Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** Drexel University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Anne Martella

4. **Grant Contact Person's Telephone Number:** (215) 895-6471

5. **Grant SAP Number:** 4100050893

6. **Project Number and Title of Research Project**: 11. *Suppression of Monocyte Migration by Inducible CCR2 Silencing*

7. **Start and End Date of Research Project:** 1/1/2010-6/30/2012

8. **Name of Principal Investigator for the Research Project:** Laura Steel, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 153,500

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Steel, Laura	Assistant Professor	42.3%	$31.727.99
Kutzler, Michele	Assistant Professor	13.7% Y1; 10.6% Y2	20,602.28
Jacobson, Jeffrey	Professor	2.5%	4,670.14
Sanghvi, Viraj	Graduate Student (PhD)	100%	29,212.02
Sylvester, Albert	Research Technician	7.4%	2,355.99

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
none		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
none		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___X____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No___X____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If

you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No___X_____

If yes, please describe your plans:

12. Future of Research Project. What are the future plans for this research project?

Although we do not currently have the resources to continue this specific project, we will be able to use some of the reagents (plasmid and viral vectors) that were generated, with only slight modifications, in ongoing projects in the laboratory that are related to the understanding of viral diseases.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes__X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male		1	1	
Female				
Unknown				
Total		**1**	**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic		1	1	
Unknown				
Total		**1**	**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White		1		
Black				
Asian				
Other			1	
Unknown				
Total		**1**	**1**	

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No___X____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes___X____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    This work has continued an active and fruitful collaboration between the laboratories of Dr. Laura Steel (P.I. on this project) in the Dept of Microbiology and Immunology and Dr. Michele Kutzler (Co-P.I.) in the Dept of Medicine at the Drexel University College of Medicine. Their complementary skills and interests in the areas of graduate education, molecular biology and immunology as applied to the development of antiviral therapeutics and vaccines, particularly for HIV-1 and HCV, will continue to be productive, as both investigators have expanded their knowledge of and access to experimental tools and systems in ways that will be invaluable in future investigations. In addition, students in both laboratories have been able to benefit from the combined knowledge base and resources of both of the investigators.

    This project contributed to the training of a very bright and motivated Ph.D. student (V. Sanghvi) who has now graduated and moved on to a post-doctoral position at Memorial Sloan-Kettering Cancer Center in New York, NY.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____X_____ No_____

If yes, please describe the collaborations:

Funding provided by this grant helped to support collaboration with investigators at the University of Pennsylvania in the laboratory of David Weiner, PhD (Department of Pathology and Laboratory Medicine) that included some technical expertise and methodology help, and supported the use of core facilities at the University of Pennsylvania, particularly in the acquisition of PBMCs.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No___X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Summary of Research Completed:

This work was designed to investigate a method to reduce the ability of HIV-1 infected immune cells to migrate into the brain by selectively silencing expression of the CCR2 receptor on the surface of HIV-1 infected monocytes. The CCR2 receptor plays a major role in the migration of monocytes across the blood-brain barrier and responds to the relatively selective monocyte chemoattractant, monocyte chemoattractant protein-1 (MCP-1 or CCL2) from astrocytes. MCP-1 secretion from astrocytes is induced by the HIV-1 protein Tat, that in turn, is released from HIV-1 infected monocyte-derived cells present in the brain. We hoped to establish a strategy to interrupt the positive feedback loop where monocytes are attracted by cytokines released from brain resident cells in infected patients, but where the attraction of HIV-1 infected monocytes serves to perpetuate or even increase the infection and its consequences within the brain. Since the response of monocytes to MCP-1 is key to many aspects of the immune response mediated by monocytes/macrophages, the selective induction of CCR2-miRNAs only in HIV-1 infected cells, and the continued normal functioning of uninfected monocytes/macrophages, is important to our overall approach.

Hypothesis: Lentiviral vectors that use an HIV-1 Tat-inducible promoter to drive the expression of CCR2-miRNAs can suppress CCR2 in transduced, HIV-1 infected monocytes, while the normal physiology and function of monocytes that are transduced, but not HIV-1 infected, will not be affected.

Aim 1: Determine whether transduction of primary human monocytes with the CK-TAR/miCCR2 lentiviral vector affects their proliferation or differentiation.

Background: Prior to beginning this project, we had constructed and characterized the CK-TAR promoter that serves as an alternative to the viral LTR as an HIV-1 Tat-inducible promoter (Sanghvi and Steel, 2010, Virus Research 155, 106). Use of the CK-TAR promoter instead of the HIV-1 LTR in lentiviral vectors reduces the potential for vector mobilization in therapeutic applications. In addition, the CK-TAR promoter is only minimally activated by a number of stimuli that might be encountered by monocytes including LPS, PMA, and TNFα; this is important since it would be undesirable to silence CCR2 expression in transduced, but uninfected, monocytes. We had also used reporter constructs to test the efficacy of several different miRNAs designed to target and knock down expression of CCR2 and inserted expression cassettes for these CCR2 miRNAs (plus controls) into lentiviral vectors.

1. Maximization of knockdown of endogenous CCR2 expression. Prior to testing the effects of transduction of CCR2-miRNA lentiviral expresson vectors on the physiology of monocytes, we needed to establish whether endogenous CCR2 could be efficiently silenced by our vectors. To do that, we sought to establish that CCR2 is expressed on the surface of U937 cells, a human monocytic cell line, and that expression could be reduced by our CCR2-miRNA. While we were successful in detecting CCR2 expression on the surface of U937 cells by FACS analysis (see Figure 1), the efficiency of transfection of these cells was too low to evaluate CCR2 knockdown by this method.

To address this problem and test our miCCR2 plasmid against CCR2 mRNA, we used HEK-293T cells that are notable for their high efficiency of transfection. These cells do not express endogenous CCR2 and therefore we transfected them with a CCR2 expression plasmid, pCMV-CCR2, together with the silencing plasmids to be tested. 48 hours after transfection, cells were reacted with antibody to CCR2 and analyzed by FACS. Results in Figure 2 show that in 293T cells, surface expression of CCR2 from the transfected plasmid can be suppressed by approximately 15% by miCCR2-D. This reduction is significant, but less than the levels of suppression (~50%) indicated in our original dual luciferase reporter assay screens of miCCR2 constructs. While this might reflect the stability of CCR2 in the cell, we constructed and tested three additional candidate CCR2-miRNAs (constructs E, F, and G). Two of these were derived from an Open Biosystems Library available to us at Drexel, and one was constructed according to a web-based algorithm. All were built into a pUC-based vector where expression of the miRNA is driven by a U6 (RNA polymerase III) promoter (described in Snyder et al. 2009. Nucl Acids Res. 37,e127). Knockdown of CCR2 mRNA was assayed in HEK-293T cells that were transfected with pCMV-CCR2, together with the silencing plasmids to be tested. RNA was isolated from cells 48 hours post-transfection and reductions in CCR2 mRNA were measured by RT-PCR. In these experiments, we confirmed the activity of miCCR2-D and identified two additional CCR2-miRNAs (constructs E and G) that showed silencing activity (see Figure 3). Since miRNAs expressed from polycistronic constructs can show increased potency compared to the constituent monocistronic constructs, we generated the combined miCCR2-D/E construct, a bicistronic construct that expresses both miCCR2-D and miCCR2-E.

2. Isolation of monocytes from human peripheral blood mononuclear cells. While working to maximize knockdown of endogenously expressed, authentic CCR2 mRNA, we proceeded to establish that we were able to isolate monocytes from human peripheral blood mononuclear cells

(PBMCs), detect expression of CCR2 on the isolated monocytes, and generate monocyte dervived macrophages (MDM) by inducing differentiation of the isolated monocytes. Methods: Human PBMCs were isolated by sequential centrifugation on Ficoll-Hypaque and Percoll gradients (Amersham Pharmacia LKB, Piscataway, NJ). For isolation of monocytes, $CD14^+$ cells were purified from PBMCs by positive enrichment using autoMACS (Miltenyi Biotec, Auburn, CA) according to the manufacturer's instructions. The purity of the monocyte cell populations was >98% as determined by FACS. Results in Figure 4 show that the $CD14^+$ monocytes are expressing CCR2, as indicated by positive staining with a CCR2 antibody.

