Chapter 14
Heterotopic Ossification Following Traumatic Blast Injury

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Abbreviations

AHO	Albright hereditary osteodystrophy
ALK	Activin receptor-like kinase
ALP	Alkaline phosphatase
ATP	Adenosine triphosphate
BMP	Bone morphogenetic protein
BNB	Blood-nerve barrier
cAMP	Cyclic adenosine monophosphate
CGRP	Calcitonin gene-related peptide
CNS	Central nervous system
COX	Cyclooxygenase
CT	Computed tomography
FDA	Food and Drug Administration
FOP	Fibrodysplasia ossificans progressiva
HIF	Hypoxia-inducible transcription factor
HO	Heterotopic ossification
IED	Improvised explosive device
IGF	Insulin-like growth factor
IL	Interleukin
IP	Interferon gamma-induced protein
MCP	Monocyte chemoattractant protein
MIP	Macrophage inflammatory protein
MMP	Matrix metalloprotein
MRSA	*Methicillin-resistant Staphylococcus aureus*

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MSC	Mesenchymal stem cell
NSAID	Non-steroidal anti-inflammatory drug
POH	Progressive osseous heteroplasia
RAR	Retinoic acid receptor
SCI	Spinal cord injury
SP	Substance P
TBI	Traumatic brain injury
TGF	Transforming growth factor
TNF	Tumour necrosis factor
VEGF	Vascular endothelial growth factor

Blast Injury

Blast is the mechanism of injury that results following explosion. Blast injuries fall into four categories [1]:

1. Primary – the wave of blast overpressure passing through the body
2. Secondary – caused by debris hitting the body
3. Tertiary – caused by the body hitting an object
4. Quaternary – all other injuries, including crushing and burns

These types of injuries have likely existed since the first utilisation of explosives [2]. The original explosive, black powder, was invented in China in the ninth century for use in rockets, eventually guns and canon in the fourteenth century, and mining in the seventeenth century [3]. A mixture of naturally occurring compounds, black powder was only replaced with the advent of organic chemistry in the nineteenth century, and the production of the infamously unstable nitroglycerin. This was followed by compounds such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), the explosives of choice today [4].

Accidental blast injuries, for example, from industrial incidents, are rare in civilian life though they do happen [5]. Explosions caused by deliberate action are far more notorious: terrorist explosive events have increased with the turn of the century, with a fourfold rise in occurrence and an eightfold increase in injury between 1999 and 2006 [6]. However, blast injuries are most common in warfare, particularly in recent conflicts. Blast accounted for only 9% of injuries in the American Civil War (1861–1865), and 35% in the Great War (1914–1918) [7]. Figures then rose in the later twentieth-century wars, until blast became the dominant injury mechanism in the recent conflicts in Iraq and Afghanistan. Between 70 and 80% of injuries to British and American soldiers in these conflicts were as a result of blast, the highest in any recent conflict [8–10]. The majority of these injuries were caused by improvised explosive devices (IEDs), which gave rise to over 70% of combat casualties in Iraq and 50% in Afghanistan, the most significant threat to the soldiers in these regions [11, 12]. In addition, 43–54% of wounds occurred in the extremities, the most commonly injured area in these conflicts [8, 9]. This is in contrast to thoracic injury, which made up only 5% of wounds in these conflicts, reduced from 13% in the Second World War.

Heterotopic Ossification

Aetiology and Epidemiology

Heterotopic ossification (HO) is the formation of bone where it ought not to exist. Etymologically, the term is derived from the Greek *hetero topos* (other place) and the Latin *ossification* (bone making). Different types of bone have been reported in HO, and indeed different types of bone may form depending on aetiology. Analysis of trauma-related HO revealed that it is composed of a heterogeneous mix of cortical and cancellous bone, in addition to fibrocartilage, with varying levels of mineralisation [13]. Like skeletal bone, the structure of which is discussed in detail in Chap. 17, HO contains arterioles, Haversian canals, and bone marrow and is subject to continuous remodelling, even after 3 years following presumed 'maturation' of the bone. These features of HO separate it from the mere calcification of tissues; HO is structured and organised at the cellular level, with a microstructure like orthotopic bone (Fig. 14.1). Macroscopically, however, HO is very different to skeletal bone. It grows polyaxially and appears floral in form, intimately associated with the soft tissue. It has also been reported to grow faster than skeletal bone, at 1.7 μm per day compared to the 1.0 μm per day of normal bone [14].

HO is not a new phenomenon; it was first described by Albucasis, the father of surgery, over a millennium ago [15]. Patin, the Doyen of the Faculty of Medicine in Paris, then described the condition in children in 1692 [16]. The disorder he described is now commonly called fibrodysplasia ossificans progressiva (FOP), a rare genetic form of HO. FOP is characterised by malformation of the hallux at birth, but is followed by gradual HO in the soft tissues, which can be exacerbated by even the smallest of traumatic events [17]. The cumulative effects of this ossification lead to gradual immobility and, ultimately, early death. Other genetic causes of HO include progressive osseous heteroplasia (POH), the intramembranous

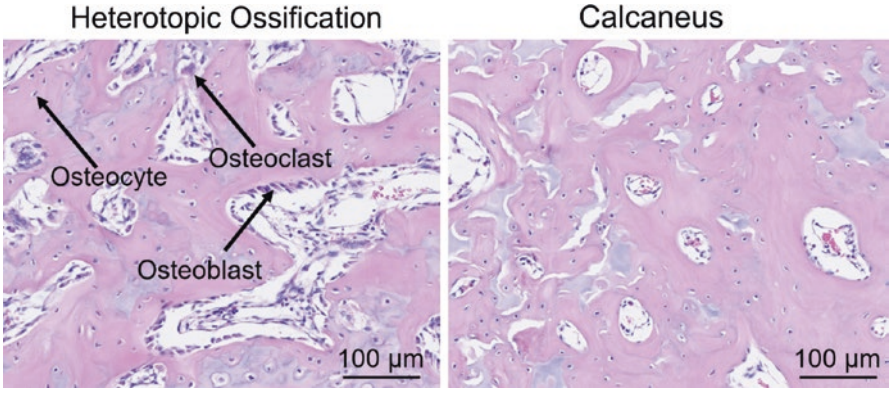

Fig. 14.1 Comparison of HO and skeletal bone (calcaneus) in a rodent, showing similar microstructure and osteocyte density but vastly increased numbers of osteoblasts and osteoclasts

ossification of dermal tissue, Albright hereditary osteodystrophy (AHO), and other similar conditions [18].

Thankfully, the genetic forms of HO are extremely rare; global incidence of FOP is one in two million [19]. However, the acquired form is far more common. HO can form following musculoskeletal trauma, including surgery, damage to the central nervous system (CNS), particularly traumatic brain injury (TBI) and spinal cord injury (SCI), and burns [20]. In addition, perhaps the most devastating cause of acquired HO is that following blast trauma. While it may be asymptomatic, HO can cause chronic pain, ulceration of the skin, particularly when the ectopic bone forms over a skin graft, ankylosis of the joints, arthrofibrosis, neurovascular entrapment, and issues with fitting and utilising prosthetic limbs [21].

