AWARD NUMBER: W81XWH-22-1-0372

TITLE: Combinatorial Helper-Dependent Adenoviral Gene Therapy for Post-Traumatic Osteoarthritis

PRINCIPAL INVESTIGATOR: Brendan Lee

CONTRACTING ORGANIZATION: Baylor College of Medicine, Houston, TX

REPORT DATE: July 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2023	ANNUAL	01Jul2022-30Jun2023

4. TITLE AND SUBTITLE

Combinatorial Helper-Dependent Adenoviral Gene Therapy for Post-Traumatic Osteoarthritis

5a. CONTRACT NUMBER
W81XWH-22-1-0372

5b. GRANT NUMBER
GRANT13457255

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Brendan Lee, MD, PhD

E-Mail: blee@bcm.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Baylor College of Medicine
One Baylor Plaza
Houston, Texas 77030-3411

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Osteoarthritis (OA) is a common clinical condition with a prevalence of 40 million individuals in the U.S. More than 80% of those over the age of 55 have radiographic evidence of OA. Among those individuals, 30% present with significant pain or disabilities. However, current treatment of osteoarthritis is limited to life style modification, analgesics and invasive procedures such as joint replacement surgery in severe cases. First, we aim to identify the most efficient gene therapy approach to joint cells (both synovium and cartilage) for transferring genes. Second, we will test molecules identified by us that have cartilage protective functions during OA development. Finally, we plan to evaluate a gene therapy approach in a large animal model, which might provide insight into possible outcomes of human clinical trials. Our specific approach is to delivery combinations of genes that will protect cartilage as well as block inflammation only when inflammation is present to delay OA progression. This research might lead to the development of novel, more efficient and long-lasting new treatments for OA.

15. SUBJECT TERMS
Osteoarthritis (OA), Destabilization of the medial meniscus (DMM) surgery, Anterior Cruciate Ligament Transection (aCLT) surgery, monotherapy, combinatorial therapy, PRG4, IL-1RA, Helper Dependent Adenovirus (HDV)

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	17	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:**

   Osteoarthritis (OA) is a degenerative joint disorder characterized by loss of articular cartilage, subchondral remodeling, inflammation with synovitis and pain. OA arising due to joint injury (termed "post-traumatic OA" or PO-OA) accounts for approximately 12% of OA incidence and corresponds to a healthcare expenditure of over $3 billion annually. In addition to post-traumatic or age-related disease, OA can occur in rare instances early in life (termed "early-onset OA" or EO-OA) as a result of skeletal dysplasias. In our proposal, we will study the effects of combinatorial gene therapy vs. monotherapy via intra-articular injection in murine models of post-traumatic (DMM or aCLT surgical models), age-related and genetic early-onset OA. Furthermore, with the validation of the efficacy determined from the murine OA models, we will employ equine carpal chip model to check the efficacy and toxicity of gene therapy. For the gene therapy, we are using helper dependent adenovirus (HDV) platform which is an effective approach for delivering due to long-term, high level gene expression with effective transduction in non-dividing cells and large capacity for delivering multi-gene therapeutic cassettes. In brief, our studies include following genes: monotherapy of 1) potential advantage of regulated expression of IL-1RA under NFκB promoter in OA; 2) chondroprotective role of PRG4 expression in OA and 3) combinatorial therapy of IL-1Ra:PRG4 vectors synergistically delaying OA progression compared with each vector alone. We will access the efficacy of the murine and equine vectors in the respective models of OA as well as the local and systemic toxicity associated with the therapy. We will assess the efficacy of gene therapy by functional behavior test and histological analysis. We will also assess the impact of pre-existing anti-Ad5 adaptive immune response to the gene therapy to overcome a potential reduction of efficacy by systemic pre-existing Ad5 immunity. Our collective work will deliver the beneficial of combinatorial gene therapy of chondroprotective role of PRG4 and anti-inflammatory role of IL1Ra in OA.

