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14. ABSTRACT HO consists of formation of ectopic bone within muscles, connective tissues and blood vessels and can cause loss of normal posture and mobility, chronic pain, prosthesis fitting problems, deep venous thrombosis or other problems. HO is induced by trauma, burns and invasive surgeries and is thus very common amongst our severely wounded service-members. HO has in fact emerged as <u>the single most important barrier to functional activity and return-to-duty</u> in recent analyses. Current treatments are not wholly effective, are fraught with complications and may actually trigger additional HO in certain circumstances. Clearly there is an urgent need to create new, effective, specific and easy-to-deliver therapies for HO. HO closely resembles the process by which endochondral bones normally form and grow during prenatal and postnatal life. Because that process requires a steep drop in activity of nuclear retinoic acid receptors (RARs), we hypothesized that acute pharmacological re-activation of the RARs could block HO. In previous studies sponsored by the USAMRMC, we did find that synthetic selective RAR agonists are potent inhibitors of surgery-induced HO in mice. One of the most effective RAR agonists we tested was R667 (Palovarotene) previously used in an FDA-approved Phase 2 trial. R667 is thus our lead compound at present.					
15. SUBJECT TERMS Heterotopic ossification; progenitor cells; chondrocytes; endochondral bone; marrow; chondrogenesis; retinoids; retinoid signaling; retinoid nuclear receptors					
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1. INTRODUCTION

Heterotopic ossification (HO) consists of formation of ectopic bone, usually endochondral bone, within muscles and other tissues, and is triggered by severe trauma, burns, neural damage and protracted immobilization. HO is particularly insidious in amputees where it can cause major complications, including prosthesis fitting problems, pain, local inflammation and pressure ulcer formation. HO has thus emerged as the most important barrier to restoration of functional activity and return-to-duty in recent studies of wounded active duty service-members conducted by this and other research groups. Because HO can be triggered by trauma, it can occur in patients in the general population undergoing invasive surgeries such as total hip arthroplasty. There is also a congenital form of it that can affect children and young adults. Surgery is often used to remove the HO lesions, but this procedure can have complications, may actually trigger another round of HO, and is not recommended for patients with recurrent HO and those suffering from congenital forms of it. Clearly, there is an urgent need to create new, effective and easy-to-deliver therapies for HO that could pave the way for a return to productive and functional life for amputees, trauma patients and other affected individuals. The formation of HO tissue masses starts with the recruitment of progenitor cells and their differentiation into chondrocytes and cartilage tissue. In our studies, we targeted this step and used retinoid agonists to block it in mouse models of HO. We found that several such drugs were effective, but agonists activating the nuclear retinoic acid receptor gamma (RAR γ) were particularly effective. The purpose of the current project at our Institution is to determine whether the RAR γ agonists also prevent HO in larger animals and whether side effects previously seen in fracture repair are reversed over time. The scope of the research is to study HO in diverse animals including rats and rabbits, analyze intramuscular and subdermal forms of HO, and examine rebound effects. The purpose of the research carried out by our collaborators at their Institution (Naval Medical Research Center) is to study the effectiveness of the drug treatment in a rat blast model of HO.

2. KEYWORDS

Heterotopic ossification; trauma; extraskeletal bone formation; combat injuries; trauma; invasive surgery; congenital heterotopic ossification; progenitor cells; chondrogenesis; chondrocytes; endochondral bone; marrow; retinoid agonists; retinoid signaling; retinoid nuclear receptors

3. OVERALL PROJECT SUMMARY

Heterotopic Ossification (HO) is a pernicious, complex, debilitating and difficult to treat musculoskeletal pathology that affects a large number of civilian and military individuals. HO involves the formation of extraskeletal bone, usually endochondral in nature, at the expense of local skeletal muscles, tendons and ligaments, other soft tissues, and the blood vessel wall (1-6). HO is triggered by trauma, burns, neural damage and other insults and can also occur in patients undergoing invasive surgeries, including total hip arthroplasty (6). HO is very common in our wounded service-members and more so than it would otherwise be expected on a pure statistical basis (7). This is likely due to the severity and encompassing nature of wounds and tissue damage suffered by the soldiers in war theaters and other conflicts. Daily function of HO patients is hampered by loss of normal posture, pain, prosthesis fitting problems, reduced mobility, formation of pressure ulcers, deep venous thrombosis or other complications (8-10). Indeed, US Army clinical studies have shown that HO represents **the single most important barrier to regain functional activity and to return to duty** in a recent analysis of wounded service-members (11). Current pharmacologic treatments -mostly involving non-steroidal anti-inflammatory drugs- are not very effective, have side effects that reduce patient compliance, and are not specifically directed against the skeletogenic process (12,13). Surgery is often used to remove the most

symptomatic HO lesions, but the procedure is fraught with complications, may actually trigger an additional round of HO, and is not recommended for those in the general population suffering from congenital forms of it (14,15). Clearly, there is an urgent need to create new, effective, specific, safe and easy-to-deliver therapies for HO, a debilitating disease that is hampering the return to productive life and service.

