ELSEVIER

Experimental Hematology 27 (1999) 956–965

EXPERIMENTAL HEMATOLOGY

How many myeloid post-progenitor cells have to be transplanted to completely abrogate neutropenia after peripheral blood progenitor cell transplantation? Results of a computer simulation

Stefan Scheding[a], Horst Franke[b], Volker Diehl[b], H.-Erich Wichmann[c], Wolfram Brugger[a], Lothar Kanz[a], and Stephan Schmitz[b]

[a]*Department of Hematology/Oncology, University of Tübingen, Tübingen, Germany;* [b]*Department of Internal Medicine, Division of Hematology/Oncology, University of Cologne, Cologne, Germany;* [c]*GSF Institute of Epidemiology, Neuherberg, Germany*

(Received 2 July 1998; revised 19 January 1999; accepted 25 January 1999)

Although hematopoietic recovery following high-dose chemotherapy (HD-CT) and peripheral blood progenitor cell (PBPC) transplantation is rapid, there is still a 5- to 7-day period of severe neutropenia which, theoretically, might be abrogated by an additional transplantation of more differentiated myeloid post-progenitor cells (MPPC). However, both the number of MPPC required to abrogate neutropenia as well as the optimum scheduling of MPPC infusions are currently unknown. Therefore, these questions were addressed by applying a computer model of human granulopoiesis. First, model calculations simulating varying levels of chemotherapy dose intensity were performed and compared with typical clinical neutrophil recovery curves. Using this approach, the data for HD-CT without PBPC transplantation could be reproduced by assuming a reduction of stem cells, committed granulopoietic progenitors and proliferating precursors to about 0.001% of normal. PBPC-supported HD-CT was reproduced by increasing the starting values to at least 0.1%, which corresponded to about 1 to 2 × 10^5/kg transplanted CFU-GM. Interestingly, reproduction of PBPC-supported HD-CT data could be observed for a wide range of starting values (0.1%–10% of normal), thus confirming the clinical observation that hematopoietic recovery after PBPCT cannot be improved by increasing the dose of transplanted cells over a certain threshold. Using the same simulation model, we then studied the effects of an additional MPPC transplantation. The results showed, that at least 5.7 × 10^8 MPPC/kg have to be provided in addition to the normal PBPC graft to avoid neutropenia <100/μL, and that MPPC are best transplanted on days 0 and 6 after HD-CT. Assuming a 100- to 120-fold cellular ex-vivo expansion rate and MPPC representing about 70% of total expanded cells, 5.7 × 10^8 MPPC/kg could be generated starting from 1 to 2 leukapheresis preparations with about 7 to 8 × 10^6 $CD34^+$ PBPC/kg. Considering furthermore, that only a fraction of ex-vivo generated cells will seed and effectively produce neutrophils in-vivo, the required number of MPPC is most likely even higher and, therefore, might be difficult to be achieved clinically. However, the validity of the model results remains to be proven in appropriate clinical studies. **© 1999 International Society for Experimental Hematology. Published by Elsevier Science Inc.**

Keywords: **Mathematical granulopoiesis model—Myeloid post-progenitor cells—Peripheral blood progenitor cell transplantation—High-dose chemotherapy**

Offprint requests to: Stefan Scheding, M.D., Department of Hematology and Oncology, University of Tübingen, Otfried Müller Str. 10, 72076 Tübingen, Germany; E-mail: stefan.scheding@t-online.de

Introduction

Peripheral blood progenitor cells (PBPC) have widely replaced bone marrow for autologous transplantation following high-dose chemotherapy (HD-CT) [1]. Hematopoietic recovery after PBPC transplantation (PBPCT) is faster when compared to bone marrow transplantation [2–7]. However, although PBPC-mediated granulopoietic recovery after HD-CT occurs within a period of 10 to 15 days, there is still a 5- to 7-day period of severe neutropenia.

Theoretically, one possible way to abrogate neutropenia after high-dose therapy might be an additional transplantation of more differentiated myeloid post-progenitor cells (MPPC; i.e., myeloblasts, promyelocytes, and myelocytes) that would lead to production of sufficient numbers of mature functional cells within this early time period. The feasibility of producing large numbers of MPPC ex-vivo in liquid culture starting from positively-selected bone-marrow or peripheral blood $CD34^+$ cells has been demonstrated by several investigators [8–11].

The question of how many MPPC are required to ameliorate post-transplant neutropenia has been previously addressed by simplified calculations based on the known kinetic parameters of human granulopoiesis [8–10]. These conven-

0301-472X/99 $–see front matter. Copyright © 1999 International Society for Experimental Hematology. Published by Elsevier Science Inc.
PII S0301-472X(99)00026-0

tional calculations suggested that about 1×10^{11} neutrophils would be required to cover a 5-day period of neutropenia that could be provided by transplantation of about 6.3×10^9 promyelocytes with every promyelocyte generating 16 neutrophils in-vivo.

Assuming an about 100-fold cellular expansion of ex-vivo cultured $CD34^+$ PBPC and that 70% of ex-vivo generated cells represent promyelocytes, a total number of 9×10^7 $CD34^+$ PBPC would be required to ex-vivo generate 6.3×10^9 promyelocytes for transplantation. In other words, expansion of about one-third of a standard leukapheresis preparation containing about 2.7×10^8 $CD34^+$ PBPC (corresponding to about 4×10^6 $CD34^+$/kg in a 70-kg patient) would be sufficient to abrogate HD-CT induced neutropenia.

However, these conventional calculations do not take into account that granulopoiesis is a highly regulated dynamical system in which changes in one cell stage inevitably influence the other compartments. Moreover, there is increasing evidence that high-dose therapy followed by transplantation causes severe damage to the hematopoietic system with regard to post-transplant cytokine production and microenvironmental function [12–17]. Consequently, cells transplanted after HD-CT may not be able to exhibit their full functional capacity as they would in an unperturbed system, as well may be the case with MPPC. This would lead to a considerable underestimation of the numbers of MPPC that are necessary to abrogate neutropenia. In addition, questions remain with regard to the design of an optimum administration schedule and the individual contribution of different post-progenitor stages towards neutrophil recovery.

In this report, we demonstrate the application of a physiologically-based mathematical model of human granulopoiesis to elucidate these clinically relevant questions. The model is based on a model of murine and canine hematopoiesis that has proven its validity in accurately simulating various experimental hematopoietic perturbations [18–28]. It comprises the entire granulopoietic lineage, multipotent progenitor cells, and granulopoietic regulation. Thus, it enables dynamical properties and possibly altered functional capacities of the granulopoietic system as well as the effects of additionally transplanted MPPC to be simulated after HD-CT and PBPC transplantation.

The results of this study show that, compared with the results of simplified calculation approaches as outlined above, a considerably higher number of MPPC cells is likely to be required for abrogation of neutropenia following PBPC-supported HD-CT.

