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TB-IRIS and remodelling of the T cell compartment in highly immunosuppressed HIV+ patients with TB: the CAPRI T (ANRS-12614) study

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Abstract

Objective—To investigate the impact of tuberculosis (TB)-associated immune reconstitution syndrome (IRIS) upon immunological recovery and the T cell compartment after initiation of TB and antiretroviral therapy (ART).

Design and methods—We prospectively evaluated T cell immunophenotypes by flow cytometry and cytokines by Luminex assays in a subset (n=154) of highly immunosuppressed HIV + patients with TB from the CAMELIA randomized clinical trial. We compared findings from patients who developed TB-IRIS to findings from patients who did not develop TB-IRIS. Data were evaluated with mixed effect linear regression, Kaplan-Meier estimates, and Wilcoxon rank sum tests, and q-values were calculated to control for multiple comparisons.

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Results—Development of TB-IRIS was associated with significantly greater pre-ART frequencies of HLA-DR+CD45RO+CD4+, CCR5+CD4+, OX40+CD4+, and Fas+ effector memory (EM) CD8+ T cells, and significantly elevated levels of plasma IL-6, IL-1β, IL-8, and IL-10 and viral load. Post-ART initiation, EM CD4+ and Fas+ EM CD4+ T cell frequencies significantly expanded, and central memory (CM) CD4+ T cell frequencies significantly contracted in patients who experienced TB-IRIS. By week 34 post-TB treatment initiation, EM/CM CD4+ T cell ratios were markedly higher in TB-IRIS versus non-TB-IRIS patients.

Conclusions—A distinct pattern of pre-ART T cell and cytokine markers appear to poise the immune response to develop TB-IRIS. Experience of TB-IRIS is then associated with long-term remodeling of the CD4+ T cell memory compartment towards an EM-dominated phenotype. We speculate that these pre- and post-ART TB-IRIS-associated immune parameters may contribute to superior immune control of TB/HIV co-infection and better clinical outcome.

Keywords

TB; HIV; TB-IRIS; immunosuppression; ART; T cells; effector memory; CCR5+CD4+; activated T cells; CAMELIA trial

INTRODUCTION

When antiretroviral treatment (ART) is initiated in rapid succession to tuberculosis (TB) therapy in HIV/TB co-infected patients, risk of immune reconstitution inflammatory syndrome (IRIS) increases [1–4]. Typically, TB-IRIS occurs after the start of TB therapy and after initial improvement of TB symptoms [5], and is characterized by clinical deterioration after ART initiation that manifests in fever, enlarged lymph nodes, and radiological features of TB disease not associated with treatment failure due to mycobacterial resistance, poor adherence to the treatment regimen, or another opportunistic infection (OI) [5, 6].

The Cambodian Early versus Late Introduction of Antiretrovirals (CAMELIA) randomized clinical trial (ANRS1295/CIPRA KH001) demonstrated that initiation of ART at 2 weeks (early arm) as compared to 8 weeks (late arm) after TB treatment initiation significantly decreased mortality by 34% in highly immunocompromised HIV-positive adults (median CD4+ T cell count = 25/mm^3) with newly diagnosed TB [1, 7]. Early ART was also associated with a significantly increased risk (2.5-fold) of TB-IRIS in the CAMELIA study [1, 7]. Notably, the survival benefit of early ART in CAMELIA was observed up to three years after the earlier ART timing intervention [1, 7].

To investigate immunological recovery in TB/HIV patients and the mechanisms underlying TB-IRIS, we nested a prospective sub-study (Cambodian Paradoxical Reaction Immune Study-T cells or “CAPRI-T” (ANRS 12164)) within the CAMELIA trial. We enrolled patients from both treatment arms at the time of their entry into the CAMELIA trial and performed extensive immunophenotypic and cytokine profiling on patient samples after TB treatment initiation (prior to the start of ART), and at several timepoints post-ART initiation up to 34 weeks after the start of TB treatment.

METHODS

Study population, design, implementation and oversight

The CAMELIA trial was a prospective, multi-center, open-label superiority trial conducted in Cambodia that enrolled HIV-positive adults with a CD4+ T cell count ≤200/mm^3 and newly diagnosed TB as confirmed by any clinical sample that was smear-positive for acid-fast bacilli [1]. Patients were randomly enrolled in the CAPRI-T substudy from both arms of the CAMELIA after enrollment in the CAMELIA (Fig 1A). After written informed consent was provided, 10 ml of blood was drawn at the timepoints shown in Fig 1B. CD4+T cell counts and HIV viral loads were determined at the Institut Pasteur in Cambodia as described [1]. Pre-ART clinical characteristics were taken from the CAMELIA database. See Supplementary Data for additional details.

Characterization of TB-IRIS

Characterization of TB-IRIS was a secondary objective of the CAMELIA trial and has been described elsewhere [1, 7]. See Supplementary Data for details.

Ethics Statement

The CAPRI-T and CAMELIA study protocols and consent forms were approved by the National Ethics Committee of Cambodia, the NIH (CSRC), and institutional review boards of the Institut Pasteur, France, and the Immune Disease Institute, (now the Program in Cellular and Molecular Medicine, Children's Hospital) of Boston, MA, USA. All work was conducted according to the principles expressed in the Declaration of Helsinki.