For FACS staining, single cell suspensions were washed in PBS (pH 7.2) containing 0.2% bovine serum albumin and 0.1% NaN_3. Cells were incubated with goat IgG to block binding of Ig to FcßR and stained with PercP, FITC, APC or PE-labeled specific mAb diluted to the optimal concentration for immunostaining for 30 min at 4°C, washed three times and analyzed by flow cytometry. Live cells were gated on the basis of their forward scatter and side scatter characteristics and analyzed directly on a BD FACsCaliber Flow Cytometer (Coulter, Hialeah, FL) using FlowJo software (TreeStar, San Carlos, CA). All samples were compared to their isotype-matched controls. In the case of dual flow cytometry individual samples treated with each isotype alone were used to determine the background levels of auto fluorescence.

Antibodies: Conjugated human mAbs to CD14, CD86, HLA class II DR were purchased from BD Biosciences Pharmingen (San Diego, CA).

Monocyte derived macrophages (MDM) were generated from monocytes prepared from PBMCs. Adherent monocytes were cultured in complete RPMI 1640 medium with 1000 U/ml of recombinant human (rh) MCSF (Peprotech, Inc.) per 10^6 cells for up to 5 days. MDM were phenotyped using HLA-DR^{+++}, $CD86^{+++}$, and $CD14^{+++}$. All cytokines were recombinant human proteins and were used at plateau concentration to induce the optimal generation of MDM. The FACS analysis shown in Figure 5 demonstrates the differentiation of monocytes into macrophages after four days of treatment with MCSF.

<u>Aim 2:</u> Determine whether HIV-1 infection of primary human monocytes produces sufficient Tat protein to induce effective silencing of CCR2 from miRNA expression vectors that use the hybrid Tat-inducible (CK-TAR) promoter.

Background: We have previously described the construction of a lentiviral vector, pLEX-GFP, that can be used for the expression of miRNAs. This lentivector was constructed by combining portions of the commercial vectors, pLEX-MCS and pGIPZ (Open Biosystems), and carries a bicistronic gene encoding turboGFP (tGFP) and puromycin resistance, driven by a CMV promoter and separated by an IRES element, so that transduction can be followed (green fluorescent cells) and integrants can be selected (puromycin resistance). In addition, the shRNAmir sequence present in pGIPZ has been eliminated, and instead, a unique XbaI site is used as a point of insertion for gene cassettes encoding miRNAs driven by a promoter of choice. The pLEX-GFP vector was used to generate VSV-G pseudotyped viruses that simutaneously express two different CCR2-miRNAs (miCCR2-D/E) under the control of a CMV promoter (for constitutive expression) or the CK-TAR promoter (for Tat-inducible expression).

1. Transduction of primary human monocytes with a lentiviral vector that expresses miRNAs that target CCR2 mRNA. As described above, we showed by FACS analysis that the CCR2 receptor is expressed on the surface of human monocytes (Figure 4), and so we attempted to downregulate this expression using VSV-G pseudotyped lentiviruses carrying miCCR2-D/E expression cassettes. The extremely low efficiency of transduction of the primary monocytes with our vectors (and additional control vectors), as indicated by detection of GFP by fluorescence microscopy, together with the modest silencing we were able to achieve with miCCR2-D/E, made it impractical to continue with these experiments. Nevertheless, we were anxious to move forward to investigate the possibility of using the Tat-inducible CK-TAR promoter to express miRNAs with anti-viral properties so that both viral and cellular transcripts might be targeted, with minimal potential of doing harm in uninfected cells. Our use of the Tat-inducible CK-TAR promoter in lentiviral vectors to suppress HIV-1 replication is described below.

2. Induction of anti-viral miGag expression in virus infected cells. We have previously tested the antiviral activity of miGag, an miRNA that targets HIV-1 transcripts in a region that encodes Gag, and found it to be a potent suppressor of viral replication when expressed from plasmids that are delivered at high copy number to cells by transient transfection. We sought to test the efficacy of this miRNA in the more clinically relevant context of delivery by lentivectors and stable incorporation of far smaller copy numbers (approximately one) into the cellular genome. Further, our initial experiments had used co-transfection with HIV-1 Tat expression plasmids to demonstrate the Tat inducibility of the CK-TAR promoter and we wanted to extend those results to ask whether enough Tat is produced in virus infected cells to induce expression of miGag to levels that can suppress viral replication.

Lentiviral vectors that express miGag were constructed, with expression driven by either the CMV or CK-TAR promoter. These lentivectors were constructed in the pLEX-GFP background described above and so express both tGFP and puromycin resistance. HeLa-CD4 cells are stably transfected to express the CD4 cell surface marker, and they are infectable by HIV-1. We transduced HeLa-CD4 cells with CMV-miGag, CK-TAR-miGag, and CMV-miFlu (control) lentiviral vectors and selected for integrants by growth in puromycin. These cells were then infected with HIV-1 (strain LAI) and virus replication was assayed two days post-infection. The results shown in Figure 6 demonstrate that transduction with the CMV-miGag lentivector suppresses viral replication as compared to the irrelevant CMV-miFlu lentivector, as expected. The CK-TAR-miGag lentivector also strongly suppresses viral replication. Since we have shown previously that the CK-TAR promoter has very low basal activity in the absence of Tat induction, these results support the interpretation that Tat produced in infected cells is sufficient to induce expression of miGag. Additional experiments are needed to verify this conclusion, and to measure the copy number of integrated vector. Although more needs to be done, these experiments provide support for a strategy where antiviral miRNAs are induced in HIV-1 infected cells so that effects on the physiology of non-infected cells may be minimal.

3. Suppression of silencing by miRNAs in HIV-1 infected cells. There have been several reports that HIV-1 gene products can globally suppress RNA silencing in infected cells and that this acts as a viral countermeasure against cellular RNAi-mediated defense mechanisms. These claims

have been controversial, but understanding the interplay between the virus and cellular RNA silencing pathways is clearly important when devising antiviral therapies based on silencing. Although the following studies were not described in our original submission of this project, we felt it was important to move forward by characterizing elements of the cellular miRNA-based antiviral response that might be affected by HIV-1 gene products present in infected cells. In particular, both the HIV-1 Tat protein and the structured TAR element, present at the 5' end of HIV-1 transcripts, have been claimed to act as global suppressors of the cellular RNA silencing machinery. Results from our experiments described below demonstrate that neither of these potential viral anti-silencing mechanisms will provide barriers to the use of miRNA-based antiviral strategies.

First, we examined whether the HIV-1 Tat protein is truly a suppressor of RNA silencing (SRS) and whether there is any indication of global suppression of RNA silencing pathways in the presence of replicating virus. Using several measures of silencing by both exogenous and endogenous miRNAs, we have shown that reported SRS activity of Tat is due to its activity as a transcriptional transactivator, and not SRS activity. In the experiment shown in Figure 7, HeLa-P4R5 cells were co-transfected with pdsEGFP (GFP expression plasmid) and a plasmid encoding miEGFP, in the presence or absence of a Tat expression plasmid, pwtTat. The suppression of EGFP expression by miEGFP appears to be diminished by co-expression of wild type Tat (lanes 1-4). However, when a transcriptionally inactive Tat mutant (K41A-Tat) was co-expressed, the apparent SRS activity disappeared (lanes 5 and 6). Further, when cells were transfected with pwtTat in the absence of the silencing plasmid, it was evident that Tat alone can substantially increase the expression of the EGFP reporter (lanes 7 and 8), although expression of the mutant K41A Tat did not affect EGFP levels (lanes 9 and 10). These results show a direct relationship between the transcriptional activity of Tat and its ability to up-regulate EGFP reporter expression, in a manner independent of silencing.

To test the effects of Tat on silencing mediated by endogenous cellular miRNAs, we used a dual luciferase reporter plasmid, psiCHECK-2, where target sequence for an miRNA is inserted into the 3'-untranslated region of a Renilla luciferase gene and firefly luciferase is expressed from an independent gene on the same plasmid to serve as a transfection efficiency control. Silencing of Renilla luciferase by miRNAs targeting the inserted sequence is reported as a reduction in the ratio of Renilla to firefly luciferase activity. Results shown in Figure 8 indicate that transfection of cells (HeLa or HeLa-P4R5) with a psiCHECK-2 reporter carrying target sequence for miR-16 (psiCH2-16T) gives no indication of reduced silencing in the presence of co-transfected Tat, again arguing against an SRS activity for Tat.