2. **KEYWORDS:** Osteoarthritis (OA), Destabilization of the medial meniscus (DMM) surgery, Anterior Cruciate Ligament Transection (aCLT) surgery, monotherapy, combinatorial therapy, PRG4, IL-1RA, Helper Dependent Adenovirus (HDV)
3. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project?**
     - **Specific Aim 1**: Does intra-articular PRG4 gene therapy protect from post-traumatic OA in an equine model?
     - *Task 1: Cloning of equine Prg4.* Timeline months 1-4 (Jul-Sept, 2022)
       - We have synthesized a codon optimized equine Prg4 cDNA using equine cartilage RNA and inserted this construct into pLPBL shuttle vector downstream of EF1 ubiquitously expressing promoter. Expression of ePRG4 vector was verified by RT-PCR. **Completed.**

     - *Task 2: Generation and in vitro characterization of HDV-EF1-ePRG4 vector.* Timeline months 5-10 (Sept-Nov 2022)
       - We have completed the transfer of pLPBL-EF1-ePRG4 cassette into pΔ28 HDV backbone to generate pHDV-EF1-ePRG4. After confirming the sequence of pHDV-EF1-ePRG4 and expression of

ePRG4, we transfected, rescued and produced large scale of HDV-EF1-ePRG4 virus. With this virus, we verified the expression of ePRG4 by RT-PCR, western blot and ELISA after infecting 293 cells. **Completed.**

- *Task 3: Efficacy and toxicity of HDV-EF1-ePRG4 for equine OA.*
  - Cornell has obtained IACUC & ACURO approval for the gene therapy studies for equine OA.
  - The first 6 horses have been enrolled in the study, operated, injected with one of 2 HDV treatments (investigators masked to treatment) and are currently undergoing treadmill exercise with tissue collection scheduled for late August. **On-going.**

- *Task 4: Long-term assessment of HDV-EF1-ePRG4 gene therapy in equine OA.*
  - Cornell has obtained IACUC & ACURO approval for the gene therapy studies for equine OA.
  - *Task 4* long-term studies are all **pending based on the results of Task 3 short-term studies and mouse study.**

- **Specific Aim 2**: Does combinatorial over-expression of PRG4 and inflammation induced expression of IL-1RA via a single helper-dependent adenoviral gene therapy vector modify surgical, age-related, and genetic models of murine OA and a surgical equine model of OA?
- *Task 1: Generation and in vitro characterization of murine and equine single combinatorial HDV-EF1-PRG4:NFκB-IL-1RA vector.* Timeline months 10-16 month (we are head of SOW - Nov.2022 – Feb. 2023)
  - We have completed cloning both pHDV-EF1-ePRG4:NFκB-eIL-1RA (equine combinatorial vector) and pHDV-EF1-mPRG4:NFκB-mIL-1RA (murine combinatorial vector) and the construct sequences were verified.
  - We have confirmed both mouse and equine combinatory vector expression by RT-PCR and ELISA using 293 cells.
  - After confirming the expression of these combinatorial vectors, we prepared the large scale of DNA of pHDV-EF1ePRG4:NFκB-eIL-1RA (equine combinatorial vector) and pHDV-EF1-mPRG4:NFκB-mIL-1RA (murine combinatorial vector). With these viral vectors, we transfected, rescued and produced HDV-EF1-mPRG4:NFκB-mIL-1RA and HDV-EF1-ePRG4:NFκB-eIL-1RA virus. We further confirmed the production of PRG4 and IL1RA in 293 cells by ELISA. **Completed.**

- *Task 2: In vivo efficacy and toxicity of a single combinatorial vector HDV-EF1-mPRG4:NFκB-mIL1-RA in surgical, age-related, and genetic models of murine OA.* Timeline months 17-42 month (about 20% completed)
    - BCM obtained IACUC and ACURO approval for gene therapy studies using mouse OA models.
    - We optimized DMM (Destabilization of the Medial Meniscus) surgery induced murine OA model using 12 weeks old FVB male mice.
    - With this established surgical OA model, we performed monotherapy (HDV-EF1-mPRG4 and NFκB-mIL-1RA) and single combinatorial therapy (HDV-EF1-mPRG4:NFκB-mIL-1RA) along with controls (HDVAd0-empty and PBS vehicle) via intra-articular injection and sham control after 2 weeks of the DMM surgery.
    - Currently, we are collecting data of the functional behavior tests (CatWalk and hot plate assay) from these 5 treatment groups. **On-going.**
    - Both knee joints from the 5 treatment groups are collected for 3D volumetric analysis using phase contrast μCT and the OARSI histological analysis at 12 weeks after gene therapy. **We are still collecting knees and analysis are pending.**
    - Overall, we performed short-term efficacy of single combinatory PRG4/IL1Ra vs. PRG4 or IL1Ra monotherapy on DMM surgical murine OA model. Currently completing the functional behavior test and 2D and 3D histological quantification of knee joints are pending.
    - Task 2 of modeling aCLT (Anterior Cruciate Ligament Transection) surgical murine OA models with monotherapy vs. single combinatorial therapy is in plan.
    - Task 2 of aging and genetic mouse models of OA with HDVs therapy is in plan and pending.

