PRS
GLOBAL OPEN

Overcoming Nuclear Winter: The Cutting-edge Science of Bone Healing and Regeneration in Irradiated Fields

Melissa Daniel, MD
Alexandra O. Luby, MD, MS
Lauren Buchman
Steven R. Buchman, MD

Background: The incidence of cancer worldwide is expected to be more than 22 million annually by 2030. Approximately half of these patients will likely require radiation therapy. Although radiotherapy has been shown to improve disease control and increase survivorship, it also results in damage to adjacent healthy tissues, including the bone, which can lead to devastating skeletal complications, such as nonunion, pathologic fractures, and osteoradionecrosis. Pathologic fractures and osteoradionecrosis are ominous complications that can result in large bone and soft tissue defects requiring complex reconstruction. Current clinical management strategies for these conditions are suboptimal and dubious at best. The gold standard in treatment of severe radiation injury is free tissue transfer; however, this requires a large operation that is limited to select candidates.
Methods: With the goal to expand current treatment options and to assuage the devastating sequelae of radiation injury on surrounding normal tissue, our laboratory has performed years of translational studies aimed at remediating bone healing and regeneration in irradiated fields. Three therapeutics (amifostine, deferoxamine, and adipose-derived stem cells) have demonstrated great promise in promoting healing and regeneration of irradiated bone.
Results: Amifostine confers prophylactic protection, whereas deferoxamine and adipose-derived stem cells function to remediate postradiation associated injury.
Conclusions: These prospective therapeutics exploit a mechanism attributed to increasing angiogenesis and ultimately function to protect or restore cellularity, normal cellular function, osteogenesis, and bone healing to nonirradiated metrics. These discoveries may offer innovative treatment alternatives to free tissue transfer with the added benefit of potentially preventing and treating osteoradionecrosis and pathologic fractures *(Plast Reconstr Surg Glob Open 2021;9:e3605; doi: 10.1097/GOX.0000000000003605; Published online 29 June 2021.)*

INTRODUCTION

According to the International Agency for Research on Cancer, the worldwide incidence of cancer is expected to reach 27.5 million by 2040, with approximately 16.3 million deaths annually.[1] Roughly half of these patients will likely require adjuvant radiation therapy (XRT), known to improve oncologic control, survivorship, and palliation of the disease.[2] In head and neck cancer (HNC), adjuvant XRT is often a critical component of treatment with recent data demonstrating improved survival rates from 30% to 80% in two decades.[3] Therefore, many HNC patients receive XRT to their tumors; however, the delivery of that radiation is associated with devastating effects on the adjacent craniofacial skeleton and the surrounding healthy tissues. These destructive complications still persist despite advancements in administration protocols and technological advances in radiation oncology[4] (Fig. 1).

Deleterious Effects of Radiation on Bone

Although XRT is highly effective in eradicating cancer, healthy tissue is inevitably injured in the process. It is well documented that bone and bone marrow is particularly vulnerable to the cytotoxic effects of XRT.[5] Radiation induced bony injury can lead to metabolically, structurally,
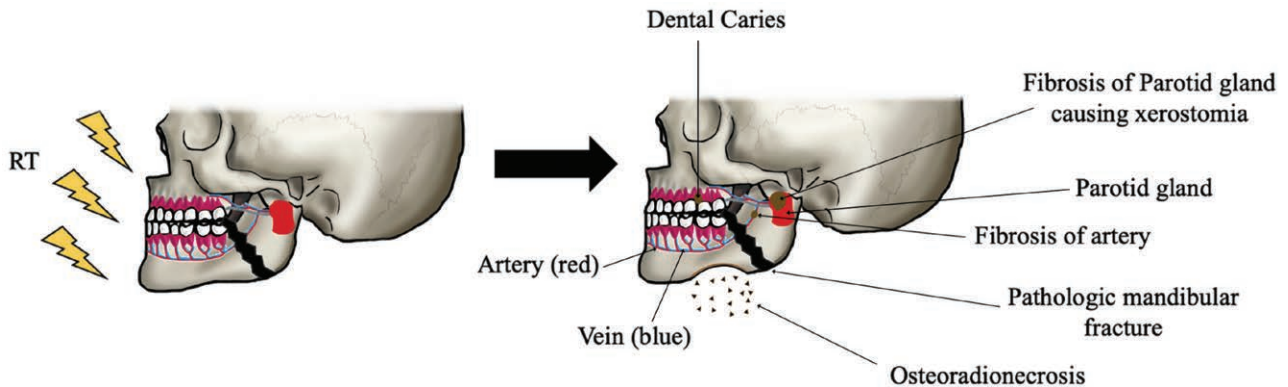
DOI: 10.1097/GOX.0000000000003605

