Statistical Analysis Plan

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Abbreviations

Abbreviation	Definition
CEC	Clinical Events Committee
CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CSR	Clinical Study Report
DCRI	Duke Clinical Research Institute
EDC	Electronic Data Capture
FU	Follow-Up
HR	Hazard Ratio
ITT	Intention-To-Treat
MI	Myocardial Infarction
mITT	Modified Intention-To-Treat
NDI	National Death Index
OMVM	Oral MultiVitamins and Multiminerals
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TACT2	Trial to Assess Chelation Therapy 2
WLW	Wei, Lin, and Weissfeld

1 Introduction

The purpose of this Statistical Analysis Plan (SAP) is to describe the analyses and data presentations that will be used for the creation of the primary manuscript of the study which will focus on the comparison of active chelation vs. placebo chelation.

1.1 Study Design

The Trial to Assess Chelation Therapy 2 (TACT2) is a randomized, double blinded, placebo-controlled 2x2 factorial clinical trial. The study treatments are edetate disodium-based chelation (weekly infusion for 40 weeks) and high-dose oral multivitamins and multi-minerals (OMVM) (twice daily for up to 5 years). Subjects with diabetes mellitus with a prior myocardial infarction (MI) are recruited from participating sites in the US and Canada and randomized in a 1:1:1:1 fashion to 4 groups:

i. Active chelation + active OMVM
ii. Active chelation + placebo OMVM
iii. Placebo chelation + active OMVM
iv. Placebo chelation + placebo OMVM.

Patients will be followed for clinical events until the earlier of database lock or five years from randomization. Clinical outcomes will be collected by study sites at each infusion visit and by the DCRI Call Center at month 6 and 12, and every 4 months afterwards with the last assessment at month 60.

TACT2 is an event driven trial with a plan to continue until at least 282 primary endpoint events are identified. The original planned sample size was 1200 participants. The sample size was later modified to 1100. The final enrollment is 1000, but with an increased follow-up time to preserve statistical power. More details on sample size and power calculation are included later in the SAP.

1.2 Study Objectives

TACT2 seeks to replicate TACT, which found a reduction of recurrent cardiovascular events most prominent in the subgroup of post-MI diabetic patients receiving edetate disodium-based chelation therapy.

1.2.1 Primary Objective

The primary objective of TACT2 is to determine if the chelation-based strategy increases the time to the first occurrence of any of the components of the primary composite endpoint of all-cause mortality, MI, stroke, coronary revascularization, or hospitalization for unstable angina compared with the placebo chelation strategy.

1.2.2 Secondary Objectives

The secondary objectives of TACT2 are to determine:

i. if the chelation-based strategy reduces the overall rate of occurrence of the events which define the primary composite endpoint of all-cause mortality, MI, stroke, coronary revascularization, or hospitalization for unstable angina events compared with the placebo chelation strategy.

ii. to determine if the chelation-based strategy increases the time to the first occurrence of a secondary composite endpoint of cardiovascular mortality, MI, or stroke compared with the placebo chelation strategy.

iii. to determine if the chelation-based strategy increases the time to all-cause mortality compared with the placebo chelation strategy.

1.2 Sample size Considerations

The study was originally designed to enroll 1200 participants with a minimum follow-up of 12 months. Since the enrollment was slower than projected, the DSMB conducted a blinded review of enrollment aggregate event rates, and projected event rates in July 2019. The study team proposed a new sample size based on the updated projected event rate, an assumed hazard ratio of 0.7 comparing chelation and placebo, and a dropout rate of 5% (Table 1). While the sample size was reduced to 1100 with an extended enrollment time by December 2020, the follow-up (FU) period was extended to 2 years to ensure to have the targeted power of 85%.

The final study enrollment was 1000. The study team evaluated the study power with a newly extended FU period of 2.5 years assuming various dropout rates (Table 2). Even with a dropout rate of 10%, the study power exceeds the targeted power of 85% with 1000 participants and 2.5 years of follow-up.

The full details around sample size adjustment and study power are included in Appendix I.

