HIV infected individuals on ART with impaired immune recovery have altered plasma metabolite profiles

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Introduction

HIV is a major global health issue, with almost 37 million people living with HIV, and approximately a million HIV/AIDS-related deaths occurring in 2017. With no cure in sight, we are heavily reliant on preventative measures and antiretroviral therapy (ART), to lessen the burden of HIV and decrease the number of AIDS-related deaths.

Some people living with HIV (PLWH) respond better than others to ART. Individuals with poor immunological recovery of CD4 T cells early on during treatment, have been shown to have increased immune depletion, as well as higher risk of both AIDS-related and non-AIDS-related conditions, independent of ART-mediated viral suppression.

Microbial translocation across the intestinal barrier is a significant driver of inflammation in PLWH and persists even with suppressive ART. Immune and inflammatory responses within the intestinal mucosa are characterized by shifts in tissue metabolism. Gut microbiota varies between HIV-infected ART suppressed and HIV uninfected people, and between PLWH immunological responders and non-responders. Recent studies have reported abnormalities involving several metabolic pathways in PLWH on ART.

In addition to bacteria, the enteric microbiome contains viruses, and low CD4 counts have been associated with increased levels of adenovirus-derived nucleic acid sequences in fecal samples independently of ART treatment.

The mechanisms associated with slow immune recovery after the initiation of ART and virus control are poorly understood and a large body of evidence has been dedicated to understanding this phenomenon from an immunological perspective. Metabolomics, the large-scale study of small molecules, intermediates and products of metabolism, is a developing diagnostic tool that has been useful in the discovery of novel biomarkers in a number of health issues including cancer and cardiovascular disease. The plasma metabolome reflects the underlying biochemical activity of the host and will reflect responses to disease. Host plasma metabolites also predict gut microbiome diversity in healthy adults.
To this end, we examined a large cohort of adult PLWH that received combination ART as their first antiretroviral regimen and achieved viral load suppression within 6-12 months of treatment initiation. The cohort consisted of two groups of HIV infected individuals, characterized by slow and fast immune recovery based on the increase in CD4 T cell numbers following 2 years of suppressive ART, as well as a group of age-, sex- and ethnically matched HIV negative individuals.

The aim of this retrospective cross-sectional study was to identify alterations in plasma metabolites in PLWH, and to see if differences in plasma metabolome signatures were associated with the diverse profiles of immune recovery following ART initiation. Indole-3-propionate (IPA), a deamination product of tryptophan produced by gut microbiota, was the most important metabolite in discriminating between HIV positive and HIV negative individuals. The most important metabolite discriminating between the two groups of PLWH was cysteine. Decreased plasma levels of cysteine in individuals with slow CD4 reconstitution suggest an association with oxidative stress and impaired immune recovery. In addition, metabolite set enrichment analysis of plasma indicated perturbation of several metabolic pathways in HIV-infected individuals compared to HIV negative controls. Bile acid biosynthesis and glycine and serine metabolism were among top de-regulated pathways in HIV positive individuals.

Methods

Study population

Individuals for the study were selected from the IL7R SNPS and Malaysian HIV and Aging (MHIVA) cohorts in Malaysia. All individuals were aged >18 years, received combination antiretroviral therapy as their first ARV regimen and achieved suppressed viral load (HIV RNA <50 copies/ml) within 6-12 months of initiating ART. There were a total of 563 people recruited to these cohorts, and individuals were selected for this study, if they displayed extreme phenotypes of immune reconstitution post ART, i.e. individuals with impaired/slow CD4 T-cell recovery were defined as...
those with a rise in CD4 T-cells less than 50 cells/year in the first 2 years following suppressive ART while efficient/fast recovery was defined as an increase in CD4 T-cell counts of >200 cells/year in the first 2 years following ART initiation. The initial study population included 75 individuals with fast immune recovery, 66 with slow immune recovery and 70 HIV negative control individuals. All samples were collected post fasting in EDTA collection tubes. Ten samples (two with fast and eight with slow immune recovery) were excluded from analysis due to low CD4 T cell counts at time of sample collection (range 0 – 85 CD4 T cells/µL). Another 10 (two fast and eight with slow immune recovery) samples were excluded from analysis due to detection of trimethoprim and sulfamethoxazole (TMP-SMZ) in samples, which might influence the metabolome 19. The HIV negative population were matched for age, sex (10 women and 60 men), and ethnicity (51 Chinese, 12 Malay and 7 Indian).

