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**Background:** Hematopoietic stem cell transplantation (HSCT) is an established and curative therapy for numerous hematological malignancies including multiple myeloma, non-Hodgkin's lymphoma, acute myelogenous leukemia, and acute lymphocytic leukemia. The predominant method of acquisition for stem cells in both autologous and allogenic HSCT settings involves mobilization of stem cells from the bone marrow into the peripheral blood. The two most commonly used mobilization strategies involve treatment with the chemokine G-CSF alone in the recovery phase following chemotherapy or in combination with chemotherapy followed by apheresis. Successful engraftment, recovery, and survival following HSCT depends on multiple factors including age and stem cell yield. Unfortunately prior to HSCT many recipients have a history of treatment with multiple high dose chemotherapies reducing overall stem cell number in the patient making collection of adequate numbers difficult (1). As a result stem cell yield is insufficient in up to 40% of treated patients depending on the type of cancer. Furthermore, poor mobilization after G-CSF treatment occurs in 25% of patients requiring extended or multiple aphaeresis which negatively impacts cost and patient outcomes most prominently in those with lymphoma's, multiple myeloma, and acute leukemia's (2). In the allogenic HSCT setting, up to 20% of healthy stem cell donors exhibit poor mobilization.

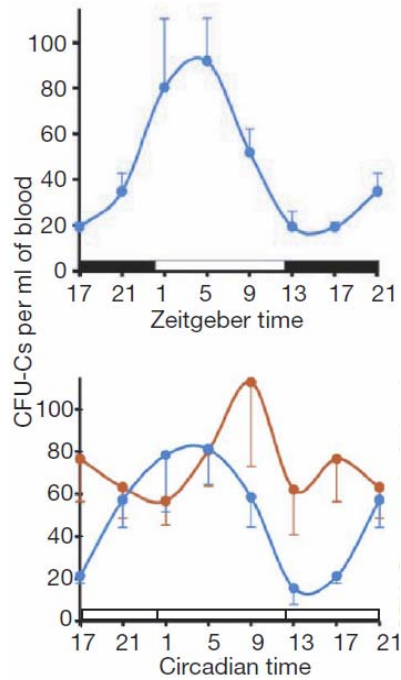
Recently it was demonstrated that circulating mouse and human hematopoietic stem cells and progenitors exhibit circadian fluctuations (Figures 1 & 2) in a species-specific manner (3, 4). Retrospective analysis of G-CSF mobilized human peripheral blood stem cells with respect to time of collection demonstrated a greater than two-fold increase in number, colony forming potential per unit of blood, and 60% increase in total yield when collection began in the evening. Practical reasons dictate that medical procedures like apheresis are performed in the morning. As HSC number oscillates in a circadian rhythm a simple adjustment in collection time has the potential for significant clinical impact. However, several critical issues remain to be addressed with respect to translating this insight into the clinical. First, it is essential to determine when HSC numbers peak in human peripheral blood of untreated and G-CSF mobilized patients. From this we can identify the optimal timing for collection to achieve the maximum potential stem cell yield. The second issue concerns the use of newer mobilizing agents, such as AMD3100, that have proved superior to G-CSF alone in enhancing stem cell yield when used in combination with G-CSF (5). It is also important to determine whether as in mice endogenous circadian rhythms influence yield independently of pharmacological mobilization. While beyond the scope of this proposal, the most critical task for translation of this insight is a prospective clinical trial to demonstrate the superiority of combined evening apheresis and pharmacological mobilization over pharmacological mobilization alone in cancer patients. We hypothesize that (a) HSC number in humans peripheral blood peaks in the evening at the beginning of the resting period independently of pharmacological mobilization, (b) pharmacologically mobilized patients that undergo apheresis during the evening when HSC number is maximized in the peripheral blood have increased HSC yields compared to apheresis in the morning.

**Experimental approach:** All human procedures and human subjects will be recruited to participate in the following study in accordance with NIH and IRB guidelines. Healthy subjects will be recruited between the ages of 18-70 years, male and female, and randomly assigned to four groups; untreated, G-CSF at 10ug/kg daily injected subcutaneously for four consecutive days in the morning, AMD3100 at 240ug/kg administered 12 hours prior to apheresis, and G-CSF plus AMD3100. Collection of peripheral blood, 5mL, will be performed at four hour intervals beginning at 8am and

