Alternative Endpoints and Analysis Techniques in Kidney Transplant Trials

Nicholas Anthony Fergusson

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements for the M.Sc. degree in Epidemiology

School of Epidemiology, Public Health, and Preventive Medicine
Faculty of Medicine
University of Ottawa

SYNOPSIS

Clinical trials in kidney transplantation suffer from several major issues including: 1) Unfeasibility due to low short-term event rates of hard outcomes and 2) Reliance on a composite outcome that consists of unequal endpoints that may generate misleading results. This thesis attempts to explore and apply methods to solve these issues and ultimately, improve kidney transplantation trials.

We present a secondary analysis of the ACE trial in kidney transplant using composites with alternative graft function surrogate endpoints. Typically, kidney transplant trials— including the ACE trial— use a time-to-event composite of death, end-stage renal disease (ESRD), and doubling of serum creatinine. Instead of doubling of serum creatinine, we investigated the use of percentage declines of estimate glomerular filtration rate (eGFR) within a time-to-event composite of death and ESRD. Additionally, we present an application of an innovative analysis method, the win ratio approach, to the ACE trial as a way of lessening concerns associated with unequal composite endpoints.

Composites of death, ESRD, and either a ≥40%, ≥30% or ≥20% decline in eGFR did not alter original ACE trial results, interpretations, or conclusions. The win ratio approach, and the presentation of a win ratio, generated very comparable results to a standard time-to-event analysis while lessening the impact of unequal composite endpoints and making fewer statistical assumptions. This research provides a novel, trial-level application of alternative endpoints and analysis techniques within a kidney transplant trial setting.

TABLE OF CONTENTS

List of Tables

List of Figures

ACKNOWLEDGMENTS

This project would not have been possible without the support of my numerous colleagues, friends, and family:

To my supervisor Dr. Greg Knoll, for his unrelenting support, mentorship, and guidance throughout this thesis and in my previous years as an undergraduate summer student. You opened a door for me, and for that, I am forever grateful.

To my co-supervisor, Dr. Tim Ramsay, for his tremendous mentorship, guidance, and effortless ability to teach and explain statistics. Your office was always open for my numerous drop-ins and for that, I am deeply thankful.

To my thesis advisory committee members, Drs. Michaël Chassé and Shane W. English, for your constant assistance and invaluable guidance throughout this project. I am fortunate to have such a great team behind me.

To my fellow classmates, not many people can say they made lifelong friends during their Master's—I can.

To my family, your support has been unconditional, your encouragement—overwhelming, and I owe so much to you. To my partner Shannon, your love makes even the most difficult seem easy. All of this work is for you.

AUTHOR CONTRIBUTIONS

The foundation and topic of this thesis arose during discussions between Dr. Greg Knoll and myself in February 2016 following work on a systematic review evaluating the effectiveness of renin angiotensin system (RAS) blockade in kidney transplantation.

Manuscript one (Chapter 3)

Nicholas A. Fergusson is the principal author and Dr. Greg A. Knoll is the senior author (primary thesis supervisor) of this manuscript. Dr. Tim Ramsay (thesis co-supervisor) contributed to study design, both statistical analysis and interpretation, and drafting of the final manuscript. Dr. Michaël Chassé (thesis advisory committee) provided assistance and advice regarding trial data logistics, statistical coding, clinical advice, and drafting of the final manuscript. Dr. Shane English (thesis advisory committee) provided clinical advice and aided in the drafting of final manuscript.

Manuscript two (Chapter 4)

Nicholas A. Fergusson is the principal author and Dr. Greg A. Knoll is the senior author (primary thesis supervisor) of this manuscript to be submitted for publication at The Journal of Clinical Epidemiology. Dr. Tim Ramsay (thesis co-supervisor) contributed to study design, both statistical analysis and interpretation, and drafting of the final manuscript. Dr. Michaël Chassé (thesis advisory committee) provided assistance and advice regarding trial data logistics, statistical coding, clinical advice and drafting of the

final manuscript. Dr. Shane W. English (thesis advisory committee) provided clinical advice and aided in the drafting of final manuscript.

Nicholas A. Fergusson was involved in all aspects of both manuscripts in their entirety. This thesis project exclusively entailed the secondary use of anonymized data originating from a closed trial (ISRCTN 78129473) and consequently, approval from the Ottawa Health Science Research Ethics Board (OHSREB) was not needed as per section 5.2.3 of the standard operating procedures (SOP). Baseline, lab, and outcome data from the trial was provided by Dr. Michaël Chassé. Drs. Knoll and Ramsay supervised all stages of this thesis in its entirety and provided invaluable advice and support regarding design, analysis, and discussion for both manuscripts.

STATEMENT OF ORIGINALITY

I, Nicholas A. Fergusson, was heavily and directly involved in all aspects of this thesis and I present this thesis as original work conducted as partial requirement for the M.Sc. degree in Epidemiology.

FUNDING

It should be acknowledged that this thesis was made possible by funding from:

1) The Frederick Banting and Charles Best Canada Graduate Scholarship awarded by the Canadian Institutes of Health Research (CIHR). This non-renewable scholarship awarded $17,500 for 12 months (May 1st, 2016 to May 1st 2017).

2) A part-time research assistantship at the Ottawa Hospital Research Institute (OHRI) within Dr. Gregory A. Knoll's clinical research team.

CHAPTER 1: INTRODUCTION AND OVERVIEW

Problems

Trial Feasibility & Endpoints

Randomized controlled trials (RCTs) designed to assess the efficacy of interventions on hard outcomes in the kidney transplantation population are often unfeasible (1). During the first 1-3 years post-transplantation there is a lack of hard events, such as death and end-stage renal disease (ESRD) (1–5). However, long-term results in kidney transplantation remain suboptimal with death and graft failure often occurring late (6). Since most interventions in kidney transplantation are administered during the first several post-transplant years, the majority of RCTs are conducted during this phase as well (1–5). Due to the low short-term event rate, a trial using hard outcomes of death and/or ESRD would require a substantial sample size and extensive follow-up (1–5) —both immense challenges to trial feasibility. This difficulty in conducting kidney transplant trials represents a significant barrier to defining, changing, and improving clinical kidney transplant practice.

When kidney transplant trials are conducted, they usually rely on a composite of death, ESRD, and graft function decline (e.g. doubling of serum creatinine) (1–5). Endpoints of graft function decline act as a surrogate endpoint for harder outcomes and they are used to increase the short-term event rate and improve trial feasibility (1–5). Historically, a doubling of serum creatinine is used as a surrogate for decline in graft function but

recently, percentage declines in estimated glomerular filtration rate (eGFR) have been validated in both the chronic kidney disease (CKD)(2) and the kidney transplant population (1). There has been a significant push to adopt alternative surrogate endpoints in kidney transplant trials in order to increase trial feasibility by increasing the number of outcome events. Yet, information regarding the trial-level impact of these endpoints is lacking.

Trial Interpretation

As mentioned, kidney transplant trials often rely on a composite of death, ESRD, and a measure of graft function decline (e.g. doubling of serum creatinine). This time to first occurrence (time-to-event) composite aims to increase the trial event rate and ideally the trial's statistical power. However, this composite also contains endpoints that are unequal in terms of clinical significance; death is a much more clinically impactful event than a decline in kidney function. Composites that possess endpoints of varying clinical significance, particularly composites that are predominantly powered by the endpoint of least clinical significance, can lead to potentially misleading and faulty trial interpretations (7–9). There is a need to improve the way kidney transplant trials are analyzed, and presented, or else we risk misinforming clinical practice.

Purpose and Rationale

The purpose of this thesis project is to investigate the application of alternative endpoints and analysis techniques in kidney transplant trials with the overarching theme of improving kidney transplant trials.

There is a need for practical, trial-level research on the usefulness and impact of using alternative endpoints, specifically percentage declines in eGFR. To the best of our knowledge there has not been a trial-level application and assessment of these endpoints within kidney transplantation. Further, since these trials use a composite of unequal endpoints, there is a need to improve the way kidney transplant trials are analyzed and presented. There is a demand for not only more, but better, trials in the field of kidney transplantation. An assessment of methods to improve the feasibility and interpretability of such trials is warranted.

Objectives

There are two objectives of this thesis, each examined in the first and second manuscripts respectively (Chapters 3 & 4).

1) Determine the impact of using alternative surrogate endpoints, specifically eGFR percentage declines of ≥40%, ≥30%, and ≥20%, and investigate whether they alter the results, interpretations, or conclusions of the ACE trial (Chapter 3).

2) Determine the impact of using an alternative analysis technique, the win ratio approach, and investigate whether this approach alters the results, interpretations, or conclusions of the ACE trial (Chapter 4).

Overview of Submitted Thesis & Manuscripts

This thesis aims to provide highly practical, trial-level information concerning the impact of using alternative surrogate endpoints and analysis techniques in a kidney transplant trial. These aims were met in the following research stages:

In the first stage, a solid foundation of current evidence was constructed. "Chapter 2: Background" situates the reader by clarifying the current environment of clinical trials in kidney transplantation, graft function decline surrogate endpoints, the win ratio approach for unequal composite endpoints and lastly, the ACE trial.

The second stage, "Chapter 3: Alternative Endpoints", introduces and presents a secondary analysis of the ACE trial using alternative graft function endpoints. A brief section introduces and summarizes the manuscript, "*The impact of using alternative graft function endpoints in kidney transplantation trials: A secondary analysis of the ACE trial*". Supporting appendices are provided.

The third stage, "Chapter 4: Alternative Analysis Techniques", introduces and presents an application of the win ratio approach to the primary outcome of the ACE trial. A brief section introduces and summarizes the manuscript submitted for publication, "*The win ratio approach for unequal composite endpoints: An application to the ACE trial in kidney transplantation*". Supporting appendices are provided. This manuscript was submitted to The Journal of Clinical Epidemiology.

The final stage, "Chapter 5: Discussion", summarizes the key findings of the thesis and reviews the implications and impact of these findings. In addition, strengths, limitations, dissemination, and future directions of this thesis project are discussed.

CHAPTER 2: BACKGROUND

Clinical Trials in Kidney Transplantation

Due to feasibility concerns, conducting trials within the kidney disease, and more specifically the kidney transplant population, is difficult. Consequently, nephrology trials only represent 2.6% of all interventional studies registered to ClinicalTrials.gov between 2007-2010, with only a fraction of those being kidney transplant trials (10). The lack of trials in kidney transplantation, presents a barrier in informing, improving, and defining clinical practice for kidney transplant recipients.

Composite Outcomes

One of the major reasons why kidney transplant trials are often unfeasible involves the scarcity of short-term hard outcomes (1–5). Hard outcomes, such as death and ESRD, are predominantly late events that occur after the first 1-3 years post-transplant (1–5). Unfortunately, the majority of interventions are given within these first several post-transplant years and consequently the majority of trials only assess this short-term period (1). In order to increase the number of events occurring, trials commonly use a time-to-event composite of death, end-stage renal disease (ESRD), and graft function decline. Death and ESRD serve as the hard outcomes, while graft function decline acts as a surrogate for these hard outcomes. Ideally, a time-to-event composite should be balanced

with respect to endpoint occurrence and endpoint clinical significance (7,8,11). In other words, events should be spread out between all component endpoints of the composite and each event should hold a similar clinical significance. For the composite of death, ESRD, and kidney function decline, there are concerns with both endpoint occurrence and endpoint clinical significance (4); concerns that are explored and addressed in this thesis project.

Graft Function Decline Surrogate Endpoints

Historically in kidney transplant trials, a doubling of serum creatinine is used as the graft function decline endpoint in a composite that also includes death and ESRD (1,2,4). Serum creatinine is a frequently used indirect estimate of kidney function (12–14). As a metabolic by-product, creatinine is produced by the body at an approximately constant rate and is excreted almost entirely by the kidneys (12–14). Thus, the concentration of serum creatinine can provide an indirect measure of kidney function with an increase of serum creatinine concentration (i.e. not being excreted) indicating a decline in kidney function. Serum creatinine can be integrated into a formula that incorporates, and adjusts for age, sex, and race to provide another measure of kidney function decline known as estimated glomerular filtration rate (eGFR) (12–16). Estimated glomerular filtration rate (eGFR) refers to the estimated flow rate through the glomerular capillaries of the kidneys and a decrease in eGFR indicates a decline in kidney function (14). A doubling of serum creatinine corresponds to roughly a 57% or greater decline in eGFR (3,4). A doubling of serum

creatinine is strongly and consistently predictive of future events of ESRD and death and therefore, it is regarded as a good surrogate endpoint for such hard outcomes (1–4). Recently, lesser kidney function decline endpoints such as ≥40% and ≥30% declines in eGFR have been explored and validated as surrogate endpoints in the chronic kidney disease (CKD) population (2). The impact of these endpoints were then assessed within two large CKD trials where they were associated with not only an increase in event rate but also a reduction in treatment effect— potentially negating any improvement in trial statistical power (17). Recently, the use of lesser eGFR percentage declines as surrogate endpoints has been validated in the kidney transplant population using the Australian and New Zealand Dialysis and Transplant Registry (1). The investigators recommended eGFR declines of ≥40% and/or ≥30% be utilized as surrogate endpoints in kidney transplant trial composites as they were strongly associated with graft failure and death while occurring more frequently than doubling of serum creatinine events (1). However, the impact of these lesser eGFR decline surrogate endpoints on kidney transplant trial results, interpretations, and conclusions has not been investigated.

Unequal Endpoints & the Win Ratio Approach

Irrespective of which graft function decline endpoint is utilized, an underlying issue with these composites is that the components are not equal with respect to clinical significance. In a time-to-event composite of death, ESRD, and kidney function decline, only the first occurrence of any event (whether death or ESRD or kidney function decline) is used in the analysis. If the composite is driven predominantly by endpoints of lower clinical significance, results can be misleading (7–9,11). For example, let's say we have patient A, who experiences a kidney function decline event (ie. doubling of serum creatinine) at 3 months' follow-up but otherwise goes event free for the remainder of the trial (48 months) and patient B, who experiences ESRD at 12 months follow-up and then dies a few months later. In a time-to-event analysis, patient B fares better as she went event-free for 12 months, whereas patient A (who is still alive at trial end) experienced an event at 3 months. If the endpoint of lesser clinical significance begins to alter or mask treatment effects on more clinically significant endpoints within the composite it may provide misleading and sometimes faulty conclusions.

The win ratio approach lessens the concern of unequal endpoints by ranking component endpoints on clinical significance and then assessing the impact of treatment sequentially by these endpoints (7). The initial concept was discussed by Buyse (18), while the full approach was proposed by Pocock et al. (7) where the authors describe both a matched and unmatched approach. The unmatched win ratio approach involves pairing each study

group participant with every other control group participant (e.g. if study group n=100 and control group n=100 we would create 100 x 100 = 10,000 pairs). Initially, each pair is assessed on the endpoint of highest clinical significance to determine if either the study group pair member or control group pair member is the 'winner'. In the situation where both pair members experienced the event, the winner is the member with the longest event-free survival time. In the situation where only one pair member has experienced an event, the winner is the event-free member but, only if his follow-up extends past that of the other pair member who has experienced an event (i.e. the win must be 'confirmed'). If follow-up does not extend past the event, or if no event occurred in either pair member the pair is labelled a 'tie' or uninformative for this event. Only these pairs are then assessed for the next most clinically significant event in the exact same manner.

At the conclusion, a win ratio, defined as the total number of pairs won by the study group divided by the total number of pairs won by the control group, is determined. The win ratio can be thought as conceptually similar to an odds ratio or a hazard ratio, as a win ratio greater than 1 indicates an increase in wins for the study group compared to the control group. An odds ratio or a hazard ratio greater than 1 indicates an increase in either the event odds or the hazard, for the study group compared to the control group. Of course, as with an odds ratio or a hazard ratio, the win ratio can be flipped (control vs. study) to provide an inverse win ratio where a value greater than 1 indicates a negative treatment effect. Further, the nonparametric unmatched win ratio approach does not rely on unrealistic statistical assumptions such as proportional hazards, unlike standard time-to-

event analyses (19,20). By ranking and sequentially assessing endpoints by decreasing severity, the win ratio approach lessens concerns of unequal endpoints while not relying on questionable statistical assumptions.

The Canadian ACE-inhibitor Trial

This analysis utilized data from the Canadian ACE-inhibitor in kidney transplant trial, henceforth—the ACE trial (21). The ACE trial was a randomized, double blind, placebo-controlled trial conducted at 14 centers in Canada and New Zealand. The trial's primary objective was to determine if ramipril treatment, a common angiotensin converting enzyme (ACE) inhibitor, would have a beneficial impact on important clinical outcomes in kidney transplant recipients with proteinuria.

Clinical Context & ACE Trial Rationale

In patients with diabetes and chronic kidney disease (CKD), particularly those with proteinuria, the use of ACE inhibitors has been a cornerstone of antihypertensive management as they reduce the risk of clinically significant events (22). However, in the kidney transplant population strong evidence for the use of ACE-inhibitors is lacking. This lack of evidence provided the rationale for the ACE trial. Even after completion of the trial there remains uncertainty concerning the use of ACE-inhibitors in the renal transplant population. A recent systematic review and meta-analysis of 8 RCTs (including the ACE

trial) concluded that the current evidence neither supports nor refutes the use of renin-angiotensin system blockade (either ACE-inhibitors or angiotensin receptor blockers [ARBs]) in the kidney transplant population (22).

Trial Characteristics

From August 23rd, 2006, to March 28th, 2012, a total of 213 kidney transplant recipients were randomized. 109 patients were allocated to placebo while 104 were allocated to ramipril treatment. Of these, 109 patients in the placebo group and 103 patients in the ramipril group were analyzed. All patients that completed the final 4-year study visit were invited to participate in a non-prespecified trial extension phase. During this extension phase (henceforth referred to as the 'extended trial') masking was maintained and patients could be followed for a maximum of 48 additional months. As a result, the primary ACE trial has a mean follow-up of 41 months while the extended ACE trial has a mean follow-up of 48 months. Further trial details including complete inclusion/exclusion criteria can be found in the published trial protocol (23) and the primary study publication (21) which is included in Appendix 1- Additional File 1.

Intervention

Eligible patients entered a short 2-week open-label trial of 5mg ramipril daily. If tolerated, patients were randomized (1:1) to either receive ramipril or placebo treatment for up to 4 years (oral 5 mg daily for 2 weeks and then 5 mg oral twice daily thereafter).

Trial Primary Outcome

The primary outcome of the trial was a time-to-event composite consisting of death, end-stage renal disease (ESRD) and doubling of serum creatinine. ESRD was defined as the date of either repeat kidney transplantation or initiation of dialysis. Doubling of serum creatinine was defined as a two-fold increase in serum creatinine (umol/L) relative to baseline and was confirmed by two consecutive tests at least 4 weeks apart by a central laboratory. Serum creatinine was measured at randomization, 1 month, 6 months, and every 6 months until trial end. All outcomes in the composite were measured as time to first occurrence endpoints and only the first event was used in the primary composite outcome. However, overall occurrences of each composite component were recorded and were reported individually.

Trial Results

No significant differences between the two groups were found in the primary composite outcome. During the initial trial period (mean 41 months follow-up) the primary outcome occurred in 19 (17%) of 109 patients in the placebo group and 14 (15%) of 103 patients in the ramipril group (hazard ratio [HR] 0.76; 95% CI, 0.38-1.51). With extended trial follow-up (mean 48 months) the primary outcome occurred in 27 (25%) in the placebo group and 25 (24%) patients in the ramipril group (HR, 0.96; 95% CI, 0.55-1.65). The intention-to-treat population was used to assess outcomes in both the primary trial and extended trial analyses. Contrary to what was previously assumed regarding ACE-inhibitor treatment in kidney transplantation, the results of the ACE trial did not support the use of ACE-inhibitors with the goal of improving clinical outcomes in kidney transplantation.

CHAPTER 3: ALTERNATIVE ENDPOINTS- MANUSCRIPT 1

Preface to Manuscript 1

In this paper, we present the results of a secondary analysis of the ACE trial using alternative graft function endpoints. The original primary composite of death, ESRD, and doubling of serum creatinine was compared to three composites that included death, ESRD, and either a ≥40%, ≥30%, or ≥20% decline in eGFR. The objective of this study was to explore the statistical impact of using alternative surrogate endpoints, specifically eGFR percentage declines of ≥40%, ≥30%, and ≥20%, and investigate whether they alter the results, interpretations, or conclusions of the ACE trial

We found that lower eGFR percentage decline thresholds resulted in a substantial increase in the number of composite events. Within the ACE trial, these lesser eGFR declines would have increased statistical power but simply because no apparent treatment effect was ever observed. The use of these alternative endpoints should be determined with respect to the intervention in question and its hypothesized treatment effect. Interestingly, regardless of whether a ≥40%, ≥30%, or ≥20% decline in eGFR was used in the composite, the trial's original clinical interpretation regarding the efficacy ACE-inhibitor treatment in kidney transplant recipients remained identical. In all composites assessed, ramipril treatment was not associated with any statistically significant differences compared to placebo.

Manuscript 1

The impact of using alternative graft function endpoints in kidney transplantation trials: A secondary analysis of the ACE trial

Nicholas A. Fergusson, BSc,[1,2] Tim Ramsay, PhD,[1,2] Michaël Chassé, MD,[3,4]

Shane W. English MD[2,5] and Greg A. Knoll, MD[1,2,5]

[1] School of Epidemiology & Public Health, University of Ottawa, Ottawa, Canada
[2] Clinical Epidemiology Program, Ottawa Hospital Research Institute, Ottawa, Canada
[3] Department of Medicine, Université de Montréal, Montréal, Canada
[4] Centre de recherche du Centre Hospitalier Universitaire de Montréal, Montréal, Canada
[5] Department of Medicine, University of Ottawa, Ottawa, Canada

Abstract

Background: Kidney transplant trials assessing the impact of interventions on end-stage renal disease (ESRD) and/or death are rarely feasible, as these are often long term outcomes. The objective of this study was to determine if using alternative eGFR percentage declines would alter results, interpretations, or conclusions of the ACE trial.

Methods: This study was a secondary analysis of the ACE trial comparing the use of a ≥40%, ≥30% or ≥20% decline in eGFR versus a doubling of serum creatinine, in a time-to-event composite of death and ESRD. Declines in eGFR were determined relative to baseline and were calculated using the CKD-EPI equation. The ACE trial enrolled 212 kidney transplant patients with proteinuria and assessed the clinical impact of ramipril treatment, a common angiotensin converting enzyme (ACE) inhibitor, versus placebo on a composite of death, ESRD, and doubling of serum creatinine.

Results: In the primary trial (mean follow-up 41 months), a composite of death, ESRD, and either a ≥40%, ≥30%, or ≥20% eGFR decline occurred in 45 (26 placebo vs. 19 ramipril), 68 (35 vs. 33), and 99 (50 vs. 49) patients respectively. Substituting these eGFR declines for doubling of serum creatinine resulted in an increase of 12, 35, and 66 composite events. In the extended trial (mean follow-up 48 months) results were similar. In all composites, ramipril treatment was not associated with any statistically significant difference in both the primary and extended trial.

Conclusions: Substituting a doubling of serum creatinine endpoint for alternative eGFR percentage declines in a composite of death and ESRD did increase the number of outcomes however, trial results, interpretations, or conclusions were not altered.

Word Count: 3784

Keywords: Kidney end point; eGFR decline; composite; kidney transplant; clinical trial; ACE-inhibitor

BACKGROUND

Clinical trials in kidney transplantation are challenged by low event rates of hard outcomes, such as death and end-stage renal disease (ESRD), particularly within the first 1-3 years post transplantation (1–5). In order to achieve statistical power and provide clinical insight, kidney transplant trials assessing the impact of an intervention on either death or ESRD require a substantial sample size and/or extended follow-up; both threats to feasibility (1–5). Although these hard, substantial events are uncommon in the first 1-3 years post-transplant the long-term clinical results continue to be suboptimal among kidney transplant patients (6) and further work to improve long-term results remains essential. To increase short-term event rate and improve trial feasibility, an endpoint of doubling of baseline serum creatinine is often incorporated into a time-to-event composite outcome that also includes death and ESRD (1–5). Doubling of serum creatinine is a marker of kidney function decline and has been shown to be an effective surrogate for both ESRD and death (1,2,7).

However, utilizing an endpoint of doubling of serum creatinine as a composite component has not solved the problem; issues of low short-term event rates and consequently, efficiency, continue to persist (8). To increase event rates and enable clinical trials of shorter duration, the use of a percentage reduction in estimated glomerular filtration rate (eGFR) as an alternative surrogate for hard outcomes was recently explored (1). In a large, multinational database of chronic kidney disease (CKD) patients, an endpoint of ≥30% decline eGFR over two years was considerably more frequent but also strongly predictive of

long-term graft failure and death in the CKD population (1). The validity of using percentage eGFR declines as surrogate endpoints in clinical trials was further validated in the kidney transplant population (2). The investigators recommended that percentage decline in eGFR should be considered for use as a surrogate endpoint in kidney transplant trials (2). To investigate the impact of such recommendations, we conducted a secondary analysis of the ACE trial (9) by incorporating endpoints of eGFR percentage decline into a composite also including death and ESRD, in order to determine their impact on trial results, interpretations, and conclusions.

METHODS

Study Design and Patients

We conducted a secondary analysis of the ACE trial (9), a double-blind, placebo-controlled, randomized trial, conducted at 14 centers in Canada and New Zealand (ISRCTN, number 78129473). Between August 23rd, 2006, and March 28th, 2012, a total of 213 high-risk kidney transplant recipients with proteinuria were enrolled. In the primary trial, 212 patients were analysed with 109 allocated to placebo and 103 allocated to ramipril. In the extended trial, all patients who completed the final 48-month study visit of the primary trial were offered to continue treatment and follow-up for up to an additional 48 months. At this point, 43 (80%) of 54 eligible patients in the placebo group and 38 (78%) of 49 eligible patients in the ramipril group participated. The extension phase of the trial was not pre-specified. Inclusion criteria specified adult renal transplant recipients who were at least 6 months post-transplant with an eGFR between 20 mL/min/1.73m^2 and 55 mL/min/1.73m^2

(calculated using the MDRD equation) and proteinuria of 0.2 g/day or greater. A protocol amendment after 58 participants were randomized included the change to enroll recipients who were at least 3 months post-transplant with an eGFR of 20 mL/min/1.73m^2 or greater. The study was approved by the local research ethics board at every participating institution and all trial participants provided written informed consent. Clinical coordination, data management, and statistical analyses for the trial were performed by the Ottawa Methods Centre at the Ottawa Hospital Research Institute (Ottawa, ON, Canada). Details of the trial protocol (10) and the primary study results (9) have been published previously.

Study Treatment

Eligible patients entered a short 2-week open-label trial of 5mg ramipril daily. If tolerated, patients were randomized (1:1) to either receive ramipril or placebo treatment for up to 4 years (oral 5 mg daily for 2 weeks and then 5 mg oral twice daily thereafter).

Outcome Assessments & Evaluation

Study visits occurred at randomization, 1 month, 6 months and then every 6 months thereafter until the duration of the trial. At each study visit, haemoglobin, serum creatinine and serum potassium concentrations were measured by a central laboratory and any event of doubling of serum creatinine, end-stage renal disease (ESRD) or death (the primary composite outcome) was determined. Additionally, after the initial visit, measured GFR was evaluated with radiolabeled ^{99m}tehcnetium-DTPA (^{99m}Tc-DTPA), 24-h urine protein and quality of life (using short-form 36) was measured. All patients that completed the

final 4-year study visit were invited to participate in a non-prespecified trial extension phase. During this extension phase, masking was maintained and patients could be followed for a maximum of 48 additional months. Study assessments were performed at 6-month intervals to determine if any event of doubling of serum creatinine, ESRD or death had occurred. Serum creatinine values during the extension phase were obtained from trial participant medical records. Laboratory testing and measured GFR calculation were not performed during this phase. Baseline data for each trial participant included age, gender, ethnic origin (White, Black, Asian or other), proteinuria (mg/day), type of donor (living or deceased), measured GFR (mL/min/1.73m^2), serum creatinine (umol/L), diabetes status (present or absent) and time post-transplantation (days).

Trial Primary Outcome

The primary outcome of the trial was a time-to-event composite consisting of death, ESRD, and doubling of serum creatinine. ESRD was defined as the date of repeat kidney transplantation or initiation of dialysis. Doubling of serum creatinine was defined as a two-fold increase in serum creatinine (μmol/L) relative to baseline and was confirmed by two consecutive tests at least 4 weeks apart by a central laboratory. All outcomes in the composite were measured as time to first occurrence of any component of the composite. However, overall total events of each composite component were recorded and were reported as individual components.

Secondary Analysis Outcomes

In this secondary analysis, in place of doubling of serum creatinine, we incorporated eGFR percentage declines of either ≥40%, ≥30%, or ≥20% into a time-to-event composite of death and ESRD. Doubling of serum creatinine corresponds to roughly a 57% decline in eGFR (3). For the primary trial, eGFR was calculated using serum creatinine values determined from our central laboratory or, in the situation where lab values were unavailable, patient charts. For the trial extension phase, eGFR was calculated using serum creatinine values from participant medical records. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (11) was used to calculate all eGFR values. Events of ≥40%, ≥30%, or ≥20% eGFR declines were determined relative to baseline eGFR and only the first occurrence was recorded as an event. All endpoints (death, ESRD or eGFR percentage decline) in each composite were measured as time-to-event and only the first event was used in the composite outcome. Overall total events of each composite component were recorded and were reported as individual components. In a sensitivity analysis, the 4-variable Modification of Diet in Renal Disease (MDRD) equation (12) was used to calculate eGFR values (Appendix; Table 4 & 5).

Statistical Analysis

The primary trial analysis included data up to 48-months post-randomization while the extended trial included all primary phase data and supplementary extended follow-up data to a maximum of 84 months. All analyses were conducted in accordance with the intention-to-treat principle. Kaplan-Meier plots assessing each time-to-event composite

over the trial period were constructed while non-parametric log-rank tests were performed as significance tests. Cox proportional hazards regression models were used to adjust for important risk factors including: age (years), diabetes (present or absent), time from transplantation (days), measured GFR (mL/min/1.73m^2), donor type (living or deceased), proteinuria (mg/24 h), and serum creatinine (umol/L). Unadjusted and adjusted Cox proportional hazard ratios along with corresponding 95% confidence intervals were created to compare outcomes between study groups. Further, 2-sided $P<0.05$ was considered to indicate statistical significance. All analyses were conducted in R statistical software version 3.3.2 (13).

