Overview of Laboratory of Molecular Virology (LMV) Research Programs

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Mission Relevance - OBRR Research Priority

Goal: Assess and promote safety and effectiveness of Transfusion-Transmitted Infectious Disease (TTID) agent donor screening and supplemental tests, and retroviral diagnostics

Objectives:

- Evaluation of screening and confirmatory technologies for detection of TTID agents for assurance and enhancement of blood safety
- Development and evaluation of reference panels for screening and confirmatory tests for TTID agents and retroviral diagnostics
- Facilitate preparedness for blood safety from emerging infectious agents and other pathogens of global significance through investigations of mechanisms of transmission and pathogenesis

LMV mission: Regulatory and Research

- Review and approval of in vitro donor screening and diagnostic tests for HIV, HTLV and retroviral agents, bacterial detection assays, and develop donor policies for Transmissible Spongiform Encephalopathies (TSE).
- Develop review criteria and standards for approval of tests, and policies related to their use in the intended setting.
- To support the regulatory mission of DETTD/OBRR, LMV performs research on:
  - HIV and related retroviruses, and co-infections in AIDS
  - Characterization of reagents for reference and lot release panels for HIV diagnostic and donor screening tests
  - Bacterial detection and methods for removal/inactivation in blood
  - Pathogenesis of TSE agents and validating detection assays

Laboratory of Molecular Virology - Organization

HIV and retrovirus section

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Projects under HIV and Retrovirus Section

- *Molecular diversity of HIV, impact on diagnosis and pathogenesis*
  - Use molecular techniques to characterize HIV diversity, impact on diagnostics, and reference reagents for HIV assays.
  - Employ OMICS to study pathogenesis and identify diagnostic/predictive host biomarkers of disease stages in HIV infection.
- *Novel, emerging diagnostic technologies, bioinformatics for retrovirus detection and characterization*
  - Evaluate NGS (next gen sequencing) techniques and related bioinformatics to detect and characterize HIV variants and blood borne pathogens.
- *Pathogen Reduction Technologies for HIV and retroviruses*

Molecular Characterization of Highly Diverse HIV-1 Viruses for reference panel development

- HIV is genetically highly diverse due to mutation and recombination.
- Unmet need for HIV RNA and DNA reference reagents of predominant diverse HIV strains to evaluate viral load, donor tests, latency assays etc. for detection sensitivity of viral variants.
  - FDA reference reagents provide common standards to evaluate different diagnostic NAT assays, especially as Class II devices.
  - For this work, highly diverse viruses from Cameroon and US, were cultured from PBMC and plasma, tested for viral load, and characterized genetically by Sanger and full genome NGS.

Zhao at al., Viruses, 2021; 13(7):14170).

Diverse Mosaic Structures of Full-Length HIV Genomes

Results:

- Multiple non-B subtypes and highly diverse recombinant mosaic forms were identified among viruses sequenced using both Sanger and full genome NGS.

- Strains included Circulating Recombinant Forms (CRFs) and non-B subtypes circulating in the US and relevant to diagnostics.

- Viral load of stocks was determined and values assigned for future use in formulation of lot release and reference panels.

Summary and Future Directions

- Repository of highly diverse HIV viruses and plasma were characterized and established at CBER as reference reagents for panel development.
- Viruses will be provided to:
  - Office of Compliance and Biologics Quality (OCBQ)/CBER for future lot release panel development for donor tests as needed.
  - External Quality Assurance Program and Oversight Laboratory (EQAPOL) of Duke University/NIAID for use as reference reagents by diagnostic test manufacturers.
- <u>Future reference panels for diagnostic and donor tests</u>
  - Highly diverse HIV DNA panel for latency assays to ensure patient and blood safety from HIV transmission by persons on PrEP, ART and cure regimens.

HIV-1 drug resistance mutation (DRM) data analysis app for review of sponsor NGS data

Rationale:

- HIV-1 drug resistance test developers use NGS platforms for product development.
- Need for in-house bioinformatics tools for FDA analysis of sponsor data.
- We developed an in-house Graphical User Interface computational tool compatible with the FDA network to establish an independent method to analyze sponsor data to support regulatory approval.
- The app can be used for review of future applications.

FDA's HIV-1 DRM app: Results and Future work

- Input Raw sequence reads into App
- Run codefreq, download codefreq output file
- Select Stanford HIVDB App for analysis.
- App was evaluated in-house by testing a virus stock at 100, 100 and 10,000 copies/ml and limited patient samples.
- CBER HIVdB app could detect all major DRMs and accessory mutations at the 5% level at an HIV-1 VL of 1000 copies/ml.
- Future efforts are aimed at transferring the app to the CBER HIVE bioinformatics platform for internal use in regulatory review of applications.

Disease stage specific host biomarkers of HIV infection

Rationale for work

- Pre-exposre prophylaxis (PrEP) and Anti-Retroviral Therapy (ART) regimens have been implemented nation-wide to reduce and /or eliminate HIV infection.
- Early treatment causes HIV markers to be undetectable in blood by current HIV diagnostic/donor screening assays posing a concern for patient and blood safety.
- Goal of research is to identify disease-stage specific, host biomarkers (eg. miRNAs and long non-coding RNAs) as surrogate markers in virally suppressed disease stages and in patients on HIV cure regimens and strategies.