- *Task 3: Effect of pre-existing anti-Ad5 antibody on intra-articular gene therapy and fiber serotype type switching.*
    - BCM obtained IACUC and ACURO approval for gene therapy studies using mouse OA models. **Completed.**
    - Task 3 will be performed next grant period reporting cycle.
- *Task 4: Combinatorial therapy in equine model of OA.*
    - **Pending based on** *Specific Aim1, Task 3.*

- **What was accomplished under these goals?**
    - Completion of large scale production of HDV virus for monotherapy and combinatorial therapy (*Specific Aim 1, Task 1&2 and Specific Aim 2, Task 1)*

      We have generated and produced a large scale of murine and equine combinatorial and monotherapy HDV (**Table 1**). We have checked the

viral infectivity and the expression of *PRG4* and *IL-1RA* by RT-PCR and ELISA.

Table 1. Monotherapy and combinatorial therapy HDV list

MONOTHERAPY			
VIRUS	**Vector**	**Species**	**Note**
BL mPRG4	pHDV-EF1-mPRG4	murine	no codon optimization
GQ mPRG4	pHDAd-EF1-mPRG4	murine	codon optimization
GQ eqPRG4	pHDAd-EF1-eqPRG4	equine	codon optimization
BL mIL-1RA	pHDAd-NFkb-mIL-1Ra	murine	no codon optimization
BL eqIL-1RA	pHDAd-NFkb-eqIL-1Ra	equine	no codon optimization
GQ mIL-1RA	pHDAd-NFkb-mIL-1Ra	murine	no codon optimization, cloned in the center of human genomic stuffer vector
GQ eqIL-1RA	pHDAd-NFkb-eqIL-1Ra	equine	not codon optimization, cloned in the center of human genomic stuffer vector
COMBINATORIAL THERAPY			
VIRUS	**Vector**	**Species**	**Note**
GQ mPRG4-mIL-1Ra	pHDAd-EF1-mPRG4:NFkB-mIL-1Ra	murine	only mPRG4 codon optimized
GQ eqPRG4-mIL-1Ra	pHDAd-EF1-eqPRG4:NFkB-eqIL-1Ra	equine	only eqPRG4 codon optimized

Each virus (10 vp/cell) was infected 293 cells for checking infectivity (**Figure 1**). Viral genomic copy number per cell showed comparable level based on the genomic human GAPDH (ghGAPDH) indicating all HDVs have similar infectivity to 293 cells.

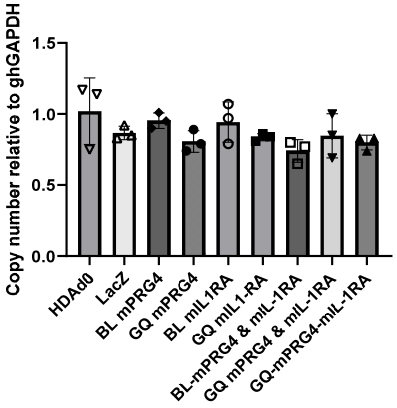

Figure 1. Viral genomic copy number of transgenes in 293 cells. 293 cells were seeded in 6-well-plate with 3x10^5 cells in 2mL of DMEM (10%FBS, 1% L-glutamine, and 1% penicillin and streptomycin) the day before infection. Infection was performed with MOI of 10 vp/cell. After 48 hrs of incubation, genomic DNA was extracted and performed qPCR and normalized using genomic human GAPDH (ghGAPDH). All HDVs have similar genomic copy numbers ranging around 1.0, suggesting a similar viral infectivity to 293 cells.