The sequestration of TRBP (an essential co-factor of Dicer in miRNA processing) by its binding to the TAR element of HIV-1 transcripts has been proposed as an additional mechanism of HIV-1 suppression of silencing. Therefore, we investigated whether products of HIV-1 replication, in addition to Tat, could suppress RNA silencing. The target site in psiCH2-16T is perfectly complementary to miR-16 and so we also constructed a second plasmid, psiCH2-cycE1, that contains the imperfectly matched sequence found in a validated miR-16 target, cycE1 mRNA, to more closely mimic natural silencing. In addition, we constructed a reporter where the miR-16 target sites were mutated by site-directed mutagenesis, to act as a control. Silencing of Renilla luciferase activity from these reporter plasmids was tested in both HEK-293T cells and in Jurkat

T-cells by co-transfection with the HIV-1 infectious molecular clone, pLAI. Results shown in Figure 9 indicate that there is no reduction in the silencing capacity of endogenous miR-16 in the presence of replicating HIV-1 in either HEK-293T cells or in Jurkat cells. The results of these and additional experiments demonstrate that HIV-1 does not act to globally suppress cellular silencing. These experiments are more fully described in a publication that resulted from this work (Sanghvi and Steel, 2011a, see Item 20, below).

4. The role of the cellular protein TRBP (TAR RNA binding protein) in promoting HIV-1 replication. Our experiments, described above, indicated that HIV-1 gene products do not suppress silencing, and the TAR element, in particular, does not suppress silencing by sequestering TRBP, an essential cellular protein in silencing pathways. Therefore, we sought to identify the mechanism through which TRBP promotes HIV-1 replication. We found that TRBP acts primarily through its known role as an inhibitor of PKR, a kinase that can play an important role in the innate antiviral response. Data presented in Figure 10 show that viral replication in 293T cells is reduced by suppression of TRBP, but only in cells where PKR can be activated. When PKR activation is inhibited by the introduction of a dominant negative mutation (PKRΔ6), suppression TRBP has no effect on HIV-1 replication. In Figure 11, we show that TRBP mRNA is expressed at relatively low levels in both Jurkat cells, a T cell line, and in primary CD4+ T cells, a natural host of HIV-1. Activation of the CD4+ cells further lowers TRBP mRNA levels. As with 293T cells, knockdown of TRBP provides no replication advantage to HIV-1 in primary CD4+ cells when PKR is suppressed. This again supports the argument that TRBP promotes HIV-1 replication through its activity as a suppressor of PKR, and not by a mechanism involving its role in miRNA processing. Additional experiments that extend and further support these findings are presented in a publication of this work (Sanghvi and Steel, 2011b, see Item 20, below).

The initial goal of this project was to explore the possibility of inducible knockdown of the CCR2 receptor on HIV-1 infected monocytes in order to prevent their migration across the blood-brain barrier and thereby reduce the progression of neurologic disease in HIV-1 infected patients. While we have successfully constructed lentiviral vectors that express miRNAs under the control of HIV-1 Tat inducible promoters, and demonstrated that these vectors can strongly downregulate HIV-1 replication in infected cells, we were unable to extend these results to the knockdown of CCR2 in human monocytic cells. This was primarily due to the very low efficiency of transduction of monocytic cells by our lentiviral vectors and was compounded by somewhat low levels of silencing shown by our individual miCCR2 constructs. We feel that the latter problem can be solved by testing further miCCR2 candidates, expressing multiple miCCR2 effectors from a single vector, and allowing a longer period of time for transduced cells to clear pre-existing receptor. However, since we could not solve the problem of poor transduction of monocytes in the timeframe of this grant, we elected to pursue experiments that characterized additional aspects of the use of miRNA-mediated silencing to reduce HIV-1 replication in infected cells.

We were able to demonstrate that Tat produced in the course of HIV-1 replication in cells (as opposed to Tat supplied by ectopic expression vectors) is sufficient to induce strong silencing of viral replication from lentiviral vectors designed to produce antiviral miRNAs from Tat-

inducible promoters. This result is important since it shows that the balance between Tat production during viral replication and that necessary for induction of antiviral miRNAs can be maintained in a way that favors the antiviral effects of the Tat inducer. This strategy remains a viable approach for the selective knockdown of viral or cellular targets in infected cells, so as to minimize potential harm from unintended effects of heterologous miRNA expression in uninfected cells.

Additionally, we have characterized aspects of the interplay between HIV-1 gene products and cellular RNA silencing pathways. This has been a controversial field and there have been many reports of mechanisms of suppression cellular RNA silencing by HIV-1 gene products that would clearly have implications for therapeutic strategies that rely on silencing. We have made contributions that support the view that HIV-1 does not suppress cellular silencing pathways. Instead, the proposed SRS activity of Tat can be explained by its function as a transcriptional transactivator, the structured TAR element present on HIV-1 transcripts does not sequester TRPB in a way that reduces processing or activity of cellular miRNAs, and the role of TRBP in promoting viral replication is primarily to inhibit the antiviral kinase, PKR.

Project Presentations:

Viraj Sangvhi was selected for an oral presentation of this work at the Drexel University College of Medicine Discovery Day, 2010.

Figures

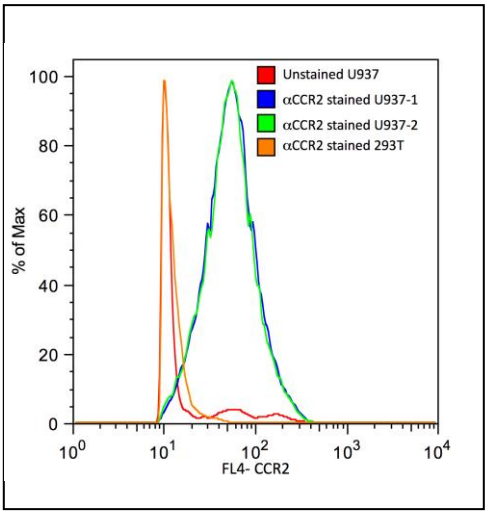


Figure 1. Cells of the monocytic line, U937, express CCR2 on their surface, as detected by FACS analysis. U937 cells or 293T (control) cells were stained with antibody to CCR2 and analyzed by FACS.

Figure 2. FACS analysis of 293T cells shows suppression CCR2 expression by miCCR2-D. 293T cells were co-transfected with pCMV-CCR2 together with no silencing plasmid, pUC-U6-miCCR2D, or pUC-U6-miEGFP, as indicated. 48 h post-transfection, cells were stained with antibody to CCR2 and analyzed by FACS.

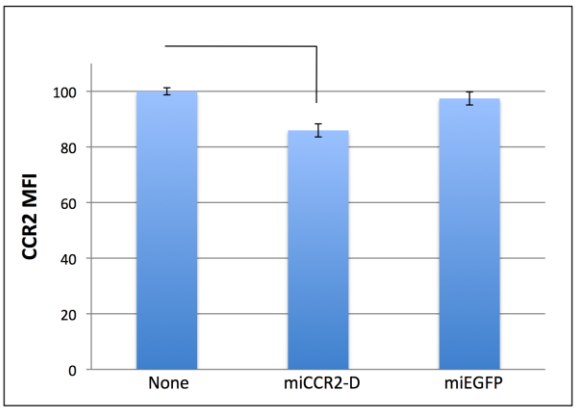
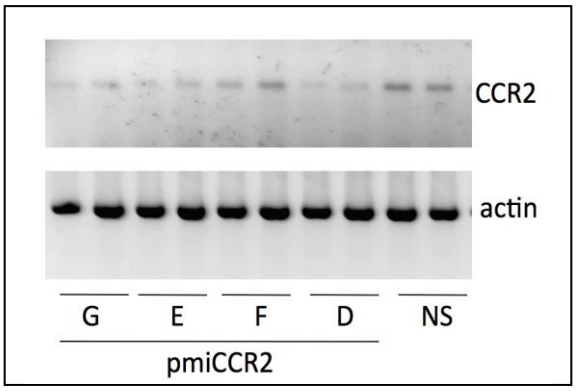


Figure 3. RT-PCR analysis of miCCR2 candidates. HEK-293T cells were transfected with pCMV-CCR2 together with pUC-U6-miCCR2-D, -E, -F, -G, or a non-specific control miRNA. Knockdown of CCR2 mRNA was assessed by RT-PCR. Actin mRNA was detected in each sample as a control. Each construct was assayed in duplicate transfections.



