Neutrophil activation and enhanced release of granule products in HIV-TB immune reconstitution inflammatory syndrome

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Abstract

Background: Tuberculosis Immune Reconstitution Inflammatory Syndrome (TB-IRIS) remains incompletely understood. Neutrophils are implicated in tuberculosis pathology but detailed investigations in TB-IRIS are lacking. We sought to further explore the biology of TB-IRIS and in particular the role of neutrophils.

Setting: Two observational, prospective cohort studies in HIV/TB co-infected patients starting antiretroviral therapy, one to analyze gene expression and subsequently one to explore neutrophil biology.

Methods: nCounter gene expression analysis was performed in TB-IRIS patients (n=17) versus antiretroviral-treated HIV/TB co-infected controls without IRIS (n=17) in Kampala, Uganda. Flow cytometry was performed in TB-IRIS patients (n=18) and controls (n=11) in Cape Town, South Africa to determine expression of neutrophil surface activation markers, intracellular cytokines and Human Neutrophil Peptides (HNP). Plasma neutrophil Elastase and HNP1-3 were quantified using ELISA. Lymph node immunohistochemistry was performed on three further TB-IRIS cases.

Results: There was a significant increase in gene expression of S100A9 (p=0.002), NLRP12 (p=0.018), COX-1 (p=0.025) and IL-10 (p=0.045) two weeks after ART initiation in Ugandan TB-IRIS patients versus controls, implicating neutrophil recruitment. IRIS patients in both cohorts demonstrated increases in blood neutrophil count, plasma HNP and elastase concentrations from ART initiation to week two. CD62L (L-selectin) expression on neutrophils increased over 4 weeks in South African controls while IRIS patients demonstrated the opposite. Intense staining for the neutrophil marker CD15 and IL-10 was seen in necrotic areas of TB-IRIS patients' lymph nodes.

Conclusion: Neutrophils in TB-IRIS are activated, recruited to sites of disease and release granule contents, contributing to pathology.

Keywords: Tuberculosis; HIV-1; neutrophils; immune reconstitution inflammatory syndrome; IRIS

Introduction

When patients with HIV-associated TB begin Antiretroviral Therapy (ART), approximately 18% develop Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome (TB-IRIS) [1]. TB-IRIS is an exaggerated immune response to *M. tuberculosis* (MTB) antigens associated with reconstitution of the immune system. It is characterized by excessive inflammatory responses and deterioration in clinical status [1, 2].

According to the International Network for the Study of HIV associated IRIS (INSHI) case definitions, two forms of TB-IRIS exist: 'paradoxical' (clinical worsening of a patient on TB treatment after starting ART) and 'unmasking' (undiagnosed TB becoming apparent after starting ART) [3].

TB-IRIS has been associated with perturbations in both the adaptive and innate immune systems [4, 5]. These include increased secretion of neutrophil-associated mediators such as S100A8/A9 and matrix metalloproteinases (MMPs) [6-8], perforin and granzyme B by CD4+ T cells [9], higher expression and imbalance of C1Q and C1-inhibitor (complement system) [10], activation of monocytes [11], inflammasome and Toll-like receptor signaling [12, 13] as well as elevated chemokine and cytokine production [14-16] with a particular role for the IL-10 family [17]. Although rapid changes in CD4+ T cell count have long been associated with all forms of IRIS, recent research has focused on these latter phenomena of inflammasome activation and release of soluble mediators from innate cells [4, 12]. However, the clinical syndromes associated with TB-IRIS, especially suppurative lymphadenitis and abscess formation, implicate neutrophils as

critical effector cells mobilized by these inflammatory signals.

To gain further understanding into the biology of TB-IRIS, we recruited and prospectively followed patients with HIV-associated tuberculosis (HIV+TB+) at risk of developing IRIS at two clinical sites, in Uganda and South Africa. First, we conducted an assessment of gene expression in putative pathways. On the basis of previous research summarized above, we chose to study the T-cell receptor, cytokine genes including the IL-10 pathway [17] and the inflammasome [12, 13]. Subsequently, in a separate cohort, we performed functional assays chosen on the basis of genes that were over-expressed in IRIS patients versus controls: these experiments focused on neutrophils which, although implicated [6], have not been extensively studied before in TB-IRIS.

Materials and Methods

Patient recruitment and study visits

Cohort 1: Patients with a confirmed diagnosis of both HIV and TB, on TB treatment (for a median [IQR] of 40 [24-59] days) and who were eligible for ART initiation according to the July 2008 Ugandan national treatment guidelines (CD4 count <250 cells/μL), were recruited in 2009 at Mulago National Tuberculosis and Leprosy clinic and the Infectious Diseases Institute in Kampala for gene expression studies, as previously described [18]; see Supplementary Table 1. Patients were reviewed at week 0 (before ART initiation), week 2 and months 1-12 (after ART initiation). Patients who developed TB-IRIS (cases) were defined according to the INSHI clinical case definitions [3] and were matched by age (<10 years difference between patients), CD4 cell count before ART initiation (mean (SD) difference, 5.3 (6.8) cells/μL) and sex with those that

did not develop TB-IRIS (non-IRIS controls). Sampling at the IRIS time-point was performed before patients received corticosteroids. All patients provided written informed consent. The Uganda National Council of~~n~~ Science and Technology, Makerere Faculty of Medicine Ethics Committee (IRB-Makerere-05_2007), Infectious Disease Scientific Review Committee, University of Antwerp Ethics Committee and the Institute of Tropical Medicine, Antwerp, Belgium (CME_UZA_7/29/157) approved the study.

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Cohort 2: Recruitment of patients for neutrophil studies took place in Cape Town, South Africa as part of the longitudinal Tissue Destruction in Tuberculosis 2 (TDTB2) study (Supplementary Table 1). Patients were recruited in 2013 at Ubuntu clinic, a primary care HIV treatment clinic in Site B, Khayelitsha. HIV-infected patients at high risk of developing TB-IRIS (CD4 count <200 cells/μL at enrolment) were followed up during anti-tuberculosis treatment and initiation of ART until twelve weeks post ART. Samples for neutrophil studies were collected at ART initiation (week 0), week two and week four of ART. TB-IRIS diagnosis was made retrospectively after week 12 by a consensus panel using the INSHI case definition; controls (non-IRIS) were those patients who were also sampled at ART initiation and Week 2 / Week 4 follow-up visits but did not develop the syndrome [3]. At the IRIS/week 2 time point, two TB-IRIS and one non-IRIS control were receiving corticosteroids. Ethical approval was obtained from the Faculty of Health Sciences Human Research Ethics Committee, University of Cape Town (HREC REF: 516/2011); all patients provided written informed consent.

Samples for detailed analysis were available from 34 patients in Cohort 1 (17 cases and 17 controls) and 29 patients in Cohort 2 (18 cases and 11 controls). Supplementary Figure 1 summarises the study design.

Sample collection and processing

For Cohort 1, venous blood (30–40 ml) was collected in EDTA tubes (BD Pharmingen, Franklin Lakes, New Jersey, USA) at week 0 and week 2 after initiation of ART. Peripheral Blood Mononuclear Cells (PBMC) were isolated by Ficoll-Hypaque gradient centrifugation and cryopreserved for further processing (see below). For Cohort 2, blood samples (30–40 ml) were collected in sodium heparin vacutainers (BD Pharmingen) at weeks 0, 2 and 4 after initiation of ART and were processed for plasma generation within two hours of collection; an aliquot (1 ml) of blood was removed for functional assays as described below.

nCounter gene expression analysis

RNA was extracted from PBMC using standard techniques (Supplementary Methods). ProbeSet sequences for the gene sets of interest (T-cell receptors, the inflammasome, IL-10 pathway and cytokines; 148 genes in total) are shown in Supplementary Table 2.

Determination of neutrophil activation and degranulation

We investigated neutrophil activation in whole blood by flow cytometry, measuring cell surface expression of CD11b, CD16, CD62L, CD66a,c,e [19] and IL-8RA. An aliquot of whole blood was stained on ice with CD11b-PE-Cy7, CD16-APC-H7, CD62L-FITC, CD66a,c,e-PE, IL-8 RA-APC (BD Pharmingen) and viability dye (eFluor 450, eBiosciences; San Diego, California, USA or ViViD, Invitrogen; Carlsbad, California, USA). After washing, the stained sample was

fixed in 2% paraformaldehyde and acquired on a Becton Dickinson Fortessa flow cytometer (BD Biosciences). Data analysis was performed with FlowJo software (FlowJo 10.1r5, Tree Star, Ashland, OR) using the gating strategy in Supplementary Figure 2~~1~~.

Determination of neutrophil elastase and Human Neutrophil Peptides (HNP1-3) in plasma

Neutrophil elastase and Human Neutrophil Peptides (HNP1-3) plasma concentrations were quantified using ELISA according to the manufacturer's instructions (Hycult Biotech; Uden, The Netherlands). Assays were performed in duplicate. The sensitivity for neutrophil elastase was 0.67 ng/ml and for HNP1-3 was 4.25 pg/ml. -The elastase assay detects both free and complexed elastase.

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Immunohistochemistry (IHC) staining of lymph nodes

Patient selection, lymph node (LN) preparation and immunohistochemistry were carried out as previously described [20] and summarized in Supplementary Methods.

Statistical analysis

Comparison between the two groups was performed using t tests (unpaired for IRIS vs non-IRIS comparisons, paired for within-group comparisons between ART initiation and later time points), the Mann-Whitney U test or Wilcoxon test for continuous variables and Fisher exact tests for categorical variables. Statistics were performed using GraphPad Prism Version 7.0 (La Jolla, California, USA) and Qlucore Omics explorer version 3.2. (Lund, Sweden) Significance was inferred below a two-tailed p-value of 0.05.

Gene expression analysis to identify discriminating transcripts between the groups (based on p-

value <0.05 and q value (False Discovery Rate-adjusted p-value) <0.1) was performed using Qlucore Omics explorer and displayed oin a heatmap. The IRIS (pink) and non-IRIS (blue) patients (columns) and genes (rows) were ordered using principal component analysis (PCA) and R statistic respectively. Gene expression at the week two time point on the heatmap was classified as high or low (relative to the entire cohort) if colored red and green respectively. A PCA plot, with the projection score and variance filtering set at 0.38 and 0.430 respectively, was used to detect strong signals within the data on gene transcript abundance. Principal Component Analysis identifies the major vectors ('components') which differentiate multi-parameter data sets. The genes were colored according to their R statistic with green and red if higher in non-IRIS controls or IRIS patients respectively, and the distance between individual genes reflects their correlation coefficient.

Results

Patient characteristics

Supplementary Table 1 summarizes demographic and basic laboratory data for both cohorts. At ART initiation, there were no statistical differences in patient characteristics between those who subsequently developed IRIS and those who did not. The median [IQR] time to IRIS presentation across both studies was 14 [10-15] days.

RNA analysis reveals higher expression of genes implicated in neutrophilic inflammation in TB-IRIS patients compared to controls

We used NanoString nCounter technology to ascertain gene expression in PBMC of IRIS and non-IRIS patients at the IRIS time-point (median of 14 days) or after 2 weeks of ART in

controls. The nCounter gene expression values obtained were log 2 transformed pre-analysis to normalize data as per standard transcriptomic analytical pathways; a false discovery rate (q-value) of 0.1 was applied to account for multiple comparisons. A heatmap to visualize the pattern of transcript abundance in IRIS patients and non-IRIS controls revealed over 70 discriminating transcripts with modest clustering of IRIS cases (pink) and non-IRIS controls (blue); there was generally lower gene expression (green) in the IRIS patients compared to the non-IRIS controls (Figure 1A). On the contrary, Cyclooxygenase-1 (COX-1), Interleukin-10 (IL-10), Nucleotide-binding domain, leucine rich repeat containing receptor (NLR) Family Pyrin Domain Containing 12 (NLRP12 / Pypaf-7), and S100 calcium-binding protein A9 (S100A9) were significantly more abundant in the IRIS cases than in the non-IRIS controls at two weeks of ART.

PCA was then used to detect correlation patterns within the discriminating transcripts. The four genes (COX-1, δ=0.96, fc=1.9, R=0.38, p=0.025, q=0.051; IL-10, δ=0.75, fc=1.7, R=0.35, p=0.045, q=0.077; NLRP12, δ=1.27, fc=2.4, R=0.40, p=0.018, q=0.042; and S100A9, δ=1.10, fc=2.1, R=0.52, p=0.002, q=0.018) which were more abundant in IRIS cases versus non-IRIS controls clearly correlated with each other and separated from the other transcripts (Figure 1B).

Next, we quantitatively analyzed these four transcripts using the log2 transformed nCounter gene expression values. As shown in Supplementary Figure 3~~2~~, S100A9 expression significantly increased at the two-week time point in the IRIS patients (median $\log_2$ expression, 16.07; IQR, 15.15–16.35) from ART initiation (median, 14.59; IQR, 14.06–15.22) and was higher at 2 weeks compared to the controls (median, 15.05; IQR, 14.12–15.50; p=0.002). NLRP-12 expression also significantly increased from ART initiation (median, 5.66; IQR, 4.12–6.77) to the two-week time

point in TB-IRIS patients (median, 6.94; IQR, 6.23–7.68), when it was higher compared to the controls (median, 6.15; IQR, 5.44–6.93; p=0.016). IL-10 significantly decreased in controls from ART initiation (median, 7.56; IQR, 6.42–7.73) to two weeks (median, 6.41; IQR, 5.38–7.02; p=0.005), and significantly greater IL-10 expression was seen in the IRIS cases (median, 6.83; IQR, 6.33–8.02) versus controls (median, 6.41; IQR, 5.38–7.02; p=0.049) at two weeks. Significantly higher COX-1 expression was also seen in the IRIS group (median, 8.93; IQR, 7.87-9.51) versus the non-IRIS controls (median, 7.94; IQR, 6.95-8.81; p=0.049) at the two-week time point.

