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. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Temple University of the Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 01/01/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat

4. **Grant Contact Person's Telephone Number:** 215.204.7655

5. **Grant SAP Number:** 4100047651

6. **Project Number and Title of Research Project:** 9 - *Control of Cellular and HIV-1 Gene Expression by Cellular CDK9, A Potential Target for HIV Therapeutics*

7. **Start and End Date of Research Project:** 1/1/2009 – 6/30/2010

8. **Name of Principal Investigator for the Research Project:** Xavier Graña, Ph.D.

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:

   $50,000

   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	Position Title	% of Effort on Project	Cost
Garriga	Collaborator	25 %	$19,000

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	Position Title	% of Effort on Project
Graña	PI	10 %
Keskin	MS Graduate Student	50 %
Kurimchak	MS Graduate Student	50 %

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__X____ No_____

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

A R21 grant with related aims was funded by the NIH and started on April 12, 2009 (5R21MH083585). The title of this grant was: "Role of Tat and human CDK9 on gene expression alterations coupled to HIV-associated dementia". The total direct cost for this grant were $275,000 for two years. This grant provided stipend funds for Alison Kurimchak and additional funds for supplies to cover work related between the two grants.

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___X*____ No____

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:
SV40 small t antigen modulates the replication machinery in human quiescent cells	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	July 2009	$1,250,000	not funded
Reactivation of pocket proteins by PP2A in endochondral bone development	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	June 2010	$1,250,000	not funded
Protein Phosphatase 2A role in activation of the retinoblastoma protein family	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	Feb. 2011 and Nov. 2011	$1,250,000	not funded
Mechanisms of PPP2R2A tumor suppression in prostate cancer	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	June 2012	$1,508,606	not funded

*Several projects in the lab have indirectly benefited from this grant. Because the preliminary data on those projects was stronger, my grant applications efforts have focused in these other projects to this point. I have submitted four different R01 applications and a DOD grant since July 2009 (see table above). Unfortunately, none of these have been funded. My current focus is on the mechanisms of PPP2R2A tumor suppression in prostate cancer, for which I received a Nodal grant for collaborative work with the Fox Chase Cancer Center aimed at fostering collaborations to submit multi-PI applications ($180,000).

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

Yes___X____ No_____

If yes, please describe your plans:
Part of the work funded by this grant and the R21 listed above is still unpublished (manuscript in preparation). The preliminary data described generated as result from these grants will be used to prepare new applications aimed at extending the work on the role of HIV Tat in HIV-associated dementia.

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

Extending the work on the role of HIV Tat in HIV-associated dementia.

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				
Female		2		
Unknown				
Total		**2**		

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic		2		
Unknown				
Total		**2**		

	Undergraduate	Masters	Pre-doc	Post-doc
White		2		
Black				
Asian				
Other				
Unknown				
Total		**2**		

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

Yes____X_____ No_____

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

Havva Keskin, earned a MS degree in my lab for this work. She came with a fellowship of the Turkish Ministry of Education to perform this work. Ms. Keskin had a B.Sc. in Biology from Ondokuz Mayis University in Samsun, Turkey. She is currently a predoctoral student at Georgia Tech.

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.

These studies led to publications in peer reviewed journals (see table in section 20), helped train two MS graduate students, who are now Ph.D. students, and resulted in the set up of experimental approaches and data analyses that increased our expertise and that of our collaborators and generated data that can be used as preliminary data for new grant applications. In addition, these bridge funds also allowed me to maintain focus in other projects that led to several publications and grant proposals:

Sotillo, E.; Garriga, J.; Padgaonkar, A; Kurimchak, A.; Cook, JG. and Graña, X. (2009) Coordinated Activation of the Origin Licensing Factor CDC6 and CDK2 in Resting Human Fibroblasts Expressing SV40 Small T Antigen and Cyclin **J. Biol. Chem.** 284, 14126 – 14135. PMC2682861.

Rivadeneira, DB.; Mayhew, CN.; Sotillo, E.; Graña, X. and Knudsen, ES. (2010) Proliferative suppression by CDK4/6 inhibition: complex function of the RB-pathway in liver tissue and hepatoma cells. **Gastoenterology.** 138(5):1920-30. PMC2860048

Jayadeva, G., Kurimchak, A,. Garriga, J., Sotillo, E., Davis, A., Haines, D. S., Mumby, M., and Graña, X. (2010). B55α PP2A Holoenzymes Modulate the Phosphorylation Status of the Retinoblastoma-related Protein p107 and Its Activation. **J. Biol. Chem.** 285: 29863-73. PMID: 20663872

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:

We established a collaboration with Dr. Olaf Kutsch Jurkat (University of Alabama at Birmingham) to perform a high throughput screen for inhibitors of HIV replication.

We also established a collaboration with Daphne Georlette from the Department of Biology that is at the main campus at Temple University (co-author in Keskin et al., 2012).

We established a collaboration with Dr. Premkumar Reddy (then the director of the Fels Institute) and Onconova Therapeutics and used their small compound library for the screen mentioned above.

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.

Control of Cellular and HIV-1 Gene Expression by Cellular CDK9, A Potential Target for HIV Therapeutics

The immediate objectives of this project were to further understand the cellular role of the cellular kinase complex, designated CDK9/cyclin T1, in the control of cellular host gene expression to determine whether there is a therapeutic window of opportunity that warrants development of drugs targeting this complex to inhibit HIV-1 replication. The project focused in two separate lines of related research aimed, in part, at generating preliminary data for submission of R01 (or comparable) grant applications. Submission of grant applications related to this research have been postponed as I have centered my application to the NIH and DOD on cell cycle, differentiation and cancer, as the preliminary data that I had for these other projects was more complete (see section 11).

Subproject#1: Control of cellular and HIV-1 gene expression by CDK9.

The CDK9/cyclin T1 complex is recruited by HIV-1 Tat to promote productive viral transcription and is essential for HIV-1 replication. CDK9 is the catalytic subunit of the positive transcription elongation factor b (P-TEFb) and is required for transcriptional elongation *in vitro*. Experiments with cells treated with flavopiridol (FVP), a CDK inhibitor that potently inhibits CDK9, have led to the proposition that CDK9 is required for the transcription of most cellular genes by RNAPII. However, our preliminary data show that the effects of FVP in gene expression are distinct to the effects of inhibiting CDK9 activity with a dnCDK9 mutant. Our hypothesis is that the role of CDK9 in cellular transcription is gene specific. A better understanding of the function of this essential HIV-1 cellular cofactor may facilitate developing of future anti-therapeutic interventions.

Specific Aim 1: To examine the selectivity of T-type cyclin/CDK9 complexes in regulation of gene expression in normal human cells. DNA microarray analysis will be performed using

normal human cells transduced with dnCDK9, shRNAs and control viruses. Our progress is summarized below.

1a. This aim was based on preliminary data obtained from a series of DNA microarray profiling experiments performed with RNA obtained from human glioblastoma T98G cells where CDK9 activity was downregulated via ectopic expression of a dominant negative form of CDK9. Unexpectedly, we found that a small number of genes/ORFs are downregulated (84) and upregulated (122), suggesting that CDK9 may both positively and negatively regulate gene expression and that the changes in gene expression are likely due to differential regulation of gene expression by CDK9 rather than a simple shutdown of general transcriptional elongation. This work was in preparation at the time of the application for this award. This study, which was only partially funded through this award, was completed and published (Garriga el al, 2010).

2b. As outlined in the original proposal we have performed microarray analysis in normal human fibroblasts using BJ fibroblasts immortalized with hTERT (human Telomerase Reverse Transcriptase) where we modulated CDK9 activity by different means. We designate these cells hT-NHF.

