Collaboratory of AIDS Researchers for Eradication (CARE)
Quantitative Viral Outgrowth Assay (Q-VOA) Resource Announcement

Purpose

The Collaboratory of AIDS Researchers for Eradication (CARE) encourages applications from investigators who wish to enter into a collaboration that will advance the study of persistent HIV-1 infection in persons treated with fully suppressive antiretroviral drug regimens. The purpose of this effort, supported by the NIAID Division of AIDS, is to afford the opportunity to investigators to utilize quantitative resting CD4+ infection assays. These Quantitative Viral Outgrown Assays (Q-VOA) will be made available to test the ability of novel agents to induce in vitro production of replication-competent HIV in resting CD4+ T cells from aviremic antiretroviral-treated (ART) patients, to measure the frequency of resting CD4+ T cell infection in such patients enrolled in clinical trials seeking to deplete latent infection, and as a benchmark comparison for other assays of latent HIV infection under development.

Background

This resource is offered based on the premise that current ART can reduce and maintain plasma viremia at levels below detection by standard assays, but do not eliminate residual, persistent viral infection. Sensitive single copy assays demonstrate that HIV-1 is still measurable in the plasma of most individuals despite long-term treatment with potent combinations of antiretroviral drugs, and intensification of such regimens with new or different drugs does not eliminate residual virus.

This quiescent reservoir of HIV is currently a major obstacle to eradication of HIV infection. The development of temporally limited therapies capable of eradicating HIV infection has recently been recognized as a high priority for the NIAID research agenda. A new treatment paradigm, utilizing small molecules to induce expression of latent HIV from the CD4+ cells of patients on suppressive ART regimens has recently shown clinical promise. Current studies suggest that this mode of virus induction may lead to advanced treatment therapies with the ability to specifically target the HIV latent reservoir in vivo. Integral to the advancement of such studies however is the ability to accurately measure the frequency of patient cells that are induced to produce replication-competent virus as a direct result of exposure to HIV-inducing compounds.

The quantitative viral outgrowth assay is currently considered the gold standard in the measurement of viral persistence. However, because this assay is demanding and resource-intensive, it is not widely implemented. Its limited use is considered a major constraint to rapid progress in the field of HIV latency. Moreover, PCR-based measures of HIV RNA or DNA using similar patient CD4+ cells have shown to greatly over-represent the frequency of cells harboring replication-competent HIV. Recent work suggests that all PCR-based assays overestimate this frequency by an average of 300 fold (S. Eriksson et al., manuscript submitted).

Therefore, by expanding the availability of the quantitative viral outgrowth assay, numerous investigators and research groups from academia and industry will benefit by having access to this definitive quantitative tool. The coordinating Principal Investigators’ laboratories will collaborate with successful applicants to provide this assay as a benchmark to test novel anti-latency agents, to quantitate latent virus in patient cells following exposure to novel HIV-
induction agents in a clinical trial, and as a means to validate novel latency assays that are in development in the growing field of HIV cure research.

References for this assay include:


Objectives and Scope

The objectives of this initiative are to:

1. Expand testing of small molecules and other novel agents for their ability to induce ex vivo production of replication-competent HIV by resting CD4+ T cells from patients on suppressive ART regimens.
2. Assess the effect of clinical interventions on the frequency of resting CD4+ T cells that harbor replication-competent HIV within resting CD4+ T cells in patients on suppressive ART regimens.
3. Compare the Q-VOA assay to other novel assays of latent and persistent HIV infection.

Criteria for Selection of Project Samples

In accordance with Objective #1:
The following preliminary data must be included in all applications from PIs wishing to submit compounds for testing. Applications lacking information will be accorded a lower priority:

1. Dose-response curves for activation of the HIV promoter or of HIV gene expression in a T cell model (e.g. J.1, J-lat, J89-GFP, a primary T cell model) over a time course of 2 to 24 hours. Data on exposures over 24 hours may be useful, depending on the mechanism of action of the compound.
2. Cytotoxicity data over a time course of 2 to 24 hours. Cytotoxicity should be measured in primary PBMCs by the investigator. At least a five-fold window between cytotoxicity and inductive activity should be demonstrated.
3. Mechanism or target of action, if known
4. Data on compound storage, purity, solubility, and stability (over time and with freeze/thaw). A compound should be documented to be soluble at testing concentrations where activity is expected. If compound is to be provided in DMSO, final concentration of DMSO following dilution of stock to achieve activity range in an experiment must be provided.
5. Evidence that the proposed compound is not a mitogen or global T cell activator. Evidence that the compound is not a histone deacetylase inhibitor is also desirable, or if the compound has HDACi activity, rationale (e.g. potency, toxicity) for its study should be provided.
In accordance with Objective #2:
All information concerning the human or animal study protocol, including all information regarding the reagent(s) used in the clinical trial must be submitted for review. The following preliminary data must be included in all applications from PIs wishing to submit patient blood samples or cells from a non-human primate or from a humanized mouse model for Q-VOA testing:

