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TITLE: Bone-Targeted Delivery of TGF-Beta Receptor Inhibitor as a Novel Treatment for Osteoarthritis

PRINCIPAL INVESTIGATOR: Gehua Zhen

CONTRACTING ORGANIZATION: Johns Hopkins University

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Background: Military personnel is highly susceptible to Osteoarthritis (OA) because of the work environment and frequent battlefield injuries. Joint pain is the primary symptom of OA that profoundly affects the quality of life and physical capabilities of the patients. Current treatments for OA pain are limited due to unacceptable side effects or short-term efficacy. Importantly, long-term treatment with pure analgesia drugs has adverse effects on OA as the primary perception of the pathological changes within the joint is blunted. Our prior studies have demonstrated the critical role of subchondral bone in maintaining the homeostasis of articular cartilage. Importantly, the subchondral bone is also a plausible source of OA pain. In the present study, we investigated whether the ALN-LY, our newly synthesized compound, possesses a synergistic effect of reversing OA pathological changes and relieving OA pain.

Our previous studies showed that high levels of transforming growth factor-beta (TGFβ) in subchondral bone initiate uncoupled subchondral bone formation and promote degeneration of articular cartilage. Inhibition of TGFβ signaling successfully improved the structure of subchondral bone and attenuated cartilage degeneration. Increased osteoclasts activity promotes the innervation of calcitonin gene-related peptide (CGRP) positive nociceptive nerve fibers into the subchondral bone and contributes to OA pain. On the other hand, TGFβ is a well-known anabolic factor for articular cartilage and has a broad spectrum of functional activity on other organs/tissues throughout the body. The potentially detrimental effect on other organs/tissues hinders the process of TGFβ inhibitor being developed as an OA drug. To this end, we developed a novel compound by conjugating the alendronate (ALN) and TβRI inhibitor (LY2109761, LY). The unique characteristic of the high bone affinity of ALN ensures the bone-targeted delivery and sustained release of LY in the bone. Moreover, inhibition of osteoclast activity by ALN holds the promise to alleviate OA pain by suppressing the nociceptive innervation into the subchondral bone.

Specific Aims: Aim 1: Evaluate safety and efficacy of ALN-LY conjugate in the post-traumatic OA mouse model. Aim 2: Determine the effect of the conjugate in alleviating joint pain and improving functional outcome in the post-traumatic OA mouse model.

Major findings: The significant alteration of subchondral bone structure in the vehicle-treated ACLT mice was observed at 1-month post-surgery by micro-CT analysis. The subchondral bone structure was substantially improved in the ALN-LY treated mice. Trap staining and phospho-Smad2/3 immunohistological staining exhibited that ACLT induced osteoclastogenesis and subsequent activation of TGFβ in subchondral bone were alleviated in ALN-LY treated group during OA progression. Safranin O-Fast green staining showed that ACLT induced loss of proteoglycan in articular cartilage observed in the ACLT-vehicle group was prevented by ALN-LY treatment at 8 weeks post-surgery. Consistently, the OARSI score of the ACLT-ALN-LY group was significantly lower than that of the ACLT-vehicle group. Additionally, immunohistological staining of matrix metallopeptidase 13 (MMP13), Type X collagen, and aggrecan showed that ACLT induced hypertrophic chondrocytes and proteoglycan loss in articular cartilage were prevented by ALN-LY treatment at 8 weeks post-surgery. ALN-LY treatment compromised subchondral bone nociceptive innervation and nociceptive neuron activation significantly. Moreover, improving subchondral bone structure by ALN-LY significantly downregulated the activity of Na^+ channel Nav1.8 in DRG neurons that innervates subchondral bone. The amplitude and frequency of the action potentials elicited in the DRG neurons after ACLT was substantially decreased by ALN-LY treatment. Therefore, ALN-LY showed a promising effect in joint pain relief in the OA mouse model.

Impact: Since there is a lack of a disease-modifying drug on the market for OA, the success of the work described in this proposal will facilitate and accelerate therapeutic development of this disease. Developing effective disease-modifying therapy for OA could greatly reduce the necessity of joint replacement surgery and medical expenditure. This proposed study directly impacts the FY18 PRMRP Area of Encouragement: "Basic and translational research to identify treatments to mitigate and/or reverse osteoarthritis, particularly in the knee, hip, ankle, and shoulder".