Fig. 1. The detrimental effects of XRT in HNC. Examples of the pathological sequelae of radiation induced complications are fibrosis of inferior alveolar artery, pathological mandibular fractures, caries, and osteoradionecrosis as well as injury of the parotid gland resulting in xerostomia.

and biomechanically compromised bone, resulting in a number of complications, including pathologic fracture, nonunion/malunion, and osteoradionecrosis (ORN). Of these, ORN is the most severe consequence. The pathophysiologic progression of ORN is typically radiation induced hypovascularity, hypoxia, and hypocellularity, leading to a chronic nonhealing wound.[6] The mandible, due to its limited vascular supply from the inferior alveolar artery, is particularly susceptible to these bony complications after XRT due to radiation-induced fibrosis of the artery.[7]

Utilizing a murine model in our laboratory, several deleterious effects of radiation on the mandible have been demonstrated. Radiation-induced fibrosis of the inferior alveolar artery was confirmed by micro-computerized tomography (micro-CT). Interestingly, vessel thickness and vessel volume fraction were also significantly decreased in irradiated hemimandibles.[8] Additionally, evaluation of the effects of radiation on osteogenic cells demonstrated significant decreases in osteocytes, increased empty lacunae, and decreased bone load tolerance in a dose-dependent relationship.[9,10] Furthermore, microdensitometric analysis showed statistically significant dose-proportional changes in irradiated bone mineral volume, quality, and maturity.[11] Finally, radiation significantly reduced the ability to achieve a bony union after fracture, consistently demonstrating a dismal union rate as low as 20%.[11]

Current Treatment Modalities for Bone Morbidities Secondary to XRT

Therapeutics targeting the prevention and mitigation of XRT induced side-effects on bone would drastically improve the quality of life and associated morbidities for cancer patients. Current therapies with limited reliable success consist of local wound care, hyperbaric oxygen therapy, surgical correction with rigid fixation, distraction osteogenesis (DO), and nonvascularized bone grafts (NVBG). The inadequacies of these treatment options have necessitated more invasive reconstructive options, such as composite osseous free flaps, that more predictably address the bone and soft tissue deficits in the aftermath of XRT. Although hyperbaric oxygen (HBO) therapy was

initially used to treat osteoradionecrosis,[12] multiple recent studies have demonstrated inconsistent results using HBO and concluded that it is *not* recommended for routine use peri-operatively.[13,14]

Surgical management remains the gold standard for ORN and pathologic fractures. There are many reconstructive options; however, the most efficacious comes in the form of free flaps for the former and rigid fixation with or without bone grafting for the latter. The current gold standard for large segments of bone necrosis secondary to XRT is the vascularized free fibula flap.[15] However, these are large, resource-intensive operations with numerous associated complications such as local infection, wound healing issues, orocutaneous fistulas, and flap loss requiring further reconstruction.[15] In addition, free flap reconstruction is often contraindicated or significantly more difficult in the elderly and infirm.

DO is another reconstructive option with potentially less morbid complications than osseous free tissue transfer. This method generates the creation of new bone by the gradual separation of 2 osteogenic fronts, resulting in an anatomical and functional replacement of deficient tissue from local substrate.[16] Interestingly, the surrounding soft tissue matrix adapts to this tensile stress and expands in conjunction with the expanding bone.[16] Mandibular bone defects can be successfully reconstructed utilizing this method; however, DO requires robust regional vasculature, which is compromised in patients who have had radiation. Multiple attempts at using DO in irradiated fields have demonstrated poor outcomes, with insufficient bone formation occurring in approximately 25% of patients according to a recent systematic review.[17]

NVBG were initially the gold standard for mandibular reconstruction starting in 1949.[18] They are typically comprised of either costochondral rib or iliac bone for small defects of the mandible. Like DO, one of the limitations of using NVBG in HNC patients is the limited locoregional vasculature secondary to radiation. Hypovascularity portends insufficient NVBG incorporation, resulting in bone graft failure rates of 46% and 56% in some clinical studies.[19] These disheartening results led to the institution of free flap procedures as the gold standard for reconstruction in

irradiated fields involving the bone, though the associated morbidity profile leaves much to be desired.

To advance the field of craniofacial oncologic reconstruction, our laboratory has dedicated the past 20 years studying bony XRT injury and investigating ways to both prophylactically protect against radiation induced bone damage as well as assuage complications after radiotherapy. Therefore, our studies have focused on clinically translational therapeutics aimed at preventing and remediating XRT damage, improving vascularity, mitigating hypoxia, and increasing cellularity of the bone to ultimately decrease the incidence of XRT-induced bony complications. Such discoveries might well permit the use of DO as a viable option for bone defects in irradiated fields as well as potentially re-introduce NVBG as a practicable addition to the reconstructive surgeons' inventory of techniques. Finally, the discoveries that prevent or treat radiation-induced injury on bone and pathologic fractures could also yield protocols that may be extended toward applications instituted to assuage associated soft tissue injuries.

