

Title: On the importance of randomized controlled trials for Ebola virus disease therapeutics: a meta-analysis from the West African outbreak

Authors

Lori E. Dodd^{1*}, Dean Follmann¹, Michael Proschan¹, Jing Wang², Denis Malvy^{3,4}, Johan van Griensven⁵, Iza Ciglenecki⁶, Peter W. Horby⁷, Rashid Ansumana^{8,9}, Jia-Fu Jiang¹⁰, Richard T. Davey¹¹, H. Clifford Lane¹², Aurelie Gouel-Cheron^{1,13}

Affiliations

¹Biostatistics Research Branch, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.

²Clinical Monitoring Research Program, Clinical Research Directorate, Leidos Biomedical Research, Inc, Frederick, MD, USA.

³Inserm, UMR 1219, Université de Bordeaux, Bordeaux, France.

⁴Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France.

⁵Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium.

⁶Operational Centre Geneva, Médecins Sans Frontières, 1211 Geneva, Switzerland.

⁷Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom.

⁸Mercy Hospital Research Laboratory, Kailand Town, Bo, Sierra Leone.

⁹School of Community Health Sciences, Njala University, Bo, Sierra Leone.

¹⁰Beijing Institute of Microbiology and Epidemiology, Beijing, China.

¹¹Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.

¹²Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.

¹³Anesthesiology and Intensive Care Department, Hopital Bichat – Claude Bernard, Assistance Publique – Hopitaux de Paris, Paris, France.

* Lori E Dodd, National Institute of Allergy and Infectious Diseases, Division of Clinical Research, Biostatistics Research Branch, 5601 Fishers Ln, Rockville, MD 20852, USA, doddl@mail.nih.gov

One Sentence Summary

Randomized controlled trials are critical for evaluating Ebola virus disease therapeutics.

Abstract

Recent Ebola virus disease outbreaks in the Democratic Republic of the Congo affirm the dire need for treatments with proven efficacy. The appropriate scientific method for establishing evidence has been a matter of debate. Randomized controlled trials remain the gold standard but can be controversial due to ethical concerns and challenging field conditions. In the absence of randomization, statistical modeling to create a control group is a possible alternative. This approach is only credible as a reference control if the modelled control represents the mortality risk of the experimental group under the counterfactual assumption that the experimental treatment was not given. One way to test this is to evaluate whether, after modelling, reasonable similarity exists across control groups, which might suggest that a future control group would be similarly homogenous. We sought to evaluate this across studies from the 2013-2016 West African outbreak. Data were obtained from eight studies, contributing data from a total of 1493 patients. Comparisons of control groups across studies revealed considerable heterogeneity. Mortality rates varied widely across control arms, from 31% to 66% ($p < 0.0001$). Models adjusting for baseline covariates (age, sex and cycle threshold, a proxy for viral load) failed to sufficiently re-calibrate these studies and showed that residual heterogeneity remained. Our study asserts the importance of randomization to accumulate reliable evidence as well as the risks of making invalid conclusions when comparing non-randomized controls and treatment arms such

as that collected under WHO's Monitored Experimental Use of Unregistered and Investigational Interventions criteria (1) .

Introduction

The 10th Ebola virus outbreak in the Democratic Republic of the Congo began in August 2018 and continues to spread as of March 2019, demonstrating the need for effective Ebola virus disease (EVD) treatments. Multiple treatments were studied during the West African Ebola outbreak of 2013-2016, but none provided definitive evidence about therapeutic efficacy. All but one of these studies relied on data from non-randomized controls, and the only randomized-controlled trial closed prior to full accrual when the outbreak ended. Nonetheless, multiple experimental agents have been given under the Monitored Experimental Use of Unregistered and Investigational Interventions outside of the context of a randomized clinical trial in the outbreak in the Democratic Republic of the Congo (1).

Conclusive evidence about experimental treatment efficacy requires an appropriate control group. Ideally, the only factor differing between the treatment and control groups is the intervention, with all other factors balanced. By balancing such factors across patients and study arms, randomization will strengthen the evidence collected in support of treatment efficacy. Randomization may not always be feasible and the ethics of randomization in this context have been the subject of debate (2, 3). In the absence of randomized groups, statistical models can attempt to equalize risk factors between the control and experimental groups. In EVD, relevant factors identified to date include baseline viral load (4–6), age (7, 8), sex (9, 10), and supportive care measures. However, this approach produces a valid reference control only if the modeling

reliably represents the risk of death in the population receiving the experimental therapy under the counterfactual assumption that the experimental therapy was not given.

Meta-analysis statistical techniques provide a framework for evaluating the validity of this assumption, through a comparison of control groups mortality proportions across studies, after adjustments for covariates. Analysis of patient-level data (as compared to the study-level summary data typically used for meta-analysis) from multiple studies offers a more powerful and comprehensive analyses of risk factors. For example, a comparison of the strength of the relationship between viral load and mortality risk across studies can characterize the heterogeneity between studies. Homogeneity of regression coefficients across studies may provide confidence that a representative control group can be generated for comparators to experimental groups. In this paper, we compare regression models across studies from the West African outbreak and evaluate the potential contribution to our understanding about experimental treatment efficacy. We note that this approach makes many assumptions, including that all relevant prognostic variables are measured and appropriately modelled in the analysis, a goal unlikely to be met in any clinical research setting. Hence, these analyses are intended to complement existing results and provide a cautionary tale about the inability to avoid randomization.

Results

Study summaries

Eight studies were identified from our extensive literature research (Figure S1). Individual patient data from all identified therapeutic intervention studies during the West African outbreak were obtained. Covariates of baseline cycle threshold (CT, a proxy measure of

viral load), age, sex were only available for six of eight studies. Intravenous-fluid (IVF) use was not available in sufficient numbers for analysis. In total, complete data were provided from 1582 subjects. However, to make studies more comparable and because of the U-shaped relationship between age and mortality (11–14), children under 6 years old were not included, resulting in data from 1493 subjects. Note that, while studies did not always use the same endpoint definition (e.g., mortality at 14 days vs mortality at 28 days), most deaths occurred within the first 14 days, making the impact of this difference negligible. Table 1 describes the data included, along with comparisons to reported results.

