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Patients by a Circulating Factor

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13. ABSTRACT (Maximum 200 Words) Some breast cancer patients are candidates for high dose therapy requiring collection of a cytokine-mobilized blood stem cell harvest for subsequent reinfusion to restore hematopoiesis and immune function. A proportion of patients respond poorly to mobilizing cytokines making collection of an adequate harvest inconvenient, prolonged and costly. The hypothesis of this project was that such patients had a circulating inhibitor of stem cell mobilization. Plasma from poorly versus vigorously mobilizing individuals was assayed in a mouse model for its ability to inhibit cytokine mobilization of stem and progenitor cells. There was a significant positive correlation between the number of CD34+ stem cells per collection and inhibition or stimulation of CD45CD34+ cells, GM-CFC and HPP-CFC progenitor cells and spleen weight of mice receiving plasma injections prior to cytokine injection. The majority of individuals who mobilized poorly (less than 10 ⁶ CD34+ cells/collection) showed inhibition of mobilization. In contrast, plasma from some vigorous mobilizers (over 5 x 10 ⁶ CD34+ cells/collection) enhanced mobilization. These data suggest that circulating inhibitor(s) and potentially, stimulator(s) of mobilization are generated as a result of cytokine injection. Genetic factors and prior therapy may influence this response. The inhibitor(s) might be TGF-beta. The identity of the stimulator(s) is unknown. Characterization and manipulation of these factors might permit adequate mobilization of all breast cancer patients.				
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FOREWORD

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“BC 961628: Inhibition of Stem Cell Mobilization in Breast Cancer Patients by a Circulating Factor”

(5) Introduction:

Statement of work revised

Year 1: In order to demonstrate proof of principle:

- a. Confirm preliminary data that injection of human plasma from poor blood stem cell mobilizers into mice prior to mobilization with cytokines (with appropriate controls) blocks mobilization (Aim #1).
- b. Commence recruitment of breast cancer patients with Stage II, 4-9+lymph nodes, Stage II-III \geq 10+lymph nodes and metastatic disease to donate a 10 ml blood sample. Plasma will be stored for subsequent assay employing normal donor plasma as the comparative control.
- c. Once the mobilization status (good, poor, intermediate) of patients recruited has been determined on the basis of the number of GM-CFC progenitor cells and CD34+ stem cells in the harvest and/or the number of leukaphereses required to achieve an adequate harvest, has been determined, patient samples will be identified for evaluation, compared to normal donor plasmas, in the mouse assay as in Work Statement #1 above (Aim #1).

(6) Body:

a. Acquisition of Clinical Samples

During the project period (08-25-97 to 08-24-99) which was extended for one year from 08-25-98 without additional funds, 89 clinical samples were acquired, in addition to 8 original samples employed to generate preliminary data, for a total of 97 samples. This includes samples from patients who were poor mobilizers or mobilized adequately as well as normal donors. This is close to the originally estimated number of 90 samples. However, since funds were awarded for only one year of three requested, it has been possible to assay only about one third of the samples, i.e. 25 patient and 10 normal donor samples. In order to maximize the potential information that could be gained, the samples to be assayed were selected by Dr. Kessinger on the basis of clinical judgement and the number of apheresis harvests required to obtain sufficient stem and progenitor cells for transplantation into the categories of having been derived from poor, adequate or good mobilizers. Detailed information on CD34+ stem content or granulocyte monocyte colony forming cells GM-CFC progenitors of the patient's harvests was not known until later when they were entered into the database. The samples selected for assay were coded upon arrival in the laboratory and consequently, were assayed blind. In the analyses of the data a sufficient range of values of the CD34+ and GM-CFC content of

the apheresis harvests was available to permit parametric correlation analyses which eliminated the need for an arbitrary characterization of mobilization vigor. At the same time clinical data (prior chemotherapies, etc.) as well as, some surrogates of genetic information were collected on a larger series of donors. The reason for the inclusion of genetic information was pre-clinical data suggesting mouse strain specific inhibitor(s) of mobilization (see Roberts et al. 1997). A larger group of patients was analyzed to determine clinical correlates because of the need for a large cohort to assess statistical significance. The correlation with the laboratory inhibitor assay was performed independently on a subgroup of these patients.

b. Laboratory Inhibitor Assay

As previously published (Sharp et al. 1998) and noted in the Interim Report, the laboratory inhibitor assay was developed and refined. This assay involves the injection of 0.2ml plasma from the patient or normal donor, collected at the time of the first apheresis and suspected to contain inhibitor, intravenously into groups of mice 10 mins prior to subcutaneous injection of the mobilizing cytokines (500 U/kg EPO + 15 µg/kg G-CSF). Twenty-four hours later the mice are necropsied to provide the following information: mononuclear cells/ml blood, spleen weight and cellularity, GM-CFC, HPP-CFC and CD45CD34+ cells per ml blood, per spleen and per femur. Each experiment is performed with a mobilized only control and an inhibitor-control (injection of plasma from part-body irradiated mice). The results are calculated in terms of mobilization following injection of inhibitor containing plasma as a percentage of the mobilization control (100%). A plasma sample, which inhibits would give a value of less than 100%. For example, the mean spleen GM-CFC \pm standard error for 6 experiments for the inhibitor control was $57 \pm 7\%$ (confidence limits 43-71 see Fig 4). Note that, in addition to detecting the presence of inhibitor, this assay might also detect enhancers of mobilization in which case assay values greater than 100% might be obtained.

c. Results of Inhibitor Assay

Analysis of the combined clinical-laboratory database presented several unanticipated challenges, which necessitate a cautious interpretation of the data.

For example, the initial classification of patients as good versus average versus poor mobilizers on the number of aphereses required to obtain an adequate harvest (proposed originally) likely underestimates the range of mobilization "vigor". The reason is that some patients can exceed the required collection of CD34+ cells or GM-CFC in a minimum of 2 aphereses whereas poor mobilizers might only meet the minimum in 4 or 5 or more aphereses. Consequently, it was necessary to calculate the average CD34+ cells or GM-CFC per apheresis for each patient.

Secondly, although there was concordance for some patients in the collection of CD34+ or GM-CFC progenitors, this concordance was not absolute (see Kessinger et al. 1999). This, therefore, presents a challenge as to which value should be selected to characterize

the harvest. It was decided to perform correlation analysis of each variable independently with the laboratory values.

From the analysis of the larger cohort of patients it was evident that both prior therapy and genetic factors correlated with the risk of poor mobilization. There was no evidence as to whether the mechanism of these effects is different and independent or the same. Furthermore, it is unclear if plasma from patients with these different backgrounds would give the same or different results in the laboratory assay. This series has too small an enrollment to analyze these questions and a much larger series of patients and normal donors will need to be studied to resolve these issues.

In the laboratory analyses, unexpected observations were changes in the mononuclear cells per ml of blood and in the spleen weights and cellularities of the recipients, which had already occurred in 24 hours. Since the coefficient of variation of the control values had only a very limited range, these changes in many instances are likely significant. The problems these differences create are that since changes are observed both in the frequency of progenitor cells and when corrected for cellularity, the total progenitor cell/ml blood or per spleen, it was not clear which value is the best measure of inhibition of mobilization. It was decided to focus on GM-CFC since these were measured in the preliminary studies of inhibition of mobilization. Additionally, high proliferative potential colony forming cells HPP-CFC > 2mm diameter represent an independent measure, since these progenitors are more primitive than GM-CFC. Finally CD45CD34+ stem cells were assayed. These are the most primitive population assayed. However, because these are rare cells, the coefficient of variation in the assay of these cells is much greater than for the other progenitor cells. Valid values could be obtained for spleen CD45CD34+ cells, but not for blood CD45CD34+ cells. The rate of positive events for the latter was too low to provide reliable data and this parameter was eliminated from the analysis. Since any inhibitors(s) could have differential effects on these populations, they were analyzed independently.

Increases in the weight and cellularity of the spleen of mice receiving cytokine injections is a surrogate of mobilization, presumably, because the mobilized cell populations are migrating to the spleen. The vigor of mobilization in the plasma donors as measured by CD34+ cells per apheresis was correlated with the mean spleen weight of groups of 5-8 murine recipients (Fig 1). There was a significant positive correlation for breast cancer patients ($P=0.012$) but not for normal donors ($P=0.48$). The correlation of spleen weight with CD34+ cells per apheresis collection was performed for the entire group of plasma donors (Fig 2). There was a highly significant positive correlation ($p \leq 0.01$) suggesting that properties of the plasma related to mobilization was a stronger influence than whether or not the plasma was obtained from a breast cancer patient or normal donor. The 95% confidence limits of the spleen weights of untreated mice, cytokine mobilized mice and mice treated with part-body radiated mouse plasma as inhibitor control are also shown.

Generally, a stem cell dose of 2×10^6 CD34+ cells/kg recipient is considered a minimum adequate transplant dose. This should be collected in two or fewer aphereses, i.e. 1×10^6

CD34+ cells/kg/apheresis. A significant proportion of the patients and a few normal donors in this study collected fewer than this number of CD34+ cells/apheresis, i.e. were poor mobilizers. However, this is not a reliable guide to the proportion of breast cancer patients or normal donors who are poor mobilizers since the plasma donors were selected to establish "proof of principle". The larger series of sequentially collected samples would need to be assayed in its entirety to make these evaluations.

The results obtained for one patient, where the recipient mice appeared to be ill, were omitted from subsequent correlation analyses. There was a significant positive correlation between CD34+ cells/collection and inhibition of CD45CD34+ cells in the spleen of plasma recipients (Fig 3, $P=0.01$). There was a positive significant correlation ($P=0.02$) with inhibition of murine spleen GM-CFC in plasma recipients (Fig 4). There was a significant correlation ($P=0.002$) between CD34+ cells/collection of the plasma donors and inhibition of spleen HPP-CFC in the murine recipients of the plasma (Fig 5).

There was no significant correlation between CD34+ cells/collection and inhibition of GM-CFC or HPP-CFC in the blood of murine recipients of human plasma (Fig 6 and Fig 7).