The association between HO and combat is not new; one of the first descriptions of acquired HO was made following observations from the American Civil War and the Great War [22]. However, prevalence of HO in soldiers has recently increased due to two key reasons. The first, as discussed above is the rising use of IEDs making blast the predominant injury mechanism of injury, and the extremities the primary zone of wounding. The second is the increased survival rate, due to improved body armour, ubiquitous tourniquet use, improved air evacuation and care, haemostatic dressings, and other modern survival innovations [23–26]. Because of this, more people with multiple limb loss are surviving their injuries [27]. It is this combination of a higher survival rate but an increased incidence of severe extremity injury which has caused the recent upsurge in HO formation in wounded combatants [28].

The prevalence of HO in combat-related amputees has been consistently reported as around 63%. Risk factors include a blast mechanism, amputation through the zone of injury, presence and severity of TBI, an age less than 30, multiple extremity injuries, delayed wound healing, a high injury severity score, and bacterial colonisation [29–31]. In contrast, the rate of HO is only around 23% in civilian (non-blast-related) amputees, and the HO was mild in 94% of these cases [32]. This corroborates the finding that blast, and not just amputation, is a risk factor for HO. Non-blast cases may also be less likely to have TBI, and other risk factors for HO (Table 14.1).

Biology

Environment/Inflammation

With the exception of POH and AHO, which are formed by intramembranous ossification, HO, including FOP, is a process of endochondral bone formation. The formation of HO therefore requires three key things: osteoprogenitor cells, capable of differentiating into endochondral bone-forming cells, the signalling pathways that induce this differentiation, and a local environment which is conducive to bone formation [33]. The disease progression pathway of acquired HO begins with an inciting event, such as a blast injury, which causes an inflammatory response and the

Table 14.1 Clinical and biological risk factors for combat-related HO

Clinical	Biological
Blast mechanism of injury	Hypoxia
Extremity injuries (presence and number)	*Serum cytokines:*
High injury severity score	IL-3
Amputation (particularly through the zone of injury)	IL-6
Traumatic brain injury (presence and severity)	IL-10
Age (<30 years)	IL-12p70
Delayed wound healing	MCP1
Bacterial colonisation	*Wound effluent cytokines:*
	IL-3
	IL-13
	IP-10
	MIP-1α

cell-signalling cascade that induces cells to differentiate and begin forming bone [34]. These cells may be local to the injury site, or recruited from circulation.

Inflammation is a common requisite to all types of HO. Macrophages, mast cells, and adaptive immune cells are known to play a role; the exact inflammatory mechanism that leads to HO remains unknown [35]. Inflammation precedes mineralisation, and as such anti-inflammatory therapies may be effective in preventing HO, but do not affect HO formation once mineralisation has begun. The inflammatory response following severe trauma is highly complex, with local and systemic components, acute and chronic factors, and an associated anti-inflammatory response [36]. Tissue analysis of combat-injured patients showed that formation of HO was associated with high levels of interleukins 3, 6, 10, and 12p70, monocyte chemoattractant protein 1 (MCP1/CCL2) in the serum, and interleukins 3 and 13, interferon gamma-induced protein 10 (IP-10/CXCL10), and macrophage inflammatory protein 1 alpha (MIP-1α/CCL3) in the wound effluent, in addition to bacterial colonisation [37, 38]. A summary of both the clinical and biological risk factors for combat-related HO is given in Table 14.1.

Hypoxia is also a prerequisite condition for the formation of HO, as it is for normal bone, that stimulates hypoxia-inducible transcription factor 1 alpha (HIF1α) [39]. HIF1α has roles in cartilage proliferation and differentiation, as well as angio- and osteogenesis, all of which are critical in osteochondral bone formation [40]. HIF1α upregulates vascular endothelial growth factor (VEGF) and transcription factor SOX9, critical for angiogenesis and chondrogenesis, respectively. Both of these factors are upregulated in cells derived from patients with high-energy combat injuries who developed HO, along with a host of others including matrix metalloprotein 9 (MMP9) and insulin-like growth factor 2 (IGF2) [41]. It has also been suggested that the increased use of tourniquets, in addition to saving lives, may contribute to the increased incidence of HO by inducing hypoxia in the residual limb [42].

Cells

In addition to the necessary environment and the signals that induce osteochondral differentiation, there needs to be a population of cells both able and available to differentiate down this lineage. Perhaps the most promising candidate is the mesenchymal stem cell (MSC), a multipotent stromal cell which can differentiate into chondroblasts, osteoblasts, and brown adipocytes and form endochondral bone when implanted in vivo [43, 44]. MSCs were found in the debrided extremity muscle of combat-injured patients, and were found to have increased alkaline phosphatase (ALP) expression and mineralised matrix production compared to bone marrow-derived MSCs, and did not terminally differentiate [45, 46]. Additionally, MSCs were found to be fewer in number and less able to differentiate with increased age, correlating with the decrease in HO seen with age [47, 48]. When pretreated in hypoxic conditions, MSCs displayed an enhanced angiogenic capacity, increased VEGF production, and decreased apoptosis, showing that MSCs thrive in the hypoxic conditions seen in HO [49].

There are a number of other cell types that have the potential to produce HO. Skeletal muscle cells, myoblasts, have been shown to dedifferentiate and progress through an osteochondral route when exposed to transforming growth factor beta (TGFβ), an inflammatory cytokine [50]. In response to bone morphogenetic protein 2 (BMP2), muscle cells were found to produce ALP and participate in HO formation, producing similar amounts of bone to MSCs [51, 52]. In addition to mature muscle cells, muscle stem cells, termed satellite cells, can also differentiate into adipocytes and osteocytes given the proper molecular cues, such as BMP2 [53]. There is also some evidence of these cells undergoing osteogenic differentiation even without BMP2 [54]. However, other studies suggest that satellite cells are terminally differentiated and that these results are due to co-contamination of other cell types [55]. A FOP model showed that smooth muscle cells don't contribute to HO, and the contribution of skeletal muscle progenitors was <5% [56].

A recently proposed source of cells is from the endoneurium. These cells have been shown to express osteogenic factors and to travel through the general circulation to the site of HO in a mouse model [57]. Either direct trauma, for example, from blast, or BMP2 can initiate the neuroinflammatory cascade. This involves the release of pain mediators, substance P (SP) and calcitonin gene-related peptide (CGRP), which recruit mast cells that in turn degranulate to release chemokines and recruit cells that open the blood-nerve barrier (BNB) [58]. This opening, which may be controlled by histamines secreted by mast cells or MMP9, allows the perineurial and endoneurial cells to cross the BNB [59]. SP has been found to be upregulated in both traumatic HO and FOP lesions, and preventing the SP signalling pathway at any point has been shown to inhibit injury-induced HO [60].

Additional potential contributors include epithelial cells, endothelial cells, and pericytes. Epithelial cells can transform into MSCs, which are known to occur during embryonic gastrulation and triggered by BMP and TGFβ [61, 62].

Both of these factors were found to be overexpressed by the epithelial cells in the HO lesions of transgenic mice [63]. Endothelial cells are similarly able to transform into MSCs when exposed to TGFβ [64]. Chondrocytes and osteoblasts in FOP lesions were found to express endothelial markers, suggesting a vascular endothelial origin, and brown adipose tissue may share this origin [65, 66]. A different study suggested that cells of endothelial origin contributed 40–50% of cells in a FOP model [56]. However, the role of these cell types may be less direct. Some studies suggest that epithelial cells do not differentiate into osteoblasts, but instead secrete factors that induce osteochondral differentiation in other cells [67]. Endothelial cells release paracrine factors that induce chondrocyte hypertrophy, which are not secreted by myoblasts, fibroblasts, or other hypertrophic chondrocytes [68]. Further, the angiogenic growth factor Ang1 enhances BMP2 signalling, osteoblast differentiation, and ectopic bone formation [69]. Endothelial cells are further important in their own right, as angiogenesis is a key requirement for HO formation. Pericytes, cells which line the outside of capillaries, have been shown to display osteogenic differentiation in vitro and in vivo [70]. However, these cells have a similar phenotype, gene expression, and differentiation potential to MSCs, making these cells and their potential role difficult to distinguish [71].