Prevail II- ZMappTM was the only randomized-controlled multi-country (Liberia, Sierra Leone, Guinea, and the United States) study of ZMappTM (n=36) versus controls (n=35), with both arms receiving optimized standard-of-care (oSOC). Per-protocol, oSOC was defined as the most optimal standard of care possible for the setting, to include “the application of aggressive fluid resuscitation, hemodynamic and respiratory support, metabolic corrections, diagnostic evaluation, and other modalities of advanced critical care that are generally available in most academic centers capable of caring for critically ill patients,” although this was not widely achieved (15). The study enrolled from March 2015 through November 2015, when new cases of EVD ceased (16).

Seven studies used non-randomized controls, with two different types of control groups. The first one corresponds to controls included from the same location over a similar time period. The *amodiaquine study* was a retrospective analysis from a natural experiment in Liberia (17). During a 12-day period in August 2014, the supply of the first-line antimalarial combination (artemether-lumefantrine) ran out, and 71 EVD patients were prescribed artesunate-amodiaquine. The amodiaquine treatment was considered experimental and was compared to the 194 control

patients prescribed artemether-lumefantrine from June through October 2014, which was considered the standard regimen.

The *convalescent whole-blood study* enrolled patients with a matching blood type in Sierra Leone from December 2014 until April 2015 (18). Patients who had a matching blood type and agreed to a transfusion (n=44) were included in the experimental group; 25 patients did not receive a transfusion and were considered controls. CT data were only available for 11 and 20 of those in the control and experimental arms, respectively.

The remaining five studies used historical controls, with either patients hospitalized in a different center outside of a trial context or during a preparatory period in the same trial center. The *convalescent-plasma study* enrolled EVD patients in Guinea from mid-February through early August 2015 (19). Control patients consisted of individuals enrolled during a preparatory period from September 2014 to January 2015. After exclusion of deceased patients within the three first days (for both experimental and control groups), a total of 84 participants received convalescent plasma; data from 418 patients were available as historical controls.

Two studies of favipiravir used historical controls: *Favi-Bai* enrolled 85 control patients in Sierra-Leone from October 10-30, 2014 and 35 experimental group patients from November 1-10, 2014 (20), while *Favi-JIKI* enrolled experimental arm participants from late-December 2014 through mid-April 2015 at three sites in Guinea (21). Historical control patients were taken from patients hospitalized at Ebola treatment centers run by Medecins Sans Frontieres (MSF) in Guinea from mid-September 2014 through mid-December 2014.

In Guinea, a small historical-controlled study of *IFN- β 1a* (Interferon) was conducted in 9 patients enrolled at an Ebola-treatment unit from late March through mid-June 2016 (22). Data from the 28 historical control subjects were not available for this meta-analysis. A study of *TKM-*

TKM-130803, a small interfering RNA (Ribonucleic Acid) molecule, enrolled 14 participants from March 1 until June 15, 2015 in a single Ebola-treatment unit in Sierra Leone (23), with a futility boundary based on historical control data on 1,820 EVD cases obtained from MSF. The study closed due to crossing a futility boundary due to the high number of deaths. CT data from controls were not available from this study.

Mortality and cycle threshold associations with calendar time

Figure 1 plots date range of enrollment relative to mortality rate and mean baseline CT, respectively. (Analysis of subject's date of enrollment was not possible). The figure might suggest a decline in mortality over time, although this visual effect is driven by Prevail II-ZMapp™ and the relationship is not statistically significant ($p=0.23$). Likewise, the mean baseline CT values did not change significantly over time ($p=0.48$). Below, we evaluate the relationship between CT and mortality, using patient-level data.

Analytic problems with pooling control arms

Standard-of-care (SOC) and symptomatic patient management measures were not always extensively described, making comparisons difficult. Among the 8 studies, all SOC measures were reported to include oral hydration, prophylactic antibiotics, antipyretics/analgesics, electrolyte supplementation (guided by a point-of-care device for some of the studies) and antimalarial therapy. Reported-IVF use varied widely among studies, ranging from 0% (Favi-Bai) to 85% or more (Favi-JIKI, PREVAIL II-ZMapp, TKM-130803 and IFN- β 1a trials). Because IVF use is thought to be an important means of fluid resuscitation in EVD patients, this is a critical missing variable to consider when evaluating the analyses. Prevail II-ZMapp allowed favipiravir as SOC in Guinea. Favi-Bai reported either artesunate or amodiaquine use (17).

Some reported use of antihelminthics, antiemetics, antidiarrheals, anticonvulsants, anxiolytics, mechanical ventilation, or corticosteroids. None of these variables were available for analysis.

Figure 2A plots the unadjusted mortality proportions from the six controlled studies, which range from 31% (Prevail II; 95% CI: 15%-51%) to 66% (Favi-Bai; 95% CI: 55%-76%). The control arm for Favi-Bai has the highest mortality rate, despite the more favorable distribution of baseline characteristics relative to the other studies.

We next adjusted mortality rates for age, sex and log-CT using logistic regression. Even with adjustment, the mortality rates varied significantly across studies ($p < 0.0001$). Furthermore, the relationship between log-CT and mortality differed markedly across studies ($p < 0.001$). Figure 2B shows expected mortality as a function of log-CT for a woman of age 34 across studies. Of note is the lack of improvement in mortality with high CTs (i.e. lower viral load) for the Favi-Bai control group compared to the others. Other control groups appear more similar but remain significantly different ($p < 0.001$). Interaction tests of study with age and sex were not significant ($p = 0.22$ and $p = 0.38$, respectively). Figure 2C is a Galbraith plot used to confirm heterogeneity. The slope of the blue line is the weighted average across studies, relating mortality to log-CT. If the relationship between log-CT and mortality did not differ by study, one would expect only 1 in 20 points to lie outside the dotted lines. Instead, 3 of 6 points were outside those boundaries. Figure 2D shows the standardized mortality rates, while Table 2 provides a tabulation of the estimated mortality rates in the control groups for a 34-year-old female for various CT values. For a CT of 30, mortality rates range from 1% to 60%, while for a CT of 20, they range from 47% to 74%.