Table 1. Original and modified sample size and study power

Sample size	Note	Power	Overall event rate (%/pt-yr)	Assumed event rate in EDTA (%/pt-yr)	Assumed event rate in Placebo (%/pt-yr)	Dropout rate	Enrollment year	FU year	Total # events
1200	Original sample size	85%	10.9%	8.97%	12.81%	2%	3	1	282
1100	Modified sample size	88%	9.2%	7.56%	10.82%	5%	4.25	2	308

Table 2. Study power with the final 1000 participants enrolled

Scenario	Overall event rate (%/pt-yr)	Assumed event rate in EDTA (%/pt-yr)	Assumed event rate in Placebo (%/pt-yr)	Overall dropout rate	Mean power	Mean # events
Projected	8.8	7.2	10.4	5%	89%	299
				7%	88%	295
				10%	87%	288

2 General Considerations for Data Analyses

2.1 Analysis Populations

2.1.1 Intention-to-treat Population (ITT)

The ITT population will include all randomized participants, including those who are later found not to meet study eligibility criteria. There were two participants who mistakenly "re-joined" the trial after infusions ended and were re-randomized in a second site, near their original site. The detail for the two participants is in Appendix II. They will only be counted once in the ITT population. Their data from the 2nd randomization will not be included the analysis.

2.1.2 Modified Intention-to-treat Infusion Population (mITT)

All participants in the ITT population who have completed at least one infusion will be included in the modified intention-to-treat population. The mITT population will be the primary analysis population.

2.2 Data Sources

The data used for analysis will come from 5 sources: the randomization data from the Interactive Voice/Web System (IXRS), the e-CRF data collected by the iMedidata Rave electronic data capture system (RAVE), the adjudicated endpoint data from the clinical events committee database (CEC), the site-level information stored in the Clinical Trial Management System (CTMS) database, and the CDC National Death Index (NDI).

Masked-source NDI search results will be used to focus a search for supporting clinical and other documentation. If supporting documentation is obtained, the data will be submitted to the CEC process prior to being used in the analysis. If needed, a Mortality Review Committee will be assigned to classify potential death events when there are insufficient/incomplete or contradictory NDI and/or Call Center data to make a reliable determination. Additional details will be described in a separate charter.

2.3 General Analysis Conventions

Statistical comparisons will be performed using two-sided significance tests. An alpha level of 0.05 will determine significance unless otherwise specified.

Continuous variables will be presented in terms of percentiles (e.g., median, 25th percentile, and 75th percentiles) along with means and standard deviations. Categorical variables will be summarized in terms of frequencies and percentages.

All programs written to create analysis datasets or perform analyses will be validated according to SOPs by the Statistical Programming group.

All statistical analysis will be performed using SAS® v9.4 or higher (Cary, NC USA) and/or other proper software, e. g. R.

2.4 Disposition of Participants

Disposition of participants (number randomized, number who received treatment, number of infusions received per patient, number of withdrawals and dropouts from the study, number lost to follow-up, length of follow-up, time since last contact, number unmasked) will be summarized by active chelation vs placebo chelation groups using a CONSORT flow diagram and subject disposition table.

Lost to follow-up among alive and non-withdrawn participants will be reported in the CONSORT diagram as described above. The number of participants lost to follow-up with a primary endpoint will be included in the total and also reported separately. Participants alive and not withdrawn or lost to follow-up will be defined as completing the study.

The definition for lost to follow-up will be:

No contact within 12 months of the end of study. End of study is defined as the 5-year informed consent expiration date or administrative end of study date (June 30, 2023). Last contact is defined as the last known alive date.

2.5 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized by active and placebo chelation groups and for the overall mITT population. These include cardiovascular risk factors, comorbidities, relevant descriptors from the history, concomitant medical care including post-MI medications, treatments for diabetes, over-the-counter supplements and herbal remedies. Physical examination, laboratory data, cardiopulmonary symptoms, and past clinical events. Blood and urine metals pre- and post-chelation are discussed in the SAP for the Trave Metals Biorepository Core Lab.

2.6 Handling of Missing Data

Every effort will be made to obtain complete data during the course of the trial. If missing data remains despite all the efforts, rules on how to handle the missing data will be implemented.