Materials and methods

Clinical data

The clinical data for neutrophil recovery after non-myeloablative HD-CT were taken from Brugger et al. [29, 30]. The data are given as the median of 6 patients with advanced solid tumors or refractory non-Hodgkin's lymphoma following high-dose VIP (VP16, 1500 mg/m^2; ifosfamide, 12,000 mg/m^2; cisplatin, 150 mg/m^2) chemotherapy without PBPC transplantation, and the median of 13 patients with advanced malignancies following high-dose VIC-E (VP16, 1500 mg/m^2; ifosfamide, 12,000 mg/m^2; carboplatin, 750 mg/m^2; epirubicin, 150 mg/m^2) chemotherapy and transplantation of a median of 2.7×10^6 chemotherapy plus rhG-CSF-mobilized $CD34^+$ PBPC/kg. We have previously shown that PBPC-supported hematopoietic recovery after non-myeloablative HD-CT (VIP, VIP-E, or VIC-E) was identical regardless of whether unmanipulated, $CD34^+$-selected or ex-vivo expanded progenitors were used for transplantation [29–31].

Mathematical model of human granulopoiesis

Structure of the model. The structure of the mathematical model of human granulopoiesis is shown in Fig. 1. Each biological cell stage is represented by a model compartment characterized by its transit time, number of mitoses and fraction of actively-proliferating cells. The model parameters (Table 1) [32–39] that provide the physiological basis of the model are taken directly from the literature or derived from published experimental data [26, 27]. Compartments are connected by cell fluxes. Changes in cell counts with time in each compartment are described mathematically by differential equations. Granulopoiesis is derived ultimately from multipotent stem cells (model abbreviation S) by differentiation into committed progenitor cells CFU-GM (CG). These cells give rise to the morphologically identifiable proliferating (myeloblasts, G1; promyelocytes, G2; myelocytes, G3) and non-proliferating bone-marrow cells (metamyelocytes, G4; bands, G5; and segmented granulocytes, G6). Circulating blood neutrophils are denoted by GRA.

Regulation. Regulation of committed granulopoietic bone marrow cells is controlled by two feedback loops via the model "hormones" CG-feedback (CG-Fdbk) and G-feedback (G-Fdbk) (Fig. 1). Reduced numbers of granulopoietic bone marrow cells induce additional mitoses in CFU-GM (CG) mainly via CG-Fdbk. The number of amplifying mitoses at the proliferating granulopoietic precursor cell stages, myeloblasts–myelocytes (G1–G3) and the transit time of the postmitotic stages, metamyelocytes-segmented granulocytes (G4–G6), are regulated by the number of peripheral granulocytes via the second model hormone G-Fdbk. Reduced numbers of peripheral granulocytes induce additional divisions of G1–G3 cells and lead to reduced transit times in G4–G6. The mitotic amplification is determined by sigmoidal dose-response curves depending on CG-Fdbk and G-Fdbk. Further details of the model and its biomathematical realization as well as model parameters are described in detail elsewhere [18–20, 26–28]. Values for normal bone marrow cell numbers and peripheral blood granulocytes were obtained (or derived) from the literature [40, 41]. Calculations in this article were based on the following numbers for normal adult hematopoiesis: myeloblasts, 0.08×10^9/kg; promyelocytes, 0.63×10^9/kg; myelocytes, 1.48×10^9/kg; peripheral blood neutrophils, 4000/μL [40, 41].

Model simulation of PBPCT

In order to allow for a comparison between model simulations and clinical data, all cell numbers are shown as percent of their normal value (normal = 100%). The effects of HD-CT were simulated by a reduction of starting cell numbers of stem, progenitor and precursor cells on Day 0. Neutrophil recovery curves were simulated beginning with the day after HD-CT (Day 0). Modeling of PBPCT

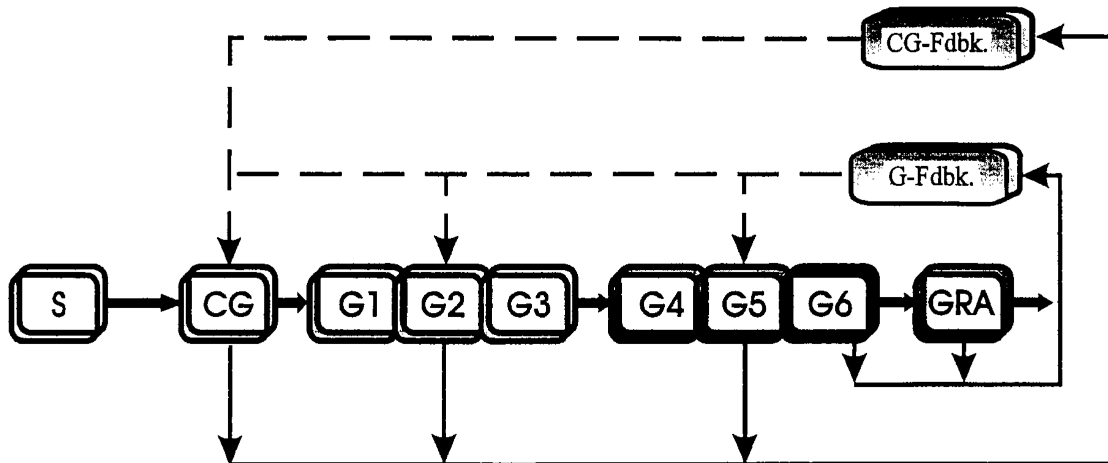

Figure 1. Mathematical model of human granulopoiesis. Committed granulopoietic precursor cells, CFU-GM (CG) descend from multipotent stem cells (S). Proliferating granulopoietic bone marrow cells consist of myeloblasts (G1), promyelocytes (G2), myelocytes (G3); maturing granulopoietic bone marrow cells are metamyelocytes (G4), bands (G5), and segmented granulocytes (G6). Circulating blood neutrophils are denoted by GRA. Granulopoietic regulation is depicted by feedback loops via the model hormones, CG-Fdbk and G-Fdbk, determining the numbers of mitoses in CG and G1–G3 as well as the post-mitotic transit time in G4–G6 depending on the number of bone marrow and blood granulocytes, respectively.

was realized by re-increasing stem and progenitor cell starting levels over those used for the simulation of HD-CT without PBPCT.

Model simulation of MPPC transplantation
Modeling of additional MPPC transplantations (G1, G2, and G3 cells) were based on the best simulation scenario for HD-CT plus PBPCT. For these model calculations, numbers of MPPC were expressed as a fraction of their normal body content; e.g., an additional transplantation of 1.6×10^8 promyelocytes/kg (normal body content: 0.63×10^9/kg) would be expressed as $0.25 \times$ G2. The number of transplanted MPPC at a given time point was added to the content of the corresponding model compartment at that time.