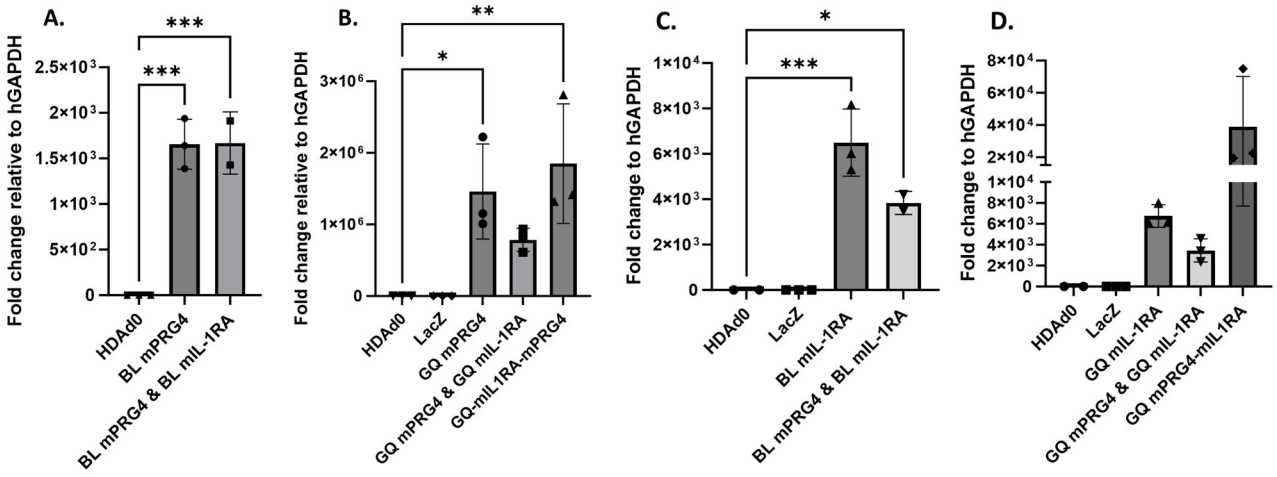

Figure 2. Transcription level of murine *PRG4* (A and B) and *IL-1Ra* (C and D) in 293 cells. 293 cells were seeded in 6-well-plate with 3x10^5 cells in 2mL of DMEM (10%FBS, 1% L-glutamine, and 1% penicillin and streptomycin) the day before infection. Infection was performed with MOI of 100 vp/cell. After 48 hrs of incubation, total RNA was extracted with Trizol and performed qRT-PCR. Murine *Prg4* (**A and B**) and *IL-1Ra* transcript levels (**C and D**) were normalized using human *GAPDH* (*hGAPDH*). One-way ANOVA with multiple comparison test were performed. N=3 for each group. **p< 0.05, **p< 0.01* and ****p< 0.001*.

We checked the transcription level of HDVs after infecting 293 cells (100 vp/cell) using qRT-PCR. Both BL mPRG4 and GQ mPRG4 (codon optimized) showed over-expression compared to negative control (HDAd0 and LacZ) (**Figure 2A and B**). GQ mPRG4-mIL-1Ra combinatorial HDV showed an additive over-expression compared to GQ mPRG4 (**Figure 2B**). Transcription levels of both mIL-1Ra HDVs (BL mIL-1Ra and GQ mIL-1Ra HDVs) were similar (6 x 10^3 folds, **Figure 2C and D**). Interestingly, we found drastic increased expression of *IL-1Ra* from GQ mPRG4-mIL-1Ra (single combinatorial HDV) compared to monotherapy virus (**Figure 2D**). Having multiple promoters (EF1 ubiquitous and NFκB regulatory promoter) in a single construct may contribute to the synergistic expression of *IL-1Ra* by creating open-chromatin structure at these regulatory regions. We further assessed the inflammation-induced transcription of murine (BL mIL-1Ra and GQ mIL-1Ra) and equine *IL-1Ra* (GQ eqIL-1Ra) under NFκB promoter by subjecting human recombinant TNFα (25ng/ ml). This *in vitro* assay mimics how IL-1Ra would be tightly regulated by the inflammation induced by OA in murine or equine model. This will allow to exert anti-inflammatory role of IL-1Ra during OA progression in vivo.