Evaluating the experimental treatments

While the large between-study variability makes combining data for a common control model problematic, it is possible to evaluate each intervention relative to each of the six control groups, in turn, adjusting for available baseline covariates (i.e., log-CT, age, and sex). If the six control groups represent the range of controls, then a consistent pattern of efficacy across the heterogeneous control groups may contribute to the evidence base. We note, however, that this assumption is not testable, and the analysis assumes model fit is adequate. Hence, we caution against over-interpretation.

All but two confidence intervals for amodiaquine compared to the control groups are below one, suggesting an association of reduced mortality with amodiaquine (Figure 3A). Confidence intervals comparing TKM-130803 include only odds ratios above 1 for comparisons with two control groups (Favi-JIKI and Prevail II-ZMapp), suggesting an association of increased mortality with TKM-130803 (Figure 3B). The confidence interval for the odds ratios for Prevail II-ZMapp relative to other control groups indicate improved survival for ZMappTM compared to all control groups, except its own control (Figure 3C).

Three of 6 confidence intervals for Favi-JIKI comparisons with controls exclude one, suggesting survival improvements (Figure 4A). Results for IFN- β 1a (Figure 3D), convalescent plasma (Figures 4B), Favi-Bai (Figure 4C), and convalescent whole blood (Figure 4D) are mixed. Supplemental figures S2 and S3 display treatment-effect odds ratios, adjusting for log-CT, age, sex on the subgroup with CT \geq 20, a reported subgroup in Favi-JIKI. Supplemental Figure S4 compares the per-protocol (A) and intent-to-treat (B) samples for TKM-130803, the only study for which this comparison could be evaluated.

Discussion

The results of our meta-analysis underscore the difficulties of conducting EVD research. The rapid course of the disease and challenging field conditions add enormous complexity to the challenge of collecting reliable, comprehensive outcome data (24). Pathogen virulence, access to diagnostics, and stage of disease at presentation may vary both across sites and over time. Additionally, background supportive care may vary across sites or time due to caseload, other resource constraints, clinician preferences, availability of treatments, and evolving medical practices gleaned from clinical observations during a trial (25, 26). Further, survivor bias may occur when the sickest patients do not live long enough to be seen in the Ebola treatment unit (27). This panoply of factors promotes a high degree of between-study variability, making it difficult to evaluate the validity of any particular non-randomized control group for a given investigational agent.

In our meta-analysis, study-specific effects were needed after adjusting for age, sex, and baseline CT. Favi-Bai had the highest mortality rate, even after adjustments, while Prevail II-ZMapp had the lowest mortality rate. Timing in the epidemic may partially explain this, although this was not shown in our study. Importantly, the relationship between CT and mortality differed by study, making estimation of a common control model, as done by Korn et al. in melanoma (28), infeasible. In the context of an outbreak, this implies that, when all factors influencing prognosis are not established or measured, the use of non-randomized cohort data, even after regression adjustments, may not be suitable.

The regression modelling approach failed to provide a common control group, so we evaluated another approach. Under an assumption that control groups broadly represent the range of controls, we hypothesized that consistency of results across such a heterogeneous collection of

control groups would add to the body of evidence for or against an investigational agent. This led to comparisons of experimental treatments relative to each available control group, adjusting for available baseline covariates. Amodiaquine analyses suggest survival improvements for three of six comparisons. While these results should be taken with caution, they may suggest a preference for amodiaquine as anti-malarial treatment in patients with acute EVD. The potential efficacy of amodiaquine against Ebola virus is debated; *in vitro* studies reported anti-Ebola virus activity (29–31), but this was not confirmed in a mouse model (32). Similar positive results were observed for Prevail II-ZMapp, which had a randomized control group. Hence, the comparison with its own control deserves primary focus. It is worth noting that, because Prevail II-ZMapp stopped prior to complete accrual due to the end of the outbreak, the study was underpowered for the targeted treatment effect. While results comparing Prevail II-ZMapp to historical control data rank considerably lower in terms of scientific evidence relative to the randomized control trial, it is reassuring that the comparisons are all conclusively in the favorable direction. We note, however, that the assumption that the control groups included in our meta-analysis represent the spectrum of variability expected in future studies is an untestable assumption.

These analyses raise several important questions. First, do consistent results across studies, with large variability, add to the evidence base for a given treatment? Perhaps a consistent pattern of benefit (or harm) in the context of this heterogeneity is reassuring. However, the heterogeneity highlights the potential risks to the validity of non-concurrent, non-randomized control groups. Another important question is whether a study's chosen control group is, in some way, more valid than those across studies. Greater similarities in patient population and management would provide a better comparator group. Further, we note that the particulars of the type of non-randomized control group are important. Some controls were based

on retrospective analysis of patients hospitalized in a center outside of a trial context, while others were collected during the preparatory period and represented prospectively enrolled patients at the same trial center. Data collection and other factors are more likely to be standardized in the latter setting. In any event, none of these considerations outweigh the value of a randomized control group.

There are many limitations to this meta-analysis. Differences between CT platforms may have contributed to between-study variability (33), but assay platform was not always recorded. Future studies should aim to standardize platforms. More consistent administration of standard-of-care measures across control arms would have strengthened this analysis, as would documentation of supportive care given. For example, if data on administration of IVF had been available, it may have helped explain more between-study variability. Additional variables that may have contributed include date of diagnosis/enrollment and measures of patient case load, which would be useful for evaluating within- and between-study time trends. The limited sample size is another weakness. Finally, the strength of conclusions from statistical models also depends on adequacy of model fit. Missing covariates and/or an incorrectly specified functional form may lead to biased estimation and inference. These potential weaknesses further limit the conclusions drawn from the analyses of experimental therapeutics.

