RESEARCH ON TAP

Working Towards an HIV Cure

September 24, 2019

bu.edu/research/events
MISSION: Reduce burden of HIV infection world-wide by supporting multidisciplinary basic, translational and clinical research.

APPROACH: Collaborative effort between Brown University/Lifespan and BU/BMC to provide services to support on-going HIV research and to encourage new investigators to explore questions relevant to HIV/AIDS with funding opportunities, services and collaboration.
ORGANIZATION AND SERVICES
Cores, Scientific Working Groups, Research Interest Groups

CORES
- Admin
- Developmental
- Bio-Behavioral Sciences
- Basic Sciences
- Substance Use Research
- BioStats

SWG
- HIV/TB

RIG
- Oral Health & HIV
- Disrupt HIV Infection
- -omics and HIV Pops
We want to understand what cells get latently infected. Resting cells, T memory cells, myeloid cells? (Agosto et al. 2018. Cell Reports)

We want to know the mechanisms that lead to latency. Chromatin? Host-Transcription Factors? Specific signaling pathways?

We want to understand at a mechanistic level of the interplay of HIV infection, inflammation and pathogenesis.

We want to identify druggable targets for HIV and associated comorbidities (Graci et al. 2017. PLoS One)
Brain Microenvironment Models for HIV Research

Tyrone Porter, PhD, Engineering
Suryaram Gummuluru, Microbiology
Boston University
A Gap in Life Expectancy Remains Between HIV-infected and HIV-uninfected Individuals

- Chronic immune activation in the central nervous system (CNS) is observed in cART-suppressed HIV+ patients
- BBB breakdown and progressive neurocognitive disorders have shown a correlation with chronic immune activation
- How does HIV evade cART in the brain, what are the mechanisms of chronic immune activation, and what strategies/therapeutics may be employed to more effectively treat and/or manage HIV infection in the brain?

**Hypothesis**

Persistent de novo expression of HIV icRNA induces innate immune activation in microglia, which contributes to neurotoxicity
Characterizing human BBB model (established in Transwells)

VE-cadherin

ZO-1

![Image of VE-cadherin and ZO-1 staining]

![Graph showing TEER (Ω x cm²) vs. Hours post seeding]

- BMEC monoculture
- BMEC pericyte coculture

TEER (Ω x cm²)

<table>
<thead>
<tr>
<th>Hours post seeding</th>
<th>43-52</th>
<th>65-73</th>
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Verdinexor Penetrated Co-BBB and Inhibited HIV-1 Production

- Co-incubation of infected MDMG with CRM-1 inhibitor verdinexor significantly reduced HIV-1 production
- Verdinexor inhibitory activity was dose-dependent
- Verdinexor penetrated Co-BBB model and suppressed HIV-1 production by infected MDMG
- Findings suggest that less than 5% of verdinexor added to luminal chamber penetrated the BBB. Additional studies are required to test this estimate and quantify $P_{app}$
Translational Approach to Understand HIV-Associated Diseases and Barriers to Cure

Nina Lin, MD

Assistant Professor of Medicine
Director of ID Clinical Research Unit (CRU)
Boston University School of Medicine
Inflammation is central in HIV pathogenesis

Multidisciplinary approach to identify factors influencing:
1) HIV residual viremia and latency
2) its effect on aging = end-organ disease
HIV translational research: Using our patients to inform us

- Substance use on HIV reactivation and latency
- Drug and alcohol use
- Low level HIV transcription on IFN responses and inflamm-aging
- Retinoic acid as mucosal immunodulatory agent to increase T cell
- Inhibitor of nuclear export on HIV-induced inflammation
- Integrase inhibitors on obesity and insulin resistance
- Abacavir on coronary flow
- Efavirenz on neurocognitive function
- Smoking cessation on lung and systemic inflammation
- Smoking
A Causal Inference Approach to HIV Research

Sara Lodi
Assistant Professor
Department of Biostatistics
BU School of Public Health
Clinical practice requires decision making
  ▪ Example: switching to integrase inhibitors: Who? When? What to switch to? Frequency of monitoring?

