Paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome after early initiation of antiretroviral therapy in a randomized clinical trial

Didier Laureillard[a], Olivier Marcy[b,c], Yoann Madec[d], Sokeo Chea[c], Sarin Chan[c,e], Laurence Borand[b], Marcelo Fernandez[c], Narom Prak[f], Chindamony Kim[g,h], Bunnet Dim[h,i], Eric Nerrienet[b], Thim Sok[c], Jean-François Delfraissy[j], Anne E. Goldfeld[c,k], François-Xavier Blanc[j], for the CAMELIA (ANRS 1295 – CIPRA KH001) Study Team

Objective: To analyze cases of paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) in the CAMbodian Early versus Late Introduction of Antiretrovirals (CAMELIA) randomized trial designed to compare early (2 weeks) versus late (8 weeks) antiretroviral therapy (ART) initiation after tuberculosis treatment onset in Cambodia (NCT00226434).

Methods: ART-naive adults with $CD4^+$ cell count of 200 cells/μl or less, newly diagnosed tuberculosis, and at least one follow-up visit after ART initiation were included in this analysis. Each case of suspected TB-IRIS was systematically validated by two physicians not involved in patients' management. Factors associated with occurrence of TB-IRIS were identified using the Cox proportional hazard model.

Results: Among 597 patients, 26% experienced TB-IRIS with an incidence rate of 37.9 cases per 100 person-years [95% confidence interval (CI) 32.4–44.4]. Main clinical manifestations included new or worsening lymphadenopathy (77.4%) and fever (68.4%). Chest radiograph revealed new or worsening abnormalities in 53.4%. Symptoms resolved in 95.5% of patients. Six deaths were directly related to TB-IRIS. Initiating ART early increased the risk of TB-IRIS by 2.61 (95% CI 1.84–3.70). Extrapulmonary or disseminated tuberculosis, $CD4^+$ cell count of 100 cells/μl or less, and HIV RNA concentration more than 6 $\log_{10}$ copies/ml were also significantly associated with higher risk of TB-IRIS.

Conclusion: Shortening the delay between tuberculosis treatment onset and ART initiation to 2 weeks was associated with an increased risk of developing TB-IRIS. However, TB-IRIS was generally easily manageable. Given the marked reported

[a]French National Agency for Research on AIDS and Viral Hepatitis (ANRS), Ho Chi Minh City, Vietnam, [b]Institut Pasteur du Cambodge, [c]Cambodian Health Committee, Phnom Penh, Cambodia, [d]Institut Pasteur, Paris, France, [e]Calmette Hospital, [f]Khmer Soviet Friendship Hospital, Phnom Penh, [g]Donkeo Provincial Hospital, Takeo, [h]Médecins Sans Frontières, Phnom Penh, [i]Siem Reap Referral Hospital, Siem Reap, Cambodia, [j]Assistance Publique - Hôpitaux de Paris, Hôpital Bicêtre, Hôpitaux Universitaires Paris Sud, Le Kremlin-Bicêtre, France, and [k]Program in Cellular and Molecular Medicine, Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA.

Correspondence to François-Xavier Blanc, MD, PhD, Service de Pneumologie, Hôpital Bicêtre, 78 Rue du Général Leclerc, 94275 Le Kremlin-Bicêtre, France.

Tel: +33 620 444 620; e-mail: xavier.blanc@bct.aphp.fr
Received: 5 May 2013; revised: 20 May 2013; accepted: 13 June 2013.

DOI:10.1097/01.aids.0000432456.14099.c7

survival advantage of early ART initiation after tuberculosis treatment onset, these data indicate that fear of TB-IRIS should not be an impediment to early ART in adults with advanced immunodeficiency in resource-limited, high burden settings.

AIDS 2013, **27**:2577–2586

Keywords: adult, antiretroviral therapy, Cambodia, HIV/AIDS, immune reconstitution disease, randomized controlled trial, tuberculosis

Introduction

Over the past two decades, potent antiretroviral therapy (ART) has dramatically reduced morbidity, AIDS progression, and mortality in patients with HIV infection [1]. However, some individuals experience clinical deterioration shortly after ART initiation despite virological efficacy. This phenomenon, known as immune reconstitution inflammatory syndrome (IRIS) [2,3], is usually linked with an exaggerated inflammatory response to either a previously diagnosed and treated infection (paradoxical IRIS) or to an unrecognized and untreated infection (unmasking IRIS) [4,5]. IRIS has been reported to occur in up to 30% of patients after ART initiation, usually within the first weeks [6–10]. This syndrome has been associated with: a large number of pathogens, including *Cryptococcus neoformans*, cytomegalovirus, varicella-zoster virus, hepatitis B and C, JC virus associated progressive multifocal leukoencephalopathy; and noninfectious diseases such as Kaposi's sarcoma, sarcoidosis and autoimmune disease [11–20]. However, *Mycobacterium tuberculosis,* a major cause of mortality among HIV-infected patients [21], is the most frequent cause of IRIS [9,22]. In high tuberculosis prevalence settings, paradoxical tuberculosis-associated IRIS (TB-IRIS) is a common complication following ART initiation and is reported to occur in 8–43% of patients [23–26]. Most descriptions of TB-IRIS have been reported from retrospective analyses or case reports, in which it has been commonly linked to extrapulmonary and disseminated tuberculosis, low baseline $CD4^+$ cell count, favorable response to ART, and a short interval between onset of tuberculosis treatment and ART initiation [27,28]. The fear of managing TB-IRIS is a common reason put forward to delay ART initiation once tuberculosis has been diagnosed. However, recent publications have shown that initiating ART early after tuberculosis treatment onset markedly reduces mortality, particularly in patients with severe immunosuppression [29–31].

Here, we report the incidence, clinical features, outcomes, and risk factors of TB-IRIS in HIV-infected adults enrolled in the CAMELIA trial (ANRS 1295/CIPRA KH001 DAIDS-ES 10425) with both newly diagnosed smear-positive tuberculosis and advanced immunodeficiency.

