Host gene expression kinetics during treatment of tuberculosis in HIV-coinfected individuals is independent of HAART therapy

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Summary of the article's main point

Transcriptional biomarkers are identified in peripheral blood discriminating active TB from latent TB infection and uninfected controls in HIV-infected individuals. These biomarkers can also be used to monitor TB treatment responses in HIV-infected individuals independent of HAART (in)eligibility and therapy.

Footnotes

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Abstract

Background: Limitations in diagnostic tools to discriminate between active and latent tuberculosis (TB) and for monitoring TB treatment responses are major challenges in TB control, especially in HIV-coinfected individuals.

Methods: Expression levels of 105 immune-related genes were determined in 131 HIV patients with either active TB (48 HIV+TB+), latent TB (37 HIV+TST+) or no TB infection (46 HIV+TST-) in Addis Ababa, Ethiopia, using focused gene expression profiling by dual-color Reverse-Transcription Multiplex Ligation-dependent Probe Amplification assay.

Results: Within the cohort of HIV+ subjects, the expression profiles of 7 genes at baseline (*FCGR1A, RAB24, TLR1, TLR4, MMP9, NLRC4* and *IL1B*) could accurately discriminate between active TB disease versus both latently infected (LTBI) and uninfected controls, largely independently of (in)eligibility for highly active antiretroviral therapy (HAART). Following 6 months anti-TB treatment (ATT), biomarker profiles of TB patients became indistinguishable from those of LTBIs and uninfected controls. Importantly, host gene expression kinetics during TB treatment in HIV-coinfected individuals was found to be independent of HAART therapy.

Conclusions: Blood transcriptomic profiles can potentially be used as biomarkers to discriminate the different clinical stages of TB in HIV-coinfected individuals and also be used to monitor TB treatment responses in both HAART treated and untreated individuals.

Keywords: Tuberculosis, HIV, HAART, Treatment response biomarkers, Gene expression profiling.

Introduction

Despite being a curable disease, tuberculosis (TB) has remained a major global health threat for centuries [1]. According to the World Health Organization, an estimated 10.4 million new TB cases and 1.8 million deaths, including 0.4 million HIV-coinfected people, occurred in 2015 [2]. In addition, 1/4 of the worldwide population is latently infected with *Mycobacterium tuberculosis* (*Mtb*), an intracellular pathogen capable of infecting and surviving within the host's mononuclear cells, particularly macrophages. Most immunocompetent individuals maintain a latent infection with only 5-10% lifelong risk of developing clinical disease [3]: the risk rises to 10% per year in HIV-coinfected individuals due to lack of effective immunity, and extra-pulmonary disease is more likely [4].

The incidence of multi-drug resistant (MDR)-TB is increasing, in part due to non-adherence to lengthy treatment regimens. HIV coinfection has been associated with, and may contribute to, the increase in MDR-TB [5, 6]. Monitoring early treatment response is essential for effective administration of anti-TB treatment to prevent drug resistance. Currently used diagnostic methods to confirm active TB disease and monitor TB treatment responses, such as sputum smear microscopy and mycobacterial culture, have low sensitivity for predicting treatment failure and relapse during TB treatment [7], especially in HIV-coinfected individuals [8] due to low cavity formation. Another disadvantage of mycobacterial culture is the several weeks delay before results become available and subsequent therapy adjustment. Existing immunological methods to diagnose TB infection, such as the tuberculin skin test (TST) and Interferon-γ release assays, cannot distinguish between latent TB infection (LTBI) and active TB disease, and have poor sensitivity in children and immune-compromised individuals including HIV-coinfected TB patients [9]. Therefore, identification of biomarkers which classify clinical stages of TB and monitor TB treatment responses in HIV-coinfected individuals is essential for improving clinical practice.

The interaction between the host immune response and *Mtb* has major roles in determining (i) clinical outcome following infection with *Mtb* and (ii) whether TB drug treatment will result in cure or treatment failure. Changes in gene expression patterns in blood resulting from the elicited immune responses could potentially be used as biomarkers to classify the different clinical outcomes of exposure to *Mtb* [10], including active disease or latent

infection, and for monitoring TB treatment responses [11-13]. However, most studies have been conducted in HIV-uninfected subjects with only very few studies showing that blood gene expression profiles can distinguish active TB from LTBI in HIV-coinfected individuals [14, 15]. Importantly, the combined effect of TB treatment and highly active anti-retroviral therapy (HAART) on host gene expression in TB-HIV-coinfected individuals has never been studied. Therefore, in this study we aimed to identify candidate host gene biomarkers that classify active TB disease in HIV-coinfected individuals, and that can be used to monitor TB treatment responses in TB-HIV patients, including those taking HAART, using focused gene expression profiling by dual-color Reverse-Transcription Multiplex Ligation-dependent Probe Amplification (dcRT-MLPA).

Materials and Methods

Ethics statement

All study participants provided written, informed consent at enrollment. Ethical clearance was obtained from the Scientific and Ethics Research Office (Ref:EHNRI 6.13/01), the Ethiopian Public Health Research Institute, and the London School of Hygiene & Tropical Medicine Ethics Review Committee (Ref:7174).

Study design and population

A longitudinal cohort study was conducted at St. Peter Specialized TB Hospital, Akaki and Kality Health Centers, Addis Ababa, Ethiopia between April 2007 and January 2011. A total of 131 adults of both sexes ranging between 15-65 years old were enrolled. Participants were subdivided into three clinical study groups at enrollment and followed up at 6 (M6) and 18 (M18) months: 1) HIV-infected active TB patients (HIV+TB+, n=48) of whom 18 were on ATT and 13 were on ATT-plus-HAART at M6; 2) HIV-infected TST positive subjects (HIV+TST+, n=37) of whom 20 were on HAART and 14 were naïve for HAART at M6; 3) HIV-infected TST negative subjects (HIV+TST-, n=46) of whom 26 were on

HAART and 16 were naïve for HAART at M6 (Supplementary Figure 1). Patients receiving HAART for <3 months or were lost during follow-up at M6 were excluded from the analysis.

At recruitment, study participants were interviewed using a standard questionnaire and demographic data were collected. Exclusion criteria for enrollment were: refusal of HIV testing, pregnancy, co-morbidity with diabetes mellitus or chronic bronchitis, receiving steroid therapy, receiving TB and/or HAART treatment (at recruitment or previously), and alcohol or drug abuse that could compromise reliability. The initiation of HAART treatment at baseline or during follow-up visits was determined by the physician at the health center using the ART treatment national guidelines [16] including immunological criteria (CD4 count<200 cells/µl). HAART was provided free of charge to eligible patients. All active TB cases confirmed at enrollment were treated according to the national guideline [17].

Diagnostic assessment

The HIV status was determined using the Determine HIV-½ (Abbott laboratories, Japan) screening test, the Capilus HIV-½ (TrinityBiotec, Ireland) confirmatory test and Unigold HIV-½ recombinant (TrinityBiotec, Ireland) as a tie breaker test [17]. The CD4 count was determined by flow cytometry using a FACS Calibur (Becton Dickinson, USA) while plasma HIV RNA viral load was determined using the NucliSensEasyQ NASBA diagnostic kit (OrganonTeknica, The Netherlands: detection range 50-3,000,000 copies/ml).

Active TB diagnosis was based on both clinical and bacteriological parameters. At least two sputum smears ("spot-early morning") were required to be positive by microscopy for Acid Fast Bacilli (AFB) using the Ziehl-Neelsen staining method (independent of the presence/absence of clinical parameters). There were no patients with dry cough in this study [17]. LTBI was determined by TST at baseline and follow-up visits for all participants except active TB patients according to the national guidelines [17]. 0.1ml tuberculin solution (RT23, State Serum Institute, Copenhagen) was injected intradermally into the dorsal surface of the forearm: TST-positivity was classified as skin induration diameter ≥5mm in HIV-infected individuals [17].

RNA Extraction

2.5ml venous blood was collected into PAXgene Blood RNA tubes (PreAnalytiX, Qiagen, Germany). RNA was extracted using the PAXgene Blood RNA extraction kit (PreAnalytiX, Qiagen) according to the manufacturer's instructions. Briefly, PAXgene tubes were centrifuged at 1800g/10 minutes, pellets were lysed, resuspended, and treated with proteinase K to remove contaminating proteins. Ethanol-precipitated nucleic acids were loaded onto spin columns, and DNA was digested using on-column RNase-free DNase (Qiagen). Purified RNA was eluted with RNase-free buffer (BR5 buffer) and quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). RNA samples with absorbance 260/280nm ratios <1.70 or >2.3 were excluded from further analyses.

Dual-color Reverse-Transcription Multiplex Ligation-dependent Probe Amplification (dcRT-MLPA)

DcRT-MLPA was performed as previously described [12]. Briefly, for each target-specific sequence, a specific RT primer was designed located immediately downstream of the left and right hand half-probe target sequence. RNA was reverse transcribed using RT-primer mix and MMLV reverse transcriptase (Promega, USA). Transcriptase activity was inactivated by heating at 98^{o}C/2 minutes. Following reverse transcription, left and right hand half-probes were hybridized to the cDNA at 60°C overnight. Annealed half-probes were ligated using ligase 65 and subsequently amplified by PCR (33 cycles of 30s/95°C, 30s/58°C, 60s/72°C, followed by 1 cycle of 20 min/72°C). Primers and probes were from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands) and MLPA reagents from MRC-Holland (Amsterdam, The Netherlands). PCR amplification products were 1:10 diluted in HiDi-formamide containing 400HD ROX size standard, denatured at 95^{o}C/5 min, ice-cooled and analyzed on an Applied Biosystems 3730 capillary sequencer in GeneScan mode (BaseClear, Leiden, The Netherlands).

