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Mini-review

Parathyroid hormone: A novel tool for treating bone marrow depletion in cancer patients caused by chemotherapeutic drugs and ionizing radiation

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Abstract

Between 1958 and the late 1970s it was learned that PTH (the parathyroid hormone) could directly stimulate the initiation of DNA replication by murine CFU-S (colony-forming unit-spleen) cells via cyclic AMP, stimulate the proliferation of normal and X-irradiated murine and rat bone marrow cells, control hematopoiesis, and increase the survival of X-irradiated mice and rats when injected any time between 18 h before and 3 h after X-irradiation. Since then, it has been shown that the hematopoietic stem cell niche consists of PTH receptor-bearing, osteoblastic trabecular bone-lining cells that maintain the stem cells' (HSCs') proliferatively quiescent 'stemness' by various gene up-regulating and down-regulating signals caused by the tight adhesion of the HSCs to the osteoblastic niche-lining cells. Stimulating the osteoblastic lining cells with recombinant human PTH-(1-34) (Forteo™) causes a cyclic AMP-mediated enlargement of the HSC pool and promotes bone marrow transplant engraftment and growth and the survival of lethally irradiated mice. But this is only the beginning of the exploitation of the PTHs for marrow engraftment. It must now be determined whether the marrow engraftment-enhancing action of this potent bone growth-stimulating PTH can be extended from mice to rats and monkeys. It must be determined whether two other PTH peptides, rhPTH-(1-84) [Preos™]and [Leu^{27}]*cyclo*(Glu^{22}–Lys^{26})hPTH-(1-31)NH$_2$ [Ostabolin-C™]) are as effective as or better than rhPTH-(1-34)(Forteo™). Since, all three peptides are on the market, or nearing the market, for safely and strongly stimulating bone growth and treating osteoporosis one or all of them may become valuable tools for safely promoting the engraftment of peripherally harvested HSCs in cancer patients whose bone marrows have been 'emptied' by chemotherapeutic drugs or ionizing radiation.

Keywords: Bone marrow; Bone marrow transplants; Chemotherapy; Hematopoietic stem cells (HSCs); HSC niche; Osteoblasts; Parathyroid hormone (PTH); Parathyroid hormone receptor 1 (PTHR1); Radiation therapy; Trabecular bone-lining cells; Transplant engraftment

1. Introduction

A life-threatening consequence of chemo- and radiotherapies is the destruction of hematopoietic stem cells, the depletion or even 'emptying' of a cancer patient's bone marrow. Thus, a drug that builds hematopoietic stem cell niches and promotes the engraftment of stem cells harvested from peripheral circulating blood would be a valuable addition to a cancer therapist's tool box. During the last half century evidence has been building up for the parathyroid hormone (PTH) being just such a marrow-stimulating tool.

During this time the native 84-amino acid PTH has spawned smaller, but still potent N-terminal peptides.

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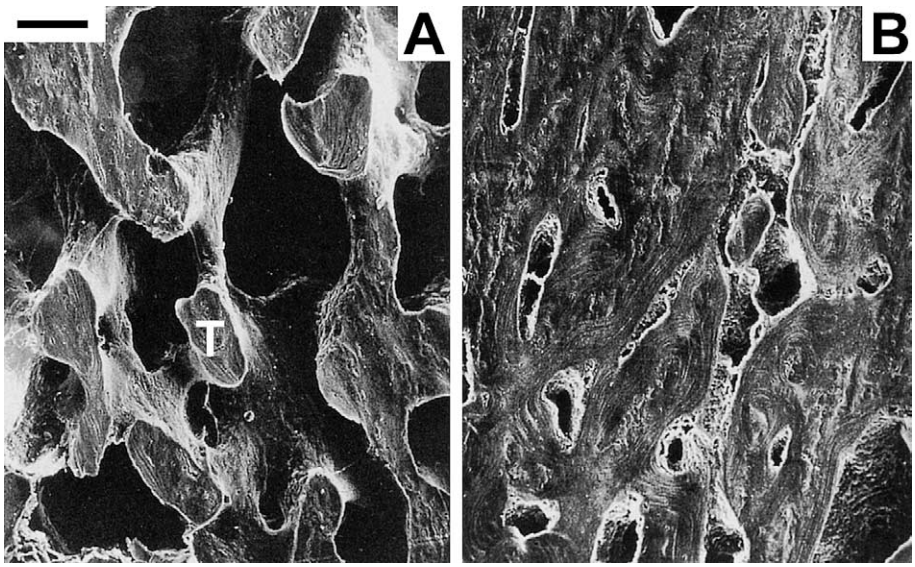

