Published in final edited form as:

Curr Opin Hematol. 2009 July ; 16(4): 235–242. doi:10.1097/MOH.0b013e32832bd0f5.

Circadian rhythms influence hematopoietic stem cells

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Abstract

Purpose of review—Hematopoiesis is tightly regulated in the bone marrow (BM) through the microenvironment, soluble factors from the circulation and neural inputs from the autonomic nervous system. Most physiological processes are not uniform but rather vary according to the time of day. There is increasing evidence showing the impact of biological rhythms in the traffic of hematopoietic stem cells (HSC) and their proliferation and differentiation capacities.

Recent findings—Recent evidence supports the role of the sympathetic nervous system (SNS) in the regulation of HSC behavior, both directly and through supporting stromal cells. In addition, the SNS transduces circadian information from the central pacemaker in the brain, the suprachiasmatic nucleus (SCN), to the BM microenvironment, directing circadian oscillations in hematopoiesis and HSC migration.

Summary—HSC traffic and hematopoiesis do not escape the circadian regulation that control most physiological processes. Clinically, the timing of stem cell harvest or infusion may impact the yield or engraftment, respectively, and may result in better therapeutic outcomes.

Keywords

circadian; clock; hematopoiesis; traffic; stem cell; chronotherapy

Introduction

Circadian (from latin *circa diem*, meaning “about a day”) rhythms allow the organism to anticipate its daily needs and accordingly modulate its physiology and behavior. The ability to anticipate future needs has provided a clear adaptive advantage, such that circadian clocks have been conserved throughout evolution from the unicellular diazotrophic cyanobacteria which exhibit daily rhythms in nitrogen fixation to more complex organisms.

In mammals, light signals detected from the retina entrain circadian rhythms to the day-light cycle that affect most physiological processes including sleep, hormone secretion, cell cycle and immunity. Other species are adapted to sense different environmental cues; for example, crustaceans and fish display circatidal activities related to rhythmic (~12h) tidal flooding and ebbing [1]. Despite its buried location inside the skeleton, the hematopoietic system is not spared from circadian influence. In this article, we will briefly review the evidence for circadian oscillations in proliferation and migration of hematopoietic stem and progenitor cells, and the possible underlying mechanisms.

The molecular clock

Circadian rhythms are sustained at the molecular level by asynchronous expression of the so-called “clock genes” that interact in feedback loops. At the organismal level, the suprachiasmatic nucleus (SCN) localized in the anterior hypothalamus is the central pacemaker that regulates oscillations in multiple peripheral tissues. The central clock is entrained by photic cues transmitted through the retinal-hypothalamic tract (RHT), allowing for the daily reset of circadian oscillations. The molecular basis for the circadian clock centers on the basic principle that in the SCN, and also in multiple peripheral tissues, the expression of a group of clock genes (*Bmal1*, *Clock*, *Npas2*) occurs asynchronously with the transcription of other genes (*Cry1*, *Cry2*, *Per1*, *Per2*, *Rev-erb-α*, *Rev-erb-β*) that form feedback loops (Figure 1 and reviewed in [2,3]). The basic mechanism of circadian gene expression involves the translation of *Bmal1* and *Clock*, which results in their heterodimerization and binding to E-boxes on the promoters of genes undergoing timed fluctuations and also other clock genes that in turn extinguish the expression of *Bmal1* and *Clock*. This yields autoregulatory loops that occur approximately in 24h cycles. Besides these transcriptional fluctuations, the expression of clock genes is also regulated by post-transcriptional, post-translational and epigenetic mechanisms, such as phosphorylation, ubiquitination, and chromatin remodeling (reviewed in [4]). In addition to the light signals, other physiological cues can regulate the central clock. For example, when food is only available during the normal sleep period, rhythms are reset in the dorsomedial hypothalamus such that the active period correspond to the period of food availability [5].

