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TITLE: Novel Approaches to Eliminating HIV Latency

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CONTRACTING ORGANIZATION: Florida International University

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Florida International University, 11200 SW 8th Street, MARC 430, Miami, FL 33199

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13. SUPPLEMENTARY NOTES

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

15. SUBJECT TERMS

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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.

Experimental set 1: We have already demonstrated that anti-7SK siRNA reactivates HIV in CNS reservoirs of HIV namely astrocytes (Figure 4 from our proposal). We tested the efficacy of this siRNA in other known reservoirs of HIV to test if siRNA mediated disruption of 7SK snRNA will reactivate HIV in diverse cell types irrespective of the mechanism by which latency was established. We first determined if our 7sK siRNA can reactivate latency in a monocyte and T-lymphoid latency models. The U1 monocyte cell line and J-LAT8.4 lymphoid cell lines were obtained from Aids reagent and reference program (aidsreagent #9847 and #165). These cell lines are routinely used as macrophage and T-cell surrogates for latency induction studies (47-49) and express negligible levels of p24 (50, 51). U1 cells were washed four times to remove any residuakl p24. Two days post-

washes, U1 cells grown in T25 flasks were transfected with 20 nM anti-7KsnRNA (2nM followed by dose response). HMBA was used for comparison. As seen in the Figure 1, 7sksiRNA transfection promotes HIV replication thereby reactivating the latent virus. The reactivation was found to exceed that observed with the P-TEFb activator HMBA. At this pont we faced some technical difficulties growing the JLat 8.4 cell line.

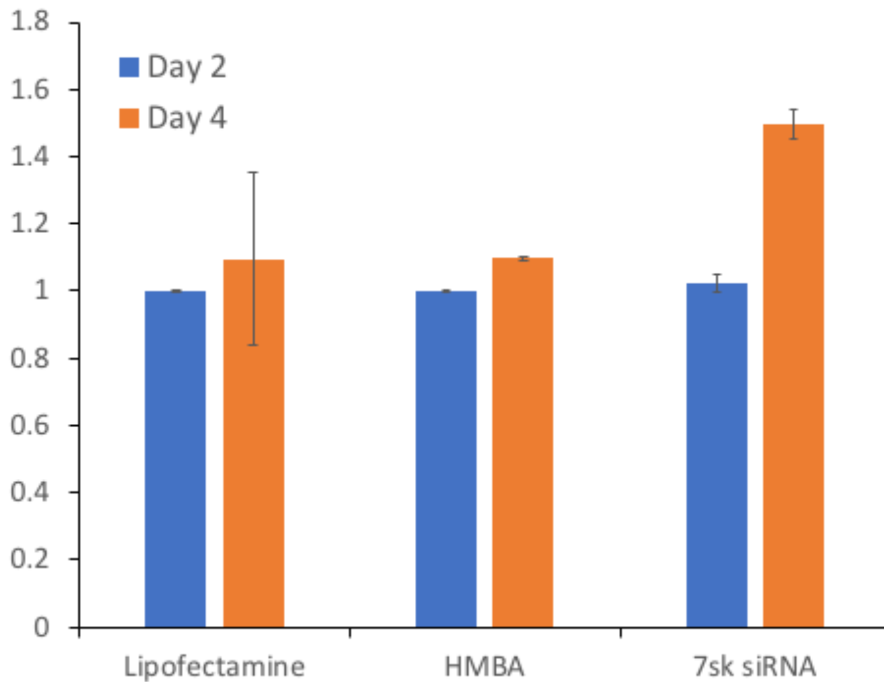


Figure 1: U1 monocytic cell line was transfected with 7sk siRNA. Lipofectamine alone was transfected as control, PTEF-b activator HMBA was used for comparison. As seen in figure 1, 7sksiRNA reactivates latency better than that observed with HMBA.

We continued Aim. 1, Experimental set 1 and initiated experimental set 2.