Fig. 1. The LTR-HSC homeland. Metaphyseal trabecular bone (T), the surface of which, when covered with osteoblastic-lining cells and their matrix components such as type I collagen and osteopontin, becomes the site of LTR-HSC niches. These images also show the potent ability of one of the PTHs, hPTH-(1-31)NH_2 (Ostabolin™), to stimulate trabecular growth in a rat femur. (A) The reduced trabecular bone and expanded marrow spaces in the distal femur of an ovariectomized Sprague–Dawley rat. The loss of estrogen caused by ovariectomy in a rat or menopause in a human might reduce the HSC niche space by causing the loss of trabecular bone. (B) The huge stimulation of trabecular bone growth caused by 6 weeks of daily subcutaneous injections of 0.8 nmol of Ostabolin™. This is an example of how much such a PTH could increase the amount of trabecular bone and with it potential LTR-HSC niche space. These are scanning electon micrographs of demineralized bone obtained with a Philips SEM-505. The bar in A represents 100 μm. These photographs are from Whitfield [1].

We now have, besides the recombinant native human hormone (rhPTH-(1-84) [Preos™]), two fragments/analogs (rhPTH-(1-34) [Forteo™] and [Leu^{27}]*cyclo*(Glu^{22}–Lys^{26})hPTH-(1-31)NH_2 [Ostabolin-C™]). These PTHs have recently become very popular because they can treat osteoporosis by strongly stimulating bone formation and strengthening bone microarchitecture in humans, monkeys and rodents with few or no side effects [1–3] (Fig. 1). One of them, Lilly's Forteo™, has been on the market for more than 2 years [1,2]. NPS Pharmaceutical's Preos™ has just finished its phase III clinical trial and should soon be on the market. And Zelos Therapeutics's [Ostabolin-C™] is in its phase II clinical trial. But they are versatile peptides. They can do a lot more than just stimulate bone growth because their PTHR1 (or PTH1R) receptor is expressed by many other cells which express the parathyroid hormone related protein (PTHrP) that also uses the PTHR1 receptor to drive the expressing cells' different maturation programs [2].

2. PTH, hematopoiesis and the survival of irradiated rodents

The story began in 1958 when Rixon et al. [4] reported that the only commercial source of PTH at that time, Eli Lilly's PTE (bovine parathyroid extract), surprisingly and significantly increased the survival of X-irradiated rats. This was the same PTE that H. Selye had used 26 years earlier for the first demonstration of PTH's potent ability to stimulate osteogenesis which laid the foundation of today's most effective treatment for established osteoporosis [5]. Three years later, Rixon and Whitfield [6], reported the results of a further 59 experiments using 1296, 300-g male hooded rats. They found that only one dorso-thoracic subcutaneous injection of PTE containing 50–200 USP units of PTH activity at any time between 18 h before and 3 h after irradiation with 7.0–8.5 Gy (700–850 rad) of 2 MeV X-rays significantly increased the number of animals surviving 30 days later. For example, 33% of vehicle-treated rats irradiated with 8.0 Gy were still living, but in poor condition, 30 days later while 73% of rats that had received PTE with 200 USP PTH units 5 min after irradiation were alive and in excellent condition by 30 days ($p \ll 0.001$). But injecting extract with as much as 200 units of PTH activity at 5 h after irradiation could not affect the 30-day survival.

