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TITLE: Novel Approaches to Eliminating HIV Latency

PRINCIPAL INVESTIGATOR: Hoshang Unwalla Ph.D,

CONTRACTING ORGANIZATION: Florida International University

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1. REPORT DATE
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8. PERFORMING ORGANIZATION REPORT NUMBER

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Fort Detrick, Maryland 21702-5012

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14. ABSTRACT

With the advent of combination antiretroviral therapy. HIV has become chronic but manageable illness. However, people living with HIV continue to suffer comorbidities due to underlying viral replication even in presence of antiretrovirals. Cessation of antiretroviral therapy or missed doses leads to a restoration of viral p24 counts in the serum suggesting a rebound of the virus from anatomical reservoirs. Moreover, low level viral replication and gene expression ensures expression and secretion of viral proteins like Tat which can by themselves contribute to HIV associated comorbidities. A number of sites have been suggested as potential anatomical reservoirs, including resting CD4 cells, monocytes, macrophages, astrocytes, etc. Eradicating HIV reservoirs can lead to a "cure" for HIV while also decreasing the overall viral burden and decreasing the incidence and severity of HIV associated comorbidities. Given that the principal mechanism by which latency is established is by sequestration of the Positive Transcription Elongation Factor-b (PTEF-b), activating P-TEFb in HIV reservoirs will lead to viral replication. Replication in presence of antiretrovirals will eliminate the infected cell and prevent denovo infection. We will couple an siRNA with an aptamer targeting an HIBV protein called gag. This will ensure that only infected cells will receive the siRNA to reactivate PTEF-b which in turn will reactivate the dormant virus. This will decrease the HIV burden in people living with HIV.

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

Combination antiretroviral therapy has made HIV a treatable disease. However, despite this progress, non-AIDS associated comorbidities have continued to remain highly prevalent among people living with HIV. VA is the largest provider of HIV care in the United States. The VACS cohort contained 34,000 veterans living with HIV in 2007. Many of these veterans also suffered from substance abuse or smoked nicotine. cART successfully suppresses viral replication but is unable to eradicate the HIV due to reactivation of the virus from latently infected anatomical reservoirs upon cessation of cART. Elimination of HIV latency will result in a sterilizing cure as well as decrease the incidence of comorbidities in veterans and people living with HIV.

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

HIV, Shock and kill, aptamers, 7sksiRNA, latency reservoirs, cure.

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.

Aim1: ***To test therapeutic leads for systemic elimination of HIV reservoirs in latency models of HIV.*** Given that PTEF-b sequestration in the 7SK snRNA complex plays a central role in establishing viral latency, 1) siRNA targeting 7SK snRNA coupled to an aptamer against HIV Gag (42), will lead to PTEF-b mobilization and reactivation only in infected cells. Gag is expressed on one of the most stringent models of HIV latency (45), 2) Reactivation of latent virus in the context of cART will eliminate HIV reservoirs in these models.

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.

In the last annual report we had completed experimental set 1, and 2 and we had attempted experimental set 3d. We had created a latency model using HIV NL-TK virus. However, gancyclovir suppression was not found to be optimal and reactivation was also observed in NL-TK clone. Another approach to eliminate HIV reservoirs is called Block and Lock whereby HIV genome is permanently placed in a transcriptionally inactive state. WE tried to determine if we can combine our shock and kill approach using anti-7sk siRNA with a block and lock approach using CRISPR based targeting to develop a more potent shock and lock approach whereby we reactivate HIV using the anti-7sk siRNA in presence of an HIV inducible CRISPR-Cas9 expression system targeting the PTEF-b component CyclinT1. HIV Tat interacts with Cyclin T1 and this interaction is critical for viral transcription. We used our previously reported HIV inducible fusion promoter to eliminate CyclinT1 only in infected cells and looked at its effect on prolonged HIV suppression.