Randomized experiments estimate causal effects, but:
  ▪ can be expensive, untimely or even unethical

Can use observational data to explicitly emulate a target trial:
  ▪ The (hypothetical) randomized trial that we would like to conduct to answer a causal question
Step 1: Specify Target Trial protocol

- Eligibility criteria
- Treatment strategies
- Outcomes
- Randomized assignment
- Analysis plan

Step 2: Emulate Target Trial protocol

- Eligibility criteria
- Treatment strategies
- Outcomes
- Identify confounders
- Analysis plan – adjusting for confounding (standard methods or g-methods)

Examples

- Effect of early ART initiation on mortality (Lodi et al. Lancet HIV 2015) and on development of drug resistance (AIDS 2018)
- Frequency of monitoring for CD4 count and viral load (Caniglia et al. JAIDS 2016)
Healthcare in Russia for HIV+ People Who Use Drugs: Role of HIV and Substance Use Stigma

Marina Vetrova, MD

General Internal Medicine
Boston University Medical Campus
Stigma and Health

- Labeling
- Exclusion
- Avoidance
Addressing Stigma

- Public education
- Provider training
- Personal empowerment:

Acceptance and commitment training

HIV-positive person

Person with substance use disorder
Addressing Tuberculosis In High HIV-Burden Settings

Helen Jenkins
Assistant Professor
Department of Biostatistics
Boston University School of Public Health
Defining the care cascade for rifampin-resistant TB patients in South Africa, geographically and by HIV/non-HIV
With Jacob Bor and Karen Jacobson

- 60% of TB cases in HIV positive patients in South Africa, no database to monitor these spatially and longitudinally
- We will combine longitudinal TB and HIV laboratory test data to identify hot-spots, track patient movement and define the care cascade
Counting kids: Detection of Pediatric TB in Ukraine
With Natasha Rybak

- Pediatric TB presents differently in children and is harder to diagnose
- Only 45% of 1 million annual incident cases are diagnosed and treated. ~250,000 annual deaths
- One third of European TB/HIV patients are in Ukraine, pediatric TB is under-diagnosed
- We aim to review autopsy records (mandatory autopsy policy in Ukraine), and clinical data to assess pediatric burden
ANal Cancer/ HSIL Outcomes Research Study
ANCHOR

Elizabeth Stier, MD
Associate Professor, Obstetrics and Gynecology
Boston University School of Medicine
Boston Medical Center

Colon-Lopez, JCO, 2017
HIV+ Individuals, 35+ Screened for HSIL (N= 17,385)

- HSIL Not Found
  - Not Enrolled
  - Enrolled & Randomized (N= 5,058)
    - HSIL Found
      - Active Monitoring Group (N= 2,529)
        - Cancer Not Found
          - Exit Study Referred for Evaluation and/or Treatment
        - Cancer Found (est 24)
      - Treatment Group (N=2,529)
        - Cancer Found (est 7)
        - Cancer Not Found
HIV & Oral Health in the Post-cART Era

Matthew Mara, DMD

Clinical Instructor
General Dentistry, Henry M. Goldman School of Dental Medicine
RESEARCH ON TAP: Working Towards an HIV Cure

Boston University Office of Research

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Specific Aims

**Aim 1.** Validate self-reported measures of periodontal disease.

**Aim 2.** Characterize the oral health status (tooth count, dental caries, periodontal disease, oral lesions) of PLWH residing in the Greater Boston area.

**Aim 3.** Establish the feasibility of participant self-collection, packing and mailing of saliva samples in a cohort of PLWH.
Determining Immune Cell Drivers of Inflammation in ART-Suppressed HIV and Aging Using Systems-Level Approaches

Jennifer Snyder-Cappione

Assistant Professor, Department of Microbiology
Director, Flow Cytometry Core Facility
Boston University School of Medicine
Premature ‘inflamm-aging’ with aviremic HIV infection?

Despite successful viral suppression, ↑‘older age’ co-morbidities in HIV+ persons

Nina Lin, M.D.
(1) Younger (≤35 years) HIV+
(2) Older (≥50 years) HIV+
(3) Younger (≤35 years) HIV-
(4) Older (≥50 years) HIV-

Projects

(1) Determine links between CD8+ T cell exhaustion, macrophage activation, and inflammation

Rahm Gummuluru Ph.D.
Nina Lin M.D., Ph.D.
Manish Sagar M.D.