Military Relevance: The proposed research project, by bone-targeted delivery of TGFβ inhibitor, holds the promise of reducing the burden of OA suffered by military Service members, Veterans, and their family members and caregivers.

15. SUBJECT TERMS NONE LISTED					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
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Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Military personnel is highly susceptible to Osteoarthritis (OA). Both articular cartilage degeneration and subchondral bone malformation are primary concerns of OA. There is no effective disease-modifying treatment for OA except joint replacement surgery. Particularly, we are lacking fundamental treatment of OA pain although pain is the most prominent symptom of OA and the common reason that military personnel are discharged from service. The project aims to develop new therapies to attenuate OA degeneration and alleviate OA pain, thereby improving the quality of life of military soldiers. The structural and functional integrity of articular cartilage highly relies on its biochemical and biomechanical interplay with the subchondral bone. We have demonstrated that high levels of TGFβ in subchondral bone initiates uncoupled subchondral bone formation and promote the degeneration of articular cartilage. Inhibition of TGFβ signaling successfully improved the structure of subchondral bone and attenuated cartilage degeneration. However, TGFβ is a well-known anabolic factor for articular cartilage and has a broad spectrum of functional activity on other organs/tissues throughout the body. The potential detrimental effect on other organs/tissues hinders the process of TGFβ inhibitor being developed as an OA drug. To reach the purpose of both osteoclast inhibition and subchondral bone tissue-specific TGFβ inhibition, we developed a new drug (ALN-LY conjugate) that links a TGFβ inhibitor LY2109761 (LY) to an osteoclast inhibitor, Alendronate (ALN), through a metabolically hydrolyzable linker. We designed experiments to test ***1)*:** Whether ALN-LY conjugate can effectively rescue subchondral bone abnormalities and attenuate AC degeneration by specifically inhibiting TGFβ signaling in bony tissue. ***2)*:** whether ALN-LY conjugate can alleviate OA symptom by suppressing subchondral bone turnover and consequent nociceptive innervation.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Alendronate; Articular cartilage; Nociceptive innervation; Osteoarthritis; Osteoclast; Pain; Subchondral bone

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

1. Local IACUC Approval and ACURO Approval	Completed (Aug 15th, 2019)
2. ACLT/Sham surgery and pharmaceutical interventions after surgery	Completed (March 30th, 2020)
3. Tissue specimen collection and specimen processing	Completed (April 30th, 2020)
4. Evaluation of treatment efficacy in reversing joint pathologies	Completed (June 15th, 2020)
5. Evaluation of functional outcome after treatment	Completed (May 30th, 2020)
6. Determine tissue distribution of conjugate	~60% of completion
7. Immunofluorescence staining of CGRP and IB4	~80% of completion
8. Examine the evoked activity of L4 DRG neurons	Completed (May 15th, 2020)

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities: Three months old C57BL/6J (WT) mice that subjected to anterior cruciate ligament transection (ACLT) or sham surgery were randomized into 6 groups: sham-vehicle, ACLT-vehicle, ACLT-ALN (100 μg/kg ALN), ACLT-ALN-LY (100 μg/kg), ACLT-LY (100 μg/kg), ACLT-ALN/LY compounds (100 μg/kg LY and 100 μg/kg ALN). All mice received the intraperitoneal injections three times a week for 1 month, or until sacrifice at 2 weeks or 4 weeks or 8 weeks after surgery. The structure of the subchondral bone was determined by micro-CT analysis, cartilage degeneration was evaluated by the OARSI grading system. Osteoclast activity was examined by tartrate-resistant acid phosphatase (Trap) staining and immunohistological staining. Immunofluorescence staining of CGRP was performed to examine the nociceptive innervation in the subchondral bone and $CGRP^+$ neuronal activation at the L4 DRG level. The function outcome was determined by pain behavior tests (Von Frey, voluntary wheel running activity, and gait analysis). The action potential of the DRG neurons were measured by patch clamp.

2) Specific objectives: examine the effect of ALN-LY conjugate in (1) improving subchondral structure, (2) attenuating articular cartilage degeneration, (3) reducing nociceptive signals transmitted by DRG neurons, (4) improving functional activity, in OA mouse model.