**Patient consent statement**

Written informed consent was obtained from all study participants. The study protocol was approved by the University of Malaya Medical Center review board (IL7R: MEC Ref. No. 896.32; MHIVA: MEC 20151-937), Malaysia.

**Metabolic profiling**

Metabolic profiling with GC-MS and LC-MS was performed at the Sweden Metabolomics Center, Umeå, Sweden (SMC). Detailed information about sample preparation, mass spectrometry, data processing and the performance of the standards and the data sets is available in supplementary information (SI) – Metabolic profiling (SI Fig 1-3).
**Statistical analysis**

A combination of univariate and multivariate bioinformatic approaches was performed using the R-script based online tool MetaboAnalyst 4.0 \(^20\). Mann-Whitney test and chi-square were used to compare differences in patient demographics for continuous and categorical parameters, respectively. Kruskal-Wallis test with post-hoc Dunn’s test was used for univariate statistics of single metabolites. Correlation was evaluated by Spearman’s rank test.

**Data normalization and transformation**

The data generated were expressed as relative response ratios, based on peak intensity. To compensate for batch analysis, samples were normalized by an injection standard (GC-MS) or internal standard (LC-MS). Normalized values were log transformed and pareto scaled before multivariate analysis in MetaboAnalyst 4.0. Differences between disease groups were further visualized by component partial least squares discriminant analysis (PLS-DA) models for each disease group comparison; HIV fast (F) vs controls (C), and HIV slow (S) vs C, for GC-MS data and LC-MS data respectively. The quality of the PLS-DA model was evaluated by Q2, R2X, R2Y and permutation test of separation distance (**SI Table 2-3**).

To investigate the biological relevance of the metabolomic differences between groups, quantitative metabolite set enrichment analysis (MSEA) was carried out. The analysis was performed using normalized values of intensity of putatively resolved metabolites in plasma using the Human Metabolome Database (HMDB) identification. Enrichment was evaluated using the Small Molecule Pathway Database in MetaboAnalyst 4.0 software.
Results

Demographics of study population

Among PLWH there were no differences in the following parameters between groups with fast and slow immune recovery: age at the initiation of ART, baseline CD4 T cell counts, sex, co-infection with hepatitis B or hepatitis C, risk category for HIV transmission, or duration of suppressive ART (Table 1). In the control group hepatitis B antigen was detected in one individual and none of the controls had positive hepatitis C serology (data not shown). In the group with slow immune recovery profiles, there was a smaller proportion of individuals of Indian ethnicity and, as expected, lower current CD4 T cell counts at the time of sample collection.

Plasma metabolomic pattern of cohorts

Mass spectrometry of the plasma samples led to the generation of three data sets that were processed separately: LC-MS negative, LC-MS positive, and GC-MS. Targeted processing approach of LC-MS data identified 180 putative metabolites (134 positive and 46 negative). Targeted processing of the GC-MS data set identified 97 putative metabolites.

Plasma metabolite profiling of HIV-infected individuals

Pair-wise analysis between HIV negative controls and the two groups of PLWH were performed to identify significantly dysregulated metabolites. As a first step in data analysis, the metabolic profiles of plasma samples from the control group were compared to that of PLWH with fast and slow immune recovery, respectively. PLS-DA analysis showed a clear discrimination of metabolomic plasma profiles of HIV infected individuals with slow or fast immune recovery, compared with that of the profiles of HIV negative controls. The explained variance of component 1 and component 2 varied between 7.8-12%, GC-MS (Fig 1A-B), and 11-19% LC-MS (Fig 2A-D). The reliability of the PLS-DA models was confirmed by 10-fold cross validation, and the best discrimination between groups was achieved using 5-7 components (SI Table 2). $R^2$ and $Q^2$ values close to 1 indicated good
performance of the model. In addition, permutation tests (1000 permutations) by separation
distance generated p-values <0.001 indicating significant differences between groups compared, i.e.
differences are unlikely to be attributable to chance or sampling bias.