Core clock gene expression is not restricted to the SCN; most cells of the body in fact express clock genes. It is thought that the central clock synchronizes these peripheral oscillators in a hierarchical fashion. It has been estimated that up to 10% of the genes in any tissue are composed of clock-controlled genes [6]. Studies using conditional repression of *Bmal1* transcription in hepatocytes have revealed that most rhythmically active genes depend on an intact liver oscillator, whereas another class of genes fluctuated from central clock signals [7]. Bone remodeling also depends on the expression of clock genes by the osteoblast, which suppress the expression of G1 cyclins and osteoblast proliferation [8]. While clock gene expression has clearly been documented in the bone marrow [9,10] and in HSCs [11,12], it is not yet clear whether, and if so how, these oscillations impact hematopoiesis.

Circadian oscillations of progenitor activity in the bone marrow

Several studies have documented circadian oscillations in the rate of DNA synthesis and the colony-forming ability in the bone marrow (BM) of humans and mice [13–27] (Table 1). In humans, BM colony-forming units-granulocyte-macrophage (CFU-GM) exhibit significant circadian oscillations that peak shortly after noon [14,17]. Interestingly, this coincides with the peak in DNA synthesis, raising the possibility that HSC proliferation and differentiation into committed progenitors may follow circadian variations. Strikingly, a 6-fold peak-trough difference in the number of CD34+ cells has been found in the human BM, with the acrophase around noon [17]. Studies in different mouse strains have shown 24 hour circadian oscillations in progenitor activity (Table 1). However, there is a wide variability across the studies, with two separate acrophases of progenitor activity: one possible peak shortly after the onset of light (the beginning of the murine rest phase) and one during the night (the latter part of the murine activity span). As in humans, these peaks appear to overlay with the acrophases of DNA synthesis in the BM (Table 1). These changes of DNA synthesis are suggested to have clinical relevance. For example, differential circadian fluctuations in DNA synthesis between host and tumor cells may maximize anti-sarcoma responses of 5-FU while minimizing toxicity to the gut and BM [28]. Moreover, a circadian variability in engraftability of congenic BM following sublethal bone marrow transplant has been described, with the nadirs associated with the highest proportion of BM cells in S phase, suggesting that the circadian regulated DNA synthesis in BM cells may result in diurnal variability in engraftability [29].

However, when analyzing circadian oscillations in the number of cells, it is important to consider the possible oscillation in the expression of the markers utilized to identify those populations. Hence, oscillations in the expression of surface markers could be erroneously interpreted as variations in BM cell numbers. Similarly, fluctuations in the expression of receptors that determine the *in vitro* responsiveness to cytokines and growth factors in colony-forming assays might impact the progenitor activity and might not reflect the *in vivo* situation. Indeed, the ability to increase murine colony yield with IL-3, G-CSF, or GM-CSF was maximal 3 hours after the onset of light when administered into CFU cultures, suggesting a circadian expression of receptors to these cytokines [26]. Therefore, in the absence of complementary *in vivo* approaches, the data regarding oscillations in cell numbers or activities in culture should be interpreted with caution.

The mechanisms behind the fluctuations of BM progenitor activity or cell cycling are unclear. As it is the case with any rhythmic activity in peripheral organs, it is important to consider the possible contribution of the sympathetic nervous system, humoral factors, or an intrinsic oscillator.

Possible contribution of the sympathetic nervous system

The SCN regulates circadian oscillations of multiple tissues through its autonomic output. Anatomically distinct pre-sympathetic and pre-parasympathetic neurons have been identified in the SCN [30]. A role for the sympathetic nervous system (SNS) is supported by the diurnal patterns of epinephrine (EPI) and norepinephrine (NE) levels in the blood and urine of humans [31–33], indicating that the activity of the SNS undergoes circadian