Additional experiment 1: ***CRISPR-mediated CyclinT1 inhibition suppresses HIV expression:*** We did a pilot experiment to identify the optimal Cyclin T1 gRNA sequence for CRISPR-based inactivation. Three cyclin T1 gRNA sequences, $gRNA_{(393-412)}$ (5'-TCCACGCCAAAACGACGGGA-3'), $gRNA_{(384-403)}$ (5'-AATAGCCCATCCCGTCGTT T-3') and $gRNA_{(191-206)}$ (5-CCTACCTCACTTCT AGTATC-3'), expressed from a U6 promoter were purchased from Genscript (https://www.genscript.com/gRNA-detail/904/CCNT1-CRISPR-guide-RNA.html). Each plasmid also expresses Cas9 from an EFS promoter. The three CyclinT1 gRNAs were tested in transient transfection assays in *LAV infected HeLa-CD4 cells* (aidsreagent# 1301). pBABE-puro-GFP plasmid was transfected as control and also served as an index of transfection efficiency. Culture supernatants were analyzed on day 6 and 8 post-transfection for p24. Experiments were terminated on Day 8 and total RNA was analyzed for HIV LTR RNA and total protein from an identically treated set was analyzed for Cyclin T1 (western blot). All three gRNAs suppressed HIV, with $gRNA_{(384-403)}$ showing the best suppression (Fig. 1A). An almost complete suppression of HIV is observed 6 days post-transfection for $gRNA_{(384-403)}$, with a small viral rebound observed on day 8.

This could be due to viral output from untransfected cells. Likewise, $gRNA_{(384-403)}$ shows the best cyclinT1 knockdown (Fig. 1B). HIV and CyclinT1 suppression was observed 8 days following transfection (by which time the transfected plasmid would be eliminated from the cultures by dilution), suggesting cyclinT1 inactivation by transient delivery of CRISPR cassette is sufficient to mediate prolonged suppression. We will co-express $gRNA_{(384-403)}$ along with Cas9 in our fusion promoter co-expression cassette for subsequent experiments. Surprisingly, CyclinT1 suppression did not affect cell viability after 8 days (Fig 1C). This could be due to a built-in redundancy for cellular functions which allows Cdk9 to also associate with other cyclins, namely Cyclin T2A, CyclinT2B and Cyclin K (3-5) thereby allowing survival of the cell harboring the locked provirus.