**Metabolites differentiating between HIV-infected individuals and HIV negative controls**

Variable importance in projection (VIP) scores were used to identify the metabolites with the highest
collection to the separation between groups (SI Fig 4A-C). Indole-3-propionate (IPA) was one of
the top metabolites discriminating between the HIV groups and the HIV negative control group. The
mean intensities of IPA and tryptophan in plasma of PLWH, were lower than that in the plasma of
HIV negative controls (SI Fig 4). Kynurenic acid (kynurenate) and tryptophan betaine (lenticin) were
other important factors, that discriminated the HIV group with slow immune recovery from the HIV
negative group (SI Fig. 4B). Univariate analysis of tryptophan metabolites showed decreased levels
of tryptophan and its bacterial metabolite IPA as well as the tryptophan 2,3-dioxygenase (TDO)/
Indoleamine-2,3-dioxygenase 1 (IDO-1) metabolites kynurenine and kynurenate (Fig. 3A). Increased
levels of fructose were also characteristic for HIV infected groups (SI Fig 4). Bile acids and lipid
compounds contributed to the separation of groups of the metabolites identified by LC-MS in
negative mode (SI Fig. 4C). In all data sets, 44 unique metabolites had VIP-score \( \geq 1.6 \) (SI Table 1). All
metabolites with VIP-score \( \geq 1.6 \) were significantly different between groups with corrected p<0.02
(SI Table 1). Among the metabolites the most common chemical subclass was amino acid (SI Table
1). Of the top discriminating metabolites, 13 were unique for separating the slow-immune recovery
HIV group from HIV negative control group and 11 for separating the fast-immune recovery group
from HIV negative controls and 20 were shared between HIV groups (SI Fig. 4D).
Plasma levels of cysteine correlates with HIV immune recovery

When the two PLWH groups were compared with each other, the PLS-DA model did not discriminate the two groups (SI Fig. 5). Instead, univariate analysis was performed to show the differences in cysteine levels between groups (Fig 3B). There was a moderate correlation ($R_s=0.3$ and $p=0.005$) between plasma intensities of cysteine and number CD4 cells at sample collection in the HIV positive populations (Fig 3C). The relative plasma-level of cysteine was 1.51 times higher, $p<0.0001$, in individuals with fast immune recovery (Fig. 3B).

Altered glycine and serine metabolism was associated with HIV infection

We next sought to identify differential biological meaningful metabolic pathways relating to immune reconstitution after initiation of ART. Quantitative metabolite set enrichment analysis was performed on normalized LC-MS and GC-MS data sets. In total, 80 metabolites were included in the enrichment analysis of the GC-MS data sets (Fig 4). Enrichment analysis revealed that glycine and serine metabolism was one of the most significantly affected pathway in both HIV groups when compared to HIV negative controls (Fig 4A-B). The glycine serine metabolism was the pathway that hit the highest number of metabolites ($n=9$) in the dataset (SI table S4). The fold enrichment was 16.7, Holm corrected $p=5.1*10^{-15}$ (F vs C) and, 12.3 Holm corrected $p=3.2*10^{-9}$ (S vs C), respectively. Other affected metabolic pathways, including metabolites other than glycine serine metabolism, were Warburg effect, Holm corrected $p=4.5*10^{-5}$ (F vs C) and $p=0.004$ (S vs C) and, urea cycle Holm corrected $p=7.6*10^{-5}$ (F vs C) and $p=1.3*10^{-7}$ (S vs C) (SI table S4). Differences were most pronounced between HIV negative controls and the group of HIV positive individuals with slow immune recovery.
Enrichments of metabolites associated with bile acid biosynthesis were associated with fast immune recovery

Enrichment analysis of the LC-MS data set included 145 mapped metabolites with an HMDB pathway assignment. Analysis revealed that bile acid biosynthesis pathway hit 10 metabolites in the data sets. It was the only affected pathway that hit more than 3 metabolites (SI table 5). Bile acid biosynthesis was significantly enriched in the plasma of PLWH with fast immune recovery compared to HIV negative controls. The fold enrichment was 4.7 and Holm corrected p=0.008 (Fig 5A). The bile acid biosynthesis pathway was not significantly enriched in PLWH with slow immune recovery (Fig 5B). Other significantly enriched pathways (Holm corrected p<0.05), i.e. phenylalanine and tyrosine metabolism, beta-alanine metabolism, and pyrimidine metabolism, only hit 2 metabolites in the LC-MS data sets. Differences were most pronounced between HIV negative controls and the group of PLWH with fast immune recovery (SI Table 5). Univariate statistics showed decreased plasma levels of the primary bile acids chenodeoxycholic acid and its derivates in HIV infected individuals (SI Fig 6A), changes were more pronounced in the fast immune recovery group. There were also differences in plasma levels of cholic acid derivates between groups (SI Fig 6B).
Discussion

In the present study, we have investigated the plasma metabolomic profiles of HIV-infected individuals who demonstrated a diverse profile of fast and slow reconstitution of CD4 T cells following ART, compared to demographically matched HIV negative individuals. Using a targeted metabolomic processing approach and applying bioinformatic analysis, we found various discriminating factors between the two HIV groups, and between the HIV positive groups and HIV negative controls. Our data analysis identified 180 putative metabolites (134 positive and 46 negative) from LC-MS, and 97 putative metabolites through GC-MS.