If the proportion of subjects with missing values in any of the covariates for the primary analysis, i.e. age, sex, and baseline insulin use, is greater than 1%, then the missing values will be imputed to the mode.

Since the clinical endpoints are adjudicated by the CEC, we don't anticipate missing values for positively adjudicated outcomes. In the very rare occasion where the event day is missing, it will be imputed as the last day of the month. If missing day and month, then December 31st of the provided year will be used. If the year is missing, no imputation will be performed.

3 Analysis of Clinical Endpoints

3.1 General

3.1.1 Estimands and Censoring Scheme

Summaries of the descriptions of the estimand attributes and censoring schemes are shown in **Table 3**. Like TACT, the primary analysis will include all identified events obtained as part of the planned follow-up from infusion visits and call center interviews.

Table 3. Key Study Estimands and Censoring Scheme.

Objective Description / Study Population	**Endpoint**	**Summary statistics**	**Events to be included in the endpoint derivation**	**Censoring date**
Primary Objective/ mITT	Time from randomization to the first occurrence of primary composite endpoint of all-cause mortality, MI, stroke, coronary revascularization, or hospitalization for unstable angina	Hazard ratio estimate based on a Cox proportional hazards model.	All positively adjudicated primary endpoint events post-randomization except those occurring after withdrawal of consent or ICF expiration. Events occurring after withdrawal consent or ICF expiration will be censored. Subjects with events after withdrawal or ICF expiration will be censored at the min(date withdrew consent, date of last contact with patient/proxy where event status was assessed).	Among subjects not experiencing endpoint of interest: Censoring date = min(date withdraw consent, date of last contact with patient/proxy where event status was assessed) Date of last contact defined as: If the last contact occurred during the study infusion phase - use last infusion visit date where survival status and event status were assessed (yes or no to event). If last contact occurred during call center follow up period – use 'complete'/'incomplete' status date by patient/proxy where survival status and hospitalization status were assessed (yes or no to hospitalization).

				For composite endpoints, date of last assessment = the earliest of the last assessment date among all components.
Secondary Objective / mITT	Overall rate of recurrent events due to all-cause mortality, MI, stroke, coronary revascularization, or hospitalization for unstable angina	Hazard ratio estimate based on a proportional intensity model by Andersen-Gill and a marginal model by Wei, Lin and Weissfeld.	*same as primary endpoint	*same as primary endpoint
Secondary Objective / mITT	Time from randomization to the first occurrence of the secondary composite endpoint of cardiovascular mortality, MI, or stroke	Cause specific hazard ratio estimate based on a cause-specific Cox proportional hazards model.	All positively adjudicated endpoint events post-randomization except those occurring after withdrawal of consent or ICF expiration date. Non-CV death or undetermined cause of death is considered a competing risk. Among subjects experiencing competing risk (non-CV death or undetermined death is a competing risk of CV death or MI/stroke if a subject died without having any	Among subjects not experiencing endpoint of interest or competing event: Censoring date = min(date withdraw consent, date of last contact with patient/proxy where event status was assessed) *Date of last contact defined in 1st row, 5th column (primary endpoint)

			of these events beforehand): Event date = non-CV or undetermined death date;	
Secondary Objective / mITT	Time from randomization to all-cause mortality	Hazard ratio estimate based on a Cox proportional hazards model.	All deaths post-randomization (including adjudicated deaths obtained from the NDI search) A death date after withdrawn consent or ICF expiration (obtained from public data source) is considered an event.	Among subjects who are alive: Censoring date = (date last known alive) Date of last known alive defined as: If the last contact occurred during the study infusion phase - use last infusion visit date. If last contact occurred during call center follow up period – use 'complete'/'incomplete' status date by patient/proxy **or ('not done' status date where source = patient)** or last alive date from Call Center where death status was assessed.
Secondary Objective / mITT	Total number of SAE and percentage of patients experiencing at least 1 SAE.	Number and proportions by treatment group. Confidence intervals will be based on the Miettinen-Nurminen method.	Randomization to min(withdraw consent, 30 days post final infusion)	N/A