T cell immunophenotyping

Phenotypic studies were performed on freshly isolated whole blood. After staining with antibodies, cells were processed by standard lyse/wash procedure and acquired on a four-color BD FACScalibur II cytometer (BD, Paris, France) on site at the Institut Pasteur in Cambodia. All flow cytometry data were analyzed using Flow-Jo 8.8.4 software (Tree Star Inc). Lymphocytes gated by light scattering were verified to be >90% pure using standard CD14/CD45 back-gating methods [8], and samples were excluded if this threshold was not met. Panels of antibody conjugates used for staining are shown in Suppl. Table 1, and a representative example of gating strategy is shown in Suppl. Fig 1.

Plasma cytokine measurements

Plasma levels of IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-17, GMCSF, IFN-γ and TNF were quantified with Bio-Plex (Bio-Rad) and Luminex (Life Technologies) assay systems. Assays were performed in duplicate, with company-provided controls, to minimize intra- and inter-assay variation.

Statistics

Mixed effect linear regression was used to characterize progression of the phenotypic markers under ART, with time 0 being ART initiation; the effects of TB-IRIS and of the early or late arm on both phenotype frequency at ART initiation and changes in its

progression were investigated. Phenotypic markers were first checked for normal distribution using Kernel density plots, and a square root transformation was used when the distribution was not normal. Based on non-parametric representations of the markers' progression over time that were inferred from the regression models we validated that the progression looked linear. The Wilcoxon rank sum test was performed on all immunophenotypes at week 34 post-TB treatment initiation (week 32 post-ART in the early arm and week 26 post-ART in the late arm, respectively) between TBIRIS and non-TB-IRIS patients. Supplementary Tables 2–5 shows p- and q-values for the different analyses.

Association of specific phenotypes with the risk of occurrence of TB-IRIS was described using Kaplan-Meier estimates after stratifying by phenotype frequency (≤ versus > median at ART initiation) and after comparison between groups was done by log-rank test.

Statistical analyses were performed using the Stata 11 (Stata Corporation, College Station, Texas, USA) and the FDR (false discovery rate) correction was performed using the R software (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org).

RESULTS

Impact of timing of ART, viral load and CD4+ T cell count on TB-IRIS

Of the 154 patients included in the CAPRI-T study, 50 developed clinically validated TB-IRIS secondary to TB treatment initiation (Fig. 1A). We note that all other potential causes of IRIS (aside from TB) were ruled out (Supplementary data). [1, 7]. None of the 104 non-TB-IRIS patients exhibited signs or symptoms of TB-IRIS at any time during their clinical course. TB-IRIS occurred at a median of 12 days (inter-quartile range (IQR): 7–24) post-ART initiation and occurred at similar times post-ART in both CAMELIA treatment arms (median of 11.5 and 16 days, respectively; p=0.27). TB-IRIS was significantly more common in the early (34/80; 42%) versus late (16/74; 22%) (p=0.003) treatment arm, consistent with findings in the overall CAMELIA cohort [1, 7].

At ART initiation, the CD4+ T cell count in TB-IRIS patients and non-TBIRIS patients was similar (median: 22/mm^3 (IQR: 11–52) and 32/mm^3 (IQR: 15–71), respectively; p=0.10), as was body mass index (BMI) (median: 17.6 (IQR 16–19) and median: 17.8 (IQR 16–20), respectively; p=0.88). However, pre-ART viral load was significantly higher in TB-IRIS patients versus non-TB-IRIS patients (median: 5.9 (IQR: 5.4–6.4) vs. 5.6 (IQR: 5.2–6.1) log copies/ml, respectively; p=0.04). Other baseline characteristics of TB-IRIS and non-TB-IRIS patients in the CAPRI-T study were similar, consistent with the overall CAMELIA cohort (Suppl. Table 6) [7].

TB-IRIS and activated and regulatory T cells

Prior to ART initiation, patients who went on to develop TB-IRIS had a significantly greater frequency of activated (CD45RO+HLA-DR+) CD4+ T cells compared to non-TB-IRIS patients (p<0.0001) (Fig 2A). This subset of cells remained significantly higher in the TB-IRIS group at week 34 post-TB therapy initiation (p=0.0003), which was week 32 and week 26 post-ART in the early and late CAMELIA treatment arms, respectively (Fig 1B). Pre-

ART, TB-IRIS patients also had significantly higher proportions of CCR5+CD4+ T cells (p=0.006) (Fig 2B), which remained markedly higher at week 34 relative to the non-TB-IRIS group (p<0.0001)(Fig 2B). By contrast, proportions of activated CD8+ (HLADR+ CD38+) T cells at the start of ART were not significantly different in the two groups (p=0.91), although there was a greater post-ART decrease in activated CD8+ T cell frequency in non-TB-IRIS patients (p=0.041; Fig 2C).

To determine whether regulatory CD4+ T cell (CD4+ Treg) frequencies differed between TB-IRIS and non-TB-IRIS patients we employed surface staining to enumerate CD25+CD127lo CD4+ T cells [9–11]. CD4+ Treg frequencies were similar in the two groups prior to ART initiation (p=0.72; Fig 2D). However, patients who experienced TB-IRIS exhibited a much greater post-ART rate of decline in CD4+ Treg frequency (p<0.0001), resulting in a significantly lower proportion of CD4+ Tregs in TB-IRIS patients as compared to non-TB-IRIS patients at week 34 post-TB therapy initiation (p=0.034) (Fig 2D).