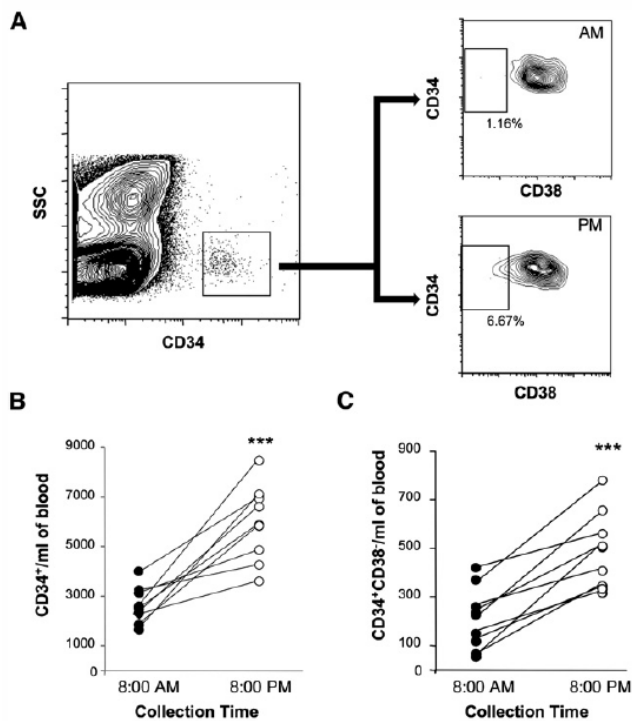
continuing until 12am the following morning. For the drug treatment groups collection will be performed on day 5 post-treatment. Human blood will be processed immediately by Ficoll-Paque gradient and ammonium-chloride mediated RBC lysis. Total cell number will be determined by counting on a hemocytometer. Flow cytometry will be performed by staining with CD34-PE and CD38-FITC primary conjugated antibodies of  $5 \times 10^6$  cells for discrimination of CD34<sup>+</sup> CD38<sup>-</sup> population on BD LSRII. Enumeration of total CD34<sup>+</sup> cells will also be performed as expression of CD34 is the clinical parameter used to determine if the minimum number of stem cells required for transplant have been collected. Colony forming potential will be determined using the CFU-C assay with established protocols (MethoCult GF+H4435). Colonies will be visualized and scored by microscopy after 7 days and 11 days in culture for CFU-G, CFU-M, CFU-E, and CFU-GM. ANOVA and spearman correlation test will be used for statistical analyses. These studies will elucidate in humans the circadian oscillation of HSC number in circulation and identify the time at which circulating HSC number is at maximum, which we expect to occur during the early evening. We will also be able to determine whether circadian oscillations in HSC number are independent of the mobilization regimen by comparing morning and evening collection times of each individual treatment groups for total stem cell number per unit volume of blood. We predict that increased circulating stem cell numbers in the evening will be observed independent of the mobilization method as seen in mouse models. These studies can be accomplished in my thesis laboratory with the aid and advice of Dr. Jamieson and members of her laboratory who have experience in human subjects research and in human tissue handling and hematopoietic assays.

Different mobilization strategies can alter the cellular and immunological profiles of the apheresis product. We will investigate whether changes are apparent in cellular composition, function, and gene expression of populations relevant to HSCT engraftment and success. After establishing the time of peak circulating HSCs, we will also establish the superiority of evening apheresis on stem cell yields for both G-CSF alone and in combination with AMD3100. This study will have direct translational impact as G-CSF is FDA approved for stem cell mobilization and G-CSF/AMD3100 combination is in phase III clinical trials and is potentially a future replacement and superior method for mobilization. Healthy subjects will be randomly assigned to two treatment groups, G-CSF alone and G-CSF/AMD3100 combination. Within each group, subjects will be assigned to either morning or optimal evening apheresis collection time as established above. Treatment will be performed as described above, with a single 15 liter apheresis to be performed on day 5 to collect stem cells. Human blood will be immediately processed for the following; enumeration of cell number and composition using flow cytometry for SSC vs CD45 expression and antibodies to lymphocyte and myeloid lineage markers, gene expression analysis by microarray of FACS sorted stem cells negative for lineage associated surface markers (Lin<sup>-</sup>) combined with CD34<sup>+</sup> and CD38<sup>-</sup> surface expression, analysis of and calculation of total stem cell yield will be performed for CD34<sup>+</sup> cells recovered per volume of blood processed per unit mass, and assessment of myeloid progenitor cell number by examining the Lin<sup>-</sup> CD34<sup>+</sup> CD38<sup>+</sup> population for IL-3Ra and CD45RA expression by flow cytometry. Sorted Lin<sup>-</sup> CD34<sup>+</sup> CD38<sup>-</sup> primitive stem cell gene expression patterns will be examined specifically for expression of maturation associated patterns and HSC transcripts associated with developmental state. The cycling status of stem cells and myeloid progenitors will also be analyzed by combined incorporation of BrdU in culture *ex vivo* with 7AAD DNA content staining of FACS sorted cells. CFU-C assays will be performed on FACS sorted CD34<sup>+</sup> stem and progenitor cells to examine differences in differentiation patterns and compared to CFU-C data of whole mononuclear cells performed above. These studies will aid in defining cellular changes associated with evening apheresis collection time

and provide a greater understanding of the potential graft as distinct functional properties and differentiation patterns are often observed between mobilization strategies.



**Figure 1.** Circadian oscillation of murine hematopoietic stem cells in circulation. Top figure: Normal oscillation of stem and progenitor cell number (blue line) is controlled by photic output. Bottom figure: Photic output controls circulating stem cell number (orange line-altered dark/light cycle).



**Figure 2.** Circadian fluctuations of human CD34<sup>+</sup> stem and progenitor cells. A. Flow cytometry of human peripheral blood cell CD34/CD38 expression. B&C. Circadian fluctuation of stem and progenitor cell number in human peripheral blood.

### References

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