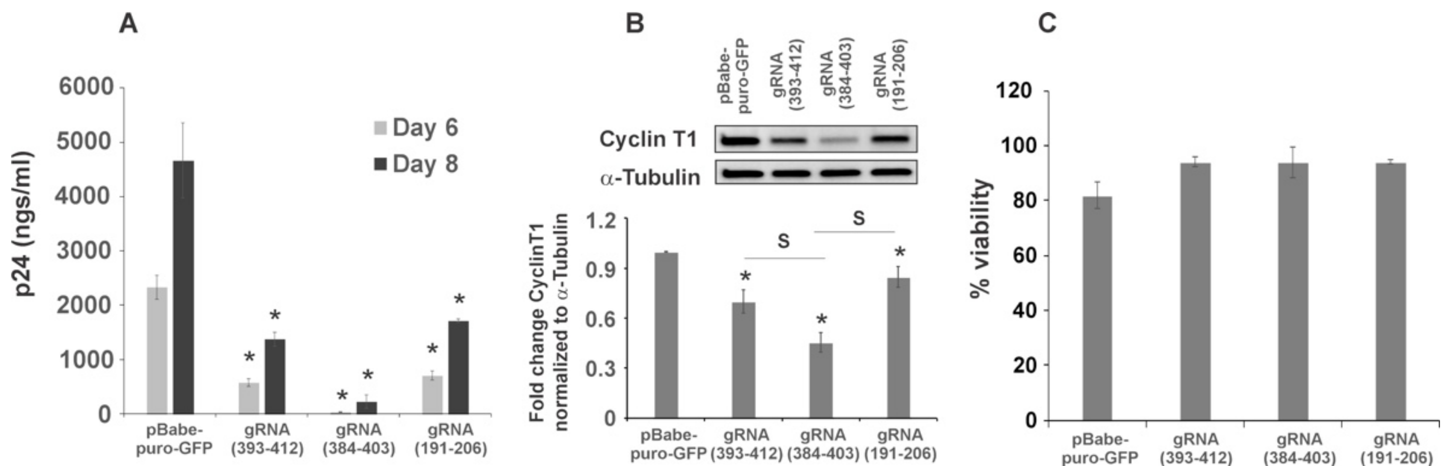

Figure 1: LAV infected HeLa-CD4 cells were transfected with 500 ngs of CRISPR cassette plasmid or pBabe-puro-GFP (as control) using lipofectamine 2000. The cells were propagated for an additional six days to dilute out the plasmids from the culture. 6 and 8 days post-transfection, culture supernatant was collected for p24 analysis. CyclinT1 gRNAs suppress HIV (panel A) and this is a function of CRISPR-mediated CyclinT1 knockdown (panel B). Viability analysis of cells using trypan blue staining shows that CyclinT1 knockdown does not affect viability (panel C). n=3; * = significant from controls, S= significant from each other ($p < 0.05$).

Additional experiment 2: Next we cloned the CRISPR Cas9 system in our HIV inducible fusion promoter system (6) as shown in Figure 2. Using precise PCR based multistep cloning processes, we cloned the CyclinT1 gRNA between the fusion promoter and the Cas9 protein downstream of the minimal polyadenylation signal sequence.

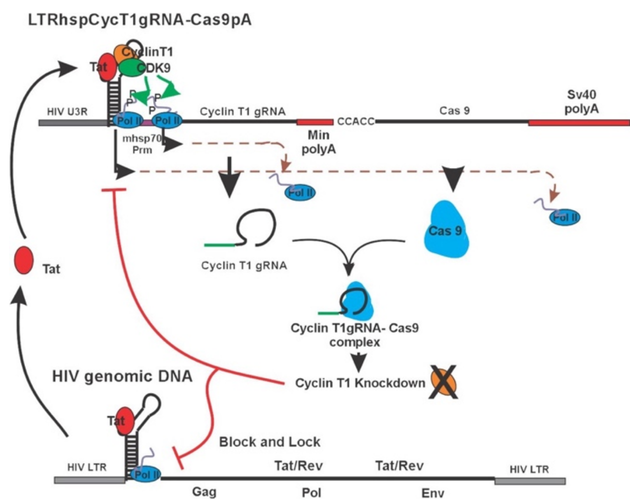

Figure 2: Schematic of LTRhspCycT1gRNA-Cas9pA cassette (not drawn to scale): In presence of HIV Tat, P-TEFb kinase will be recruited to the fusion promoter initiating transcription from both promoters. Most of the transcription from the minimal hsp70 promoter will terminate at the minimal polyA to express the Cyclin T1 gRNA while transcriptional read-through will encode the Cas9. The strong eukaryotic translation initiation signal CCACC ensures that the first ATG after this sequence is used for translation initiation. Given the critical importance of PTEF-b (Cyclin T1-CDK9) for HIV transcription, inactivation of Cyclin T1 will irreversibly block all transcription from HIV. Since the fusion promoter also requires Tat-Cyclin T1 interaction for co-expression of Cyclin T1 gRNA and Cas9, once Cyclin T1 is knocked down, transcription from both, the HIV proviral DNA as well as the fusion promoter will be completely inhibited thereby affecting a functional cure and limiting any further expression from the fusion promoter.

HIV LTRhsp70 fusion promoter drives HIV inducible expression of Cas9: We first tried to determine HIV specific expression of Cas9 in our constructs. LAV infected HeLa-CD4 cells were transfected with our LTRhspCycT1gRNA-Cas9pA. Uninfected Hela cells were transfected as control. As seen in Figure 3, LTRhspCycT1gRNA-Cas9pA demonstrates Cas9 expression only in presence of HIV infection whereas uninfected HeLa cells demonstrated barely detectable levels of cas9. Surprisingly, deleting the NF-kb sites completely abolished Cas9 expression from the fusion promoter suggesting that NF-kb is essential for transcription from the HIV LTR. We also found that the full length LTR demonstrated no expression of cas9 in absence of HIV, while the SP1 deletion construct showed very low levels of Cas9 expression.