3) Key outcomes: (1) We first tested if the conjugate can successfully inhibit TGF-β signaling in vitro. We treated human MSCs with the conjugate and found that the TGF-β signaling was effectively inhibited as evidenced by a significant reduction of pSMAD2/3. Then we examined the efficacy in preventing the development and progression of OA in anterior cruciate ligament transection (ACLT) mouse model. Trap staining and phospho-Smad2/3 immunohistological staining exhibited that ACLT induced osteoclastogenesis and subsequent activation of TGFβ in subchondral bone were alleviated in ALN-LY treated group during OA progression (at 2 weeks and 4 weeks post-surgery). The effect of ALN and ALN/LY treatment on inhibiting osteoclast activity was comparable to that of the ALN-LY treatment. However, none of the ACLT groups except the ALN-LY mitigate the abnormal bone formation and over-activation of TGFβ signaling (**Fig. 1**).

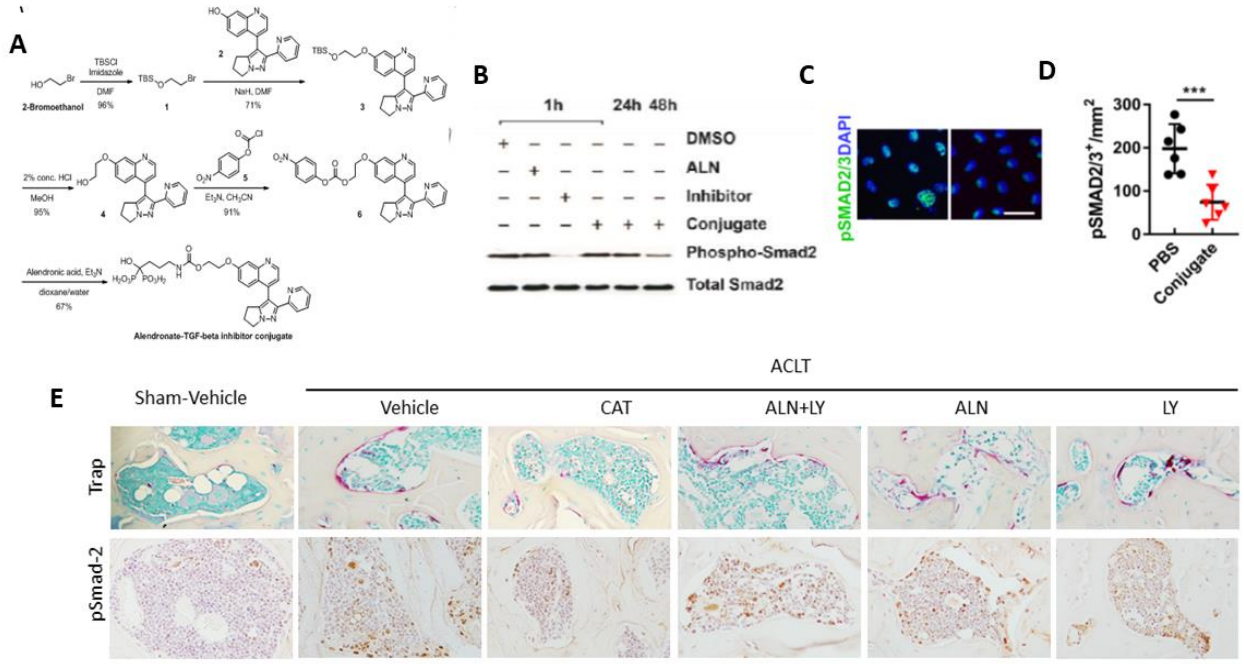

Fig 1. The ALN-LY conjugate inhibits TGF-β signaling. (A) Synthesis and chemical structure of the conjugate. **(B)** Western-blot analysis of pSmad2 in the cell lysate of mesenchymal stem cells. **(C-D)** Immunofluorescence staining of pSmad2 in mesenchymal stem cells, left: PBS treated group, right: conjugate treated group. **(E)** Trap staining (purple) and pSmad2 staining (brown) in the tibia subchondral bone of the mice at 2 weeks (trap staining) or 4 weeks (pSmad2 staining) post surgery.