The fraction of rats that were still living by 30 days after receiving an X-ray dose between 7.0 and 8.5 Gy would have depended on the fraction of their hematopoietic stem cells and the stem cells' transit-amplifying progeny that survived the irradiation

and repopulated the bone marrow above the threshold for survival. In 1967, Perris et al. [7] showed that injecting 200 USP units of the PTE immediately after irradiation of rats with 3 Gy of γ-radiation from ^{60}Co did significantly increase the flow of femoral bone marrow cells into mitosis starting as soon as 4 h later. Therefore, PTE's survival-enhancing action in the irradiated rats was due to it somehow stimulating a timely repopulation of their radiation-depleted bone marrows. But was it the PTH activity in the PTE extract that saved the irradiated rats in 1958 and 1961?

There was no pure bovine PTH in the early 1960s which can answer this question. The now popular osteogenic hPTH-(1-34)OH (teriparatide; Lilly's Forteo™) did not appear until the early 1970s, but was not yet commercially available [8,9]. So we had to use the animals' own PTH. We did this by injecting the Ca^{2+}-chelating EDTA or the Ca^{2+}-binding Na-caseinate, either of which would lower the circulating free (ionic) Ca^{2+} concentration. This circulating Ca^{2+} drop would silence the parathyroid cells' Ca^{2+}-monitoring CaSRs (Ca^{2+}-sensing receptors [10,11]) and thus cause the cells to fire a bolus of PTH into the circulation within seconds to restore the normal circulating Ca^{2+} concentration.

In 1967, Perris and Whitfield [12] reported that intraperitoneally injecting 125-g male Sprague–Dawley rats with EDTA almost halved the circulating free Ca^{2+} concentration within 10 min and significantly increased the flow of femoral bone marrow cells into mitosis by 4 h. Then Rixon and Whitfield [13] showed that injecting enough Na-caseinate to halve the circulating free Ca^{2+} concentration in normal female CF_1 mice doubled the proliferation of their femoral bone marrow cells and tripled the proliferative activity in the femoral bone marrows of mice irradiated with 6.0 Gy (600 rad) of 300 kVp X-rays. Moreover, the Na-caseinate significantly increased the 30-day survival of the irradiated mice from 45 to 80%. Na-caseinate also stimulated femoral bone marrow cell proliferation in normal, but not TPTXed (thyroparathyroidectomized), male Sprague–Dawley rats, which indicated the mediation of the proliferative response by endogenous PTH [13].

Then, Rixon and Whitfield [14] showed that removing just the parathyroid glands (PTX) or the whole thyroid–parathyroid complex (TPTX) from male Sprague–Dawley rats greatly reduced the mitotic activity in the femoral bone marrow. This drop was followed within 8 days by a 40% drop in the marrow's total nucleated cell population which included a dramatic (ca. 70%) reduction of the erythroid and lymphoid subpopulations. As expected, the dramatic erythroid hypoplasia in the bone marrows of HPX and TPTX rats was accompanied by a 68% drop in the reticulocyte fraction of the non-nucleated marrow cell population, an equivalent reduction of ^{59}Fe incorporation into peripheral erythrocytes and an increase in the post-hemorrhage hematocrit restoration time from the normal 5 days to 9 days [15,16]. Erythropoiesis in these parathyroidectomized rats and the speed of hematocrit restoration after hemorrhage were restored to the normal values by daily subcutaneous injections of purified native bovine PTH purchased from the now long-gone Wilson Laboratories in Chicago. It should also be added in passing that PTX reduced, and the purified bovine PTH restored, the primary immune response of rats to injection of sheep erythrocytes [17]. Thus, it was the PTH activity in the Lilly PTE that had saved Rixon et al.'s heavily X-irradiated rats and that PTH somehow controls hematopoiesis in mice and rats. But did the hormone act directly on hematopoietic progenitors or work only indirectly by raising the blood Ca^{2+} concentration?