Trace data were analyzed using GeneMapper software 5 (Applied Biosystems). The areas of each assigned peak (arbitrary units) were exported for analysis in Microsoft Excel. Data were normalized to GAPDH and signals below the threshold value for noise cutoff in GeneMapper (log2 transformed peak area 7.64) were assigned the threshold value for noise cutoff. Finally, the normalized data were log2 transformed for statistical analysis.

RT primers and half-probes were designed by Leiden University Medical Centre (LUMC, Leiden, The Netherlands) [12, 18] and comprised sequences for 4 housekeeping genes and 105 selected genes to profile the innate and adaptive immune responses (Supplementary Table 1).

Statistical analysis

A non-parametric Kruskal-Wallis test was performed to compare data between more than two clinical groups, while a two-tailed Wilcoxon rank-sum (Mann-Whitney) test for unpaired data or a Wilcoxon signed-rank test for paired data was performed to compare data between two clinical groups. A Chi^2 test was used to compare the different proportions of dichotomous variables amongst the groups. All data analysis was done using Inter cooled STATA version 11.0 (College Station, Texas, USA). The statistical significance cut-off level was $p<0.05$.

Results

Characteristics of the study population at baseline.

Altogether, 131 study participants categorized into 48 HIV+TB+ (active TB cases), 37 HIV+TST+ (LTBI individuals) and 46 HIV+TST- (TB uninfected controls), were included. Baseline demographic, clinical and laboratory data of each study group is shown in Supplementary Tables 2A and 2B. The mean age (±S.D.) of HIV+TB+, HIV+TST+ and HIV+TST- subjects was 32.6±7.8, 34.2±8.02 and 32.8±6.8 respectively while 50% of HIV+TB+, 61% of HIV+TST+ and 72% of HIV+TST- subjects were female.

Statistically significant differences between groups were observed in the proportion of patients with malnutrition (Body Mass Index (BMI) <18.50 kg/m^2) [19] and HIV-1 viral load (Supplementary Table 2A). HIV+TB+ patients presented with lower BMI (p=0.0001 and 0.0013), lower CD4+ T-cell counts (p=0.0335 and 0.0185), and

higher HIV RNA levels (p=0.028 and 0.046) than HIV+TST+ and HIV+TST- subjects, respectively, whereas no significant differences in these parameters were observed between HIV+TST+ and HIV+TST- individuals.

Gene expression profiles of clinical stages of TB in HIV-infected individuals.

Whole blood gene expression profiles of 48 HIV+TB+, 37 HIV+TST+ and 46 HIV+TST- individuals were analyzed by dcRT-MLPA using probe sets for 105 selected genes to profile innate and adaptive immune responses (Supplementary Table 1). 36 out of 105 genes examined were differentially expressed between the three clinical groups and 26 of these genes discriminated between active disease (HIV+TB+) and latent infection (HIV+TST+) in HIV-coinfected individuals (Table 1). 21 genes were significantly more highly expressed during active disease than latent infection (*PTPRCv2, IL1B, IL15, IL4δ2, IL9, IL22RA1, TGFB1, FLCN1, TNFRSF1A, FPR1, CLEC7A, NLRC4, NLRP12, MMP9, LTF, TLR1, TLR2, TLR4, TLR8, FCGR1A* and *RAB24*), while five genes (*CD4, PTPRCv1, TLR3, BLR1* and *ZNF331*) exhibited significantly lower expression in HIV+TB+ than HIV+TST+ subjects. The lower levels of *CD4* transcripts in HIV+TB+ patients compared to HIV+TST+ (and also HIV+TST-) individuals is consistent with the lower CD4+ T-cell counts observed in active TB cases (Supplementary Table 2A).

With the exception of *TGFB1*, all other 35 genes were differentially expressed between HIV-infected TB cases and HIV-infected TST- controls. 24 genes had significantly higher expression in HIV+TB+ than HIV+TST- controls (*IL15, IL22RA1, FPR1, PTPRCv2, IL1B, IL4δ2, IL9, FLCN1, TNFRSF1A, CLEC7A, NLRC4, NLRP12, MMP9, LTF, TLR1, TLR2, TLR4, TLR8, FCGR1A, RAB24, TLR6, CCL19, SPP1* and *TIMP2*), whereas 11 genes had significantly lower expression in TB cases compared to uninfected controls (*CD3E, CD4, IL7R, PTPRCv1, TBX21, GZMB, GNLY, CCL5, TLR3, BLR1* and *ZNF331*). 25 of the 35 genes that were differentially expressed between active TB cases and uninfected controls also discriminated between active TB cases and LTBIs (*PTPRCv2, IL1B, IL15, IL4δ2, IL9, IL22RA1, FLCN1, TNFRSF1A, FPR1, CLEC7A, NLRC4, NLRP12, MMP9, LTF, TLR1, TLR2, TLR4, TLR8, FCGR1A, RAB24, CD4, PTPRCv1, TLR3, BLR1* and *ZNF331*) (Table 1), suggesting that these biomarkers are strongly associated with TB disease in HIV-coinfected individuals.

As expected, only a limited number of genes distinguished between LTBIs and TST- controls in HIV-infected individuals (Table 1), in line with our previous studies in HIV-uninfected individuals [12]. From the 10 genes that were differentially expressed, 4 genes (*IL15, FPR1, TLR6* and *SPP1*) had significantly higher expression in HIV+TST+ compared to HIV+TST- individuals, whereas 6 genes (*CD3E, IL7R, TGFB1, GNLY, GZMB* and *CCL5*) had significantly lower expression.

Non-parametric Receiver Operator Characteristic (ROC) curves to determine the discriminatory potential of single genes identified *TGFB1, FCGR1A, RAB24, PTPRCv2, TLR1, TLR4, MMP9, NLRC4* and *IL1B,* with Area Under the Curve (AUCs) of 1.00, 0.81, 0.79, 0.79, 0.77, 0.76, 0.75, 0.75 and 0.75 respectively, as genes with the most powerful classifying potential to discriminate between active TB disease and latent TB infection in HIV-coinfected individuals (Figure 1A). The expression profiles of these signature genes are displayed in Figure 2. Genes that could best classify HIV+TB+ and HIV+TST- were *FCGR1A, NLRC4, TLR1, RAB24, TLR4, TLR2, MMP9, FPR1* and *IL1B,* with AUCs of 0.90, 0.82, 0.81, 0.80, 0.80, 0.78, 0.76, 0.75 and 0.75 respectively (Figure 1B). Transcriptomic profiles of genes that markedly classified HIV+TB+ and HIV+TST- individuals are displayed in Figure 2. Seven of nine single genes that could discriminate between active TB cases and uninfected controls also discriminated between active TB cases and LTBIs, again suggesting these coinciding biomarkers are strongly associated with TB disease in HIV-coinfected individuals. Genes that were differentially expressed between LTBIs and TST- controls in HIV-infected individuals had limited classifying value (AUCs of <0.70), as expected.

Transcriptomic profiles of anti-TB treatment (ATT) responses in HIV-coinfected TB patients are independent of HAART therapy.

To determine if (in)eligibility for HAART might be a confounding parameter in the analysis of TB treatment responses, study groups (HIV+TB+, HIV+TST+, HIV+TST-) were stratified at baseline as either eligible or ineligible for HAART, and gene expression profiles were compared (Table 2A). Clearly, very few genes were found to be differentially expressed within each study group when individuals were stratified based on in(eligibility) for HAART.

From the 36 genes that were differentially expressed among the study groups before stratification, only 1 gene (*IL4δ2*) was differentially expressed between HAART-eligible and -ineligible HIV+TB+, 5 genes (*TNFRSF1A, TLR4, NLRC4, LTF* and *BLR1*) were differentially expressed between HAART-eligible and -ineligible HIV+TST+ and 3 genes (*IL7R, GZMB* and *TLR6*) were differentially expressed between HAART-eligible and -ineligible HIV+TST-. Together, these data indicate that stratification based on in(eligibility) for HAART is not a dominant confounding factor in the analysis of TB treatment responses.

Similarly, following 6 months of ATT and/or HAART therapy, only a limited number of genes was found to be differentially expressed between ATT and ATT-plus-HAART treated HIV+TB+ patients, HAART treated and untreated HIV+TST+ subjects, and HAART treated and untreated HIV+TST- individuals (Table 2B). From the 36 genes that were found to be differentially expressed among the study groups before stratification based on HAART in(eligibility), only 2 genes (*TGFB1* and *BLR1*) were differentially expressed between ATT and ATT-plus-HAART treated HIV+TB+ patients, 2 genes (*GNLY* and *MMP9*) were differentially expressed between HAART treated and untreated HIV+TST+ subjects and 4 genes (*IL7R, CCL19, TLR6* and *MMP9*) were differentially expressed between HAART treated and untreated HIV+TST- individuals, suggesting that treatment with HAART merely affects the direct *ex vivo* RNA expression profile in peripheral blood of HIV-infected individuals.