TB-IRIS is characterized by neutrophilia

The most up-regulated gene in TB-IRIS identified in our expression analysis was S100A9, which is implicated in neutrophil accumulation in tuberculosis [21]. Similarly, NLRP12 (Pypaf-7) is crucial for neutrophil recruitment in other models of infection [22], including to the lungs [23], while (among its other actions) COX-1 generates eicosanoids which activate neutrophils [24]. We have also shown that neutrophil markers strongly co-localise with IL-10 in human tuberculous granulomas [20]. Our gene expression data therefore suggested a role for neutrophils in TB-IRIS pathogenesis and we examined this in another patient cohort, subsequently recruited in Cape Town. Supplementary Table 1 details participant characteristics.

The IRIS cases in both cohorts demonstrated an increase in peripheral neutrophil counts from ART initiation to the IRIS time-point / week 2 (Cohort 1 median [IQR] 1.77 [1.04–2.37] $x10^9$/L to 2.91 [2.29–5.56] $x10^9$/L, p=0.049, Figure 2A; Cohort 2 median [IQR] 2.45 [1.48–4.00] $x10^9$/L to 5.00 [3.35–7.23] $x10^9$/L, p=0.001, Figure 2B). There were no changes in non-IRIS controls

from ART initiation to two weeks. At two weeks, IRIS patients in Cohort 1 had significantly higher neutrophil counts versus the controls (median [IQR] 2.91 [2.29–5.56] $x10^9$/L) and median [IQR] 1.70 [0.97–2.52] $x10^9$/L respectively, p=0.003, Figure 2A).

There were no differences between IRIS patients and controls' total lymphocyte and monocyte counts at either baseline or at the two week / IRIS time point.

TB-IRIS patients demonstrate activation of neutrophils, as defined by surface marker expression

Neutrophil cell surface activation markers (CD11b, CD16, CD62L and CD66a,c,e) were analyzed in whole blood from a subset of patients in Cohort 2 (n=6 per group) using flow cytometry. There was a significant linear trend towards decreased expression of CD62L, as defined by median fluorescence intensity, on TB-IRIS patients' neutrophils over the first four weeks from ART initiation (p=0.014), with a significant difference between neutrophil CD62L expression at ART initiation (mean, 3881; SD, 2746) versus four weeks (mean, 1229; SD, 483; p=0.042; Figure 3A). Significantly higher expression of CD62L was observed in non-IRIS controls (mean, 3422; SD, 1196) compared to TB-IRIS cases (mean, 1269; SD, 483; p=0.005; Figure 3A) at week four, consistent with significantly increased CD62L expression on non-IRIS controls' neutrophils from ART initiation (mean, 1596; SD, 427) to two weeks (mean, 2387; SD, 517; p=0.003) and further to four weeks (mean, 3422; SD, 1196; p=0.009; Figure 3A).

Supplementary Figure 2B presents representative CD62L MFI at the Week 2 / IRIS time point.

A similar pattern was seen for CD16 expression (Figure 3B) although comparisons did not reach statistical significance. Median fluorescence intensity of CD11b decreased in the control group

from ART initiation (mean, 12130; SD, 4253) to Week 4 (mean, 5562; SD, 2584; p=0.047; Figure 3C) but no difference was seen in the IRIS group. No differences were seen in CD66a,c,e expression (Figure 3D), nor in IL-8 RA (data not shown).

TB-IRIS patients exhibit increased Neutrophil Elastase and Human Neutrophil Peptide 1-3 plasma concentrations

Neutrophil elastase is implicated in inflammation and tissue damage [25], and we measured this marker in plasma samples from Cohort 2. ~~We analysed plasma n~~Neutrophil elastase concentration~~s in Cohort 2, finding a significant increase~~ increased significantly in TB-IRIS patients between ART initiation (median 154 ng/mL; IQR, 122.5–191.3) and week two (median 274 ng/mL; IQR, 228–324; p=0.0004; Figure 4A). At two weeks after ART initiation, there was a significantly ~~lower~~ higher plasma neutrophil elastase ~~plasma~~ concentration in TB-IRIS patients compared to non-IRIS controls (median, 274 ng/mL; IQR, 228–324 versus median, 175 ng/mL; IQR, 119–253~~) versus TB-IRIS patients (median, 274 ng/mL; IQR, 228–324;~~ p=0.005; Figure 4A).

Analysis of plasma Human Neutrophil Peptide (HNP) 1-3 concentrations in Cohort 2 revealed an increase in TB-IRIS patients from ART initiation (median, 0 pg/mL; IQR, 0–1775) to the week two-time point (median, 2675 pg/mL; IQR, 990–11353; p=0.005; Figure 4B). In Cohort 1, HNP1-3 concentrations also increased from week 0 (median, 7153 pg/mL; IQR, 5998–8896) to week two (median, 13821 pg/mL; IQR, 7271–22975; p=0.001), when they were higher compared to controls (median, 7510 pg/mL; IQR, 6007–8751; p=0.038; Figure 4C).

Analysis of a wider cohort recruited identically in Uganda confirmed significant differences in HNP concentration between TB-IRIS patients and non-IRIS controls at the IRIS time-point / Week 2, with resolution of these differences by later time points (Supplementary Figure 43).

Lymph node granulomas from IRIS patients show significant neutrophil infiltration and IL-10 production.

We proceeded to characterize neutrophil infiltration and accumulation in lymph nodes of TB-IRIS patients *in situ*, using immunohistochemistry. There was intense staining in the centre of the biopsies for the neutrophil marker CD15, correlating with areas of significant necrosis (Figure 5). Lymph nodes from patients with TB-IRIS also stained strongly for IL-10, largely correlating with neutrophils, as previously shown [20].

Discussion

TB-IRIS immunopathogenesis remains incompletely defined and a lack of predictive markers makes its diagnosis and treatment complex. Given the temporal association of IRIS with reconstitution of CD4+ T lymphocyte numbers on antiretroviral therapy, many studies have focused on Th1 cells [26, 27]. However, TB-IRIS is not explained simply by a change in CD4 numbers, and innate cells are also implicated in the syndrome [5, 12]. Neutrophils are increasingly recognised in tuberculosis pathology [28-30], as we have previously described in TB-meningitis IRIS [6], but they had not previously been studied in this detail.

We recruited HIV+TB+ patients at risk of developing IRIS (Cohort 1) and investigated transcript abundance of genes relating to inflammasome, T-cell receptor, cytokines and their receptors. The

gene transcripts that were most abundant in IRIS patients versus non-IRIS controls, and clearly discriminatory on a PCA plot, were S100A9, IL-10, NLRP-12 and COX-1. Increased expression of inflammasome and neutrophil-associated genes in TB-IRIS is consistent with previous results [12, 31], but the lower abundance of TCR-associated genes in TB-IRIS patients was unexpected and deserves further analysis. This may reflect poor reconstitution of normal T cell function in TB-IRIS and again supports the concept that the phenomenon is driven by innate inflammation without an orchestrated acquired immune response.

Among the more abundant transcripts, S100A9 contributes to inflammation in tuberculosis due to its role in neutrophil recruitment [6, 21, 32] and it has been proposed as a promising biomarker for TB diagnosis [33, 34]. NLRP-12 also plays an important role in neutrophil recruitment [22, 23]. We have reported increased levels of the IL-10 cytokine family in IRIS [17] and observed significant IL-10 staining in tuberculous granulomas where it associates with neutrophil markers and necrosis [20]. The source of IL-10 in TB-IRIS remains unclear, with conflicting data on whether regulatory T cell populations are expanded (reviewed in [4]). Again, it may be that innate cells are responsible for the production of immunosuppressive cytokines. Gene expression data therefore suggested a role of neutrophils in the development of TB-IRIS and we recruited a further cohort to perform neutrophil functional assays.

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In both cohorts, we first demonstrated that patients meeting INSHI criteria for IRIS exhibited an increase in neutrophil count from ART initiation. We observed that neutrophils accumulate intensely at sites of pathology in TB-IRIS and associate with areas of necrosis. IRIS patients' neutrophils were activated, shedding their CD62L/L-Selectin over time with a significant drop

from ART initiation to four weeks (despite the initiation of corticosteroids in three patients); the reverse pattern being observed in controls. A similar trend to CD62L was seen for CD16. We have previously shown that at ART initiation, neutrophils in antiretroviral-naïve HIV-infected patients are activated, rapidly undergo cell death and their ability to kill *M. tuberculosis* is impaired compared to HIV-uninfected controls [18]. Our data confirms that abnormal activation is reversed on ART in patients with an uncomplicated clinical course (undergoing protective immune reconstitution), while in IRIS the neutrophil dysfunction becomes exaggerated (these patients undergo pathogenic immune reconstitution).

We did not see differences between the groups in other activation markers, including CD11b and CD66a,c,e. However, loss of CD16 and CD62L occurs preferentially as neutrophils progress to cell death [35]. Collectively, these data suggest that neutrophil activation and presumably early cell death is a hallmark of TB-IRIS [28, 30]. Increased neutrophil influx and death at disease sites will lead to release of cytotoxic granule contents causing local tissue damage and amplifying inflammatory responses [29, 36], consistent with necrotic abscesses and lymphadenopathy often observed in TB-IRIS.

Compatible with this conclusion, we found an increased neutrophil elastase concentration in the plasma of TB-IRIS patients versus non-IRIS controls two weeks after initiation of ART in cohort 2. There was also an increase from ART initiation in the South African TB-IRIS patients' elastase concentration, and an increase in HNP 1-3 in both cohorts. The difference in neutrophil elastase concentration between IRIS patients and controls was seen despite no significant difference in absolute neutrophil count in Cohort 2, suggesting that plasma concentrations of this

296 granule product might represent more than simply a higher number of circulating neutrophils.
297
298 Notably, some activation parameters in the patients developing IRIS tended to be less abnormal
299 at ART initiation. This is consistent with observations by others [14, 37, 38] that TB-IRIS may
300 be heralded by lower cytokine concentrations at ART initiation but subsequent large magnitude
301 changes.
302
303 Limitations of our study include relatively small group sizes. We were unable to perform
304 neutrophil functional assays including phagocytosis, mycobacterial killing and cell death in
305 sufficient numbers, as few samples met our stringent pre-specified neutrophil purity and viability
306 criteria of >90%. Differences in HNP concentrations between the cohorts might be due to
307 differences in pre-analytical handling; in Cohort 1 blood was collected in Uganda and assays
308 performed in Belgium, whereas South African samples were analysed locally. We also note a
309 difference in neutrophil and CD4 counts between the two cohorts, likely to reflect the clinical
310 realities of treating HIV-TB co-infection in Uganda in 2009 compared to South Africa in 2013,
311 as well as differences in analysis platforms and racial background. However, the fact that we
312 could demonstrate a role for neutrophils in two geographically different cohorts increases the
313 generalizability of our findings.
314 A strength of our analysis was the inclusion of both peripheral blood and lymph node samples,
315 although longitudinal analyses were conducted exclusively in peripheral blood which may not be
316 representative of the tissue environment. However, as peripheral blood does exhibit significant
317 perturbations in TB-IRIS, is easily accessible for serial measurements and contains many
318 components of both the innate and acquired immune systems, we believe that analysis of this

compartment is informative.

In conclusion, our data suggest that TB-IRIS is characterized by aberrant immunological recovery with inflammasome activation and neutrophil recruitment instead of reconstitution of normal T cell receptor function. Within the context of local and systemic inflammation, recruited neutrophils are activated, are likely to undergo rapid cell death and will release cytotoxic granule contents. This drives tissue damage and further inflammation, paradoxically associated with immunosuppressive IL-10 release which may compromise host control of any remaining viable mycobacteria. As neutrophils are likely to be key effector cells mediating pathological damage in TB-IRIS, it seems logical to consider host-directed therapies to reduce neutrophil recruitment (eg CXCR2 inhibitors [39] and anti-C5a inhibitors [40]) or to promote neutrophil apoptosis (eg statins [41]): these questions require further research. ~~neutrophil influx, activation with probable rapid cell death and release of cytotoxic granule contents at the site of disease is likely to underlie tissue damage seen in TB-IRIS and suggest that neutrophils play a major role in the pathology of TB-IRIS.~~

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Figure legends

Figure 1: **Gene expression analysis in PBMCs from patients with HIV-associated TB-IRIS and HIV/TB co-infected controls without clinical IRIS: A**. 100 ng of total RNA was used to obtain values for gene expression analysis using nCounter technology. Unsupervised hierarchical clustering of transcript abundance data from TB-IRIS (pink) (n = 17) and non-IRIS (blue) (n = 17) patients at week two/IRIS-time point was performed using a heatmap in Qlucore Omics explorer v3.2. The columns represent patients while the rows are genes identified as discriminatory ($p<0.05$, $q<0.1$). Relative gene expression compared to the entire cohort was classified as low (green) and high (red) respectively. Genes were ordered according to their R statistic between IRIS and non-IRIS patients. **B.** Discriminatory genes were visualized on a PCA plot. The genes (variables) were colored according to their R statistic; green for the lowest (implying greater abundance in non-IRIS vs IRIS) and red if the highest (implying greater abundance in IRIS vs non-IRIS). The genes with the highest expression in IRIS were COX-1, IL-10, NLRP-12 and S100A9.

Abbreviations: ASC; Apoptosis-associated speck-like protein containing a Caspase Recruitment Domain (CARD); CD, Cluster of Differentiation; COX-1/PTGS, Cyclooxygenase-1/prostaglandin-endoperoxide synthase; CTLA4, Cytotoxic T Lymphocyte-associated protein 4 (CD152); GATA3, Glycine, Alanine, Thymine, Alanine binding protein 3; ICOS, Inducible T-cell costimulator; IFN-ϒ, Interferon gamma; IL, Interleukin; IL-7R, Interleukin-7 receptor; ITK, Interleukin-2-inducible T-cell kinase; pypaf-7, PYRIN-containing Apaf-1-like proteins; S100A9, S100 calcium-binding protein A9; Tbet, T-box transcription factor; TRAC, T-cell Receptor alpha constant; TRAV, T-cell Receptor alpha variable; TRBC, T-cell Receptor beta constant; TRBV,

T-cell Receptor beta variable; TRDV, T-cell Receptor delta variable; TRGC, T-cell Receptor gamma constant; TRGV; T-cell Receptor gamma variable.