It was Gallien-Lartigue and Carrez [18] who answered this question. To do this they used Till and McCulloch [19]'s spleen colony assay in which mouse femoral bone marrow is removed, its cells are suspended in a suitable medium and an appropriate number of them are then injected into lethally irradiated mice. Ten days later the numbers of colonies with variously differentiated cells that were formed in the irradiated animals' spleens by pluripotent hematopoietic stem cells and early-stage precursor cells in the injected marrow suspension are counted [18–20]. Gallien-Lartigue and Carrez also used the 'thymidine suicide' technique [18,21–24] to find out whether the pure native bovine PTH they obtained from Calbiochem could directly stimulate CFU-S cell proliferation. They incubated the suspended donors' femoral bone marrow cells for 30 min in medium containing a relatively high radioactivity (e.g. 200 μCi [7.4 MBq]/ml) from high-specific activity ^{3}H-thymidine and then determined the number of colonies in the spleens of the recipient irradiated animals. If the suspended CFU-S cells were stimulated to make DNA by the PTH, the number of spleen colonies in the recipient animals' spleens would have been reduced relative to the number produced in the recipient spleens by injecting untreated cells because the DNA-synthesizing cells would have incorporated ^{3}H-thymidine from the medium into their DNA and been killed by the β-particles from the ^{3}H.

Gallien-Lartigue and Carrez [18] did indeed find that the PTH stimulated CFU-S cells in the donor bone

marrow to suicidally start replicating DNA within just a couple of hours as indicated by the significantly fewer colonies that appeared in the spleens of the lethally irradiated mice injected with PTH-treated donor marrow cell suspensions instead of untreated marrow cell suspensions. In fact these colony-forming cells responded to the PTH as fast as the flow of bone marrow cells into mitosis in PTH-treated rats. Thus, PTH could at least stimulate the initiation of DNA replication by a population of cycling CFU-S cells that had exited the primitive quiescent, G_0, niche-bound LTR ('long-term repopulating')-HSC cell state and paused in a late G_1 (i.e. prereplicative) state [18] (Fig. 2). Therefore, these CFU-S cells had PTH receptors and could promptly start replicating DNA, enter mitosis and presumably start generating lymphoid and myeloid progenitors and their progeny in response to signals from these receptors (Fig. 2).

What are these PTH-responsive CFU-S cells? They are pluripotent, but they have only a limited proliferation potential [23,24]. This is not 'stemness'. True stem cells nestle in their niches guarding their proliferatively quiescent G_0 state in order not to lose their self-renewing and marrow-repopulating potential with unnecessary cycling (Fig. 2). When a niche-bound stem cell does receive a signal to initiate and complete a growth-division cycle, one daughter cell stays in the niche and lapses back into a G_0 state while the other leaves home. If it cannot find another niche, it will keep its cell cycle engine running as a CFU-S cell, the rapidly proliferating, transient amplifying progeny of which lose a fraction of stemness with each round of replication. After a limited number of rounds of replication the CFU-S progeny move along trails marked with appropriate chemokines, cytokines and adhesives to find vascular endothelial cell niches where they terminally mature and from which they enter the blood.

