Effectiveness of protease inhibitor/nucleos(t)ide reverse transcriptase inhibitor-based second-line antiretroviral therapy for the treatment of HIV-1 infection in sub-Saharan Africa: systematic review and meta-analysis

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Running title:
Second-line ART in sub-Saharan Africa

Summary
In sub-Saharan Africa, second-line ritonavir-boosted protease inhibitor-based antiretroviral therapy led to virological suppression in 69.3% at week 48 and 61.5% at week 96, based on an intention-to-treat meta-analysis of 4558 participants (14 studies) and 2145 participants (8 studies) respectively.

ABSTRACT

Background

In sub-Saharan Africa, 25.5 million people are living with HIV, representing 70% of the global total. The need for second-line antiretroviral therapy (ART) is projected to increase in the next decade in keeping with the expansion of treatment provision. Outcome data are required to inform policy.

Methods

We performed a systematic review and meta-analysis of studies reporting the virological outcomes of protease inhibitor (PI)-based second-line ART in sub-Saharan Africa. The primary outcome was virological suppression (HIV-1 RNA <400 copies/ml) after 48 and 96 weeks of treatment. The secondary outcome was the proportion of patients with PI resistance. Pooled aggregate data were analysed using a DerSimonian-Laird random effects model. PROSPERO registration: CRD42016048985.

Results

By intention-to-treat, virological suppression occurred in 69.3% (95% confidence interval 58.2, 79.3) at week 48 (4558 participants, 14 studies), and in 61.5% (47.2, 74.9) at week 96 (2145 participants, 8 studies). Pre-existing resistance to the nucleos(t)ide reverse transcriptase inhibitors (NRTIs) increased the likelihood of virological suppression. Major protease resistance mutations occurred in median 17% (IQR 0-25) of the virological failure population and increased with duration of second-line ART.

Conclusions

One third of patients receiving PI-based second-line ART with continued NRTI use in sub-Saharan Africa did not achieve virological suppression although among viraemic patients protease resistance was infrequent. There remain significant challenges in implementation of viral load monitoring. Optimising definitions and strategies for management of second-line ART failure is a research priority.

Keywords:

HIV; second-line antiretroviral therapy; protease inhibitor; sub-Saharan Africa; drug resistance

INTRODUCTION

The number of people receiving ART in sub-Saharan Africa increased from 7.5 million in 2010 to 17 million in 2015[1], and expanded treatment access has led to substantial gains in life expectancy.[2] UNAIDS aspires to further, fast-tracked improvements, with a target for 90% of patients knowing their HIV status, 90% being on ART, and 90% showing virological suppression by 2020.[1] The World Health Organisation (WHO) has advocated a public health approach to HIV control in sub-Saharan Africa, centred on standardised regimens for first-line and second-line therapy, and since 2015, on prompt ART initiation regardless of CD4 cell counts.[3] Recommended first-line regimens comprise two NRTIs such as tenofovir disoproxil fumarate (TDF) and lamivudine, and a non-nucleoside reverse transcriptase inhibitor (NNRTI), principally efavirenz.[3] Current recommended second-line regimens include two NRTIs such as zidovudine with lamivudine, and a boosted PI, with lopinavir/ritonavir or atazanavir/ritonavir preferred. A recent network meta-analysis has highlighted the current lack of evidence for alternative second-line regimens, besides lopinavir/ritonavir with raltegravir.[4] As NRTIs are continued in second-line ART, NRTI resistance acquired during first-line ART might represent an important determinant of efficacy.[5, 6]

In 2013 WHO recommended adoption of plasma viral load monitoring to enable early identification of treatment failure and appropriately guide treatment changes.[3] The level of implementation varies across the region, and even in settings with access to routine viral load testing, delays in switching to second-line ART are common.[7] With further expansion in ART use, an increasing number of people in sub-Saharan Africa are at risk of treatment failure and drug resistance.[8]

In order to inform policy related to treatment selection, monitoring, patient management, and access to third-line therapy, systematically collated data on outcomes of second-line ART, impact of prior NRTI resistance, and risk of emergent protease resistance are needed. The aim of this study is to provide a comprehensive overview of data on effectiveness of second-line ART in sub-Saharan Africa and to present pooled estimates of virological and resistance outcomes.

METHODS

Search strategy and selection criteria

PubMed, EMBASE, the Cochrane Register of Controlled Trials, Scopus and Web of Science were searched for articles published from 1 January 1996 to 28 July 2017 according to a predefined strategy (Supplementary table 1). References cited in the selected articles and abstracts from the International AIDS Society Conference (2014-2016) and the Conference on Retroviruses and Opportunistic Infections (2014-2016) were also reviewed. We contacted the authors of 15 studies to clarify definitions, obtain additional data, and remove duplications.

Type of studies

We included randomised controlled trials (RCTs) and observational studies that reported the outcomes of second-line ART in sub-Saharan Africa with viral load measured at least annually. We excluded studies with fewer than 20 participants to avoid small sample size bias, and participants outside sub-Saharan Africa in international trials. We excluded studies without defined criteria for switching to second-line ART. For studies reporting the prevalence of drug resistance at second-line ART failure, we required that an unbiased selection method for resistance testing was applied, whereby either all patients meeting a defined viral load threshold, or a random selection were tested.

Types of participants

Eligible studies investigated HIV-1 positive participants aged >10 years[3] who received first-line ART with two NRTIs and one NNRTI for ≥6 months prior to switching to second-line ART, defined as ≥2 NRTIs with a ritonavir-boosted PI. Clinical, immunological or virological criteria for switching to second-line ART were accepted. provided the criteria were clearly defined.

Analyses

The intention to treat (ITT) analysis described outcomes for all patients commencing second-line ART. Participants without virological data were categorised as: lost to follow up (no contact for ≥90 days since the last visit); died; transferred to another care provider; or missing data. The on-treatment (OT) analysis provided outcomes for participants that remained under follow-up with available viral load results. For participants of observational studies that had

commenced second-line ART but had not been in the study long enough to reach the virological analysis window, outcomes were imputed in proportion to the remaining participants in the cohort using a missing-at-random assumption. Data prior to imputation are presented in Supplementary Tables 2-3.