Endochondral HO lesions contain several tissue types, in addition to the soft tissue(s) it is formed in, including bone, cartilage, brown fat, and vasculature. The formation of HO thus requires all of the cell types found in these tissues, precisely located in both spatially and temporally. For example, the hypoxic conditions required for chondrogenesis and neovascularisation must precede and be separate from the normoxia required for osteogenesis. It is clear that several cell types have the potential to differentiate into HO forming cells, given the proper cues; however, elucidating which cells play a part in blast-related HO is far more complicated. There may be more than one source for the cells found in HO, and the different cell types that make up HO may have the same of differing precursor cells. It is also important to consider the cells that do not give rise to HO tissue, but have an indirect role by secreting paracrine factors. The cell types required for HO, and potential progenitor cells, are summarised in Table 14.2. Overall, it is clear that HO is a complex biological process, and it is likely that there are several pathways that can lead to it.

Table 14.2 Summary of cell types found in HO tissue and potential progenitor cells

Cell types found in HO	Potential progenitor cells
Osteoblasts	Mesenchymal stem cells
Osteoclasts	Myoblasts
Osteocytes	Satellite cells
Chondroblasts	Perineurial cells
Chondrocytes	Endoneurial cells
Brown adipocytes	Epithelial cells
Endothelial cells	Endothelial cells
	Pericytes

Diagnosis, Prevention, and Treatment

Diagnosis

The initial stages of HO diagnosis are rooted in clinical examination, which can provide some important information. Swelling, stiffness, warmth, and redness are all early signs of HO; however these symptoms are not specific to the condition, and may also indicate thrombophlebitis, cellulitis, myelitis, or a tumour [72]. Once HO is suspected, plain X-ray radiographs are the most common modality to image and monitor lesions. X-ray computed tomography (CT) is more expensive and time-consuming, but provides a more detailed 3D picture, which is a valuable perioperative tool. However, both X-ray modalities can only detect mineralised tissue and thus can only detect HO once mineralisation has begun [73]. Bone scintigraphy is a currently used technique for diagnosis, which can detect HO within 3 weeks of injury, several weeks earlier than radiographs. This technique can also be used to detect lesion maturity in order to correctly time excision surgery and to detect recurrence [74]. Elevation of serum ALP has been suggested as a marker of HO; however, ALP levels are dependent on hepatic and renal function, which may differ in blast-injured patients [75]. In HO caused by SCI, it was found that less than half of patients displayed elevated ALP levels [76]. It has also been proposed that the serum cytokines upregulated in HO may be used for early detection. However, these cytokines may differ by patient and wound type, and may be upregulated by the severe injury experienced by blast-injured patients, rather than specifically indicating HO [20].

New techniques are thus required in order to detect HO earlier, in order to begin a prophylactic regime as soon as possible. Given the issues with detecting serum markers of HO, focus is instead placed on imaging modalities. In Achilles tenotomy plus burn models, ultrasound, near-infrared, and Raman modalities were able to detect HO within a week of injury, which was only visible in microCT after several weeks [77–79]. Ultrasound was found to detect HO in 88.9% of afflicted SCI patients; however, at 62 days the mean interval was similar to confirmation of the condition by CT at a mean interval of 64 days [80]. Near-infrared imaging, though useful, requires the injection of a fluorescent tracer that may make it less attractive than Raman, which does not. In an ex vivo study of tissue from combat-wounded patients, Raman spectroscopy was able to differentiate between uninjured and injured muscle, unmineralised and mineralised HO lesions [81]. This technique can thus measure mineral maturity to aid surgical timing, but also may be used during the operation to identify lesion boundaries.

Current Preventions

Given the historical lack of mechanistic insight into HO formation, current preventions are based around non-specific anti-inflammatories. The exceptions to this are bisphosphonates, molecules with a P-C-P bridge that imitate the role of pyrophosphate in vivo but are not broken down by ALP. This is the only FDA-approved

medication to prevent or treat HO [31]. Bisphosphonates prevent the formation and aggregation of calcium phosphate crystals, and act as crystal poisons after adsorbing to the surfaces, in addition to interfering with biochemical processes when internalised by osteoclasts [82]. However, their efficacy in preventing HO is inconsistently reported. Etidronate, a first-generation bisphosphonate, was reported to lower the rate of HO following TBI and SCI [20]. However, etidronate was also shown to increase incidence of HO in burns patients [83]. A more consistent report, however, is that bisphosphonates only delay mineralisation, which recommences when treatment is stopped [84]. Another consideration is that bisphosphonates may delay fracture union, a common complication following blast injury [85]. However, this only appears to be following prolonged use of the drug, e.g. for osteoporosis, and rarely affects fracture healing when used for the first time following injury [86]. One interesting finding is that nitrogen-containing bisphosphonates hastened HO maturity, leading to prompter surgical excision [87]. However, due to the lack of clear evidence for efficacy, bisphosphonates are rarely administered for prevention of HO.

Commonly utilised prophylaxes for HO include non-steroidal anti-inflammatory drugs (NSAIDs) and radiotherapy. Both of these modalities are most commonly studied, and frequently administered clinically, for the prevention of HO in the hip. NSAIDs inhibit cyclooxygenase-2 (COX2), a key factor required for endochondral ossification, regulating the differentiation of MSCs and preventing angiogenesis [88]. Additionally, NSAIDs have been shown to suppress proliferation and induce apoptosis in osteoblasts and chondrocytes [89]. The efficacy of NSAIDs in preventing HO in the hip is generally taken to be good, though some studies dispute this [90, 91]. However the side effects of NSAIDs, including postoperative bleeding, hepatic and renal toxicity and failure, haematochezia, asthma, gastrointestinal bleeding, and other effects, often lead to discontinuation even in relatively healthy patients [91–94]. Blast-injured patients typically display severe systemic polytrauma, complex contaminated wounds, skeletal fractures, TBI, renal impairment, gastritis, and bleeding, which make the side effects of NSAIDs intolerable [95]. Primary prophylaxis against HO is therefore utilised rarely in combat-related amputees. However, there are attempts to curb these side effects. Local delivery of indomethacin, the most commonly prescribed NSAID for HO prophylaxis, was shown not to inhibit wound healing [96]. Local delivery allows a high concentration at the site, but a low systemic drug concentration, reducing side effects. The majority of NSAIDs utilised, including indomethacin, are non-selective, in that they inhibit both COX1 and COX2. Celecoxib is a selective COX2 inhibitor, which displayed equal efficacy to indomethacin but with fewer gastrointestinal side effects [97]. However, there are concerns about the effect of selective COX2 inhibitors on the cardiovascular system. Despite this, a small clinical trial of celecoxib in blast-injured patients showed a decrease in HO formation [21].