TB-IRIS and T cell memory

To evaluate the impact of TB-IRIS on the reconstitution of antigen experienced T cell subsets, we next studied changes in effector memory (EM) and central memory (CM) CD4+ T cell proportions and their association with TBIRIS. At ART initiation, there was no appreciable difference between EM (CD62L-CD45RA-) and CM (CD62L+CD45RA-) CD4+ T cell frequencies in patients who did or did not develop TB-IRIS (p=0.96 and p=0.48, respectively) (Figs 3A and B). Strikingly, however, EM and CM CD4+ T cell frequencies diverged significantly in the two patient groups after ART initiation. The EM CD4+ T cell frequency rose, and the CM CD4+ T cell frequency declined, at significantly greater rates in TB-IRIS patients (p=0.001 and p=0.004, respectively), and by week 34 TB-IRIS patients had significantly higher EM CD4+ T cell proportions (p=0.002) and significantly lower CM CD4+ T cell proportions (p=0.011) (Fig 3A and 3B).

The frequency of Fas+ (CD95+) EM CD4+ T cells (CD27-CD45RO+), indicative of late-stage EM cells, was also similar in non-TB-IRIS and TB-IRIS patients at ART initiation (p=0.40). Post-ART, however, the frequency of Fas+ EM CD4+ T cells rose significantly in TB-IRIS patients (p=0.005) and at week 34 was markedly higher in this group (p=0.003) (Fig 3C).

The proportion of Fas+ EM (CD27-CD45RA-) CD8+ T cells was significantly higher in TB-IRIS patients at ART initiation (p<0.0001; Fig 3D), although the overall EM CD8+ T cell frequency was similar between the groups at week 0 (p=0.06). The proportion of CD8+ T cells with a CM/early EM or ‘transitional’ phenotype [12–14] (CD27+CD45RA-) was also significantly lower in TB-IRIS patients (p=0.017) (Fig 3F). By week 34 there were no differences between TB-IRIS and non-TB-IRIS patients in proportions of Fas+ EM CD8+ T cells (p=0.075), EM CD8+ T cells (p=0.067), or CM/early EM transitional memory CD8+ T cells (p=0.072).

TB-IRIS and T cell co-stimulatory signals

We next evaluated a panel of CD4+ or CD8+ co-stimulatory markers. We found no differences in proportions of ICOS+CD4+ cells or CD28+CD8+ or PD-1+CD8+ cells prior to ART or at week 34 (Suppl. Figs 2A–C). By contrast, OX40+CD4+ T cell proportions were significantly elevated (p=0.013) at ART initiation in TB-IRIS relative to non-TB-IRIS patients (Fig 4A), and post-ART this cellular subpopulation declined to a much greater extent in the TB-IRIS group, leading to similar OX40+CD4+ T cell frequencies in the two groups (p=0.15).

When we divided the 154 patients into two groups based on median OX40+CD4+ T cell frequency at ART initiation (median=7.4%), Kaplan-Meier analysis revealed that a pre-ART OX40+CD4+ T cell frequency >7.4% was associated with a significantly greater risk of developing TB-IRIS as compared to a frequency ≤7.4% (p=0.036) (H.R=1.8, 95% C.I. 1.03–3.36) (Fig. 4B). No other cellular phenotype was associated with TB-IRIS risk after similar stratification by median pre-ART phenotype frequency.

Cellular and cytokine changes between ART initiation and TB-IRIS

In order to evaluate early post-ART immune parameters associated with TB-IRIS development, we evaluated changes from week 0 of ART to the time of TB-IRIS in a randomly selected subgroup of TB-IRIS patients (n=32) who experienced TB-IRIS within 3 weeks (median of 10 days, IQR: 6–14) post-ART. As controls, we evaluated the same parameters in a randomly selected subgroup (n=28) of non-TB-IRIS patients who had a week 2 post-ART timepoint available (see Supplementary data). The net increase in CD4+ T cell frequency was similar between the two groups (Fig 5A); however, the net increase in HLA-DR+CD45RO+ and CCR5+ CD4+ T cell frequencies from ART initiation to the TB-IRIS timepoint was significantly greater (p=0.0023 and p=0.0035, respectively) than to the 2-week post-ART timepoint in non-TB-IRIS patients (Figs 5B and 5C). No differences in activated CD8+ and CD4+ Treg frequency increases were detected (Figs 5D and 5E). Among other subsets examined, only CD8+ T cells, but not CD4+ T cells, bearing a CD27+CD95-CD45RO− phenotype increased to a greater extent in the TB-IRIS group versus the non-TB-IRIS group (p=0.0085) (Suppl. Figs 3A–B).

We also measured plasma levels of 12 different cytokines by Luminex, which revealed that the net increases in IL-6 and IL-1β were significantly greater at the TB-IRIS event than at the 2-week post-ART timepoint in non-TB-patients (IL-6: p=0.0066 and IL-1β: p=0.026 (Figs 5F and 5G). Other cytokine differences included: (i) IL-1β, IL-6, IL-8 and IL-10 higher prior to ART and at the TB-IRIS timepoint; (ii) IL-17, IL-12, and TNF similar at ART initiation but higher at the time of TB-IRIS; and (iii) IL-2, IL-7, GM-CSF and IFN-γ similar at all timepoints (Suppl. Fig 3 and Supplementary Data).

DISCUSSION

We have identified distinct immunological patterns that differentiate TBIRIS and non-TB-IRIS patients both prior to ART and post-ART. Pre-ART, significantly higher frequencies of cellular subsets associated with T cell activation, including HLA-DR+/CD45RO+, CCR5+,

and OX40+ CD4+ T cells and Fas+ EM CD8+ T cells were associated with TB-IRIS. Furthermore, prior to ART initiation, higher viral loads and significantly elevated levels of circulating IL-1β, IL-6, IL-8 and IL-10 were present in patients who developed TB-IRIS. Post-ART, EM CD4+ T cells and Fas+ EM CD4+ T cells increased, and CM CD4+ T cells decreased in TB-IRIS patients relative to non-TB-IRIS patients, and these differences remained evident even two months after completion of TB treatment and over 4 months after the vast majority of TB-IRIS events, when the last blood collection timepoint occurred.