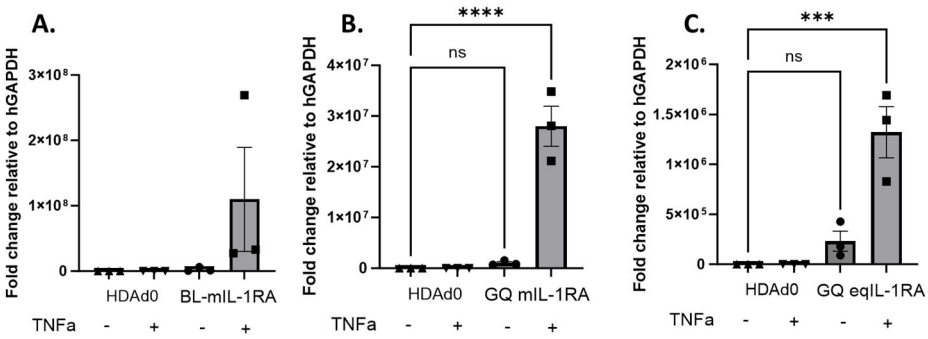

Figure 3. TNFα stimulation induced expression of IL-1Ra. 293 cells were seeded in 6-well-plate with 3x10^5 cells the day before infection. Infection was performed with MOI of 100 vp/cell. After 24hrs, cells were treated with hrTNFα (25ng/ ml). After 48 hrs, total RNA was extracted and performed qRT-PCR. Normalized using human *GAPDH* (*hGAPDH*). One-way ANOVA with multiple comparison test was performed. N=3 for each group. ****p< 0.001* and *****p< 0.0001*.

We found 10 to 100 folds induction of both murine (**Figure 3A and B**) and equine *IL-1Ra* (**Figure 3C**) upon TNFα treatment compared to the baseline (w/o TNFα).

The protein level of murine IL-1Ra was quantified by ELISA using 293 cell supernatant harvested post 48 hrs infection (100 vp/cell) (**Figure 4**). We found increased murine IL-1Ra protein level from GQ mPRG4-mIL-1Ra infected 293 cells (single combinatorial HRV) compared to GQ mIL-1Ra (monotherapy HRV). Interestingly, mIL-1Ra protein level from BL mIL-1Ra HDV was very low compared to GQ although their transcriptional levels were comparable (**Figure 2C and D**). We plan to repeat this experiment with subjecting human recombinant TNFα.

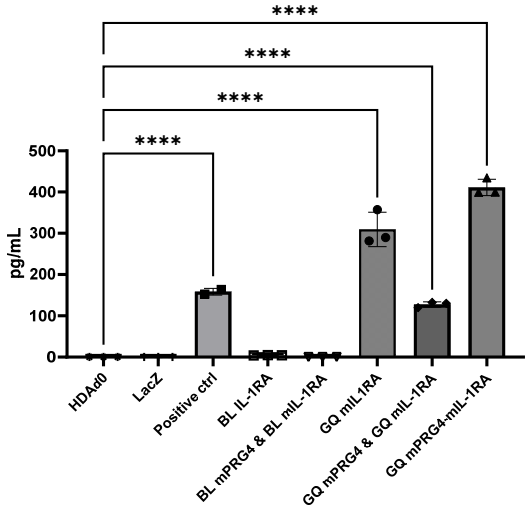

Figure 4. Quantification of protein level of IL-1Ra by ELISA using 293 cell. 293 cells were seeded in 6-well-plate with 3x10^5 cells the day before infection. After 48 hrs of infection, supernatant was harvested from each group and performed ELISA (R&D Systems, Catalog no/ MRA00) One- way ANOVA with multiple comparison test was performed. N=3 for each group. ****$p< 0.0001$.

We have also generated additional HDV virus of murine PRG4 and IL-1Ra with different insertional cloning orientations (Toward to ITR and Away from ITR in pLPBL shuttle vector) which known to affect the expression level of inserted gene. In addition, to assess the chondroprotective function of Hemopexin-like domain of mPRG4 *in vivo*, we have generated pHDAd-EF1-mPRG4 (999) with also two different orientations. These additional HDV virus (**Table 2**) will be employed to further understand the therapeutic efficacy with our existing HDV.

