LABORATORY OF RETROVIRUSES
DIVISION OF VIRAL PRODUCTS

VRBPAC, December 12, 2024

LAB OVERVIEW

LR UNITS

Hana Golding, Ph.D. (PI and Lab Chief)

Unit of Viral Immunology and Pathogenesis

Development of New Immunological Assays and Animal Models

Evaluate Vaccine Safety and Efficacy

Additional FTEs:

Marina Zaitseva Ph.D. (Staff Scientist GS-14)

Surender Khurana Ph.D. (Staff Scientist GS-14)

Jody Manischewitz, M.S.,

Lisa King, B.A.

David Acosta, B.A

Training Fellows: 5-6 postdoc, post-bacc, contracts / year

LR UNITS

Arifa Khan, Ph.D. (PI)

Unit of Molecular Retrovirology

Development of Sensitive Virus Detection Assays for Safety of Vaccines and Other Biologics and Evaluation of their Potential Threat for Human Infections

Additional FTEs:

Hailun Ma, Ph.D. (Staff Scientist)

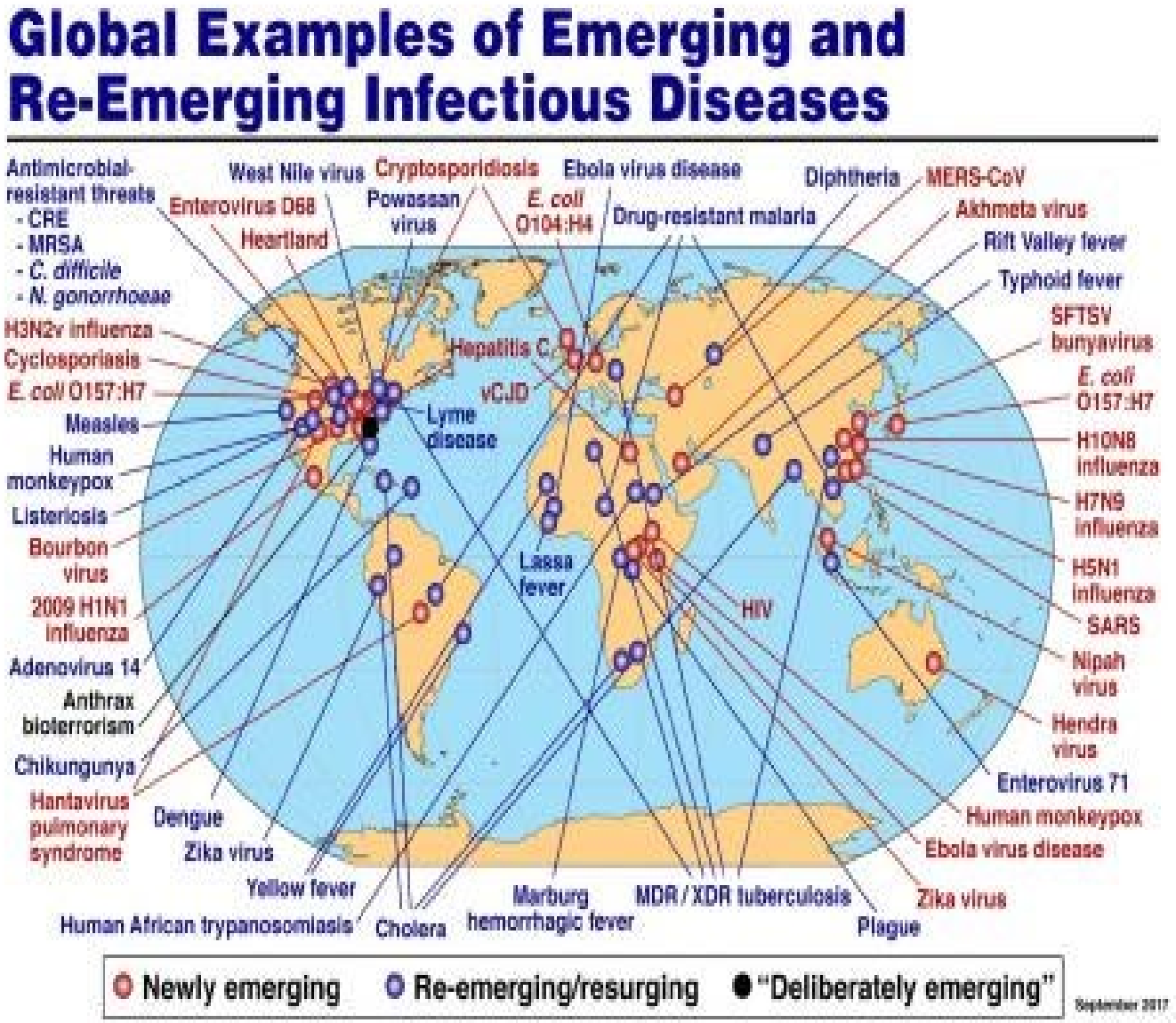
Andrea Kennard Ph.D. (Staff Fellow)