Since, injecting CFU-S cells can save lethally irradiated mice [24,25], increasing the size of the quiescent LTR-HSC pool, increasing the proliferation and preventing the apoptosis of their transit-amplifying progeny were ways by which the PTH activity in the Lilly PTE could have saved Rixon et al.'s [4,6] irradiated rats. Obviously by 1974 it had become important to locate the LTR-HSC niches in the bones

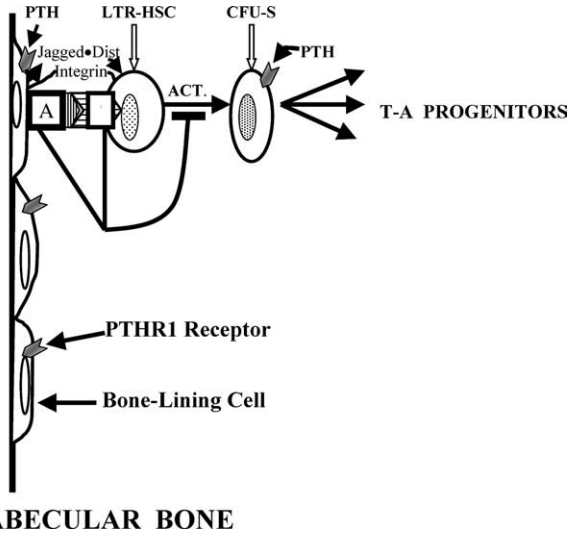

Fig. 2. In the living human or rodent the surfaces of trabeculae, such as those in Fig. 1, are covered with bone-lining cells which are 'retired' osteoblasts that have stopped making collagen and other matrix components and paracrine factors. But they have retained a few PTHR1 receptors from their PTHR1 receptor-rich osteoblast days [1,2]. When stimulated by a PTH they pick up their bone-making tools again and go back to work making bone [52]. These lining cells are armed with Ang-1 which if it contacts a LTR-HSC will lock onto the HSC cell by causing the HSC to express Tie-2 which binds to the lining cell's Ang-1. But that is very far from all! The lining cells also grab the LTR-HSC with N-cadherin which binds to the LTR-HSC's β-catenin. Then there are signals generated by the binding of the lining cell's PTH/cyclic AMP-stimulable Jagged 1 to the LTR-HSC's Distal-1 receptor. Not illustrated to avoid congestion, are the signals from Wnt·Frizzled·L/LRP complexes which send β-catenin into nucleus to combine with LEF/TCF and affect the expression of a set of target genes. The β-catenin accumulating in the LTR-HSC's cytoplasm will also bind to the lining cell's N-cadherin to make N-cadherin·β-catenin mooring cables. But the LTR-HSC is also anchored to the matrix by Ang-1·Tie-2-stimulated β1 integrins which, when activated by attaching to the matrix, send still more signals into the HSC. The result of all of this signaling from this still incompletely characterized nano-tangle is a stemness-preserving package of gene expressions that stick the LTR-HSC to the lining cell and suppress activation (ACT) and the transit from LTR-HSC to CFUs and transit-amplifying (T-A) progenitors but still allows the cells to be activated (ACT) for generating transit-amplifying (T-A) progenitor cells in response to marrow depletion.

and how PTH might control their maintenance and numbers in these niches. But we had to wait for a further 30 years to find out.

3. Osteoblastic trabecular bone-lining cells, the HSCs' niche cells

It was suspected for the last 30 or so years that the LTR-HSC cells' bone marrow 'niches' were on the surfaces of bones lined with the osteoblastic cells [23,24,26–29]. For example, Visnjic et al. [29] demonstrated the linkage of bone marrow hematopoiesis to osteoblasts using transgenic mice with a ganciclovir-inhibitable herpes virus thymidine kinase (HVTK) gene under the control of a fragment of the collagen gene's Col α1 type 1 promoter (Col2.3Δ TK). In these animals only the collagen α1-expressing osteoblasts express the Col α1 type 1 promoter-driven HVTK and are killed by ganciclovir. The selective ganciclovir-induced loss of osteoblasts caused a progressive loss of bone, a loss of bone marrow cellularity and early hematopoietic progenitors, and reversion of hematopoiesis from the adult bone sites to the former fetal sites in liver and spleen. But when the osteoblast-killing ganciclovir treatment was stopped, hematopoiesis returned to the bones as osteoblasts reappeared along with pockets of hematopoiesis at sites of new bone formation.