Virological outcomes

The primary outcome was virological suppression, defined as plasma HIV-1 RNA <400 copies/ml after 48 and 96 weeks of second-line ART, with a 24-week window period to allow for variations across studies (e.g., measurements taken between weeks 36 and 60 were accepted for the 48 week outcome). The 400 copies/ml threshold was chosen to reflect the most commonly used definition of virological suppression in studies from the region. Outcomes were further categorised as low level viraemia (400-1000 copies/ml) and virological failure as per WHO-definition (>1000 copies/ml).[3]

A secondary analysis explored how detection of NRTI resistance prior to starting second-line ART influenced virological outcomes at week 48. We included studies with available data using an OT analysis. The overall activity of the second-line regimen was scored as either full or partial using the Stanford Resistance algorithm (v8.2).[9]

Resistance

The prevalence of major protease resistance mutations according to the Stanford Resistance algoirthm (v8.2)[9] after 48 and 96 weeks was calculated as a proportion of the population that underwent resistance testing at failure.

Data extraction

Following the literature search and removal of duplicate citations, two reviewers (AS, MS) independently screened the abstracts of retrieved records to include all potentially relevant articles, and then independently reviewed the full text of the remaining articles. Disputes about inclusion of articles were resolved through discussion with recourse to a third reviewer (AG). AS and MS independently extracted data from the studies.

Quality assessment

We conducted this study according to recommendations from the Preferred reporting Items for Systematic Reviews and Meta-Analyses.[10] The quality of included articles was assessed

using a modified version of a quality appraisal tool (Supplementary Material). The review was registered with PROSPERO (CRD42016048985).

Statistical analysis

Agreement between reviewers was assessed using Cohen's kappa statistic. Confidence intervals (CIs) were calculated using the Wilson method. Proportions were stabilised using the Freeman-Tukey arcsine square root transformation and a pooled proportion calculated using the DerSimonian-Laird random effects model.[11] To assess the effect of pre-existing NRTI resistance on virological outcomes, we calculated the odds ratio (OR) of pooled rates of virological suppression at 48 weeks among patients receiving fully active regimens compared to those on partially active regimens, using a DerSimonian-Laird random effects model. We reported the I^2 statistic, where I^2 is interpreted as the proportion of variability in the treatment estimate attributable to between-study heterogeneity rather than sampling error. We assessed potential publication bias by visual inspection of funnel plots and by Egger's test.[12]

To determine the effect on virological outcomes of study design (randomised vs. observational), median CD4 cell count, year of study and duration of first-line ART, we performed meta-regression analysis using a restricted maximum-likelihood estimator mixed effects model. Analyses were conducted in Stata version 14.2 (College Station, USA).

RESULTS

Data selection and quality assessment: virological outcome studies

Following removal of duplicates, we screened 3525 abstracts and selected 206 full articles for review; the selection showed good agreement between reviewers (Cohen's kappa 0.70 [95% CI 0.63, 0.76]). Twenty articles describing 15 studies met the inclusion criteria (Figure 1), comprising five RCTs,[6, 13-18] five prospective observational studies,[19-25] and five retrospective observational studies.[26-31] Six studies reported from multinational cohorts.[13-17, 19, 23] Data were available from 11/48 (23%) sub-Saharan African countries, with study locations in west, central, east and southern Africa (Figure 2, Table 1).

Assessment of study quality is shown in Supplementary Table 4. The size of the initial first-line ART population, the rate of first-line ART failure, and the rate of switching to second-line ART were poorly described. The NRTIs used in first- and second-line regimens were inconsistently reported. The rate of adverse events and the contribution of tolerability to treatment discontinuation were not reported in most studies. In one study, criteria for starting second-line ART were at risk of performance bias as they included a requirement for regular attendance at clinic.[20] Sensitivity analysis excluding this trial from the ITT and OT analyses did not significantly alter pooled estimates. There was no evidence of publication bias on inspection of funnel plots and by Egger's test of asymmetry at 48 or 96 weeks (p=0.16 and 0.19 respectively) (Supplementary Figure 1).

Outcomes of second-line ART

The median duration of first-line ART prior to starting second-line ART varied from 13 to 49 months (Table 1). Estimates of the rate of switching from first to second-line ART were calculable for 8 studies and ranged from 6 to 47 per 1000 patient-years. All studies used twice-daily lopinavir/ritonavir; one RCT randomised one third of participants to ritonavir-boosted darunavir (800mg once daily).[15] By ITT, virological suppression rates were 69.3% (95% CI 58.2, 79.3) among 4558 participants from 14 studies at week 48, and 61.5% (47.2, 74.9) among 2145 participants from 8 studies at week 96 (Figure 3, Supplementary Tables 2-3). In the OT analysis, suppression rates were 82.7% (76.9, 87.8) among 3626 participants from 15 studies at week 48, and 84.8% (78.8, 89.9) among 1090 participants from 8 studies at week 96 (Figure 4, Supplementary Table 5) (Figure 4). The rate of virological failure according to the WHO

definition (>1000 copies/ml), ranged between 2.5 and 26.6% of participants at 48 weeks and between 4.1 and 11.1% at 96 weeks, while low level viraemia occurred in 0 to 3.3% at 48 weeks and 0 to 5.0% at 96 weeks (Supplementary Tables 2-3).

Rates of virological suppression were significantly higher among participants of RCTs compared to observational cohorts at both week 48 (85.7% [95% CI 80.6, 90.2] vs. 58.2% [48.2, 68.0]; p<0.001) and week 96 (76.5% [72.8, 80.4] vs. 55.7 [43.1, 67.8]; p<0.001). After exclusion of missing viral load data, the difference between RCTs and observational cohorts persisted (p<0.0001 and p=0.001 at 48 and 96 weeks respectively) and estimates of virological suppression rates did not significantly change (p=0.39 and p=0.58 at 48 and 96 weeks respectively). By meta-regression analysis, neither median CD4 cell count, nor median duration of first-line ART at the time of starting second-line, nor the year of study recruitment were significantly associated with virological suppression, after adjustment for study design (p=0.37, p=0.83 and 0.95 at week 48; p=0.91, p=0.74 and p=0.28 at week 96, respectively).