PHARMACEUTICAL AND CELLULAR-BASED THERAPEUTICS

Amifostine

Amifostine was approved by the United States Federal Drug Administration for the prevention of xerostomia in HNC patients undergoing XRT.[20] Amifostine selectively protects normal cells, acts as a free-radical scavenger, and upregulates DNA repair and inhibition of apoptosis.[20,21] In clinical studies, prophylactic use of amifostine before radiation resulted in statistically significant reduction in 1 and 2 year incidence of xerostomia compared with untreated controls.[22,23] Most importantly, there were no differences of tumor recurrence or survival benefit in patients receiving amifostine, demonstrating the oncologic safety of amifostine and refuting concerns about its potential to be tumor protective.[20,23]

Given the cytoprotective properties exhibited by amifostine in soft tissues, the therapy was investigated for its potential applications as a radioprotective agent for the bone. In a murine model of irradiated pathologic fracture, pre-treatment with amifostine preserved osteocyte number and function in irradiated bone to that of nonirradiated bone.[21] Additionally, prophylactic amifostine significantly improved bony union rates to 57%–100% compared with the 20%–25% union rate of irradiated controls.[24,25] On Raman spectroscopy, amifostine prophylaxis protected the mineral and organic matrix of irradiated bone.[26] Radiation induced hyper-mineralization demonstrated increases in bone volume fraction (BVF, ratio of new bone volume to total volume of the gap), bone mineral density (BMD, ratio of new bone mineral content to total volume of the gap), and tissue mineral density (TMD, ratio of tissue mineral content to bone volume of the gap) in the irradiated control group. Amifostine prophylaxis, however, also maintained native bone structure to the level of the nonirradiated controls 8 weeks after radiation.[27] Furthermore, amifostine pre-treatment preserved bone mineralization to the level of the nonirradiated control 18 weeks following radiotherapy, as there was no statistical difference between the mineral density levels and mineral to collagen ratios between groups.[27,28] These results, corroborated with micro-CT analysis, demonstrated that amifostine prophylaxis maintained mineralization metrics (BVF, BMD, and TMD) of irradiated bone to that of the nonirradiated controls.[24]

Recognizing that bone healing and repair requires a robust blood supply, further studies investigated the capability of amifostine to preserve regional vascularity at irradiated sites. Findings confirmed that amifostine prophylaxis preserved the vascular network of irradiated fractured bone to that of nonirradiated fractured controls and doubled the union rate from 25% to 57%[25] (Figs. 2, 3). These results were supported in a nonfractured irradiated model, where amifostine prophylaxis also demonstrated statistically significant angioprotection based on micro-CT vascular analysis compared with the irradiated controls.[29]

Despite these benefits, clinical utilization of prophylactic amifostine has not been widely adopted in HNC radiation treatment. This may be due in part to its intravenous (IV) infusion administration approximately 30 minutes before each fractionated radiation session. Although only occurring in approximately 5% of patients, its most common IV formulation is associated with episodes of hypotension, in addition to nausea and emesis.[30] When given subcutaneously, 15% of patients can exhibit symptoms such as asthenia, fever, or rash.[30] Therefore, despite the potential significant improvement in quality of the bone and surrounding soft tissues as well as the associated

Fig. 2. Gross images of hemimandible fracture healing. Gross images of the left hemimandible are shown for the (A) nonirradiated control, (B) irradiated control, and (C) irradiation + amifostine. Note the distinct brittle features and persistent fracture of irradiated control compared with amifostine-treated irradiated specimen and noncontrol. Reprinted with permission from Sarhaddi D, Tchanque-Fossuo C, Poushanchi B, et al. Amifostine protects vascularity and improves union in a model of irradiated mandibular fracture healing. Reprinted with permission from *Plastic and Reconstructive Surgery* 2013;132:6.
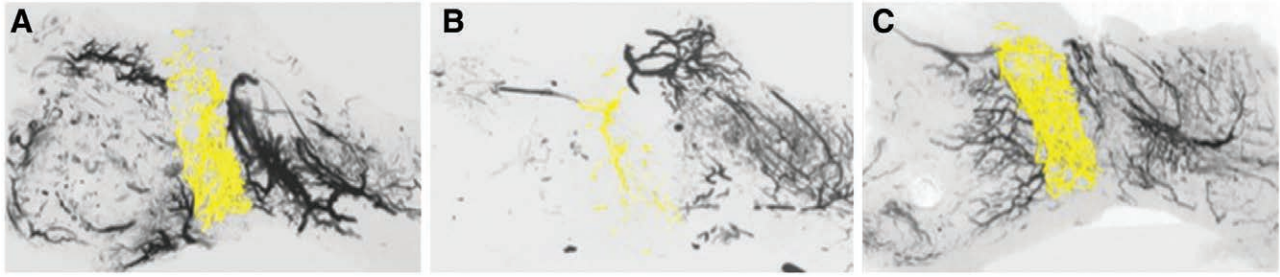
Fig. 3. Micro-computed tomography vasculature of hemimandible. Micro-computed tomographic images demonstrating qualitative evidence of differences in vascularity at the fracture line for (A) nonirradiated control, (B) irradiated control, and (C) amifostine + irradiation hemimandible. Note the paucity of vascularity secondary to irradiation and the robust vascular network resulting from amifostine treated bone. Reprinted with permission from Sarhaddi D, Tchanque-Fossuo C, Poushanchi B, et al. Amifostine protects vascularity and improves union in a model of irradiated mandibular fracture healing. Reprinted with permission from *Plastic and Reconstructive Surgery* 2013;132:6.