Results

Model simulation of HD-CT either with or without PBPCT
First, the model was used to simulate neutrophil recovery data following either PBPC-supported or non-PBPC-supported HD-CT. The comparison of model simulations with clinical neutrophil recovery data is shown in Fig. 2. The changes in circulating neutrophil levels following non-myeloablative VIP HD-CT without PBPCT (Fig. 2a, left panel) could be modeled by assuming a severe reduction of stem cells, progenitor cells, and proliferating granulopoietic cells ranging from 0.00001% to 0.001% of normal (Fig. 2a, right panel, curves 1–3) with non-proliferating cells (G4–G6) being damaged to a lesser degree, resulting in an overall 80% reduction of bone marrow granulopoiesis on Day 0. When Day 0 values for stem and progenitor cells were increased in order to simulate transplantation of PBPC, this resulted in accelerated neutrophil recovery curves, some quite comparable to the course of the clinical data for HD-CT with PBPCT (Fig. 2b, right panel). Here, starting values in the range of 0.1% to 10% of normal for stem and progenitor cells (curves 5 to 7) reproduced the data, with higher Day 0 values (10% of normal, curve 7) resulting in a shortening of the neutropenic period as well as an increase of neutrophil nadirs. However, even in the latter case (Fig. 2b, right panel, curve 7) peripheral blood neutrophils still decreased below 2.5% of normal, which corresponds to an absolute number of < 100 neutrophils/μL.

Taken together, these results showed that, in fact, the model is capable of adequately reproducing the clinical data for HD-CT with or without PBPC support, thus setting the stage for the simulation of an additional myeloid post-progenitor cell transplantation.

Model simulation of HD-CT and PBPCT plus additional MPPC transplantation on Day 0
Based on the scenario that best reproduced HD-CT with PBPCT (Fig. 2b, right panel, curve 5), the effects of an additional transplantation of myeloblasts (G1), promyelocytes

Table 1. Model parameters of human granulopoiesis

Cell stage	Average transit time (hours) (half-life/log2)	Mitoses
Bone marrow		
CG (CFU-GM)*	112	6
G1 (myeloblasts)*	22	1
G2 (promyelocytes)*	24	1
G3 (myelocytes)*	102	2
G4 (metamyelocytes)**	30	0
G5 (bands)**	50	0
G6 (segmented)**	72	0
Blood		
GRA (granulocytes)**	5	0

References *[32–37]; **[33, 38, 39].

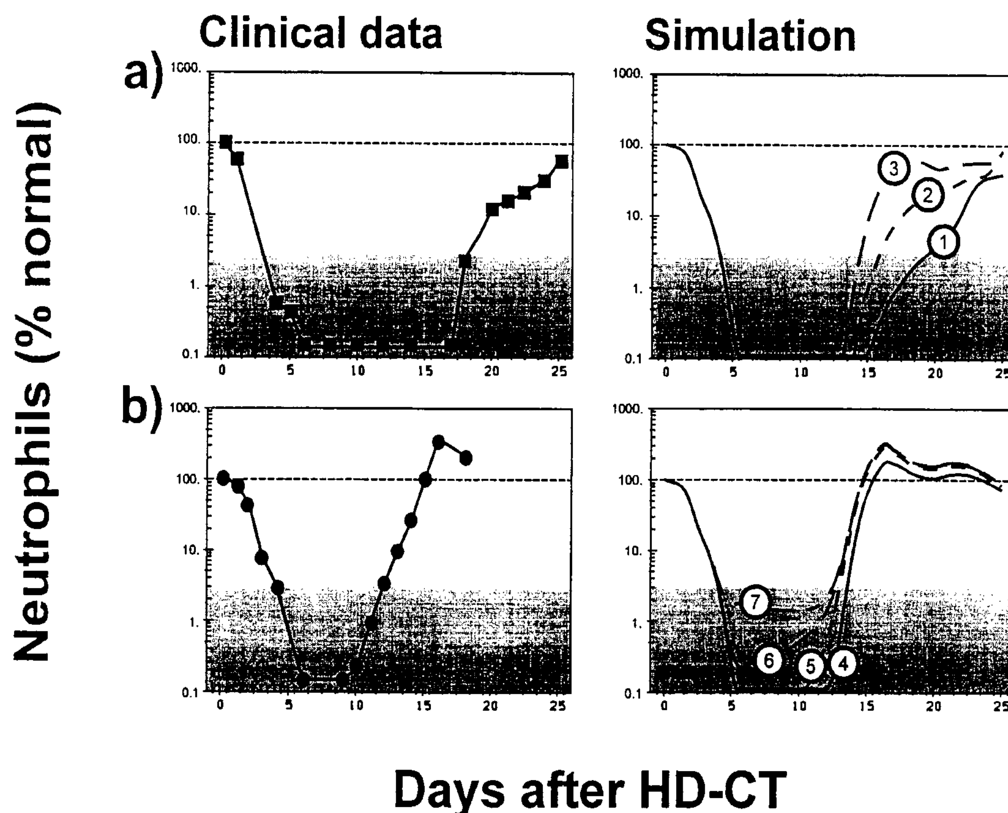

Figure 2. Neutrophil recovery following non-myeloablative HD-CT with and without PBPC. Clinical data (left panels) show the recovery of blood neutrophils following HD-CT without (**a**) and with PBPCT (**b**). The right panels illustrate model simulations assuming different Day 0 starting values for stem and progenitor cells, ranging from 0.00001% (curve 1), to 10% of normal (curve 7) in increments of 1 decade from curve 1 to 7. The shaded area indicates neutrophil levels below 2.5% of normal, corresponding to < 100 neutrophils/μL. Clinical data: ■ Brugger et al. [29]; ● Brugger et al. [30].

(G2), and myelocytes (G3) (MPPC transplantation) after HD-CT and PBPCT were modeled as shown in Fig. 3. The additional administration of 0.22×10^9 MPPC/kg to the PBPCT (curve 1) was not sufficient to totally abrogate neutropenia after HD-CT. This number of MPPC cells is equivalent to about 10% of the total body content of G1+G2+G3 cells. Only when higher numbers of MPPC were used in the model, could neutrophil levels be maintained over 2.5% of normal (> 100 neutrophils/μL) (Fig. 3, curves 2–4). Neutrophil nadirs after HD-CT and PBPCT plus MPPCT were <1%, 1.2%, 6%, 12%, and 36% following the addition of MPPC doses of 0, 0.22, 2.22, 5.5, and 22×10^9 MPPC/kg to the PBPCT. The minimum number of G1+G2+G3 MPPC required to ensure a constant neutrophil count of > 100/μL was 0.57×10^9 MPPC/kg (G1+G2+G3). This number of MPPC cells is equivalent to about 1/4 of the total body content of G1+G2+G3 cells.