An array of factors can influence ART induced immune recovery in HIV infected individuals. HIV infection and ART are associated with perturbation of lipid profiles \(^{21,22}\). In addition to low levels of HIV replication, the antiviral therapy itself can induce alteration in the HIV infected individuals as shown for protease inhibitor-based ART, which can give lipid alterations due to inflammation, hepatic function, and microbial translocation \(^{24}\). In our study, the difference in metabolic profile seen between the fast and slow immune recovery groups should be due to biological differences and not metabolic changes given that the antiretroviral drugs received were similar between the groups. This is supported by the comparable plasma lipidomic profile induced by darunavir-based or integrase inhibitor-based antiretroviral therapy \(^{25}\). Host metabolic factors have been associated with poor CD4 recovery in HIV. It has been suggested that adipose tissue may affect the recovery of the peripheral CD4 T cells and that hyperactivation of glycolysis in CD4 T cells may drive CD4 T cell depletion in HIV infection \(^{26,27}\), changes that are likely to be reflected in the plasma metabolite profiles.

One of the metabolites separating PLWH from the HIV negative controls, was IPA, a strong antioxidant microbial metabolite \(^{28}\), which is produced when tryptophan is deaminated \(^{29}\). IPA is anti-inflammatory and important for intestinal barrier integrity and immune cell function \(^{30}\). Disruption of the gastrointestinal barrier, i.e. dysbiosis, is a hallmark of many intestinal and chronic inflammatory diseases, including HIV infection \(^{31}\). In addition, decreased plasma levels of IPA have been reported...
to correlate with reduced gut microbial diversity. Thus, reductions in circulating IPA may reflect changes in gut microbial activity, dysbiosis and the persistent low grade of inflammation in ART-treated HIV-infected individuals, as previously reported.

Decreased plasma levels of tryptophan found in both HIV groups, are unlikely to be attributed to increased TDO/IDO-1 activity, since plasma kynurenine and kynurenate also were decreased in the HIV groups. Differences in uptake of tryptophan in the small intestine could explain the differences. Dietary preferences should be of minor importance in this study, given the relatively large groups and that controls are matched for ethnicity. Studies on germ-free animals have shown that gut microbiota directly regulates circulating tryptophan and altering gut microbiota through the administration of probiotics in human supplementation trials has also influenced circulating tryptophan levels. Hence, alterations in gut microbial activity can have contributed to the changes in plasma levels of tryptophan and its metabolites seen in HIV positive individuals.

We observed elevated levels of cysteine in the plasma of the fast-immune recovery HIV group, compared to that of the slow-immune recovery HIV group. Levels of cysteine were also elevated in the fast-immune recovery HIV group compared to the non-infected controls. Moreover, several pathways; homocysteine degradation, cysteine metabolism and taurine and hypotaurine metabolism were more enriched in the fast-immune recovery group. There was also a positive correlation in CD4 T cell numbers and cysteine levels. The correlation of cysteine with CD4 T cell numbers is in contrast to previous findings in HIV infected youths, and could be due to a different composition of ethnicity and/or age of the cohorts. Cysteine provides the redox-active thiol group of glutathione, which is one of the most important endogenous anti-oxidants. Signs of oxidative stress have been observed in individuals with HIV infection. T cell proliferation drives the production of reactive oxygen species and is cysteine dependent. It can be hypothesized, that elevated plasma levels of cysteine may contribute to T cell proliferation and improved anti-viral T cell responses, by facilitating glutathione synthesis. In vitro studies recently showed that N-
acetylcysteine may regulate antitumor immunity. All these pathways may collectively improve T-cell function and favor efficient immune reconstitution following ART.