To eliminate the effects of transduction as a cause of differential effects in gene expression, all cells were transduced identically using the inducible Adeno-X TM Tet-Off system from Clontech carrying a dnCDK9 transgene under a tetracycline repressible promoter. hT-NHF were transduced with the same sets of adenoviruses (Ad-X-dnCDK9 and Ad-Tet-off). Tetracycline was added to the media of the control cells, as well as cells to be treated with FVP to prevent expression of dnCDK9, while cells selected to express dnCDK9 were allowed to grow in the absence of tetracycline (see methods). Under these experimental conditions, the baseline gene expression was the same in all samples prior to inhibition of CDK9 by different means. 48 h post-transduction, we analyzed the effects of CDK9 inhibition on phosphorylation of RNAPII on serine position 2 (Ser-2) and 5 (Ser-5) using specific antibodies (Fig 1A). CDK9 is thought to phosphorylate Ser-2 during transcription elongation, while CDK7 phosphorylates Ser-5 at initiation. However, and consistently with our previous observations using T98G cells, both Ser-2 and Ser-5 were found to be clearly dephosphorylated in hT-NHF expressing dnCDK9 (Fig. 1A). hT-NHF transduced in parallel in the presence of tetracycline were treated with 300 nM FVP for the indicated last 4, 8 and 24 h prior to the 48 h collection time. FVP inhibited Ser-2 and Ser-5 phosphorylation at all time points more efficiently than dnCDK9. Consistently, with its global inhibitory effect on transcription, the levels of endogenous CDK9 and, less prominently, cyclin T1 were also downregulated by the FVP treatment (Fig. 1A). We also verified the inhibitory effects of dnCDK9 and FVP on the expression of *HEXIM1*, a gene known to be modulated by P-TEFb, which is very sensitive to CDK9 inhibition (He *et al*., 2006). As shown in Fig. 1B, *HEXIM1* mRNA levels are downregulated by both dnCDK9 and FVP. The effects of overexpressing cyclin T1 and CDK9 were also monitored. No major effects were noticed in the phosphorylation of the CTD of RNAPII or the expression of *HEXIM1* mRNA, as compared to control cells infected with Ad-Cre. We next performed global gene expression profiling of the effects of inhibiting CDK9 in hT-NHF by using Affymetrix Human Gene 1.0 ST DNA arrays and total RNAs from the samples described above in triplicate. These arrays contain

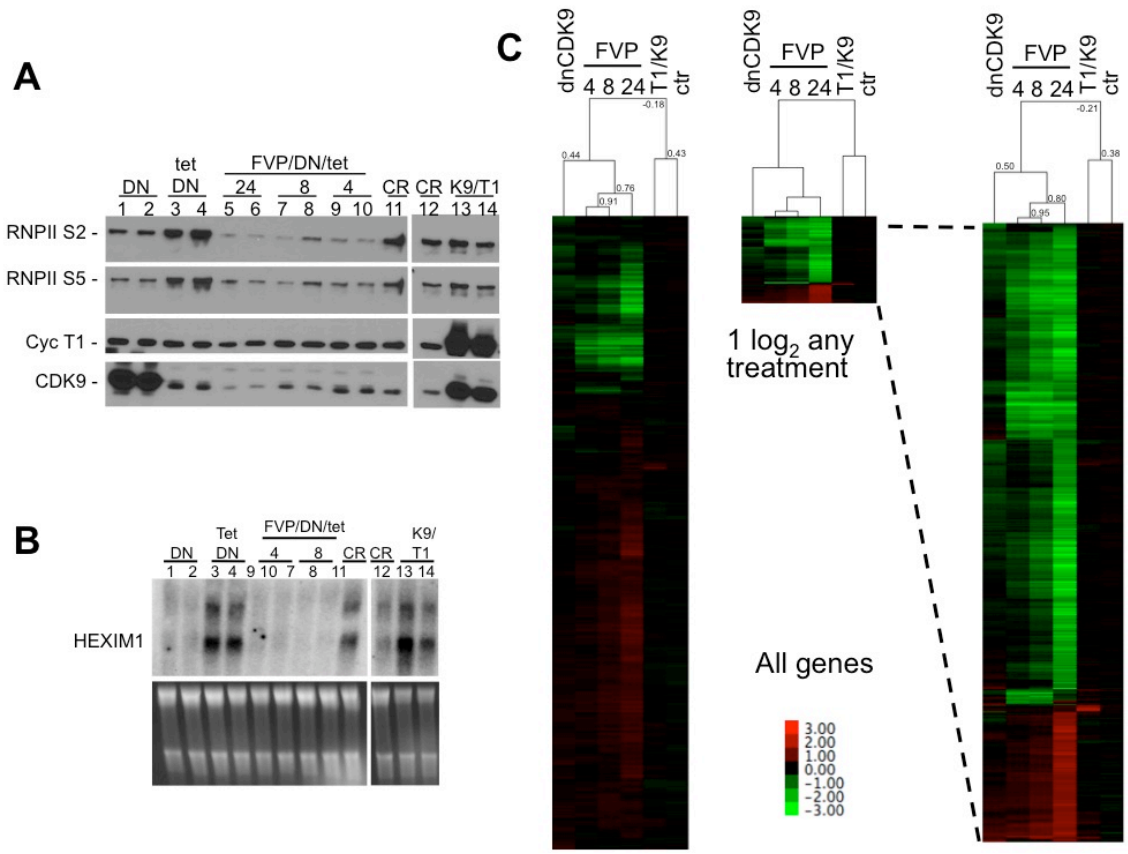


Fig. 1. Effects of CDK9 inhibition on the phosphorylation of RNAPII and the expression of genes in hTERT-immortalized normal human fibroblasts (NHF). Ectopic expression of dnCDK9 or treatment with FVP inhibit both RNAPII Ser-2 and Ser-5 phosphorylation (A) and the expression of Hexim1 (B), a known CDK9 target gene, in NHF. (C) Global gene expression effects of ectopic expression of dnCDK9 or FVP treatment in NHF. Normalized Affymetrix microarray data (log2 ratios) were analyzed by hierarchical clustering (Cluster 3.0, correlation uncentered, average linkage). See text for details.

probes representing 28,869 different genes. RNAs were labeled, hybridized to microarrays and scanned as described in Garriga *et al*., 2010. Raw transcript intensity data were normalized and the expression value log2 ratio for each gene was computed between its treatment and corresponding control sample. We represented the average gene expression value between replicates as the average log2 ratio. Next, hierarchical cluster analysis was performed with the log2 ratios and visualized using by Java Tree View (Fig. 1C). Gene array ratios for the three FVP treatments clustered together (correlation: 0.76-0.91), but exhibited

much lower correlation with the dnCDK9 gene array ratios (correlation: 0.44). If only the probe sets, which change two fold with at least one treatment are considered, this correlation increases slightly (0.50)(Fig. 1C, right clusters). Interestingly, ectopic expression of P-TEFb subunits resulted in the marked upregulation of a small subset of genes, with mostly no effect on genes dramatically upregulated/downregulated by FVP or dnCDK9. As expected, very little difference was observed in the expression profile of cells transduced with Ad-X-dnCDK9/Ad-Tet and Ad-Cre (Fig. 1C, right array in all clusters).

