Busch: Validation of Existing and Ultra-Sensitive Assays for Quantifying HIV Persistence

OVERVIEW

The HIV Reservoir Assay Validation and Evaluation Network (RAVEN) project will facilitate the development and rigorous evaluation of performance characteristics (sensitivity, specificity, limits of detection and quantitative dynamic ranges) for blood-based assays to detect and quantify HIV persistence, by establishing a large and well-characterized repository of samples from HIV-infected participants on suppressive ART therapy, initiated either early or late following viral acquisition.

A major barrier to the discovery and development of curative interventions for HIV is the lack of validated, high-throughput assays that reliably quantify the size of the viral reservoir (total body burden) of replication-competent HIV. Multiple assays are in development, but there is an immediate need for a rigorous head-to-head comparative evaluation to establish which HIV reservoir assays have the best performance characteristics, and hence should be "scaled up", optimally by commercial manufacturers, and employed in prospective cure research protocols. To do this, academic and commercial laboratories require high volume plasma/leukocyte sample procurement, processing to capture or concentrate virus/infected cells, and ultimately HIV RNA/DNA/protein detection and characterization. The present project is an international collaboration that seeks to validate established and novel molecular methods for quantifying HIV persistence that are blood-based, high-throughput, and applicable for use in cure research protocols and eventually routine clinical application.

Apheresis collections were performed serially on 50 ART-suppressed participants in San Francisco (HIV-1 clade B) and 25 in South Africa (HIV-1 clade C) and the HIV reservoirs characterized with respect to low level plasma viremia, cell-associated HIV DNA and RNA and quantitative viral outgrowth assays (QVOA). Cryopreserved PBMC, CD4+ T cells and plasma aliquots were coded andassembled into "qualification" and "evaluation" panels that are being distributed to academic and commercial labs focused on developing or performing HIV reservoir assays. These panels are being used to evaluate induced viral outgrowth assays, molecular assays for plasma and cell-associated HIV DNA and RNA, HIV sequence based assays and immunological assays that indirectly reflect reservoir size. Blinded panels of analytical and clinical samples are provided to laboratories, and the RAVEN team is responsible for decoding results and final data analysis.

RAVEN administration originates under Vitalent Research Institute (formerly Blood Systems Research Institute) in San Francisco; the RAVEN team includes two international clinical sites (University of California, San Francisco; and South African NationalBlood Service), a Steering Committee, senior laboratory scientists and staff, apheresis technicians, data management specialists, regulatory and

fiscal oversight, and numerous collaborating laboratories. As a collaboratory network, RAVEN includes an evolving roster of both academic and industry laboratories with expertise in viral quantification, assay development, and/or active discovery pipelines in support of HIV cure research.

The work is a collaborative effort led by Michael Busch, MD, PhD (Vitalant Research Institute in San Francisco), Steven Deeks, MD (University of California, San Francisco), John W. Mellors, MD (University of Pittsburgh), Douglas Richman, MD (University of California, San Diego) and Charlotte Ingram, MD (South African National Blood Service), The award was received in October, 2014 with an initial agreement length of 4 years.

Grant at a Glance

Principal Investigator
Michael Busch, MD, PhD

Grantee Institution
Vitalant (formerly Blood Systems, Inc., San Francisco, USA

Project Title
Validation of Existing and Ultra-Sensitive Assays for Quantifying HIV Persistence

OPPID
1115400

Grant Award
Up to $4.6 million, awarded in October, 2014

Collaborating Institutions
- University of California, San Francisco, USA
- South African National Blood Service, South Africa
- University of Pittsburgh, Pittsburgh, USA
- University of California, San Diego, USA

RESEARCH OBJECTIVES

1.) Conduct a validation study of the current and next generation of viral outgrowth assays;

2.) Rigorously evaluate the performance characteristics (e.g., sensitivity, specificity, reproducibility and precision) of currently established and novel molecular and immunological assays for HIV reservoir detection and quantitation using coded panels of well characterized analytical control samples and low-level HIV positive clinical samples for which large volumes of plasma and leukocytes will be procured

3.) Compare performance of these assays when applied to clinical samples from representative cohorts of ART-suppressed patients with clade B and C HIV-1 infections.