Figure 2: **TB-IRIS patients exhibit a rise in neutrophil count after two weeks of ART. A:** Neutrophil counts from TB-IRIS (n = 10 at ART initiation, n = 17 at Week 2 (W2)) and non-IRIS (n=12 at ART initiation, n = 17 at W2) patients (Cohort 1) are presented at ART initiation and at the Week 2 (W2) time point. **B**: Neutrophil counts from TB-IRIS (n =18 at ART initiation, n = 16 at W2) and non-IRIS (n =11 at ART initiation, n = 10 at W2) patients (Cohort 2) are presented at initiation of ART and at Week 2 (W2). Mann Whitney and Wilcoxon tests were used (* $p < 0.05$, ** $p < 0.01$).

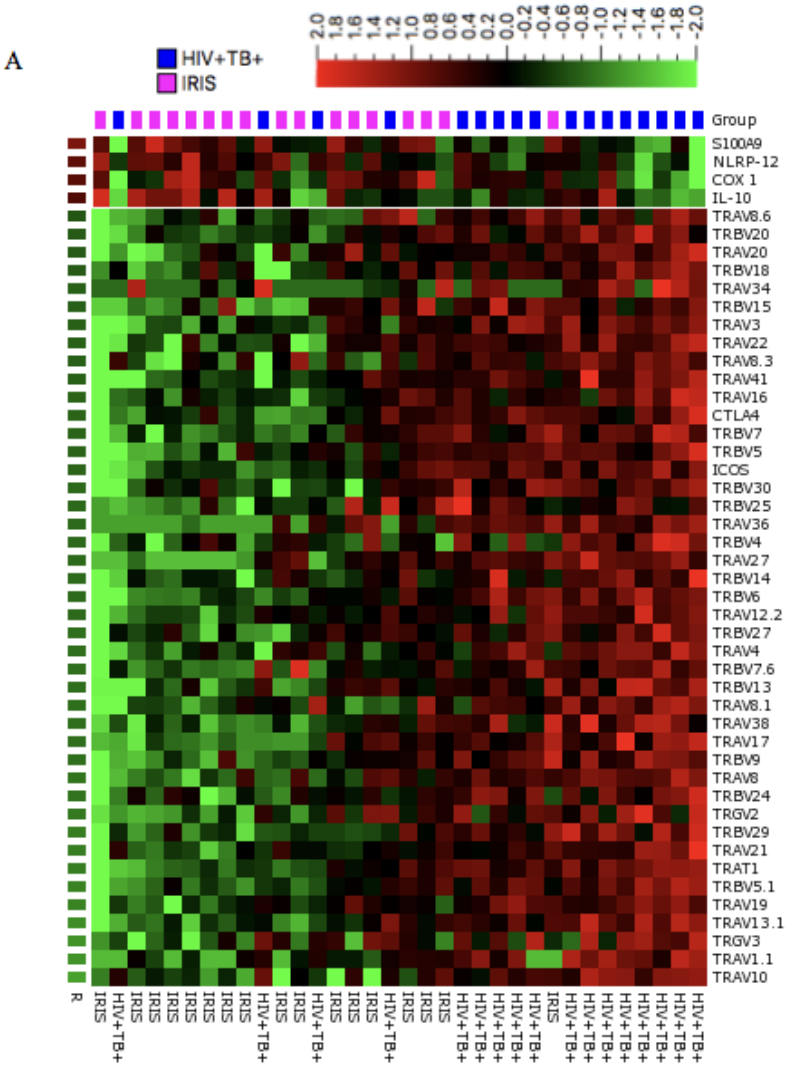
Figure 3: **Neutrophil activation in TB-IRIS patients and Non-IRIS controls:** The Median Fluorescence Intensity of CD62L (A), CD16 (B), CD11b (C) and CD66a,c,e (D) on neutrophils in fresh whole blood is shown for TB-IRIS patients (red, n=6) and non-IRIS controls (black, n=6 at ART initiation (Week (W) 0), n = 4 at W2, n = 3 at W4). Lines represent means and p-values (* $p < 0.05$, ** $p < 0.01$) were derived from unpaired and paired t tests.

Figure 4: **Analysis of plasma levels of neutrophil elastase and HNP1-3 in patients with TB-IRIS and non-IRIS controls. A**. Neutrophil Elastase (TB-IRIS patients (red, n = 18 at ART initiation, n = 15 at W2) and non-IRIS controls (black n = 11)) plasma concentrations were quantified using ELISA in Cohort 2. **B**. Human Neutrophil Peptide (HNP) 1-3 (TB-IRIS patients (red, n = 18 at ART initiation, n = 16 at W2) and non-IRIS controls (black n = 11)) plasma concentrations were quantified using ELISA in Cohort 2. **C**. Human Neutrophil Peptide (HNP)

1-3 plasma concentrations were quantified using ELISA in Cohort 1 (TB-IRIS patients (n =15 at ART initiation, n = 16 at W2) and non-IRIS controls (n = 8)). Lines represent medians and p-values (** $p < 0.01$, *** $p < 0.001$) were derived from Mann-Whitney and Wilcoxon tests.

Figure 5: **Neutrophil infiltration in the lymph nodes of TB-IRIS patients.** Caseous granulomas from consecutive cross-sectional lymph node sections of TB-IRIS patients (n = 3) that were stained with Hematoxylin and Eosin (H&E) (**A**), CD15 (neutrophils, **B**), or IL-10 (**C**). Intense neutrophil staining localizes within most of these caseous granulomas. IL-10 staining was diffuse but did localize within and near caseous granulomas. Black bars represent 200 µm.

Figure 1


A
HIV+TB+
IRIS
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
Group
S100A9
NLRP-12
COX 1
IL-10
TRAV8.6
TRBV20
TRAV20
TRBV18
TRAV34
TRBV15
TRAV3
TRAV22
TRAV8.3
TRAV41
TRAV16
CTLA4
TRBV7
TRBV5
ICOS
TRBV30
TRBV25
TRAV36
TRBV4
TRAV27
TRBV14
TRBV6
TRAV12.2
TRBV27
TRAV4
TRBV7.6
TRBV13
TRAV8.1
TRAV38
TRAV17
TRBV9
TRAV8
TRBV24
TRGV2
TRBV29
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TRAV1.1
TRAV10
R
IRIS
HIV+TB+

B

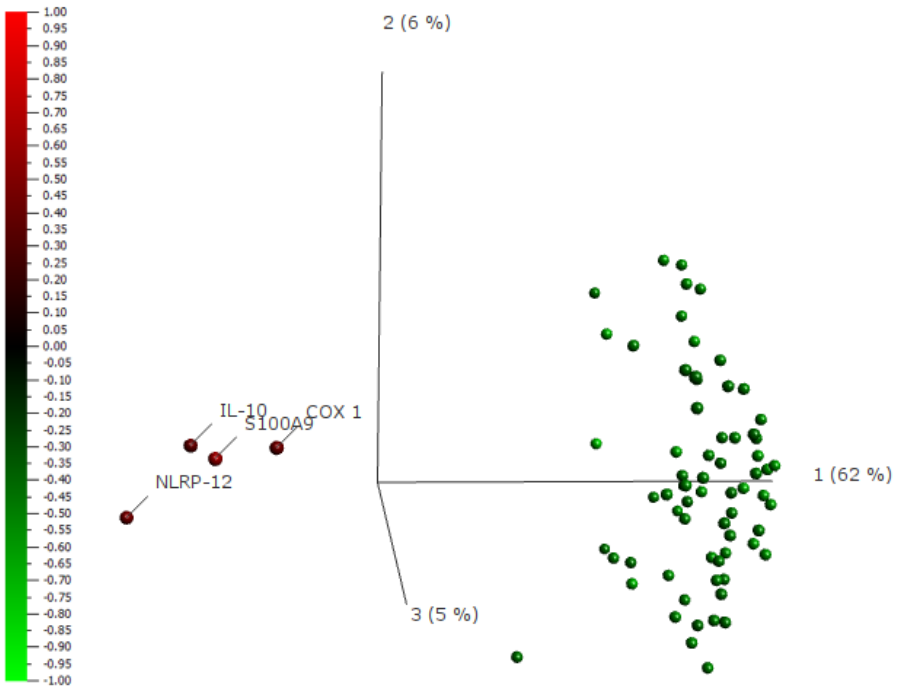

1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
-0.35
-0.40
-0.45
-0.50
-0.55
-0.60
-0.65
-0.70
-0.75
-0.80
-0.85
-0.90
-0.95
-1.00
2 (6 %)
IL-10
S100A9
COX 1
NLRP-12
1 (62 %)
3 (5 %)

Figure 2

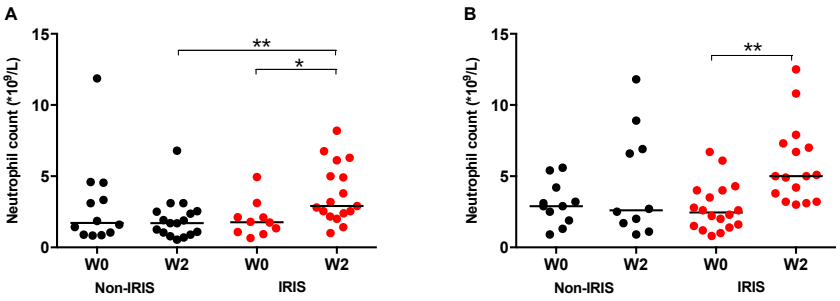


Figure 3

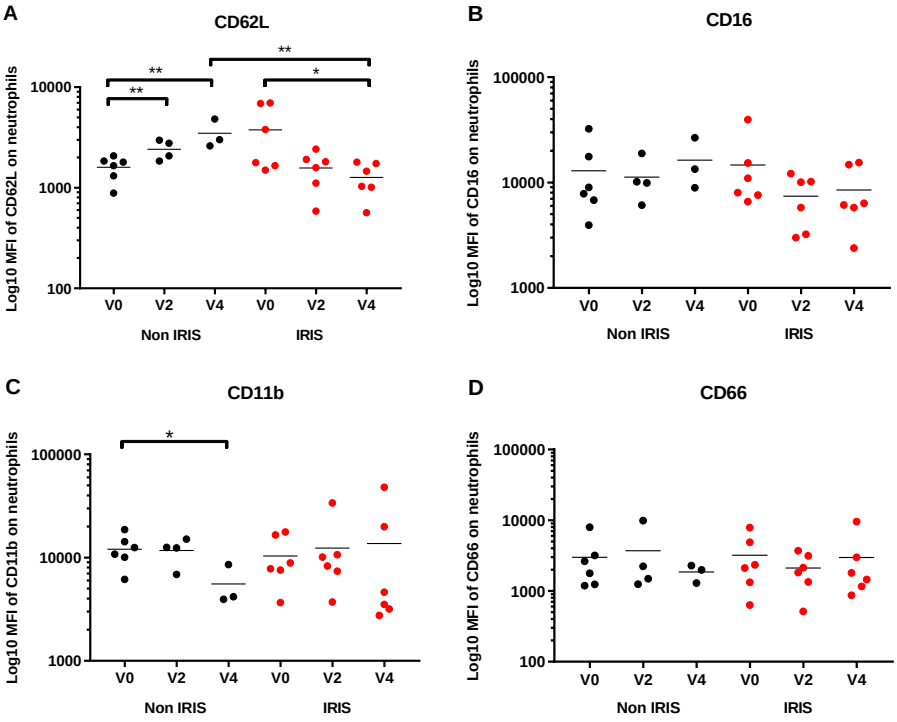

A
CD62L
Log10 MFI of CD62L on neutrophils
10000
1000
100
**
**
**
*
V0
V2
V4
V0
V2
V4
Non IRIS
IRIS
B
CD16
Log10 MFI of CD16 on neutrophils
100000
10000
1000
V0
V2
V4
V0
V2
V4
Non IRIS
IRIS
C
CD11b
Log10 MFI of CD11b on neutrophils
100000
10000
1000
*
V0
V2
V4
V0
V2
V4
Non IRIS
IRIS
D
CD66
Log10 MFI of CD66 on neutrophils
100000
10000
1000
100
V0
V2
V4
V0
V2
V4
Non IRIS
IRIS