Analysis of the probe sets that change approximately two fold or more upon dnCDK9 expression showed that 93 probe sets were downregulated and 32 were upregulated (Fig. 2). The highest downregulation corresponded to Hexim1 (log2=-1.72043), confirming the sensitivity of Hexim1 to CDK9 inhibition. As expected, FVP also potently inhibited Hexim 1 expression (log2=-1.72494 to -3.22926). The highest probe set intensity in the dnCDK9 array was CDK9, an obvious expected result due to the ectopic expression of dnCDK9, as the probe set does not discriminate between dnCDK9 and endogenous CDK9. Of note, FVP inhibited the expression of endogenous CDK9. The correlation between the FVP arrays was 0.95 for the 4 and 8 h treatments and 0.84 with the 24 h treatment. It was also clear that the increase/decrease in gene expression with FVP was time dependent. The correlation between the FVP and dnCDK9 arrays was 0.57, noticeably higher than we had seen in our previous study using T98G cells (Garriga *et al.*, 2010). The expression change range for FVP was much higher than for dnCDK9. Thus, one cannot conclude that the effects of dnCDK9 are more or less potent than any of the FVP treatments for this set of genes but rather different.

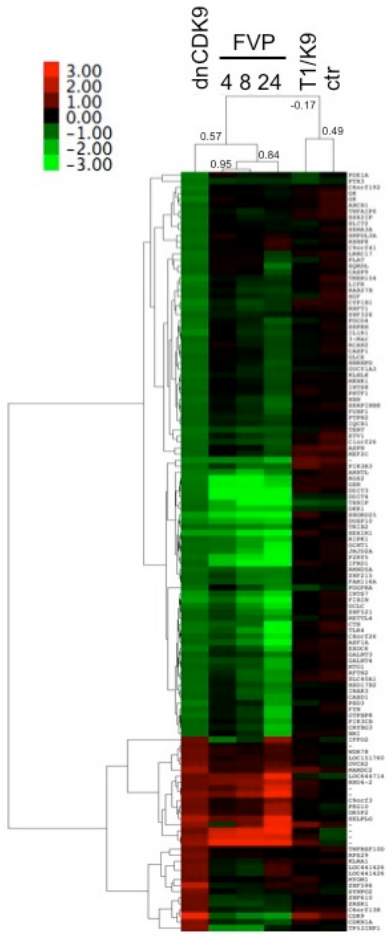


Fig. 2. Effects of FVP treatment on genes modulated by dnCDK9 in hTERT-immortalized normal human fibroblasts. Affymetrix microarray expression ratios for transcripts changing +/-1 log2 with ectopic expression of dnCDK9 were clustered with the corresponding values of the FVP treatments using hierarchical clustering analysis (Cluster 3.0, correlation uncentered, average linkage).

The observation that many of the genes more sensitive to dnCDK9 or FVP treatment are different, suggest that one or both methods lack selectivity or have off-target effects. For instance, FVP may inhibit other CDKs with roles in transcription in addition to CDK9, even at the concentrations used in this study. One could also envision dnCDK9 having additional effects unrelated to CDK9 inhibition resulting from squelching P-TEFb partners. Alternatively, the differences could be explained by the difference in kinetics of inhibition of FVP compared to dnCDK9. FVP acts very rapidly and potently, while inhibition by dnCDK9 occurs slowly, depending on

expression and replacement of active complexes by inactive ones. In view of these results, we decided to broaden our study by reducing CDK9 expression via siRNA. This is *a priori* a very specific and selective method, as no other RNA sequences are targeted and, thus, other RNAPII CDKs should not be directly affected. hT-NHF were transfected twice within 24 h with two independent siRNAs (103 and 104) and collected 72 h after the first transfection. We next determined the effect of each siRNA and their combination on CDK9 levels. Both, 103 and 104 siRNAs efficiently knocked-down CDK9 (Fig. 3A), with little additional downregulation when both siRNAs were used in combination. As expected, scramble siRNAs had no effect on CDK9 levels. However, in contrast to FVP and dnCDK9 treatments, phosphorylation of Ser-2 and Ser-5 of the CTD of RNAPII were not visibly affected by either siCDK9 or their combination (Fig. 3A). The effects of knocking-down CDK9 in global gene expression in hT-NHF were determined using Affymetrix Human Gene 1.0 ST DNA arrays similarly as described for dnCDK9 and FVP treated cells. Cells were transfected in duplicate with each siCDK9 or combination or with scramble siRNA. Cells were collected 72 h later to generate RNA probes (see Material and Methods). About 650 probe sets exhibited changes in gene expression equal or larger than two fold. A cluster analysis of genes that changed two fold with siCDK9 or dnCDK9 is shown in Fig. 3B. The correlation between siCDK9 treatments was high (0.86-0.95). Both the 103 and 104 siRNAs exhibit some unique set of genes that were more potently affected, with the combination appearing to be additive for those genes. However, for genes that were commonly downregulated or upregulated by both siRNA separately, the combination mostly lacked additive effects (Fig 3B, side grey bars). This may reflect that both siRNAs knocked-down CDK9 to similar levels even when used in combination, as the total siRNA concentration was the same in all transfections. Surprisingly clustering analysis also revealed major differences between the effect of the siRNAs and dnCDK9 (correlation 0.15). For instance a large set of genes upregulated by dnCDK9 are not affected by either siRNA (bottom large cluster in the array). Similarly, the top of the array shows several genes potently downregulated by dnCDK9 that show minimal downregulation by siRNA. In addition, most of the genes upregulated by either 103, 104 or the combination of

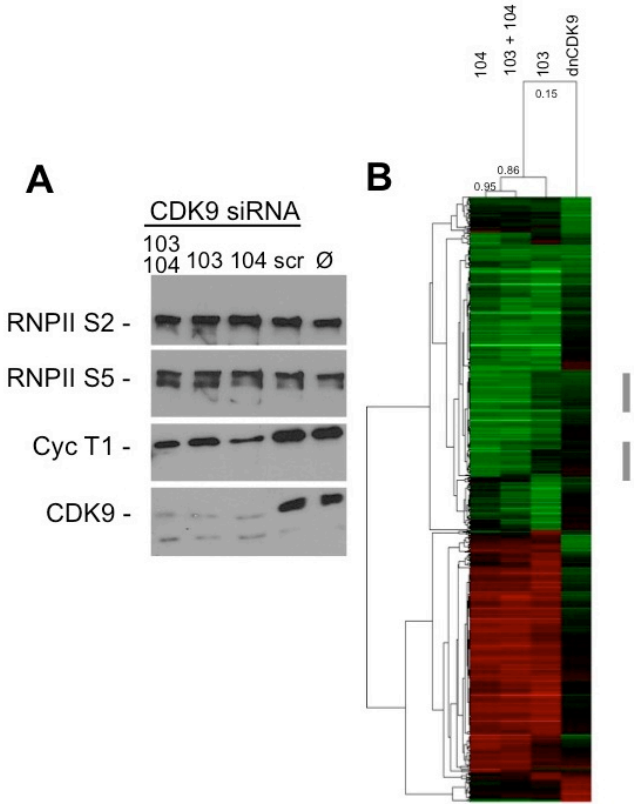


Fig. 3. Very limited correlation among genes modulated by siCDK9 and dnCDK9 in hTERT immortalized NHF.
(A) Knockdown of CDK9 results in reduced cyclin T1 expression, but does not affect RNAPII phosphorylation noticeably.
(B) log2 expression ratios for transcripts which levels changed +/-1 log2 ratio with siCDK9 or dnCDK9 expression were hierarchically clustered using Cluster 3.0 (correlation uncentered, average linkage)(~650 transcripts are shown). The correlation among array is show on top.

both are either not affected or downregulated by dnCDK9. While there is more agreement within the genes downregulated by the siRNAs, the overall comparison exhibits little similarity.

