Immune reconstitution inflammatory syndrome in HIV late presenters starting integrase inhibitor containing antiretroviral therapy.

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Submitted.

ABSTRACT

Introduction

Integrase inhibitors (INI) induce a rapid HIV-RNA decrease and CD4 T-lymphocyte recovery. Both characteristics are also associated with immune reconstitution inflammatory syndrome (IRIS). Whether the use of INI-containing cART increases the risk for IRIS is unknown.

Methods

Observational study within the Dutch ATHENA cohort. HIV-1 late presenters initiating cART after March 2009 were included, if they had CD4 T-lymphocytes < 200 cells/mm^3 *and* were diagnosed with an opportunistic infection. IRIS was defined either according to the criteria by French et al ($IRIS_{FRENCH}$) or by a clinical IRIS diagnosis of the physician ($IRIS_{CLINICAL}$). The primary outcomes were the association between INI and the occurrence of $IRIS_{FRENCH}$ and $IRIS_{FRENCH+CLINICAL}$ in multivariable logistic regression and Cox regression models.

Results

672 patients with a median CD4 T-lymphocyte count of 35 cells/mm^3 were included. Treatment with INI was independently associated with $IRIS_{FRENCH}$ as well as $IRIS_{FRENCH+CLINICAL}$ (OR 2.43, 95% CI 1.45 – 4.07, and OR 2.17, 95% CI 1.45 – 3.25), which was confirmed by Cox regression (HR 1.71, 95% CI 1.17 – 2.49, and HR 2.45, 95% CI 1.52 – 3.96). Raltegravir (HR 4.10, 95% CI 2.30–7.29), but not dolutegravir (HR 1.30; 95% CI 0.77–2.19), was associated with IRIS. Elvitegravir was too infrequently used in this patient category to draw conclusions on IRIS risk. Steroid initiation for IRIS was more likely in those who initiated INI versus non-INI, but no increased hospital (re)admission or mortality rates were observed.

Conclusions

In HIV late presenters from a resource rich setting, treatment initiation including raltegravir but not dolutegravir increased the risk of IRIS. This association needs to be further explored to exclude residual confounding.

INTRODUCTION

Treatment with an integrase inhibitor (INI)-containing combination antiretroviral therapy (cART) regimen is recommended as the preferred first-line cART in current treatment guidelines for HIV-1 infected patients.[1,2] The use of INI-containing cART is associated with a faster decline of plasma HIV-RNA compared with a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing cART regimen, and in some studies INI are also associated with a faster recovery of CD4 T-lymphocytes.[3–5] However, a steep decline of HIV-RNA and a fast CD4-T-lymphocyte recovery are also risk factors for the immune reconstitution inflammatory syndrome (IRIS).[6–8] Therefore, HIV-1 late presenters are at a particularly high risk for IRIS.[8–10]

IRIS is an excessive, pathological inflammatory response against antigens of opportunistic infections (OI).[11,12] In view of the abovementioned arguments, the incidence of IRIS might be higher in patients initiating INI-containing cART than in patients starting a non-INI-containing cART regimen. However, most of the randomized phase 3 trials, comparing INI-containing cART regimens with non-INI-containing cART, were not suited to answer this question. Indeed, by excluding patients with an active OI at the start of cART, the number of patients at risk for IRIS in these studied was very limited. In fact, patients with an active OI were often explicitly excluded from being enrolled in these trials.[4,5] In contrast, large prospective observational HIV cohort studies typically include a significant number of HIV-1 late presenters and can therefore be useful to assess the IRIS risk in these patients.[13] IRIS can be difficult to diagnose and is associated with significant morbidity as well as (re)hospitalization. Occasionally IRIS can be lethal, in particular in patients with an intracranial infection. We hypothesize that the initiation of INI-containing cART is an independent risk factor for development of IRIS in HIV-1 late presenters.

METHODS

Study design and participants

This was a retrospective analysis conducted using data from the prospective Dutch nationwide observational HIV cohort maintained by the HIV Monitoring Foundation (Stichting HIV Monitoring, SHM), also known as the "AIDS Therapy Evaluation in the Netherlands" (ATHENA) cohort.[14] The ATHENA cohort comprises all patients in care for HIV in one of the 26 Dutch HIV treatment centers, who consented to have their data collected in ATHENA. IRIS has not been systematically collected in ATHENA. Therefore, we performed chart reviews in 22 of the 26 centers to retrospectively diagnose and collect information on IRIS. The chart reviews were limited to patients starting their first-line cART in the INI era and to those at

increased risk for the development of IRIS: we therefore included treatment naive adult HIV patients who initiated cART and who were included in the ATHENA cohort as of March 2009 (the date that raltegravir (RAL) became available in the Netherlands). Also, included patients needed to have (1) a CD4 T-lymphocyte count below 200 cells/mm^3 and (2) a diagnosis of an OI prior to or within 12 months after initiation of cART. To improve case finding of unmasking IRIS, we also included all patients fulfilling criterium 1 who had received corticosteroids within 12 months after cART initiation (as a proxy for severe unmasking IRIS). Finally, we included all patients who had died within 12 months after initiation of cART to be certain that we reviewed all patients in which IRIS might have contributed to their death. Patients with no clinical data available after the start of cART were excluded. The patient files of all patients who were identified with this strategy were reviewed on site by one of the investigators as described below.

Study procedures

All relevant data available in the ATHENA database (e.g. use of cART, CD4 T-lymphocyte counts, HIV viral loads, diagnosis and treatment of OI, concomitant medication, hospital admissions, mortality) were retrieved. All clinical data required to verify whether a patient fulfilled the predefined definitions of IRIS (see below) were collected/verified on site from the individual patient files by IEAW, AMP, VCMB, and GB using a standardized case report form (CRF, see Figure S1). If based on the predefined IRIS definitions the suspicion of a potential case of IRIS arose, each CRF was discussed with IEAW and BJAR until a unanimous decision on the presence of IRIS was made. By design, blinding the investigators for the cART regimen was not possible.

Definitions of IRIS:

Two definitions of IRIS were used: IRIS according to the criteria described by French et al[15] ($IRIS_{FRENCH}$) and a broader clinical definition ($IRIS_{CLINICAL}$). $IRIS_{CLINICAL}$ included all patients with IRIS documented as the most likely diagnosis in the patient file by the treating physician or if IRIS was mentioned in the differential diagnosis and immunosuppressive therapy for IRIS was initiated. For a more detailed description of the IRIS definitions see supplementary appendix, page 1 and Table S1. Information on all OIs that were diagnosed before or after the start of cART was collected. Detailed information on OI and what was considered appropriate therapy in relation to the diagnosis of IRIS are described in the supplementary appendix, page 1.