amelioration in pain, suffering, and quality of life, the requirement of increased medical personnel, healthcare costs, and the side effect profile have all contributed to the lack of widespread adoption.

With the goal to improve administration and alleviate side effects, an oral formulation of amifostine was initiated and investigated. Early results from preliminary studies of oral amifostine demonstrated effective delivery to the upper gastrointestinal tract (specifically the jejunum) with 61.5% bioavailability and decreased peak levels of amifostine in the blood. It is postulated that by decreasing peak drug levels, there may be a possible decrease in the side effect profile.[31] However, further studies are required to investigate the radioprotective effects with this formulation.

Deferoxamine

Deferoxamine (DFO) is another pharmaceutical that has been extensively investigated for its applications in wound healing, radiation injury, and angiogenesis. Although initially developed as an iron chelating agent to treat pathologic iron overload, it was subsequently found to improve vascularity at fractured bone sites and enhance bone healing.[32] By inhibiting the degradation of hypoxia inducing factor 1-α, deferoxamine upregulates genes and proteins involved in vascular tubule formation and osteogenic factors. In the setting of irradiated fractured bone, deferoxamine treatment markedly improved bony union from 25% to 67% and improved local vascularity compared with the irradiated control.[33] Additionally, on histologic evaluation, there were significantly more osteocytes and filled lacunae in the bone treated with DFO compared with the irradiated control.[34] These results were corroborated by findings that deferoxamine administration restored bone callus size, mineralization, and biomechanical strength to nonirradiated control levels after XRT[35] (Fig. 4). However, given the potent angiogenic properties of deferoxamine, concerns for potential tumorigenesis arose regarding its use in cancer patients. Interestingly, studies found DFO to be anti-tumorigenic, suggesting its potential for use as a chemotherapeutic agent in addition to its applications as a potent mitigator of radiation injury.[36,37]

Further studies have focused on innovative methods to improve the efficacy of deferoxamine due to its promising characteristics. With the creation of a composite particle formulation of deferoxamine (HA-DFO), our murine model demonstrated a remarkable bony union rate of 91% compared with a dismal 20% union rate in irradiated controls. This 91% union rate showed a concomitant improvement in biomechanical strength and also represented a 24% increase in bone healing over the 67% union rate demonstrated by standard deferoxamine therapy alone.[38,39]

Mesenchymal Stem Cells

Mesenchymal stem cells are adult stem cells with the potential to differentiate into a multitude of cell lineages, including osteocytes and chondrocytes. Bone marrow mesenchymal stem cells (BMSC) and adipose derived stem cells (ASCs) have emerged as promising therapeutics for bone tissue engineering. Although BMSCs have demonstrated greater efficacy in activating osteogenic differentiation in some studies,[40] ASCs exhibit both osteogenic and vasculogenic activating capabilities and are also more abundant, easier, and less invasive to harvest compared with BMSCs.[41,42]

Studies directly comparing BMSC and ASC applications in a murine irradiated bone healing model resulted in significant improvement of bony union rates. BMSCs achieved bony union rates of 66%, whereas ASCs reached bony union rates of 94% compared with the 23% union rate in the irradiated control.[43] Additionally, ASC treated bone demonstrated significant improvement in biomechanical strength compared with the irradiated controls.[44] Interestingly, ASCs demonstrated significant upregulation of vasculogenesis when co-cultured with human umbilical vein endothelial cells in vitro compared with irradiated controls, tripling tubule formation and increasing vasculogenic molecules, suggesting that ASC's likely enhance bone healing indirectly by increasing vascularity at irradiated sites.[42,45]

Given the immense potential that ASCs have to promote irradiated bone healing, further studies focused on improving the translation and feasibility of utilizing ASCs in the clinical setting. Current ASC harvest protocols require onerous laboratory processing techniques and expansion utilizing cell culture, which remains a major regulatory hurdle to clinical translation. The development of noncultured,