Fig 8 shows a highly significant correlation ($P \leq .0005$) between the number of CD34+ cells/collection and GM-CFC progenitors/collection. This is not unexpected. However, such a strong correlation has not been observed in all studies, potentially because if the harvests are replete with both stem and progenitor cells, the correlation is performed over a narrow range of values. Because of the wide range of harvests evaluated in this study, including very poor as well as very vigorous mobilizers, the extent of the correlation may be enhanced. Despite this strong correlation and the correlations of CD34+ cells/collection with some measures of inhibition of mobilization vigor, e.g. HPP-CFC per spleen, (above) the only significant correlation of GM-CFC/collection and any of the measures of inhibition of mobilization vigor (Figs 9-13) was with CD43CD34+ cells/spleen ($P=0.01$, Fig 9).

For some of the poor mobilizers the weight of the spleens of plasma recipients was lower than those of the mobilized controls. The other significant correlations also provided values of less than 100% of those of the controls. However, in several of the assays, e.g. inhibition of GM-CFC/spleen, HPP-CFC/spleen and the values obtained were greater than 100%, i.e. stimulation, not inhibition. This suggests that some of the human plasma samples enhanced cytokine mobilization in mice. Therefore, enhancers of mobilization, in addition to inhibitors, are present in the plasma of some humans receiving cytokines.

These results are compatible with, but do not establish that administration of cytokines to breast cancer patients or normal donors induces secondary cytokines or chemokines that are actually responsible for mobilization. In some cases inhibitors are generated, in others, enhancers of mobilization are generated. This balance may be influenced by genetic factors and prior therapy. The overall balance of these factors dictates the vigor of mobilization.

Clearly, there would be a great benefit to defining both the nature of the inhibitors and the enhancers of mobilization since this information should be exploitable to improve the results of mobilization for all patients and normal donors.

d. Identification of Inhibitors

Because of the shortened duration of the funded project, characterization of the inhibitor, which was original Technical Objective #3 and not included in the revised, more limited Statement of Work, has not been possible in a comprehensive fashion. In the Interim Report, assay of serum amyloid A showed no differences indicating that TNF, IL1 or IL6 were likely not involved. In addition, plasma containing inhibitor was subjected to three different, but potentially complementary assays: two dimensional gel electrophoresis; ELISA assay for TGF beta and Western blot analysis with immunoprecipitation of three TGF beta isotypes (beta 1, 2 and 3). The results showed that inhibitor containing plasma had approximately 8 µg/ml TGF-beta versus about 2µg/ml in non-inhibiting plasma. However, these values are at the limits of sensitivity of the assay and need confirmation.

Plasma which had been assayed with the murine method described earlier to demonstrate it contained inhibitor, compared to normal donor plasma which did not inhibit mobilization, was fractionated biochemically. The initial step was dialysis to segregate soluble small molecules from larger less soluble molecules which were precipitated by this step. The plasma containing inhibitor showed a much more extensive precipitate suggesting an increase in (a) large, somewhat insoluble molecule(s). Subsequently the soluble molecules were acidified resulting in essentially similar precipitated and soluble fractions. Heating the inhibitor-containing plasma too greater than 56°C gelled the protein. However, inhibitor could still be assayed after one or two thawing cycles.

Inhibitor-containing plasma versus normal non-inhibiting plasma samples were subjected to two-dimensional gel electrophoresis (Figure 14). Certain bands were present in the normal control gel and absent in the other and vice versa (identified by asterisks in Figure 14). The gels also showed several differentially expressed proteins (identified by diamonds in Figure 14). The plasma sample which contained inhibitor appeared to contain at least two proteins which were not present in the non-inhibiting plasma. Of particular interest, given the increased precipitation of a large poorly soluble protein in the preliminary biochemical analysis, is the large protein with intermediate charge.** If the association of this protein with inhibition can be established, it appears to be present in the gels in a sufficient quantity to be sequenced directly.

Figure 15a and 15b represent a gel analysis and immunoblotting for TGFβ isoforms 1,2 and 3 employing antibodies and antigens from Santa Cruz Biotechnology Inc. Fig. 15a shows analysis of normal, plasma and Fig. 15b of proven inhibitor-containing plasma.

The key presents the contents of each lane. Lane A is plasma alone. Lane B is the most important lane for the assessment of TGFβ in control and inhibitor containing plasma. No major differences between the gels were evident and no TGFβ was detected at this level of sensitivity in either plasma sample. The remaining lanes are control lanes. Equal

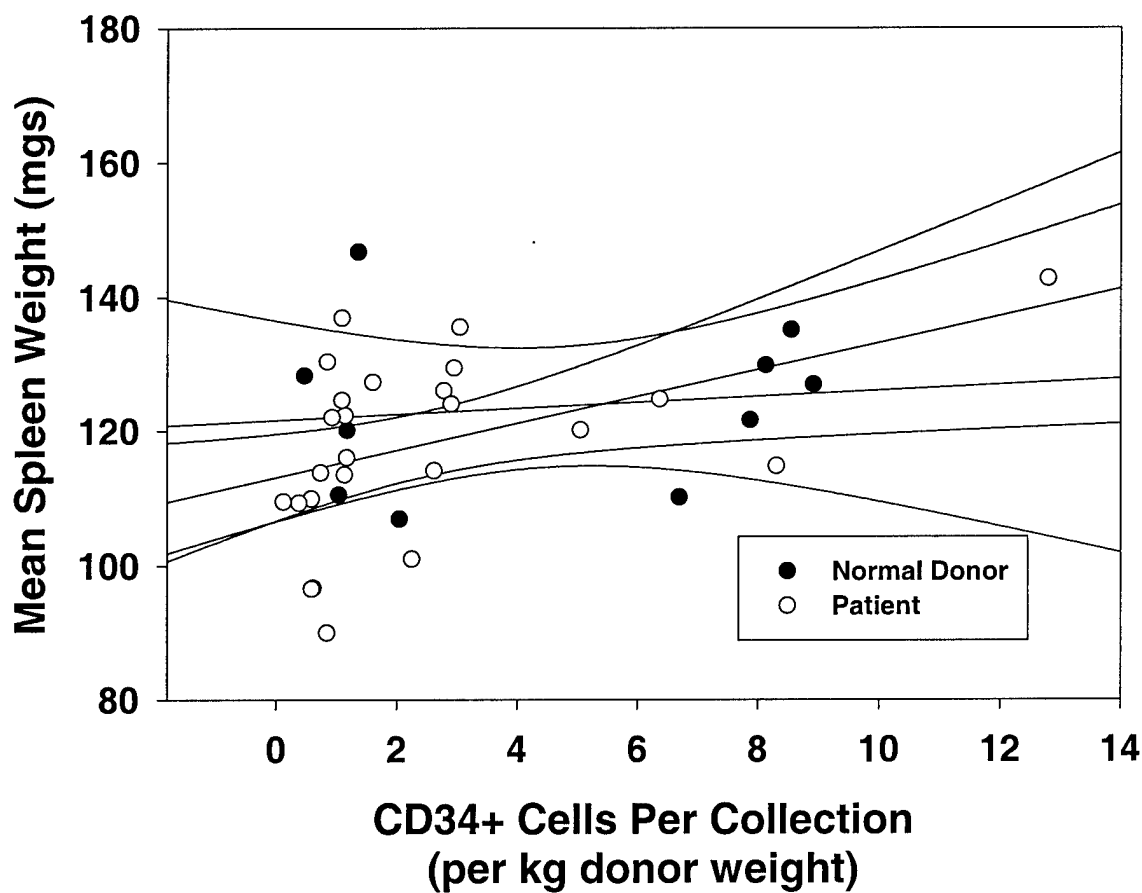
amounts of TGF β 1 + β 2 + β 3 (50 ng each) were added to plasma and mixed antibody to TGF β 1 + β 2 + β 3 in lane C or run with mixed antibody alone in lane D. TGF β is detected in both lanes. The antibody mixture was added together with 50ng TGF β 1 (lane E), TGF β 2 (lane F), and TGF β 3 (lane G). Each isoform is detected by the mixed antibodies but the efficiency of detection is best for TGF β 2 (including a higher molecular weight component), intermediate for TGF β 3 and poor for TGF β 1. Consequently, for this assay and these antibodies, detection sensitivity is greater for TGF β 2, lower for TGF β 3, and poor for TGF β 1.

A minor difference was evident between the gels which might be highly significant given the other preliminary results (the 2-D gels and fractionation analysis). At the top of lane B in Fig. 15b there is a large protein that did not migrate in the gel. This is not present in Fig. 15a.

The lack of detectable TGF β in normal plasma using this assay was anticipated because of sensitivity issues. Greatly elevated TGF β would likely have been seen. These data indicate that if TGF β was the inhibitory factor, the level in normal plasma is below the level of sensitivity of this assay which has differential sensitivity for TGF β with β 2 >> β 3 >> β 1. In terms of sensitivity of detection, TGF β 1 was barely detected at 50 ng (anticipated level in undiluted plasma is estimated to be 30-90 ng/ml in man). These data highlight the necessity, not only to detect the inhibitor, but demonstrates that the levels in inhibitor-containing plasma are physiological as regards inhibition of blood stem cell mobilization. Whether TGF β inhibits blood stem cell mobilization at these levels is unknown. However, based on the above results, it has to be considered as a candidate in patients exhibiting poor blood stem cell mobilization.

The nature of the factor(s) which enhance mobilization are completely unknown.