Figure 4

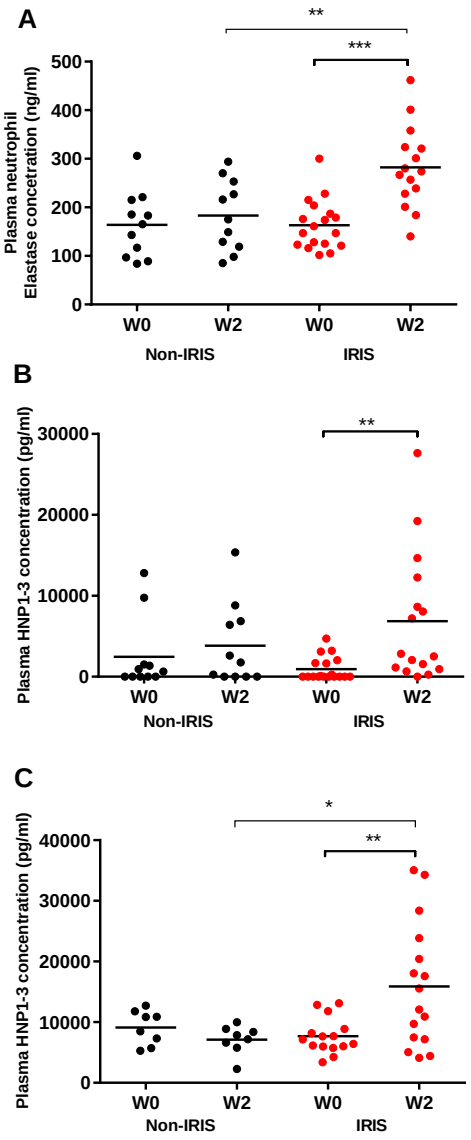
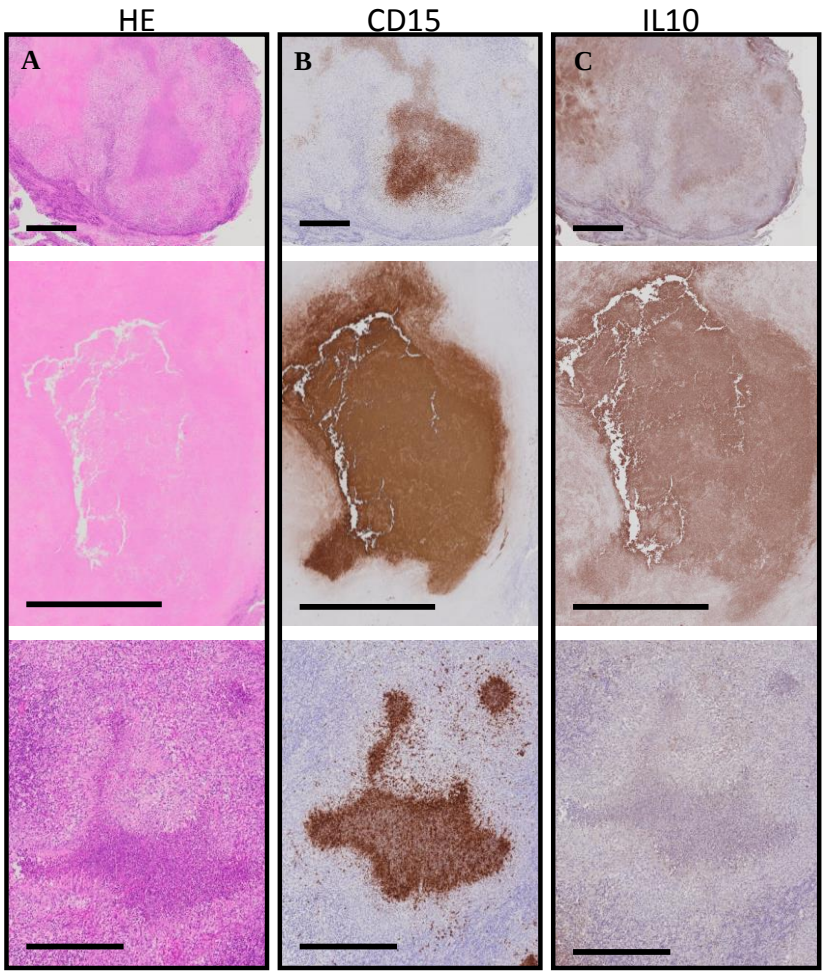

A
**
***
Plasma neutrophil Elastase concetration (ng/ml)
500
400
300
200
100
0
W0
W2
W0
W2
Non-IRIS
IRIS
B
**
Plasma HNP1-3 concentration (pg/ml)
30000
20000
10000
0
W0
W2
W0
W2
Non-IRIS
IRIS
C
*
**
Plasma HNP1-3 concentration (pg/ml)
40000
30000
20000
10000
0
W0
W2
W0
W2
Non-IRIS
IRIS

Figure 5



Supplementary Material

Supplementary Methods

RNA extraction

RNA was extracted from PBMC using Trizol (Invitrogen, Carlsbad, CA, USA) [1] coupled with glycogen as a carrier during RNA precipitation. The RNA cleanup protocol [2] was used to remove residual Trizol while concentrating RNA. RNA concentration and purity were determined using the Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, Delaware, USA). A cut-off of OD260nm/280nm ratio >1.8 ('pure RNA') was used for the downstream nCounter assays. In addition, RNA integrity (RIN >7) was assessed using a Bioanalyser 2100 (Agilent, Santa Clara, California, USA). An aliquot of 100ng of total RNA was used for NanoString nCounter analysis (www.nucleomics.be) according to the manufacturer's instructions and as described [3].

ELISA

Briefly, 100 µl of the standard, samples, or controls were transferred in duplicate into appropriate wells and the plate was incubated for one hour at room temperature. The plate was washed, 100 µl of diluted tracer added and incubated for one hour at room temperature. After another wash, 100 µl of diluted streptavidin-peroxidase was pipetted onto the plate and incubated at room temperature for an hour. The plate was washed and 100 µl of TMB substrate was added. The reaction was stopped with 100 µl of stop solution after a thirty-minute incubation at room temperature. The plate was read at 450 nm using a plate reader, following the manufacturer's instructions.

Immunohistochemistry of lymph node sections

Methods for immunohistochemistry were previously reported [4]. Briefly, patients with a diagnosis of TB lymphadenitis HIV+ IRIS+ (cases, n=3) were included into the study. TB-IRIS was defined using the INSHI criteria [5]. Formalin fixed paraffin embedded (FFPE) block sections (4-6 micron thickness) of LN biopsies underwent standard hematoxylin and eosin staining. Further sections underwent polyclonal rabbit anti-human IL-10 (1:2000; Abcam) or

monoclonal mouse anti-human CD15 (LeuM1, 1:50; Abcam) staining followed by either horseradish peroxidase–conjugated polyclonal goat anti-mouse or anti-rabbit (Dako) secondary antibodies (Dako). Chromogenic DAB staining (Liquid DAB+; Dako) was used to visualize the antibodies. The slides were mounted onto an Olympus VS120 Scanning Microscope (Olympus, Tokyo, Japan) and the respective objectives as indicated were used to capture the images.

References

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Supplementary Table 1: Baseline characteristics of included patients

Median Value (IQR)[a]

Cohort 1 (recruited for NanoString nCounter gene expression analysis

Characteristic	Non-IRIS Controls (n =17)	TB-IRIS Patients (n = 17)	p value[b]
Age, years	36 (28-40)	32 (30-43)	0.43
Sex (Male), No. (%)	9 (52.9)	10 (58.8)	>0.99
Temperature (^{0}C)	36.0 (35.5-36.8)	36.5 (35.7-37.1)	0.27
IRIS episode presentation, days	n/a	14 (10-15)	
CD4 count, cells/μL[c]	24 (12-57)	20 (11-54)	0.60
HIV viral load, log10[c]	5.52 (5.05-5.58)	5.36 (5.26-5.70)	0.56
Neutrophil count, $\times10^9$/L[c]	1.72 (0.93-4.24)	1.77 (1.04-2.37)	0.82
Monocyte count, $\times10^9$/L[c]	0.41 (0.27-0.44)	0.25 (0.18-0.52)	0.39
Lymphocyte count, $\times10^9$/L[c]	0.87 (0.63-1.36)	0.68 (0.44-0.90)	0.33
C-reactive protein, mg/L[c]	9.53 (3.71-27.39)	9.64 (5.30-31.01)	0.68
Antiretroviral regime: Zidovudine/Lamivudine/ Efavirenz, n (%)	13 (76.5)	10 (58.8)	
Zidovudine/Lamivudine/ Nevirapine, n (%)	1 (5.9)	1 (5.9)	
Stavudine/Lamivudine/ Efavirenz, n(%)	3 (17.6)	6 (35.3)	

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Median Value (IQR)[a]

Cohort 2 (recruited for neutrophil assays)

Characteristic	Non-IRIS Controls (n =11)	TB-IRIS Patients (n = 18)	p value[b]
Age, years	39 (31-43)	35 (29-40)	0.33
Sex (Female), No. (%)	4 (36)	7 (39)	>0.99
Temperature (^{0}C)	35.7 (35.3-36.5)	36.2 (36.0-36.5)	0.092
IRIS episode presentation, days	n/a	14 (10-15)	
CD4 count, cells/μL[c]	125 (68-154)	119 (90-188)	0.41
HIV viral load, log10[c]	5.40 (4.85-5.75)	5.80 (5.08-6.00)	0.18
Neutrophil count, $\times10^9$/L[c]	2.90 (1.90-4.20)	2.45 (1.48-4.00)	0.51

Monocyte count, $\times 10^9$/L[c]	0.24 (0.19 – 0.29)	0.32 (0.23 – 0.36)	0.07
Lymphocyte count, $\times 10^9$/L[c]	1.18 (0.83 – 1.48)	0.72 (0.52 – 1.36)	0.19
C-reactive protein, mg/L[c]	5.90 (2.50-26.70)	13.55 (5.70-42.80)	0.28
Antiretroviral regime: Tenofovir, Lamivudine/ Emtricitabine, Efavirenz, n (%)	11 (100)	18 (100)	

Abbreviations: IRIS, Immune Reconstitution Syndrome; CD4, Cluster of differentiation 4; TB, tuberculosis; HIV, human immunodeficiency virus; IQR, interquartile range.
[a] Values represent medians (IQRs) unless otherwise specified.
[b] P values were calculated for comparisons between groups, using the Mann Whitney U test for continuous variables and the Fisher exact test for categorical variables.
[c] Values presented are from time of ART initiation

Formatted Table

Supplementary Table 2: nCounter™ CodeSet Design for the gene expression study

Gene	Accession	NSID	Targeted Region	Target Sequence	Tm CP	Tm RP	PN(CP;RP)
ASC(PYCARD)	NM_013258.3	NM_013258.3:714	714-814	ATGCGGAAGCTCTTCAGTTTCAC ACCAGCCTGGAACTGGACCTGC AAGGACTTGCTCCTCCAGGCCCT AAGGGAGTCCCAGTCCTACCTG GTGGAGGACC	85	86	315895;215895
Caspase-1	NM_033292.2	NM_033292.2:575	575-675	ACAGGCATGACAATGCTGCTACA AAATCTGGGGTACAGCGTAGATG TGAAAAAAAATCTCACTGCTTCG GACATGACTACAGAGCTGGAGG CATTTGCAC	82	80	310120;210120
Caspase-5	NM_004347.1	NM_004347.1:580	580-680	TGCAATACAAAGTTTGATCACCT GCCTGCAAGGAATGGGGCTCAC TATGACATCGTGGGGATGAAAAG GCTGCTTCAAGGCCTGGGCTAC ACTGTGGTTG	80	81	301377;201377
CD14	NM_000591.2	NM_000591.2:885	885-985	GCCCAAGCACACTCGCCTGCCTT TTCCTGCGAACAGGTTCGCGCCT TCCCGGCCCTTACCAGCCTAGAC CTGTCTGACAATCCTGGACTGGG CGAACGCG	82	83	309575;209575
CD163	NM_004244.4	NM_004244.4:163 0	1630-1730	CATCTGTGATTCGGACTTCTCTC TGGAAGCTGCCAGCGTTCTATGC AGGGAATTACAGTGTGGCACAGT TGTCTCTATCCTGGGGGGAGCTC ACTTTGGA	83	82	309101;209101
COX 1(PTGS1)	NM_000962.2	NM_000962.2:700	700-800	ACCCCCAAGGCACCAACCTCATG TTTGCCTTCTTTGCACAACACTTC ACCCACCAGTTCTTCAAAACTTC TGGCAAGATGGGTCCTGGCTTCA CCAAGGC	81	79	306892;206892
COX 2 (PTGS2)	NM_000963.1	NM_000963.1:495	495-595	GCTACAAAAGCTGGGAAGCCTTC TCTAACCTCTCCTATTATACTAGA GCCCTTCCTCCTGTGCCTGATGA TTGCCCGACTCCCTTGGGTGTCA AAGGTAA	79	81	300048;200048
IFNg	NM_000619.2	NM_000619.2:970	970-1070	ATACTATCCAGTTACTGCCGGTT TGAAAATATGCCTGCAATCTGAG CCAGTGCTTTAATGGCATGTCAG ACAGAACTTGAATGTGTCAGGTG ACCCTGAT	76	79	301672;201672
IL-10	NM_000572.2	NM_000572.2:230	230-330	AAGGATCAGCTGGACAACTTGTT GTTAAAGGAGTCCTTGCTGGAGG ACTTTAAGGGTTACCTGGGTTGC CAAGCCTTGTCTGAGATGATCCA GTTTTACC	79	79	301231;201231
IL-18	NM_001562.2	NM_001562.2:48	48-148	GACAGTCAGCAAGGAATTGTCTC CCAGTGCATTTTGCCCTCCTGGC TGCCAACTCTGGCTGCTAAAGCG GCTGCCACCTGCTGCAGTCTACA CAGCTTCG	84	85	310105;210105
IL-1b	NM_000576.2	NM_000576.2:840	840-940	GGGACCAAAGGCGGCCAGGATA TAACTGACTTCACCATGCAATTT GTGTCTTCCTAAAGAGAGCTGTA CCCAGAGAGTCCTGTGCTGAATG TGGACTCAA	81	82	300980;200980
IL-6	NM_000600.1	NM_000600.1:220	220-320	TGACAAACAAATTCGGTACATCC TCGACGGCATCTCAGCCCTGAG AAAGGAGACATGTAACAAGAGTA	79	80	300038;200038