Our ability to detect previously unidentified immunological parameters associated with risk of TB-IRIS, TB-IRIS pathogenesis, and post-TB-IRIS immune reconstitution was facilitated by our study design. The nesting of the prospective CAPRI-T study within the CAMELIA trial provided strategic advantages over other studies that were observational or retrospective in nature. These advantages included: (i) the ability to prospectively evaluate patients who went on to develop TB-IRIS and 'control' patients who avoided TB-IRIS from a single large cohort of treatment-naïve patients of similar ethnicity, all with profound HIV-associated immunosuppression and newly diagnosed AFB smearpositive TB; (ii) clinical TB-IRIS validation or exclusion by an experienced clinical team; and (iii) the sampling design, which included long-term follow-up, allowing us to evaluate the impact of TB-IRIS on ART-mediated T cell reconstitution.

Previous studies have reported increased global CD4+ T cell activation at the time of, or following, TB-IRIS [15, 16], or during IRIS precipitated by diverse opportunistic infections [17]. This is the first report, however, to demonstrate that activated CD4+ T cell frequencies are elevated *prior* to ART in TB+/HIV+ patients who go on to develop TB-IRIS. Furthermore, this pre-ART CD4+ T cell activation was accompanied by a significantly higher OX40+CD4+ T cell frequency, and the latter phenotype was predictive of TB-IRIS risk. We also found that the activated CD4+ T cell frequency rises more dramatically post-ART in the TB-IRIS group, confirming a previous report [16]. Taken together, these findings underscore the critical role of CD4+ T cells in the development of TB-IRIS, and clearly demonstrate that the pre-ART CD4+ T cell compartment is distinct in the subset of TB+/HIV+ patients who subsequently develop TB-IRIS. In agreement with other reports [16, 20, 21], pre-ART CD4+ Treg proportions were similar in both TB-IRIS and non-TB-IRIS patients, although there was a relatively greater post-ART decline in this CD4+ subpopulation in TB-IRIS patients.

Our finding that an elevated pre-ART CCR5+CD4+ T cell frequency was also associated with TB-IRIS development, combined with the relatively higher pre-ART viral loads in TB-IRIS patients, provides a novel link between pre-ART CCR5+CD4+ T cell levels, viral load, and TB-IRIS occurrence. Although a recent small study reported that CCR5+CD4+ T cell proportions were higher in TB-IRIS versus non-TB-IRIS patients at week 6 post-ART [22], only 7 TB-IRIS patients were analyzed, and there was no indication when TB-IRIS occurred in these patients relative to ART initiation. In our patient cohort, which included 50 TBIRIS patients, we found that CCR5+CD4+ T cell proportions increased dramatically in the first weeks post-ART relative to non-TB-IRIS patients, and remained significantly higher six months later. CCR5 is a critical homing receptor for Th1 cells to peripheral inflammatory sites, including the lungs and the central nervous system [23–26]. Thus, the rapid post-ART

rise in CCR5+CD4+ T cell frequency in TB-IRIS patients may help explain certain clinical manifestations of TB-IRIS, including pleural effusion and neurological symptoms [4, 7, 27, 28]. In addition, since CCR5 is a major co-receptor for HIV [29], the higher pre-ART CCR5+ CD4+ T cell frequency in patients who develop TB-IRIS may help drive the higher viral loads observed in these patients.

Although other innate immune cell types, including NK cells and γ/δ T cells, have been linked to TB-IRIS development [15, 31], it is becoming increasingly clear that myeloid cells play a major part in this syndrome [32]. Our finding that plasma IL-1β levels are elevated pre-ART and increase significantly post-ART initiation in TB-IRIS patients relative to non-TB-IRIS patients provides the first clear indication that this critical pro-inflammatory mediator plays a role in TB-IRIS. We also found that circulating IL-6 levels were higher prior to ART in the TB-IRIS group and increased more dramatically in the TB-IRIS patients once ART began, and that plasma IL-8, IL-12, and TNF (which is also produced by activated T cells [33]) were all significantly higher at the time of TB-IRIS, confirming previous reports that found higher plasma levels of these proinflammatory mediators prior to ART and/or at the time of TB-IRIS.

While other studies have found elevated MTb antigen-induced IFN-γ production by T cells from TB-IRIS patients stimulated *ex vivo* [15, 16, 22, 42, 44– 46], and higher levels of IFN-γ in plasma of TB-IRIS patients [42], we saw no difference in plasma IFN-γ levels between TB-IRIS and non-TB-IRIS patients. We did observe that circulating IL-10 levels were significantly higher in TB-IRIS patients both pre-ART and at the time of TB-IRIS, similar to what was observed in a South African patient cohort [42, 43]. Thus, the relatively elevated IL-10 levels in the TB-IRIS group might have suppressed IFN-γ production.