Sensitivity Analysis of the Primary Outcome / Bayesian analysis using TACT results as one of the priors / mITT	*Same as primary analysis	Treatment effect mean posterior hazard ratio based on a Bayesian time to event model	*Same as primary analysis	*Same as primary analysis
Sensitivity Analysis of the Secondary All-Cause Mortality Outcome / Bayesian analysis using TACT results as one of the priors / mITT	*Same as primary analysis	Treatment effect mean posterior hazard ratio based on a Bayesian time to event model	*Same as primary analysis	*Same as primary analysis
Sensitivity Analysis of the Primary Outcome / **ITT**	*Same as primary analysis	*Same as primary analysis	*Same as primary analysis	*Same as primary analysis
Sensitivity Analysis of the Primary Outcome / mITT with imputed event status for subjects withdrew /lost to follow up	*Same as primary analysis	*Same as primary analysis	*Same as primary analysis except that the event status will be imputed for subjects who withdrew consent or lost to follow up before study completion	*Same as primary analysis

Sensitivity Analysis of the Primary Outcome/ mITT with the exclusion of positively adjudicated events > 1 year after the last contact where all components of the composite were assessed	*Same as primary analysis	*Same as primary analysis	*Same as primary analysis but exclude positively adjudicated events more than 1 year from the most recent study contact where all components of the composite endpoint were assessed.	Among subjects not experiencing endpoint of interest or experiencing first CEC event > 1 year after event assessment date: Censoring date = min(date withdraw consent, date of last contact with patient/proxy where event status was assessed) *Date of last contact defined in 1st row, 5th column (primary endpoint)
Sensitivity Analysis of the Secondary Composite Outcome/ Where undetermined death is considered CV-death / mITT	*same as secondary composite endpoint	*same as secondary composite endpoint, where: Undetermined death is analyzed as CV-death.	*same as secondary composite endpoint	*same as secondary composite endpoint
Sensitivity Analysis of the Secondary Composite Objective / Cause of death based on best available data (from CEC or CRF if CEC is unavailable) / mITT	*Same as secondary composite analysis	*Same as secondary composite analysis	*Same as secondary composite analysis, where: Cause of death is determined based on best available data. If cause of death from CEC is available, use CEC cause of death. Otherwise, if CRF cause of death available, use CRF cause of death.	*Same as secondary composite analysis

			If cause of death is unavailable in CEC and CRF, use NDI cause of death.	
Sensitivity Analysis of the Secondary All-Cause Mortality Outcome / Censor at the date of NDI assessment / mITT	*Same as secondary all-cause mortality analysis	*Same as secondary all-cause mortality analysis	*Same as secondary all-cause mortality analysis	Among subjects who are alive: Censoring date = max(date of NDI assessment, last known alive date) Date of NDI assessment is defined as: The last date that NDI results were assessed. This will be consistent for all participants without an event.

*See section 2.1 for the definition of ITT and mITT.

One participant who was randomized twice and received infusions from the second site will be censored at the second randomization. The other participant who was lost to follow-up at the first site and then was randomized at the second site will be censored at the time of lost to follow-up at the first site.

3.1.2 Event Data

Analyses of the clinical endpoints will use the CEC-adjudicated data for death, MI, stroke, coronary revascularization, or hospitalization for unstable angina.

3.1.3 Assessment of Model Assumptions

The validity of the proportional hazards assumption will be examined using a standard graphical method such as Schoenfeld residual plots. If the assumption holds, the survival curves should be approximately parallel to each other. An additional analytical method that includes the supremum test as implemented by using the ASSESS statement in PROC PHREG in SAS version 9.4 may be utilized. A *P*-value of < 0.05 indicates violation of the proportional hazards assumption. If there is evidence of non-proportionality, a time dependent covariate will be included in the model, or a restricted mean survival time model will be performed.

3.1.4 Multiple Comparisons and Composite Endpoints

With the number of primary hypotheses and the various secondary endpoints to be analyzed and compared, there is an increased probability that at least one of the comparisons could be "significant" by chance. We have pre-specified the primary and secondary outcome variables to help avoid over-interpretation and to reduce the problems inherent with multiple testing. We will be conservative in the interpretation of these analyses, taking into account the degree of significance, and looking for consistency across endpoints.