Sandra Fuentes Ph.D. (Microbiologist)

Pei-Ju Chin, Ph.D (Staff Fellow)

Training Fellows: 2-4 postdoc, post-bacc, contracts / year

Ever shifting pandemic threats around the globe (Fauci, 2017)



CBER/OVRR Response: facilitate rapid deployment of vaccines against emerging diseases

- **Goals: Identify regulatory and scientific gaps in knowledge, methods for vaccine release, and correlates of protection**
  - **LR researcher-regulators provide CMC expertise and readiness to re-direct their scientific programs to meet the challenges of emerging diseases, including the use of new cell substrates, manufacturing platforms, novel immunogen/adjuvant design, and clinical protocols.**
- **Develop advanced technologies for improved analyses of:**
  - **Known and emerging viruses for evaluation of cell substrate and product safety**
  - **Humoral immune responses post-infection**
  - **Immune response to novel viral vaccines**
  - **Adjuvant safety and mode of action**
  - **Vaccine potency assays**
  - **Animal models for preclinical evaluation of vaccines including safety and effectiveness**

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Regulatory Responsibilities

- **Vaccines against human pathogens**
  - HIV, Influenza, RSV, SARS-CoV-2, adjuvanted vaccines

  Platforms
  - Non replicating and replicating viral vectors : Poxviruses, NDV, PIV
  - DNA Vaccines
  - mRNA vaccines
  - Live attenuated vaccines
  - Recombinant proteins, peptide-based vaccines, nanoparticles

- **Novel Adjuvants, vaccine delivery systems/routes (IM, SC, ID, Mucosal)**

- **Universal Influenza Vaccines**

- **Novel cell substrates and detection of adventitious agents using next generation sequencing technologies (NGS)**
  - Mammalian tumorigenic and non tumorogenic cell lines
  - Insect cell lines for baculovirus expression vectors
  - Avian cell lines

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Regulatory Work since last Site Visit

	GOLDING Zaitseva Khurana	KHAN Kennard Ma Fuentes Chin	COMBINED
Pre-IND	**68**	**50**	**118** (164%)
IND/*Original*	**124**	**76**	**200** (198%)
IND/*Amend*.	**1716**	**765**	**2481** (250%)
BLA/*Original*	**4**	**4**	**6** (150%)
BLA/*Suppl*.	**41**	**4**	**45**

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Other Regulatory Activities

GUIDANCE DOCUMENTS

- ICH, WHO, EDQM, and USP Guidelines on the implementation of NGS technologies for enhancing safety of vaccines and cell substrates *(Khan)*
- WHO Guidelines on Nonclinical Safety Evaluation of Vaccine Adjuvants and Adjuvanted Preventive Vaccines for Infectious Disease Indications *(Golding, Zaitseva*)
- FDA Guidance for Industry: Pharmacogenomic Data Submissions. *(Khurana)*

WHO CONSULTATIONS, BARDA PRESENTATIONS

(Golding, Khan, Khurana)

CROSS-OFFICE AND CROSS-CENTER CONSULTS

(Golding, Khan, Zaitseva, Khurana, Chin)

Golding Lab:
Scientific projects

- Elucidation of humoral immune responses following Ebola and Marburg infection and vaccination (*Khurana*)
- SARS-CoV-2 pathogenesis
- Antibody responses following SARS-CoV-2 infections vs. vaccination in different cohorts (adults, pediatrics, MISC, immune-compromised).
- Elucidation of humoral immune responses following RSV infection or vaccination
- Influenza vaccines; seasonal, pandemic, next generation/universal vaccines
- Mucosal vaccines
- Adjuvant safety: *In vitro* human cell-based assays for testing of novel adjuvants: Primary monocytes, differentiated macrophages, broncho-epithelial cells grown under Liquid-Air-Interphase (ALI) (*Zaitseva)*