We now know that the mouse bone marrow LTR-HSC cells' niches are on trabecular bone surfaces (Fig. 1). There the LTR-HSC cells are tethered to trabecular bone-lining cells with a dense nano-tangle of receptor·ligand complexes, signaling wires, matrix-mooring cables and matrix components such as collagen and osteopontin [30–36], only a few of which could be included in Fig. 2. The osteoblastic-lining cells make Ang-1 (Angiopoietin-1) which in turn stimulates the HSCs to make Ang-1's Tie-2 tyrosine kinase receptor which 'ties' the HSCs to the trabecular bone-lining cells [30,31,35,36] (Fig. 2). The signals sent into the LTR-HSC cells from the Ang-1·Tie-2 complex do an extremely important thing for the maintenance of LTR-'stemness'. As with epidermal basal keratinocyte stem cells [37], the HSCs' self-renewing LTR-'stemness' and the associated proliferative quiescence in a G_0 state depend in part on matrix adhesion-stimulated signaling from β1-integrins which are lost when the LTR-HSCs start cycling and disconnecting their various tethers to the lining cells. Repeated cycling would deplete the pool of LTR-HSC cells which would irreversibly lose their abilites to re-enter the G_0 quiescent state and to self-renew when starting to cycle [38]. So the signals from Ang-1·Tie-2 complexes prevent the HSCs from cycling, keep them sticking to the bone surface, keep their β1-integrin signaling from fading, keep them resistant to apoptosis, but also still keep them able to generate rapidly proliferating transit-amplifying progenitors when needed [30,31] (Fig. 2).

Although the signals from lining cell-Ang-1·HSC Tie-2 complexes and β1-integrins are major keys to the maintenance of the LTR-HSC pool, there are several other important players in LTR-HSC control. There is the HSCs' Wnt-activated Frizzled/LRP (low density lipoprotein receptor-related protein) 5/6 receptor mechanism [34]. The signals triggered by the Wnt glycoprotein stop cytoplasmic β-catenin from being phosphorylated, ubiquitinylated, and dumped into the proteasome for proteolysis. This enables β-catenin to build up and enter the nucleus where it combines with LEF/TCF protein to form a transcription factor that affects the expression of several genes, the products of which contribute to the maintenance of the LTR-HSC pool by promoting LTR-HSC self-renewal rather than LTR-HSC-depleting conversion to transit-amplifying progenitors and terminal differentiation [39–44]. The accumulating cytoplasmic β-catenin also contributes to the mooring of HSCs to the lining cells by binding to the bone-lining cells' N-cadherins [34]. But, as we shall see further on, the niche has yet another important pair of denizens—Notch1 and its activator Jagged 1 which collaborate with the Wnt receptor-driven mechanism in maintaining the LTR-HSC cells' self-renewing 'stemness' [45].

4. How does PTH affect this complex system?

It has been shown that all of the PTHs listed in Section 1 can strongly stimulate trabecular bone growth with bursts of adenylyl cyclase activity and cyclic AMP production by activating PTH receptors on bone-lining cells and osteoblasts [1] (Fig. 1). Since, the size of the LTR-HSC pool depends on the available trabecular bone niche space [34,35], the osteogenic PTHs should increase the LTR-HSC pool size and with this the effectiveness of the response to bone marrow injury. Since, proliferatively quiescent (i.e. G_0-phase), Ang-1·Tie-2-restrained LTR-HSCs' have been shown to resist apoptosis, and since intravenously injecting Ang-1 protein or *ang-1* gene-bearing adenovirus into mice increases the total survival of 5-flurouracil-treated mice from 0 to 40% and to prolong the time required for X-irradiated mice to die by about 30% [30], a PTH-induced increase of the apoptosis-resistant LTR-HSC

pool and thus the mobilizable reserve of transit-amplifying progenitors should also increase the resistance of an animal or human to myelosuppressive drugs and ionizing radiation. The PTHs might also promote the attachment of LTR-HSC cells to the expanding niches by stimulating the bone-lining cells to make more N-cadherin for binding to the LTH-HSC cells' β-catenin [46,47].

