

## Meta-Analysis and Rationale Regarding the Effect of Protease Inhibitors on Survival in the HIV-1-Infected Population

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### Abstract

One of the most effective ways to decrease the replication of HIV and viral load in HIV-infected patients is to inhibit the protease enzyme system. Medications that inhibit the HIV protease enzyme system have been shown to decrease mortality. Protease inhibitors attack a vital point in the viral replication cycle of HIV. Medical research has shown that this class of drugs increases survival and decreases morbidity in the HIV-infected population. However, protease inhibitors have specific side effects, adversely affecting patient compliance and increasing comorbidities and mortality. The first-generation of HIV drugs (and treatment regimes) have been replaced by newer drugs and drug combinations, resulting in fewer side effects and higher compliance rates. This paper presents the results and rationale of a meta-analysis regarding the effect of protease inhibitors on survival in the HIV-1-infected population. The current use of protease inhibitors—although not a cure for HIV/AIDS—is beneficial in controlling HIV and decreasing associated mortality and morbidity.

**Keywords:** AIDS; Immunodeficiency; HIV; Human Immunodeficiency Virus; Mortality; Protease Inhibitor; Survival

### Abbreviations

AIDS: Acquired Immunodeficiency Syndrome; ART: Antiretroviral Treatment; ART-CC: Antiretroviral Therapy Cohort Collaboration; ARV: Antiretroviral; CDC: The Centers for Disease Control and Prevention; CMA: Comprehensive Meta-Analysis; HAART: Highly Active Antiretroviral Therapy; HIV: Human Immunodeficiency Virus; NRTI: Nucleoside Reverse Transcriptase Inhibitor; PI: Protease Inhibitor; RR: Risk Ratio; RT: Reverse Transcriptase; USDHHS: U.S. Department of Health and Human Services; WHO: World Health Organization

### Preface

Research has shown an improvement in survival for persons living with HIV infection. This improvement is partly due to better medication compliance, improvements in medical care, and drug development. Although mortality in the HIV-1-infected population has decreased over the years, it remains higher than the general non-HIV population. A foundation for this study involved evaluating antiretroviral medication regimens containing protease inhibitors to determine if survival rates increased using such regimes.

Data were sourced from eligible studies (Africa, China, France, Malaysia, Rwanda, the United Kingdom, and the United States). Eleven studies met eligibility criteria (total n = 459,703). The random model was used for effect size. The risk ratio (RR) for death was 0.116 (95% CI: 0.115 - 0.117, p = 0.04). Out of 459,703 people, 39,912 (8.7%) died, and 419,791 (91.3%) survived while on antiretroviral therapy. The Kaplan-Meier survival probability range was 0.61 to 0.95. Heterogeneity for the analysis was determined by Tau-squared and I-squared (0.727 and 99.95, respectively). The Odd ratio was 0.008 (95% CI: 0.003 to 0.020).

The foundational research indicated an improved survival rate with antiretroviral therapy that included a protease inhibitor for people living with HIV-1. Protease inhibitors seem to have fewer adverse effects, resulting in improved patient compliance and diminished resistance (in the long term), which may contribute to an increased survival rate among HIV-1-infected individuals.

### Introduction

This review utilized a meta-analysis methodology to seek answers to the following questions: Do protease inhibitors (PIs) increase survival in the HIV-1-infected population? To what effect size degree do PIs reduce mortality in the HIV-1-infected population?

### Inclusion criteria

Articles were included that monitored survival, adverse drug reactions, and risk factors for drug reactions for HIV persons on ART, including at least one PI. The search was global (not limited to the United States). No countries were excluded. All articles were considered, except those conducted in a medical institution, which prevented confounding entrapment of having a sicker population (in a medical facility) mixed with healthier patients.

### Exclusion criteria

The study excluded health care providers, children, and those with special health status, such as diabetes or pregnancy. Healthcare workers tend to have a higher medical knowledge base and compliance level than the general population. Articles with children were excluded for ethical reasons and the difficulty of determining life expectancy in individuals less than 18 years of age. Also, medication adherence in children is more challenging to ascertain.

Special health conditions were excluded, such as pregnancy, renal failure, and diabetes. People with such conditions were eliminated due to ethical considerations (pregnancy) and increased mortality rates (diabetes and renal failure). Diabetic and renal failure patients often die from disease-related complications of their primary disease, making it challenging to ascertain survival longevity. The aim of these exclusions was to minimize or control for confounding.

### Information search

Published articles were sourced from peer-reviewed journals indexed in ProQuest, PsycArticles, Academic Search Complete, EBSCOhost, MEDLINE, SOCindex, ERIC, Ovid, Google Scholar, and PsycINFO (dated between January 1, 1997 and July 31, 2017), using the prominent search terms “protease inhibitors”, “adverse drug reactions”, and “mortality or survival”; the keywords “HIV risk behaviors”, “HIV risk factors”, “HIV risk networks”, “HIV infections”, “HIV seropositivity”, “HIV seroprevalence”, “human immunodeficiency virus”, “human-immuno deficiency virus”, “life expectancy”, “morbidity”, and or “quality of life”.

A total of 123 articles had the potential to be included in the meta-analysis. Of these, 105 studies (85%) were excluded because the studies did not include primary interventions. Instead, they were discussions or reviews of the literature. Three studies (2.1%) were excluded because the intervention reports did not state an effect size [1]. Five studies (4 %) were excluded because of duplication across different databases (Figure 1).

### Research questions

- Aim 1: To determine the effectiveness of protease inhibitors on mortality and morbidity.
- Aim 2: To determine the impact of protease inhibitors on long-term survival.

