AWARD NUMBER: **W81XWH-22-1-0893**

TITLE: **Commandeering an RNA-Editing Enzyme to Correct DNA Nonsense Mutations Causing Duchenne Muscular Dystrophy**

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REPORT DATE: **October 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
OCTOBER 2023	Annual	15SEPT2022 - 14SEPT2023

SUBTITLE: Commandeering an RNA-Editing Enzyme to Correct DNA Nonsense Mutations Causing Duchenne Muscular Dystrophy

5a. CONTRACT NUMBER
W81XWH-22-1-0893

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California, Davis
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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

The main objective of this Idea Development Award is to lay the foundation in establishing an innovative DNA base editor that ameliorates the drawbacks and difficulties of current base-editing systems by commandeering a human RNA editing enzyme and redeploying it to serve as a DNA adenosine base editor, which would be able to correct all DMD-causing nonsense mutations. Based on our ADAR structures, we hypothesized that ADAR can deaminate adenosine in DNA, if the DNA strand is complexed with RNA, creating the requisite A-form duplex. Preliminary data does indeed show that a DNA:RNA hybrid can react with ADAR to deaminate 'A' in DNA creating deoxy-inosine (dI), a guanosine analog. Then, as demonstrated in the existing base-editors, during the next cycle of DNA replication this edit would exchange an A:T base pair to G:C. Therefore, since all stop codons contain A and T bases, by editing the 'A' in either the coding or non-coding strand of DNA, all nonsense mutations can be converted to a translatable codon, which would produce a full-length dystrophin protein and likely restore normal muscle function

15. SUBJECT TERMS

NONE LISTED

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
U	U	U	UU	12	19b. TELEPHONE NUMBER *(include area code)*

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1.) Introduction:

Duchenne muscular dystrophy (DMD) is an X-linked recessive disease that affects 1 in ~4,000 new-born males. Over 600 DNA point mutations have been documented to cause DMD, of which, 451 are nonsense mutations resulting in a truncated dystrophin protein causing disease in roughly 15% of all DMD cases. Genetically correcting these nonsense mutations in muscle or myoblast cells holds great promise as a potential cure and treatment for Duchenne muscular dystrophy. The main objective of this Idea Development Award is to lay the foundation in establishing an innovative DNA base editor, which ameliorates the drawbacks and difficulties of current base-editing systems, by commandeering a human RNA editing enzyme and redeploying it to serve as a DNA adenosine base editor, which would be able to correct all DMD-causing nonsense mutations. Based on our ADAR structures (Adenosine Deaminase Acting on RNA), we hypothesized that ADAR can deaminate adenosine in DNA, if the DNA strand is complexed with RNA, creating the requisite A-form double helix. Preliminary data does indeed show that a DNA:RNA hybrid can react with ADAR to deaminate 'A' in DNA creating deoxy-inosine (dI), a guanosine analog. Then, as demonstrated in the existing base-editors, during the next cycle of DNA replication this edit would exchange an A:T base pair to G:C. Therefore, since all stop codons contain A and T bases, by editing the 'A' in either the coding or non-coding strand of DNA, all nonsense mutations can be converted to a translatable codon, which would produce a full-length dystrophin protein and likely restore normal muscle function.

2.) Keywords:

DNA editing, Base-editor, Nonsense mutation, RNA editing, Adenosine Deaminase acting on RNA, ADAR, Stop codon.

3.) Accomplishments:

Major Goals: The major goals for the first year of this exploratory two-year grant is to (1) design RNA/LNA mixmers for DNA strand-invasion and editing, (2) test DNA strand-invasion using the RNA/LNA mixmers in both linear and plasmid DNA, (3) test ADAR editing efficiency of DNA in the DNA:RNA hybrids.

Major Goal Accomplishments: While there has been a slow start to the progress because of supply chain issues and searching for a qualified postdoctoral scholar, many encouraging accomplishments have been achieved this first year for Major Goals 1 and 3 above. We have been focusing our efforts on DNA base-editing to correct four common nonsense mutations in the dystrophin gene, *DMD*, which cause Duchenne muscular dystrophy. The four nonsense mutations in the coding strand of DNA are: Q45X, Q568X, Q1087X, and Q2182X. All these DMD-causing mutations have a glutamine codon of CAG where the C is mutated to a T to generate the premature stop codon of TAG (UAG in RNA). One goal is to administer guide-RNA/LNA mixmer oligos to complement to the DNA strand, facilitate DNA strand invasion creating an R-LOOP where the DNA:RNA/LNA mixed hybrid will serve as a substrate for ADAR, which we have shown in preliminary data for sample DNA sequences. Below we show great progress in editing the coding strand DNA in a DNA/RNA hybrid. However, one concern of targeting the DNA coding strand is that the guide-RNA oligo will also complement the mRNA of the *DMD* gene transcript, which may sequester much of the targeting guide-RNA preventing the oligo mixmer to enter the nucleus to strand-invade and edit the coding strand of DNA. Therefore, we have been focusing more effort on targeting the non-coding DNA strand of the DMD-causing nonsense mutations. All stop codons contain an A in the noncoding (or negative-sense) strand of DNA– the DNA compliment of TAG stop codon is 5'-CTA-3', where the 'A' in the non-coding strand will be targeted, which would revert the TAG stop codon to CAG, the original Glutamine codon, creating a full-length wild-type dystrophin protein.