Radiotherapy is the other primary prophylaxis for HO, inhibiting proliferation and inducing terminal differentiation of MSCs [98]. In the hip, pre- and postoperative radiotherapy are equally as effective, though the total dose is usually higher when given in several fractions postoperatively compared to the single preoperative dose [99–101]. Preoperative radiotherapy is usually preferred as it reduces patient

burden following the procedure. However, radiotherapy in the elbow has been shown to have no effect on HO formation, but significantly increased non-union [102]. Additional side effects of radiotherapy include compromised soft tissue healing and detrimental effects on immunological functions [103]. There are also logistical limitations to the use of radiotherapy. Though timing guidelines are inconsistent, it is generally accepted that radiotherapy must be administered within 48–72 hours of injury. This may be unfeasible for blast-injured patients, particularly in combat where radiotherapy is not available in far-forward medical facilities [31]. Another concern of radiotherapy is carcinogenesis. There is thus far no evidence of carcinogenesis following radiotherapy in the hip [104]. However, given the discrepancy in the average age between combat-wounded patients and those who undergo procedures in the hip, and the potentially decades-long latency period following radiotherapy, this is a risk which must be considered in younger patients [105].

Comparisons between radiotherapy and administration of NSAIDs for HO prophylaxis in the hip reveal near-equal efficacy, with a slight leaning towards radiotherapy because of dose-dependent efficacy, fewer side effects, and greater patient compliance [106–108]. However, neither are suitable for the majority of blast-injured patients. In addition, neither modality showed prophylactic efficacy in a rodent blast model of HO [109, 110]. There is a clear need for new prophylaxes for HO with greater efficacy and fewer side effects which, combined with improved early diagnosis, can successfully be implemented in combat-injured patients.

Current Treatment

Thankfully, HO is often asymptomatic, even with large lesions, or only transiently symptomatic after prolonged activity or mechanical irritation, which may subside with maturation of the HO and the associated inflammation. The first line of treatment is always conservative and includes rest, physical therapy, stretching, dynamic splinting, injections, nerve ablations, pain medication, and prosthetic sock adjustment and padding [31, 73]. However, if symptoms persist, excision surgery is the only current treatment for HO; this is required for 41% of transfemoral and 15% of transtibial combat-related amputees [111]. Complete marginal excision of the ectopic bone lesions is recommended, and surgery should take place at least 180 days post-injury to allow the HO to mature, to reduce the risk of recurrence and re-excision [112]. In addition to HO excision, amputation revision, quadricepsplasty, contracture release, and excision of neuroma or skin graft are often required [95]. Excision surgery is technically demanding, with risk of haemorrhage, infection, wound complication, and neurovascular damage [112]. This surgery can be made more difficult by the HO changing the native anatomy and incarcerating important nerves and blood vessels [73]. Because of this preoperative planning is crucial, and CT is often utilised for both planning before and reference during surgery [95]. NSAIDs are routinely used as secondary prophylaxis to prevent recurrence; radiotherapy is only used in high-risk cases, because of concerns about impairment of wound healing [31].

Novel Therapies

Given the lack of safe and effective prophylaxes against HO for combat-injured patients, it is clear that new therapies are needed to prevent patients having to go through surgical excision. As the biological mechanisms behind HO are being elucidated, new druggable targets are emerging. Perhaps the most exciting potential new prophylaxis is through retinoic acid receptor γ (RARγ) agonism. Retinoid signalling is a strong inhibitor of chondrogenesis, and thus endochondral bone formation. RARγ agonists were shown to prevent chondrogenic differentiation in vitro and prevent HO in traumatic animal models [113]. The treatment was shown to inhibit BMP2 signalling and to stop cells from differentiating into chondroblasts even when subsequently exposed to BMP2 or implanted into otherwise osteogenic environments in vivo. However, a delay in fracture repair was seen, a clear contraindication for blast-injured patients, though the investigators suggest a window of opportunity for treatment after stabilisation but prior to healing [113].

Palovarotene, a RARγ agonist, was examined further; while not the most potent of the molecules studied, palovarotene was already in clinical trials for emphysema [114, 115]. In a FOP model, palovarotene was shown to prevent HO and restore long bone growth, and in a complex combat blast injury model, it also significantly reduced HO but may delay wound healing especially in the presence of bacteria [116, 117]. However a further study, while confirming that palovarotene prevents chondrogenic differentiation and reduces HO, showed deleterious effects on the skeleton including overgrowth of synovial joints and long bone growth plate ablation [118]. Regardless, palovarotene was taken to clinical trial for FOP. A 28% reduction in HO was seen in phase two; 65% was the benchmark, though there was some dispute as the drug was only administered for flare-ups above a certain threshold [119]. Despite this, a phase three trial is ongoing, which is scheduled to end in 2020 [120].

Another potential strategy is inhibition of activin receptor-like kinase-2 (ALK2), a BMP receptor. Activated receptors phosphorylate the SMAD 1, 5, and 8 pathways that lead to bone formation; constitutive ALK2 activation is the genetic defect that leads to FOP. Thus, by inhibiting ALK2 with LDN-193189, a study has shown inhibition of HO formation in a FOP model [121]. The same ALK2 inhibitor also inhibited HO in an Achilles tenotomy plus burn model of HO [122]. Interestingly this study also showed that, by applying apyrase to the burn site, remote hydrolysis of ATP also inhibited HO, by decreasing extracellular ATP and increasing intracellular cyclic adenosine monophosphate (cAMP), an inhibitor of SMAD 1, 5, and 8 phosphorylation.

As discussed above HIF1α plays a crucial role in osteochondral bone formation, by upregulating VEGF and SOX9, which are critical for angiogenesis and chondrogenesis, respectively. It has also been shown to increase the intensity and duration of BMP signalling and that inhibiting it restores normal BMP2 signalling and reduced HO formation in a FOP model [123]. In addition to a genetic model, treatment with PX-478 or rapamycin was shown to inhibit HIF1α

and prevent HO in a trauma model [124]. Another HIF1α inhibitor is the antibiotic echinomycin, which was shown to prevent HO in an Achilles tenotomy model [125]. Other antibiotics have also been shown to inhibit HO; vancomycin was shown to prevent HO in a complex blast with infection model [126]. Though presumed that this was due to antimicrobial action, vancomycin also inhibited HO even in the absence of MRSA infection. The authors postulate that this is due to upregulation of tissue necrosis factor alpha (TNFα), IL-6, and IL-10 and thus that vancomycin alters the immune response pathway to reduce HO.

Several other potential prophylaxes are also under investigation. Macrophages contribute to the inflammatory process and release factors, including BMP, which support differentiation and maturation of osteoblasts. By utilising clodronate to deplete macrophages, HO has been shown to be reduced in genetic and spinal cord injury plus cardiotoxin injection models [127, 128]. Cells transduced to produce Noggin, a BMP antagonist, decreased HO in Achilles tenotomy and demineralised bone matrix implantation models [129]. Pulsed electromagnetic fields, by increasing blood flow and preventing hypoxia, have been shown to reduce HO in hip and SCI patients [130, 131].

Outlook

Despite being described for over a millennium, HO is still a significant problem today. The increasing frequency of terrorist incidents and the growing prevalence of high-energy extremity injuries in combat mean that blast-related HO is likely to continue to be an issue in the future. Inconsistent efficacy and side effects that are intolerable in a blast-injured population mean that current prophylaxes for HO are unsuitable, leaving excision surgery as the only option for many. Promisingly, the biological processes behind blast-related HO are gradually being elucidated, revealing the critical biological pathways and new druggable targets. Many of these new therapies have shown great success in various animal models of HO. Nevertheless, the translation of new prophylaxes into the clinic is thus far lacking. This is, in part, due to the currently diminished combat leading to low numbers of new blast-related HO patients. However, there is still work to be done in order to illuminate the entire biological network behind blast-related HO. In addition, few studies utilise blast-injury models for HO, and it may be that new models are required in which to test potential therapeutics. A multidisciplinary approach is thus called for, in order to fully uncover the pathways behind the condition, design therapeutics to target these pathways, develop delivery systems and models to test these therapies, and finally to translate these therapies through trials and into the clinic. This work is ongoing, in the hope that when a major conflict next occurs, there will be a therapy waiting so that blast-injured patients do not have to suffer HO.

