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# Persistent Immune Activation in Persons with HIV-2 During Antiretroviral Therapy

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## Background

Human Immunodeficiency Virus Type 2 (HIV-2) is a lentivirus with structure and replication cycle similar to HIV-1. Infection with HIV-1 or HIV-2 is lethal, but the two viruses differ in origin, epidemiology, pathogenesis, disease progression, and therapeutics. HIV-2, a zoonotic transmission from monkeys, emerged from West Africa in the 1970s-80s. There are currently 1-2 million people with HIV-2 (PWH2), residing largely in West Africa and countries with social, cultural, and economic ties to that region (Fig.1). In the United States, there are approximately 300 PWH2 including 11 dual HIV-1/HIV-2 or probable HIV-2 have been identified since 2000.[1-2] Prior to initiating combination antiretroviral therapy (ART), HIV-2 RNA levels may be low or absent, and disease progression to AIDS is at least twice as long as HIV-1.[3] Immune responses remain substantial in PWH2, however, with increased levels of soluble (sCD14, HIV-2 antibody), and cellular immune activation and exhaustion markers.[4-6] Levels of cellular immune activation markers have been linked to levels of viremia prior to ART and have been reported to be lower than those measured in persons with HIV-1.[7] Levels of immune activation in PWH2 undergoing ART have not been well-characterized. Here, we investigated viral and immune characteristics in PWH2 prior to and following introduction of ART and in matched otherwise healthy controls.

### Fig. 1 HIV-2 Spread and Prevalence




c. 1-2 million PWH2 worldwide[8]

## HYPOTHESES

- Pretherapy levels of activated CD8 cell subsets will be elevated in viremic, but not aviremic PWH2 compared with matched otherwise healthy controls.
- Levels of activated CD8 cell subsets will normalize after introduction of ART in all PWH2 and will not be significantly different than matched otherwise healthy controls.

## METHODS

### Study Participants

#### Persons with HIV-2

Persons with HIV-2 were identified and clinical data abstracted from clinical records of participants enrolled in NIH protocols studying immunology and virology of persons with HIV infection: ALLHANDS 13-N-0149, BIOINCO 09-I-0013, IDFU 95-I-0072, LYMPH NODE 92-I-0125, RASFB 91-I-0140, RPHI 02-I-0202, and ZEUS 000866. All PWH2 had reactive HIV 1/2 ELISA and HIV-2 positive supplemental testing documented at NIH.

#### Healthy Controls (HC)

Otherwise healthy individuals were recruited into the NIH volunteer program and provided phlebotomy and clinical information for demographic characteristics. Individuals were matched for age (±5 years), sex, and race.

Peripheral blood was obtained by phlebotomy and plasma and peripheral blood mononuclear cells (PBMC) were obtained by standard Ficoll-Hypaque isolation.

### Human Subjects Protection Oversight

All participants provided informed consent for collection of samples and demographic information. All protocols were approved by NIH Institutional Review Boards (NIH FWA00005897).

### Laboratory Analyses

PBMC underwent immunophenotyping using fluorescence-activated cell analysis and CD4, CD8, $CD8^+DR^+$, and $CD8^+CD38^+DR^+$ subsets quantified.

HIV-2 proviral DNA levels were measured in 2.4-6 million PBMC.

### Statistical Analyses

Unpaired *t* tests were used to analyze immunophenotyping data.