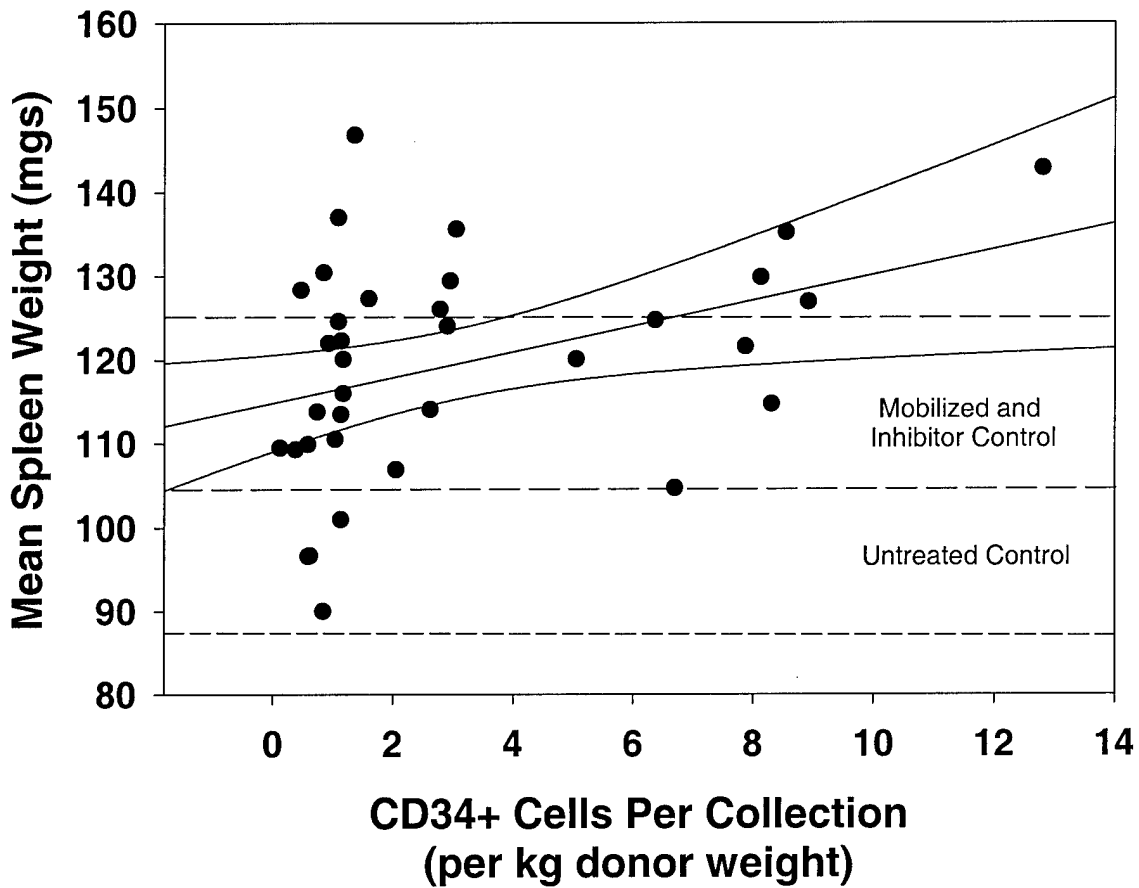
Figure 1: Correlation Between Patient or Normal Donor CD34+ Cells Per Collection in Individuals and Mean Spleen Weight of Mice in Inhibition Assay



Curve 1:
 nd34/coll, ndsplwt:
 Coefficients:
 b[0], 121.5548119884
 b[1], 0.446483696
 r², 0.0172196163
 p = 0.48

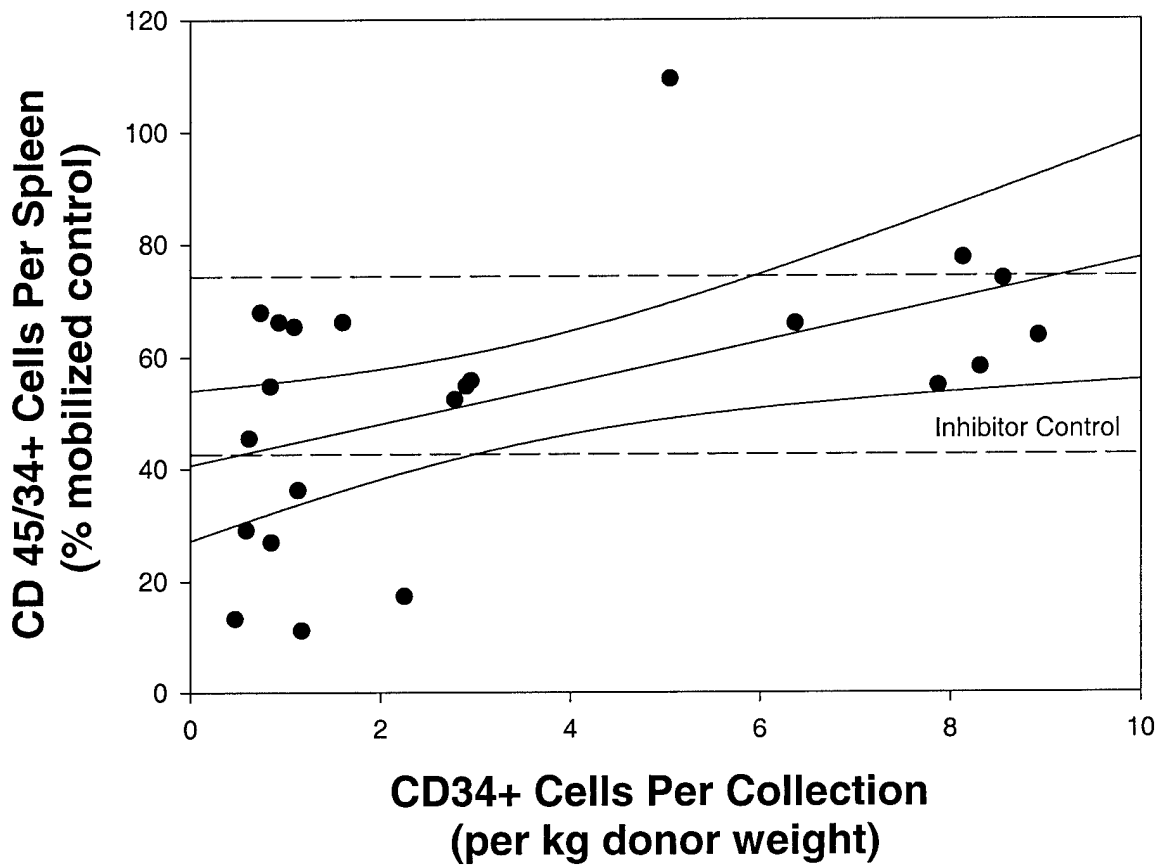
Curve 2:
 pt34/col, ptsplwt:
 Coefficients:
 b[0], 113.0622953758
 b[1], 2.0130543994
 r², 0.1996425119
 p = 0.012

Figure 2: Correlation Between CD34+ Cells Per Collection in Individuals and Mean Spleen Weight of Mice in Inhibition Assay



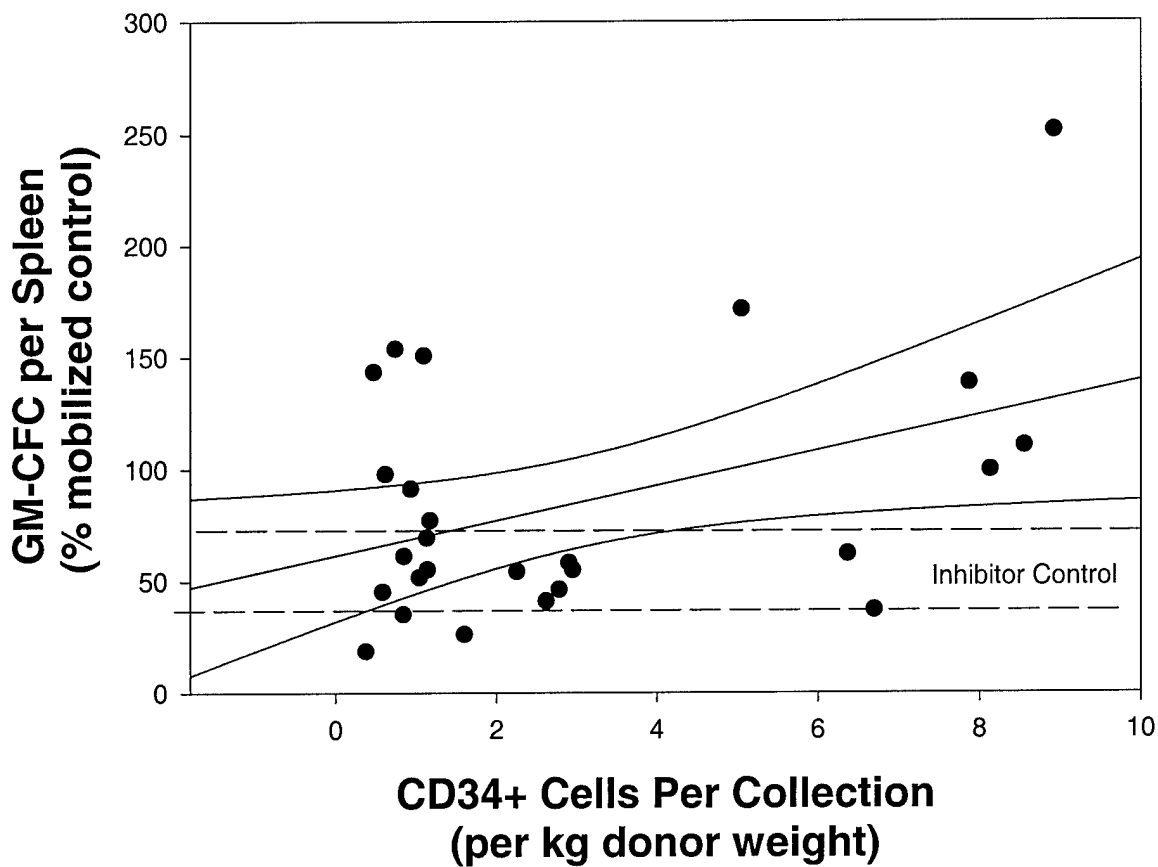
Curve 1:
 34/coll, splwt:
 Coefficients:
 b[0], 114.7942384378
 b[1], 1.58427095
 r², 0.1571267381
p = 0.01

Figure 3: Correlation Between CD34+ Cells per Collection in Individuals and Spleen CD45/34+ Cells of Mice in Inhibition Assay