Table 2. HDV virus list

VIRUS	Vector	Species	Note
BL PN mPRG4 - 3276-1	pHDAd-EF1-mPRG4 (3276)-1	murine	no codon optimized, full Length mPRG4, orientation-toward ITR
BL PN mPRG4 - 3276-2	pHDAd-EF1-mPRG4 (3276)-2	murine	no codon optimized, full Length mPRG4, orientation-away from ITR
BL PN mPRG4 - 999-1	pHDAd-EF1-mPRG4 (999)-1	murine	mPRG4 hemopexin domain, toward ITR
BL PN mPRG4 - 999-2	pHDAd-EF1-mPRG4 (999)-2	murine	mPRG4 hemopexin domain, away from ITR
BL. PN mIL1RA-1	pHDAd-NFkb-mIL-1Ra-1	murine	Toward ITR
BL.PN mIL1RA -2	pHDAd-NFkb-mIL-1Ra-2	murine	Away ITR

- *In vivo* efficacy and toxicity of monotherapy and single combinatorial therapy in surgical, age-related, and genetic models of murine OA. (*Specific Aim 2 Task 2)*

To examine the efficacy of monotherapy and single combinatorial therapy in OA progression, we performed DMM surgery on both knees of 12 weeks old FVB male mice and HDV virus were delivered via intra-articular (IA) injection at post 2 wks of DMM surgery (**Figure 5A**). HDV virus of BL mIL-1RA, GQ mIL-1RA, BL mPRG4, GQ mPRG4 or GQ mPRG4 mIL-1RA (10^9 vp/ each knee) were injected into both knees. To access the functional outcome of monotherapy vs. single combinatorial therapy on OA, we have performed behavior test (CatWalk for gait analysis and Hotplate for pain assessment). In our preliminary data of CatWalk analysis, mPRG4 monotherapy groups (BL mPRG4, N=12 and GQ mPRG4, N=11) showed significantly elevated Right Hindlimb (RH) MaxContact-MaxIntensity-Mean compared to DMM surgery group (HDAd0, N=12) (**p< 0.01) and comparable level to Sham group (**Figure 5B**). Similar result was observed from the Left Hindlim (LH) MaxContact-MaxIntensity-Mean (**Figure 5C**). This preliminary result is consistent with our published data demonstrating the chondroprotective role of PRG4 in articular cartilage in OA progression. **We are still collecting additional CatWalk data of single combinatorial therapy group (GQ mPRG4 mIL-1RA).** Additional behavioral, molecular, cellular and histological analysis (Phase contrast μCT and OARSI score) **is all pending.**