Aptamer-7sk siRNA chimera reactivates HIV latency:

Experimental set 1: In the first quarterly progress report we had demonstrated that anti-7SK siRNA reactivates latency of R5-tropic HIV in the U1 monocytic cell line. The reactivation exceeded that observed with the small molecule PTEF-b activator HMBA. However, we had some technical difficulties growing the J-lat 8.4 cell line. We believed we had resolved this as the cells started dividing and we were able to split them. However after a few passages before we could initiate transfections of these cells using the 7SK-siRNA, we faced the same problems with decreasing viability of the cells. We have now tried using the LAV infected Hela cells (aidsreagent.org# 1301). LAV is a dual tropic virus that can infect both macrophages and T-cells. While these cells divide robustly, one of the issues is that these are not latency models and produce enormous amounts of p24. However, we tested our anti-7SK siRNA in these cells by transfecting LAV infected Hela cells. Cells were washed four times to remove any residual p24. Two days post-washes, LAV infected Hela cells grown in 12-well plates were transfected with 20 nM anti-7KsnRNA. HMBA was used for comparison. As seen in the Figure 2, 7sksiRNA increases p24 output suggesting that the siRNA is capable of knocking down 7sk snRNA to relieve PTEF-b suppression. A much higher 9-fold increase in viral RNA is observed in 7sk siRNA treated cells. This is because our probe measures all species of HIV RNA compared to only the full length RNA that encodes the viral p24. We will be reordering J-LAT8.4 lymphoid cell line from the AIDS repository. It is possible that the batch of freeze downs were defective. We will also attempt our experiments with JLAT6.3 (aidsreagent # 9846). JLat6.3 also expresses GFP and reactivation can be quantitated by GFP expression as well. We would like to establish latency reactivation for T-cell tropic HIV as experimental set 3 specifically proposes using T-cells as reservoirs.

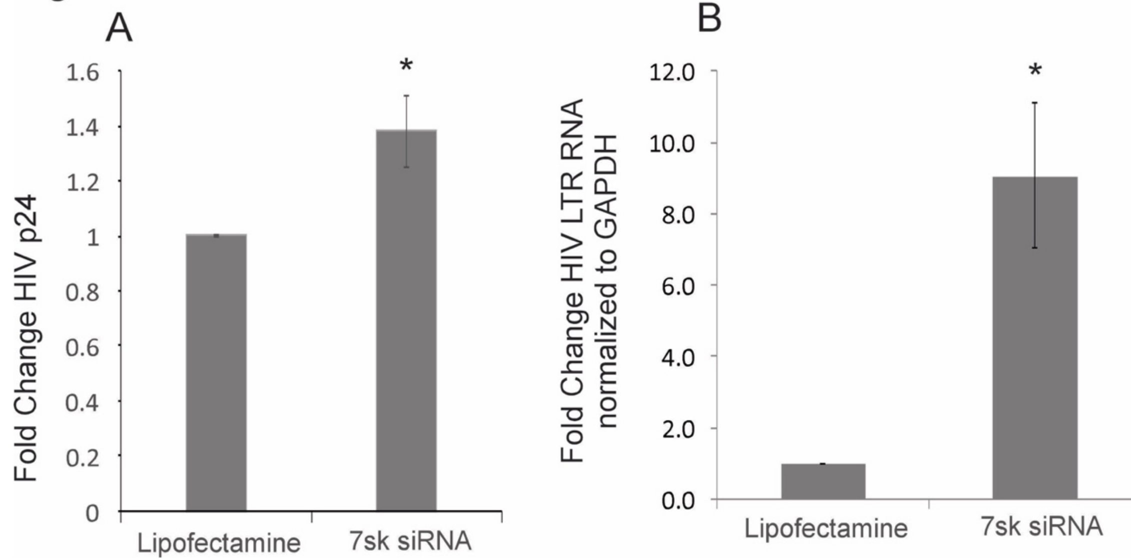
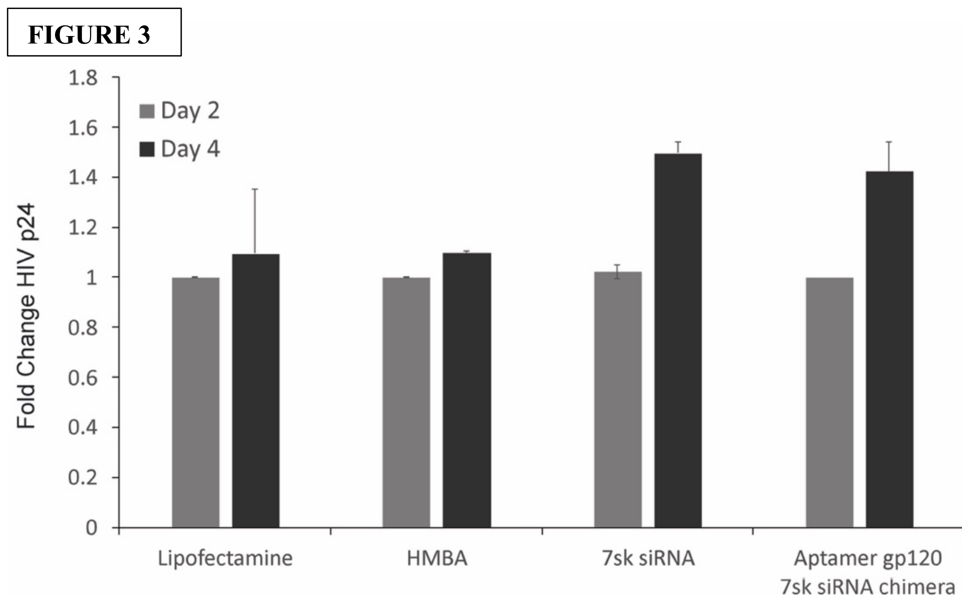