Importantly, while baseline biomarker profiles were identified in HIV-infected individuals that markedly discriminated between active TB cases versus LTBIs and uninfected controls (Table 1), longitudinal follow-up analysis showed that biomarker profiles of treated TB patients became indistinguishable from those of LTBIs and uninfected controls at the end of 6 months ATT therapy. This outcome was independent of HAART (in)eligibility and HAART treatment (Tables 3A and B and Supplementary Figure 2). In summary, these data show that transcriptomic profiles of ATT treatment responses in HIV-coinfected TB patients are independent of HAART therapy and normalize to levels observed in latently TB-infected and uninfected controls after completion of ATT.

Discussion

Identification of biomarkers that can discriminate between active and latent TB is essential for controlling the spread of TB worldwide, especially in HIV-infected subjects. Furthermore, biomarkers that can monitor TB treatment responses and evaluate ATT efficacy could potentially reduce *de* novo drug resistance development [20]. Here, we used a focused gene expression profiling platform, dcRT-MLPA [12], targeting innate and adaptive immune response genes, to analyze RNA expression levels of 105 pre-selected genes in peripheral blood of HIV-infected individuals and identified biomarkers with excellent discriminatory capacity. At baseline, expression levels of 7 single genes (*FCGR1A, RAB24, TLR1, TLR4, MMP9, NLRC4* and *IL1B*) could accurately discriminate between active TB versus both LTBIs and uninfected controls with AUCs ≥75, indicating these interrelating biomarkers are strongly associated with TB disease in HIV-coinfected individuals. In contrast, all genes that were differentially expressed between LTBIs and uninfected controls had limited classifying value (AUC<70), in accordance with previous studies in HIV-uninfected populations [12].

The observed higher expression of immune-related genes in HIV-infected individuals with active TB may be due to an increased load of bacterial antigens from actively replicating bacilli, triggering a vigorous host immune response. In contrast, lower expression of T-cell associated genes (e.g. *CD4* and *PTPRCv1*) in these TB patients may reflect HIV-related cell depletion and/or cell migration to the lungs as the primary infection site. This peripheral T-cell depletion may be one underlying mechanism by which TB disease aggravates HIV-infection progression [21].

TGFB1 expression was significantly higher in HIV-infected participants with active TB disease compared to latent TB infection, in contrast to previous reports in HIV-uninfected participants in South Africa and Malawi [15]. TGFB1 is involved in the induction of fibrosis, a hallmark presentation of tuberculosis disease, and excessive TGFB1 during tuberculosis may enhance viral activity, thereby accelerating HIV disease [22]. Concentrations of TGFB1 cytokine were about twofold higher in PPD-induced culture supernatants of HIV-infected patients with tuberculosis [23]. Therefore, elevation of *TGFB1* gene expression levels in TB-HIV patients may result in enhanced T-cell suppression and HIV as well as TB propagation.

Within our HIV-infected cohort, *FCGR1A* was amongst the strongest differentially expressed genes, with marked discriminatory power between active TB cases versus both LTBIs and uninfected controls. This is consistent with gene expression profiles of PBMCs and whole blood from TB patients and healthy donors in HIV-uninfected populations of different ethnic backgrounds [24-26]. Moreover, Sutherland *et.al*. recently reported transcript levels of *FCGR1A* as the most reliable classifier of active TB compared to latent TB and *Mtb*-uninfected controls regardless of HIV status and genetic background [15]. FCGR1A is an essential component of interferon signaling and plays a central role in endocytosis, phagocytosis, antibody-dependent cellular toxicity, cytokine release, and superoxide generation [27] but may participate in TB pathogenesis.

Transcript levels of *TLR1, TLR2, TLR4, RAB24* and *MMP9* were also higher in HIV+TB+ patients compared to HIV+TST+ and HIV+TST- subjects, in agreement with published data in HIV-uninfected subjects [28, 29], suggesting a biomarker signature encompassing these genes and also including *FCGR1A* may be useful in classifying different clinical stages of TB both in HIV-uninfected and HIV-infected individuals. Although these genes display comparable expression patterns between study groups and similar expression kinetics during ATT therapy, their functional roles during TB infection is diverse. RAB24 is involved in intracellular trafficking [30] and the increase in RAB24 expression in TB-infected monocytes has been correlated to increased autophagic activity [29]. Pattern recognition receptors are the first line of recognition of *Mtb* bacilli and have a critical role in initiating innate and subsequent adaptive immune responses. TLR1 and TLR2 play an important role in host defense against mycobacteria, especially by mediating responses to mycobacterial triacylated lipopeptides [31]. In addition, the ability of *Mtb* to inhibit major histocompatibility complex Class II expression and antigen presentation through stimulation of TLR2 and TLR1 creates a niche for survival of *Mtb* in macrophages and promotes its evasion of recognition by CD4+ T-cells [28]. Finally, higher expression levels of MMP9 have been shown to correlate with dysregulation of cellular apoptosis in HIV-TB-coinfected patients [32] and with early dissemination of *Mtb* followed by recruitment of macrophages, induction of Th1-type immunity and granuloma formation [33].

The LTBI patients recruited into this study likely reflect a heterogeneous group of individuals, including some with quiescent *Mtb* infection and others with asymptomatic subclinical disease [34]: future work could test whether

the gene expression signatures are able to discriminate these subgroups. Additionally, the current study only included microbiologically confirmed active TB cases: future work could determine the utility of the gene expression in a wider range of clinically suspected TB.

Most genes that were differentially expressed at baseline, between HIV-infected subjects with active TB versus those with latent TB infection or uninfected controls, normalized during ATT treatment of (HAART-treated) TB patients to levels observed in (HAART-treated) LTBIs and uninfected control subjects. Normalization of gene expression profiles in TB patients during 6 months anti-TB chemotherapy has previously been reported in HIV-uninfected TB patients [10, 35, 36] and correlates with clinical cure [11].

Interestingly, only a few genes were differentially expressed at baseline between study groups eligible and ineligible for HAART, excluding (in)eligibility for HAART as a confounding factor when analyzing the kinetic response of TB treatment at the transcriptomic level. Moreover, HAART therapy of eligible HIV+TB+, HIV+TST+, and HIV+TST- subjects only marginally affected transcriptomic profiles. This suggests that a similar biomarker signature can be used to monitor ATT treatment efficacy in TB-HIV patients both eligible and ineligible for HAART.

In conclusion, the expression of several single genes (*FCGR1A, RAB24, TLR1, TLR4, MMP9, NLRC4* and *IL1B*) in peripheral blood can discriminate active TB from latent TB infection and uninfected controls in HIV-infected individuals. These biomarkers can also be used to monitor TB treatment responses in HIV-infected individuals independent of HAART (in)eligibility and therapy. Potentially, in the future, the identified biomarkers could be applied to facilitate evaluation of new tuberculosis chemotherapeutic regimens and for vaccines trials, regardless of HAART.

Figure legends

Figure 1. Identification of single genes with discriminatory power to classify study groups.

Receiver operator characteristics (ROC) curves showing the accuracies of individual genes in discriminating (A) HIV+TB+ versus HIV+TST+ subjects and (B) HIV+TB+ versus HIV+TST- subjects. AUC = Area under the curve.

Figure 2. Gene expression profiles of signature genes.

Median gene expression levels (peak areas normalized to GAPDH and log2-transformed) of the indicated genes are shown as box-and-whisker plots (5-95 percentiles). Significant differences among the groups and between study groups were determined using Kruskal-Wallis H test and Wilcoxon Mann-Whitney test respectively. Shown are individual genes that were found to have the best discriminatory power to distinguish between active TB cases (HIV+TB+) versus latently infected (HIV+TST+) and uninfected (HIV+TST-) controls. (* = P-value ≤0.05, ** = P-value ≤0.01, *** = P-value ≤0.001, **** = P-value ≤0.0001).

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Table 1. Gene expression profiles differentiating between study groups at baseline (Month 0).