				ACATGTGTGAAAGCAGCAAAGAGGCACTGGCA			
IL-7	NM_000880.2	NM_000880.2:38	38-138	AATAACCCAGCTTGCGTCCTGCACACTTGTGGCTTCCGTGCACACATTAACAACTCATGGTTCTAGCTCCCAGTCGCCAAGCGTTGCCAAGGCGTTGAGA	83	85	312873;212873
IL-7R (CD127)	NM_002185.2	NM_002185.2:1610	1610-1710	TTGCTTTGACCACTCTTCCTGAGTTCAGTGGCACTCAACATGAGTCAAGAGCATCCTGCTTCTACCATGTGGATTTGGTCACAAGGTTTAAGGTGACCCA	78	79	302928;202928
IL-8 (CXCL8)	NM_000584.2	NM_000584.2:25	25-125	ACAGCAGAGCACACAAGCTTCTAGGACAAGAGCCAGGAAGAAACCACCGGAAGGAACCATCTCACTGTGTGTAAACATGACTTCCAAGCTGGCCGTGGCT	82	81	300981;200981
IL-10Ra	NM_001558.2	NM_001558.2:150	150-250	TGCCCAGCCCTCCGTCTGTGTGGTTTGAAGCAGAATTTTTCCACCACATCCTCCACTGGACACCCATCCCAAATCAGTCTGAAAGTACCTGCTATGAAGT	79	80	310129;210129
IL-10Rb	NM_000628.3	NM_000628.3:1760	1760-1860	TTCTACCAGATTATGGATGGACTGATCTGAAAATCGACCTCAACTCAAGGGTGGTCAGCTCAATGCTACACAGAGCACGGACTTTTGGATTCTTTGCAGT	82	81	316760;216760
IL-12A	NM_000882.2	NM_000882.2:775	775-875	CTTTCTAGATCAAAACATGCTGGCAGTTATTGATGAGCTGATGCAGGCCCTGAATTTCAACAGTGAGACTGTGCCACAAAAATCCTCCCTTGAAGAACCG	82	82	311545;211545
IL-12RB1	NM_005535.1	NM_005535.1:1292	1292-1392	AGGAAAAGTGTTACTACATTACCATCTTTGCCTCTGCGCACCCCGAGAAGCTCACCTTGTGGTCTACGGTCCTGTCCACCTACCACTTTGGGGGCAATGC	83	86	315839;215839
IL-12RB2	NM_001559.2	NM_001559.2:1315	1315-1415	CCTCCGTGGGACATTAGAATCAAATTTCAAAAGGCTTCTGTGAGCAGATGTACCCTTTATTGGAGAGATGAGGGACTGGTACTGCTTAATCGACTCAGAT	79	83	316775;216775
IP-10 (CXCL-10)	NM_001565.1	NM_001565.1:40	40-140	GCAGAGGAACCTCCAGTCTCAGCACCATGAATCAAACTGCGATTCTGATTTGCTGCCTTATCTTTCTGACTCTAAGTGGCATTCAAGGAGTACCTCTCTC	80	78	301917;201917
IPAF(NLRC4)	NM_021209.3	NM_021209.3:840	840-940	GCTCTGACCAAGTTCAAATTCGTCTTCTTCCTCCGTCTCAGCAGGGCCCAGGGTGGACTTTTTGAAACCCTCTGTGATCAACTCCTGGATATACCTGGCA	78	80	300813;200813
MCP-1 (CCL2)	NM_002982.3	NM_002982.3:0	0-100	GAGGAACCGAGAGGCTGAGACTAACCCAGAAACATCCAATTCTCAAACTGAAGCTCGCACTCTCGCCTCCAGCATGAAAGTCTCTGCCGCCCTTCTGTGC	80	82	301314;201314
NALP-1(NLRP1)	NM_033004.2	NM_033004.2:2135	2135-2235	CCTGATGCAGCAGATGAAGCGGAAGGAAAAACTCACACTGACTTCCAAGACCACCACAACCCTCTGTCTACATTACCTTGCCCAGGCTCTCCAAGCTCAG	80	82	301508;201508
NALP-3(NLRP3)	NM_001079821.2	NM_001079821.2:415	415-515	AGTGGGGTTCAGATAATGCACGTGTTTCGAATCCCACTGTGATATG	79	82	307148;207148

				CCAGGAAGACAGCATTGAAGAG GAGTGGATGGGTTTACTGGAGTA CCTTTCGAG			
PGDH(HPGD)	NM_001145816.1	NM_001145816.1:570	570-670	AGGTGAAGGCGGCATCATTATCA ATATGTCATCTTTAGCAGGACTC ATGCCCGTTGCACAGCAGCCGG TTTATTGTGCTTCAAAGCATGGC ATAGTTGGA	80	80	347104;247104
Pypaf-7 (NLRP12)	NM_033297.1	NM_033297.1:1030	1030-1130	TTCAAGCAGACCAGAGAGGACC GTTCTGCTGGACGCCTACAGTGA ACATCTGGCAGCGGCCCTGTGC ACCAATCCAAACCTGATAGAGCT GTCTCTGTAC	83	82	301511;201511
S100A9	NM_002965.2	NM_002965.2:75	75-175	AACATAGAGACCATCATCAACAC CTTCCACCAATACTCTGTGAAGC TGGGGCACCCAGACACCCTGAA CCAGGGGGAATTCAAAGAGCTG GTGCGAAAAG	81	82	300798;200798
SLAM (SLAMF1)	NM_003037.2	NM_003037.2:580	580-680	GTGTCTCTTGATCCATCCGAAGC AGGCCCTCCACGTTATCTAGGAG ATCGCTACAAGTTTTATCTGGAG AATCTCACCCTGGGGATACGGG AAAGCAGGA	82	81	308992;208992
SOCS1	NM_003745.1	NM_003745.1:1025	1025-1125	TTAACTGTATCTGGAGCCAGGAC CTGAACTCGCACCTCCTACCTCT TCATGTTTACATATACCCAGTATC TTTGCACAAACCAGGGGTTGGG GGAGGGTC	83	82	301664;201664
SOCS3	NM_003955.3	NM_003955.3:1870	1870-1970	GGAGGATGGAGGAGACGGGACA TCTTTCACCTCAGGCTCCTGGTA GAGAAGACAGGGGATTCTACTCT GTGCCTCCTGACTATGTCTGGCT AAGAGATTC	82	82	301356;201356
TLR1	NM_003263.3	NM_003263.3:545	545-645	TCAACCAGGAATTGGAATACTTG GATTTGTCCCACAACAAGTTGGT GAAGATTTCTTGCCACCCTACTG TGAACCTCAAGCACTTGGACCTG TCATTTAA	80	79	315332;215332
TLR2	NM_003264.3	NM_003264.3:180	180-280	CTGCTTTCAACTGGTAGTTGTGG GTTGAAGCACTGGACAATGCCAC ATACTTTGTGGATGGTGTGGGTC TTGGGGGTCATCATCAGCCTCTC CAAGGAAG	80	83	300995;200995
TLR4	NM_138554.2	NM_138554.2:2570	2570-2670	ACTCAGAAAAGCCCTGCTGGATG GTAAATCATGGAATCCAGAAGGA ACAGTGGGTACAGGATGCAATTG GCAGGAAGCAACATCTATCTGAA GAGGAAAA	79	79	306332;206332
TNFa	NM_000594.2	NM_000594.2:1010	1010-1110	AGCAACAAGACCACCACTTCGAA ACCTGGGATTCAGGAATGTGTGG CCTGCACAGTGAAGTGCTGGCA ACCACTAAGAATTCAAACTGGGG CCTCCAGAA	83	81	301235;201235
TRAC	TRAC.1	TRAC.1:126	126-226	ATATCACAGACAAAACTGTGCTA GACATGAGGTCTATGGACTTCAA GAGCAACAGTGCTGTGGCCTGG AGCAACAAATCTGACTTTGCATG TGCAAACGC	85	86	349448;249448
TRAV1-1	TRAV1_1.1	TRAV1_1.1:243	243-343	AGTCGCTCTGATAGTTATGGTTA CCTCCTTCTACAGGAGCTCCAGA TGAAAGACTCTGCCTCTTACTTC TGCGCTGTGAGAGACACAGTGA CTATGAGGC	82	85	349416;249416

TRAV1-2	TRAV1_2.1	TRAV1_2.1:187	187-287	TGTCTTACAATGTTCTGGATGGT TTGGAGGAGAAAGGTCGTTTTTC TTCATTCCTTAGTCGGTCTAAAG GGTACAGTTACCTCCTTTTGAAG GAGCTCCA	73	79	349409;249409
TRAV2	TRAV2.1	TRAV2.1:67	67-167	CAGAAAGCAAGGACCAAGTGTTT CAGCCTTCCACAGTGGCATCTTC AGAGGGAGCTGTGGTGGAAATC TTCTGTAATCACTCTGTGTCCAAT GCTTACAA	84	81	349453;249453
TRAV3	TRAV3.1	TRAV3.1:163	163-263	ATGTTCAATACCCCAACCGAGGC CTCCAGTTCCTTCTGAAATACAT CACAGGGGATAACCTGGTTAAAG GCAGCTATGGCTTTGAAGCTGAA TTTAACAA	81	82	349426;249426
TRAV4	TRAV4.1	TRAV4.1:159	159-259	CAACAGTTTCCCAGCCAAGGACC ACGATTTATTATTCAAGGATACAA GACAAAAGTTACAAACGAAGTGG CCTCCCTGTTTATCCCTGCCGAC AGAAAGT	78	83	349463;249463
TRAV5	TRAV5.1	TRAV5.1:169	169-269	AGCAAGAACCTGGAGCAGGTCT CCAGTTGCTGACGTATATTTTTTC AAATATGGACATGAAACAAGACC AAAGACTCACTGTTCTATTGAATA AAAAGGA	78	81	349442;249442
TRAV6	TRAV6.1	TRAV6.1:128	128-228	CAACTATACAAACTATTCCCCAG CATACTTACAGTGGTACCGACAA GATCCAGGAAGAGGCCCTGTTTT CTTGCTACTCATACGTGAAAATG AGAAAGAA	81	81	349443;249443
TRAV7	TRAV7.1	TRAV7.1:136	136-236	ACTCTGTCAGTCGTTTTAACAATT TGCAGTGGTACAGGCAAAATACA GGGATGGGTCCCAAACACCTATT ATCCATGTATTCAGCTGGATATG AGAAGCA	78	82	349464;249464
TRAV8	TRAV8_2.1	TRAV8_2.1:207	207-307	ACATCAGCGGCCACCCTGGTTAA AGGCATCAACGGTTTTGAGGCTG AATTTAAGAAGAGTGAAACCTCC TTCCACCTGACGAAACCCTCAGC CCATATGA	84	85	349404;249404
TRAV8-1	TRAV8_1.1	TRAV8_1.1:109	109-209	CACTGGAGTTGGGATGCAACTAT TCCTATGGTGGAACTGTTAATCT CTTCTGGTATGTCCAGTACCCTG GTCAACACCTTCAGCTTCTCCTC AAGTACTT	80	82	349465;249465
TRAV8-3	TRAV8_3.1	TRAV8_3.1:49	49-149	CTGCCAGAGCCCAGTCAGTGAC CCAGCCTGACATCCACATCACTG TCTCTGAAGGAGCCTCACTGGAG TTGAGATGTAACTATTCCTATGG GGCAACACC	85	84	349419;249419
TRAV8-6	TRAV8_6.1	TRAV8_6.1:207	207-307	TTATCAGGATCCACCCTGGTTAA AGGCATCAACGGTTTTGAGGCTG AATTTAACAAGAGTCAAACTTCCT TCCACTTGAGGAAACCCTCAGTC CATATAA	79	80	349412;249412
TRAV9-1	TRAV9_1.1	TRAV9_1.1:41	41-141	GTTTGGGGGAATCAATGGAGATT CAGTGGTCCAGACAGAAGGCCA AGTGCTCCCCTCTGAAGGGGATT CCCTGATTGTGAACTGCTCCTAT GAAACCACA	85	84	349431;249431
TRAV9-2	TRAV9_2.1	TRAV9_2.1:4	4-104	ACTATTCTCCAGGCTTAGTATCT CTGATACTCTTACTGCTTGGAAG AACCCGTGGAAATTCAGTGACCC	80	86	349466;249466

				AGATGGAAGGGCCAGTGACTCT CTCAGAAGA			
TRAV10	TRAV10.1	TRAV10.1:164	164-264	GTGGTATAAGCAAGATACTGGGA GAGGTCCTGTTTCCCTGACAATC ATGACTTTCAGTGAGAACACAAA GTCGAACGGAAGATATACAGCAA CTCTGGAT	84	82	349449;249449
TRAV12-1	TRAV12_1.1	TRAV12_1.1:140	140-240	CAACAGTGCTTCTCAGTCTTTCTT CTGGTACAGACAGGATTGCAGG AAAGAACCTAAGTTGCTGATGTC CGTATACTCCAGTGGTAATGAAG ATGGAAGG	80	83	349410;249410
TRAV12-2	TRAV12_2.1	TRAV12_2.1:128	128-228	CAACTGCACTTACAGTGACCGAG GTTCCCAGTCCTTCTTCTGGTAC AGACAATATTCTGGGAAAAGCCC TGAGTTGATAATGTTCATATACTC CAATGGT	84	77	349415;249415
TRAV12-3	TRAV12_3.1	TRAV12_3.1:100	100-200	GTGTTCCAGAGGGAGCCATTGTT TCTCTCAACTGCACTTACAGCAA CAGTGCTTTTCAATACTTCATGTG GTACAGACAGTATTCCAGAAAAG GCCCTGA	82	79	349411;249411
TRAV13-1	TRAV13_1.1	TRAV13_1.1:233	233-333	CCAACGAATTGCTGTTACATTGA ACAAGACAGCCAAACATTTCTCC CTGCACATCACAGAGACCCAACC TGAAGACTCGGCTGTCTACTTCT GTGCAGCA	80	86	349408;249408
TRAV13-2	TRAV13_2.1	TRAV13_2.1:229	229-329	GGCAAGGCCAAAGAGTCACCGT TTTATTGAATAAGACAGTGAAACA TCTCTCTCTGCAAATTGCAGCTA CTCAACCTGGAGACTCAGCTGTC TACTTTTG	80	82	349450;249450
TRAV14	TRAV14.1	TRAV14.1:232	232-332	ATGCAACAGAAGGTCGCTACTCA TTGAATTTCCAGAAGGCAAGAAA ATCCGCCAACCTTGTCATCTCCG CTTCACAACTGGGGGACTCAGCA ATGTATTT	82	83	349451;249451
TRAV16	TRAV16.1	TRAV16.1:223	223-323	TCAAAGGCTTCACTGCTGACCTT AACAAAGGCGAGACATCTTTCCA CCTGAAGAAACCATTTGCTCAAG AGGAAGACTCAGCCATGTATTAC TGTGCTCT	84	83	349452;249452
TRAV17	TRAV17.1	TRAV17.1:181	181-281	GAGGCCTTGTCCACCTAATTTTA ATACGTTCAAATGAAAGAGAGAA ACACAGTGGAAGATTAAGAGTCA CGCTTGACACTTCCAAGAAAAGC AGTTCCTT	78	80	349437;249437
TRAV18	TRAV18.1	TRAV18.1:198	198-298	CTGAAAAGTTCAGAAAACCAGGA GACGGACAGCAGAGGTTTTCAG GCCAGTCCTATCAAGAGTGACAG TTCCTTCCACCTGGAGAAGCCCT CGGTGCAGC	85	85	349425;249425
TRAV19	TRAV19.1	TRAV19.1:113	113-213	TGTGACCTTGGACTGTGTGTATG AAACCCGTGATACTACTTATTACT TATTCTGGTACAAGCAACCACCA AGTGGAGAATTGGTTTTCCTTATT CGTCGG	77	78	349438;249438
TRAV20	TRAV20.1	TRAV20.1:136	136-236	ACACAGTCAGCGGTTTAAGAGG GCTGTTCTGGTATAGGCAAGATC CTGGGAAAGGCCCTGAATTCCTC TTCACCCTGTATTCAGCTGGGGA AGAAAAGGA	85	85	349417;249417
TRAV21	TRAV21.1	TRAV21.1:177	177-277	GGGAAAGGTCTCACATCTCTGTT GCTTATTCAGTCAAGTCAGAGAG	82	83	349454;249454