Objectives

The primary objective of this study was to evaluate whether the use of INI-containing cART is an independent risk factor for a combined endpoint of both types of IRIS combined ($IRIS_{FRENCH+CLINICAL}$) as well as for $IRIS_{FRENCH}$. Secondary objectives were to evaluate whether the use of INI-containing cART is associated with an increased risk of the use of corticoste-

roids for IRIS, hospital (re)admission after initiation of cART and death. Endpoints were assessed within 12 months of cART initiation. The occurrence of all endpoints together up to 12 months after cART initiation was evaluated as composite endpoint.

Statistical analyses

The risk of IRIS was compared between the INI-containing cART and non-INI-containing cART by Kaplan Meier analysis and by calculating odds ratios (OR) with 95% confidence intervals (CI) by univariable logistic regression analysis. We performed multivariable logistic regression and Cox regression models to identify independent risk factors for IRIS. We tested for interactions of INI-use with risk factors that may also be associated with IRIS. Patients were censored when any of the following occurred: a switch from an INI- to a non-INI-containing cART regimen or vice versa, death or lost to follow up of the patient. Potential risk factors for IRIS, that could confound the association between the use of INI and risk of IRIS, were investigated in the multivariable models. These were demographic, immunological and virological parameters, cART and OI-characteristics including use of corticosteroids as part of OI-treatment. A full list of these variables can be found in the supplementary appendix, Table S2. The analyses were done using SAS statistical software version 9.4 (SAS Institute, Cary, NC, USA).

Ethical considerations

All patients were enrolled in the ATHENA cohort and had consented to have their data used by the SHM. The study protocol was approved by the scientific review board of the SHM.

RESULTS

Of 25.564 patients registered in the ATHENA-cohort by May, 2016, 25.306 were adults and consented to data collection.[14] Of them, 727 were included in the study based on the selection criteria. In total, 55 patients were subsequently excluded for various reasons identified during the chart review (these were mainly patients who, after additional full chart review, did not fulfill the inclusion criteria). Therefore, 672 patients were included in the analyses (Figure 1). Of these 672 patients, 155 initiated an INI-containing cART-regimen and 517 initiated a non-INI containing cART-regimen.

Baseline characteristics of patients who initiated an INI-containing- ('INI') and a non-INI-containing ('non-INI') first-line cART-regimen are listed in Table 1. The two groups were well balanced for most of the baseline characteristics. For obvious reasons, patients starting an INI-containing cART entered HIV-care in later years than patients from the non-INI group (2014 versus 2011, $p<0.001$). In the INI group, 60 (38.7%), 21 (13.6%), and 74 (47.7%)

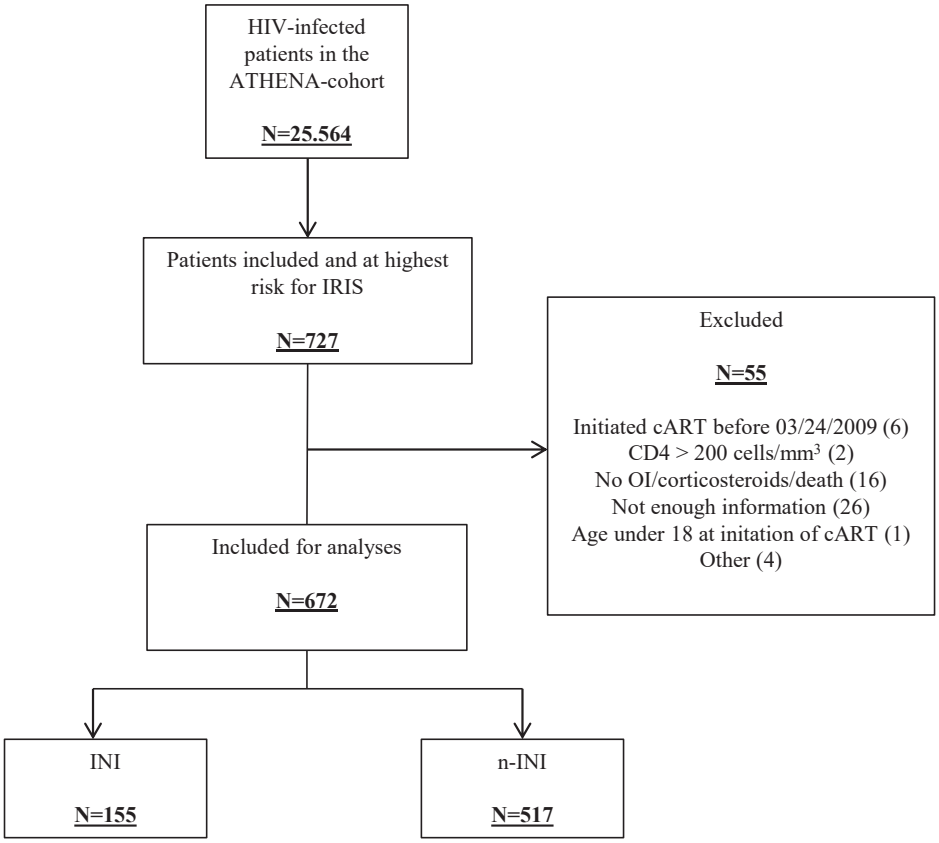

Figure 1. Patient disposition in the study.

initiated a RAL-, elvitegravir- (EVG), and dolutegravir- (DTG) containing cART-regimen, respectively, whereas in the non-INI group a comparable number of patients initiated a PI- (267/517, 51.6%) or an NNRTI-containing (250/517, 48.4%) regimen.