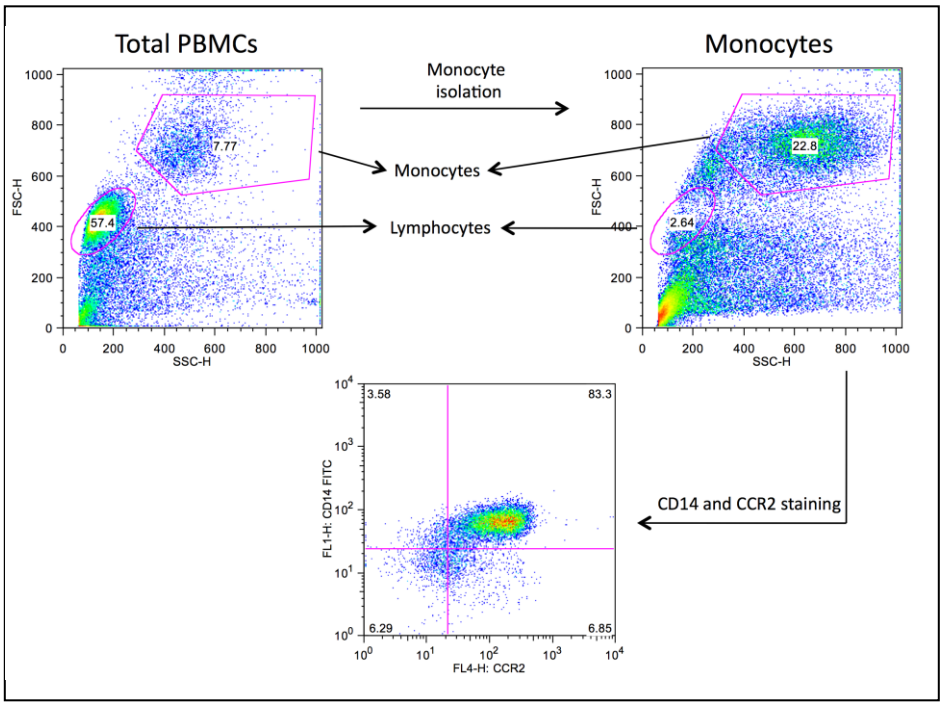


Figure 4. CCR2 is expressed on the surface of isolated human monocytes. The monocytic cell population was isolated from human PBMCs as described in the text. The identity of monocytes was confirmed by the marker CD14; CCR2 expression on the monocytes was demonstrated by anti-CCR2 staining.

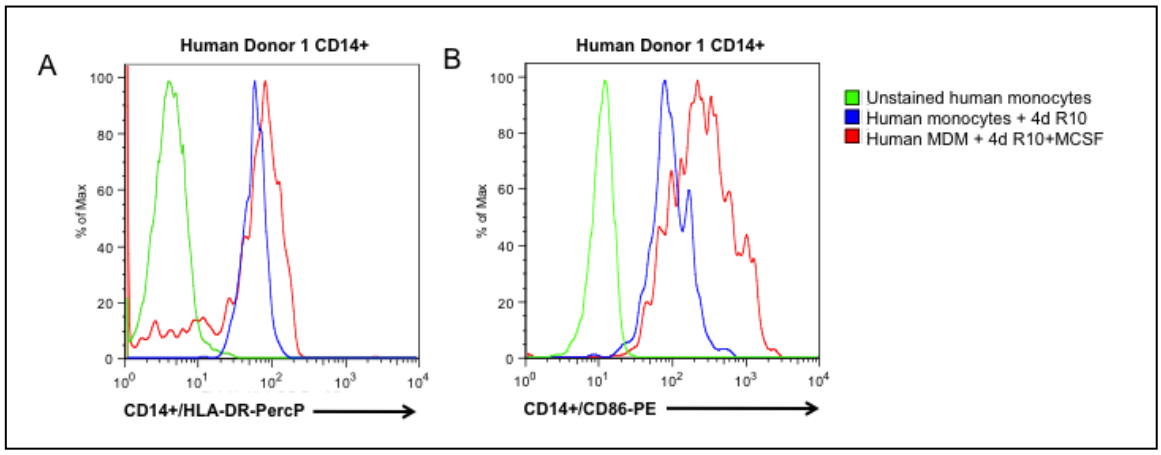


Figure 5. FACS analysis demonstating differentiation of isolated human monocytes into macrophages (MDM) as determined by HLA-DR+ and CD86+ staining on CD14+ cells following 4 days of treatment with M-CSF.

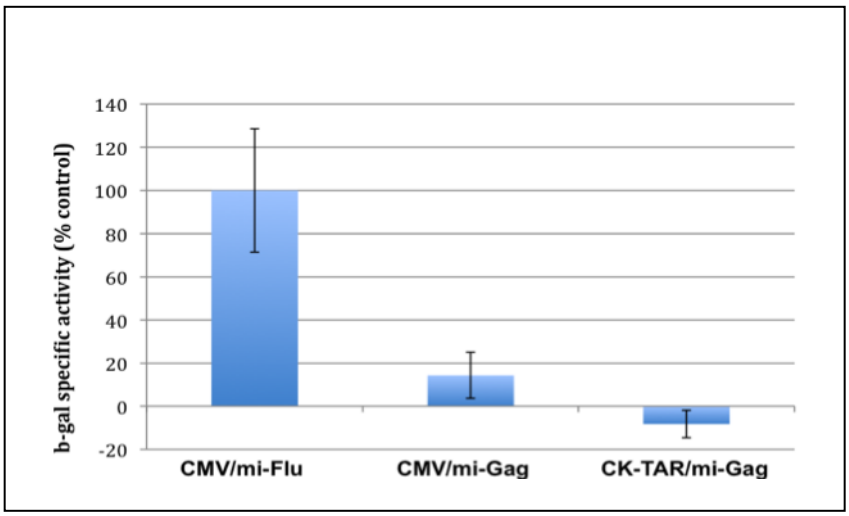


Figure 6. Suppression of viral replication by Tat-induced expression of miGag in virus infected cells. Stable HeLa-CD4 transductants were prepared from cells transduced with CMV-miFlu, CMV-miGag, and CK-TAR-miGag lentivectors. Cells were infected with HIV-1 (LAI) virus and extensively washed. Two days post-infection, supernatant was collected and viral replication was quantified by an infectivity assay where HeLa-P4R5 indicator cells were treated with equal amounts of supernatant from each of the infected cell samples. Infectious virus was measured by the induction of β-galactosidase in the indicator cells. Results are presented as percent of activity from the control CMV/miFlu cells and error bars show the standard deviation from 8 experimental replicates.

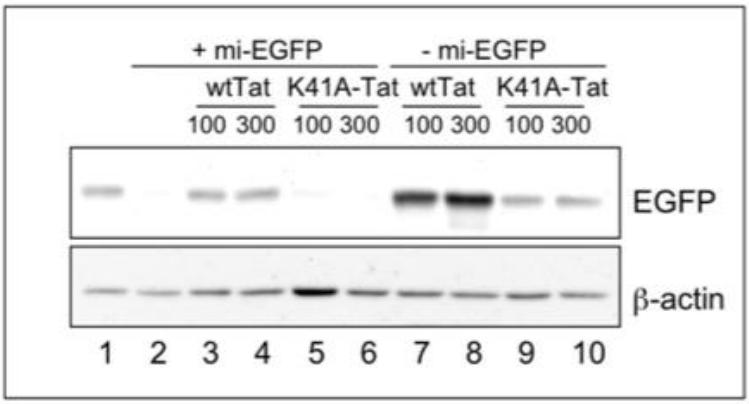


Figure 7. Apparent SRS activity of HIV-1 Tat protein is due to transcriptional activation of reporter expression. Immunoblot analysis of EGFP expression in the presence or absence of a silencing plasmid (mi-EGPP) and wt-Tat or K41A-Tat plasmids, as indicated. Detection of β-actin serves as a loading control.

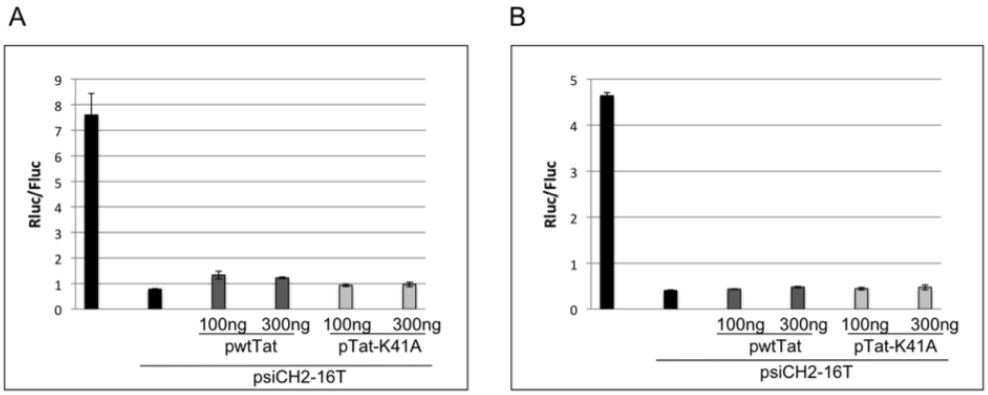


Figure 8. Tat overexpresssion does not reduce silencing mediated by endogenous miR-16. A) HeLa cells, B) HeLa-P4R5 cells.

