Published in final edited form as:

Bone. 2014 June ; 0: 87–94. doi:10.1016/j.bone.2014.02.018.

Consequences of irradiation on bone and marrow phenotypes, and its relation to disruption of hematopoietic precursors

Danielle E. Green¹ and Clinton T. Rubin¹

¹Department of Biomedical Engineering, Stony Brook University, Stony Brook, New York 11794-5281

Abstract

The rising levels of radiation exposure, specifically for medical treatments and accidental exposures, have added great concern for the long term risks of bone fractures. Both the bone marrow and bone architecture are devastated following radiation exposure. Even sub-lethal doses cause a deficit to the bone marrow microenvironment, including a decline in hematopoietic cells, and this deficit occurs in a dose dependent fashion. Certain cell phenotypes though are more susceptible to radiation damage, with mesenchymal stem cells being more resilient than the hematopoietic stem cells. The decline in total bone marrow hematopoietic cells is accompanied with elevated adipocytes into the marrow cavity, thereby inhibiting hematopoiesis and recovery of the bone marrow microenvironment. Poor bone marrow is also associated with a decline in bone architectural quality. Therefore, the ability to maintain the bone marrow microenvironment would hinder much of the trabecular bone loss caused by radiation exposure, ultimately decreasing some comorbidities in patients exposed to radiation.

Keywords

Ionizing radiation; bone marrow; bone architecture; mesenchymal stem cells; hematopoietic stem cells; trabecular bone; cortical bone

Introduction

It has been well established that radiation exposure causes a drastic deficit to bone marrow populations, but the severity of even sub-lethal doses will lead to irreparable tissue damage of the bone tissue. As cancer patients continue to undergo whole body and localized radiation therapy, it becomes imperative to determine a means to rescue their bone marrow population and maintain their bone architecture, to prevent the risk of bone fractures long term. Understanding the relationship between the cell phenotypes within the bone marrow

© 2014 Elsevier Inc. All rights reserved.

Corresponding Author: Danielle Green, Ph.D., Department of Biomedical Engineering, Stony Brook University, Stony Brook, New York 11794-5281, Phone: (631) 632 1489, Fax: (631) 632 8577, Danielle.Green@stonybrook.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and the bone architecture could lead to new insights on repairing the bone quality and immunological health. We will review the effects of irradiation on bone marrow phenotypic populations and the bone structure, and current treatment methods used to mitigate bone loss.

Bone Marrow Failure Induced by Ionizing Radiation

Bone Marrow Failure and Current Treatment Methods

Bone marrow failure is defined as the inability to produce the proper number of blood cells necessary to control immune function. There are many different causes of bone marrow failure syndromes, of which some are acquired and some are inherited. Those that are acquired are typically due to exposure to chemicals, radiation, viruses, or other toxins. Both acquired and inherited disorders often lead to aplastic anemia [1], which is the failure to produce blood cells in the bone marrow. Acquired bone marrow failure disorders include aplastic anemia, hypoplastic myelodysplastic syndrome, myelodysplastic syndrome, myeloproliferative disorders, acquired pure red cell aplasia, amegakaryocytic thrombocytopenia, and chronic acquired neutropenia [2]. The inherited bone marrow disorders include Fanconi's Anemia, Diamond-Blackfan Anemia, Shwachman-Diamond Syndrome, Dyskeratosis Congenita, Severe Congenital Neutropenia, Thrombocytopenia Absent Radii, Amegakaryocytic Thrombocytopenia, and Pearson's Syndrome [1]. Patients who have these acquired or inherited bone marrow syndromes are at high risk of developing cancer and leukemia during their lifetimes. All types of bone marrow failure syndromes result in the inability to produce or maintain proper blood cell numbers and function in the bone marrow. Treatment options are dependent on the type of bone marrow failure syndrome. Often times patients will undergo hematopoietic stem cell transplantation from a donor who has the matching human leukocyte antigen, in many cases a sibling, and/or pharmacologic treatments including immunosuppressants [2], with the ultimate goal of maintaining a healthy bone marrow population to support a healthy immune response. Prior to transplantation though, ionizing radiation or chemotherapy are used to ablate the cancerous and the immune cells.

Ionizing Radiation

Exposure to high doses of ionizing radiation will lead to bone marrow failure and eventually death. Sub-lethal doses of irradiation will cause bone marrow suppression, which is a less severe case of bone marrow failure, and will leave a patient immunosuppressed due to an abnormal number of functional blood cells. One of the more common types of radiation that humans are exposed to is γ -irradiation. Gamma irradiation is a result of nuclear decay and penetrates through tissue in an exponential fashion. The absorbed dose is measured in units of Gray (Gy), which is a measure of 1 joule of energy per kilogram of matter (J/kg). Alternatively, radiation can be measured using "equivalent dose," which is primarily used to describe the dose of radiation to a fixed amount of biological tissue, and uses the Sievert (Sv) unit. To convert from the absorbed dose measured in gray to the equivalent dose measured in Sv, it is necessary to use a radiation weighting factor which is specific to the type of ionizing radiation. For γ -rays and x-rays, this weighting factor is 1, therefore 1 Gy of γ -irradiation is equivalent to 1 Sv to the biological tissue.

Human and Mouse Exposure to Ionizing Radiation

Ionizing radiation occupational exposure limits are heavily regulated in the United States by the United States Nuclear Regulatory Commission (U.S.NRC) and are categorized by dose equivalents for eye exposure, organ exposure, and whole body exposure. The annual limit for the total effective dose equivalent to the whole-body is 50 mSv/yr, where 1 Sv is the dose equivalent of a 1 Gy absorbed dose of γ -irradiation. To put this into perspective, a CT scan of the chest is approximately 5 mGy and exposure from a dental x-ray exam is approximately 0.2 mGy. Even if one avoids irradiation from medical imaging techniques, everyone receives minimal doses of radiation from the natural environment which includes radon, cosmic, and terrestrial sources.

In 2006, the average person in the United States was exposed to approximately 6.2 mGy annually [3], which is presumed to be below a harmful dose. The lethal dose of radiation though is species dependent. For instance, the median lethal dose of total body γ -irradiation within 60 days (LD50/60) in humans falls between 2.5-5 Gy [4], whereas in C57BL/6 mice, the median lethal dose within 30 days (LD50/30) is approximately 8 Gy [5]. The region surrounding the Fukushima Daiichi reactors sustained radiation levels that could have certainly been harmful to humans, with doses estimated as high as 400 mGy/year [6], and well above the 50 mGy/yr U.S.NRC limit. It is likely that it will take decades to decay without proper cleanup, therefore people who return to settle or work in the evacuated regions receiving 100 mGy/yr could receive a cumulative dose up to 1 Gy over a 10 year period, which could pose a large biological hazard.

While some argue that there are beneficial effects of low doses of radiation, such a platform remains controversial [7, 8]. However, high doses of radiation exposure are often beneficial for pursuing medical treatments. Many patients with hematological disorders and cancers of the bone marrow undergo elective radiation therapy, where they are exposed to 12 Gy of total body irradiation in order to ablate the marrow prior to bone marrow transplantation [9]. Patients with tumors can also undergo localized radiotherapy and are often administered doses as high as 66 Gy of γ -irradiation [10]. Many of these patients undergoing radiation therapy are exposed to fractionated doses of radiation [11], allowing the radiation dose to be spread out over a period of time. The purpose of fractionation is to allow for the repopulation and repair of non-cancerous cells while still providing the toxic effect to the cancerous cells. For tumor ablation, the fractionated doses range between 2 Gy/fraction to 20 Gy/fraction, and can be administered daily or even twice a day, up to a few weeks. The fractionated regime is dependent on the type of tumor being ablated.