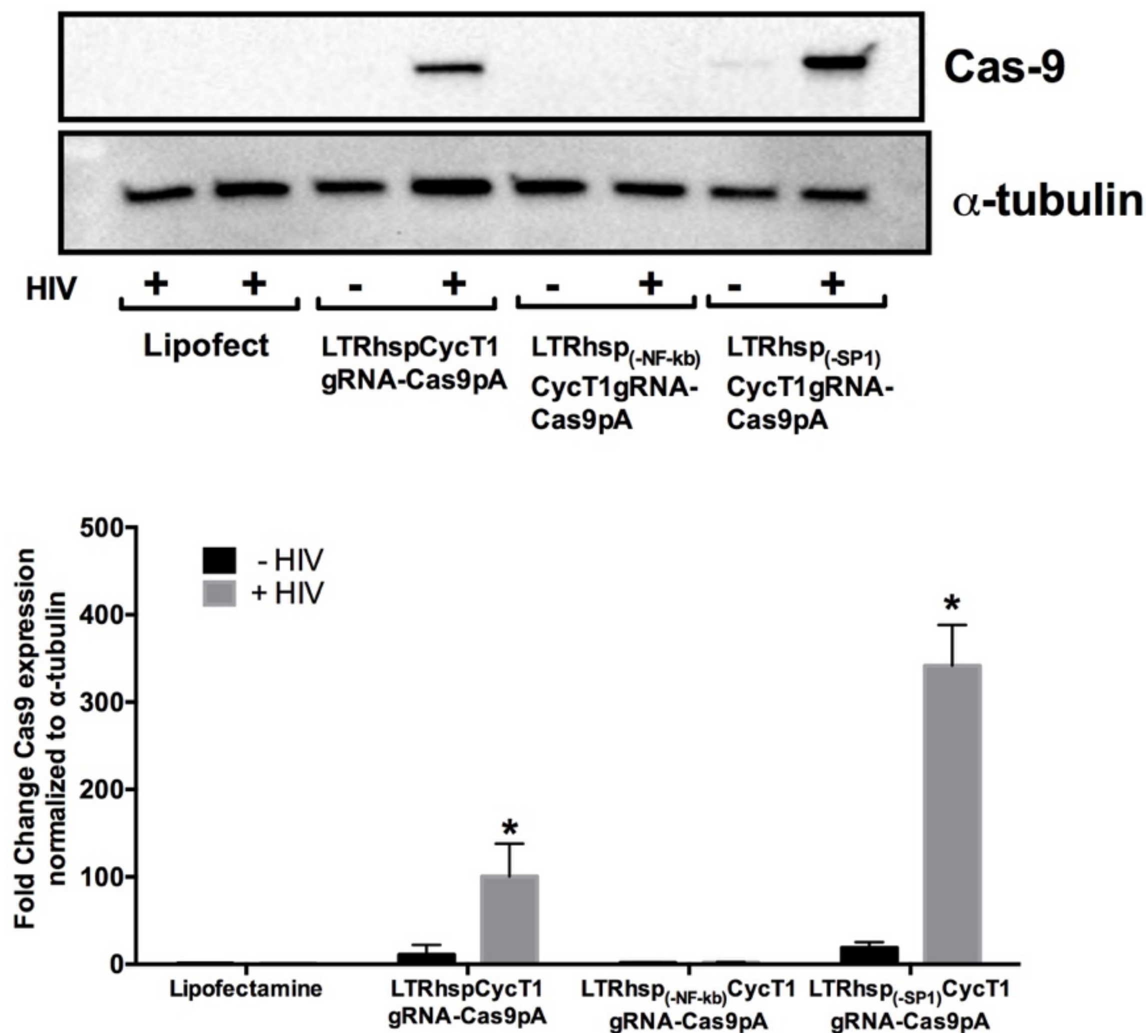

Figure 3: HIV inducible expression of Cas-9 from the HIVLTR-hsp70 fusion promoter. LAV infected HeLa-CD4 cells were transfected with our LTRhspCycT1gRNA-Cas9pA, $LTR_{(-NF-kb)}$CycT1gRNA-Cas9pA or $LTR_{(-SP1)}$CycT1gRNA-Cas9pA. Uninfected Hela cells were transfected as control. No expression is observed when the NF-kb sites are deleted. However, HIV inducible expression of Cas-9 is observed with both, LTRhspCycT1gRNA-Cas9pA and $LTR_{(-SP1)}$CycT1gRNA-Cas9pA. The full length HIV LTR in the LTRhspCycT1gRNA-Cas9pA did not demonstrate any leaky expression. Some leaky expression was observed for $LTR_{(-SP1)}$CycT1gRNA-Cas9pA even in absence of HIV.