Fig. 4. Micro-computed tomography during fracture healing. Micro-computed tomographic images illustrating the differences among (A) nonirradiated fracture healing, (B) irradiated fracture healing, and (C) deferoxamine + irradiated fracture healing. Note the fragmented healing vs stable union of irradiated control compared with deferoxamine treated specimen and controls. Reprinted with permission from Donneys A, Ahsan S, Perosky J, et al. Deferoxamine restores callus size, mineralization, and mechanical strength in fracture healing after radiotherapy. Reprinted with permission from *Plastic and Reconstructive Surgery* 2013;131:711e-719e.

minimally processed ASCs (MP-ASC) represent significant progress toward a more clinically translatable cellular therapy. Irradiated fractures treated with MP-ASCs demonstrated a union rate that was 3 times that of irradiated controls (60% versus 20%) and improved the biomechanical strength of the bone.[46]

MP-ASCs represent an innovative advancement for the application of cell therapeutics in irradiated bone healing, as it minimizes laboratory processing and eliminates the need for cell culture. Continued optimization of this technique is promising for clinical translation of this technology from the bench to the bedside.

Combination Therapy

Although amifostine, deferoxamine, and ASCs have demonstrated improved bone healing as sole therapeutics, the combination of these therapies has been investigated to assess for potential synergistic effects. One such combination investigated was the dual use of ASCs and DFO. In 1 study, irradiated fractures treated with ASCs at the time of surgical fracture repair and postoperatively with deferoxamine demonstrated a 93% union rate with enhanced mineralization and biomechanical strength.[47] Another combination therapy involved prophylactic treatment of amifostine before radiation therapy and postradiation administration of deferoxamine. Their effects were found to be synergistic as amifostine prophylaxis preserved the number of residual osteocytes in irradiated bone and deferoxamine promoted angiogenesis, while preserving early and late mineralization and remediating mechanical strength to the level of the nonirradiated control.[48] Our laboratory continues to investigate combination therapies that may provide a synergistic effect to mitigate bony XRT injury.

Enhancing Surgical Techniques with Adjuvant Therapeutics for Mandibular Reconstuction

Amifostine, deferoxamine, and ASCs have demonstrated the unique ability to prevent or remediate the detrimental effects of XRT on bone, namely through their effects on angiogenesis and osteogenesis. With the goal of increasing the repertoire of reconstructive procedures previously contraindicated in an irradiated, hypovascular field, our laboratory utilized these efficacious bone healing enhancing therapeutics with either NVBG or DO.

Distraction Osteogenesis

As previously described, DO allows for the formation of endogenous tissue regeneration through mechano-transduction pathways and enhances vascularity at the osteotomy site.[49,50] However, irradiated mandibles have demonstrated a significant decrease in creation of bony bridges across the distraction gap with concurrent extensive cell death and empty lacunae compared with the non-irradiated control.[51]

To potentiate DO healing capacity, prophylactic amifostine was utilized in distracted irradiated bone, which demonstrated improved bony union rates of 84% compared with the 40% union of irradiated distracted controls.[52] In another study, the mandible was distracted after radiation and then DFO was administered to enhance the dismal success rate of untreated controls. DFO demonstrated a 92% union rate compared with the irradiated distracted control union rate of 11% combined with restoration of biomechanical parameters.[53]

Clinically, deferoxamine was utilized in a first in human proof of concept case to demonstrate successful DO for midfacial hypoplasia after radiotherapy for retinoblastoma. The use of DFO resulted in an increased area of bone formation and bone density compared with the untreated control, which happened to be the contralateral side, confirming the ability of DFO to improve the feasibility of DO as a reconstructive option in irradiated fields.[54]

Finally, BMSCs were investigated for their potential to improve the application of DO. BMSC's significantly augmented bone regeneration and the quality of new bone formation in irradiated mandibular DO.[55,56] BMSCs improved union rates to 80% compared with 0% union rate in the irradiated, distracted controls.[55,56] The findings of these studies support the potential application of DO in irradiated oncologic reconstruction when combined with pharmaceutical or cellular therapeutics.

Nonvascularized Bone Grafts

NVBG offer distinct advantages in maxillomandibular reconstruction. They do not require extensive microsurgical techniques and have a high success rate when placed in a well vascularized bed.[57] Additionally, they provide greater precision in bone symmetry, allowing for improved facial contour.[57] However, these grafts are associated with significantly higher failure rates when utilized in postradiation reconstruction due to the vascular depletion and loss of cellular function at the recipient site.[58] The decreased morbidity, diminished cost, and ease of use that an efficacious, predictable, and durable NVBG can contribute as a reconstructive option for the replacement of bone after oncologic surgical extirpation and XRT cannot be overstated.