Patients undergoing radiation therapies though have been shown to suffer from bone loss, and have elevated fracture risk [12-14], which is quite problematic due to the vast number of people undergoing radiation therapy annually and the difficulty in managing radiation associated fractures [15]. According to the 2013 Surveillance, Epidemiology, and End Results Program statistics, there were 12.8 cases of leukemia diagnosed in every 100,000 people in the United States. Furthermore, there are more than 1,129,800 people living with or in remission from leukemia, myeloma, non-Hodgkin's lymphoma, and Hodgkin's lymphoma [16], for all of which whole body irradiation therapy is a common treatment, and often used in combination with chemotherapy. The fracture rate in these patients is believed

to be elevated and has been shown in children with acute lymphoblastic leukemia, but data has not been collected quantifying the overall fracture risk of leukemia patients on a mass scale [13, 14, 17]. In 2006 alone, there were 17,875 people in North America and 50,417 people worldwide who underwent a bone marrow transplant, primarily due to leukemia or another lymphoproliferative disorder, and many of them were exposed to total body irradiation prior to transplantation [18]. Now that there is a 60% survival rate for leukemia patients compared to a 10% survival in the 1950's, a 72% survival for non-Hodgkin's lymphoma compared to 33% in the 1950's, an 89% survival for Hodgkin's lymphoma compared to a 30% survival in the 1950's and a 45% survival for myeloma compared to only a 6% survival in the 1950's [16], these increased survival rates inevitably increase the complications due to radiation exposure, including osteopenia and osteoporosis [12]. Therefore the ability to protect and/or regenerate bone and reduce the risk for bone fractures is of increasing importance, particularly considering patients are now living longer following diagnosis of the disease and radiation associated fractures are difficult to treat [15].

Relationship between Mesenchymal Stem Cells and Hematopoietic Stem Cells Within the Bone Marrow

The bone marrow is situated internal to the skeleton and serves as a principal home for two multipotent stem cell populations, the hematopoietic (HSC) and mesenchymal stem cells (MSC). The HSCs control the immune cell lineages and the MSCs give rise to bone, cartilage, muscle, tendon, ligament and fat cells; both stem cells and their functions will be described in detail below.

Hematopoietic stem cells give rise to the myeloid and lymphoid lineages and are responsible for maintaining the immune system [19, 20]. Myeloid cells include neutrophils, eosinophils, basophils, monocytes, macrophages, erythrocytes and platelets, whereas the lymphoid cells include T-cells, B-cells and natural killer cells [21]. Myeloid cells are responsible for defending the body against infection. Specifically, monocytes migrate to the site of infection and differentiate into macrophages to phagocytose the infected cells, and the neutrophils, the most common granulocyte, target primarily fungal and bacterial infections [22]. Erythrocytes deliver oxygen through the blood circulation and are responsible for maintaining oxygen levels within the biological tissues. T-cells are responsible for the cell mediated immune response and they originate in the bone marrow, mature in the thymus and circulate throughout the body [21]. Helper T-cells, which are positive for the CD4 antigen, assist in B-cell maturation and cytotoxic T-cell activation [23]. Cytotoxic T-cells are positive for the CD8 antigen, and are responsible for removing harmful cells by binding to their major histocompatibility complex I (MHC I) antigen [24]. Memory T-cells can be positive for either CD8 or CD4 antigens and remain in circulation to prevent future exposure to previous infections [25]. Regulatory T-cells are positive for the CD4 antigen and they halt activation of the immune response once an infection is adequately removed from the system. The primary purpose of B-cells is to produce specific antibodies to protect against antigens at the site of infection [26]. Therefore there are many B-cells each expressing different B-cell receptors that are appropriate to bind to specific antigens. Plasma B-cells secrete

antibodies following antigen exposure whereas memory B-cells proliferate in order to quickly produce antibodies for antigens that were previously present [27]. B-cells are created in the bone marrow, move to the spleen to mature, and circulate through the body to be close to a site of infection. Although osteoclasts are not necessarily involved in maintaining the immune system, they too are derived from the hematopoietic lineage, and are responsible for bone resorption during the active bone turnover process [28].

The remaining non-hematopoietic cells found in the bone marrow originate from the mesenchymal stem cell lineages. Mesenchymal stem cells are multipotent cells that have the potential to differentiate into numerous lineages, including adipocytes, osteoblasts, and chondrocytes [29]. Their differentiation potential is often dependent on the biological environment *in vivo* and MSCs can be promoted to different lineages *in vitro* when exposed to the appropriate growth factors [30-33].

HSCs and MSCs share the same niche in the bone marrow, and many of their lineage cells are responsible for maintaining the niche. Although MSCs and HSCs are found within the bone marrow compartment, they are not uniformly distributed and they localize into particular regions. The HSCs typically surround the sinusoid blood vessels and the endosteal surface [34, 35], and the MSCs are found within close proximity to the HSCs [36]. These two stem cell populations are dependent on one another for survival [36, 37]. In fact, it has been shown during bone marrow transplantation that HSCs are more capable of engrafting when they are co-transplanted with MSCs [38]. It is believed that bone marrow MSCs have HSC regulatory factors that control the HSC proliferative capacity [36, 39]. *In vitro* studies have also shown the ability of MSCs to suppress cells from the HSC lineage due to their secretion of immunomodulatory factors [40, 41], which ultimately leads to a decline in T-cell activity [42, 43], natural killer cell activation [44, 45], and B-cell proliferation [46]. Therefore one would assume that in order to maintain proper immunological health, it is necessary to have healthy and balanced interaction between the HSCs and MSCs. Bone marrow macrophages, which are derived from the hematopoietic lineage, contributed to maintaining the HSC population within the bone marrow by acting directly on MSCs [47]. The depletion of macrophages will disrupt the niche leading to mobilization of hematopoietic stem cells into the blood and a loss of bone cells, osteoblasts [48]. *In vitro* and *in vivo* studies have also shown the contribution of monocytes and macrophages in maintaining osteoblast survivability and activity [48, 49].

As discussed above, MSCs are capable of differentiating into either osteoblasts or adipocytes amongst other cells, and they modulate HSC activity. Osteoblasts have recently been shown to modulate hematopoietic cells including erythrocyte production [50] and B-cell cell differentiation [51, 52]. For example, Ding *et al.* altered the osteoblast phenotype by deleting the CXCL12 chemokine from strictly the osteoblasts [53]. CXCL12 is most well-known for maintaining the HSC population and lymphoid progenitors within the bone marrow [54]. The CXCL12 reduction from the osteoblast cells led to a decline in lymphoid precursors, indicating the importance of osteoblasts in maintaining early lymphoids [53]. However the deletion of CXCL12 from the osteoblasts did not lead to a depletion of the HSC or myeloerythroid progenitors [53, 55]. Adipocytes on the other hand have shown to be negative regulators of hematopoietic progenitors cells [56]. Naveiras *et al.* showed that

adipocytes occupy the bone marrow of lethally irradiated mice following wild-type transplantation, but mice with compromised adipogenesis (A-Zip/F1), had fewer adipocytes in their bone marrow following transplantation when compared to the wild-type mice. Of the donor cells, the A-Zip/F1 mice had a significantly greater number of hematopoietic progenitor cells [56] indicating the ability of adipocytes to suppress HSC progenitor proliferation. In addition, the A-Zip/F1 mice had increased osteogenesis three weeks following transplantation which was marked by a fivefold increase in trabecular bone [56]. Therefore it was necessary to ablate, using radiation, the hematopoietic stem cells and the adipocytes in the bone marrow, and suppress future adipogenic formation in order to increase osteogenesis. This shows the co-dependency of the adipogenic, osteogenic and hematopoietic cells.

Both the HSCs and MSCs produce cells critical to the bone turnover process, namely osteoclasts (HSC) and osteoblasts (MSC). Therefore, it is believed that a deficit to these stem cell populations in the bone marrow, such as one caused by radiation exposure, will ultimately compromise the bone architecture and structural integrity likely due to a deficit in osteoprogenitor cells and overall decline in HSC self-renewal [57].

Radiation Effects in the Bone Marrow

The depletion of cells in the bone marrow [58] and peripheral blood [59-61], as well as the drop in weight of lymphoid organs [62] following irradiation has been widely shown throughout the literature, and is dependent on the dose received by the biological tissue [62]. The decline in erythrocytes in the blood following irradiation [62] exposure leads to anemia, which is commonly seen in patients undergoing radiation treatment and chemotherapy. To mitigate anemia, blood cell transfusions and erythropoietin may be administered to cancer patients [63].