Figure 2: LAV infected Hela CD4 cells were transfected with lipofectamine alone or 7sk siRNA**.** Two days post-transfection, culture supernatant was collected for p24 analysis. Total RNA was isolated from cells and analyzed for HIV RNA. As seen in the figure, 7sK siRNA increases HIV p24 output (panel A). The magnitude of RNA upregulation is much higher than p24 (panel B). This could be because only a fraction of RNA encodes p24 while most HIV RNA is used to transcribe other genes and also for packaging.

Experimental set 2: Given our promising results with U1 monocytic cell line we proceeded with experimental set 2 for these cell lines. We tried to determine if an aptamer specifically directed against HIV infected cells can reactivate latency in HIV reservoir models. Given the reactivation observed with our anti-7sk siRNA in reactivating latency in U1 cells, we chemically synthesized an aptamer targeted against HIV gp120 and coupled with the sense strand of siRNA. Before adding to cells equimolar quantities of aptamer-sense strand were annealed with the antisense strand of the siRNA (synthesized separately) by heating at 90^0C followed by cooling.

U1 cells were washed four times to remove any residual p24. Two days post-washes, U1 cells grown in T25 flasks were treated with aptamer-siRNA chimera (20nM). After a further forty-eight hours culture supernatants were analyzed for HIV p24 as an index of viral output. We plotted this data along with our previous experiment. As seen in figure 3 reactivation with the Aptamer-siRNA chimera was comparable to that observed with siRNA transfection suggesting that HIV gp120 aptamer can be used for delivery of siRNA specifically to HIV infected cells.

FIGURE 3



<u>Figure 3:</u> HIV infected U1 monocytic cell lines were treated as desci cells were transfected with lipofectamine alone or 7sk siRNA**.** Two days post-transfection, culture supernatant was collected for p24 analysis. Total RNA was isolated from cells and analyzed for HIV RNA. As seen in the figure, 7sK siRNA increases HIV p24 output (panel A). The magnitude of RNA upregulation is much higher than p24 (panel B). This could be because only a fraction of RNA encodes p24 while most HIV RNA is used to transcribe other genes and also for packaging.

Aptamer-7sk siRNA chimera reactivates HIV latency:

<u>Experimental set 1:</u> In the first and second quarterly progress report we had demonstrated that anti-7SK siRNA reactivates latency of R5-tropic HIV in the U1 monocytic cell line. The reactivation exceeded that observed with the small molecule PTEF-b activator HMBA. However, we had some technical difficulties growing the J-lat 8.4 cell line. Hence we used LAV infected Hela cells (aidsreagent.org# 1301). LAV is a dual tropic virus that can infect both macrophages and T-cells. While these cells divide robustly, one of the issues is that these are not latency models and produce enormous amounts of p24. Anti-7sk siRNA delivered using lipofectamine 2000 led to a dramatic increase in p24 output suggesting that the siRNA is capable of knocking down 7sk snRNA to relieve PTEF-b suppression. A much higher 9-fold increase in viral RNA is observed in 7sk siRNA treated cells. This is because our probe measures all species of HIV RNA compared to only the full length RNA that encodes the viral p24. In this period we tried to determine if aptamer-siRNA chimera will reactivate HIV expression in HIV infected Hela CD4 cells. Cells were washed four times to remove any residual p24. Two days post-washes, LAV infected Hela cells grown in 12-well plates were treated with gp120-aptamer-anti7sk siRNA chimera. HMBA and 7sk siRNA delivered by lipofectamine were used for comparison. As seen in Figure 4A, a very modest upregulation was observed by gp120-aptamer-anti7sk siRNA chimera while the anti-7sk siRNA delivered by lipofectamine still showed reactivation of gene expression. Total RNA extracted from these cells was analyzed for HIV LTR. RNA expression analysis showed that the aptamer-siRNA chimera did increase viral transcription but activation by lipofectamine delivered siRNA was still several fold higher (Figure 4B). Hence our chimera does increase viral RNA expression. A suboptimal p24 output could be due to additional restriction factors that might be affecting viral release.