Methods development: Virus Neutralization Assays

Influenza

- Hemaglutinaion inhibition assay (HI)
- Microneutralization assays using all available vaccine strains (CDC protocol)

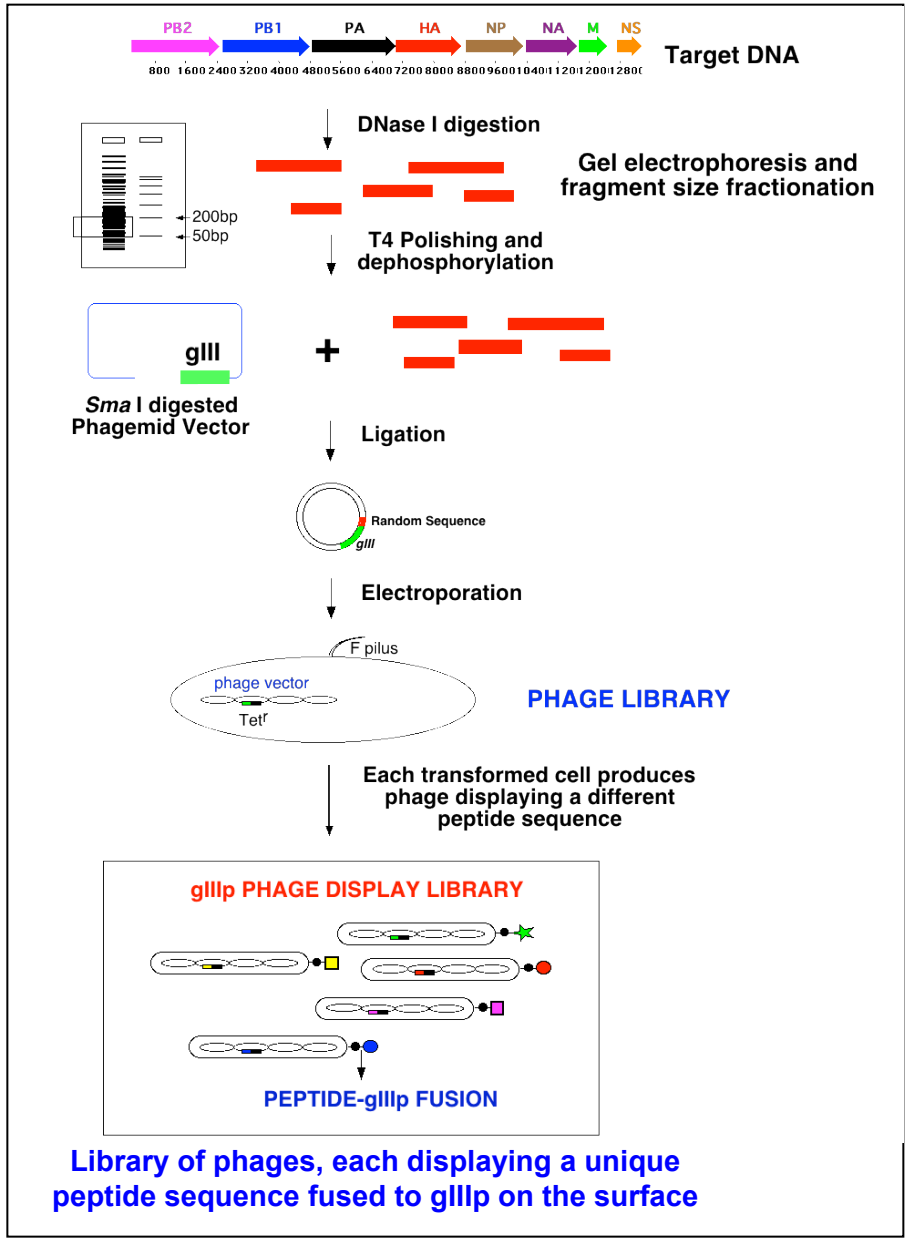
RSV (A/B)