The overall effects of radiation on MSCs are only beginning to be examined *in vitro* and *in vivo*, and they are believed to be more resilient to radiation compared to hematopoietic cells [64-68]. The radioresistance of these MSCs is explained by Chen *et al.* as an increase in oxidative stress resistance in MSCs compared to other cell phenotypes [67] because oxidative stress is often what leads to DNA damage and cell death following irradiation, as previously reviewed by Azzam *et al.* [69]. However, the MSCs are capable of repairing double stranded breaks by homologous recombination and nonhomologous end joining following irradiation [69]. Studies performed by Cao *et al.* do not show complete radioresistance of the MSC population following localized irradiation to mouse bone, as indicated by an absence of MSCs in the colony forming unit fibroblast (CFU-F) assay [70]. Interestingly, 4 weeks following irradiation, MSCs showed recovery within the irradiated bone [70] which could imply that damage to the MSC population is only temporary. Few clinical studies have assessed MSC survivability following radiation exposure. However, there are clinical studies showing MSC radioresistance in the bone marrow of lethally irradiated patients who were subsequently given bone marrow or peripheral blood stem cell transplants [71, 72]. Following transplantation, the MSCs present within the patients are from the host rather than the donor, indicating that the MSCs cells survived irradiation [71, 72].

According to *in vitro* studies, MSCs are also capable of retaining their differentiation capacity following ionizing radiation [67, 70, 73], however it is still unclear whether this holds true *in vivo*. The resiliency of the MSCs to radiation does not necessarily imply that the mature cells from the MSC lineage, such as osteoblasts and adipocytes are also resilient. In fact, mice exposed to lethal (12 Gy) and sub-lethal (5 Gy) doses of γ -irradiation exposure had significantly elevated numbers of adipocytes occupying the bone marrow following irradiation, because radiation depletes the bone marrow population, allowing space for adipocytes to occupy the marrow as early as 10 days following irradiation [56, 74], and perhaps even earlier (Figure 1). Increased levels of adipocytes in the marrow are also associated with a decline in hematopoietic progenitor cells [56]. Therefore it is possible that MSCs are preferentially differentiating towards adipocytes instead of osteoblasts following radiation exposure. This could ultimately hinder the proper bone formation process, and in turn lead to disorders associated with bone loss, such as osteoporosis, and the increased adipocyte content leading to hematopoietic progenitor cell depletion. Taken together, this would compromise immunologic health and compromise the major organs such as the bone marrow, thymus, and spleen, associated with HSCs.

Numerous preclinical studies report the beneficial effects of MSC transplantation following injury [75-78]. Lin *et al.* recently demonstrated that following localized radiation exposure to mice femurs, MSCs transplanted intra-arterially will engraft at the site of the injury induced by irradiation [75]. Even 6 weeks following exposure, the MSCs continued to proliferate ultimately showing long term engraftment capabilities [75]. Chang *et al.* report that MSC transplantation into rats from radiation-induced abdominal injury will lead to an increased rate of recovery as evidenced by neovascularization, epithelium homeostasis, and a decline in inflammation [76]. MSC transplantation has also shown to facilitate the recovery of damaged skin following localized injury caused by irradiation [77].

Radiation exposure, including cumulative doses of radiation exposure, can significantly compromise proper function of the bone marrow population. Atomic bomb survivors exposed to 200 mSv of radiation at Hiroshima and Nagasaki had greater incidence of chronic and acute myelogenous leukemia [79, 80], which typically developed 2-3 years following exposure [81]. However an overwhelming number of studies show there was no significant increase in leukemia cases of Chernobyl victims, who experienced an average of 410 mSv over a 2 year period (but ranged to 1000 mSv) [82, 83], perhaps because the prolonged dose exposure was not sufficiently high to induce bone marrow failure. The overall decrease in the number of cells in the bone marrow accompanying a change in the phenotypic makeup of the marrow in a dose dependent fashion leads not only to a compromised immune system, but poor bone architectural quality. The deficit to the bone architecture was believed to be a cell dependent response caused by increased osteoclast activity following irradiation [84, 85], implicating that osteoclasts, like MSCs, are more resilient to irradiation. Additionally, a decline in the number of long term HSCs (identified as side population, Lin⁻, Sca1⁺, C-kit⁺; SPLSK) and progenitor HSCs can alter immune function because there may be insufficient number of cells available to create mature leukocytes necessary for a proper immune response [86, 87]. These changes in the HSC,

MSC, and leukocyte populations can be monitored in the blood as well as the bone marrow and are often monitored following radiation therapy.

Radiation Effects on Bone Architecture

The Bone Matrix

Bone is a complex tissue as it serves to provide structural support to the body, houses the bone marrow, is critical to locomotion, protects vital organs, and serves as the reservoir for minerals such as calcium and phosphorus to maintain homeostatic levels throughout a range of physiologic systems [88, 89]. The cellular components of bone, include osteocytes and osteoblasts, which coordinate bone growth, and osteoclasts which are the cells responsible for resorption of tissue during remodeling.

Bone, including cortical and trabecular bone, is composed of mineral (50-70%), an organic matrix (20-40%), water (5-10%), and lipids (<3%) [90]. The organic matrix, intended to provide elasticity and flexibility, is composed of collagen, primarily type I collagen, and non-collagenous proteins. Collagen is a triple helical structure composed of three α -chains, where two of the three α -chains are typically identical. The primary amino acid component for the α -chains is glycine, and is often followed by proline or hydroxyproline [91]. The inorganic mineralized matrix is intended to provide strength and rigidity and is composed primarily of hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ crystals. This mineral is deposited into the “hole” regions, which is the spacing between individual strands of collagen fibrils [92]. The more mature bone has greater number and larger mineralized crystals.

Deficit to Bone Architecture Due to Radiation Exposure

Exposure to ionizing radiation leads to a dramatic decline in trabecular bone with minor, at most, apparent consequences to cortical bone [93]. Interestingly, it has been reported that the decline occurs in a dose but not age dependent fashion, and the different animal studies are their outcomes are summarized in Table 1 [84, 94-97]. The specific mechanism(s) leading to radiation-induced increase in fracture risk is still questionable, but primary suspects would include the suppression of bone formation, an elevation of bone resorption, or even the destruction of the acellular components of the bone matrix including its organic and inorganic components.

A suppression of bone formation is certainly likely, as radiation suppresses the bone marrow population in a dose dependent fashion, putting the MSC reservoir at risk. Elevated resorption is also possible [74, 84], with the goal of osteoclast recruitment prioritized to remove damaged bone matrix. However bone loss occurs so rapidly following radiation exposure, taking fewer than 10 days [85, 95], and with the marrow compromised so severely, a non-biological process may also contribute to this loss of matrix [74].

Patients undergoing total body or localized radiation therapy are at greater risk of developing a fracture because of a decline in bone mechanical strength [98], but little is known about the change in bone quality as clinical evidence of a decline in bone mineral density following radiotherapy remains inconclusive [10, 99, 100]. Interestingly, patients who undergo radiation therapy often have fractures that are not associated with trauma and

may go undetected until radiographic analysis [10, 101, 102]. Animal models become a good means to quantify the impact of radiation on trabecular and cortical bone quantity and quality, as well as the biological and physical factors responsible for this loss. Using micro-CT, we have shown a drastic decline to trabecular bone in irradiated mice 8 weeks following 5 Gy of γ -irradiation (Figure 2). Kondo *et al.* exposed 18 week old C57BL/6 mice to 1 Gy and 2 Gy doses of sub-lethal ^{137}Cs γ -irradiation at 0.915 Gy/min and observed declines in trabecular microarchitecture including a 20% decline in bone volume fraction (BV/TV) as early as 3 days following 2 Gy of irradiation, which further declined by 43% of baseline by 10 days [85]. They justify this compromise in trabecular bone architecture as a 44% increase in osteoclast activity within the bone marrow which was present just after 3 days [84, 85]. The mice exposed to a lower 1 Gy dose did not show a significant decline in BV/TV until 10 days and it was 28% lower than the age-matched controls. It is difficult to reconcile how the decline in bone volume could be due strictly to an increase in osteoclasts, particularly because there was probably a 30% depletion of the total number of hematopoietic cells in the bone marrow following 1 Gy of irradiation [103]. Therefore, it may be possible that the 20% decline in BV/TV observed after just 3 days is attributable to factors beyond cell-driven resorption. Perhaps another process, such as physicochemical, contributes to this rapid depletion of bone.