References

1. Jorolemon MR, Krywko DM. Blast injuries: StatPearls Publishing; Treasure Island, Florida, USA; 2019.
2. Clemedson C-J. Blast Injury. Physiol Rev. 1956;36:336–54.
3. Meyers S, Shanley ES. Industrial explosives – a brief history of their development and use. J Hazard Mater. 1990;23:183–201.
4. Chatterjee S, Deb U, Datta S, Walther C, Gupta DK. Common explosives (TNT, RDX, HMX) and their fate in the environment: emphasizing bioremediation. Chemosphere. 2017;184:438–51.
5. Kulla M, Maier J, Bieler D, Lefering R, Hentsch S, Lampl L, Helm M. Zivile Explosionstraumata – ein unterschätztes Problem? Unfallchirurg. 2016;119:843–53.
6. Wolf SJ, Bebarta VS, Bonnett CJ, Pons PT, Cantrill SV. Blast injuries. Lancet. 2009;374:405–15.
7. Hoencamp R, Vermetten E, Tan ECTH, Putter H, Leenen LPH, Hamming JF. Systematic review of the prevalence and characteristics of battle casualties from NATO coalition forces in Iraq and Afghanistan. Injury. 2014;45:1028–34.
8. Penn-Barwell JG, Roberts SAG, Midwinter MJ, Bishop JRB. Improved survival in UK combat casualties from Iraq and Afghanistan: 2003-2012. J Trauma Acute Care Surg. 2015;78:1014–20.
9. Owens BD, Kragh JF, Wenke JC, Macaitis J, Wade CE, Holcomb JB. Combat wounds in operation Iraqi freedom and operation enduring freedom. J Trauma Inj Infect Crit Care. 2008;64:295–9.
10. Schoenfeld AJ, Belmont PJ. Traumatic Combat Injuries. In: Musculoskelet. New York: Inj. Mil. Springer New York; 2016. p. 11–23.
11. Wilson C . Improvised explosive devices (IEDs) in Iraq and Afganistan: effects and countermeasures. CRS Rep Congr. 2007; 1–6.
12. Ramasamy A, Hill AM, Clasper JC. Improvised explosive devices: pathophysiology, injury profiles and current medical management. J R Army Med Corps. 2009;155:265–72.
13. Isaacson BM, Brown AA, Brunker LB, Higgins TF, Bloebaum RD. Clarifying the structure and bone mineral content of heterotopic ossification. J Surg Res. 2011;167:e163–70.
14. Isaacson BM, Potter BK, Bloebaum RD, Epperson RT, Kawaguchi BS, Swanson TM, Pasquina PF. Link between clinical predictors of heterotopic ossification and histological analysis in combat-injured service members. J Bone Jt Surg. 2016;98:647–57.
15. Al-Zahrāwī A Abū al-Qāsim Khalaf ibn, Spink M, Lewis G. Albucasis on surgery and instruments. A definitive edition of the Arabic text with English translation and commentary by M. S. Spink and G. L. Lewis. 1973.
16. Zaman SR. Heterotopic ossification of the elbows in a major petrol burn. Case Rep. 2012;2012:bcr0320126027.
17. Kaplan FS, Glaser DL, Pignolo RJ, Goldsby RE, Kitterman JA, Groppe J, Shore EM. Fibrodysplasia ossificans progressiva. Best Pract Res Clin Rheumatol. 2008;22:191–205.
18. Kaplan FS, Shore EM. Progressive osseous heteroplasia. J Bone Miner Res. 2000;15:2084–94.
19. Pignolo RJ, Shore EM, Kaplan FS. Fibrodysplasia ossificans progressiva: clinical and genetic aspects. Orphanet J Rare Dis. 2011;6:80.
20. Brady RD, Shultz SR, McDonald SJ, O'Brien TJ. Neurological heterotopic ossification: current understanding and future directions. Bone. 2018;109:35–42.
21. Hoyt BW, Pavey GJ, Potter BK, Forsberg JA. Heterotopic ossification and lessons learned from fifteen years at war: a review of therapy, novel research, and future directions for military and civilian orthopaedic trauma. Bone. 2018;109:3–11.
22. Dejerne A, Ceillier A. Para-osteo-arthropathies des paraplegiques par lesion medullaire; etude clinique et radiographique. Ann Med. 1918;5:497.
23. Eskridge SL, Macera CA, Galarneau MR, Holbrook TL, Woodruff SI, MacGregor AJ, Morton DJ, Shaffer RA. Injuries from combat explosions in Iraq: injury type, location, and severity. Injury. 2012;43:1678–82.