We selected a number of genes for validation whose changes in expression were among the highest in at least one of the conditions tested by using Taqman qRT-PCR. We found close agreement in expression changes detected by both microarray and q-RT-PCR. The expression patterns in primary fibroblasts confirmed and extended some of our striking observations made previously with transformed astrocytes, which are now published (Garriga et al., 2010), for instance: the unexpected upregulation of some inducible genes, many classified as primary response genes (PRGs). This was even more surprising in light of recent work that shows that P-TEFb is recruited to the promoters of primary response genes in response to LPS signaling and required for the process (Hargreaves et al., 2009). In addition, we also observed little agreement in expression patterns between CDK9 knockdown and the dnCDK9 mutant. However, these differences could potentially be the result of differences in the kinetics of CDK9 inhibition.

3b. Having seen potent upregulation of FOS, JUN and EGR1 transcripts upon FVP treatment for 8 and 24 h, we considered the possibility that the early effects of FVP on the expression of these genes could be in sharp contrast to what is seen at late time points. Therefore we treated BJ-T cells with FVP and collected cells at early time points (0, 0.5, 1 and 2 hrs) in addition to the time points that we had used in the microarray expression analysis. Transcript levels were measured using q-RT-PCR and specific Taqman probes. Interestingly, we found that most primary response genes tested are downregulated very rapidly upon FVP treatment. However, the expression of some of these transcripts recovered and were expressed potently at later time points (i.e. EGR1, JUNB and GADD45B). We also observed primary and secondary response genes with unchanged or upregulated expression by FVP at all time points. This was unexpected for secondary response genes whose expression is thought to be dependent on the expression of primary response genes that are expressed early and sharply downregulated. Finally, there were also secondary response genes that were downregulated less sharply, but their levels remained low through the remaining time course. With these results, we performed a new set of experiments with focus on genes that are known to be induced by mitogens as primary or secondary genes. BJ-T cells were serum starved for 3 days and subsequently stimulated with serum in the presence of FVP or vehicle (control). We observed that the FVP treatment effectively blocked cell cycle re-entry from quiescence (data not shown). We also observed that FVP effectively blocked the expression of several primary response genes though the 22 h time course. However, other genes including *JUNB*, *FOS*, *EGR1* and *GADD45B* are rapidly downregulated within the first 30 min and drop 5-10 fold in expression by 2 h (Keskin et al., 2012). However, the expression of these genes is up to normal levels by 4-8 hours and subsequently upregulated 3-4 fold by 24 hours. Since our microarray analyses measured changes in expression at 4, 8 and 24 h, we had missed the rapid and deep downregulation induced by pharmacologic inhibition of CDK9. In contrast, other genes such as HEXIM1 or MCL1 are downregulated with slower kinetics and remain down through the 24-hour time course. These data suggested that PRG could be effectively expressed in the presence of a potent CDK9 inhibitor, and thus we followed up this observation, which ultimately led to a publication in Cell Division (Keskin et al., 2012) and

the M.S. Thesis of Ms. Keskin (2011).

Subproject#2: To develop a high throughput cellular reporter assay to identify inhibitors of HIV-1 transcription with no cellular toxicity.

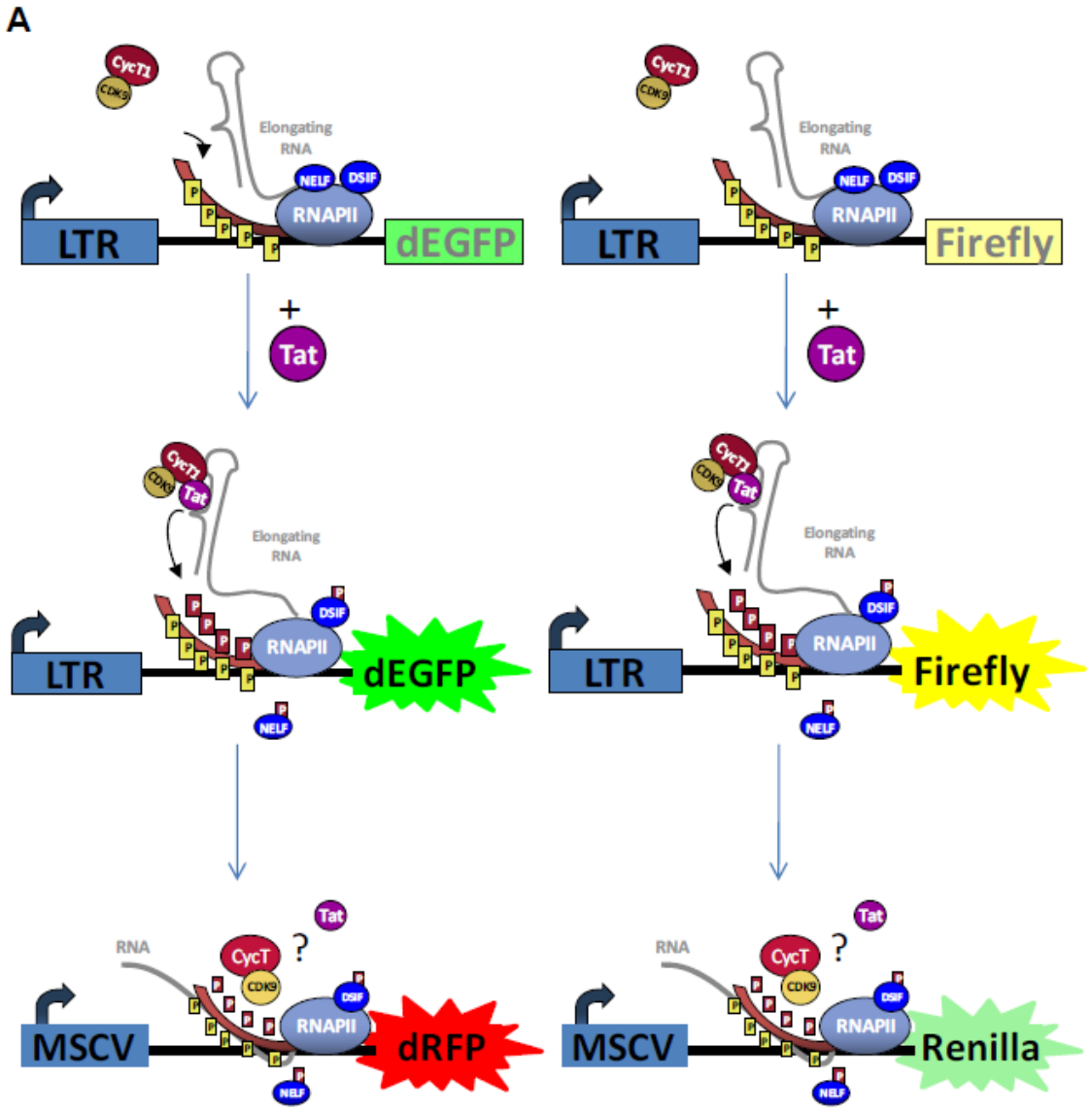
HIV-1 transcription is an essential step in the viral cycle and is dependent on viral and host transcription factors. We hypothesize that selective inhibition of HIV-1 transcription, and hence replication, should be feasible if the small compound specifically targets a viral protein essential for productive transcription (i.e.: Tat), a required cellular cofactor non-essential for cellular transcription, or if it interferes with the interactions among viral and cellular cofactors without altering cellular functions.

Specific Aim 2: To generate and characterize dual reporter cell lines to simultaneously monitor HIV-1 and cellular transcription in vivo in multi-well tissue culture plates.