Although it was previously not widely accepted, there is some evidence that radiation directly induces physical damage to collagen, undermining the bone's architectural structure; Acil *et al.* irradiated porcine jaws *in vitro* with a combined 60 Gy dose of ^{60}Co γ -irradiation, which is a comparable to doses seen in the clinic for localized radiotherapy, and observed an increase in hydroxyllysylpyridinoline and lysylpyridinoline ultra-filtrates in the irradiated bones compared to the non-irradiated controls, implying increased split collagen in the exposed bones [104]. We also report through Fourier Transform Infrared imaging a decrease in the mineral composition of trabecular bone as early as 2 days following sub-lethal irradiation in mice, which persisted even 10 days following exposure [74]. Similarly, using Raman spectroscopy, Gong *et al.* show that mice exposed to 20 Gy of localized radiation have an alteration in the collagen crosslinking and the mineralized matrix as early as 1 week following irradiation exposure [105]. These results suggest that radiation leads to dissolution of the bone matrix, and therefore bone loss is due to a combination of both biological and physicochemical processes. Considering the consequences of irradiation on the skeleton, there is a clear need to improve our understanding of what causes this loss of bone (e.g., consequences to the cellular component, the matrix, or a combination thereof), and with this understanding, perhaps improve our ability to protect or recover bone quality.

Pharmacologic Treatments to Restore the Bone Marrow and Bone Architecture

Since bone loss occurs following high doses of irradiation-induced bone marrow suppression, therapeutic treatments, including both pharmacologic and non-pharmacologic treatments, may be necessary to prevent osteopenia and restore bone loss [106-108].

Numerous therapeutic methods are currently used to maintain and restore bone in order to prevent fractures. These include pharmacological approaches such as the administration of

bisphosphonates, vitamin D, calcitonin, and hormone therapy [109-114]. Bisphosphonates, such as alendronate, zoledronic acid, and risedronate, are intended to treat bone disorders including osteoporosis and Paget's disease, thereby preserving the bone structure [115], and could have applications for skeletal maintenance following radiotherapy. In fact, zoledronic acid was recently shown to increase bone density in irradiated mice, but was unable to maintain its mechanical properties [116] which may be from its inability to restore physiological bone collagen cross linking levels following radiation exposure [117]. Risedronate too was capable of preventing radiation-induced bone loss in a mouse model [118]. The bisphosphonates inhibit bone resorption by altering osteoclast activity. This is done by preventing osteoclast recruitment to the bone surface [119], inhibiting its activity on the bone surface [120], and potentially inducing osteoclast apoptosis [121, 122]. In addition to decreasing osteoclast activity, bisphosphonates decrease bone turnover all together, by decreasing bone formation as well [122, 123]. Since bisphosphonates work to protect the bone structure by acting primarily on the osteoclasts, they may be insufficient to prevent bone loss due to radiation exposure if in fact there is a large physicochemical contribution to bone loss. Rather than acting to inhibit osteoclasts, Vitamin D supplementation increases calcium absorption into the intestines and is currently recommended for fracture prevention [124, 125]. Vitamin D is a steroid hormone that interacts with the vitamin D receptor within osteoblasts to increase receptor activator of nuclear factor κ - β ligand (RANKL) [126]. RANK on the preosteoclast membrane binds to RANKL, activates the osteoclasts, and leads to active bone turnover [126]. This in turn mobilizes calcium from the skeleton to circulate through the blood stream in order to maintain serum calcium levels, but to our knowledge, studies have not been conducted that assess the ability of Vitamin D to maintain bone following radiotherapy. Parathyroid Hormone (PTH), is anabolic to bone when administered intermittently [127] because it elevates the rate of bone resorption thereby increasing the rate of the bone remodeling process. Alternatively, PTH can be catabolic to bone at chronic levels because of increased rate of bone resorption [128, 129]. PTH acts by binding to its receptor PTH receptor 1 found on osteoblasts [130], ultimately increasing RANKL expression. Osteoprotegerin (OPG), a decoy receptor for RANKL, binds to RANKL, and blocks the RANKL and RANK interaction between osteoblasts and osteoclasts. However, the increase in osteoblastogenesis leads to elevated RANKL expression [131], and therefore the preosteoclasts expressing RANK bind to the RANKL inducing osteoclastogenesis, and overall bone remodeling. Preclinical studies report that PTH can be used to prevent radiation induced-bone loss [132], and PTH in combination with zoledronic acid have the ability to increase bone mineral density following radiation therapy beyond the use of zoledronic acid alone [133]. These results indicate that bisphosphonates and PTH could be promising therapies for fracture prevention following radiation exposure.

Although the pharmacological treatments mentioned above increase bone mineral density, they are not necessarily suitable options for all osteoporotic patients. For instance, patients with allergic reactions to bisphosphonate or those with kidney disease should avoid bisphosphonates and Vitamin D [134]. Bisphosphonates can also lead to side effects including osteonecrosis of the jaw, gastroesophageal irritation, ocular inflammation, and musculoskeletal pain [134]. PTH can lead to side effects such as nausea and muscle weakness, and is not suitable for pregnant women. Therefore non-pharmacologic treatments,

such as physical activity [135-137], could be beneficial for many osteopenic and osteoporotic patients and ultimately decrease their risk of fractures [138] following irradiation.

Conclusion

In summary, exposure to ionizing radiation, even in sub-lethal doses will lead to drastic declines of both the bone marrow and bone architecture. The ability to maintain the bone marrow during and after exposure to ionizing radiation may be an insufficient method to prevent bone architectural destruction because of the potentially non-biological process leading to bone dissolution. The elevation of adipocytes within the bone marrow following irradiation may contribute to the inability of the bone marrow to repair its phenotypic populations following injury, ultimately leading to a deficit to the bone architecture. A better understanding of the role of hematopoietic and mesenchymal stem cells in disease etiology will pronounce their role in pathways to treat bone marrow disorders and poor bone quality.

Acknowledgments

This work was supported by the National Institutes of Health AR043498, EB14351, and RR23782.

References

1. Alter BP. Diagnosis, Genetics, and Management of Inherited Bone Marrow Failure Syndromes. ASH Education Program Book 2007. 2007;29–39.
2. Gordon-Smith, EC. Els, *Bone Marrow Failure*. John Wiley & Sons, Ltd; 2001.
3. National Council on Radiation Protection and Measurements, No. 160. Ionizing Radiation Exposure of the Population of the United States: 2009.
4. Centers for Disease Control and Prevention. Acute Radiation Syndrome: A Fact Sheet for Physicians. Atlanta: 2005.
5. Okunieff P, Wu T, Huang K, Ding I. Differential Radioprotection of Three Mouse Strains by Basic or Acidic Fibroblast Growth Factor. *Br. J. Cancer Suppl.* 1996; 27:S105–8. [PubMed: 8763859]
6. Burgess, J.; Cox, A.; Pecanha, S. The New York Times. The New York Times Company; New York: 2011.
7. Wyngaarden KE, Pauwels EKJ. Hormesis: Are Low Doses of Ionizing Radiation Harmful or Beneficial? *Eur. J. Nucl. Med. Mol. I.* 1995; 22:481–486.
8. Karim L, Judex S. Low Level Irradiation in Mice Can Lead to Enhanced Trabecular Bone Morphology. *J. Bone Miner. Metab.* 2013
9. Inagaki J, et al. Tbi and Melfalan Followed by Allogeneic Hematopoietic Sct in Children with Advanced Hematological Malignancies. *Bone Marrow Transpl.* 2011; 46:1057–1062.
10. Holt GE, et al. Fractures Following Radiotherapy and Limb-Salvage Surgery for Lower Extremity Soft-Tissue Sarcomas. *J. Bone. Joint .Surg. Am.* 2005; 87:315–319. [PubMed: 15687153]
11. Kallman RF. The Phenomenon of Reoxygenation and Its Implications for Fractionated Radiotherapy. *Radiology.* 1972; 105:135–142. [PubMed: 4506641]
12. Savani BN, et al. Increased Risk of Bone Loss without Fracture Risk in Long-Term Survivors after Allogeneic Stem Cell Transplantation. *Biol. Blood Marrow Transpl.* 2007; 13:517–520.
13. van der Sluis IM, van den Heuvel-Eibrink MM, Hählen K, Krenning EP, de Muinck Keizer-Schrama S. Altered Bone Mineral Density and Body Composition, and Increased Fracture Risk in Childhood Acute Lymphoblastic Leukemia. *J. Pediatr.* 2002; 141:204–210. [PubMed: 12183715]
14. Halton JM, et al. Altered Mineral Metabolism and Bone Mass in Children During Treatment for Acute Lymphoblastic Leukemia. *J. Bone Miner. Res.* 1996; 11:1774–1783. [PubMed: 8915786]