In terms of histopathogenesis, HO initiates and progresses through the multiple steps by which endochondral bone normally forms and grows

during prenatal and postnatal skeletal development, morphogenesis and elongation (14,16). The process initiates with recruitment and condensation of preskeletal progenitor cells at specific sites that are followed by differentiation of the progenitors into chondrocytes (17). This differentiation step requires a steep drop in activity of nuclear retinoic acid receptors -RAR alpha

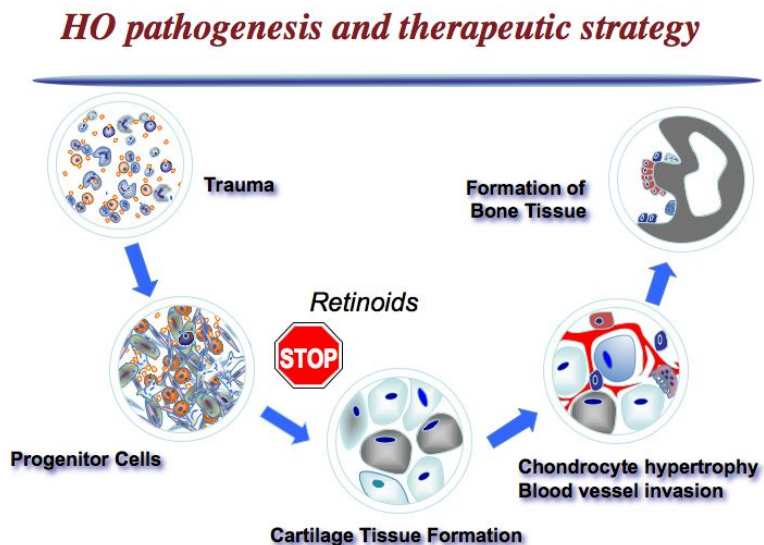


Fig. 1. Schematic depicting the major key phases in HO formation following an initial inciting step –trauma in this case- and the use of retinoids to stop HO at the chondrogenesis phase.

(RAR α), RAR beta (RAR β) and RAR gamma (RAR γ)- (18-21). Thus, we hypothesized that acute pharmacological re-activation of the RARs (22,23) with retinoid-based drugs could inhibit chondrogenic cell differentiation and in turn prevent HO all together. To test this hypothesis, we carried out studies sponsored by the USAMRMC in which we used mouse models of HO mimicking surgery-induced subcutaneous or intramuscular HO (24). As we reported previously, we found that synthetic selective agonists for RAR α or RAR γ did block these forms of HO, with the RAR γ agonists being superior in terms of effectiveness (25,26). The drugs had no major side effects on overall systemic physiology in the mice except for a transient delay in bone fracture healing. Notably, the drugs also inhibited HO in a mouse model of Fibrodysplasia Ossificans Progressiva (FOP), a congenital, severe and often fatal form of HO (27). One of the most effective RAR γ agonists we used was R667 (Palovarotene), previously tested for long-term treatment of a chronic disease (emphysema) in a FDA-approved Phase 2 clinical trial (28). Thus, this is a case of drug repurposing, and R667 has been and is our lead compound.

The goals of the current CDMRP project are to: establish the general validity of a retinoid agonist-based therapy against HO by testing additional animal models; gain insights into dose effectiveness and regimens; and verify safety. In the past second year of funding, we have made progress at multiple levels toward these central goals. We have also had the opportunity to test additional aspects and implications of the findings that could represent follow-up studies for subsequent projects toward clinical studies.

Given that the research goals of the project include comparison of animal models and treatment strategies, our focus during the past year of support was to carry out experiments to complete studies on window of opportunity and efficacy of treatment in both mice and rats. Our postdoctoral researcher Dr. S. Sinha (under the supervision of Dr. M. Iwamoto) carried out experiments in mice first to establish the effectiveness of R667 treatment started at different time points from the onset of HO formation. Accordingly, 2 month-old female CD-1 mice were implanted with 250 μ l aliquots of growth factor-reduced Matrigel containing 1 μ g rhBMP2 at 2 subcutaneous locations in the abdominal area. The injected Matrigel quickly solidifies at body temperature and its round-shaped mass is large enough to protrude out onto the skin in the form of local swelling. In addition, because Matrigel contains a number of matrix components and is filled with recombinant human BMP-2, it very likely elicits local