Gene Symbol	HIV+TB+ (n=47)	HIV+TST+ (n=37)	HIV+TST- (n=46)	HIV+TB+ vs HIV+TST+ P-value	HIV+TB+ vs HIV+TST- P-value	HIV+TST+ vs HIV+TST- P-value
T cell subset markers						
CD3E	13.0 (11.8-13.5)	13.2 (12.1-13.6)	13.6 (12.6-13.9)	0.2324	**0.0015**	**0.0232**
CD4	11.4 (10.5-12.1)	11.8 (11.2-12.6)	12.1 (11.6-12.5)	**0.0025**	**0.0002**	0.8940
IL7R	11.7 (10.6-12.2)	12.0 (11.1-12.7)	12.3 (11.7-13.3)	0.2137	**0.0017**	**0.0373**
PTPRCv1	10.5 (9.8-11.3)	11.0 (10.5-11.5)	11.4 (10.9-11.9)	**0.0285**	**0.0005**	0.1213
PTPRCv2	13.3 (12.8-13.6)	12.4 (11.9-12.9)	12.8 (12.2-13.0)	**0.0000**	**0.0002**	0.2776
Th1/2/9/17 associated genes/Treg associated genes						
IL1B	11.4 (10.9-11.8)	10.5 (9.9-11.4)	10.5 (9.7-11.1)	**0.0002**	**0.0000**	0.6785
IL15	7.9 (7.6-8.3)	7.6 (7.6-8.0)	7.6 (7.6-7.6)	**0.0326**	**0.0000**	**0.0290**
TBX21	7.6 (7.6-7.6)	7.6 (7.6-7.6)	7.6 (7.6-8.1)	0.0971	**0.0011**	0.0976
IL4δ2	7.6 (7.6-11.2)	7.6 (7.6-7.6)	7.6 (7.6-7.6)	**0.0136**	**0.0004**	0.2313
IL9	9.9 (9.5-10.4)	9.5 (8.6-10.0)	9.5 (8.9-9.9)	**0.0041**	**0.0004**	0.8087
IL22RA1	7.7 (7.6-8.5)	7.6 (7.6-7.6)	7.6 (7.6-7.6)	**0.0027**	**0.0002**	0.6352
TGFB1	14.4 (14.2-14.6)	11.6 (11.1-12.1)	14.3 (14.1-14.5)	**0.0000**	0.4134	**0.0000**
Cytotoxicity genes						
GNLY	13.9 (12.6-14.3)	13.7 (13.1-14.3)	14.5 (13.4-15.2)	0.9685	**0.0032**	**0.0023**
GZMB	12.7 (12.2-13.0)	12.6 (11.8-13.0)	13.1 (12.3-13.7)	0.7003	**0.0208**	**0.0097**
Apoptosis/survival						
FLCN1	7.7 (7.6-8.3)	7.6 (7.6-7.6)	7.6 (7.6-7.8)	**0.0037**	**0.0306**	0.3708
TNFRSF1A	13.3 (13.1-13.8)	12.7 (12.0-13.4)	12.8 (12.2-13.3)	**0.0024**	**0.0002**	0.9253
Myeloid associated genes						
CCL5	14.6 (14.0-14.9)	14.7 (14.3-14.9)	15.0 (14.4-15.3)	0.2865	**0.0050**	**0.0330**
FPR1	13.9 (13.3-14.4)	13.4 (12.6-14.0)	12.7 (12.2-13.7)	**0.0220**	**0.0001**	**0.0401**
Chemokines						
CCL19	8.8 (8.0-9.2)	8.6 (8.2-9.0)	8.1 (7.6-8.8)	0.3645	**0.0158**	0.0992
Pattern recognition receptors						
CLEC7A	12.9 (12.3-13.4)	12.4 (11.7-12.9)	12.2 (11.6-12.7)	**0.0041**	**0.0001**	0.4127
TLR1	12.0 (11.3-12.3)	11.1 (9.9-11.6)	10.9 (10.0-11.3)	**0.0000**	**0.0000**	0.4592
TLR2	10.0 (9.3-10.6)	9.0 (8.0-10.0)	9.0 (8.4-9.3)	**0.0005**	**0.0000**	0.7898
TLR3	10.3 (9.7-10.8)	10.9 (10.0-11.3)	11.0 (9.9-11.4)	**0.0199**	**0.0212**	0.6497
TLR4	10.6 (10.0-11.0)	9.8 (9.0-10.4)	9.6 (8.9-10.2)	**0.0001**	**0.0000**	0.5439
TLR6	10.1 (9.6-10.4)	9.8 (9.5-10.2)	9.6 (9.1-9.9)	0.1524	**0.0012**	**0.0373**
TLR8	11.7 (10.4-12.3)	10.7 (8.5-11.5)	10.7 (10.0-11.1)	**0.0017**	**0.0011**	0.7779
Inflammasome components						
NLRC4	9.8 (9.5-10.2)	9.2 (8.8- 9.5)	9.0 (8.8-9.4)	**0.0001**	**0.0000**	0.3587
NLRP12	8.2 (7.9-8.6)	7.8 (7.6-8.3)	7.9 (7.6-8.4)	**0.0158**	**0.0248**	0.8823
IFN signalling genes						
FCGR1A	11.4 (10.8-11.8)	9.9 (8.5-10.8)	9.2 (8.7-9.8)	**0.0000**	**0.0000**	0.0554
Inflammation						

MMP9	10.5 (9.2-11.3)	9.2 (7.6-9.8)	8.3 (7.6-9.7)	**0.0001**	**0.0000**	0.6271
SPP1	7.6 (7.6-8.1)	7.6 (7.6-7.8)	7.6 (7.6-7.6)	0.4728	**0.0038**	**0.0442**
TIMP2	14.2 (13.5-15.0)	14.1 (13.3-14.5)	13.5 (12.8-14.4)	0.1414	**0.0045**	0.2865
Other						
RAB24	11.6 (11.2-12.0)	10.7 (9.9-11.3)	10.6 (9.9-11.1)	**0.0000**	**0.0000**	0.8051
LTF	9.2 (8.3-10.4)	7.9 (7.6-9.0)	7.9 (7.6-9.5)	**0.0015**	**0.0058**	0.7537
ZNF331	7.6 (7.6-8.7)	8.5 (7.6-9.5)	8.5 (7.6-9.3)	**0.0185**	**0.0306**	0.7857
BLR1	9.5 (9.0-9.9)	10.0 (9.6-10.7)	10.2 (9.5-10.8)	**0.0001**	**0.0002**	0.9764

Median (inter quartile range) gene expression values (peak areas normalized for GAPDH and log2-transformed) are shown at baseline and significant differences between study groups were determined using Kruskal-Wallis H and Wilcoxon Mann-Whitney test. In pink: genes are indicated that were more highly expressed in the test group compared to the reference/control group. In blue: genes are indicated that had lower expression in the test group compared to the reference/control group. Only genes whose expression level significantly differed between any of the study groups are listed.

Table 2A. Baseline (Month 0) gene expression profiles after stratification by HAART (in)eligibility.

Gene Symbol	HIV+TB+ HAART- (n=18)	HIV+TB+ HAART+ (n=13)	HIV+TB+ HAART- vs HIV+TB+ HAART+ P-value	HIV+TST+ HAART- (n=14)	HIV+TST+ HAART+ (n=20)	HIV+TST+ HAART- vs HIV+TST+ HAART+ P-value	HIV+TST- HAART- (n=16)	HIV+TST- HAART+ (n=26)	HIV+TST- HAART- vs HIV+TST- HAART+ P-value
T cell subset markers									
CD3E	13.0 (12.5-13.4)	13.0 (12.3-13.7)	0.9833	13.2 (12.1-13.6)	13.3 (12.4-13.7)	0.7021	13.8 (13.6-13.8)	13.2 (12.5-13.9)	0.3635
CD4	11.5 (10.8-12.0)	11.7 (10.7-12.2)	0.5994	11.9 (11.0-13.0)	12.3 (11.5-12.5)	0.9419	12.2 (11.9-12.7)	12.1 (11.4-12.3)	0.3227
IL7R	11.9 (10.9-12.2)	11.6 (10.8-12.3)	0.7220	12.1 (11.4-12.9)	11.8 (10.6-12.7)	0.4663	13.2 (12.2-13.5)	11.7 (10.8-12.5)	**0.0006**
PTPRCv1	10.1 (9.8-11.5)	10.9 (10.5-11.4)	0.2330	11.0 (10.5-11.5)	11.0 (10.5-11.8)	0.6887	11.6 (11.1-12.2)	11.2 (10.8-11.7)	0.2042
PTPRCv2	13.4 (13.0-13.7)	13.3 (12.9-13.4)	0.5165	12.1 (11.1-12.7)	12.5 (12.0-12.9)	0.1173	12.9 (12.7-13.2)	12.7 (11.8-13.1)	0.1606
Th1/2/9/17 associated genes/Treg associated genes									
IL1B	11.6 (11.2-11.8)	11.3 (11.0-11.5)	0.2170	10.0 (9.6-11.3)	10.8 (10.2-11.5)	0.1663	10.5 (9.9-11.1)	10.2 (9.7-11.4)	0.6885
IL15	7.8 (7.6-8.2)	7.9 (7.6-8.3)	0.6467	7.6 (7.6-7.8)	7.6 (7.6-8.1)	0.3317	7.6 (7.6-7.6)	7.6 (7.6-7.6)	0.8620
TBX21	7.6 (7.6-7.6)	7.6 (7.6-7.6)	0.4452	7.6 (7.6-7.6)	7.6 (7.6-8.1)	0.4977	7.6 (7.6-7.8)	7.6 (7.6-8.2)	0.3015
IL4δ2	10.5 (7.6-12.1)	7.6 (7.6-7.6)	**0.0311**	7.6 (7.6-7.6)	7.6 (7.6-7.6)	0.8585	7.6 (7.6-7.6)	7.6 (7.6-7.6)	0.8824
IL9	10.3 (9.6-10.5)	10.0 (9.7-10.2)	0.4388	9.5 (8.4-10.0)	9.6 (9.2-10.2)	0.3816	9.5 (8.7-9.7)	9.5 (9.1-10.2)	0.1378
IL22RA1	7.8 (7.6-9.0)	7.6 (7.6-7.9)	0.2097	7.6 (7.9-7.6)	7.6 (7.6-7.6)	0.6260	7.6 (7.6-7.6)	7.6 (7.6-7.6)	0.5428
TGFB1	14.4 (14.2-14.5)	14.5 (14.4-14.7)	0.2170	11.4 (9.2-12.0)	11.6 (11.4-12.1)	0.3721	14.2 (14.1-14.6)	14.3 (14.0-14.5)	0.9787
Cytotoxicity genes									
GNLY	14.0 (12.8-14.3)	13.9 (13.5-14.3)	0.4898	13.3 (13.0-14.1)	14.0 (13.7-14.4)	0.0492	14.5 (14.2-15.2)	14.4 (13.2-15.2)	0.4383
GZMB	12.6 (12.1-13.0)	12.8 (12.6-13.0)	0.1738	12.7 (11.8-13.2)	12.7 (12.2-13.0)	0.8842	12.6 (11.8-13.4)	13.4 (12.6-13.8)	**0.0111**
Apoptosis/survival									
FLCN1	7.6 (7.6-8.2)	8.2 (7.8-8.5)	0.1003	7.6 (7.6-7.6)	7.6 (7.6-7.7)	0.5611	7.6 (7.6-8.0)	7.6 (7.6-7.7)	0.9098
TNFRSF1A	13.4 (13.2-13.8)	13.4 (13.1-13.7)	0.8506	12.2 (11.9-12.9)	13.2 (12.5-13.4)	**0.0414**	12.8 (12.4-13.2)	12.7 (12.2-13.2)	0.4383
Myeloid associated genes									
CCL5	14.6 (14.0-14.8)	14.8 (14.6-15.0)	0.1874	14.7 (13.9-14.9)	14.7 (14.5-15.0)	0.3625	14.8 (14.2-15.1)	15.0 (14.7-15.4)	0.1211
FPR1	14.0 (13.2-14.4)	13.9 (13.6-14.4)	0.6909	12.7 (12.5-14.0)	13.4 (12.9-14.0)	0.2438	12.9 (12.4-13.5)	12.8 (12.2-13.8)	0.6885
Chemokines									
CCL19	8.9 (8.4-9.2)	8.3 (7.6-8.9)	0.1242	8.6 (8.2-9.1)	8.6 (7.6-9.0)	0.4868	7.9 (7.6-8.7)	8.2 (7.6-9.0)	0.2875
Pattern recognition receptors									
CLEC7A	13.1 (12.8-13.5)	12.9 (12.6-13.5)	0.4144	12.0 (10.6-13.0)	12.5 (12.1-12.9)	0.2293	12.1 (11.5-12.6)	12.3 (11.5-12.7)	0.7996
TLR1	12.0 (11.6-12.6)	12.0 (11.5-12.3)	0.8835	10.6 (9.0-11.4)	11.2 (10.8-11.6)	0.1172	10.9 (10.3-11.4)	10.9 (9.8-11.2)	0.7182
TLR2	10.1 (9.3-10.6)	9.9 (9.6-10.5)	0.6603	8.9 (7.6-9.4)	9.1 (8.3-10.2)	0.0935	8.8 (8.3-9.1)	9.1 (8.5-9.7)	0.1527
TLR3	10.3 (10.2-10.7)	10.2 (9.8-10.7)	0.5721	10.9 (10.4-11.4)	10.9 (9.8-11.3)	0.8555	11.0 (10.6-11.4)	10.8 (9.9-11.6)	0.5470
TLR4	10.4 (10.2-11.0)	10.6 (10.2-11.0)	0.9833	9.4 (8.3-10.1)	10.1 (9.3-10.4)	**0.0414**	9.8 (9.1-10.2)	9.5 (8.8-10.2)	0.6689
TLR6	9.9 (9.6-10.2)	10.1 (9.8-10.4)	0.7536	9.9 (9.5-10.3)	9.8 (9.5-10.2)	0.6620	9.8 (9.6-10.2)	9.4 (9.1-9.9)	**0.0304**
TLR8	11.8 (10.7-12.4)	11.4 (10.0-12.2)	0.4898	10.6 (7.6-11.0)	11.0 (10.2-11.8)	0.0933	10.9 (10.2-11.2)	10.4 (8.4-10.8)	0.0726