FIGURE 4

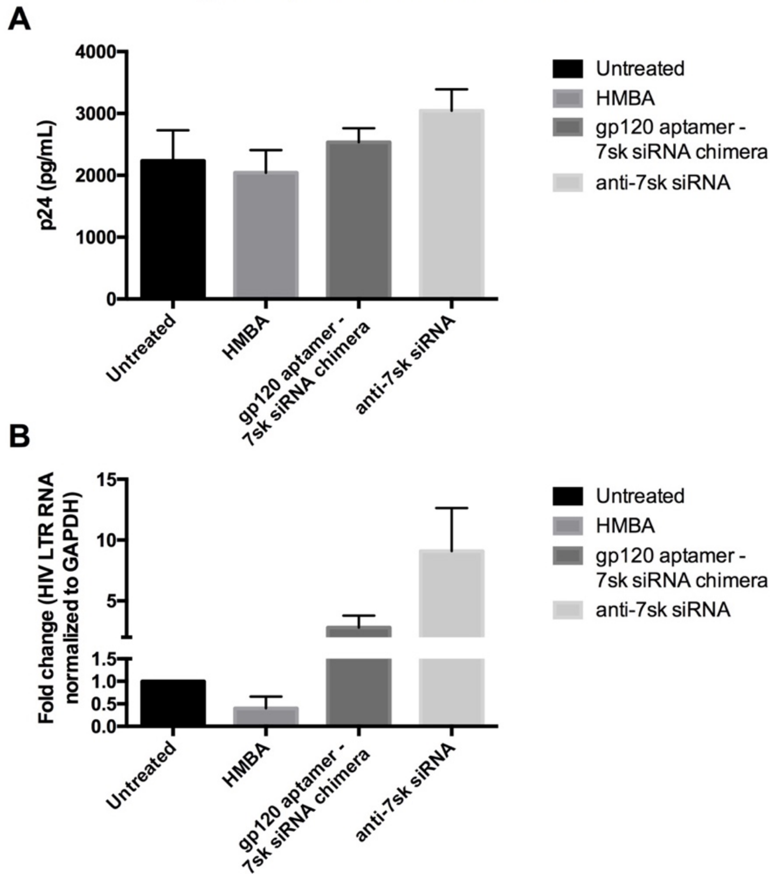


Figure 4: LAV infected Hela CD4 cells were treated with gp120 aptamer-anti-7sk siRNA chimera**.** Two days post-transfection, culture supernatant was collected for p24 analysis. Total RNA was isolated from cells and analyzed for HIV RNA. As seen in the figure, lipofectamine delivered 7sK siRNA increases HIV p24 output (panel A). The magnitude of RNA upregulation is much higher than p24 (panel B). However, the chimera only showed a modest upregulation for p24. Upregulation for HIV RNA was more robust but still less than that observed for lipofectamine delivered siRNA. Data is mean +/- SEM from 3 different experiments.

Experimental set 2: In this project we have observed

1) significant reactivation of HIV latency in HIV infected U1 monocytic cell line reservoir models (progress report 1 and 2).
2) Suboptimal effects of the chimera on HIV LAV infected Hela CD4 model.

We again tried to determine reactivation in J-LAT8.4 lymphoid cell line obtained from the NIH repository. We were able to grow these cells by changing serum concentration in the media. Cells were washed four times to remove any residual p24. Two days post-washes, JLAT8.4 cells were treated with gp120-aptamer-anti7sk siRNA chimera. HMBA and 7sk siRNA delivered by lipofectamine were used for comparison. RNA analysis demonstrates that the aptamer-siRNA chimera is as efficient as lipofectamine delivered 7sk siRNA even though the reactivation of transcription was less than that observed with HMBA (Figure 5).