Our findings that elevated CCR5+CD4+ and OX40+CD4+ T cell frequencies, and circulating IL-1β and IL-6 levels, are present pre-ART in patients who go on to develop TB-IRIS point to possible therapeutic interventions to reduce TB-IRIS incidence and/or severe or complicated clinical presentations of TB-IRIS. Although the recently completed CADIRIS trial found no benefit from inclusion of the CCR5 blocker maraviroc at ART initiation in reducing IRIS incidence due to multiple etiologies, the patients included in this trial had CD4 counts >100/mm^3 and viral loads as low 10^3 copies/ml [47]. It is possible therefore that CCR5 antagonists would have some benefit in patients with more advanced immunosuppression and higher pre-ART CCR5+CD4+ T cell frequencies, like those analyzed here. We note that IL-1β and IL-6 antagonists are also in clinical use for diverse inflammatory conditions [48], and OX40/OX40L antagonists are currently in development [49].

Due to our study design we could evaluate the impact of TB-IRIS on ART-mediated T cell reconstitution up to two months after TB therapy completion/TB cure. Here, we have shown that EM and Fas+ EM CD4+ T cell frequencies expanded, and CM CD4+ T cell frequencies contracted, at significantly greater rates post-ART in TB-IRIS patients versus non-TB-IRIS patients, and that these remained evident at week 34. This strongly suggests that the TB-IRIS event is associated with a dramatic and persistent shift in EM/CM CD4+ T cell ratios either as a precipitant or as a reflection of this inflammatory host response. Our findings

differ from a previous retrospective study of patients with IRIS caused by diverse opportunistic pathogens, which found similar kinetics of EM CD4+ T cell expansion/contraction between IRIS and non-IRIS patients post-ART [17].

TB-IRIS incidence was significantly higher in CAPRI-T patients from the early treatment arm (ART at 2 weeks post-TB treatment) versus the late treatment arm (ART at 8 weeks post-TB treatment), as it was in the overall CAMELIA cohort [1, 7]. We speculate that the steep post-ART rise in EM/CM CD4+ T cell ratio in the TB-IRIS group, which was especially apparent after week 8 when the vast majority of TB-IRIS cases had resolved, reflected an MTb-specific response, and that this response was amplified in early treatment arm patients due to relatively higher bacterial burden at the time of ART initiation. In this scenario, early ART facilitated the expansion of MTb-specific CD4+ T cell clones in patients who were already predisposed to respond strongly to infection once immune reconstitution began, as reflected by their enhanced pre-ART CD4+ T cell activation profiles. By contrast, delay of ART to 8 weeks in patients with a similarly “primed” CD4+ T cell compartment led to a more muted and less robust MTb-specific response due to the relatively lower bacterial burden by this timepoint, which may have been exacerbated by greater CD4+ T cell death due to the absence of ART for 6 additional weeks in the context of very high viremia. Intriguingly, EM CD4+ T cells are elevated in individuals with latent TB as compared to BCG-vaccinated individuals, consistent with this memory subpopulation playing a critical role in the long-term control of TB reactivation [56]. Indeed, it has been argued that induction of “frontline” EM T cells that can respond to pathogens that establish chronic infections at sites of entry (like the lung for MTb) should be a major goal of HIV, malaria, and TB vaccines [57].

Taken together, it is interesting to speculate that the post-ART/TB-IRIS shift of the CD4+ T cell memory compartment to an EM-dominated phenotype may help in controlling acute TB infection during the early stages of ART-mediated immune restoration and help in conferring long-term enhanced protection from MTb reinfection/reactivation/relapse. In this scenario, TB-IRIS is a clinical manifestation at the end of a spectrum of desirable MTb-driven innate and CD4+ T cell responses that are associated with better treatment outcome in TB/HIV co-infected patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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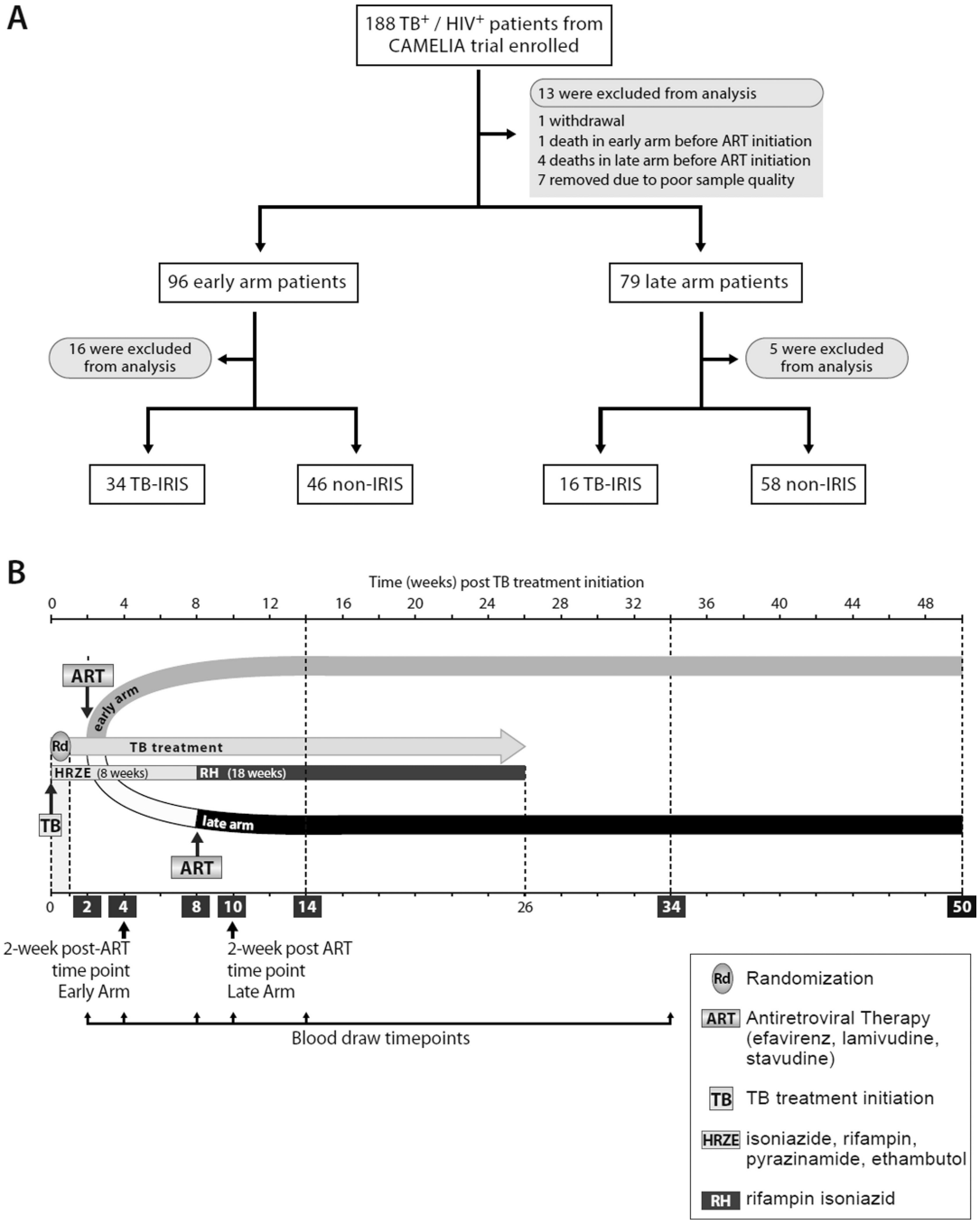