We made several attempts to generate HeLa cells with dual reporters directed by a Tat-dependent HIV-l LTR promoter and a housekeeping like promoter (MSCV promoter). As reporters we utilized dEGFP and RFP for strategy 1, and firefly and renilla luciferases for strategy 2. We were successful in generating clones that expressed dEGFP or firefly luciferase under the control of the HIV-1 promoter in a Tat dependent manner. This was accomplished by the sequential introduction of appropriate plasmids as follow. First, transfection of the LTR-reporter plasmid, followed by selection of clones resistant to antibiotics but lacking reporter activity in the absence of exogenously transduced/transfected Tat. Second, transfection with a Tat expression plasmid, followed by antibiotic selection and identification of clones with reporter activity. Third, transfection of the second reporter under the control of the MSCV promoter (Fig. 4A).

We were successful in introducing and selecting clones for the first two steps (Fig. 4 and Fig. 5), but in both strategies introduction of the MSCV-reporter (RFP or renilla luciferase) resulted in toxicity leading to slow growth and inability to maintain stable clones. While these problems could have likely been worked out by reducing plasmid copy numbers, we obtained in collaboration with Dr. Olaf Kutsch Jurkat cells with the desired properties. These cells were generated as described in strategy 1 but using retroviral transduction strategies followed by cell sorting. It appears that Jurkat cells are better suited than HeLa cells to stably co-express several exogenous proteins. We also established a collaboration with Dr. Kutsch to perform two high throughput screens aimed at identifying small compounds with the ability to inhibit HIV-1 transcription, without altering cellular transcription. We dispensed in 96 well plates a library of about 3200 small compounds generated by our collaborator Dr. Premkumar Reddy. Alison Kurimchak, the graduate student assigned to this project aim traveled to Dr. Kutsch's lab (University of Alabama at Birmingham), where she performed the two fluorescent-based screens. One screen was flow cytometry-based and the other utilized a fluorescence plate reader. The screens were performed in Dr. Kutsch lab, as he had the required flow cytometric equipment and had extensive expertise in this innovative type of screen. The screens were completed within a week, and the data was analyzed in our lab. While the control compound, Ro24-7429, a known inhibitor of HIV-transcription

validated both screens, we did not find potent inhibitors of HIV-1 transcription that were not also inhibitors of cellular transcription or toxic to cells. However, the library of small compounds that we used was relatively small. Thus, these results are not completely unexpected. Given the successful validation of both screens, it appears feasible to screen larger libraries of small compounds (>100,000). Submission of a grant application to support high throughput screens with large libraries of small compounds is being evaluated.



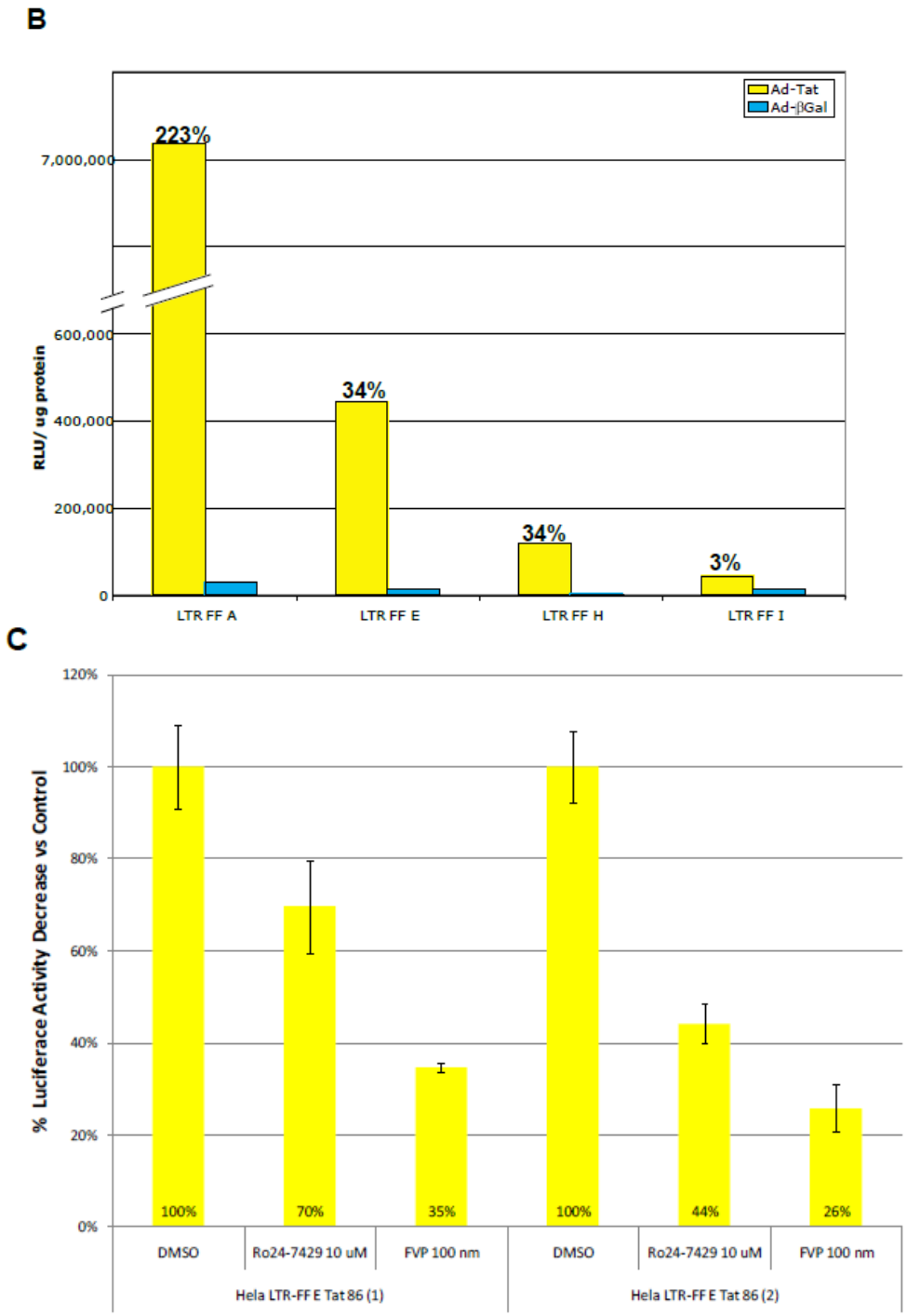


Figure 4. Generation of Cell Lines. (A) Schematic for generating HeLa based fluorescent and luciferase reporter cells. (B) Percent induction of LTR-FF activity in the absence or presence of Tat. Cells were infected with 50 MOI Ad-Tat or Ad-beta-gal and harvested 48 hours later and lysed. Firefly luciferase assays were performed, activity is normalized per microgram of protein. Percentages indicate fold increase over beta-gal control. (C) Effect of HIV transcriptional inhibitors in HeLa LTR-FF Tat dependent clones. Cells were treated with the indicated inhibitors and concentrations in duplicates for 48 hours, then harvested and lysed. Luciferase assays were performed using five micrograms of protein per sample. Chart shows percent decrease in luciferase activity over the control.