	INI (N=155)	non-INI (N=517)	p-value (test)
Male sex, N (%)	123 (79.4)	433 (83.8)	0.41 (CS)
Age, mean (SD)	44 (11)	44 (11)	0.60 (UT)
Year of HIV-diagnosis, median (Q1,Q3)	2014 (2011,2015)	2011 (2010,2013)	<0.0001 (WRS)
HIV-RNA at HIV-diagnosis, log_{10} c/mL, median (Q1,Q3)	5.5 (5.1,6.0)	5.5 (5.1,5.8)	0.38 (WRS)
CD4 T-lymphocytes at HIV-diagnosis, cells/mm^3, median (Q1,Q3)	39 (13,100)	33 (18,80)	0.33 (WRS)
Mode of transmission, N (%)			0.25 (CS)
HSX	58 (37.4)	197 (38.1)	
MSM	54 (34.8)	205 (39.7)	
Unknown	19 (12.3)	56 (10.8)	
Other	24 (15.5)	59 (11.4)	
Region of origin, N (%)			0.76 (CS)
NL	84 (54.2)	297 (57.5)	
Europe	10 (6.5)	40 (7.7)	
Africa	22 (14.2)	75 (14.5)	
South America and Caribbean	16 (10.3)	57 (11.0)	
Other	23 (14.8)	48 (9.3)	
Type of INI, N (%)			
RAL	60 (38.7)	*	
EVG	21 (13.6)	*	
DTG	74 (47.7)	*	
Type of cART initiated, N(%)			
INI + 2 NRTI	136 (87.7)	*	
INI + PI + 2 NRTI	13 (8.4)	*	
INI + NNRTI + 2 NRTI	6 (3.9)	*	
NNRTI + 2 NRTI	*	241 (46.6)	
PI + 2 NRTI	*	267 (51.6)	
NNRTI + PI + 2 NRTI	*	9 (1.8)	

Table 1. Baseline characteristics of patients who initiated INI-containing cART versus patients who initiated non-INI-containing cART. INI=INtegrase Inhibitor (containing cART), non-INI=non-INtegrase Inhibitor (containing cART), HSX=HeteroSeXual, MSM=Men having Sex with Men, NL=the Netherlands, RAL=Raltegravir, EVG=Elvitegravir, DTG=Dolutegravir, CS=Chi square test, UT=Unpaired T-test, WRS=Wilcoxon Rank Sum test, *=not applicable.

The baseline characteristics of the patients starting RAL, EVG, or DTG are listed in Table 2. Differences were observed with more female patients starting RAL than DTG, ($p<0.001$) and patients on RAL starting cART in earlier calendar years than patients on EVG or DTG ($p<0.001$).

A total of 231 OIs were diagnosed in the 155 INI users, whereas 780 OIs were diagnosed in the 517 patients in the non-INI group. The most frequently diagnosed OIs were *Pneumocystis jirovecii* pneumonia (PJP), candidiasis, mycobacterial infections, and kaposi sarcoma (KS). For a complete overview of the distribution of the different OIs in both groups, see Table 3.

	RAL (N=60)	EVG (N=21)	DTG (N=74)	p-value
Male sex, N (%)	41 (68.3)	17 (8.0)	65 (87.8)	0.006 (CS)
Age, mean (SD)	42 (11)	46 (10)	46 (12)	0.73 (OWA)
Year of HIV-diagnosis, median (Q1,Q3)	2011 (2009,2013)	2014 (2014,2015)	2015 (2015,2016)	<0.0001 (KW)
HIV-RNA at HIV-diagnosis, log_{10} c/mL, median (Q1,Q3)	5.6 (5.0,6.1)	5.6 (5.0,5.9)	5·4 (5.1,5.8)	0.55 (KW)
CD4-T-lymphocytes at HIV-diagnosis, cells/mm^3, median (Q1,Q3)	30 (10,79)	50 (20,115)	40 (12,105)	0.34 (KW)
Mode of transmission, N (%)				0.36 (CS)
HSX	32 (53.3)	5 (23.8)	21 (28.4)	
MSM	15 (25.0)	10 (47.6)	29 (39.2)	
Unknown	6 (10.0)	4 (19.0)	9 (12.2)	
Other	7 (11.7)	2 (9.5)	15 (20.3)	
Region of origin, N (%)				0.80 (CS)
NL	28 (46.7)	11 (52.4)	45 (60.8)	
Europe	6 (10.0)	0 (0.0)	4 (5.4)	
Africa	13 (21.7)	3 (14.3)	6 (8.1)	
South America and Caribbean	6 (10.0)	3 (14.3)	7 (9.5)	
Other	7 (11.7)	4 (19.0)	12 (16.2)	

Table 2. Baseline characteristics of users of different types of INI. HSX=HeteroSeXual, MSM=Men having Sex with Men, NL=the Netherlands, RAL=Raltegravir, EVG=Elvitegravir, DTG=Dolutegravir, CS=Chi square test, OWA=One way ANOVA, KW=Kruskal Wallis test.

	INI (N=231 in 155 pts)	non-INI (N=780 in 517 pts)
Pneumocystis jirovecii pneumonia, N (% of events)	60 (26.0)	250 (32.1)
Candidiasis, N (%)	59 (25.5)	236 (30.1)
Mycobacterial infections, N (%)		
Mycobacterium tuberculosis	34 (14.7)	54 (6.9)
Mycobacterium avium complex or	18 (7.8)	31 (4.0)
Mycobacterium kansasii	11 (4.8)	15 (1.9)
Other	5 (2.2)	8 (1.0)
Kaposi's sarcoma, N (%)	17 (7.4)	60 (7.7)
Cytomegalovirus disease, N (%)	12 (5.2)	39 (5.0)
Toxoplasmosis gondii (cerebral), N (%)	10 (4.3)	34 (4.4)
Cryptococcosis, N (%)	4 (1.7)	14 (1.8)
Other, N (%)	35 (15.2)	93 (11.9)

Table 3. Disposition of diagnosed opportunistic infections. INI=INtegrase Inhibitor, n-INI=non-INtegrase Inhibitor, pts=patients.

During the 52 weeks of follow-up, $IRIS_{FRENCH}$ was diagnosed in 18.1% (28/155) of patients in the INI group, and in 8.3% (43/517) of patients in the non-INI group (OR 2.43, 95% CI 1.45 – 4.07, p=0.0010). The incidence of $IRIS_{FRENCH+CLINICAL}$ was 32.3% (50/155) and 18.0% (93/517) respectively (OR 2.17, 95% CI 1.45 – 3.25, p=0.0003). The multivariable logistic regression analyses showed that the use of an INI-containing cART and a diagnosis of *Mycobacterium avium* complex before cART initiation were independent risk factors for both $IRIS_{FRENCH}$ and $IRIS_{FRENCH+CLINICAL}$, while no significant associations were found for baseline plasma HIV-1 RNA, use of corticosteroids as part of the treatment for the OI, and the time between the start of the OI treatment and the start of cART (Table 4). In the model for the combined endpoint of $IRIS_{FRENCH+CLINICAL}$, also a pre-cART diagnosis of cryptococcal meningitis, tuberculosis, and KS were significantly associated with an increased risk for IRIS. The pre-cART CD4 T-lymphocyte count showed a trend towards significance Apart from INI, none of the other drug classes or individual antiretroviral agents were significantly associated with IRIS although a trend was observed for efavirenz exposure and a lower risk of $IRIS_{FRENCH+CLINICAL}$.