RESULTS

A total of 212 patients were analyzed and both groups were well balanced at baseline (Table 1). Mean follow-up was 41 months (range 1-48) in the primary trial and 48 months (range 1-84) in the extended trial.

A composite consisting of death, ESRD, and either a ≥40%, ≥30%, or ≥20% eGFR decline occurred in a total of 45 (26 placebo vs. 19 ramipril), 68 (35 vs. 33), and 99 (50 vs. 49) patients respectively. Ramipril treatment was not associated with a statistically significant hazard ratio (HR) in any these time-to-event composites (Table 2). The time to occurrence of each composite did not differ significantly between groups (Fig. 1).

In the extended trial, a composite consisting of death, ESRD, and either a ≥40%, ≥30%, or ≥20% eGFR decline occurred in a total of 61 (33 placebo vs. 28 ramipril), 82 (42 vs. 40), and 111 (58 vs. 53) patients respectively. Ramipril treatment was not associated with a statistically significant hazard ratio (HR) in any these time-to-event composites (Table 2). The time to occurrence of each composite did not differ significantly between groups (Fig. 2). After adjustment, the effects of ramipril on each composite in both the primary trial and extended trial were comparable to the unadjusted hazards (Appendix; Table 6).

Regarding graft function endpoints solely (without inclusion in a composite), a total of 14 participants (9 placebo vs. 5 ramipril) experienced a doubling of serum creatinine (Table 3). An eGFR decline of ≥40%, ≥30%, or ≥20% occurred in a total of 28 (17 placebo vs. 11 ramipril), 57 (30 vs. 27), and 90 (46 vs. 44) respectively (Table 3). Ramipril treatment was not associated with a statistically significant hazard ratio (HR) in any these graft function outcomes (Table 3).

In the extended trial, graft function endpoints (without inclusion in a composite), a total of 25 participants (12 placebo vs. 13 ramipril) experienced a doubling of serum creatinine (Table 3). An eGFR decline of ≥40%, ≥30%, or ≥20% occurred in a total of 38 (19 placebo vs. 19 ramipril), 65 (33 vs. 32), and 98 (50 vs. 48) respectively (Table 3). With extended follow-up, ramipril treatment was not associated with a statistically significant hazard ratio (HR) in any of these graft function outcomes (Table 3). After adjustment, the effects of ramipril

on each graft function surrogate endpoint in both the primary trial and extended trial were comparable to the unadjusted hazards (Appendix; Table 7).

There were 14 deaths (5 placebo vs. 9 ramipril) in the primary trial. The unadjusted HR for death was 1.97 (95% CI, 0.66-5.89). Further, 15 patients experienced ESRD (9 placebo vs. 6 ramipril) yielding an unadjusted HR of 0.67 (95% CI, 0.24-2.90). In the extended trial, there were 25 total deaths (11 placebo vs. 14 ramipril) resulting in an unadjusted HR of 1.45 (95% CI, 0.66-3.21). An overall total of 23 patients experienced ESRD (12 placebo vs. 11 ramipril) with an unadjusted HR of 0.94 (95% CI, 0.41-2.13).

DISCUSSION

We found that a time-to-event composite of death, ESRD, and percentage eGFR declines (either ≥40%, ≥30%, or ≥20%) did not lead to any significant change from the original conclusions of the primary trial or the extended trial. In each time-to-event composite, no statistically significant differences between the ramipril and placebo groups could be identified. Further, no statistically significant differences were observed between the ramipril and placebo groups when eGFR declines were assessed on their own (without inclusion in a composite).

The effectiveness of percentage eGFR decline endpoints as surrogates for ESRD and death has been demonstrated in chronic kidney disease (CKD) patients using the large, multi-national Chronic Kidney Disease Prognosis Consortium dataset (1). A National Kidney

Foundation (NKF) and Federal Drug Administration (FDA) Scientific Workshop concluded that a ≥40% eGFR decline over 2-3 years was broadly acceptable as a substitute for doubling of serum creatinine in CKD progression trials, while a ≥30% eGFR decline may be acceptable in certain cases (4). Stemming from this workshop, *Badve et al.* conducted a review and meta-analysis and concluded that the decision to use a ≥30% or ≥40% eGFR decline as a surrogate endpoint in CKD progression trials should be determined on a trial-by-trial basis depending on the intervention being assessed and it's hypothesized impact on eGFR percentage decline (4). However, these appraisals involved the CKD population, not the kidney transplant population. *Clayton et al*. (2) has recently investigated the relationship between eGFR percentage declines and hard outcomes, like death and ESRD, in 7949 kidney transplants from the Australia and New Zealand Dialysis and Transplant Registry. They determined that both ≥30% and ≥40% eGFR declines not only occurred more frequently than doublings of serum creatinine, but were still strongly associated with both subsequent death and ESRD (2). A doubling of serum creatinine corresponds to a ~57% decrease in eGFR and thus, as the threshold is lowered, we should expect to see an increase in both the number and rate of events (3). However, although these eGFR percentage endpoints will likely increase event rate in kidney transplant trials, evidence of their trial-level impact on statistical power and trial feasibility within the kidney transplant setting is lacking (2). A post-hoc analysis of the RENAAL and IDNT trials in ESRD found that these lesser eGFR decline endpoints do increase the number of events but, they also attenuate treatment effect (8). If the impact of treatment on these lesser eGFR decline

endpoints is diminished, they may not increase statistical power or feasibility at all, and could even lead to improper trial interpretations.

Our results demonstrate that using a threshold percentage of eGFR (≥40%, ≥30%, and ≥20%), instead of a doubling of serum creatinine, in a time-to-event composite of death and ESRD substantially increases the number and rate of events observed. We define events with respect to events observed solely in the time-to-first-occurrence composite, not overall events. As expected, the number of composite events, and the rate at which they occurred, increased as the percentage threshold was lowered (Table 2, Figures 1 & 2). In the primary trial (41 months mean follow-up), using ≥40% eGFR decline, instead of doubling of serum creatinine in the composite outcome resulted in an additional 12 composite events (7 in the placebo group vs. 5 in the ramipril group) which is a 36% relative increase in events. The composite using a ≥30% eGFR decline resulted in an additional 35 total events (16 in the placebo group vs. 19 events in the ramipril group), a relative increase of 106%. Lastly, the composite using a ≥20% eGFR decline resulted in an additional 66 total events (31 in the placebo group vs. 35 events in the ramipril group), a relative increase of 200%. In the extended trial (48 months mean follow-up), similar but lesser increases were observed, with a ≥40% eGFR decline endpoint increasing the total composite events by 9 additional events (6 and 3 events in the placebo and ramipril groups respectively) for a 17% relative increase in events, while a ≥30% eGFR endpoint added a total of 30 events (15 events in both the placebo and ramipril groups) a 58% relative increase. The composite using a ≥20% eGFR decline endpoint resulted in an additional 59 events (31 and 28 events in the placebo

and ramipril groups respectively) for a relative increase of 113%. These increases in trial event rate did lead to narrower 95% confidence intervals and indicates that within the ACE trial there would have been an increase in statistical power as no treatment effect was demonstrated. Increasing trial statistical power would depend on whether or not the intervention in question exhibits a similar treatment effect on endpoints of percentage eGFR decline. If the event rate increases, and the treatment effect is similar, trial statistical power would increase and the same conclusions could be drawn using less patients or a shorter duration.

In our analysis, the original composite of death, ESRD and doubling of serum creatinine (HR, 0.76; 95% CI, 0.38-1.51) was closely replicated with the composite incorporating a ≥40% eGFR decline (HR, 0.74; 95% CI, 0.41-1.34) while increasing the number of total events by 36%. In the primary trial, when ≥30% eGFR decline was incorporated into the composite, no treatment effect was demonstrated (HR, 1.01; 95% CI, 0.63-1.62; Table 2). Similarly, this was observed when ≥20% eGFR decline was used in the composite (HR, 1.04; 95% CI, 0.70-1.54; Table 2). No treatment effect was observed in the extended trial with respect to the original composite of death, ESRD and doubling of serum creatinine. This lack of treatment effect in the extended trial was consistently observed regardless of whether a ≥40%, ≥30% or ≥20% eGFR decline was substituted for doubling of serum creatinine in the composite (Table 2).

While certainly increasing the number of events, there may be concerns of endpoint imbalance within the composites using ≥30% or ≥20% eGFR declines. Within these composites, the more clinically significant components of ESRD and death are significantly outweighed in number by ≥30% and ≥20% eGFR decline endpoints, which accounted for 75% or more of the total events experienced in both the primary and extended trial. This raises some concern as a composite outcome powered by the least clinically significant component may result in misleading conclusions (14–17). This was less of a concern with the composite of ≥40% eGFR decline, ESRD, and death as the endpoint of ≥40% eGFR decline represented around half of the total events in both the primary and extended trial.

Ramipril treatment did not lead to any statistically significant differences in any of the time-to-event composites used even with high risk patients and extended follow-up. Although substantially increasing the number and rate of events, when eGFR declines of ≥40%, ≥30% or ≥20% were incorporated into the composite they did not produce any indication of a treatment effect. The principal conclusion of the ACE trial with respect to ramipril treatment in the kidney transplant population remains unchanged.

This secondary analysis is a direct and trial-level assessment of lesser eGFR percentage decline surrogates in a typical kidney transplant trial. Using original trial data, we were able to directly compare the impact of using a surrogate endpoint of eGFR percentage declines versus doubling of serum creatinine in a time-to-event composite outcome. Limitations of this secondary analysis should be noted. During the original trial, any

suspected doubling of serum creatinine event was confirmed with a subsequent laboratory test at least 4 weeks later. As a secondary analysis relying on retrospectively collected data, any percentage eGFR decline event determined was not able to be confirmed by subsequent laboratory tests. In addition, the generalizability of these results to other kidney transplant trials may be limited as our patient population only included high risk recipients with proteinuria. In the ACE trial, ACE-inhibitor treatment was expected to have an impact on endpoints of graft function, including the alternative eGFR percentage declines assessed in this secondary analysis. Within the ACE trial, the fact that less conservative eGFR declines did not alter the interpretation and principal conclusions is consistent with a lack of a treatment effect. The lack of any clear treatment effect within the ACE trial made it impossible to assess whether lesser eGFR percentage decline endpoints would mirror the treatment effects on a doubling of serum creatinine. In trials assessing an intervention where a treatment effect is present, these alternative percentage declines in eGFR may be useful in increasing the short-term event rate and improving trial efficiency. This depends entirely on the hypothesized magnitude of an intervention's effect on graft function. Depending on the treatment being assessed, graft function decline endpoints may be inappropriate.

CONCLUSION

Using alternative graft function endpoints of ≥40%, ≥30% and ≥20% eGFR declines in a time-to-event composite of death and ESRD did not alter the ACE trial's results, interpretations, or conclusions. We found that lower eGFR percentage decline thresholds resulted in an increase in the number of composite outcomes. Within the ACE trial, these lesser eGFR declines would have increased statistical power but only because no treatment effect was ever observed. As a result, an assessment of whether lesser eGFR declines would attenuate treatment effect was not possible. The use of these alternative endpoints should be determined with respect to the intervention in question and its hypothesized treatment effect. We conclude that using alternative graft function endpoints of ≥40%, ≥30% and ≥20% eGFR declines in a time-to-event composite of death and ESRD resulted in no deviations from original ACE trial conclusions surrounding ACE-inhibitor use in kidney transplant recipients.

Author Contributions

NAF and GAK were responsible for conceiving and designing the project. NAF performed all statistical analyses with assistance from TR and MC. NAF drafted the manuscript while all authors contributed to, and critically revised, the final report.

Conflicts of Interest

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Table 1. Baseline characteristics from the Canadian ACE inhibitor in transplant trial

	Placebo (n=109)	Ramipril (n=103)
Age (years)	54.5 (11.4)	52.4 (13.3)
Sex		
Women	29 (27%)	32 (31%)
Men	80 (73%)	71 (69%)
BMI (kg/m^2)	29.5 (7.4)	29.3 (6.5)
Ethnic Origin		
White	88 (81%)	88 (85%)
Black	3 (3%)	5 (5%)
Asian	9 (8%)	4 (4%)
Other	9 (8%)	6 (6%)
Medical History		
Hypertension	101 (93%)	97 (94%)
Hyperlipidemia	74 (68%)	67 (65%)
Diabetes	42 (39%)	48 (47%)
Angina	15 (14%)	12 (12%)
PCI or CABG	13 (12%)	14 (14%)
Myocardial infarction	10 (9%)	6 (6%)
Peripheral vascular disease	4 (4%)	11 (11%)
Congestive heart failure	11 (10%)	2 (2%)
TIA or stroke	5 (5%)	4 (4%)
Cancer	24 (22%)	19 (18%)
Current Smoker	20 (18%)	16 (16%)
Primary Cause of renal disease		
Glomerulonephritis	24 (22%)	24 (23%)
Diabetes mellitus	19 (17%)	23 (22%)
Polycystic kidney disease	9 (8%)	10 (10%)
Hypertension	13 (12%)	6 (6%)
Other	36 (33%)	34 (33%)
Unknown	8 (7%)	6 (6%)
Type of donor		
Living	45 (41%)	47 (46%)
Deceased	64 (59%)	56 (54%)
Primary transplant	96 (88%)	89 (86%)
Antihypertensive use		
Calcium channel blocker	63 (58%)	59 (57%)
Beta-blocker	59 (54%)	58 (56%)
Diuretic	34 (31%)	32 (31%)
Alpha-blocker	8 (7%)	6 (6%)
Vasodilator	1 (1%)	6 (6%)
Measured DTPA GFR (mL/min)	65.1 (27.6)	65.9 (25.0)
Corrected (mL/min/1.73m^2)	58.6 (24.1)	59.8 (21.9)

Blood pressure		
Systolic blood pressure (mm Hg)	135 (17)	135 (16)
Diastolic blood pressure (mm Hg)	78 (10)	77 (9)
<130/80	32 (29%)	35 (34%)
Serum creatinine (umol/L)	142 (54)	138 (51)
Proteinuria (mg per day)	400 (270-720)	430 (270-813)

Data are mean (SD), n (%), or median (IQR). PCI = percutaneous coronary intervention. CABG = coronary artery bypass grafting. DTPA GFR = glomerular filtration rate measured using ^{99m}technetium-diethylene triamine pentacetate

Table adapted from Knoll et al. (12).

Table 2. Time-to-event composite outcomes

	Primary Trial			Extended Trial		
	Placebo (n=109)	Ramipril (n=103)	Hazard ratio (95% CI)	Placebo (n=109)	Ramipril (n=103)	Hazard ratio (95% CI)
Total follow-up (months)	41.8	41.4	..	48.6	47.8	..
Composite of:						
Doubling of serum creatinine	9	3		11	9	
Return to dialysis	6	3		8	4	
Repeat transplant	0	0		0	0	
Death	4	8		8	12	
Total*	19	14	0.76 (0.38-1.51)	27	25	0.96 (0.55-1.65)
Composite of:						
≥40% eGFR decline	16	10		18	15	
Return to dialysis	6	2		7	3	
Repeat transplant	0	0		0	0	
Death	4	7		8	10	
Total*	26	19	0.74 (0.41-1.34)	33	28	0.89 (0.54-1.48)
Composite of:						
≥30% eGFR decline	29	26		32	30	
Return to dialysis	5	1		6	1	
Repeat transplant	0	0		0	0	
Death	1	6		4	9	
Total*	35	33	1.01 (0.63-1.62)	42	40	1.06 (0.68-1.63)
Composite of:						
≥20% eGFR decline	45	43		49	45	
Return to dialysis	4	1		5	1	
Repeat transplant	0	0		0	0	
Death	1	5		4	7	
Total*	50	49	1.04 (0.70-1.54)	58	53	0.96 (0.66-1.40)

eGFR= estimated glomerular filtration rate. *Only the first event per patient is included in the total for each composite. Note that events for each composite component are only included if it was the first event. eGFR was calculated using the CKD-EPI equation (11).

Table 3. Total number of trial participants experiencing a graft function surrogate endpoint by study group and trial phase.

	Primary Trial			Extended Trial		
	Placebo (n=109)	Ramipril (n=103)	Hazard ratio (95% CI)	Placebo (n=109)	Ramipril (n=103)	Hazard ratio (95% CI)
Doubling of serum creatinine	9 (8.3%)	5 (4.9%)	0.59 (0.20-1.77)	12 (11%)	13 (13%)	1.11 (0.51-2.44)
eGFR Decline:						
≥40%	17 (16%)	11 (11%)	0.62 (0.28-1.35)	19 (17%)	19 (18%)	1.04 (0.55-1.97)
≥30%	30 (28%)	27 (26%)	0.96 (0.57-1.62)	33 (30%)	32 (31%)	1.06 (0.65-1.73)
≥20%	46 (42%)	44 (43%)	1.01 (0.68-1.53)	50 (46%)	48 (47%)	1.01 (0.67-1.50)

eGFR= estimated glomerular filtration rate. eGFR was calculated using the CKD-EPI equation (11).
Primary trial mean follow-up was 41 months while extended trial mean follow-up was 48 months.

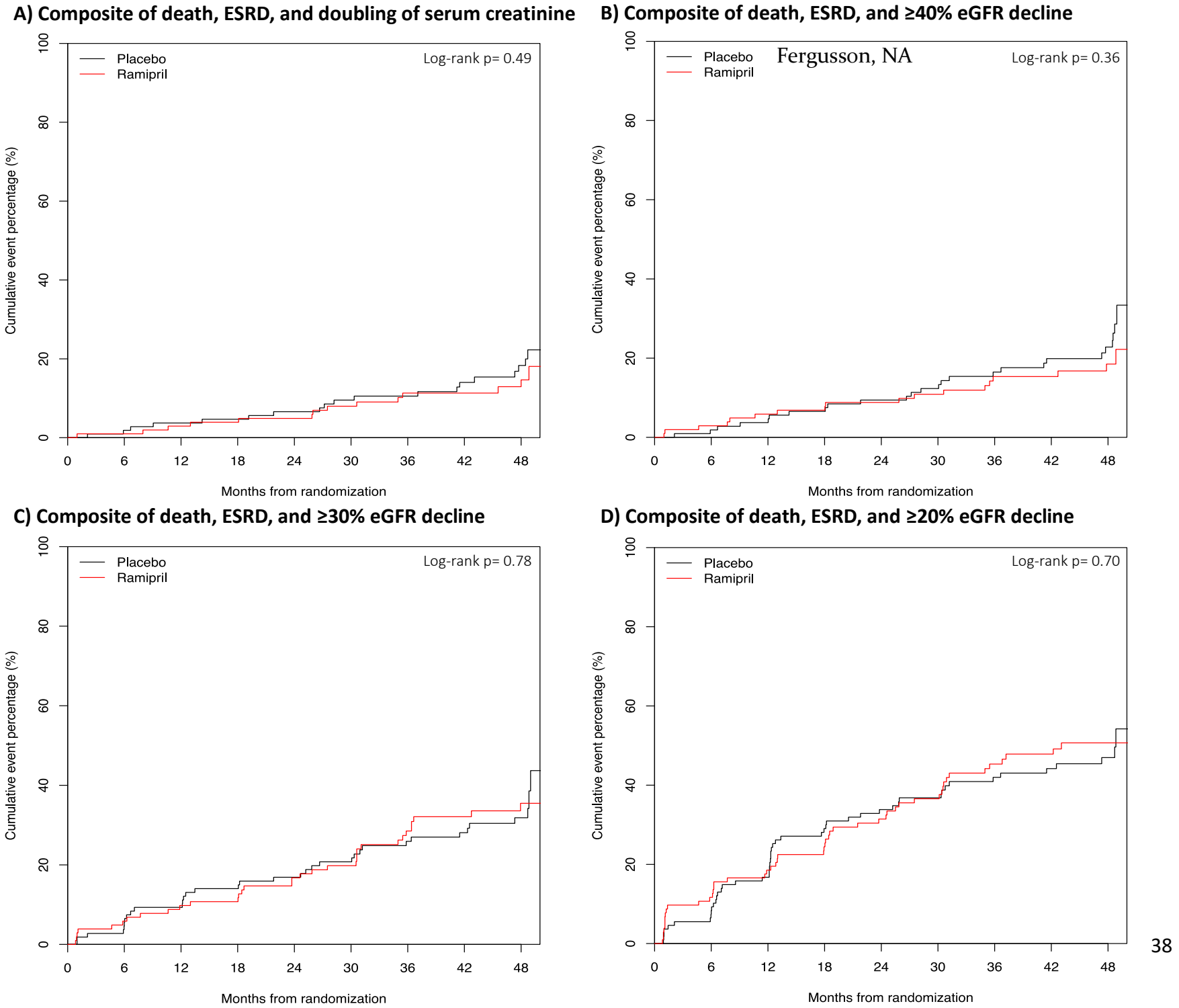


Figure 1. Cumulative hazard plots of composite outcomes in the primary trial (mean follow-up 41 months). eGFR= estimated glomerular filtration rate.

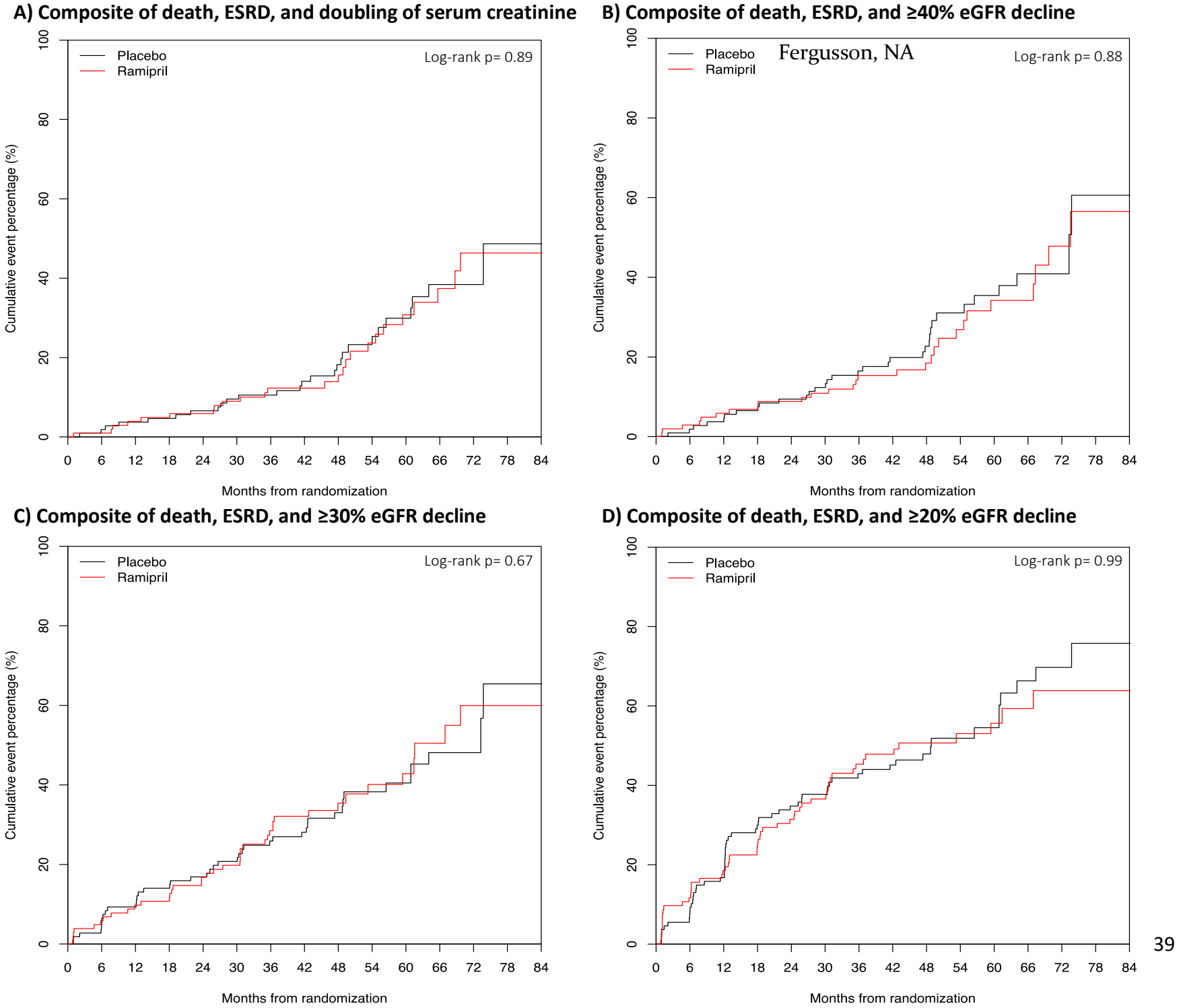


Figure 2. Cumulative hazard plots of composite outcomes in the extended trial (mean follow-up 48 months). eGFR= estimated glomerular filtration rate.

APPENDIX

Table 4. Time-to-event composite outcomes using the MDRD equation to calculate eGFR

	Primary Trial			Extended Trial		
	Placebo (n=109)	Ramipril (n=103)	Hazard ratio (95% CI)	Placebo (n=109)	Ramipril (n=103)	Hazard ratio (95% CI)
Total follow-up (months)	41.8	41.4	..	48.6	47.8	..
Composite of:						
Doubling of serum creatinine	9	3		11	9	
Return to dialysis	6	3		8	4	
Repeat transplant	0	0		0	0	
Death	4	8		8	12	
Total*	19	14	0.76 (0.38-1.51)	27	25	0.96 (0.55-1.65)
Composite of:						
≥40% eGFR decline	17	9		19	13	
Return to dialysis	6	2		7	3	
Repeat transplant	0	0		0	0	
Death	4	7		8	10	
Total*	27	18	0.66 (0.36-1.21)	34	26	0.79 (0.47-1.32)
Composite of:						
≥30% eGFR decline	28	20		30	24	
Return to dialysis	5	1		6	1	
Repeat transplant	0	0		0	0	
Death	1	7		5	10	
Total*	34	28	0.85 (0.51-1.40)	41	35	0.90 (0.57-1.42)
Composite of:						
≥20% eGFR decline	45	43		49	45	
Return to dialysis	4	1		5	1	
Repeat transplant	0	0		0	0	
Death	1	5		4	7	
Total*	50	49	1.05 (0.71-1.55)	58	53	0.98 (0.67-1.42)

eGFR= estimated glomerular filtration rate. *Only the first event per patient is included in the total for each composite. Note that events for each composite component are only included if it was the first event. eGFR was calculated using the MDRD equation (12).

Table 5. Graft function surrogate endpoints using the MDRD equation to calculate eGFR

	Primary Trial			Extended Trial		
	Placebo (n=109)	Ramipril (n=103)	Hazard ratio (95% CI)	Placebo (n=109)	Ramipril (n=103)	Hazard ratio (95% CI)
Doubling of serum creatinine	9 (8.2%)	5 (4.9%)	0.59 (0.20-1.77)	12 (11%)	13 (13%)	1.11 (0.51-2.44)
eGFR Decline:						
≥40%	18 (17%)	10 (9.7%)	0.52 (0.23-1.16)	20 (18%)	16 (16%)	0.81 (0.42-1.57)
≥30%	29 (27%)	21 (20%)	0.75 (0.43-1.31)	31 (28%)	26 (25%)	0.88 (0.52-1.48)
≥20%	46 (42%)	44 (43%)	1.02 (0.67-1.54)	50 (46%)	48 (47%)	1.02 (0.69-1.52)

eGFR= estimated glomerular filtration rate. eGFR was calculated using the MDRD equation (12)

Table 6. Time-to-event composite outcomes with adjusted hazard ratios

	Primary Trial			Extended Trial		
	Placebo (n=109)	Ramipril (n=103)	Adjusted¶ Hazard ratio (95% CI)	Placebo (n=109)	Ramipril (n=103)	Adjusted¶ Hazard ratio (95% CI)
Follow-up (months)	41.8	41.4	..	48.6	47.8	..
Composite of:						
Doubling of serum creatinine	9	3		11	9	
Return to dialysis	6	3		8	4	
Repeat transplant	0	0		0	0	
Death	4	8		8	12	
Total*	19	14	0.85 (0.41-1.77)	27	25	0.97 (0.56-1.68)
Composite of:						
≥40% eGFR decline	16	10		18	15	
Return to dialysis	6	2		7	3	
Repeat transplant	0	0		0	0	
Death	4	7		8	10	
Total*	26	19	0.72 (0.36-1.35)	33	28	0.85 (0.51-1.41)
Composite of:						
≥30% eGFR decline	29	26		32	30	
Return to dialysis	5	1		6	1	
Repeat transplant	0	0		0	0	
Death	1	6		4	9	
Total*	35	33	0.98 (0.61-1.59)	42	40	1.04 (0.67-1.61)
Composite of:						
≥20% eGFR decline	45	43		49	45	
Return to dialysis	4	1		5	1	
Repeat transplant	0	0		0	0	
Death	1	5		4	7	
Total*	50	49	1.09 (0.74-1.63)	58	53	0.98 (0.67-1.43)

eGFR= estimated glomerular filtration rate. * Only the first event per patient is included in the total for each composite. ¶Cox proportional hazards were adjusted for the following variables: age (years), diabetes (present or absent), time post-transplantation (days), measured GFR (per mL/min/1.73 m^2), donor type (living or deceased), proteinuria (per mg/24 h), and serum creatinine (per μ mol/L).