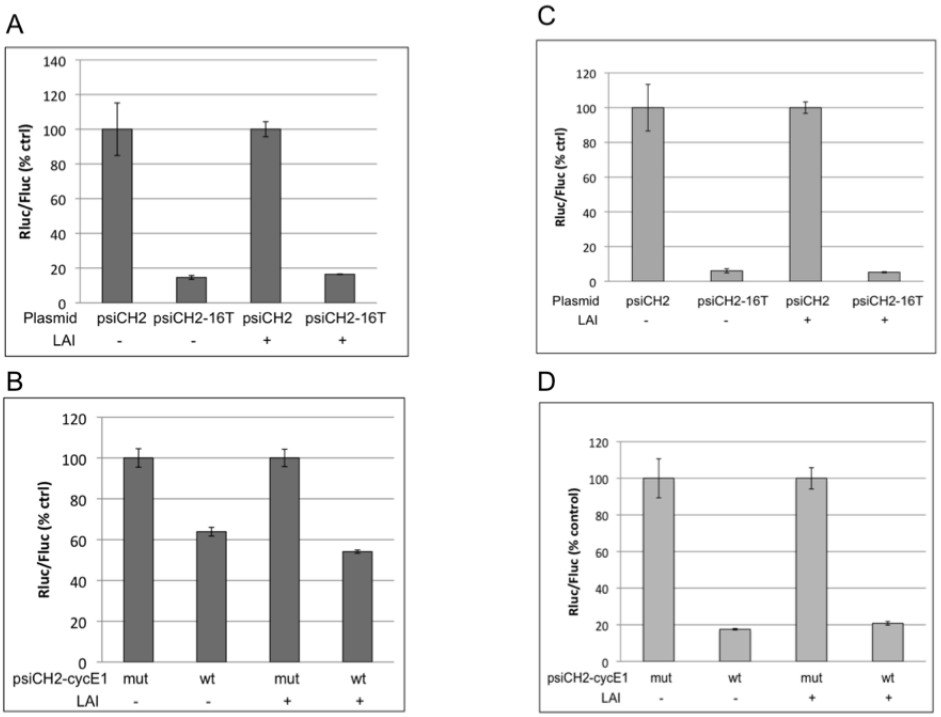


Figure 9. Silencing efficacy of endogenous miR-16 is not affected by the presence of replicating HIV-1. A) 293T cells were transfected with a reporter plasmid, either psiCH2 (empty plasmid) or psiCH2-16T, together with or without pLAI, as indicated. Cell lysates were prepared and luciferase activity was assayed 2d post-transfection. Data are normalized to the respective psiCH2 control. B) 293T cells were transfected with either psiCH2-wtE1 or psiCH2-mutE1, in the presence or absence of pLAI, as indicated. Cells lysates were analyzed for luciferase activity as in (A). Data are normalized to the respective psiCH2-E1 control. C) Jurkat cells were transfected and analyzed for the efficacy of miR-16 silencing as in (A). D) Jurkat cells were transfected and analyzed as in (B). Error bars represent standard deviation from 4 replicates.

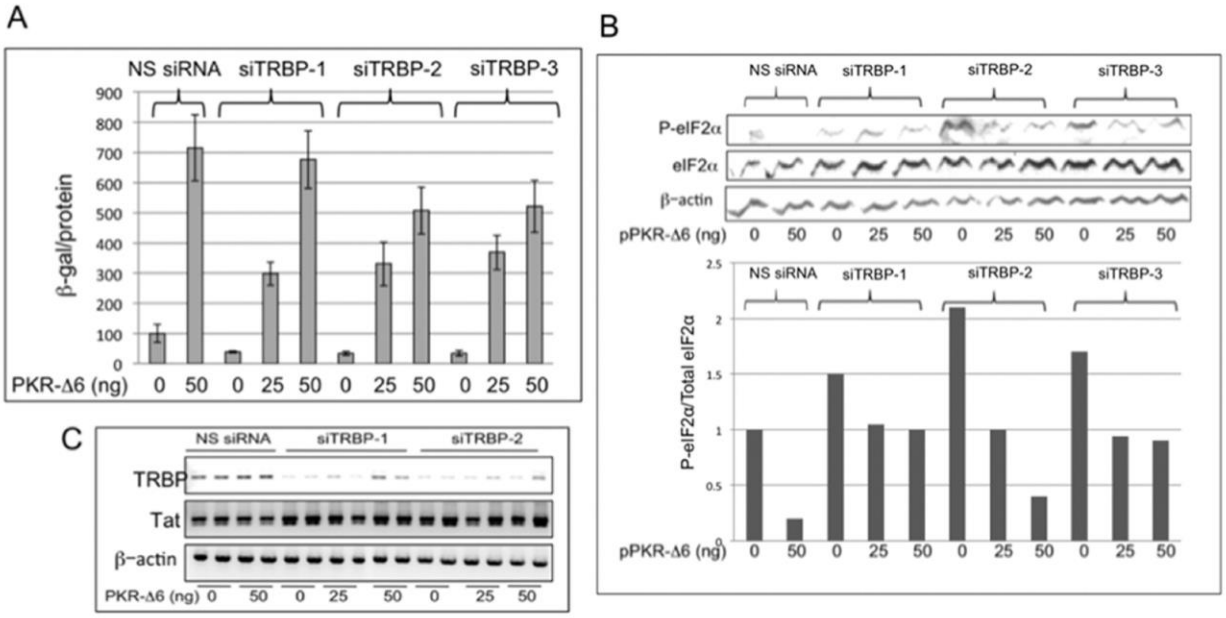


Figure 10. Inhibition of PKR activation prevents the suppression of HIV-1 replication by TRBP knockdown in 293T cells. A) 293T cells were transfected with non-specific (NS) siRNA, siTRBP-1, siTRBP-2, or siTRBP-3, together with a constant amount of pLAI and different amounts of pPKR-Δ6, as indicated. Two days post-transfection, infectious virus released into the supernatant was determined by P4R5 infection assay. Data are represented as β-galactosidase specific activity in the P4R5 indicator cells and shown as percent of control (NS siRNA, no pPKR-Δ6). Error bars represent standard deviation derived from a total of 4 replicates. B) Upper panel: Total protein extracts prepared from cells transfected in panel (A) were immunoblotted for detection of P-eIF2α, total eIF2α, and actin. Lower panel: Relative expression of P-eIF2α to total eIF2α as determined by densitometry analysis of immunoblot shown in the upper panel. C) Total RNA isolated from cells transfected as in panel (A) was analyzed by RT-PCR using primers specific for HIV-1 Tat mRNA, TRBP mRNA, and β-actin mRNA with a limited number of PCR cycles to enable detection of quantitative differences in input RNA. Replicate samples are shown for each experimental condition.