Applying MSEA to annotated compounds revealed statistically significant alterations in several metabolic pathways when the two groups of PLWH were compared to non-infected people. One of the most pronounced changes associated with HIV were detected in glycine and serine metabolism. The conversion of serine to glycine is catalyzed by serine hydroxymethyltransferase. Glycine, in turn, refuels one-carbon metabolism that directly controls the levels of methionine, serine and glycine. The levels of all these amino acids were decreased in HIV positive individuals with fast immune recovery. One-carbon metabolism is a complex process that also indirectly controls cysteine levels and plays an important role in the maintenance of cellular redox balance. Hence, altered serine and glycine metabolism may contribute to efficient immune recovery, by balancing inflammation induced oxidative stress, in HIV infected individuals.

Bile acids are the final products of the catabolism of cholesterol and facilitate the absorption of dietary fat. Bile acid deconjugation and the conversion of primary bile acids to secondary bile acids are catalyzed by bacterial enzymes. Among metabolites detected by LC-MS, bile acid biosynthesis was the most enriched pathway in the fast HIV infection group. In contrast, this pathway was not significantly enriched in the slow HIV infection group. Bile acid metabolites function as biological detergents and bile acid tolerance is an important property of gut colonization bacteria. The interplay between bile acid metabolism and gut microbiota is complex. Accumulation of toxic bile acids may cause inflammation, but bile acids may also prevent epithelial deterioration and bacterial translocation by activation of the nuclear farnesoid X receptor. Targeting the bile acids-microbiota axis may be a potential approach to improve immune function in HIV.

Our study has several limitations. Most importantly, information about how longitudinal changes in metabolites reflect immune reconstitution during ART suppression is lacking due to the retrospective cross-sectional design of the study. In addition, the diet and nutritional status of the
cohorts could influence the metabolic profiles of the cohorts. On the other hand, the large number of the cohorts and the similarities in age, gender and ethnicity may compensate for individual differences in nutritional intake. The HIV negative control group was not matched for sexual behavior, which is a weakness since differences in gut microbiome has been observed in a study of men who have sex with men (MSM) independent of HIV-status. However, in one of our previous studies MSM was not found to have an independent influence on gut microbiota and it was recently reported that HIV-associated gut microbiota features correlate with disease progression and immune activation rather than sexual preferences. Also, MSM are subjected to punitive actions in Malaysia and disclosures of sexual preferences are in general highly unreliable. The strength of the study is the selection of participants with extreme profiles of immune recovery following comparable baseline demographics and HIV-related characteristics at ART initiation. This approach allowed us to identify important host-driven metabolic factors which could potentially modulate immune reconstitution.

In conclusion, we present a view of the plasma metabolic changes related to efficient and slow, i.e. poor immune recovery following suppressive ART in a large cohort of HIV-infected individuals. Decreased plasma levels of the bacterial metabolites IPA and changes in bile acid composition in PLWH may reflect microbial dysbiosis. We also show that efficient immune recovery is associated with elevated levels of plasma cysteine that may be the result of altered serine and glycine metabolism and pointing towards a role for redox balance in CD4 T cell reconstitution in HIV infection. This study highlights several biomarkers and metabolic pathways that could facilitate the understanding and monitoring of immune recovery after initiation of ART in HIV infected individuals.
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Potential conflicts of interests

None of the authors have any conflict of interest to declare.
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Table 1. Demographics and clinical characteristics

|                                      | Efficient/fast (n=71) | Impaired/poor (n=50) | p-valuea |
|--------------------------------------|-----------------------|----------------------|----------|
|                                      | Median (IQR)          | Median (IQR)         |          |
| Age at cART initiation (years)       | 36 (32-42)            | 37 (31-43)           | 0.927    |
| Time to cART initiation (months)     | 1.9 (0.9-14)          | 1.9 (0.9-12)         | 0.770    |
| Duration on suppressive cART (years)| 6 (4-11)              | 7 (5-11)             | 0.086    |
| Baseline CD4 T-cell count (cells/ul) | 107 (30-246)          | 165 (40-336)         | 0.317    |
| Current CD4 (cells/ul)              | 751 (578-877)         | 428 (264-537)        | <0.001   |
| Baseline CD4 T-cell count (cells/ul) | 107 (30-246)          | 165 (40-336)         | 0.317    |
| **Baseline CD4 T-cell counts**       |                       |                      | 0.803b   |
| <200 cells/ul                        | 47 (60%)              | 32 (40%)             |          |
| >200 cells/ul                        | 24 (57%)              | 18 (43%)             |          |
| **Sex, n (%)**                       |                       |                      | 0.174b   |
| Male                                 | 62 (61%)              | 39 (39%)             |          |
| Female                               | 9 (45%)               | 11 (55%)             |          |
| Ethnicity, n (%) |  |
|-----------------|---|
| Chinese         | 45 (51%) | 43 (49%) |
| Indian          | 10 (91%) | 1 (9%)   |
| Malay           | 15 (75%) | 5 (25%)  |
| Others          | 1 (50%)  | 1 (50%)  |