Table 7. Graft function surrogate endpoints with adjusted hazard ratios

	Primary Trial			Extended Trial		
	Placebo (n=109)	Ramipril (n=103)	Adjusted* Hazard ratio (95% CI)	Placebo (n=109)	Ramipril (n=103)	Adjusted* Hazard ratio (95% CI)
Doubling of serum creatinine	9 (8.3%)	5 (4.9%)	0.56 (0.19-1.70)	12 (11%)	13 (13%)	1.05 (0.47-2.33)
eGFR Decline:						
≥40%	17 (16%)	11 (11%)	0.59 (0.27-1.29)	19 (17%)	19 (18%)	1.01 (0.53-1.91)
≥30%	30 (28%)	27 (26%)	0.91 (0.54-1.55)	33 (30%)	32 (31%)	1.02 (0.63-1.67)
≥20%	46 (42%)	44 (43%)	1.05 (0.69-1.59)	50 (46%)	48 (47%)	1.03 (0.69-1.53)

eGFR= estimated glomerular filtration rate. *Cox proportional hazards were adjusted for the following variables: age (years), diabetes (present or absent), time post-transplantation (days), measured GFR (per mL/min/1.73 m^2), donor type (living or deceased), proteinuria (per mg/24 h), and serum creatinine (per μ mol/L).

References

1. Coresh J, Turin TC, Matsushita K, Sang Y, Ballew SH, Appel LJ, et al. Decline in Estimated Glomerular Filtration Rate and Subsequent Risk of End-Stage Renal Disease and Mortality. JAMA [Internet]. 2014;311(24):2518–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24892770
2. Clayton PA, Lim WH, Wong G, Chadban SJ. Relationship between eGFR Decline and Hard Outcomes after Kidney Transplants. J Am Soc Nephrol [Internet]. 2016;1–7. Available from: http://www.jasn.org/cgi/doi/10.1681/ASN.2015050524%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/27059513
3. Inker LA, Lambers Heerspink HJ, Mondal H, Schmid CH, Tighiouart H, Noubary F, et al. GFR Decline as an Alternative End Point to Kidney Failure in Clinical Trials: A Meta-analysis of Treatment Effects from 37 Randomized Trials. Am J Kidney Dis [Internet]. Elsevier Inc; 2014;64(6):848–59. Available from: http://dx.doi.org/10.1053/j.ajkd.2014.08.017
4. Badve S V., Palmer SC, Hawley CM, Pascoe EM, Strippoli GFMM, Johnson DW. Glomerular filtration rate decline as a surrogate end point in kidney disease progression trials. Nephrol Dial Transplant [Internet]. 2016 Sep [cited 2017 May 6];31(9):1425–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26163881
5. R.R., Alloway, N.A., Pilch DC. Evidence-based medicine and clinical trials in renal transplantation - UpToDate [Internet]. UpToDate. 2015 [cited 2016 Jul 18]. Available from: http://www.uptodate.com/contents/evidence-based-medicine-and-clinical-trials-in-renal-transplantation
6. Hiremath S, Fergusson DA, Fergusson N, Bennett A, Knoll GA. Renin-Angiotensin System Blockade and Long-term Clinical Outcomes in Kidney Transplant Recipients: A Meta-analysis of Randomized Controlled Trials. Am J Kidney Dis [Internet]. Elsevier Inc; 2016;5–12. Available from: http://dx.doi.org/10.1053/j.ajkd.2016.08.018
7. Jun M, Turin TC, Woodward M, Perkovic V, Lambers Heerspink HJ, Manns BJ, et al. Assessing the Validity of Surrogate Outcomes for ESRD: A Meta-Analysis. J Am Soc Nephrol [Internet]. 2015;1–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25556165
8. Lambers Heerspink HJ, Weldegiorgis M, Inker LA, Gansevoort R, Parving HH, Dwyer JP, et al. Estimated GFR decline as a surrogate end point for kidney failure: A post hoc analysis from the reduction of end points in non-insulin-dependent diabetes with the Angiotensin II Antagonist Losartan (RENAAL) study and Irbesartan Diabetic Nephropathy Trial (IDNT). Am J Kidney Dis [Internet]. Elsevier Inc; 2014;63(2):244–50. Available from: http://dx.doi.org/10.1053/j.ajkd.2013.09.016
9. Knoll GA, Fergusson D, Chasse M, Hebert P, Wells G, Tibbles LA, et al. Ramipril versus placebo in kidney transplant patients with proteinuria: a multicentre, double-blind, randomised controlled trial. The Lancet Diabetes Endocrinol [Internet]. 2016;4(4):318–26. Available from:

http://www.thelancet.com/article/S221385871500368X/fulltext
10. Knoll GA, Cantarovitch M, Cole E, Gill J, Gourishankar S, Holland D, et al. The Canadian ACE-inhibitor trial to improve renal outcomes and patient survival in kidney transplantation - Study design. Nephrol Dial Transplant. 2008;23(1):354–8.
11. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med [Internet]. 2009;150(9):604–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19414839%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2763564
12. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, et al. Expressing the Modification of Diet in Renal Disease Study Equation for Estimating Glomerular Filtration Rate with Standardized Serum Creatinine Values. Clin Chem [Internet]. 2007 Apr 1 [cited 2017 Mar 22];53(4):766–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17332152
13. Team RC. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2016. Available from: https://www.r-project.org/
14. Pocock SJ, Ariti CA, Collier TJ, Wang D. The win ratio: A new approach to the analysis of composite endpoints in clinical trials based on clinical priorities. Eur Heart J. 2012;33(2):176–82.
15. Busse JW, Bhandari M, Ferreira-González I, Montori VM, Guyatt GH. Use and Interpretation of Composite End Points in Orthopaedic Trials. J Bone Jt Surg [Internet]. 2012;94(Suppl 1(E)):65. Available from: http://jbjs.org/cgi/doi/10.2106/JBJS.K.01287
16. Ferreira-González I, Alonso-Coello P, Solà I, Pacheco-Huergo V, Domingo-Salvany A, Alonso J, et al. [Composite endpoints in clinical trials]. Rev española Cardiol [Internet]. 2008;61(3):283–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18361902
17. Luo X, Qiu J, Bai S, Tian H. Weighted win loss approach for analyzing prioritized outcomes. Stat Med [Internet]. 2017;(November 2016). Available from: http://doi.wiley.com/10.1002/sim.7284

CHAPTER 4: ALTERNATIVE ANALYSIS TECHNIQUES- MANUSCRIPT 2

Preface to Manuscript 2

In Chapter 3 (Manuscript 1), we presented a secondary analysis assessing the impact of utilizing lesser percentage eGFR decline endpoints on the ACE trial's results, interpretations, and conclusions. This secondary analysis investigated the use of these eGFR decline endpoints as they may offer the ability to increase trial event rate and ideally, statistical power, resulting in trials that require less patients and/or shorter duration. However, with respect to the ACE trial's primary composite outcome of death, ESRD, and doubling of serum creatinine, there remains the lingering, and underlying, issue of unequal endpoints.

In the next manuscript, we present an application of an alternative analysis technique, the win ratio approach (7,18), to the ACE trial. The win ratio approach was applied to the ACE trial's original primary composite of death, ESRD, and doubling of serum creatinine. Although no major concerns of disproportionality were apparent within the ACE trial composite, the presence of composite components trending in opposite directions and highly unexpected ACE trial results, the impact of the win ratio approach on the ACE trial's results, interpretations, and conclusions is worth exploring.

We performed the unmatched version of the win ratio approach as described by *Pocock et al*. (7). The objective of this study was to determine the statistical impact of using an

alternative analysis technique, the win ratio approach, and investigate whether this approach alters the results, interpretations, or conclusions of the original ACE trial.

The unmatched win ratio approach involves the creation of pairs that consist of one study (ramipril) patient and one control (placebo) patient. Each ramipril patient (n=103) was paired with every other placebo patient (n=109) to create a total of 11,227 pairs (103 x 109). The components within the primary composite were then ranked with respect to clinical significance in the following manner: 1) Death, 2) ESRD, 3) Doubling of serum creatinine. Each pair was then initially assessed on the endpoint of death (since it was ranked the highest) and it was determined whether the ramipril or placebo pair member 'won' (Manuscript 1, Figure 1). Afterwards, the next most clinically significant endpoint was assessed in the same manner but only if the pair was labelled uninformative (a 'tie'). The definition of 'wins' and 'ties' are displayed in Figure 1 of the manuscript while the entire process is outlined in Figure 2.

Upon completion, a win ratio, defined as the total number of pairs won by the ramipril pair member (ramipril wins) divided by the total number of pairs won by the placebo pair member (placebo wins), was determined. The win ratio can be thought of as conceptually similar to an odds ratio or a hazard ratio as depicted in Appendix 4 of this thesis. A win ratio greater than 1 indicates a positive treatment effect while a hazard ratio greater than 1 indicates a negative treatment effect. R code to determine the win ratio and its 95% confidence intervals are provided in Appendix 5-Additional File 1 & 2 of this thesis.

The win ratio approach produced comparable results to a standard time-to-event analysis within the ACE trial while maximizing information derived from outcomes of greater clinical significance. A win ratio comparing ramipril treatment to placebo was not statistically significant in both the primary (win ratio 1.21 [95% CI, 0.55-2.59]) and extended (win ratio 1.02 [95% CI, 0.54-1.83]) ACE trial. Within a kidney transplant trial setting, the win ratio approach is able to reproduce time-to-event results while taking into account the relative clinical significance of endpoints and minimizing unrealistic statistical assumptions. Due to its ability to lessen concerns of unequal endpoints its application requires further investigation and consideration in kidney transplant trials using composites of unequal endpoints.

Manuscript 2

The win ratio approach for unequal composite endpoints: An application to the ACE trial in kidney transplantation

Nicholas A. Fergusson, BSc,[1,2] Tim Ramsay, PhD,[1,2] Michaël Chassé, MD,[3,4]

Shane W. English MD[2,5] and Greg A. Knoll, MD[1,2,5]

[1] School of Epidemiology & Public Health, University of Ottawa, Ottawa, Canada
[2] Clinical Epidemiology Program, Ottawa Hospital Research Institute, Ottawa, Canada
[3] Department of Medicine, Université de Montréal, Montréal, Canada
[4] Centre de recherche du Centre Hospitalier Universitaire de Montréal, Montréal, Canada
[5] Department of Medicine, University of Ottawa, Ottawa, Canada

Abstract

Objective: Determine the statistical impact of using the win ratio approach and investigate whether this approach alters the results, interpretations, or conclusions of the ACE trial.

Study Design and Setting: We present an application of the win ratio approach in a kidney transplant trial. The ACE trial assessed the clinical effectiveness of ramipril treatment versus placebo on a primary composite of death, end-stage renal disease (ESRD) and doubling of serum creatinine. We compare the win ratio (total pairs won for ramipril divided by total pairs won for placebo) to a conventional hazard ratio. A win ratio greater than 1 indicates a positive treatment effect, while a hazard ratio less than 1 indicates a positive treatment effect.

Results: On the primary composite ramipril treatment resulted in a win ratio of 1.21 (95% CI, 0.55-2.59) versus a hazard ratio of 0.76 (95% CI, 0.38-1.51). With extended follow-up (mean 48 months), ramipril was associated with a win ratio of 1.02 (95% CI, 0.54-1.83) versus a hazard ratio of 0.96 (95% CI, 0.55-1.65).

Conclusion: The win ratio approach confirmed the time-to-event findings of the ACE trial while lessening concerns of unequal composite endpoints.

Key words: Win ratio; composite endpoints; kidney transplant; ACE-inhibitor
Word Count: 3845

1. Introduction

Trials in kidney transplantation regularly suffer from low short-term event rates and low efficiency [1]; both obstacles to improving clinical practice. In order to increase event rate, often an endpoint of graft function decline, acting as a surrogate for hard outcomes, is incorporated into a time-to-first-event composite that also includes death and end-stage renal disease (ESRD) [1–5]. Doubling of serum creatinine is commonly used as the graft function decline endpoint and is strongly predictive of both death and ESRD [3].

Although a time-to-first-event composite of death, ESRD, and graft function decline, may increase the event rate and therefore, reduce sample size and trial duration it also possesses components with unequal levels of clinical significance— patient death has much more clinical significance than a doubling of serum creatinine. It can be difficult to interpret composite outcomes that are driven by a particular component, especially if that component is the least clinically significant (most often the case as less significant events occur more frequently) [6–9]. Further, if a less clinically significant component is going in the opposite direction of a more clinically significant component it may weaken the ability to derive confident conclusions from the trial [6]. Using a time-to-first-event analysis on composites with endpoints of unequal significance and frequency can produce potentially misleading and faulty trial interpretations [7,8,10].

The win ratio method proposed by Buyse [11] and Pocock et al. [7] attempts to mitigate concerns resulting from unequal endpoints. The win ratio involves pairing control arm

patients with study arm patients and working from the top-ranked, most clinically significant endpoint downwards towards the least clinically significant endpoint. For each pair a 'winner' is found by assessing which patient experienced the most clinically significant endpoint first, although pairs can be uninformative with no 'winner' present. Ranking and sequentially assessing endpoints by clinical importance may offer a win-win scenario by lessening the concerns of unequal composite components and statistical assumptions while maintaining an increase in trial event rate and efficiency.

The ACE trial [12] compared the impact of ramipril, an angiotensin converting enzyme (ACE) inhibitor, to placebo on important clinical outcomes in renal transplant patients with proteinuria. The primary outcome of the trial was a time-to-event composite of death, ESRD, and doubling of serum creatinine. The trial concluded that compared to placebo, ramipril treatment did not lead to any significant differences in the primary composite outcome [12]. Although no major concerns of disproportionality were apparent within the primary composite, the presence of composite components trending in opposite directions and highly unexpected ACE trial results, the impact of the win ratio approach on the ACE trial's conclusions and interpretations is worth exploring. The objective of this study is to explore the statistical impact of the win ratio approach and investigate whether it alters the results, interpretations, or conclusions of the ACE trial.

2. Methods

2.1 Overview of the ACE Trial

The ACE trial evaluated the impact of ramipril versus placebo on clinically important outcomes in kidney transplant recipients with proteinuria. Complete trial design and detailed inclusion/exclusion criteria are available in the original trial publication and trial protocol [12,13], and the trial is registered (ISRCTN, number 78129473). In summary, between August 23rd, 2006, and March 28th, 2012, a total of 213 kidney transplant recipients were randomized (1:1) to either receive ramipril or placebo treatment for a maximum of 4 years (oral 5 mg daily for 2 weeks and then 5 mg oral twice daily thereafter). A total of 109 patients were allocated to placebo while 104 were allocated to ramipril, of whom 109 patients in the placebo group and 103 patients in the ramipril group were analyzed. Because of lower than expected recruitment rate, and in order to increase the number of events and trial power, all patients that completed the final 4-year study visit were invited to participate in a non-prespecified trial extension phase (extended trial). In the extended trial, all patients finishing the final 48-month study visit of the primary trial were offered to continue treatment and follow-up for up to an additional 48 months. At this point, 43 (80%) of 54 eligible patients in the placebo group and 38 (78%) of 49 eligible patients in the ramipril group agreed to participate. During this extension phase masking was maintained. Trial conduct and participant safety were overseen by a data safety monitoring board responsible for reviewing data every 6 months. The study was approved by the local research ethics board at every participating institution and all trial participants provided written informed consent.

2.2 Trial Data Collection

Study visits occurred at randomization, 1 month, 6 months and then every 6 months thereafter for the duration of the trial. At each study visit, haemoglobin, serum creatinine and serum potassium concentrations were measured by a central laboratory and any event of the primary composite outcome (doubling of serum creatinine, end-stage renal disease or death) was determined. End-stage renal disease (ESRD) was defined as the date of repeat kidney transplantation or initiation of dialysis. Doubling of serum creatinine was defined as a two-fold increase in serum creatinine (umol/L) relative to baseline and was confirmed by two consecutive tests at least 4 weeks apart by a central laboratory. In the extended trial, study assessments were performed at 6-month intervals to determine if any event of primary composite outcome (doubling of serum creatinine, return to dialysis or death) had occurred. Serum creatinine values during the extension phase were obtained from trial participant medical records. Laboratory testing and measured GFR calculation were not performed during this phase.

2.3 Outcomes

The original primary outcome of the ACE trial was a time-to-first event composite consisting of death, ESRD, and doubling of serum creatinine. For the win ratio, individual component endpoints were ranked based on clinical significance in the following order: 1) Death, 2) ESRD and 3) Doubling of serum creatinine.

2.4 Analysis

2.4.1 Time-to-event analysis

Cox proportional hazards regression models were used to adjust for important risk factors including: age (years), diabetes (present or absent), time post-transplantation (days), measured GFR (per mL/min/1.73m2), donor type (living or deceased), proteinuria (per mg/24 h), and serum creatinine (per umol/L). Unadjusted and adjusted Cox proportional hazard ratios with corresponding 95% confidence intervals were created to compare the time-to-event composite outcome between study groups.

2.4.2 Unmatched win ratio analysis

The unmatched win ratio approach was conducted in accordance with the original methods described by *Pocock et al*. [7], as illustrated in Fig. 1 & 2 (a matched approach is also described however, we decided that individually matching patients in this trial's specific population, and limited sample size, would not be realistic). Each patient in the ramipril group (n=103) was paired with every patient in the placebo group (n=109) yielding a total of 11,227 pairs. For each pair, it was determined whether the study group (ramipril) member 'won' or if the control group (placebo) member 'won' (Fig 1.) using the following methodology (Fig 2.):

First off, each pair was assessed in terms of time to death, the most clinically significant endpoint. If death occurred in both the ramipril and placebo patient, the winner is defined as the patient with the longest survival. If only one patient in the pairing has died,

the surviving patient was labelled a winner but only if they had equal or greater follow-up (Fig 1.). If follow-up was not equal to or greater the comparison was labeled a 'tie' and the analysis shifted to the next ranked endpoint (Fig 1.). As well, if both patients had not died, the pair was labelled a 'tie' and the comparison shifted to the second most clinically important endpoint, which was ESRD (Fig. 2). Only pairs labelled as 'ties', or uninformative, for death were assessed for ESRD. The analysis repeated itself in identical fashion for ESRD and only pairs labelled as ties for both death and ESRD were assessed on doubling of serum creatinine. Finally, the analysis was repeated for doubling of serum creatinine until each pair eventually fitted into one of the seven categories:

a) Ramipril patient won on death
b) Placebo patient won on death
c) Ramipril patient won on ESRD
d) Placebo patient won on ESRD
e) Ramipril patient won on doubling of serum creatinine
f) Placebo patient won on doubling of serum creatinine
g) Uninformative (tied on all endpoints)

All 11,227 total pairs were eligible to enter categories a) or b) and as a result, the endpoint of death had an inbuilt priority over all other endpoints. Pairs were only classified into categories *c)* or *d)* if they could not be classified into categories *a)* or *b)*. In other words, pairs only entered categories *c)* or *d)* if they were uninformative for the endpoint of

patient death. Similarly, patients were only classified into groups *e)* and *f)* if they could not be entered into categories *a), b), c)* or *d)*. If it could not be determined if a pair has won or lost on any of the endpoints they entered category *g)* and were labelled uninformative ('ties'). The number of matched pairs in categories *a), b), c), d), e), f) and g)* can be represented as N_a, N_b, N_c, N_d, N_e, N_f and N_g respectively.

The win ratio was then defined as the total number of wins in the ramipril study group ($N_a + N_c + N_e$) divided by the total number of wins in the placebo control group ($N_b + N_d + N_f$). The win ratio can be thought of as similar to an odds ratio or a hazard ratio. Instead of comparing the odds, or the hazard of an event in the study group to the control group, the win ratio compares the number of wins in the study group to the control group. Since the number of wins is an indicator of positive treatment effect (we want a treatment to increase the number of wins) a win ratio above 1 indicates a positive treatment effect, while a win ratio below 1 indicates a negative treatment effect. This would be the same of an odds ratio or hazard ratio measuring the odds or hazard of a positive outcome. The direction of the win ratio depends on how the study and control groups are being compared. In this study, we are assessing whether ramipril treatment increases the number of wins compared to the placebo group. This is similar to trying to assess whether ramipril decreases the event hazard compared to placebo group. A win ratio was determined for both the primary and extended trial. Corresponding 95% confidence intervals for each win ratio were calculated using bootstrapping [14]. Using bootstrapping, a total of one thousand replications were simulated using random sampling with

replacement and confidence intervals were determined from this simulation. A win ratio is declared statistically significant if tis 95% confidence interval excludes 1. All analyses were conducted in R statistical software version 3.3.2. R code to conduct the win ratio approach is available from nfergusson09@gmail.com.

3. Results

In the primary trial, a total of 212 patients were assessed with 109 allocated to placebo and 103 allocated to ramipril treatment. In the primary trial, mean follow-up was 41 months (range 1-48) while in the extended trial mean follow-up was 48 months (range 1-84) The groups were well balanced at baseline (Appendix; Table 1).

Using the win ratio approach, a total of 11,227 (109x103) pairs each consisting of one placebo and one ramipril patient were assessed. In the primary trial, ramipril treatment was associated with a win ratio of 1.21 (95% CI, 0.55-2.59, Table 1). This indicates that patients randomized to ramipril treatment won 21% more pairs than patients randomized to placebo. The ratio of ramipril wins to placebo wins for the components of death, ESRD, and doubling of serum creatinine were 410:840, 640:361, and 414:14 respectively (Table 2) with 8,548 (76%) pairs remaining uninformative ('tied') at the end of the analysis.

In the extended trial (mean follow-up 48 months), ramipril treatment was associated with a win ratio of 1.02 (95% CI, 0.54-1.83), Table 1). The ratio of ramipril wins to placebo wins for the components of death, ESRD, and doubling of serum creatinine were 542:995,

695:571 and 430:74 respectively (Table 2) with 7,920 (71%) pairs remaining uninformative ('tied') at the end of the analysis.

In the time-to-event analysis assessing the effect of ramipril treatment on the primary composite of death, ESRD, and doubling of serum creatinine yielded unadjusted hazard ratios (HR) of 0.76 (95% CI, 0.38-1.51; Table 1) in the primary trial, and 0.97 (95% CI, 0.56-1.67; Table 1) in the extended trial. The number of events for the primary composite outcome and its components for the primary trial and extended trial are listed in Table 2 & 3 respectively. Adjusted HRs did not differ substantially from unadjusted HRs (Appendix; Table 2).

4. Discussion

Applying the win ratio method for composite outcomes to the ACE trial in kidney transplantation yielded comparable results to standard time-to-event analyses while taking unequal endpoints into account and not relying on unrealistic statistical assumptions such as proportional hazards.

These results are in agreeance with results from a recent application of the win ratio to the EVOLVE trial [10] in end-stage renal disease (ESRD) patients with moderate to severe secondary hyperparathyroidism (sHPT). The EVOLVE trial was a substantially large trial (n=3883) assessing the impact of cinacalcet therapy on a primary composite of death, non-fatal myocardial infarction (MI), hospitalization with unstable angina, heart failure

or peripheral vascular event [10]. The authors concluded that the win ratio method corroborated the conventional time-to-event analysis findings of the EVOLVE trial while mitigating issues of unequal endpoints. This approach allows investigators the flexibility to modify the order in which endpoints are assessed. The EVOLVE trial was a large-scale trial enrolling over 3,000 patients while the majority of trials in nephrology are often much smaller in scale [15]. In our analysis, we applied the unmatched win ratio approach in a typically sized nephrology trial enrolling kidney transplant recipients.

Compared to a standard time-to-event analysis, the win ratio approach generated comparable results. The original primary composite of death, ESRD, and doubling of serum creatinine, ramipril treatment was associated with a HR of 0.76 (95% CI, 0.38-1.51) and a win ratio of 1.21 (95% CI, 0.55-2.59) in the primary trial. These results are directly comparable, as a 24% decrease in hazard is consistent with a 21% increase in wins for patients randomized to ramipril treatment, however neither of these results were statistically significant. These parallels were seen within the breakdown of the composite, as death occurred twice as often in the ramipril group versus placebo group (8 vs. 4), and similarly the ramipril group only had half the number of wins as the placebo group (410 vs. 840). Within the composite, the ramipril group experienced half the amount of ESRD events (3 vs. 6) and won twice as often on ESRD (640 vs. 361). For the doubling of serum creatinine component, the effect of ramipril was similar in interpretation but different in magnitude when comparing the standard time-to-event analysis to the win ratio approach. The ramipril group experienced one third of the doubling of serum creatinine

events as the placebo group (3 vs. 9), but substantially more wins (414 vs. 14) than the placebo group. By prioritizing more clinically significant endpoints of death and ESRD, ramipril's reduction of doubling of serum creatinine events appears to increase however, it does not alter the overall win ratio and trial interpretations. Here lies one of the strengths of the win ratio, as it derives the bulk of its information from the more clinically significant events. The win ratio derived 84% of its information from the endpoints of death and ESRD in the primary and extended ACE trial. The win ratio approach maximizes data from the more clinically significant endpoints, lessening the concern of lesser clinically significant endpoint overpowering the composite.

These interpretations were confirmed in the extended trial, as ramipril treatment was associated with a HR of 0.96 (95% CI, 0.55-1.65) and a win ratio of 1.02 (95% CI, 0.54-1.83) with respect to the primary composite. Again, results are comparable, as a 4% decrease in hazard is consistent with a 2% increase in wins for patients randomized to ramipril. Within the composite, the impact of ramipril treatment on death was comparable, although slightly amplified using the win ratio approach compared to a time-to-event analysis. About 50% more deaths occurred in the ramipril group compared to placebo (12 vs. 8 deaths) while the ramipril group had half as many wins as the placebo group (542 vs. 995 wins). On the other hand, the impact of ramipril treatment on ESRD reduction was minimized to some degree in the win ratio analysis (695 ramipril vs. 571 placebo wins) compared to the time-to-event analysis (4 events vs. 8 events). Lastly, and similar to the primary trial results, the effect of ramipril on reducing doubling of serum creatinine

events was more pronounced in the win ratio analysis (430 vs. 74 wins) than in the time-to-event analysis (9 events vs. 11 events). Yet, the overall win ratio derived the bulk of its information from the endpoints of higher clinical significance resulting in an overall win ratio that indicates no impact of ramipril treatment.

In order to be feasible, kidney transplant trials often have to utilize an endpoint of graft function decline, such as a doubling in serum creatinine. Short-term events of death and graft failure are scarce, while long term outcomes for kidney transplantation patients remain suboptimal with death and graft failure often occurring more than 5 years post-transplantation [1–3,5,16]. A kidney transplant trial investigating a treatment's impact on death and ESRD over the first several post-transplant years would require a substantial sample size and extensive follow-up [1–3,5]. Since the majority of kidney transplant treatments, and consequently, trials, target the first 1-3 years post-transplant there is a need to increase the short term event rate and improve trial efficiency and as a result kidney transplant trials must rely on composites with unequal endpoints [1].

Death and ESRD are both events that hold a very high level of clinical significance and direct patient value. Meanwhile, a doubling of serum creatinine is a surrogate endpoint, not a clinical endpoint that a patient may be directly concerned with [5]. It should be noted that within the ACE trial's primary composite outcome there were no major issues with the doubling of serum creatinine component overpowering the other components. However, in the primary trial, the components of doubling of serum creatinine and ESRD

seemed to be trending in the opposite direction of death (more deaths in the ramipril group). In the extended trial, the occurrence of a doubling of serum creatinine was nearly identical in both groups while ESRD was trending in the opposite direction of death. The win ratio approach is preferable in these situations as it maximizes all the information available with respect to events of higher clinical significance. In the primary trial, a doubling of serum creatinine occurred as the first composite event in 9 (8%) placebo patients and 3 (3%) ramipril patients while death occurred as the first composite event in 4 (4%) placebo patients and 8 (8%) ramipril patients. These components are trending in opposite directions and if these endpoints had a similar clinical importance, it would not be as much of a concern– simply a 'trade-off'. Yet, they are not equally significant and it would be misleading for events of doubling of serum creatinine to completely cancel out events of death. Instead, it would be preferable to assess those patients whose first event was a doubling of serum creatinine and see how they fared with respect to death and this, in essence, is what the win ratio approach accomplishes. The win ratio approach still considers events of doubling of serum creatinine to be significant, as they are strongly predictive ESRD and death however, they should be assessed following death and ESRD.

Applied research assessing the impact of using graft function surrogate endpoints in kidney transplantation trials is lacking, despite its importance. In this study, the graft function endpoint assessed was a doubling of serum creatinine, which corresponds to about a 57% decrease in estimated glomerular filtration rate (eGFR) [3]. Recently, the use of alternative graft function endpoints such as a ≥40% or ≥30% decline in eGFR have been

validated in the chronic kidney disease (CKD) [2] and kidney transplant population [1]. If these lesser declines in eGFR can lead to a greater increase in short-term events while maintaining surrogacy and similar treatment effects, they may present an appealing way of improving trial feasibility [1,2,5]. It is here that the win ratio approach may be particularly useful, as it limits the impact of unequal composite components but still includes them to increase the trial event rate.

An additional strength of the unmatched win ratio approach is that, unlike a conventional time-to-event analysis, it does not depend on the often-unrealistic assumption of proportional hazards [17,18]. Assuming proportional hazards can often be questionable, particularly when follow-up extends over several years [17,18]. The win ratio amplifies the impact of these events and so the uncertainty observed in these endpoints is reflected in the confidence intervals. In general, the confidence level surrounding the win ratio was comparable to that of the hazard ratio and consequently there was no indication of a significant reduction of statistical power. Limitations of win ratio approach should be noted, including the current lack of a priori statistical power calculations for the win ratio. Kidney transplant trials can still utilize conventional time-to-event analyses while also incorporating the unmatched win ratio approach, particularly if using a composite with unequal component endpoints. An additional limitation is the inability to easily control for covariates however, within a randomized trial setting this should not be concerning. Further, the win ratio approach can be stratified on factors believed to be significant as illustrated in the EVOLVE trial [10].