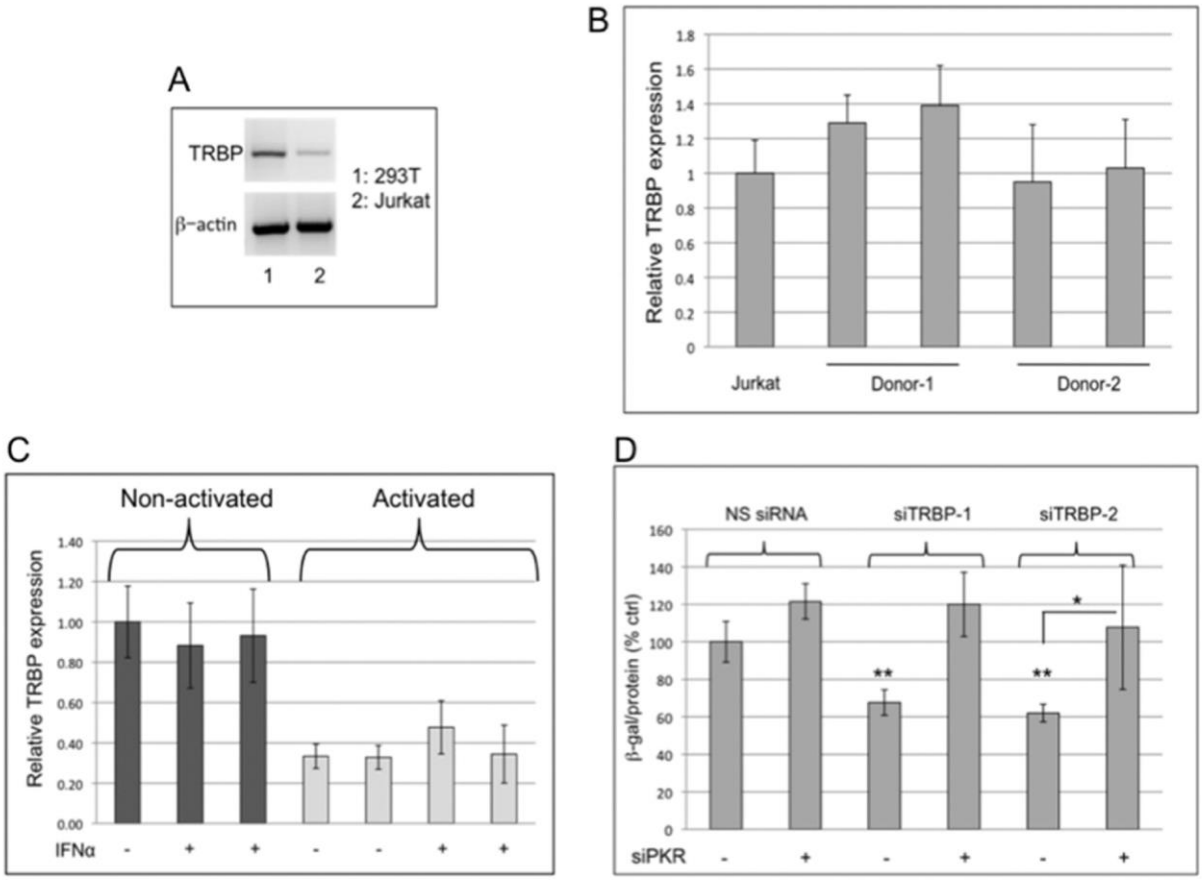


Figure 11. TRBP promotes HIV-1 replication in primary CD4$^+$ T cells only when the cells express functional PKR. A) RNA isolated from 293T and Jurkat cells was subjected to RT-PCR using primers specific for either TRBP mRNA or actin mRNA. B) TRBP expression was analyzed by qRT-PCR in non-activated primary CD4$^+$ T cells isolated from two different donors. Data are normalized to actin and represented as fold change relative to TRBP mRNA expression in Jurkat cells. C) Total RNA was isolated from non-activated and activated CD4$^+$ T cells, treated or not treated with 1000 U/mL IFNα for 24 hr, and subjected to qRT-PCR analysis to examine TRBP expression. Data are normalized to actin and represented as fold change relative to non-activated, untreated T cells. D) Activated primary CD4$^+$ T cells were transfected with indicated siRNAs and 24 hr post-transfection, cells were infected with HIV-LAI (4 ng p24). Production of infectious virus was determined by P4R5 infection assays 2 d post-infection. β-galactosidase specific activity is shown as a percent of the NS siRNA/no siPKR control. Error bars represent standard deviation from four replicates. (**) denotes p-value<0.005 (relative to NS siRNA/no siPKR control) and (*) represents p-value<0.05.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:
This project did not involve human embryonic stem cells.

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one

publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. A re-examination of global suppression of RNA interference by HIV-1	Sanghvi, V.R. and Steel, L.F.	PLoS One 2011 6(2), e17246 Epub Feb 28, 2011 PMID 21386885	11/2010	☐Submitted ☐Accepted ☒Published
2. The cellular TAR RNA binding protein, TRBP, promotes HIV-1 replication primarily by inhibiting the activation of double-stranded RNA-dependent kinase PKR	Sanghvi, V. R., and L. F. Steel	Journal of Virology 2011 85:12614-21. PMID 21937648 Epub Sept 21 2011	05/2011	☐Submitted ☐Accepted ☒Published
3. RNA silencing as a cellular defense against HIV-1 infection: progress and issues	Sanghvi, V. R., and L. F. Steel	FASEB J 2012 26(10):3937-45. PMID 22751007 Epub Jul 2 2012	04/2012	☒Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No___X____

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis,

or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

This work has provided support for the eventual feasibility of a novel approach to HIV-1 therapy that selectively targets HIV-1 infected cells while preserving the function of uninfected cells.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Our work utilizes a new approach to antiviral therapy that is based on gene silencing technologies. This is an active area of new research that shows great promise, although problems remain to be solved before the strategy can be implemented. For instance, HIV-1 is able to escape from silencing reagents because of the high mutation rate of this virus. Our research has sought to develop a method that will silence cellular genes that are necessary for viral replication or pathogenesis, and thereby avoid the problem of rapid viral evolution. Our project is at an early, proof-of-concept stage that represents the basic investigation that must be completed in order to develop new treatments for disease.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___X____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Laura F. Steel, Ph.D.	Assistant Professor
eRA COMMONS USER NAME STEEL1	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Michigan, Ann Arbor, MI	--	1967-69	
Yale University, New Haven, CT	B.A.	1971	Biology
Cornell University, Ithaca, NY	Ph.D.	1977	Genetics
Univ of Mass. Medical School, Worcester, MA	post-doc	1977-81	Molecular Biology

Positions and Honors

1981-1987	Instructor, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA
1982-1984	Scientist/Consultant under contract to Cetus Corporation, Emeryville, CA. Work performed at Univ. of Mass. Medical School, Worcester, MA
1987-1989	Assistant Professer (Research), Department of Molecular Genetics and Microbiology, Univ. of Mass. Medical School, Worcester, MA
1989-1991	Assistant Professor, Fels Institute for Cancer Research and Molecular Biology, Temple University, Philadelphia, PA
1991-1996	Assistant Professor, Jefferson Cancer Institute, Thomas Jefferson University, Philadelphia, PA. (In 1991, members of the Fels Institute relocated to Thomas Jefferson University.)
1996-1998	Lecturer, Biology Department, University of Pennsylvania, Philadelphia, PA Instructor, College of Graduate Studies, Thomas Jefferson University
1998-2004	Assistant Professor (Research), Department of Biochemistry and Molecular Pharmacology, Jefferson Center for Biomedical Research, Thomas Jefferson University, Doylestown, PA
2004-present	Assistant Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA (In 2004, the Jefferson Center switched its academic affiliation to Drexel University.)
1971-1977	NIH Pre-doctoral Fellow
1977-1979	American Cancer Society Post-doctoral Fellow
1979-1981	Muscular Dystrophy Association Post-doctoral Fellow

Selected Publications (selected publications)

Steel, L.F. and Jacobson, A. (1991). Sequence elements that affect mRNA translational activity in developing *Dictyostelium* cells. Devel. Genet. 12,98-103. PMID: 2049885

Steel, L.F., Telly, D.L., Leonard, J., Rice, B.A., and Sawicki, J.A. (1996). Elements in the murine c-*mos* messenger RNA 5'-untranslated region repress translation of downstream coding sequences. Cell Growth & Differentiation 7, 1415-1424. PMID: 8891345

Steel, L.F., Mattu, T.S., Mehta, A., Hebestreit, H., Dwek, R., Evans, A., London, W.T., and Block, T. (2001). A proteomic approach for the discovery of early detection markers of hepatocellular carcinoma. Disease Markers 17, 179-189. PMID: 11790885

Steel, L.F., Shumpert, D., Trotter, M., Seeholzer, S.H., Evans, A.A., London, W.T., Dwek, R., and Block, T.M. 2003. A strategy for the comparative analysis of serum proteomes for the discovery of biomarkers for hepatocellular carcinoma. Proteomics 3, 601-609. PMID: 12748940

Steel, L.F., Trotter, M.G., Nakajima, P.B., Mattu, T.S., Gonye, G., and Block, T.M. 2003. Efficient and specific removal of albumin from human serum samples. Molec Cell Proteomics 2.4, 262-270. PMID: 12754305

Comunale, M.A., Mattu, T.S., Lowman, M., Evans, A.A., London, W.T., Drake, R., Semmes, J., **Steel, L.F.**, Block, T.M., and Mehta, A. 2003. Comparative proteomic analysis of de-N-glycosylated serum from hepatitis B carriers reveals polypeptides that appear to correlate with disease status. Proteomics 4, 826-838. PMID: 14997503