Methods

Study population

CAMELIA was a randomized trial designed to determine the optimal timing of ART initiation after tuberculosis treatment onset in Cambodia. From January 2006 to May 2009, we enrolled 661 HIV-infected ART-naive adults with a positive smear for acid-fast bacilli and $CD4^+$ cell count less than or equal to 200 cells/μl, as described elsewhere [29]. Following initiation of a standard 6-month tuberculosis treatment, patients were randomly assigned to initiate ART at either 2 weeks (early-ART group) or 8 weeks (late-ART group) with a combination of stavudine, lamivudine, and efavirenz. Randomization was stratified according to study site and $CD4^+$ cell count at enrollment ($\leq$50 or 51–200 cells/μl). The trial demonstrated that early initiation of ART significantly reduced the risk of mortality by 34% as compared to a later initiation ($P<0.006$) [29]. Patients included in the current analysis are those with both documented tuberculosis (defined here as a positive culture for *M. tuberculosis* or positive smear without evidence of nontuberculous mycobacteria in culture), and at least one follow-up visit after having initiated ART.

Tuberculosis-associated immune reconstitution inflammatory syndrome case definition

Characterization of TB-IRIS was a secondary objective of the CAMELIA trial. TB-IRIS was defined as unexplained worsening or emergence of symptoms or signs of tuberculosis (e.g. fever, cough, shortness of breath, lymph node, or exacerbation of disease at other extrapulmonary sites) occurring after ART initiation, in agreement with previously published definitions [2,3,27,32,33]. Differential diagnoses such as poor adherence, drug-related side-effects, and other associated diseases were systematically considered and excluded. Each case of suspected TB-IRIS reported by on-site treating physicians was subsequently validated by at least two experienced physicians, who were members of the study team not involved in the day-to-day management of the patients. The validation process was not blinded and reviewers had full access to medical record documents and outcomes.

Ethics

The trial was approved by the National Ethics Committee for Health Research of the Cambodian Ministry of

Health, the Ethical Review Board of Médecins sans Frontières, and the Institutional Review Board of Immune Disease Institute (Boston, Massachusetts, USA), and conducted according to the principles expressed in the Declaration of Helsinki. All patients enrolled in the study gave written, informed consent to participate. All patient information was entered into a database using coded identification numbers. No information that could reveal patient identity was entered into the database.

Statistical analysis

Baseline characteristics were compared between patients who did and who did not experience TB-IRIS using χ^2 test for categorical variables or Student's *t*-test for continuous variables. Delay to TB-IRIS was described using Kaplan–Meier estimates. Factors associated with occurrence of TB-IRIS were identified using Cox proportional hazard model. The proportional hazards assumption was checked with the use of a test based on Schoenfeld residuals and was found to be valid for all factors investigated. All factors associated with occurrence of TB-IRIS in univariate analysis with a *P* value less than 0.20 were entered in the multivariate model, and a backward stepwise procedure was used to identify factors that remained significant. A sensitivity analysis was conducted considering only patients with culture-confirmed and drug-sensitive *M. tuberculosis* strains. We investigated the association between occurrence of TB-IRIS and mortality using a Cox proportional hazard model, occurrence of TB-IRIS being considered as a time-dependent cofactor. Again, a test based on Schoenfeld residuals was used to check that the proportional hazard assumption was valid. Statistical analyses were performed using STATA 11 (Stata Corp. College Station, Texas, USA). The CAMELIA trial is registered with ClinicalTrials.gov, number NCT00226434.

Results

Study population

Among the 661 patients enrolled in the CAMELIA trial, 597 individuals (308 in the early-ART group and 289 in the late-ART group) were considered in this analysis. The remaining 64 patients were excluded for the following reasons: 16 patients were infected with nontuberculous mycobacteria as identified by culture, 37 died, and two withdrew before ART initiation, whereas nine did not have a follow-up visit after ART initiation. As shown in Table 1, male patients represented nearly two-thirds of the study population. The majority of tuberculosis cases were pulmonary tuberculosis (69.7%), with 12.7% of extrapulmonary tuberculosis and 17.6% disseminated tuberculosis. Tuberculosis was confirmed by culture in 88.8% of cases. At ART initiation, mean age was 36 years [standard deviation (SD): 7.9], median $CD4^+$ cell count was 26 cells/μl [interquartile range (IQR): 12–62], and median plasma HIV RNA concentration was 5.6 $\log_{10}$ copies/ml (IQR: 5.2–6.0). Furthermore, patients from the early-ART group had lower hemoglobin level ($P<0.001$), Karnofsky score ($P<0.001$), and BMI ($P<0.001$) and higher alanine aminotransferase level ($P=0.021$) than patients from the late-ART group (Table 1).

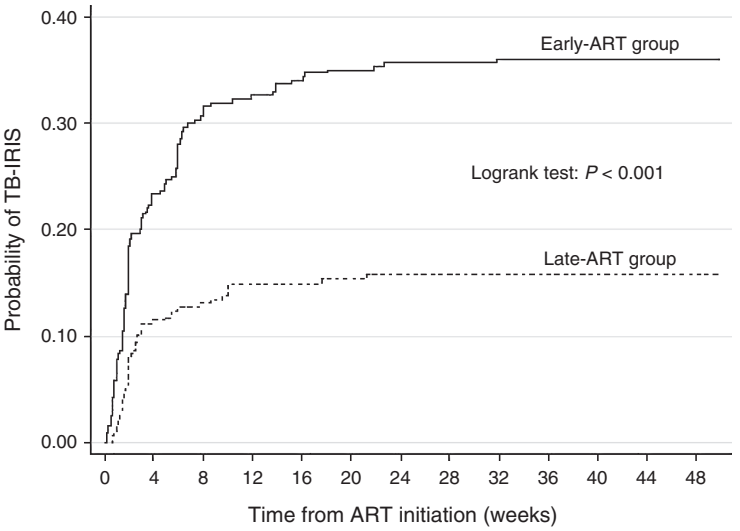
Incidence and time of occurrence of tuberculosis-associated immune reconstitution inflammatory syndrome

Among the 597 patients included in this analysis who were followed for a median time of 26 months

Table 1. Distribution of patient characteristics at antiretroviral therapy initiation according to CAMELIA study arm.