24. Kragh JF, Littrel ML, Jones JA, Walters TJ, Baer DG, Wade CE, Holcomb JB. Battle casualty survival with emergency tourniquet use to stop limb bleeding. J Emerg Med. 2011;41:590–7.
25. Mabry RL, Apodaca A, Penrod J, Orman JA, Gerhardt RT, Dorlac WC. Impact of critical care-trained flight paramedics on casualty survival during helicopter evacuation in the current war in Afghanistan. J Trauma Acute Care Surg. 2012;73:S32–7.
26. Bennett BL, Littlejohn L. Review of new topical hemostatic dressings for combat casualty care. Mil Med. 2014;179:497–514.
27. Dougherty PJ, McFarland LV, Smith DG, Esquenazi A, Blake DJ, Reiber GE. Multiple traumatic limb loss: a comparison of Vietnam veterans to OIF/OEF service members. J Rehabil Res Dev. 2010;47:333.
28. Daniels CM, Pavey GJ, Arthur J, Noller M, Forsberg JA, Potter BK. Has the proportion of combat-related amputations that develop heterotopic ossification increased? J Orthop Trauma. 2018;32:283–7.
29. Potter BK, Burns TC, Lacap AP, Granville RR, Gajewski DA. Heterotopic ossification following traumatic and combat-related amputations: prevalence, risk factors, and preliminary results of excision. J Bone Jt Surg Ser A. 2007;89:476–86.
30. Forsberg JA, Pepek JM, Wagner S, Wilson K, Flint J, Andersen RC, Tadaki D, Gage FA, Stojadinovic A, Elster EA. Heterotopic ossification in high-energy wartime extremity injuries: prevalence and risk factors. J Bone Jt Surg. 2009;91:1084–91.
31. Alfieri KA, Forsberg JA, Potter BK. Blast injuries and heterotopic ossification. Bone Joint Res. 2012;1:192–7.
32. Matsumoto ME, Khan M, Jayabalan P, Ziebarth J, Munin MC. Heterotopic ossification in civilians with lower limb amputations. Arch Phys Med Rehabil. 2014;95:1710–3.
33. Kaplan FS, Glaser DL, Hebela N, Shore EM. Heterotopic ossification. J Am Acad Orthop Surg. 2004;12:116–25.
34. Davies OG, Grover LM, Eisenstein N, Lewis MP, Liu Y. Identifying the cellular mechanisms leading to heterotopic ossification. Calcif Tissue Int. 2015;97:432–44.
35. Matsuo K, Chavez RD, Barruet E, Hsiao EC. Inflammation in fibrodysplasia ossificans progressiva and other forms of heterotopic ossification. Curr Osteoporos Rep. 2019. https://doi.org/10.1007/s11914-019-00541-x.
36. Vanzant EL, Lopez CM, Ozrazgat-Baslanti T, et al. Persistent inflammation, immunosuppression, and catabolism syndrome after severe blunt trauma. J Trauma Acute Care Surg. 2014;76:21–30.
37. Evans KN, Forsberg JA, Potter BK, Hawksworth JS, Brown TS, Andersen R, Dunne JR, Tadaki D, Elster EA. Inflammatory cytokine and chemokine expression is associated with heterotopic ossification in high-energy penetrating war injuries. J Orthop Trauma. 2012;26:e204–13.
38. Forsberg JA, Potter BK, Polfer EM, Safford SD, Elster EA. Do inflammatory markers portend heterotopic ossification and wound failure in combat wounds? Clin Orthop Relat Res. 2014;472:2845–54.
39. Merceron C, Ranganathan K, Wang E, et al. Hypoxia-inducible factor 2α is a negative regulator of osteoblastogenesis and bone mass accrual. Bone Res. 2019;7:7.
40. Araldi E, Schipani E. Hypoxia, HIFs and bone development. Bone. 2010;47:190–6.
41. Davis TA, O'Brien FP, Anam K, Grijalva S, Potter BK, Elster EA. Heterotopic ossification in complex orthopaedic combat wounds: quantification and characterization of osteogenic precursor cell activity in traumatized muscle. J Bone Jt Surg Ser A. 2011;93:1122–31.
42. Isaacson B, Swanson T, Potter K, Pasquina P. Tourniquet use in combat-injured service members: a link with heterotopic ossification? Orthop Res Rev. 2014;6:27.
43. Downey J, Lauzier D, Kloen P, Klarskov K, Richter M, Hamdy R, Faucheux N, Scimè A, Balg F, Grenier G. Prospective heterotopic ossification progenitors in adult human skeletal muscle. Bone. 2015;71:164–70.
44. Scotti C, Tonnarelli B, Papadimitropoulos A, Scherberich A, Schaeren S, Schauerte A, Lopez-Rios J, Zeller R, Barbero A, Martin I. Recapitulation of endochondral bone formation

using human adult mesenchymal stem cells as a paradigm for developmental engineering. Proc Natl Acad Sci U S A. 2010;107:7251–6.
45. Nesti LJ, Jackson WM, Shanti RM, Koehler SM, Aragon AB, Bailey JR, Sracic MK, Freedman BA, Giuliani JR, Tuan RS. Differentiation potential of multipotent progenitor cells derived from war-traumatized muscle tissue. J Bone Jt Surg. 2008;90:2390–8.
46. Jackson WM, Aragon AB, Bulken-Hoover JD, Nesti LJ, Tuan RS. Putative heterotopic ossification progenitor cells derived from traumatized muscle. J Orthop Res. 2009;27: 1645–51.
47. Asumda FZ, Chase PB. Age-related changes in rat bone-marrow mesenchymal stem cell plasticity. BMC cell biology. 2011;12:44. https://doi.org/10.1186/1471-2121-12-44.
48. Efimenko A, Dzhoyashvili N, Kalinina N, Kochegura T, Akchurin R, Tkachuk V, Parfyonova Y. Adipose-derived mesenchymal stromal cells from aged patients with coronary artery disease keep mesenchymal stromal cell properties but exhibit characteristics of aging and have impaired angiogenic potential. Stem Cells Transl Med. 2014;3:32–41.
49. Liu L, Gao J, Yuan Y, Chang Q, Liao Y, Lu F. Hypoxia preconditioned human adipose derived mesenchymal stem cells enhance angiogenic potential via secretion of increased VEGF and bFGF. Cell Biol Int. 2013;37:551–60.
50. Mu X, Li Y. Conditional TGF-β1 treatment increases stem cell-like cell population in myoblasts. J Cell Mol Med. 2011;15:679–90.
51. Bosch P, Musgrave DS, Lee JY, Cummins J, Shuler F, Ghivizzani SC, Evans C, Robbins PD, Huard J. Osteoprogenitor cells within skeletal muscle. J Orthop Res. 2000;18:933–44.
52. Gao X, Usas A, Tang Y, et al. A comparison of bone regeneration with human mesenchymal stem cells and muscle-derived stem cells and the critical role of BMP. Biomaterials. 2014;35:6859–70.
53. Asakura A, Rudnicki MA, Komaki M. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation. 2001;68:245–53.
54. Hashimoto N, Kiyono T, Wada MR, Umeda R, Goto Y, Nonaka I, Shimizu S, Yasumoto S, Inagawa-Ogashiwa M. Osteogenic properties of human myogenic progenitor cells. Mech Dev. 2008;125:257–69.
55. Starkey JD, Yamamoto M, Yamamoto S, Goldhamer DJ. Skeletal muscle satellite cells are committed to myogenesis and do not spontaneously adopt nonmyogenic fates. J Histochem Cytochem. 2011;59:33–46.
56. Lounev VY, Ramachandran R, Wosczyna MN, Yamamoto M, Maidment ADA, Shore EM, Glaser DL, Goldhamer DJ, Kaplan FS. Identification of progenitor cells that contribute to heterotopic skeletogenesis. J Bone Joint Surg Am. 2009;91:652–63.
57. Lazard ZW, Olmsted-Davis EA, Salisbury EA, Gugala Z, Sonnet C, Davis EL, Beal E, Ubogu EE, Davis AR. Osteoblasts have a neural origin in heterotopic ossification. Clin Orthop Relat Res. 2015;473:2790–806.
58. Davis EL, Davis AR, Gugala Z, Olmsted-Davis EA. Is heterotopic ossification getting nervous?: the role of the peripheral nervous system in heterotopic ossification. Bone. 2018;109:22–7.
59. Gugala Z, Olmsted-Davis EA, Xiong Y, Davis EL, Davis AR. Trauma-induced heterotopic ossification regulates the blood-nerve barrier. Front Neurol. 2018;9:408.
60. Kan L, Lounev VY, Pignolo RJ, et al. Substance P signaling mediates BMP-dependent heterotopic ossification. J Cell Biochem. 2011;112:2759–72.
61. Hay ED. An overview of epithelio-mesenchymal transformation. Acta Anat (Basel). 1995;154:8–20.
62. Xu J, Lamouille S, Derynck R. TGF-β-induced epithelial to mesenchymal transition. Cell Res. 2009;19:156–72.
63. Maroulakou IG, Shibata M-A, Anver M, et al. Heterotopic endochondrial ossification with mixed tumor formation in C3(1)/Tag transgenic mice is associated with elevated TGF-beta1 and BMP-2 expression. Oncogene. 1999;18:5435–47.