Effect of pre-existing NRTI resistance

Resistance test results (by conventional sequencing) were available for six studies.[6, 14, 18, 20, 21, 23, 30] The likelihood of virological suppression at week 48 was lower (OR 0.31 [95% CI 0.14, 0.70]; p=0.020) among participants lacking evidence of NRTI resistance and therefore predicted to be receiving fully-active second-line ART, relative to those with NRTI resistance receiving partially-active second-line ART (Figure 5). Pre-existing NRTI resistance comprised predominantly the lamivudine mutation M184V (67.0 – 92.7% of participants) and thymidine analogue mutations (12.5 – 74.3%) (Supplementary Table 6).

Protease resistance at failure of second-line ART

Resistance test results (by conventional sequencing) were available from 649 participants from 13 studies, including five prospective [14, 15, 18, 23, 30] and eight cross-sectional studies.[32-39] The threshold for resistance testing ranged from 400 to 5000 copies/ml. Duration of second-line ART at the time of sequencing ranged from 6 to 37 months. Major protease resistance mutations were present in median 17% (IQR 0-25, range 0-66.7%) of patients that underwent resistance testing (Table 2). An association between the prevalence of protease resistance mutations and median duration of second line ART was observed (0-11.8% at 6-12

months to 0-28.9% at 16-24 months, and 16.7-66.7% at 27-37 months; r^2=0.75, p<0.001). (Figure 6).

DISCUSSION

By 2030, the number of patients requiring second-line ART in sub-Saharan Africa is estimated to exceed 4 million.[8] Our pooled ITT estimates for virological suppression after 48 and 96 weeks of second-line ART were 69.3% and 61.5% respectively, demonstrating reasonable efficacy of PI-based therapy with continued NRTI use in these treatment-experienced populationsEmploying similar analytical methodologies, studies from India, China, and Cambodia reported virological suppression rates ranging from 70% to 85.7% over 48-96 weeks of second-line ART.[40-42] RCTs using lopinavir/ritonavir in high-income settings reported comparable virological suppression rates among treatment-experienced patients.[43] Rates of virological suppression with first-line ART in low and middle-income countries were similar: 67.3% and 64.6% at week 48 and week 96, respectively.[44] Thus first- and second-line ART regimens show overall comparable efficacy in sub-Saharan Africa, despite the widely held assumption that sub-optimal adherence may drive first-line failure and continue to reduce responses after patients start second-line ART. Importantly, these rates fall considerably short of the 90% UNAIDS target for virological suppression. Using a high-genetic barrier regimen in first line ART (eg. with dolutegravir) may be required to meet this targets.[45] Whilst options for first-line ART are expanding, evidence is presently limited for alternative second-line options.[4]

One third of participants did not achieve virological suppression. An important reason in the ITT analysis, and a source of significant heterogeneity between studies, was the proportion of missing viral load data (excluding death or loss to follow-up) which varied from 0 to 30%, despite accepting a 24-week window. This finding implies substantial challenges in implementation of viral load monitoring. . Consistent with this observation, virological outcomes were significantly better and loss to follow-up was lower among RCT participants compared to observational studies; a finding that persisted after exclusion of missing viral load data. In the EARNEST trial, therapy was delivered in a manner designed to replicate typical programme settings with broadly generalizable entry criteria, predominantly nurse-led care and without real-time viral load monitoring.[18] Outcomes were comparable to other trials with more restrictive entry criteria that used real-time viral load monitoring. Enhanced attention to patient retention, improving staffing and provision of a constant drug supply are important for

ensuring improved treatment outcomes and are likely to account for the observed differences between RCTs and observational studies.

Emergence of drug resistance is common after failure of first-line ART, and is typically characterised by mutations affecting both NNRTIs and and NRTIs .[46-51] Interestingly, detection of NRTI resistance, and specifically thymidine analogue mutations (TAMs) prior to starting second-line ART, predicted significantly higher odds of virological suppression.[5, 14, 20, 21, 23, 30] An explanation is that patients who develop resistance at failure of first-line ART may have overall higher levels of adherence (and therefore greater drug selective pressure), than subjects who experience failure in the absence of resistance.[5] Importantly, the NRTIs commonly included in second-line regimens, such as zidovudine or TDF plus lamivudine, retain significant residual activity in the presence of TAMs and this is enhanced by continuation of lamivudine.[52, 53] Data from the SECOND-LINE and EARNEST studies demonstrate that apparent paradoxical benefit of NRTI resistance persists at 96-144 weeks.[5, 6]

Current reports of HIV epidemic control do not differentiate between first- and second-line ART provision and rates of second-line failure are not included among metrics of epidemic control or ART programme performance.[54] Yet between 2 and 26% of recipients of second-line ART experienced virological failure by 48 weeks. The optimal public health management of second-line failure has not been adequately defined. In South Africa, 64% of patients experiencing viraemia >400 copies/ml (median 3.5 $\log_{10}$ copies/ml) while on second-line ART regained virological suppression 2-4 months after targeted adherence counselling.[55] This rate of re-suppression is consistent with our finding that major protease resistance mutations were uncommon at virological failure, particularly in the first 18 months of second-line ART. Emphasis on adherence is therefore necessary for second-line recipients. This should be differentiated from first-line failure where rapid emergence of NNRTI resistance is likely to limit the impact of adherence support. Effective adherence interventions may include weekly SMS reminders and targeted counselling.[56] In cohort studies from Cambodia[57], India[40] and Vietnam[58] higher rates (42-68%) of major protease mutations were observed at failure of second-line ART. This higher rate may reflect differences in adherence, duration of failing regimens or an effect of viral subtypes. In our analysis rates of PI resistance were strongly associated with increasing duration of second-line ART, suggesting duration of PI failure is an

important determinant of the need for third-line ART. Optimising the frequency of viral load monitoring and the definition of virological failure for second-line ART and defining appropriate regimens for third-line ART represent clear research priorities.