(2) The significant alteration of subchondral bone structure in the vehicle-treated ACLT mice was observed at 1-month post-surgery by micro-CT analysis. The subchondral bone structure was substantially improved in the ALN-LY treated mice as evidenced by decreased total tissue volume (TV), lowered trabecular pattern factor (Tb.pf), and decreased thickness of subchondral bone plates (SBP Th), as compared to that of the vehicle-treated mice post ACLT. The sole treatment of ALN, LY, or ALN/LY did not show a significant beneficial effect in reversing the ACLT induced pathological changes in the subchondral bone (**Fig. 2**).

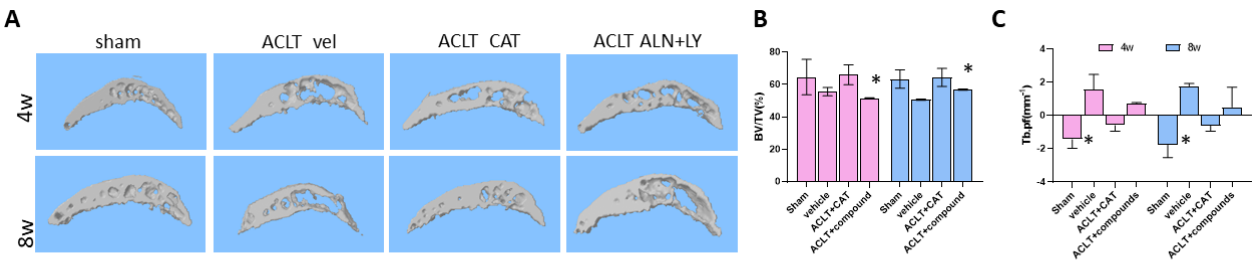

Fig 2. ALN-LY conjugate treatment prevented ACLT induced subchondral bone deterioration in OA mouse model. (A) Representative image of 3D reconstructed μCT images. Vel: PBS, CAT: conjugate, compounds: ALN+LY. **(B-C)** Quantitative analysis of (A).

(3) Safranin O-Fast green staining showed that ACLT induced loss of proteoglycan in articular cartilage observed in the ACLT-vehicle group was prevented by ALN-LY treatment at 8 weeks post-surgery. Consistently, the OARSI score of the ACLT-ALN-LY group was significantly lower than that of the ACLT-vehicle group. The ALN, LY, or ALN/LY treated group slightly decreased the OARSI score relative to that of the ACLT-vehicle group but did not achieve a statistical difference. Additionally, immunohistological staining of matrix metallopeptidase 13 (MMP13), Type X collagen, and aggrecan showed that ACLT induced hypertrophic chondrocytes and proteoglycan loss in articular cartilage were prevented by ALN-LY treatment at 8 weeks post-surgery. Treatment of ALN, LY, or ALN/LY did not show a convincing beneficial effect in protecting ACLT induced degradation of articular cartilage (**Fig. 3**).