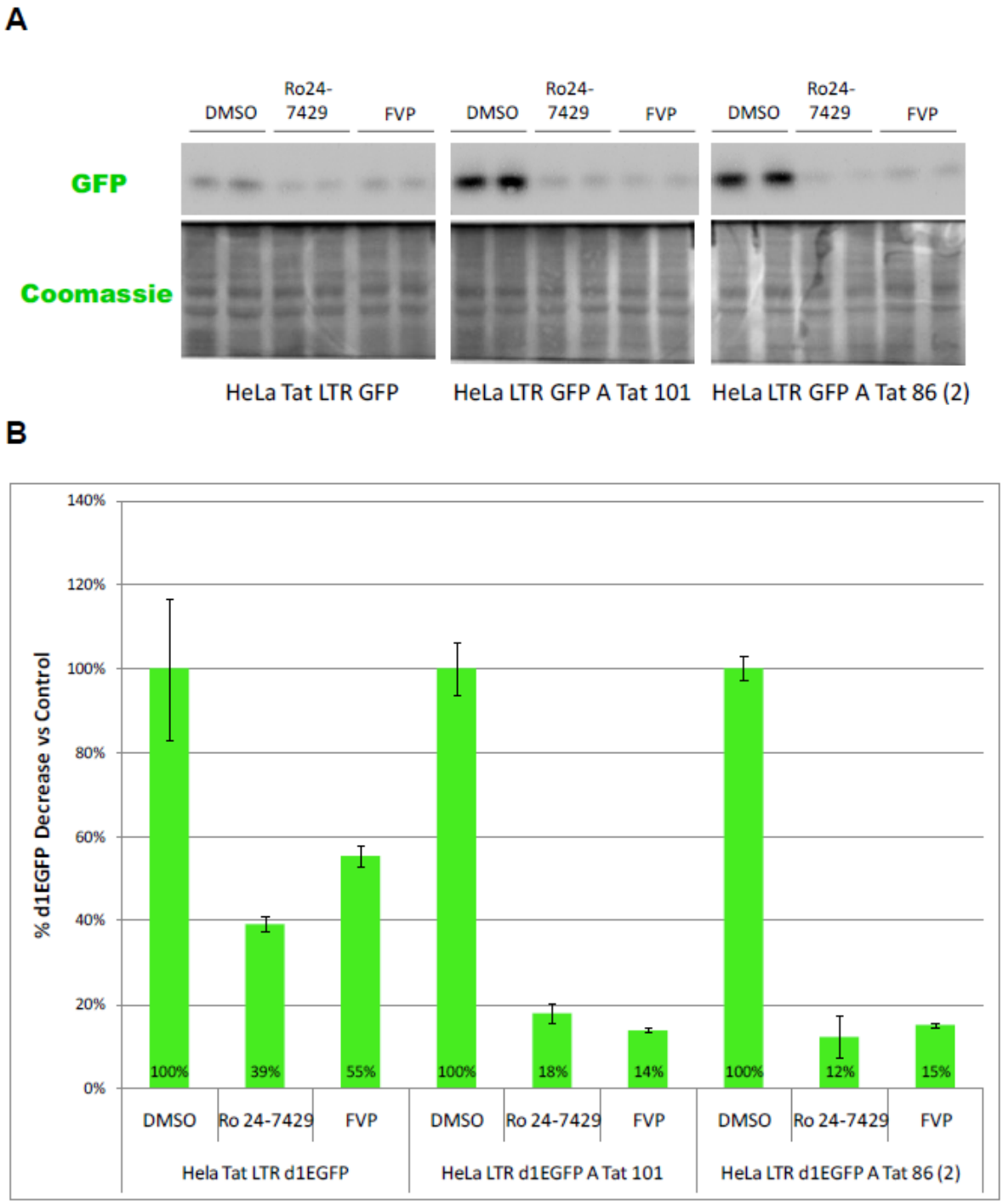


Figure 5. Effects of HIV-1 Transcription Inhibitors in HeLa Tat LTR d1EGFP and HeLa LTR d1EGFP Tat Cells. (A) d1EGFP western blot analysis of three cell lines treated with 10 microM Ro24-7429 or 100 nm flavopiridol. The cells were harvested and lysed. Twenty micrograms of protein were resolved in a 10% SDS-PAGE gel, transferred to a PVDF membrane and probed for d1EGFP. Bottom panels show Coomassie stained membranes for loading control. (B) Densitometric analysis of d1EGFP western blot bands shown in (A) normalized to the DMSO control using AlphaChemQ software.

References

Garriga, J., Xie, H., Obradovic, Z., and Graña, X. (2010). Selective control of gene expression by CDK9 in human cells. J Cell Physiol 222, 200-208.

Hargreaves, D. C., Horng, T., and Medzhitov, R. (2009). Control of inducible gene expression by signal-dependent transcriptional elongation. Cell 138, 129-145.

He N, Pezda AC, Zhou Q. 2006. Modulation of a P-TEFb functional equilibrium for the global control of cell growth and differentiation. Mol Cell Biol 26:7068–7076.

Keskin, H., Garriga, J., Georlette, D. & Graña, X. (2012) Complex effects of flavopiridol on the expression of primary response genes. Cell Div. 7.

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:

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, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

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. Selective control of gene expression by CDK9	Garriga, J.; Xie, H.; Obradovic, Z. & Graña, X.	J Cell Physiol	June 2009	☐Submitted ☐Accepted ☒Published
2. Complex effects of flavopiridol on the expression of primary response genes.	Keskin, H., Garriga, J., Georlette, D. & Graña, X.	Cell Division	December 2011	☐Submitted ☐Accepted ☒Published

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

Yes____X___ No_____

If yes, please describe your plans:

Only a part of the results described above have been reported in Keskin et al., 2011. We plan to describe the rest of the results for aim 1 in a separate publication, which is under preparation.

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.

None

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.

The two screens performed did not identify new compounds with anti-HIV replication activity.

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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME: **Xavier Graña, Ph.D.**

POSITION TITLE: **Associate Professor of The Fels Institute for Cancer Research**
Associate Professor of Biochemistry

eRA COMMONS USER NAME (credential, e.g., agency login): xgrana

EDUCATION/TRAINING:

University of Barcelona School of Biology, Spain	B.Sc	1982-1987	Biology
University of Barcelona Sch of Medicine, Spain	Ph.D.	1987-1991	Mol. Biol. & Biochem.
Cold Spring Harbor Laboratory, NY	Postdoctoral	1991-1992	Cell cycle control in normal and cancer cells
Fels Institute, Temple University Sch of Medicine,	Postdoctoral	1992-1994	

A. Personal Statement

Dr. Xavier Graña is a cancer biologist who has worked in the field of cell cycle regulation and control of gene expression since the initiation of his postdoctoral training at Cold Spring Harbor Laboratory in 1991. As a Fulbright Postdoctoral Fellow he identified two novel cyclin dependent kinases, now designated CDK9 and CDK10, and was a key member of one of the teams that identified the retinoblastoma related pocket protein p130. In 1994, Dr. Graña established his own laboratory and discovered that p130 was regulated by phosphorylation at the cell cycle entry exit transitions and during the cell cycle. He was awarded a NIH First Award (R29) that funded subsequent studies on the characterization of the CDKs targeting p130 and the deregulation of pocket protein phosphorylation and stability in normal and transformed cells. In 1998, his work on CDK9 and its cyclin T1 regulatory subunit in T cells resulted in the award of an R01; and a K02 Career Award in 2000 to continue his work on CDK9 in T cell activation and HIV-1 replication. This work has continued to this day with NIH funding through STTR and R21 awards, as well as a project in a NIH Program Project aimed at studying the mechanisms that regulate the decision to enter or exit the cell division cycle and their bypass by cellular and viral oncogenes, such as SV40 small t antigen and cyclin E. Dr. Graña's laboratory discovered that cellular insults that compromise the activity of CDKs result in rapid dephosphorylation of pocket proteins, and demonstrated that the serine/threonine phosphatase PP2A was implicated. He postulated a novel model for modulation of the steady state phosphorylation of pocket proteins by a dynamic equilibrium between CDKs and PP2A holoenzymes, challenging previous models that only considered PP1. PP2A is not a simple enzyme, but rather a collection of trimeric holoenzymes that consist of regulatory (B), catalytic (C) and scaffold (A) subunits. His recent work has led to the identification of a B subunit of the B55 family (B55〈) that targets PP2A to the retinoblastoma family of proteins and plays a critical role in cell cycle exit. B55〈 is encoded by the *PPP2R2A* gene, which is recurrently deleted in prostate cancer, and preliminary work suggests it might be a tumor suppressor gene. Dr. Graña is the director of the Molecular Biology and Genetics Graduate Program at the Fels institute and the Head of the Cluster in Cancer Biology and Genetics for the new Bioscience Graduate Program. He has trained several postdoctoral fellows and graduate studies. Many are currently independent investigators, or in postdoctoral training. Dr. Graña's work is internationally known and has been cited extensively.