	$IRIS_{FRENCH}$ OR (95% CI), p-value	$IRIS_{FRENCH+CLINICAL}$ OR (95% CI), p-value
Use of INI	2.46 (1.45-4.18), 0.0009	1.83 (1.16-2.87), 0.009
Diagnosed with Cryptococcal meningitis		2.83 (1.05-7.66), 0.040
Diagnosed with TB		2.43 (1.26-4.69), 0.008
Diagnosed with MAC	2.96 (1.29-6.80), 0.011	2.89 (1.37-6.09), 0.005
Diagnosed with KS		2.05 (1.15-3.67), 0.016
CD4 T cell count prior to start cART (per 10 cells/mm^3 increment)	0.96 (0.91-1.02), 0.17	0.96 (0.93-1.00), 0.062
Plasma HIV RNA prior to start cART (/log_{10} c/mL)	0.97 (0.68-1.38), 0.85	1.10 (0.83-1.46), 0.51
Use of corticosteroids prior to IRIS diagnosis	0.67 (0.40-1.14), 0.14	1.02 (0.67-1.54), 0.94
No OI treatment / no AIDS prior to start cART	Ref	ref
>2 weeks between start OI treatment and start cART	1.80 (0.88-3.66), 0.11	1.11 (0.66-1.87), 0.71
<2 weeks between start OI treatment and start cART	1.91 (0.99-3.67), 0.053	0.87 (0.52-1.45), 0.60
Use of efavirenz		0.62 (0.37-1.01), 0.057

Table 4. multivariable logistic regression analysis of possible risk factors for occurrence of $IRIS_{FRENCH}$ and $IRIS_{FRENCH+CLINICAL}$. Univ.=univariate analysis, Multiv.=multivariable analysis, OR=Odds Ratio, INI=INtegrase Inhibitor.

No significant interaction was found between the use of INI and any of the other risk factors tested in the models. The results of the Cox regression analysis confirmed the findings of the logistic regression analyses; the use of an INI-containing cART was again independently associated with $IRIS_{FRENCH+CLINICAL}$ as well as $IRIS_{FRENCH}$ (HR 1.71, 95% CI 1.17 – 2.49, and HR 2.45, 95% CI 1.52 – 3.96), table 5 and figures 2 and 3. Eight patients with IRIS events were excluded from analyses because they occurred after patients switched from INI to non-INI cART or vice versa. They were censored at the time of switch. These events included seven IRIS occurring after switching to an INI-containing cART regimen, and one IRIS occurring after switching to a non-INI containing cART.

When we investigated the three different INI separately, only RAL remained significantly associated with $IRIS_{FRENCH}$ (HR 4.10, 95% CI 2.30–7.29) as well as $IRIS_{FRENCH+CLINICAL}$ (HR 2.63 95% CI 1.63–4.24) while no significant associations were found between the use of DTG and $IRIS_{FRENCH}$ or $IRIS_{FRENCH+CLINICAL}$ (HR 1.30 (95% CI 0.77–2.19) and 1.79 (95% CI 0.90–3.57)). The number of patients starting EVG was too small (N=21) to draw meaningful conclusions in multivariable analyses.

	$IRIS_{FRENCH}$ HR (95% CI), p-value	$IRIS_{FRENCH+CLINICAL}$ HR (95% CI), p-value
Use of INI	2.·45 (1.52-3.96), 0.0003	1.71 (1.17-2.49), 0.006
Diagnosed with Cryptococcal meningitis		2.05 (0.97-4.35), 0.060
Diagnosed with TB		2.36 (1.38-4.06), 0.002
Diagnosed with MAC	2.81 (1.38-5.72), 0.004	2.28 (1.31-3.95), 0.003
Diagnosed with KS		1.65 (1.03-2.64), 0.037
CD4 count prior to start cART (/10 cells/mm^3)	0.96 (0.91-1.01), 0.15	0.96 (0.93-0.99), 0.033
Plasma HIV RNA prior to start cART (/log_{10} c/mL)	0.99 (0.70-1.39), 0.95	1.17 (0.90-1.51), 0.24
Use of corticosteroids prior to IRIS diagnosis	0.71 (0.43-1.16), 0.17	0.99 (0.70-1.40), 0.93
No OI treatment / no AIDS prior to start cART	ref	Ref
>2 weeks between start OI treatment and start cART	1.71 (0.88-3.32), 0.11	1.04 (0.67-1.62), 0.86
<2 weeks between start OI treatment and start cART	1.73 (0.93-3.19), 0.082	0.81 (0.52-1.26), 0.35
Use of efavirenz		0.61 (0.39-0.95), 0.027

Table 5. Multivariable analysis of prognostic factors for occurrence of IRIS using a Cox proportional hazards model. HR=Hazard Ratio, CI=Confidence Interval.

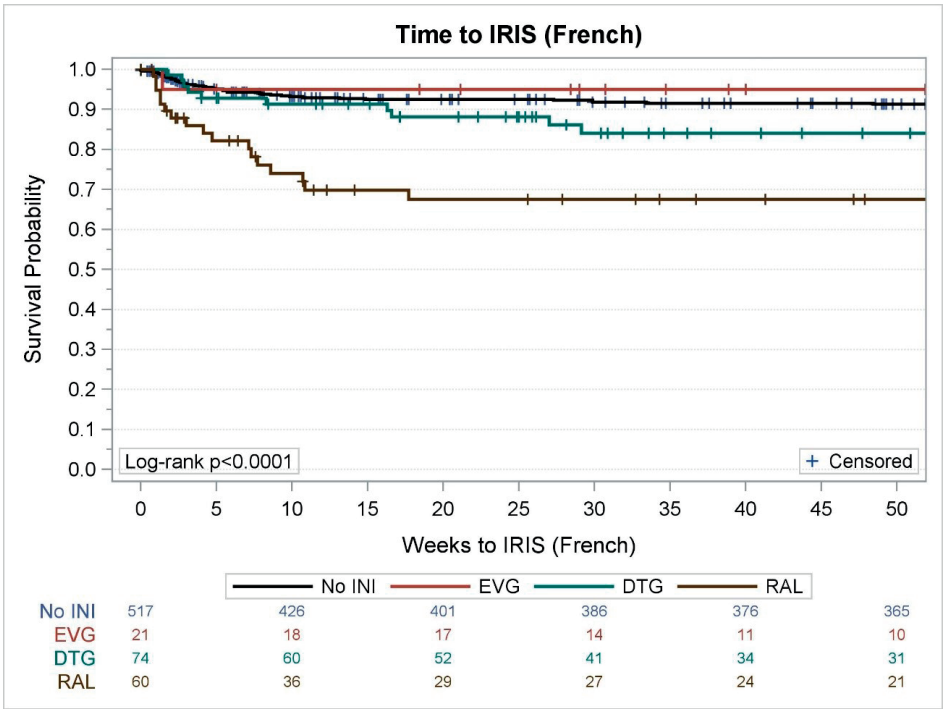

Figure 2. Kaplan-Meier analysis of occurrence of $IRIS_{FRENCH}$ in users of integrase inhibitor-containing cART versus non-integrase inhibitor-containing cART.