3.2 Primary Endpoint Analysis

The primary endpoint is the composite of all-cause mortality, MI, stroke, coronary revascularization, or hospitalization for unstable angina.

If two of the component events of the primary composite endpoint occur on the same day, the following hierarchy will be used when reporting the components of the primary endpoint. For example, a patient with stroke and all-cause mortality on the same date will be considered as having stroke when summarizing individual events within the primary composite outcome. This hierarchy will only be used for summarizing events within the primary composite outcome and will not affect the primary analysis.

i. MI
ii. Stroke
iii. Hospitalization for unstable angina
iv. Coronary revascularization
v. All-cause mortality

3.2.1 Primary Analysis

The primary objective is to determine if the chelation-based strategy increases the time to composite endpoint compared with the placebo chelation strategy. The primary composite endpoint will be analyzed using a Cox proportional hazards regression model of time from randomization to the first occurrence of all-cause mortality, MI, stroke, coronary revascularization, or hospitalization for unstable angina.

The primary analysis will be based on a Cox model which includes indicator variables for the active chelation as the primary variable of interest and active OMVM groups, age, sex, and baseline insulin use as covariates. Age will be included in the model in the form of cubic splines using three knots at 10^{th}, 50^{th}, and 90^{th} percentiles. Two separate manuscripts will explore the effect of active OMVM and the effects of chelation by OMVM.

The chelation treatment effect will be summarized using hazard ratios and associated 95% CIs. A hazard ratio of < 1 would suggest a benefit for the chelation-based therapy. Kaplan Meier survival estimates will be constructed based on the time from randomization to the first

primary event occurrence. Events occurring after withdrawal of consent or informed consent expiration will be censored.

The OMVM treatment effect from the Cox model will be estimated using the Cox model described above. A hazard ratio of < 1 would suggest a benefit for OMVM treatment. Given the focus on chelation therapy, the interpretation of the OMVM treatment effect will be considered exploratory. A full set of analyses of the 2x2 design will be reported in a separate manuscript.

Four interim analyses were conducted at 25%, 40%, 60%, and 80% of expected information using a Haybittle-Peto-type boundary for efficacy (i.e. p<=0.001, Z=3). For this reason, the adjusted two-sided significance level for the final analysis is 0.05 (i.e., the corresponding critical value, Z = -1.96).

Formal statistical hypothesis testing will follow a hierarchy based on statistical significance of the prior endpoint. If the prior endpoint is statistically significant at the 0.05 level, the next endpoint's p-value will be tested for significance. The outcome hierarchy will be evaluated as follows:

1) Primary composite outcome
2) Secondary all-cause mortality outcome (with conservative censoring)
3) Secondary composite outcome of cardiovascular mortality, MI, or stroke
4) Secondary recurrent event analysis (Andersen & Gill method)

3.2.2 Sensitivity Analyses of the Primary Outcome

The primary hypothesis for the primary composite endpoint will be tested using the ITT population as a sensitivity analysis.

A sensitivity analysis was initially planned to investigate the potential effect of the COVID-19 outbreak on the primary analysis. Instead, the potential effect of COVID-19 may be explored in a separate manuscript.

A sensitivity analysis will use Bayesian methods to estimate the primary outcome treatment effect posterior distribution using the first TACT trial results and other related information to develop a set of priors for the treatment effect (Zampieri, 2020). As suggested by Zampieri, these analyses will include at least one prior distribution for the effect of chelation vs. placebo in each of the following categories: skeptical, pessimistic, and optimistic. The priors will be assumed to follow a normal distribution and will represent the log hazard ratio from the Cox model. There will be two skeptical (non-informative) priors for the log hazard ratio with a mean of zero and differing standard deviations (e.g., one standard deviation will be 10 and the other will be 100). Two pessimistic priors for the log hazard ratio will also be included, both will be centered around zero and allow for low probability of a favorable treatment effect (e.g., 0.5%

and 2.5% probability of the hazard ratio for chelation of 0.80 compared to placebo). The two optimistic priors will include the overall treatment effect from TACT (e.g., HR of 0.82 with a 95% CI of 0.69 to 0.99) and diabetes subgroup treatment effect from TACT (e.g., HR of 0.59 with a 95% CI of 0.44 to 0.79). In all of the above models, the priors for the parameters associated with other model covariates will be non-informative with large standard deviations (e.g., standard deviation of 100) and center at zero for the associated log hazard ratios. The chelation treatment effect will be summarized using mean posterior hazard ratios and associated 95% highest posterior density credible intervals. A mean posterior hazard ratio of < 1 would suggest a benefit for the chelation-based therapy.