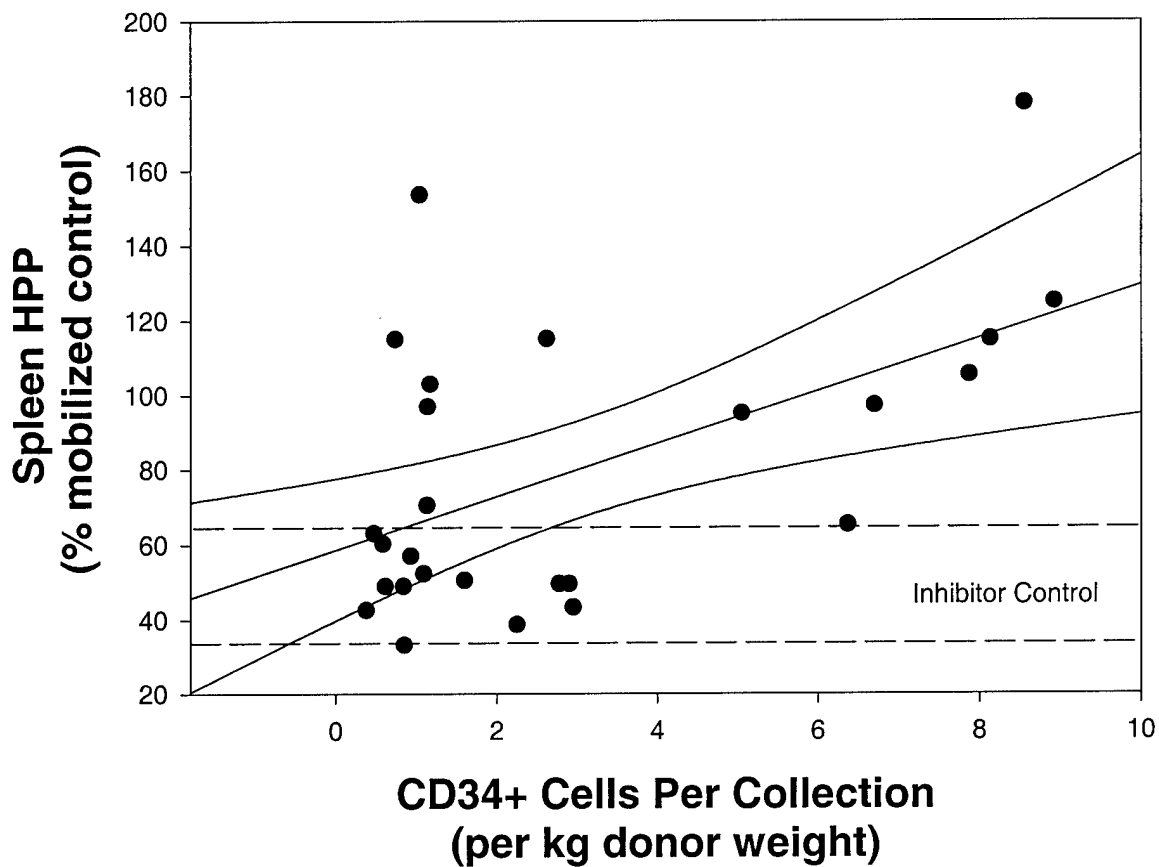
Curve 1:
 cd34/colB, 34/45 spl:
 Coefficients:
 b[0], 49.5828212264
 b[1], 2.5001011736
 r², 0.0577546441
p=0.01

Figure 4: Correlation between CD34+ Cells Per Collection in Individuals and GM-CFC Per Spleen (% mobilized control) of Mice in Inhibition Assay



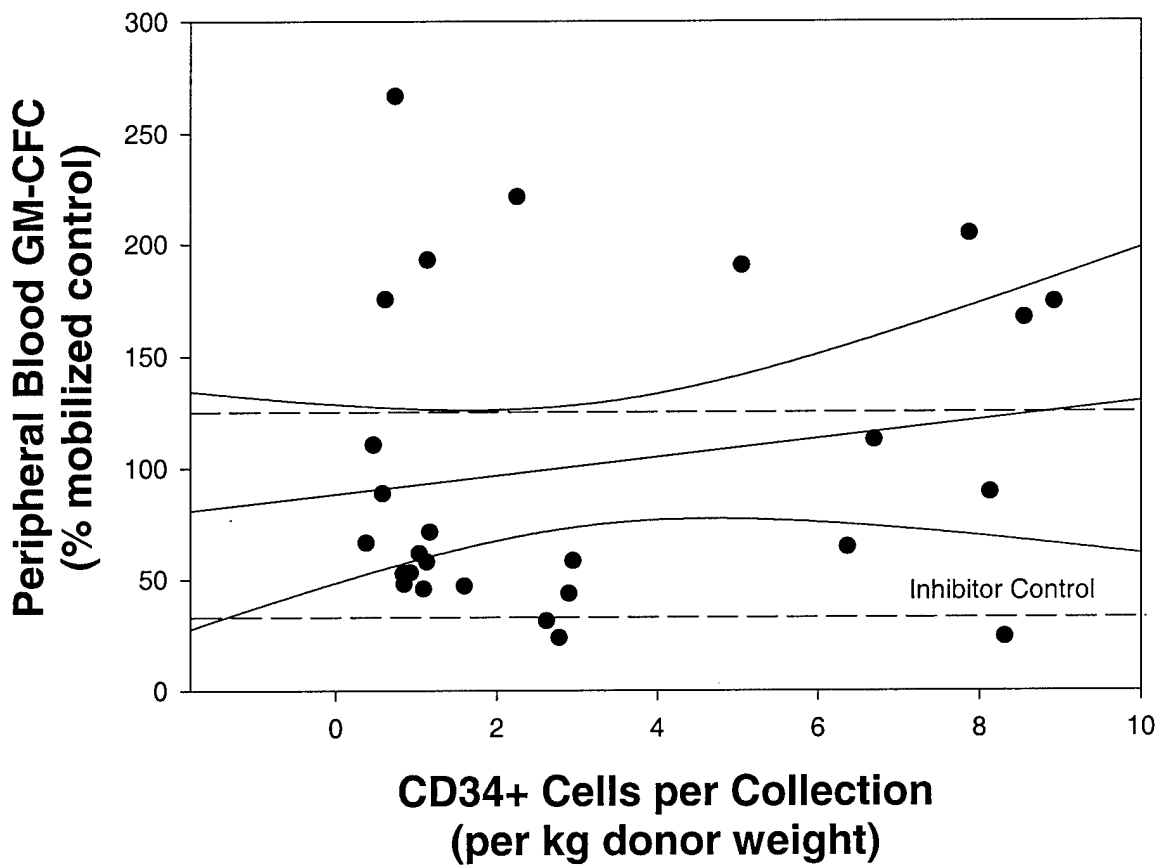
Curve 1:
 cd34/colB, spl gm % mob co:
 Coefficients:
 b[0], 65.2045725788
 b[1], 5.2141549134
 r², 0.0793656158
 p=0.02

Figure 5: Correlation Between CD34+ Cells Per Collection in Individuals and Spleen HPP (% mobilized control) of Mice in Inhibition Assay



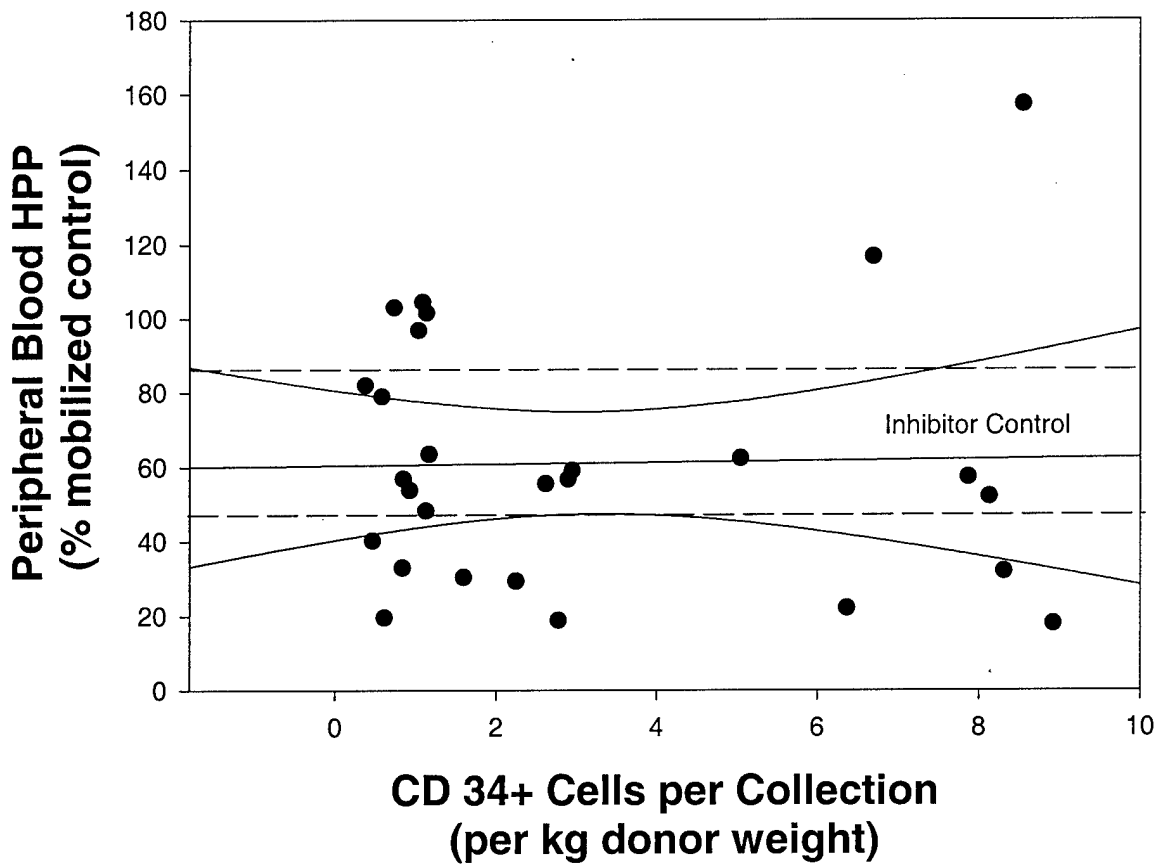
Curve 1:
 cd34/colB, spl hpp % mob c:
 Coefficients:
 b[0], 62.0859592137
 b[1], 4.71117013624
 r², 0.127730316
 p=0.002