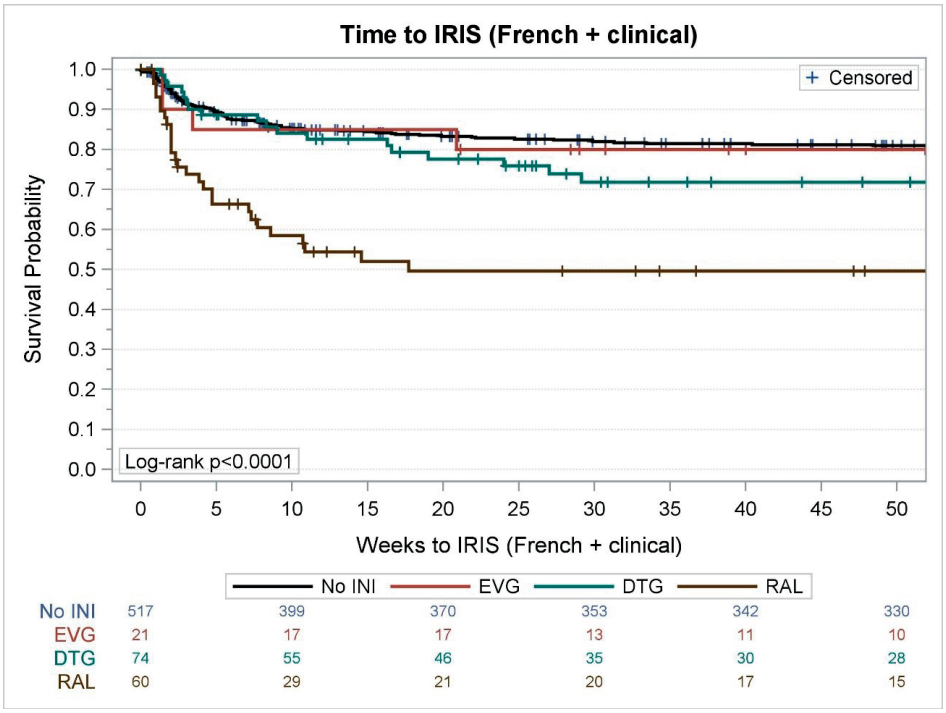

Figure 3. Kaplan-Meier analysis of occurrence of $IRIS_{FRENCH+CLINICAL}$ in users of integrase inhibitor-containing cART versus non-integrase inhibitor-containing cART.

When we compared the use of corticosteroids as therapy for IRIS, an increased use of corticosteroids for IRIS was more likely to be observed in the INI group compared to the non-INI group (OR 1.56, 95% CI 0.95–2.58). The hospital (re)admission rates after cART-initiation were comparable in both groups, being 60/155 (39%) in the INI versus 181/517 (35%) in the non-INI group (OR 1.17, 95% CI 0.81–1.70). Similarly, the mortality rate within 12 months was comparable at 11.6% (18/155) and 8.7% (45/517) in the INI and non-INI groups respectively (OR 1.38, 95% CI 0.77–2.46).

DISCUSSION

The primary goal of this study was to examine whether initiating INI-containing cART in HIV-1 late presenters is a risk factor for IRIS. This was indeed the case, although the risk was mostly driven by RAL exposure while the observed association of IRIS with the use of DTG was not significant. Additionally, our analysis confirmed that a lower pre-cART CD4 T-lymphocyte count and a pre-cART diagnosis of *Mycobacterium avium* complex infection, tuberculosis, cryptococcal meningitis, and KS increased the risk for IRIS. Our models

did not show any evidence that our observation was confounded by differences in patient, OI-related, and HIV-related characteristics at the start of cART. Although we incorporated all available measures of disease severity and presence of specific OI in our multivariable models, it remains possible that unmeasured confounders caused the observed association. Indeed, our study was non-randomized and observational. As such, RAL may have been used preferentially in the sickest patients and/or to patients in whom drug-drug-interactions with concomitant medication had to be avoided.

Only two recent studies have previously described an association between INI and IRIS. However, these studies were small with relatively few IRIS cases, did not report IRIS outcomes or lacked a comparison between different INI.[16,17] Therefore, the major strengths of our study are its larger sample size, the availability of a substantial amount of clinical data systematically registered in the ATHENA cohort, combined with additional on-site data extraction using a detailed IRIS CRF. This allowed us to check for IRIS according to two predefined IRIS definitions, one of which also included the clinical diagnosis of IRIS by the treating physician.

Our study has clear limitations. This was an unblinded study for the investigators. We used several methods to avoid possible association bias by making the adjudication of IRIS as objective as possible with the IRIS definitions described by French et al. In addition, we excluded IRIS in relation to progressive multifocal leukoencephalopathy (PML) because the clinical course of PML is too variable to distinguish PML-IRIS from clinical progression of PML without IRIS. Similarly, strict criteria were used to diagnose paradoxical IRIS in a patient with KS (see methods in the online supplement) to avoid a subjective interpretation. The fact that the treating physician was aware of the type of prescribed cART to the patient may be considered a limitation as well. Indeed, in theory, an increasing awareness of a possible association between INI and IRIS over the years may have caused clinicians to avoid INI in particular in those patients starting cART in the more recent INI era with DTG available and specifically in patients considered to be at very high risk for IRIS (e.g. patients with cryptococcal meningitis or a proven or suspected mycobacterial infection). However, no such trend could be identified when we evaluated the type of cART given to patients with a mycobacterial infection or cryptococcal meningitis between 2009 and 2017. Actually the opposite was true; from 2009 to 2017, the use of INI progressively increased from 28% for mycobacterial and 0% for cryptococcal infection in the years 2009-2011 to 60% and 100% in the years 2015-2017. Therefore, we have no clear explanation for the observed difference in the risk for IRIS with RAL and DTG. Finally, the study's observational design made us rely on the diagnostics that the treating physician had used for the clinical IRIS diagnosis and on its documentation in the patient files.