15. Cannon CP, Lin PP, Lewis VO, Yasko AW. Management of Radiation-Associated Fractures. *Journal of the American Academy of Orthopaedic Surgeons*. 2008; 16:541–549. [PubMed: 18768711]
16. Howlader, N., et al. *Seer (Surveillance, Epidemiology and End Results) Cancer Statistics Review, 1975-2010*. National Cancer Institute; Bethesda, MD: 2013.
17. Höglér W, et al. Incidence of Skeletal Complications During Treatment of Childhood Acute Lymphoblastic Leukemia: Comparison of Fracture Risk with the General Practice Research Database. *Pediatr. Blood Cancer*. 2007; 48:21–27. [PubMed: 16317756]
18. Gratwohl A, et al. Hematopoietic Stem Cell Transplantation. *J. Am. Med. Assoc*. 2010; 303:1617–1624.
19. Wu AM, Till JE, Siminovitch L, McCulloch EA. Cytological Evidence for a Relationship between Normal Hematopoietic Colony-Forming Cells and Cells of the Lymphoid System. *J. Exp. Med*. 1968; 127:455–464. [PubMed: 5636553]
20. Wu AM, Till JE, Siminovitch L, McCulloch EA. A Cytological Study of the Capacity for Differentiation of Normal Hemopoietic Colony-Forming Cells. *J. Cell. Physiol*. 1967; 69:177–184. [PubMed: 6033948]
21. Khan, FH. *The Elements of Immunology*. Dorling Kindersley Pvt. Ltd.; Delhi: 2009.
22. Geissmann F, et al. Blood Monocytes: Distinct Subsets, How They Relate to Dendritic Cells, and Their Possible Roles in the Regulation of T-Cell Responses. *Immunol. Cell Biol*. 2008; 86:398–408. [PubMed: 18392044]
23. Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological Decision-Making: How Does the Immune System Decide to Mount a Helper T-Cell Response? *Immunology*. 2008; 123:326–338. [PubMed: 17983439]
24. Fung-Leung W-P, et al. Cd8 Is Needed for Development of Cytotoxic T but Not Helper T Cells. *Cell*. 1991; 65:443–449. [PubMed: 1673361]
25. Kaech SM, Wherry EJ, Ahmed R. Effector and Memory T-Cell Differentiation: Implications for Vaccine Development. *Nat. Rev. Immunol*. 2002; 2:251–262. [PubMed: 12001996]
26. Sayles PC, Gibson GW, Johnson LL. B Cells Are Essential for Vaccination-Induced Resistance to Virulent *Toxoplasma Gondii*. *Infect. Immun*. 2000; 68:1026–1033. [PubMed: 10678903]
27. Delves PJ, Roitt IM. The Immune System. *N. Engl. J. Med*. 2000; 343:108–117. [PubMed: 10891520]
28. Miyamoto T, Suda T. Differentiation and Function of Osteoclasts. *Keio J. Med*. 2003; 52:1–7. [PubMed: 12713016]
29. Pittenger MF, et al. Multilineage Potential of Adult Human Mesenchymal Stem Cells. *Science*. 1999; 284:143–147. [PubMed: 10102814]
30. Cassiede P, Dennis JE, Ma F, Caplan AI. Osteochondrogenic Potential of Marrow Mesenchymal Progenitor Cells Exposed to Tgf-B1 or Pdgf-Bb as Assayed in Vivo and in Vitro. *J. Bone Miner. Res*. 1996; 11:1264–1273. [PubMed: 8864901]
31. Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult Rat and Human Bone Marrow Stromal Cells Differentiate into Neurons. *J. Neurosci. Res*. 2000; 61:364–370. [PubMed: 10931522]
32. Mackay AM, et al. Chondrogenic Differentiation of Cultured Human Mesenchymal Stem Cells from Marrow. *Tissue Eng*. 1998; 4:415–428. [PubMed: 9916173]
33. Jaiswal RK, et al. Adult Human Mesenchymal Stem Cell Differentiation to the Osteogenic or Adipogenic Lineage Is Regulated by Mitogen-Activated Protein Kinase. *J. Biol. Chem*. 2000; 275:9645–9652. [PubMed: 10734116]
34. Kiel MJ, et al. Slam Family Receptors Distinguish Hematopoietic Stem and Progenitor Cells and Reveal Endothelial Niches for Stem Cells. *Cell*. 2005; 121:1109–21. [PubMed: 15989959]
35. Wilson A, et al. Hematopoietic Stem Cells Reversibly Switch from Dormancy to Self-Renewal During Homeostasis and Repair. *Cell*. 2008; 135:1118–1129. [PubMed: 19062086]
36. Mendez-Ferrer S, et al. Mesenchymal and Haematopoietic Stem Cells Form a Unique Bone Marrow Niche. *Nature*. 2010; 466:829–834. [PubMed: 20703299]
37. Chan CKF, et al. Endochondral Ossification Is Required for Haematopoietic Stem-Cell Niche Formation. *Nature*. 2009; 457:490–494. [PubMed: 19078959]