Inflammasome components									
NLRC4	9.9 (9.6-10.2)	9.8 (9.6-10.0)	0.3909	9.0 (8.8-9.2)	9.4 (9.0-9.8)	**0.0492**	9.2 (8.9-9.4)	9.1 (8.8-9.4)	0.4074
NLRP12	8.5 (8.0-8.7)	8.2 (8.0-8.6)	0.5860	7.7 (7.6-7.9)	8.1 (7.6-8.3)	0.0967	7.9 (7.6-8.4)	7.9 (7.6-8.3)	0.8341
IFN signalling genes									
FCGR1A	11.4 (10.8-11.8)	11.4 (11.0-11.9)	0.5165	9.7 (7.7-10.4)	9.9 (9.3-11.2)	0.2292	9.1 (8.2-9.4)	9.4 (8.9-10.4)	0.1521
Inflammation									
MMP9	10.9 (8.7-11.4)	10.2 (9.1-10.5)	0.2566	7.6 (7.6-9.3)	9.3 (7.7-9.8)	0.0620	8.9 (7.6-10.0)	8.0 (7.6-9.4)	0.4486
SPP1	7.6 (7.6-8.2)	7.6 (7.6-7.6)	0.3308	7.6 (7.6-7.9)	7.6 (7.6-7.8)	0.7103	7.6 (7.6-7.6)	7.6 (7.6-7.6)	1.0000
TIMP2	14.2 (13.7-14.9)	14.5 (13.9-15.3)	0.5165	13.3 (13.0-14.5)	14.2 (13.4-14.5)	0.1260	13.4 (12.8-14.1)	13.5 (13.0-14.8)	0.3778
Other									
RAB24	11.6 (10.8-12.0)	11.9 (11.5-12.0)	0.2170	10.2 (8.2-11.3)	10.7 (10.2-11.5)	0.1172	10.6 (10.3-11.0)	10.8 (9.9-11.2)	0.9148
LTF	9.1 (8.4-10.7)	8.8 (7.6-9.7)	0.3445	7.6 (7.6-7.7)	8.2 (7.6-9.9)	**0.0100**	7.6 (7.6-10.4)	8.1 (7.6-9.7)	0.3749
ZNF331	7.6 (7.6-9.1)	7.8 (7.6-8.0)	0.8737	9.3 (7.6-10.3)	7.9 (7.6-9.2)	0.0860	8.2 (7.6-9.1)	8.7 (7.6-9.3)	0.7538
BLR1	9.6 (9.3-9.9)	9.4 (9.1-10.1)	0.9499	10.3 (10.0-11.1)	9.8 (9.6-10.7)	**0.0414**	10.2 (9.7-10.8)	10.1 (9.5-10.6)	0.4871

Median (inter quartile range) gene expression values (peak areas normalized for GAPDH and log2-transformed) are shown at baseline. Significant differences within each study group after stratification by HAART (in)eligibility were determined using Wilcoxon Mann-Whitney test. In pink: genes are indicated that were more highly expressed in the HAART eligible group compared to the HAART ineligible group. In blue: genes are indicated that had lower expression in the HAART eligible group compared to the HAART ineligible group. Genes listed in this table were

Table 2B. Effect of HAART on the ATT induced treatment response transcriptomic profiles (Month 6).