				AGCAAACAAGTGGAAGACTTAATGCCTCGCTGGATAAATCATCAGGACGTAGTA			
TRAV22	TRAV22.1	TRAV22.1:113	113-213	TTCCACGCTGCGGTGCAATTTTTCTGACTCTGTGAACAATTTGCAGTGGTTTCATCAAAACCCTTGGGGACAGCTCATCAACCTGTTTTACATTCCCTCA	77	78	349455;249455
TRAV23	TRAV23_DV6.1	TRAV23_DV6.1:70	70-170	AGGAGAAAAGTGACCAGCAGCAGGTGAAACAAAGTCCTCAATCTTTGATAGTCCAGAAAGGAGGGATTTCAATTATAAACTGTGCTTATGAGAACACTGC	84	81	349439;249439
TRAV24	TRAV24.1	TRAV24.1:84	84-184	AGTCCTCAGTCACTGCATGTTCAGGAGGGAGACAGCACCAATTTCACCTGCAGCTTCCCTTCCAGCAATTTTTATGCCTTACACTGGTACAGATGGGAAA	85	78	349456;249456
TRAV25	TRAV25.1	TRAV25.1:99	99-199	GGAGAGGACTTCACCACGTACTGCAATTCCTCAACTACTTTAAGCAATATACAGTGGTATAAGCAAAGGCCTGGTGGACATCCCGTTTTTTTGATACAGT	81	79	349457;249457
TRAV26_1	TRAV26_1.1	TRAV26_1.1:92	92-192	AGGAAGAGCTGCAAACCTGCCTTGTAATCACTCTACCATCAGTGGAAATGAGTATGTGTATTGGTATCGACAGATTCACTCCCAGGGGCCACAGTATATC	84	81	349458;249458
TRAV26_2	TRAV26_2.1	TRAV26_2.1:25	25-125	TACTCCTATCTTTGGGTATTATGGGTGATGCTAAGACCACACAGCCAAATTCAATGGAGAGTAACGAAGAAGAGCCTGTTCACTTGCCTTGTAACCACTC	82	84	349459;249459
TRAV27	TRAV27.1	TRAV27.1:69	69-169	CAGAGCCCTCAGTTTCTAAGCATCCAAGAGGGAGAAAATCTCACTGTGTACTGCAACTCCTCAAGTGTTTTTTCCAGCTTACAATGGTACAGACAGGAGC	83	80	349460;249460
TRAV29	TRAV29_DV5.1	TRAV29_DV5.1:0	0-100	ATGGCCATGCTCCTGGGGGCATCAGTGCTGATTCTGTGGCTTCAGCCAGACTGGGTAAACAGTCAACAGAAGAATGATGACCAGCAAGTTAAGCAAAATT	85	82	349418;249418
TRAV30	TRAV30.1	TRAV30.1:236	236-336	AAAAATATCTGCTTCATTTAATGAAAAAAAGCAGCAAAGCTCCCTGTACCTTACGGCCTCCCAGCTCAGTTACTCAGGAACCTACTTCTGCGGCACAGAG	78	86	349440;249440
TRAV34	TRAV34.1	TRAV34.1:169	169-269	AAAAGTATGGTGAAGGTCTTATCTTCTTGATGATGCTACAGAAAGGTGGGGAAGAGAAAAGTCATGAAAAGATAACTGCCAAGTTGGATGAGAAAAAGCA	80	84	349427;249427
TRAV35	TRAV35.1	TRAV35.1:216	216-316	TTGACCTCAAATGGAAGACTGACTGCTCAGTTTGGTATAACCAGAAAGGACAGCTTCCTGAATATCTCAGCATCCATACCTAGTGATGTAGGCATCTACT	84	81	349461;249461
TRAV36	TRAV36_DV7.1	TRAV36_DV7.1:172	172-272	AGCAGGAAAAGAAAGCTCCCACATTTCTATTTATGCTAACTTCAAGTGGAATTGAAAAGAAGTCAGGAAGACTAAGTAGCATATTAGATAAGAAAGAACT	78	79	349428;249428

TRAV38	TRAV38_1.1	TRAV38_1.1:218	218-318	TTATAAGCAACAGAATGCAACGG AGAATCGTTTCTCTGTGAACTTC CAGAAAGCAGCCAAATCCTTCAG TCTCAAGATCTCAGACTCACAGC TGGGGGAC	78	84	349429;249429
TRAV39	TRAV39.1	TRAV39.1:62	62-162	AGTGGAACAAAACCCTCTGTTCC TGAGCATGCAGGAGGGAAAAAA CTATACCATCTACTGCAATTATTC AACCACTTCAGACAGACTGTATT GGTACAGG	85	79	349462;249462
TRAV40	TRAV40.1	TRAV40.1:149	149-249	TTTCTGGTATGTGGAATACCCCA GCAAACCTCTGCAGCTTCTTCAG AGAGAGACAATGGAAAACAGCAA AAACTTCGGAGGCGGAAATATTA AAGACAAA	83	83	349441;249441
TRAV41	TRAV41.1	TRAV41.1:236	236-336	AAGATTAATTGCCACAATAAACAT ACAGGAAAAGCACAGCTCCCTG CACATCACAGCCTCCCATCCCAG AGACTCTGCCGTCTACATCTGTG CTGTCAGA	84	86	349430;249430
TRBC1	TRBC1.1	TRBC1.1:29	29-129	GTCGCTGTGTTTGAGCCATCAGA AGCAGAGATCTCCCACACCCAAA AGGCCACACTGGTGTGCCTGGC CACAGGCTTCTTCCCCGACCACG TGGAGCTGA	86	86	349413;249413
TRBV2	TRBV2.1	TRBV2.1:221	221-321	AGAGAAGTCTGAAATATTCGATG ATCAATTCTCAGTTGAAAGGCCT GATGGATCAAATTTCACTCTGAA GATCCGGTCCACAAAGCTGGAG GACTCAGCC	77	85	349446;249446
TRBV3-1	TRBV3_1.1	TRBV3_1.1:228	228-328	GAAACAGTTCCAAATCGCTTCTC ACCTAAATCTCCAGACAAAGCTC ACTTAAATCTTCACATCAATTCCC TGGAGCTTGGTGACTCTGCTGTG TATTTCT	79	77	349474;249474
TRBV4	TRBV4_2.1	TRBV4_2.1:244	244-344	GCTTCTCACCTGAATGCCCCAAC AGCTCTCACTTATTCCTTCACCTA CACACCCTGCAGCCAGAAGACT CGGCCCTGTATCTCTGTGCCAGC AGCCAAGA	83	87	349402;249402
TRBV5	TRBV5_8.1	TRBV5_8.1:263	263-363	CCCTAATTATAGCTCTGAGCTGA ATGTGAACGCCTTGGAGCTGGA GGACTCGGCCCTGTATCTCTGTG CCAGCAGCTTGG	80	87	349401;249401
TRBV5-1	TRBV5_1.1	TRBV5_1.1:172	172-272	CAGGACAGGGCCTTCAGTTCCTC TTTGAATACTTCAGTGAGACACA GAGAAACAAAGGAAACTTCCCTG GTCGATTCTCAGGGCGCCAGTTC TCTAACTC	83	84	349475;249475
TRBV6	TRBV6_1.1	TRBV6_1.1:55	55-155	TGAATGCTGGTGTCACTCAGACC CCAAAATTCCAGGTCCTGAAGAC AGGACAGAGCATGACACTGCAG TGTGCCCAGGATATGAACCATAA CTCCATGTA	79	88	349400;249400
TRBV6-4	TRBV6_4.1	TRBV6_4.1:102	102-202	GGACGGAGCATGACACTGAGAT GTACCCAGGATATGAGACATAAT GCCATGTACTGGTATAGACAAGA TCTAGGACTGGGGCTAAGGCTC ATCCATTATT	86	84	349476;249476
TRBV7	TRBV7_2.1	TRBV7_2.1:77	77-177	CCCCAGTAACAAGGTCACAGAGA AGGGAAAGGATGTAGAGCTCAG GTGTGATCCAATTTCAGGTCATA CTGCCCTTTACTGGTACCGACAG AGCCTGGGG	85	85	349424;249424

TRBV7-6	TRBV7_6.1	TRBV7_6.1:44	44-144	GACAGATCACACAGGTGCTGGA GTCTCCCAGTCTCCCAGGTACAA AGTCACAAAGAGGGGACAGGAT GTAGCTCTCAGGTGTGATCCAAT TTCGGGTCAT	86	85	349403;249403
TRBV7-9	TRBV7_9.1	TRBV7_9.1:144	144-244	AACCGCCTTTATTGGTACCGACA GACCCTGGGGCAGGGCCCAGAG TTTCTGACTTACTTCCAGAATGAA GCTCAACTAGAAAAATCAAGGCT GCTCAGTG	85	83	349414;249414
TRBV9	TRBV9.1	TRBV9.1:180	180-280	GGCCTCCAGTTCCTCATTCAGTA TTATAATGGAGAAGAGAGAGCAA AAGGAAACATTCTTGAACGATTC TCCGCACAACAGTTCCCTGACTT GCACTCTG	82	79	349435;249435
TRBV10-2	TRBV10_2.1	TRBV10_2.1:69	69-169	ACCCAGAGCCCAAGATACAAGAT CACAGAGACAGGAAGGCAGGTG ACCTTGATGTGTCACCAGACTTG GAGCCACAGCTATATGTTCTGGT ATCGACAAG	86	83	349420;249420
TRBV10-3	TRBV10_3.1	TRBV10_3.1:179	179-279	TGGGCTGAGGCTGATCCATTACT CATATGGTGTTAAAGATACTGAC AAAGGAGAAGTCTCAGATGGCTA TAGTGTCTCTAGATCAAAGACAG AGGATTTC	83	81	349421;249421
TRBV11	TRBV11_1.1	TRBV11_1.1:225	225-325	GATTCACAGTTGCCTAAGGATCG ATTTTCTGCAGAGAGGCTCAAAG GAGTAGACTCCACTCTCAAGATC CAGCCTGCAGAGCTTGGGGACT CGGCCATGT	81	84	349423;249423
TRBV12	TRBV12_3.1	TRBV12_3.1:162	162-262	AGACAGACCATGATGCGGGGAC TGGAGTTGCTCATTTACTTTAACA ACAACGTTCCGATAGATGATTCA GGGATGCCCGAGGATCGATTCT CAGCTAAGA	83	81	349422;249422
TRBV12-5	TRBV12_5.1	TRBV12_5.1:116	116-216	AATGAGATGTCAGCCAATTTTAG GCCACAATACTGTTTTCTGGTAC AGACAGACCATGATGCAAGGACT GGAGTTGCTGGCTTACTTCCGCA ACCGGGCT	78	86	349432;249432
TRBV13	TRBV13.1	TRBV13.1:91	91-191	CTGGAGTCATCCAGTCCCCAAGA CATCTGATCAAAGAAAAGAGGGA AACAGCCACTCTGAAATGCTATC CTATCCCTAGACACGACACTGTC TACTGGTA	85	85	349444;249444
TRBV14	TRBV14.1	TRBV14.1:244	244-344	ATCGATTCTTAGCTGAAAGGACT GGAGGGACGTATTCTACTCTGAA GGTGCAGCCTGCAGAACTGGAG GATTCTGGAGTTTATTTCTGTGC CAGCAGCCA	83	85	349467;249467
TRBV15	TRBV15.1	TRBV15.1:31	31-131	TTTGTCTCCTTGGAACAGGTCAT GGGGATGCCATGGTCATCCAGA ACCCAAGATACCAGGTTACCCAG TTTGGAAAGCCAGTGACCCTGAG TTGTTCTCA	85	82	349468;249468
TRBV18	TRBV18.1	TRBV18.1:150	150-250	GTTTACTGGTATCGGCAGCTCCC AGAGGAAGGTCTGAAATTCATGG TTTATCTCCAGAAAGAAAATATCA TAGATGAGTCAGGAATGCCAAAG GAACGAT	80	83	349445;249445
TRBV19	TRBV19.1	TRBV19.1:166	166-266	AGGACCCAGGGCAAGGGCTGAG ATTGATCTACTACTCACACATAGT AAATGACTTTCAGAAAGGAGATA	85	85	349469;249469