5. Conclusion

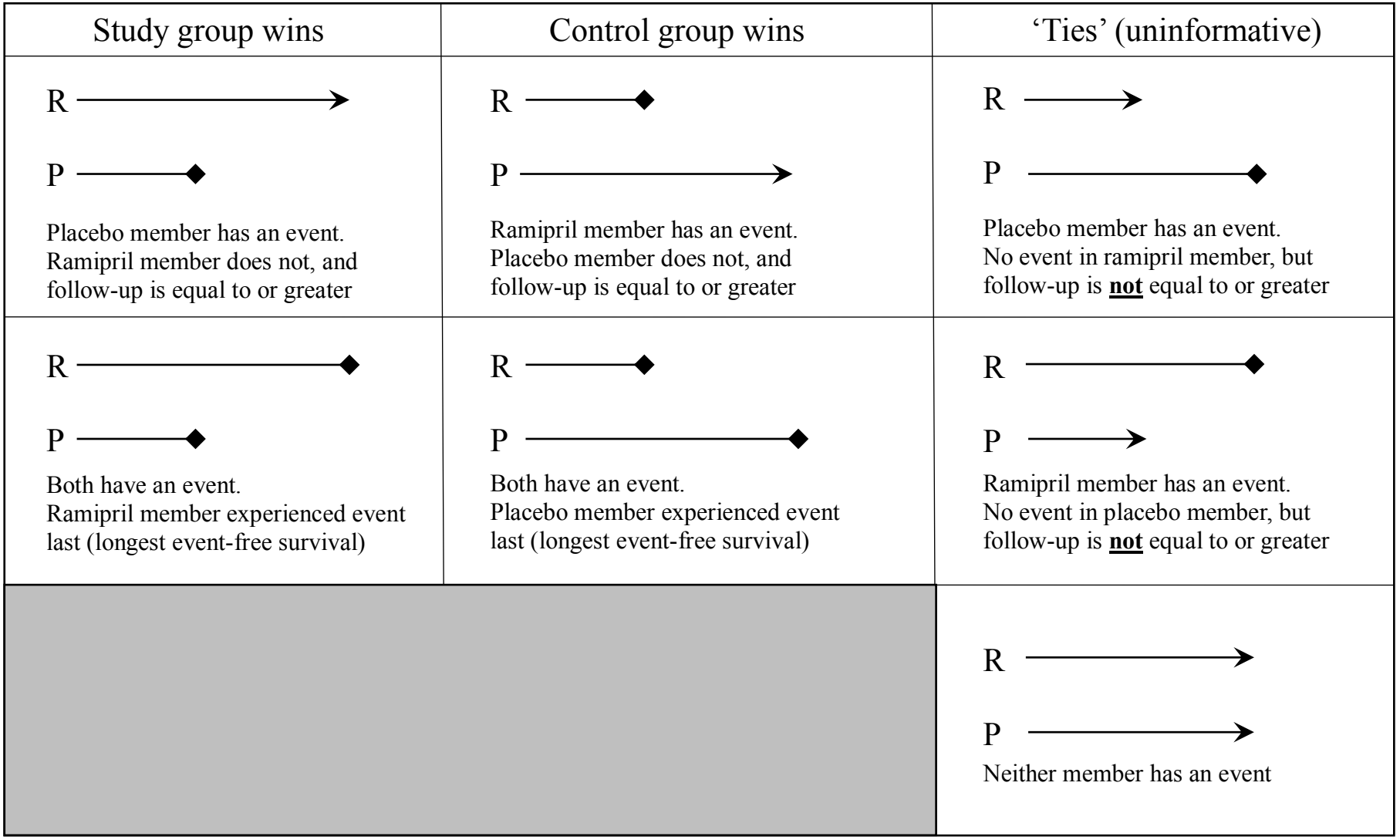
In this study, we investigated the impact of using the win ratio method on the primary composite outcome of the ACE trial. With respect to the impact of ramipril treatment on the time-to-first-event composite outcome of death, ESRD, and doubling of serum creatinine, the win ratio was directly comparable to a hazard ratio produced with standard time-to-event analyses. We applied the win ratio to explain and illustrate its methodology within kidney transplant trials. Within a kidney transplant trial setting, the win ratio method is able to reproduce time-to-event results while taking into account the relative clinical significance of endpoints and minimizing unrealistic statistical assumptions. Going forward, the win ratio approach may offer an optimized way of analyzing kidney transplant trials and its utility should be further explored.

Conflict of interest

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.



R: pair member randomized to ramipril (study group), P: pair member randomized to placebo (placebo group)

→ : follow-up period from participant's first day in the trial until trial completion or trial exit (not due to an outcome event)

◆ : Event

Figure 1. Win ratio scenarios. Potential scenarios for determining whether the study (ramipril) or control (placebo) group pair member won for a particular endpoint. The most clinically significant endpoint of death would be assessed first. Only pairs classified as uninformative for death would be assessed for ESRD. Similarly, only pairs classified as uninformative for both death and ESRD would be assessed for doubling of serum creatinine. Table adapted from Pocock et al. [7] & Abdalla et al. [10].

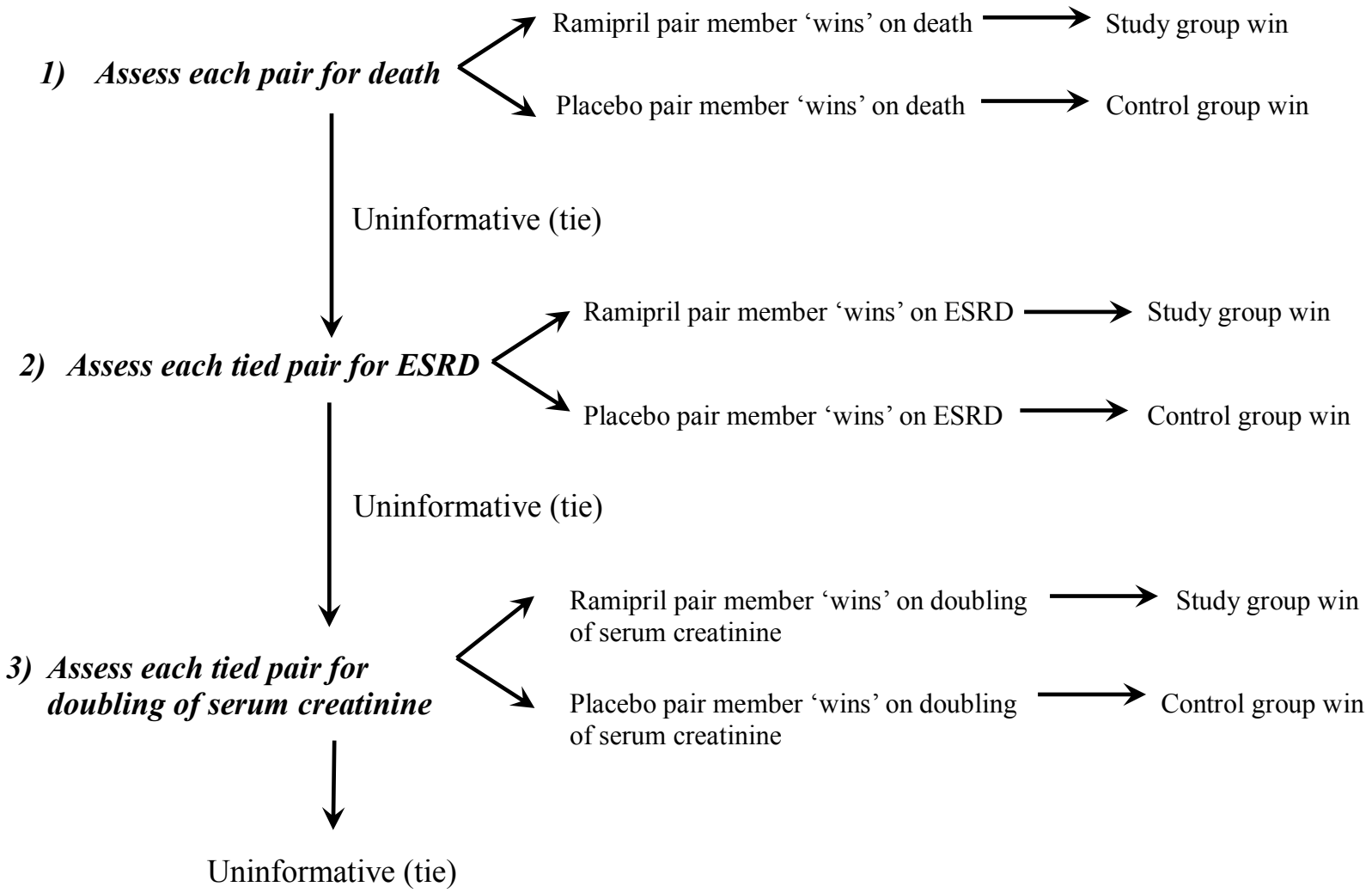


Figure 2. Win ratio process. Schematic illustrating the win ratio method. All pairs (n=11,227) are initially assessed on the endpoint of death. Pairs uninformative on death are then assessed on ESRD, and pairs remaining uninformative on ESRD are finally assessed on doubling of serum creatinine. Pairs remaining uninformative on doubling of serum creatinine are labelled uninformative. The win ratio is the total number of study group wins divided by control group wins.

Table 1. Composite outcome results using the win ratio method versus a standard time-to-event analysis in the primary and extended trial.

	Win Ratio Approach		Time-to-event Analysis
	Win Ratio[a] (95% CI)	Inverse Win Ratio (95% CI)	Hazard Ratio[b] (95% CI)
Primary Trial (mean follow-up 41 mo.)			
Composite of death, ESRD, & doubling of serum creatinine	1.21 (0.55-2.59)	0.83 (0.40-1.68)	0.76 (0.38-1.51)
Extended Trial (mean follow-up 48 mo.)			
Composite of death, ESRD, & doubling of serum creatinine	1.02 (0.54-1.83)	0.98 (0.53-1.75)	0.96 (0.55-1.65)

[a] Win ratio greater than 1 indicates a positive treatment effect, while a win ratio less than 1 indicates a negative treatment effect.
[b] Hazard ratio less than 1 indicates a positive treatment effect, while a hazard ratio greater than 1 indicates a negative treatment effect.
ESRD=end-stage renal disease, defined as either return to dialysis or repeat transplantation.

Table 2. Breakdown of primary and extended trial results from the time-to-event and win ratio analyses

	Time-to-event Analysis			Win Ratio Approach				
	Events Placebo (n=109)	Events Ramipril (n=103)	Hazard Ratio (95% CI)	Events Placebo (n=109)	Events Ramipril (n=103)	Wins Ramipril	Wins Placebo	Win ratio (95% CI)
Primary Trial (mean follow-up 41 mo.)								
Primary composite[b]*:*								
Death	4	8		5	9	410	840	
ESRD	6	3		9	6	640	361	
Doubling of serum creatinine	9	3		9	5	414	14	
Total[a]	19	14	0.76 (0.38-1.51)	23[c]	20[c]	1,464	1,215	1.21 (0.55-2.59)
Extended Trial (mean follow-up 48 mo.)								
Primary composite[b]:								
Death	8	12		11	14	542	995	
ESRD	8	4		12	11	695	571	
Doubling of serum creatinine	11	9		12	13	430	74	
Total[a]	27	25	0.96 (0.55-1.65)	35[c]	38[c]	1,667	1,640	1.02 (0.54-1.83)

[a] Only the first event per patient is included in the total for each composite.
[b]Note that events for each composite component are only included if it was the first event.
[c] Note that the win ratio approach does not necessarily utilize all events that have occurred but it is able to if needed. It is not reliant on only the first occurrence.
ESRD=end-stage renal disease, defined as either return to dialysis or repeat transplantation.

APPENDIX

Table 3. Baseline characteristics from the Canadian ACE inhibitor in transplant trial

	Placebo (n=109)	Ramipril (n=103)
Age (years)	54.5 (11.4)	52.4 (13.3)
Sex		
Women	29 (27%)	32 (31%)
Men	80 (73%)	71 (69%)
BMI (kg/m2)	29.5 (7.4)	29.3 (6.5)
Ethnic Origin		
White	88 (81%)	88 (85%)
Black	3 (3%)	5 (5%)
Asian	9 (8%)	4 (4%)
Other	9 (8%)	6 (6%)
Medical History		
Hypertension	101 (93%)	97 (94%)
Hyperlipidemia	74 (68%)	67 (65%)
Diabetes	42 (39%)	48 (47%)
Angina	15 (14%)	12 (12%)
PCI or CABG	13 (12%)	14 (14%)
Myocardial infarction	10 (9%)	6 (6%)
Peripheral vascular disease	4 (4%)	11 (11%)
Congestive heart failure	11 (10%)	2 (2%)
TIA or stroke	5 (5%)	4 (4%)
Cancer	24 (22%)	19 (18%)
Current Smoker	20 (18%)	16 (16%)
Primary Cause of renal disease		
Glomerulonephritis	24 (22%)	24 (23%)
Diabetes mellitus	19 (17%)	23 (22%)
Polycystic kidney disease	9 (8%)	10 (10%)
Hypertension	13 (12%)	6 (6%)
Other	36 (33%)	34 (33%)
Unknown	8 (7%)	6 (6%)
Type of donor		
Living	45 (41%)	47 (46%)
Deceased	64 (59%)	56 (54%)
Primary transplant	96 (88%)	89 (86%)
Immunosuppression		
Prednisone	101 (93%)	92 (89%)
Tacrolimus	59 (54%)	60 (58%)
Cyclosporine	40 (37%)	29 (28%)
Mycophenolate mofetil or sodium	83 (76%)	80 (78%)
Azathioprine	22 (20%)	12 (12%)

Sirolimus	7 (6%)	13 (13%)
Antihypertensive use		
Calcium channel blocker	63 (58%)	59 (57%)
Beta-blocker	59 (54%)	58 (56%)
Diuretic	34 (31%)	32 (31%)
Alpha-blocker	8 (7%)	6 (6%)
Vasodilator	1 (1%)	6 (6%)
Other drugs		
Aspirin	33 (30%)	32 (31%)
Clopidogrel	2 (2%)	3 (3%)
Warfarin	7 (6%)	8 (8%)
Statin	77 (71%)	66 (64%)
Fibrate	0	2 (2%)
Ezitimibe	3 (3%)	1 (1%)
Measured DTPA GFR (mL/min)	65.1 (27.6)	65.9 (25.0)
Corrected (mL/min/1.73m2)	58.6 (24.1)	59.8 (21.9)
Blood pressure		
Systolic blood pressure (mm Hg)	135 (17)	135 (16)
Diastolic blood pressure (mm Hg)	78 (10)	77 (9)
<130/80	32 (29%)	35 (34%)
Serum potassium (mmol/L)	4.3 (0.5)	4.3 (0.6)
Serum creatinine (umol/L)	142 (54)	138 (51)
Hemoglobin (g/L)	129 (17)	131 (14)
Proteinuria (mg per day)	400 (270-720)	430 (270-813)

Data are mean (SD), n (%), or median (IQR). PCI = percutaneous coronary intervention. CABG = coronary artery bypass grafting. DTPA GFR = glomerular filtration rate measured using 99mtechnetium-diethylene triamine pentacetate

Table adapted from Knoll et al. [12]

Table 4. Composite outcome results using the win ratio method versus a standard time-to-event analysis in the primary and extended trial.

	Win Ratio Approach		Time-to-event Analysis
	Win Ratio[a] (95% CI)	Inverse Win Ratio (95% CI)	Adjusted[b] Hazard Ratio[c] (95% CI)
Primary Trial (mean follow-up 41 mo.)			
Composite of death, ESRD, and doubling of serum creatinine	1.21 (0.55-2.95)	0.83 (0.40-1.68)	0.85 (0.41-1.77)
Extended Trial (mean follow-up 48 mo.)			
Composite of death, ESRD, and doubling of serum creatinine	1.02 (0.55-1.95)	0.98 (0.53-1.75)	0.97 (0.56-1.68)

[a] Win ratio greater than 1 indicates a positive treatment effect, while a win ratio less than 1 indicates a negative treatment effect.
[b] Cox proportional hazards were adjusted for the following variables: age (years), diabetes (present or absent), time post-transplantation (days), measured GFR (per mL/min/1.73 m^2), donor type (living or deceased), proteinuria (per mg/24 h), and serum creatinine (per μ mol/L).
[c]Adjusted Hazard ratio less than 1 indicates a positive treatment effect, while an adjusted hazard ratio greater than 1 indicates a negative treatment effect.
ESRD=end-stage renal disease, defined as either return to dialysis or repeat transplantation.

References

[1] Clayton PA, Lim WH, Wong G, Chadban SJ. Relationship between eGFR Decline and Hard Outcomes after Kidney Transplants. J Am Soc Nephrol 2016;27:3440–6. doi:10.1681/ASN.2015050524.

[2] Coresh J, Turin TC, Matsushita K, Sang Y, Ballew SH, Appel LJ, et al. Decline in Estimated Glomerular Filtration Rate and Subsequent Risk of End-Stage Renal Disease and Mortality. JAMA 2014;311:2518–31. doi:10.1001/jama.2014.6634.

[3] Inker LA, Lambers Heerspink HJ, Mondal H, Schmid CH, Tighiouart H, Noubary F, et al. GFR Decline as an Alternative End Point to Kidney Failure in Clinical Trials: A Meta-analysis of Treatment Effects from 37 Randomized Trials. Am J Kidney Dis 2014;64:848–59. doi:10.1053/j.ajkd.2014.08.017.

[4] R.R., Alloway, N.A., Pilch DC. Evidence-based medicine and clinical trials in renal transplantation - UpToDate. UpToDate 2015. http://www.uptodate.com/contents/evidence-based-medicine-and-clinical-trials-in-renal-transplantation (accessed July 18, 2016).

[5] Badve S V., Palmer SC, Hawley CM, Pascoe EM, Strippoli GFMM, Johnson DW. Glomerular filtration rate decline as a surrogate end point in kidney disease progression trials. Nephrol Dial Transplant 2016;31:1425–36. doi:10.1093/ndt/gfv269.

[6] Luo X, Qiu J, Bai S, Tian H. Weighted win loss approach for analyzing prioritized outcomes. Stat Med 2017. doi:10.1002/sim.7284.

[7] Pocock SJ, Ariti CA, Collier TJ, Wang D. The win ratio: A new approach to the analysis of composite endpoints in clinical trials based on clinical priorities. Eur Heart J 2012;33:176–82. doi:10.1093/eurheartj/ehr352.

[8] Busse JW, Bhandari M, Ferreira-González I, Montori VM, Guyatt GH. Use and Interpretation of Composite End Points in Orthopaedic Trials. J Bone Jt Surg 2012;94:65. doi:10.2106/JBJS.K.01287.

[9] Ferreira-González I, Alonso-Coello P, Solà I, Pacheco-Huergo V, Domingo-Salvany A, Alonso J, et al. [Composite endpoints in clinical trials]. Rev Española Cardiol 2008;61:283–90. doi:10.1016/S1885-5857(08)60116-4.

[10] Abdalla S, Montez-Rath ME, Parfrey PS, Chertow GM. The win ratio approach to analyzing composite outcomes: An application to the EVOLVE trial. Contemp Clin Trials 2016;48:119–24. doi:10.1016/j.cct.2016.04.001.

[11] Buyse M. Generalized pairwise comparisons of prioritized outcomes in the two-sample problem. Stat Med 2010;29:3245–57. doi:10.1002/sim.3923.

[12] Knoll GA, Fergusson D, Chasse M, Hebert P, Wells G, Tibbles LA, et al. Ramipril versus placebo in kidney transplant patients with proteinuria: a multicentre, double-blind, randomised controlled trial. Lancet Diabetes Endocrinol 2016;4:318–26. doi:10.1016/S2213-8587(15)00368-X.

[13] Knoll GA, Cantarovitch M, Cole E, Gill J, Gourishankar S, Holland D, et al. The Canadian ACE-inhibitor trial to improve renal outcomes and patient survival in kidney transplantation - Study design. Nephrol Dial Transplant 2008;23:354–8. doi:10.1093/ndt/gfm574.

[14] Carpenter J, Bithell J. Bootstrap confidence intervals: when, which, what? A

practical guide for medical statisticians. Stat Med 2000;19:1141–64. doi:10.1002/(SICI)1097-0258(20000515)19:9<1141::AID-SIM479>3.0.CO;2-F.

[15] Inrig JK, Califf RM, Tasneem A, Vegunta RK, Molina C, Stanifer JW, et al. The landscape of clinical trials in nephrology: A systematic review of clinicaltrials.gov. Am J Kidney Dis 2014;63:771–80. doi:10.1053/j.ajkd.2013.10.043.

[16] Hiremath S, Fergusson DA, Fergusson N, Bennett A, Knoll GA. Renin-Angiotensin System Blockade and Long-term Clinical Outcomes in Kidney Transplant Recipients: A Meta-analysis of Randomized Controlled Trials. Am J Kidney Dis 2016:5–12. doi:10.1053/j.ajkd.2016.08.018.

[17] Valsecchi MG, Miller ME, Hui SL. Evaluation of long-term survival: use of diagnostic and robust estimators with Cox's proportional hazards model. Stat Med 1996;15:2763–80. doi:3.0.CO;2-O.

[18] Royston P, Parmar MKB. Flexible parametric proportional-hazards and proportional-odds models for censored survival data, with application to prognostic modelling and estimation of treatment effects. Stat Med 2002;21:2175–97. doi:10.1002/sim.1203.

CHAPTER 5: DISCUSSION

Summary

This thesis project investigated the use of alternative graft function endpoints and analysis techniques within the setting of a kidney transplant trial. The current issues regarding kidney transplant trial feasibility and interpretation were first discussed in Chapter 1. Detailed background information regarding the field of kidney transplantation trials and the ACE trial specifically was presented in Chapter 2. A secondary analysis of the ACE trial using alternative eGFR decline endpoints was conducted and the impact of these endpoints on ACE trial results, interpretations, and conclusions was discussed (Chapter 3, Manuscript 1). Next, an alternative analysis technique, the win ratio approach, was applied to the ACE trial in order to determine the statistical impact and whether this approach alters the results, interpretations, and conclusions of ACE trial (Chapter 4, Manuscript 2). This discussion will highlight novel findings, strengths and limitations, dissemination, and future directions of this project.

Novel Findings

To the best of our knowledge, this is the first-time endpoints of lesser eGFR decline (e.g. ≥40%, ≥30%, or ≥20%) have been applied and investigated in a kidney transplant trial. The potential of these endpoints to increase the short-term event rate of kidney trials seem promising however, statistical power can only be increased if these lesser eGFR decline endpoints are in fact valid surrogates and maintain similar treatment effects to a

doubling of serum creatinine. Within the ACE trial, ACE-inhibitor treatment (ramipril) was not associated with any statistically significant differences on any of the secondary composites. The use of composites incorporating eGFR declines of ≥40%, ≥30%, or ≥20%, produced similar results as the original trial composite– reconfirming ACE trial results, interpretations, and conclusions.

The win ratio approach for unequal endpoints was developed in cardiology trials (7) and later applied to the EVOLVE trial in end-stage-renal disease (ESRD) (9). This approach had yet to be applied in kidney transplant trials, where composites of unequal endpoints are commonly used. The application of the win ratio approach to the ACE trial's primary composite did not alter the results, interpretations, or conclusions. However, this approach does maximize data from endpoints of higher clinical significance and reduces the probability of a less significant endpoint masking important treatment effects. Further, the win ratio approach does not rely on unrealistic statistical assumptions, such as proportional hazards.

Limitations

There are several limitations of this thesis project that are worth discussing. These limitations have been previously discussed in Chapter 3 (Manuscript 1) and Chapter 4 (Manuscript 2) and will be summarized in this section.

Limitations of the ACE trial secondary analysis (Manuscript 1) include the inability to confirm events of percentage eGFR decline, as these events were determined post-hoc. There may be limitations with respect to generalizability as the ACE trial enrolled high risk transplant recipients with proteinuria. Furthermore, within the ACE trial, ACE-inhibitor treatment was expected to have an impact of kidney function decline however, that may not be case for other interventions. Depending on the treatment being assessed, graft function decline endpoints may be inappropriate. Additionally, the lack of any clear treatment effect within the ACE trial made it impossible to assess whether treatment effects on the doubling of serum creatinine endpoint were mirrored in the lesser eGFR percentage decline endpoints.

Limitations of the win ratio approach utilized in Manuscript 2 include the inability to perform apriori statistical power calculations for the win ratio. However, if the win ratio approach is utilized in complementation with a standard time-to-event analysis, a priori power calculations can be based on the time-to-event analysis. An additional limitation of this approach is the inability to easily control for covariates yet, with randomization this should not be pertinent. Further, the win ratio approach can be stratified on factors believed to be significant as illustrated in the EVOLVE trial (9).

Future Directions

The next steps of this research include an application of the win ratio approach to the composites incorporating lesser eGFR declines (≥40%, ≥30%, or ≥20%) that were constructed in the secondary analysis (Manuscript 1). Based on the results of this secondary analysis, there appears to significant concerns of disproportionality as these lesser eGFR declines seem to drive the composite outcome and overpower the other components of death and ESRD. Our secondary analysis results indicated that within the ACE trial, if a time-to-event composite of ≥30% eGFR decline, ESRD, and death were used, more than 75% of the information would stem from the ≥30% eGFR component. The win ratio approach offers the ability reduce the impact of the eGFR decline component and maximize the information derived from ESRD and death components. If kidney transplant trials begin to adopt the use of lesser percentage eGFR decline endpoints within their composites, the win ratio approach may be highly useful. This study will offer crucial guidance and information on this developing topic.

Dissemination

The concept of this thesis project was presented at the annual uOttawa School of Epidemiology and Public Health Student Research Day in March, 2016. In the following year, preliminary results of this thesis project were presented at the uOttawa School of Epidemiology and Public Health, Student Research Day in March, 2017. Additionally, preliminary results were presented at the OHRI Centre for Transfusion Research meeting

in April, 2017. I plan on presenting results from this thesis project at several upcoming conferences in nephrology, kidney transplantation, and clinical trials.

This thesis project has produced at least 2 manuscripts that have been, or will be, submitted for publication in peer-reviewed journals in 2017. An additional third manuscript is currently being drafted:

1) NA Fergusson, T Ramsay, M Chassé, SW English, GA Knoll. *The impact of using alternative graft function endpoints in kidney transplantation trials: A secondary analysis of the ACE trial*

2) NA Fergusson, T Ramsay, M Chassé, SW English, GA Knoll. *The win ratio approach for unequal composite endpoints: An application to the ACE trial in kidney transplantation*

3) NA Fergusson, T Ramsay, M Chassé, SW English, GA Knoll. *The win ratio approach for composites using eGFR decline as an endpoint* (currently being drafted)

Conclusion

In conclusion, alternative endpoints of lesser eGFR decline (e.g. ≥40%, ≥30%, or ≥20%) did noticeably increase the event rate of the ACE trial's primary composite but, they did not alter ACE trial results, interpretations, or conclusions. In all composites assessed, ramipril treatment was not associated with any statistically significant differences compared to placebo. Additionally, the use of an alternative analysis technique, the win ratio approach, when applied to the ACE trial's primary composite outcome did not alter trial results, interpretations, or conclusions. However, this approach did lessen concerns of unequal composite endpoints by maximizing data from endpoints of higher clinical significance and reducing the probability of a less significant endpoint masking significant treatment effects. As trials in kidney transplantation continue exploring the use of lesser eGFR decline endpoints, the win ratio approach may become a valuable analysis tool.

APPENDICES

Appendix 1- Original Publication of the ACE Trial

Additional File 1: The following is the original publication of the ACE trial in The Lancet, Diabetes & Endocrinology.

Reference:

Knoll GA, Fergusson D, Chasse M, Hebert P, Wells G, Tibbles LA, et al. Ramipril versus placebo in kidney transplant patients with proteinuria: a multicentre, double-blind, randomised controlled trial. The Lancet Diabetes & Endocrinology. 2016;4(4):318–26

Ramipril versus placebo in kidney transplant patients with proteinuria: a multicentre, double-blind, randomised controlled trial

Greg A Knoll, Dean Fergusson*, Michaël Chassé, Paul Hebert, George Wells, Lee Anne Tibbles, Darin Treleaven, David Holland, Christine White, Norman Muirhead, Marcelo Cantarovich, Michel Paquet, Bryce Kiberd, Sita Gourishankar, Jean Shapiro, Ramesh Prasad, Edward Cole, Helen Pilmore, Valerie Cronin, Debora Hogan, Tim Ramsay, John Gill*

Summary

Background Angiotensin-converting enzyme inhibitors have been shown to reduce the risk of end-stage renal disease and death in non-transplant patients with proteinuria. We examined whether ramipril would have a similar beneficial effect on important clinical outcomes in kidney transplant recipients with proteinuria.

Methods In this double-blind, placebo-controlled, randomised trial, conducted at 14 centres in Canada and New Zealand, we enrolled adult renal transplant recipients at least 3-months post-transplant with an estimated glomerular filtration rate (GFR) of 20 mL/min/1·73m² or greater and proteinuria 0·2 g per day or greater and randomly assigned them to receive either ramipril (5 mg orally twice daily) or placebo for up to 4 years. Patients completing the final 4-year study visit were invited to participate in a trial extension phase. Treatment was assigned by centrally generated randomisation with permuted variable blocks of 2 and 4, stratified by centre and estimated GFR (above or below 40 mL/min/1·73 m²). The primary outcome was a composite consisting of doubling of serum creatinine, end-stage renal disease, or death in the intention-to-treat population. The principal secondary outcome was the change in measured GFR. We ascertained whether any component of the primary outcome had occurred at each study visit (1 month and 6 months post-randomisation, then every 6 months thereafter). This trial is registered with ISRCTN, number 78129473.

Findings Between Aug 23, 2006, and March 28, 2012, 213 patients were randomised. 109 were allocated to placebo and 104 were allocated to ramipril, of whom 109 patients in the placebo group and 103 patients in the ramipril group were analysed and the trial is now complete. The intention to treat population (placebo n=109, ramipril n=103) was used for the primary analysis and the trial extension phase analysis. The primary outcome occurred in 19 (17%) of 109 patients in the placebo group and 14 (14%) of 103 patients in the ramipril group (hazard ratio [HR] 0·76 [95% CI 0·38–1·51]; absolute risk difference –3·8% [95% CI –13·6 to 6·1]). With extended follow-up (mean 48 months), the primary outcome occurred in 27 patients (25%) in the placebo group and 25 (24%) patients in the ramipril group (HR 0·96 [95% CI 0·55–1·65]); absolute risk difference: –0·5% (95% CI –12·0 to 11·1). There was no significant difference in the rate of measured GFR decline between the two groups (mean difference per 6-month interval: –0·16 mL/min/1·73m² (SE 0·24); p=0·49). 14 (14%) of patients died in the ramipril group and 11 (10%) in the placebo group, but the difference between groups was not statistically significant (HR 1·45 [95% CI 0·66 to 3·21]). Adverse events were more common in the ramipril group (39 [38%]) than in the placebo group (24 [22%]; p=0·02).