	Total ($n=597$)	Early-ART group ($n=308$)	Late-ART group ($n=289$)	*P*
Men *n* (%)	385 (64.5)	200 (64.9)	185 (64.1)	0.81
Age (years) mean (SD)	36 (7.9)	36 (7.8)	36 (8.1)	0.67
$CD4^+$ cell count (cells/μl) median (IQR)	26 (12–62)	27 (12–62)	26 (13–61)	0.26
Viral load ($\log_{10}$ copies/ml) median (IQR)	5.6 (5.2–6.0)	5.6 (5.2–6.0)	5.6 (5.2–6.0)	0.71
Hemoglobin (g/l) median (IQR)	101 (82–117)	87 (72–105)	111 (100–123)	<0.001
Karnofsky score *n* (%)				<0.001
≥80	329 (55.1)	100 (32.4)	229 (79.2)	
50–70	252 (42.2)	196 (63.6)	56 (19.4)	
≤40	16 (2.7)	12 (3.9)	4 (1.4)	
BMI (kg/m^2) mean (SD)	17.6 (2.5)	17.1 (2.3)	18.2 (2.7)	<0.001
ALT >2.50 ULN *n* (%)	10 (1.7)	9 (2.9)	1 (0.3)	0.021
Tuberculosis location *n* (%)				0.32
Pulmonary	416 (69.7)	213 (69.1)	203 (70.2)	
Extrapulmonary	76 (12.7)	35 (11.4)	41 (14.2)	
Disseminated[a]	105 (17.6)	60 (19.5)	45 (15.6)	
Diagnosis of tuberculosis *n* (%)				0.37
Culture confirmed	530 (88.8)	270 (87.7)	260 (90.0)	
AFB smear +, culture –	67 (11.2)	38 (12.3)	29 (10.0)	

AFB, acid-fast bacilli; ALT, alanine aminotransferase; ART, antiretroviral therapy; IQR, interquartile range; SD, standard deviation; ULN, upper limit normal.
[a]Defined as the presence of both pulmonary and extrapulmonary tuberculosis.



Weeks	0	4	12	24	36
No. at risk					
Early-ART group	308	235	200	187	186
Late-ART group	289	255	241	223	218
No. of TB-IRIS*					
Early-ART group		72	100	109	110
Late-ART group		33	43	45	45

* Cumulative

Fig. 1. Kaplan–Meier estimates of occurrence of tuberculosis-associated immune reconstitution inflammatory syndrome by study arm. ART, antiretroviral therapy; TB-IRIS, tuberculosis-associated immune reconstitution inflammatory syndrome.

(IQR: 15–36), 155 (26%) experienced TB-IRIS. In the early-ART group, 110 of 308 (36%) patients experienced TB-IRIS as compared with 45 of 289 (16%) in the late-ART group ($P<0.001$). The overall TB-IRIS incidence rate was 37.9 cases per 100 person-years [95% confidence interval (CI) 32.4–44.4]. As shown by the Kaplan–Meier estimate (Fig. 1), occurrence of TB-IRIS was significantly higher in the early-ART group than in the late-ART group with incidences of 58.2 cases per 100 person-years (95% CI 48.3–70.2) and 20.4 cases per 100 person-years (95% CI 15.3–27.4), respectively ($P<0.001$).

The median time between ART initiation and TB-IRIS was 14 days (IQR: 10–42) and did not differ between patients enrolled in the early and late-ART groups ($P=0.53$). In the majority of patients (143/155), TB-IRIS occurred during the first 3 months of ART. Only 11 patients experienced TB-IRIS between 3 and 6 months after ART initiation (nine in the early-ART group and two in the late-ART group) and one after 6 months. Interestingly, this later patient was lost to follow-up, stopped ART for several months, and developed TB-IRIS within the first month of ART reintroduction.

Clinical and radiological features of tuberculosis-associated immune reconstitution inflammatory syndrome

The most frequent clinical manifestations of TB-IRIS were emergence or worsening of lymphadenopathy (120 patients), and fever (106 patients; Table 2). Abdominal manifestations were also common, including abdominal pain (44 patients), hepatomegaly (16 patients), and ascites (15 patients). Neurological manifestations were observed in eight patients. At the time of TB-IRIS, a chest radiograph was performed in 103 patients. New or worsening chest radiograph abnormalities were observed in 55 patients, the most common being parenchymal opacities (36 patients), mediastinal lymph node enlargement (27 patients), and pleural effusion (17 patients). There were no differences in clinical symptoms of TB-IRIS between patients in the early and late-ART group, with the exception of more frequent enlargement of mediastinal lymph nodes in the late-ART group ($P=0.016$; Table 2).

Tuberculosis-associated immune reconstitution inflammatory syndrome treatment and outcome

The median duration of TB-IRIS symptoms was 7.4 weeks (IQR: 4.0–19.8). Of the 155 TB-IRIS cases,

Table 2. Manifestations, treatment, and outcome of tuberculosis-associated immune reconstitution inflammatory syndrome according to the timing of antiretroviral therapy initiation.

	Total ($n=155$)	Early-ART group ($n=110$)	Late-ART group ($n=45$)	*P*
Symptoms at the time of TB-IRIS, *n* (%)				
Lymph nodes	120 (77.4)	83 (75.5)	37 (82.2)	0.36
Peripheral lymph nodes	94 (60.6)	68 (61.8)	26 (57.8)	0.64
Abdominal lymph nodes	23 (14.8)	18 (16.4)	5 (11.1)	0.40
Mediastinal lymph nodes	27 (17.4)	14 (12.7)	13 (28.9)	0.016
Fever	106 (68.4)	74 (67.3)	32 (71.1)	0.64
Abdominal pain	44 (28.4)	30 (27.3)	14 (31.1)	0.61
Hepatomegaly	16 (10.3)	12 (10.9)	4 (8.9)	0.71
Ascites	15 (9.7)	9 (8.2)	6 (13.3)	0.33
Neurological symptoms	8 (5.2)	6 (5.5)	2 (4.4)	0.80
Chest radiograph performed at the time of TB-IRIS, *n* (%):	103 (66.5)	74 (67.3)	29 (64.4)	0.10
New or worsening signs	55 (53.4)	36 (48.7)	19 (65.5)	
Unchanged	30 (29.1)	26 (35.1)	4 (13.8)	
Improved	18 (17.5)	12 (16.2)	6 (20.7)	
Treatment received during TB-IRIS, *n* (%)				0.76
None	41 (26.5)	30 (27.3)	11 (24.4)	
NSAID	55 (35.5)	41 (37.3)	14 (31.1)	
Steroids	23 (14.8)	15 (13.6)	8 (17.8)	
NSAID + steroids	36 (23.2)	24 (21.8)	12 (26.7)	
Delay from TB-IRIS to NSAID/steroids initiation, days: median (IQR)	5 (1–14)	5 (1–14)	3.5 (0–10)	0.30
Duration of TB-IRIS (weeks): median (IQR)	7.2 (4.0–18.1)	7.2 (4.1–20.0)	7.4 (4.0–14.9)	0.36
Outcome of TB-IRIS, *n* (%)				0.33
Cured	144 (92.9)	100 (90.9)	44 (97.8)	
TB-IRIS-related death	6 (3.9)	6 (5.5)	0 (0.0)	
Death not directly attributed to TB-IRIS	5 (3.2)	4 (3.6)	1 (2.2)	

ART, antiretroviral therapy; IQR, interquartile range; TB-IRIS, paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome.