### Variables

#### Independent variables

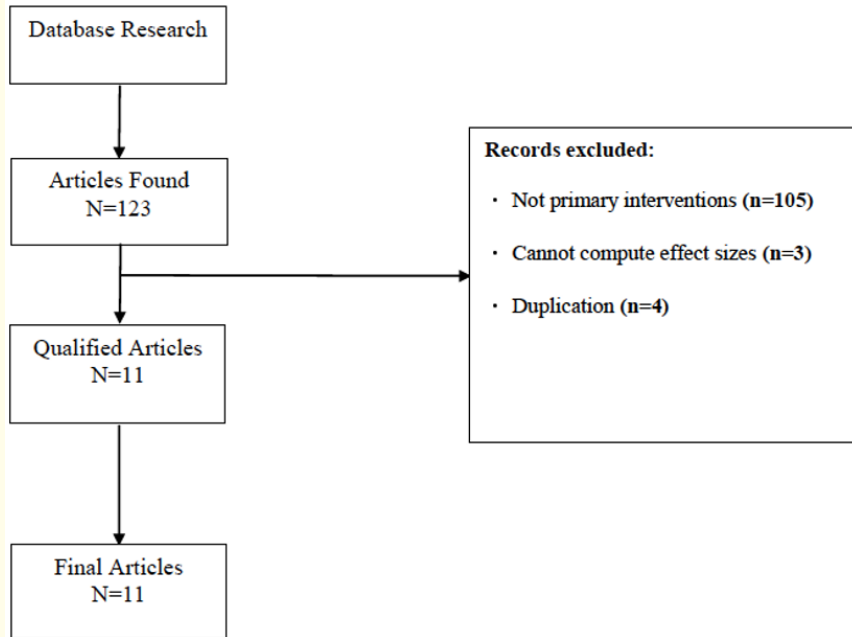


Figure 1: Flowchart of the literature search.

- Age, HIV status.

**Dependent variable**

- CD4.

**Control variables**

- Economic income status
- Educational background
- Gender.

**Research format and purpose**

The study utilized a meta-analysis design to determine the effectiveness of protease inhibitors in reducing mortality in HIV-1-infected patients.

**Methods**

**Sample screening**

This meta-analysis collected data from eleven studies on HIV survival in individuals on ART with a protease inhibitor. Heterogeneity was determined using a Forest plot and I-squared. The chance of survival was calculated based on Kaplan-Meier estimates for survival probability. DerSimonian and Laird random-effects model was used due to the diversity of individual studies and the small number of studies included.

The Knapp-Hartung method was utilized to adjust the standard error of the estimated coefficients. All statistical analyses were conducted using Comprehensive Meta-Analysis (CMA) software Version 3.

**Article coding**

To successfully conduct meta-analysis research, a reliable and valid coding process must be in place to pull data from the primary articles [2]. The following section defines the coding categories for this research.

**Descriptive code sheet**

Descriptive information, such as names, locations, race, duration, drug side effects, and year, was coded with the article information.

Study ID Number (1) \_\_\_\_\_  
 Author \_\_\_\_\_  
 Title \_\_\_\_\_  
 Journal \_\_\_\_\_  
 Year \_\_\_\_\_ Vol. \_\_\_\_\_ No. Pages \_\_\_\_\_

**Methodological Features**  
 Publication date \_\_\_\_\_ (2)  
 Source of reference \_\_\_\_\_ (3)  
 Blinding of experimenter \_\_\_\_\_ (4)  
 Assignment to groups \_\_\_\_\_ (5)  
 Experimental mortality (%) from treatment group \_\_\_\_\_ (6)  
 Experimental mortality (%) from comparison group \_\_\_\_\_ (7)  
 Overall mortality rate (%) \_\_\_\_\_ (8)  
 Research design \_\_\_\_\_ (9)

**Study Quality**  
 Study design \_\_\_\_\_ (10)  
 Selection and specification of study sample \_\_\_\_\_ (11)  
 Specification of illness or condition \_\_\_\_\_ (12)  
 Definition of outcome construct \_\_\_\_\_ (13)  
 Outcome measure \_\_\_\_\_ (14)

**Intervention Characteristics**  
 Type of intervention strategy \_\_\_\_\_ (15)  
 Contact frequency \_\_\_\_\_ (16)  
 Length of each contact \_\_\_\_\_ (17)  
 Total length of the intervention \_\_\_\_\_ (18)  
 Contact frequency \_\_\_\_\_ (19)  
 Length of each contact \_\_\_\_\_ (20) [2]

Figure 2: Code sheet.

**Data extraction and effect size calculation**

This research aimed to extract useful data that could be utilized for enhanced understanding of PI effectiveness in the HIV patient as it relates to decreased mortality. To gain meaningful information from the research, internal, measurement, external, and statistical conclusion validity had to be protected [3].

Internal validity exposes any confounding variables that may have led the research to a false conclusion [4]. Measurement validity authenticates the measurement characteristics and intent of the study. External validity defines whether the necessary information extracted matches the intent [5]. Statistical conclusion validity answers the questions of whether the results represent the relationships between characteristics and outcomes studied [6].

To protect external measurement and internal validity, an emphasis was placed on effect size. The effect size is defined as the standardized difference between a pair of means between the experimental and control group, which reveals the relationship between treatment and outcome variables or the magnitude of treatment effects [7]. According to Chow (1988) and Cohen (1965,1969,1988), the effect size amount can be categorized as: 0.2 > = small, 0.5 > = medium, 0.8 > = Large [8-11]. Four mathematical methods can be used to calculate effect size: standardized mean difference, chi-square, analysis of variance (ANOVA)/regression, and Pearson correlation [8-11] (Figure 3-6).

- Population effect size  $\Delta = \frac{\mu_1 - \mu_2}{\sigma}$
- Sample effect size  $d = \frac{\bar{X}_1 - \bar{X}_2}{\sigma_{pooled}}$
- Small=.20, Medium=.50, Large=.80

**Figure 3:** Standard mean difference. This mathematical manipulation utilizes the mean between pooled groups to determine if an effect is realized.