B. Positions and Honors:

Positions and Employment

1994-1996 Research Assistant Professor, Fels Institute for Cancer Research, TUSM. Philadelphia.
1996-2000 Assistant Professor of Fels Institute for Cancer Research, TUSM.
Assistant Professor of Biochemistry
1999-2009 Co-Director of the Molecular Biology and Genetics Graduate Program

2000-2001 Associate Professor of Biochemistry, TUSM.
2000-Present Associate Professor of Fels Institute for Cancer Research
2001-Present Associate Professor of Biochemistry, Tenured. TUSM. Philadelphia.
2009-Present Director of the Molecular Biology and Genetics Graduate Program
2012-Present Director, Cluster in Cancer Biology and Genetics, Bioscience Graduate Program

Other Experience and Professional Memberships

1994-present Ad hoc reviewer: *Oncogene, Proc. Natl. Acad. Sci. USA, EMBO J, EMBO Rep., Mol. Cell. Biol., J. Biol. Chem, Can. Res., Virology, Gene, J Cell Biochem, Mol. Hum. Rep., J. Cell Phys., CMLS, J. Immunol., Hepatology, Mol. Carcinogenesis, Trends Pharmacol, Mol. Biol. Rep., Gene, PLOSone* and *Future Virol.*
1996-1999 Member, American Cancer Society/Institutional Research Grant Review Committee.
1997-1998 Managing Editor for *Frontiers in Bioscience.*
1998 Association for International Cancer Research, Ad hoc, peer reviewer.
1998,1999 John Sealy Memorial Endowment Fund for Biomedical Research, ad hoc reviewer.
1999, 2001 Ohio Cancer Research Associates. Adhoc, peer reviewer
2000-2003 NIH Cell Development and Function 6/F05 study section, frequent, ad hoc reviewer.
2000, 2001 NIH/NCI R21 review panel “Molecular Targets Drug Discovery,” member
2003-2007 NIH Cell Signaling & Dynamics study section (previously CDF 3), member.
2005-present BMC Cell Division, Associate Editor
2010-present Gene, Member of the Editorial Board

Honors

1989-1990 Fellowship from CIRIT (Interdep. Commission Res. Technol Improvement). Spain.
1991-1993 Fulbright/MEC (Ministry of Education and Science of Spain) postdoctoral fellowship.
1993-1994 Lady Tata Memorial Award for Research in Leukemia and Allied conditions.
1994-1995 Fellowship from CIRIT. Generalitat de Catalunya. Spain.
1996 Individual allocation from American Cancer Society. "Regulation of p130” PI.
1996-1997 Wendy Will Case Grant: "Regulation of p130 During the Cell Cycle and Quiescence" PI
1996-2002 NIH First Award (R29) : "Regulation of p130 During the Cell Cycle and Quiescence" PI.
1998-2002 WW Smith Charitable Trust Grant: "T-type cyclin/CDK9 in HIV replication." PI.
2000-2005 NIH/NIAID Career Development Award (K02).
2013 Dawn B. Marks Memorial Award in Graduate Education, TUSM.

C. Selected Peer-reviewed Publications: (selected from 56 peer-reviewed publications)**.**

1. Mayol, X., Garriga, J. and Graña, X. (1995) Cell cycle-dependent phosphorylation of the retinoblastomarelated protein p130. **Oncogene** 11, 801-808. PMID: 7651744.
2. Mayol, X., Garriga, J. & Graña, X. (1996). G1 Cyclin/CDK-independent phosphorylation and accumulation of p130 during the transition from G1 to G0 lead to its association with E2F-4. **Oncogene** 13, 237-246.
3. Parreño, M., Garriga, J., Limón, A., Mayol, X., Beck, G.R.Jr., Moran, E. and Graña, X. (2000) E1A blocks hyperphosphorylation of p130 and p107 without affecting the phosphorylation status of the retinoblastoma protein. **J. Virol.** 74, (7), 3166-3176. PMC111817.
4. Calbó, J.; Parreño, M.; Sotillo, E.; Yong, T.; Mazo, A.; Garriga, J. and Graña, X. (2002) G1 cyclin/CDK coordinated phosphorylation of endogenous pocket proteins differentially regulates their interactions with E2F4 and E2F1 and gene expression. **J. Biol. Chem**. 277, 50263-50274. PMID: 12401786.
5. Bhattacharya, S.; Garriga, J., Calbó, J., Yong, T.; Haines, D.S. and Graña, X. (2003) SKP2 associates with p130 and accelerates p130 ubiquitylation and degradation in human cells. **Oncogene** 22, 2443-51.