The corresponding DNA sequences for both the coding and noncoding DNA strands for the four *DMD* nonsense mutations under study are given in **Table A** of the appendix. This table list the 150 nucleotide gBlock DNAs we have created centered around each nonsense mutation for both the coding and noncoding strand. We first tested the ability of ADAR (a RNA-editing enzyme that deaminates adenosines to inosine in regions of dsRNA) to edit the DNA strand of a mixed DNA:RNA (or LNA) hybrid. ADARs have been shown to display some sequence specificity so we first want to confirm that human ADARs can actually edit the disease-causing DNA mutation of the DNA strand in a DNA:RNA hybrid.

Using the gBlock 150mer-DNA strands (**Tables A & B** in Appendix), we formed the appropriate DNA:RNA hybrid by hybridizing the DNA 150mer to a 60mer RNA complementary sequence. For DNA:RNA hybrid substrates, single-turnover deamination assays were performed in 15 mM Tris-HCl pH 7.5, 3% glycerol, 60 mM KCl, 1.5 mM EDTA, 0.003% NP-40, 3 mM MgCl2, 160 U/mL RNAsin, 1 µg/mL yeast tRNA, 10 nM DNA/RNA hybrid, and 500 nM hADAR2. Each reaction was incubated at 30˚C for 30 minutes before the addition of ADAR enzyme. Reactions were then incubated at 30˚C for two hours prior to quenching with 190 µL of 95˚C nuclease-free water with timepoints taken at 0 minutes, 60 minutes, and 120 minutes. At 0 minutes, the reaction was quenched without protein. Reaction products were amplified using PCR (NEB Hot Start Taq Polymerase), then purified through a DNA clean and concentrator kit (Zymo) and subjected to Sanger Sequencing using Genewiz (Azenta).

Figure 1 shows the result of the deamination reaction for the Q45X noncoding DNA strand after a timepoint of 2 hours. As can be seen in the top of Figure 1 (zero time point), the TAT sequence is read by Sanger sequencing as TAT, but after two hours using the full-length human ADAR2 hyperactive mutant of E488Q, the Sanger sequencing shows a very large peak corresponding to the “G” nucleotide, and a small peak of the “A” nucleotide at this position. The ratio of the peak heights suggests that about 83% of the DNA sequence was edited by full-length human ADAR2 (E488Q mutant). **Figure 2** shows the results of editing the DNA coding strand for the Q2182X *DMD* mutation. After 2 hours, nearly 90% of the “A” is edited to inosine, which is read as “G” in Sanger sequencing. **This is the first known example of using ADAR to edit a DNA sequence corresponding to the *DMD* gene.** This is very encouraging news because it demonstrates that indeed ADARs can edit DNA of the *DMD* gene in a pre-formed DNA:RNA hybrid!