FIGURE 5

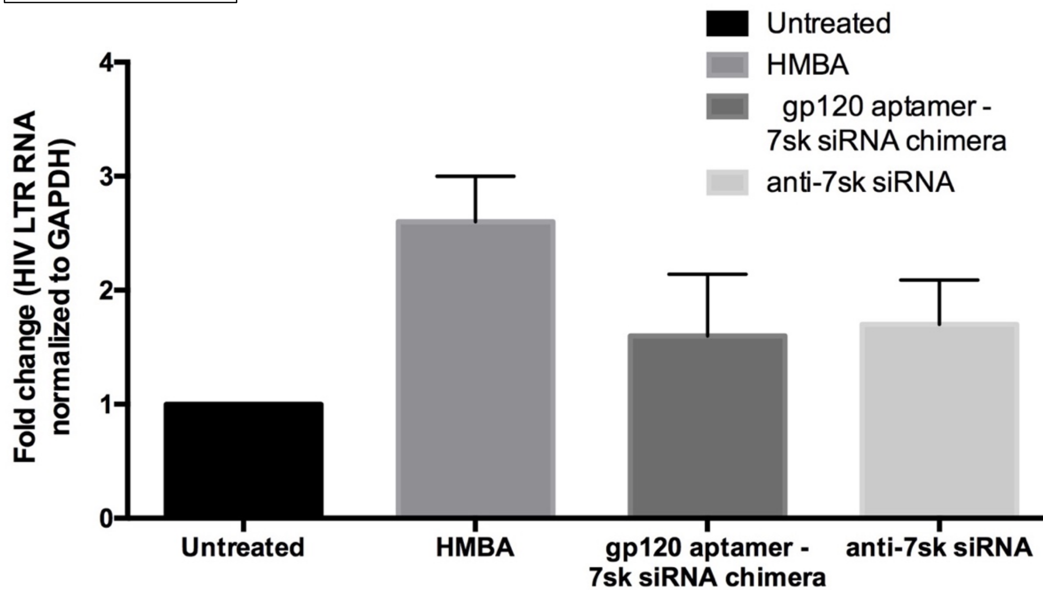


Figure 5: HIV infected J-Lat8.4 T-lymphoid cell lines were treated as described cells were transfected with lipofectamine alone or 7sk siRNA**.** Two days post-transfection, culture supernatant was collected for p24 analysis. Total RNA was isolated from cells and analyzed for HIV RNA. As seen in the figure 2, 7sK siRNA increases HIV p24 output comparable to that observed with lipofectamine delivered siRNA. The magnitude of RNA upregulation is much higher with HMBA. Data is mean +/- SEM from 3 different experiments.

In our pitfalls and solutions we have discussed if suboptimal reactivation is observed then we will try to reactivate using a combination of PTEF-b activator HMBA and out aptamer-siRNA chimera. We tried to determine if reactivation using a combinatorial approach has a synergistic effect. This would be expected as reactivation by HMBA will upregulate gp120 allowing more aptamer-siRNA chimera to bind and get internalized. Cells were washed four times to remove any residual p24. Two days post-washes, culture supernatant was collected for initial p24 analysis. JLAT8.4 cells were treated with gp120-aptamer-anti7sk siRNA chimera alone, HMBA alone, or a combination of aptamer-siRNA chimera and HMBA. After another 2 days (day 4), culture supernatant was collected for p24 analysis to determine any reactivation. Cells were then lysed and total RNA was analyzed for HIV RNA. As seen in Figure 3, a combined effect of HMBA and aptamer-siRNA chimera results in a synergistic reactivation that is higher than that observed by HMBA or aptamer-chimera alone. Reactivation was observed for both HIV p24 analysis (Figure 6A) as well as HIV RNA (Figure 6B). Hence these data demonstrate using a combination of HMBA and chimera would be optimal for reactivation of latent HIV from reservoirs. We will test this combined approach in U973 HIV infected monocytic cell line. We anticipate a similar synergistic effect using the chimera in combination with HMBA. In experimental set 3 for latency reactivation in primary cells we will now use this combinatorial approach.