- Population effect size  $\phi = \sqrt{\sum_j \frac{(\pi_{oj} - \pi_{oj})^2}{\pi_{oj}}}$
- Sample effect size  $w = \sqrt{\frac{\chi^2}{N}}$
- Small=.10, Medium=.30, Large=.50

**Figure 4:** Chi-square test. Chi-square compares group differences to find an effect.

- ANOVA  $\hat{f} = \sqrt{\frac{(k-1)F}{N}}$
- Regression (test of R<sup>2</sup>)  $\hat{f}^2 = \frac{R^2}{1-R^2} = \frac{signal}{noise}$
- Regression (test of R<sup>2</sup> change)  $\hat{f}^2 = \frac{\Delta R^2}{1-R_L^2} = \frac{\Delta signal}{remaining\ noise}$

**Figure 5:** ANOVA and regression. ANOVA and regression determine variation between groups, revealing if the means of several groups are equal.

$$z_r = .5 \log_e \left[ \frac{1+r_{xy}}{1-r_{xy}} \right]$$

- Small=.10, Medium=.30, Large=.50

**Figure 6:** Correlation. Correlation shows the relationship of dependence or predictive events between groups [8-11].

### Data entry

Comprehensive Meta-Analysis (CMA) software Version 3 program was used for data manipulation [6]. The CMA allows for different types of effect size data entry formats. The data formats included risk ratio, tau-squared, I-squared, odds ratio, and random-effect model.

### Statistical design and analysis

Studies are rarely identical replications of each another. Studies conducted on the same topic often use different methodologies, samples, and populations. One aspect of heterogeneity in meta-analysis denotes the variation in outcomes between studies. To compensate for this issue, tests for heterogeneity were performed to determine if the percentage of variance across studies was due to heterogeneity rather than chance. Thus, it had to be determined whether to use a random-effects model or fixed-effects model to conduct the meta-analysis.

If the variation between studies is small, the heterogeneity will be low. Thus, a fixed-effects model would be appropriate. For a fixed-effects model, each study must be conceptualized as a different “random sample” from the same population that produces different results [12]. If done incorrectly, sampling errors occur.

The studies selected were assumed to be similar in population base; hence, the variance between studies is in their ability to detect the outcome of interest. In contrast, the random-effects model permits the study outcomes to vary in a normal distribution between studies [1]. In this model type, each study is considered to come from a different population. The actual population effect size is variable. The research goal was to estimate the average of all population effect sizes and clarify the variability in the population effect sizes. More priority was given to those studies with larger sample sizes because of less sampling error. A common error when using the random-effects model is the assumption that studies themselves are sampled from a population of studies [13].

The random-effects model was selected for this study because a high degree of heterogeneity was present among the studies. The high degree of heterogeneity might partly be explained by the variety of studies chosen from different countries. The variability in population, design, and outcomes might be due to the region of the world in which the research was conducted [14].

Sensitivity analysis was used to determine whether results would be affected by shifts in study inclusion/exclusion criteria [15]. A sensitivity analysis was done to answer the study questions: “Should studies be included if most individuals in the study meet the viral load requirement?”; “Should studies that made gender comparisons of men and women be included?”; “Should studies that follow different HIV treatment guidelines than the WHO, CDC, and USDHHS be included?”; and “What assumptions should be made about missing outcomes?”

For example, one study in the analysis included participants under the age of 18, but mean age of all the participants in that study was twenty-seven. Subgroup analysis involves splitting participant data into subgroups, allowing comparisons to be made between them. Subgroup analyses can be done for subsets of participants. Subgroup analyses can also be used for investigating heterogeneous results [16].

Moderator analysis can be used when there is considerable heterogeneity in effect sizes across studies. Subgroup and moderator analysis can be used to address research questions, such as: “What is the correlation between protease inhibitors’ effectiveness and mortality/morbidity?” or “Is the impact of protease inhibitors on long-term suppression of RNA viral load over other HIV medications related to survival?” Characteristics include: age, gender, HIV status, economic income status, educational background, and medication regimen.

### Statistical procedures

According to Moher, *et al.* (2009) and Shubber, *et al.* (2013), the statistical procedure necessary for complicated meta-analysis includes the following steps:

- Inverse weight variance
- Heterogeneity of effects (fixed vs. random)
- Funnel plot and trim and fill to assess the impact of publication bias
- Small sample effects cumulative forest plot
- Sensitivity analysis to assess the impact of missing data
- Subgroup analysis to assess within-group effects
- Moderator analysis to assess between-group effects
- Cumulative analysis by year to assess change over time [17,18].

All necessary analyses were performed to determine the size of the effect.

CMA Version 3 was used to perform each of the statistical tests for this study. The CMA software is an Excel coding program used to enter data that can be extrapolated for statistical manipulation. The CMA program can convert different types of entered data into a common metric to appreciate effect size.

Heterogeneity was determined using a Forest plot and I-squared (% of the total variability in effect measure that is attributable to heterogeneity—not chance). The survival probability for each study was calculated based on Kaplan-Meier estimates. DerSimonian and Laird random-effects models were used due to the diversity of individual studies and the small number of studies included.

### Study selection

Only studies published in English were considered for this review. Given the advancements in HIV treatment since the introduction of highly active antiretroviral therapy (HAART) in 1996, studies conducted before this date were excluded because they would not have included a PI. No restrictions were placed on study design (except that qualitative studies were not included), study duration, and sample size.

The study population of interest was adults with HIV infection irrespective of viral load, clinical status, or prior treatment experience. However, many studies included children as young as 14 years. This research covered many different countries worldwide, and the definition of a child varied among cultures and groups. Thus, only individuals older than 18 years of age were included; however, some studies did include persons younger than 18 years of age into their data. All ethnic groups and genders were included.

Studies conducted exclusively in populations of specific risk or demographic groups were excluded from the analysis. For example, health care providers, renal failure patients, pregnant women, and diabetics were excluded. The abstract and title were used for the possible inclusion of articles according to the eligibility criteria. During the screening stage, divergences were reconciled by a study's inclusion of a protease inhibitor in its treatment regimen.

Data were entered into Microsoft Excel. Extracted data included details of study design, author, study time range, number of patients, age range, ART regimen, and outcomes. Determinant variables were compared across studies before meta-analysis. Nevertheless, studies that reported on the relationship between mortality/survival, morbidity, and ART treatment were included in the meta-analysis.

Research amalgamation and multiple meta-analyses of data were performed to investigate 1) the correlation between CD4 count and the effectiveness of protease inhibitors on mortality and morbidity and 2) the impact of protease inhibitors on long-term survival compared to no antiretroviral medication.