In summary, non-randomized controls are only credible reference comparators if they represent the risk of death in the population who received experimental treatment under the counterfactual condition that the experimental treatment was not given. We sought to test this by evaluating the homogeneity between control groups from the West African outbreak. This analysis revealed considerable heterogeneity, which was not removed after statistical modelling. Therefore, we cannot rely on non-randomized controls as a valid benchmark for efficacy

evidence. While this analysis applies to EVD trials, results are likely to be similar in other emerging infectious disease outbreak settings.

Materials and Methods

Literature search / Study design

We conducted a meta-analysis to include all studies evaluating experimental interventions during the West African outbreak from 2013-2016. Eligible studies included those with control groups from the following three categories were eligible: randomized controls, historical controls, concurrent but non-randomized controls, such as those who refused or were ineligible for experimental treatments. Studies reporting case-fatality rates over time were not eligible for inclusion. To identify studies, we conducted a literature review following PRISMA guidelines (34). A comprehensive search of the Medline/PubMed, EMBASE, Scopus and Web of Science databases was performed for papers published from December 2013 until February 2017. Additionally, Cochrane Central Register of Controlled Trials and ClinicalTrials.gov were searched. Results were further restricted to those evaluating curative treatments, either in the context of a randomized controlled trial or with external control data. Independent literature searches were performed by LED and AGC. De-identified data to include mortality, age, sex, baseline polymerase chain reaction (PCR) CT as a proxy for viral load, and IVF administration were requested under multiple data-sharing agreements.

Statistical methods

Logistic regression models were fitted, using the covariates age, sex, log-CT, and study. Statistical significance of interaction terms for age, sex, and log-CT. Likelihood ratio tests were

applied to compare evaluate significance of additional covariates to models.. A Galbraith plot was used to illustrate between-study heterogeneity (35). All tests were two-sided with statistical significance set to $\alpha=0.05$; 95% confidence intervals were two-sided. Weighted-least squares logistic regression methods were applied to summary-level (as opposed to patient-level) regression models to evaluate the relationship between mortality rate, mean CT, and study timing (evaluated as mid-point of study enrollment). Mixed effects logistic regression models were estimated but failed had convergence problems arising from the small number of studies relative to the amount of between-study variability.

Supplementary Materials

Table S1. Model estimates from weighted least squares regressions for figure 1 analyses.

Table S2. Model estimates from logistic regression models for figure 2D

Tables S3A-S3D. Model estimates from logistic regression models for Figures 3A-3D

Tables S4A-S4D. Model estimates from logistic regression models for Figures 4A-4D

Fig. S1. PRISMA 2009 Flow Diagram.

Fig. S2. Adjusted odds ratios for amodiaquine (a), TKM-130803 (b), Prevail II-ZMapp (c) and IFN- β 1a (d) treatment arms for patients with a CT ≥ 20 relative to each control group.

Fig. S3 Adjusted odds ratios for Favi-JIKI (a), Convalescent Plasma (b), Favi-Bai (c), and Convalescent Whole Blood (d) treatment arms for patients with a CT ≥ 20 relative to each control group.

Fig S4. Adjusted odds ratios for treatment relative to each of 6 control groups for patients of the TKM-130803 study, from the a/ per-protocol and b/ intent-to-treat samples.

Data file S1. Bibliography research criteria.

Data file S2. PRISMA 2009 Checklist.

References

1. WHO, in *Guidance For Managing Ethical Issues In Infectious Disease Outbreaks.*, (WHO Library Cataloguing-in-Publication Data, Geneva, Switzerland, 2016).
2. C. Adebamowo, O. Bah-Sow, F. Binka, R. Bruzzone, A. Caplan, J.-F. Delfraissy, D. Heymann, P. Horby, P. Kaleebu, J.-J. M. Tamfum, P. Olliaro, P. Piot, A. Tejan-Cole, O. Tomori, A. Toure, E. Torreele, J. Whitehead, Randomised controlled trials for Ebola: practical and ethical issues, *Lancet* **384**, 1423–1424 (2014).
3. E. Cox, L. Borio, R. Temple, Evaluating Ebola Therapies — The Case for RCTs, *N Engl J Med* **371**, 2350–2351 (2014).
4. O. Faye, A. Andronico, O. Faye, H. Salje, P. Y. Boelle, N. Magassouba, E. I. Bah, L. Koivogui, B. Diallo, A. A. Diallo, S. Keita, M. K. Konde, R. Fowler, G. Fall, S. Cauchemez, A. A. Sall, Use of Viremia to Evaluate the Baseline Case Fatality Ratio of Ebola Virus Disease and Inform Treatment Studies: A Retrospective Cohort Study, *PLoS Med* **12**, e1001908 (2015).
5. J. R. Spengler, A. K. McElroy, J. R. Harmon, U. Ströher, S. T. Nichol, C. F. Spiropoulou, Relationship Between Ebola Virus Real-Time Quantitative Polymerase Chain Reaction–Based Threshold Cycle Value and Virus Isolation From Human Plasma, *J Infect Dis* **212**, S346–S349 (2015).
6. C. Samuel J., J. M. Matthew, K. Solomon, E. Bobbie Rae, C. Megan, K. Barbara, K. John, F. Joyce, H. Darren, H. Veerle, A. Jay, M. C. Grazia, H. Michel Van, G. A. César, A. Brian, B. Alison Jane, B. Scott, A. B. Jessica, B. Eric, B. Dianna, C. B. Aaron, C. Shelley, F. Mike, G. Aridh, G. Christin, M. Laura, D. P. Christopher, R. Brandy, S. S. Johanna, S. Angela, S. Tara, W. David, S. Gbessay, T. Alhajie, T. N. Stuart, S. T. Jonathan, Prognostic Indicators for Ebola Patient Survival, *Emerg Infect Dis* **22**, 217 (2016).
7. J. Y. Wong, W. Zhang, D. Kargbo, U. Haque, W. Hu, P. Wu, A. Kamara, Y. Chen, B. Kargbo, G. E. Glass, R. Yang, B. J. Cowling, C. Liu, Assessment of the severity of Ebola virus disease in Sierra Leone in 2014–2015, *Epidemiol Infect* **144**, 1473–1481 (2015).