There are a number of limitations in our analysis. First, there was substantial variation in both the duration of first-line ART at the time of switching to second-line ART, and the rate of switching to second-line ART among each cohort, which was only reported in 8 studies. The lack of consistency may represent a source of reporting bias. The variation in rate of switching we observed across studies (range 6 to 47 per 1000 person years) is consistent with other low- and middle-income settings.[7] In programmes with routine viral load monitoring, rates of switching are three times higher, suggesting potentially different outcomes in programmes without monitoring.[7] Second, our analysis used aggregate rather than individual patient data and therefore it was not possible to analyse the contribution of individual risk factors to outcomes. Third, most studies applied a viral load <400 copies/ml to denote suppression. Data from South Africa demonstrate a continuum of risk of virological failure even with the lowest level of viraemia (50-199 copies/ml), indicating that low-level viraemia should trigger adherence interventions and repeat viral load. [59] Fourth, zidovudine and stavudine, previously common components of ART regimens in sub-Saharan Africa, have now been replaced by TDF, and impact on NRTI resistance profiles and second-line ART efficacy is to be demonstrated.[60]

In summary, reported rates of virological suppression among patients receiving second-line PI-based ART in sub-Saharan Africa are similar to those observed with first-line ART and comparable to the outcomes of similar regimens in Asian and Western settings. There is a significant gap in achieving the third 90 of the WHO 90-90-90 strategy for epidemic control. Reporting of second-line ART provision and rates of virological suppression among recipients is crucial to understanding of epidemic control and should be strongly encouraged. Given that over one-third of patients did not achieve virological suppression, defining the optimal definition and management of second-line ART failure, both with and without PI resistance, in this setting is an urgent research priority.

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Potential conflicts of interest.

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Table 1: Characteristics of included outcome studies

Reference	Design	Year	Location	N	Age, median years (IQR)	Gender (% F)	Duration first-line ART, median months (IQR)	Frequency viral load monitoring	Second-line ART				
									Switch rate/1000 PY	Reason for switch	PI	CD4 count at start, median cells/mm^3 (IQR)	Viral load at start, median log_{10} copies/ml (IQR)
La Rosa[17]	RCT	2012-13	Kenya, Malawi, South Africa, Tanzania, Zimbabwe	162	38 (33-43)	50	48 (26-72)	6m	NA	VF	LPV	182 (160)[a]	4.5 (0.9)[a]
Ciaffi[15]	RCT	2010-13	Cameroon, Senegal, Burkina Faso	451	38 (32-46)	72	49 (33-69)	3m	NA	VF	LPV 64% DRV 34%	183 (87-290)	4.5 (4.0-51)
Paton[18]	RCT	2010-14	Uganda, Kenya, Malawi, Zimbabwe, Zambia	426	37 (31-43)	62	48 (34-65)	None	NA	VF	LPV	72 (29-143)	4.8 (4.4-5.2)
Boyd[14]/ Amin[13]	RCT	2010-14	Nigeria, South Africa	100	38 (33-45)	65	29 (19-50)	3m	NA	VF	LPV	199 (64-284)	4.2 (3.5-4.9)
Gross[16]	RCT	2009-11	Botswana, South Africa, Uganda, Zambia, Zimbabwe	132	38 (34-45)	50	34 (20- 55)	3m	NA	VF	LPV	183 (94-271)	4.3 (3.8-4.9)
Osinusi-Adekanmbi[22]	POC	2008-11	Nigeria	73	35 (30-41)	67	24 (16-32)	6m	NA	VF	LPV	121	NA
Shearer[31]	ROC	2004-12	South Africa	1150	38 (33-44)	59	19 (13-31)	6m	NA	VF	LPV	203 (114-305)	4.2 (3.6-4.8)
Schoffelen[29]	ROC	2004-10	South Africa	156	35 (29-41)	72	19 (11-31)	6m	8	VF	LPV	187 (93-299)	4.0 (3.4-4.5)
Adetunji[26]	ROC	2006-09	Nigeria	225	34 (29-40)	65	16 (12-23)	6m	11	VF	LPV	139 (58-235)	4.6 (3.9-5.2)
Wandeler[24]	POC	2006-12	South Africa	971	38 (32-45)	56	27 (17-38)	6m	NA	C/I/VF	LPV	172 (95-267)	NA
Boender[19]/ Sigaloff[23]	POC	2007-11	Kenya, Nigeria, Uganda, South Africa, Zambia, Zimbabwe	243	38 (34-45)	50	27 (15-44)	12m	32	C/I/VF	LPV	126 (66-205)	4.2 (3.2-5.0)
Murphy[28]	ROC	2006-10	South Africa	136	36 (31-43)	65	13 (7-20)	6m	10	VF	LPV	153 (89-232)	4.5 (3.8-4.9)
Johnston[27]	ROC	2003-08	South Africa	417	36 (31-44)	35	23 (15-34)	6m	6	VF	LPV	169 (97-235)	4.6 (4.1-5.1)
Hosseinipour[21]	POC	2006-08	Malawi	101	38 (32-46)	55	35 (25-49)	3m	8	VF	LPV	65 (22-173)	4.7 (4.1-5.2)
Castelnuovo[20]	POC	2004-06	Uganda	40	39 (36-43)	50	22 (19-23)	6m	47	VF	LPV	108 (43-205)	4.8 (4.0-5.4)

[a]Mean (SD) Abbreviations: PY= patient years; POC= prospective observational cohort; ROC= retrospective observational cohort, RCT= randomised controlled trial, NA= data not available, ART= antiretroviral therapy, VF= virological failure, C=clinical failure, I=immunological failure, LPV=lopinavir with ritonavir, DRV=darunavir with ritonavir, IQR=interquartile range