55 patients (35.5%) were treated with NSAIDs for a median time of 12 days (IQR: 9–24) and 59 patients (38%) received steroids for a median time of 40 days (IQR: 24–70; Table 2). Forty-one patients (26.5%) did not receive any anti-inflammatory treatment. Overall, more than 92% (144/155) of patients who presented with TB-IRIS experienced a favorable outcome with regression of symptoms.

Out of 597 patients, 95 (15.9%) died during the overall follow-up. There were 23 deaths among the 155 patients (14.8%) who experienced TB-IRIS and 72 deaths among the 442 patients (16.3%) who did not experience TB-IRIS throughout the study. In a Cox proportional hazard model, there was no association between occurrence of TB-IRIS and mortality [crude hazard ratio: 0.97 (95% CI: 0.60–1.57); $P=0.91$]. Out of the 23 deaths that occurred in the 155 patients who experienced TB-IRIS, 12 were definitively not related to TB-IRIS and occurred several weeks or months after resolution of TB-IRIS (voluntary prolonged interruption of follow-up and ART for four patients, lactic acidosis for four patients, two sepsis, one gastrointestinal hemorrhage, and one hemoptysis). Six deaths were directly attributed to TB-IRIS, all in patients from the early-ART group: two had central neurological disorders (one meningitis and one tuberculoma) and four presented with severe abdominal symptoms related to enlarged inflammatory lymph nodes, ascites, and abscess. The five remaining deaths could not be directly attributed to TB-IRIS: stroke was the cause of death in a 60-year-old man, while acute severe hepatotoxicity and intestinal obstruction were noticed in two patients. Another two patients were found dead at home a few weeks after initial improvement of TB-IRIS and hospital discharge. For these five patients, TB-IRIS might have contributed to the fatal outcome despite not being the direct cause of death.

$CD4^+$ cell count gain and viral load suppression

At ART initiation, $CD4^+$ cell count did not differ between TB-IRIS and non-TB-IRIS patients [median (IQR): 27 (12–52) cells/μl and 25 (12–63) cells/μl, respectively; $P=0.06$]. After 6 months of tuberculosis treatment, which corresponded to 24 and 18 weeks of ART in the early-ART and late-ART groups, respectively, the median $CD4^+$ cell count gain was significantly higher in patients who experienced TB-IRIS than in those who did not ($P=0.01$; Fig. 2). This difference persisted at one year of follow-up ($P=0.02$), but not at later time points measured (up to 48 months). Among patients with TB-IRIS, median $CD4^+$ cell count gain was similar at 6 and 12 months of ART in those who received corticosteroids [120 cells/μl (66–156) and 175 cells/μl (111–222), respectively] and those who did not [123 cells/μl (83–197) and 176 cells/μl (122–258), respectively] ($P=0.30$ and $P=0.59$, respectively). The proportion of patients with undetectable HIV RNA did not differ between those who experienced TB-IRIS and those who did not at anytime after ART initiation (Fig. 2).

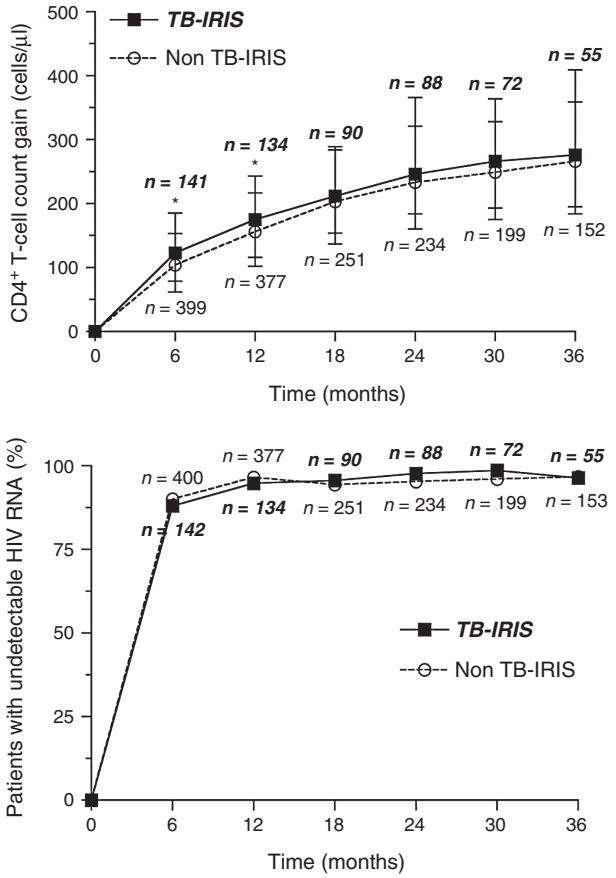


Fig. 2. Time course of immunological (upper panel) and virological (lower panel) outcomes over a 3-year period. For each time point, the number of patients who experienced a tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) is written in bold italic. *: $P < 0.05$ (Student's *t*-test).

Risk factors of tuberculosis-associated immune reconstitution inflammatory syndrome

In multivariate analysis, factors independently associated with an increased risk of TB-IRIS were early ART initiation ($P < 0.001$), extrapulmonary or disseminated tuberculosis ($P < 0.001$), mediastinal lymphadenopathy on chest radiograph at enrollment ($P = 0.004$), $CD4^+$ cell count of 100 cells/µl or less ($P = 0.048$), and HIV RNA more than 6 $\log_{10}$ copies/ml at ART initiation ($P = 0.031$; Table 3). A sensitivity analysis considering only cases with culture positive and drug-sensitive tuberculosis did not affect these findings (data not shown).