(2) Investigate role of γδ T cells in intestinal permeability

Anna Belkina, M.D., Ph.D.
Nina Lin, M.D., Ph.D.
Luis Agosto, Ph.D.,
Irina Vonovoy, M.D.

Readouts

(1) 33-color flow cytometry (blood lymphocytes)
(2) 20+ markers of inflammation (plasma)
(3) 30+ parameter tissue analysis via Imaging Mass Cytometry (gut biopsies)

How can we analyze these enormous datasets?
Data Analyses: Multivariate Algorithmic Approach
Anna Belkina, M.D., Ph.D.

Bioinformatic analysis tools:
- opt-SNE*
- Citrus
- SPADE
- PLSR
- PLS-DA...

Enable:
1. Unbiased, comprehensive analysis of all collected data
2. Determination of how different parameters co-vary to better understand biological systems as a whole
3. Measurement of what happens when conditions collide (HIV and Aging)

*Belkina et al, Nature Communications, in press

(1) Citrus
TIGIT

placed subjects into correct groups (+/- HIV) with 89% accuracy

Belkina et al, Front Immuno, Dec. 2018

(2) PLS-DA

Conclusions:
1. γδ T cells involved in/impacted by inflammaging
2. Age, HIV infection distinctly impact immune/inflammatory signatures in blood
Humoral Immunity, Cell by Cell

Fumi Aihara

Kepler Lab
Boston University
What We’re Interested In

Affinity Maturation

How do antibodies improve?

Antibody Repertoire

How diverse are a pool of antibodies?

B cell

Antig

B cell
Techniques

Surface Plasmon Resonance

Analysis and Modeling

RNA Sequencing
Reference Gene
Clone 1
Clone 2
Clone 3

Single Cell B Cell Culture
Feeder Cell

Single Memory B Cell

https://www.google.com/search?rlz=1C1GCEU_enUS847US848&tbm=isch&q=Computer&chips=q:computer,g_1:desktop:p9fvRxODGhw%3D&usg=AI4_-kRIbhK8EMb9OFXqxkOwgzV9FprlOw&sa=X&ved=0ahUKEwiXxv3p1OfkAhXMUt8KHSmnDhgQ4lYILCgA&biw=1413&bih=703&dpr=1#imgrc=cATyzr-TLHQQLM:
Affinity Maturation From a Single B Cell Clone

\[ K_D = \frac{K_{off}}{K_{on}} \]

where \( K_D \) is the dissociation constant measured in nM.

Clone founder

670

23 aa diff

2.9

36

3.0

5.7

4.2

6.7

: vaccine 4.

All others, vaccine 3
Antibodies and HIV-1 Mucosal Transmission

Manish Sagar, MD

Associate Professor
Department of Medicine
Boston University School of Medicine
Selection of HIV-1 variants during mucosal transmission
Role of antibodies in preventing transmission and eliminating infected cells
Antiretroviral Strategies Based on Artificial Virus Nanoparticles

Björn Reinhard
Professor
Chemistry, College of Arts and Sciences
Artificial Virus Nanoparticles Target Glycoprotein-Independent, Lipid-Mediated Virus Recognition

A. Image of virus nanoparticle with annotations:
- octadecanethiol layer
- lipid layer

B. Graph showing MFI (norm to blank) for different liposome types:
- Blank Cer
- Gal
- GM3
- GM1
- GQ1b

C. Graph showing HIV_LaEnv (norm to blank) for different liposome inputs:
- Blank
- GM3
- Gal
- GM1
- GQ1b

D. Graph showing Gag-eGFP (norm to blank) for different liposome inputs:
- Blank
- GM3
- Gal
- GM1
- GQ1b

References:
Artificial Virus Nanoparticles Recapitulate CD169-dependent HIV-1 Uptake and Trafficking

Nanoparticles Escape Endocytosis and Co-localize with Virus Like Particles in Non-Endolysosomal Compartments

Targeting of CD169-expressing myeloid cells in Mouse Lymph Node


Boston University Office of Research
Towards Nanoparticles for Long-Term Release of Antiretroviral Compounds