One hundred twenty-seven studies were included in the search. Eleven studies reported the necessary data for inclusion in the meta-analysis. The criterion for statistical significance for all analyses was  $p \leq 0.05$ . The total number of men analyzed was 247,806; the total number of women analyzed was 212,449, for a total of 459,703 after adjusting for dropouts. These individuals came from nine countries worldwide.

## Results

### Study characteristics

Baseline demographic characteristics of each treatment group are shown in Table 1. The data collected represented a diverse group of men and women. Depending on the country, individuals came from an economically challenged or affluent healthcare system. The diversity in patients from different economic healthcare systems helped to limit confounding. For example, if protease inhibitors are beneficial to HIV patients, economic status should not influence the outcome. The educational background was also diverse.

This research aimed to determine if the class of medications, known as the protease inhibitors, increased survival regardless of economic background or education level. The number of men and women included in this research was 247,806 men and 212,449 women. Thus, the groups are relatively even in representation. Depending on the country, many individuals were on various antiretroviral regimen combinations. Thus, there were too many to include every combination in the research. To facilitate the goal of studying protease inhibitors, it was determined that all studies included at least one protease inhibitor in its treatment regimen.

Of the eleven studies included in the research, eight studies had a median age range of 34 to 38 years in treated individuals. Two studies had ranges outside the other studies. The medium age range for individuals in China was 48.3 years. Rwanda had a medium treatment age of 27 years (Table 1).

The average CD4 count had a wide range of variability according to the country of origin. France, Malaysia, Thailand, Uganda, the United Kingdom, and the United States, all chose from a lower CD4 count population of individuals at research origin. The average CD4



Study	Men	Women	Median CD4 Count mm/cells	RNA Viral count copies/ml
Zhigang, <i>et al.</i> (2016)	7492	3234	344	Not reported
ART-CC (2017)	66747	21757	230	480,000
Teeraananchi, <i>et al.</i> (2017)	93,119	108,569	109	Not reported
Lewden, <i>et al.</i> (2002)	271	844	288	440,000
Shah, <i>et al.</i> (2012)	1271	208	81	138,000
Chirowze, <i>et al.</i> (2014)	1067	214	43	460,000
ART-CC (2008)	31,605	11,750	>200	<500,000
Brechtl, <i>et al.</i> (1999)	54	16	66	286,000
Mills, <i>et al.</i> (2011)	6823	15,492	126	Not reported
Nsanumana, <i>et al.</i> (2015)	26,055	46,006	348	Not reported
May, <i>et al.</i> (2011)	13,302	4359	165	480,000
Total	247,806	212,449		

**Table 1:** Study demographic characteristics.

count for patients in the Thailand study at origin was 109 mm/cells. France started with a CD4 count of 43 mm/cells. Malaysia started with a CD4 count of 81mm/cells. Uganda started with a CD4 count of 126 mm/cells. The United Kingdom started with a CD4 count of 165 mm/cells, and the United States with a CD4 count of with 66 mm/cells. The other countries started with an average CD4 count of greater than 200 mm/cells for its participants.

Although RNA viral load was recorded for some studies, not all of the studies included this parameter in the research. The countries of China, Malaysia, Rwanda, and Uganda did not report RNA viral load levels. No explanation was given for this exclusion of data. However, the cost of such data gathering might have been a factor as these countries are economically limited by comparison.

Some of the studies included were via government-sponsored HIV treatment programs. Government programs are limited in their treatment protocols. Many patients received a standard formulary HIV ART regimen, depending on the locale and country. There was no genetic testing for resistance or use of newer ART medications, such as integrase inhibitors on the formulary. All patients throughout the eleven studies received an NNRTI, PI, and NRTI in various combinations. All studies were retrospective, except for two prospective cohort studies conducted by Brechtl, *et al.* (2001) and Chirowze, *et al.* (2015) [19,20].

**Risk of bias within the studies**

Each study had the potential for bias or confounding. Although not explicit in its intentions, it was necessary to highlight potential shortcomings of each study’s data. In Table 2, the listings of potential risk for bias from each study are displayed. This sensitivity analysis intended to obtain a better understanding of the results and limitations of each study. This sensitivity summary helped determine if a study should be excluded from the research. The intention was to minimize confounding in each study to observe a true effect size.

**Results of the individual studies**

All studies showed an increase in life expectancy or survival after initiating ART therapy. Accordingly, variable rates of survival depended on medication combinations, economic resources, drug compliance, country of origin, and the number of people in each study. All studies reported an increase in survival years for individuals on HIV medication that included a protease inhibitor.

Study	Risk of Bias
Zhigang, <i>et al.</i> 2016	No mention of comorbidities, deaths unrelated to HIV/AIDS: tuberculosis, hepatitis, coinfection, IDU, and late and early initiation of ART.
ART-CC., <i>et al.</i> 2017	Results can only be applied to people living in high-income counties (Europe and North America).
Teeraananchai, <i>et al.</i> 2017	The study failed to mention behavior factors that might influence life expectancy such as smoking, alcohol consumption, exercise, and adherence to ART.
Lewden, <i>et al.</i> (2002)	The study did not note problems of adherence, which may have contributed to an artificial lower number of participants included in the study.
Shah, <i>et al.</i> (2012)	Some patients had reduced compliance with follow-up appointments and inadequate family support. There were some missing data and no mention of disease staging at enrollment, which may have caused bias in the assessment of the progression to morbidity and mortality.
Chirowze, <i>et al.</i> (2014)	It was not determined if comorbidities, such as atherosclerosis or pathologies linked with aging, were the cause of death versus HIV/AIDS.
ART-CC., <i>et al.</i> (2008)	The study was limited by under-reporting of deaths by some cohorts, that were not linked to administrative records. This under-reporting may have confounded the statistical analysis regarding mortality and survival. The reporting methods among the cohorts participating in this study were different. Some cohorts used record linkages by vital statistics, while others used self-reporting systems.
Brechtl, <i>et al.</i> (1999)	Difficult to estimate the magnitude of the effect (improved QoL, increase adherence, and survival) in this brief study period.
Mills, <i>et al.</i> (2011)	A total of 3817 patients did not have baseline CD4 cell count evaluations. Likewise, no routine viral-load assessments were done. Thus, caution about inferences about risks for treatment failure on life expectancy must be noted.
Nganumana, <i>et al.</i> (2015)	Absence of the critical baseline variable (viral load); was not done. This variable could have served as a marker of treatment adherence. It was noted that viral load testing remains limited in Rwanda to HIV-positive patients on antiretroviral therapy. Also, adjustments for mortality follow-up adjusted using a conservative proportion for mortality. Thus, the proportion used may have been too high, which could have caused underestimated life expectancy.
May, <i>et al.</i> (2011)	Did not include untreated patients. Excluded patients with a history of use of injected drugs.