Given that NF-kb mutant did not demonstrate any Cas9 expression, we restricted all our experiments with the full length LTRhspCycT1gRNA-Cas9pA and the SP1 deletion construct, $LTR_{(-SP1)}$CycT1gRNA-Cas9pA. LAV HeLa CD4 cells were transfected with LTRhspCycT1gRNA-Cas9pA or $LTR_{(-SP1)}$CycT1gRNA-Cas9pA. Transfection with gRNA384-403 was used for comparison. 48 hours post-transfection, experiments were terminated and total protein was analyzed for CyclinT1 suppression by western blot analysis. As seen in Figure 4, LTRhspCycT1gRNA-Cas9pA suppressed CyclinT1 protein expression comparable to that observed with gRNA384-403. The suppression was more robust than that observed with $LTR_{(-SP1)}$CycT1gRNA-Cas9pA.

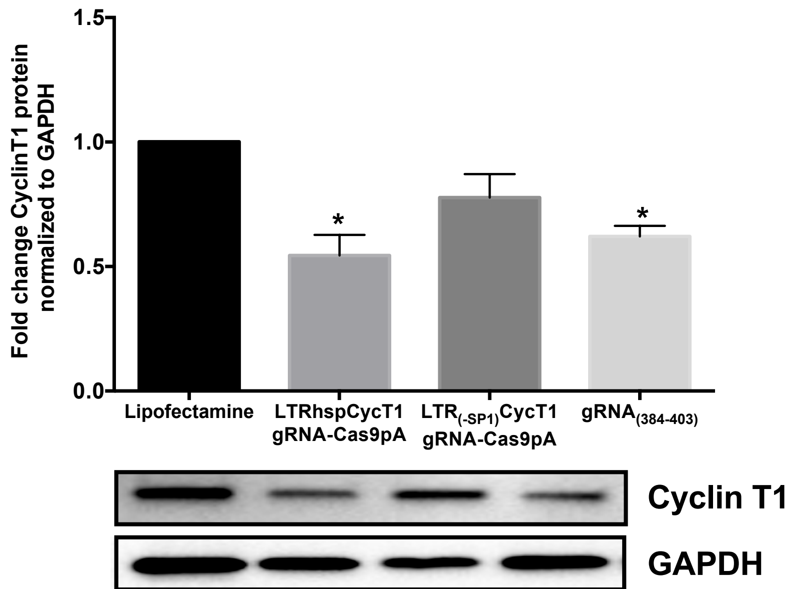

Figure 4: LAV infected HeLa-CD4 cells were transfected with 500 ngs of LTRhspCycT1gRNA-Cas9pA and the SP1 deletion construct, LTR(-SP1)CycT1gRNA-Cas9pA using lipofectamine 2000. 48 hours post-transfection, tota protein was analyzed for Cyclin T1 suppression by western blot analysis. LTRhspCycT1gRNA-Cas9pA suppressed CyclinT1 protein expression comparable to that observed with gRNA384-403. n=3; * = significant from controls, S= significant from each other ($p < 0.05$).

Next we tested our the LTRhspCycT1gRNA-Cas9pA for its ability to suppress HIV in Hela-CD4 cells stably infected with HIV. Hela-CD4 cells were transfected with 500 ngs of our LTRhspCycT1gRNA-Cas9pA plasmid. Culture supernatants were collected every 24 hours for a period of 72 hours and analyzed for HIV p24 as an index of HIV infection. As seen in Figure 5, The LTRCycT1gRNA-Cas9pA demonstrates over 90% suppression of HIV. The extent of suppression remains stable over three days suggesting that this could be a consequence of transfection efficiency.