rhythms. NE can be secreted directly from nerve terminals or from the adrenal medulla, whereas EPI is only synthesized in the adrenal medulla (4:1 ratio over NE). SNS responses typically show regional variability, with the sympathetic outflow to some organs being activated but to other regions unchanged or inhibited [34]. A role for the SNS in the relay of signals from the SCN to peripheral tissues was first demonstrated in the liver, where electrical stimulation of the sympathetic nerves or adrenaline injection caused an elevation of bioluminescence in the liver area of transgenic mice carrying the luciferase gene downstream of the *mPer1* promoter [35]. It was first suggested in 1972 that β -adrenergic signaling is required for circadian oscillations in the DNA synthesis of putative HSCs [36]. In the mouse BM, NE and dopamine, but not EPI levels, display a circadian rhythmicity, peaking at night, that is sensitive to pharmacological sympathectomy with 6-hydroxydopamine (6OHDA), and is positively correlated with the proportion of BM in G2/M and S phase [37]. It is important to point out that plasma or tissue levels of NE are influenced by NE clearance, reuptake and degradation and therefore do not directly reflect SNS activity [34]. Measurements of regional spillover of NE from organs to venous circulation and microneurography, which are more accurate estimates of organ-specific SNS function [38], have been not been performed in the BM. Administration of NE is able to protect mice from an otherwise lethal dose of carboplatin by stimulating BM proliferation [39]. This indicates that the central SCN may regulate hematopoiesis through β -adrenergic receptor signaling, and that the acrophases in BM DNA synthesis may result from peaks in sympathetic tone. However, it should also be noted that earlier studies have reported that α -adrenergic receptors are present in the BM and that administration of NE causes a tonic inhibition of myelopoiesis [40,41], reversible with α 1-adrenergic antagonists. This tonic inhibition by NE was confirmed by suppression of CFU-GM. These opposite effects of NE on hematopoiesis have not been clarified further.

Possible contribution of humoral factors

There are numerous candidates that could theoretically affect progenitor growth and behavior in the BM. One obvious class of hormones is the glucocorticoids, which are produced in the adrenal cortex and regulate homeostatic functions in many vertebrate tissues, including the response to stress. Pharmacological dexamethasone treatment induces the expression of clock genes in cultured human and murine bone marrow stromal cells [42], rat fibroblasts and also phase-shifts expression in the liver, kidney, and heart of mice, but not in SCN neurons [43]. These findings suggest that glucocorticoids may be a physiologically important entraining molecule of peripheral clock genes. Interestingly, adrenalectomized rats placed in a 12-hour phase shift of light, or jet lag, demonstrated a more rapid adjustment and restoration of rhythms [44]. Thus, under constant lighting conditions, the authors concluded that corticosterone serves to stabilize photoperiod variations in the circadian timing system. This might represent an evolutionary advantage to prevent mammalian clocks from being entrained too easily by transient stimuli. In humans, the inflammatory cytokines IFN- γ , TNF- α , IL-1, and IL-12 were found to peak in circulation in the early morning, prior to the acrophase of cortisol that counteracts their effects whereas GM-CSF peaked in the evening [45,46]. However, whether the circadian rhythm of DNA synthesis and colony forming capability in the bone marrow are modulated by glucocorticoids or soluble cytokines has not been reported using loss- or gain-of-function

analyses. Interestingly, there is some evidence that melatonin, a neuroendocrine hormone synthesized from a tryptophan precursor in the pineal gland in the latter part of the night in both humans and rodents, might affect progenitor capability of the rat BM. These authors found that pinealectomy performed in the previous afternoon ablated the biphasic bone marrow CFU-GM peaks at 06:00 and 18:00, and the oscillations were rescued by administration of melatonin [47].