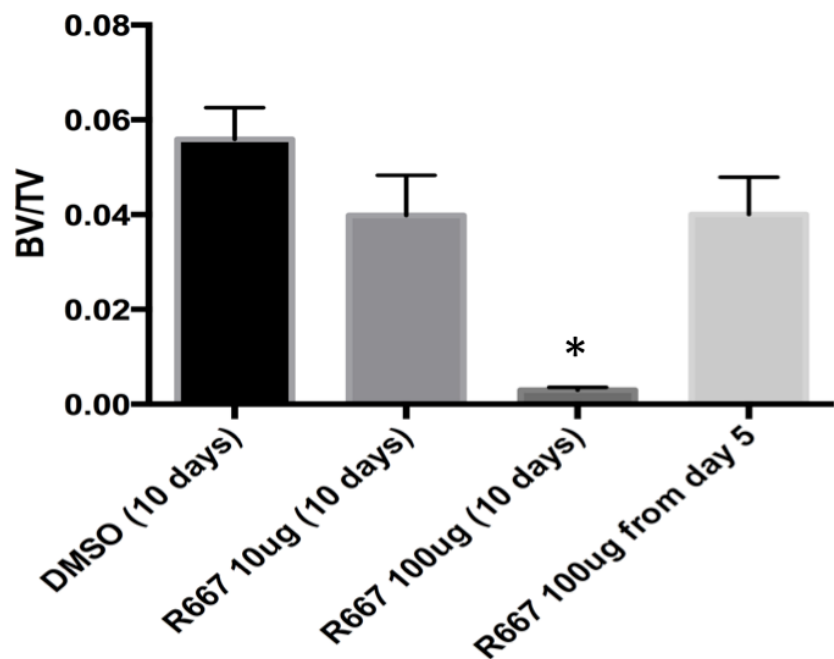


Fig. 2: Summarized data representing BV/TV values of ectopic masses isolated on day 12 from different groups of mice. N=6 in each group. p value represents < 0.05

inflammation in coordination with skin swelling, thus mimicking the initial inflammatory situation that typically leads to HO. Mice were randomly subdivided in groups of 6 mice each, and each group was then treated with drug vehicle (corn oil), 10 μ g R667 or 100 μ g R667 by oral gavage (30) starting at different time points. Doses are per average 25 gr mouse, and were adjusted to actual weight. In one set, mice received gavage treatment from day 1 of injection, while in companion sets the gavage treatment was started at later time points. On day 12 from the initial implantation, ectopic tissue masses forming within the Matrigel scaffold were harvested and processed for analyses including bone volume/total volume determination by μ CT. The data showed that R667 was very effective when treatment started soon after induction of HO by Matrigel implantation, but its effectiveness decreased progressively when treatment started later than day 4 (Fig. 2). Importantly, we established that the effectiveness of R667 was maximal when given at 100 μ g/day/25 gr mouse.

To extend these studies to a larger animal model, we carried out experiments in rats and determined responsiveness to, and effectiveness of, R667. As we reported last year, our initial experiments with this model elicited a moderate level of variability that we explained by some difficulties in delivering the exact same amount of Matrigel to each animal, using analogous anatomical implantation sites, administering the same amount of drug without damaging the esophagus, and physically handling the animals in the same gentle manner. These problems have now been overcome after solving each of these possible problems. Accordingly, in the most recent experiments, juvenile 300 gr female Wistar rats were carefully injected with 300 μ l aliquots of growth factor reduced Matrigel containing 3 μ g rhBMP2 at two prescribed subdermal ventral sites. This higher dose of rhBMP-2 turned out to be needed for induction of HO in a maximal and reproducible manner from animal to animal, in agreement with a recent rat HO study (29). Rats were randomly divided into three groups (6 rats per group) and were treated with vehicle (corn oil), 200 μ g R667 or 400 μ g R667 by daily gavage for a total of 11 days. On day 12, ectopic skeletal tissue masses forming within the Matrigel scaffold were collected and were analyzed by histology, histomorphometry and μ CT. In vehicle-treated control rats, the ectopic tissues displayed characteristic histology, structure and arrangements. Most notably, an appreciable amount of cartilage was present and displayed many chondrocytes undergoing maturation and hypertrophy, clearly indicating the formation of the ectopic skeletal tissues was following the endochondral ossification pathway. Indeed, in addition to hypertrophying cartilage, the ectopic masses displayed clear areas of endochondral bone and marrow. Because hypertrophic cartilage and endochondral bone were almost always contiguous, it appears that the two tissues formed in a coordinated manner as part of an endochondral process, implying that there was minimal concurrent intramembranous ossification in this model. In rats treated with R667, however, histopathological analysis showed that the tissues present on day 12 were largely composed of fibrogenic and connective tissue cells and matrix, but the amounts of appreciable cartilage and bone were minimal (Fig. 3A). These histomorphometric data were verified by μ CT imaging showing that large masses of mineralized tissues were present in vehicle-treated control rats, but far less in R667-treated animals (Fig. 3B). This analysis indicated also that maximal effectiveness was obtained at the higher drug dose.