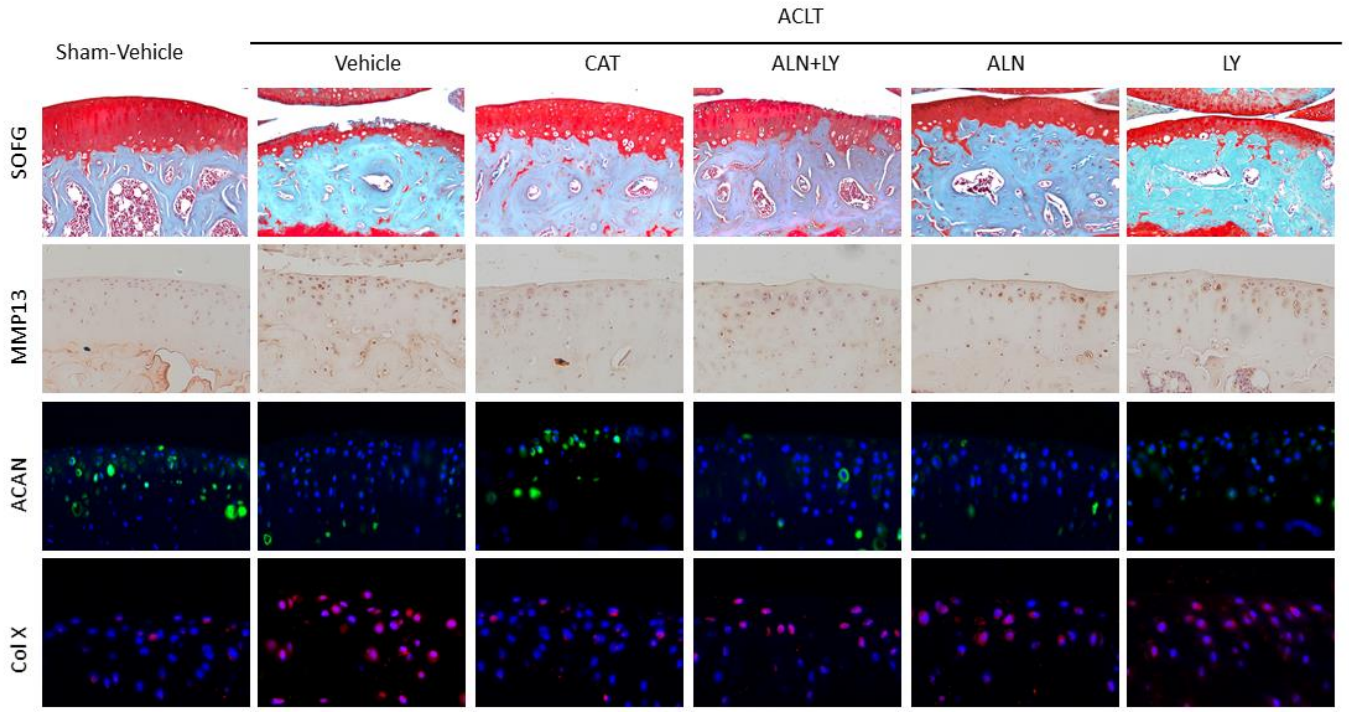

Fig 3. ALN-LY conjugate treatment attenuated articular cartilage degeneration in ACLT mice. Mice were sacrificed at 2 months post ACLT or sham surgery. Safranin O fast green (SOFG), MMP13 (brown), aggrecan (ACAN, green) and type X collagen (Col X, red) staining were conducted in articular cartilage of tibia epiphysis.

(4) Finally, increased nociceptive innervation to subchondral bone and activation of DRG nociceptive neuron were observed in ACLT vehicle-treated mice as compared to that of the sham-operated mice. The ALN, LY, ALN/LY treatment did not reverse the ACLT induced nociceptive innervation and activation. On the contrary, ALN-LY treatment compromised subchondral bone nociceptive innervation and nociceptive neuron activation significantly (**Fig. 4**).

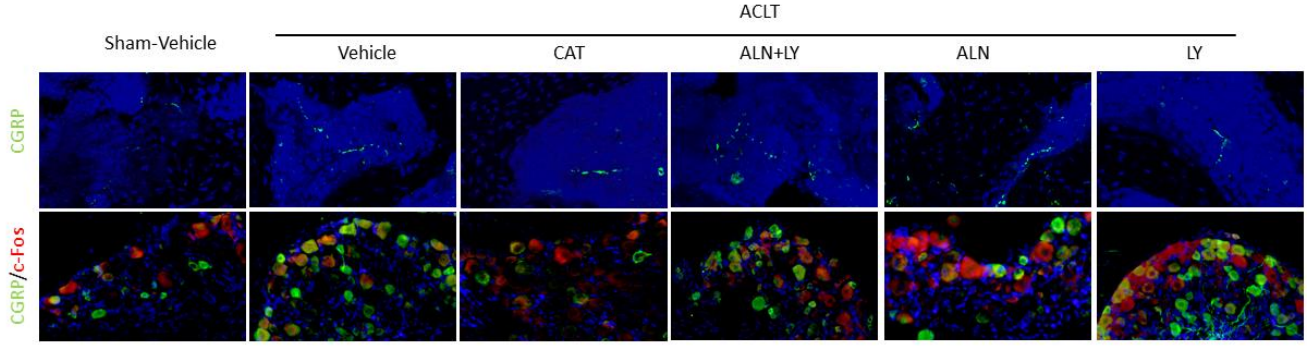

Fig 4. ALN-LY conjugate treatment attenuated articular cartilage degeneration in ACLT mice. CGRP (green, top row) staining in tibia subchondral bone and co-staining of CGRP (green) and c-Fos (red) in L4 DRG (bottom row) were conducted in mice sacrificed at 1 month post ACLT or sham surgery.