Possible contribution from an intrinsic oscillator

It has been reported that murine CFU-GM activity in culture peaked at CT 09:00 independently of the time of harvest [27]. In addition, ablation of the SCN did not perturb the twenty-four hour rhythms of BM cell cycle distribution or progenitor activity [21]. Taken together, these two studies suggest that an autonomous and self-sustaining BM oscillator contributes to circadian variations in BM activity. Oscillations in *Per1*, *Per2*, and *Cry2* expression have been described in human CD34⁺ progenitor cells [48]. Recent studies have analyzed the expression of clock genes in the murine BM. Quantitative real-time RT-PCR studies have demonstrated the expression of clock genes in HSC-enriched Hoechst 33342-excluding side population [12] and sorted Lin⁻ Sca-1⁺ c-kit⁺ (LSK) cells (S. M.-F. and P. S. F., unpublished observations). However, expression of clock genes in the BM does not seem to follow rigorously organized circadian oscillations [10^{**},11]. Although our studies have shown an oscillatory trend for *Bmal1*, *Clock*, *Per1*, *Per2*, *Cry1*, and *Rev-erb-α* mRNA levels in total BM, these fluctuations were not statistically significant. This might be attributable to the heterogeneity in the BM compartment, with heterogeneous patterns in different cell types. Nonetheless, the pattern of mRNA oscillations were noticeably different when photic cues were changed from 12:12 light-darkness (LD) to constant light or 12:12 DL (jet lag), suggesting that the peripheral oscillators in the BM are entrained by the SCN. Conclusive studies with selective modification of clock gene expression in the BM will be needed to distinguish the respective roles of the central clock and a putative BM oscillator.

Circadian oscillations in HSC / progenitor egress from the bone marrow

Previous studies from our group have demonstrated that the SNS is required for both physiological and enforced egress of HSC / progenitors from the BM. G-CSF-induced mobilization of progenitor cells from the BM is dependent on SNS-mediated downregulation of *Cxcl12* and is enhanced by β 2-adrenergic agonists [49]. Human CD34⁺ cell migration, proliferation and mobilization are promoted by NE and EPI through β 2-adrenergic activation directly on CD34⁺ cells [50]. Unforced, physiological release of HSCs into the circulation is not random or steady but instead follows circadian oscillations. These oscillations are dependent on sympathetic signaling through the β 3-adrenergic receptors expressed by BM stromal cells, which leads to *Cxcl12* mRNA downregulation probably via degradation of the transcription factor Sp1 (Figure 2) [10^{**}]. These circadian oscillations are regulated by the molecular clock since *Bmal1*^{-/-} mice did not show fluctuations in circulating progenitors, and normal oscillations were entrained by light. Pharmacological sympathectomy with 6OHDA abrogated fluctuations of circulating HSCs and local denervation of the BM abolished fluctuations in the expression of *Cxcl12* in the BM. The *Cxcl12* gene promoter contains consensus E-box sequences, which raised the possibility of

direct regulation by a peripheral oscillator. However, primary BM stromal cultures from *Bmal1*^{-/-} and *Per1*^{-/-} *Per2*^{m/m} mice downregulated *Cxcl12* in response to stimulation with isoproterenol (a non-selective β -adrenergic agonist), similarly to control cultures. The mouse *Cxcl12* promoter also contains binding sites for Sp transcription factors, which are highly conserved in humans. Mutations in the proximal Sp site in the human [51] or mouse (D. Lucas and P.S.F., unpublished) promoters ablated constitutive *Cxcl12* transcription, suggesting a key role for this family of transcription factors in steady-state *Cxcl12* levels in the BM. Indeed, treatment of primary BM stromal cultures and the reticular fibroblastic stromal cell line MS-5 with mithramycin A, an inhibitor of the binding of Sp transcription factors to DNA, significantly downregulated *Cxcl12* expression. Pharmacological activation of β -adrenergic receptors rapidly reduced Sp1 nuclear content in MS-5 cells [10^{**}], an effect that was only partially prevented with the proteasome inhibitor MG132 (S. M.-F. and P. S. F., unpublished). Interestingly, other studies have revealed that Sp1 degradation induced by the treatment with the progenitor-mobilizing agent lipopolysaccharide (LPS) also leads to *Cxcl12* downregulation that is mediated by a trypsin-like serine protease [52]. In conclusion, these studies indicate that rhythmic HSC release is likely controlled by clock genes in the SCN and transduced by the SNS innervation into the BM.