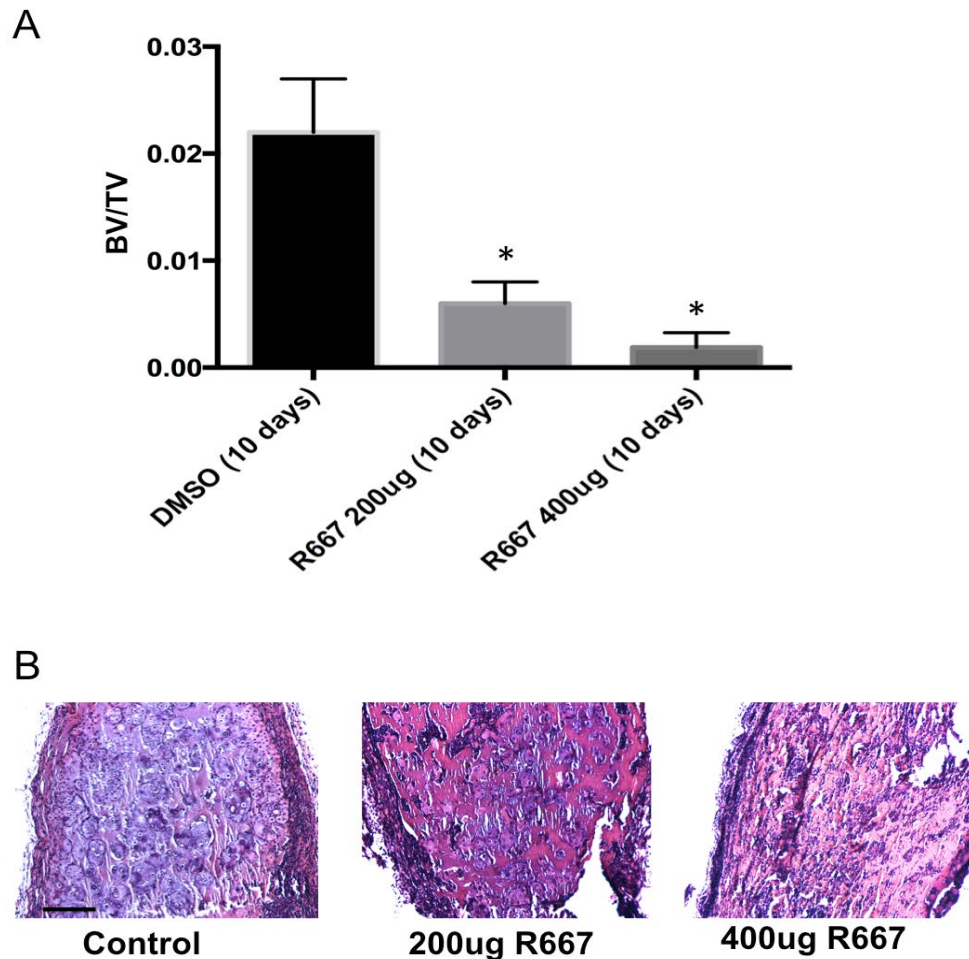


Fig. 3: (A): Summarized data representing BV/TV values of ectopic masses isolated on day 12 from Wistar rats. (B): H & E staining demonstrating chondrogenesis in control rats which are inhibited in the presence of two different concentrations of R667. N=7 in each group. Samples were analyzed using one-way analysis of variance

Current common pharmacologic treatments for HO -steroidal and non-steroidal anti-inflammatory drugs- have considerable side effects that limit their effectiveness and applicability and reduce patient compliance (12,13). When new drug treatments are being tested, it is then very important to assess whether they have unwanted side effects. Thus, we monitored both mice and rats undergoing treatment with R667 by a number of established criteria that include overall animal behavior and activity, body weight, ease of motion, fur/skin condition, food and water consumption, etc. Such analyses were conducted over several independent experiments and by independent investigators and included animals treated with different doses of R667 at different time points and endpoint. Animals appeared to respond well to drug treatment and we observed no obvious changes in behavior. Weight and food consumption were normal. The only occasional side effect also seen in our previous published studies was skin redness around the oral cavity that may be due to systemic effects or inadvertent local administration during gavage (25,26). To verify these observations, we carried out blood chemistry analyses. As shown in Fig. 4, there were no major changes in overall levels of multiple standard markers for organ function and blood homeostasis. Thus, as seen in the phase 2 clinical trial with patients with emphysema, R667 continues to display a very safe treatment profile.