Table 2: Protease inhibitor resistance at failure of second-line ART

Reference	Study design	Year	Location	Total population, n	Second-line ART duration, months, median (IQR)	Viral load threshold for sequencing (copies/ml)	Failure population, n (%)	Resistance analysis population. n (%)[a]	Protease resistance, n (% of at-risk population)[b]	Protease resistance, n (% of those sequenced)	Major protease mutation (n)
Prospective studies											
Paton[18]	RCT	2010-14	Uganda, Kenya, Malawi, Zimbabwe, Zambia	426	24	1000	46 (10.7)	41 (89.1)	8 (2.1)[c]	8 (19.5)[c]	M46I (8) I54V (7) L76V (3) V82AF (6)
Boyd[14]	RCT	2010-14	Nigeria, South Africa	100	12	500	8 (8.0)	8 (100)	0 (0)	0 (0)	-
Ciaffi[15][d]	RCT	2010-13	Cameroon, Burkina Faso, Senegal	451	12	1000 x 2	29 (6.4)	5 (17.2)	0 (0)	0 (0)	-
Boender[25]	POC	2007-11	Kenya, Nigeria, South Africa, Uganda, Zambia, Zimbabwe	205	12	1000	21 (10.2)	17 (81.0)	2 (1.2)	2 (11.8)	M46I (2) I54V (2) L76V(1) V82A (2) L90M (1)
				177	24	1000	26 (14.7)	21 (80.8)	6 (4.2)	6 (28.6)	M46I (5) I54V (4) L76V(2) V82A (4) I84V (1)
				90	36	1000	8 (8.9)	3 (37.5)	2 (5.9)	2 (66.7)	M46I (2) I50V (1) I54V (1) V82A (2)
Johnston[30]	POC	2003-8	South Africa	417	12	400	112 (26.8)	15 (13.4)	0 (0)	0 (0)	-
Cross-sectional observational studies											
Schramm[39]	CS	2014-15	Kenya	355	27 (23-36)	500	65 (18.3)	65 (100)	16 (4.5)	16 (24.6)	NA
Inazule[38]	CS	2010-15	Kenya	NS	37 (23-55)	1000	126 (-)	123 (97.6)	39 (-)	39 (31.7)	M46I/L (30) I54V (27) V82ATFS (25)
Court[32]	CS	2009-13	South Africa	NS	20 (13-34)	1000	164 (-)	134 (81.7)	28 (-)	28 (20.9)	M46I (22) I47VA (2) I50V (1) I54VTALM (24), L76V (19) V82A (22) I84V (2) L90M (1)
Maiga[34]	CS	2012	Mali	913	24 (6-48)	500	106 (11.6)	93 (87.7)	23 (2.9)	23 (24.7)	M46I (15) I47V/A (6) I54V (12) L76V(11) V82A (8) I84V (10) L90M (3)
Ndahimana[37]	CS	2012	Rwanda	74	31 (18-46)	1000	35 (47.3)	30 (85.7)	5 (7.9)	5 (16.7)	L33F (2) M46I (4) I54V (5) L76V (2) V82A (4) I84V (2)
Levison[33]	CS	2009	South Africa	322	17 (18)[c]	1000 x 2	43 (13.3)	33 (76.7)	0 (0)	0 (0)	-
Reynolds[35]	CS	2004-9	Uganda	65	6 (6-14)	2000	8 (12.3)	6 (75.0)	0 (0)	0 (0)	-
Wallis[36]	CS	2008	South Africa	NS	16 (7-18)	5000 x 2	75 (-)	75 (100)	5 (-)	5 (6.7)	L33F (2) M46I (4) I54SV (2) L76V (2) V82A (1) I84V (2) L90M (1)

.Abbreviations: NS not specified; RCT randomised controlled trial; VL viral load; PI protease inhibitor; NA genotype not available

[a] As proportion of failure population; [b]As proportion of total at-risk population; adjusted for proportion who underwent sequencing. Major protease resistance mutations as defined by the

Stanford HIV drug resistance database[9] [c]Resistance refers to intermediate or high level resistance to lopinavir only [d]All patients received lopinavir/ritonavir apart from Ciaffi et al; 33% were randomised to darunavir/ritonavir and the remainder received lopinavir/ritonavir [e]Standard deviation

Figure 1: Flow diagram of search strategy

Figure 2: Map of included studies

Figure 3: Forest plot of virological suppression at 48 and 96 weeks: intention to treat analysis, random effects model

Figure 4: Forest plot of virological suppression at 48 and 96 weeks: on-treatment analysis, random effects model