6. Garriga, J.; Bhattacharya, S.; Calbó, J.; Marshall RM.; Truongcao, M.; Haines, DS. and Graña, X. (2003). CDK9 is constitutively expressed throughout the cell cycle and its steady-state expression is independent of SKP2. **Mol. Cell. Biol.** 23, 5165-5173. PMC165719.
7. Garriga, J.; Jayaraman, AL.; Limón, A.; Jayadeva, G.; Sotillo, E.; Patsialou, A.; Truongcao, M.; Wadzinski, BE. and Graña, X. (2004) A dynamic equilibrium between kinases and PP2A modulates phosphorylation of pRB, p107 and p130. **Cell Cycle** 3, 1320-1330. PMID: 15467457. http://www.landesbioscience.com/journals/cc/article/1183/
8. Marshall, R.; Salerno, D.; Garriga, J. and Graña, X. (2005) Cyclin T1 expression is regulated by multiple signaling pathways and mechanisms during activation of human peripheral blood lymphocytes. **J. Immunol.** 175: 6402–6411. PMID: 16272292 (Article highlighted in JI, 2005, 175: 6251-6252.
9. Reddy, HKDL; Mettus, MV; Rane, SG; Graña, X.; Litvin, J. and Reddy, EP (2005) Cdk4 expression is essential for Neu-induced breast tumorigenesis. **Cancer Res.** 65(22):10174-10178.
10. Carbone, C.; Graña, X.; Reddy, EP and Haines, DS (2007). p21 loss cooperates with INK4 inactivation facilitating immortalization and Bcl-2 mediated anchorage independent growth of oncogene-transduced primary mouse fibroblasts. **Cancer Res.** 67(9):4130-7. PMID: 17483323.
11. Sotillo, E.; Garriga, J.; Kurimchak, A. and Graña, X. (2008) Cyclin E and SV40 small t antigen cooperate to bypass quiescence and contribute to transformation by activating cdk2 in human fibroblasts **J. Biol. Chem.** 283(17):11280-92. PMC2431056. http://www.jbc.org/content/283/17/11280.long
12. Sotillo, E.; Garriga, J.; Padgaonkar, A; Kurimchak, A.; Cook, JG. and Graña, X. (2009) Coordinated Activation of the Origin Licensing Factor CDC6 and CDK2 in Resting Human Fibroblasts Expressing SV40 Small T Antigen and Cyclin **J. Biol. Chem.** 284, 14126 –14135. PMC2682861. http://www.jbc.org/content/284/21/14126.long
13. Rivadeneira, DB.; Mayhew, CN.; Sotillo, E.; Graña, X. and Knudsen, ES. (2010) Proliferative suppression by CDK4/6 inhibition: complex function of the RB-pathway in liver tissue and hepatoma cells. **Gastoenterology.** 138(5):1920-30. PMC2860048
14. Garriga, J.; Xie, H.; Obradovic, Z. & Graña, X. (2009) Selective control of gene expression by CDK9. **J Cell Physiol.** 2009 Sep 24;222(1):200-208. PMC2783197. http://onlinelibrary.wiley.com/doi/10.1002/jcp.21938/full
15. Jayadeva, G., Kurimchak, A,. Garriga, J., Sotillo, E., Davis, A., Haines, D. S., Mumby, M., and Graña, X. (2010). B55〈 PP2A Holoenzymes Modulate the Phosphorylation Status of the Retinoblastoma-related Protein p107 and Its Activation. **J. Biol. Chem.** 285: 29863-73. PMID: 20663872 http://www.jbc.org/content/285/39/29863.long
16. Keskin, H., Garriga, J., Georlette, D. & Graña, X. (2012) Complex effects of flavopiridol on the expression of primary response genes. **Cell Div.** 7. PMID: 22458775. http://www.celldiv.com/content/7/1/11
17. Kurimchak, A; Haines, D; Garriga, J; Wu, S; Deluca, F.; Sweredoski; Deshaies, RJ; Hess, S. and Graña, X. (2013). Activation of p107 by FGF, which is essential for chondrocyte cell cycle exit, is mediated by the PP2A/B55〈 holoenzyme **Mol. Cell. Biol.** (doi:10.1128/MCB.00082-13, published online ahead of print). http://mcb.asm.org/content/early/2013/06/11/MCB.00082-13.full.pdf+html
18. Cherrier, T; Le Douce, V, Eilebrecht, S; Riclet, R; Marban, C; Dequiedt, F, Goumon, Y; Paillart, JC; Mericskay, M; Parlakian, A; Bausero, P; Abbas, W; Herbein, G; Kurdistani, S; Graña, X.; Van Driessche,B; Candolfi, E; Schwartz, C; Benecke, AG; Van Lint, C and Rohr, O (2013) CTIP2 is a negative regulator of PTEFb. **Proc. Natl. Acad. Sci. USA.** (in press).

Invited reviews on the topic (in chronological order, partial list)

1. Graña, X., Garriga, J. and Mayol, X. (1998) Role of the retinoblastoma protein family, pRB, p107 and p130 in the negative control of cell growth. **Oncogene** 17, 3365-3384. PMID: 9916999
2. Graña, X. (2008). Downregulation of the Phosphatase Nuclear Targeting Subunit (PNUTS) triggers pRB dephosphorylation and apoptosis in pRB positive tumor cell lines. **Can. Biol and Ther**. 14 (7) 824-826. P

3. Sotillo, E. and Graña, X. (2009) Escape from quiescence. In Cell Cycle Deregulation in Cancer. **Springer Publishing** Ed. G. Enders. pp 3-22.
4. Kurimchak, A. & Graña, X. (2012) PP2A holoenzymes negatively and positively regulate cell cycle progression by dephosphorylating pocket proteins and multiple CDK substrates. **Gene** 499, 1-7. http://www.sciencedirect.com/science/article/pii/S037811191200217X
5. Kurimchak, A. & Graña, X. (2013) PP2A counterbalances phosphorylation of pRB and mitotic proteins by multiple CDKs: Potential implications for PP2A disruption in cancer. **Genes and Cancer** 499, 1-
7. http://gan.sagepub.com/content/early/2013/02/27/1947601912473479.full

D. Research Support:

1. Elucidation of a novel mechanism of haploinsufficient tumor suppression in Prostate Cancer.
Source: **Temple University-Fox Chase Cancer Center SEED Nodal Grant Program**
Role on Project: **PI, CoPI Vladimir Kolenko**
Date of entire project: 01/01/2013– 12/31/2014.
Specific aims of project:
Aim 1. To determine if B55α expression levels found in cells with hemizygous PPP2R2A deletions confer haploinsufficient tumor suppression properties to immortalized prostate epithelial cells engineered to mimic PCa alterations in culture and in a xenograft mouse model.
Aim 2. To identify the substrates of B55α implicated in suppressing tumorigenicity in prostate cells and establish their deregulation in human tumor samples.
Aim 3. To determine the effect of pharmacological activation of PP2A holoenzymes and their dependence on B55α in PCa cells in culture and in a mouse PCa xenograft model.
Successful progress on these aims will unveil the potential mechanisms of PPP2R2A haploinsufficiency tumor suppression in PCa and establish a mouse model suitable for testing PP2A activity modulating drugs, hence providing solid preliminary for a multi-PI application to the NCI and DoD, as well as foundations.

2. To gain regulatory insight on the key changes in the p107 interactome and the p107-activating phosphatase during FGF-induced chondrocyte maturation.
Source: **Fels Institute (seed money)**
Role on Project: **PI**
Date of entire project: 07/01/2012– 06/31/2013.
Specific aims of project:
1. To confirm the B55α and p107 interactomes in RCS cells. 2: To determine what subsets of the B55α and p107 interactomes are regulated by FGF1 using stable isotope labeling by amino acids in culture (SILAC) and solution-based LC-MS/MS analysis.

Recently Completed Research Support
1. Title: Tat and CDK9 on gene expression alterations coupled to HIV-1 Associated Dementia
Source: **NIH (1R21MH083585-01A2)**
Role on Project: **PI**
Date of entire project: 04/15/2009– 03/31/2011. No cost Extension 03/31/2012.
Specific aims of project:
1. To determine the role of cellular CDK9 in the alterations on gene expression triggered by HIV-1 Tat in astrocytes and neurons.
2. To determine whether the effects of Tat in gene expression can be reverted by restoring normal cellular CDK9 function in Tat expressing astrocytes and neurons.

2. Title: Regulation of p130 by CDKs at the G1/S transition
Source: **NIH P01 (CA095569-01A1)** "G1 cell cycle in Tumorigenesis and Senescence" (PI: Reddy, EP).
Role on Project: **leader for project #2**.
Date of entire project: 02/14/03-01/31/08. No cost Extension 01/31/09.
Specific aims of project:
1. Specific aims of project: First, to identify the regulatory mechanism(s) that tag p130 for cellular depletion as cells progress through the mid-late G1 and S phases of the cell cycle. Second, to determine the biochemical pathway that mediates cell cycle dependent degradation of the p130 protein. Third, to characterize p130 functions other than its ability to repress E2F-dependent transcription by using a genetic approach.

3 Title: Identification of Small Pharmacologic Inhibitors of HIV-1 Replication
Source: **NIH STTR (1R41MH082775-01)**
Role on Project: **PI**
Date of entire project: 09/13/2007– 09/12/2008.
Specific aims of project:
1. To complete an ongoing screening of the Onconova Therapeutics' proprietary library of styryl sulfones and related molecules for inhibitors of cyclin T1/CDK9 activity. We have
2. To characterize and expand the chemical structure activity relationship (SAR) of the subset of leading cyclin T1/CDK9 inhibitors identified during the initial screening of the above-mentioned library as well as other compounds with similar or enhanced activity, which predictably will be identified as we complete the screens. We propose: (a) To establish the selectivity of these leading. 3. To perform an unbiased high throughput-amenable cell-based HIV transcription screening of this library with the goal of identifying inhibitors of HIV-transcription with no cytotoxicity.