Gene Symbol	HIV+TB+ HAART-	HIV+TB+ HAART+	HIV+TB+ HAART- vs	HIV+TST+ HAART-	HIV+TST+ HAART+	HIV+TST+ HAART- vs	HIV+TST- HAART-	HIV+TST- HAART+	HIV+TST- HAART- vs
T cell subset markers									
CD3E	14.1 (13.6-14.2)	14.1 (13.5-14.6)	0.5462	14.1 (13.7-14.4)	13.5 (13.2-14.2)	0.0987	14.2 (13.9-14.4)	14.0 (13.9-14.3)	0.2732
CD4	11.5 (10.9-12.0)	11.5 (11.0-12.2)	0.7452	11.8 (11.4-12.2)	11.4 (10.8-11.9)	0.1077	11.8 (11.4-12.3)	11.5 (10.7-12.0)	0.0872
IL7R	12.8 (11.9-13.3)	12.6 (11.9-13.5)	0.9630	13.2 (12.7-13.5)	12.7 (11.8-13.3)	0.2803	13.5 (13.2-13.7)	13.0 (12.6-13.5)	**0.0422**
PTPRCv1	11.3 (10.6-11.9)	12.0 (11.3-12.3)	0.0569	11.6 (10.9-12.2)	11.5 (10.8-12.4)	0.7995	12.0 (11.3-12.5)	11.4 (11.1-11.8)	0.0543
PTPRCv2	12.6 (11.9-13.0)	12.8 (12.6-12.9)	0.5459	13.0 (12.7-13.2)	12.6 (12.5-12.9)	0.1077	12.5 (12.0-13.0)	12.8 (12.6-13.1)	0.2189
Th1/2/9/17 associated genes/Treg associated genes									
IL1B	9.6 (9.2-10.5)	10.0 (9.6-10.5)	0.5459	10.3 (9.8-10.9)	10.2 (9.9-10.4)	0.8989	10.0 (9.8-10.3)	10.2 (9.6-10.7)	0.7484
IL15	7.6 (7.6-7.7)	7.6 (7.6-7.9)	0.4647	7.6 (7.6-8.1)	7.6 (7.6-7.6)	0.0972	7.6 (7.6-7.6)	7.6 (7.6-7.6)	0.3844
TBX21	7.6 (7.6-7.7)	7.7 (7.6-8.1)	0.1402	7.6 (7.6-8.0)	7.6 (7.6-7.6)	0.4035	7.6 (7.6-8.2)	7.6 (7.6-7.8)	0.9515
IL4δ2	9.7 (9.1-10.1)	8.9 (7.6-10.1)	0.3518	9.2 (7.8-10.0)	8.4 (7.6-9.9)	0.3698	9.1 (7.6-9.6)	9.3 (9.1-10.0)	0.1122
IL9	9.8 (9.2-10.2)	10.0 (9.3-10.2)	1.0000	9.9 (9.6-10.1)	9.4 (8.8-9.8)	0.0826	9.5 (8.8-9.8)	9.7 (9.4-9.8)	0.4073
IL22RA1	7.6 (7.6-8.0)	7.6 (7.6-7.9)	0.9784	7.7 (7.6-7.8)	7.7 (7.6-8.1)	0.5659	7.6 (7.6-7.8)	7.6 (7.6-7.8)	0.4293
TGFB1	14.4 (14.1-14.7)	14.7 (14.5-14.9)	**0.0326**	14.6 (14.4-14.7)	14.6 (14.5-14.7)	0.8324	14.7 (14.5-14.8)	14.6 (14.4-14.8)	0.5930
Cytotoxicity genes									
GNLY	15.1 (14.2-15.7)	15.3 (14.7-15.5)	0.7805	13.6 (10.1-14.3)	14.8 (13.7-15.0)	**0.0132**	14.9 (14.6-15.7)	15.1 (14.5-15.3)	0.7283
GZMB	13.1 (11.9-13.8)	13.7 (13.3-14.0)	0.0776	12.8 (12.4-13.4)	12.6 (12.2-13.1)	0.4717	12.8 (12.5-13.5)	13.1 (12.7-13.6)	0.5212
Apoptosis/survival									
FLCN1	7.6 (7.6-7.6)	7.6 (7.6-7.9)	0.2444	7.6 (7.6-8.2)	7.6 (7.6-7.9)	0.9049	7.6 (7.6-7.8)	7.6 (7.6-7.7)	0.6273
TNFRSF1A	13.5 (13.3-13.8)	13.5 (13.4-13.8)	0.9260	13.5 (13.2-13.8)	13.5 (13.4-13.7)	0.8324	13.3 (13.1-13.7)	13.7 (13.2-13.9)	0.5481
Myeloid associated genes									
CCL5	14.5 (13.6-15.0)	15.2 (14.7-15.5)	0.0511	14.9 (14.3-15.2)	14.8 (14.1-15.2)	0.8989	14.8 (14.6-15.2)	15.0 (14.6-15.3)	0.3635
FPR1	13.8 (13.6-14.0)	13.8 (12.7-14.3)	1.0000	13.4 (13.0-14.4)	13.7 (13.1-13.9)	0.7995	13.8 (13.0-14.1)	13.6 (13.1-14.0)	0.6885
Chemokines									
CCL19	9.5 (9.3-9.8)	9.6 (9.4-10.0)	0.2185	9.5 (9.3-9.9)	9.5 (9.3-9.7)	0.5534	9.7 (9.5-9.9)	9.5 (9.3-9.7)	**0.0325**
Pattern recognition receptors									
CLEC7A	12.6 (11.9-13.0)	12.2 (12.0-12.5)	0.1499	12.6 (12.3-13.1)	12.3 (12.1-12.6)	0.0987	12.4 (12.1-12.6)	12.1 (11.9-12.7)	0.2850
TLR1	11.1 (9.6-11.5)	11.1 (10.8-11.2)	0.8526	11.2 (10.7-11.8)	10.9 (10.7-11.0)	0.3096	10.7 (10.2-11.1)	10.7 (10.4-11.4)	0.4074
TLR2	9.0 (8.2-9.7)	9.1 (8.6-9.6)	0.9629	9.0 (8.1-9.7)	9.3 (9.0-9.8)	0.3302	9.2 (8.7-9.4)	9.2 (8.7-9.7)	0.4150
TLR3	9.8 (8.9-10.6)	9.7 (9.6-10.1)	0.9260	10.1 (9.2-10.8)	10.1 (9.8-10.8)	0.8324	10.2 (9.8-10.6)	10.3 (9.9-10.6)	0.8726
TLR4	9.9 (8.8-10.3)	10.1 (9.5-10.3)	0.4574	10.0 (9.8-10.2)	10.1 (9.6-10.4)	0.5254	9.6 (9.3-10.1)	10.0 (9.3-10.2)	0.6689
TLR6	9.3 (8.2-9.6)	9.4 (9.2-9.7)	0.5303	9.4 (9.1-10.0)	9.7 (9.4-10.0)	0.3096	9.1 (8.7-9.5)	9.6 (9.2-9.9)	**0.0480**
TLR8	10.7 (7.6-11.5)	10.1 (7.6-11.2)	0.5711	9.9 (8.5-11.0)	10.5 (9.4-11.1)	0.3738	10.5 (9.0-11.3)	9.9 (8.8-10.9)	0.3425

Inflammasome components									
NLRC4	9.1 (8.9-9.3)	9.0 (8.9-9.2)	0.4297	9.3 (9.2-9.6)	9.4 (9.1-9.6)	0.8989	9.3 (9.2-9.4)	9.3 (9.1-9.5)	0.9361
NLRP12	8.0 (7.6-8.7)	8.0 (7.7-8.7)	0.5125	7.9 (7.6-8.3)	8.4 (7.7-8.6)	0.2152	7.8 (7.6-8.1)	7.8 (7.6-8.4)	0.4592
IFN signalling genes									
FCGR1A	10.8 (10.3-11.2)	10.9 (10.5-11.6)	0.5462	11.1 (10.4-11.8)	10.6 (10.3-10.2)	0.1624	10.7 (10.5-11.3)	10.6 (9.7-11.4)	0.5212
Inflammation									
MMP9	9.6 (8.7-11.1)	9.5 (9.0-10.5)	0.8527	8.5 (8.3-9.0)	9.4 (8.9-10.1)	**0.0178**	9.4 (8.8-10.0)	8.8 (8.4-9.3)	**0.0215**
SPP1	7.7 (7.6-8.2)	7.7 (7.6-9.1)	0.5516	7.8 (7.6-7.9)	8.3 (7.6-8.5)	0.0606	8.0 (7.6-8.6)	7.6 (7.6-8.2)	0.1816
TIMP2	14.3 (14.0-14.6)	14.1 (13.8-14.4)	0.2458	14.1 (13.9-14.7)	14.2 (14.0-14.3)	0.8324	14.2 (13.9-14.6)	14.1 (13.8-14.3)	0.3227
Other									
RAB24	11.7 (11.5-12.0)	11.8 (11.3-12.0)	0.7103	11.7 (11.5-12.1)	11.5 (11.3-11.8)	0.2530	11.4 (11.2-11.7)	11.3 (11.0-11.6)	0.3496
LTF	9.5 (8.5-10.1)	9.2 (8.1-9.6)	0.3764	8.7 (8.0-9.5)	8.5 (7.9-9.7)	0.9662	9.0 (8.2-9.7)	8.6 (8.1-9.2)	0.2612
ZNF331	8.2 (7.7-8.8)	8.7 (7.9-9.6)	0.1902	8.9 (8.2-9.4)	9.3 (8.5-9.7)	0.3302	8.7 (8.2-9.3)	9.0 (8.6-9.6)	0.1995
BLR1	9.8 (8.8-10.5)	10.8 (9.9-11.5)	**0.0121**	10.1 (9.6-11.0)	10.5 (10.0-11.1)	0.4212	10.5 (10.1-11.0)	10.6 (9.8-10.9)	0.7688

Median (inter quartile range) gene expression values (peak areas normalized for GAPDH and log2-transformed) are shown at 6 months following treatment initiation. Significant differences between ATT and ATT plus HAART treated HIV+TB+ patients, HAART treated and untreated HIV+TST+ subjects, and HAART treated and untreated HIV+TST- individuals were determined using Wilcoxon Mann-Whitney test. In pink: genes are indicated that were more highly expressed in the HAART treated group compared to the HAART untreated group. In blue: genes are indicated that had lower expression in the HAART treated group compared to the HAART untreated group. Genes listed in this table were differentially expressed between any of the study groups before stratification by HAART (in)eligibility (Table 1).

Table 3A. Kinetic profiling of the ATT treatment response at the transcriptomic level in HAART treated HIV+TB+ patients compared to HAART eligible HIV+TB+, HAART treated HIV+TST+ and HAART treated HIV+TST- subjects.