Interpretation Treatment with ramipril compared with placebo did not lead to a significant reduction in doubling of serum creatinine, end-stage renal disease, or death in kidney transplant recipients with proteinuria. These results do not support the use of angiotensin-converting enzyme inhibitors with the goal of improving clinical outcomes in this population.

Funding Canadian Institutes of Health Research.

Introduction

Although a major therapeutic advance, kidney transplantation leaves many patients with complications of chronic kidney disease such as proteinuria, hypertension, and anaemia.[1] Depending on the definition, proteinuria occurs in up to 45% of kidney transplant recipients and is strongly associated with graft loss, cardiovascular disease, and death.[2] In non-transplant patients with proteinuria, treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) is recommended because these drugs reduce the risk of clinically important outcomes, such as doubling of serum creatinine, kidney failure, or death.[3]

In kidney transplant recipients, evidence supporting the use of ACE inhibitors or ARBs for proteinuric chronic kidney disease is lacking. Two observational studies have shown conflicting results, with one showing clear benefit[4] and the other finding no benefit[5] with use of ACE inhibitors or ARBs with respect to patient or graft survival. Systematic reviews reported that ACE inhibitors or ARBs were associated with a significant reduction in

Lancet Diabetes-Endocinol 2015

Published Online
October 23, 2015, 2015
http://dx.doi.org/10.1016/S2213-8587(15)00368-X

See Online/Comment
http://dx.doi.org/10.1016/S2213-8587(15)00415-5

*Contributed equally

Department of Medicine, University of Ottawa, Ottawa, ON, Canada (Prof G A Knoll MD, Prof D Fergusson PhD); **Clinical Epidemiology Program, Ottawa Hospital Research Institute and Department of Epidemiology and Community Medicine, University of Ottawa, Ottawa, ON, Canada** (Prof G A Knoll, Prof D Fergusson, M Chassé MD, Prof G Wells PhD, D Hogan, T Ramsay PhD); **Kidney Research Centre, Ottawa Hospital Research Institute and University of Ottawa, Ottawa, ON, Canada** (Prof G A Knoll, V Cronin); **Centre de Recherche, Centre Hospitalier de l'Université de Montréal, Montréal, QC, Canada** (Prof P Hebert MD); **Division of Nephrology, Department of Medicine, University of Calgary, Calgary, AB, Canada** (L A Tibbles MD); **Division of Nephrology, Department of Medicine, McMaster University, Hamilton, ON, Canada** (D Treleaven MD); **Division of Nephrology, Department of Medicine, Queen's University, Kingston, ON, Canada** (Prof D Holland MD, C White MD); **Division of Nephrology, Department of Medicine, Western University, London, ON, Canada** (Prof N Muirhead MD); **Division of Nephrology, Department of Medicine, McGill University, Montreal, QC, Canada** (Prof M Cantarovich MD); **Division of Nephrology, Department of Medicine, Centre Hospitalier de l'Université de Montréal,**

Montréal, QC, Canada (M Paquet MD)**; Division of Nephrology, Department of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada** (Prof B Kiberd MD)**; Division of Nephrology, Department of Medicine, University of Alberta, Edmonton, AB, Canada** (S Gourishankar MD)**; Division of Nephrology, Department of Medicine, Vancouver General Hospital, Vancouver, BC, Canada** (Prof J Shapiro MD)**; Division of Nephrology, Department of Medicine, St Michael's Hospital, Toronto, ON, Canada** (R Prasad MD)**; Division of Nephrology, Department of Medicine, University Health Network, Toronto, ON, Canada** (Prof E Cole MD)**; Department of Renal Medicine, Auckland City Hospital and Department of Medicine, Auckland University, Auckland, New Zealand** (H Pilmore MD)**; and Division of Nephrology, Department of Medicine, St Paul's Hospital, Vancouver, BC, Canada** (Prof J Gill MD)

Correspondence to:
Prof Greg Knoll,
The Ottawa Hospital,
Ottawa, ON K1H 7W9, Canada
gknoll@toh.on.ca

Research in context

Evidence before this study

We searched MEDLINE (1966, and February, 2005) and the Cochrane Library (2004, issue 3) for literature examining renin–angiotensin system blockade in kidney transplantation with the following search terms: "kidney transplantation", "Angiotensin II", "Receptors, Angiotensin" and "Angiotensin Converting Enzyme Inhibitors" as well as the names of all known ACE inhibitors and angiotensin II receptor blockers (eg, ramipril, losartan), and identified 22 randomised controlled trials and no systematic reviews or meta-analyses. 13 of 22 trials were crossover studies and nine were parallel-group design. The trials were small with a median sample size of 17 for the crossover trials and 37 for the parallel group trials. The treatment duration was short with a median of only 4 weeks for the crossover trials and 12 months for the parallel-group trials. Most of the studies were designed to measure changes in surrogate markers such as blood pressure or haemoglobin; none were designed to assess important clinical outcomes such as transplant failure or death. We published this systematic review in 2007 (Hiremath et al, 2007). Since then, two other systematic reviews have been published. One review examined early (<12 weeks post-transplant) use of ACE inhibitors (Jennings et al, 2008). They concluded that ACE-inhibitors were safe soon after transplant, but no long-term data were available for clinical outcomes such as patient or graft survival. The other review was done by the Cochrane Renal Group and examined all classes of antihypertensives in kidney transplantation (Cross et al, 2009). Similar to our review, they noted that ACE inhibitors significantly reduced proteinuria, but also reduced glomerular filtration rate and haemoglobin. Patient or graft survival was reported in only five trials and there were too few events (eg, only five deaths across four trials) to draw any firm conclusions. Thus, the trials so far have been restricted by small sample size or short duration, have focused on surrogates rather than clinically important endpoints (eg, transplant failure), or have not studied a population likely to benefit from ACE inhibitors such as those with proteinuria. These findings emphasise the need for long-term trials examining clinically relevant patient-important outcomes in kidney transplant recipients with proteinuria.

Added value of this study

Our study is the first randomised trial, to our knowledge, specifically designed to assess the effect of ACE inhibitors on long-term, patient-important outcomes in kidney transplant recipients with proteinuria. We have shown that treatment with ramipril does not reduce the risk of doubling serum creatinine, end-stage renal disease, or death in this population. Even with extended follow-up past 4 years, the absolute difference between the groups was only 0·5%, suggesting that if any effect is present, its magnitude is likely small. Another important finding was the absence of benefit of ramipril with respect to preservation of GFR.

Implications of all the available evidence

Despite robust evidence that ACE inhibitors can reduce the risk of clinically important outcomes in non-transplant patients with proteinuria, our findings suggest that these drugs do not have similar efficacy in the kidney transplant population. Differences could be due to the fact that post-transplant proteinuria is multifactorial in nature, whereas non-transplant trials included more homogenous populations (eg, all patients with diabetes). Future research might need to focus on select transplant patients, such as those with recurrent glomerulonephritis, to determine if ACE inhibitors have a role in managing post-transplant proteinuria in certain patient populations.

proteinuria, glomerular filtration rate (GFR), and haemoglobin concentration in kidney transplant patients, but there was insufficient evidence regarding the effect on patient or graft survival.[6–8] To determine if ACE inhibitors are effective at improving important clinical outcomes, we aimed to compare ramipril with placebo in kidney transplant recipients with proteinuria.

Methods

Study design and participants

In this multicentre, double-blind, randomised, placebo-controlled trial conducted at 14 academic centres in Canada and New Zealand, we initially included renal transplant recipients who were at least 6 months post-transplantation with an estimated GFR (eGFR) between 20 mL/min/1·73m² and 55 mL/min/1·73m² (calculated with the Modification of Diet in Renal Disease study equation) and proteinuria 0·2 g per day or greater. To increase enrolment, the protocol was amended on Feb 25, 2008 (58 participants had been randomised up to this point) to include recipients who were at least 3 months post-transplant with an eGFR 20 mL/min/1·73m² or greater. We excluded patients for the following reasons: unable to provide informed consent; younger than 18 years old; pregnant; angio-oedema from an ACE inhibitor or ARB or other known reaction to an ACE inhibitor (such as rash, neutropenia, or cough); serum potassium greater than 5·5 mmol/L on two or more occasions in the preceding 3 months for those not on an ACE inhibitor or ARB or serum potassium greater than 5·9 mmol/L on two or more occasions in the preceding 3 months for those on an ACE inhibitor or ARB; left ventricular dysfunction that required an ACE inhibitor or an ARB; severe comorbidity with life expectancy less than 3 months; immunosuppressive drug that changed within 3 months before study entry; acute coronary syndrome, stroke, or transient ischaemic attack in the 3 months before the study entry; currently on an ACE inhibitor or an

ARB and patient or physician unwilling to stop the drug; acute rejection episode in the 3 months before study entry; and currently taking four or more antihypertensives and have an average blood pressure over three previous visits of greater than 150/100.

Trial conduct and participant safety were overseen by a data safety monitoring board that reviewed the data every 6 months. Clinical coordination, data management, and statistical analyses were done by the Clinical Epidemiology Program at the Ottawa Hospital Research Institute (Ottawa, ON, Canada). The trial protocol is published[9] and available in the appendix. The study was approved by the local research ethics board at every participating institution and all patients gave written informed consent. The study was designed by the authors, who vouch for the accuracy and completeness of the data and for the fidelity of this report to the study protocol. No one who is not an author contributed to the writing of the report. The trial and is registered with ISRCTN, number 78129473.

Randomisation and masking

Treatment was randomly assigned (1:1) by centrally generated randomisation with permuted variable blocks of 2 and 4, stratified by centre and eGFR (above or below 40 mL/min/1·73 m²). Treatment allocation was concealed from physicians, nurses, investigators, and research staff to prevent selection bias. An independent statistician prepared the randomisation schedule. Only the independent statistician and a designated research pharmacist at the coordinating centre had knowledge of the randomisation schedule. We reencapsulated ramipril to be identical in appearance to the placebo capsule. Physicians, nurses, investigators, and research staff were masked to administered treatments.

Procedures

Eligible patients entered a 2-week open-label trial of oral ramipril 5 mg daily. If tolerated, we randomised patients to receive ramipril or placebo capsule for up to 4 years (oral 5 mg daily for 2 weeks and then 5 mg oral twice daily thereafter). Study visits occurred at randomisation, 1 month, 6 months, and every 6 months thereafter until trial completion. At every visit, haemoglobin, serum creatinine, and serum potassium concentrations were measured and we ascertained whether any component of the primary outcome had occurred. At each study visit (except the 1 month visit), we measured GFR with radiolabelled ^{99m}technetium-DTPA (^{99m}Tc-DTPA); 24-h urine protein; and quality of life score with the short-form 36. We recommended that blood pressure and dyslipidaemia be managed according to published guidelines.[10,11] Specifically, the target blood pressure for all participants was less than 130/80. Investigators could use any drug to control blood pressure except those that blocked the renin–angiotensin system (eg, ACE inhibitors). We also recommended that centres follow usual care practices for the management of immunosuppression, diabetes, and cardiovascular risk reduction strategies such as weight loss, smoking cessation, and aspirin use. We ascertained whether patients should be in the per-protocol analysis based on the medications recorded on the case report forms—for example, if the study drug was stopped and they were placed on an ACE inhibitor by their physician, they were excluded from the per-protcol analysis

We invited all patients that completed the final 4-year study visit to participate in a non-prespecified trial extension phase, in which masking was maintained and patients could be followed for up to a maximum of 48 additional months. For this phase of the trial, a second informed consent was signed. Assessments were done at 6-month intervals to determine the primary outcome had occurred. Serum creatinine values during this phase were obtained from medical records. Study-mandated laboratory testing and measured GFR were not done during the trial extension phase. Apart from components of the primary outcome, adverse events were not recorded during the extension phase.

See Online for appendix

Outcomes

The primary outcome was a composite measure consisting of a doubling of serum creatinine, end-stage renal disease, or death. Doubling of serum creatinine was confirmed by two consecutive tests at least 4 weeks apart at our central laboratory. End-stage renal disease was defined as the date of repeat kidney transplantation or initiation of dialysis. The principal secondary outcome was the change in measured GFR (^{99m}Tc-DTPA).[12] Other secondary outcomes included quality of life, proteinuria, blood pressure, cardiovascular events, admissions to hospital, hyperkalaemia, and haemoglobin concentration.

Statistical analysis

We estimated that 528 patients would be needed for the trial to have 80% power to detect a 12% absolute difference in the occurrence of the primary endpoint at 4 years. The underlying assumptions for this estimate are reviewed in detail in our trial protocol.[9] Briefly, we assumed the following: a placebo group 4-year survival (freedom from the primary endpoint) of 70%; a 2-year accrual period; a 4-year study duration; a two-sided α=0·05; and a 5% non-compliance rate. The original trial duration was 4 years with follow-up ranging between 2 years and 4 years depending on time of randomisation. Because of slower than expected recruitment, we extended follow-up to 4 years for all participants to increase statistical power.

We analysed the primary trial according to the prespecified plan in the trial protocol (appendix) and included all data up to 48 months post-randomisation. In the extension phase, the primary trial analysis was supplemented with the use of all available follow-up data. We did all analyses, including the analysis of the extension

phase, based on the intention-to-treat principle. We used a non-parametric log-rank test, stratified by centre, to compare the time to occurrence of the primary outcome between ramipril and placebo groups. We constructed Kaplan-Meier survival curves to visually assess the incidence of the primary composite outcome and mortality by group over time. A Cox proportional hazards regression model was used for the primary outcome to adjust for important risk factors: age (years), diabetes (present or absent), time post-transplantation (days), measured GFR (per mL/min/1·73m²), donor type (living or deceased), proteinuria (per mg/24 h), and serum creatinine (per µmol/L). Cox proportional hazard ratios with corresponding 95% CIs were calculated for each of the individual items of the primary outcome. The secondary outcomes were compared with mean differences for continuous outcomes or risk ratios for dichotomous variables. The continuous secondary endpoints (measured GFR, serum creatinine, blood pressure, proteinuria, and haemoglobin) were also analysed with generalised linear regression models to assess differences between groups and compare changes over time. Exploratory analyses were done on the following clinically important subgroups: age, sex, diabetes, blood pressure, measured GFR, and proteinuria. We also did a per-protocol analysis that only included patients who remained on their allocated treatment throughout the entire study period. All tests of statistical inference reflect a 2-sided α=0·05. Analyses were done with SAS (version 9.4; SAS Institute, Cary, NC, USA).

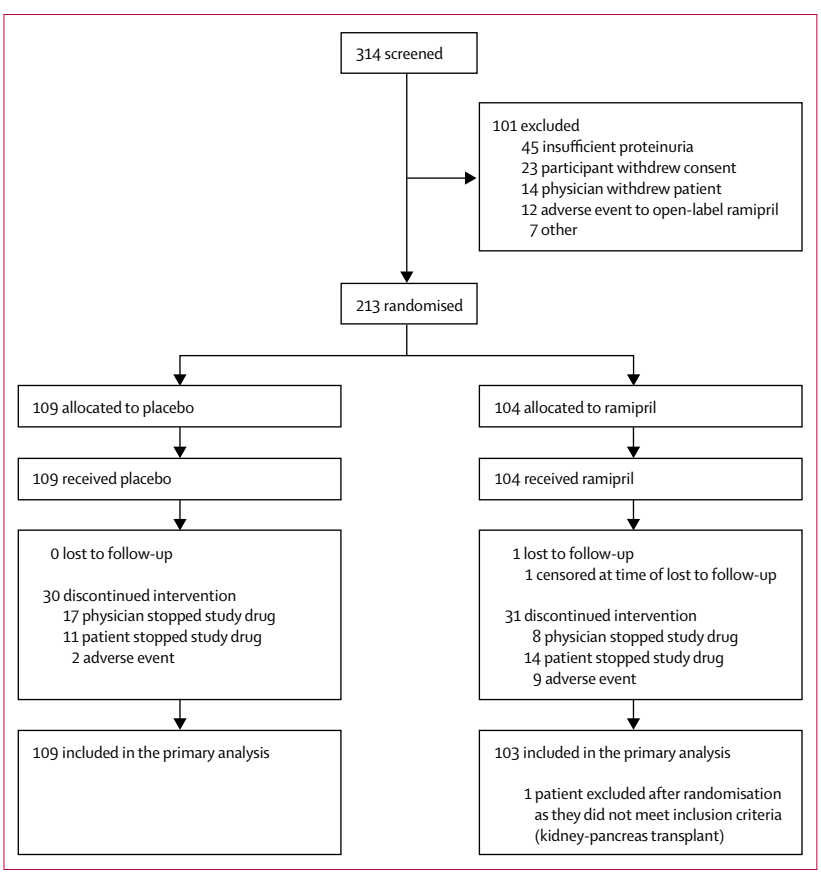


Figure 1: **Trial profile**

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 23, 2006, and March 28, 2012, 213 patients were randomised. 109 were allocated to placebo and 104 were allocated to ramipril, of whom 109 and 103 patients, respectively, were analysed. The trial was terminated due to resource constraints. The main reasons for screen failures were insufficient proteinuria (n=45) and patient (n=23) or physician (n=14) refusal to continue with the study (figure 1). In the extension phase, 43 (80%) of 54 eligible patients in the placebo and 38 (78%) of 49 eligible patients in the ramipril group participated. Mean follow-up was 41 months (range 1–48) in the primary trial and 48 months (1–84) in the extension phase. There were 8826 patient-months of follow-up time in the primary trial and 10 176 patient-months by the end of the extension phase.

The groups were well balanced at baseline (table 1). A third of patients were women and 176 (83%) were white. Hypertension was present in 198 (93%), 90 (42%) had diabetes, and proteinuria was similar between the groups. Dose reductions occurred in 14 (14%) of 103 in the ramipril group and seven (6%)of 109 in the placebo group (p=0·11).

In the ramipril group, 31 patients permanently discontinued the study drug a mean of 410 days (SD 140) post-randomisation, with 15 patients stopping at a timetpoint beyond year 1 (14 patients' decision, 8 physicians' decision, 9 adverse events). In the placebo group, 30 patients permanently discontinued study drug a mean of 651 days (SD 389) post-randomisation, with 23 patients stopping beyond year 1 (11 patients' decision, 17 physician's decision, 2 adverse events; appendix p 4).

The primary endpoint occurred in 19 patients (17%) in the placebo group and 14 patients (14%) in the ramipril group (hazard ratio [HR] 0·76 [95% CI 0·38–1·51]; table 2) The time to occurrence of the primary outcome did not differ significantly between the groups (log-rank p=0·44; appendix p 7). After adjustment, the effect of ramipril on the primary endpoint was similar (adjusted HR 0·85 [95% CI 0·41–1·77]; p=0·67). The absolute risk difference of the primary outcome was –3·8% ([95% CI –13·6 to 6·1]; appendix p 8).

In the trial extension phase, the primary outcome occurred in 27 patients (25%) in the placebo group and 25 patients (24%) in the ramipril group (HR 0·96 [95% CI 0·55–1·65]; table 2). The time to occurrence of the primary outcome did not differ significantly between the

groups (log-rank p=0·89; figure 2. The absolute risk difference of the primary endpoint was –0·5% (95% CI –12·0 to 11·1; appendix p 8).There was no difference in any component of the primary outcome when death was treated as a competing event (appendix p 5).

Overall death did not differ significantly between groups during the primary trial (HR 1·97 [95% CI 0·66 to 5·89]; p=0·22; table 2; appendix p 9) or the extension phase (1·45 [0 ·66 to 3·21]; p=0·36; table 2; appendix p 10). There was no difference in the causes of death between the groups (appendix p 6). Adverse events were more common in the ramipril group (39 [38%]) than in the placebo group (24 [22%]; p=0·02; table 3) and more patients in the ramipril group discontinued study drug because of an adverse event (9 [9%] in the ramipirl group vs 2 [2%] in the placebo group; p=0·03).

The per-protocol analysis of the primary outcome, which only included patients who remained on their allocated treatment, showed no difference between ramipril and placebo (adjusted HR 0·80 [95% CI 0·32 to 1·99]; p=0·63). No interactions were seen between ramipril and clinically important subgroups (age, sex, diabetes, blood pressure, measured GFR, and proteinuria) with respect to the occurrence of the primary endpoint (appendix p 11).

There was a significant decline in measured GFR in both group (–1·19 mL/min/1·73 m² per 6-months in the ramipril group and –1·03 mL/min/1·73 m² per 6-months in the placebo), but there was no significant between-group difference (mean difference –0·16 mL/min/1·73 m² [SE 0·24]; p=0·49)

There was a decline in systolic and diastolic blood pressure for both groups (appendix p 13), but the decline was greater for the ramipril group than for the placebo

	Placebo (n=109)	Ramipril (n=103)
Age (years)	54·5 (11·4)	52·4 (13·3)
Sex		
Women	29 (27%)	32 (31%)
Men	80 (73%)	71 (69%)
BMI (kg/m²)	29·5 (7·4)	29·3 (6·5)
Ethnic origin		
White	88 (81%)	88 (85%)
Black	3 (3%)	5 (5%)
Asian	9 (8%)	4 (4%)
Other	9 (8%)	6 (6%)
Medical history		
Hypertension	101 (93%)	97 (94%)
Hyperlipidaemia	74 (68%)	67 (65%)
Diabetes	42 (39%)	48 (47%)
Angina	15 (14%)	12 (12%)
PCI or CABG	13 (12%)	14 (14%)
Myocardial infarction	10 (9%)	6 (6%)
Peripheral vascular disease	4 (4%)	11 (11%)
Congestive heart failure	11 (10%)	2 (2%)
TIA or stroke	5 (5%)	4 (4%)
Cancer	24 (22%)	19 (18%)
Current smoker	20 (18%)	16 (16%)
Primary cause of renal disease		
Glomerulonephritis	24 (22%)	24 (23%)
Diabetes mellitus	19 (17%)	23 (22%)
Polycystic kidney disease	9 (8%)	10 (10%)
Hypertension	13 (12%)	6 (6%)
Other	36 (33%)	34 (33%)
Unknown	8 (7%)	6 (6%)
Type of donor		
Living	45 (41%)	47 (46%)
Deceased	64 (59%)	56 (54%)
Primary transplant	96 (88%)	89 (86%)
Immunosuppression		
Prednisone	101 (93%)	92 (89%)
Tacrolimus	59 (54%)	60 (58%)
Cyclosporine	40 (37%)	29 (28%)
Mycophenolate mofetil or sodium	83 (76%)	80 (78%)
Azathioprine	22 (20%)	12 (12%)
Sirolimus	7 (6%)	13 (13%)
(Table 1 continues in next column)		
(Continued from previous column)		
Antihypertensive use		
Calcium channel blocker	63 (58%)	59 (57%)
Beta-blocker	59 (54%)	58 (56%)
Diuretic	34 (31%)	32 (31%)
Alpha-blocker	8 (7%)	6 (6%)
Vasodilator	1 (1%)	6 (6%)
Other drugs		
Aspirin	33 (30%)	32 (31%)
Clopidogrel	2 (2%)	3 (3%)
Warfarin	7 (6%)	8 (7.8)
Statin	77 (71%)	66 (64%)
Fibrate	0	2 (2%)
Ezitimibe	3 (3%)	1 (1%)
Measured DTPA GFR (mL/min)	65·1 (27·6)	65·9 (25·0)
Corrected (mL/min/1·73m²)	58·6 (24·1)	59·8 (21·9)
Blood pressure		
Systolic blood pressure (mm Hg)	135 (17)	135 (16)
Diastolic blood pressure (mm Hg)	78 (10)	77 (9)
<130/80	32 (29%)	35 (34%)
Serum potassium (mmol/L)	4·3 (0·5)	4·3 (0·6)
Serum creatinine (umol/L)	142 (54)	138 (51)
Haemoglobin (g/L)	129 (17)	131 (14)
Proteinuria (mg per day)	400 (270–720)	430 (270–813)

Data are mean (SD), n (%), or median (IQR). PCI=percutaneous coronary intervention. CABG=coronary artery bypass grafting. DTPA GFR=glomerular filtration rate measured using ^{99m}technetium-diethylene triamine pentacetate.

Table 1: **Baseline characteristics**

	Primary trial (n=212)			Trial extension phase (n=212)		
	Placebo (n=109)	Ramipril (n=103)	Hazard ratio (95% CI)	Placebo (n=109)	Ramipril (n=103)	Hazard ratio (95% CI)
Follow-up (months)	41·8 (11·2)	41·4 (11·1)	..	48·6 (18·3)	47·8 (17·7)	..
Primary endpoint*: doubling of serum creatinine, return to dialysis, repeat transplantation or death	19 (17%)	14 (14%)	0·76 (0·38–1·51)	27 (25%)	25 (24%)	0·96 (0·55–1·65)
Components of primary endpoint†						
Doubling of serum creatinine	9 (8%)	3 (3%)	0·35 (0·09–1·30)	11 (10%)	9 (9%)	0·82 (0·34–1·99)
Return to dialysis	6 (6%)	3 (3%)	0·45 (0·11–1·82)	8 (7%)	4 (4%)	0·52 (0·16–1·73)
Repeat transplantation	0	0	..	0	0	
Death	4 (4%)	8 (8%)	2·14 (0·64–7·13)	8 (7%)	12 (12%)	1·56 (0·64–3·84)
Overall graft failure‡	9 (8%)	6 (6%)	0·67 (0·24–1·90)	12 (11%)	11 (11%)	0·94 (0·41–2·13)
Overall death¶	5 (5%)	9 (9%)	1·97 (0·66–5·89)	11 (10%)	14 (14%)	1·45 (0·66–3·21)

Data are n (%), unless otherwise stated. *Only the first event per patient is included in the composite primary endpoint. †Components of primary endpoint included only if it was the first event. ‡Includes any participant that had allograft failure (repeat transplantation or return to dialysis) as a primary endpoint as well as those with graft failure after doubling of serum creatinine. ¶Includes any participant that died and was counted as a primary endpoint as well as those who died after they had doubling of serum creatinine, repeat transplantation, or return to dialysis.

Table 2: **Clinical outcomes**

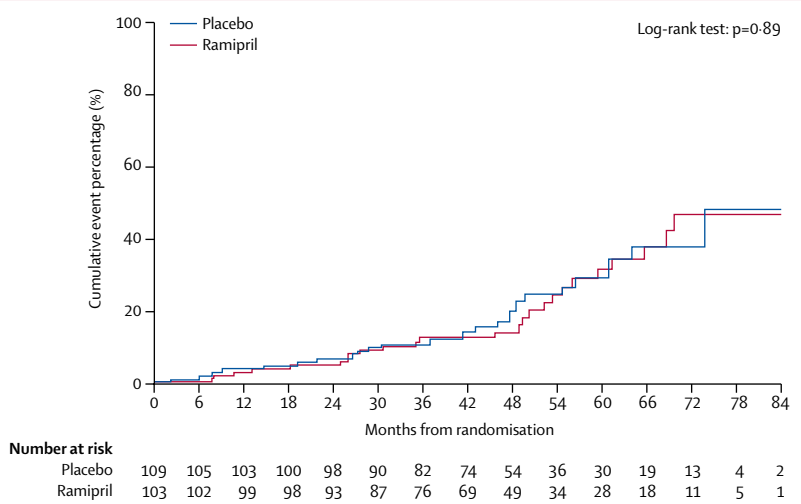


Figure 2: **Time to the primary outcome of doubling serum creatinine, end-stage renal disease or death during the extension phase of the study**

	Placebo (n=109)	Ramipril (n=103)	p value
Total	24 (22%)	39 (38%)	0·02
Angioedema	0	1 (1%)	0·49
Cough	0	4 (4%)	0·05
Hyperkalemia*	1 (1%)	5 (5%)	0·11
Anemia*	22 (20%)	25 (24%)	0·51
Other	1 (1%)	4 (4%)	0·20

*Hyperkalemia defined as serum potassium ≥6·0 mmol/L; anaemia defined as haemoglobin ≤100 g/L.

Table 3: **Adverse events**

group: the mean between-group difference for systolic blood pressure was –5·3 mm Hg (SE 1·7; p=0·001), and for diastolic blood pressure, it was –3·4 mm Hg (SE 1·1; p=0·002). During most study visits, the proportion of patients with blood pressure less than 130/80 was higher in the ramipril group than in the placebo group (table 4). In both groups, there was a significant decline in all SF-36 domains (except bodily pain and role emotional) over time (appendix pp 14–21). The only significant between-group difference was in the role emotional domain; at 6 months and 48 months the placebo group had a higher quality of life score than the ramipril group (6 months: 85·0 *vs* 77·2 [p=0·04]; 48 months: 83·9 *vs* 68·8 [p=0·02]).

Overall, there was a significant rise in serum creatinine (3·4 umol/L per 6-month interval [SE 0·9]; p<0·0002). However, there was no significant between-group difference for ramipril versus placebo (–3·1 µmol/L [SE 1·8] per 6-month interval; p=0·09; appendix p 22). Proteinuria declined in the ramipril group; by 18 months, the mean difference in the decline of proteinuria was –202 g/24 h per 6-month interval (SE 69) for ramipril compared with placebo (p=0·003). There was a significant decline in haemoglobin in the ramipril group (–0·96 g/L [SE 0·23] per 6-month interval; p<0·001), but not in the placebo group (0·36 g/L [SE 0·24] per 6-month interval; p=0·14; appendix p 24). At the end of the trial, mean haemoglobin in the placebo group was 135 g/L (SD 16) compared with 121 g/L (17) in the ramipril group (mean difference –14 g/L (95% CI –22 to –7). There was no difference in admissions to hospital or cardiovascular events between the groups (table 4).

Discussion

We noted no significant difference between ramipril and placebo groups in the occurrence of the primary composite outcome (doubling of serum creatinine, end-stage renal disease, or death) in adult kidney transplant recipients with proteinuria. There was also no significant difference

in measured GFR over time, our principal secondary outcome. By increasing average follow-up to 48 months, we observed substantially more events, but were still unable to detect important decreases in our primary outcome with ramipril compared with placebo. Extrapolating our observed event rates to a sample size of 528 still showed a non-significant difference in the primary endpoint (risk difference –3 8% (95% CI –10·4 to 2·0). Although speculative, this suggests that if there is a benefit of ramipril in this population its magnitude is likely small.