Model simulation of HD-CT and PBPCT plus additional MPPC transplantation

Is there an influence of transplantation schedule? In order to test for the best administration schedule with regard to MPPCT, model simulations assuming different application regimens were computed. The model curves illustrated in Fig. 4 assumed a total additional transplantation of 2.5×10^9 MPPC/kg, which would be predicted to certainly abrogate a significant neutropenia. The results of the model simulations indicated that the majority of MPPC had to be given at or shortly after PBPC transplantation (curve 2 and 3) to avoid significant neutropenia (< 2.5% of normal). Any further delay of the MPPC transplantation (e.g., MPPCT on Day 4, curve 1) resulted in a steep decrease of neutrophils to levels below 100/μL within the first days. The second nadir that was observed when MPPC were only transplanted on Day 0 (Fig. 4, curve 2), could be ameliorated by splitting the MPPC transplant such that 3/4 of the total MPPC transplant was given on Day 0 with the remainder given on Day 6 (Fig. 4, curve 3).

Which of the post-progenitor cells are important? In order to test which of the transplanted MPPC were responsible for an effective abrogation of neutropenia, model simulations were computed assuming the transplantation of different combinations of G1, G2, and G3 cells (Fig. 5). MPPC trans-

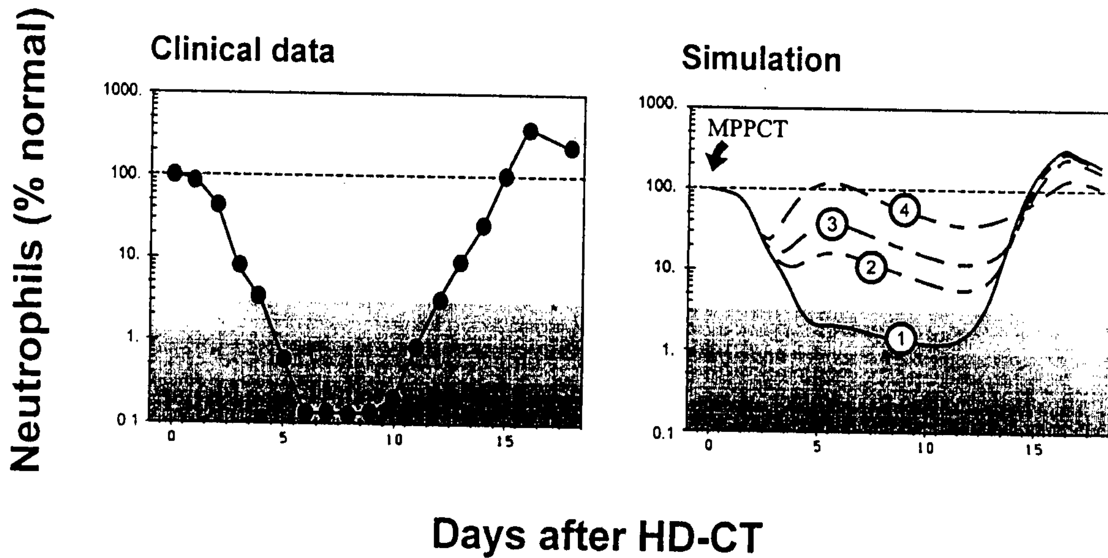

Figure 3. Neutrophil recovery following HD-CT and PBPCT plus MPPC transplantation. Clinical data (● Brugger et al. [30], left panel) show the recovery of blood neutrophils following HD-CT with PBPC transplantation only (as in Fig. 2b, left panel). The right panel illustrates model simulations assuming an additional transplantation of MPPC on Day 0. Four scenarios with increasing numbers of MPPC are shown corresponding to the transplantation of 0.22 (curve 1), 2.22 (curve 2), 5.5 (curve 3), and 22 (curve 4) × 10^9 MPPC/kg. The shaded area indicates neutrophil levels below 2.5% of normal corresponding to < 100 neutrophils/μL.

plantation of myelocytes (G3) either alone or in combination with myeloblasts (G1) and promyelocytes (G2) was essential for abrogation of neutropenia, whereas the transplantation of myeloblasts or promyelocytes either alone or in combination with each other was not sufficient.

Discussion

Despite the use of peripheral blood progenitor cells, administration of HD-CT is inevitably linked to a period of severe neutropenia, which is the underlying cause for serious treatment-related infectious complications. Therefore, patients

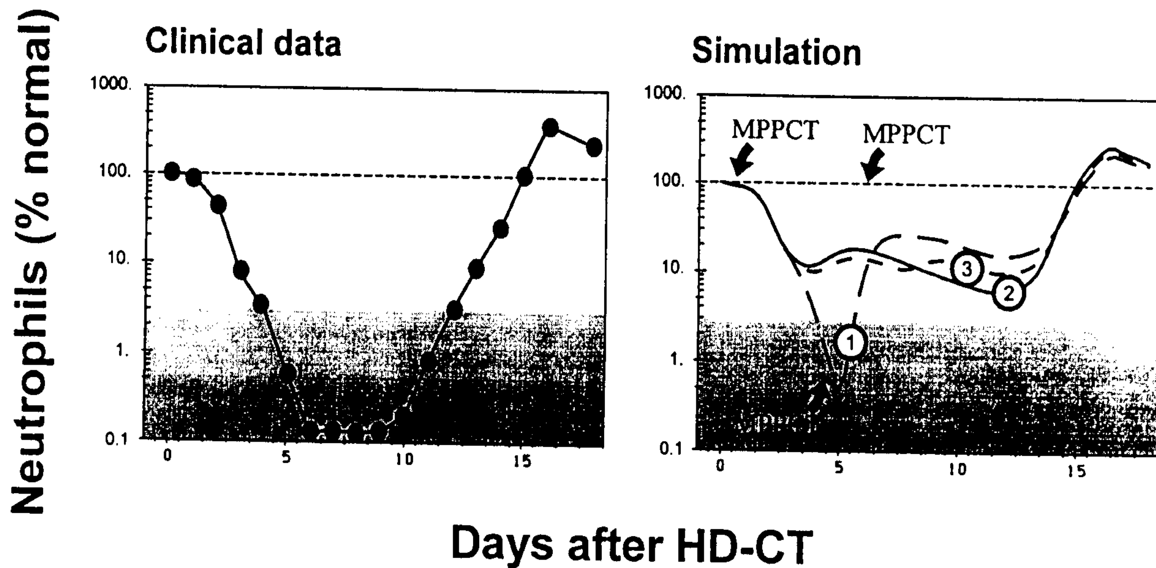

Figure 4. Neutrophil recovery following HD-CT and PBPCT plus MPPC transplantation. Clinical data (● Brugger et al. [30], left panel) show the recovery of blood neutrophils following HD-CT with PBPC transplantation. The right panel illustrates model simulations assuming an additional transplantation of a total of 2.5 × 10^9 MPPC/kg (G1+G2+G3) on different days. Curve 1: 1.9 × 10^9 MPPC/kg on Day 4 and 0.6 × 10^9 MPPC/kg on Day 6; curve 2: 2.5 × 10^9 MPPC/kg on Day 0 ; curve 3: 1.9 × 10^9 MPPC/kg on Day 0 and 0.6 × 10^9 MPPC/kg on Day 6. The shaded area indicates neutrophil levels below 2.5% of normal corresponding to < 100 neutrophils/μL.