				TAGCTGAAGGGTACAGCGTCTCT CGGGAGAA			
TRBV20	TRBV20_1.1	TRBV20_1.1:41	41-141	TGGTGCTGTCGTCTCTCAACATC CGAGCAGGGTTATCTGTAAGAGT GGAACCTCTGTGAAGATCGAGTG CCGTTCCCTGGACTTTCAGGCCA CAACTATG	84	84	349470;249470
TRBV24	TRBV24_1.1	TRBV24_1.1:24	24-124	GGGGCCTTTTATCTCCTGGGAAC AGGGTCCATGGATGCTGATGTTA CCCAGACCCCAAGGAATAGGAT CACAAAGACAGGAAAGAGGATTA TGCTGGAAT	85	86	349433;249433
TRBV25	TRBV25_1.1	TRBV25_1.1:161	161-261	TCAACAAGATCCAGGAATGGAAC TACACCTCATCCACTATTCCTATG GAGTTAATTCCACAGAGAAGGGA GATCTTTCCTCTGAGTCAACAGT CTCCAGA	85	81	349471;249471
TRBV27	TRBV27.1	TRBV27.1:163	163-263	GACAAGACCCAGGGCTGGGCTT AAGGCAGATCTACTATTCAATGA ATGTTGAGGCGACTGATAAGGGA GATGTTCCTGAAGGGTACAAAGT CTCTCGAAA	86	86	349472;249472
TRBV28	TRBV28.1	TRBV28.1:45	45-145	GTAGGCCTCGTAGATGTGAAAGT AACCCAGAGCTCGAGATATCTAG TCAAAAGGACGGGAGAGAAAGTT TTTCTGGAATGTGTCCAGGATAT GGACCATG	83	83	349434;249434
TRBV29	TRBV29_1.1	TRBV29_1.1:52	52-152	TCATCTCTCAAAAGCCAAGCAGG GATATCTGTCAACGTGGAACCTC CCTGACGATCCAGTGTCAAGTCG ATAGCCAAGTCACCATGATGTTC TGGTACCG	86	82	349473;249473
TRBV30	TRBV30.1	TRBV30.1:192	192-292	TTCTACTCCGTTGGTATTGGCCA GATCAGCTCTGAGGTGCCCCAG AATCTCTCAGCCTCCAGACCCCA GGACCGGCAGTTCATCCTGAGTT CTAAGAAGC	84	86	349447;249447
TRDC	TRDC.1	TRDC.1:773	773-873	AGGCTCTGCTCAACTGAGCACTA GATTTGCTACAAACCAGCATCAT CTTCTTCCTCCTGTCCTCACGGC TTGTCCCACCCTCTATGTTCACTT CAGGAGC	84	84	349477;249477
TRDV1	TRDV1.1	TRDV1.1:225	225-325	CAGAATGCAAAAAGTGGTCGCTA TTCTGTCAACTTCAAGAAAGCAG CGAAATCCGTCGCCTTAACCATT TCAGCCTTACAGCTAGAAGATTC AGCAAAGT	80	79	349478;249478
TRDV2	TRDV2.1	TRDV2.1:213	213-313	AAGGACATCTATGGCCCTGGTTT CAAAGACAATTTCCAAGGTGACA TTGATATTGCAAAGAACCTGGCT GTACTTAAGATACTTGCACCATC AGAGAGAG	80	83	349479;249479
TRDV3	TRDV3.1	TRDV3.1:203	203-303	GGATAACAGCAGATCAGAAGGT GCAGATTTTACTCAAGGACGGTT TTCTGTGAAACACATTCTGACCC AGAAAGCCTTTCACTTGGTGATC TCTCCAGTA	79	81	349480;249480
TRGC1	TRGC1.1	TRGC1.1:182	182-282	ACCATGAAGACTAACGACACATA CATGAAATTTAGCTGGTTAACGG TGCCAGAAAAGTCACTGGACAAA GAACACAGATGTATCGTCAGACA TGAGAATA	83	83	349407;249407
TRGV2	TRGV2.1	TRGV2.1:59	59-159	CAACTTGGAAGGGAGAACGAAG TCAGTCATCAGGCAGACTGGGTC	85	83	349405;249405

				ATCTGCTGAAATCACTTGTGATC TTGCTGAAGGAAGTAACGGCTAC ATCCACTGG			
TRGV3	TRGV3.1	TRGV3.1:210	210-310	TCCACCGCAAGGGATGTGTTGG AATCAGGACTCAGTCCAGGAAAG TATTATACTCATACACCCAGGAG GTGGAGCTGGATATTGAGACTGC AAAATCTAA	85	85	349406;249406
TRGV8	TRGV8.1	TRGV8.1:232	232-332	AATCAGGAATCAGTCGAGAAAAG TATCATACTTATGCAAGCACAGG GAAGAGCCTTAAATTTATACTGG AAAATCTAATTGAACGTGACTCT GGGGTCTA	82	77	349436;249436
TRGV9	TRGV9.1	TRGV9.1:66	66-166	CACCTAGAGCAACCTCAAATTTC CAGTACTAAAACGCTGTCAAAAA CAGCCCGCCTGGAATGTGTGGT GTCTGGAATAACAATTTCTGCAA CATCTGTAT	82	80	349481;249481
CD3D	NM_000732.4	NM_000732.4:110	110-210	TATCTACTGGATGAGTTCCGCTG GGAGATGGAACATAGCACGTTTC TCTCTGGCCTGGTACTGGCTACC CTTCTCTCGCAAGTGAGCCCCTT CAAGATAC	82	83	308874;208874
CD3E	NM_000733.2	NM_000733.2:75	75-175	AAGTAACAGTCCCATGAAACAAA GATGCAGTCGGGCACTCACTGG AGAGTTCTGGGCCTCTGCCTCTT ATCAGTTGGCGTTTGGGGGCAA GATGGTAATG	81	83	305395;205395
CD3G	NM_000073.2	NM_000073.2:515	515-615	AGAGCTTCAGACAAGCAGACTCT GTTGCCCAATGACCAGCTCTACC AGCCCCTCAAGGATCGAGAAGAT GACCAGTACAGCCACCTTCAAGG AAACCAGT	83	82	308886;208886
CD247	NM_198053.1	NM_198053.1:149 0	1490-1590	TGGCAGGACAGGAAAAACCCGT CAATGTACTAGGATACTGCTGCG TCATTACAGGGCACAGGCCATG GATGGAAAACGCTCTCTGCTCTG CTTTTTTTCT	81	80	302943;202943
HLA-DRA	NM_019111.3	NM_019111.3:335	335-435	GGCCAACATAGCTGTGGACAAA GCCAACCTGGAAATCATGACAAA GCGCTCCAACTATACTCCGATCA CCAATGTACCTCCAGAGGTAACT GTGCTCACG	81	81	308873;208873
CD2	NM_001767.2	NM_001767.2:140 0	1400-1500	TGGGTCTCACTACAAGCAGCCTA TCTGCTTAAGAGACTCTGGAGTT TCTTATGTGCCCTGGTGGACACT TGCCCACCATCCTGTGAGTAAAA GTGAAATA	81	80	301282;201282
CD4	NM_000616.3	NM_000616.3:835	835-935	AGACATCGTGGTGCTAGCTTTCC AGAAGGCCTCCAGCATAGTCTAT AAGAAAGAGGGGGAACAGGTGG AGTTCTCCTTCCCACTCGCCTTT ACAGTTGAA	82	82	308876;208876
CD8A	NM_001768.5	NM_001768.5:132 0	1320-1420	GCTCAGGGCTCTTTCCTCCACAC CATTCAGGTCTTTCTTTCCGAGG CCCCTGTCTCAGGGTGAGGTGC TTGAGTCTCCAACGGCAAGGGAA CAAGTACTT	83	83	306921;206921
CD8B	NM_004931.3	NM_004931.3:440	440-540	CAGCTGAGTGTGGTTGATTTCCT TCCCACCACTGCCCAGCCCACC AAGAAGTCCACCCTCAAGAAGAG AGTGTGCCGGTTACCCAGGCCA GAGACCCAGA	82	82	308866;208866

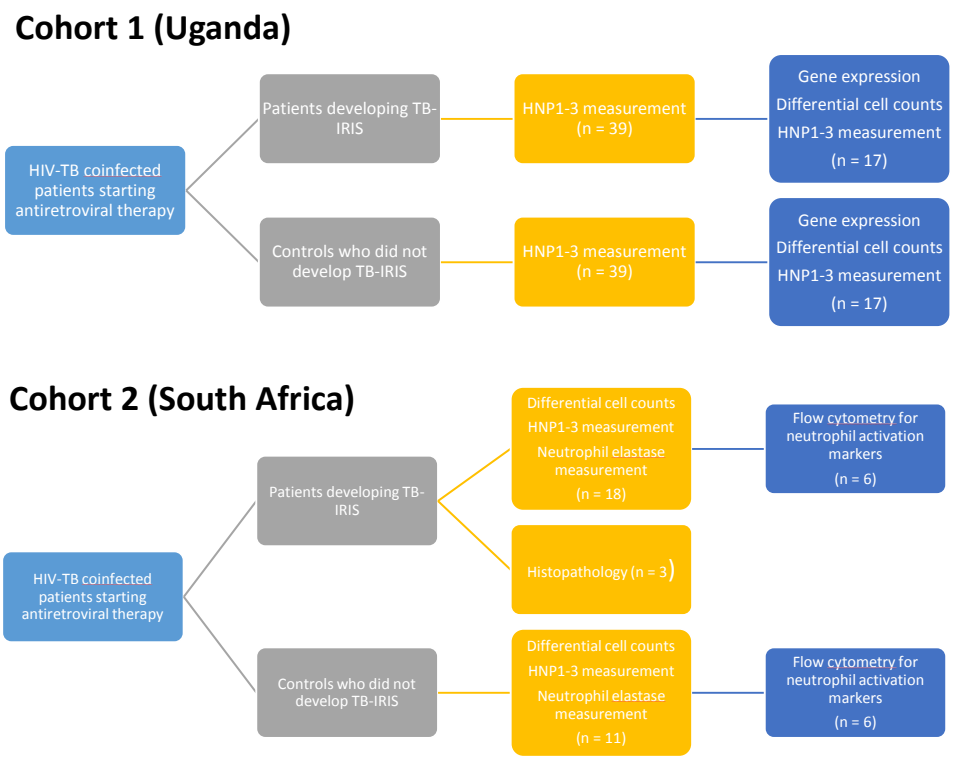
ICAM1	NM_000201.1	NM_000201.1:1990	1990-2090	GAAATACTGAAACTTGCTGCCTATTGGGTATGCTGAGGCCCACAGACTTACAGAAGAAGTGGCCCTCCATAGACATGTGTAGCATCAAAACACAAAGGCC	81	80	300024;200024
ITGAL(LFA-1)	NM_002209.2	NM_002209.2:3905	3905-4005	GTGAGGGCTTGTCATTACCAGACGGTTCACCAGCCTCTCTTGGTTTCCTTCCTTGGAAGAGAATGTCTGATCTAAATGTGGAGAAACTGTAGTCTCAGGA	80	83	311572;211572
CTLA4	NM_005214.3	NM_005214.3:405	405-505	AGTCTGTGCGGCAACCTACATGATGGGGAATGAGTTGACCTTCCTAGATGATTCCATCTGCACGGGCACCTCCAGTGGAAATCAAGTGAACCTCACTATC	82	81	302935;202935
PTPRC	NM_002838.2	NM_002838.2:2340	2340-2440	CTCGATGTGAAGAAGGAAACAGGAACAAGTGTGCAGAATACTGGCCGTCAATGGAAGAGGGCACTCGGGCTTTTGGAGATGTTGTTGTAAAGATCAACCA	79	78	306033;206033
ITK	NM_005546.3	NM_005546.3:3430	3430-3530	GCCAGTAAAGAAGTCAGTATAGAACCACTAGCGAATAGTGTTGCTCTGGCACAGACCACTGTGGTTGATGGCATGGCCCTCCAACTTGGAATAGGATTTT	78	82	302936;202936
TRAT1	NM_016388.2	NM_016388.2:770	770-870	ACAGAGGACACAGAAGGACTTGGCAGCAGGGTGATGACCTGATCATTTGTTGATGGGATGGTGGCTTACCTCTTATTCACAGCTTACACTTATGCATGCC	81	82	302941;202941
PRR7	NM_001174101.1	NM_001174101.1:393	393-493	CGTGCCGCCGCCATGGTGATGTCCCAGGGCACCTACACGTTCCTCACGTGCTTCGCCGGCTTCTGGCTCATCTGGGGTCTCATCGTCCTGCTCTGCTGCT	84	83	349399;249399
ICOS	NM_012092.2	NM_012092.2:640	640-740	AACTCTGGCACCCAGGCATGAAGCACGTTGGCCAGTTTTCCTCAACTTGAAGTGCAAGATTCTCTTATTTCCGGGACCACGGAGAGTCTGACTTAACTAC	81	79	302939;202939
CD28	NM_006139.1	NM_006139.1:305	305-405	GCTTGTAGCGTACGACAATGCGGTCAACCTTAGCTGCAAGTATTCCTACAATCTCTTCTCAAGGGAGTTCCGGGCATCCCTTCACAAAGGACTGGATAGT	78	81	301415;201415
CD80	NM_005191.3	NM_005191.3:1288	1288-1388	AAAGATCTGAAGGTCCCACCTCCATTTGCAATTGACCTCTTCTGGGAACTTCCTCAGATGGACAAGATTACCCCACCTTGCCCTTTACGTATCTGCTCTT	83	81	315973;215973
CD86	NM_006889.3	NM_006889.3:146	146-246	TATGGGACTGAGTAACATTCTCTTTGTGATGGCCTTCCTGCTCTCTGGTGCTGCTCCTCTGAAGATTCAAGCTTATTTCAATGAGACTGCAGACCTGCCA	82	82	315980;215980
ACTB	NM_001101.2	NM_001101.2:1010	1010-1110	TGCAGAAGGAGATCACTGCCCTGGCACCCAGCACAATGAAGATCAAGATCATTGCTCCTCCTGAGCGCAAGTACTCCGTGTGGATCGGCGGCTCCATCCT	87	87	301013;201013
B2M	NM_004048.2	NM_004048.2:25	25-125	CGGGCATTCCTGAAGCTGACAGCATTCGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGC	82	81	301358;201358