8. Y. Furuse, M. Fallah, H. Oshitani, L. Kituyi, N. Mahmoud, E. Musa, A. Gasasira, T. Nyenswah, B. Dahn, L. Bawo, Analysis of patient data from laboratories during the Ebola virus disease outbreak in Liberia, April 2014 to March 2015, *PLoS Negl Trop Dis* **11**, e0005804 (2017).
9. WHO Ebola Response Team. Ebola Virus Disease among Male and Female Persons in West Africa, *N Engl J Med* **374**, 96–98 (2016).
10. Y. L. Haaskjold, H. A. Bolkan, K. Ø. Krogh, J. Jongopi, K. M. Lundebj, S. Mellesmo, P. S. J. Garcés, O. Jøsendal, Å. Øpstad, E. Svensen, L. M. Z. Fuentes, A. S. Kamara, M. Riera, J. Arranz, D. P. Roberts, P. D. Stamper, P. Austin, A. J. Moosa, D. Marke, S. Hassan, G. E. Eide, Å. Berg, B. Blomberg, Clinical Features of and Risk Factors for Fatal Ebola Virus Disease, Moyamba District, Sierra Leone, December 2014–February 2015, *Emerg Infect Dis* **22**, 1537–1544 (2016).
11. J. S. Schieffelin, J. G. Shaffer, A. Goba, M. Gbakie, S. K. Gire, A. Colubri, R. S. G. Sealfon, L. Kanneh, A. Moigboi, M. Momoh, M. Fullah, L. M. Moses, B. L. Brown, K. G. Andersen, S. Winnicki, S. F. Schaffner, D. J. Park, N. L. Yozwiak, P.-P. Jiang, D. Kargbo, S. Jalloh, M. Fonnies, V. Sinnah, I. French, A. Kovoma, F. K. Kamara, V. Tucker, E. Konuwa, J. Sellu, I. Mustapha, M. Foday, M. Yillah, F. Kanneh, S. Saffa, J. L. B. Massally, M. L. Boisen, L. M. Branco, M. A. Vandi, D. S. Grant, C. Happi, S. M. Gevaio, T. E. Fletcher, R. A. Fowler, D. G. Bausch, P. C. Sabeti, S. H. Khan, R. F. Garry, Clinical Illness and Outcomes in Patients with Ebola in Sierra Leone, *N Engl J Med* **371**, 2092–2100 (2014).
12. J. Li, H.-J. Duan, H.-Y. Chen, Y.-J. Ji, X. Zhang, Y.-H. Rong, Z. Xu, L.-J. Sun, J.-Y. Zhang, L.-M. Liu, B. Jin, J. Zhang, N. Du, H.-B. Su, G.-J. Teng, Y. Yuan, E.-Q. Qin, H.-J. Jia, S. Wang, T.-S. Guo, Y. Wang, J.-S. Mu, T. Yan, Z.-W. Li, Z. Dong, W.-M. Nie, T.-J. Jiang, C. Li, X.-D. Gao, D. Ji, Y.-J. Zhuang, L. Li, L.-F. Wang, W.-G. Li, X.-Z. Duan, Y.-Y. Lu, Z.-Q. Sun, A. B. J. Kanu, S. M. Koroma, M. Zhao, J.-S. Ji, F.-S. Wang, Age and Ebola viral load correlate with mortality and survival time in 288 Ebola virus disease patients, *Int J Infect Dis* **42**, 34–39 (2016).

13. M. A. Smit, I. C. Michelow, J. Glavis-Bloom, V. Wolfman, A. C. Levine, Characteristics and Outcomes of Pediatric Patients With Ebola Virus Disease Admitted to Treatment Units in Liberia and Sierra Leone: A Retrospective Cohort Study, *Clin Infect Dis* **64**, 243–249 (2017).
14. WHO Ebola Response Team. Ebola Virus Disease among Children in West Africa, *N Engl J Med* **372**, 1274–1277 (2015).
15. A. Sterck, Médecins sans Frontières. Filovirus haemorrhagic fever guideline ([www .medbox .org/ filovirus-haemorrhagic-fever-guideline/ download .pdf](http://www.medicins-sans-frontieres.org/filovirus-haemorrhagic-fever-guideline/download.pdf))., (2008).
16. The PREVAIL II Writing Group. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection, *N Engl J Med* **375**, 1448–1456 (2016).
17. E. Gignoux, A. S. Azman, M. de Smet, P. Azuma, M. Massaquoi, D. Job, A. Tiffany, R. Petrucci, E. Sterk, J. Potet, M. Suzuki, A. Kurth, A. Cannas, A. Bocquin, T. Strecker, C. Logue, T. Pottage, C. Yue, J. C. Cabrol, M. Serafini, I. Ciglenecki, Effect of Artesunate-Amodiaquine on Mortality Related to Ebola Virus Disease, *N Engl J Med* **374**, 23–32 (2016).
18. F. Sahr, R. Ansumana, T. A. Massaquoi, B. R. Idriss, F. R. Sesay, J. M. Lamin, S. Baker, S. Nicol, B. Conton, W. Johnson, O. T. Abiri, O. Kargbo, P. Kamara, A. Goba, J. B. Russell, S. M. Gevaio, Evaluation of convalescent whole blood for treating Ebola Virus Disease in Freetown, Sierra Leone, *J Infect* **74**, 302–309 (2017).
19. J. van Griensven, T. Edwards, X. de Lamballerie, M. G. Semple, P. Gallian, S. Baize, P. W. Horby, H. Raoul, N. Magassouba, A. Antierens, C. Lomas, O. Faye, A. A. Sall, K. Fransen, J. Buyze, R. Ravinetto, P. Tiberghien, Y. Claeys, M. De Crop, L. Lynen, E. I. Bah, P. G. Smith, A. Delamou, A. De Weggheleire, N. Haba, Evaluation of Convalescent Plasma for Ebola Virus Disease in Guinea, *N Engl J Med* **374**, 33–42 (2016).
20. C. Q. Bai, J. S. Mu, D. Kargbo, Y. B. Song, W. K. Niu, W. M. Nie, A. Kanu, W. W. Liu, Y. P. Wang, F. Dafaie, T. Yan, Y. Hu, Y. Q. Deng, H. J. Lu, F. Yang, X. G. Zhang, Y. Sun, Y. X. Cao, H. X. Su, Y. Sun, W. S. Liu, C. Y. Wang, J. Qian, L. Liu, H. Wang, Y. G. Tong, Z. Y. Liu, Y. S. Chen, H. Q. Wang, B. Kargbo, G. F. Gao, J. F. Jiang, Clinical and Virological