Figure 6: Correlation Between CD34+ Cells per Collection in Individuals and Peripheral Blood GM-CFC (% mobilized control) of Mice in Inhibition Assay



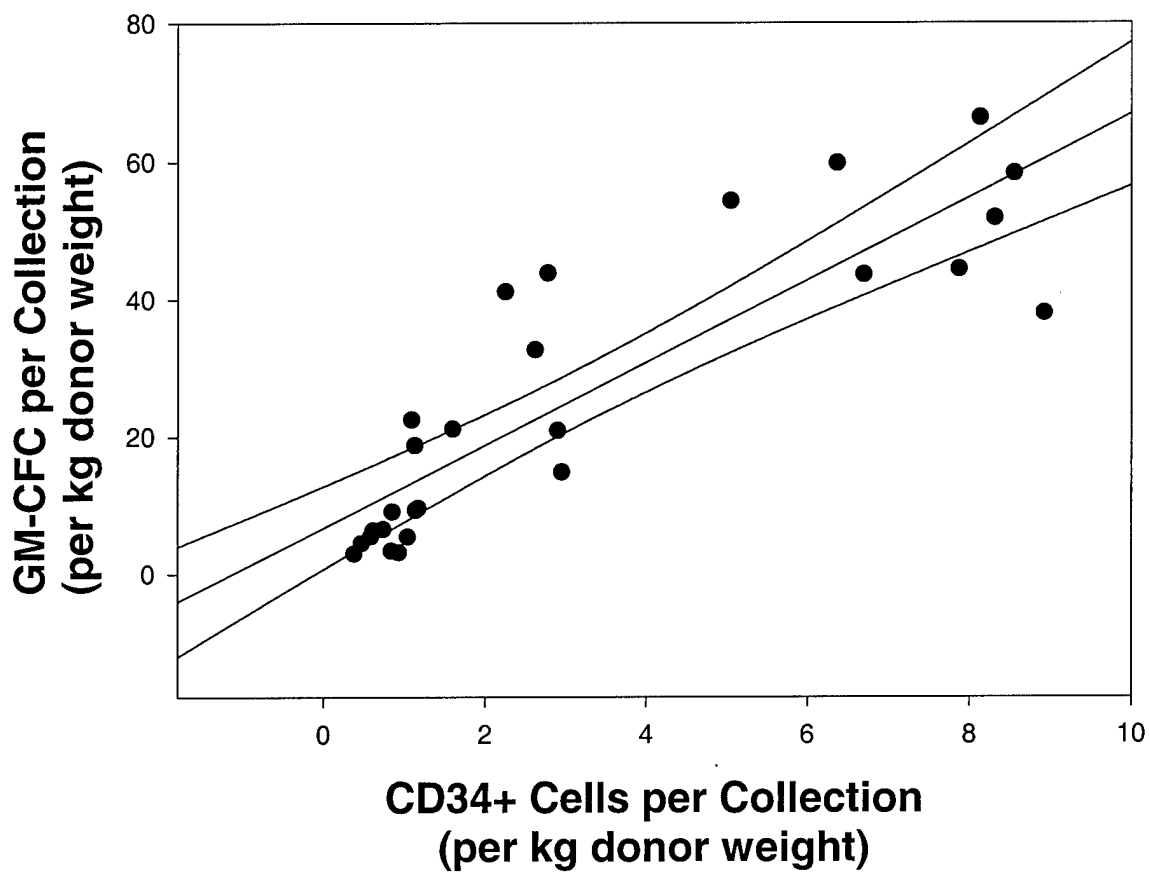
Curve 1:
 cd34/colB, % pb gm inhib:
 Coefficients:
 b[0], 88.32283705
 b[1], 4.1464576047
 r², 0.031998848
 p=0.24 N.S.

Figure 7: Correlation Between CD34+ Cells per Collection in Individuals and Peripheral Blood HPP (% mobilized control) of Mice in Inhibition Assay



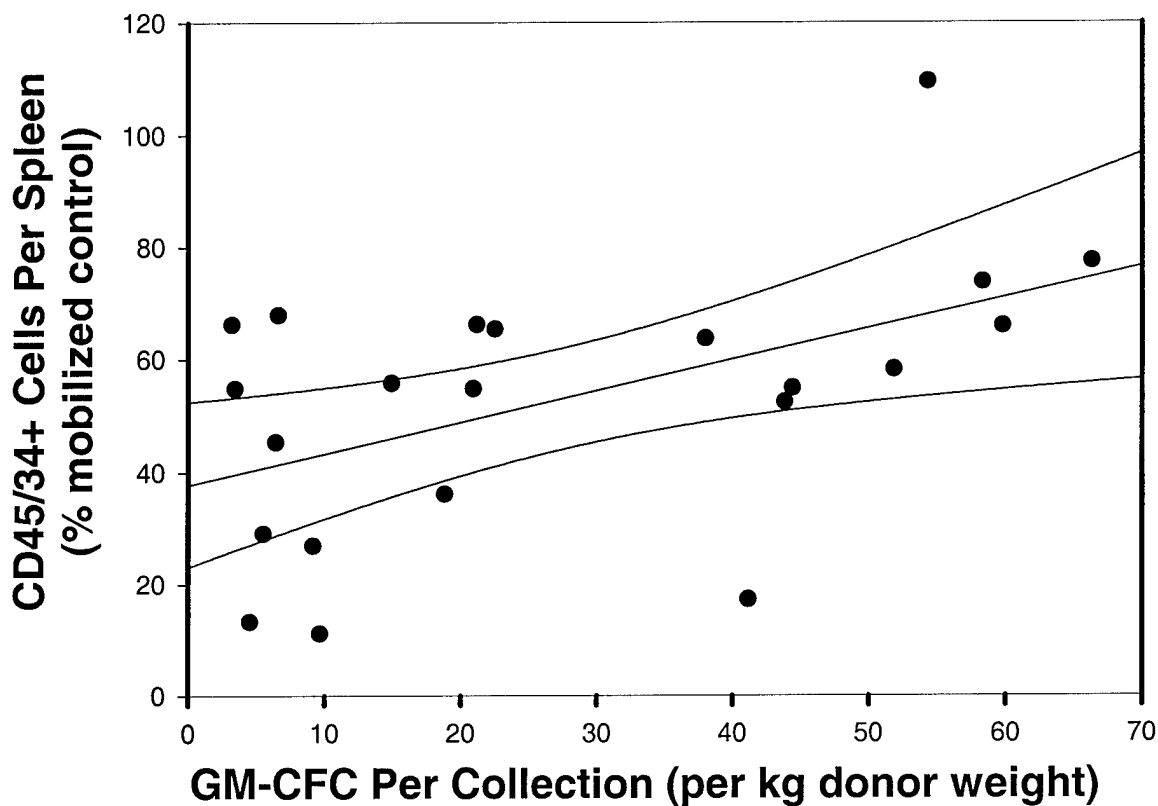
Curve 1:
 cd34/colB, % pb hpp inhib:
 Coefficients:
 b[0], 60.3802110388
 b[1], 0.2086038867
 r^2 , 3.3181284247e-4
p= 0.46 N.S.

Figure 8: Correlation Between CD34+ Cells and GM-CFC per Collection in Individuals



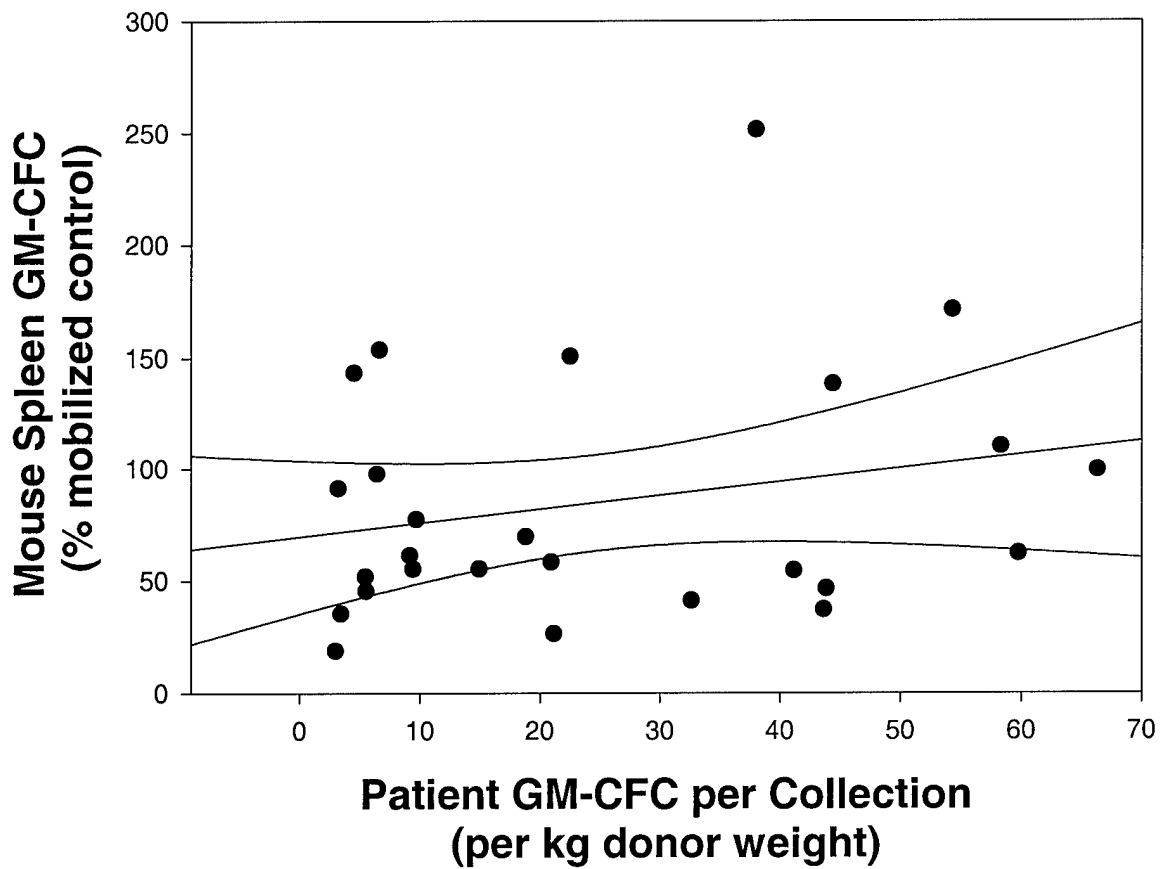
Curve 1:
 cd34/colB, cfugm/coll:
 Coefficients:
 b[0], 6.7574002767
 b[1], 6.0043001004
 r², 0.7513223156
p= 0.0005