Steel, L.F., Haab, B.B., and Hanash, S.M. 2005. Methods of comparative proteomic profiling for disease diagnosis. J. Chromatogr. B. 815,275-284. PMID: 15652816

Schwegler, E.E., Cazares, L., **Steel, L.F.**, Adam, B.L., Johnson, D.A., Semmes, O.J., Block, T.M., Marrero, J.A., Drake, R.R. 2005. SELDI-TOF-MS profiling of serum for detection of the progression of chronic hepatitis C to hepatocellular carcinoma Hepatology 41, 634-642. PMID: 15726646

Block, T.M., Comunale, M.A., Lowman, M., **Steel, L.F.**, Romano, P.R., Fimmel, C., Tennant, B.C., London, W.T., Evans, A.A., Blumberg, B.S., Dwek, R.A., Mattu, T.S., and Mehta, A.S. 2005, Use of targeted gylcoproteomics to identify serum glycoproteins that correlate with liver cancer in woodchucks and humans. Proc. Natl. Acad. Sci. 102, 779-784. PMID: 15642945

Marrero, J.A., Romano, P.R., Nikolaeva, O., **Steel, L.**, Mehta, A., Fimmel, C.J., Comunale, M.A., D'Amelio, A., Lok, A.S., and Block, T.M. 2005. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. J Hepatol. 43, 1007-1012.

Snyder, L.C., Esser, J.E., Pachuk, C.J., and **Steel, L.F**. 2008. Vector design for liver specific expression of multiple interfering RNAs that target hepatitis B virus transcripts. Antiviral Research 80, 36-44. PMID: 18499277

Snyder, L.C., Ahmed, I., and **Steel, L.F.** 2009. RNA polymerase III can drive polycistronic expression of functional interfering RNAs designed to resemble microRNAs. Nucl Acids Res. 37,e127. PMID: 19679642

Sanghvi, V.R. and **Steel, L.F.** 2011. Expression of interfering RNAs from an HIV-1 Tat-inducible chimeric promoter. Virus Res. 155, 106-111. PMID: 20851721

Sanghvi, V.R. and **Steel, L.F.** 2011. A re-examination of global suppression of RNA interference by HIV-1. PLoS One 6(2), e17246. PMID: 21386885

Sanghvi, V.R. and **Steel, L.F.** 2011. The cellular TAR RNA binding protein, TRBP, promotes HIV-1 replication primarily by inhibiting the activation of double-stranded RNA-dependent kinase PKR. J Virol 85**:**12614-21. PMID: 21937648.

Sanghvi, V. R. and **Steel, L.F.** 2012. RNA silencing as a cellular defense against HIV-1 infection: progress and issues. FASEB J 26, 3937-3945. Epub ahead of print Jul 2. PMID: 22751007.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.

NAME	POSITION TITLE
Michele Wetzel Kutzler, Ph.D.	tenure track
eRA COMMONS USER NAME (credential, e.g., agency login) MKUTZLER	Assistant Professor of Medicine Assistant Professor of Microbiology and Immunology Assistant Dean of Faculty Affaris and Professional Develop.

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and*

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Temple University	B.S.	1995	Biology/Education
Temple University School of Medicine	Ph.D.	2001	Opioid immunomodulation of HIV-1
University of Pennsylvania *Postdoctoral Fellow*		2001-2006	HIV-1 DNA vaccines and IL15 molecular adjuvant
University of Pennsylvania *Research Associate*		2006-2007	HIV-1, Flu DNA vaccine/Mucosal chemokine molecular adjuvants
Drexel University College of Medicine *Assistant Dean of Faculty Affairs and Professional Develop.* *Assistant Professor of Medicine* *Assistant Professor of Micro/Immuno*		2007-present	DNA based HIV-1 vaccines and mucosal adjuvants *C. difficile* DNA vaccine/vaccine immunity in elderly/animal models

A. Personal Statement. Dr. Kutzler is an Assistant Professor of Medicine, with her primary appointment in the Division of Infectious Disease & HIV Medicine, and she holds a secondary appointment in the Department of Microbiology and Immunology.

B. Positions and Honors

1993-1996	**Honors Research in Biology** Temple University, Philadelphia, PA.
1994, 1995, 1996	**Henry R. Sklar Ambassador Award for academic achievement, leadership and dedication,** Temple University, Philadelphia, PA.
1994	**National Science Foundation Summer Honors Research Program**. Temple U Philadelphia, PA.
1995	**Mark Berger Prize for outstanding research in neurobiology.** Temple U Philadelphia, PA.
1995	**Graduation with Distinction in Biology major.** Temple University Philadelphia, PA.
1996	**PhD graduate student** in Microbiology/Immunology, Temple University School of Medicine
1998	**Young Investigator Award**. The sixth annual symposium for Drugs of Abuse, Immunomodulation and AIDS
2001 to 2006	**Postdoctoral fellow**, The University of Pennsylvania
2005	**Outstanding Lecturer Award**. Fourth Annual Postdoctoral Symposium. The University of Pennsylvania School of Medicine, Philadelphia, PA
2006	**Research Associate**, The University of Pennsylvania

2007	**Assistant Professor,** Tenure Tack, Division of Infectious Diseases & HIV Medicine, Department of Medicine, Drexel University College of Medicine
2010	**Young Investigator Conference Scholarship Recipient**, AIDS Vaccine 2010, Atlanta GA
2011	Professional Enrichment Growth Grant Award Recipient (DUCOM faculty Affairs)
2011-2013	***Chair*, Women in Academic Medicine Committee**, DUCOM
2013	Faculty **Young Investigator Award**, DUCOM Discovery Day 2013

C. Selected peer-reviewed publications (selected from 40 articles, Wetzel is *maiden* name)

1. Kathuria N., Kraynyak K.K., Carnathan, D., Betts, M., Weiner, D.B., and **Kutzler, M.A**. Generation of Antigen-Specific Immunity Following Systemic Immunization with DNA vaccine encoding CCL25 Chemokine Immunoadjuvant. Human Vaccines & Immunothera. **2012** Nov 1;8(11).
2. Reuter M.A., Yuan, S., Marx, P.A., **Kutzler, M.A.,** Weiner, D.B., Betts, M. R. DNA-based HIV vaccines do not induce generalized activation in mucosal tissue T cells. Human Vac & Immunother. **2012** Oct 30;8(11).
3. K. A Kraynyak, **M.A. Kutzler**, N. J Cisper, A.S. Khan, R. Draghia-Akli; N.Y. Sardesal, M. G. Lewis, J.Yan, D.B. Weiner. **2010**. Systemic Immunization with CCL27/CTACK Modulates Immune Responses at Mucosal Sites in Mice and Macaques. *Vaccine* 28:1942-1951.
4. **Kutzler, M.A.,** Kimberly A. Kraynyak, Sarah J. Nagle, Rose M. Parkinson, Darya Zharikova, Michael Chattergoon, Henry Maguire, Karuppiah Muthumani, Kenneth Ugen and David B. Weiner. **2010.** Plasmids encoding the mucosal chemokines CCL27 and CCL28 are effective adjuvants in eliciting antigen-specific immunity *in vivo*. *Gene Therapy* Jan;17(1);72-82. Epub, 2009:Oct 22.
5. Kimberly A. Kraynyak, **M.A. Kutzler**, Neil J. Cisper, Dominick J. Laddy, Matthew P. Morrow, Thomas A.Waldmann, and David B. Weiner. **2009.** Plasmid-Encoded IL-15Rα Adjuvants Specific Immune Responses Induced by a DNA Vaccine *in vivo*. *Human Gene Therapy* Oct 20(10):1143-56.
6. **Kutzler, M.A**. and D.B. Weiner. **2008**. DNA Vaccines: Ready for Prime Time? *Nature Reviews Genetics*. Vol.9:1-13.
7. **Kutzler, M.A.**, and J.M. Jacobson. **2008.** Treatment Interruption as a tool to measure changes in immunologic response to HIV-1. *Current Opinion in HIV and AIDS* Vol.3 (2):131-135.
8. **Kutzler, M.A.,** Ugen, K. E., and Weiner, D.B. Smallpox: Pathogenesis and Host Immune Responses Relevant to Vaccine and Therapeutic Strategies. Microorganisms and Bioterrorism. Series: Infectious Agents and Pathogenesis. Friedman, Herman; Anderson, Burt; Bendinelli, Mauro (Eds.) **2006**, p. 63-81. Illus. ISBN: 978-0-387-28156-8 (print) 978-0-387-28159-9 (online).
9. **Kutzler, M. A.,** T. M. Robinson, M. A. Chattergoon, D. K. Choo, A. Y. Choo, P. Y. Choe, M. P. Ramanathan, R. Parkinson, S. Kudchodkar, Y. Tamura, M. Sidhu, V. Roopchand, J. J. Kim, G. N. Pavlakis, B. K. Felber, T. A. Waldmann, J. D. Boyer, and D. B. Weiner. **2005.** Coimmunization with an optimized IL-15 plasmid results in enhanced function and longevity of CD8 T cells that are partially independent of CD4 T cell help. *J Immunol* 175:112-123.
10. **Kutzler, M. A.,** and D. B. Weiner. **2004**. Developing DNA vaccines that call to dendritic cells. *The Journal of Clinical Investigation* 114:1241-1244.