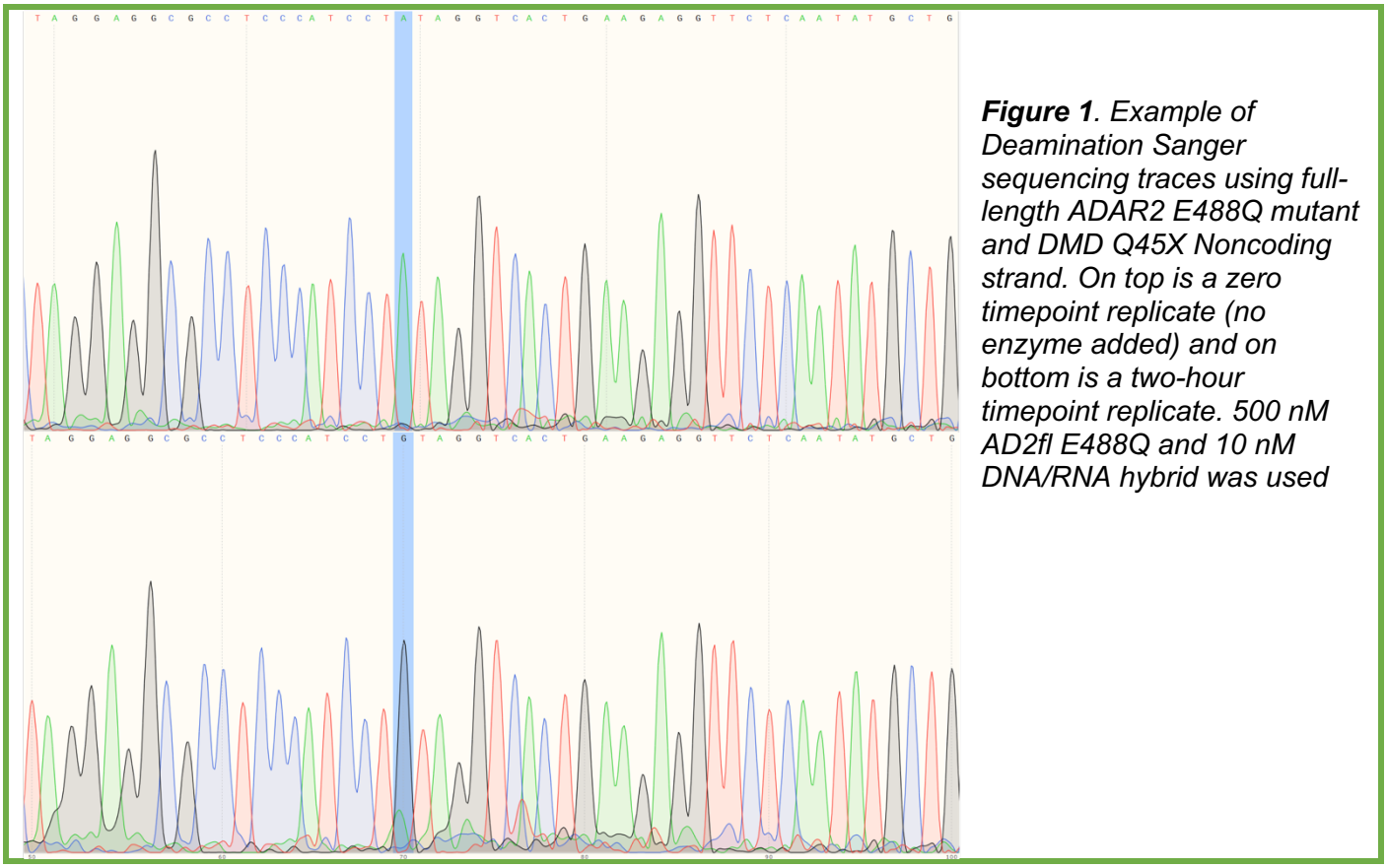

***Figure 1**. Example of Deamination Sanger sequencing traces using full-length ADAR2 E488Q mutant and DMD Q45X Noncoding strand. On top is a zero timepoint replicate (no enzyme added) and on bottom is a two-hour timepoint replicate. 500 nM AD2fl E488Q and 10 nM DNA/RNA hybrid was used*

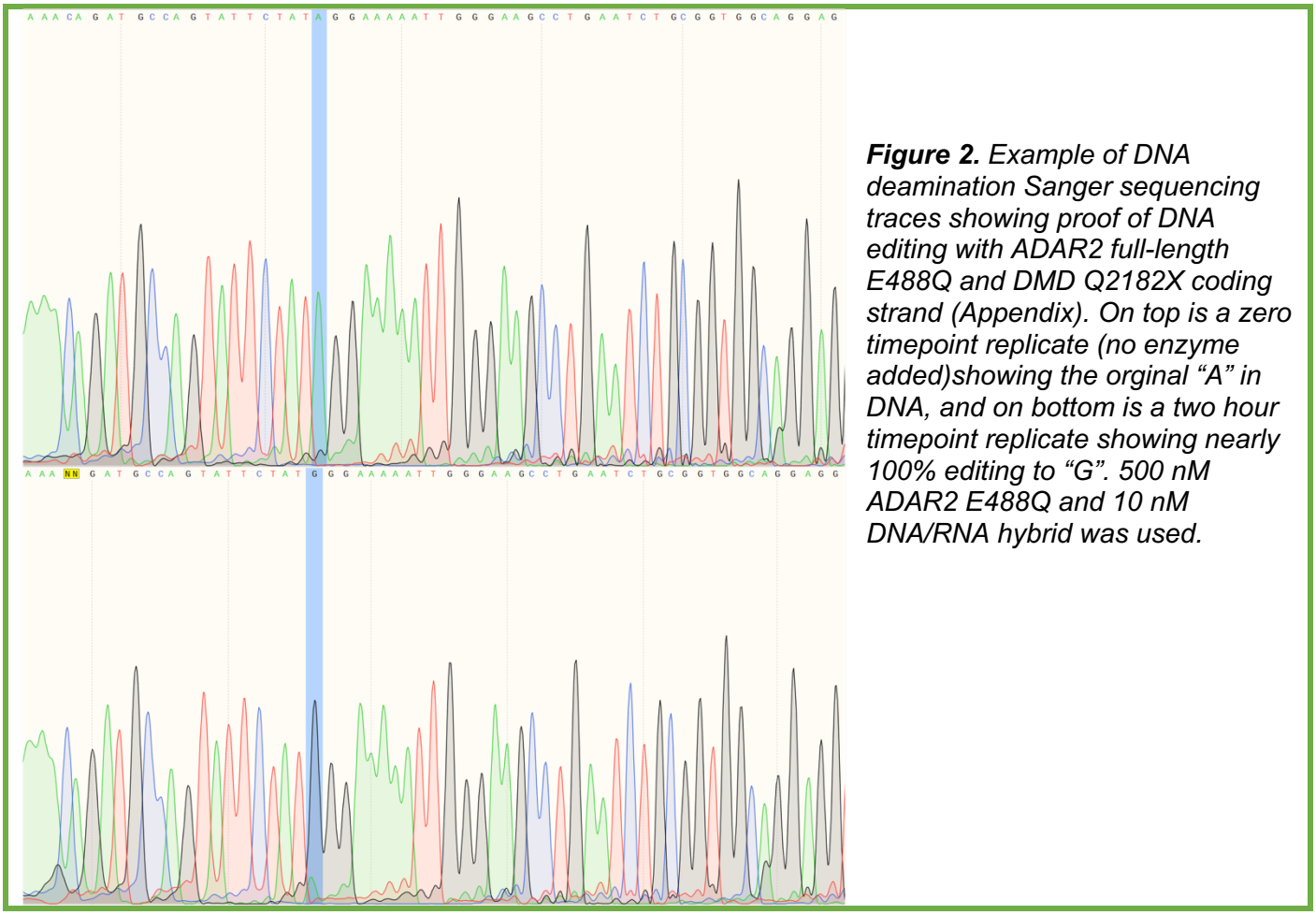

Figure 2. *Example of DNA deamination Sanger sequencing traces showing proof of DNA editing with ADAR2 full-length E488Q and DMD Q2182X coding strand (Appendix). On top is a zero timepoint replicate (no enzyme added)showing the orginal "A" in DNA, and on bottom is a two hour timepoint replicate showing nearly 100% editing to "G". 500 nM ADAR2 E488Q and 10 nM DNA/RNA hybrid was used.*

Thus far we have tried using ADARs for DNA editing on the coding and noncoding strands of DMD nonsense mutations that cause muscular dystrophy: Q568X, Q1087X and Q2182X. Using full-length human ADAR2 (hyperactive E488Q mutant), we have quantitated the level of editing to be 27%, 33% and 88%, respectively, for the three different *DMD* nonsense mutations on the coding strand (**Table 1**). We have also tested to edit the noncoding DNA strand (negative sense) for these mutations. So far, we have used ADAR2 to edit the Q45X and Q2182X mutations on the noncoding strand resulting in 83% and 33% "A" to "G" editing, respectively, after 2 hours (**Table 1**).

Table 1. DNA editing of DMD mutations by ADAR.

DMD Nonsense Mutation	**Percent DNA base-edited by human full-length ADAR2 E488Q**
Q568X (Coding strand)	27 ± 4
Q1087X (Coding strand)	33 ± 2
Q2182X (Coding strand)	88 ± 1
Q45X (Noncoding strand)	88 ± 1
Q2182X (Noncoding strand)	33 ± 6

All these assays used ADAR2 hyperactive E488Q mutant, which has been shown to have higher editing efficiency *[1, 2]*. We next tested if wild type ADAR2 would be able to also edit the DNA strands of the

noncoding sequences because this is what would naturally occur in the cells. Unfortunately, the two sequences we tested, corresponding to the Q45X and Q2182X *DMD* mutations, did not yield any editing after 2 hours. But this was one experiment, and we plan to repeat the experiment, and to also test if human ADAR1 can edit these mutations. ADAR1 is more highly expressed in muscle cells. Additionally, previously we have identified that using a nucleotide analog opposite the targeted A base to be edited can increase editing using wild type ADARs. We identified that using Benner's base Z opposite the targeted "A", in what we call the "orphan" position increases editing of RNA *[3]*. We will test if incorporating Benner's base Z in our guide RNA will also increase DNA editing with wild type ADAR1 and ADAR2.

Opportunities for Training: This research proposal has provided an excellent opportunity to train scientists at all levels, including Undergraduate students, Graduate students, and Postdoctoral researchers. This grant directly pays the salaries of the Graduate student: Jeff Cheng and Postdoctoral scholar: Abdul Shiraj. Additionally, one undergraduate student, Katherine Htut, is working with Jeff on running some of the DNA editing deamination reactions. My graduate student Jeff Cheng has had the opportunity to present his work at some local conferences here on campus, our Departmental Chemical Biology Retreat, and the Miller Symposium. At both events Jeff presented a poster on the DMD work funded by this grant. My postdoc, Abdul, started working in the lab a few months ago, so has not had the opportunity yet to present at any research conferences or workshops. Jeff has mentored one of my undergraduate students, Katherine, to increase her skill set. Katherine is planning to apply to Graduate School this coming Fall. Finally, all three of the trainees give presentations at our group meetings to update their research progress, and all have attended many other seminars offered on campus, throughout this past year for additional training.

Dissemination of Results: Nothing to report yet in publications. It is expected that publications will follow soon. Some of the initial results were presented at a Gordon Conference on RNA editing by PI, Andrew Fisher, who was an invited speaker.

Plans for the Next Year: The plans for the coming year are outlined in the original grant proposal. In this first year we have indeed shown that ADARs can selectively base-edit DNA of DMD-causing mutations when it is complexed with a guide strand of RNA. The next steps in the project are to create the strand-invading "mixmers" which are RNA oligomers that contain locked nucleic acids (LNAs), and we will also explore using Peptide-nucleic acids (PNAs), which have been reported to also help facilitate DNA strand invasion *[4, 5]*. The recently hired postdoc (Abdul), has significant experience in synthesizing oligomers that contain LNAs and PNAs. Unfortunately, there have been some supply chain issues with obtaining the phosphoramidites of the LNAs and of the PNA monomers required to synthesize the oligo-mixmers. But we have started to synthesize some LNA-containing RNA oligos to test for DNA strand invasion to create an R-loop.

The mixmers containing both RNA and LNA in the guide RNA oligos are currently being synthesized against the Q2182X DMD nonsense mutation because this displayed good editing with ADAR. **Figure 3** shows the 14 different mixmers of RNA and LNA combinations that we are presently synthesizing. We will determine which mixmers exhibit the best DNA strand invasion by measuring LNAs on the 5'end, 3'end or flanking both ends of the targeted A to be edited. The efficiency of DNA strand invasion will be monitored by CD spectroscopy as well as RNaseH digestion, and using anti DNA:RNA hybrid S9.6 antibodies for gel-shift experiments. Once we confirm strand invasion and R-loop formation, we will test ADAR's ability to edit the DNA strand of the DNA:RNA hybrid as outlined above.

DMD Q2182X Nonsense Mutation

```
Target DNA (-ve sense)  5/-…GCT TCC CAA TTT TTC CTA TAG AAT ACT CGG ATC…-3/
          Guide RNA     3/-…CGA AGG GUU AAA AAG GAC AUC UUA UGA GCC UAG…-5/
```

Target LNA-RNA mixmers (RNA=A/G/U/C**, LNA=a/g/t/c, C= orphan base)**

```
Mixmer 1    3/-…CGA AGG GUU AAA AAG GAC atc tta tga gcc tag…-5/
Mixmer 2    3/-…CGA AGG GUU AAA AAG GAC atc tta tga gcC Uag…-5/
Mixmer 3    3/-…CGA AGG GUU AAA AAG GAC Atc tta tga gcc tag…-5/
Mixmer 4    3/-…CGA AGG GUU AAA AAG GAC Atc tta tga gcC Uag…-5/
Mixmer 5    3/-…cga agg gtt aaa AAG GAC AUC UUA UGA GCC UAG…-5/
Mixmer 6    3/-…cga agg gtt aaA AAG GAC AUC UUA UGA GCC UAG…-5/
Mixmer 7    3/-…cga agg gtt aAA AAG GAC AUC UUA UGA GCC UAG…-5/
Mixmer 8    3/-…cga agg gtU AAA AAG GAC AUC UUA UGA GCC UAG…-5/
Mixmer 9    3/-…cga agg gtt aAA AAG GAC Atc tta tga gcc tag…-5/
Mixmer 10   3/-…cga agg gtt aAA AAG GAC Atc tta tga gcC Uag…-5/
Mixmer 11   3/-…cga agg gtt AAA AAG GAC AUc tta tga gcc uag…-5/
Mixmer 12   3/-…cga agg gtt AAA AAG GAC AUc tta tga gcC Uag…-5/
Mixmer 13   3/-…cga agg gtt aaa AAG GAC AUc tta tga gcc tag…-5/
Mixmer 14   3/-…cga agg gtt aaa AAG GAC AUc tta tga gcC Uag…-5/
```

Figure 3. *Sequence of the DMD Q2182X nonsense mutation. The top strand is the negative sense DNA strand harboring the "A" mutation causing the stop codon (yellow highlight). The 14 RNA/LNA mixmers being synthesized are shown. Uppercase letters are RNA, bold lowercase letters are LNA nuclotides. All will contain a "C" in the orphan position but will also test Benners base Z.*

Additionally, we will also incorporate some PNAs as part of the design both as part of the "origami" designed anti-sense oligonucleotides (**Figure 4**). Next, we will test if the R-loop created from the strand invasion will also be edited by ADARs. Given results we obtained in this past year showing editing of simple DNA/RNA mixed hybrids, we expect that the R-loop DNA mutation will be edited as well. We will test using both human ADAR1 and ADAR2, both wild type and hyperactive mutants. We will also explore using the Benner's base Z in the orphan position to determine if this increases editing using wildtype ADAR.

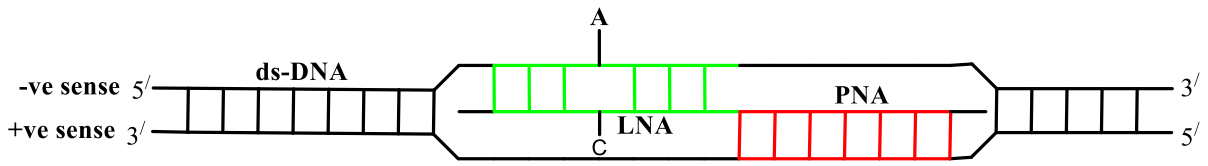

Figure 4. *Origami design using both RNA/LNA mixmer (bottom green strand), tethered to PNA (red strand), to help in DNA strand invasion. PNAs can also be designed against the top negative sense DNA strand to help facilitate strand invasion.*

Finally, we will collaborate with our colleague Dr. Keith Baar (UC-Davis), to transfect the most promising RNA/LNA/PNA mixmers (antisense oligonucleotides, ASOs) that display the most significant *in vitro* editing, and measure the level of in cellular DNA editing on the corresponding DMD mutations in C2C12 cells and primary MDX myoblast cells. Dr. Baar, has expertise on these cell lines.

4.) Impact:

Impact on Development of Principal Discipline: While in the first year of this grant there has not been any direct impact in the principal discipline of this proposal, there is tremendous promise that the results of this proposal can make a significant impact on the Duchenne muscular dystrophy field in the years to come. If one can edit the DNA mutations causing Duchenne muscular dystrophy, this could result in essentially curing this genetic disorder for many individuals that suffer from the disease because they harbor one of the many disease-causing nonsense mutations. With DNA genomic sequencing becoming very accessible and affordable, DMD patients can have more personalized treatment for the disease. The DMD gene of the patient can be sequenced to see if they possess a point mutation that causes the debilitating disease. Then based on the sequence of gene around the mutation, specific guide RNA mixmers can be synthesized that would target the patient's own mutation and with the use of the cell's ADAR enzymes, the DNA can be genetically changed at a single nucleotide to correct the mutation, allowing for the patient's muscle cells to make the full-length dystrophin protein, resulting in the likely hood of normal muscle development.

Impact on other Disciplines: The results of this proposal have tremendous promise to make a significant impact in many disciplines beyond Duchenne muscular dystrophy field. It is estimated that almost 60% of genetic diseases are caused by a single point mutation. Additionally, it is also estimated that roughly half of

these point mutations can be corrected if an adenosine nucleotide can be changed to a guanosine nucleotide *[6]*. This proposal's goal is to carry out this genetic alteration, to change an "A" to a "G" at a precise location in the genome. Therefore, this precise base-editing tool being developed with this funding, holds enormous promise to cure many other diseases that are caused by a point mutation.