Gene Symbol	HIV+TB+ (M0)	HIV+TB+ (M6)	HIV+TST+ (M6)	HIV+TST- (M6)	HIV+TB+ (M6) vs HIV+TB+ (M0)	HIV+TB+ (M6) vs HIV+TST+ (M6)	HIV+TB+ (M6) vs HIV+TST- (M6)
T cell subset markers							
CD3E	13.0 (12.3-13.7)	14.1 (13.5-14.6)	13.5 (13.2-14.2)	14.0 (13.9-14.3)	**0.0022**	0.1384	0.6732
CD4	11.7 (10.7-12.2)	11.5 (11.0-12.2)	11.4 (10.8-11.9)	11.5 (10.7-12.0)	0.4328	0.4717	0.6732
IL7R	11.6 (10.8-12.3)	12.6 (11.9-13.5)	12.7 (11.8-13.3)	13.0 (12.6-13.5)	**0.0150**	1.0000	0.3636
PTPRCv1	10.9 (10.5-11.4)	12.0 (11.3-12.3)	11.5 (10.8-12.4)	11.4 (11.1-11.8)	**0.0150**	0.7670	0.1194
PTPRCv2	13.3 (12.9-13.4)	12.8 (12.6-12.9)	12.6 (12.5-12.9)	12.8 (12.6-13.1)	**0.0060**	0.4717	0.7456
Th1/2/9/17 associated genes/Treg associated genes							
IL1B	11.3 (11.0-11.5)	10.0 (9.6-10.5)	10.2 (9.9-10.4)	10.2 (9.6-10.7)	**0.0022**	0.2899	0.5376
IL15	7.9 (7.6-8.3)	7.6 (7.6-7.9)	7.6 (7.6-7.6)	7.6 (7.6-7.6)	**0.0681**	0.1087	0.1489
TBX21	7.6 (7.6-7.6)	7.7 (7.6-8.1)	7.6 (7.6-7.6)	7.6 (7.6-7.8)	0.2038	0.0863	0.2795
IL4δ2	7.6 (7.6-7.6)	8.9 (7.6-10.1)	8.4 (7.6-9.9)	9.3 (9.1-10.0)	0.1127	0.4528	0.5356
IL9	10.0 (9.7-10.2)	10.0 (9.3-10.2)	9.4 (8.8-9.8)	9.7 (9.4-9.8)	0.2721	0.0687	0.1943
IL22RA1	7.6 (7.6-7.9)	7.6 (7.6-7.9)	7.7 (7.6-8.1)	7.6 (7.6-7.8)	0.7122	0.2580	0.6121
TGFB1	14.5 (14.4-14.7)	14.7 (14.5-14.9)	14.6 (14.5-14.7)	14.6 (14.4-14.8)	0.2393	0.2899	0.2428
Cytotoxicity genes							
GNLY	13.9 (13.5-14.3)	15.3 (14.7-15.5)	14.8 (13.7-15.0)	15.1 (14.5-15.3)	**0.0342**	0.0655	0.4173
GZMB	12.8 (12.6-13.0)	13.7 (13.3-14.0)	12.6 (12.2-13.1)	13.1 (12.7-13.6)	**0.0096**	**0.0035**	**0.0410**
Apoptosis/survival							
FLCN1	8.2 (7.8-8.5)	7.6 (7.6-7.9)	7.6 (7.6-7.9)	7.6 (7.6-7.7)	0.0544	0.7688	0.7481
TNFRSF1A	13.4 (13.1-13.7)	13.5 (13.4-13.8)	13.5 (13.4-13.7)	13.7 (13.2-13.9)	0.4802	0.8324	0.9483
Myeloid associated genes							
CCL5	14.8 (14.6-15.0)	15.2 (14.7-15.5)	14.8 (14.1-15.2)	15.0 (14.6-15.3)	0.0844	0.2196	0.3468
FPR1	13.9 (13.6-14.4)	13.8 (12.7-14.3)	13.7 (13.1-13.9)	13.6 (13.1-14.0)	0.3465	0.7670	0.4957
Chemokines							
CCL19	8.3 (7.6-8.9)	9.6 (9.4-10.0)	9.5 (9.3-9.7)	9.5 (9.3-9.7)	**0.0076**	0.1892	0.3304
Pattern recognition receptors							
CLEC7A	12.9 (12.6-13.5)	12.2 (12.0-12.5)	12.3 (12.1-12.6)	12.1 (11.9-12.7)	**0.0037**	0.4982	0.6732
TLR1	12.0 (11.5-12.3)	11.1 (10.8-11.2)	10.9 (10.7-11.0)	10.7 (10.4-11.4)	**0.0029**	0.3740	0.4754
TLR2	9.9 (9.6-10.5)	9.1 (8.6-9.6)	9.3 (9.0-9.8)	9.2 (8.7-9.7)	**0.0150**	0.3302	0.6851
TLR3	10.2 (9.8-10.7)	9.7 (9.6-10.1)	10.1 (9.8-10.8)	10.3 (9.9-10.6)	0.1823	0.1894	0.1119
TLR4	10.6 (10.2-11.0)	10.1 (9.5-10.3)	10.1 (9.6-10.4)	10.0 (9.3-10.2)	**0.0186**	0.6721	0.6265
TLR6	10.1 (9.8-10.4)	9.4 (9.2-9.7)	9.7 (9.4-10.0)	9.6 (9.2-9.9)	**0.0186**	0.0515	0.2700
TLR8	11.4 (10.0-12.2)	10.1 (7.6-11.2)	10.5 (9.4-11.1)	9.9 (8.8-10.9)	**0.0096**	0.3506	0.9222

Inflammasome components							
NLRC4	9.8 (9.6-10.0)	9.0 (8.9-9.2)	9.4 (9.1-9.6)	9.3 (9.1-9.5)	**0.0022**	**0.0111**	**0.0297**
NLRP12	8.2 (8.0-8.6)	8.0 (7.7-8.7)	8.4 (7.7-8.6)	7.8 (7.6-8.4)	0.4802	0.9662	0.3468
IFN signalling genes							
FCGR1A	11.4 (11.0-11.9)	10.9 (10.5-11.6)	10.6 (10.3-10.2)	10.6 (9.7-11.4	**0.0281**	0.3627	0.3145
Inflammation							
MMP9	10.2 (9.1-10.5)	9.5 (9.0-10.5)	9.4 (8.9-10.1)	8.8 (8.4-9.3)	0.5303	0.5254	**0.0104**
SPP1	7.6 (7.6-7.6)	7.7 (7.6-9.1)	8.3 (7.6-8.5)	7.6 (7.6-8.2)	0.1482	0.6960	0.4162
TIMP2	14.5 (13.9-15.3)	14.1 (13.8-14.4)	14.2 (14.0-14.3)	14.1 (13.8-14.3)	0.1361	0.7670	0.8457
Other							
RAB24	11.9 (11.5-12.0)	11.8 (11.3-12.0)	11.5 (11.3-11.8)	11.3 (11.0-11.6)	0.1361	0.4212	0.0980
LTF	8.8 (7.6-9.7)	9.2 (8.1-9.6)	8.5 (7.9-9.7)	8.6 (8.1-9.2)	0.7828	0.7026	0.2761
ZNF331	7.8 (7.6-8.0)	8.7 (7.9-9.6)	9.3 (8.5-9.7)	9.0 (8.6-9.6)	**0.0202**	0.3738	0.2842
BLR1	9.4 (9.1-10.1)	10.8 (9.9-11.5)	10.5 (10.0-11.1)	10.6 (9.8-10.9)	**0.0121**	0.4717	0.2700

Median (inter quartile range) gene expression values (peak areas normalized for GAPDH and log2-transformed) are shown. Significant differences between HAART eligible HIV+TB+ patients at baseline (Month 0) and 6 months following HAART and ATT treatment initiation (Month 6) were determined using Wilcoxon signed-rank test. Significant differences between the different HAART treated study groups at the 6 month time point was determined using Wilcoxon Mann-Whitney test. In pink: genes are indicated that were more highly expressed in the test group compared to the reference/control group. In blue: genes are indicated that had lower expression in the test group compared to the reference/control group. Genes listed in this table were differentially expressed between any of the study groups before stratification by HAART (in)eligibility (Table 1).

Table 3B. Kinetic profiling of the ATT treatment response at the transcriptomic level in HAART untreated HIV+TB+ patients compared to HAART ineligible HIV+TB+, HAART untreated HIV+TST+ and HAART untreated HIV+TST- subjects.