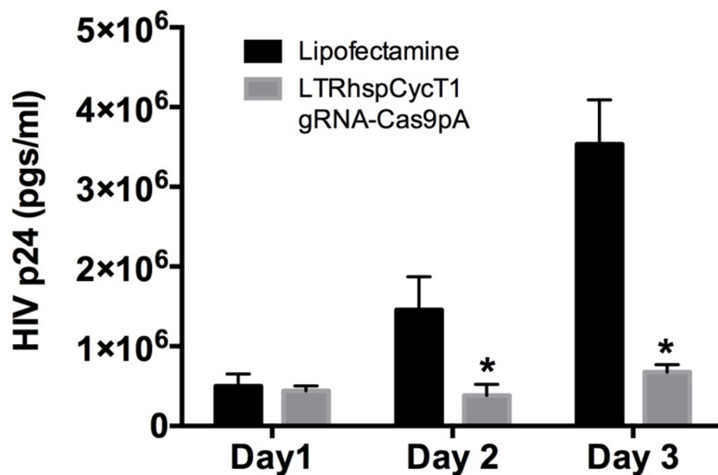

Figure 5: Inhibition of HIV by LTRhspCycT1-gRNA-Cas9pA: LAV infected HeLa-CD4 cells were transfected with 500 ngs of LTRhspCycT1-gRNA-Cas9pA plasmid using lipofectamine 2000 (Lipofectamine 2000 alone as control). Culture supernatant was collected every 24 hours and analyzed for HIV p24. LTRhspCycT1-gRNA-Cas9pA suppresses HIV p24 and the viral p24 output remains constant suggesting that cells that the suppression depends on transfection efficiency.

Next we tried to determine if sequential transfections will increase the number of cells transfected thereby also increasing HIV suppression. LAV infected HeLa-CD4 cells were transfected with LTRCycT1gRNA-Cas9pA (lipofectamine alone as control). On Day 3 cells were transfected a second time with LTRCycT1gRNA-Cas9pA (lipofectamine alone as control). On day 6 following transfection, culture supernatants were collected and analyzed for HIV p24. Figure 6 shows that a second transfection showed suppression similar to that observed with one transfection alone. This suggests that a complete suppression for HIV may require further improvement of the LTRhspCycT1gRNA-Cas9pA construct.

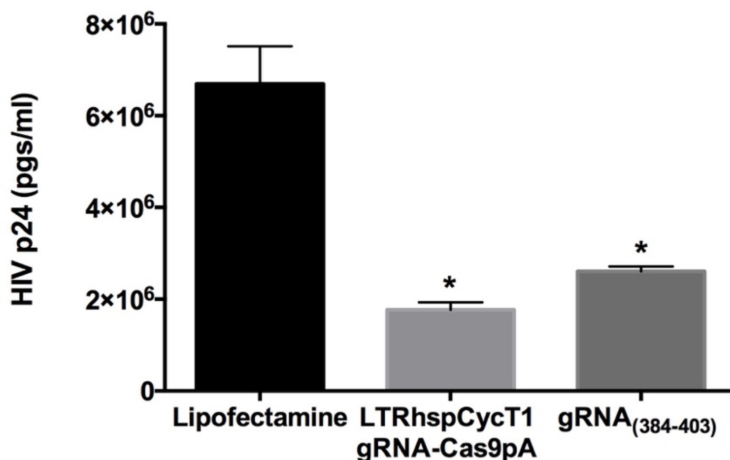

Figure 3: Inhibition of HIV by LTRhspCycT1-gRNA-Cas9pA: LAV infected HeLa-CD4 cells were transfected with 500 ngs of LTRhspCycT1-gRNA-Cas9pA plasmid using lipofectamine 2000 (Lipofectamine 2000 alone as control) on day 0 and then again on day 3. Culture supernatant was collected on day 6 and analyzed for HIV p24. LTRhspCycT1-gRNA-Cas9pA suppresses HIV p24. Suppression is observed to a similar extent as observed with single transfection (figure 2). This data suggest that failure to achieve complete suppression could be sue to a consequence of gRNA being exported to the cytoplasm instead of being retained in the nucleus.

