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AAO Foundation Final Report Form (a/o 5/30/2021)

In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Please prepare a report that addresses the following:

Type of Award: Biomedical Research Award

Name(s) of Principal Investigator(s): Jin Hee Kwak, DDS, MS

Institution: University of California, Los Angeles

Title of Project: BP-NELL-PEG systemic therapy regenerates spaceflight-induced bone loss in the craniofacial skeleton

Period of AAOF Support (e.g. 07-01-2021 to 06-30-2022): 7-1-2018 to 6-30-2020 (with a NCE)

Amount of Funding: \$30,000

Detailed results and inferences: See below Summary/Abstract. The manuscript is in active preparation for publication.

Summary/Abstract:

Introduction: NELL-1 is a potent osteogenic and anti-osteoclastic growth factor that was most studied for its capacity to regenerate appendicular and axial (postcranial) bones as a *local* therapeutics in various animal models. Recently, we demonstrated that the PEGylation of NELL-1 (NELL-PEG) significantly enhances the pharmacokinetics of NELL-1 as a potential *systemic* osteogenic therapy. To meet the technical demands of our recently completed rodent research in space, we further enhanced the pharmacokinetics of NELL-PEG by conjugating with *bioinert* bisphosphonate (BP-NELL-PEG) to target-deliver NELL-1 to bone tissues, including the craniofacial bones.

Osteoporosis has been proven to be associated with craniofacial bone degeneration, particularly in the mandibular condyles and the alveolar bones of the jaws, and to consequently complicate dental treatments. To date, there is no concrete evidence that answers the popular question of whether currently available osteoporosis therapies can also effectively and safely regenerate bone in the jaws. As such, the current study aims to find a therapy that could prevent and reverse bone loss in both the craniofacial and postcranial skeleton in extreme osteoporosis conditions, with the *central hypothesis* that BP-NELL-PEG systemic therapy can prevent spaceflight-induced osteoporotic bone loss in both skeletal types.

Methods: 8 months old skeletally mature BALB/c female mice were used (n=10/group). Flight groups were flown to orbit by SpaceX-11 rocket and housed onboard the International Space Station (ISS). Matching Ground control groups were housed in the Kennedy Space Center (KSC), FL, under the same environmental settings as the Flight groups with a 3 days off-set. Starting at approximately 1 week in orbit and every 2 weeks thereafter, mice received either BP-NELL-PEG or PBS injections intraperitoneally. DXA scan was performed before launch, at 4.5 weeks post-launch and at terminal timepoint to longitudinally assess the BMD. At 9 weeks post-launch, all animals were euthanized, and frozen carcasses were later returned to UCLA for dissection and analyses. Femurs, tibiae, lumbar vertebrae and craniofacial bones underwent microCT, histology and IHC analyses.

Results: Postcranial bones showed a significant bone loss by spaceflight, although not as much as we had expected due to the addition of enrichment hut in the animal housing system, and a significant increase of bone quantity and quality by BP-NELL-PEG treatment. Craniofacial bones did not show effects of spaceflight or BP-NELL-PEG treatment in the calvaria, orbit and dentoalveolar bones. However, the mandibular condyle in temporomandibular joint (TMJ) complex showed a significant change. This is the first study to demonstrate changes in the TMJ resulted by spaceflight and BP-NELL-PEG treatment.

Based on histology findings, 9 weeks of spaceflight microgravity resulted in increased subchondral bone porosity in the mandibular condyle, but did not affect the articular cartilage. In addition, in both Ground and Flight groups, BP-NELL-PEG treatment drastically increased the mature and hypertrophic zones of the condylar cartilage, which were found to be calcified based on microCT. Further studies are necessary to determine whether the changes occurred from BP-NELL-PEG treatment are favorable to TMJ health.