Characteristics of Ebola Virus Disease Patients Treated With Favipiravir (T-705)-Sierra Leone, 2014, *Clin Infect Dis* **63**, 1288–1294 (2016).

21. D. Sissoko, C. Laouenan, E. Folkesson, A. B. M'Lebing, A. H. Beavogui, S. Baize, A. M. Camara, P. Maes, S. Shepherd, C. Danel, S. Carazo, M. N. Conde, J. L. Gala, G. Colin, H. Savini, J. A. Bore, F. Le Marcis, F. R. Koundouno, F. Petitjean, M. C. Laham, S. Diederich, A. Tounkara, G. Poelart, E. Berbain, J. M. Dindart, S. Duraffour, A. Lefevre, T. Leno, O. Peyrouset, L. Irengue, N. Bangoura, R. Palich, J. Hinzmann, A. Kraus, T. S. Barry, S. Berette, A. Bongono, M. S. Camara, V. Chanfreau Munoz, L. Doumbouya, H. Souley, P. M. Kighoma, F. R. Koundouno, L. Rene, C. M. Loua, V. Massala, K. Moumouni, C. Provost, N. Samake, C. Sekou, A. Soumah, I. Arnould, M. S. Komano, L. Gustin, C. Berutto, D. Camara, F. S. Camara, J. Colpaert, L. Delamou, L. Jansson, E. Kourouma, M. Loua, K. Malme, E. Manfrin, A. Maomou, A. Milinouno, S. Ombelet, A. Y. Sidiboun, I. Verreckt, P. Yombouno, A. Bocquin, C. Carbonnelle, T. Carmoi, P. Frange, S. Mely, V. K. Nguyen, D. Pannetier, A. M. Taburet, J. M. Treluyer, J. Kolie, R. Moh, M. C. Gonzalez, E. Kuisma, B. Liedigk, D. Ngabo, M. Rudolf, R. Thom, R. Kerber, M. Gabriel, A. Di Caro, R. Wolfel, J. Badir, M. Bentahir, Y. Deccache, C. Dumont, J. F. Durant, K. El Bakkouri, M. Gasasira Uwamahoro, B. Smits, N. Toufik, S. Van Cauwenberghe, K. Ezzedine, E. D'Ortenzio, L. Pizarro, A. Etienne, J. Guedj, A. Fizet, E. Barte de Sainte Fare, B. Murgue, T. Tran-Minh, C. Rapp, P. Piguet, M. Poncin, B. Draguez, T. Allaford Duverger, S. Barbe, G. Baret, I. Defourny, M. Carroll, H. Raoul, A. Augier, S. P. Eholie, Y. Yazdanpanah, C. Levy-Marchal, A. Antierrens, M. Van Herp, S. Gunther, X. de Lamballerie, S. Keita, F. Mentre, X. Anglaret, D. Malvy, Experimental Treatment with Favipiravir for Ebola Virus Disease (the JIKI Trial): A Historically Controlled, Single-Arm Proof-of-Concept Trial in Guinea, *PLoS Med* **13**, e1001967 (2016).

22. M. K. Konde, D. P. Baker, F. A. Traore, M. S. Sow, A. Camara, A. A. Barry, D. Mara, A. Barry, M. Cone, I. Kaba, A. A. Richard, A. H. Beavogui, S. Gunther, M. Pintilie, E. N. Fish, Interferon beta-1a for the treatment of Ebola virus disease: A historically controlled, single-arm proof-of-concept trial, *PLoS One* **12**, e0169255 (2017).

23. J. Dunning, F. Sahr, A. Rojek, F. Gannon, G. Carson, B. Idriss, T. Massaquoi, R. Gandhi, S. Joseph, H. K. Osman, T. J. Brooks, A. J. Simpson, I. Goodfellow, L. Thorne, A. Arias, L.