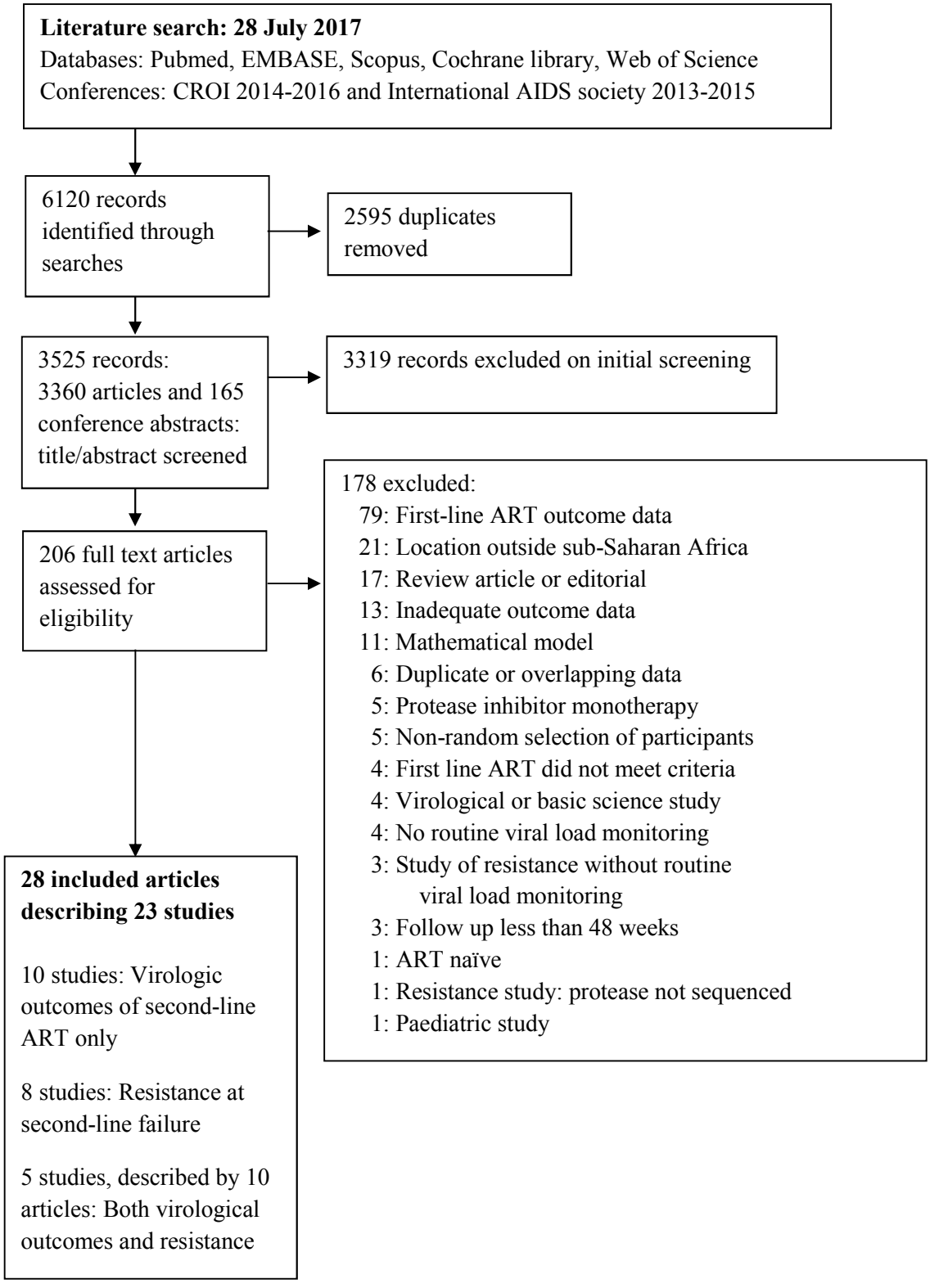
Figure 5: Forest plot: Odds ratio for virological suppression at 48 weeks among participants with fully-active compared to partially-active second-line ART

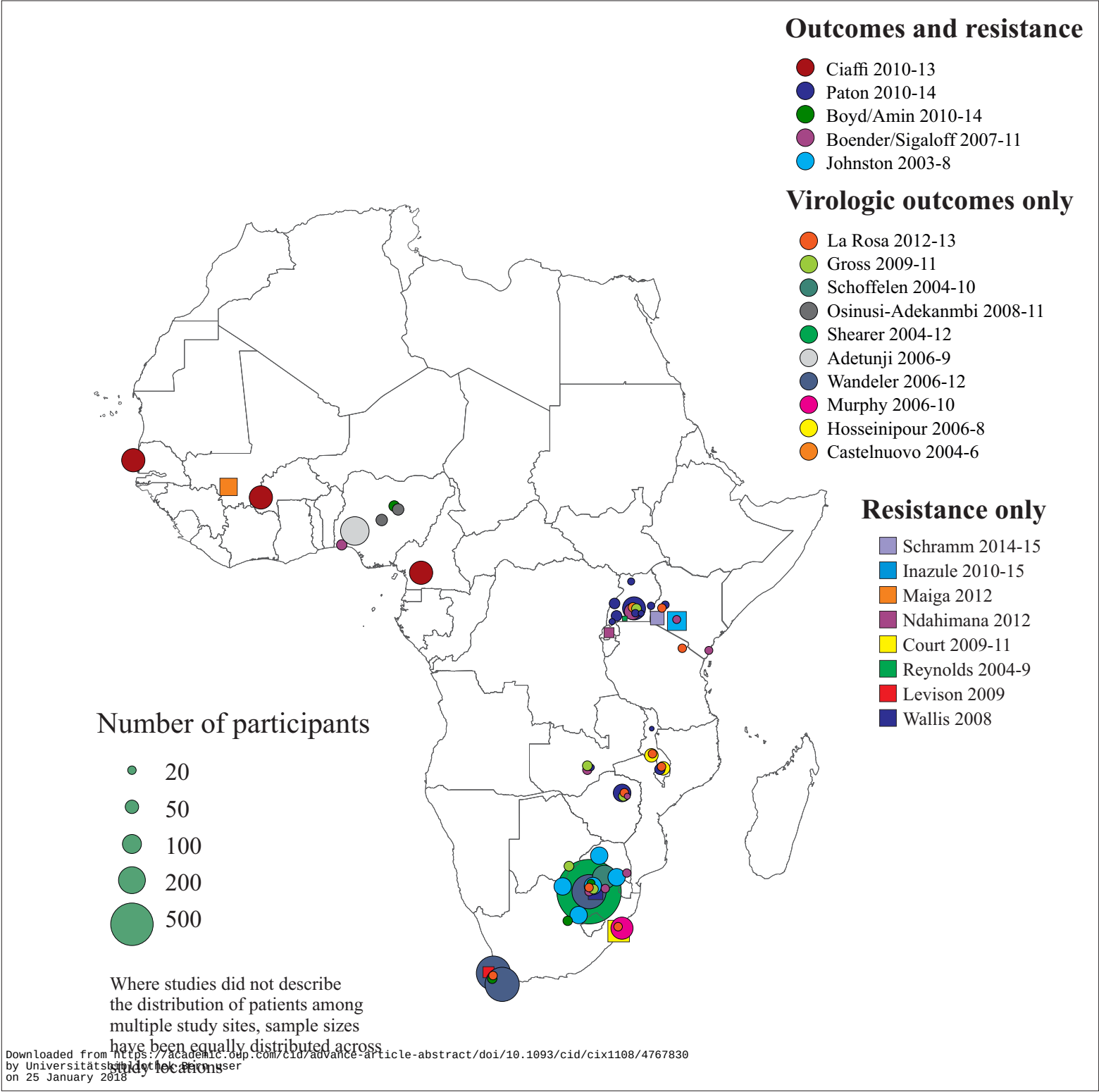
Partially active ART is defined as level low level or greater resistance to any component of second-line ART (Stanford database version 8.2)[9] Abbreviations: VL<400 viral load less than 400 copies/ml; OR odds ratio; CI confidence interval