| Hepatitis C Ab, n (%) |  |
|-----------------------|---|
| Yes                   | 2 (67%) | 1 (33%) |
| No                    | 67 (60%) | 44 (40%) |
| Not recorded          | 2 (29%)  | 5 (71%)  |

| Hepatitis B Ag, n (%) |  |
|-----------------------|---|
| Yes                   | 2 (29%) | 5 (71%) |
| No                    | 68 (62%) | 42 (38%) |
| Not recorded          | 1 (25%)  | 3 (75%)  |

| Receiving Bactrim at cART initiation, n (%) |  |
|---------------------------------------------|---|
| Yes                                         | 38 (55%) | 31 (45%) |
| No                                          | 13 (54%)  | 11 (46%) |

| HIV transmission risk category, n (%)      |  |
|--------------------------------------------|---|
| MSM                                        | 20 (69%) | 9 (31%) |
| IDU                                        | 2 (67%)   | 1 (33%) |
| Heterosexual                               | 33 (49%)  | 34 (51%) |
| Others          | 15 (71%) | 6 (29%) |
|-----------------|----------|---------|

**History of AIDS defining illness at cART initiation, n (%)**  

|   | Yes       | 40 (64%) | 23 (36%) |
|---|-----------|----------|----------|
| No| 31 (53%)  | 27 (47%) |

**Baseline cART regimen, n (%)**  

|   | NNRTI-based | 69 (59.5%) | 47 (40.5%) |
|---|-------------|------------|------------|
| PI-based    | 2 (40%)    | 3 (60%)    |
Figure legends

**Figure 1. Partial least squares-discriminant analysis (PLS-DA) of GC-MS plasma metabolites.** GC-MS data, comparing the plasma metabolites of HIV positive individuals with fast immune recovery (fast, n=70) and HIV negative controls (control, n=65) (A) and HIV positive individuals with slow immune recovery (slow, n=50) and HIV negative controls (B).

**Figure 2. Partial least squares-discriminant analysis (PLS-DA) of LC-MS plasma metabolites.** LC-MS positive mode data, comparing the plasma metabolites of HIV positive individuals with fast immune recovery (fast, n=71) and HIV negative controls (control, n=70) (A) and HIV positive individuals with slow immune recovery (slow; n=50) and HIV negative controls (B). LC-MS negative mode data (C, D).

**Figure 3. Plasma levels of single metabolites and CD4 T cell numbers.** Univariate statistical analysis of plasma indole-3-propionic acid (IPA), tryptophan, kynurenine and kynurenate (A). Cysteine levels presented as arbitrary units (AU) (B). Spearman correlation of cysteine plasma intensity and CD4 number in HIV positive individuals (B). Medians and 5-95 percentiles are shown. ***p<0.001, Kruskal-Wallis test with Dunn’s post-test. Fast n=70, slow n=50 and controls n=65.

**Figure 4. Metabolite set enrichment analysis GC-MS.** Summary plot of over-representation analysis of plasma metabolites in HIV individuals with fast immune recovery (A) and slow immune recovery (B) compared to HIV negative controls.

**Figure 5. Metabolite set enrichment analysis LC-MS.** Summary plot of over-representation analysis of plasma metabolites in HIV individuals with fast immune recovery (A) and slow immune recovery (B) compared to HIV negative controls.
Figure 1

(A) GCMS

Component 2 (11.6%)

Component 1 (7.8%)

Fast

Controls

(B) GCMS

Component 2 (8.8%)

Component 1 (9.9%)

Slow

Controls
Figure 3

(A) IPA, Tryptophan, Kynurenine, and Kynurenic acid levels in controls, Fast, and Slow groups.

(B) Cysteine levels in controls, Fast, and Slow groups.

(C) Correlation between Cysteine levels and number of CD4+ T cells. R² = 0.3, p = 0.005.
Figure 5

A

Metabolite Sets Enrichment Overview

LC-MS
Fast vs Controls

B

Metabolite Sets Enrichment Overview

LC-MS
Slow vs Controls