64. van Meeteren LA, ten Dijke P. Regulation of endothelial cell plasticity by TGF-β. Cell Tissue Res. 2012;347:177–86.
65. Medici D, Shore EM, Lounev VY, Kaplan FS, Kalluri R, Olsen BR. Conversion of vascular endothelial cells into multipotent stem-like cells. Nat Med. 2010;16:1400–6.
66. Tran K-V, Gealekman O, Frontini A, et al. The vascular endothelium of the adipose tissue gives rise to both white and brown fat cells. Cell Metab. 2012;15:222–9.
67. Rutherford RB, Racenis P, Fatherazi S, Izutsu K. Bone formation by BMP-7-transduced human gingival keratinocytes. J Dent Res. 2003;82:293–7.
68. Bittner K, Vischer P, Bartholmes P, Bruckner P. Role of the subchondral vascular system in endochondral ossification: endothelial cells specifically derepress late differentiation in resting chondrocytesin vitro. Exp Cell Res. 1998;238:491–7.
69. Jeong B-C, Kim H-J, Bae I-H, et al. COMP-Ang1, a chimeric form of Angiopoietin 1, enhances BMP2-induced osteoblast differentiation and bone formation. Bone. 2010;46:479–86.
70. Doherty MJ, Ashton BA, Walsh S, Beresford JN, Grant ME, Canfield AE. Vascular pericytes express osteogenic potential in vitro and in vivo. J Bone Miner Res. 1998;13:828–38.
71. Crisan M, Corselli M, Chen C-W, Péault B. Multilineage stem cells in the adult: a perivascular legacy? Organogenesis. 2011;7:101–4.
72. Vanden Bossche L, Vanderstraeten G. Heterotopic ossification: a review. J Rehabil. 2005;37:129–36.
73. Richards JT, Overmann A, Forsberg JA, Potter BK. Complications of combat blast injuries and wounds. Curr Trauma Reports. 2018;4:348–58.
74. Shehab D, Elgazzar AH, Collier BD. Heterotopic ossification. J Nucl Med. 2002;43:346–53.
75. Hsu J, Keenan M. Current review of heterotopic ossification. Univ Pennsylvania Orthop J. 2010;20:126–30.
76. Citak M, Grasmücke D, Suero EM, Cruciger O, Meindl R, Schildhauer TA, Aach M. The roles of serum alkaline and bone alkaline phosphatase levels in predicting heterotopic ossification following spinal cord injury. Spinal Cord. 2016;54:368–70.
77. Ranganathan K, Hong X, Cholok D, et al. High-frequency spectral ultrasound imaging (SUSI) visualizes early post-traumatic heterotopic ossification (HO) in a mouse model. Bone. 2018;109:49–55.
78. Perosky JE, Peterson JR, Eboda ON, Morris MD, Wang SC, Levi B, Kozloff KM. Early detection of heterotopic ossification using near-infrared optical imaging reveals dynamic turnover and progression of mineralization following Achilles tenotomy and burn injury. J Orthop Res. 2014;32:1416–23.
79. Peterson JR, Okagbare PI, De La Rosa S, et al. Early detection of burn induced heterotopic ossification using transcutaneous Raman spectroscopy. Bone. 2013;54:28–34.
80. Rosteius T, Suero EM, Grasmücke D, Aach M, Gisevius A, Ohlmeier M, Meindl R, Schildhauer TA, Citak M. The sensitivity of ultrasound screening examination in detecting heterotopic ossification following spinal cord injury. Spinal Cord. 2017;55:71–3.
81. Crane NJ, Polfer E, Elster EA, Potter BK, Forsberg JA. Raman spectroscopic analysis of combat-related heterotopic ossification development. Bone. 2013;57:335–42.
82. Russell RGG. Bisphosphonates: the first 40 years. Bone. 2011;49:2–19.
83. Shafer DM, Bay C, Caruso DM, Foster KN. The use of etidronate disodium in the prevention of heterotopic ossification in burn patients. Burns. 2008;34:355–60.
84. Haran MJ, Bhuta T, Lee BSB. Pharmacological interventions for treating acute heterotopic ossification. Cochrane Database Syst Rev. 2004; https://doi.org/10.1002/14651858.cd003321.pub4.
85. Yue B, Ng A, Tang H, Joseph S, Richardson M. Delayed healing of lower limb fractures with bisphosphonate therapy. Ann R Coll Surg Engl. 2015;97:333–8.
86. Kates SL, Ackert-Bicknell CL. How do bisphosphonates affect fracture healing? Injury. 2016;47:S65–8.
87. Sinha S, Biernaskie JA, Nickerson D, Gabriel VA. Nitrogen-containing bisphosphonates for burn-related heterotopic ossification. Burn Open. 2018;2:160–3.

88. Liu H, Zhao J-G, Li Y, Xia J, Zhao S. Non-steroidal anti-inflammatory drugs for preventing heterotopic bone formation after hip arthroplasty. Cochrane Database Syst Rev. 2017; https://doi.org/10.1002/14651858.CD012861.
89. Chang J-K, Li C-J, Liao H-J, Wang C-K, Wang G-J, Ho M-L. Anti-inflammatory drugs suppress proliferation and induce apoptosis through altering expressions of cell cycle regulators and pro-apoptotic factors in cultured human osteoblasts. Toxicology. 2009;258:148–56.
90. Kurz AZ, LeRoux E, Riediger M, Coughlin R, Simunovic N, Duong A, Laskovski JR, Ayeni OR. Heterotopic ossification in hip arthroscopy: an updated review. Curr Rev Musculoskelet Med. 2019;12:147–55.
91. Karunakar MA, Sen A, Bosse MJ, Sims SH, Goulet JA, Kellam JF. Indometacin as prophylaxis for heterotopic ossification after the operative treatment of fractures of the acetabulum. J Bone Joint Surg Br. 2006;88–B:1613–7.
92. Beckmann JT, Wylie JD, Kapron AL, Hanson JA, Maak TG, Aoki SK. The effect of NSAID prophylaxis and operative variables on heterotopic ossification after hip arthroscopy. Am J Sports Med. 2014;42:1359–64.
93. Kan S-L, Yang B, Ning G-Z, Chen L-X, Li Y-L, Gao S-J, Chen X-Y, Sun J-C, Feng S-Q. Nonsteroidal anti-inflammatory drugs as prophylaxis for heterotopic ossification after total hip arthroplasty: a systematic review and meta-analysis. Medicine (Baltimore). 2015;94:e828.
94. Bozimowski G. A review of nonsteroidal anti-inflammatory drugs. AANA J. 2015;83:425–33.
95. Potter BK, Forsberg JA, Davis TA, et al. Heterotopic ossification following combat-related trauma. J Bone Jt Surg Ser A. 2010;92:74–89.
96. Rivera JC, Hsu JR, Noel SP, Wenke JC, Rathbone CR. Locally delivered nonsteroidal antiinflammatory drug: a potential option for heterotopic ossification prevention. Clin Transl Sci. 2015;8:591–3.
97. Macfarlane RJ, Han Ng B, Gamie Z, El Masry MA, Velonis S, Schizas C, Tsiridis E. Pharmacological treatment of heterotopic ossification following hip and acetabular surgery. Expert Opin Pharmacother. 2008;9:767–86.
98. Trott KR, Kamprad F. Radiobiological mechanisms of anti-inflammatory radiotherapy. Radiother Oncol. 1999;51:197–203.
99. Gregoritch SJ, Chadha M, Pelligrini VD, Rubin P, Kantorowitz DA. Randomized trial comparing preoperative versus postoperative irradiation for prevention of heterotopic ossification following prosthetic total hip replacement: preliminary results. Int J Radiat Oncol Biol Phys. 1994;30:55–62.
100. Seegenschmiedt MH, Keilholz L, Martus P, Goldmann A, Wölfel R, Henning F, Sauer R. Prevention of heterotopic ossification about the hip: final results of two randomized trials in 410 patients using either preoperative or postoperative radiation therapy. Int J Radiat Oncol Biol Phys. 1997;39:161–71.
101. Seegenschmiedt MH, Makoski HB, Micke O. Radiation prophylaxis for heterotopic ossification about the hip joint – a multicenter study. Int J Radiat Oncol Biol Phys. 2001;51:756–65.
102. Hamid N, Ashraf N, Bosse MJ, Connor PM, Kellam JF, Sims SH, Stull DE, Jeray KJ, Hymes RA, Lowe TJ. Radiation therapy for heterotopic ossification prophylaxis acutely after elbow trauma. J Bone Jt Surg. 2010;92:2032–8.
103. Juarez JK, Wenke JC, Rivera JC. Treatments and preventative measures for trauma-induced heterotopic ossification: a review. Clin Transl Sci. 2018;11:365.
104. Pakos EE, Papadopoulos D V, Gelalis ID, Tsantes AG, Gkiatas I, Kosmas D, Tsekeris PG, Xenakis TA. Is prophylaxis for heterotopic ossification with radiation therapy after THR associated with early loosening or carcinogenesis? HIP Int. 2019. 112070001984272.
105. Baird EO, Kang QK. Prophylaxis of heterotopic ossification – an updated review. J Orthop Surg Res. 2009;4:12.
106. Pakos EE, Ioannidis JPA. Radiotherapy vs. nonsteroidal anti-inflammatory drugs for the prevention of heterotopic ossification after major hip procedures: a meta-analysis of randomized trials. Int J Radiat Oncol. 2004;60:888–95.