- RSV-Luc-Neut – reporter-based neutralization assay
- PRNT

SARS-CoV-2

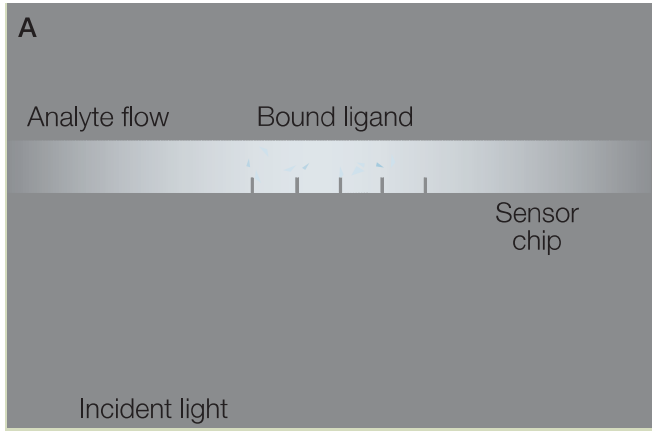
- Lentivirus based pseudovirus neutralization assay (PsVNA) – against all circulating strains and variants of concern (VOC)

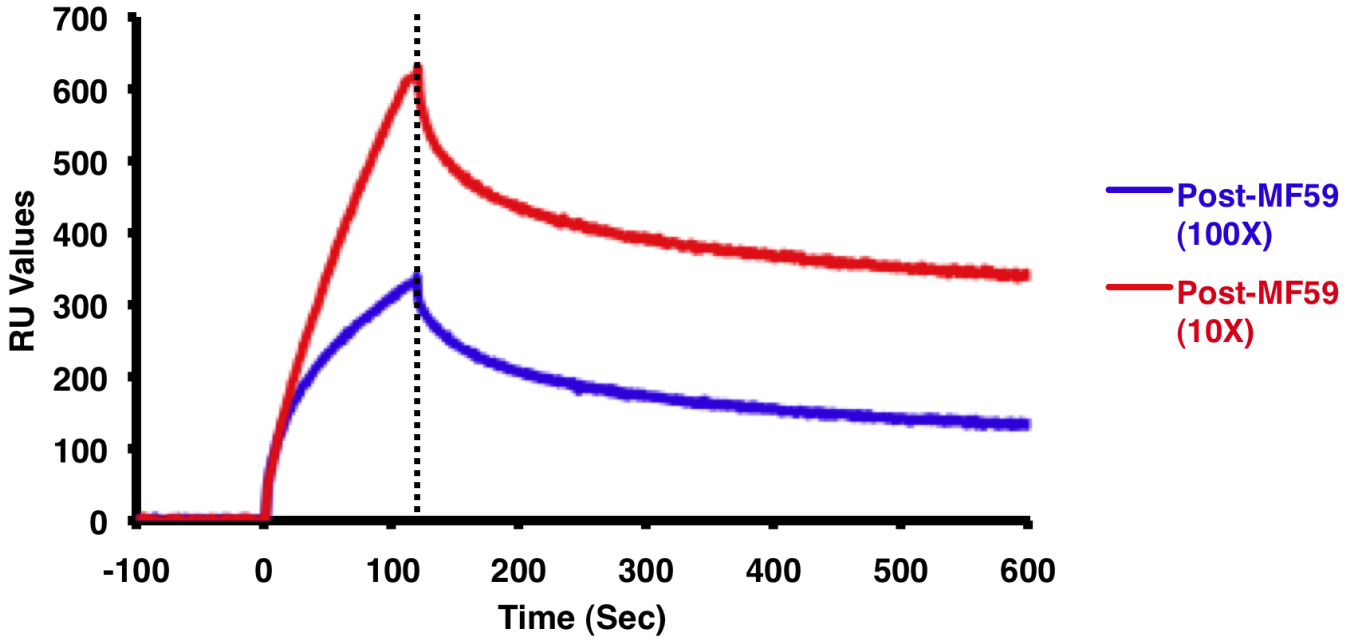
CONSTRUCTION OF GENE-FRAGMENT PHAGE DISPLAY LIBRARY (GFPDL) OF VIRAL GENOME



H5N1
H7N7
H7N9
pH1N1
H3N2
RSV
Ebola
Marburg
Zika
SARS-CoV-2

ANTIBODY BINDING AND AVIDITY MEASUREMENTS IN POLYCLONAL SAMPLES USING SPR REAL TIME KINETICS ASSAY





-Total Ab binding
-Isotype distribution
-Antibody Off-rates /avidity
-Capture properly folded proteins