**Table 2:** Risk bias per study.

The variability and range of countries covered throughout the world added heterogeneity to the overall analysis. Likewise, the studies provided a variety of study subjects. No study included only homosexual males, intravenous drug users, or acquisition of HIV through maternal transmission. The analysis used a mixture of populations of people living with HIV. Using these inclusion criteria, a final tally of men and women included 247,806 and 212,449, respectively. Table 3 lists the results for each study included in the meta-analysis.

**Amalgam of results**

A meta-analysis was performed on various data. The total number of individuals included in all studies totaled 459,704. From this total, 39,912 (8.7%) died and 419,791 (91.3%) survived (Table 4).

A Kaplan-Meier was performed for each study to determine the probability of survival for HIV patients on ART therapy (Table 5). The range from the content study was 0.61 to 0.95. Three out of the eleven studies were below the ninety-percentile for survival probability. Those studies were Zhigang, Teeraananchai, and Brechtl, with survival probabilities of 0.61, 0.89, and 0.84, respectively.

Study	Location	Study Period	Study Design	No# of Patients	Age Range years	ART Regimen	Outcome
Zhigang et al. 2016	China	2010-21014	Cohort	10,726	15-66	NNRTI, PI, NRTI	ART reduced mortality by 38.6% over 60
ART-CC	Europe & North America	1996-2013	Retrospective Cohort	88,504	16 to >60	NNRTI, PI, NRTI	Life expectancy in >20 yrs patient starting ART increased life by 9 yrs in women & 10 yrs in men
Teeraananchai et al. 2017	Thailand	2008-2014	Cohort	201,688	15-55	NNRTI, PI, NRTI	Increased life expectancy with ART for patient with high CD4+ count
Lewden et al. 2002	France	1997-1998	Cohort	1155	18 to >50	NNRTI, PI, NRTI	Increased survival based on haem, CD4, transaminase & low education
Shah et al. 2012	Malaysia	1997-2008	Retrospective Cohort	1479	>18	NNRTI, PI, NRTI	Decreased mortality on HAART
Chirowze et al. 2014	France	2000-2010	Prospective Cohort	1281	>18	NNRTI, PI, NRTI	Viraemia copy yrs was associated with mortality outcomes positively & negatively
ART-CC 2008	North America & Europe	1996-2005	Retrospective Cohort	43,355	16 to >50	NNRTI, PI, NRTI	Life expectancy increased from 1996-2005
Brechtel et al. 1999	United States	1997-1998	Prospective Cohort	70	>18	NNRTI, PI, NRTI	HAART improved QOL & survival
Mills et al. 2011	Africa (Uganda)	2000-2009	Cohort	22,315	>14	NNRTI, PI, NRTI	Increased survival with ART
Nsanumana et al. 2015	Rwanda	1997-2008	Retrospective Observational Cohort	72,061	>15	NNRTI, PI, NRTI	ART improves life expectancy by 11.3 yrs in Rwanda
May et al. 2011	United Kingdom	1996-2008	Cross Sectional Cohort	17,661	>20	NNRTI, PI, NRTI	ART increases life expectancy: 30 yrs (1996-1999), 43.8 yrs (2006-2008), 39.5 yrs (1996-2006)

Table 3: Individual study summaries.

Study	ART Survival	Total Patients	ART Deaths	Total Patients	Study Period Length (months)	Median CD4 Count (cell/ul)	RNA Viral Load Count (copies/ml)
Zhigang, et al. (2016)	6593	10726	4133	10726	48	344	Not reported
ART-CC (2017)	84096	88504	4408	88504	204	230	480000
Teeraananchai, et al. (2017)	179667	201688	22021	201688	48	109	Not reported
Lewden, et al. (2002)	1107	1155	48	1155	12	288	440000
Shah, et al. (2012)	831	887	56	887	144	81	138000
Chirowze, et al. (2014)	1204	1281	77	1281	120	43	460000
ART-CC (2008)	41305	43355	2050	43355	100	200	500000
Brechtel, et al. (1999)	59	70	11	70	12	66	286000
Mills, et al. (2011)	20372	22315	1943	22315	108	126	Not reported
Nsanumana, et al. (2015)	68144	72061	3917	72061	204	348	Not reported
May, et al. (2011)	16413	17661	3917	17661	144	165	480000
Total	419791	459703	39912	459703			

Table 4: Survival versus death.

Study	Study Time (months)	Number at Risk (NT)	Number of Deaths (DT)	Number Censored "survival" (pt)	Proportion Surviving (NT-DT/NT)	Survival Probability $Pt*S(t-1)$
Zhigang, <i>et al.</i> (2016)	48	10726	4133	6593	0.61	0.61
ART-CC 2017	204	88504	4408	84096	0.95	0.95
Teeraananchai, <i>et al.</i> (2017)	48	201688	22021	179667	0.89	0.89
Lewden, <i>et al.</i> (2002)	12	1155	48	1107	0.95	0.95
Shah, <i>et al.</i> (2012)	144	887	56	831	0.93	0.93
Chirowze, <i>et al.</i> (2014)	120	1281	77	1204	0.93	0.93
ART-CC (2008)	100	43355	2050	41305	0.95	0.95
Brechtel, <i>et al.</i> (1999)	12	70	11	59	0.84	0.84
Mills, <i>et al.</i> (2011)	108	22315	1943	20372	0.91	0.91
Nsanumana, <i>et al.</i> (2015)	204	72061	3917	68144	0.94	0.94
May, <i>et al.</i> (2011)	144	17661	1248	16413	0.93	0.93

Table 5: Kaplan-Meier life probability chart.