Discussion

Our study reports the largest published series of TB-IRIS in the context of a randomized clinical trial conducted in severely immunocompromised patients (median $CD4^+$ cell count: 26 cells/µl). The proportion of paradoxical TB-IRIS observed during the CAMELIA trial is among the highest reported. Starting ART early after tuberculosis treatment onset was the strongest risk factor for developing TB-IRIS. $CD4^+$ cell count below or equal to 100 cells/µl, viral load higher than 6 $\log_{10}$ copies/ml, extrapulmonary or disseminated tuberculosis also significantly increased this risk.

The CAMELIA trial design allowed us to demonstrate that the occurrence of TB-IRIS was strongly influenced by the timing of ART initiation in adults with advanced immunodeficiency. In multivariate analysis, enrollment in the early-ART group nearly tripled the risk of developing TB-IRIS. It might be due, at least in part, to a higher residual amount of mycobacterial antigen at the time of early ART initiation. This finding is consistent with data reported from the Starting Antiretroviral Therapy at Three Points in Tuberculosis (SAPIT) and AIDS Clinical Trials Group Study A5221 (STRIDE) trials, which enrolled less immunocompromised patients who experienced TB-IRIS less frequently [34,35]. A practical implication of our results is that clinicians and patients should be prepared to face a temporary worsening after starting ART early in patients with tuberculosis.

The frequency of TB-IRIS reported in this study is high, but it is consistent with previous reports [25,36–39]. Reported factors that are often associated with TB-IRIS include extrapulmonary or disseminated tuberculosis (presumably because of a high antigen burden), low $CD4^+$ cell count, and high viral load at ART initiation [24,40,41]. The majority of our patients (87.3%) had a $CD4^+$ cell count below 100 cells/µl, which nearly doubled the risk of developing TB-IRIS when compared with a $CD4^+$ cell count ranging from 100 to 200 cells/µl. Similarly, the risk of developing TB-IRIS was doubled in cases of extrapulmonary or disseminated tuberculosis, or when HIV RNA was above 10^6 $\log_{10}$ copies/ml. Thus, physicians who initiate ART in adults with both $CD4^+$ cell count below 100 cells/µl and extrapulmonary or disseminated tuberculosis should be particularly vigilant regarding potential TB-IRIS. Moreover, detailed and systematic information about its high frequency and management should be part of pre-ART counseling in such patients, similar to what is currently done for adherence and drug side-effects.

In our study, the time between ART initiation and TB-IRIS occurrence was similar in the early-ART and late-ART groups. More than two-thirds of TB-IRIS (105/155) occurred within the first month of ART and 92% (143/155) occurred during the first 3 months. These data are consistent with the definition published by the International Network for the Study of HIV-associated IRIS while our study was ongoing, and that set a limit of 3 months between the initiation of ART and the onset of TB-IRIS manifestations [27]. However, TB-IRIS cases

Table 3. Risk factors associated with tuberculosis-associated immune reconstitution inflammatory syndrome (Cox model).

	N	TB-IRIS *N* (%)	Crude HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Study arm				<0.001		<0.001
Early ART initiation	308	110 (35.7)	2.58 (1.82–3.65)		2.61 (1.84–3.70)	
Late ART initiation	289	45 (15.6)	1		1	
Sex				0.037		
Male	385	111 (28.8)	1			
Female	212	44 (20.7)	0.70 (0.49–0.99)			
Age (years)				0.64		
≤29	142	41 (28.9)	1			
30–39	262	66 (25.2)	0.84 (0.57–1.24)			
≥40	193	48 (24.8)	0.84 (0.56–1.28)			
BMI at ART initiation (kg/m^2)				0.84		
≤16	160	43 (26.9)	1.16 (0.77–1.75)			
16–17	99	27 (27.3)	1.18 (0.74–1.89)			
17–18.5	132	36 (27.7)	1.16 (0.75–1.78)			
>18.5	206	49 (23.8)	1			
Karnofsky score at ART initiation				0.013		
≥80	329	71 (21.6)	1			
50–70	252	80 (31.7)	1.62 (1.18–2.23)			
≤40	16	4 (25.0)	1.26 (0.46–3.45)			
CD4$^+$ cell count at ART initiation (cells/μl)				0.038		0.048
≤25	296	74 (25.0)	1.70 (0.92–3.12)		1.58 (0.85–2.91)	
26–50	118	39 (33.0)	2.37 (1.24–4.53)		2.35 (1.23–4.51)	
51–100	107	30 (28.0)	1.95 (1.00–3.91)		1.80 (0.92–3.55)	
>100	76	12 (15.8)	1		1	
Viral load at ART initiation ($\log_{10}$ copies/ml)				0.014		0.031
≤5.00	115	24 (20.9)	1		1	
5.01–6.00	330	79 (23.9)	1.18 (0.75–1.87)		1.15 (0.73–1.82)	
>6.00	152	52 (34.1)	1.86 (1.15–3.02)		1.74 (1.06–2.84)	
Hemoglobin at ART initiation (g/l)				<0.001		
≤70	76	31 (40.8)	2.58 (1.67–3.99)			
70–85	94	30 (31.9)	1.88 (1.21–2.92)			
85–100	116	35 (30.2)	1.69 (1.12–2.57)			
>100	311	59 (19.0)	1			
ALT at ART initiation				0.20		
≤2.50 ULN	587	154 (26.2)	1			
>2.50 ULN	10	1 (10.0)	0.35 (0.05–2.48)			
Tuberculosis location				0.001		<0.001
Pulmonary	416	89 (21.4)	1		1	
Extrapulmonary	76	28 (36.8)	1.89 (1.23–2.89)		2.06 (1.34–3.17)	
Disseminated	105	38 (63.2)	1.94 (1.33–2.84)		1.88 (1.28–2.77)	
Tuberculosis drug resistance at enrollment				0.44		
No	486	129 (26.5)	1			
Yes	101	24 (23.8)	0.89 (0.58–1.38)			
Yes, MDR	9	1 (11.1)	0.36 (0.05–2.61)			
Chest radiograph at enrollment				0.44		
Normal	113	24 (21.2)	1			
1 abnormality	211	54 (25.6)	1.22 (0.75–1.97)			
≥2 abnormalities	273	77 (28.2)	1.34 (0.85–2.12)			
Mediastinal adenopathy at enrollment				0.003		0.004
No	380	83 (21.8)	1		1	
Yes	217	72 (33.2)	1.61 (1.17–2.21)		1.62 (1.17–2.23)	
Initial tuberculosis culture				0.68		
Negative	67	15 (22.4)	1			
Positive for *Mycobacterium tuberculosis*	530	140 (26.4)	1.35 (0.33–5.46)			

ALT, alanine aminotransferase; ART, antiretroviral therapy; CI, confidence interval; HR, hazard ratio; MDR, multidrug-resistant; TB-IRIS, paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome; ULN, upper limit normal.

have been reported to occur later, even beyond 6 months post-ART initiation [6,23,37,40,41]. In our study, 11 patients experienced TB-IRIS after 3 months of ART and none after 6 months. The only 'very late presentation' occurred 6 weeks after ART reinitiation in a patient who had been lost to the trial for 5 months. On the basis of these findings, we suggest that the timing for consensus definition of TB-IRIS should be extended up to 6 months.