Another sensitivity analysis is planned to assess how the primary analysis would be affected by various assumptions regarding the occurrence of primary endpoint events among those who withdrew consent or were lost to follow-up, as was performed and published in TACT. Baseline characteristics by treatment group will be assessed for the patients who did versus did not withdraw consent or were lost to follow-up. Events will be imputed by simulation only for the consent withdrawal or lost patients who did not have a documented occurrence of one of the primary events prior to the withdrawn consent/lost to follow-up. The different percentages of the withdrawn or lost patients will be assumed to have an event at their censoring time. The event rates among patients that withdrew or were lost to follow-up in each treatment group will be varied across a broad spectrum and include scenarios that were markedly unfavorable to chelation. The imputed event rates for patients who withdrew consent or were lost to follow-up will be combined with the observed event rates of patients who completed the study to assess the treatment effect via a hazard ratio. These analyses will focus on scenarios in which events among withdrawn or lost patients in the active arm are assumed to occur at a higher rate than withdrawn or lost patients in the placebo arm.

Another sensitivity analysis is planned for the primary endpoint to censor, at the point of last contact, those deaths that were identified more than one year following the most recent study contact.

3.3 Analysis of the Secondary Endpoints

The three secondary endpoints are:

i. Recurrent events of the primary composite endpoint
ii. All-cause mortality
iii. Composite of cardiovascular mortality, MI, or stroke

3.3.1 Recurrent-event analysis of the primary endpoint

Recurrent event analyses include all positively adjudicated primary endpoint events attributed to a participant: all-cause mortality, recurrent MI, stroke, coronary revascularization, or hospitalization for unstable angina events. The number of patients with a primary composite endpoint and the frequency of the primary composite endpoint per each component will be

summarized by active chelation and placebo. We analyze this endpoint using two statistical techniques:

i. The generalization of the Cox model to handle recurrent events developed by Andersen & Gill (1982) is the most frequently used approach to estimate treatment differences with total and recurrent events. This approach models gap time (time from enrollment to first event or from one event to the next) and assumes independence of observed event times within the same subject with the adjustment of the covariates. Our modeling will use robust standard errors to account for individual patients' heterogeneity and covariance across event times. The treatment effect will be presented as a hazard ratio (EDTA vs. placebo) and associated 95% CIs and *P*-value. It will be the primary analysis for the total number of all-cause mortality, recurrent MI, stroke, coronary revascularization, or hospitalization for unstable angina events endpoint.

ii. The stratified marginal Cox modeling approach developed by Wei, Lin, and Weissfeld (WLW) (1989) considers each event recurrence separately and models all the available data for the specific event. Therefore, each subject is considered to be at risk for all events, regardless of how many events each subject actually experienced. A different baseline hazard function is assumed for each event, through use of strata for each event. The WLW will model the marginal hazard of each event's failure time. The time interval for each event for each subject starts at date of randomization and ends at the time of the specific event or censoring. A treatment effect will be presented as a hazard ratio (EDTA vs. placebo) and a 95% CI and *P*-value. This will be considered as a sensitivity analysis.

Both analyses will be adjusted for age, sex, and baseline insulin use. In either analysis, a hazard ratio of < 1 would suggest a benefit for the chelation-based therapy.

An event rate of the endpoint for each treatment group will be calculated as percentage per 100 patient years, accounting for differential follow-up duration. A 95% CI for the event rates will be calculated as

$$(\frac{r_C}{T_C}) \pm 1.96\sqrt{\frac{\overline{r_C}}{T_C^2}},$$

where subscript *C* denotes the chelation arm, and *r* is the total number of event occurrences, and *T* is the total follow-up time among all participants.

3.3.2 Time to Event Analysis of the Secondary Endpoints

The analyses for the time to the first occurrence of secondary endpoints (1) time to the first occurrence of the composite of cardiovascular mortality, MI, or stroke; 2) time to all-cause

mortality) will be performed in the same manner as the primary analysis in Section 4.1, replacing the primary composite endpoint with secondary endpoints.

Competing risk of non-CV death or undetermined death will be taken into account for the secondary composite endpoint (time to the first occurrence of the composite of cardiovascular mortality, MI, or stroke). Follow-up will be censored at the time of non-CV or undetermined death if there is no prior composite event. For this endpoint, the treatment effect will be presented as a cause-specific hazard ratio.

All deaths post-randomization will be included in the secondary all-cause mortality endpoint. For this endpoint, a death date after withdrawal of consent or ICF expiration (obtained from public data sources) is considered an event.

3.3.3 Sensitivity Analyses of the Secondary Outcomes

A sensitivity analysis is planned for the secondary composite endpoint where undetermined death will be classified as CV-death.

Another sensitivity analysis is planned for the secondary composite endpoint where cause of death is determined based on the best available data. Cause of death will first be determined based on the data from the CEC. If cause of death from the CEC is unavailable, cause of death will be determined from the CRF data. If cause of death from the CEC and CRF is unavailable, cause of death will be determined based on the NDI data.

A sensitivity analysis is planned for the secondary all-cause mortality endpoint where participants with no event will be censored at the last date that NDI results were assessed. The censoring date will be consistent for all participants without an event.

A sensitivity analysis will use Bayesian methods to estimate the secondary all-cause mortality outcome treatment effect posterior distribution. These analyses will be performed in an analogous manner as the Bayesian analysis of the primary outcome (section 3.2.2).

3.4 Subgroup Analyses

To examine the heterogeneity of the treatment effect, subgroup analyses for the primary and secondary endpoints will be performed using interaction terms within the Cox proportional hazards model. The pre-specified key subgroups of interest are following.

- Sex: female vs. male
- Race: White vs. Black vs. Other
- Ethnicity: Hispanic vs. non-Hispanic (unknown ethnicity is considered missing)
- Age: ≤ 70 years vs. > 70 years
- MI location: anterior MI vs. non-anterior MI

- Pharmacologic treatment of diabetes (two subgroups analyses: no insulin vs. insulin, SGLT2i or GLP-1a vs. neither)
- Known peripheral artery disease at baseline: yes vs. no
- Tertiles of lead (in blood) and cadmium (in urine)

For categorical variables, if the proportional hazards assumption is violated, we will include the subgroup factor as a stratification variable within the Cox regression model. This model structure will decrease the reliance on the proportional hazards assumption. Event rates by treatment and HRs with 95% CIs will be reported for each subgroup. Forest plots will be generated displaying the estimated hazard ratios and 95% CIs for each subgroup. For subgroups defined using continuous variables, the analysis based on the continuous form will be considered primary but for display purposes these variables can also be categorized.

To further analyze the heterogeneity of treatment effects at the patient level, a separate manuscript will use the PATH statement approach for subgroup analyses.

The use of statin treatment at baseline will be evaluated as a subgroup analysis in the OMVM manuscript.

3.5 Safety Analyses

Using the mITT population, the total number of serious adverse events (SAEs) and percentage of participants experiencing at least one SAE from randomization to 30 days post final infusion in each patient group will be compared with confidence intervals based on the Miettinen-Nurminen method. Whether SAEs were associated with treatment and required hospitalization (inpatient hospitalization or prolongation of existing hospitalization) will be summarized per Body System or Organ Class and Dictionary-Driven Term.