In humans, progenitors were also reported to fluctuate in a circadian manner, with an initial report suggesting a morning acrophase but subsequent studies indicating that the peak was the evening [10^{**}, 53–57^{**}] (Table 2). Thus, nocturnal mice and diurnal humans have inverted cycles of progenitors, with peaks coinciding with their resting period. These rhythms are conserved in mice after enforced mobilization with G-CSF [57^{**}]. In addition, expression levels of *Cxcr4*, the cognate receptor for CXCL12, also fluctuated on HSCs and these oscillations depended on *Bmal1* expression. These data therefore suggest that a coordinated expression of chemokine/ligands regulate the steady-state traffic of HSCs.

Conclusion

These studies clearly demonstrate circadian changes in HSC /progenitor cell proliferation and trafficking in the bone marrow. Why does HSC function oscillate in a circadian manner? Although there is no definitive answer to this question, the peak of circulating HSC counts in the resting period of both mice (light phase) and humans (darkness) suggest a possible role in the regeneration of the stem cell niche and/or perhaps, more broadly, of extramedullary tissues. Interestingly, HSCs and progenitors constitutively traffic in the bloodstream to multiple tissues and have been suggested to differentiate in extramedullary tissues [58–60]. Additionally, the rhythmic proliferation patterns likely have physiological importance since *Per2*-deficient mice have a high frequency of lymphomas attributed to reduced apoptosis in damaged cells [61]. Even if much remains to be learned about the biological function and mechanisms, there are important practical and clinically relevant conclusions that can be drawn from these studies and hopefully soon applied to the clinic. For example, the circadian effects on cell proliferation may have important implications in cancer therapy. Over 30 anticancer agents have been studied for chronobiological oscillations in efficacy and toxicity in rodents [62], and clinical trials using circadian guided infusions of chemotherapies have reported promising results (reviewed in [3]). Based on recent studies, we would expect that the egress of human HSCs from the BM is maximal in

the evening and the return (homing) to the BM is predicted to peak in the morning. Consistent with this possibility, a retrospective analysis of G-CSF-mobilized patients has revealed a higher stem cell yield in patients harvested later during the day [57^{**}]. Thus, prospective studies are needed to assess whether the timing of the stem cell harvest and the subsequent infusion can impact the yield and engraftment, respectively. As one says, time will tell.

Acknowledgments

We are grateful to the National Institutes of Health (R01 grants CA112100, HL086899 to M.M. and DK056638, HL69438, AI069402 to P.S.F.), and the Department of Defense (Idea Development Award PC060271 to P.S.F.) for their support. S.M.-F. is the recipient of a Scholar Award from the American Society of Hematology. P.S.F. is an Established Investigator of the American Heart Association.

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Figure 1. Basic Schematic of the Molecular Circadian Oscillating Network In Mammals

Transcripts of the central clock genes *Bmal1* and *Clock*, or its related protein *Npas2*, are transcribed, transported to the cytosol, translated, and then form heterodimers. These BMAL1:CLOCK heterodimers translocate back into the nucleus and bind to canonical E-box sequences (CACCTG) to activate transcription of clock-controlled genes (ccg) which mediate circadian processes. BMAL1:CLOCK activation of retinoic acid orphan receptor (*Ror*) transcription and translation feeds-forward the circadian clock by binding the retinoic acid receptor elements (RRE) on the promoter of *Bmal1*. Negative feedback occurs by 1) initiating transcription of the clock genes *Period* (*Per*) and *Cryptochrome* (*Cry*), whose protein products heterodimerize and thwart BMAL1:CLOCK interaction in the nucleus and 2) activating transcription of *Rev-erb*, whose protein product competes with the positive regulator ROR for RRE binding. Maroon arrows depict feed-forward loops, while pink arrows label negative feedback loops. Not depicted above are the post-translational modifications that regulate protein stability and nuclear translocation that are critical for maintenance of 24 hour rhythms.