Study Design for identification of plasma miRNA biomarkers of early HIV-1 infection

- **Stage1: Biomarker discovery phase**
  - Three samples from each category (controls, RNA+, Ag+ and Ag+Ab+) were screened using PCR Arrays to identify differentially expressed miRNAs. Potential miRNAs were identified and selected for further investigation.
- **Stage2: Biomarker validation phase**
  - Candidate miRNAs were assessed for the predictive performance. Validation of candidate miRNAs were carried out using quantitative RT-PCR.
- **Stage 3: Panel development phase**
  - Validated miRNAs were grouped together (using multivariate analysis) for the derivation of a diagnostic panel using multivariate analysis. This panel of miRNAs were evaluated as potential biomarkers.
- **Stage 4: Blinded test phase.**
  - Diagnostic panel was further evaluated using blinded panels (panels of specimen in which the clinical diagnostic information is blinded to the investigators).

S. Biswas et al.. EBioMedicine 43 (2019) 307–316

Summary and Future Directions

Results:

- microRNA panel PeHIV-1 (miR-223-1, miR-16-2, miR-195-1, miR-20b-1) that detects early HIV-1 infection with a high degree of sensitivity and specificity was identified.
- PeHIV-1 could accurately detect samples from patients in the early stage of HIV-1 with undetectable plasma HIV markers (RNA and HIV-1 p24).

Future Studies:

- Validate miRNAs with additional patient samples.
- Develop a diagnostic miRNA panel to identify HIV-1 infection in latently infected or ART patients with undetectable viral markers (RNA and HIV-1 p24).
- Identify and validate other small RNAs including non-coding RNAs and transfer RNAs for various HIV disease stages.

Studies on Pathogen Reduction Technologies (PRT): HIV-1 inactivation in plasma by 405nm visible blue light

Rationale:

- Current PRTs are known to have adverse effects on coagulation factors of plasma, and platelet functions.

- 405 nm blue light was studied as a potential alternative to existing PRT as it is lethal at all levels to microorganisms, without adverse effects on the host cell.

Ragupathy et al, Pathogens 2022

Summary and Future Work

- Five individual plasma samples were treated with 405 nm light for varying lengths of time.
- Samples treated for 5 hours showed significant ($p<0.001$) reduction in infectivity HIV-1 (~2 log HIV-1 p24) compared to controls or samples treated for 30 min.
- 405 nm light inactivated HIV-1 effectively in multiple plasma donor samples.
- Future work is aimed at studying mechanism of inactivation and evaluating other PRT for effects on HIV-1 infectivity.

Bacterial and TSE Section
Research goals

- To develop a blood assay to detect transmissible spongiform encephalopathy (TSE) agents and to maintain safety of therapeutic products including blood

- To develop novel methods of bacterial reduction and detection in blood and blood components to improve safety of blood transfusions

Rationale for TSE Research

- TSEs (prion diseases) are incurable neurodegenerative diseases that have occasionally been transmitted to recipients of therapeutical products
- Theoretical risk exists of sporadic Creutzfeldt-Jakob disease (sCJD) transmission from human cellular and tissue-derived products used in regenerative medicine
- Need for a rapid, sensitive, and specific sCJD blood test
- Unmet need for relevant reagents to develop and validate sCJD blood tests
- We addressed this unmet need by producing blood from macaques infected with variant CJD (vCJD) as a surrogate for human sCJD blood

Results and Future Work

- Generated vCJD-infected macaque blood as reagent for assay development
- Used blood to detect abnormal prion protein, PrP^{TSE}, and other biomarkers suitable for pre-clinical sCJD diagnostics
- PrP^{TSE} and neurofilament light-chain (NfL) are in macaque blood months before clinical onset
- Macaque blood materials are now available as reagents to develop and validate blood tests for PrP^{TSE} and non-PrP^{TSE}

- **Future work**: complete the work on detection of PrP^{TSE} in pre-clinical macaque blood and evaluate sCJD and familial CJD blood samples

Rationale for Bacterial Research

- Methods of pathogen reduction are available for plasma and platelets but not for red blood cell concentrates and whole blood
- Research gap to develop novel pathogen reduction methods for all blood components
- We addressed this research need with a new project focused on capture of bacteria spiked in platelets and whole blood using affinity ligands

Results and Future Work

- Selected nylon filaments as the solid support of ligands
- Produced a panel of nylon materials containing different functional groups (charged, polar, hydrophobic) to test as binders of bacteria
- Screened dyed nylons with *E. coli* and *S. epidermidis* and identified best binders

- **Future work:**
  - Bacteria spiked in platelets (and whole blood)
  - Detection of bacteria concentrated on dyed nylon (diagnostics)
  - Investigate quality of platelets and whole blood after treatment

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