The most common presentation of TB-IRIS in CAMELIA included new-onset or enlarging lymph nodes, fever, and abdominal symptoms (abdominal pain, ascites, and hepatomegaly). Although these symptoms are easy to identify, they are nonspecific and are frequently reported in HIV-infected adults with tuberculosis in resource-limited settings, which underscores the difficulty of ruling out potential competing diagnoses such as

the occurrence of another opportunistic infection or the progression of HIV disease. However, we note that neurological TB-IRIS occurred in only eight patients of our series, which is lower than what has been previously reported [42].

In our study, there was no association between occurrence of TB-IRIS and mortality. TB-IRIS was not a common direct cause of death: six (3.9%) of patients who developed TB-IRIS died without another clear cause of death. Even including the five others in whom TB-IRIS itself might have contributed to a fatal outcome despite not being the direct cause of death, case fatality rate reached 7%, which is relatively low in this very advanced immunosuppressed population. Notwithstanding this, we do note that the six TB-IRIS events that were directly assigned as a direct cause of death all occurred in patients enrolled in the early-ART arm.

We observed a greater $CD4^+$ cell count gain in patients with TB-IRIS compared with those without TB-IRIS at 6 and 12 months of follow-up, consistent with other studies [23,37]. However, $CD4^+$ cell gain was similar in the two groups at the end of follow-up. Of interest, we did not observe a lower reconstitution of $CD4^+$ cell count in patients treated with steroids, unlike what has been reported [43]. Furthermore, we found the same high percentage of patients with undetectable plasma viral load in patients with TB-IRIS compared with those without TB-IRIS during the study duration, up to 90% from the first year.

A potential limitation of our study is that some forms of TB-IRIS may not have been recognized by site clinicians, especially those presentations without fever, lymph node enlargement, or major clinical symptoms. We note that these minor forms of TB-IRIS do not require any specific medical management and spontaneously resolve. Furthermore, although it was clearly stated in the study procedures, chest radiograph was not performed in all suspected cases of TB-IRIS, making it difficult to draw definite conclusions on the proportion of new or worsening radiological abnormalities. However, among the two-thirds of patients who had a chest radiograph at the time of TB-IRIS, 53% presented new or worsening radiological abnormalities, whereas at least 29% of patients with TB-IRIS had no chest radiograph modification between tuberculosis diagnosis and the occurrence of TB-IRIS. Although the absence of worsening or new radiological finding does not rule out the diagnosis of TB-IRIS, our data suggest that this simple and noninvasive examination be performed when an event occurs during the first weeks following ART initiation, given the reported high frequency of new-onset or worsening respiratory symptoms and increased lymphadenopathy in TB-IRIS [44]. Another limitation of our study was the lack of established criteria to initiate NSAID or steroids for TB-IRIS. This could explain the relatively high percentage of patients treated with these drugs as the decision to initiate a specific treatment course was not standardized across study sites and largely depended on the site physician's previous experience, expert opinion, or data from observational studies [43,45]. NSAIDs were largely prescribed by clinicians and steroids were added when the symptoms did not resolve quickly, or were initiated to treat life-threatening manifestations. We note that it remains difficult to provide clear therapeutic recommendations as there are no clinical data about efficacy of NSAID in TB-IRIS while steroids have only been shown to reduce morbidity and hospitalization in a single randomized clinical trial [46]. However, the management of TB-IRIS in our series was generally successful and led to more than 90% of events resolution without any sequelae.

Shortening the delay to initiate ART to 2 weeks after tuberculosis treatment onset in severely immunocompromised HIV-infected adults increased the risk of developing TB-IRIS, especially when tuberculosis was extrapulmonary or disseminated. However, TB-IRIS was clinically manageable and outcome was generally favorable. As initiating ART early at 2 weeks after tuberculosis treatment onset provides a marked survival advantage in severely immunocompromised HIV-infected adults [29–31] and that there is no association between occurrence of TB-IRIS and mortality, healthcare managers should reinforce TB-IRIS training in both HIV and tuberculosis programs to strengthen the recognition of this frequent complication by clinicians. Moreover, pre-ART counseling should include systematic information about TB-IRIS. Finally, our data indicate that TB-IRIS should not be deterrent to initiate ART early in resource limited-settings.

Acknowledgements

The authors would like to thank all patients who participated in the CAMELIA trial, the health workers of the five hospitals, Cambodian health authorities, particularly the National HIVAIDS (NCHADS) and the National Tuberculosis (CENAT) Programs, Médecins Sans Frontières Belgium, and the Organisation Franco-Cambodgienne de Pneumologie.

D.L. wrote the CAMELIA protocol, implemented the trial, supervised clinical issues, reviewed data, interpreted analyses, edited and approved the report. O.M. implemented the trial, supervised clinical issues, reviewed data, interpreted analyses, and edited the report. Y.M. analyzed data, and edited the report. So.C. enrolled and followed patients, reviewed data, and interpreted analyses concerning TB-IRIS. Sa.C. implemented the trial, supervised clinical issues, and reviewed data. L.B. implemented the CAMELIA trial, supervised clinical research issues,

and edited the report. M.F. implemented the trial, enrolled patients, and supervised clinical issues. N.P., C.K., and B.D. enrolled and followed patients. E.N. implemented the trial, supervised biological issues, interpreted analyses, and edited report. T.S. implemented the trial, supervised clinical issues, edited and approved the report. J.F.D. wrote the CAMELIA protocol, edited and approved the report. A.E.G. wrote the CAMELIA protocol, reviewed data, interpreted analyses, edited and approved the report. F.X.B. wrote the CAMELIA protocol, supervised clinical issues, reviewed data, interpreted analyses, edited and approved the report. The first draft of this article was written by D.L. and F.X.B. with assistance from A.E.G. The article was edited by D.L., O.M., Y.M., J.F.D., A.E.G., and F.X.B. The final version of the article was approved by all authors.