				GCTACTCTCTCTTTCTGGCCTGG AGGCTATCCA			
GAPDH	NM_002046.3	NM_002046.3:35	35-135	TCCTCCTGTTCGACAGTCAGCCG CATCTTCTTTTGCGTCGCCAGCC GAGCCACATCGCTCAGACACCAT GGGGAAGGTGAAGGTCGGAGTC AACGGATTT	82	82	300988;200988
TBP	NM_003194.3	NM_003194.3:25	25-125	CGCCGGCTGTTTAACTTCGCTTC CGCTGGCCCATAGTGATCTTTGC AGTGACCCAGCAGCATCACTGTT TCTTGGCGTGTGAAGATAACCCA AGGAATTG	79	78	300993;200993
UBC	NM_021009.3	NM_021009.3:187 5	1875-1975	TGCAGATCTTCGTGAAGACCCTG ACTGGTAAGACCATCACTCTCGA AGTGGAGCCGAGTGACACCATT GAGAATGTCAAGGCAAAGATCCA AGACAAGGA	82	81	306338;206338
RPL13A	NM_012423.2	NM_012423.2:720	720-820	AGTCCAGGTGCCACAGGCAGCC CTGGGACATAGGAAGCTGGGAG CAAGGAAAGGGTCTTAGTCACTG CCTCCCGAAGTTGCTTGAAAGCA CTCGGAGAAT	92	79	304775;204775
Tbet (TBX21)	NM_013351.1	NM_013351.1:890	890-990	ACACAGGAGCGCACTGGATGCG CCAGGAAGTTTCATTTGGGAAAC TAAAGCTCACAAACAACAAGGGG GCGTCCAACAATGTGACCCAGAT GATTGTGCT	81	80	301952;201952
GATA3	NM_00100229 5.1	NM_001002295.1: 2835	2835-2935	AAGAGTCCGGCGGCATCTGTCTT GTCCCTATTCCTGCAGCCTGTGC TGAGGGTAGCAGTGTATGAGCTA CCAGCGTGCATGTCAGCGACCC TGGCCCGAC	81	81	302821;202821
RORC	NM_00100152 3.1	NM_001001523.1: 1350	1350-1450	CTCATCAATGCCCATCGGCCAGG GCTCCAAGAGAAAAGGAAAGTAG AACAGCTGCAGTACAATCTGGAG CTGGCCTTTCATCATCATCTCTG CAAGACTC	79	82	302252;202252
FOXP3	NM_014009.3	NM_014009.3:123 0	1230-1330	GGGCCATCCTGGAGGCTCCAGA GAAGCAGCGGACACTCAATGAG ATCTACCACTGGTTCACACGCAT GTTTGCCTTCTTCAGAAACCATC CTGCCACCTG	81	79	310104;210104
Vitamin D receptor (VDR)	NM_000376.2	NM_000376.2:438 5	4385-4485	GCTAACTGGAAGCATGTAGGAGA ATCCAAGCGAGGTCAACAGAGAA GGCAGGAATGTGTGGCAGATTTA GTGAAAGCTAGAGATATGGCAGC GAAAGGAT	82	79	301901;201901

Abbreviations: NSID-Nanostring probe ID; Tm CP-melting temperature of capture probe; Tm RP-melting temperature of reporter probe; TRAC, T-cell Receptor alpha constant; TRAV, T-cell Receptor alpha variable; TRBC, T-cell Receptor beta constant; TRBV, T-cell Receptor beta variable; TRDV, T-cell Receptor delta variable; TRGC, T-cell Receptor gamma constant; TRGV; T-cell Receptor gamma variable; IPAF, Ice protease-activating factor/NLR family Card domain containing 4 (NLRC4); NLRP12Nucleotide-binding domain, leucine rich repeat containing receptor (NLR) Family Pyrin Domain Containing 12; TLR, Toll-like receptor; IL-7(R), Interleukin-7 (receptor); SOCS1, Suppressor Of Cytokine Signaling 1; NALP-1, NACHT, LRR and PYD domains-containing protein 1**;** ASC, Apoptosis-associated speck-like protein containing a CARD (Caspase activation and recruitment domains)**;** IL-10, Interleukin-10; COX-1/PTGS, Cyclooxygenase-1/prostaglandin-endoperoxide synthase; pypaf-7, PYRIN-containing Apaf-1-like proteins; S100A9, S100 calcium-binding protein A9; IFN-ϒ, Interferon gamma; CD, Cluster of Differentiation; COX-1/PTGS, Cyclooxygenase-1/prostaglandin-endoperoxide synthase; CTLA4, Cytotoxic T Lymphocyte-associated protein 4 (CD152); GATA3, Glycine, Alanine, Thymine, Alanine binding protein 3; ICOS, Inducible T-cell costimulator; ITK, Interleukin-2-inducible T-cell kinase;; Tbet, T-box transcription factor.

Supplementary Figures

Supplementary Figure 1



Flow chart indicating study designs and numbers of participants in each analysis.

Supplementary Figure 2

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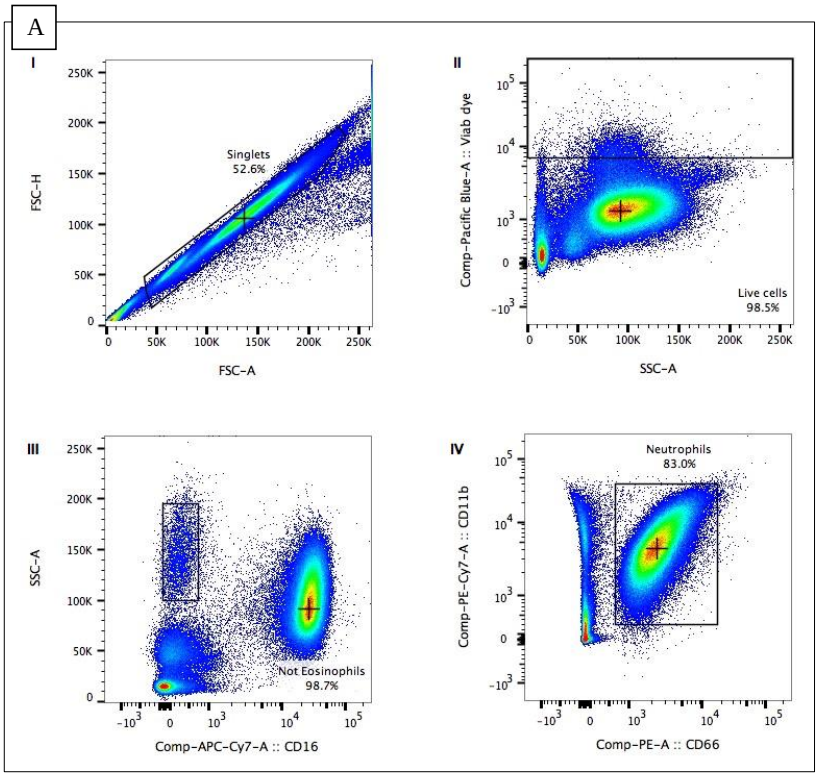
A



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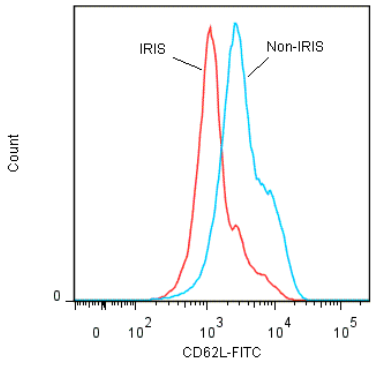
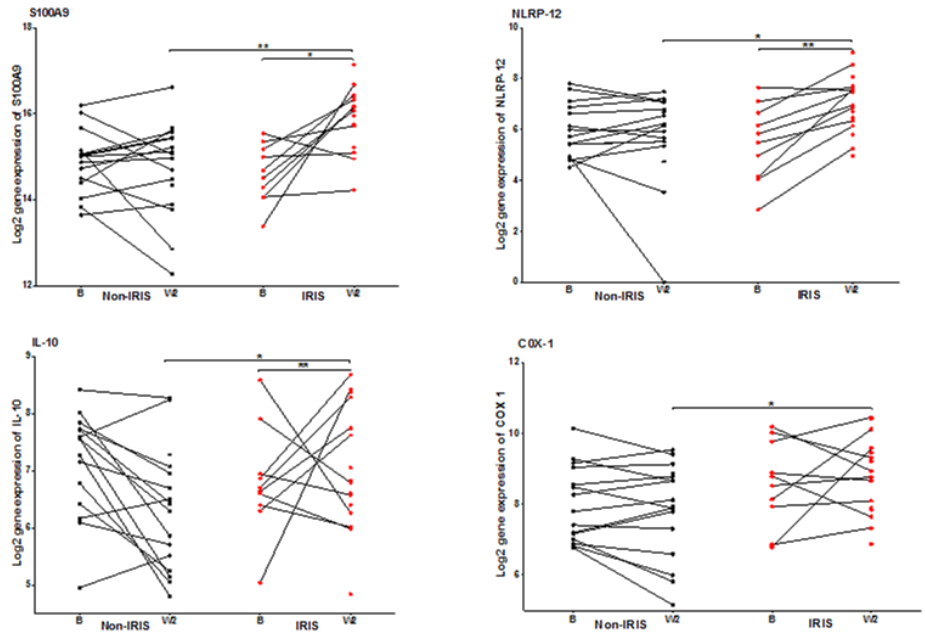


Analysis strategy for neutrophil activation measurement via flow cytometry: A. An aliquot of whole blood (200mcl) was stained with fluorochromes, red cells were lysed and the samples were fixed before acquisition on a BD Fortessa flow cytometer. **I**. Singlet signals were gated by Forward Scatter (FSC) Area versus FSC Height. **II**. Dead cells were excluded using eFluor450 Viability Dye (or ViViD viability dye) versus Side Scatter (SSC). **III**. Eosinophils, defined as CD16-negative with high SSC, were excluded. **IV**. Neutrophils were defined as CD66a,c,e-PE/CD11b-PE-Cy7 positive events. The CD11b, CD16, CD62L, CD66a,c,e and IL-8RA MFI of neutrophils was then determined.
B. Representative histogram of CD62L MFI in one IRIS patient and one non-IRIS control at Week 2 time-point.

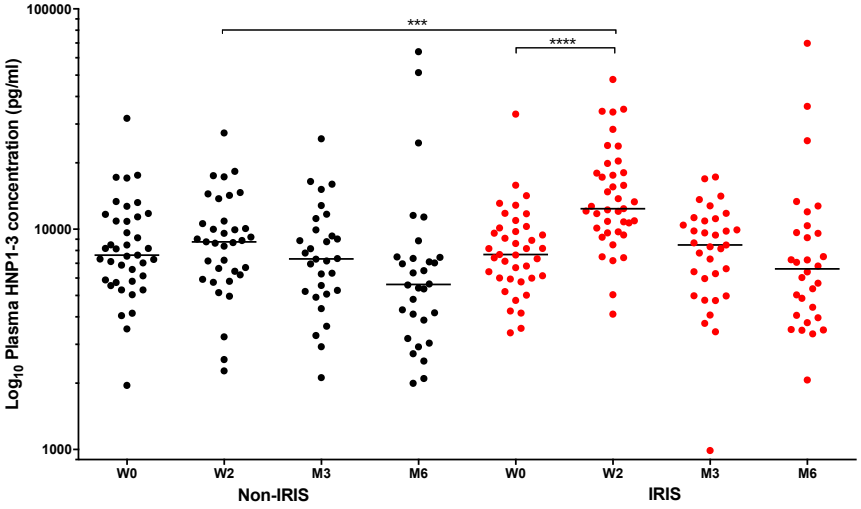
Supplementary Figure 3



Increased expression of genes in TB-IRIS patients compared to the non-IRIS controls: Graphs show the changes from ART initiation to two weeks of the four most over-expressed genes in TB-IRIS patients (n = 10 at baseline/ART initiation (B), n = 17 at Week 2 (W2)) (red) versus non-IRIS controls (n = 15 at baseline/ART initiation, n = 17 at Week 2) (black). Values were obtained from 100 ng of total RNA using nCounter technology and were log2 transformed. Mann Whitney and Wilcoxon tests were used and p values < 0.05 were considered significant (* $p < 0.05$, ** $p < 0.01$).

Abbreviations: ASC; Apoptosis-associated speck-like protein containing a Caspase Recruitment Domain (CARD); IL-10, Interleukin-10; COX-1/PTGS, Cyclooxygenase-1/prostaglandin-endoperoxide synthase; NLRP-12//pypaf-7, NOD-like receptor (NLR) family pyrin domain containing 12/ PYRIN-containing Apaf-1-like proteins; S100A9, S100 calcium-binding protein A9.

Supplementary Figure 4



Analysis of plasma HNP1-3 levels in TB-IRIS and Non IRIS controls for the whole Ugandan cohort. Human Neutrophil Peptides (HNP) 1-3 plasma concentrations were quantified using ELISA in the whole Ugandan cohort (TB-IRIS patients (n =39 at ART initiation, n = 39 at W2, n = 33 at Month (M) 3 and n = 30 at M6) and non-IRIS controls (n =39 at ART initiation, n = 35 at W2, n = 30 at Month (M) 3 and n = 30 at M6)). Lines represent medians and p-values (*** $p < 0.001$, **** $p < 0.0001$) were derived from Mann-Whitney and Wilcoxon tests.