HETEROGENOUS SAMPLE MODEL

$$L_1 + A \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} L_1A \quad and \quad L_2 + A \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} L_2A$$

$$L + A_1 \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} LA_1 \quad L + A_2 \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} LA_2 \quad LA_1 + A_2 \underset{k}{\overset{k_x}{\rightleftharpoons}} LA_2 + A_1$$

Khan Lab:
Scientific projects

- **Evaluation of High-Throughput/Next-Generation Sequencing (HTS/NGS) technologies for adventitious virus detection in biologics**
  - **Generating reference materials for validation of high-throughput sequencing (HTS)**
    - Development of WHO virus standards for viromics
    - Development of virus-infected cell standards for genomics and transcriptomics
    - Refinement and annotation of the Reference Virus Database (RVDB) (***Pei-Ju Chin***)
  - **Determining the sensitivity and breadth of virus detection by short-read and long-read HTS technologies**
- **Investigating adventitious and endogenous viruses for safety of cell lines used for manufacturing of biologics**
  - **Sf9 insect cells used for baculovirus-expressed products**
  - **Chinese hamster ovary (CHO) cells used for recombinant protein production**
- ***In vitro* cell culture and *in vivo* animal models to assess potential outcomes of simian foamy virus (SFV) infection in humans**
  - **Characterization of SFV expression in infected human A549 cell clones**
  - **Identification of SFV miRNAs as potential biomarkers of virus infection**
  - ***In vitro* studies of SFV replication and genome analysis to elucidate factors influencing virus expression**

Khan Lab: Advancing HTS as a Rapid Adventitious Virus Detection Assay for Safety of Biologics

❑ Development of Reference Viruses for HTS Implementation

- CBER NGS Virus Reagents to support NGS development and advancement (*NIAID BEI cat no. NR-59622*) (*previous WHO reference reagents established based on CBER collaborative study in 2020*)
- First WHO International Reference Panel for Adventitious Virus Detection in Biological Products for NGS qualification and validation studies ((NIAID BEI cat. no. NR-59630) (*established based on CBER collaborative study in 2024*)
- Publicly available for distribution; Free of charge (except minimal shipping cost in the US); To be used as a panel to demonstrate breadth and sensitivity of virus detection for HTS viromics (*e.g. viral seeds, viral vector preparations, unprocessed bulk harvests*)

❑ Providing a Reference Virus Database (RVDB) for Detection of Known, Emerging, and Novel viruses by HTS

- With high diversity of viral sequences for broad virus detection, with reduced nonspecific cellular hits resulting in less computational time and reducing cost of unnecessary follow up work to verify a true virus signal
- Freely available at https://rvdb.dbi.udel.edu/ (*collaboration with U. Delaware*) Maintained and regularly updated by the Khan Lab

Khan Lab: Continuing Efforts on HTS Implementation

- **Generating in-house data and by external collaborations to fill knowledge gaps for using HTS as a routine assay**
  - Developing optimized protocols by analyzing HTS short-read and long-read platforms
  - Determining LOD for virus detection by HTS in different matrices relevant to biological materials during manufacturing for developing general regulatory and industry expectations
  - Developing virus-infected cell standards for HTS genomics and transcriptomics (*e.g. cell substrates, cell therapies, unprocessed bulk harvests*)

- **Introduced HTS in international guidelines** [ICH Q5A(R2) and new Ph. Eur. chapter 2.4.61]
  - To replace the *in vivo* assays and PCR assays and to replace or supplement the *in vitro* cell culture assays (*resulting in general acceptance*).

- **Organized international HTS training webinars and workshops**
  - To facilitate establishment of HTS in LMICs and other regions considering use of HTS to replace the conventional assays for adventitious virus detection
    - PDA - June 28, 2024
    - IABS – July 23, 2022; Sept 19, 2023; Sept 24-25, 2024; Dec 3, 2024