Figure 1. Screening, enrollment, follow-up

A. Flow chart of CAMELIA patient recruitment for the CAPRI-T sub-study. Of the 188 CAMELIA patients who provided informed consent for participation in the CAPRI-T study, 13 were excluded for the reasons shown in the figure, and an additional 21 were excluded after initial suspicion of TB-IRIS, which was not subsequently clinically validated.

B. Schema showing how the CAPRI-T sub-study was nested within the CAMELIA clinical trial and the timing of blood samples relative to initiation of TB treatment and ART. The

week 2 post-ART blood collection timepoint in the early and late CAMELIA treatment arms is indicated in the figure.

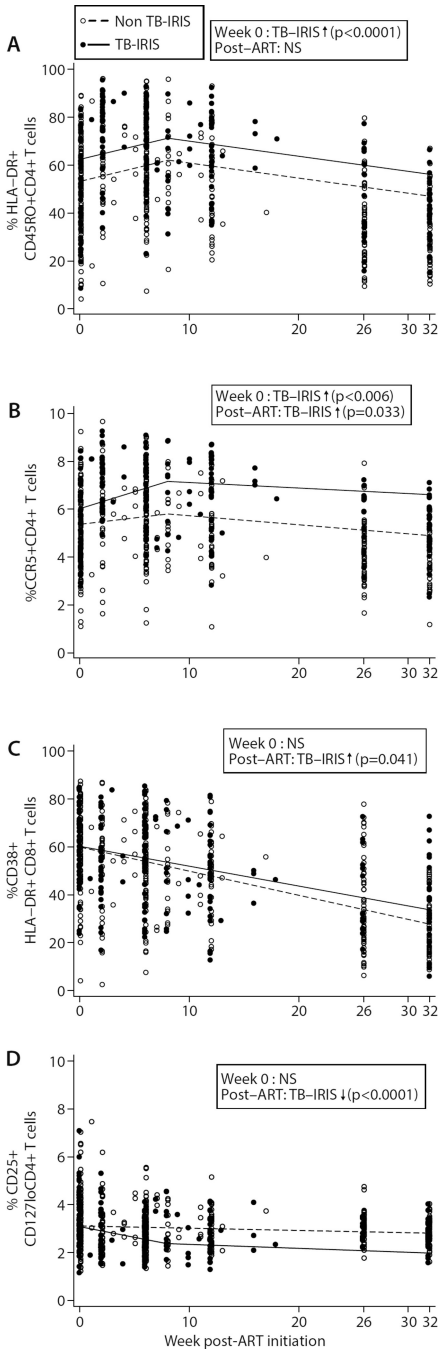

Figure 2. Markers of CD4+ and CD8+ T cell activation and regulatory activity in TB-IRIS and non-TB-IRIS patients

T cell immunophenotypes were obtained on whole blood samples. Lines depict mean progression over time deduced from mixed effect linear regression models in each patient group for the T cell subset shown. TB-IRIS patients (filled circles and solid line) and non-TB-IRIS patients (open circles and dashed line) are shown at the actual time of sample analysis post-ART initiation. Significant differences obtained from regression analysis in the frequency of each T cell subset at week 0 of ART and/or its rate of change post-ART initiation are indicated in each figure (see supplementary tables 2 and 3 for full list of p- and

q-values). NS= not significant; up arrow = higher in TB-IRIS; down arrow = lower in TB-IRIS. For CCR5+CD4+ T cells and CD4+ Tregs, regression plots were generated with square root transformation due to non-normal distribution. A) Activated (CD45RO+HLA-DR+) CD4+ T cells; B) CCR5+CD4+ T cells; C) Activated (CD38+HLA-DR+) CD8+ T cells; D) CD4+ regulatory T cells. So the spread of values in each subgroup (TB-IRIS vs. non-TB-IRIS) can be better appreciated, the two patient groups are shown immediately adjacent to one another at weeks 0, 2, 6, 8, 26, and 32 post-ART in Suppl. Figs 4A–D.