Figure 9: Correlation Between GM-CFC Per Collection in Individuals and CD45/34+ Cells in Spleens of Mice in Inhibition Assay



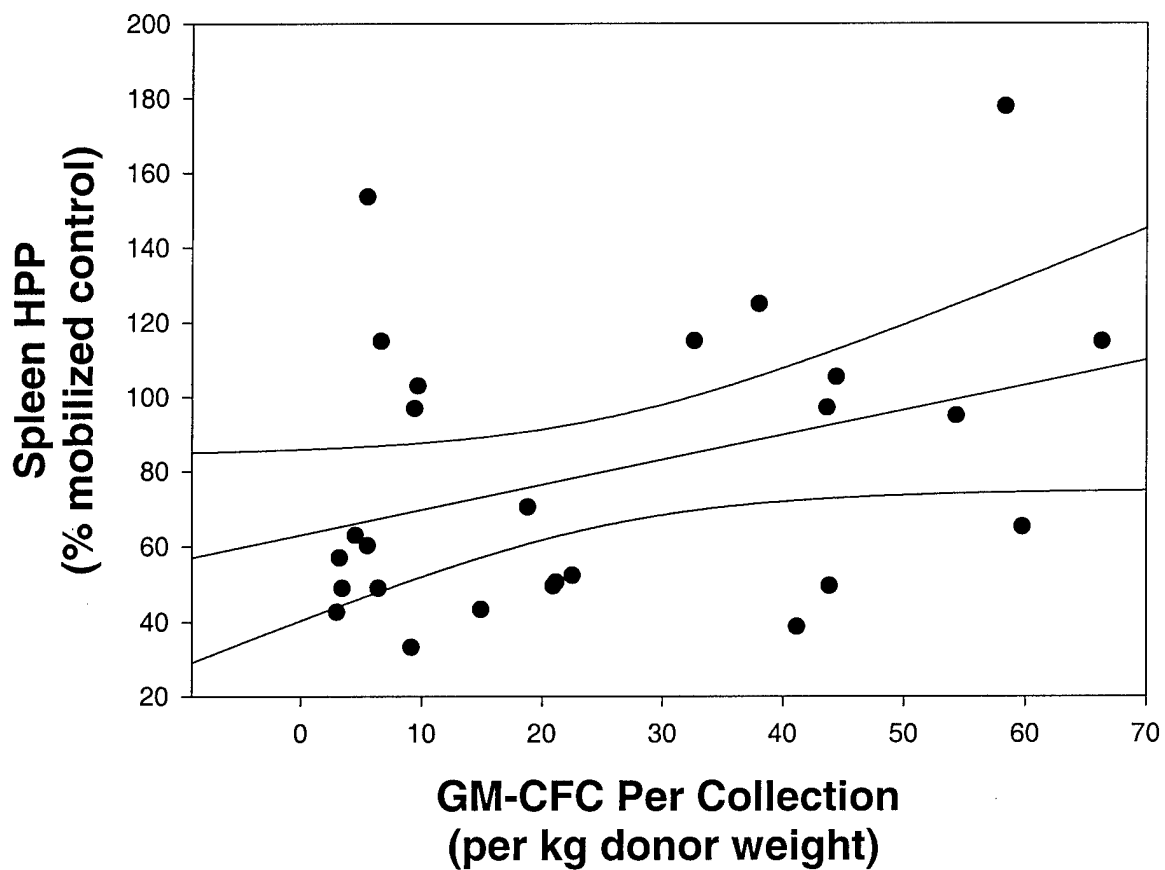
Curve 1:
 cfugm/coll, 34/45 spl:
 Coefficients:
 b[0], 37.756803517
 b[1], 0.5553837812
 r², 0.2613592173
 p=0.01

Figure 10: Correlation Between GM-CFC per Collection in Individuals and GM-CFC in Splens of Mice in Inhibition (% mobilized control)



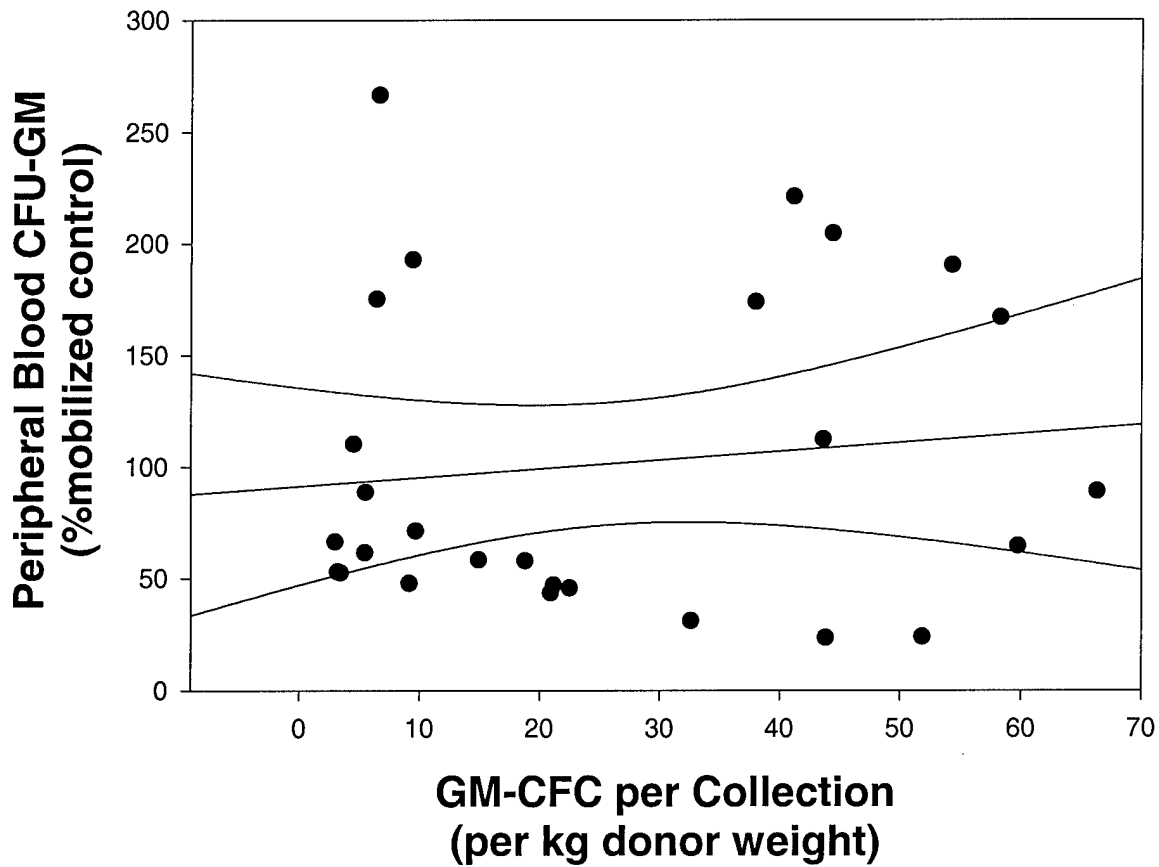
Curve 1:
 cfugm/coll, spl gm % mob co:
 Coefficients:
 b[0], 71.4939364684
 b[1], 0.3985697074
 r², 0.0222521305
 p =0.23 N.S.

Figure 11: Correlation Between GM-CFC Per Collection in Individuals and Spleen HPP (% mobilized control) of Mice in inhibition Assay