Carbovir

Integrase inhibitor

Rilpivirine

Non-nucleoside reverse transcriptase inhibitor

https://en.wikipedia.org/wiki/Rilpivirine

https://en.wikipedia.org/wiki/Cabotegravir

Drug release (%)

Time (h)

Drug release (%)

Time (h)

% Infection

log(nM)

RPV

Cabotegravir

RPV:Cab (40:500)

RPV+Cab 1

RPV+Cab 2

RPV+Cab 3

RPV+Cab 4
Identification of Novel Transcription Factors Regulating HIV Expression

Juan Fuxman Bass

Assistant Professor
Biology Department, CAS
eY1H assays to identify novel TFs binding to the HIV-1 and HIV-2 LTRs

Grow in absence of histidine

Blue compound from X-gal
eY1H screening against 1,086 human TF array

**Boston University** Office of Research
KLF2 and KLF3 repress HIV-1 transcription in CD4+ T cells

TF overexpression

TF knockdown

ChIP

HIV Expression (Ratio to Vector)

HIV mRNA Relative to siCtrl

% Input

siRNA Target

ChIP Target

Vector GABPA KLF2 KLF3

+EFV siCtrl siKLF2 siKLF3

mIgG KLF2 KLF3

p=0.014 p=0.014

rCD4T aCD4T

KLF2 and KLF3 repress HIV-1 transcription in CD4+ T cells
Inflammatory Mechanisms Driving HIV Persistence

Rahm Gummuluru, PhD

Professor,
Department of Microbiology
School of Medicine
Chronic Innate Immune activation and HIV persistence

- HIV/AIDS is an inflammatory disease

- Current anti-retroviral therapies (ART) do not suppress inflammation

- Chronic inflammation is the driver of HIV-associated co-morbidities, even in virologically-suppressed individuals
  - Cardiovascular atherosclerosis, neurocognitive dysfunctions, diabetes mellitus, non-AIDS cancer, osteoporosis and renal disorders
  - HIV-infected individuals on ART still have shorter life-spans

- Inflammation promotes HIV persistence in secondary lymphoid tissues
  - Reactivation of latent viral reservoirs; reseeding of reservoirs
  - Promote clonal amplification and homeostatic proliferation of HIV DNA+ cells
  - Persistent antigenemia → immune exhaustion resulting in degradation of innate and adaptive immune responses

- Hypothesis: Persistent HIV RNA expression in tissue resident macrophages nucleates inflammatory signaling cascades and promotes virus persistence
  - Tissue-resident macrophages (TRMs): embryoid-origin, long-lived productively infected cells
  - Latently-infected TRMs have been observed
  - Stochastic mechanisms might lead to virus reactivation without virus spread
  - Induction of innate responses and immune activation?
HIV intron-containing RNA (icRNA) Expression Activates Innate Immune Responses in Macrophages: Driver of Chronic Inflammation in cART-Suppressed HIV+ Individuals

Low levels of IFN-I
ISG up-regulation
Proinflammatory cytokines

Persistent expression of HIV icRNA (even from deleted, hypermutated, non-infectious proviruses) may contribute to chronic inflammation in cART-suppressed patients

Self/Non-self Discrimination of HIV icRNA from host icRNAs
- Viral intron identity,
- Secondary structure,
- Altered localization and/or diversity of RNA binding proteome

Sensing of icRNA

CRM1 dependent export

Not targeted by current cART

Akiyama et al 2018 Nat Comm

De-repression of Viral Transcription

Therapeutic Goals: Suppress aberrant viral transcription, HIV icRNA export or sensing of viral icRNA
UPCOMING EVENTS

Learn more & RSVP: bu.edu/research/events
Topic ideas & feedback: research@bu.edu

RESEARCH ON TAP

Human Capital and Global Development
October 29, 2019 | 4-6pm

Light as Medicine
November 20, 2019 | 4-6pm

RESEARCH HOW-TO

Ending Sexual Harassment in Academia
September 26, 2019 | 3-5pm

Media Training & Messaging Workshop for Faculty
October 2, 2019 | 1-5pm