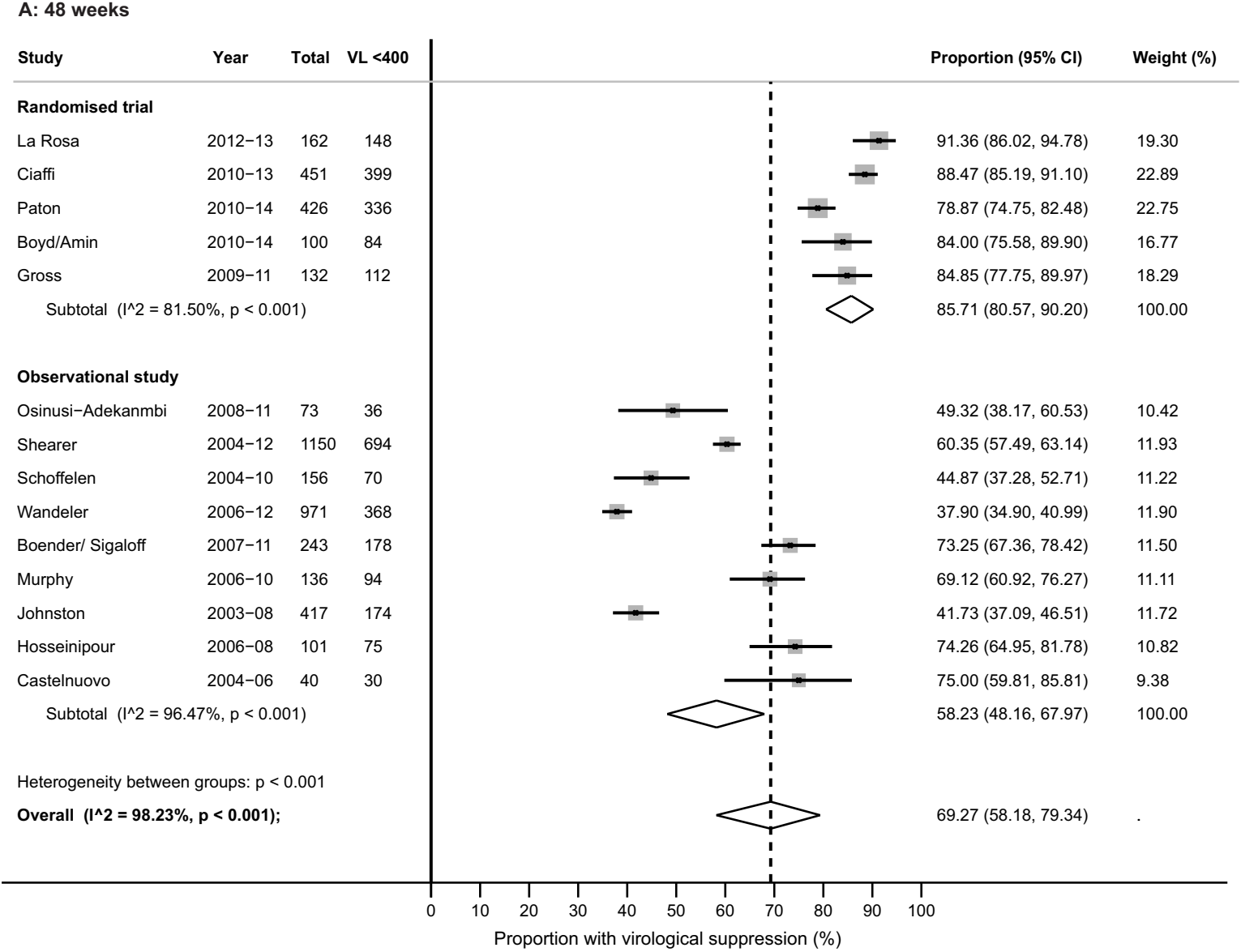
Figure 6: Proportion of participants with major protease mutations according to duration of second-line ART at virological failure [a]