Figure 2. Model for circadian release of HSC from the bone marrow

Photic cues are transmitted from the eye to the central pacemaker in the brain, the suprachiasmatic nucleus (SCN) through the retinal-hypothalamic tract (RHT). The signals are transduced to the BM through the SNS which releases norepinephrine (NE) rhythmically in the BM microenvironment. NE binding to β_3 -adrenergic receptors on stromal cells triggers Sp1 degradation and Cxcl12 downregulation in a circadian manner, triggering rhythmic HSPC release from the BM to the bloodstream (Reproduced from [10**] with permission).

Table 1

Acrophases of Bone Marrow DNA Synthesis and Progenitor Numbers in Mice and Men

	Study	Population	Variable	Method	Acrophase	Statistical Significance
Humans	Mauer, 1965 [13]	4 healthy men	Total DNA synthesis	Tritiated thymidine	CT 11:21 *	Not analyzed
	Smaaland R, 1992 [14]	16 healthy men	Total DNA Synthesis	Flow cytometry with Propidium Iodide every 4 hr	CT 12:28	ANOVA (p=0.004) and Cosinor (p=0.002)
	Abrahamsen JF, 1997 [15]	19 healthy men	Mononuclear DNA Synthesis	Flow cytometry with Propidium Iodide every 4 hr	CT 11:53	ANOVA (p<0.001) and Cosinor (p=0.001)
Progenitors	Smaaland R, 2002 [16]	35 healthy men	Total DNA Synthesis	Flow cytometry with Propidium Iodide every 4 hr	CT 13:16	ANOVA (p=0.018) and Cosinor (p=0.018)
	Smaaland R, 1992 [14]	16 healthy men	Granulocyte/macrophage progenitors	CFU-GM every 4 hr	CT 12:09	ANOVA (p<0.001) and Cosinor (p<0.001)
	Abrahams en JF, 1998 [17]	5 healthy men	CD34+ Cells	Magnetic bead separation every 5 hr	CT 12:36	ANOVA (p=0.02) and Cosinor (p=0.02)
DNA Synthesis	Pizzarello DJ, 1970 [18]	Swiss webster male	Total DNA Synthesis	Tritiated thymidine every 4 hr	ZT 15:00	Not analyzed
	Burns ER, 1979 [19]	BDF1 male	Total DNA Synthesis	Tritiated thymidine every 3 hr	ZT 20:00	Not analyzed
	Wood PA, 1998 [20]	CD2F1 female	Total DNA Synthesis	Flow cytometry with Propidium Iodide every 4 hr	ZT 10:10 and 22:10	Cosinor (p=0.044 for 12 hr period)
Mice		CD2F1 female	Myeloid Proliferation	Flow cytometry with BRDU every 4 hr	ZT 02:70	Cosinor (p<0.001)
	Filipski E, 2004 [21]	B6D2F1 male	Total DNA Synthesis	Flow cytometry with Propidium Iodide every 4 hr	ZT 19:05	Cosinor (p=0.0003)
	Granda TG, 2005 [22]	C3H/HeN male	Total DNA Synthesis	Flow cytometry with Propidium Iodide every 4 hr	ZT 04:10	Cosinor (0.071)
Progenitors	Aardal NP, 1983 [23]	C3H-SPF female	Multipotent progenitors	CFU-S every 3 hr	ZT 6:00 and 21:00	Not analyzed
	Haus E, 1983 [24]**	BDF1 male and CD2F1 male + female	Granulocyte progenitors	GCFU -C every 4 hr	ZT 6:52	Cosinor (p<0.001)
		BDF1 male and CD2F1 male + female	Granucocyte progenitors	GCFU -C every 4 hr	ZT 3:44	Cosinor (p=0.015)
		BDF1 male and CD2F1 male + female	Granucocyte progenitors	GCFU -C every 4 hr	ZT 21:08	Cosinor (p=0.035)
	Levi F, 1988 [25]	B6D2F1 male	Committed progenitors	CFU-C every 4 hr	ZT 20:40	ANOVA (p<0.01) and Cosinor (p<0.01)
	Perpoint B,1995 [26]	B6D2F1 male	Committed progenitors	CFU w/ different CSFs every 4 hr	ZT 3:24 (24 hr period) and 3:52 (12 hr period)	Cosinor (p<0.0001 for 24 and 12 hr period)
	Wood PA, 1998 [20]	CD2F1 female	Multipotent progenitors	CFU-total every 4 hr	ZT 07:30 and 19:30	Cosinor (p=0.007) for 12 hr
			Granulocyte/macrophage progenitors	CFU-GM every 4 hr	ZT 07:50 and 19:50	Cosinor (p=0.039) for 12 hr
			Macrophage progenitors	CFU-M every 4 hr	ZT 07:20 and 19:20	Cosinor (p=0.012) for 12 hr
			Granulocyte progenitors	CFU-G every 4 hr	ZT 9:10 and 21:20	Cosinor (p=0.002) for 12 hr