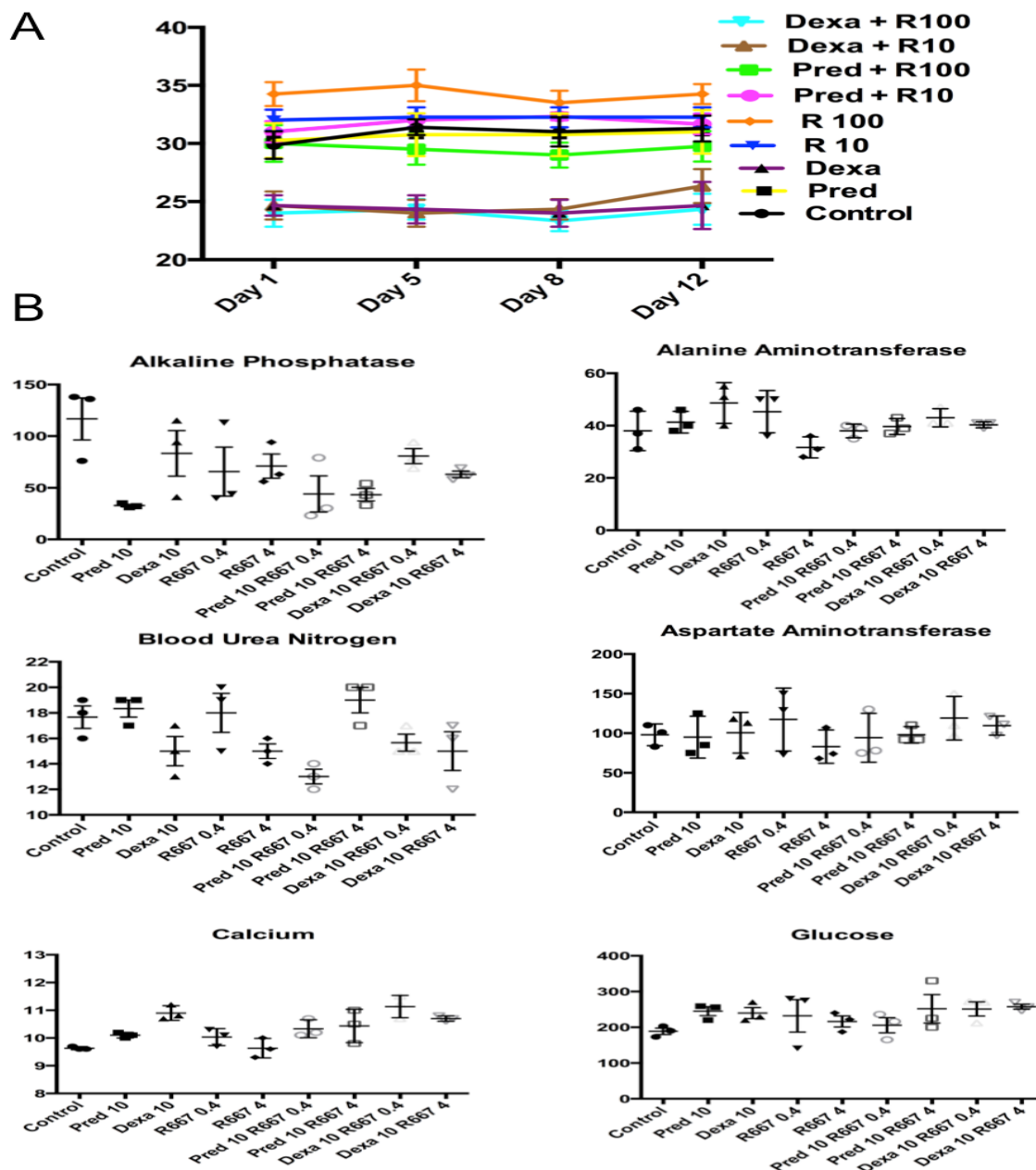


Fig. 4: (A): Summarized data illustrating body weight of CD-31 mice during the time of treatment (B): Graph indicating the blood parameters of CD-1 females during different treatments of drugs.

The process of bone fracture repair is quite similar to HO, and both involve initial inflammation, recruitment of progenitors, formation of cartilage tissue and replacement by endochondral bone. Thus, it was reasonable to predict that a possible side effect of R667 treatment could be a delay in fracture repair. We did observe this delay in our previous mouse studies, and our goals in this project include a study of fracture repair in larger animals. To this end, our postdoctoral researcher Dr. Sinha had to master the fracture repair procedures for which she had no previous experience. The surgery required to induce fracture repair is delicate and complex given the small size of the fibula (used as our model skeletal structure for bone

repair since it does not require external or internal long bone fixation). In addition, collateral damage to surrounding tissues and bones must be minimized. Likewise, each fracture needs to be done in a consistent and reproducible manner such that animals in each experimental group and animals from different groups and different experiments can be evaluated and compared directly, without possible problems due to variability of procedure and severity/orientation of the fracture. In addition, the imaging analytical procedures to monitor fracture healing include soft x-ray imaging in 2 different orientations for initial evaluation of union formation, μ CT analysis for quantification of soft and hard callus, histomorphometry and pathology that need to be consistent and accurate from specimen to specimen. Dr. Sinha has honed her surgical procedures and skills (Fig. 5). Experiments are currently ongoing on CD-1 mice (8 wks old) and will be followed by experiments with rats. In addition to morphological and imaging procedures, the fracture repair process will be monitored with molecular tools to decipher mechanisms that may be selectively affected by the drug treatment. We have carried out preliminary experiments using various RNA extraction methods from bone to make sure that the RNA quality is high and that cDNA libraries can be produced. These will be used to carry out gene arrays analyses using different sets of plates for distinct signaling pathways or different regulatory machineries including nuclear ligand receptors and transcription factors. As data are gathered, they will be described in the quarterly reports.

