EMBO Molecular Medicine

Compounds Producing an Effective Combinatorial Regimen For Disruption of HIV-1 Latency

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 19 October 2017

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now heard back from the three referees whom we asked to evaluate your manuscript.

As you will see from the comments below, the three referees are enthusiastic about the study, even though recommendations are made to provide more mechanistic insights into the mode of action, that I would like to encourage you to address, at least in part.

We would welcome the submission of a revised version within three months for further consideration. Please note that EMBO Molecular Medicine strongly supports a single round of revision and that, as acceptance or rejection of the manuscript will depend on another round of review, your responses should be as complete as possible.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

Please also contact us as soon as possible if similar work is published elsewhere. If other work is published we may not be able to extend the revision period beyond three months.

Please read below for important editorial formatting and consult our author's guidelines for proper formatting of your revised article for EMBO Molecular Medicine.

I look forward to receiving your revised manuscript.

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System):

The work is interesting and correctly performed. The authors first screened about 180,000 compounds to identify molecules reactivating a latent HIV provirus in a model cell line. They selected 5 compounds that were further characterized, either alone or in combination with known molecules. One compound, termed PH02, reactivates viral replication in CD4+ T cells from HIV-1 infected individuals under successful antiretroviral treatment. The compound works synergistically with previously characterized compounds, including PEP005.
The novelty resides in the identification of PH02 and other compounds, and their synergistic activity against the viral reservoir.

Referee #2 (Comments on Novelty/Model System):

The manuscript by Hashemi et al. deals with the issue of identifying compounds that, in combination, could lead latent HIV-1 proviruses out of their latent state. In particular, the authors have identified 5 compounds by high throughput screening (HTS) that reverse latent HIV-1 infection without causing a generalized T cell activation. These novel compounds synergize with previously identified latency reversing agents (LRA), particularly with Ingenol in both cell lines and primary resting CD4+ T cells isolated from pts. receiving cART showing synergy by the standard Q-VOA in the case of the combination between PH02 and PEP005.

The paper is very clearly written and represents and impressive thorough analysis of candidate novel LRA up to their validation in resting CD4 T cells of patients under suppressive cART.

Referee #2 (Remarks):

impressive work! it would be impossible to reduce it to a short report. The main limitation is the concept of "shock and kill" as a whole; however, within this hypotethical model this study has been really well conducted.

Referee #3 (Comments on Novelty/Model System):

Phenotypic screen for novel latency reactivating agents is wel performed and well described.

Referee #3 (Remarks):

Authors report on a phenotypic screen for novel latency reactivating agents. The screen is performed well and the hits are interesting. The paper is very well written. Unfortunately, although some epigenetic characteristics are tested, the true mechanism of action of the novel compounds is not elucidated. I find this a prerequisite for a paper in EMBO Molecular Medicine.

1st Revision - authors' response 10 November 2017

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Referee #3: Phenotypic screen for novel latency reactivating agents is well performed and well described.

(Remarks): Authors report on a phenotypic screen for novel latency reactivating agents. The screen is performed well and the hits are interesting. The paper is very well written. Unfortunately, although some epigenetic characteristics are tested, the true mechanism of action of the novel compounds is not elucidated. I find this a prerequisite for a paper in Embo Molecular Medicine.

We thank all three referees for their encouraging comments; several of the referees commented that this was "impressive work" and overall the screen was well performed. All of the referees have commented that the writing was good, and therefore we have made only minor corrections to spelling and grammar in most of the text throughout the revisions. Referee #2 alludes to "limitations of shock and kill", but without mentioning specifics in their comments. We, like others working in this field, recognize the steep incline that potential shock and kill therapies face for effective treatment, and consequently we have noted this in the Introduction in the paragraph ending "Therefore, it is likely that more advanced combination strategies must be used to produce efficient provirus induction for eradication of cells that produce replication-competent viruses using the shock and kill strategy."

Referee #3, notes that we have not described a mechanism for reactivation of HIV latency by thecompounds. We believe it important to point out that this study represents the first analysis of completely synthetic compound libraries where novel compounds were identified that can reactivate HIV latency in cell lines, as well as infected PBMCs from patients, and importantly can also cause induction of replication competent virus from latently infected samples from patients. One previous study describes a screen using a similar number of compounds (~200,000), but this study failed to identify compound s capable of reactivating expression of replication competent virus from patient samples (Micheva-Viteva et al., 2011). Furthermore, we note that most comparable previous screens were performed with much smaller "known drug" libraries or libraries of natural products where mechanistic activity had already been identified or at least suspected.

We agree that it will be important to understand the biochemical effect(s) of the compounds we have identified, and we have spent considerable effort over the past several months towards this goal. Unfortunately none of the most obvious possible mechanisms that could reactivate HIV from latency seem to be affected. We provide additional evidence in the revised manuscript showing that PH02 does not affect global histone acetylation and also does not affect expression of key transcription factors regulating activation from the HIV enhancer. In additional experiments, not described in the manuscript, we have determined, unfortunately, that none of the PH compounds inhibit growth or other obvious phenotypes in yeast. From these efforts we believe that the compounds must affect previously uncharacterized mechanisms for maintenance of viral latency. Identification of mechanism of action for compounds identified in synthetic small molecule libraries is never trivial, and will require detailed analysis of responses using proteomics and genomics, which seems beyond the scope of the present manuscript.

2nd Editorial Decision 17 November 2017

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed reports from the referees that were asked to re-assess it. As you will see

the reviewers are now globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending final amendments.

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

The manuscript has been improved and the authors have addressed my concerns

Referee #3 (Remarks for Author):

The lack of MOA dampens my enthusiasm but the work deserves publication so the work can be shared and continued.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Dr. Ivan Sadowski
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EMM-2017-08193

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The sample sizes were chosen to obtain a 95% confidence interval of statistical significance between and within the groups of samples.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	No.
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes, for every figure with statistical tests, there were sufficient numbers of replicates to perform these tests.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes, a ratio paired t-test and a one-way analysis of variance (ANOVA) were used when appropriate.
Is there an estimate of variation within each group of data?	Yes, the error bars were reported as standard errors from the standard deviation.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Yes.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	SP1- Abcam 13370, NF-kB p65- Abcam 7970, IkB- Abcam 32518, GAPDH- Abcam 9484, H3K9-ac-Abcam 10812, H3K9-trimethylation- Abcam 8898, H3- Abcam 1791.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	All of the cell lines originated from Jurkat-tat cells (obtained from the National Institutes of Health [NIH]-AIDS reagent resource, cat # 1399) and J-Lat Tat-GFP (A1) cells (obtained from NIH-AIDS reagent resource , cat # 9852) were reported negative for mycoplasma, bacteria and fungi.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	UBC Clinical Research Ethics Board (CREB) # H13-00357
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	HIV-infected patient samples were provided through the British Columbia Centre for Excellence in HIV/AIDS at St. Paul's Hospital, Vancouver. All participants provided written, informed consent prior to their inclusion in this study.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	All blood samples were used following a strict pre-approval protocol, following all ethical guidelines associated with using human samples. There is no restriction on publishing the data reported in the manuscript.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No.