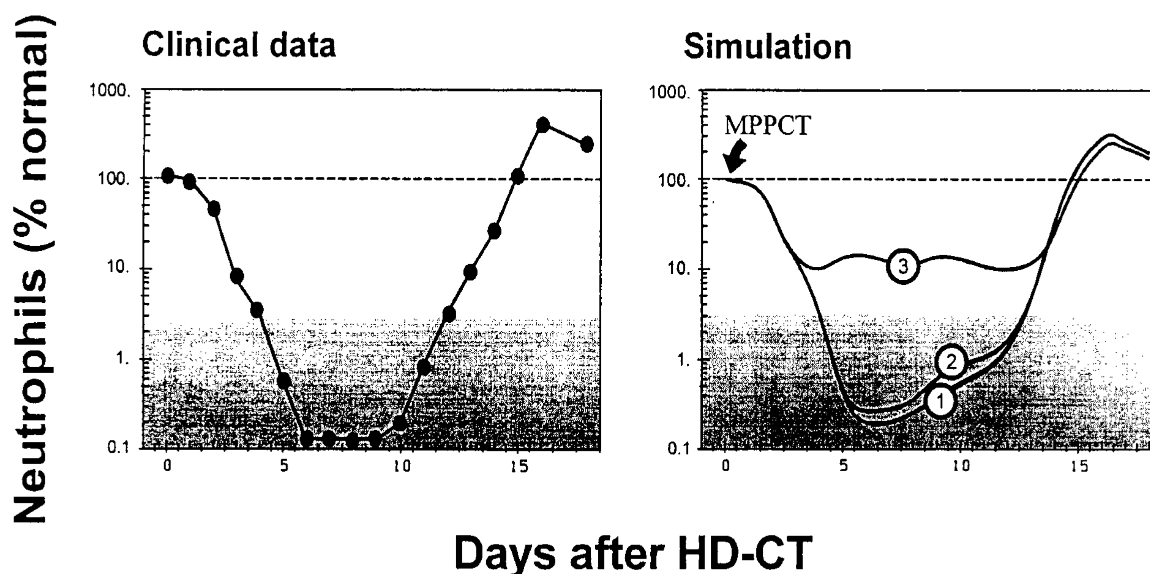

Figure 5. Neutrophil recovery following HD-CT and PBPCT plus MPPC transplantation. Clinical data (● Brugger et al. [30], left panel) show the recovery of blood neutrophils following HD-CT with PBPC transplantation. The right panel illustrates model simulations assuming an additional MPPC transplantation. The different curves represent transplantation of the 1.1-fold total body content of different combinations of G1, G2, and G3 cells. Curve 1: no MPPC transplantation or transplantation of G1 cells only (curves overlap); curve 2: transplantation of G2 or G1+G2 cells (curves overlap); curve 3: transplantation of G3, G2+G3, or G1+G2+G3 cells (curves overlap). Note that the amelioration of neutropenia could only be achieved when G3 MPPC were included in the transplant. The transplantation of G1 and G2 cells whether alone or in combination resulted in recovery curves comparable to those without additional MPPCT.

would greatly benefit if methods could be devised that would lead to a shortening or even abrogation of neutropenia. Additional transplantation of more differentiated, so-called MPPC has been suggested to possibly offer a feasible approach to this problem [8–10, 42]. However, until now, successful clinical transplantation of MPPC has not been convincingly reported.

Here, we demonstrate how a mathematical model of human granulopoiesis can be used to define the conditions that have to be met for a successful application of post-progenitor transplantations.

In a first step, clinical neutrophil recovery data following non-myeloablative VIP HD-CT without and with PBPCT were simulated. Non-PBPCT-supported HD-CT neutrophil recovery kinetics were reproduced by assuming a severe reduction of stem, progenitor, and proliferating precursors (Fig. 2a). The effects of PBPC transplantation could then be sufficiently simulated by simply increasing stem and progenitor Day 0 starting values to 0.1% to 10% of normal (Fig. 2b). Notably, these model results closely matched several clinical findings. (A) It is well known that a threshold dose of about 1 to 2 $\times$ 10^5 CFU-GM/kg exists for PBPC transplants, defining the minimum number of transplanted cells that will ensure rapid hematopoietic recovery after HD-CT. This threshold dose corresponds to about 0.15% to 0.3% of the normal total CFU-GM body content (calculated as the product of the total bone marrow cellularity [41] and the frequency of CFU-GM in the marrow [43]). Transplantation of numbers lower than the threshold leads to a prolonged neutropenic period. Since not all of the transplanted CFU-GM would be expected to seed in the bone marrow, the model simulations reproduced the clinical finding reasonably well, with an increase in Day 0 values over 0.1% of normal leading to a shortening of the neutropenic period comparable to the clinical data for HD-CT plus PBPCT. (B) Furthermore, the clinical data for HD-CT plus PBPCT could be reproduced over a broad range of starting values for stem and progenitor cells (0.1%–10%), thus reflecting the clinical observation that hematopoietic recovery cannot be improved by increasing the dose of transplanted PBPC over that threshold. Here, the model clearly demonstrated that even transplantation of a cell dose approximately 100-fold that of the threshold dose and corresponding to about 1 $\times$ 10^7 CFU-GM/kg or 1 $\times$ 10^8 $CD34^+$/kg PBPC was not sufficient to avoid neutropenia below 100/μL (Fig. 2, curve 7).

In accordance with our results, Fliedner et al. [44] reported that blood granulocyte recovery patterns of four patients with acute myeloid leukemia undergoing HD-CT and autologous PBPCT could be successfully simulated by a mathematical model of granulopoiesis assuming that the PBPC transplantation resulted in the initial filling of the stem cell pool of 0.6%–3.9% of normal.