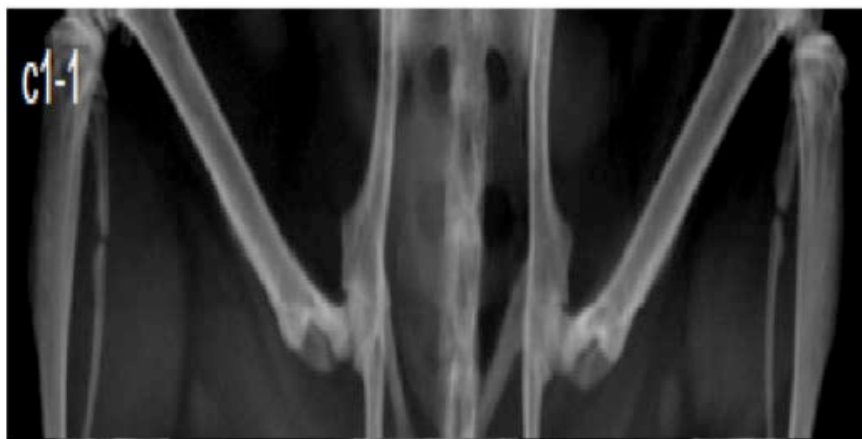


Fig. 5: Representative soft X-ray image depicting the fibula fracture in CD-1 mice

While these fracture repair studies are ongoing, we have received some insights through our collaboration with Dr. J. Forsberg at the Naval Medical Research Center in Silver Spring, MD who is a co-Principal Investigator in this project. His group member Dr. Thomas Davies is in charge of HO experiments in a rat blast model that mimics conditions occurring in the theaters of war. The model involves air-pressure blast to one hind limb of anesthetized rats that is followed by surgical amputation of the affected limb. The model elicits HO with nearly 100% penetrance and as described in an abstract presented at a recent symposium (in which we are co-authors), R667 treatment prevented HO extremely efficiently in this blast model as well. Because the model involves initial amputation of the damaged limb at the level of the tibia and fibula, the distal ends of those amputated bones would need to heal and undergo a bone fracture repair process. Ongoing histological analyses of those distal ends in control versus R667-treated rats have indicated that the healing/repair process was effective and apparent in control animals at the 3 or 4 week time point, but appeared to be delayed somewhat in the treated animals. In controls, the tissues were thick and compact and were fully covering the bone ends. In the treated animals, the repair tissues were thinner and uneven (Dr. Davies,

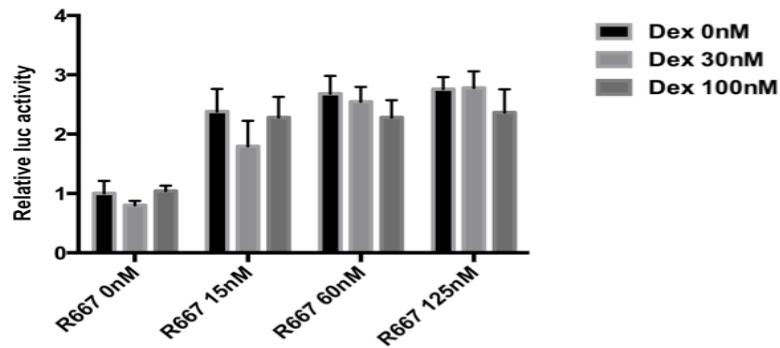
personal communication). These initial observations need to be extended to later time points to determine whether the apparent delay in tissue healing is transient or permanent. Our prediction is that the delay is transient –as we have observed in our previous fracture repair studies- and that the healing process would resume as soon as the initial side effects of drug treatment dwindle with time. We will use these observations as a guide in our own rat bone repair studies.

Though the retinoid-based treatment of HO appears to be quite effective in experimental animals, we cannot predict with absolute confidence that it will turn out to be as affective in patients. In addition and as pointed out above, HO is currently treated mostly with anti-inflammatory drugs that though not very effective, represent the current standard of care. Thus, it is important to begin to clarify whether anti-inflammatory drugs may interact with the retinoid treatment and whether they enhance or decrease retinoid effectiveness. If they enhance it or at least do not interfere with it, then a combination therapy with R667 plus an anti-inflammatory drug could turn out to be even more potent and maybe even safer (if each drug could be used at lower doses while still eliciting maximal anti-HO effectiveness). For these initial and exploratory experiments, we used the subcutaneous HO model in mice that as shown above, is our most rapid, most efficient and least expensive assay. Accordingly, 6-8 week-old female CD-1 mice were implanted with two 250 μ l aliquots of Matrigel/1.0 μ g rhBMP2 as above. Mice were returned to their cages and randomized. On day 1 from Matrigel injection, groups of 4 to 6 mice each were subjected to gavage administration of R667 (0.4 or 4.0 mg/kg), prednisone (10 mg/kg) or prednisone plus R667 at 0.4 or 4.0 mg/kg. Control mice received vehicle. The resulting 6 groups of mice then received daily doses of drug or vehicle and were sacrificed on day 12 at which point the ectopic tissue masses were harvested and subjected to μ CT analyses. As expected, samples from vehicle-treated control mice displayed HO masses, reaffirming the efficiency of HO formation in this model. Treatment with R667 alone at the lower moderately inhibited the extent of HO, but the retinoid was more effective at the higher dose. Prednisone by itself elicited a significant inhibition of HO, which was not significantly increased in combination with R667. This is likely due to the fact that in these initial experiments, we used the maximal dose of prednisone that was used previously in mouse HO experiments by others, including those using a mouse model of the severe, usually fatal and congenital form of HO which is called Fibrodysplasia Ossificans Progressiva (3). Though not completed, these preliminary data indicate that R667 and prednisone do not obstruct each other effectiveness against HO and could thus be used in combination (albeit at lower doses to avoid possible side effects).