38. Masuda S, et al. Cotransplantation with Mscs Improves Engraftment of Hscs after Autologous Intra-Bone Marrow Transplantation in Nonhuman Primates. *Exp. Hematol.* 2009; 37:1250–1257.e1. [PubMed: 19638293]
39. Omatsu Y, et al. The Essential Functions of Adipo-Osteogenic Progenitors as the Hematopoietic Stem and Progenitor Cell Niche. *Immunity.* 2010; 33:387–399. [PubMed: 20850355]
40. Nombela-Arrieta C, Ritz J, Silberstein LE. The Elusive Nature and Function of Mesenchymal Stem Cells. *Nat. Rev. Mol. Cell Biol.* 2011; 12:126–131. [PubMed: 21253000]
41. Meirelles, L.d.S.; Fontes, AM.; Covas, DT.; Caplan, AI. Mechanisms Involved in the Therapeutic Properties of Mesenchymal Stem Cells. *Cytokine Growth Factor Rev.* 2009; 20:419–427. [PubMed: 19926330]
42. Nicola MD, et al. Human Bone Marrow Stromal Cells Suppress T-Lymphocyte Proliferation Induced by Cellular or Nonspecific Mitogenic Stimuli. *Blood.* 2002; 99:3838–3843. [PubMed: 11986244]
43. Krampera M, et al. Bone Marrow Mesenchymal Stem Cells Inhibit the Response of Naive and Memory Antigen-Specific T Cells to Their Cognate Peptide. *Blood.* 2003; 101:3722–3729. [PubMed: 12506037]
44. Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M. Interactions between Human Mesenchymal Stem Cells and Natural Killer Cells. *Stem Cells.* 2006; 24:74–85. [PubMed: 16099998]
45. Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal Stem Cell-Natural Killer Cell Interactions: Evidence That Activated Nk Cells Are Capable of Killing Mscs, Whereas Mscs Can Inhibit Il-2-Induced Nk-Cell Proliferation. *Blood.* 2006; 107:1484–1490. [PubMed: 16239427]
46. Corcione A, et al. Human Mesenchymal Stem Cells Modulate B-Cell Functions. *Blood.* 2006; 107:367–372. [PubMed: 16141348]
47. Chow A, et al. Bone Marrow Cd169+ Macrophages Promote the Retention of Hematopoietic Stem and Progenitor Cells in the Mesenchymal Stem Cell Niche. *J. Exp. Med.* 2011; 208:261–71. [PubMed: 21282381]
48. Winkler IG, et al. Bone Marrow Macrophages Maintain Hematopoietic Stem Cell (Hsc) Niches and Their Depletion Mobilizes Hscs. *Blood.* 2010; 116:4815–4828. [PubMed: 20713966]
49. Chang MK, et al. Osteal Tissue Macrophages Are Intercalated Throughout Human and Mouse Bone Lining Tissues and Regulate Osteoblast Function in Vitro and in Vivo. *The Journal of Immunology.* 2008; 181:1232–1244. [PubMed: 18606677]
50. Rankin EB, et al. The Hif Signaling Pathway in Osteoblasts Directly Modulates Erythropoiesis through the Production of Epo. *Cell.* 2012; 149:63–74. [PubMed: 22464323]
51. Wu JY, et al. Osteoblastic Regulation of B Lymphopoiesis Is Mediated by Gs{Alpha}-Dependent Signaling Pathways. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:16976–81. [PubMed: 18957542]
52. Zhu J, et al. Osteoblasts Support B-Lymphocyte Commitment and Differentiation from Hematopoietic Stem Cells. *Blood.* 2007; 109:3706–12. [PubMed: 17227831]
53. Ding L, Morrison SJ. Haematopoietic Stem Cells and Early Lymphoid Progenitors Occupy Distinct Bone Marrow Niches. *Nature.* 2013; 495:231–235. [PubMed: 23434755]
54. Tokoyoda K, Egawa T, Sugiyama T, Choi B-I, Nagasawa T. Cellular Niches Controlling B Lymphocyte Behavior within Bone Marrow During Development. *Immunity.* 2004; 20:707–718. [PubMed: 15189736]
55. Greenbaum A, et al. Cxcl12 in Early Mesenchymal Progenitors Is Required for Haematopoietic Stem-Cell Maintenance. *Nature.* 2013; 495:227–30. [PubMed: 23434756]
56. Naveiras O, et al. Bone-Marrow Adipocytes as Negative Regulators of the Haematopoietic Microenvironment. *Nature.* 2009; 460:259–263. [PubMed: 19516257]
57. Bonyadi M, et al. Mesenchymal Progenitor Self-Renewal Deficiency Leads to Age-Dependent Osteoporosis in Sca-1/Ly-6a Null Mice. *Proc. Natl. Acad. Sci. U. S. A.* 2003; 100:5840–5845. [PubMed: 12732718]
58. Till JE, McCulloch EA. Direct Measurement of Radiation Sensitivity of Normal Mouse Bone Marrow Cells. *Radiat. Res.* 1961; 14:213–222. [PubMed: 13776896]

59. Fuks Z, et al. Long-Term Effects of Radiation on T-Lymphocytes and B-Lymphocytes in Peripheral-Blood of Patients with Hodgkins-Disease. *J. Clin. Investig.* 1976; 58:803–814. [PubMed: 135001]
60. Matsumoto K, Ramsey MJ, Nelson DO, Tucker JD. Persistence of Radiation-Induced Translocations in Human Peripheral Blood Determined by Chromosome Painting. *Radiat. Res.* 1998; 149:602–613. [PubMed: 9611099]
61. Scott D, Lyons CY. Homogeneous Sensitivity of Human Peripheral Blood Lymphocytes to Radiation-Induced Chromosome Damage. *Nature.* 1979; 278:756–758. [PubMed: 431733]
62. Gridley DS, Pecaut MJ, Dutta-Roy R, Nelson GA. Dose and Dose Rate Effects of Whole-Body Proton Irradiation on Leukocyte Populations and Lymphoid Organs: Part I. *Immunol. Lett.* 2002; 80:55–66. [PubMed: 11716966]
63. Dusenbery KE, et al. Erythropoietin Increases Hemoglobin During Radiation Therapy for Cervical Cancer. *Int. J. Radiat. Oncol. Biol. Phys.* 1994; 29:1079–1084. [PubMed: 8083076]
64. Li J, Kwong DLW, Chan GCF. The Effects of Various Irradiation Doses on the Growth and Differentiation of Marrow-Derived Human Mesenchymal Stromal Cells. *Pediatr. Transplant.* 2007; 11:379–387. [PubMed: 17493217]
65. Singh S, et al. Mesenchymal Stem Cells Show Radioresistance in Vivo. *J. Cell. Mol. Med.* 2012; 16:877–887. [PubMed: 21762375]
66. Mussano F, et al. Differential Effect of Ionizing Radiation Exposure on Multipotent and Differentiation-Restricted Bone Marrow Mesenchymal Stem Cells. *J. Cell. Biochem.* 2010; 111:322–332. [PubMed: 20506196]
67. Chen MF, et al. The Sensitivity of Human Mesenchymal Stem Cells to Ionizing Radiation. *Int. J. Radiat. Oncol. Biol. Phys.* 2006; 66:244–253. [PubMed: 16839703]
68. Sugrue T, Lowndes NF, Ceredig R. Mesenchymal Stromal Cells: Radio-Resistant Members of the Bone Marrow. *Immunol. Cell Biol.* 2013; 91:5–11. [PubMed: 23165606]
69. Azzam EI, Jay-Gerin J-P, Pain D. Ionizing Radiation-Induced Metabolic Oxidative Stress and Prolonged Cell Injury. *Cancer Lett.* 2012; 327:48–60. [PubMed: 22182453]
70. Cao X, et al. Irradiation Induces Bone Injury by Damaging Bone Marrow Microenvironment for Stem Cells. *Proceedings of the National Academy of Sciences.* 2011
71. Rieger K, et al. Mesenchymal Stem Cells Remain of Host Origin Even a Long Time after Allogeneic Peripheral Blood Stem Cell or Bone Marrow Transplantation. *Exp. Hematol.* 2005; 33:605–11. [PubMed: 15850839]
72. Dickhut A, et al. Mesenchymal Stem Cells Obtained after Bone Marrow Transplantation or Peripheral Blood Stem Cell Transplantation Originate from Host Tissue. *Ann. Hematol.* 2005; 84:722–727. [PubMed: 16132912]
73. Sugrue T, Brown JA, Lowndes NF, Ceredig R. Multiple Facets of the DNA Damage Response Contribute to the Radioresistance of Mouse Mesenchymal Stromal Cell Lines. *Stem Cells.* 2013; 31:137–45. [PubMed: 22961695]
74. Green DE, et al. Altered Composition of Bone as Triggered by Irradiation Facilitates the Rapid Erosion of the Matrix by Both Cellular and Physicochemical Processes. *PLoS ONE.* 2013; 8:e64952. [PubMed: 23741433]
75. Lin P, et al. Serial Transplantation and Long-Term Engraftment of Intra-Arterially Delivered Clonally Derived Mesenchymal Stem Cells to Injured Bone Marrow. *Mol. Ther.* 2013
76. Chang P, et al. Multi-Therapeutic Effects of Human Adipose-Derived Mesenchymal Stem Cells on Radiation-Induced Intestinal Injury. *Cell Death Dis.* 2013; 4:e685. [PubMed: 23788042]
77. Kotenko K, et al. Successful Treatment of Localised Radiation Lesions in Rats and Humans by Mesenchymal Stem Cell Transplantation. *Radiat. Prot. Dosimetry.* 2012; 151:661–5. [PubMed: 23024175]
78. Hu KX, Sun QY, Guo M, Ai HS. The Radiation Protection and Therapy Effects of Mesenchymal Stem Cells in Mice with Acute Radiation Injury. *British Journal of Radiology.* 2010; 83:52–58. [PubMed: 20139249]
79. Preston DL, et al. Cancer Incidence in Atomic Bomb Survivors. Part Iii: Leukemia, Lymphoma and Multiple Myeloma, 1950–1987. *Radiat. Res.* 1994; 137:S68–S97. [PubMed: 8127953]