- Merson, L. Castle, R. Howell-Jones, R. Pardinaz-Solis, B. Hope-Gill, M. Ferri, J. Grove, M. Kowalski, K. Stepniewska, T. Lang, J. Whitehead, P. Olliaro, M. Samai, P. W. Horby, Experimental Treatment of Ebola Virus Disease with TKM-130803: A Single-Arm Phase 2 Clinical Trial, *PLoS Med* **13**, e1001997 (2016).
24. E. National Academies of Sciences, Medicine, *Integrating Clinical Research into Epidemic Response: The Ebola Experience* (The National Academies Press, Washington, DC, 2017).
25. J. M. Grimshaw, I. T. Russell, Effect of clinical guidelines on medical practice: a systematic review of rigorous evaluations, *Lancet* **342**, 1317–1322 (1993).
26. A. Hallstrom, L. Friedman, P. Denes, C. Rizo-Patron, M. Morris, Do arrhythmia patients improve survival by participating in randomized clinical trials?: Observations from the Cardiac Arrhythmia Suppression Trial (CAST) and the Antiarrhythmics Versus Implantable Defibrillators Trial (AVID), *Control Clin Trials* **24**, 341–352 (2003).
27. M. Lipsitch, C. A. Donnelly, C. Fraser, I. M. Blake, A. Cori, I. Dorigatti, N. M. Ferguson, T. Garske, H. L. Mills, S. Riley, M. D. V. Kerkhove, M. A. Hernán, Potential Biases in Estimating Absolute and Relative Case-Fatality Risks during Outbreaks, *PLoS Negl Trop Dis* **9**, e0003846 (2015).
28. E. L. Korn, P.-Y. Liu, S. J. Lee, J.-A. W. Chapman, D. Niedzwiecki, V. J. Suman, J. Moon, V. K. Sondak, M. B. Atkins, E. A. Eisenhauer, W. Parulekar, S. N. Markovic, S. Saxman, J. M. Kirkwood, Meta-Analysis of Phase II Cooperative Group Trials in Metastatic Stage IV Melanoma to Determine Progression-Free and Overall Survival Benchmarks for Future Phase II Trials, *JCO* **26**, 527–534 (2008).
29. P. B. Madrid, S. Chopra, I. D. Manger, L. Gilfillan, T. R. Keepers, A. C. Shurtleff, C. E. Green, L. V. Iyer, H. H. Dilks, R. A. Davey, A. A. Kolokoltsov, R. C. Jr, J. L. Patterson, S. Bavari, R. G. Panchal, T. K. Warren, J. B. Wells, W. H. Moos, R. L. Burke, M. J. Tanga, A Systematic Screen of FDA-Approved Drugs for Inhibitors of Biological Threat Agents, *PLoS One* **8**, e60579 (2013).

30. J. Kouznetsova, W. Sun, C. Martínez-Romero, G. Tawa, P. Shinn, C. Z. Chen, A. Schimmer, P. Sanderson, J. C. McKew, W. Zheng, A. García-Sastre, Identification of 53 compounds that block Ebola virus-like particle entry via a repurposing screen of approved drugs, *Emerg Microbes Infect* **3**, e84 (2014).
31. L. Zilbermintz, W. Leonardi, S.-Y. Jeong, M. Sjodt, R. McComb, C.-L. C. Ho, C. Retterer, D. Gharaibeh, R. Zamani, V. Soloveva, S. Bavari, A. Levitin, J. West, K. A. Bradley, R. T. Clubb, S. N. Cohen, V. Gupta, M. Martchenko, Identification of agents effective against multiple toxins and viruses by host-oriented cell targeting, *Sci Rep* **5**, 13476 (2015).
32. S. L. Bixler, A. J. Duplantier, S. Bavari, Discovering Drugs for the Treatment of Ebola Virus, *Curr Treat Options Infect Dis* **9**, 299–317 (2017).
33. L. Cnops, J. van Griensven, A. N. Honko, D. G. Bausch, A. Sprecher, C. E. Hill, R. Colebunders, J. C. Johnson, A. Griffiths, G. F. Palacios, C. S. Kraft, G. Kobinger, A. Hewlett, D. A. Norwood, P. Sabeti, P. B. Jahrling, P. Formenty, J. H. Kuhn, K. K. Ariën, Essentials of filoviral load quantification, *Lancet Infect Dis* **16**, e134–e138 (2016).
34. D. Moher, A. Liberati, J. Tetzlaff, D. G. Altman, Prisma Group, Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement, *PLoS Med* **6**, e1000097 (2009).
35. R. F. Galbraith, The radial plot: Graphical assessment of spread in ages, *International Journal of Radiation Applications and Instrumentation. Part D. Nuclear Tracks and Radiation Measurements* **17**, 207–214 (1990).

Figure 1. Mortality and cycle threshold by enrollment date.

Figure 2. Control group comparisons. (A) Raw mortality proportions from the six controls studies, (B) Expected mortality as a function of log-CT for a woman of age 34 across studies, (C) Galbraith plot confirm between-study heterogeneity, (D) Standardized mortality proportions (for a female of age 34 across the possible range of CT values).

Figure 3. Standardized odds ratios for treatment effect for (A) amodiaquine, (B) TKM-130803, (C) Prevail II-ZMapp and (D) IFN- β 1a treatment arms relative to each control group. For each standardized odds ratio, a logistic regression model was fit using data from two studies: the row-labelled control group and the experimental arm (indicated at the top of the graph). The odds ratios for the models are reported in the supplementary materials.

Figure 4. Standardized odds ratio for treatment effect for (A) Favi-JIKI, (B) Convalescent Plasma, (C) Favi-Bai, and (D) Convalescent Whole Blood treatment arms relative to each control group. . For each standardized odds ratio, a logistic regression model was fit using data from two studies: the row-labelled control group and the experimental arm (indicated at the top of the graph). The odds ratios for the models are reported in the supplementary materials.

Table 1. Demographic summary of included studies by treatment group

Table 2. Expected study-specific mortality rates for control patients by cycle threshold (CT), adjusting for sex and age based on a logistic regression model for a 34-year old female.