Gene Symbol	HIV+TB+ (M0)	HIV+TB+ (M6)	HIV+TST+ (M6)	HIV+TST- (M6)	HIV+TB+ (M6) vs HIV+TB+ (M0)	HIV+TB+ (M6) vs HIV+TST+ (M6)	HIV+TB+ (M6) vs HIV+TST- (M6)
T cell subset markers							
CD3E	13.0 (12.5-13.4)	14.1 (13.6-14.2)	14.1 (13.7-14.4)	14.2 (13.9-14.4)	**0.0045**	0.6425	0.2744
CD4	11.5 (10.8-12.0)	11.5 (10.9-12.0)	11.8 (11.4-12.2)	11.8 (11.4-12.3)	0.3343	0.2458	0.1223
IL7R	11.9 (10.9-12.2)	12.8 (11.9-13.3)	13.2 (12.7-13.5)	13.5 (13.2-13.7)	**0.0076**	0.2856	**0.0075**
PTPRCv1	10.1 (9.8-11.5)	11.3 (10.6-11.9)	11.6 (10.9-12.2)	12.0 (11.3-12.5)	0.4265	0.3898	**0.0499**
PTPRCv2	13.4 (13.0-13.7)	12.6 (11.9-13.0)	13.0 (12.7-13.2)	12.5 (12.0-13.0)	**0.0064**	0.1431	0.8801
Th1/2/9/17 associated genes/Treg associated genes							
IL1B	11.6 (11.2-11.8)	9.6 (9.2-10.5)	10.3 (9.8-10.9)	10.0 (9.8-10.3)	**0.0008**	0.2011	0.2276
IL15	7.8 (7.6-8.2)	7.6 (7.6-7.7)	7.6 (7.6-8.1)	7.6 (7.6-7.6)	0.0719	0.2669	0.0915
TBX21	7.6 (7.6-7.6)	7.6 (7.6-7.7)	7.6 (7.6-8.0)	7.6 (7.6-8.2)	0.2313	0.4012	0.5059
IL4δ2	10.5 (7.6-12.1)	9.7 (9.1-10.1)	9.2 (7.8-10.0)	9.1 (7.6-9.6)	0.7982	0.4712	0.0582
IL9	10.3 (9.6-10.5)	9.8 (9.2-10.2)	9.9 (9.6-10.1)	9.5 (8.8-9.8)	0.3627	0.9222	0.0631
IL22RA1	7.8 (7.6-9.0)	7.6 (7.6-8.0)	7.7 (7.6-7.8)	7.6 (7.6-7.8)	0.1704	0.5540	0.7782
TGFB1	14.4 (14.2-14.5)	14.4 (14.1-14.7)	14.6 (14.4-14.7)	14.7 (14.5-14.8)	0.7764	0.3293	0.0545
Cytotoxicity genes							
GNLY	14.0 (12.8-14.3)	15.1 (14.2-15.7)	13.6 (10.1-14.3)	14.9 (14.6-15.7)	0.1398	**0.0091**	0.6784
GZMB	12.6 (12.1-13.0)	13.1 (11.9-13.8)	12.8 (12.4-13.4)	12.8 (12.5-13.5)	0.8203	0.7451	0.9399
Apoptosis/survival							
FLCN1	7.6 (7.6-8.2)	7.6 (7.6-7.6)	7.6 (7.6-8.2)	7.6 (7.6-7.8)	**0.0299**	0.0767	0.1371
TNFRSF1A	13.4 (13.2-13.8)	13.5 (13.3-13.8)	13.5 (13.2-13.8)	13.3 (13.1-13.7)	0.1914	0.8527	0.3230
Myeloid associated genes							
CCL5	14.6 (14.0-14.8)	14.5 (13.6-15.0)	14.9 (14.3-15.2)	14.8 (14.6-15.2)	0.6496	0.3176	0.1870
FPR1	14.0 (13.2-14.4)	13.8 (13.6-14.0)	13.4 (13.0-14.4)	13.8 (13.0-14.1)	0.9547	0.5157	0.7919
Chemokines							
CCL19	8.9 (8.4-9.2)	9.5 (9.3-9.8)	9.5 (9.3-9.9)	9.7 (9.5-9.9)	**0.0007**	0.3777	**0.0418**
Pattern recognition receptors							
CLEC7A	13.1 (12.8-13.5)	12.6 (11.9-13.0)	12.6 (12.3-13.1)	12.4 (12.1-12.6)	**0.0268**	0.5612	0.5717
TLR1	12.0 (11.6-12.6)	11.1 (9.6-11.5)	11.2 (10.7-11.8)	10.7 (10.2-11.1)	**0.0012**	0.4431	0.5717
TLR2	10.1 (9.3-10.6)	9.0 (8.2-9.7)	9.0 (8.1-9.7)	9.2 (8.7-9.4)	**0.0090**	0.8523	0.9398
TLR3	10.3 (10.2-10.7)	9.8 (8.9-10.6)	10.1 (9.2-10.8)	10.2 (9.8-10.6)	0.1118	0.4574	0.3459
TLR4	10.4 (10.2-11.0)	9.9 (8.8-10.3)	10.0 (9.8-10.2)	9.6 (9.3-10.1)	**0.0090**	0.5929	0.8505
TLR6	9.9 (9.6-10.2)	9.3 (8.2-9.6)	9.4 (9.1-10.0)	9.1 (8.7-9.5)	**0.0008**	0.2455	0.9099
TLR8	11.8 (10.7-12.4)	10.7 (7.6-11.5)	9.9 (8.5-11.0)	10.5 (9.0-11.3)	**0.0146**	0.7789	0.8649

Inflammasome components							
NLRC4	9.9 (9.6-10.2)	9.1 (8.9-9.3)	9.3 (9.2-9.6)	9.3 (9.2-9.4)	**0.0064**	0.1143	0.0898
NLRP12	8.5 (8.0-8.7)	8.0 (7.6-8.7)	7.9 (7.6-8.3)	7.8 (7.6-8.1)	0.1704	0.5686	0.2119
IFN signalling genes							
FCGR1A	11.4 (10.8-11.8)	10.8 (10.3-11.2)	11.1 (10.4-11.8)	10.7 (10.5-11.3)	0.4265	0.2856	0.9399
Inflammation							
MMP9	10.9 (8.7-11.4)	9.6 (8.7-11.1)	8.5 (8.3-9.0)	9.4 (8.8-10.0)	0.3201	**0.0274**	0.5465
SPP1	7.6 (7.6-8.2)	7.7 (7.6-8.2)	7.8 (7.6-7.9)	8.0 (7.6-8.6)	0.7979	0.9815	0.3652
TIMP2	14.2 (13.7-14.9)	14.3 (14.0-14.6)	14.1 (13.9-14.7)	14.2 (13.9-14.6)	0.6092	0.5775	0.5718
Other							
RAB24	11.6 (10.8-12.0)	11.7 (11.5-12.0)	11.7 (11.5-12.1)	11.4 (11.2-11.7)	**0.0199**	1.0000	**0.0458**
LTF	9.1 (8.4-10.7)	9.5 (8.5-10.1)	8.7 (8.0-9.5)	9.0 (8.2-9.7)	0.6092	0.1250	0.2740
ZNF331	7.6 (7.6-9.1)	8.2 (7.7-8.8)	8.9 (8.2-9.4)	8.7 (8.2-9.3)	0.5887	0.0850	0.0541
BLR1	9.6 (9.3-9.9)	9.8 (8.8-10.5)	10.1 (9.6-11.0)	10.5 (10.1-11.0)	0.5321	0.2649	**0.0215**

Median (inter quartile range) gene expression values (peak areas normalized for GAPDH and log2-transformed) are shown. Significant differences between HAART ineligible HIV+TB+ patients at baseline (Month 0) and 6 months following ATT treatment initiation (Month 6) were determined using Wilcoxon signed-rank test. Significant differences between the different HAART untreated study groups at the 6 month time point was determined using Wilcoxon Mann-Whitney test. In pink: genes are indicated that were more highly expressed in the test group compared to the reference/control group. In blue: genes are indicated that were lower expressed in the test group compared to the reference/control group. Genes listed in this table were differentially expressed between any of the study groups before stratification by HAART (in)eligibility (Table 1).

Figure 1.

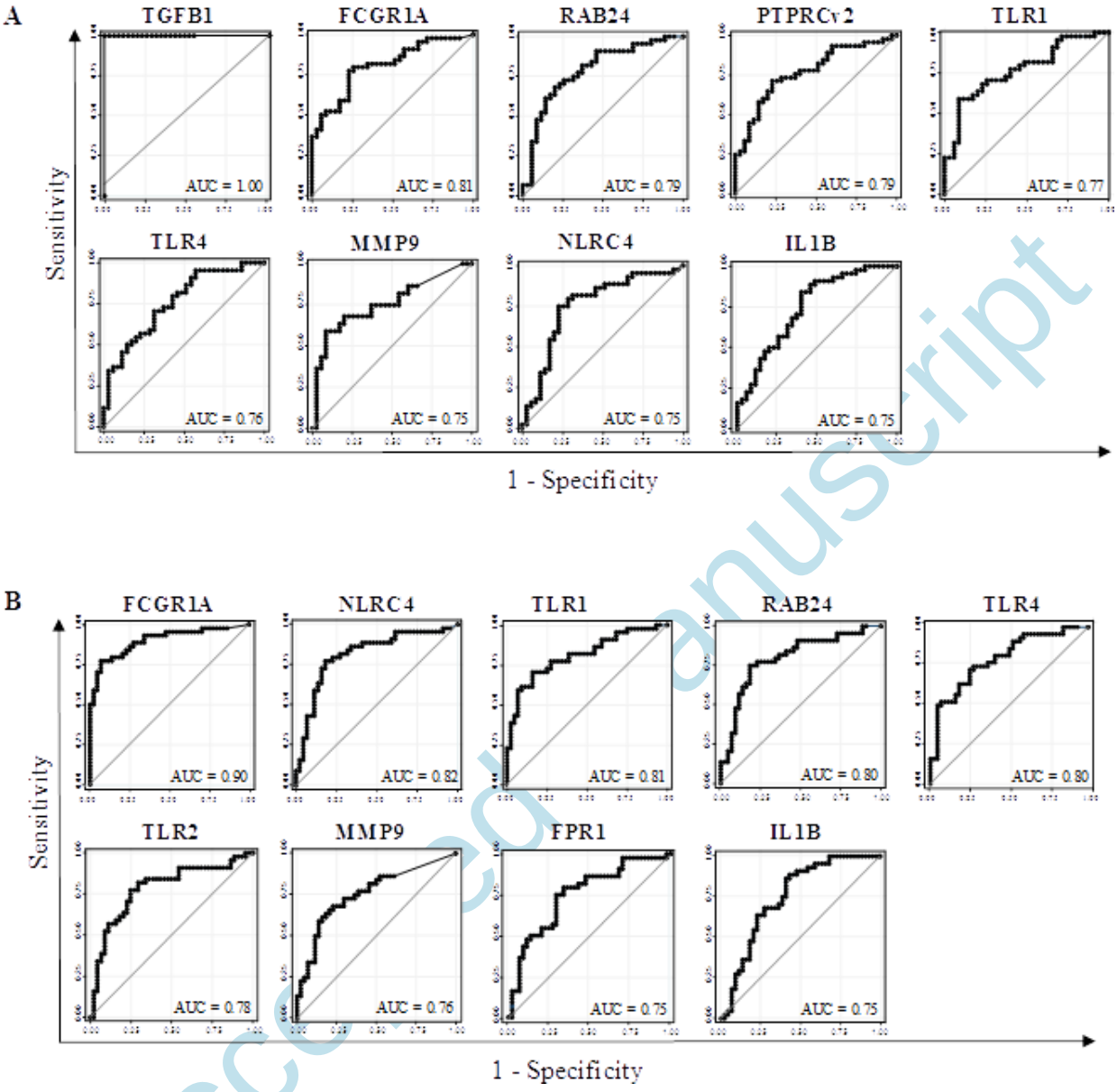

Figure 2.