The observed association should not be considered causal until it is confirmed in other studies. Ideally, these should be prospective studies in which a large number of HIV-1 late presenters are included, started on cART as soon as possible and randomized to an INI- or a non-INI-containing cART regimen. This should be possible now that the rollout of DTG-containing cART in resource-limited settings is starting. The ongoing ADVANCE trial (NCT03122262) and the completed REALITY trial may provide valuable data to this regard. However, not only is the clinical setting of the REALITY trial very different, also its factorial design with multiple interventions simultaneously, studying the addition of RAL to various NNRTI based cART regimens next to enhanced OI prophylaxis and supplementary food, might very well hinder definite conclusions regarding IRIS despite its randomized design.[13]

In conclusion, INI-containing cART was an independent risk factor for IRIS, and this was mainly driven by patients on RAL. A well-designed randomized controlled trial specifically aiming at a prospective uniform assessment of IRIS is needed to confirm or refute our findings.

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SUPPLEMENTARY DATA CHAPTER 6

Detailed description of the definition of IRIS

As IRIS is a very heterogeneous syndrome, we used two predefined definitions of IRIS: IRIS according to the criteria described by French et al[S1] ($IRIS_{FRENCH}$) and a broader clinical definition ($IRIS_{CLINICAL}$). Briefly, an IRIS case was meeting the definition of $IRIS_{FRENCH}$ if there was an atypical presentation of OI or tumour in a patient responding to cART with either a decrease in plasma HIV-RNA by at least $1.0 log_{10}$ c/mL or an increase in CD4 T-lymphocytes of at least 50 cells/mm^3. For a detailed overview of the criteria for IRIS according to French et al, see table S1. A tentative IRIS case was considered to be 'confirmed' when it was meeting major criterion A plus B or major criterion A plus two minor criteria. A tentative IRIS case was considered as 'probable IRIS' when it is meeting major criterion A and one minor criterion. For the endpoint of this study, we considered both a confirmed and a probable IRIS as $IRIS_{FRENCH}$. A tentative IRIS case was considered to meet the definition of $IRIS_{CLINICAL}$ if the treating physician had documented IRIS as the most likely diagnosis in the patient file or if IRIS was mentioned in the differential diagnosis and immunosuppressive therapy for IRIS (almost always corticosteroids) was initiated. Every patient and IRIS-endpoint were counted only once. As we considered $IRIS_{FRENCH}$ to be more specific than $IRIS_{CLINICAL}$, patients with IRIS meeting both definitions were counted as $IRIS_{FRENCH}$. Diagnosed cases of IRIS were additionally classified as paradoxical or unmasking IRIS. Paradoxical IRIS is characterized by an initial clinical improvement of OI-related symptoms after initiation of cART, followed by a clinical deterioration with recurrence of symptoms of the previously diagnosed OI. Unmasking IRIS is characterized by a clinical deterioration with symptoms of an OI, after initiation of cART and with the absence of characteristic symptoms of the OI at the moment cART was initiated.[S2,S3]

Diagnosis and appropriate therapy for an opportunistic infection

All diagnosed OIs prior or after the initiation of cART were collected. OI were diagnosed according to the criteria of the Centers for Disease Control and Prevention.[S4] Because suboptimal treatment of an OI can result in a relapse of a previously diagnosed OI after this inappropriate therapy is discontinued (e.g. pneumocystis pneumonia relapse four weeks after a ten day course of cotrimoxazole), tentative IRIS cases were only classified as $IRIS_{FRENCH}$ if appropriate therapy for the OI had been given according to established guidelines.[S5] Due to the very variable presentation, course and prognosis of PML and malignant lymphoma after cART initiation and therefore the intrinsic difficulty to distinguish the natural course of these diseases with an atypical presentation (which is part of the $IRIS_{FRENCH}$ definition) we never diagnosed $IRIS_{FRENCH}$ in relation to PML or a lymphoma. For Kaposi's sarcomata (KS) we only considered KS to be an atypical presentation (and therefore $IRIS_{FRENCH}$) if the number of KS lesions increased or when KS lesions became larger after initiation of cART and subsequently resolved spontaneously without specific antineoplastic treatment.

RISING study

IRIS as complication of integrase inhibitor containing cART in HIV-1 infected patients

Version 5.5

Patient identification number **M**_____

Center (initial registered) _____

Date of today |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

Contact:
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RISING study **Patient Identification Number:**

1. IN- AND EXCLUSION CRITERIA

Inclusion criteria:

1. First line cART started on 24-03-2009 or later	O No O Yes
2. CD4 ≤ 200 cells/mm^3 at start cART	O No O Yes
3a. OI present prior to start cART	O No O Yes
AND/OR	
3b. Use of oral steroids/thalidomide ≤12 months after start cART	O No O Yes
AND/OR	
3c. Death ≤12 months after start cART	O No O Yes

Exclusion criteria:

1. Age < 18 years at start cART	O No O Yes
2. The above criteria could not be checked	O No O Yes

2. PATIENT-SPECIFIC INFORMATION

Date of birth	|__|__| / |__|__| / |__|__|__|__| (Day) (Month) (Year)
HIV diagnosis	|__|__| / |__|__| / |__|__|__|__| (Day) (Month) (Year)
CD4 nadir	|__|__|__| cells/mm^3
CD4 count closest to start date cART	|__|__|__| cells/mm^3

3. CART REGIMEN, OPPORTUNISTIC INFECTIONS

Start date cART	**cART regimen** O NRTI Type:_____
Note switch date in cART treatment and the reason for the switch.	Type:_____ O NNRTI Type:_____ Type:_____ O PI Type:_____ Type:_____ O INI Type:_____ Type:_____

RISING study **Patient Identification Number:**

	O Other Type:_____ Type:_____

Use the date on which the patient actually started with cART, not the date on which cART was prescribed

Type of OI present prior to start cART	Date OI diagnosis	Therapy OI	Start date – end date
		Dose: _____ Administration: _____	
		Dose: _____ Administration: _____	
		Dose: _____ Administration: _____	
		Dose: _____ Administration: _____	
		Dose: _____ Administration: _____	