Then there is the Jagged–Notch 1 couple (Fig. 2). Calvi et al. [48] have shown that injecting the adenylyl cyclase-stimulating hPTH-(1-34) (Forteo™) or the potent adenylyl cyclase-stimulator forskolin, into mice or producing mice with osteoblasts expressing permanently switched-on mutant PTHR1 receptors (which do not need a PTH to activate them) enlarges the LTR-HSC pool. And it does this by causing the bone-lining cells to make large amounts of Jagged 1 which binds to and activates the cleavage of the attached LTR-HSC cells' Notch 1 receptors by ADAM metalloproteinases and γ-secretases. The released intracellular ICN portions of Notch 1 receptors then move from the cell surface into nucleus. There they combine with the DNA-binding CSL/RBP-J protein to form the ICN·CSL/RBP-J gene transactivator [49]. It seems that PTH-enhanced Jagged 1·Notch 1 signaling will collaborate with the Ang-1·Tie-2 signals and the Wnt cascade to give LTR-HSCs a complete stemness maintenance package by stimulating β1 integrin and N-cadherin expression, cell cycle suppression and nuclear β-catenin·LEF/TCF and ICN·CSL/RBP-J transactivators to suppress the expression of transit-amplifying progenitors' gene expressions [45,48–50].

Finally, as found by Gallien-Lartigue and Carrez [18], the adenylyl cyclase-stimulating native PTH can directly stimulate a late-G_1 population of CFU-S cells to start cycling [18] (Fig. 2). Moreover, another adenylyl cyclase stimulator, the β-adrenergic agonist isoproterenol and dibutyryl-cyclic AMP itself also stimulate mitotic activity in rat bone marrow and the proliferation of murine CFU-S cells [22,51].

5. The PTHs, promising tools to promote HSC transplant engraftment

These observations extending from those of Rixon et al. in 1958 to the present suggest that the osteogenic and osteoporosis-reversing PTHs are likely to be tools to promote the repopulation by HSCs of cancer patients' bone marrows that have been emptied by ionizing radiation and cytotoxic chemotherapeutic drugs. If so, they would enhance harvested HSC engraftment and bone marrow repopulation in at least three ways: first by making more trabecular bone to accommodate more LTR-HSCs; second, by expanding transplanted LTR-HSC pools after the injected HSCs have set up their niches with bone-lining cells; and third by stimulating transit-amplifying progenitor proliferation until at least an adequately functional bone marrow has been established. A tantalizing glimpse of the PTHs' potential for enhancing transplant engraftment and repopulating bone marrow has been provided by Calvi et al. [48]. They lethally X-irradiated mice and then injected just enough bone marrow cells from normal donor animals to allow 27% of the irradiated animals to survive for at least 28 days. But when the irradiated animals also received daily injections of hPTH-(1-34) (Forteo™), all of them survived with larger loads of transplanted marrow cells in their hind limbs.

In conclusion, our exploration of the abilities of adenylyl cyclase-stimulating PTHs for the engraftment of whole bone marrow and HSCs has only just re-started. We must learn more about molecular basis of the PTHs interactions with the dauntingly complex nano-tangle of things connecting HSC cells to the bone-lining cells in their niche. We must also find out whether Forteo™, Ostabolin-C™ and Preos™, all of which are known to be clinically safe when subcutaneously injected in an osteogenic dose (e.g. 20 μg of rhPTH-(1-34) injected once each day for 2 years) for treating osteoporotic humans, can also promote the engraftment of peripherally harvested HSCs as well as whole bone marrow and stimulate subsequent bone marrow repopulation in rats, monkeys and ultimately cancer patients as effectively as Forteo™ appears to do in mice.

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