We identified three relevant randomised trials that have examined the effect of ACE inhibitors or ARBs on clinical outcomes in the kidney transplant population.[13–16] The largest such trial (SECRET study) assessed the effect of candesartan on a composite outcome consisting of cardiovascular events, transplant failure, or death.[13] This trial differed substantially from our study because proteinuria was not an inclusion criterion, those with ≥2 g/day proteinuria were excluded, and baseline values of proteinuria were in the normal range.[13] After a mean follow-up of about 20 months, there was no significant difference between the candesartan and placebo groups with respect to the occurrence of the primary outcome measure. Ibrahim and colleagues[14] showed that losartan did not improve end-stage renal disease from interstitial fibrosis or cortical interstitial volume expansion on biopsy assessment or a composite measure consisting of a doubling serum creatinine, transplant failure, or death. Similar to our study, they also reported no significant benefit of losartan on measured GFR after 5 years of follow-up.[14] In kidney transplant patients without diabetes, Paoletti and colleagues[16] showed that lisinopril significantly reduced the composite endpoint of doubling serum creatinine, renal transplant failure, major cardiovascular event, or death. However, these findings were primarily affected by differences in cardiovascular events because only six patients died or sustained transplant failure after 9 years of follow-up.[16] Our study adds to these findings by showing that even with high-risk patients and extended follow-up, ramipril has no beneficial effect in the kidney transplant population.

Although we had a target blood pressure of less than 130/80 for all patients, the ramipril group had a lower blood pressure compared with the control group. Differences appeared early post-randomisation and were maintained throughout the trial. A similar pattern of improved blood pressure was seen in the candesartan group of the SECRET trial.[13] We should also note that blood pressure values were excellent in both groups compared with usual clinical practice. About 50% of patents given placebo had a blood pressure less than 130/80 compared with only 27% in a recent report of nearly 10 000 transplant patients from the UK Renal Registry.[17] The tight blood pressure control does not, however, take away from the generalisability of our findings. Strict blood pressure control to a target less than 130/80 was mandated for all study participants and reminders about blood pressure were repeatedly sent to investigators and coordinators throughout the course of the trial. Such tight control probably decreased our overall event rates for the primary outcome.

This trial had notable strengths. Unlike other published trials, we enrolled a very high-risk population that included a substantial number of patients with diabetes (43%), hyperlipidaemia (67%), and history of cardiovascular disease (25%), as well as older patients (34% >60 years), and those with substantial proteinuria (43% >500 mg/day). In view of the fact that that ramipril had no effect in patients at high risk for renal failure and

	Placebo (n=109)	Ramipril (n=103)	Risk ratio or mean difference (95% CI)
Blood pressure <130/80			
1 month	48 (47%)	65 (66%)	1·42 (1·11 to 1·83)
12 months	47 (51%)	49 (60%)	1·17 (0·90 to 1·53)
24 months	45 (53%)	49 (65%)	1·23 (0·95 to 1·60)
36 months	33 (52%)	43 (74%)	1·42 (1·07 to 1·87)
48 months	17 (43%)	23 (68%)	1·59 (1·04 to 2·44)
Admissions to hospital			
Patients with a least one admission	60·0 (55%)	46·0 (45%)	0·80 (0·61 to 1·06)
Days admitted	10·4 (15·4)	16·2 (24·7)	5·8 (–0·01 to 11·6)
Cardiovascular events			
Myocardial infarction	6 (6%)	2 (2%)	0·35 (0·07 to 1·69)
TIA or stroke	4 (4%)	1 (1%)	0·27 (0·04 to 1·79)
Amputation	0	4 (4%)	..
Congestive heart failure	2 (2%)	3 (3%)	1·57 (0·27 to 9·22)
Revascularisation events	5 (5%)	7 (7%)	1·47 (0·48 to 4·48)
PCI or CABG	4 (4%)	2 (2%)	0·52 (0·10 to 2·80)
Peripheral	0	5 (5%)	..
Cerebral	1 (1%)	0	..
Measured GFR (mL/min/1·73m^2)			
6 months	61 (24)	56 (22)	–5 (–12 to 2)
12 months	56 (23)	55 (23)	–1 (–8 to 6)
18 months	56 (22)	56 (23)	0 (–7 to 7)
24 months	56 (22)	54 (23)	–2 (–10 to 5)
30 months	56 (21)	55 (26)	–1 (–9 to 7)
36 months	55 (22)	57 (27)	2 (–7 to 11)
42 months	55 (18)	53 (26)	–2 (–11 to 7)
48 months	49 (20)	47 (23)	–2 (–13 to 9)
Proteinuria (mg/24 h*)			
6 months	430 (285–775)	310 (180–560)	0·006
12 months	400 (214–730)	340 (140–610)	0·09
18 months	400 (156–930)	200 (90–560)	0·02
24 months	415 (60–900)	280 (34–700)	0·31
30 months	440 (160–750)	195 (43–570)	0·02
36 months	390 (49–660)	250 (43–560)	0·26
42 months	326 (70–540)	185 (50–510)	0·58
48 months	200 (61–550)	200 (40–670)	0·86

Data are n (%), mean (SD), or median (IQR). *Proteinuria data were not normally distributed; therefore, Wilcoxon rank sum test was reported instead of mean difference. PCI=percutaneous coronary intervention. CABG=coronary artery bypass grafting. DTPA GFR=glomerular filtration rate measured using ^{99m}technetium-diethylene triamine pentacetate.

Table 4: **Secondary outcomes**

death, it is improbable that a benefit would be seen in transplant patients at lower risk. We also had considerable patient follow-up to allow sufficient time for clinical endpoints to occur. In the landmark trials assessing ARBs and ACE inhibitors in non-diabetic and diabetic nephropathy, the separation of treatment effect became evident by just 24 months post-randomisation.[18–22] Finally, our renal outcomes were very accurate and reliable in view of the fact that we used radioisotopic methods for measuring GFR and a central laboratory was used to measure serum creatinine concentration.

Limitations of our study should also be noted. First, we did not achieve our target sample size. This was mainly due to the evolution of physician practice since trial inception: the use of ACE inhibitors and ARBs in the transplant population has increased substantially over the past decade and directly hampered our recruitment. Indeed, many clinicians caring for the patients were reluctant to discontinue these drugs to allow participation despite the absence of evidence for their use and reviews and guidelines supporting the need for this trial.[23–28] Second, ramipril was dosed at 10 mg daily based on trials done in the non-transplant population.[29,30] With a larger dose, we might have noted more clinical benefit; however, the increased ramipril dosage would probably result in even more adverse events. Third, the cause of proteinuria was not known because baseline kidney transplant biopy samples were not obtained at trial entry. Ramipril might have been effective if entry was restricted to a more homogeneous population such as those with recurrent glomerulonephritis, although this would have restricted patient recruitment even further.

Despite the limitations noted, this trial provides important findings for the management of kidney transplant patients with proteinuria. Previously, clinicians had to extrapolate from non-transplant trials to make treatment recommendations for these patients. Our study is the first randomised trial of ACE inhibitors that provides long-term data about safety and clinically important outcomes in kidney transplant recipients with proteinuria. We believe that this will be useful information to guide patient-clinician discussions on therapy. Perhaps the key message is the relative absence of expected clinical benefit and an increased risk of adverse events including mortality in the ramipril group. Unlike the positive response noted in non-transplant ACE inhibitor trials,[29,31] we found no significant effect of ramipril on measured GFR. This is important in view of the fact that declining GFR is directly on the clinical pathway to end-stage renal disease, and transplant patients with reduced GFR are at increased risk for metabolic complications, cardiovascular disease, and death.[23,24,35] In addition to the absence of benefit on GFR, we noted no significant between-group differences in patient-reported quality of life. With respect to safety, there were more adverse events and drug discontinuations due to side-effects in those receiving ramipril. This included a clinically significant reduction in haemoglobin for patients in the ramipril group. Anaemia is a well-documented risk factor for cardiovascular disease, transplant failure, and death in kidney transplantation, which could contribute to worsening outcomes with long-term ramipril therapy.[32,33] Although the numbers were small and the difference between groups non-significant, we noted more deaths in the ramipril group than in the placebo group, and not fewer as we had hypothesised. There were more deaths due to sepsis and cardiac causes in the ramipril group than in the placebo group. These are the two most frequent causes of death in the kidney transplant population, so it is difficult to determine the significance of this finding.[34] In the SECRET trial, there were more cardiovascular morbidity events in the candesartan group than in the placebo group, but no difference in overall mortality.[13] Current guidelines make an ungraded recommendation that ACE inhibitors or ARBs be considered as first-line antihypertensives in transplant patients with proteinuria.[35] The data from our trial suggests that ramipril use in transplant patients with proteinuria is associated with more adverse events and probably no significant improvement in clinical outcomes. Treatment guidelines should be reconsidered given the findings of our trial.

In conclusion, the ACE inhibitor ramipril did not significantly prevent the occurrence of clinically important events including a doubling of serum creatinine, transplant failure, cardiovascular events, or death in kidney transplant patients with proteinuria. Additionally, there was no significant benefit with respect to measured GFR over time, despite the inclusion of patients at high risk for renal progression. Although ramipril improved blood pressure compared with placebo, in renal transplant recipients with proteinuria it should not be used with the goal of improving clinical outcomes in this population.

Contributors

GAK, DF, PH, and GW conceived and designed the study. GAK, LAT, DT, DH, CW, NM, MC, MP, BK, SG, JS, RP, EC, HP, VP, DH, and JG collected the data. GAK, DF, MC, LAT, DT, DH, CW, NM, MC, MP, BK, SG, JS, RP, EC, HP, PH, TR, GW, and JG analysed and interpreted the data. GAK, DF, and MC drafted the report. All authors critically revised the report for important intellectual content. MC, DF, GAK, TR, and GW analysed the data. GAK, DF, PH, GW, MC, JG, SG, BK, NM, RP, and DT obtained the funding. DH and VC provided administrative, technical, and material. GAK, DF, and DH supervised the study.

Declaration of interests

GAK has received investigator-initiated research grants from Astellas Canada, Pfizer Canada, Roche Canada, and Novartis Canada unrelated to this study. LAT has received funds from Astellas for an advisory board. JS has received grants from Astellas Pharma outside of the submitted work. RP has received an investigator-initiated research grant and funds from an advisory board from Astellas Canada, both unrelated to this work. All other authors declare no competing interests.

Acknowledgments

This project was funded by the Canadian Institutes of Health Research (CIHR grant # MCT-78844). We acknowledge the work of the members of the data and safety monitoring board: Andreas Laupacis (chair), University of Toronto, ON, Canada; Brendan Barrett, Memorial

University, St John's, NL, Canada; Braden Manns, University of Calgary, Calgary, AB, Canada; and Joel Singer, University of British Colombia, Vancouver, BC, Canada.

References

1 Karthikeyan V, Karpinski J, Nair RC, Knoll G. The burden of chronic kidney disease in renal transplant recipients. *Am J Transplant* 2004; **4:** 262–69.

2 Knoll G. Proteinuria in kidney transplant recipients: prevalence, prognosis and evidence-based management. *Am J Kidney Dis* 2009; **54:** 1131–44.

3 Kidney Disease: Improving Global Outcomes (KDIGO) Blood Pressure Work Group. KDIGO clinical practice guideline for the management of blood pressure in chronic kidney disease. *Kidney Int Suppl* 2012; **2:** 337–414.

4 Heinze G, Mitterbauer C, Regele H, Kramar R, Winkelmayer W, Curhan G, Oberbauer R. Angiotensin-converting enzyme inhibitor or angiotensin II type 1 receptor antagonist therapy is associated with prolonged patient and graft survival after renal transplantation. *J Am Soc Nephrol* 2006; **17:** 889–99.

5 Opelz G, Zeier M, Laux G, Morath C, Döhler B. No improvement of patient or graft survival in transplant recipients treated with angiotensin-converting enzyme inhibitors or angiotensin II type 1 receptor blockers: a collaborative transplant Study Report. *J Am Soc Nephrol* 2006; **17:** 3257–3262.

6 Hiremath S, Fergusson D, Doucette S, Mulay A, Knoll GA. Renin angiotensin system blockade in kidney transplantation: a systematic review of the evidence. *Am J Transplant* 2007; **7:** 2350–60.

7 Cross, NB, Webster A, Masson P, O'Connell P, Craig JC. Antihypertensives for kidney transplant recipients: systematic review and meta-analysis of randomized controlled trials. *Transplantation* 2009; **88:** 7–18.

8 Jennings DL, Taber DJ. Use of renin–angiotensin–aldosterone system inhibitors within the first eight to twelve weeks after renal transplantation. *Ann Pharmacother* 2008; **42:** 116–20.

9 Knoll G, Cantarovitch M, Cole E, et al. The Canadian ACE-inhibitor trial to improve renal outcomes and patient survival in kidney transplantation: study design. *Nephrol Dial Transplant* 2008; **23:** 354–58.

10 Kidney Disease Outcomes Quality Initiative (K/DOQI). K/DOQI clinical practice guidelines on hypertension and antihypertensive agents in chronic kidney disease. *Am J Kidney Dis* 2004; **43:** S1–S290.

11 Kidney Disease Outcomes Quality Initiative (K/DOQI). K/DOQI clinical practice guidelines for managing dyslipidemias in kidney transplant patients. *Am J Transplant* 2004; **4:** 13–53.

12 White C, Akbari A, Doucette S, et al. Estimating GFR using serum beta trace protein: accuracy and validation in kidney transplant and pediatric populations. *Kidney Int* 2009; **76:** 784–89.

13 Philipp T, Martinez F, Geiger H, et al. Candesartan improves blood pressure control and reduces proteinuria in renal transplant recipients: results from SECRET. *Nephrol Dial Transplant* 2010; **25:** 967–76.

14 Ibrahim HN, Jackson S, Connaire J, et al. Angiotensin II blockade in kidney transplant recipients. *J Am Soc Nephrol* 2013; **24:** 320–27.

15 Paoletti E, Cassottana P, Amidone M, Gherzi M, Rolla D, Cannella G. ACE inhibitors and persistent left ventricular hypertrophy after renal transplantation: a randomized clinical trial. *Am J Kidney Dis* 2007; **50:** 133–42.

16 Paoletti E, Bellino D, Marsano L, Cassottana P, Rolla D, Ratto E. Effects of ACE inhibitors on long-term outcome of renal transplant recipients: a randomized controlled trial. *Transplantation* 2013; **95:** 889–95.

17 Rao A, Pitcher D, Farrington K. UK Renal Registry 16th Annual Report: Chapter 11, Blood Pressure Profile of Prevalent Patients receiving Renal Replacement Therapy in 2012: National and Centre-specific Analyses. *Nephron Clin Pract* 2013; **125:** 209–18.

18 Lewis EJ, Hunsicker LG, Bain RP, Rohde RD for the Collaborative Study Group. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. *N Engl J Med* 1993; **329:** 1456–62.

19 Brenner BM, Cooper ME, de Zeeuw D, et al, for the RENAAL Study Investigators. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; **345:** 861–69.

20 Lewis EJ, Hunsicker LG, Clarke WR, et al, for the Collaborative Study Group. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 2001; **345:** 851–60.

21 Ruggenenti P, Perna A, Gherardi G, et al. Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria. *Lancet* 1999; **354:** 359–64.

22 Maschio G, Alberti D, Janin G, et al. Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group. *N Engl J Med* 1996; **334:** 939–45.

23 Kidney Disease: Improving Global Outcomes Group. Kidney disease: improving global outcomes (KDIGO) clinical practice guideline for the management of blood pressure in chronic kidney disease. *Kidney Int Suppl* 2012; **2:** 337–414.

24 Weir MR, Burgess E, Cooper JE, et al. Assessment and management of hypertension in transplant patients. *J Am Soc Nephrol* 2015; published online Feb 4. DOI:10.1681/ASN.2014080834.

25 Cravedi P, Perico N, Remuzzi G. Non-immune interventions to protect kidney allografts in the long term. *Kidney Int Suppl* 2010; **78:** S71–75.

26 Mariat C. Early use of renin-angiotensin-aldosterone system inhibitors after renal transplantation. *Nat Clin Pract Nephrol* 2008; **4:** 306–07.

27 Morath C, Schmied B, Mehrabi A, et al. Angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers after renal transplantation. *Clin Transplant* 2009; **23:** 33–36.

28 Wadei HM, Textor SC. Hypertension in the kidney transplant recipient. *Transplant Rev (Orlando)* 2010; **24:** 105–20.

29 Agodoa LY, Appel L, Bakris GL, et al. Effect of ramipril vs amlodipine on renal outcomes in hypertensive nephrosclerosis: a randomized controlled trial. *JAMA* 2001; **285:** 2719–28.

30 The Heart Outcomes Prevention Evaluation Study Investigators. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000; **342:** 145–53.

31 The GISEN Group. Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. *Lancet* 1997; **349:** 1857–63.

32 Jones H, Talwar M, Nogueira JM, et al. Anemia after kidney transplantation; its prevalence, risk factors, and independent association with graft and patient survival: a time-varying analysis. *Transplantation* 2012; **93:** 923–28.

33 Garrigue V, Szwarc I, Giral M, et al. Influence of anemia on patient and graft survival after renal transplantation: results from the French DIVAT cohort. *Transplantation* 2014; **97:** 168–75.

34 Saran R, Li Y, Robinson B, et al. US Renal Data System 2014 annual data report: epidemiology of kidney disease in the United States. *Am J Kidney Dis* 2015; **66:** S1–306.

35 Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9:** S1–S157.

Appendix 2- Cumulative Hazard Plots for the Primary ACE Trial

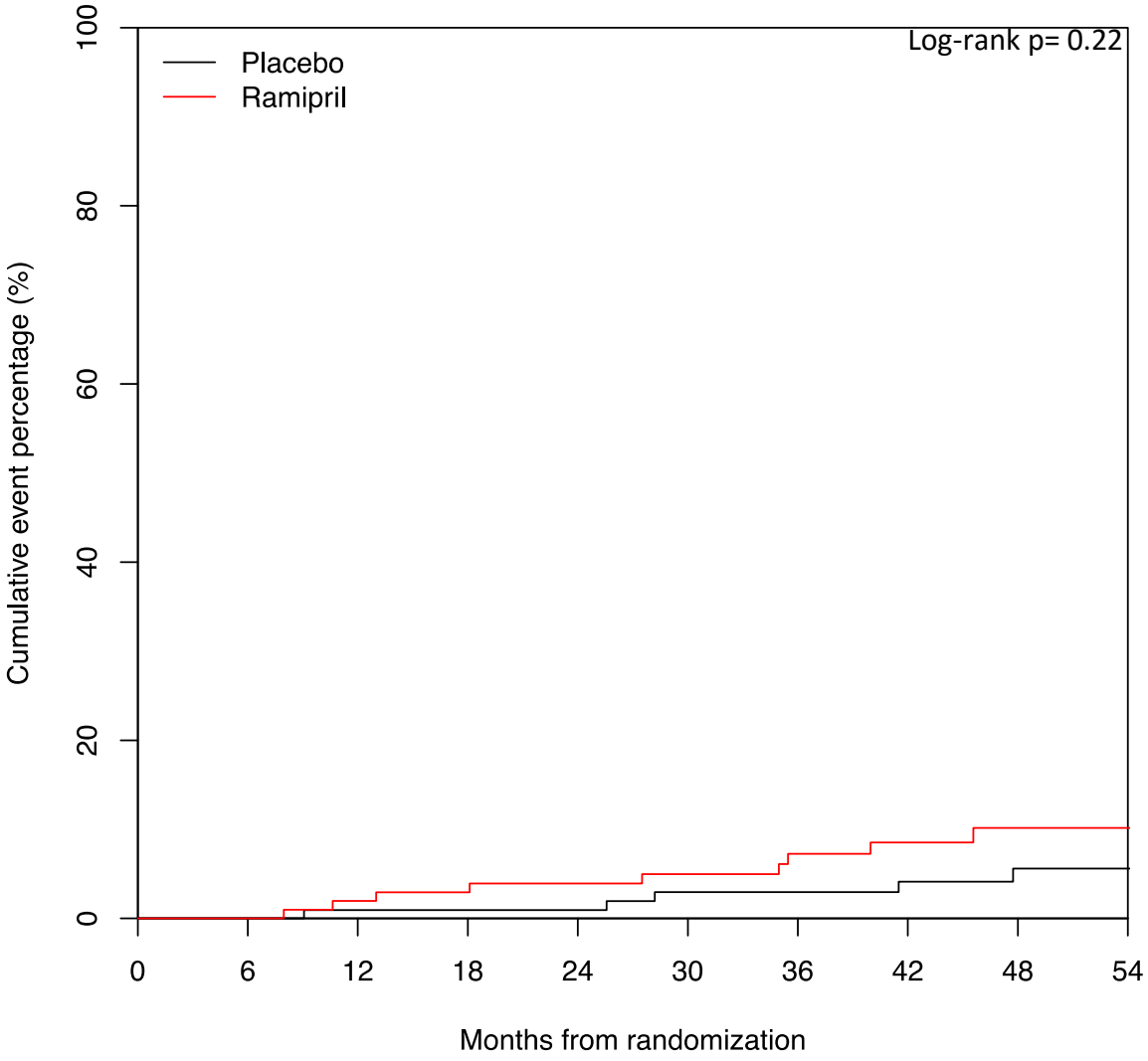


Figure 1. Time to death in the primary trial.

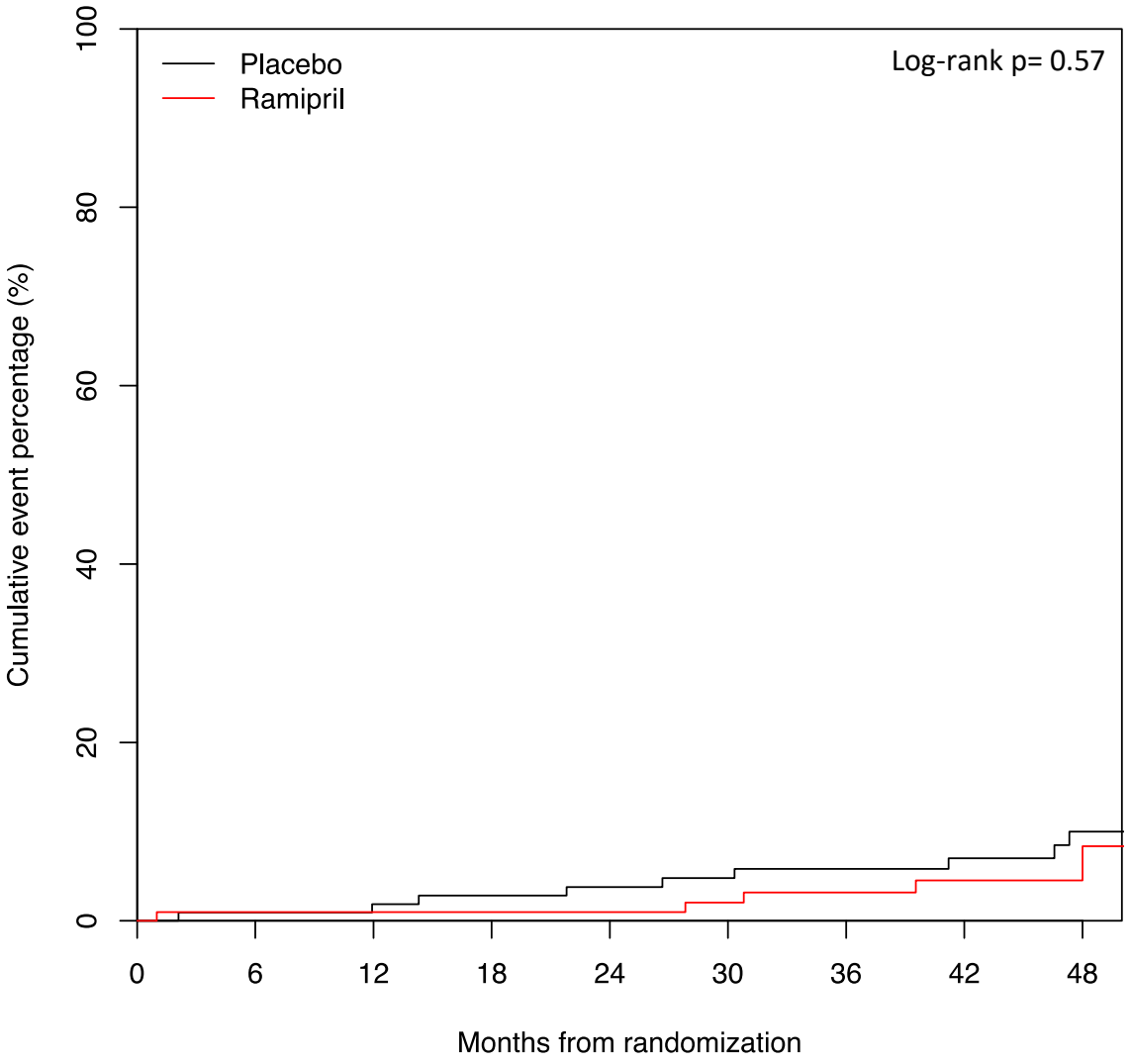


Figure 2. Time to ESRD in the primary trial.

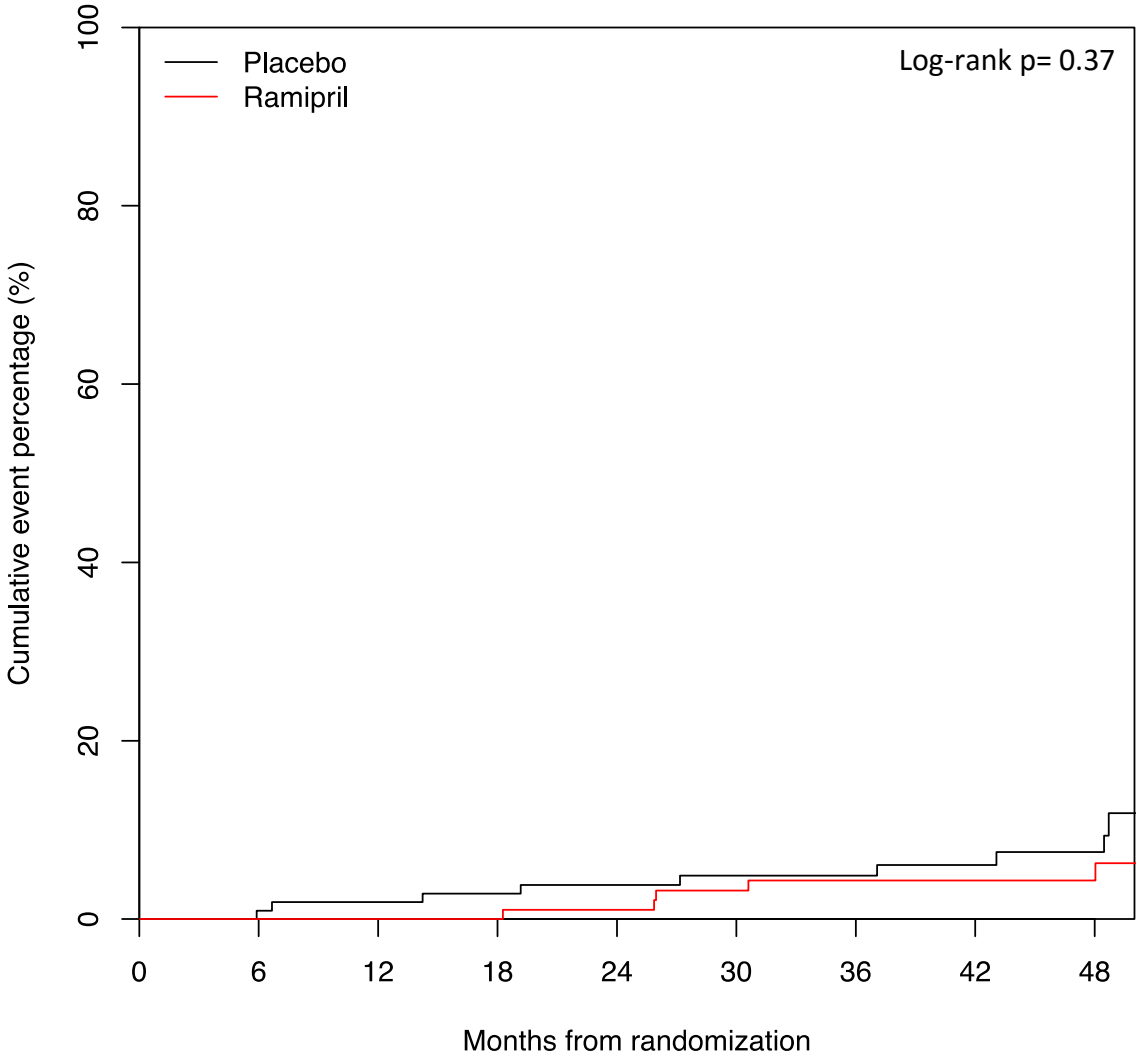


Figure 3. Time to doubling of serum creatinine in the primary trial.