4 References

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5 SAP Revision

Revision Date MMM dd, yyyy	Section	Summary of Revision	Reason for Revision
SEP 12, 2023	2.2 Data sources, 2.6 Missing data, 3.1.1 Estimands and censoring, 3.2.1 Primary Analysis, 3.2.2, Sensitivity, 3.3.1 Recurrent events, 3.4 Subgroup analyses, Appendix III	Added NDI as a data source. Updated imputation for missing data. Added sensitivity analyses. Added hierarchy for formal hypothesis testing. Added event rate equation for recurrent event analysis. Updated subgroup analyses. Added Bayesian sensitivity analysis.	Additional information on NDI. Missing dates in the data. Additional sensitivity analyses requested. Requests from PIs.

6 Appendices

Appendix I. TACT2 Sample size and study power projections as of March 1, 2021

1. **Enrollment and timeline**
    a. 1000 patients enrolled.

Last rand subject infusion	Last rand subject Follow-up	Last endpoint adjudicated	Database lock
December 2021	June 2023	August 2023	October 2023

2. **Event rate in the TACT diabetic cohort (n=633)**
    a. EDTA: 7.8%/pt-yr = 80 events / 1031 patient-years
    b. Placebo: 13.2%/pt-yr = 117 events / 886 patient-years
    c. Hazard ratio: 0.59

3. **Original and modified sample size and power**

Sample size	Enrollment	FU	Power	Hazard ratio (EDTA/placebo)	Overall event rate (%/pt-yr)	Assumed event rate in EDTA (%/pt-yr)	Assumed event rate in Placebo (%/pt-yr)	Total # events
1200	3 years	1 year	85%	0.7	10.9	8.97	12.81	282
1100	3 years + 15 months	2 years	88%	0.7	9.2	7.56	10.82	308

4. **Statistical power with 1000 enrolled participants as of March 1, 2021**
    a. As of February 23, 2021, 137 patients with at least one adjudicated primary endpoint, 1990 patient-years from 1000 enrolled patients
        i. Proportion of reported events adjudicated as primary endpoints = 69%
        ii. Projected overall event rate: If 39 patients had an adjudicated primary endpoint out of 57 additional patients with at least one reported event, 176/1990 = 8.8%/pt-yr
        iii. Minimum overall event rate: 137/1990 = 6.9%/pt-yr
        iv. Optimistic overall event rate: If 45 patients had an adjudicated primary endpoint out of 57 additional patients with at least one reported event, 182/1990 = 9.1%/pt-yr
    b. Assumptions for simulations with 10K iterations per scenario
        i. No re-consent for those who run out 5 years from the initial consent
        ii. Constant parameters: a two-sided alpha=0.05, hazard ratio of EDTA to placebo=0.7, sample size=1000, FU=2.5 years
        iii. Overall dropout rate: 5, 7, 10%
        iv. Overall event rate: 6.9, 8.8, 9.1%/pt-yr

Scenario	Overall event rate (%/pt-yr)	Assumed event rate in EDTA (%/pt-yr)	Assumed event rate in Placebo (%/pt-yr)	Overall dropout rate	Mean power	Mean # events
Projected	8.8	7.2	10.4	5%	89%	299
				7%	88%	295
				10%	87%	288
Minimum	6.9	5.7	8.1	5%	78%	244
				7%	77%	240
				10%	76%	235
Optimistic	9.1	7.5	10.7	5%	88%	307
				7%	87%	303
				10%	86%	297

Appendix II. Details of two participants who were randomized from two different sites

Double randomization case 1

One participant was randomized again in a neighboring site right after completing 40 infusions from the first site where randomized for the first time. The study team found out the second randomization after the participant finished 35 infusions at the second site. This participant has been actively followed up by the Call Center. This participant will be censored at the time of the second randomization for any analyses using the ITT or mITT populations.

Double randomization case 2

The other participant was lost to follow up after the third infusion at the first site and then randomized again in a neighboring site after a break of longer than a year. The participant did not receive any infusion from the second site. The Call Center was not able to locate the participant for any follow-up calls. This participant will be censored at the time of lost to follow-up at the first site for any analyses using the ITT or mITT populations.

Appendix III. TACT2 Statistical Team

Team	Name	Email	Attends DSMB Meetings
Blinded Statistical Team	Hayley Nemeth	Hayley.nemeth@duke.edu	X
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Metals Manuscript	Zhen Huang	zhen.huang@duke.edu	X
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