The CAMELIA trial was funded by the French National Agency for Research on AIDS and viral hepatitis (ANRS 1295) and the U.S. National Institutes of Health/National Institute of Allergy and Infectious Diseases, Division of AIDS (CIPRA KH001/DAID-ES ID 10425). The funders had no role in the design of the study, data collection and interpretation, decision to publish, and preparation of the article. The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

Conflicts of interest

J.F.D. reports serving on the international board for HIV treatment of Bristol-Myers Squibb, GlaxoSmithKline, Merck Sharp & Dohme Chibret, and Gilead. All other authors do not report any conflict of interest.

A summary of the results was presented at the 6th International AIDS Conference, Rome, Italy, 20 July 2011.

References

1. Mocroft A, Ledergerber B, Katlama C, Kirk O, Reiss P, d'Arminio Monforte A, *et al*. **Decline in the AIDS and death rates in the EuroSIDA study: an observational study.** *Lancet* 2003; **362**:22–29.
2. French MA, Price P, Stone SF. **Immune restoration disease after antiretroviral therapy.** *AIDS* 2004; **18**:1615–1627.
3. Shelburne SA, Montes M, Hamill RJ. **Immune reconstitution inflammatory syndrome: more answers, more questions.** *J Antimicrob Chemother* 2006; **57**:167–170.
4. French MA. **HIV/AIDS: immune reconstitution inflammatory syndrome – a reappraisal.** *Clin Infect Dis* 2009; **48**:101–107.
5. Kelley CF, Armstrong WS. **Update on immune reconstitution inflammatory syndrome: progress and unanswered questions.** *Curr Infect Dis Rep* 2009; **11**:486–493.
6. Shelburne SA, Visnegarwala F, Darcourt J, Graviss EA, Giordano TP, White AC Jr, *et al*. **Incidence and risk factors for immune reconstitution inflammatory syndrome during highly active antiretroviral therapy.** *AIDS* 2005; **19**:399–406.
7. Ratnam I, Chiu C, Kandala NB, Easterbrook PJ. **Incidence and risk factors for immune reconstitution inflammatory syndrome in an ethnically diverse HIV type 1-infected cohort.** *Clin Infect Dis* 2006; **42**:418–427.
8. Murdoch DM, Venter WD, Feldman C, Van Rie A. **Incidence and risk factors for the immune reconstitution inflammatory syndrome in HIV patients in South Africa: a prospective study.** *AIDS* 2008; **22**:601–610.
9. Muller M, Wandel S, Colebunders R, Attia S, Furrer H, Egger M. **Immune reconstitution inflammatory syndrome in patients starting antiretroviral therapy for HIV infection: a systematic review and meta-analysis.** *Lancet Infect Dis* 2010; **10**:251–261.
10. Novak RM, Richardson JT, Buchacz K, Chmiel JS, Durham MD, Palella FJ, *et al*. **Immune reconstitution inflammatory syndrome: incidence and implications for mortality.** *AIDS* 2012; **26**:721–730.
11. Murdoch DM, Venter WD, Van Rie A, Feldman C. **Immune reconstitution inflammatory syndrome (IRIS): review of common infectious manifestations and treatment options.** *AIDS Res Ther* 2007; **4**:9.
12. Achenbach CJ, Harrington RD, Dhanireddy S, Crane HM, Casper C, Kitahata MM. **Paradoxical immune reconstitution inflammatory syndrome in HIV-infected patients treated with combination antiretroviral therapy after AIDS-defining opportunistic infection.** *Clin Infect Dis* 2012; **54**:424–433.
13. Bicanic T, Meintjes G, Rebe K, Williams A, Loyse A, Wood R, *et al*. **Immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis: a prospective study.** *J Acquir Immune Defic Syndr* 2009; **51**:130–134.
14. Bower M, Nelson M, Young AM, Thirlwell C, Newsom-Davis T, Mandalia S, *et al*. **Immune reconstitution inflammatory syndrome associated with Kaposi's sarcoma.** *J Clin Oncol* 2005; **23**:5224–5228.
15. Feurle GE, Moos V, Schinnerling K, Geelhaar A, Allers K, Biagi F, *et al*. **The immune reconstitution inflammatory syndrome in whipple disease: a cohort study.** *Ann Intern Med* 2010; **153**:710–717.
16. Letang E, Almeida JM, Miro JM, Ayala E, White IE, Carrilho C, *et al*. **Predictors of immune reconstitution inflammatory syndrome-associated with kaposi sarcoma in mozambique: a prospective study.** *J Acquir Immune Defic Syndr* 2010; **53**:589–597.
17. Lortholary O, Fontanet A, Memain N, Martin A, Sitbon K, Dromer F. **Incidence and risk factors of immune reconstitution inflammatory syndrome complicating HIV-associated cryptococcosis in France.** *AIDS* 2005; **19**:1043–1049.
18. Safdar A, Rubocki RJ, Horvath JA, Narayan KK, Waldron RL. **Fatal immune restoration disease in human immunodeficiency virus type 1-infected patients with progressive multifocal leukoencephalopathy: impact of antiretroviral therapy-associated immune reconstitution.** *Clin Infect Dis* 2002; **35**:1250–1257.
19. Foulon G, Wislez M, Naccache JM, Blanc FX, Rabbat A, Israel-Biet D, *et al*. **Sarcoidosis in HIV-infected patients in the era of highly active antiretroviral therapy.** *Clin Infect Dis* 2004; **38**:418–425.
20. Knysz B, Bolanowski M, Klimczak M, Gladysz A, Zwolinska K. **Graves' disease as an immune reconstitution syndrome in an HIV-1-positive patient commencing effective antiretroviral therapy: case report and literature review.** *Viral Immunol* 2006; **19**:102–107.
21. WHO. Global Tuberculosis Report 2012. Geneva: World Health Organization; 2012. Available: http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf. [Accessed 5 April 2013].
22. Lawn SD, Bekker LG, Miller RF. **Immune reconstitution disease associated with mycobacterial infections in HIV-infected individuals receiving antiretrovirals.** *Lancet Infect Dis* 2005; **5**:361–373.
23. Breton G, Duval X, Estellat C, Poaletti X, Bonnet D, Mvondo Mvondo D, *et al*. **Determinants of immune reconstitution inflammatory syndrome in HIV type 1-infected patients with tuberculosis after initiation of antiretroviral therapy.** *Clin Infect Dis* 2004; **39**:1709–1712.
24. Lawn SD, Myer L, Bekker LG, Wood R. **Tuberculosis-associated immune reconstitution disease: incidence, risk factors and impact in an antiretroviral treatment service in South Africa.** *AIDS* 2007; **21**:335–341.
25. Elliott JH, Vohith K, Saramony S, Savuth C, Dara C, Sarim C, *et al*. **Immunopathogenesis and diagnosis of tuberculosis and tuberculosis-associated immune reconstitution inflammatory syndrome during early antiretroviral therapy.** *J Infect Dis* 2009; **200**:1736–1745.