FIGURE 6

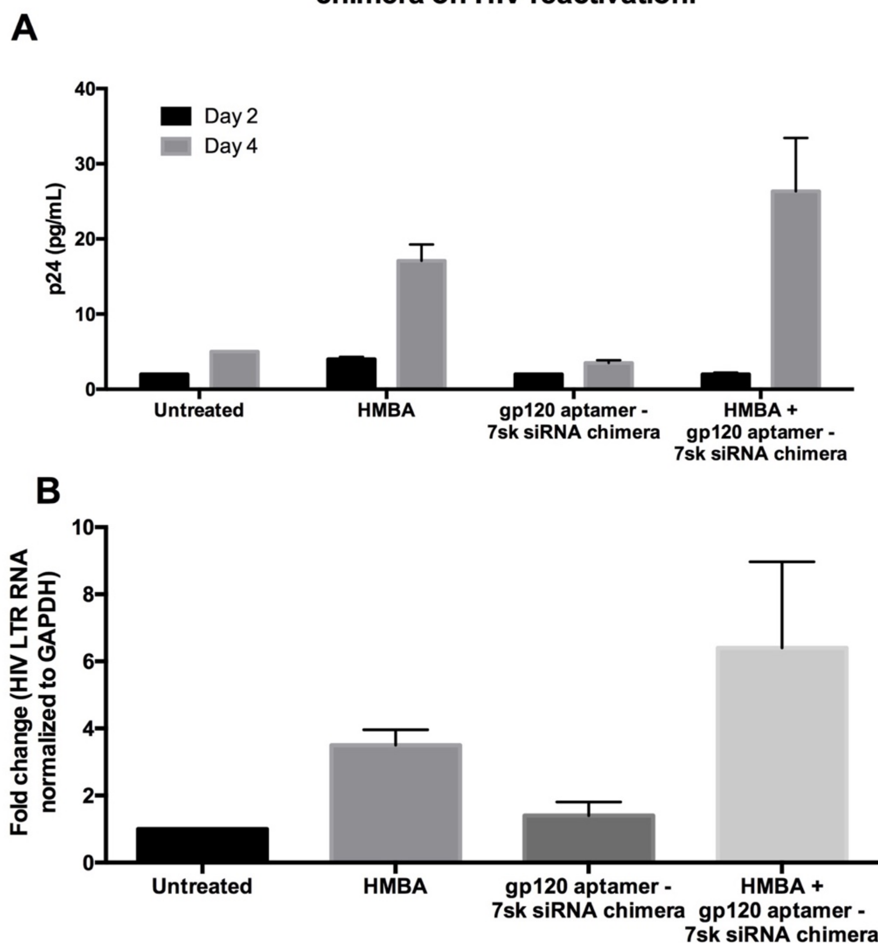


Figure 6: J-Lat8.4 cells were treated as described. The aptamer-siRNA chimera when used in combination with HMBA demonstrated a synergistic increase in HIV p24 (panel A). HIV RNA analysis using qRT-PCR demonstrates a similar synergistic reactivation of HIV transcription.

Experimental set 3: We will create a latency model using HIV NL-TK virus. pNL-TK (AIDS reagent Cat # 4120) is a replication competent HIV clone. HIV-NL-TK was packaged with HIV IIIB envelope plasmid using cotransfection of four different plasmids in HEK 293T cells. The pseudotyped lentivirus was isolated and concentrated as demonstrated in our earlier reports. We initiated Set 3d as we already had differentiated primary bronchial epithelial cells. Primary bronchial epithelial cells redifferentiated at the airliquid interface were infected with NL-TK virus. 72-hours post-infection, productively infected cells will be eliminated by treating the cells with ganciclovir (5nmol/L) based on our prior work. 72 hours post-gancyclovir treatment, cells will be washed four times with PBS to remove any residual ganciclovir and adherent virus. The last wash will be collected for pre-reactivation treatment p24 analysis. Cultures were treated with either, Aptamer siRNA or Aptaer-siRNA supplemented with the PTEF-b activator HMBA. Following reactivation, culture supernatant was analyzed for p24 expression. Culture supernatant was also used to establish a second round of activation in presence of the antiretroviral Tenofovir to demonstrate the shock and kill approach. As seen in Figure 7, NHBE ALI cultures demonstrate HIV p24. However, following gancyclovir treatment, all p24 output is eliminated suggesting that gancyclovir kills all cells actively expressing NL-TK virus leaving only latently infected cells. Thus we were successful in establishing the latency model. Following reactivation, we observed that both the chimera as well as chimera + HMBA demonstrate reactivation. Some reactivation was also observed in primary bronchial epithelial cells.