Future plans: Our experiments have shown the efficacy of CyclinT1 suppression and HIV inducible Cas-9 expression when expressed form our HIV LTR-hsp70 fusion promoter. This is the first report of a complete CRISPR system expressed from a Pol II promoter specifically in HIV infected cells. Our proposal involved the shock and kill approach. However, this approach will kill the infected cell and allow live virus to escape the cell which will then be neutralized by antivirals. The new concept of Shock and Lock will activate HIV using the aptamer-7sk siRNA chimera which will reactivate latency and induce Tat. Tat will promote expression of the anti-cyclinT1 gRNA and Cas9 which will prevent any further HIV gene expression. Thus the viral DNA will be permanently inactivated and the cell will survive. This approach will also prevent release of infectious virus from the cell. We will test these in Hela CD4 cells in preliminary experiments in combination with our anti-7sk siRNA gp120 aptamer chimera and then implement the combination in T-cells, macrophages and microglia as outlined in the proposal.

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.

This project has provided one-on-one mentoring to Dr. Chinnapaiyan who has now been promoted to Visiting Assistant Professor in the laboratory and Mr. Rajib Dutta who was a graduate student and has now successfully defended his thesis. He is now a post-doctoral fellow at the University of Miami. Both have learnt the techniques and biology of HIV latency.

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.

We are currently in the process of completing experiments that would allow us to eradicate HIV latency using our fusion promoter. We are preparing the manuscript, "A shock and lock approach to eliminating HIV latency". Following completion of experiments, the manuscript will be submitted for publication.

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.

We are currently in the process of completing experiments that would allow us to eradicate HIV latency using our fusion promoter. We are preparing the manuscript, "A shock and lock approach to eliminating HIV latency". Following completion of experiments, the manuscript will be submitted for publication.

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).

The ability to eradicate HIV reservoirs is the cornerstone of HIV therapy. As yet, none of the studies have demonstrated eradication of HIV reservoirs, our approach exploits a focal point in the HIV silencing to reactivate HIV in presence of antivirals. We anticipate that our approach will significantly decrease viral burden and improve the quality of life of people living with HIV.

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.*

We are in the process of using our fusion promoter to co-express a cyclinT1 targeting gRNA along with cas9 only in HIV infected cells. We anticipate that upon successful demonstration of its anti-HIV activity we will be filing an invention disclosure.

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 yet but we anticipate that upon completion of project and follow on proposals, we will significantly decrease the viral burden and improve the quality of life of people living with HIV.

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.

Our approach was designated "Shock and Kill" whereby, the latent HIV will be reactivated using aptamer-siRNA chimeras and then eliminated with anti-retrovirals. We found that gancyclovir suppression was not found to be optimal and reactivation was also observed in NL-TK clone. Hence we have modified this approach to "shock and lock" whereby we target Cyclin T1 only in HIV infected cells. The aptamer-siRNA chimera will reactivate the virus as well as a targeting approach. This would completely inactivate the virus in latent cells.

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 have faced delays due to the pandemic. We have continued to perform experiments. However, lab closures as well as social distancing guidelines in the laboratory has required us to minimize the number of individuals in the laboratory at any given time. In the last one year this has created delays. We anticipate that while we will continue to perform experiments, the pandemic effects will continue till a vaccine is widely available.

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.

Not applicable

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

Not applicable

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

We continue to work with live HIV virus.

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).*

We are currently in the process of completing experiments that would allow us to eradicate HIV latency using our fusion promoter. We are preparing the manuscript, "A shock and lock approach to eliminating HIV latency". Following completion of experiments, the manuscript will be submitted for publication.

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).*

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.*

If the pandemic permits, we would like to present our work at the 2021 meeting of the American Society of Gene Therapy.

- **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.

NA

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

We have adapted our previously reported fusion promoter to co-express a gRNA and Cas9 to target a cellular factor that plays a pivotal role in HIV replication. These anti-HIV molecules are expressed only in infected cells.

- **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.

Not yet but we intend to file an invention disclosure if our fusion promoter successfully eliminates HIV latency.

- **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: Hoshang Unwalla
Project role: PI
Nearest person months: 3
Contribution to project: Dr. Unwalla was involved in overall planning, analyses and execution

Name: *Srinivasan Chinnapaiyan*
Project Role: *Post-doctoral Fellow*
Researcher Identifier (e.g. ORCID ID): srinic
Nearest person month worked: 4
Contribution to Project: *performed experiments outlined in the proposal.*

Name: *Rajib Dutta*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: *performed experiments outlined in the proposal.*

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.

Nothing to report

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:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (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:

QUAD CHARTS:

APPENDICES: