1.0 SPECIFIC AIMS

Coronary artery disease (CAD) is the most frequent cause of morbidity and mortality in the United States. At present, standard therapies for CAD include lifestyle modifications, medical therapies and invasive interventions. Despite the ready availability of a large number and variety of treatments of proven benefit, a national survey has shown that over one third of all patients also seek out and receive alternative therapies[1], including chelation therapy with ethylenediaminetetraacetic acid (EDTA). The National Center for Complementary and Alternative Medicine (NCCAM) and the National Heart, Lung, and Blood Institute (NHLBI) have recognized the clinical and public health need for a large-scale trial of chelation therapy and have issued a Request for Application. Chelation therapy, as practiced in the community, includes the use of high dose antioxidant vitamin and mineral supplements. Thus, any clinical benefit of chelation therapy may be due either to the effect of EDTA chelation, or high dose supplements, or both. We are proposing a randomized clinical trial (Trial to Assess Chelation Therapy [TACT]) with a 2 X 2 factorial design to independently test the effects of the standard chelation solution recommended by the American College for Advancement in Medicine (ACAM) versus placebo solution, and the effects of a high-dose supplementation, versus a low dose regimen to simply replace chelation-related losses. TACT has the following Specific Aims:

- To determine if chelation or high-dose supplements in patients with CAD improve event-free survival;
- To determine if chelation and high-dose supplements are safe in patients with CAD;
- To determine if chelation or high-dose supplements in patients with CAD improve quality of life;
- To determine if chelation or high-dose supplements in patients with CAD are cost effective;
- To determine if chelation or high-dose supplements in patients with CAD improve vascular function.

The primary endpoint of this trial will be a composite of: all cause mortality, myocardial infarction, stroke, hospitalization for angina and hospitalization for congestive heart failure. This primary endpoint is consistent with the RFA. A blinded Clinical Events Committee will adjudicate all events. TACT will have over 90% power to detect a 25% reduction, and over 80% power to detect a 20% reduction in this primary endpoint. Major secondary endpoints will include (1) a combined endpoint of cardiac death, or nonfatal myocardial infarction, or nonfatal stroke; (2) the individual components of the primary endpoint; (3) coronary revascularization; (4) safety of the interventions including indices of renal, hepatic, and hematological function, as suggested by the RFA; (5) health-related quality-of-life, as suggested by the RFA; (6) cost and cost-effectiveness, as suggested by the RFA; (7) brachial artery flow-mediated endothelial function, as suggested by the RFA; (8) plasma markers of oxidative stress and anti-oxidant protection, as suggested by the RFA; and (9) plasma markers of endothelial activation and inflammation, as suggested by the RFA.

TACT has been designed and will be conducted in collaboration with the American College for Advancement in Medicine (ACAM), the world's largest and most respected organization of physicians who employ chelation therapy. ACAM has trained over 4000 active chelation practitioners and has developed and published a standardized multi-component protocol that includes oral antioxidant vitamins and minerals. The chelation infusion, and vitamin and mineral supplements being tested in TACT have been developed in collaboration with ACAM, based on current clinical practice.

TACT is a multicenter and multidisciplinary collaboration of the following centers under the leadership of Gervasio A. Lamas MD:

The Clinical Coordinating Center (the Grant Awardee; CCC) including the Clinical Units, the Central Pharmacy, and the Central Lab, led by Gervasio Lamas MD, Study Chairman (Mount Sinai Medical Center);
The Data Coordinating Center (DCC, Appendix 1-3), led by Kerry Lee PhD (Duke Clinical Research Institute);
The Economics and Quality of Life Coordinating Center (EQOL Coordinating Center, Appendix 4) led by Daniel Mark MD (Duke Clinical Research Institute).
The Vascular Function Core Laboratory (Appendix 5), led by Joseph Vita MD (Boston Medical Center).

We propose to enroll 1600 patients 50 years of age or older with a prior myocardial infarction. Following baseline assessments, patients will be randomly assigned to receive either the chelation or placebo solution. Thus, 800 patients will receive active chelation solution and 800 will receive placebo solution. Each of these 2 groups will additionally be randomized to receive high-dose supplements versus low-dose supplements. Thus, 800 patients will receive high-dose supplements, and 800 will receive low-dose supplements. If both therapies

are effective, this factorial design will permit the estimation of the contribution of each to the overall effect. All patients will be followed for clinical events until the end of the trial. The average length of follow up for clinical events will be 3 years. The investigators proposing this trial believe that plausible biological mechanisms of benefits and published case series[2] suggesting clinical benefits, combined with the lack of data from large-scale, randomized trials, and widespread utilization of EDTA chelation therapy have led to a state of equipoise[3]. Specifically, we believe there is sufficient belief to justify exposing half the patients at random to this therapy, as well as sufficient doubt to justify withholding therapy from the other half. The major elements of this proposal for a randomized trial of chelation therapy are already being used in a pilot trial for TACT discussed in section 3.5 and Appendix 6.

As stated in the RFA, this proposal will "strengthen the knowledge base regarding efficacy and safety of EDTA chelation therapy for persons with CAD through the use of rigorous trial design and validated outcomes measures"[4]. The results of TACT will provide either a significant positive result or an informative null result upon which rational clinical decision-making and health policy can be based.

2.0 BACKGROUND AND SIGNIFICANCE

2.1 Alternative medicine and chelation therapy in the United States

Alternative medicine constitutes a large number and wide range of practices. However, a broad[1] definition would include those therapies that are not taught widely at US medical schools, nor are generally available at US hospitals. A carefully performed national survey, and other more restricted local surveys[5 6 7] all find the practice of alternative medicine to be widespread. Eisenberg et al[1] conducted a random national survey of 1539 adults and found that 34% reported using at least one alternative therapy in the last year, and a third of these saw providers for alternative therapy. These patients had made an average of 19 visits to such providers within the past year. The estimated cost of these practices in 1990 dollars for the US as a whole was $13.7 billion, the majority of which was paid out-of-pocket[1]. Thus, alternative medical practices are common, and constitute a significant and generally hidden health care cost for patients.

NCCAM estimated that more than 800,000 visits for chelation therapy were made in the U.S. in 1997[4]. The estimated 1-year cost for these visits is about $60 million, which is almost 3 times the cost of this 5-year trial to definitively test the benefit to risk and benefit to cost ratios.

2.2 Clinical studies: case reports and case series

The majority of the clinical literature describing benefits of chelation therapy in patients with CAD or peripheral arterial, non-carotid disease is in the form of case reports and case series[8]. Cranton[9] reported that by 1993, there were over 4600 published case reports and case series supporting potential benefits of chelation therapy. These descriptive studies are useful to formulate hypotheses and thus raise the possibility of benefits of chelation therapy. They cannot, however, be used as reliable evidence of either effectiveness or safety. EDTA chelation has also been used in patients with cerebrovascular disease. The claims of efficacy with EDTA therapy in this population are based on subjective clinical improvement and in some studies, improved cerebral perfusion or reduction in degree of carotid stenosis.[10 11 12 13 14 15 16 17 18 19] Typically, however, the patient populations were small and had a variety of cerebral diseases. Most importantly, the studies were without appropriate controls, and in some, there was criticism of methodology. Particularly in the older data, the observations tended to be subjective and descriptive. Thus, there are no appropriate randomized trials of EDTA in the treatment of cerebrovascular disease. Altogether, there are no substantial data to support claims of efficacy.

2.3 Clinical studies: randomized trials

There have been 3 interpretable randomized trials of EDTA chelation for patients with atherosclerotic vascular disease. The 2 additional very small trials of 9 and 10 patients are not interpretable and not reviewed here. A fourth trial, our Pilot to Assess Chelation Therapy (PACT), is currently ongoing and is described in the Preliminary Results section and in Appendix 6.

The first trial by Guldager et al[20] enrolled 159 patients with stable intermittent claudication for at least 12 months, and excluded patients with underlying conditions such as renal insufficiency, cardiac disease, or diabetes. The treatment regimen consisted of 20 infusions administered over 5 to 9 weeks. Patients also

received oral supplements of multivitamins and magnesium. There were no differences in any parameters studied in the EDTA-treated group compared to placebo.

Van Rij reported the second trial in 1994[21]. There were 32 patients recruited who had peripheral vascular disease confirmed by angiography. Diabetics were excluded, and patients were required to stop smoking. The active infusion consisted of 3.0 g of EDTA 0.76g magnesium chloride, and 0.84 g sodium bicarbonate in normal saline, to a total volume of 500 ml. The placebo infusion was 500 ml of normal saline. Both groups received parenteral vitamin supplements. There were no significant differences reported in pain-free walking distance, or total walking distance when the EDTA-treated group was compared to the placebo group. At 3 months after treatment, however, resting ankle-brachial index showed some improvement in the chelation group in both legs, with a significant between-groups effect favoring chelation. An extensive analysis of quality of life also was performed in the research subjects, with mixed results. Although there were no differences in scales relating to general health and effect of poor circulation on life activities, chelation patients scored better on 2 scales that rated the level of physical activity ($p<0.05$ for between-groups differences) 3 months after therapy.

Wyse and colleagues at the American College of Cardiology Scientific Sessions presented the third and most recent trial in March of 2001. The Program to Assess Alternative Treatment Strategies to Achieve Cardiac Health (PATCH) was a 6-month randomized trial that measured exercise capacity in 84 stable angina patients randomized to receive either EDTA treatment or placebo. Patients were eligible to participate in the trial if they were over the age of 21, had proven CAD, stable angina pectoris, and $\geq$ 1 mm ST-segment depression within 2-14 minutes on a gradually ramping treadmill test. A total of 39 patients were ultimately randomized to the treatment groups, receiving 40 mg/kg up to a maximum of 3 g, or placebo. Both were administered in an IV saline solution over a 3-hour period, 2 times per week over 15 weeks, then once per month for 3 months, for a total of 33 treatments. All patients were given oral multivitamins. There were no significant differences in clinical outcomes between the treatment groups. There were no deaths, no MIs, and 9 hospitalizations for worsening angina (6 in the chelation group and 3 in the placebo group). Both groups were able to increase their exercise times approximately 1 minute, an improvement that the investigators attributed to placebo or "training" effect. The investigators concluded that a trial of far larger sample size was necessary to reach any definitive conclusions.

2.4 Biological mechanisms

TACT has been designed with the concept that regardless of which anti-atherosclerotic mechanism of chelation therapy predominates; a reduction of clinical events will be the principal index of effectiveness. However, as suggested by the RFA, the study also provides an opportunity to gain insight into mechanism(s). The original hypothesis underlying the use of chelation therapy was that EDTA would remove calcium from atheromatous plaques producing favorable effects[22]. Unfortunately, there are little data to support the decalcifying hypothesis. Other postulated mechanisms of EDTA action include a) inhibition of platelet aggregation[23], b) stimulation of parathormone (PTH) release that in turn mobilizes calcium from plaques and reduces progressive calcification, c) an antioxidant effect by complexing with transitional metals thus interfering with free radical production and lipid peroxidation, d) effects on serum iron[24], and e) transient lowering of serum cholesterol. Some of these hypotheses may be valid, but there are no confirmatory mechanistic studies. On the basis of emerging knowledge reviewed below, we hypothesize that chelation therapy reduces oxidative stress in the vascular wall leading to improved vascular function, reduced inflammation, and, as a result, reduced risk for cardiovascular disease events. To investigate this hypothesis and to be responsive to the requirements of the RFA, we will assess vascular function (endothelium-dependent and -independent vasodilation), markers of oxidation status (8-epi-$PGF_{2\alpha}$ and ascorbic acid), and circulating markers of inflammation (C-reactive protein and soluble ICAM-1) in a subset of 400 TACT participants.

2.4.1 Vascular Function and Cardiovascular Disease

The vascular endothelium plays a key role in the regulation of vascular homeostasis by releasing paracrine factors that influence vascular tone, blood fluidity, inflammation, and the cellular and matrix composition of the vessel wall.[25] A prototypical and relevant endothelial product is nitric oxide (NO), which is a vasodilator and an inhibitor of platelet activity, leukocyte adhesion, and smooth muscle cell proliferation. NO production is stimulated by several factors including acetylcholine, bradykinin, catecholamines, and most importantly for this

application, shear stress resulting from increased flow.[26] It is well established that NO-dependent flow-mediated dilation can be detected in the brachial artery using the non-invasive methodology proposed for the present study.[27 28] Loss of the biologic activity of endothelium-derived NO is important in both the early[29] and later[30] stages of atherosclerosis. The clinical relevance of endothelial dysfunction due to this abnormality is supported by several recent studies demonstrating a link between endothelial dysfunction and events.[31 32 33] In general, the healthy endothelium maintains normal vascular tone, opposes thrombosis, and prevents entry of inflammatory cells into the intimal space. However, when dysfunctional, endothelial cells contribute to a vasospastic, pro-thrombotic, growth promoting, and pro-inflammatory state that is relevant to lesion formation, hypertrophy, and plaque rupture.

Mechanistic studies have linked the dysfunctional endothelial phenotype to increased oxidative stress.[34] Increased production of reactive oxygen species, components of oxidized LDL, and other aspects of increased oxidative stress have all been shown to impair NO bioactivity in conditions relevant to atherosclerosis.[35 36] Oxidative stress also is implicated in other aspects of endothelial dysfunction including adhesion molecule and PAI-1 expression.[37] There is growing recognition that endothelial dysfunction and inflammation are inter-related and play a critical role in the pathogenesis of atherosclerotic events[38 39]. To explore the effects of the TACT interventions on these mechanisms, plasma markers of lipid peroxidation (8-epi-$PGF_{2\alpha}$), antioxidant status (ascorbic acid), and inflammation (C-reactive protein and soluble ICAM) will be examined before and after therapy.

A number of interventions proven to reduce cardiovascular risk have been shown to reverse endothelial dysfunction. For example, lipid-lowering therapy,[40 41 42] ACE inhibitor therapy,[43 44 45] and exercise[46 47 48] all have this effect. Notably, estrogen also improves endothelial function[49], but failed to have a beneficial effect in a randomized trial.[50] Although this exception likely reflects the complex effects of estrogen on the vasculature, in general, interventions that improve vascular function also tend reduce cardiovascular risk. TACT will seek to gain evidence for a similar relation for chelation therapy and antioxidant vitamin and mineral supplements.

2.4.2 Effects of chelation therapy on vascular function

Chelation therapy may improve vascular function by a number of mechanisms. One possibility is direct removal of calcium from the vascular wall. Studies in animal models, suggest a relationship between endothelial function and arterial calcification.[51] As discussed above, removal of calcium from lesions and the vessel wall by EDTA chelation therapy has been questioned. However, if operative, such an effect would likely result in an improved response to both endothelium-dependent and -independent vasodilators, as will be measured in TACT.

Another potential mechanism of benefit is chelation of redox active transition metals such as iron and copper. Transition metals ions are a well-recognized source of oxidative stress in the vasculature.[52] Iron is a catalyst for the formation of the highly reactive hydroxyl radical via the Fenton reaction and similar chemistry exists for copper[52]. Free copper and iron are also known to induce oxidation of lipids and proteins.[53 54] On the basis of these observations, investigators have proposed that transition metals may contribute to atherogenesis by stimulating LDL oxidation. In support of this proposal is the observation that human atherosclerotic lesions contain redox active iron and copper,[55 56] while normal tissue does not.[57]

In addition to impairing endothelial function by stimulating LDL oxidation and forming reactive oxygen species, metal ions may also have direct effects that contribute to atherogenesis and vascular dysfunction. For example, iron contributes to NFκB activation[58] and to the expression of VCAM-1 in endothelial cells.[59] Iron also directly binds NO, as evidenced by the reaction of NO with heme iron in guanylyl cyclase. Removing redox active iron from atherosclerotic lesions would, thus, be expected to enhance the bioavailability of NO. Recently, iron chelation with intravenous deferoxamine was shown to improve NO-dependent vasodilation in the coronary arteries of patients with diabetes mellitus[60]. Dr. Vita observed a similar effect in patients with angiographically proven coronary artery disease (see Appendix 5).[61]

Table 1. Effect of Chelation Therapy on Forearm Endothelial Function.

	Response to ACH 40 μg/min (ml/min/dl)
Baseline	11.3 ± 1.8
After EDTA	15.2 ± 3.6
After EDTA + vitamins	19.7 ± 3.2*

* $p<0.05$ compared to baseline. ±SD.

It is important to point out that EDTA is a less effective chelator of iron and copper than deferoxamine and other more specific agents. Furthermore, there is data to suggest that iron, but not copper, may remain in a redox active state when bound to EDTA.[92] Importantly, this effect does not occur when there is a molar excess of EDTA,[93] as is the case in plasma following high-dose EDTA infusion. Thus, EDTA chelation therapy to reduce vascular transition metals clearly has the potential to improve vascular function.

One prior study examined the effect of chelation therapy on endothelial function.[94] In that unblinded study (n=8), endothelium-dependent forearm blood flow responses to acetylcholine were examined at baseline, after 10 session of chelation therapy with EDTA alone, and after 10 additional sessions with EDTA plus standard additives (magnesium, thiamine (B_1), riboflavin (B_2), pyridoxine (B_6), and vitamin B_{12}). As shown in Table 1, there was a trend toward improved endothelium-dependent vasodilation after EDTA alone, and significantly improved responses after the additional course of treatment with EDTA plus B vitamins. Although the sample size was small, the findings support the central hypothesis of this application.

In summary, endothelial cell dysfunction contributes to the pathogenesis of cardiovascular disease and is modified by risk reduction therapies. We hypothesize that chelation therapy and antioxidant vitamin and mineral supplements will reduce cardiovascular events by reducing oxidative stress and inflammation, resulting in an improvement in vascular function. As suggested by the RFA, this mechanistic hypothesis will be tested in a subset of the TACT population.

2.5 Current totality of evidence

- Biologically plausible mechanisms of benefit of chelation therapy in CAD exist and require further exploration based on our current understanding of the pathophysiology of the atherosclerotic plaque.
- The very large number of published case reports and case series support a hypothesis that EDTA chelation therapy provides clinical benefits in atherosclerotic vascular disease.
- A very small number of trials of very small sample size have randomized 275 patients in aggregate, a number far too small to reliably detect or exclude the most plausible benefits.

The TACT proposal seeks to resolve the current state of equipoise about the clinical value of chelation therapy. TACT provides a comprehensive response to the NCCAM/NHLBI RFA that calls for the performance of a definitive trial to measure the effect of chelation therapy on clinical and physiological endpoints.

3.0 PRELIMINARY STUDIES

In this section, we review the vast experience of our multidisciplinary investigative leadership at the CCC, DCC, EQOL, and Vascular Function Core Lab. In addition, we review our 3-year experience in developing numerous collaborations for a pilot phase and large-scale trial of chelation therapy.

3.1 Experience of the leadership team at the CCC

Gervasio Lamas MD, the Study Chairman, is Associate Professor of Medicine at the University of Miami School of Medicine (Mount Sinai), a Board Certified cardiologist, and meets the requirements of the RFA for Principal Investigator of the trial. Dr. Lamas has extensive experience in the design, conduct, analysis, and interpretation of a large number of randomized trials, largely multicenter, in the treatment and management of cardiac disease, including CAD. Dr. Lamas was the Study Chairman of the Pacemaker Selection in the Elderly trial, a 38-site, 407-patient (100% of projected enrollment) randomized trial of pacemaker selection whose results were published in the New England Journal of Medicine [95]. Dr. Lamas was also Study Chairman of the Rate Modulated Pacing and Quality of Life trial, a 35-site trial comparing DDD and DDDR pacing in 406 patients (>100% of projected enrollment). Dr. Lamas was also the Study Chairman of MOST[96], a 100-site, 2010-patient (100% of projected enrollment) NHLBI-sponsored trial comparing clinical events in patients paced VVIR versus DDDR. MOST is the largest trial of pacemaker therapy in patients with sinus node dysfunction. Dr. Lamas serves as Study Co-Chair of the Occluded Artery Trial (OAT), an NHLBI-sponsored clinical trial of late opening of the infarct artery. OAT has recruited 400 patients to date. Dr. Lamas also serves as the Study Chairman on the Advanced Elements of Pacing Trial (ADEPT), a randomized trial that will assess the quality of life benefits of several advanced features in modern cardiac pacemakers. ADEPT has randomized 700 patients out a projected 950 patients to date. Thus, as required by the RFA, Dr. Lamas has demonstrated ability to enroll patients in randomized clinical trials. Furthermore, in all these trials patient adherence and compliance has been excellent. Dr. Lamas served for 4 years as a member of the NHLBI Clinical Trials

Review Committee, and for an additional year as the Committee Chair. Dr. Lamas serves as Director of Cardiovascular Research and Academic Affairs at Mount Sinai Medical Center – Miami Heart Institute and has recently recruited Dr. Charles Hennekens as his Associate Director. Dr. Lamas is working closely with Dr. Hennekens on a randomized trial of hormones, aspirin, and C-reactive protein. Dr. Lamas has worked closely with Dr. Lee on MOST and ADEPT, and with Dr. Mark on OAT and ADEPT. Thus, the investigative team is highly experienced in clinical trials, and they and their staff have years of experience working together. Finally, the teams of Senior Investigators that Drs. Lamas, Hennekens, Lee, and Mark have developed over the years will form the nucleus of the Enrolling Sites that will enroll patients into TACT.

Charles H. Hennekens MD DrPH, *the trial Co-Principal Investigator*, is Associate Director of Cardiovascular Research at Mount Sinai Medical Center; Visiting Professor of Medicine and Epidemiology and Public Health at the University of Miami School of Medicine; and Visiting Fellow, Green College, University of Oxford, United Kingdom. Dr. Hennekens is the former Braunwald Professor of Medicine at Harvard Medical School and Chief of Preventive Medicine at the Brigham and Women's Hospital. Dr. Hennekens was the Principal Investigator of the landmark Physician's Health Study[87], and the PI of the cardiovascular component of the Women's Health Study[88] . Furthermore, Dr. Hennekens has a long-standing interest in the rigorous evaluation of anti-oxidant vitamins and has served as PI or co-PI of a number of megatrials of vitamin supplementation totaling over 100,000 patients. Dr. Hennekens serves as Chair of the worldwide Antioxidant Vitamin Trialists Collaboration. Dr. Hennekens delivered the Linus Pauling Lecture and was the recipient of the 1997 Prevention Award from ACAM. Dr. Hennekens has served as both a member and Chair of the Clinical Applications and Prevention Advisory Committee to NHLBI.

Martin Dayton MD DO, the trial chelation consultant, is an Assistant Clinical Professor at Nova Southeastern College of Osteopathic Medicine, the former Director of the Scientific Research Committee of ACAM, and a chelation clinician with a busy practice in Miami. He is Board Certified in Family Practice and has additional Board certification in Chelation Therapy and Clinical Nutrition. Dr. Dayton has clinical experience with over 75,000 chelation infusions.

Rachel Eidelman MD, will serve as Trial Manager for TACT. As required by the RFA, Dr. Eidelman has experience directing an ongoing multi-site clinical trial. Currently, Dr. Eidelman serves as Project Director with Drs. Lamas and Hennekens of the randomized trial of hormones, aspirin, and C-reactive protein. This is a multicenter trial funded by Bayer. Dr. Eidelman has had experience in training physicians and coordinators in the protocol details and in regulatory requirements, and developing enrollment-enhancing strategies. Dr. Eidelman received her M.D. from the University of Texas Medical Branch at Galveston and completed a residency in Internal Medicine at Yale-New Haven Hospital. She recently concluded a year of research with Drs. Lamas and Hennekens. She is currently a cardiology fellow at Mount Sinai Medical Center & Miami Heart. Should the grant be awarded, Dr. Eidelman will defer her clinical training to complete this project.

Alan Ackermann DO will serve as Trial Co-Manager. Dr. Ackermann completed Osteopathic Medical School at Nova Southeastern University and Internal Medicine training at Franklin Square Hospital Center in Baltimore (U of Maryland affiliate), and has experience with the role of Trial Manager in the ongoing pilot trial. In that role, he has been instrumental in effectively managing enrollment problems, IRB issues at Mount Sinai and at collaborating institutions, personnel changes in clinical units and other routine management problems encountered in clinical trials. Furthermore, Dr. Ackermann has acquired unique experience in the clinical management of chelation patients, and in the implementation of the Internet-based data entry and prescription system that was developed in the pilot study for expansion into TACT. Finally, Dr. Ackermann has published on chelation therapy with the Study Chairman, and has made invaluable contacts in the chelation community. Thus, Dr. Ackermann is a valuable resource in general for TACT, and specifically for the Trial Manager Dr. Eidelman. In the unlikely event that the Trial Manager is unavailable, the Trial Co-Manager will respond immediately to clinical and protocol questions from clinical sites. In fact, as suggested in the RFA, if Dr. Eidelman were unable to continue her role, Dr. Ackermann would assume the role of Trial Manager.

3.2 Experience of the Principal Investigator at the DCC

Kerry Lee PhD is Senior Statistician and Associate Professor of Biostatistics at Duke University School of Medicine. Dr. Lee and the Duke group have accumulated extensive experience in coordinating multicenter collaborative clinical trials. Commencing in 1985, Dr. Lee and Dr. Robert Califf at Duke established the Data Coordinating Center for the Thrombolysis and Angioplasty in Myocardial Infarction (TAMI) clinical trials study group[69 70 71]. Nine multicenter trials involving the TAMI collaborative group were successfully conducted between 1985 and 1992. Dr. Lee was the biostatistical director of the TAMI Coordinating Center. The clinical trials experience of Dr. Lee and the Duke group was greatly enhanced and expanded through coordinating the large international megatrial, GUSTO-I, a four-arm study of thrombolytic therapies in which 41,021 acute myocardial infarction (MI) patients were enrolled from 1,081 hospitals in 15 different countries[72 73 74]. Patient enrollment for that trial was completed in February, 1993, the initial results were announced in April 1993, and the first study report appeared in the New England Journal of Medicine in September 1993[72]. Dr. Lee directed the world-wide statistical and data management functions in GUSTO-I. He also provided the statistical leadership for GUSTO IIb, a 12,000 patient trial of anti-thrombin therapy (heparin versus hirudin) in patients with acute coronary syndromes (conducted during 1994-96, and for GUSTO III, a 15,000 patient international trial of reteplase (r-PA) versus accelerated-dose t-PA in acute myocardial infarction (conducted during 1996-98). Dr. Lee served as principal investigator for the Data Coordinating Center of an NIH-funded trial of electrophysiologic-guided antiarrhythmic therapy in patients with coronary artery disease, left ventricular dysfunction, and nonsustained ventricular tachycardia[75 76]. This study, known as MUSTT (Multicenter UnSustained Tachycardia Trial), involved 85 clinical sites in the United States and Canada and included a large patient registry as well as a randomized trial. The primary study report from that trial, which Dr. Lee co-authored, recently appeared in the New England Journal of Medicine[76]. Dr. Lee was also principal investigator of the Data Coordinating Center for the 100 site, 2,010 patient, NIH-sponsored pacemaker mode selection trial (MOST) designed to assess the effects of single chamber versus dual chamber pacing on survival and quality of life in patients with sinus node dysfunction. Dr. Lamas was the study chairman of MOST, and through this trial, Dr. Lee and Dr. Lamas have established a productive collaborative working relationship. This productive relationship has been recently reinforced in the industry-sponsored study ADEPT (Advanced Elements of Pacing Trial), a 100-site trial of quality of life in pacemaker patients in which Dr. Mark leads the assessment of quality of life and the cost-effectiveness of the therapies being tested. Thus, Drs. Lamas, Lee, and Mark have a close working relationship that will assure the successful completion of TACT. Dr. Lee also served four years as a member of the NHLBI Clinical Trials Review Committee. In summary, Dr. Lee and his colleagues at the Duke Clinical Research Institute have many years of accumulated experience in collaborative clinical research and in coordinating multicenter clinical studies. They are well prepared to undertake the coordination of this large and important clinical trial.