Recent experiments in our laboratory aimed to remediate the destructive effects of XRT to improve NVBG incorporation and healing. Adjuvant use of a novel HA-DFO composite in NVBG implanted at an irradiated site demonstrated a 90% union rate of bone gaps compared with the 25% union rate in the irradiated control group. Perhaps even more impressive, radiomorphometric and biomechanical analysis of HA-DFO treated NVBG demonstrated no statistical differences when compared with NVBG in nonirradiated controls.[57] (Fig. 5) The potent angiogenic properties of HA-DFO appears to have the remarkable capability to remediate the corrosive effects of XRT. Utilizing this new model, future studies will focus on taking these important scientific discoveries and moving them from the bench to the bedside to treat some of the most challenging reconstructive dilemmas with the ultimate goal of restoring form and function in patients after oncologic resection.

CONCLUSIONS

Throughout the years, studies have investigated pharmaceutical and cell-based therapeutics to enhance bone healing and bone regeneration after radiotherapy. Applications of amifostine, deferoxamine, and mesenchymal stem cells offer tremendous promise in the setting of pathologic irradiated fracture healing and osteoradionecrosis as well as improving the reliability, feasibility, and utility of distraction osteogenesis and nonvascularized bone grafts after radiotherapy. The recent innovative strides and discoveries made in our laboratory hold enormous promise and impact that cutting-edge science can have on bone healing and regeneration in the quixotic efforts to overcome the nuclear winter imposed on wounds subject to the devastating effects of radiation therapy.

Fig. 5. Micro-computed tomography of bone graft healing. Micro-computerized tomography images of (A) control nonvascularized bone graft, (B) radiated nonvascularized bone graft, and (C) radiated nonvascularized bone graft + HA-DFO after 60 days. Note the incomplete graft incorporation in the radiated nonvascularized bone graft as opposed to the control and HA-DFO treated nonvascularized bone graft. Reprinted with permission from *Plastic and Reconstructive Surgery* 2017;5(9S):195–196.

Steven R. Buchman, MD
Buchman Craniofacial Research Laboratory
C&W 4-730, E. Hospital Dr
Ann Arbor, MI 48109
E-mail: sbuchman@med.umich.edu

REFERENCES

1. American Cancer Society. *Global Cancer Facts & Figures*. 4th ed. Atlanta: American Cancer Society; 2018.
2. Aggarwal A, Lewison G, Rodin D, et al. Radiation therapy research: a global analysis 2001-2015. *Int J Radiat Oncol Biol Phys*. 2018;101:767–778.
3. Chen HHW, Kuo MT. Improving radiotherapy in cancer treatment: promises and challenges. *Oncotarget*. 2017;8:62742–62758.
4. Gooi Z, Fakhry C, Goldenberg D, et al; Education Committee of the American Head and Neck Society (AHNS). AHNS series: do you know your guidelines? Principles of radiation therapy for head and neck cancer: a review of the National Comprehensive Cancer Network guidelines. *Head Neck*. 2016;38:987–992.
5. Mauch P, Constine L, Greenberger J, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. *Int J Radiat Oncol Biol Phys*. 1995;31:1319–1339.
6. Marx RE, Johnson RP. Studies in the radiobiology of osteoradionecrosis and their clinical significance. *Oral Surg Oral Med Oral Pathol*. 1987;64:379–390.
7. Kuhnt T, Stang A, Wienke A, et al. Potential risk factors for jaw osteoradionecrosis after radiotherapy for head and neck cancer. *Radiat Oncol*. 2016;11:101.
8. Deshpande SS, Donneys A, Farberg AS, et al. Quantification and characterization of radiation-induced changes to mandibular vascularity using micro-computed tomography. *Ann Plast Surg*. 2014;72:100–103.
9. Tchanque-Fossuo CN, Monson LA, Farberg AS, et al. Dose-response effect of human equivalent radiation in the murine mandible: part I. A histomorphometric assessment. *Plast Reconstr Surg (1963)* 2011;128:114–121.
10. Tchanque-Fossuo CN, Monson LA, Farberg AS, et al. Dose-response effect of human equivalent radiation in the murine mandible: part II. A biomechanical assessment. *Plast Reconstr Surg (1963)*. 2011;128:480e–487e.
11. Monson LA, Jing XL, Donneys A, et al. Dose-response effect of human equivalent radiation in the mandible. *J Craniofac Surg*. 2013;24:1593–1598.
12. Marx RE. A new concept in the treatment of osteoradionecrosis. *J Oral Maxillofac Surg*. 1983;41:351–357.
13. Annane D, Depondt J, Aubert P, et al. Hyperbaric oxygen therapy for radionecrosis of the jaw: a randomized, placebo-controlled, double-blind trial from the ORN96 study group. *J Clin Oncol*. 2004;22:4893–4900.
14. Sultan A, Hanna GJ, Margalit DN, et al. The use of hyperbaric oxygen for the prevention and management of osteoradionecrosis of the jaw: a dana-farber/brigham and women's cancer center multidisciplinary guideline. *Oncologist (Dayton, Ohio)*. 2017;22:343–350.
15. Cheriex KC, Nijhuis TH, Mureau MA. Osteoradionecrosis of the jaws: a review of conservative and surgical treatment options. *J Reconstr Microsurg*. 2013;29:69–75.
16. Swennen G, Schliephake H, Dempf R, et al. Craniofacial distraction osteogenesis: a review of the literature. Part 1: clinical studies. *Int J Oral Maxillofac Surg*. 2001;30:89–103.
17. Momeni A, Januszyk M, Wan DC, et al. Is distraction osteogenesis of the irradiated craniofacial skeleton contraindicated? *J Craniofac Surg*. 2017;28:1236–1241.
18. Blocker TG Jr, Stout RA. Mandibular reconstruction, World War II. *Plast Reconstr Surg (1946)*. 2019;4:153–156.
19. Handschel J, Hassanyar H, Depprich RA, et al. Nonvascularized iliac bone grafts for mandibular reconstruction–requirements and limitations. *In Vivo (Athens)*. 2011;25:795–799.
20. Andreassen CN, Grau C, Lindegaard JC. Chemical radioprotection: a critical review of amifostine as a cytoprotector in radiotherapy. *Semin Radiat Oncol*. 2003;13:62–72.
21. Donneys A, Tchanque-Fossuo CN, Blough JT, et al. Amifostine preserves osteocyte number and osteoid formation in fracture healing following radiotherapy. *J Oral Maxillofac Surg*. 2014;72:559–566.
22. Brizel DM, Wasserman TH, Henke M, et al. Phase III randomized trial of amifostine as a radioprotector in head and neck cancer. *J Clin Oncol*. 2000;18:3339–3345.
23. Wasserman TH, Brizel DM, Henke M, et al. Influence of intravenous amifostine on xerostomia, tumor control, and survival after radiotherapy for head-and- neck cancer: 2-year follow-up of a prospective, randomized, phase III trial. *Int J Radiat Oncol Biol Phys*. 2005;63:985–990.
24. Tchanque-Fossuo CN, Donneys A, Sarhaddi D, et al. The effect of Amifostine prophylaxis on bone densitometry, biomechanical strength and union in mandibular pathologic fracture repair. *Bone*. 2013;57:56–61.
25. Sarhaddi D, Tchanque-Fossuo CN, Poushanchi B, et al. Amifostine protects vascularity and improves union in a model of irradiated mandibular fracture healing. *Plast Reconstr Surg (1963)*. 2013;132:1542–1549.
26. Tchanque-Fossuo CN, Gong B, Poushanchi B, et al. Raman spectroscopy demonstrates amifostine induced preservation of bone mineralization patterns in the irradiated murine mandible. *Bone*. 2012;52:712–717.