Impact on Technology Transfer: Nothing to report during this reporting period. However, in the future the results of this research will likely result in a patent if proven successful. Additionally, the results could result in a start-up company designing these oligomer-mixmers to target genetic mutations to cure potentially many genetic diseases.

Impact on Society Beyond Science and Technology: Currently, there is nothing to announce during this reporting period. But the future holds promise to increase public knowledge and improve attitudes to genetically modifying somatic cell tissue, like muscle, to treat genetic disorders.

5.) Changes/Problems:

Changes in Approach: The only minor change to report from the original proposal, is to test the ability of using peptide-nucleic acids (PNAs) with the planned use of RNA/LNA mixmers to see if this improves DNA strand invasion efficiency. PNAs are nucleic acids with a peptide backbone, compared to the normal ribose-phosphate backbone in RNA and DNA. This peptide backbone offers many advantages because it is neutral charge allowing for better cellular uptake, offers greater stability because not prone to hydrolysis like RNA, and gamma-modified PNAs have recently been shown to display duplexed DNA strand invasion *[4, 5, 7]*. Therefore, my recently hired postdoc, Abdul, who has extensive knowledge and skills working the PNAs will incorporate PNAs in the mixmers (**Figure 4**) and explore this design and compared to normal RNA/LNA mixmers in dsDNA strand invasion efficiency, and ADAR's efficiency in DNA editing.

Actual/Anticipated Problems: There has been some issues with supply chain, specifically in ordering the phosphoramidites of LNAs to incorporate them in our mixmer synthesis. We have finally received them after being on backorder for extended time. Additionally, the PNA monomers must be synthesized by an outside company, and they have been delayed too because of supply chain issues. Additionally, it has been challenging to find a qualified postdoc to work on this project. After interviewing two exceptional candidates, both delayed in deciding and ultimately turned down the offer, because they did not want to relocate to Davis California. However, I was finally able to interview and offer a position to Abdul Shiraj, someone with expertise in RNA and PNA synthesis and characterization. But he did not join the lab until the end of June 2023, nine months into the first year. These two major issues delayed some output for the first year. To help resolve the issue, I hired a junior specialist who will start working in the lab next week. This BS level position will help contribute to the project in many ways.

Changes in Expenditures: As mentioned above, there has been a delay in hiring a postdoc to help work on this project. A postdoc was budgeted in the proposal, but after two qualified prospective postdocs delayed in deciding and finally declining the offer, I finally hired Abdul Shiraj as a postdoc, but he started nine months into the first budgeting year. Some of the funds will be used to pay a junior specialist, to help meet the objectives of the proposal.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to report.

6.) Products:

Journal publications: Nothing to report.

Books or other non-periodical, one-time publications: Nothing to report.

Other publications, conference papers, and presentations: The PI, Andrew Fisher presented some work funded by this grant at the 2023 Gordon Research Conference on: "RNA and DNA Editing and Epitranscriptomics Across Biological Systems", in Ventura California.

Website(s) or other Internet site(s): Nothing to report.

Technologies or techniques: Nothing to report.

Inventions, patent applications, and/or licenses: Nothing to report.

Other Products: Nothing to report.

7.) Participants & Other Collaborating Organizations:

Individuals worked on the Project:

Name: Andrew J. Fisher

Project Role: Principal Investigator

Research Identifier: ORCID: 0000-0003-3488-6594

Contribution to Project: Oversaw entire project and helped train graduate student and postdoc.

Funding Support: University of California, and one calendar month from this grant (W81XWH-22-1-0893)

Name: Jeff Cheng

Project Role: Graduate Student

Research Identifier: ORCID: 0000-0002-2846-3205

Contribution to Project: Graduate Student performing all the DNA editing assays using ADAR.

Funding Support: This grant (W81XWH-22-1-0893)

Name: Abdul Shiraj

Project Role: Postdoctoral Scholar

Research Identifier: ORCID: 0009-0002-7368-483X

Contribution to Project: Making the RNA/LNA and PNA oligos used for the guide strand in the DNA editing.

Funding Support: This grant (W81XWH-22-1-0893)

Change in the active other support of the PD/PI(s) or senior/key personnel: Nothing to Report.

Other organizations/partners involved: Nothing to Report.

8.) Special Reporting Requirements:

Nothing to report.

9.) Appendices:

Table A. DMD Mutation Target Sequences for DNA base-editing deamination reactions 5'-3' (150 nt). In **bold**, the target codon. In blue, the C>T mutation. In red, the target "A".