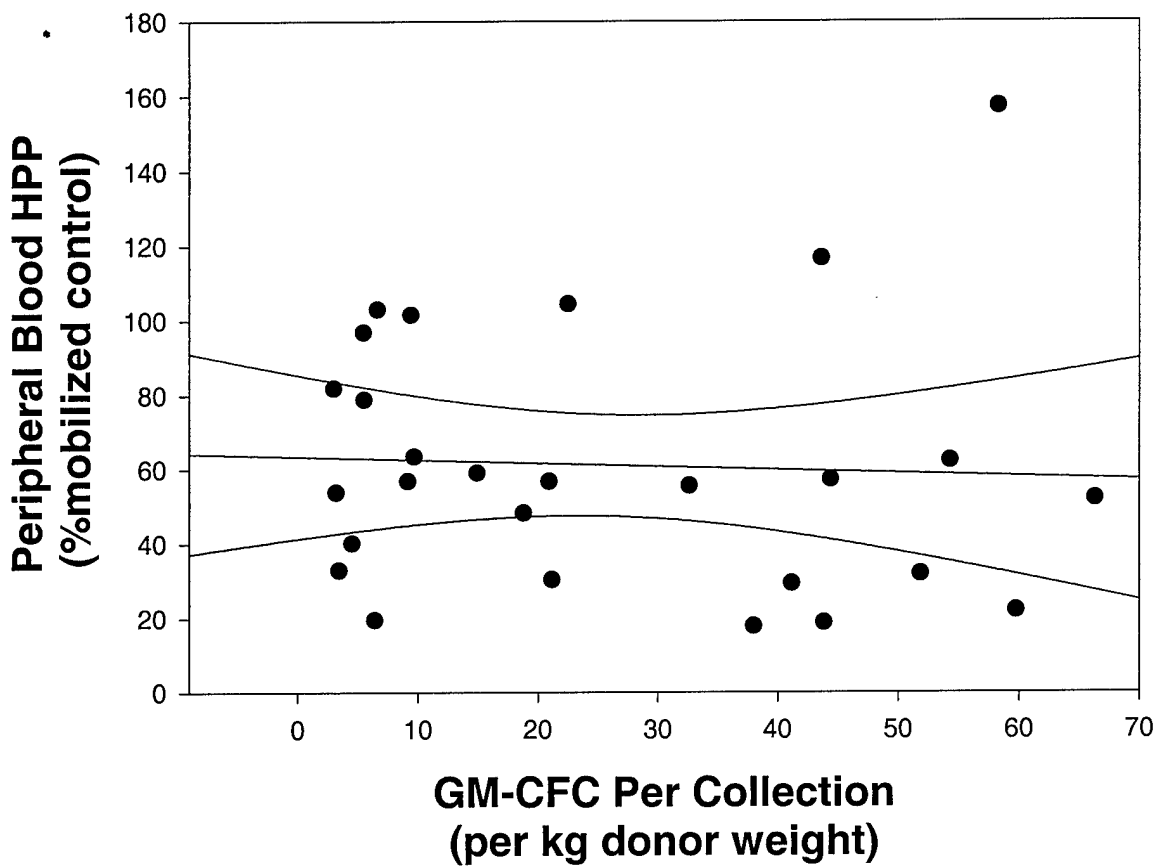
Curve 1:
 cfugm/coll, spl hpp % mob c:
 Coefficients:
 b[0], 65.0274867391
 b[1], 0.466119462
 r², 0.0599834499
 p= 0.12 N.S.

Figure 12: Correlation Between GM-CFC per Collection in Individuals and Peripheral Blood GM-CFC (% mobilized control) of Mice in Inhibition Assay



Curve 1:
 cfugm/coll, % pb gm inhib:
 Coefficients:
 b[0], 91.2921470724
 b[1], 0.3954899633
 r², 0.0139684851
 p= 0.28 N.S.

Figure 13: Correlation Between GM-CFC Collected in Individuals and Peripheral Blood HPP (% mobilized control) of Mice in Inhibition Assay



Curve 1:
 cfugm/coll, % pb hpp inhib:
 Coefficients:
 b[0], 63.3418767394
 b[1], -0.0887855236
 r², 2.8842387837e-3
 p = 0.40 N.S.

Figure 14

a. Normal Plasma

b. Inhibitor Containing Plasma

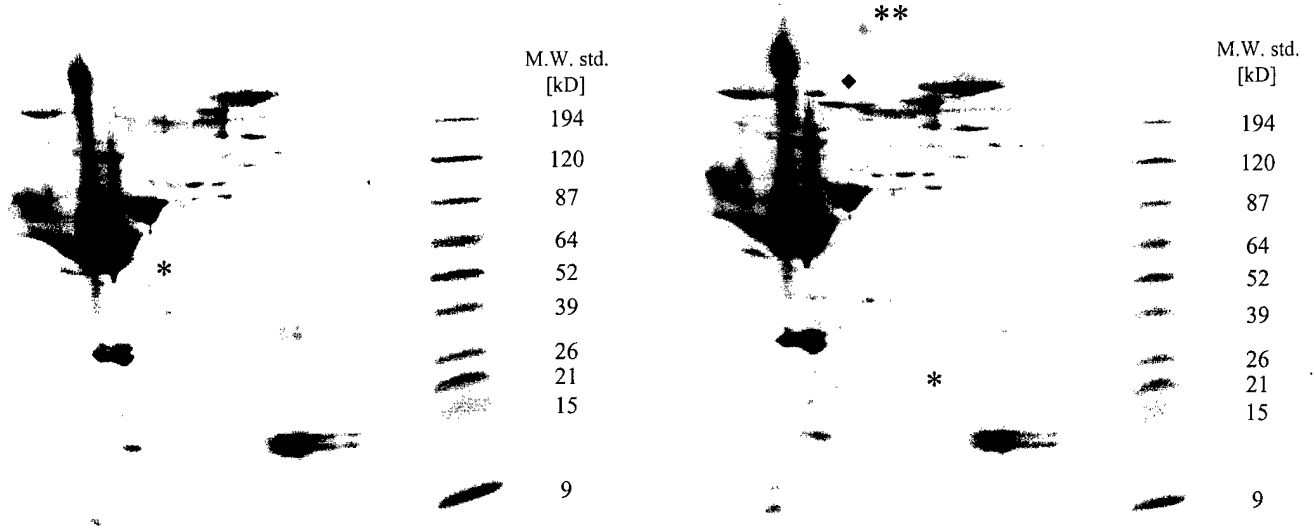
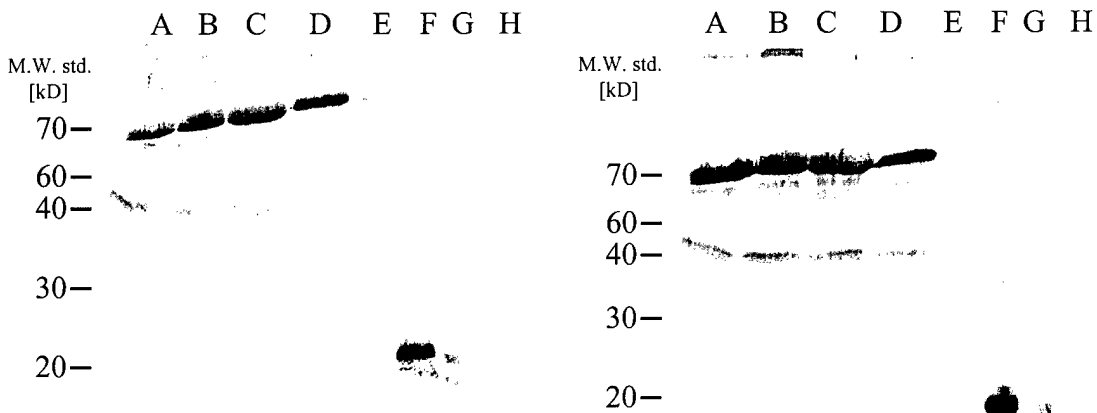


Figure 15

a. Normal Unirradiated Mouse Plasma

b. Part-Body Irradiated Mouse Plasma



Lane/ Content	A	B	C	D	E	F	G	H
Plasma	+	+	+	-	-	-	-	-
Antibody ($\alpha\beta_1+\beta_2+\beta_3$)	-	+	-	+	+	+	+	-
TGF ($\beta_1+\beta_2+\beta_3$)	-	-	+	+	-	-	-	-
TGF β_1	-	-	-	-	+	-	-	-
TGF β_2	-	-	-	-	-	+	-	-
TGF β_3	-	-	-	-	-	-	+	-

(7) **Key Research Accomplishments:**

- 97 patient or normal donor samples obtained for inhibitor assay. 25 patient and 10 normal donor samples assayed.
- Significant correlations of CD34+ cells/collection/kg donor weight and spleen weight and inhibition or lack of inhibition of CD45CD34+ cells, GM-CFC and HPP-CFC progenitors in the spleens of murine recipients of plasma from these individuals. A significant correlation of GM-CFC/collection/kg donor weight and CD45CD34+ cells in the spleen of murine recipients of plasma from these individuals.
- No significant correlations of GM-CFC/collection/kg donor weight and GM-CFC or HPP-CFC in spleen or blood of murine recipients of plasma from these individuals. No significant correlation of CD34+ cells/collection/kg donor weight and GM-CFC or HPP-CFC in blood of murine recipients of plasma.
- In addition to plasma samples from poorly mobilizing individuals which inhibited mobilization in the mouse, some plasma samples from vigorous mobilizers enhanced cytokine mobilization of stem and progenitor cells in the mouse.
- The inhibitor(s) or stimulator(s) are not TNF, IL1 or IL6 (Interim Report) however preliminary data suggests that TGF-beta may be present in plasma that inhibits mobilization.

(8) **Reportable Outcomes:**

Sharp JG, A Kessinger, SR Clausen, SL Mann, B O'Kane-Murphy. Concurrent Partial Body Radiation Prevents Cytokine Mobilization of Blood Progenitor Cells: An Effect Mediated by a Circulating Factor. *Journal of Hematotherapy* 7:343-349 (1998).

Kessinger A, JG Sharp. Mobilization of Blood Stem Cells. *Stem Cells* 16 (Suppl1):139-143 (1998).

Kessinger A, S Mann, J Lynch, B O'Kane-Murphy, JG Sharp. Patterns of Mobilization. Presented at Peripheral Blood Stem Cell Transplantations for the 10th Anniversary of the Symposium on Autologous PBSCT, September 5-8th, 1999, Mulhouse, France.

Kessinger A, S Mann, J Lynch, B O'Kane-Murphy, JG Sharp. Patterns of Hematopoietic Stem/Progenitor Cell Mobilization. Submitted to American Society of Hematology, December 1999.

Kessinger A, JC Lynch, K Petersen, JG Sharp. Patterns of Mobilization in Autologous Peripheral Blood Stem Cell Donors. Submitted to American Society of Hematology, December 1999.

Patents: Technology Disclosure entitled "Circulating factor which inhibits cytokine mobilization of stem cells" UNMC Disclosure #96017 filed June 4, 1999.

Degrees: None

Development of Cell Lines, Tissue or Serum Repositories: 97 plasma samples have been stored from this project.

Informatics: This project has validated the application of a mouse model to detect circulating inhibitor(s) and stimulator(s) of blood stem cell mobilization.

Funding Applied for Based on this Work: NIH Grant CA83935 entitled: Circulating inhibitors of blood stem cells mobilization, was recently submitted but not funded.