- Dose-response curves for activation of the HIV promoter or of HIV gene expression in a T cell model (e.g. J.1. J-lat, J89-GFP, a primary T cells model, etc.) over a time course of 2 to 24 hours. Data on exposures over 24 hours may be useful, depending on the mechanism of action of the compound.
- Cytotoxicity data over a time course of 2 to 24 hours.
- Mechanism or target of action, if known.
- Compound storage, solubility, and stability data.
- Biomarker data, if available, to validate that the reagents under study have achieved effective in vivo exposure.
- Relevant virologic and clinical data on study subjects (if applicable), e.g. duration of therapy and suppression, recent CD4 count, CD4 nadir, therapy during acute and/or chronic infection.
- PK/PD data available for the compounds to be evaluated.
- A schedule indicating when and how many blood samples would be sent for analysis.
- Plans for obtaining blood, tissue, or leukopheresis samples, and shipping to the coordinating PIs’ laboratories.
- Documentation of IRB or IACUUC approval for the study. If approved for analysis by the Q-VOA resource, evidence of consent for the performance of these assays will be required from human studies participants.

In accordance with Objective #3: Applications to submit assays for comparative assessment or to send or receive patient blood samples for the same purpose must contain the following information:

- Methodological details of the novel assay and preliminary assay performance data in a cell line, primary cell model of HIV latency, or patients’ cells.
- Plans for a) receiving blood, cells, tissue, or leukopheresis samples from the coordinating PIs’ laboratories for evaluation, or b) sending blood, tissue, or leukopheresis samples to the coordinating PIs’ laboratories for evaluation.

Application Submission
All applications must be submitted electronically as a single pdf file through the Q-VOA Resource page found on the CARE website, www.delaneycare.org

Applicants will be notified of the status of their proposal within 10 days after the committee’s review. If rejected, reapplication may be considered on a case-by-case basis if substantial new information can be presented that will significantly strengthen the application.

Application Selection Criteria
The decision to select a compound for testing in the Q-VOA assay or for choosing an assay for comparison to the Q-VOA will be made based on the overall strength of the application as
determined by a comprehensive assessment of the preliminary data provided, including evidence that interpretable testing data can be obtained with reasonable effort, and that testing of a compound will advance general knowledge in the field of HIV latency.

Similar review and selection criteria will be used in the decision to accept patient blood samples, cells from a non-human primate or from a humanized mouse model for Q-VOA testing.

**Application Review**

An internal review committee consisting of the coordinating PIs, NIAID and DAIDS program representatives as well as other ad hoc reviewers as necessary will review all applications.

Applications will be scored based on their overall merit and feasibility using a 9-point scale. A score of 1 indicates an exceptionally strong application. A score of 9 indicates an application with serious weaknesses. Scores are in whole numbers with no decimal ratings.

Applications will be given a numeric score together with one of three possible adjectival ratings: 1. **Accepted** 2. **Deferred**, with the requirement for additional information if reapplication is desired, or 3. **Rejected** without further consideration.

**Confidentiality**

All applications submitted to the Q-VOA resource are considered to contain proprietary information and will considered confidential at all times. No one outside of the Q-VOA review committee will be allowed to disclose information about the contents of an application or about the nature of review discussions or recommendations.

In the event that an application is not selected by the Q-VOA resource, the application will be properly discarded immediately following applicant notification in order to safeguard the confidentiality of the material contained within the application.

Confidentiality agreements and Material Transfer Agreements (CDAs and MTAs) are available for downloading on the CARE website if applicants wish to have these legal and binding documents in place prior to application submission. However, submission of a signed CDA or MTA will in no way influence the review of an application.

**Co-Authorship and Q-VOA Data**

Publications that utilize data generated by the Q-VOA resource will be required to include the name of the coordinating PI and laboratory personnel as well as the U19 funding source.

If you have more questions about this resource or the submission process, please contact:

qvoa@delaneycare.org