FIGURE 7

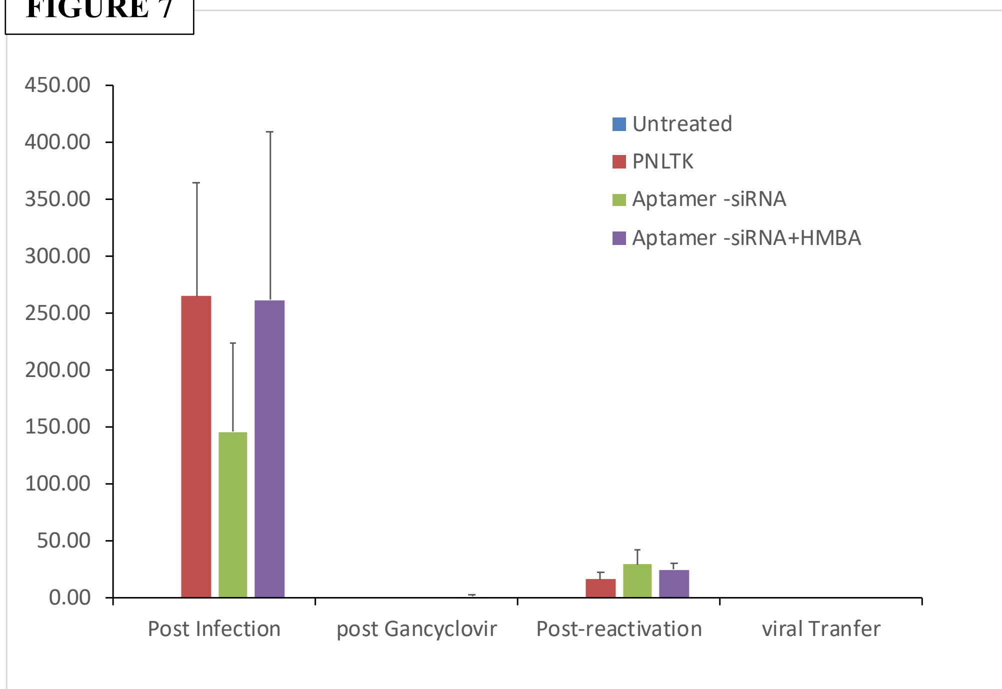


Figure 7: NHBE Air liquid interface cultures were infected with NL-TK virus enveloped with HIV IIIB gp120. Gancyclovir treatment completely abolishes HIV p24 suggesting that only uninfected and latently infected cells survive. Following gancyclovir removal and reactivation, NHBE ALI cultures again express HIV p24 suggesting the reactivation of latent virus. Viral transfer to Jurkat cells in presence of Tenofovir demonstrates complete suppression of p24.

Experimental set 3d: In this experimental subset, we have observed

1) Establishment of latency model using NL-TK virus in combination with gancyclovir.
2) Reactivation of virus by aptamer-siRNA chimera and HMBA

We will optimize this experiment to eliminate HIV in gancyclovir treated cells by increasing the duration of gancyclovir treatment. We will also increase the aptamer-siRNA chimera dose to improve reactivation. We will be analyzing DNA from Jurkat cells to confirm complete inhibition of HIV transfer.

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 was a post-doctoral fellow in the laboratory and Mr. Rajib Dutta who is a graduate student. 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 preparing the manuscript, "Use of aptamer-7sk siRNA chimera to eliminate 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 preparing the manuscript, "Use of aptamer-7sk siRNA chimera to eliminate 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 this 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.*

Nothing to report yet

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

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 faced technical issues reviving JLat 8.4 cells that delayed the completion of experimental set 1. WE tried several alternative cell lines to complete the experimental set. WE also faced disruptions due to hurricane. My post-doctoral fellow was out on paternity leave. WE will be requesting a no-cost extension.

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

Not applicable

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 preparing the manuscript, "Use of aptamer-7sk siRNA chimera to eliminate 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.*

In the next year we will be presenting our data at the American Society of Gene Therapy conference.

- **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.

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

- **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.

- **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.*

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.

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.

New funded proposal:

1 R01 HL147715-01 (PI: Unwalla) 09/01/2019 – 08/31/2022 2.4
Calendars NHLBI $356,475.00
Mechanisms of defective mitophagy and cellular senescence in HIV associated COPD

The major goals of this proposal are to determine the role of altered microRNAome and its impact on mitophagy and cellular senescence on chronic inflammation and COPD in HIV smokers/non-smokers.
No overlap with DOD grant

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.*

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*