27. Felice PA, Ahsan S, Perosky JE, et al. Prophylactic amifostine preserves the biomechanical properties of irradiated bone in the murine mandible. *Plast Reconstr Surg (1963)*. 2014;133:314e–321e.

28. Felice PA, Gong B, Ahsan S, et al. Raman spectroscopy delineates radiation-induced injury and partial rescue by amifostine in bone: a murine mandibular model. *J Bone Miner Metab*. 2015;33:279–284.

29. Page EE, Deshpande SS, Nelson NS, et al. Prophylactic administration of Amifostine protects vessel thickness in the setting of irradiated bone. *J Plast Reconstr Aesthet Surg*. 2015;68:98–103.

30. Kouvaris JR, Kouloulias VE, Vlahos LJ. Amifostine: the first selective-target and broad-spectrum radioprotector. *Oncologist*. 2007;12:738–747.

31. Ranganathan K, Simon E, Lynn J, et al. Novel formulation strategy to improve the feasibility of amifostine administration. *Pharm Res*. 2018;35:99.

32. Wan C, Gilbert SR, Wang Y, et al. Activation of the hypoxia-inducible factor-1 pathway accelerates bone regeneration. *Proc Natl Acad Sci*. 2008;105:686–691.

33. Donneys A, Weiss DM, Deshpande SS, et al. Localized deferoxamine injection augments vascularity and improves bony union in pathologic fracture healing after radiotherapy. *Bone*. 2013;52:318–325.

34. Donneys A, Nelson NS, Page EE, et al. Targeting angiogenesis as a therapeutic means to reinforce osteocyte survival and prevent nonunions in the aftermath of radiotherapy. *Head Neck*. 2015;37:1261–1267.

35. Donneys A, Ahsan S, Perosky JE, et al. Deferoxamine restores callus size, mineralization, and mechanical strength in fracture healing after radiotherapy. *Plast Reconstr Surg (1963)*. 2013;131:711e–719e.