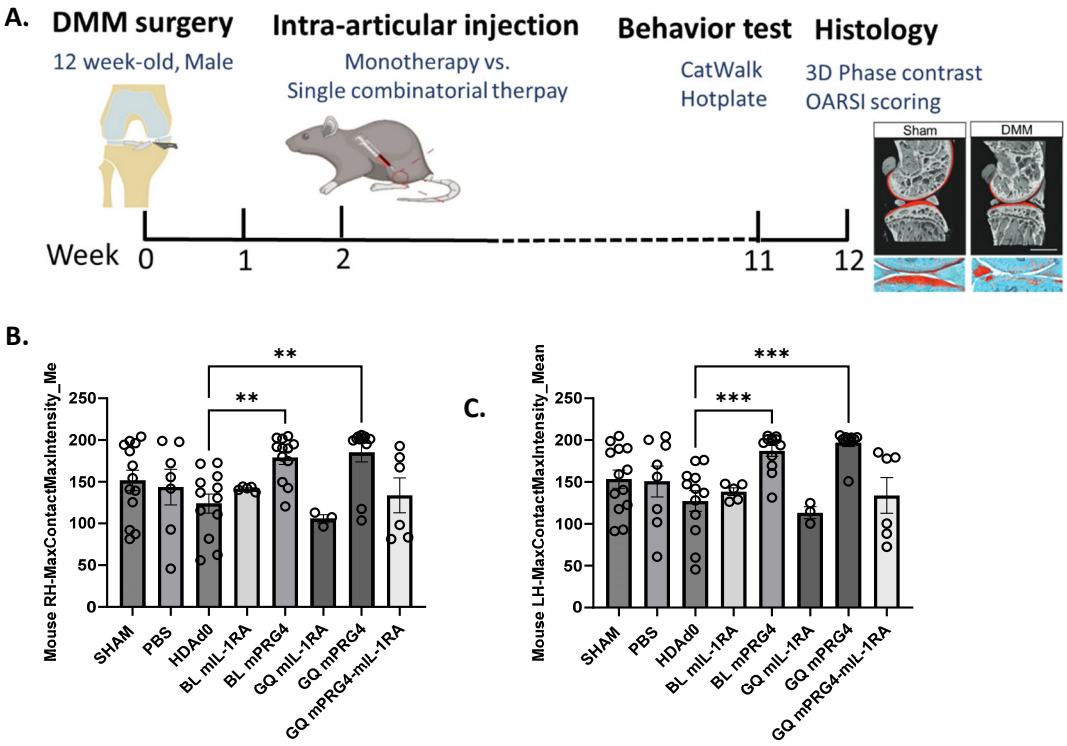

Figure 5. *In vivo* experimental scheme and CatWalk analysis. (A). DMM surgery was performed on twelve-week-old FVB male mice. Two weeks post-surgery, mice were received HDV via intra-articular injection into both knees (1e9 vp at each knee). Behavioral tests (Catwalk and hotplate) were performed during week 12 post surgery, followed by knee collection for histological and phase contrast μCT analysis. **(B and C).** At week 12 post surgery, mice were tested by Catwalk XT system in Transgenic Mouse Facility-Behavioral Core V007 at Baylor College of Medicine, with the MaxContactMaxIntensity_Mean value automatically recorded. (N=13 for SHAM, N=7 for PBS, N=12 for HDAd0, N=5 for BL mIL-1RA, N=12 for BL mPRG4, N=3 for GQ mIL-1RA, N=11 for GQ mPRG4, N=6 for GQ mPRG4-mIL-1RA, sample collection is still ongoing). Bars represent average ± SEM, and each point represents each animal. MaxContactMaxIntensity_Mean of DMM mice treated by BL mPRG4 and GQ mPRG4 were significantly higher than the HDAd0 control in both left and right hind limbs, suggesting a functional restoration effect. LH=Left Hindlimb, RH=Right Hindlimb. One-way ANOVA with multiple comparison test was performed. N=3 for each group. **p< 0.01* and ****p< 0.001*.

- **What opportunities for training and professional development has the project provided?**

  Nothing to Report

- **What opportunities for training and professional development has the project provided?**

  Nothing to report

- **How were the results disseminated to communities of interest?**

  Nothing to report

- **What do you plan to do during the next reporting period to accomplish the goals?**
  - For Specific Aim1, Task 3: Cornell plans to enroll an additional 18 horses in the short-term efficacy and toxicity study evaluating HDV-EF1-ePRG4, HDV-NFκB-IL-e1RA, HDV-Empty, and Vehicle treatments in the equine osteochondral chip surgery model in the next reporting period.

  - For Specific Aim2, Task 2: DMM surgical murine OA model does not recapitulate the OA progression in female mouse, which limits understanding the predominant OA population of aged woman. Therefore, we are currently optimizing the aCLT surgical murine OA models using FVB and C57BL6 male and female mice. We plan to assess the OA progression of aCLT model by functional behavior test and histological analysis including OARSI scoring and phase contrast μCT. We will use our DMM surgery result as a benchmark to compare the OA progression in aCLT model. We plan to perform IA injection of monotherapy (HDV-EF1-mPRG4 and HDV-NFκB-mIL-1RA) and single combinatorial therapy (HDV-EF1-mPRG4:NFκB-mIL-1RA) along with controls (HDVAd0-empty and PBS vehicle) to test efficacy and toxicity.
  - We plan to complete the functional behavior test of HDV gene therapy cohort with DMM surgery. We are currently collecting the CatWalk data from IL-1RA gene therapy group.
  - For joint pain measurement, we will also employ PAM (Pressure Application Measurement) test which is designed for measuring mechanical pain threshold on joint directly. This devise will allow to assess the knee joint hypersensitivity of mouse by OA progression and outcome of gene therapy. We are currently optimizing the use of PAM and data analysis of PAM.
  - The isoform function of PRG4 in the context of OA are not well understood, therefore, we have generated HDV expressing only Hemopexin-like domain of mPRG4. We will compare the *in vivo* efficacy of full length mPRG4 vs. Hemopexin-like domain of mPRG4 using DMM surgery.