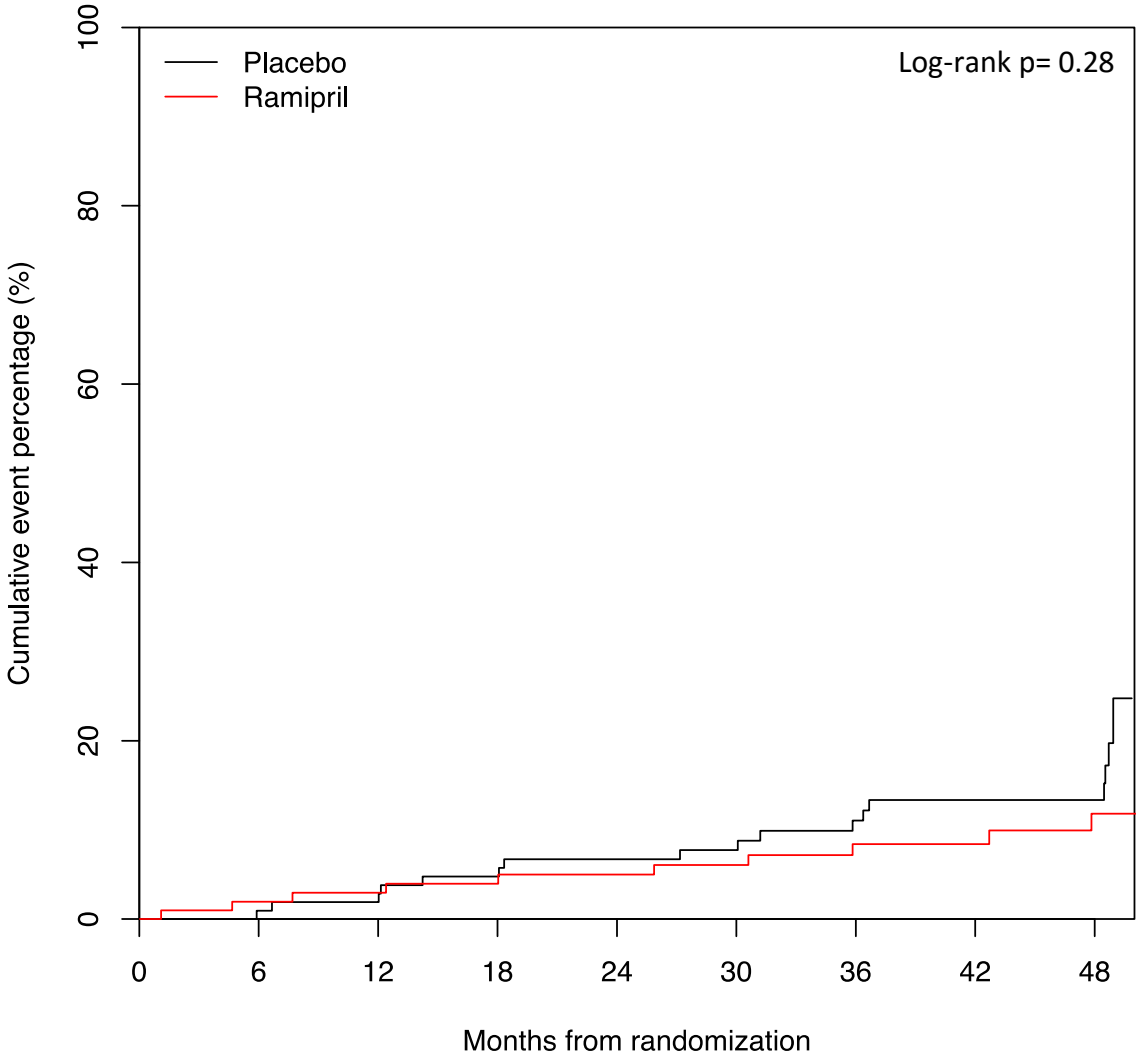


Figure 4. Time to ≥40% eGFR decline in the primary trial.

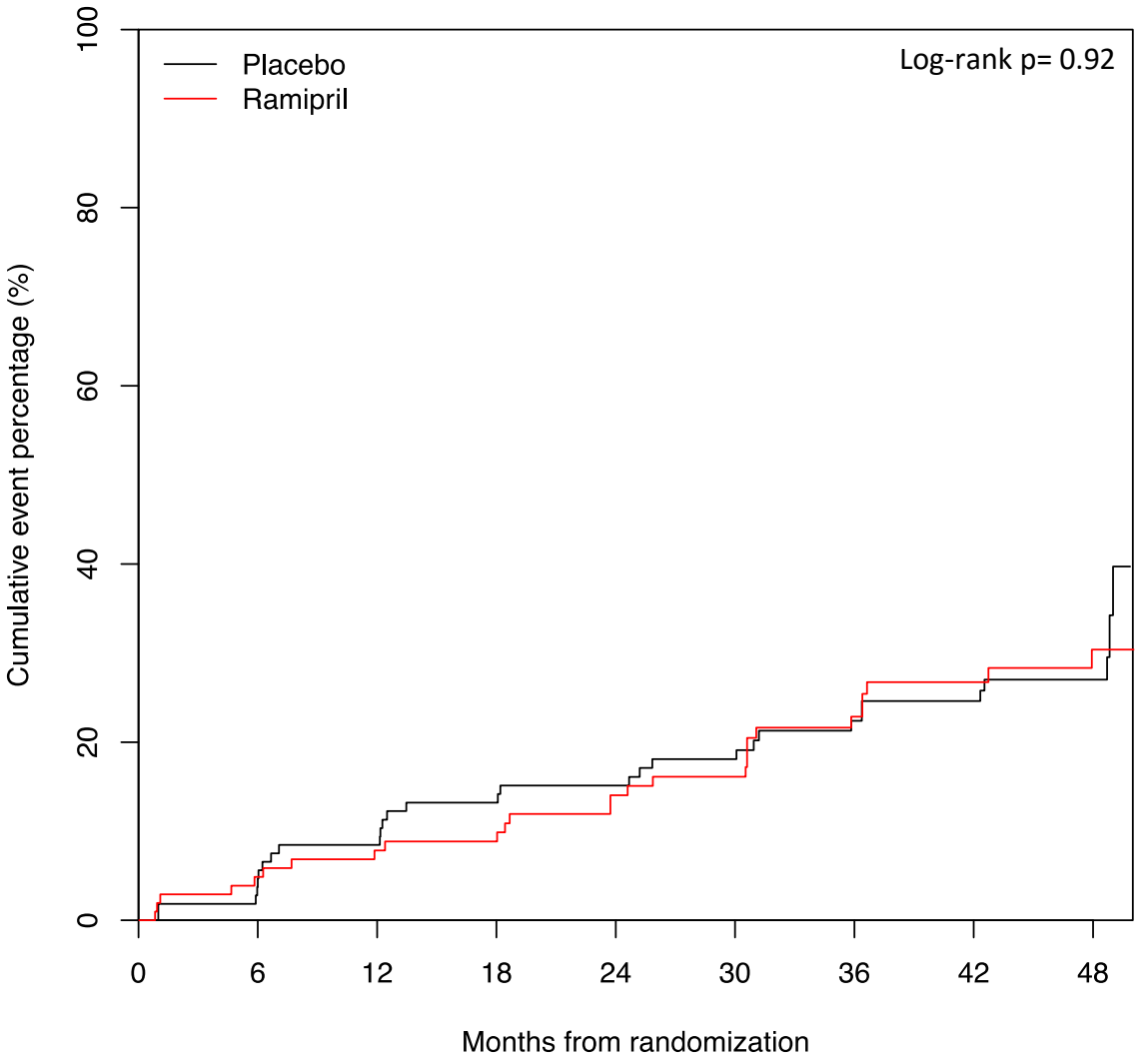


Figure 5. Time to ≥30% eGFR decline in the primary trial.

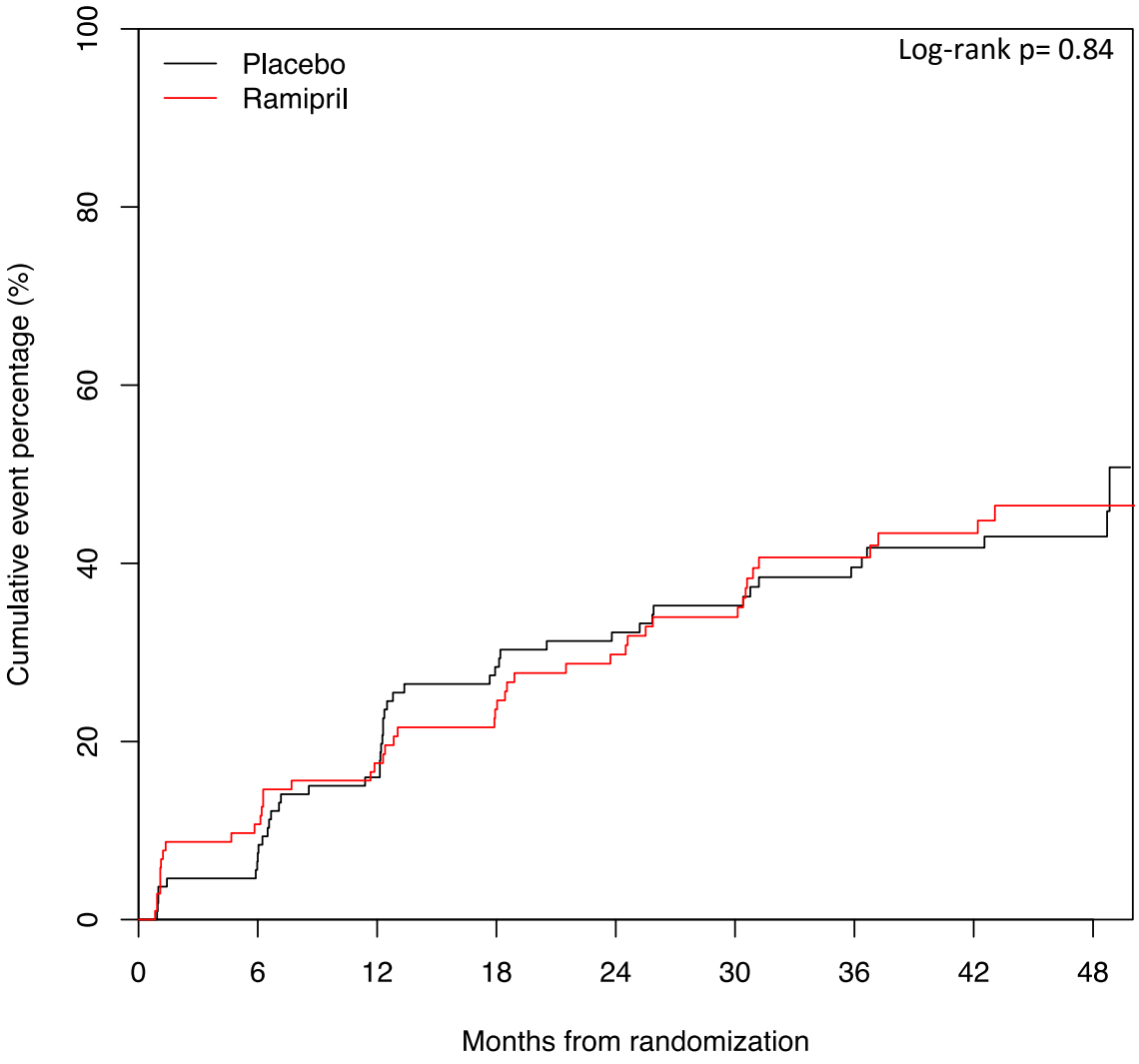


Figure 6. Time to ≥20% eGFR decline in the primary trial.

Appendix 3- Cumulative Hazard Plots for the Extended ACE Trial

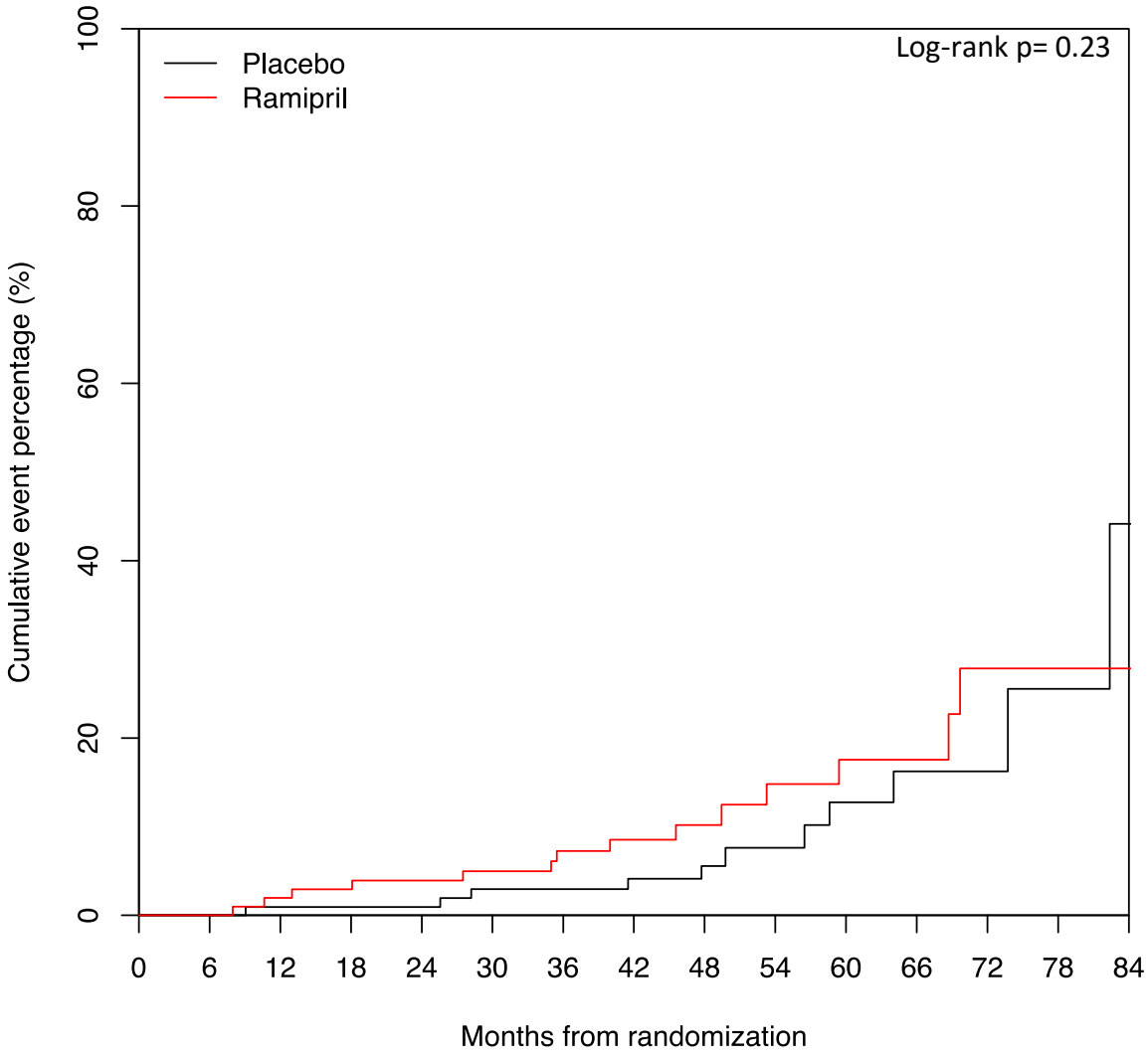


Figure 1. Time to death in the extended trial.

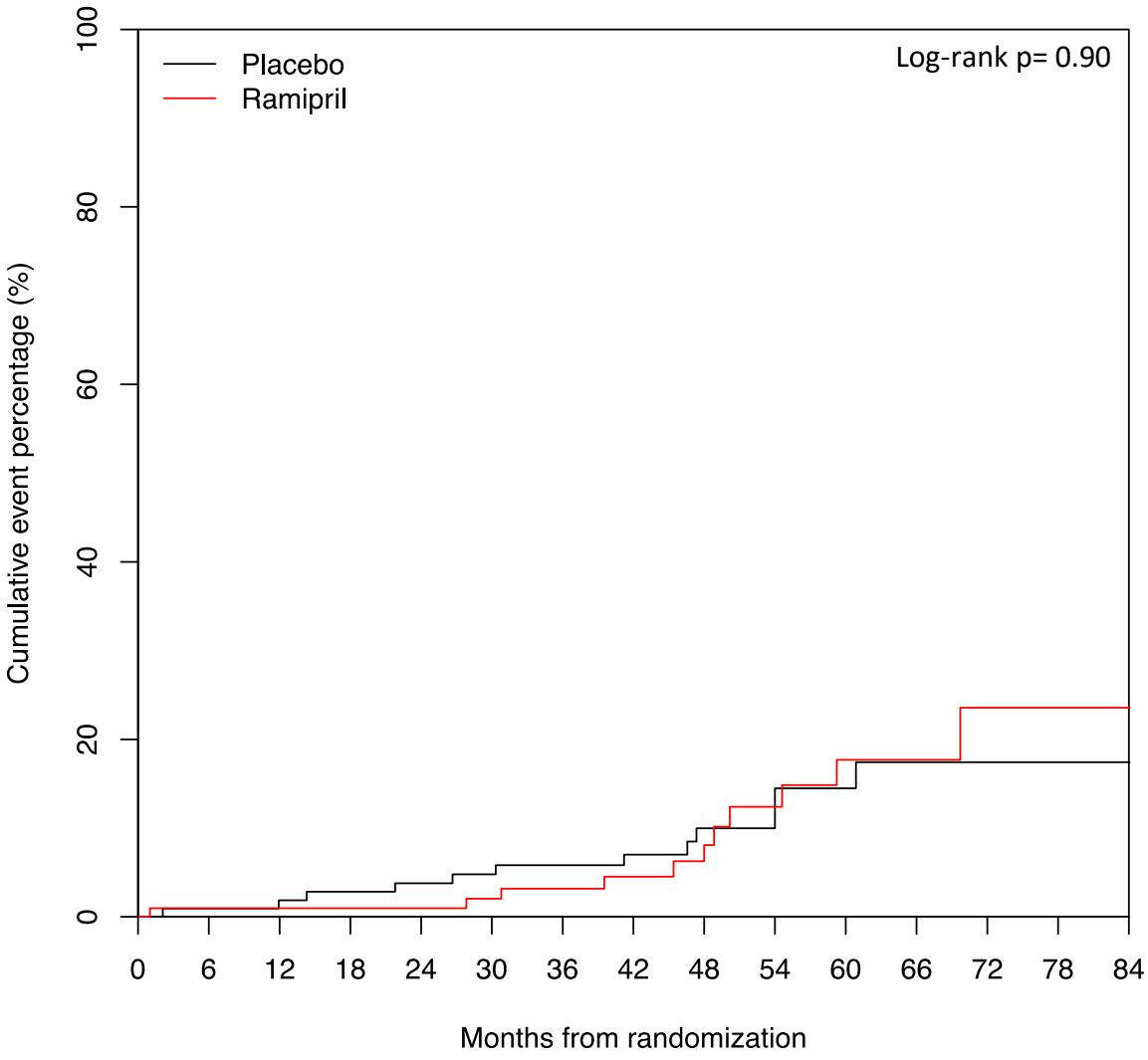


Figure 2. Time to ESRD in the extended trial.

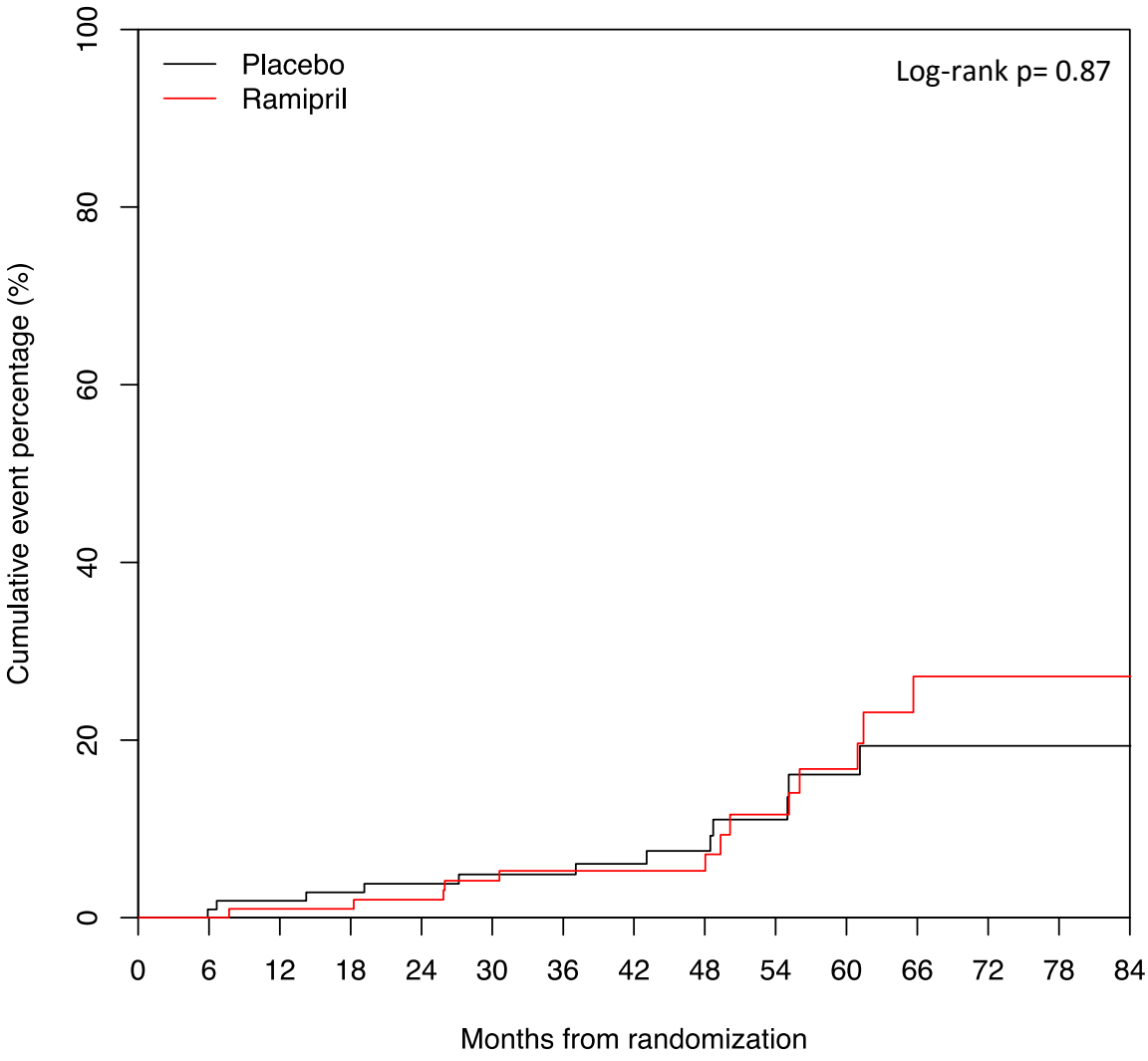


Figure 3. Time to doubling of serum creatinine in the extended trial.

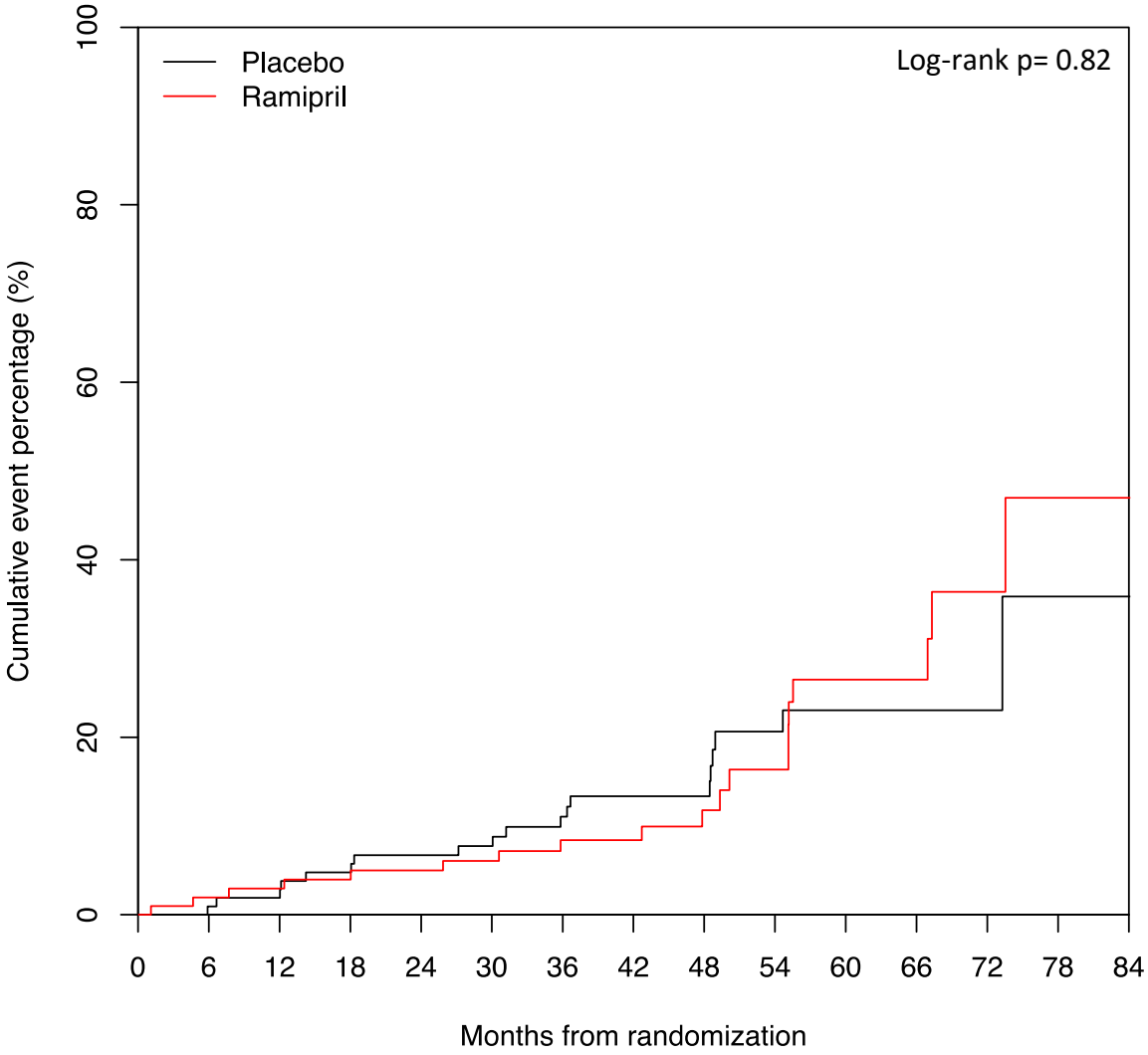


Figure 4. Time to ≥40% eGFR decline in the extended trial.

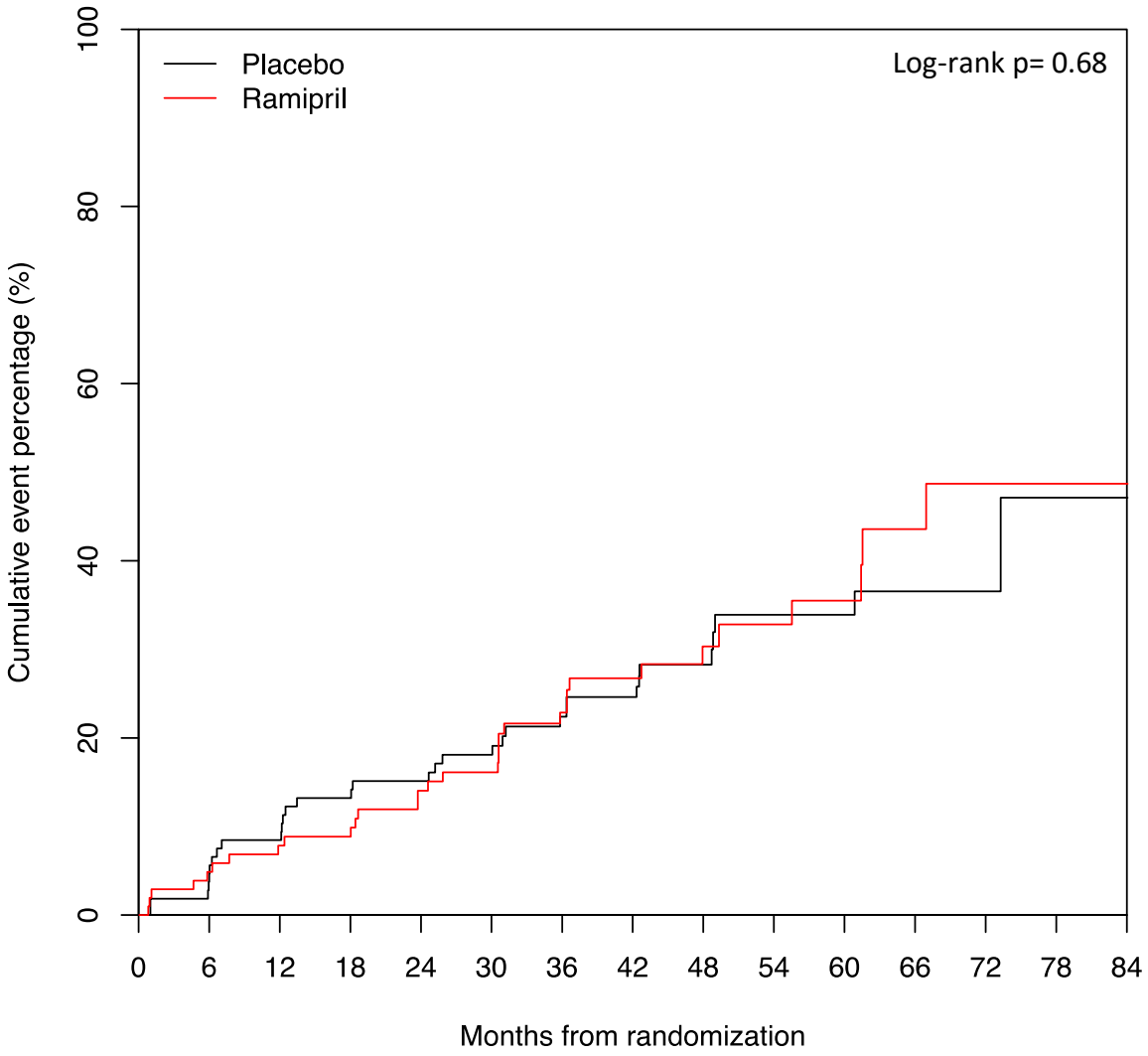


Figure 5. Time to ≥30% eGFR decline in the extended trial.