80. Ron E, Preston DL, Mabuchi K, Thompson DE, Soda M. Cancer Incidence in Atomic-Bomb Survivors .4. Comparison of Cancer Incidence and Mortality. *Radiat. Res.* 1994; 137:S98–S112. [PubMed: 8127954]
81. Folley JH, Borges W, Yamawaki T. Incidence of Leukemia in Survivors of the Atomic Bomb in Hiroshima and Nagasaki, Japan. *Am. J. Med.* 1952; 13:311–321. [PubMed: 12985588]
82. Auvinen A, et al. Fallout from Chernobyl and Incidence of Childhood Leukaemia in Finland, 1976-92. *Br. Med. J. (Clin. Res. Ed.)*. 1994; 309:151–154.
83. Parkin DM, et al. Childhood Leukaemia in Europe after Chernobyl: 5 Year Follow-Up. *Br. J. Cancer.* 1996; 73:1006–1012. [PubMed: 8611419]
84. Willey JS, et al. Early Increase in Osteoclast Number in Mice after Whole-Body Irradiation with 2 Gy X Rays. *Radiat. Res.* 2008; 170:388–392. [PubMed: 18763868]
85. Kondo H, et al. Total-Body Irradiation of Postpubertal Mice with Cs-137 Acutely Compromises the Microarchitecture of Cancellous Bone and Increases Osteoclasts. *Radiat. Res.* 2009; 171:283–289. [PubMed: 19267555]
86. Linton PJ, Dorshkind K. Age-Related Changes in Lymphocyte Development and Function. *Nat. Immunol.* 2004; 5:133–139. [PubMed: 14749784]
87. Trigg ME. Hematopoietic Stem Cells. *Pediatrics.* 2004; 113:1051–1057. [PubMed: 15060199]
88. Bauer W, Aub JC, Albright F. Studies of Calcium and Phosphorus Metabolism. V. A Study of the Bone Trabeculae as a Readily Available Reserve Supply of Calcium. *J. Exp. Med.* 1929; 49:145–U19. [PubMed: 19869533]
89. Carter D. Mechanical Loading Histories and Cortical Bone Remodeling. *Calcif. Tissue Int.* 1984; 36:S19–S24. [PubMed: 6430518]
90. Clarke B. Normal Bone Anatomy and Physiology. *Clin. J. Am. Soc. Nephro.* 2008; 3:S131–S139.
91. Fietzek PP, Kuhn K. The Primary Structure of Collagen. *Int. Rev. Connect. Tiss.* 1976; 7:1–60.
92. Landis WJ. The Strength of a Calcified Tissue Depends in Part on the Molecular Structure and Organization of Its Constituent Mineral Crystals in Their Organic Matrix. *Bone.* 1995; 16:533–544. [PubMed: 7654469]
93. Nyaruba MM, Yamamoto I, Kimura H, Morita R. Bone Fragility Induced by X-Ray Irradiation in Relation to Cortical Bone-Mineral Content. *Acta Radiol.* 1998; 39:43–46. [PubMed: 9498868]
94. Willey JS, et al. Bone Architectural and Structural Properties after 56Fe26+ Radiation-Induced Changes in Body Mass. *Radiat. Res.* 2009; 170:201–207. [PubMed: 18666808]
95. Green DE, Adler BJ, Chan ME, Rubin CT. Devastation of Adult Stem Cell Pools by Irradiation Precedes Collapse of Trabecular Bone Quality and Quantity. *J. Bone Miner. Res.* 2012; 27:749–759. [PubMed: 22190044]
96. Bandstra ER, et al. Musculoskeletal Changes in Mice from 20-50 Cgy of Simulated Galactic Cosmic Rays. *Radiat. Res.* 2009; 172:21–29. [PubMed: 19580504]
97. Bandstra ER, et al. Long-Term Dose Response of Trabecular Bone in Mice to Proton Radiation. *Radiat. Res.* 2009; 169:607–614. [PubMed: 18494551]
98. Sugimoto M, et al. Changes in Bone after High-Dose Irradiation. *Biomechanics and Histomorphology. J Bone Joint Surg Br.* 1991; 73:492–7. [PubMed: 1670456]
99. Baxter NN, Habermann EB, Tepper JE, Durham SB, Virnig BA. Risk of Pelvic Fractures in Older Women Following Pelvic Irradiation. *J. Am. Med. Assoc.* 2005; 294:2587–2593.
100. Hopewell JW. Radiation-Therapy Effects on Bone Density. *Med. Pediatr. Oncol.* 2003; 41:208–211. [PubMed: 12868120]
101. Cannon CP, et al. Complications of Combined Modality Treatment of Primary Lower Extremity Soft-Tissue Sarcomas. *Cancer.* 2006; 107:2455–61. [PubMed: 17036354]
102. Lin PP, Boland PJ, Healey JH. Treatment of Femoral Fractures after Irradiation. *Clin. Orthop. Relat. Res.* 1998:168–78. [PubMed: 9678045]
103. Wambi C, et al. Dietary Antioxidants Protect Hematopoietic Cells and Improve Animal Survival after Total-Body Irradiation. *Radiat. Res.* 2008; 169:384–396. [PubMed: 18363433]
104. Açil Y, et al. Proof of Direct Radiogenic Destruction of Collagen in Vitro. *Strahlenther. Onkol.* 2007; 183:374–379. [PubMed: 17609870]

105. Gong B, Oest ME, Mann KA, Damron TA, Morris MD. Raman Spectroscopy Demonstrates Prolonged Alteration of Bone Chemical Composition Following Extremity Localized Irradiation. *Bone*. 2013; 57:252–258. [PubMed: 23978492]
106. Prince RL, et al. Prevention of Postmenopausal Osteoporosis. *N. Engl. J. Med.* 1991; 325:1189–1195. [PubMed: 1922205]
107. Liberman UA, et al. Effect of Oral Alendronate on Bone Mineral Density and the Incidence of Fractures in Postmenopausal Osteoporosis. *N. Engl. J. Med.* 1995; 333:1437–1444. [PubMed: 7477143]
108. Riggs BL, Melton LJ. The Prevention and Treatment of Osteoporosis. *N. Engl. J. Med.* 1992; 327:620–627. [PubMed: 1640955]
109. Reid IR, et al. Intravenous Zoledronic Acid in Postmenopausal Women with Low Bone Mineral Density. *N. Engl. J. Med.* 2002; 346:653–661. [PubMed: 11870242]
110. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of Calcium and Vitamin D Supplementation on Bone Density in Men and Women 65 Years of Age or Older. *N. Engl. J. Med.* 1997; 337:670–676. [PubMed: 9278463]
111. Chesnut CH, et al. A Randomized Trial of Nasal Spray Salmon Calcitonin in Postmenopausal Women with Established Osteoporosis: The Prevent Recurrence of Osteoporotic Fractures Study. *Am. J. Med.* 2000; 109:267–276. [PubMed: 10996576]
112. Neer RM, et al. Effect of Parathyroid Hormone (1-34) on Fractures and Bone Mineral Density in Postmenopausal Women with Osteoporosis. *N. Engl. J. Med.* 2001; 344:1434–1441. [PubMed: 11346808]
113. Rodan GA, Fleisch HA. Bisphosphonates: Mechanisms of Action. *J. Clin. Investig.* 1996; 97:2692–2696. [PubMed: 8675678]
114. Tang BMP, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of Calcium or Calcium in Combination with Vitamin D Supplementation to Prevent Fractures and Bone Loss in People Aged 50 Years and Older: A Meta-Analysis. *The Lancet*. 2007; 370:657–666.
115. Whitaker M, Guo J, Kehoe T, Benson G. Bisphosphonates for Osteoporosis--Where Do We Go from Here? *N. Engl. J. Med.* 2012; 366:2048–2051. [PubMed: 22571168]
116. Keenawinna L, Oest ME, Mann KA, Spadaro J, Damron TA. Zoledronic Acid Prevents Loss of Trabecular Bone after Focal Irradiation in Mice. *Radiat. Res.* 2013; 180:89–99. [PubMed: 23772924]
117. Acil Y, et al. Effects of Zoledronate on Irradiated Bone in Vivo: Analysis of the Collagen Types I, V and Their Cross-Links Lysylpyridinoline, Hydroxylslypyridinoline and Hydroxyproline. *Calcif. Tissue Int.* 2013; 92:251–260. [PubMed: 23179106]
118. Willey JS, et al. Risedronate Prevents Early Radiation-Induced Osteoporosis in Mice at Multiple Skeletal Locations. *Bone*. 2010; 46:101–111. [PubMed: 19747571]
119. Schmidt A, et al. Protein-Tyrosine Phosphatase Activity Regulates Osteoclast Formation and Function: Inhibition by Alendronate. *Proc. Natl. Acad. Sci. U. S. A.* 1996; 93:3068–3073. [PubMed: 8610169]
120. Murakami H, et al. A Possible Mechanism of the Specific Action of Bisphosphonates on Osteoclasts: Tiludronate Preferentially Affects Polarized Osteoclasts Having Ruffled Borders. *Bone*. 1995; 17:137–144. [PubMed: 8554921]
121. Hughes DE, et al. Bisphosphonates Promote Apoptosis in Murine Osteoclasts in Vitro and in Vivo. *J. Bone Miner. Res.* 1995; 10:1478–1487. [PubMed: 8686503]
122. Storm T, Steiniche T, Thamsborg G, Melsen F. Changes in Bone Histomorphometry after Long-Term Treatment with Intermittent, Cyclic Etidronate for Postmenopausal Osteoporosis. *J. Bone Miner. Res.* 1993; 8:199–208. [PubMed: 8442438]
123. Boyce RW, Paddock CL, Gleason JR, Sletsema WK, Eriksen EF. The Effects of Risedronate on Canine Cancellous Bone Remodeling: Three-Dimensional Kinetic Reconstruction of the Remodeling Site. *J. Bone Miner. Res.* 1995; 10:211–221. [PubMed: 7754801]
124. Bischoff-Ferrari H, et al. Fracture Prevention with Vitamin D Supplementation: A Meta-Analysis of Randomized Controlled Trials. *J. Am. Med. Assoc.* 2005; 293:2257–2264.