The three studies that showed a lower than 90% probability of survival had a higher rate of deaths compared to the number of participants in the study (Figure 7). This discrepancy can be explained by the variety of patients entering the study in various stages of HIV infection. Retrospective research might not always offer the condition of the person being studied. For instance, the Zhigang, *et al.* (2016) study was created by government records, which often have vast amounts of data missing [21]. Accordingly, these participants could have had comorbidities that contributed to their death.

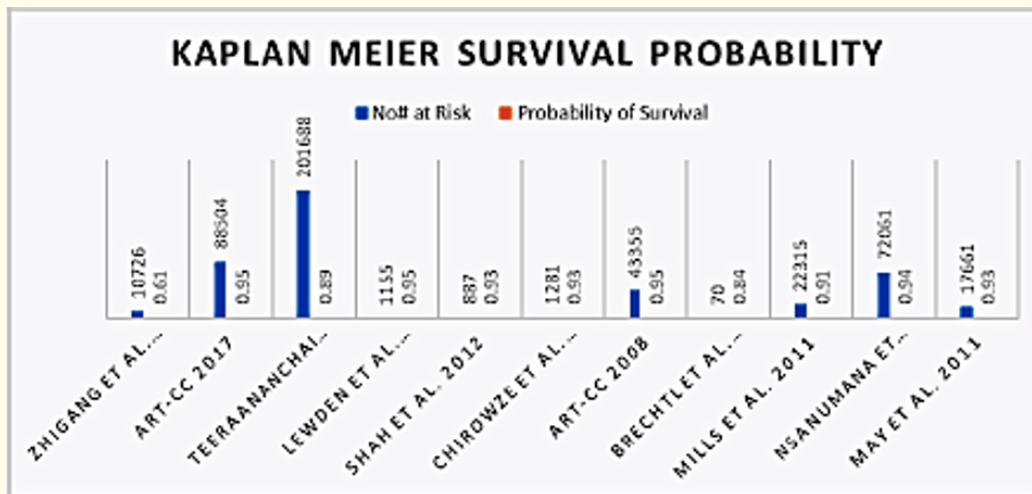


Figure 7: Kaplan-Meier survival probability.

An analysis was run comparing survival among participants in each study who took ART with a protease inhibitor for HIV infection. A risk ratio was performed to determine if ART decreased the chance of death. Geographically, the studies encompassed five continents with heterogeneous populations. Thus, the risks could vary by region. Meta-analysis was used to determine if protease inhibitor usage was beneficial in decreasing mortality across various populations (Figure 8). According to the risk ratio, taking ART was associated with a reduction in the risk of death for HIV patients.

	Study name	ART Events	ART Total N	Censored Events	Censored Total N	Risk ratio	Log risk ratio	Std Err	Variance
1	Zhigang et al. 2016	4133	10726	6593	10726	0.627	-0.467	0.014	0.000
2	ART-CC 2017	4408	88504	84096	88504	0.052	-2.949	0.015	0.000
3	Teeraananchai et	22021	201688	179667	201688	0.123	-2.099	0.006	0.000
4	Lewden et al. 2002	48	1155	1107	1155	0.043	-3.138	0.141	0.020
5	Shah et al. 2012	56	887	831	887	0.067	-2.697	0.130	0.017
6	Chirowze et al.	77	1281	1204	1281	0.064	-2.750	0.111	0.012
7	ART-CC 2008	2050	43355	41305	43355	0.050	-3.003	0.022	0.000
8	Brechtel et al. 1999	11	70	59	70	0.186	-1.680	0.282	0.079
9	Mills et al. 2011	1943	22315	20372	22315	0.095	-2.350	0.022	0.000
10	Nsanumana et al.	3917	72061	68144	72061	0.057	-2.856	0.016	0.000
11	May et al. 2011	1248	17661	16413	17661	0.076	-2.577	0.027	0.001

Figure 8: Risk ratio.

ART events equaled the number of patients who died; censored events equaled the number of patients who survived. A risk ratio of less than one suggested a reduced risk in the exposed group. All studies met this standard. Thus, it was consistent with other research in this area, demonstrating that protease inhibitor usage reduced mortality in the HIV population. The confidence interval for this analysis was set at 95%. The underlying assumption was that the samples were normally distributed.

**Additional analysis**

The outlier in the group was the Zhigang., *et al.* (2016) study that had a risk ratio closest to one [21] (Figure 9). Consequently, this study had the greatest amount of deaths compared to other studies.

Model	Study name	Statistics for each study				Risk ratio and 95% CI				
		Risk ratio	Lower limit	Upper limit	p-Value	0.01	0.10	1.00	10.00	100.00
	Zhigang et	0.627	0.609	0.645	0.000			+		
	ART-CC	0.052	0.051	0.054	0.000		+			
	Teeraananc	0.123	0.121	0.124	0.000			+		
	Lewden et	0.043	0.033	0.057	0.000		+			
	Shah et al.	0.067	0.052	0.087	0.000		+			
	Chirowze et	0.064	0.051	0.079	0.000		+			
	ART-CC	0.050	0.048	0.052	0.000		+			
	Brechtel et	0.186	0.107	0.324	0.000			+		
	Mills et al.	0.095	0.091	0.100	0.000			+		
	Nsanumana	0.057	0.056	0.059	0.000			+		
	May et al.	0.076	0.072	0.080	0.000			+		
Fixed		0.116	0.115	0.117	0.000			+		

Figure 9: Risk ratio statistics.

The Zhigang, *et al.* (2016) study had a Kaplan-Meier survival probability of only 0.61, while the other studies in the meta-analysis were all greater than 0.80 [21]. The authors of the study did not give the reason for this discrepancy. However, when a CD4 count (Figure 4) was superimposed on death events, this mean CD4 count was higher at the onset of the research than in some other studies (Figure 10). This discrepancy might be due to comorbidities at the start of the study’s research period.