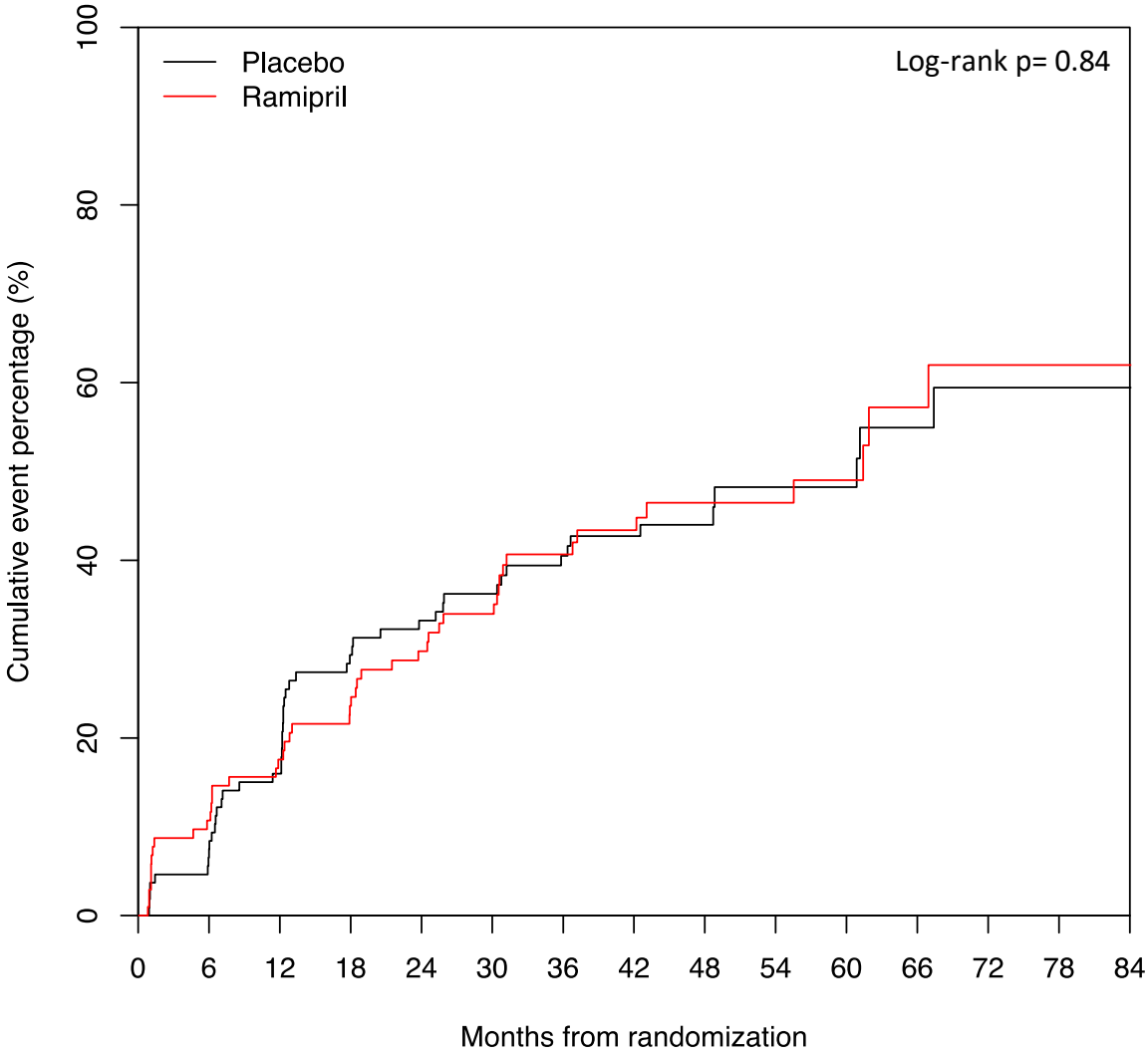


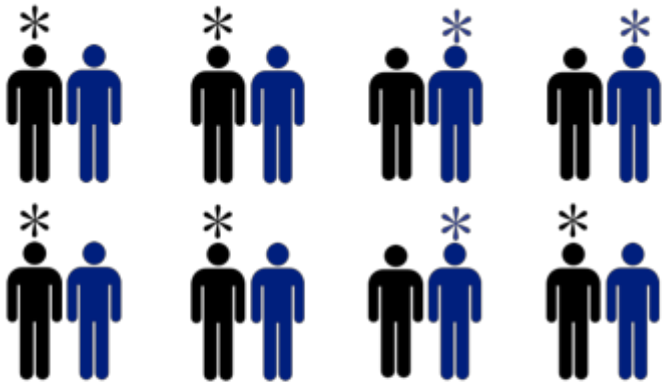
Figure 6. Time to ≥20% eGFR decline in the extended trial.

Appendix 4- Win Ratio Illustration

$$\frac{\text{Odds of event in study group}}{\text{Odds of event in control group}} = \text{Odds ratio}$$

$$\frac{\text{Hazard of event in study group}}{\text{Hazard of event in control group}} = \text{Hazard ratio}$$

*No. pairs = Study group (N) * Control group (N)*



$$\frac{\text{* No. of wins for study group}}{\text{* No. of wins for control group}} = \text{Win ratio}$$

* Study group patient won

* Control group patient won

Figure 1. Schematic demonstrating how the win ratio is conceptually similar to other epidemiological measures

Appendix 5- R Code to Conduct Win Ratio Approach

Additional File 1- R Code for the 'get.winratio' Function to Determine the Win Ratio

```
get.winratio <- function(No.1, No.2, No.3) {

#This function determines the win ratio for composite consisting of 3 endpoints of varying
clinical significance.


#INPUT:
#            No.1 = A dataset of the event ranked #1 on clinical significance. *Must
include columns labelled "pt_id", "group", "timeTo", and "Event".

#            No.2 = A dataset of the event ranked #2 on clinical significance. *Must
include columns labelled "pt_id", "group", "timeTo", and "Event".

#            No.3 = A dataset of the event ranked #3 on clinical significance. *Must
include columns labelled "pt_id", "group", "timeTo", and "Event".



#NOTE-**IMPORTANT** Within these datasets, the group column should be a factor and
described as "A" for the control group and "B" for study (treatment) group. Event column
should be numeric with 1 indicating an event and 0 no event. timeTo column should be
numeric, while pt_id should be a factor.



#OUTPUT:

#            Win.ratio = Win ratio for study group vs. control group. The win ratio is
equal to the total amount of pairs won by the study group divided by the total number of
pairs won by the control group.


#            Event.1.win.study = pairs won for study group (B) on #1 clinically
significant event
#            Event.1.win.cont = pairs won for control group (A) on #1 clinically
significant event
#            Event.1.tie = pairs tied on #1 clinically significant event

#            Event.2.win.study = pairs won for study group (B) on #2 clinically
significant event
#            Event.2.win.cont = pairs won for controlgroup (A) on #2 clinically
significant event
#            Event.2.tie = pairs tied on #2 clinically significant event

#            Event.3.win.study = pairs won for group (B) on #3 clinically significant
event
```

```
#             Event.3.win.cont = pairs won for control group (A) on #3 clinically
significant event
#             Event.3.tie = pairs tied on #3 clinically significant event



Event.1 <- No.1[,c(grep(c("pt_id"),colnames(No.1)), grep(c("group"),colnames(No.1)),
grep(c("timeTo"),colnames(No.1)),grep(c("Event"),colnames(No.1)))]

Event.2 <- No.2[,c(grep(c("pt_id"),colnames(No.2)), grep(c("group"),colnames(No.2)),
grep(c("timeTo"),colnames(No.2)),grep(c("Event"),colnames(No.2)))]

Event.3 <- No.3[,c(grep(c("pt_id"),colnames(No.3)), grep(c("group"),colnames(No.3)),
grep(c("timeTo"),colnames(No.3)),grep(c("Event"),colnames(No.3)))]



compare<-function(cont,study) {
# This function compares one control patient to one study patient to see who wins on the
event of interest.
#INPUT:
#    cont = 4-vector with first entry equal to pt_id, second entry equal to group, third
equal to time, and fourth entry equal to event; refers to placebo patients
#    study = same as cont; refers to study group patients
# OUTPUT:
#     win = -1 if control group patient wins, 0 if neither, 1 if study group patient wins

if (cont[4]==1 & study[4]==1) {
      if (cont[3]>study[3]) {win<--1}  else
      if (cont[3]<study[3]) {win<-1} else
      {win<-0}
}
if (cont[4]==1 & study[4]==0) {
      if (cont[3]<=study[3]) {win<-1} else {win<-0}
}
if (cont[4]==0 & study[4]==1) {
      if (cont[3]>=study[3]) {win<--1} else {win<-0}
}
if (cont[4]==0 & study[4]==0) {win<-0}
win
}

#Creates an index list for control and study patients
cont.ind<-(1:nrow(Event.1))[Event.1[,2]=="A"]
study.ind<-(1:nrow(Event.1))[Event.1[,2]=="B"]

#Creates a matrix of length study by length control and fills in with NAs
No.1.win<-matrix(NA,length(study.ind),length(cont.ind))

#For every study patient and for every control patient plug in the respective row # into
the compare function and then import that result into the No.1.win matrix
for (i in 1:length(study.ind)) {
for (j in 1:length(cont.ind)) {
      No.1.win[i,j]<-compare(Event.1[cont.ind[j],],Event.1[study.ind[i],])
}
}
```

```
#This step fills in all the No.1 win ratio "answers", so anything that isn't 0 gets filled
 in as we will not be assessing it for this step


No.2.win <- ifelse(No.1.win==0,0,"Event1")

#Same description as above BUT with an added a "if" statement relating to if "i,j" are 0.
 If so then we need to look at them at this step. If not 0 then they have already been dealt
 with

for (i in 1:length(study.ind)) {
 for (j in 1:length(cont.ind)) {
       if (No.2.win[i,j]==0) {
       No.2.win[i,j]<-compare(Event.2[cont.ind[j],],Event.2[study.ind[i],])
       }
 }
}


No.3.win <- ifelse(No.2.win==0,0,"Event2")

#Same description as above BUT with an added a "if" statement relating to if "i,j" are 0.
 If so then we need to look at them at this step. If not 0 then they have already been dealt
 with

for (i in 1:length(study.ind)) {
 for (j in 1:length(cont.ind)) {
       if (No.3.win[i,j]==0) {
       No.3.win[i,j]<-compare(Event.3[cont.ind[j],],Event.3[study.ind[i],])
       }
 }
}


#Total number of study group 'wins', control group 'wins', and 'ties' with respect to
 number 1 clinically significant event
Event.1.win.study <-sum(No.1.win==1)
Event.1.win.cont <-sum(No.1.win==-1)
Event.1.tie <- sum(No.1.win==0)

#Total number of study group 'wins', control group 'wins', and 'ties' with respect to
 number 2 clinically significant event
Event.2.win.study <- sum(No.2.win==1)
Event.2.win.cont <- sum(No.2.win==-1)
Event.2.tie <- sum(No.2.win==0)

#Total number of study group 'wins', control group 'wins' and 'ties' with respect to number
 3 clinically significant event
Event.3.win.study <- sum(No.3.win==1)
Event.3.win.cont <- sum(No.3.win==-1)
Event.3.tie <- sum(No.3.win==0)


#win ratio
```

```
Win.ratio <- (Event.1.win.study + Event.2.win.study + Event.3.win.study) /
 (Event.1.win.cont + Event.2.win.cont + Event.3.win.cont)



output <- cbind.data.frame(Win.ratio, Event.1.win.study, Event.1.win.cont, Event.1.tie,
 Event.2.win.study, Event.2.win.cont, Event.2.tie, Event.3.win.study, Event.3.win.cont,
 Event.3.tie )

output

}
```

Additional File 2- R Code for the 'CI.winratio' Function to Calculate 95% Confidence Intervals for the Win Ratio

```
CI.winratio <- function(No.1, No.2, No.3) {

#CI.winratio function, bootstraps 95% CIs for the win ratio

#INPUT:
 #            No.1 = A dataset of the event ranked #1 on clinical significance. *Must
 include columns labelled "pt_id", "group", "timeTo", and "Event".

 #            No.2 = A dataset of the event ranked #2 on clinical significance. *Must
 include columns labelled "pt_id", "group", "timeTo", and "Event".

 #            No.3 = A dataset of the event ranked #3 on clinical significance. *Must
 include columns labelled "pt_id", "group", "timeTo", and "Event".



 #NOTE-**IMPORTANT** Within these datasets, the group column should be a factor and
 described as "A" for the control group and "B" for study (treatment) group. Event column
 should be numeric with 1 indicating an event and 0 no event. timeTo column should be
 numeric, while pt_id should be a factor.



#OUTPUT:
#            95% Confidence interval for the win ratio



No.1 <- No.1[,c(grep(c("pt_id"),colnames(No.1)), grep(c("group"),colnames(No.1)),
 grep(c("timeTo"),colnames(No.1)),grep(c("Event"),colnames(No.1)))]

No.2 <- No.2[,c(grep(c("pt_id"),colnames(No.2)), grep(c("group"),colnames(No.2)),
 grep(c("timeTo"),colnames(No.2)),grep(c("Event"),colnames(No.2)))]

No.3 <- No.3[,c(grep(c("pt_id"),colnames(No.3)), grep(c("group"),colnames(No.3)),
 grep(c("timeTo"),colnames(No.3)),grep(c("Event"),colnames(No.3)))]
```

```
random <- function(No.1, No.2, No.3) {

#random function, creates a random sample from original trial data using replacement. Then
 using this random sample data it derives a win ratio.

#INPUT:

 #            No.1 = A dataset of the event ranked #1 on clinical significance. *Must
 include columns labelled "pt_id", "group", "timeTo", and "Event".

 #            No.2 = A dataset of the event ranked #2 on clinical significance. *Must
 include columns labelled "pt_id", "group", "timeTo", and "Event".

 #            No.3 = A dataset of the event ranked #3 on clinical significance. *Must
 include columns labelled "pt_id", "group", "timeTo", and "Event".

#OUTPUT:
#             Win ratio derived from a random sample (n= patients in the trial) from
 original trial data (using replacement)


#'tagging' the three input datasets


colnames(No.1)[grep(c("timeTo"),colnames(No.1))] <- "timeTo1"

colnames(No.2)[grep(c("timeTo"),colnames(No.2))] <- "timeTo2"

colnames(No.3)[grep(c("timeTo"),colnames(No.3))] <- "timeTo3"

colnames(No.1)[grep(c("Event"),colnames(No.1))] <- "Event1"

colnames(No.2)[grep(c("Event"),colnames(No.2))] <- "Event2"

colnames(No.3)[grep(c("Event"),colnames(No.3))] <- "Event3"


#combining these datasets

all <- cbind.data.frame(No.1, No.2, No.3)

all <- all[,c(1:4,7,8,11,12)]


#separating by "A" (control group) and "B" (study group)

cont <- all[all[,"group"]=="A",]
study <- all[all[,"group"]=="B",]

#random sample of equal length to the original trial groups, with replacement
 "replace=TRUE"

rand_cont <- cont[sample(1:nrow(cont),nrow(cont),replace=TRUE),]
rand_study <- study[sample(1:nrow(study),nrow(study),replace=TRUE),]
```

```
#combining these

all_random <- rbind.data.frame(rand_cont, rand_study)

all_random


#subsetting by event type

No.1.random <- all_random[,1:4]

No.2.random <- all_random[,c(1,2,5,6)]

No.3.random <- all_random[,c(1,2,7,8)]

#finding the win ratio of this 'random sample' data

winratio <- get.winratio(No.1.random, No.2.random, No.3.random)

winratio[1]

}


#this creates the bootstrap vector

bootstrap <- replicate(999, random(No.1, No.2, No.3), simplify="vector")


CI <- function(data) {

 #This function orders the bootstrap output and takes the 25th value and the 975th value
 creating the 95% confidence interval

bootstrap <- as.numeric(data)

bootstrap <- sort(unlist(bootstrap))

output <- c(bootstrap[25],bootstrap[975])

output
}


CI(bootstrap)

}
```

Appendix 6 Win Ratio Plots for the Primary and Extended ACE Trial

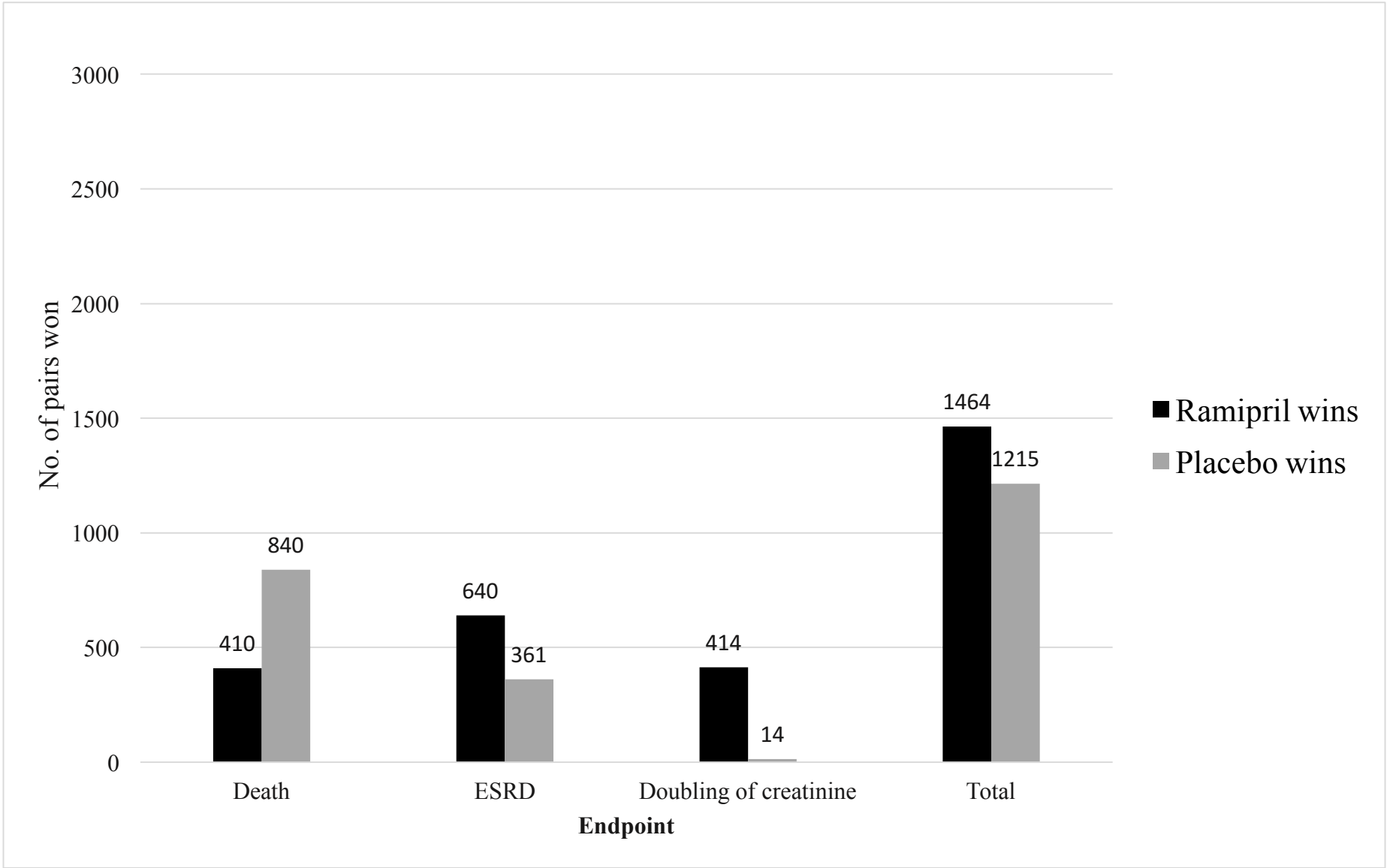


Figure 1. Win ratio component breakdown for primary trial. Number of wins (pairs won) for ramipril and placebo groups for the composite of death, ESRD, and doubling of serum creatinine in the primary trial. The win ratio is equal to the total wins of ramipril divided by the total wins of placebo.

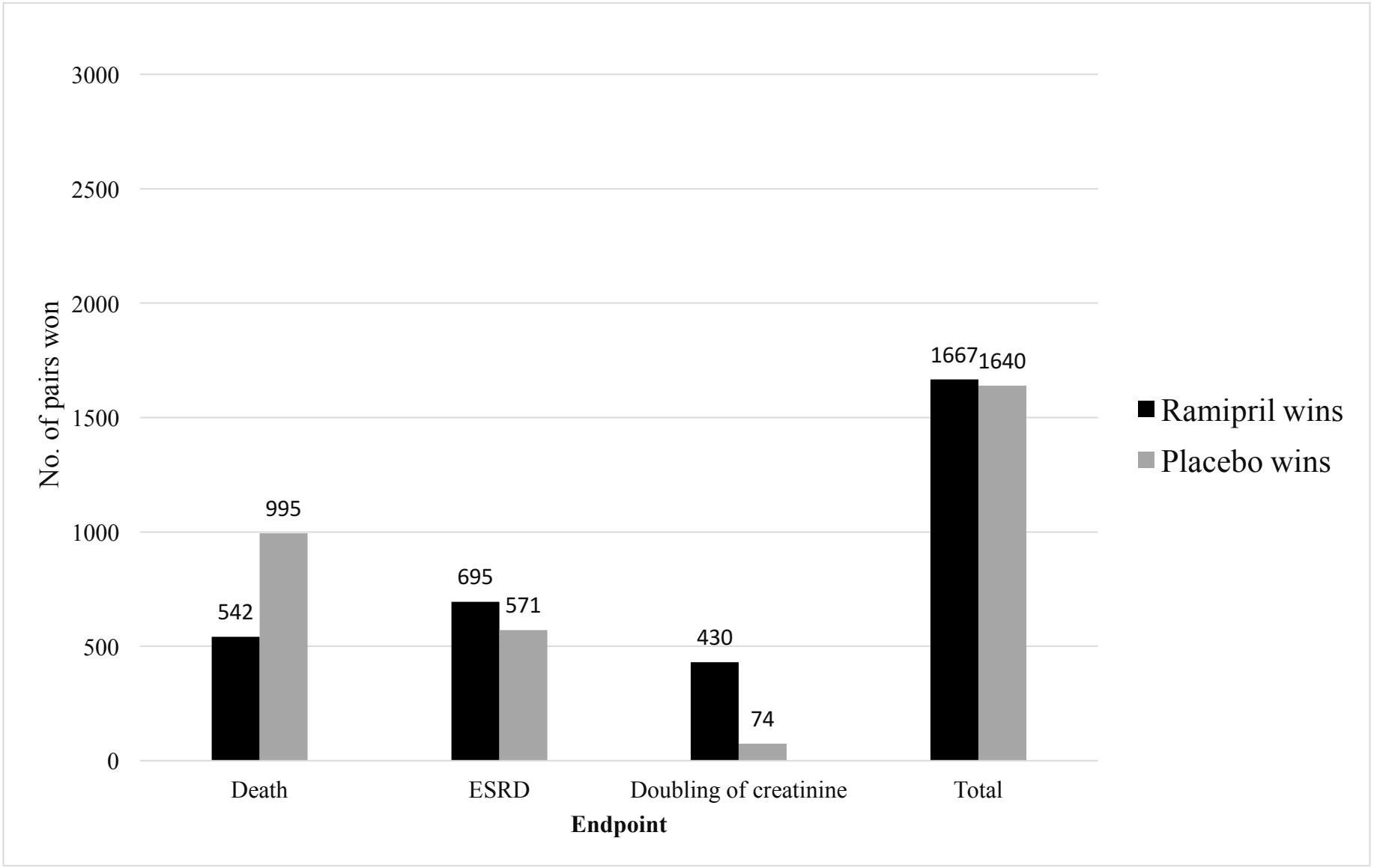


Figure 2. Win ratio component breakdown for extended trial. Number of wins (pairs won) for ramipril and placebo groups for the composite of death, ESRD, and doubling of serum creatinine in the extended trial. The win ratio is equal to the total wins of ramipril divided by the total wins of placebo.

1. Clayton PA, Lim WH, Wong G, Chadban SJ. Relationship between eGFR Decline and Hard Outcomes after Kidney Transplants. J Am Soc Nephrol [Internet]. 2016;1–7. Available from: http://www.jasn.org/cgi/doi/10.1681/ASN.2015050524%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/27059513
2. Coresh J, Turin TC, Matsushita K, Sang Y, Ballew SH, Appel LJ, et al. Decline in Estimated Glomerular Filtration Rate and Subsequent Risk of End-Stage Renal Disease and Mortality. JAMA [Internet]. 2014;311(24):2518–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24892770
3. Inker LA, Lambers Heerspink HJ, Mondal H, Schmid CH, Tighiouart H, Noubary F, et al. GFR Decline as an Alternative End Point to Kidney Failure in Clinical Trials: A Meta-analysis of Treatment Effects from 37 Randomized Trials. Am J Kidney Dis [Internet]. Elsevier Inc; 2014;64(6):848–59. Available from: http://dx.doi.org/10.1053/j.ajkd.2014.08.017
4. Badve S V., Palmer SC, Hawley CM, Pascoe EM, Strippoli GFMM, Johnson DW. Glomerular filtration rate decline as a surrogate end point in kidney disease progression trials. Nephrol Dial Transplant [Internet]. 2016 Sep [cited 2017 May 6];31(9):1425–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26163881
5. R.R., Alloway, N.A., Pilch DC. Evidence-based medicine and clinical trials in renal transplantation - UpToDate [Internet]. UpToDate. 2015 [cited 2016 Jul 18]. Available from: http://www.uptodate.com/contents/evidence-based-medicine-and-clinical-trials-in-renal-transplantation
6. Saran R, Robinson B, Abbott KC, Agodoa LYC, Albertus P, Ayanian J, et al. US Renal Data System 2016 Annual Data Report: Epidemiology of Kidney Disease in the United States [Internet]. American Journal of Kidney Diseases. 2017 [cited 2017 Apr 12]. p. A7–8. Available from: http://www.sciencedirect.com.proxy.bib.uottawa.ca/science/article/pii/S027263861630703X
7. Pocock SJ, Ariti CA, Collier TJ, Wang D. The win ratio: A new approach to the analysis of composite endpoints in clinical trials based on clinical priorities. Eur Heart J. 2012;33(2):176–82.
8. Busse JW, Bhandari M, Ferreira-González I, Montori VM, Guyatt GH. Use and Interpretation of Composite End Points in Orthopaedic Trials. J Bone Jt Surg [Internet]. 2012;94(Suppl 1(E)):65. Available from: http://jbjs.org/cgi/doi/10.2106/JBJS.K.01287
9. Abdalla S, Montez-Rath ME, Parfrey PS, Chertow GM. The win ratio approach to analyzing composite outcomes: An application to the EVOLVE trial. Contemp Clin Trials. 2016;48:119–24.
10. Inrig JK, Califf RM, Tasneem A, Vegunta RK, Molina C, Stanifer JW, et al. The landscape of clinical trials in nephrology: A systematic review of clinicaltrials.gov. Am J Kidney Dis. 2014;63(5):771–80.
11. Ferreira-González I, Alonso-Coello P, Solà I, Pacheco-Huergo V, Domingo-Salvany A, Alonso J, et al. [Composite endpoints in clinical trials]. Rev española Cardiol [Internet]. 2008;61(3):283–90. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/18361902
12. Tsai SF, Shu KH, Ho HC, Chen CH, Wu MJ, Wen MC. Doubling of serum creatinine as an outcome after renal transplant - Pathological evidence of graft biopsy. Ann Transplant. 2013;18(1):471–81.
13. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for Improving Serum Creatinine Measurement: A Report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem [Internet]. 2006 [cited 2017 Apr 19];52(1). Available from: http://clinchem.aaccjnls.org.proxy.bib.uottawa.ca/content/52/1/5.long
14. Stevens LA, Coresh J, Greene T, Levey AS. Assessing Kidney Function — Measured and Estimated Glomerular Filtration Rate. N Engl J Med [Internet]. Massachusetts Medical Society ; 2006 Jun 8 [cited 2017 Apr 19];354(23):2473–83. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMra054415
15. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med [Internet]. 2009;150(9):604–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19414839%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2763564
16. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, et al. Expressing the Modification of Diet in Renal Disease Study Equation for Estimating Glomerular Filtration Rate with Standardized Serum Creatinine Values. Clin Chem [Internet]. 2007 Apr 1 [cited 2017 Mar 22];53(4):766–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17332152
17. Lambers Heerspink HJ, Weldegiorgis M, Inker LA, Gansevoort R, Parving HH, Dwyer JP, et al. Estimated GFR decline as a surrogate end point for kidney failure: A post hoc analysis from the reduction of end points in non-insulin-dependent diabetes with the Angiotensin II Antagonist Losartan (RENAAL) study and Irbesartan Diabetic Nephropathy Trial (IDNT). Am J Kidney Dis [Internet]. Elsevier Inc; 2014;63(2):244–50. Available from: http://dx.doi.org/10.1053/j.ajkd.2013.09.016
18. Buyse M. Generalized pairwise comparisons of prioritized outcomes in the two-sample problem. Stat Med. 2010;29(30):3245–57.
19. Valsecchi MG, Miller ME, Hui SL. Evaluation of long-term survival: use of diagnostic and robust estimators with Cox's proportional hazards model. Stat Med [Internet]. 1996;15(July 1994):2763–80. Available from: http://dx.doi.org/3.0.CO%5Cn2-O
20. Royston P, Parmar MKB. Flexible parametric proportional-hazards and proportional-odds models for censored survival data, with application to prognostic modelling and estimation of treatment effects. Stat Med. 2002;21(15):2175–97.
21. Knoll GA, Fergusson D, Chasse M, Hebert P, Wells G, Tibbles LA, et al. Ramipril versus placebo in kidney transplant patients with proteinuria: a multicentre, double-blind, randomised controlled trial. lancet Diabetes Endocrinol [Internet]. 2016;4(4):318–26. Available from: http://www.thelancet.com/article/S221385871500368X/fulltext
22. Hiremath S, Fergusson DA, Fergusson N, Bennett A, Knoll GA. Renin-Angiotensin

System Blockade and Long-term Clinical Outcomes in Kidney Transplant Recipients: A Meta-analysis of Randomized Controlled Trials. Am J Kidney Dis [Internet]. Elsevier Inc; 2016;5–12. Available from: http://dx.doi.org/10.1053/j.ajkd.2016.08.018

23. Knoll GA, Cantarovitch M, Cole E, Gill J, Gourishankar S, Holland D, et al. The Canadian ACE-inhibitor trial to improve renal outcomes and patient survival in kidney transplantation - Study design. Nephrol Dial Transplant. 2008;23(1):354–8.