Conclusions: For the first time, the current study investigated the effects of space microgravity and BP-NELL-PEG systemic therapy on temporomandibular joint (TMJ). The impact of this study is immense as TMJ is a non-weight-bearing joint and allows us to assess spaceflight effects independent of mechanical loading, which is known to be the primary cause of osteoporosis in space and in severe disuse atrophy on Earth. Here, we discovered that TMJ osteoporosis can occur in space, independent of mechanical loading, indicating that microgravity has a direct effect on osteoblast and osteocytes. In addition, we report a significant anti-osteoporosis effect of BP-NELL-PEG systemic therapy in both the mandibular condyle and

postcranial bones. Finally, we demonstrated the utility of bioinert bisphosphonate as a platform technology to deliver protein drugs systemically without introducing unwanted anti-osteoclastic effects.

Respond to the following questions:

1. Were the original, specific aims of the proposal realized?
- Yes
2. Were the results published?
- The manuscript is in active preparation for publication. AAOF support is acknowledged.
3. Have the results of this proposal been presented?

All of below abstracts and presentations acknowledged the current AAOF award. In total, 17 oral presentations have been made with 8 awards, and 10 poster presentations have been made with an award.

• **Oral Presentations and Awards:**

1. Velicu D.B.*, Liu T., Maram S.S., Ting K., Soo B.C., **Kwak J.H. (Senior author)** “Spaceflight-Induced Osteoarthritis in Non-Weight-Bearing Bone and Treatment with BP-NELL-PEG”. UCLA School of Dentistry Research Day, Jan 2021, Virtual event. ** 1st Place Winner in Master’s Students and Residents Category.
2. Velicu, D.B.*, Liu, T., Dong, S., Low, J., Tran, L., Duong, T., Shi, J., Nguyen, T., **Kwak J.H. (Senior author)** “Development of TMJ-OA in spaceflight and treatment with BP-NELL-PEG therapy”. 8th Edition of the Virtual World Congress of Dental Students, Zagreb, Croatia (held online due to COVID-19 pandemic), May 2020. ** Winner of Postgraduate scientific Program.
3. Dong, N.*, Velicu, D., Low, J., Tran, L., Duong, T., Shi, J., Nguyen, T., **Kwak, J.H. (Senior author)**. Development of TMJ-OA in spaceflight and treatment with BP-NELL-PEG therapy. IADR/AADR/CADR General Session, March 2020. Washington D.C. *Accepted for oral presentation. ** Selected as a finalist for the Craniofacial Biology Junior Awards Competition. Competition canceled due to COVID-19 pandemic.*
4. Invited lecture: **Kwak, J.H.** “An Update To: Taking Craniofacial Patient Discoveries to the International Space Station”. University of Southern California (USC) Department of Orthodontics, July 25th, 2019. Los Angeles, CA.
5. Shi, J. (presenter), Ha, P., Pan, H.C., Kim, J.K., Zhang, Y., Chen, E., Duong, T., Baik, L., ***Kwak, J.H. (co-senior author)**, * Ting, Kang., Soo, C. “Inactivated-bisphosphonate boosts bone-targetedness of systemic NELL-1 therapy for spaceflight-induced osteoporosis”. IADR/AADR/CADR General Session, Vancouver, BC, Canada, June

2019.

** 1st Place winner in IADR Unilever Hatton Competition Senior Category (Basic Science). AND 2nd Place winner in AADR Hatton Competition Senior Category.