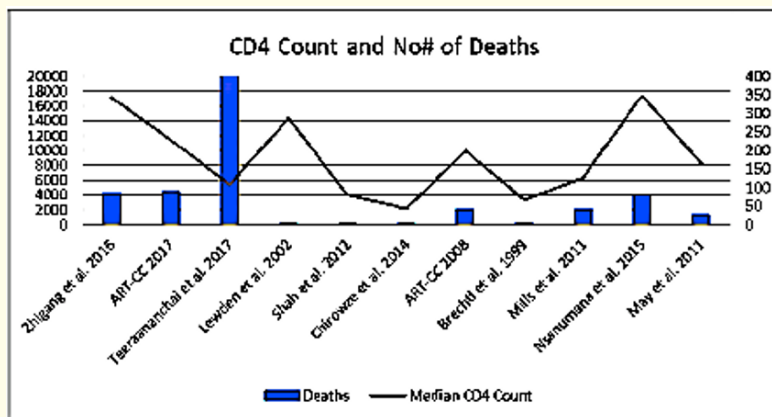


Figure 10: CD4 count and the number of deaths.

The reason for the increase in death events is unknown. A higher starting CD4 count typically corresponds to fewer risks for HIV related mortality, which is why comorbidities (such as diabetes and heart or renal disease) may have played a part. A random-model analysis was performed to determine effect size (95% confidence interval), the test of null (2-tail), heterogeneity (I-squared), and tau-squared (Figure 11). The risk ratio significance was  $p < 0.04$ . Heterogeneity among studies was present.

Model	Number Studies	Effect size and 95% interval			Test of null (2-tail)		Heterogeneity			Tau-squared				
		Point estimate	Lower limit	Upper limit	Z-value	P-value	Q-value	Df(Q)	P-value	I <sup>2</sup>	Tau <sup>2</sup>	Std. Error	Tau	
Fixed	11	0.116	0.115	0.117	-450.53	0.004	2017.32.39	10	0.000	99.95	0.727	0.539	0.290	0.853
Random	11	0.089	0.054	0.148	-9.337	0.040								

Figure 11: Random model analysis.

In a forest plot of the research studies, ten of the eleven studies were significantly below the number one. The study that was closest to one was Zhigang, *et al.* (2016) [21]. Consequently, the p-value for all the studies was significant ( $p < 0.05$ ), indicative of a significant outcome, which was decreased mortality while on ART containing a protease inhibitor (Figure 12).

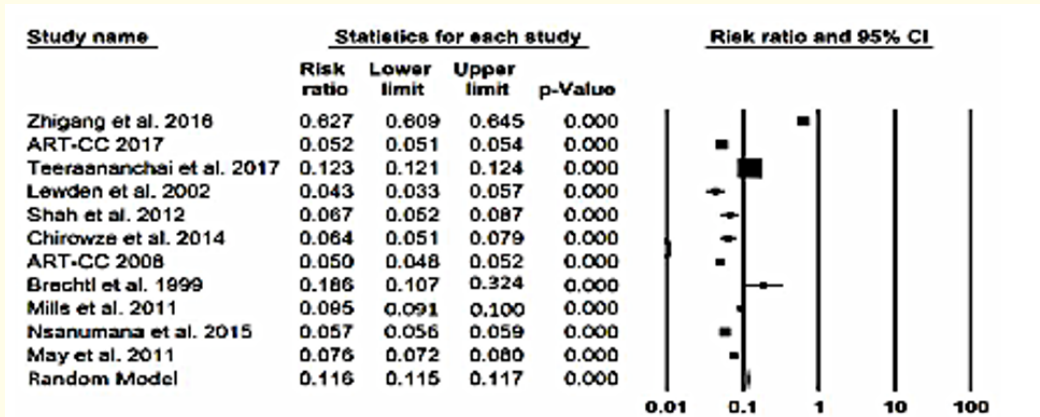


Figure 12: Forest plot of survival with ART regime of protease inhibitors.

In addition to the risk ratio, an odds ratio was performed. Odds ratios were used to compare the relative odds of the occurrence of the outcome of interest (e.g. death), given exposure to the variable of interest (e.g. antiretroviral HIV therapy). The odds ratio scale was as follows:

- OR = 1 Exposure did not affect the odds of outcome.
- OR >1 Exposure associated with higher odds of outcome.
- OR < 1 Exposure associated with lower odds of outcome.

The odds ratio (Figure 13) was less than one on the scale indicating exposure to ART was associated with lower odds of the outcome (death).

Model	Effect size and 95% interval				Test of null (P=Ia)		Heterogeneity			Tau-squared				
	Number Studies	Point estimate	Lower limit	Upper limit	Z-value	P-value	I <sup>2</sup> -value	d [I <sup>2</sup> ]	P-value	I-squared	Tau Squared	Standard Error	Yainence	Tes
Fixed	11	0.011	0.011	0.011	-534.825	0.000	25488.955	10	0.000	99.962	2.310	1.714	2.936	1.527
Random	11	0.008	0.003	0.020	12.174	0.000								

Figure 13: Odds ratio.

Antiretroviral medications increased survival in HIV individuals. According to the studies included in this meta-analysis, life-years were gained using ART for HIV infections. Although life expectancy was still short of that in the general population, it steadily increased [22].

### Summary

According to this meta-analysis, antiretroviral therapy is associated with a decrease in mortality. Multiple studies were used for this meta-analysis to determine if there is a variance according to the geographical location in ART therapy. Amalgam of the data from eleven studies worldwide indicated a correlation between ART therapy with protease inhibitor use and increased survival for HIV patients.

This research aimed to determine if ART therapy that includes a protease inhibitor will increase survival in the HIV individual. Further, the aim was to determine the impact of protease inhibitors on long-term survival. The meta-analysis included eleven studies covering 459,703 individuals. The results revealed that ART correlated with a decrease in mortality for people living with HIV.