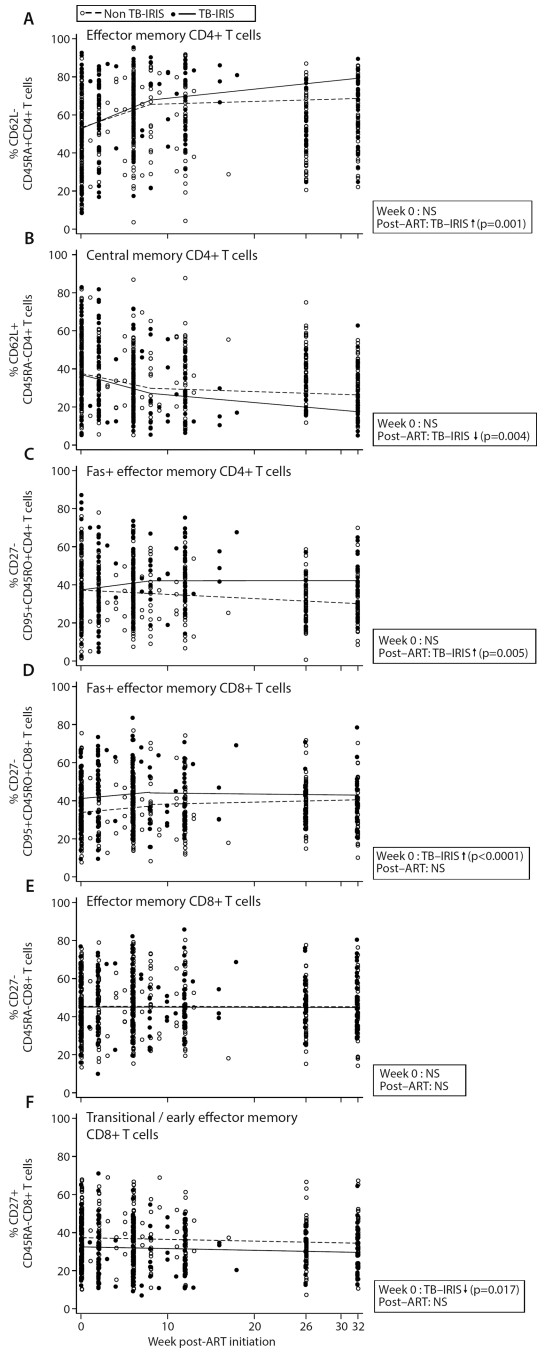

Figure 3. Markers of CD4+ and CD8+ T cell memory differentiation in TBIRIS and non-TB-IRIS patients

T cell immunophenotypes were obtained on whole blood samples. Lines depict mean progression over time deduced from mixed effect linear regression models in each patient group for the T cell subset shown. TB-IRIS patients (filled circles and solid line) and non-TB-IRIS patients (open circles and dashed line) are shown at the actual time of sample analysis post-ART initiation. Significant differences obtained from regression analysis in the frequency of each T cell subset at week 0 of ART and/or its rate of change post-ART initiation are indicated in each figure (see supplementary tables 2 and 3 for full list of p- and

qvalues). NS= not significant; up arrow = higher in TB-IRIS; down arrow = lower in TB-IRIS A) Effector memory (CD62L-CD45RA-) CD4+ T cells; B) Central memory (CD62L +CD45RA−) CD4+ T cells; C) Fas+ effector memory (CD27−CD95+CD45RO+) CD4+ T cells; D) Fas+ effector memory (CD27−CD95+CD45RO+) CD8+ T cells; E) Effector memory (CD27−CD45RA−) CD8+ T cells; F) Transitional/early effector memory (CD27+CD45RA−) CD8+ T cells. So the spread of values in each subgroup (TB-IRIS vs. non-TB-IRIS) can be better appreciated, the two patient groups are shown immediately adjacent to one another at weeks 0, 2, 6, 8, 26, and 32 post-ART in Suppl. Figs 5A–F.