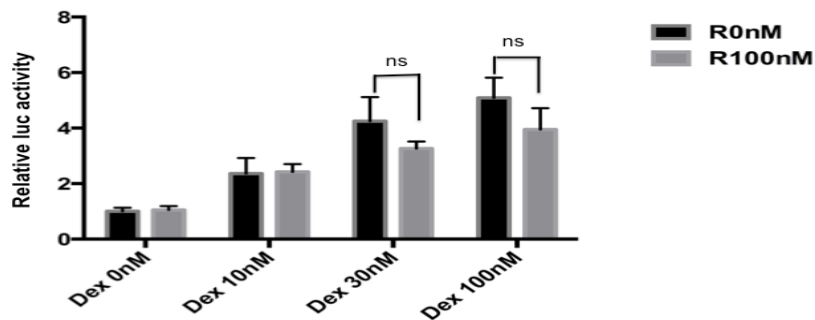
Because both R667 and prednisone blocked HO in our subcutaneous model, this raised the issue of whether the drugs acted at similar or dissimilar levels to exert that effect. Both drugs operate at the nuclear level whether they interact with their respective nuclear receptors and elicit effects on their respective target genes in cooperation with specific nuclear proteins. So, how could such diverse drugs elicit similar effects on HO? One possibility is that they each acted independently on a common mechanism which may be essential for the initiation of HO. Thus, we asked whether such common denominator could be inflammation. To obtain preliminary evidence that this may be the case, we carried out reporter assays on skeletal progenitor cells in vitro. We transfected the cells with reporter plasmids that monitor transcriptional activity of retinoids (*RARE-Luc*), corticosteroids (*GRE-Luc*) or inflammation effectors (we used *NF-kB-Luc* that measures the activity of such common inflammatory nuclear effector). We found that R667 and prednisone stimulated transcriptional activity of their respective reporter plasmid but did not affect each other's reporter, reaffirming that each was acting specifically at the transcriptional level (Fig. 6A and 6B). Very interestingly, however, each drug was able to reduce the activity of the *NF-kB-Luc* reporter, and this inhibition was nearly complete when the drugs were used in combination (Fig. 6C). Thus, the data do support the interesting possibility that despite their distinctiveness and specificity of nuclear action, R667

and prednisone act at a common denominator level –very likely inflammation- to exert their inhibitory effects on HO.

A



B



C

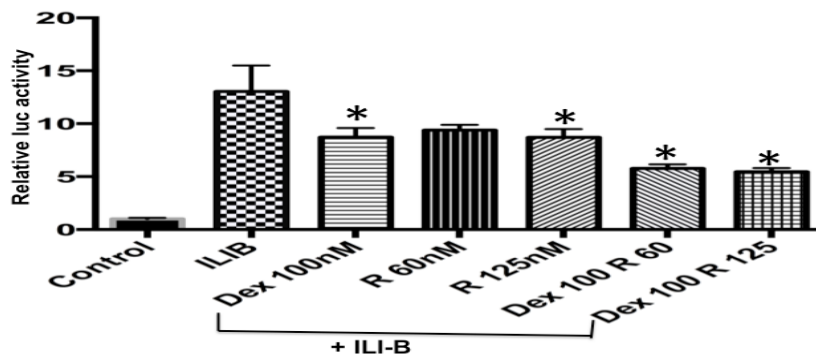


Fig. 6: Relative luciferase activity of retinoid activator response elements (RARE)(A), glucocorticoid response elements (GRE) (B) and nuclear factor kappa beta (C) in presence and absence of combination of steroids and retinoids transfected in bone marrow stromal cells (BMSCs). $p < 0.05$

4. KEY RESEARCH ACCOMPLISHMENTS OVER THE LAST TWO YEARS

- Establish and obtain approval of new rat and rabbit HO protocols from IACUC
- Submit and receive approval of those protocols by ACURO
- Establish a NCRADA between our Institution and the NMRC

- Organize meetings between the two research groups here and in Bethesda to review plans, share information on research protocols and procedure, coordinate work at respective sites, and establish lines of communication and coordination
- Recruit and train new postdoc personnel
- Perform in vivo experiments to establish effectiveness and reproducibility of HO animal model and insure expertise
- Carry out sets of preliminary experiments in the new animal model –rat-
- Document effectiveness of drug treatment by histological and imaging procedures
- Present data and accomplishments at several national and international symposia and workshops
- Prepare manuscripts for submission for publications
- Maintain close contacts with our collaborators on both drug effectiveness and side effects
- Explore combination drug regimens

5. CONCLUSIONS

The data obtained so far strongly indicate that the retinoid agonist treatment is quite effective in preventing subcutaneous Matrigel/rhBMP2-driven HO in mice and rats and appears to be as effective in a blast model also. These observations are a major step forward in our quest to establish the general validity of this experimental therapy and move it ever closer to the clinics. In the coming year, we plan to extend these experiments to solidify the observations and in particular to exclude irreversible side effects on processes such as fracture repair. Based on our previous studies, we think this is most unlikely and the retinoid therapy will turn out to be not only effective but also safe.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

a.

