁸²	9	Unknown	7.4	Yes
Thomas <i>et al.</i> (1964) ⁴⁰⁸	9	Unknown	6.1	Yes

In view of these favourable results it seems justifiable to recommend a resumption of therapeutic trials with homologous marrow as soon as criteria become established for the selection of comparatively histocompatible (or at least incompatible) bone marrow donors. Cautious pretreatment of the recipient with immune suppressive drugs may then even be considered.

Production of chimaerism as a preparation for organ transplantation

The discovery that the successful transplantation of foreign bone marrow allows the permanent survival of other tissue grafts from the same donor—or in the case of inbred animals from the donor strain—has immediately given rise to the hope that this principle would be applicable in clinical work. However, the severity of the secondary syndrome which has so far always complicated the continued proliferation of homologous bone marrow in human patients seems to prohibit this approach at present, even if the technical difficulties involved in obtaining sufficient bone marrow as well as a kidney from the same donor could be overcome. In dogs, where the donor material provides no problems, this approach has been largely unsuccessful. Only a single dog has been described²⁴⁸ in which the kidney homograft remained intact until the time of death, 49 days after the transplantation. This animal was subjected to 1300 r of whole-body irradiation and received a fresh homologous bone marrow graft 8 days later. On the 24th day after irradiation, transplantation of a kidney from the same donor was performed. At that time the peripheral blood counts were increasing and donor type (female) leucocytes were present. The animal died from pneumonia on the 73rd day after irradiation. A similar experiment performed by Calne⁷² failed, probably because the bone marrow graft was rejected. Following whole-body irradiation with a dose of 900 r, the recipient's own marrow recovered and the kidney graft showed a typical homograft reaction when explored 10 days after the transplantation (37 days after irradiation).

Murray *et al.*²⁸⁷ subjected a patient to similar treatments prior to the transplantation of a homologous kidney. Following whole-body irradiation with a dose of 600 r the patient received 107×10^9 pooled marrow cells from 17 donors among which were siblings, unrelated adults and the kidney donor (an unrelated infant). Three days later the kidney was grafted. Death from pulmonary infection and haemorrhage occurred on the 32nd day after irradiation; there was

no evidence of rejection of the kidney graft nor of a take of the bone marrow.

Because of the extreme dangers involved in lethal whole-body irradiation and in view of the consistent failures to induce haemopoietic chimaerism in man, this method of pre-treating kidney recipients has not been pursued. Sublethal irradiations in combination with prolonged treatment with so-called immunosuppressive drugs have been found to be more successful in preventing the homograft reaction. As yet the stability of the "tolerance" induced in this way towards the kidney homograft, once the drugs have been withdrawn, is uncertain. Equally uncertain is the question of whether this form of treatment will preclude further attempts to facilitate organ transplantation in human patients by changing them into radiation chimaeras. Obviously, this will depend on how much progress is made in overcoming the difficulties and hazards involved in the production and maintenance of haemopoietic chimaerism in man.