(5) We also performed a series of behavior tests to determine whether ALN-LY treatment successfully prevents the functional decline of mobility. In CatWalk gait analysis, the ACLT-vehicle group showed decreased maximum intensity, print area, and increased swing phase of the ipsilateral limb than the contralateral limb after 8 weeks of ACLT surgery. The disparity between the contralateral and ipsilateral hind limbs was significantly reduced in the ALN-LY group post ACLT as compared to that of the ACLT-vehicle group. No significant differences in maximum intensity, print area, and swing phase were observed between

the hind limbs in the ALN-LY treated mice. Consistently, the assessment of voluntary wheel running activity indicates that the daily well-being of ALN-LY treated mice was remarkably improved than vehicle-treated mice at 8 weeks post ACLT. The total traveled distance, active time, mean speed, and maximum speed in the ALN-LY group were significantly greater than ALN, LY, or ALN/LY treated group post ACLT. The secondary mechanical allodynia and hyperalgesia of the mice were measured by Von Frey analysis. We found that the paw withdraw frequency increased significantly at 4weeks and 8 weeks after ACLT relative to the sham-operated group whereas ALN-LY treated group showed significantly decreased paw withdraw frequency compared to the vehicle-treated group. Taken together, ALN-LY substantially attenuated ACLT induced articular cartilage damage and subchondral bone structural alteration. Importantly, ALN-LY showed a promising effect in joint pain relief in the OA mouse model (**Fig. 5**).