36. Donneys A, Subramanian C, Lynn J, et al. Abstract 86: altered iron-mediator expression identifies chemotherapeutic effects of deferoxamine on head and neck squamous cell carcinoma. *Plast Reconstr Surg*. 2019;7:60–61.

37. Donneys A, Lynn JV, Urlaub K, et al. Abstract 105: deferoxamine: a regenerative therapeutic with antitumorigenic potential. *Plast Reconstr Surg*. 2018;6:84–84.

38. Ettinger R, Donneys A, Nelson N, et al. Abstract 5: a novel implantable angiogenic nanotechnology improves bone mineralization, biomechanical strength, and union rates in irradiated fractures. *Plast Reconstr Surg*. 2016;4:37.

39. Donneys A, Yang Q, Forrest ML, et al. Implantable hyaluronic acid-deferoxamine conjugate prevents nonunions through stimulation of neovascularization. *NPJ Regen Med*. 2019;4:11.

40. Ranganathan K, et al. Abstract 48. Stem cells harvested from bone marrow and adipose tissue demonstrate differential healing mechanisms and union rates in a murine model of irradiated mandibular fracture repair. *Plast Reconstr Surg*. 2017;5:46.

41. Luby AO, Ranganathan K, Lynn JV, et al. Stem cells for bone regeneration: current state and future directions. *J Craniofac Surg*. 2019;30:730–735.

42. Snider AE, et al. Abstract: adipose derived stem cells enhance vascularity in an irradiated murine mandibular fracture model. *Plast Reconstr Surg*. 2016;4(9S Suppl):3–4.

43. Ranganathan K, et al. Abstract 69: stem cells harvested from bone marrow and adipose tissue demonstrate equivalent healing but through different mechanisms in a murine model of irradiated mandibular fracture healing. *Plast Reconstr Surg*. 2017;5(4 Suppl.):52.

44. Ettinger RE, et al. Abstract: adipose derived stem cells improve union rates and biomechanical strength following fracture in the irradiated murine mandible. *Plast Reconstr Surg*. 2016;4(9S Suppl):4–5.

45. Rinkinen JR, et al. Abstract P30 : the role of anatomical region and hypoxia on induction of angiogenesis and proliferation in adipose-derived stem cells. *Plast Reconstr Surg (1963)*, 2013;131:178.

46. Lynn JV, et al. Noncultured minimally processed adipose-derived stem cells improve radiated fracture healing. *Ann Plast Surg*. 2020;85:83–88.

47. Donneys A, Blough JT, Nelson NS, et al. Translational treatment paradigm for managing non-unions secondary to radiation injury utilizing adipose derived stem cells and angiogenic therapy. *Head Neck*. 2016;38(Suppl 1):E837–E843.

48. Donneys A, Nelson NS, Perosky JE, et al. Prevention of radiation-induced bone pathology through combined pharmacologic cytoprotection and angiogenic stimulation. *Bone*. 2016;84:245–252.

49. Yu JC, Fearon J, Havlik RJ, et al. Distraction osteogenesis of the craniofacial skeleton. *Plast Reconstr Surg (1963)*. 2004;114:1E–20e.

50. Donneys A, Tchanque-Fossuo CN, Farberg AS, et al. Bone regeneration in distraction osteogenesis demonstrates significantly increased vascularity in comparison to fracture repair in the mandible. *J Craniofac Surg*. 2012;23:328–332.

51. Deshpande SS, Monson LA, Cavaliere CM, et al. Distraction osteogenesis following low-dose hyperfractionated irradiation in the rat mandible. *J Oral Maxillofac Surg*. 2013;71:1465–1470.

52. Tchanque-Fossuo CN, Donneys A, Deshpande SS, et al. Radioprotection with amifostine enhances bone strength and regeneration and bony union in a rat model of mandibular distraction osteogenesis. *Ann Plast Surg*. 2018;80:176–180.

53. Felice PA, Ahsan S, Donneys A, et al. Deferoxamine administration delivers translational optimization of distraction osteogenesis in the irradiated mandible. *Plast Reconstr Surg (1963)*. 2013;132:542e–548e.

54. Momeni A, Rapp S, Donneys A, et al. Clinical use of deferoxamine in distraction osteogenesis of irradiated bone. *J Craniofac Surg*. 2016;27:880–882.

55. Deshpande SS, Gallagher KK, Donneys A, et al. Stem cell therapy remediates reconstruction of the craniofacial skeleton after radiation therapy. *Stem Cells Dev*. 2013;22:1625–1632.

56. Donneys A, et al. Abstract: implantable deferoxamine facilitates non-vascularized grafting in irradiated bone. *Plast Reconstr Surg*. 2017;5:195–196.

57. van Gemert JT, van Es RJ, Van Cann EM, et al. Nonvascularized bone grafts for segmental reconstruction of the mandible—a reappraisal. *J Oral Maxillofac Surg*. 2009;67:1446–1452.