(1) Our research has been publicized by our Institution for a lay audience at the following web sites:

<http://www.research.chop.edu/blog/experts-research-leads-rare-disease-clinical-trial/>

<http://www.research.chop.edu/btob/breakthrough-work-leads-to-rare-bone-disease-trial/>

b. List of presentations:

(1) "Signaling pathways regulating chondrocyte function and heterotopic ossification". Montefiore-Einstein University Medical Center, New York City, NY, October 2013

(2) "FOP therapy: steps ahead and implications for the treatment of related diseases". IFOPA Association, Bologna, Italy, April 2014

(3) "Drug repurposing for the treatment of heterotopic ossification and Fibrodysplasia Ossificans Progressiva". Musculoskeletal and Engineering Gordon Conference, Andover, NH, August 2014

(4) "Pharmacological prevention of heterotopic ossification". Annual meeting of the American Society for Bone and Mineral Research, Houston, TX, September 2014.

(5) "Retinoid agonist effectiveness against blast-induced heterotopic ossification". Orthopaedic Trauma Association meeting, San Diego, CA, September 2015

(6) "Analysis of retinoid agonist-steroid interactions in heterotopic ossification therapy". Annual meeting of the American Society for Bone and Mineral Research, Seattle, WA, October 2015.

7. INVENTIONS, PATENTS AND LICENSES

Nothing to report

8. REPORTABLE OUTCOMES

Manuscripts are being completed to report the data described above.

9. OTHER ACHIEVEMENTS

Nothing to report

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11. APPENDICES

Nothing to report

Preventive Therapeutics for Heterotopic Ossification

OR120056P1 Eighth Quarter Quad Chart 6/30/15-9/29/15
W81XWH-13-2-0076



PI: Maurizio Pacifici

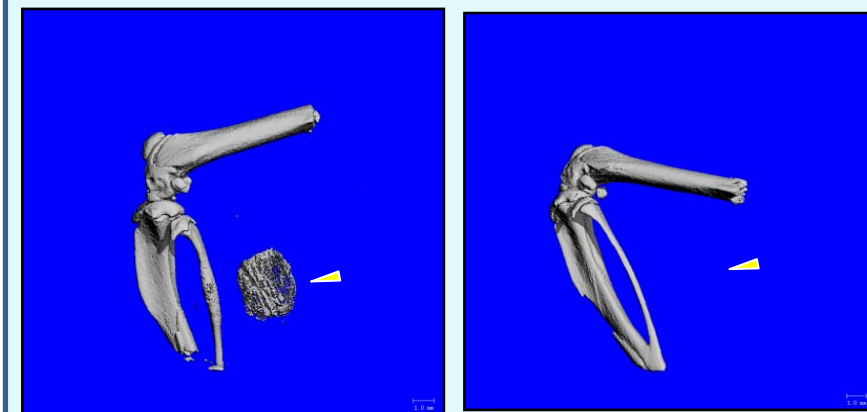
Org: The Children's Hospital of Philadelphia

Award Amount: \$766,275

Study/Product Aim(s)

- To determine whether retinoid agonists prevent HO in larger animals
- To assess whether side effects on fracture healing are fully reversible.
- To determine whether the retinoid agonists block blast- and combat-related HO

We will test the anti-HO retinoid therapy in rats and rabbits and in a novel blast-induced rat HO model that mimics the physiologic and pathologic traumatic conditions triggered by a blast injury. We will also test whether the relatively mild side effects previously seen in retinoid-treated mice occur in rats and rabbits as well and if they do, will disappear over time or by drug regimen modulation. Importantly, we will follow up preliminary data indicating that while the retinoids block HO, they stimulate the repair of neighboring surgery-damaged skeletal muscles.



The goal of this grant is: to determine if RARg agonists prevent HO in larger animals (pictured here is our previous success with HO mouse models), to assess the drug effects on fracture healing, and to explore the drug's effect in combat injury related HO.

Timeline and Cost

Activities	CY	13	14	15	16
We will compare the anti-HO effectiveness of different RARg agonists (including R667)					
Assessment of fracture healing models for reversibility of side effects					
Estimated Budget (\$K)		\$000	\$144,302	\$153,162	\$158,586

Updated: 07/09/15

Goals/Milestones (Example)

CY14 Goals – Dosing

- ☐ Effective doses for maximal HO suppression by each agonist in rats

- ☐ Dosing “window of opportunity” for HO suppression

CY15 Goal – Assessment of drug treatment

- ☐ Rebound effects (if any) and HO formation upon drug withdrawal.
- ☐ Drug/dose effectiveness in rabbits

CY16 Goal – Assessment of fracture healing

- ☐ Analyze fibula fracture healing in agonist-treated rats and rabbits.
- ☐ Test fractures structurally and mechanically
- ☐ Compare effectiveness of local drug delivery.

Comments/Challenges/Issues/Concerns

Budget Expenditure to Date

Projected Expenditure: \$257,030 total 2015 award

Actual Expenditure: \$57,931 in combined direct and indirect costs this quarter