**Elevated immune activation is observed in PWH2 despite long-term ART and low or undetectable levels of HIV-2 cell-associated DNA.**

## RESULTS

| Study ID | Age* | Sex | Race | Country of Origin ☆ | Comorbidities & Coinfections | Current ART Regimen | Total ART duration (years)*** |
|---|---|---|---|---|---|---|---|
| BIOINCO P162 | 27 | Male | Black | Liberia | | ART-naïve | N/A |
| IDFU2 85 | 45 | Male | Black | Ghana | Type 2 Diabetes<br>Hypertension<br>Hyperlipidemia<br>Shingles<br>Recurrent genital herpes | BIC/TAF/FTC | 18.7 |
| IDFU2 187 | 57 | Male | Black | Côte d'Ivoire | Hypertension<br>Cerebrovascular Accident<br>Chronic kidney insufficiency<br>Gout<br>Eczema | BIC/TAF/FTC | 16.1 |
| RPHI CI345 | 58 | Male | Black | Ghana | Hypertension | ART-naïve | N/A |
| RASFB 773 | 61 | Female | Black | Ghana | Type 2 Diabetes<br>Hypertension<br>Strongyloides | ART-naïve | N/A |
| IDFU2 C18 | 67 | Male | Black | Liberia & Ghana | Chronic renal failure<br>Hypertension<br>Hyperlipidemia<br>Cataracts | ABC + 3TC+ ATV + RAL | 3.4 |

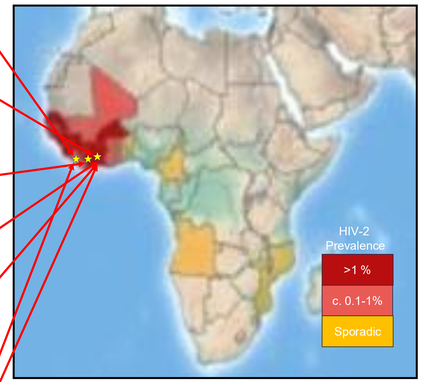



**Table 1. PWH2 Demographic Information**
*At time of study enrollment
**From medical records
ART Abbreviations: 3TC=Lamivudine, ABC=Abacavir, ATV=Atazanavir, BIC=Bictegravir, FTC=Emtricitabine, RAL=Raltegravir, TAF=Tenofovir Alafenamide

| Study ID | Pretherapy HIV-2 viral plasma RNA levels (c/mL) | HIV-2 caRNA | HIV-2 caDNA | Total CD4 (mean) (%) | Total CD8 (mean) (%) | CD4/CD8 (mean) | $CD8^+38^+DR^+$ (mean, %) | $CD8^+DR^+$ (mean,%) |
|---|---|---|---|---|---|---|---|---|
| **total participants, N=6** | | | | | | | | |
| **pre-ART, N=4** | | | | | | | | |
| RPHI CI345 | | | | 696.70 (30.30) | 696.70 (49.30) | 0.62 | 49.70 | 68.30 |
| BIOINCO P162 | | | | 585 (29) | 787(39) | 0.74 | 33 | 48 |
| RASFB 773 | | <0.24 in 4.5x$10^6$ cells | <0.20 in 5.4x$10^6$ cells | 891.30 (44.70) | 466.40 (23.30) | 1.91 | 24.40 | 63.30 |
| IDFU2 85 | 47,700; 65,900 | | | 429.60 (30.80) | 497.30 (25.90) | 0.86 | 19.30 | 35.60 |
| **on ART, N=3** | | | | | | | | |
| IDFU2 C18 | | 2.40 in 5x$10^6$ cells | <0.18 in 6x$10^6$ cells | 115 (7) | 950 (58) | 0.12 | 65 | 73 |
| IDFU2 187 | | 19 in 270,000 cells | 7.10 in 3.2x$10^6$ cells | 245.50 (20.70) | 460 (39) | 0.53 | 24.70 | 70.60 |
| IDFU2 85 | | <0.52 in 2x$10^6$ cells | <0.43 in 2.4x$10^6$ cells | 429.60 (30.80) | 497(35.90) | 0.86 | 19.30 | 35.60 |

**Table 2. Immune Profile of PWH2**
PWH2 (N=6) were studied. One individual had measured HIV-2 plasma viral RNA levels. Proviral DNA levels were not detected in 2/3 individuals undergoing ART, and levels in 1 PWH2 were low (7 c/million PBMC). In one individual, HIV-2 RNA and proviral DNA could not be detected pre-ART despite significant CD8 subset elevations. Total CD4 w/ percentage, total CD8 w/ percentage, CD4/CD8, $CD8^+38^+DR^+$%, and $CD8^+DR^+$% were reported as averages for individuals with multiple samples. One individual, IDFU2 85, had available pre-ART and on ART samples that were analyzed.