Individual study periods ranged from one year to seventeen years. Over the various periods, patients were started on antiretroviral medications that included at least one protease inhibitor. The eleven studies measured survival or increased years of life. Ten of the eleven studies showed a survival probability of greater than 80% when performed under a Kaplan-Meier analysis. The Zhigang, *et al.* (2016) study generated a Kaplan-Meier survival probability of only 0.61. Consequently, this study had the highest proportion of deaths to the number of people entering the study. This study originated in China and did not explain the high mortality rate among its study subjects. One can propose that individuals entering their research were sicker than other participants entering the study.

Individuals entering the Zhigang, *et al.* (2016) study could have had comorbidities that contributed to increased mortality. Consequently, this study came the closest to one in the risk ratio (RR = 0.627,  $p = 0.00$ ). The risk ratio ( $1 - 0.627 = 0.373$ ) for death in this study was 37.3%, which was higher than the other studies. None of the studies' risk ratios crossed the number one matrix. Thus, all eleven studies reached statistical significance for reducing the risks of mortality when taking ART ( $p = 0.00$ , 95% CI).

The results of the meta-analysis demonstrated that ART with a protease inhibitor increases survival. The total risk ratio for all studies combined in this meta-analysis was 0.116,  $p = 0.00$ . Geographically, the eleven studies encompassed five continents. Each study contained heterogeneous populations. The meta-analysis revealed that ART with protease inhibitor usage is beneficial across various populations, increasing survival. Due to the vast heterogeneity, the random effect model was utilized.

Heterogeneity was determined by using I-squared and Tau-squared as measurement parameters. I-squared is the percentage of total variation across studies due to heterogeneity, not chance. It was calculated using the formula  $100\% \times (Q - df)/Q$  (Q is the Cochran's heterogeneity statistic). Tau-squared is an absolute measure of heterogeneity. The square root is a measurement of the standard deviation of effect sizes across studies. For the random-effects model, more data is required to achieve the same statistical power as a fixed-effects model. This research included 459,703 individuals with the aim of achieving this goal.

In the random-effects model, it is assumed that the effect is not the same in all studies. This variation might explain the I-squared value ( $I^2 = 99.95$ ) for this research as being higher than usual. Heterogeneity is to be expected in a meta-analysis. It is challenging and problematic for multiple studies—performed by different research teams in different places with different methods—to all have the same fundamental parameters. Eleven studies from nine different countries are a perfect example. Heterogeneity is inevitable. Nevertheless, heterogeneity seems to be an area of ambiguity within research circles [6].

The challenge in this research was determining the most appropriate way to analyze different heterogeneous studies from different populations and cultures. Hence, heterogeneity was assumed to represent a normal distribution of subjects for each study. A common practice among researchers is to examine the sensitivity of heterogeneity metrics to exclude questionable studies. Although effective, this approach may not be the best decision when looking at the overall effect of multiple studies. According to Borenstein, *et al.* (2010), the actual implementation in terms of I-squared can be flawed [6]. Researchers offer no rationale for seeking to reduce I-squared below an arbitrary number.



It is commonly believed that I-squared is a measure of the degree between-study heterogeneity or an estimate between-study heterogeneity. However, I-squared is neither. According to DerSimonian, *et al.* (2007), I-squared is the approximate proportion of total variability in point estimates attributed to heterogeneity [14]. Consequently, total dissimilarity depends on the within-study precisions (the sample sizes of the individual studies); therefore, so does I-squared. As a result, I-squared should not represent a meaningful parameter but rather a descriptive statistic.

Tau-squared is a superior parameter for heterogeneity point estimates among study variance of actual effects [14]. Thus, I-squared should be used as the proportion of unpredictability in point estimates due to Tau-squared rather than within-study error. The Tau-squared number for this research is 0.727. This Tau-squared number is below the parameter of one. Hence, it was significant for the effect.

The odds ratio was used to compare the relative odds of the occurrence of the outcome of interest (e.g. death), given exposure to antiretroviral HIV therapy. The odds ratio was less than one (0.008), indicating that exposure to ART is associated with lower odds of the outcome (death). For example, the odds ratio of 0.008 means that in one group, the outcome is 92% less likely to occur. In this case, it would be death ( $1 - 0.008 = 0.92 \times 100\% = 92\%$ ) less likely. This result was consistent with the other findings that ART with a protease inhibitor increased the HIV patient's survival.

### Limitations of the Study

Another challenge of this meta-analysis was that it often focused on the summary of effect and neglected the fact that the treatment effect might vary from study to study. The goal of a meta-analysis is to process the effect size. If the effects are normalized, the analysis will show good effect across all studies. This study had significant dispersion; thus, the focus shifted from the summary effect to the heterogeneity of the studies [16].

Publication bias is a common problem with meta-analysis research. Likewise, meta-analytic data can only be discerned in research that represents the population of people in the studies. This research found evidence of publication bias. For instance, some researchers had been conducting studies on HIV medication use for several years, making retrieval of all the literature a challenge. The Art-CC, *et al.* (2017) study was done over seventeen years, making retrieval of all information impossible [23]. Furthermore, all studies chosen for this analysis were published in English. Many of the studies were conducted in non-English-speaking countries. Unfortunately, pertinent information or biases could have been introduced during translation. Also, some of the studies like Mills, *et al.* (2011) (Uganda) and Zhigang, *et al.* (2016) (China) were taken from government records. Archives from governments tend to have data missing, which could have further contributed to publication bias [21,24]. Finally, significant heterogeneity was found that could not be accounted for, despite efforts to prevent confounding. It is postulated that including studies from various countries with different standards of research conduction might have contributed to a large amount of heterogeneity.

### Conflict of Interest Statement

The authors declare that this paper was written in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

### Supplementary Information

The authors intend to publish three interdependent papers on this topic—this being the third and final paper— a meta-analysis of ART in HIV, in particular life expectancy or survival; the first being the “search for the most efficacious engagement point in the campaign against HIV”; and the second being on ART (pharmacology, compliance, and drug combinations), highlighting the protease inhibitors. The three papers will be made available through E-Cronicon of the United Kingdom by the same team of researchers and authors. This paper is based, in part, on prior research: Pruitt, KD. (2018). Allopathic Medicine and Effectiveness of Protease Inhibitors in HIV/AIDS Survival (unpublished doctoral dissertation).

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