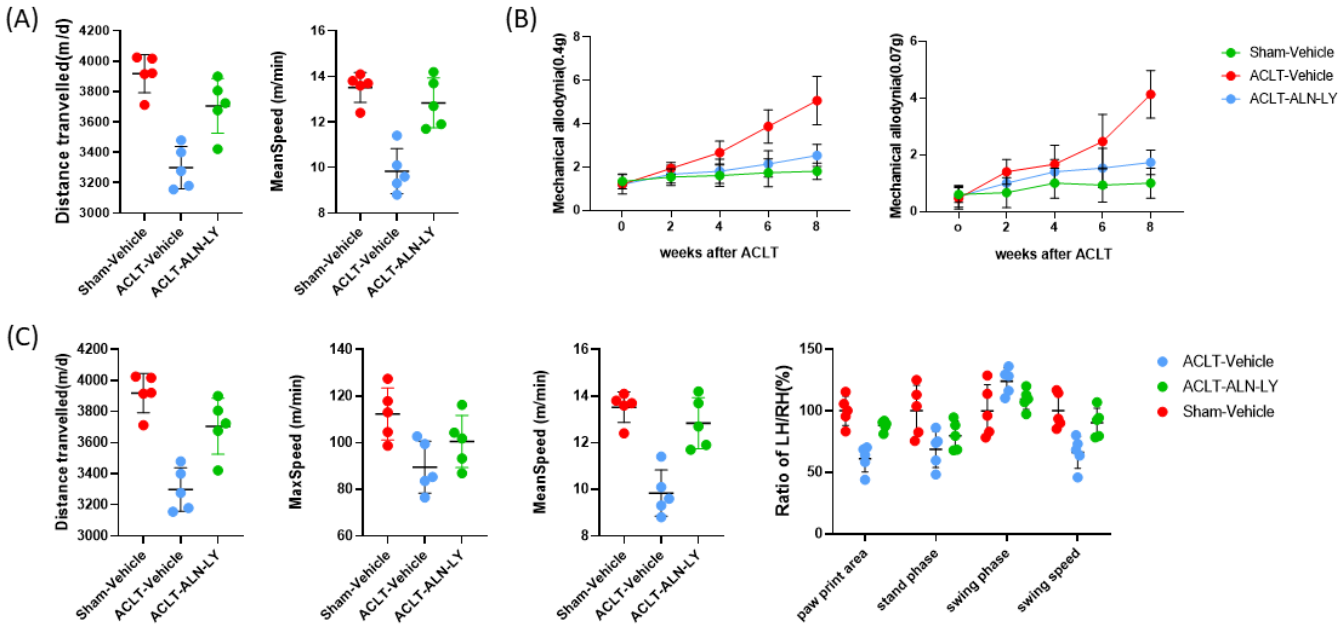

Fig 5. ALN-LY conjugate treatment attenuated articular cartilage degeneration in ACLT mice. Mice were sacrificed at 2 months post ACLT or sham surgery. (A) Spontaneous wheel running, (B) Von Frey tests, and (C) Gait analysis were performed in mice in different groups as indicated.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the next stage of study, we will complete the experiments identifying the nociceptive axons growth in subchondral bone by immunofluorescence staining of CGRP and IB4. We will also complete the pharmacokinetic examination of the ALN-LY in mice.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Since there is a lack of a disease-modifying drug on the market for OA, the success of the work described in this proposal will facilitate and accelerate therapeutic development of this disease. Developing effective disease-modifying therapy for OA could greatly reduce the necessity of joint replacement surgery and medical expenditure. The proposed research project, by bone-targeted delivery of TGFβ inhibitor, holds the promise of reducing the burden of OA suffered by military Service members, Veterans, and their family members and caregivers.

The goal of the proposed study is to determine the therapeutic efficacy of the conjugate in rescuing osteoarthritic joint pathologies as well as alleviating symptoms. The results are expected to provide a strong technological and theoretical foundation for future clinical trials. Moreover, because of the time limitation, we are not able to substantially investigate the toxicology and side-effect of the conjugate in the proposed study. The detailed pharmacokinetic profile and toxicology study are necessary for the next step drug development. Finally, at the late-stage OA when large cartilage defect already develops, a combination of multiple therapeutic approaches may be needed. The unique pharmacological characteristic of the conjugate makes it be a promising drug candidate to be tested in improving biomechanical microenvironment for AC when combined with cartilage regeneration techniques. The proposed project will enable us to generate preliminary data, optimize hypothesis and experimental design for the next step of investigations.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We requested NCE due to some of the experiments were delayed due to the pandemic of Covid-19. We have completed majority of the experiment during the period when our institute resumed operation.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Zhen G, Guo Q, Li Y, Wu C, Zhu S, Wang R, Guo XE, Kim BC, Huang J, Hu Y, Dan Y, Wan M, Ha T, An S, Cao X. Mechanical stress determines the configuration of TGFβ activation in articular cartilage. Nat Commun. 2021 Mar 17;12(1):1706. doi: 10.1038/s41467-021-21948-0. PubMed PMID: 33731712; PubMed Central PMCID: PMC7969741; Status of publication: Published; Acknowledgment of federal support (Yes).
2. Zhu J, Zhen G, An S, Wang X, Wan M, Li Y, Chen Z, Guan Y, Dong X, Hu Y, Cao X. Aberrant subchondral osteoblastic metabolism modifies $Na_V1.8$ for osteoarthritis. Elife. 2020 May 22;9. doi: 10.7554/eLife.57656. PubMed PMID: 32441256; PubMed Central PMCID: PMC7308086. Status of publication: Published; Acknowledgment of federal support (Yes).

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Targeted inhibition of TGF-β signaling in subchondral bone alleviates osteoarthritis pain. Qiaoyue Guo, Xu Cao, Gehua Zhen. 2021 MHSRS, Aug 23-26, 2021, Kissimmee Florida

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	Qiaoyue Guo
Project Role:	Graduate Student
Nearest person month worked:	6
Contribution to Project:	Ms. Guo has performed animal surgery, behavior tests, CT analysis and immunostaining.
Funding Support:	NIH R01 program.
Name:	Gehua Zhen
Project Role:	PI
Nearest person month worked:	1.2
Contribution to Project:	Dr. Zhen is responsible for overall scientific management, animal surgery, immunostaining, manuscripts/reports writing
Funding Support:	NIH P01 program; NIH R01 program.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

The PI, Dr. Gehua Zhen obtained a new funding support from NIH started at Jan 1st, 2021. In the present project, she had accomplished majority portion of the experiments she in charged. Therefore, she requested to reduce her effort to 10% during NCE, and her request had been approved by CDMRP.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*