26. Cohen K, Meintjes G. **Management of individuals requiring antiretroviral therapy and TB treatment.** *Curr Opin HIV AIDS* 2010; **5**:61–69.
27. Meintjes G, Lawn SD, Scano F, Maartens G, French MA, Worodria W, *et al*. **Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings.** *Lancet Infect Dis* 2008; **8**:516–523.
28. Gray JM, Cohn DL. **Tuberculosis and HIV coinfection.** *Semin Respir Crit Care Med* 2013; **34**:32–43.
29. Blanc FX, Sok T, Laureillard D, Borand L, Rekacewicz C, Nerrienet E, *et al*. **Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis.** *N Engl J Med* 2011; **365**:1471–1481.
30. Abdool Karim SS, Naidoo K, Grobler A, Padayatchi N, Baxter C, Gray AL, *et al*. **Integration of antiretroviral therapy with tuberculosis treatment.** *N Engl J Med* 2011; **365**:1492–1501.
31. Havlir DV, Kendall MA, Ive P, Kumwenda J, Swindells S, Qasba SS, *et al*. **Timing of antiretroviral therapy for HIV-1 infection and tuberculosis.** *N Engl J Med* 2011; **365**:1482–1491.
32. Colebunders R, John L, Huyst V, Kambugu A, Scano F, Lynen L. **Tuberculosis immune reconstitution inflammatory syndrome in countries with limited resources.** *Int J Tuberc Lung Dis* 2006; **10**:946–953.
33. Robertson J, Meier M, Wall J, Ying J, Fichtenbaum CJ. **Immune reconstitution syndrome in HIV: validating a case definition and identifying clinical predictors in persons initiating antiretroviral therapy.** *Clin Infect Dis* 2006; **42**:1639–1646.
34. Naidoo K, Yende-Zuma N, Padayatchi N, Naidoo K, Jithoo N, Nair G, *et al*. **The immune reconstitution inflammatory syndrome after antiretroviral therapy initiation in patients with tuberculosis: findings from the SAPiT trial.** *Ann Intern Med* 2012; **157**:313–324.
35. Luetkemeyer A, Kendall M, Nyirenda M, Wu X, Ive P, Andersen J, *et al*. **Severity and timing of paradoxical TB-IRIS in a 48-week multicenter randomized trial of immediate vs early ART in patients with $CD4^+ < 250$ cells/mm^3 starting TB treatment: A5221 STRIDE study.** *19th Conference on Retroviruses and Opportunistic Infections* 2012 Abstract 145.
36. Dibyendu D, Sarkar RN, Phaujdar S, Bhattacharyya K, Pal HK. **Incidence and risk factors of immune reconstitution inflammatory syndrome in HIV-TB coinfected patients.** *Braz J Infect Dis* 2011; **15**:553–559.
37. Michailidis C, Pozniak AL, Mandalia S, Basnayake S, Nelson MR, Gazzard BG. **Clinical characteristics of IRIS syndrome in patients with HIV and tuberculosis.** *Antivir Ther* 2005; **10**:417–422.
38. Worodria W, Menten J, Massinga-Loembe M, Mazakpwe D, Bagenda D, Koole O, *et al*. **Clinical spectrum, risk factors and outcome of immune reconstitution inflammatory syndrome in patients with tuberculosis-HIV coinfection.** *Antivir Ther* 2012; **17**:841–848.
39. van der Plas H, Meintjes G, Schutz C, Goliath R, Myer L, Baatjie D, *et al*. **Complications of antiretroviral therapy initiation in hospitalised patients with HIV-associated tuberculosis.** *PLoS One* 2013; **8**:e54145.
40. Burman W, Weis S, Vernon A, Khan A, Benator D, Jones B, *et al*. **Frequency, severity and duration of immune reconstitution events in HIV-related tuberculosis.** *Int J Tuberc Lung Dis* 2007; **11**:1282–1289.
41. Manosuthi W, Kiertiburanakul S, Phoorisri T, Sungkanuparph S. **Immune reconstitution inflammatory syndrome of tuberculosis among HIV-infected patients receiving antituberculous and antiretroviral therapy.** *J Infect* 2006; **53**:357–363.
42. Pepper DJ, Marais S, Maartens G, Rebe K, Morroni C, Rangaka MX, *et al*. **Neurologic manifestations of paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome: a case series.** *Clin Infect Dis* 2009; **48**:e96–107.
43. Breton G, Bourgarit A, Pavy S, Bonnet D, Martinez V, Duval X, *et al*. **Treatment of tuberculosis-associated immune reconstitution inflammatory syndrome in 34 HIV-infected patients.** *Int J Tuberc Lung Dis* 2012; **16**:1365–1370.
44. Zumla A, Raviglione M, Hafner R, von Reyn CF. **Tuberculosis.** *N Engl J Med* 2013; **368**:745–755.
45. Breen RA, Smith CJ, Bettinson H, Dart S, Bannister B, Johnson MA, *et al*. **Paradoxical reactions during tuberculosis treatment in patients with and without HIV co-infection.** *Thorax* 2004; **59**:704–707.
46. Meintjes G, Wilkinson RJ, Morroni C, Pepper DJ, Rebe K, Rangaka MX, *et al*. **Randomized placebo-controlled trial of prednisone for paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome.** *AIDS* 2010; **24**:2381–2390.