Employment or Research Opportunities: None

(9) **Conclusions:**

Poorly mobilizing breast cancer patients have a plasma inhibitor that can be assayed by its ability to inhibit cytokine mobilization of stem and progenitor cells to the spleen of mice. Plasma from some individuals who are vigorous mobilizers enhanced cytokine induced mobilization of stem and progenitor cells to the spleen of mice. The inhibitor might be TGF-beta. The identity of the stimulator is unknown. These results suggest that injections of cytokines into breast cancer patients or normal donors to mobilize stem cells into the blood are associated with the generation or release of additional factors which can either inhibit or stimulate blood stem cell mobilization. Obviously, the next step would be to identify these factors. This would permit manipulation of mobilization i.e. removal of inhibitor, provision of stimulator, so that all breast cancer patients and normal donors would mobilize adequately.

(10) **References:**

Sharp JG, A Kessinger, SR Clausen, SL Mann, B O'Kane-Murphy. Concurrent Partial Body Radiation Prevents Cytokine Mobilization of Blood Progenitor Cells: An Effect Mediated by a Circulating Factor. *Journal of Hematotherapy* 7:343-349 (1998).

Kessinger A, JC Lynch, K Petersen, JG Sharp Patterns of Mobilization in Autologous Peripheral Blood Stem Cell Donors. Submitted to American Society of Hematology, December, 1999.

Roberts AW, S Foote, WS Alexander, C. Scott, L. Robb and D. Metcalf. Genetic influences determining progenitor cell mobilization and leukocytes induced by granulocyte colony-stimulating factor. *Blood* 89:2736-2744 (1997).

(11) **Appendices:**

Copies of the two manuscripts referred to in Section 8 Reportable Outcomes were submitted with the Interim (Annual) Report.

Copies of the remaining three abstracts submitted referred to in Section 8 Reportable Outcomes are appended.

(12) **Binding:**

Report is stapled upper left-hand corner.

(13) **Final Report:**

For bibliography see Section 8: Reportable Outcomes

Personnel receiving pay from this research effort:

J.G. Sharp Ph.D.
A. Kessinger M.D.
Sally Mann M.S.
Barb Murphy M.S.

PATTERNS OF MOBILIZATION A. Kessinger*, S. Mann, J. Lynch, B. O'Kane-Murphy, J.G. Sharp, University of Nebraska Medical Center, Omaha, Nebraska, USA

Untreated DBA, Balb/c and C57B1 mice demonstrate strain-specific patterns of vigorous to poor CFU-GM mobilization to blood when treated with identical doses of G-CSF. Mobilization in cross-bred progeny of these strains showed at least a partial genetic inheritance of poor mobilization (Metcalf and colleagues). Poor mobilization occurs in up to 30% of cancer patients undergoing autologous blood stem cell (BSC) collection and was attributed to prior myelotoxic therapy, but since 10% of normal donors also exhibited poor mobilization, other explanations seem necessary. Plasma from poorly mobilizing donors or from partially irradiated mice (simulating prior cytotoxic therapy) injected into Balb/c mice prior to mobilizing cytokine dosing inhibited mobilization. (Sharp et al) This and other evidence suggested a circulating inhibitor could be responsible for poor mobilization. Therefore, the poorly mobilizing C57B1 strain was investigated for the presence of a circulating inhibitor. When plasma from an untreated C57B1 animal was injected into a Balb/c mouse prior to administration of mobilizing cytokines, the Balb/c mouse exhibited mobilization inhibition, suggesting that a species specific circulating inhibitor was responsible for the poor mobilization of the C57B1 mouse. To examine this question in man, mobilization patterns (increasing, decreasing or constant) of 89 consecutive patients who underwent G-CSF mobilization and autologous BSC collection were investigated. 23 patients were excluded because too few collections were performed to determine a slope and standard error. The remaining collections showed two predominant patterns of mobilization: either a constant (58%) or a decreasing pattern (42%) for CD34+ cells, a constant (52%) decreasing (58%) or increasing (3%) pattern for CFU-GM precursors, and a constant (40%) or decreasing (56%) pattern for mononuclear cells (MNC). The concordance of the estimated slopes across all 3 cell types was all decreasing 24%, all constant 26% and discordant 50%. There was no statistically significant relationship between slope concordance and age, gender, diagnosis or prior radiation. The variable of fewer prior chemotherapy regimens was statistically associated with concordance. The number of prior chemotherapy regimens was significantly related to the pattern of MNC collection but not to the CD34 pattern or the CFU-GM pattern. Poor mobilization may be the result of a genetically determined or therapeutically induced circulating inhibitor of mobilization. If all individuals have some level of circulating inhibitor, removal might result in sufficient mobilization to obviate apheresis for BSC collection.

PATTERNS OF HEMATOPOIETIC STEM/PROGENITOR CELL MOBILIZATION A.
Kessinger, S. Mann, J. Lynch, B. O'Kane-Murphy, J.G. Sharp, University of Nebraska Medical
Center, Omaha, Nebraska, USA

Untreated DBA, Balb/c and C57B1 mice demonstrate strain-specific patterns of vigorous to poor CFU-GM mobilization to blood when treated with identical doses of G-CSF. Mobilization in cross-bred progeny of these strains showed at least a partial genetic inheritance of poor mobilization (Roberts et al, Blood 1997;89:2736). Poor mobilization occurs in up to 30% of cancer patients undergoing autologous blood stem cell (BSC) collection and was attributed to prior myelotoxic therapy, but since 10% of normal donors also exhibited poor mobilization, other explanations seem necessary. Plasma from part-body irradiated Balb/c mice (simulating prior cytotoxic therapy) injected into Balb/c mice prior to mobilizing cytokine dosing inhibited mobilization. (J Hematother 7:343--349, 1998). This and other evidence suggested a circulating inhibitor could be responsible for poor mobilization. Therefore, the presence and effectiveness of a circulating inhibitor of mobilization was investigated in Balb/c, DBA and C57B1 untreated mice. Controls included Balb/c mice injected with plasma collected from part-body irradiated Balb/C mice 10 minutes before injection of mobilizing cytokines (EPO and G-CSF) and Balb/c mice injected with plasma collected from normal Balb/c mice 10 minutes before injection of identical doses of EPO and G-CSF. Identical volumes of plasma from either untreated C57B1 mice, known to be poor mobilizers or DBA mice known to be vigorous mobilizers were also injected into Balb/c mice prior to EPO + G-CSF injections. Balb/c mice injected with plasma from part-body irradiated Balb/c mice demonstrated an inhibition of mobilization of splenic CFU-GM and HPP colonies ($25.8 \pm 2\%$ and $36.4 \pm 5.6\%$) compared with Balb/c mice injected with plasma from non-irradiated Balb/c mice. The Balb/c mice injected with plasma from untreated DBA mice demonstrated an inhibition of mobilization of splenic CFU-GM and HPP colonies ($76.9 \pm 5.0\%$ and $83.5 \pm 8.9\%$) while those injected with plasma from untreated C57B1 mice demonstrated an inhibition of mobilization of splenic CFU-GM and HPP colonies of $27.2 \pm 7.3\%$ and $33.8 \pm 7.7\%$. These results suggest that a species specific circulating inhibitor was responsible for the poor mobilization of the C57B1 mouse. If all individuals have some level of circulating inhibitor, removal of this substance(s) might result in sufficient mobilization in the clinical arena to obviate apheresis for BSC collection.

PATTERNS OF MOBILIZATION IN AUTOLOGOUS PERIPHERAL BLOOD STEM CELL DONORS A. Kessinger, J.C. Lynch, K.Petersen, J.G. Sharp, University of Nebraska Medical Center, Omaha, Nebraska, USA

Mice exhibit strain-specific patterns of vigorous to poor CFU-GM mobilization to blood in response to administration of identical doses of G-CSF. Mobilization in cross-bred progeny of these strains showed at least a partial genetic inheritance of poor mobilization (Roberts et al. Blood 1997;89:2736). Poor mobilization occurs in up to 30% of cancer patients undergoing autologous blood stem cell (BSC) collection and was attributed to prior myelotoxic therapy, but since 10% of normal donors also exhibited poor mobilization, other explanations seem necessary. Previous studies (Sharp et al, J Hematother 1998;7:343 and Kessinger et al, Stem Cells 1998; 16 (suppl 1): 191) suggested a circulating inhibitor could be responsible for poor mobilization. To begin to examine whether genetic factors have a role in determining the vigor of mobilization in response to a constant dose of G-CSF, records of 52 consecutive patients who underwent G-CSF mobilization and autologous BSC collection using the same mobilization and collection protocol were investigated to determine if patterns of mobilization (increasing, decreasing or constant numbers of CD34+ and CFU-GM progenitors in successive apheresis products) could be recognized. Three distinct patterns were observed. Eight percent of patients exhibited increasing numbers of CFU-GM, CD34+ cells and mononuclear cells (MNC) in the successive apheresis products, 46% exhibited a decreasing number of all three cell types and 8% exhibited a constant number of all three cell types. The remaining patients had a discordant pattern among the three cell types. Patients who expressed HLA allele A 2 were more likely to exhibit a concordant pattern ($p = 0.012$), while patients who had previously received radiation therapy were more likely to exhibit a discordant pattern ($p = 0.042$). No other associations with the concordance of CD34+, CFU-GM and MNC patterns of mobilization were identified. If only CFU-GM and CD34+ cell numbers were considered, 13% exhibited an increase in the number of both cell types collected as the aphereses progressed, 48% a decreasing number and 21% a constant number. The remaining 17% showed discordance between the CD34+ and CFU-GM cell numbers. Patients who expressed HLA allele A 1 ($p = 0.032$) or allele A 24 ($p = 0.0089$) were more likely to exhibit discordance of CD34+ and CFU-GM mobilization patterns, but no other associations with concordance of CD34+ and CFU-GM mobilization patterns were identified. The identification of these patterns and associations suggest the possibility that mobilization vigor may be the result of a genetically determined or therapeutically induced inhibitor of mobilization.