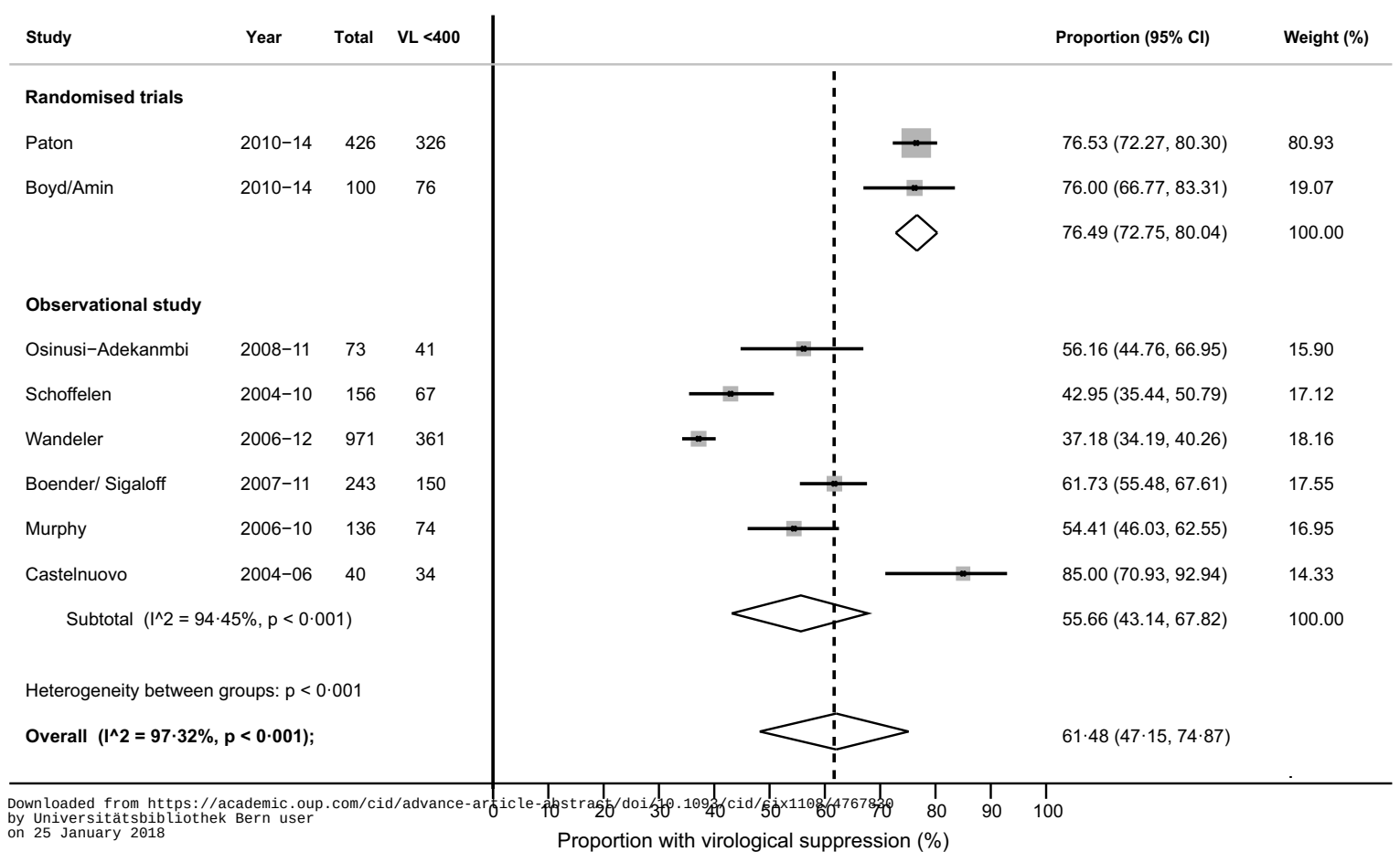
[a] Area of circles are proportional to size of cohort failing second line. Red and dashed line are quadratic line of best fit and 95% confidence interval respectively. Major protease resistance mutations according to the Stanford HIV resistance database v8.2[9]

Figure 1: Flow diagram of search strategy

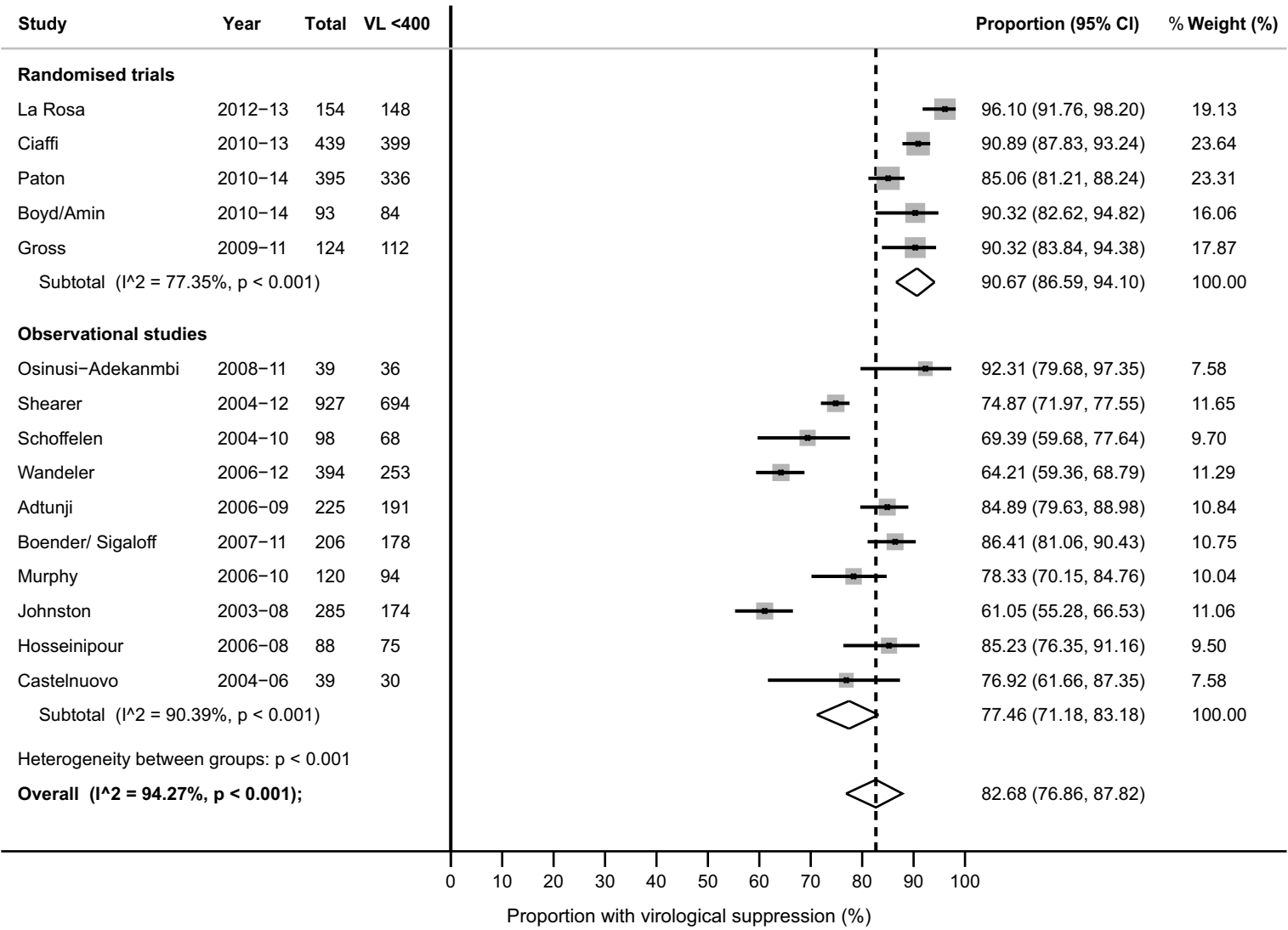








A: 48 weeks



B: 96 weeks