		Study	Population	Variable	Method	Acrophase	Statistical Significance
		Bourin P, 2002 [27]	BALB/c male	Granulocyte/macrophage progenitors after <i>in vitro</i> culture	CFU-GM every 3 hr	ZT3:15	Cosinor (p<0.003)

Circadian Time (CT) refers to standard military time in which CT0 = midnight and CT12 = noon. Zeitgeber Time (ZT) refers to hours after the onset of light for animals in 12L:12D cycle. All cosinor analyses were performed for 24 hr period, unless otherwise indicated.

* Acrophase calculated in Smaaland R, 2002.

** Haus E, 1983 cited results form three different experiments.

Table 2**Acrophases of Hematopoietic Stem and Progenitor Cell Numbers in Mice and Men**

	Study	Population	Variable	Method	Acrophase	Statistical Significance
Humans	Ross DD, 1980 [53]	9 healthy men	Committed progenitors	CFU-C every 3 hr	CT 9:00	T-test ($p<0.01$)
	Verma DS, 1980 [54]	15 healthy men and women	Committed progenitors	CFU-C at 8AM, 11AM, 3PM, 8AM	CT 15:00	Wilcoxon Rank Test ($p=0.002$)
	Lasky LC, 1983 [55]	6 healthy men and women	Multipotent progenitors	CFU-GEMM at 8AM and 4PM	CT 16:00	Paired T-test ($p<0.025$)
	Morra L, 1984 [56]	45 healthy men and women	Granulocyte/Macrophage progenitors	Ratio of CFU-GM:Neutrophils at 8AM and 3PM	CT 15:00	Wilcoxon Rank Test ($p=0.02$) and Paired T-test ($p=n.s$)
	Lucas D, 2008 [57*]	8 healthy men and women	HSC-enriched fraction	CD34+CD38- cells at 8AM and 8PM	CT 20:00	Paired T-test ($p<0.001$)
			Committed progenitors	CFU-C at 8AM and 8PM	CT 20:00	Paired T-test ($p<0.001$)
Mice	Mendez-Ferrer S, 2008 [10**]	C57BL/6 male mice	Committed progenitors	CFU-C every 4 hr	ZT 05:00	ANOVA ($p=0.005$)
		C57BL/6 male mice	HSC	LSK cells and competitive reconstitution assays at ZT5 and ZT13	ZT 05:00	T-test ($p<0.05$)

Circadian Time (CT) refers to standard military time in which CT0 = midnight and CT12 = noon. Zeitgeber Time (ZT) refers to hours after the onset of light for animals in 12L:12D cycle.