RISING study **Patient Identification Number:**

4. OPPORTUNISTIC INFECTIONS – POSSIBLE CONFOUNDERS

O Cryptococcosis

O Intracerebral
O Extra cerebral
O Pulmonary
O Other:_____

O Cerebrospinal fluid leukocyte cell count |__|__|__| * 10^6 cells/L

O Opening pressure in cerebrospinal fluid |__|__|__| cm H_2O

Was adjuvant corticosteroid therapy given for increased intracranial pressure before cART was initiated? O No O Yes
Dose: _____

Start date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

End date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

Highest pathogen load before start of therapy (in serum) |__|__|__|__|__|__| copies/ml

Pathogen load closest to start date of cART (in serum) |__|__|__|__|__|__| copies/ml

Highest pathogen load before start of therapy (in CSF) |__|__|__|__|__|__| copies/ml

Pathogen load closest to start date of cART (in CSF) |__|__|__|__|__|__| copies/ml

O Cytomegalovirus

O Retinitis
O Colitis
O Oesophagitis
O Viremia with fever, without organ damage
O Other:_____

Highest pathogen load before therapy (in plasma) |__|__|__|__|__|__| copies/ml

Pathogen load closest to start date of cART (in plasma) |__|__|__|__|__|__| copies/ml

O Kaposi sarcoma

RISING study **Patient Identification Number:**

O Skin
O Pulmonary
O Gastrointestinal
O Other:_____

Stage Kaposi sarcoma

O T0 (localized)[2]	O T1 (widespread)[3]
O I0 (CD4 count ≥ 150 cells/mm^3)	O I1 (CD4 count < 150 cells/mm^3)
O S0 (No systemic illness present)[4]	O S1 (systemic illness present)[5]

[2] KS only in skin and/or lymph nodes or a small amount of disease on the palate

[3] Edema, ulcerations, extensive oral KS, KS in organs other than lymph nodes

[4] No history of opportunistic infections or thrush AND no B-symptoms present AND no diarrhoea AND Karnofsky performance status ≥ 70

[5] History of opportunistic infections or thrush OR B-symptoms present OR diarrhoea OR Karnofsky performance status < 70 OR other HIV-related illness present

Type of therapy
O cART only
O Chemotherapy
O Interferon
O Radiotherapy
O Other:_____

Dose: _____

Start date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

End date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

O Mycobacterium

O Mycobacterium avium complex
O Mycobacterium kansasii
O Mycobacterium tuberculosis
O Mycobacterium other species/unidentified species

For Mycobacterium tuberculosis only:
O Pulmonary
O Disseminated (positive blood culture or multiple sites involved):_____
O Extrapulmonary

RISING study **Patient Identification Number:**

O Lymph nodes
O Other:_____

Was corticosteroid therapy given as part of the mycobacterium therapy?
O No O Yes Dose: _____

Start date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

End date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

Was anti-mycobacterial therapy initiated?
O No O Yes Dose: _____
Start date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

End date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

O Pneumocystis jirovecii pneumonia

Lowest pO_2 during PJP illness |__|__|__| mm Hg

Lowest saturation before administration oxygen |__|__|__| %

Was corticosteroid therapy given as part of the PJP therapy?
O No O Yes Dose: _____

Start date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

End date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

O Toxoplasmosis

Was adjuvant corticosteroid therapy given before cART was initiated?
O No O Yes Dose: _____

Start date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year)

End date therapy |__|__| / |__|__| / |__|__|__|__|
(Day) (Month) (Year

RISING study **Patient Identification Number:**

5. IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME

IRIS diagnosis #1 (related OI) O Unmasking O Paradoxical	**Date IRIS diagnosis**
Therapy IRIS O No specific therapy for IRIS was started O Antimicrobial therapy Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O Antifungal therapy Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O cART only Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O Chemotherapy O Corticosteroids Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ O Interferon O Radiotherapy O Thalidomide O Other:_____	**Start date – end date therapy IRIS**
What was the clinical presentation of the IRIS? O Unexplained fever ≥ 38.3 °C for at least 6 days O Progressive seborrheic dermatitis O New onset lymphadenopathy (unrelated to new or earlier diagnosed ADE) O Other:	
Describe outcome of the patient after IRIS (residual damage, etc.)	

RISING study **Patient Identification Number:**

IRIS diagnosis #2 (related OI) O Unmasking O Paradoxical	**Date IRIS diagnosis**
Therapy IRIS O No specific therapy for IRIS was started O Antimicrobial therapy Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O Antifungal therapy Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O cART only Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O Chemotherapy O Corticosteroids Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ O Interferon O Radiotherapy O Thalidomide O Other:_____	**Start date – end date therapy IRIS**
What was the clinical presentation of the IRIS? O Unexplained fever ≥ 38.3 °C for at least 6 days O Progressive seborrheic dermatitis O New onset lymphadenopathy (unrelated to new or earlier diagnosed ADE) O Other:	
Describe outcome of the patient after IRIS (residual damage, etc.)	

RISING study **Patient Identification Number:**

IRIS diagnosis #3 (related OI) O Unmasking O Paradoxical	**Date IRIS diagnosis**
Therapy IRIS O No specific therapy for IRIS was started O Antimicrobial therapy Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O Antifungal therapy Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O cART only Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O Chemotherapy O Corticosteroids Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ O Interferon O Radiotherapy O Thalidomide O Other:_____	**Start date – end date therapy IRIS**
What was the clinical presentation of the IRIS? O Unexplained fever ≥ 38.3 °C for at least 6 days O Progressive seborrheic dermatitis O New onset lymphadenopathy (unrelated to new or earlier diagnosed ADE) O Other:	
Describe outcome of the patient after IRIS (residual damage, etc.)	