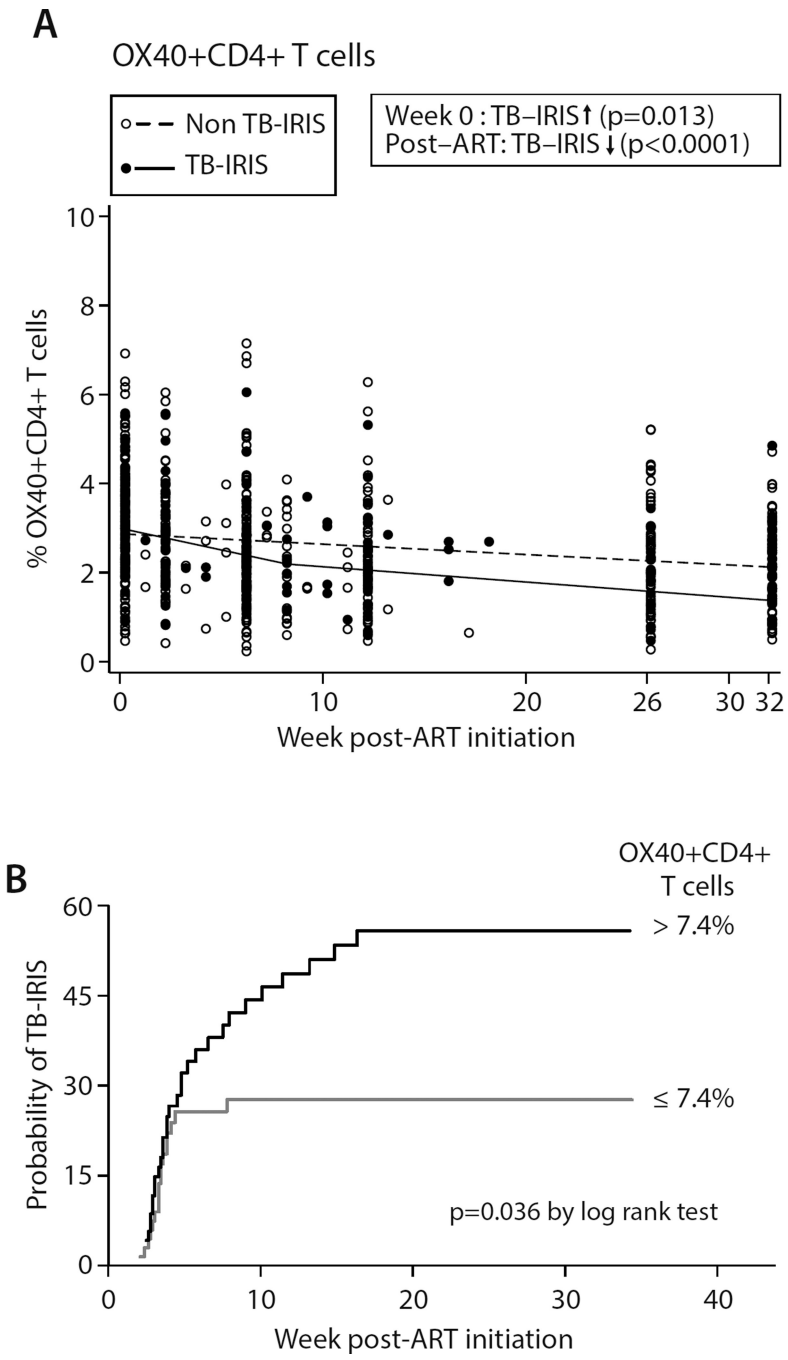

Figure 4. Differences in OX40+CD4+ T cell frequencies between TB-IRIS and non-TB-IRIS patients

OX40+CD4+ T cell frequencies were measured in whole blood samples. Lines depict mean progression over time deduced from mixed effect linear regression models. TB-IRIS patients (filled circles and solid line) and non-TB-IRIS patients (open circles and dashed line) are shown at the actual time of sample analysis post-ART initiation in the left panel. Significant differences obtained from regression analysis in the frequency of OX40+CD4+ T cells at week 0 of ART and/or its rate of change post-ART initiation are indicated (see supplementary tables 2 and 3 for full list of p- and q-values). Up arrow = higher in TB-IRIS;

down arrow = lower in TB-IRIS. Since OX40+CD4+ T cell frequencies were not normally distributed they were plotted using square root transformation. So the spread of values in each subgroup (TB-IRIS vs. non-TB-IRIS) can be better appreciated, the two patient groups are shown immediately adjacent to one another at weeks 0, 2, 6, 8, 26, and 32 post-ART in Suppl. Fig 6.

B. Plot depicting Kaplan–Meier estimates for probability of developing TBIRIS depending on pre-ART frequency of OX40+CD4+ T cells. Black line: patients with >7.4% OX40+CD4+ T cells at ART initiation; grey line: patients with ≤ 7.4% OX40+CD4+ T cells at ART initiation.

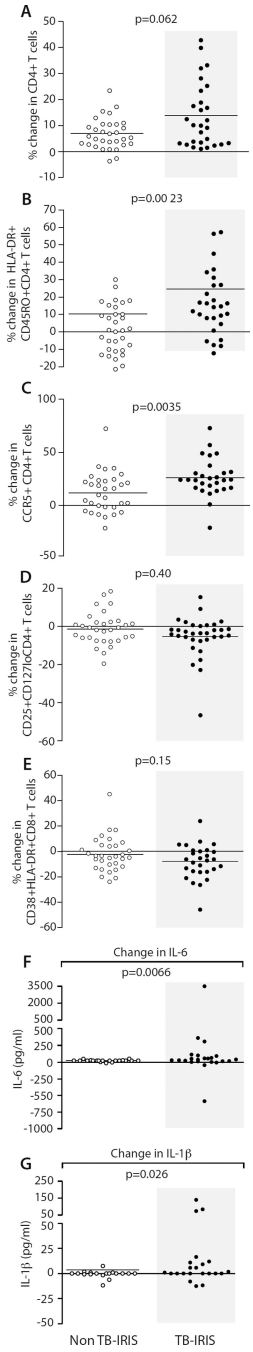

Figure 5. Net changes in cellular phenotypes and plasma cytokines early post-ART in TB-IRIS and non-TB-IRIS patients

The Wilcoxon rank sum test was used to compare the net change in cellular phenotype frequency (n=28) and plasma cytokine level (n=19) in non-TBIRIS patients from week 0 to week 2 and cellular phenotype frequency (n=32) and plasma cytokine level (n=23) in TB-IRIS patients from week 0 to the TB-RIIS event. P and q values for these analyses are presented in Suppl. Tables 4 and 5. A) CD3+CD4+ T cells; B) HLA-DR+CD45RO+CD4+

T cells; C) CCR5+CD4+ T cells; D) HLA-DR+CD38+CD8+ T cells; E) CD4+ regulatory T cells; F) plasma IL-6; and G) plasma IL-1β