6. **Kwak, J.H.**, “Taking Craniofacial Patient Discoveries to the International Space Station”, California Dental Association (CDA) meeting, Anaheim, CA, May 2019.
7. Shi, J. (presenter), Ha, Pin, Pan, H.C., Kim, J.K., Zhang, Y., Chen, E., Duong, T, Baik, L., ***Kwak, J.H. (co-senior author)**, *Ting, K. “Inactivated-bisphosphonate boosts bone-targetedness of systemic NELL-1 therapy for spaceflight-induced osteoporosis”. Oral and poster presentations. UCLA Research Day Feb 2019. ** 1st place winner in master/residents category.
8. **Kwak, J.H.**, Shi, J., Ha, P., Pan, H.C., Zhang, Y., Chen, E., Stodieck, L., Kim, J.K., Zhang, X., Wu, B., Ting, K., Soo, C. “NELL-1-based Systemic Therapy Prevents Long-duration Spaceflight-induced Osteoporosis In Rodents”. Orthopaedic Research Society (ORS). Oral and poster presentation in 2019 ORS Annual Meeting, Austin, TX, Feb 2019.
** Winner of the Research Section Award in the Preclinical Models Section (podium).
9. **Kwak, J.H.**, “NELL-1-based therapy prevents spaceflight-induced osteoporosis in rodents”, Ground Rounds, UCLA & Orthopaedic Institute for Children, Department of Orthopaedic Surgery, UCLA. Dec 2018.
10. **Kwak, J.H.**, Shi, J., Ha, P., Pan, H.C., Zhang, Y., Chen, E., Stodieck, L., Kim, J.K., Zhang, X., Wu, B., Ting, K., Soo, C. “NELL-1-based Systemic Therapy Prevents Long-duration Spaceflight-induced Osteoporosis In Rodents”. Chosen as Finalist for New Investigator Recognition Award (NIRA), Orthopaedic Research Society (ORS). Oral and poster presentation in 2019 ORS Annual Meeting, Feb 2019, Austin, TX.
11. **Kwak, J.H.**, “NELL-1-based therapy prevents spaceflight-induced osteoporosis in rodents”, Musculoskeletal Research Seminar Series, Orthopaedic Hospital Research Center, UCLA. Nov 2018.
12. **Kwak, J.H.**, “Taking Craniofacial Patient Discoveries to the International Space Station”, RAC Research Seminar, UCLA School of Dentistry, Nov 2018.
13. Bedree, J.K., Huang, Y., Shi, J., Ha, P., Pan, H.C., Stodieck, L., Kim, J.K., He, X., Wu, B., **Kwak, J.H.**, Ting, K., Soo, C., Shi, W. “Microgravity induces alpha diversity increase in the gut microbiome of rodents”. American Society for Gravitational and Space Research (ASGSR) Oct-Nov 2018. Bethesda, MD.
14. Stodieck, L. **Kwak, J.H.** et al. “Four weeks of recovery after 30 days of flight on board the ISS restores strength but only partially restores muscle size in mouse hindlimbs”. American Society for Gravitational and Space Research (ASGSR) Oct-Nov 2018. Bethesda, MD.

15. **Kwak, J.H.**, Shi, J., Ha, P., Pan, H.C., Zhang, Y., Chen, E., Stodieck, L., Kim, J.K., Ding, C., Zhang, X., Wu, B., Ting, K., Soo, C. “Rodent Research 5 mission: NELL-1-based therapy prevents spaceflight-induced osteoporosis in rodents”. American Society for Gravitational and Space Research (ASGSR) Oct-Nov 2018. Bethesda, MD.
16. **Kwak, J.H.** Keynote presentation: “Taking Craniofacial Patient Discoveries to the International Space Station”. 2nd Orthopedic Research Society (ORS) SoCal Symposium, Oct 12th, 2018. Cedars Sinai, Los Angeles, CA.
17. **Kwak, J.H.** “Briefing on Rodent Research 5 Mission: NELL-1-based therapy prevents long-duration spaceflight-induced osteoporosis in rodents”. ISS R&D Conference, July 2018. San Francisco, CA. ** Winner of the 2018 ISS Innovation Award from the American Astronautical Society.