4. **IMPACT:**
    - **What was the impact on the development of the principal discipline(s) of the project?** Nothing to report
    - **What was the impact on other disciplines?** Nothing to report
    - **What was the impact on technology transfer?** Nothing to report
    - **What was the impact on society beyond science and technology?** Nothing to report

5. **CHANGES/PROBLEMS:** Nothing to report
    - **Changes in approach and reasons for change**
    - **Actual or anticipated problems or delays and actions or plans to resolve them**
    - **Changes that had a significant impact on expenditures**
    - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
    - **Significant changes in use or care of human subjects**
    - **Significant changes in use or care of vertebrate animals.**
    - **Significant changes in use of biohazards and/or select agents**

6. **PRODUCTS:** Nothing to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
    - **What individuals have worked on the project?**

Name:	Brendan Lee
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0001-8573-4211
Nearest person month worked:	2.52
Contribution to Project:	Responsible for the overall implementation of the project, communication, and reporting of the grant
Funding Support:	NIH UC2AE0822200-01 Neuronal anatomy, connectivity and phenotypic innervation of the knee joint

Name:	Masataka Suzuki
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.20
Contribution to Project:	Investigate innate and acquired immune response in OA gene therapy
Funding Support:	No new support to report since submission

Name:	Philip Ng
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.80
Contribution to Project:	HDV vector platform design and virus production
Funding Support:	No new support to report since submission

Name:	Yangjin Bae
Project Role:	Faculty
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	8.82
Contribution to Project:	Assist overall management of the project, experimental design, data analysis, and reporting of the experiments in the grant
Funding Support:	No new support to report since submission

Name:	Zelong Dou
Project Role:	Post-doctoral associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	10.96
Contribution to Project:	Perform experiments proposed in the grant, generate data analysis data and report the data
Funding Support:	No new support to report since submission

Name:	Shamika Ketkar
Project Role:	Faculty
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	.87
Contribution to Project:	Perform experiments proposed in the grant, generate data analysis data and report the data
Funding Support:	No new support to report since submission

Name:	Donna Palmer
Project Role:	Research technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.97
Contribution to Project:	Assist experiments proposed in the grant, perform histology and imaging data analysis, generate data and report data
Funding Support:	No new support to report since submission

Name:	Lisa Yuva
Project Role:	Research technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.48
Contribution to Project:	Assist mouse surgery proposed in the grant, perform histology, generate data and report data
Funding Support:	No new support to report since submission

Name:	Racel Cela
Project Role:	Lab manager
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	10,71
Contribution to Project:	Assist mouse surgery proposed in the grant, overseeing the aliquoting and distribution of HDV. Manage animal protocol and animal husbandry.
Funding Support:	No new support to report since submission

Name:	Amanda Rosewell Shaw
Project Role:	Lab assistant
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3.60
Contribution to Project:	Assist mouse tissue harvesting and oversee histology core and perform histology proposed in the grant
Funding Support:	No new support to report since submission

Name:	Heidi Reesink
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.15
Contribution to Project:	Assist in the performance of surgical procedures and post-operative care for the equine model. Responsible for providing a preliminary report to the principal investigator for inclusion within the final report
Funding Support:	Cornell Sub, No new support to report since submission

Name:	Char Panek
Project Role:	Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.84
Contribution to Project:	Will assist with animal husbandry, veterinary care and ex vivo laboratory work
Funding Support:	Cornell Sub, No new support to report since submission

Name:	Lisa Thorson
Project Role:	Veterinary Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.42

Contribution to Project:	Responsible for horse husbandry and will assist with veterinary care and exercising horses
Funding Support:	Cornell Sub, No new support to report since submission

Name:	Isabella Joy Cervero
Project Role:	Undergraduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.30
Contribution to Project:	Assist with equine peri-operative care and record keeping.
Funding Support:	Cornell Sub, No new support to report since submission

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** Nothing to report.
- **What other organizations were involved as partners?** Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS** - None
9. **APPENDICES** - None