Table 1. Demographic summary of included studies by treatment group

Study	Meta-analysis data set ^{a,b}						Published study results		
	Arm (N)	Enrollment dates	Age Mean (SD)	Females	CT Mean (SD)	Mortality	N	Mortality	IVF use
Prevail II-ZMapp (16)	Ctrl (29)	3/1/2015-11/1/2015	33 (13.1)	41.4%	22.9 (3.7)	31.0%	35	37%	63%
	Trt (31)	3/1/2015-11/1/2015	27.6 (17.5)	58.1%	24.1 (5.2)	19.4%	36	22%	61%
Amodiaquine (17)	Ctrl (169)	6/5/2014-10/24/2014	31.1 (14.8)	48.5%	20.2 (4.1)	62.1%	194	64.4%	32%
	Trt (58)	8/18/2014-8/30/2014	29.5 (18.3)	46.6%	19.9 (4.2)	51.7%	71	50.7%	35.2%
ConvWBlood (18)	Ctrl (11)	12/1/2014-4/30/2015	35.8 (13.0)	36.4%	27.7 (7.2)	54.5%	25	44%	NA
	Trt (20)	12/1/2014-4/30/2015	26.2 (11.5)	60%	25.4 (5.0)	20%	43	28%	100%
ConvPlasma (19)	Ctrl (382)	9/1/2014-1/1/2015	33.2 (15.8)	49.5%	26.4 ^e (4.1)	36.9%	418	38%	NA
	Trt (78)	1/1/2015-7/7/2015	31.6 (14.8)	56.4%	27.9 ^e (4.1)	32.1%	84	31%	NA
Favi-Bai ^c (20)	Ctrl (78)	10/10/2014-10/30/2014	29.6 (15.0)	53.8%	26.0 (4.6)	65.4% ^c	85	64.7%	0%
	Trt (38)	11/1/2014-11/10/2014	31.7 (17.8)	47.4%	27.3 (5.4)	42.1% ^c	39	43.6%	0%
Favi-JIKI (21)	Ctrl (478)	9/15/2014-12/15/2014	36.0 (17.2)	50.6% ^e	20.8 (4.2)	56.9%	540	58%	NA
	Trt (99)	12/17/2014-4/8/2015	37.6 (16.7)	63.6% ^e	21.0 (4.3)	51.5%	99	51.5%	92%

IFN-β1a (22)	Ctrl (NA)	3/26/2015- 6/12/2015	NA	NA	NA	NA	21	81.0%	NA
	Trt (9)	3/26/2015- 6/12/2015	33.9 (14.6)	55.6%	23.5 (4.4)	33.3%	9	33.3%	100%
TKM-130803 ^d (23)	Ctrl (NA)	NA	NA	NA	NA	NA	1820	55%	NA
	Trt (13)	3/11/2015- 6/15/2015	41.7 (18.1)	38.5%	22.8 (3.9)	76.9%	12	75%	100%

a. Patients below 6 years of age were excluded from this meta-analysis. b. Three studies had subjects with missing cycle threshold (CT) values: amodiaquine (n=9), Favi-Bai (n=1) and TKM-130803 (n=1) c. In the published manuscript, primary analyses were reported excluding patients who were transferred out of the Ebola treatment units, leading to 18 control and 17 cases, with a 72% and 35% mortality rate (p=.044), respectively. The data provided for the meta-analysis included all patients, including those transferred d. In the paper, reported mortality rates eliminated deaths within the first 48 hours. However, in the meta-analysis, all deaths were included to line up with the other studies. e. Indicates a statistically significant difference at p<0.05 (Favi-JIKI trial: proportion females; ConvPlasma: CT). IVF: Intravenous Fluid.

Table 2. Expected study-specific mortality rates for control patients by cycle threshold (CT), adjusting for sex and age based on a logistic regression model for a 34-year old female.

	Prevail II-ZMapp (16)	Amodiaquine (17)	ConvWBlood (18)	ConvPlasma (19)	Favi-Bai (20)	Favi-JIKI (21)
CT 20	0.47 (0.23,0.72)	0.55 (0.37,0.71)	0.83 (0.31,0.98)	0.61 (0.49,0.71)	0.74 (0.54,0.87)	0.57 (0.5,0.64)
CT 25	0.06 (0.01,0.28)	0.15 (0.04,0.45)	0.55 (0.22,0.84)	0.35 (0.29,0.42)	0.66 (0.54,0.76)	0.18 (0.13,0.24)
CT 30	0.01 (0,0.17)	0.04 (0,0.29)	0.29 (0.06,0.7)	0.19 (0.14,0.25)	0.59 (0.43,0.73)	0.05 (0.03,0.08)

Acknowledgments

We would like to thank the reviewers for excellent comments and suggestions. Michael Piziali and Wade Green (Technology Transfer and Intellectual Property Office, National Institute of Allergy and Infectious Diseases, National Institutes of Health) for assistance with establishing data sharing agreements. We are also grateful to Professor Eleanor Fish (Department of Immunology, University of Toronto, Canada) to have shared online all the data from the IFN- β 1a trial. We are also thankful to Judith Welsh (Clinical Informationist, NIH Library, Office of Research Services, National Institutes of Health) for her help with the extensive bibliography research.

Funding: None.

Author contributions

LED and AGC designed the study. LED, DM, JG, IC, PH, RA, JFJ, RTD provided patient data. LED, DF, MP, JW performed the analysis. LED, DF, MP, AGC drafted the manuscript. DM, JG, IC, PH, RA, JFJ, RTD, HCL reviewed the manuscript.

Competing interests: None.

Data were made available under multiple data sharing agreements between NIAID and the following institutions:

- [MSF \(Geneva, Switzerland\) \(17\),](#)
- [Institute of Tropical Medicine, Clinical Sciences, Antwerp, Belgium \(19\)](#)
- [Beijing Institute of Microbiology and Epidemiology, Beijing, China \(20\)](#)
- [INSERM, Paris, France \(21\)](#)
- [The Infectious Diseases Data Observatory, University of Oxford, Oxford, UK \(23\)](#)

We are grateful to Professor Eleanor Fish (Department of Immunology, University of Toronto, Canada) to have shared online all the data from the IFN- β 1a trial (22) and to Dr Ansumana (Mercy Hospital Research Laboratory, Kulanda Town, Bo, Sierra Leone) for the data of the convalescent whole-blood study that were also available on their paper (18). Data from the Prevail II- ZMapp trial was under the possession of NIAID and of the first author of this meta-analysis (15).