125. Holick MF. Sunlight and Vitamin D for Bone Health and Prevention of Autoimmune Diseases, Cancers, and Cardiovascular Disease. *Am. J. Clin. Nutr.* 2004; 80:1678S–1688S. [PubMed: 15585788]
126. Khosla S. Minireview: The Opg/Rankl/Rank System. *Endocrinology.* 2001; 142:5050–5055. [PubMed: 11713196]
127. Neer RM, et al. Effect of Parathyroid Hormone (1-34) on Fractures and Bone Mineral Density in Postmenopausal Women with Osteoporosis. *N. Engl. J. Med.* 2001; 344:1434–1441. [PubMed: 11346808]
128. O'Brien CA, et al. Control of Bone Mass and Remodeling by Pth Receptor Signaling in Osteocytes. *PLoS ONE.* 2008; 3:e2942. [PubMed: 18698360]
129. Parisien M, et al. The Histomorphometry of Bone in Primary Hyperparathyroidism: Preservation of Cancellous Bone Structure. *J. Clin. Endocrinol. Metab.* 1990; 70:930–8. [PubMed: 2318948]
130. Liu B-Y, et al. Conditionally Immortalized Murine Bone Marrow Stromal Cells Mediate Parathyroid Hormone-Dependent Osteoclastogenesis in Vitro. *Endocrinology.* 1998; 139:1952–1964. [PubMed: 9528982]
131. Lee S-K, Lorenzo JA. Parathyroid Hormone Stimulates Trance and Inhibits Osteoprotegerin Messenger Ribonucleic Acid Expression in Murine Bone Marrow Cultures: Correlation with Osteoclast-Like Cell Formation. *Endocrinology.* 1999; 140:3552–3561. [PubMed: 10433211]
132. Chandra A, et al. Pth Prevents the Adverse Effects of Focal Radiation on Bone Architecture in Young Rats. *Bone.* 2013; 55:449–457. [PubMed: 23466454]
133. Arrington SA, Fisher ER, Willick GE, Mann KA, Allen MJ. Anabolic and Antiresorptive Drugs Improve Trabecular Microarchitecture and Reduce Fracture Risk Following Radiation Therapy. *Calcif. Tissue Int.* 2010; 87:263–272. [PubMed: 20563797]
134. Kennel KA, Drake MT. Adverse Effects of Bisphosphonates: Implications for Osteoporosis Management. *Mayo Clin. Proc.* 2009; 84:632–638. [PubMed: 19567717]
135. Snow-Harter C, Bouxsein ML, Lewis BT, Carter DR, Marcus R. Effects of Resistance and Endurance Exercise on Bone Mineral Status of Young Women: A Randomized Exercise Intervention Trial. *J. Bone Miner. Res.* 1992; 7:761–769. [PubMed: 1642145]
136. Wallace BA, Cumming RG. Systematic Review of Randomized Trials of the Effect of Exercise on Bone Mass in Pre- and Postmenopausal Women. *Calcif. Tissue Int.* 2000; 67:10–18. [PubMed: 10908406]
137. Nelson ME, et al. Effects of High-Intensity Strength Training on Multiple Risk Factors for Osteoporotic Fractures: A Randomized Controlled Trial. *J. Am. Med. Assoc.* 1994; 272:1909–1914.
138. Klibanski A, et al. Osteoporosis Prevention, Diagnosis, and Therapy. *J. Am. Med. Assoc.* 2001; 285:785–795.

Highlights

- Sub-lethal doses of irradiation lead to drastic bone loss
- Trabecular bone shows minimal recovery following irradiation
- There are long term risks of bone fracture following hematopoietic depletion

Figure 1.

Increased Adiposity in Mouse Bone Marrow Following 5 Gy of Irradiation. These histological sections are from the proximal tibia. Scale bar corresponds to 1.0 mm. Image adapted from Green, D.E. et al. Altered Composition of Bone as Triggered by Irradiation Facilitates the Rapid Erosion of the Matrix by Both Cellular and Physicochemical Processes. *PLoS ONE* **8**, e64952 (2013).

Figure 2.

Micro-CT Images Displaying Trabecular Bone Loss in the Proximal Tibiae of Mice 8 Weeks Following 5 Gy of Total Body Irradiation. Scale bar corresponds to 1.0 mm.

Table 1**Studies Evaluating Bone Architecture Following Radiation Exposure**

Study	Dose	Age	Species	Dose Rate	Source	Outcome
Açil et al. (2007)	60Gy	-	porcine	2Gy/d, 5fractions/wk, 0.734Gy/min	Co-60	elevated levels of split collagen in jaw
Green et al. (2012)	5Gy	8wk & 16wk	mouse	0.6Gy/min	Cs-137	40% decline in trabecular bone volume fraction 10 days following irradiation which extended to more than a 45% decline by 56 days in the tibiae. elevated osteoclast expression
Kondo et al. (2009)	1Gy, 2Gy	18wk	mouse	0.915Gy/min	Cs-137	20% decline in trabecular bone volume fraction 3 days following 2Gy irradiation which extended to 43% by 10 days in the tibiae 25% decline in trabecular bone volume fraction 10 days following 1Gy irradiation in the tibiae. elevated osteoclast expression
Willey, Grilley, et al. (2008)	1Gy, 2Gy, 4Gy	9wk	rat	1.5Gy/min	Fe ⁺	20% decline in trabecular bone volume fraction 9 months following 2Gy irradiation 26% decline in trabecular bone volume fraction 9 months following 4Gy irradiation elevated osteoclast expression Trabecular bone architecture was altered in shielded bones
Willey, Lloyd, et al. (2008)	2Gy	13wk	mouse	1.37Gy/min	X-Ray	44% more osteoclasts 3 days following 2Gy irradiation
Bandstra, Thompson, et al. (2009)	0.18Gy, 0.47Gy	16wk	mouse	4Gy/min	Fe ⁺	17% decline in trabecular bone volume fraction 9 weeks following 0.47Gy irradiation in the humeri changes to skeletal muscle morphology
Bandstra, Pecaut, et al. (2009)	0.5Gy, 1Gy, 2Gy	8wk	mouse	0.7Gy/min	Proton	20% decline in trabecular bone volume fraction 4 months following 2Gy irradiation in the tibiae. 13% decline in trabecular bone volume fraction 4 months following 1Gy irradiation in the tibiae. no changes in trabecular bone volume fraction to the tibiae were evident 4 months following 0.5Gy irradiation