RISING study **Patient Identification Number:**

6. IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME FRENCH CRITERIA

Major criteria:

O **A**: atypical presentation of 'opportunistic infections or tumours' in patients responding to ART

- O Localized disease, e.g. lymph nodes, liver, spleen
- O Exaggerated inflammatory reaction, e.g.
  - O Fever ≥38.3 for >6 days,[6] with exclusion of other causes
  - O Painful lesions
- O Atypical inflammatory response in affected tissues, e.g.
  - O Granulomas, suppuration, necrosis
  - O Perivascular lymphocytic inflammatory cell infiltrate
- O Progression of organ dysfunction or enlargement of pre-existing lesions after definite clinical improvement with pathogen-specific therapy prior to the commencement of ART and exclusion of treatment toxicity and new diagnoses, e.g.
  - O Development or enlargement of cerebral space occupying lesions after treatment for cerebral cryptococcosis, toxoplasmosis or tuberculoma
  - O Progressive pneumonitis or the development of organizing pneumonia after treatment for pulmonary tuberculosis or PJP
  - O New onset or worsening of uveitis/vitritis after the resolution of CMV retinitis
  - O Fever and cytopenia after treatment for disseminated MAC
  - O Enlargement of Kaposi's sarcoma lesions and subsequent resolution or partial regression without commencement of radiotherapy, systemic chemotherapy or intralesional therapy
- O Other atypical presentation:_____

[6] This means, documented fever ≥38.3 for at least 7 days (=at least 6 days in between 2 measurements of 38.3C)

RISING study **Patient Identification Number:**

O **B:** decrease in plasma HIV RNA level by > 1 log_{10} copies/ml[7]

Date measurement HIV RNA (first measurement must be most recent HIV RNA *before* start cART)	**Result (copies/ml)**

[7] Date of documentation of plasma HIV-1 RNA decline of >1 log_{10} has to precede or be no later than 4 weeks after the date of IRIS

Minor criteria:

O Increased blood CD4 T-cell count after the start of cART

Date measurement CD4 T-cell count (first measurement must be most recent CD4 T-cell count *before* start cART)	**Result (cells/mm^3)**

O Increase in an immune response specific to the relevant pathogen

O Spontaneous resolution of disease without specific antimicrobial therapy or tumour chemotherapy with continuation of ART

Diagnosis IRIS according to French criteria:

O None
O Probable (criterion A and one of the minor criteria)
O Confirmed (both major criteria OR criterion A and two minor criteria)

RISING study **Patient Identification Number:**

7. IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME CLINICAL CRITERIA

Criteria clinical diagnosis IRIS:

IRIS is mentioned as most likely diagnosis in patient status/ letter O No O Yes

OR

IRIS is mentioned in the differential diagnosis AND therapy for IRIS was started O No O Yes

Corticosteroids prescribed for another reason than IRIS	**Start date – end date corticosteroids**
O No Administration:_____ O Yes Dose:_____ **Reason:** O The opportunistic infection itself O Another illness O Side-effects of received medication O Other:_____	

Corticosteroids prescribed for another reason than IRIS	**Start date – end date corticosteroids**
O No Administration:_____ O Yes Dose:_____ **Reason:** O The opportunistic infection itself O Another illness O Side-effects of received medication O Other:_____	

Did death occur within 12 months after start cART? O No O Yes	**Date of death**
Cause of death	
Did IRIS contribute to the death of the patient? O No O Yes	

RISING study **Patient Identification Number:**

8. HOSPITALIZATION

Hospitalization #1 after HIV diagnosis O No O Yes	**Reason for hospitalization** O HIV O Opportunistic infection O IRIS O Other:_____
Therapy during hospitalization O No specific therapy was started O Antimicrobial therapy Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O cART only Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O Chemotherapy O Corticosteroids Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ O Interferon O Radiotherapy O Thalidomide O Other:_____	**Admission date – discharge date hospital**

Hospitalization #2 after HIV diagnosis O No O Yes	**Reason for hospitalization** O HIV O Opportunistic infection O IRIS O Other:_____
Therapy during hospitalization O No specific therapy was started O Antimicrobial therapy Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O cART only Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____	**Admission date – discharge date hospital**

RISING study **Patient Identification Number:**

O Chemotherapy O Corticosteroids Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ O Interferon O Radiotherapy O Thalidomide O Other:_____	

Hospitalization #3 after HIV diagnosis O No O Yes	**Reason for hospitalization** O HIV O Opportunistic infection O IRIS O Other:_____
Therapy during hospitalization O No specific therapy was started O Antimicrobial therapy Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O cART only Type:_____ Dose:_____ Type:_____ Dose:_____ Type:_____ Dose:_____ O Chemotherapy O Corticosteroids Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ Type:_____ Dose:_____ Administration:_____ O Interferon O Radiotherapy O Thalidomide O Other:_____	**Admission date – discharge date hospital**

CRF – RISING study
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Figure S1. The Case Report Form used for standardized data collection.

Major criteria
A. Atypical presentation of OI or tumours in patients responding to cART Localized disease, eg. lymph nodes, liver, spleen. Exaggerated inflammatory reaction, eg. severe fever with exclusion of other causes, painful lesions. Atypical inflammatory response in affected tissues, eg. granulomas, suppuration, necrosis, perivascular lymphocytic inflammatory cell infiltrate. Progression of organ dysfunction or enlargement of pre-existing lesions after definite clinical improvement with pathogen-specific therapy prior to the commencement of ART and exclusion of treatment toxicity and new diagnoses, eg: development or enlargement of cerebral space occupying lesions after treatment for Cerebral cryptococcosis or toxoplasmosis, progressive pneumonitis or the development of organizing pneumonia after treatment for pulmonary MTB or PCP, new onset or worsening of uveitis/vitritis after the resolution of CMV retinitis, fever and cytopenia after treatment for disseminated MAC, enlargement of Kaposi's sarcoma lesions and subsequent resolution or partial regression without commencement of radiotherapy, systemic chemotherapy or intralesional therapy. **B.** Decrease in plasma HIV-RNA by >1$\log_{10}$ c/mL.
Minor criteria
Increased blood CD4 T-lymphocyte count after cART. Increase in an immune response specific to the relevant pathogen, eg. DTH response to mycobacterial antigens. Spontaneous resolution of disease without specific antimicrobial therapy or tumour chemotherapy with continuation of anti-retroviral therapy.

Table S1. Criteria for the definition of IRIS, as proposed by French et al.

Demographic	Virological and immunological	cART	OI
Sex	Log_{10} HIV-RNA at moment of initiation of cART	Type of cART (NNRTI/ bPI/INI-containing)	Type of OI(s) at HIV diagnosis: pneumocystis jirovecii pneumonia, tuberculosis, mycobacterium avium complex, other mycobacterial infection, cerebral toxoplasmosis, Kaposi's sarcoma, cryptococcal meningitis, and cytomegalovirus.
Age	Date of HIV diagnosis		Treatment of OIs: type of antimicrobial agents, chemotherapy, steroids: dosage, duration.
Mode of HIV acquisition	CD4 T-lymphocyte count and CD4/8-ratio at moment of initiation of cART		Corticosteroids as part of OI treatment
Region of origin			

Table S2. Variables included in univariable analysis on risk factors for development of IRIS. NNRTI=non nucleoside reverse transcriptase inhibitor; bPI=boosted protease inhibitor; INI=integrase inhibitor; OI=opportunistic infection.

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