Two important issues deserve mention at this point. First, because clinical raw data were required to provide the basis for optimum comparison with the model simulations, only those clinical data published by our group were used. We are confident that they represent typical neutrophil recovery curves because: (a) we have observed them in several hun-

dred patients undergoing autologous PBPCT at our institution, and (b) they are quite comparable with data published by numerous other groups. Second, it has to be emphasized that the mathematical model was generic and not specifically designed to simulate any particular set of data. When compared to the standard model of human granulopoiesis [26, 28] it was only necessary to assume a reduced mitotic responsiveness of the immature granulopoietic bone marrow cells following transplantation (i.e., the maximum amplification of proliferating granulopoietic bone marrow cells was reduced to 1/3 of normal at Day 0 and slowly recovered to normal thereafter). Without this reduced mitotic responsiveness neutrophil recovery curves could be reproduced only with extremely low Day 0 starting values (model simulations not shown). These low numbers, however, were in clear contrast to what is known about the numbers of PBPC usually used for autografting, even when assuming only a minimum seeding efficiency. The need for the assumption of a reduced mitotic responsiveness of model granulopoiesis might be merely due to inherent computational requirements, however, it might also possibly reflect a HD-CT-induced defect of the hematopoietic system and the microenvironment, respectively. In fact, there is increasing evidence that high-dose therapy causes severe damage to the hematopoietic system in terms of post-transplant cytokine production and microenvironmental function [12–17]. Interestingly, data reported by Anklesaria et al. [45] showed in a murine model that co-transplantation of a sufficient number of stromal cells resulted in considerably faster hematopoietic recovery when compared to mice transplanted with only bone marrow. Furthermore, El-Badri et al. [46] recently demonstrated that co-transplantation of osteoblasts facilitated the engraftment of purified murine hematopoietic progenitor cells across the major histocompatibility antigen barrier. In addition to animal studies, human autologous mesenchymal (stromal) progenitor cells have been isolated from bone marrow aspirates, culture-expanded in vitro, and either infused without prior conditioning therapy into patients or successfully co-transplanted following HD-CT [47,48]. The transplantation of ex-vivo expanded mesenchymal progenitor cells was not associated with any toxicity and appeared to have a positive impact on hematopoietic recovery when compared to historic control patients [48]. Therefore, co-transplantation of stromal elements might represent an promising approach to improve post-high-dose therapy microenvironmental function, which in turn might result in a faster hematopoietic recovery, and, possibly, might also improve the proliferative responsiveness of additionally transplanted ex-vivo generated MPPC. Other approaches to improve post-transplant hematopoietic function include the use of novel hematopoietic growth factors, such as Flt-3 ligand [49], however, no sufficient data are currently available that would allow for a final conclusion.

In summary, the mathematical model utilized was highly effective in reproducing typical neutrophil recovery data PBPC-supported HD-CT, thus setting the stage for the second step, i.e., simulation of an additional transplantation of myeloid post-progenitor cells.

Based on the scenario that best reproduced HD-CT with PBPCT, the effects of an additional transplantation of myeloblasts (G1), promyelocytes (G2), and myelocytes (G3) (MPPC transplantation) after HD-CT and PBPCT were modeled (Fig. 3). Model analysis of additional MPPC transplantation showed that at least 5.7×10^8 G1+G2+G3 MPPC/kg were required to ensure a constant neutrophil count of >2.5% of normal (100/μL). In other words, we would predict that at least about 1/4 of the normal body content of G1+G2+G3 cells would have to be transplanted in addition to the standard PBPC graft to completely abrogate neutropenia. Assuming (a) an average patient weight of 70 kg, and (b) an ex-vivo expanded MPPC preparation consisting of 70% of G1+G2+G3 cells, a minimum total of about 5.7×10^{10} ex-vivo generated nucleated cells would have to be additionally transplanted into individual patients. Assuming that $CD34^+$ PBPC ex-vivo expansion leads to a 100- to 120-fold increase of mononuclear cells, such a large number of MPPC could be produced from a 16- to 19-L culture (at 3×10^4 cells/mL) starting with 4.75 to 5.7×10^8 $CD34^+$ cells (6.8 to 8.1×10^6 $CD34^+$/kg, corresponding to about two standard leukapheresis preparations).

The model simulations of additional MPPC administration were calculated based on the assumption that differentiated myeloid (non-stem and non-progenitor) cells are capable of seeding and generating functional end cells in-vivo after transplantation, an assumption that has yet to be proven for humans. However, early studies by Fliedner et al. [50] demonstrated that H^3-TDR labeled dividing bone marrow cells transplanted into rats or dogs following total body irradiation reached the marrow and divided and matured at normal speed as evidenced by the fact that labeled metamyelocytes, bands and segmented neutrophils were found in this sequence at expected time intervals. The majority of labeled transplanted cells, however, was trapped in the lungs with only a small fraction of cells reaching the bone marrow and, therefore, one can conjecture that the minimum number of MPPC required for abrogation of neutropenia identified by the model is most likely still too low. Human studies utilizing autologous transplantation of H^3-TDR labeled cells obtained from 300–400 mL of bone marrow blood mixture furthermore showed, that labeled cells in the peripheral blood were only found 2 hours after transplantation, but in none of the subsequent cell smears prepared daily thereafter [50]. However, these transplants were frozen, stored, and thawed for transplantation, a procedure that was demonstrated to prevent the emergence of labeled granulocytes after autologous transplantation in a canine model [50].

Williams et al. [42] recently reported that the additional transplantation of up to 12 billion ex-vivo generated cells containing a mean of 70% neutrophil precursors did not re-

sult in a clear beneficial effect with regard to shortening or abrogation of post-transplant neutropenia. Our model analysis demonstrated that at least about five times as many cells are needed. This is a minimum estimate since clearly only a fraction of the transplanted MPPC seeds to an environment that supports and promotes the production and maturation of neutrophils. Additional model simulations furthermore addressed the issues of (a) identification of the best administration schedule with regard to MPPCT (Fig. 4) and (b) determination of the relative contribution of the different post-progenitor cell stages towards neutrophils recovery (Fig. 5). Model analysis clearly demonstrated that the majority of MPPC had to be given at or shortly after PBPC transplantation to avoid significant neutropenia (< 100 neutrophils/μL). Furthermore, the results showed that a possible second nadir following MPPC transplantation could be avoided by splitting the MPPC transplant such that 3/4 of the total MPPC transplant was given on Day 0 with the remainder given on Day 6. It is generally believed that myeloblasts and promyelocytes are most able among MPPC to abrogate post-transplant neutropenia because (a) they retain their proliferative activity and (b) require only 6 to 10 days to mature to bands or segmented neutrophils under steady-state conditions [32–39]. This latter maturation period is furthermore known to be considerably shortened under maximum granulopoietic stimulation [51–53]. However, damage to hematopoiesis incurred by HD-CT may influence the relative contribution of MPPC to neutrophil recovery. Interestingly, computer analysis revealed that, in contrast to what one might expect, myelocytes (G3) were most important whereas transplantation of myeloblasts or promyelocytes either alone or in combination with each other was not sufficient (Fig. 5).