107. Blokhuis TJ, Frölke JPM. Is radiation superior to indomethacin to prevent heterotopic ossification in acetabular fractures?: a systematic review. Clin Orthop Relat Res. 2009;467:526–30.
108. Burd TA, Hughes MS, Anglen JO. Heterotopic ossification prophylaxis with indomethacin increases the risk of long-bone nonunion. J Bone Joint Surg Br. 2003;85:700–5.
109. Robertson AD, Chiaramonti AM, Nguyen TP, et al. Failure of indomethacin and radiation to prevent blast-induced heterotopic ossification in a Sprague-dawley rat model. Clin Orthop Relat Res. 2019;477:644–54.
110. Pellegrini VJD. Heterotopic ossification following extremity blast amputation: an animal model in the Sprague Dawley Rat. 2016.
111. Tintle SM, Shawen SB, Forsberg JA, Gajewski DA, Keeling JJ, Andersen RC, Potter BK. Reoperation after combat-related major lower extremity amputations. J Orthop Trauma. 2014;28:232–7.
112. Pavey GJ, Polfer EM, Nappo KE, Tintle SM, Forsberg JA, Potter BK. What risk factors predict recurrence of heterotopic ossification after excision in combat-related amputations? Clin Orthop Relat Res. 2015;473:2814–24.
113. Shimono K, Tung W-E, Macolino C, et al. Potent inhibition of heterotopic ossification by nuclear retinoic acid receptor-γ agonists. Nat Med. 2011;17:454–60.
114. Hind M, Stinchcombe S. Palovarotene, a novel retinoic acid receptor gamma agonist for the treatment of emphysema. Curr Opin Investig Drugs. 2009;10:1243–50.
115. Stolk J, Stockley RA, Stoel BC, et al. Randomised controlled trial for emphysema with a selective agonist of the γ-type retinoic acid receptor. Eur Respir J. 2012;40:306–12.
116. Chakkalakal SA, Uchibe K, Convente MR, Zhang D, Economides AN, Kaplan FS, Pacifici M, Iwamoto M, Shore EM. Palovarotene inhibits heterotopic ossification and maintains limb mobility and growth in mice with the human ACVR1 R206H fibrodysplasia ossificans progressiva (FOP) mutation. J Bone Miner Res. 2016;31:1666–75.
117. Pavey GJ, Qureshi AT, Tomasino AM, et al. Targeted stimulation of retinoic acid receptor-γ mitigates the formation of heterotopic ossification in an established blast-related traumatic injury model. Bone. 2016;90:159–67.
118. Lees-Shepard JB, Nicholas S-AE, Stoessel SJ, Devarakonda PM, Schneider MJ, Yamamoto M, Goldhamer DJ. Palovarotene reduces heterotopic ossification in juvenile FOP mice but exhibits pronounced skeletal toxicity. elife. 2018;7:e40814. https://doi.org/10.7554/eLife.40814.001.
119. Clementia Pharmaceuticals. An efficacy and safety study of palovarotene to treat preosseous flare-ups in FOP subjects. 2017. In: ClinicalTrails.gov. https://clinicaltrials.gov/ct2/show/NCT02190747. Accessed 24 Jun 2019.
120. Clementia Pharmaceuticals. An efficacy and safety study of palovarotene for the treatment of FOP. 2019. In: ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT03312634. Accessed 24 Jun 2019.
121. Yu PB, Deng DY, Lai CS, et al. BMP type I receptor inhibition reduces heterotopic ossification. Nat Med. 2008;14:1363–9.
122. Peterson JR, De La Rosa S, Eboda O, et al. Treatment of heterotopic ossification through remote ATP hydrolysis. Sci Transl Med. 2014;6:255ra132.
123. Wang H, Lindborg C, Lounev V, et al. Cellular hypoxia promotes heterotopic ossification by amplifying BMP signaling. J Bone Miner Res. 2016;31:1652–65.
124. Agarwal S, Loder S, Brownley C, et al. Inhibition of Hif1α prevents both trauma-induced and genetic heterotopic ossification. Proc Natl Acad Sci U S A. 2016;113:E338–47.
125. Zimmermann SM, Würgler-Hauri CC, Wanner GA, Simmen HP, Werner CML. Echinomycin in the prevention of heterotopic ossification – an experimental antibiotic agent shows promising results in a murine model. Injury. 2013;44:570–5.
126. Seavey JG, Wheatley BM, Pavey GJ, et al. Early local delivery of vancomycin suppresses ectopic bone formation in a rat model of Trauma-induced heterotopic ossification. J Orthop Res. 2017;35:2397–406.

127. Kan L, Liu Y, McGuire TL, Berger DMP, Awatramani RB, Dymecki SM, Kessler JA. Dysregulation of local stem/progenitor cells as a common cellular mechanism for heterotopic ossification. Stem Cells. 2009;27:150–6.
128. Genêt F, Kulina I, Vaquette C, et al. Neurological heterotopic ossification following spinal cord injury is triggered by macrophage-mediated inflammation in muscle. J Pathol. 2015;236:229–40.
129. Hannallah D, Peng H, Young B, Usas A, Gearhart B, Huard J, Surgery J. Retroviral delivery of noggin inhibits the formation of heterotopic ossification induced by BMP-4. J Bone Jt Surg. 2004;86:80–91.
130. Kocić M, Lazović M, Kojović Z, Mitković M, Milenković S, Cirić T. Methods of the physical medicine therapy in prevention of heterotopic ossification after total hip arthroplasty. Vojnosanit Pregl. 2006;63:807–11.
131. Durović A, Miljković D, Brdareski Z, Plavšić A, Jevtić M. Pulse low-intensity electromagnetic field as prophylaxis of heterotopic ossification in patients with traumatic spinal cord injury. Vojnosanit Pregl. 2009;66:22–8.