DMD Q45X Coding	TTCACAAAATGGGTAAATGCACAATTTTCTAAGTTTGGGAAGCAGCATATTGAGAACCTCTTCA GTGACCTA**TAG**GATGGGAGGCGCCTCCTAGACCTCCTCGAAGGCCTGACAGGGCAAAAACTGCC AAAAGAAAAAGGATCCACAAGA
DMD Q45X Noncoding	TCTTGTGGATCCTTTTTCTTTTGGCAGTTTTTGCCCTGTCAGGCCTTCGAGGAGGTCTAGGAGG CGCCTCCCATCC**TAT**AGGTCACTGAAGAGGTTCTCAATATGCTGCTTCCCAAACTTAGAAAATT GTGCATTTACCCATTTTGTGAA
DMD Q568X Coding	TGTAGATGGACAGAAGACCGCTGGGTTCTTTTACAAGACATCCTTCTCAAATGGCAACGTCTTA CTGAAGAA**TAG**TGCCTTTTTAGTGCATGGCTTTCAGAAAAAGAAGATGCAGTGAACAAGATTCA CACAACTGGCTTTAAAGATCAA
DMD Q568X Noncoding	TTGATCTTTAAAGCCAGTTGTGTGAATCTTGTTCACTGCATCTTCTTTTTCTGAAAGCCATGCA CTAAAAAGGCAC**TAT**TCTTCAGTAAGACGTTGCCATTTGAGAAGGATGTCTTGTAAAAGAACCC AGCGGTCTTCTGTCCATCTACA
DMD Q1087X Coding	TGGATGGCTGAAGTTGATGTTTTTCTGAAGGAGGAATGGCCTGCCCTTGGGGATTCAGAAATTC TAAAAAAG**TAG**CTGAAACAGTGCAGACTTTTAGTCAGTGATATTCAGACAATTCAGCCCAGTCT AAACAGTGTCAATGAAGGTGGG
DMD Q1087X Noncoding	CCCACCTTCATTGACACTGTTTAGACTGGGCTGAATTGTCTGAATATCACTGACTAAAAGTCTG CACTGTTTCAGC**TAC**TTTTTTAGAATTTCTGAATCCCCAAGGGCAGGCCATTCCTCCTTCAGAA AAACATCAACTTCAGCCATCCA
DMD Q2182X Coding	GTTGTCAGAACATTGAATGCAACTGGGGAAGAAATAATTCAGCAATCCTCAAAAACAGATGCCA GTATTCTA**TAG**GAAAAATTGGGAAGCCTGAATCTGCGGTGGCAGGAGGTCTGCAAACAGCTGTC AGACAGAAAAAAGAGGCTAGAA
DMD Q2182X Noncoding	TTCTAGCCTCTTTTTTCTGTCTGACAGCTGTTTGCAGACCTCCTGCCACCGCAGATTCAGGCTT CCCAATTTTTCC**TAT**AGAATACTGGCATCTGTTTTTGAGGATTGCTGAATTATTTCTTCCCCAG TTGCATTCAATGTTCTGACAAC

Table B. DMD Guide-RNA Sequences 5'-3'for *in vitro* DNA base-editing deamination reactions (60 nt)

DMD Q45X Coding	UCGAGGAGGUCUAGGAGGCGCCUCCCAUCCCAUAGGUCACUGAAGAGGUUCUCAAUAUGC
DMD Q45X Noncoding	AGCAUAUUGAGAACCUCUUCAGUGACCUACAGGAUGGGAGGCGCCUCCUAGACCUCCUCG
DMD Q568X Coding	UUUCUGAAAGCCAUGCACUAAAAAGGCACCAUUCUUCAGUAAGACGUUGCCAUUUGAGAA
DMD Q568X Noncoding	UUCUCAAAUGGCAACGUCUUACUGAAGAACAGUGCCUUUUUAGUGCAUGGCUUUCAGAAA
DMD Q1087X Coding	CACUGACUAAAAGUCUGCACUGUUUCAGCCACUUUUUUAGAAUUUCUGAAUCCCCAAGGG
DMD Q1087X Noncoding	CCCUUGGGGAUUCAGAAAUUCUAAAAAAGCAGCUGAAACAGUGCAGACUUUUAGUCAGUG
DMD Q2182X Coding	ACCGCAGAUUCAGGCUUCCCAAUUUUUCCCAUAGAAUACUGGCAUCUGUUUUUGAGGAUU
DMD Q2182X Noncoding	AAUCCUCAAAAACAGAUGCCAGUAUUCUACAGGAAAAAUUGGGAAGCCUGAAUCUGCGGU

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