    Wetzel, M. A., A. D. Steele, E. E. Henderson, and T. J. Rogers. **2002.** The effect of X4 and R5 HIV-1 on C, C-C, and C-X-C chemokines during the early stages of infection in human PBMCs.*Virology* 292:6.
11. **Wetzel, M. A.**, A. D. Steele, T. K. Eisenstein, M. W. Adler, E. E. Henderson, and T. J. Rogers. **2000.** Mu-opioid induction of monocyte chemoattractant protein-1, RANTES, and IFN-gamma-inducible protein-10 expression in human peripheral blood mononuclear cells. *J Immunol* 165:6519-6524.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Jeffrey M. Jacobson, M.D.	POSITION TITLE Chief, Division of Infectious Diseases and HIV Medicine; Professor of Medicine, Microbiology and Immunology
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Cornell University (Arts and Science) Ithaca, NY	B.A.	1973	Biology
Cornell University Medical College, NY, NY	M.D.	1977	Medicine
University of Wisconsin Hospitals, Madison, WI	Internship	1977-1978	Medicine
Mount Sinai Medical Center, New York, NY	Residency	1978-1980	Medicine
Mount Sinai Medical Center, New York, NY	ID Fellowship	1980-1982	Infectious Diseases

Positions and Honors

Positions and Employment

1981-1997 Chief, Infectious Diseases Section, Medical Service, VA Medical Center, Bronx, NY
1981-1997 Chairman, Infection Control Committee, VA Medical Center, Bronx, NY
1994-1997 Chairman, Human Subjects Subcommittee, VA Medical Center, Bronx, NY
1994-2002 Associate Professor of Medicine, Mount Sinai School of Medicine, New York, NY
1997-2002 Director, AIDS Center, Mount Sinai Medical Center, New York, NY C.
2002-2006 Assistant Chief and Fellowship Program Director, Division of Infectious Diseases, Beth Israel Medical Center, New York, NY
2002-2006 Professor of Medicine, Albert Einstein College of Medicine, Bronx, NY
2006- Chief, Division of Infectious Diseases and HIV Medicine, Drexel University College of Medicine, Philadelphia, PA
2006- Professor of Medicine, Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA.

ACTG Activities (since 2004)

2004-2005 Chair, Immunology Research Agenda Committee, NIH-AIDS Clinical Trials Group
2004-2007 Member, Scientific Agenda Steering Committee, NIH-AIDS Clinical Trials Group
2004-2005 Chair, Experimental Therapeutic Research Agenda Committee, NIH-AIDS Clinical Trials
2005-2007 Chair, Translational Research & Drug Development Committee, NIH-AIDS Clinical Trials Group
2008-2011 Member, Translational Research & Drug Development Committee, NIH-AIDS Clinical Trials Group
2008-2011 Co-chair, Long-Acting Drug Task Force
2011- Member, End Organ Disease and Inflammation Transformative Science Group, NIH-AIDS Clinical Trials Group

C. Selected Peer-Revised Publications

1. **Jacobson, J. M.,** Colman, N., Ostrow, N., Simson, R. W., Tomesch, D., Marlin, L., Rao, M. E., Mill, J., Clemens, J., and A. M. Prince. Passive immunotherapy in the treatment of advanced HIV infection. Journal of Infectious Diseases, 168: 298-305, 1993.
2. **Jacobson, J. M.,** Davidian, M., Rainey, R., et al. Pyrimethamine pharmacokinetics in HIV- infected patients seropositive for Toxoplasma gondii. Antimicrobial Agents and Chemotherapy, 40: 1360-1365, 1996.
3. **Jacobson, J. M.,** Greenspan, J. S., Spritzler, J., et al. Thalidomide for the treatment of oral aphthouulcers in patients with human immunodeficiency virus infection. New England Journal of Medicine, 336: 1487-93, 1997.
4. **Jacobson, J. M.**, Spritzler, J., Fox, L., et al., and the NIAID AIDS Clinical Trials Group. Thalidomide for the treatment of esophageal aphthous ulcers in patients with human immunodeficiency virus infection. Journal of Infectious Diseases, 180: 61-67, 1999.
5. **Jacobson, J. M.**, Lowy, I., Fletcher, C. V., et al. Single-dose safety, pharmacology and antiviral activity of the human immunodeficiency virus (HIV) type 1 entry inhibitor PRO 542 in HIV-infected adults. Journal of Infectious Diseases, 182: 326-329, 2000.
6. **Jacobson, J. M.**, Hafner, R., Holden-Wiltze, J., Luft, B. J., and the NIAID AIDS Clinical Trials Group. A dose-escalation, phase I/II study of oral azithromycin and pyrimethamine for the treatment of toxoplasmic encephalitis in patients with AIDS. AIDS, 15: 583-589, 2001.
7. **Jacobson, J. M.**, Greenspan, J. S., Spritzler, J., et al., and the NIAID AIDS Clinical Trials Group. Thalidomide in low intermittent doses does not prevent HIV-associated aphthous ulcer recurrence. Journal of Infectious Diseases, 183: 343-346, 2001.
8. **Jacobson. J. M.,** Lederman, M. M., Spritzler, J., et al. and the NIAID AIDS Clinical Trials Group. Granulocyte-macrophage colony stimulating factor induces modest increases in plasma HIV-RNA and CD4 lymphocyte counts in patients with uncontrolled HIV infection. Journal of Infectious Diseases, 188: 1804-1814, 2003.
9. Kuritzkes, D. R., **Jacobson, J. M.**, Powderly, W. G., et al. Antiretroviral activity of the anti-CD4 monoclonal antibody TNX-355 in patients infected with human immunodeficiency virus type 1. Journal of Infectious Diseases, 189: 286-291, 2004.
10. **Jacobson, J. M.**, Israel, R. J., Lowy, I., Ostrow, N. A., et al. Treatment of advanced human immunodeficiency virus type 1 disease with the viral entry inhibitor PRO 542. Antimicrobial Agents and Chemotherapy, 48: 423-429, 2004.
11. **Jacobson, J. M.**, Bucy, R. P., Spritzler, J., et al. Evidence that intermittent structured treatment interruption, but not immunization with ALVAC-HIV vCP1452, promotes host control of HIV replication: the results of AIDS Clinical Trials Group 5068. Journal of Infectious Diseases, 194: 623-32, 2006.
12. **Jacobson, J. M.**, Saag, M. S., Thompson, M. A., et al. Antiviral activity of single-dose PRO 140, a CCR5 monoclonal antibody, in HIV-infected adults. Journal of Infectious Diseases, 198: 1345-52, 2008.
13. **Jacobson, J. M.,** Kuritzkes, D. R., Godofsky, E., et al. Safety, Pharmacokinetics, and Antiretroviral Activity of Multiple Doses of Ibalizumab (formerly TNX-355), an Anti-CD4 Monoclonal Antibody, in HIV-1 Infected Adults. Antimicrobial Agents and Chemotherapy, 53: 450-457, 2009. PMC2630626.
14. Gandhi, R. T., O'Neill, D., Bosch, R. J., and **J. M. Jacobson**. A randomized therapeutic vaccine trial of canarypox-HIV-pulsed dendritic cells vs. canarypox-HIV alone in HIV-1-infected patients on antiretroviral therapy. Vaccine, 27: 6088-94, 2009. PMCID: PMC2820102
15. **Jacobson J.M.**, et. Al.. Anti-HIV activity of weekly or bi- weekly treatment with subcutaneous PRO140, a CCR5 monoclonal antibody. J Infect Dis, 201: 1481-7,
2010. PMCID: PMC2856743.