The ratios of the different myeloid progenitor cells generated by current ex-vivo expansion protocols have been demonstrated to be considerably influenced by culture conditions. The use of fetal calf serum, for example, results in the generation of more mature granulopoietic precursor cells whereas ex-vivo cultured cells remain at more immature stages of differentiation when applying serum-free conditions [10]. Smith et al. [54] reported that repetitive addition of G-CSF (every 3 days) induced a significant increase in cell proliferation and a preferred production of CD15 pos./CD11b pos. cells (58.5% ± 6.5% in a 12-day culture stimulated with PIXY321) representing mainly myelocytes and metamyelocytes. When G-CSF was added only on Days 0 and 6, the percentage of CD15 pos./CD11b pos. was lower (38.2% ± 7.3%), whereas the fraction of CD15 pos/CD11b neg. cells (promyelocytes) was increased. We have shown that the addition of Flt3-ligand to CD34$^+$ PBPC cultured in serum-free medium stimulated by SCF, Synthokine, IL-6, and G-CSF induced a shift to the generation of earlier MPPC, i.e., CD15 pos./CD11b neg. and CD16 neg./CD11b low cells, whereas CD16 neg./CD11b high cells were considerably decreased ([11] and own unpublished results). Taking furthermore into account that with increasing culture periods ex-vivo generated MPPC progressively mature to more differentiated cells, current ex-vivo expansion protocols might be modified such that predominantly myelocytes are being produced, thereby allowing to test our model hypothesis.

In summary, these results demonstrated that the physiologically-based mathematical model applied proved successful in reproducing clinical data for HD-CT without and with PBPC support. Furthermore, and importantly, the model results were well in line with several important biological principles in PBPC transplantation biology. Moreover, the model enabled us to predict the effects of an additional transplantation of more differentiated myeloid post-progenitor cells, calculate the minimum MPPC number required for abrogation of neutropenia, as well as to identify the optimum administration schedule and define the relative importance of the various MPPC cell stages.

We believe these model results to possibly provide a useful tool for clinical researchers to not only better interpret clinical data but also point to fruitful discretions for future experimentation of clinical protocols. However, we are well aware that due to the theoretical nature of this work, the results presented in this article must remain somewhat speculative until they have been tested directly by appropriate clinical trials, some of which are currently ongoing in our laboratories.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft, Germany, grant No. Wi610/3 and funds from the University of Tübingen fortüne research program (grant no. 139 awarded to SS, WB, and LK). The authors wish to thank Dr. A. Nakeff for critically reviewing the manuscript. This article is dedicated to Horst Franke, Ph.D., a devoted scientist and a trusted friend who passed away. We will miss him.

References

1. Kessinger A, Armitage JO (1991) The evolving role of autologous peripheral stem cell transplantation following high dose chemotherapy for malignancies. Blood 77:101
2. To LB, Roberts MM, Haylock DN, et al. (1992) Comparison of hematological recovery times and supportive care requirements of autologous recovery phase peripheral blood stem cell transplants, autologous bone marrow transplants and allogeneic bone marrow transplants. Bone Marrow Transplant 9:277
3. Bregni M, Siena S, Magni M, et al. (1991) Circulating hematopoietic progenitors mobilized by cancer chemotherapy and by rhGM-CSF in the treatment of high-grade non-Hodgkin's lymphoma. Leukemia 5(Suppl 1):123
4. Elias AD, Ayash L, Anderson KC, et al. (1992) Mobilization of peripheral blood progenitor cells by chemotherapy and granulocyte-macrophage colony-stimulating factor for hematopoietic support for breast cancer. Blood 79:3036
5. Bensinger W, Singer J, Appelbaum F, et al. (1993) Autologous transplantation with peripheral blood mononuclear cells collected after administration of recombinant granulocyte stimulating factor. Blood 81:3158
6. Peters WP, Rosner G, Ross M, et al. (1993) Comparative effects of

granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte stimulating factor (G-CSF) on priming peripheral blood progenitor cells for use with autologous bone marrow after high-dose chemotherapy. Blood 81:1709
7. Schmitz N, Linch DC, Dreger P, et al. (1996) Randomized trial of filgrastim-mobilized peripheral blood progenitor cell transplantation versus autologous bone marrow transplantation in lymphoma patients. Lancet 347:353
8. Haylock DN, To LB, Dowse TL, Juttner CA, Simmons PJ. (1992) Ex vivo expansion and maturation of peripheral blood CD34$^+$ cells into the myeloid lineage. Blood 80:1405
9. Smith SL, Bender JG, Maples PB, et al. (1993) Expansion of neutrophil precursors and progenitors in suspension cultures of CD34$^+$ cells enriched from human bone marrow. Exp Hematol 10:870
10. Lill MC, Lynch M, Fraser JK, et al. (1994) Production of functional myeloid cells from CD34$^+$-selected hematopoietic progenitor cells using a clinically relevant ex vivo expansion system. Stem Cells 12:626
11. Scheding S, Meister B, Baum C, Mc Kearn J, Bühring HJ, Ziegler B, Bock T, Brugger W, Kanz L (1997) Flt-3 ligand promotes the ex-vivo generation of granulopoietic post-progenitor cells for clinical use after peripheral blood progenitor cell transplantation (abstr). Exp Hematol 25:805
12. Emerson SG, Sieff CA, Gross RG, et al. (1987) Decreased hematopoietic accessory cell function following bone marrow transplantation. Exp Hematol 15:1013
13. Atkinson K, Seymour R, Altavilla N, Cooley M, Biggs J (1992) Cytokine activity after allogenic bone marrow transplantation. IV. Production of mRNA for IL-3 and GM-CSF by mitogen-stimulated circulating mononuclear cells. Bone Marrow Transplant 9:175
14. Cairo MS, Gillan ER, Weinthal J, et al. (1993) Decreased endogenous circulating steel factor (SLF) levels following allogeneic and autologous BMT: lack of an inverse correlation with post-BMT myeloid engraftment. Bone Marrow Transplant 11:155
15. Domenech J, Gihana E, Truglio D, et al. (1994) Haemopoiesis of transplanted patients with autologous marrows assessed by long-term marrow culture. Br J Haematol 88:488
16. Testa NG, Hendry JH, Molineux G (1994) Long-term bone marrow damage in experimental systems and in patients after irradiation or chemotherapy. Anticancer Res 5:101
17. Domenech J, Linassier C, Gihana E, et al. (1995) Prolonged impairment of hematopoiesis after high-dose therapy followed by autologous bone marrow transplantation. Blood 85:3320
18. Wichmann HE, Loeffler M (1985) Mathematical modeling of cell proliferation. In: Stem cell regulation in hemopoiesis, vols 1 and 2. Boca Raton, FL: CRC Press
19. Wichmann HE, Loeffler M, Schmitz S (1988) A concept of hemopoietic regulation and its biomathematical realization. Blood Cells 14:411
20. Loeffler M, Pantel K, Wulff H, Wichmann HE (1989) A mathematical model of erythropoiesis in mice and rats. Part 1: Structure of the model. Cell Tissue Kinet 22:13
21. Wichmann HE, Loeffler M, Pantel K, Wulff H (1989) A mathematical model of erythropoiesis in mice and rats. Part 2: Stimulated erythropoiesis. Cell Tissue Kinet 22:31
22. Wulff H, Wichmann HE, Pantel K, Loeffler M (1989) A mathematical model of erythropoiesis in mice and rats. Part 3: suppressed erythropoiesis. Cell Tissue Kinet 22:51
23. Scheding S, Loeffler M, Anselstetter V, Wichmann HE (1992) A mathematical approach to Benzo[a]pyrene-induced hematotoxity. Arch Toxicol 66:546
24. Scheding S, Loeffler M, Schmitz S, Seidel HJ, Wichmann HE (1992) Hematotoxic effects of benzene analyzed by mathematical modeling. Toxicology 72:265
25. Schmitz S, Loeffler M, Jones JB, Lange RD, Wichmann HE (1990) Synchrony of bone marrow proliferation and maturation as the origin of cyclic haemopoiesis. Cell Tissue Kinet 23:425
26. Schmitz S, Franke H, Brusis J, Wichmann HE (1993) Quantification of the cell kinetic effects of G-CSF using a model of human granulopoiesis. Exp Hematol 10:755
27. Schmitz S, Franke H, Loeffler M, Wichmann HE, Diehl V (1994) Reduced variance of bone-marrow transit time of granulopoiesis—a possible pathomechanism of human cyclic neutropenia. Cell Prolif 27:655
28. Schmitz S, Franke H, Wichmann HE, Diehl V (1995) The effect of continuous G-CSF application in human cyclic neutropenia: a model analysis. Br J Haematol 90:41
29. Brugger W, Birken R, Bertz H, et al. (1993) Peripheral blood progenitor cells mobilized by chemotherapy plus granulocyte-colony stimulating factor accelerate both neutrophil and platelet recovery after high-dose VP16, ifosfamide and cisplatin. Br J Haematol 84:402
30. Brugger W, Henschler R, Heimfeld S, Berenson RJ, Mertelsmann R, Kanz L (1994) Positively selected autologous blood CD34$^+$ cells and unseparated peripheral blood progenitor cells mediate identical hematopoietic engraftment after high-dose VP16, ifosfamide, carboplatin, and epirubicin. Blood 84:1421
31. Brugger W, Heimfeld S, Berenson RJ, Mertelsmann R, Kanz L (1995) Reconstitution of hematopoiesis after high-dose chemotherapy by autologous progenitor cells generated ex vivo. N Engl J Med 333:283
32. Killmann SA, Cronkite EP, Fliedner TM, Bond VP (1962) Mitotic indices of human bone marrow cells. I. Number and cytologic distribution of mitoses. Blood 19:743
33. Cartwright GE, Athens JW, Wintrobe MM (1964) The kinetics of granulopoiesis in normal man. Blood 24:780
34. Cronkite EP, Fliedner TM (1964) Granulocytopoiesis. N Engl J Med 270:1347
35. Cronkite EP, Fliedner TM, Stryckmans P, et al. (1965) Flow patterns and rates of human erythropoiesis and granulocytopoiesis. Ser Haematol 5:51
36. Cronkite EP, Vincent PC (1969) Granulocytopoiesis.Ser Haematol 4:3
37. Pike BL, Robinson WA (1970) Human bone marrow colony growth in agar-gel. J Cell Physiol 76:77
38. Donohue DM, Gabrio BW, Finch CA, Hanson ML, Conroy L (1958) Quantitative measurement of hematopoietic cells of the marrow. J Clin Invest 37A:1564
39. Cronkite EP, Bond VP, Fliedner TM, Killmann SA (1960) The use of tritiated thymidine in the study of hematopoietic cell proliferation. In: GEW Wolstenholme, M O'Connor (eds) Cell production and its regulation. Proceedings of the Ciba Foundation Symposium on Haemopoiesis, London: J&A Churchill, p 70
40. Lee GR, Bithell TC, Foerster J, Athens JW, Lukens JN (1993) Wintrobe's clinical hematology. Philadelphia: Lea & Febiger
41. Finch CA, Harker LA, Cook JD (1977) Kinetics of the formed elements of human blood. Blood 50:699
42. Williams SF, Lee WJ, Bender JG, et al. (1996) Selection and expansion of peripheral blood CD34$^+$ cells in autologous stem cell transplantation for breast cancer. Blood 87:1687
43. Metcalf D (1984) The hemopoietic colony stimulating factors. London: Elsevier Science
44. Fliedner TM, Tibkken B, Hofer EP, Paul W (1996) Stem cell responses after radiation exposure: A key to the evaluation and prediction of its effects. Health Physics 70:787
45. Anklesaria P, Kase K, Glowacki J, Holland CA, Sakakeeny MA, Wright JA, FitzGerald TJ, Lee CY, Greenberger JS (1987) Engraftment of a clonal bone marrow cell line in vivo stimulates hematopoietic recovery from total body irradiation. Proc Natl Acad Sci U S A 84:7681
46. El-Badri NS, Wang BY, Cherry, Good RA (1998) Osteoblasts promote engraftment of allogeneic hematopoietic stem cells. Exp Hematol 26:110
47. Lazarus HM, Haynesworth SE, Gerson SL, Rosenthal NS, Caplan AI (1995) Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells) [MPCs]: Implications for therapeutic use. Bone Marrow Transplant 16:557
48. Koc ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan A, Tainer N, Lazarus HM (1998) Rapid hematopoietic recovery af-

ter co-infusion of autologous culture expanded human mesenchymal stem cells (hMSCs) and PBPCs in breast cancer patients receiving high dose chemotherapy (abstr). Blood 92:274a

49. Lyman SD (1998) Biologic effects and potential clinical applications of Flt3 ligand. Curr Opin Hematol 5:192
50. Fliedner TM, Thomas ED, Meyer LM, Cronkite EP (1964) The fate of transfused H^3 thymidine-labeled bone-marrow cells in irradiated recipients. Ann New York Acad Sciences 114:510
51. Perry S, et al. (1958) Rates of appearance and disappearance of white blood cells in normal and various disease states. J Lab Clin Med 51:501
52. Fliedner TM, Cronkite EP, Killmann SA, Bond VP (1964) Granulocytopoiesis II. Emergence and pattern of labeling neutrophilic granulocytes in humans. Blood 24:683
53. Lord BI, Bronchud MH, Owens S, et al. (1989) The kinetics of human granulopoiesis following treatment with granulocyte colony-stimulating factor in vivo. Proc Natl Acad Sci U S A 86:9499
54. Smith SL, Bender JG, Berger C, et al. (1997) Neutrophil maturation of $CD34^+$ cells from peripheral blood and bone marrow in serum-free culture medium with PIXY321 and granulocyte-colony stimulating factor (G-CSF). J Hematother 6:323