| CD8 Subset | All Participants | | Pre-ART | | Undergoing ART | |
|---|---|---|---|---|---|---|
| $CD8^+38^+DR^+$% | All PWH2 | Matched Healthy Controls | Pre-ART | Matched Healthy Controls | ART | Matched Healthy Controls |
| mean | 22.8 | 14.24 | 35.31 | 12.34 | 20.83 | 11.25 |
| SD | 12.36 | 7.1 | 13.54 | 10.53 | 11.02 | 9.82 |
| p | <0.0001 | | <0.0001 | | <0.0001 | |
| $CD8^+DR^+$% | | | | | | |
| mean | 49 | 24.2 | 62.15 | 23.6 | 46.93 | 22.95 |
| SD | 18.62 | 12.77 | 6.26 | 12.97 | 19.08 | 12.01 |
| p | <0.0001 | | <0.0001 | | <0.0001 | |

**Table 3. Unpaired *t*-test results comparing activated CD8 cell subsets**
**Unpaired *t*-test total controls, N=62** Mean percentages of activated CD8 cell subsets between various groups were compared through performing unpaired t-tests. Between PWH2 and HC's, levels of CD8 cell subsets were significantly elevated in PWH2 even on ART.

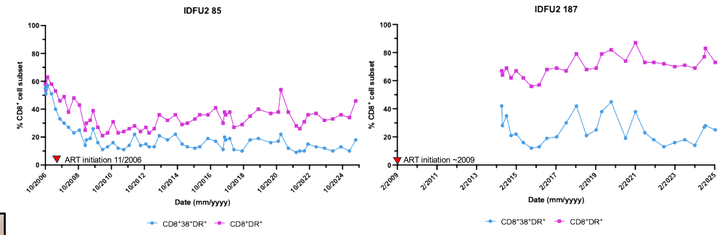



**Figure 2. Activated CD8 cell subsets over time for PWH2 on long-term ART**
Percentages of activated CD8 cell subsets are graphed over time for 2 PWH2 with multiple samples. Approximate date of ART initiation is marked on each graph. IDFU2 85 had two pre-ART samples.

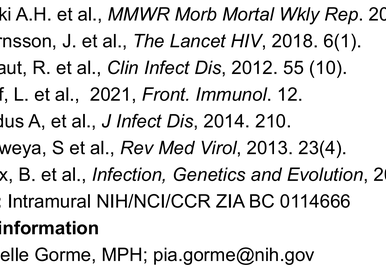



**Figure 3. Activated CD8 cell subsets over time for ART-naïve individual with HIV-2**
Percentages of activated CD8 cell subsets are graphed over time for participant RASFB 773. This participant had elevated activated CD8 cell subsets over time despite no levels of HIV-2 caRNA or caDNA detected.

## SUMMARY

- Elevated immune activation persists despite long-term ART and low or undetectable levels of HIV-2 DNA.
- Immune activation in PWH2 can be elevated in some PWH2 even in the absence of viral RNA in plasma or DNA in cells.
- Drivers of immune activation in PWH2 undergoing ART are uncertain. Viral sources of immune activation may not be based in blood.

## FUTURE DIRECTIONS

- Evaluating for HIV DNA and RNA in tissues, e.g. colonic biopsies may provide additional insight into immune activation in PWH2.
- Perform further studies evaluating immune activation in more PWH2, especially women
- Investigate the role of immune activation in morbidity and mortality of PWH2 undergoing ART

## ADDITIONAL KEY INFORMATION

**Thank you to our participants!**

**References**

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## PLAIN LANGUAGE SUMMARY

Activated immune cell subsets are elevated in PWH2 during ART.