- **Poster Presentations and Awards:**

1. Liu T., Velicu D.B., Dong S., Tran L., Maram S.S., Low J., Shi J., Ting K., Soo C., **Kwak J.H.** Spaceflight-induced osteoporosis in non-weight-bearing bone and treatment with BP-NELL-PEG. ASBMR Annual Meeting, 11-15 September 2020, Virtual event.
2. Dong N*, Velicu D, Low J, Tran L, Duong T, Shi J, Kwak J.H. (Senior author) BP-NELL-PEG systemic therapy for spaceflight-induced osteoporosis in craniofacial vs. postcranial bones. Student Table Clinic presentation. 2020 AAO Annual Session, Atlanta, GA, USA. May 1-3, 2020. Virtual event.
3. Dong, N.*, Velicu, D., Low, J., Tran, L., Duong, T., Shi, J., Nguyen, T., **Kwak, J.H. (Senior author)**. Development of TMJ-OA in spaceflight and treatment with BP-NELL-PEG therapy. UCLA School of Dentistry Research Day. March 2020.
4. Tran, L.*, Luo, X., Wu, S., Dong, N., Pham, E., Lee, M., Lee, T., Tran, P., Hom, B., Soo, C., **Kwak, J.H. (Co-senior author)**. Nell-1 Inactivation in Osteoblast Causes Hemifacial Microsomia and Postcranial Osteoporosis. IADR/AADR/CADR General Session, March 2020. Washington, D.C.
5. Maram, S.* , Ha, P., Shi, J., Tran, L., Dong, N., Zhang, Y., Pan, H.C., Kim, J.K., Easley, J., Ting, K., Soo, C., **Kwak, J.H. (Co-senior author)**. Systemic therapy of bisphosphonate conjugated NELL-PEG reverses osteoporosis in sheep. IADR/AADR/CADR General Session, March 2020. Washington, D.C. *Accepted for poster presentation. ** Selected as finalist in the IADR Innovation Award for Excellence in Orthodontics Research.*
6. Wu, S.* , Tran, L., Luo, X., Dong, N., Pham, E., Lee, M., Lee, T., Tran, P., Hom, B., Soo, C., **Kwak, J.H. (Co-senior author)**. Nell-1 Inactivation in Osteoblast Causes Hemifacial Microsomia and Postcranial Osteoporosis. ORS Annual Meeting, Feb 2020, Phoenix, AZ.
7. Low, J., Velicu, D., Tran, L., Shi, J., Duong, T., **Kwak, J.H. (co-senior author)**, Soo, C. “Development of TMJ-OA in space microgravity and treatment with the novel compound

BP-NELL-PEG”. Poster Fair for STTP, David Geffen School of Medicine, UCLA. July 2019.

8. Shi, J. (presenter), Ha, Pin, Pan, H.C., Kim, J.K., Zhang, Y., Chen, E., Duong, T, Baik, L., ***Kwak, J.H. (co-senior author)**, *Ting, K. “Inactivated-bisphosphonate boosts bone-targetedness of systemic NELL-1 therapy for spaceflight-induced osteoporosis”. Oral and poster presentations. UCLA Research Day Feb 2019. ** 1st place winner in master/residents category
 9. **Kwak, J.H.**, Shi, J., Ha, P., Pan, H.C., Zhang, Y., Chen, E., Stodieck, L., Kim, J.K., Zhang, X., Wu, B., Ting, K., Soo, C. “NELL-1-based Systemic Therapy Prevents Long-duration Spaceflight-induced Osteoporosis In Rodents”. Chosen as Finalist for New Investigator Recognition Award (NIRA), Orthopaedic Research Society (ORS). Oral and poster presentation in 2019 ORS Annual Meeting, Feb 2019, Austin, TX.
 10. Jiayu Shi, Hsin Chuan Pan, Pin Ha, Jong Kil Kim, Yulong Zhang, Eric Chen, Lloyd Baik, ***Jin Hee Kwak (co-senior author)**, *Kang Ting. Innovative Systemic BP-NELL-PEG Therapy for Microgravity-induced Osteoporosis. PCSO Annual Meeting, Oct 2018, Monterey, CA.
4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?

The AAOF BRA allowed us to focus the time, effort and resources to the analysis of craniofacial bones specifically in the large-scale Rodent Research 5 spaceflight mission funded by several sponsors including NIH, CASIS and NASA, Colgate and AAOF. In addition, the AAOF BRA was an incredible resource for Dr. Kwak’s career development and student education.

This project provided educational and training opportunities for the following four students at UCLA: (1) Diana Beatrix Velicu, an orthodontic resident enrolled in Oral Biology Master’s program, UCLA School of Dentistry, (2) Josiah Low and Timothy Liu, medical students at UCLA School of Medicine, and (3) Luan Tran, a PhD student in Oral Biology, UCLA School of Dentistry. They contributed significantly in performing experiments and presenting data in scientific conferences and school research meetings.

Accounting for Project; (i.e.), any leftover funds, etc.

As per the budget plan, the funds were used for supplies and other expenses (core services, statistician and travel). Remaining fund: \$6,049.87.