3.3 Experience of the Principal Investigator at the Economics and Quality of Life Coordinating Center
Daniel B. Mark MD, who will be a collaborator in this project and will serve as director of the Economics and Quality of Life Coordinating Center, brings to this study a wealth of clinical trials and clinical research experience. Dr. Mark is Professor of Medicine, Duke University School of Medicine, and Director of the Outcomes Research and Assessment Group in the Duke Clinical Research Institute. He has participated in numerous international clinical trials coordinated at Duke, and has focused on assessing cost effectiveness and quality of life in these trials. Major multi-center completed trials in which Dr. Mark has directed research on economic and quality of life endpoints include GUSTO-I, GUSTO-IIb, EPIC, EPILOG, EPISTENT, IMPACT II, PURSUIT, SYMPHONY, 2nd SYMPHONY, PARAGON B. Dr Mark is Principal Investigator of NHLBI-funded research investigating economic and quality of life outcomes in OAT (with Dr Lamas, see above) and SCD-Heft (with Dr Lee, see above.) Dr Mark is also working with Dr Lamas on the ADEPT randomized trial and co-directs (with Dr Lee) the Coordinating Center for this trial. Additional details concerning his extensive clinical trials experience and innovative leadership in economic and quality of life studies are provided in Appendix 4, the Economics and Quality of Life Coordinating Center appendix.

3.4 Experience of the Principal Investigator at the Vascular Function Core Lab
Dr. Vita is Professor of Medicine, Boston University School of Medicine and Director of Clinical Research for the Department of Medicine, Boston Medical Center. Dr. Vita has 15 years of experience with the clinical assessment of endothelial function in human subjects and is a recognized expert in this field[77 78]. His research

program focuses on the relation between oxidative stress and endothelial dysfunction in atherosclerosis and on the clinical implications of vascular dysfunction. He has a particular interest in the effects of transition metals on vascular function and oxidative stress[61]. He is the principal investigator of a Program Project Grant entitled "Vitamin C and GSH: Roles in Vascular Function" (PO1-HL60888) and the principal investigator of the clinical project of a SCOR on Ischemic Heart Disease in Blacks (P50 HL55993). He directs a clinical research unit at Boston University School of Medicine and has studied over 2,000 subjects using the ultrasound methodology and plasma/urine markers of oxidative stress proposed in this application. Dr. Vita is the Principal Investigator of the Endothelial Function and Oxidative Stress Core Laboratory for the ongoing Pilot Study to Assess Chelation Therapy (PACT). Dr. Vita is also a co-investigator on RO1 grants entitled "Inflammation: Correlates and Prognosis in Framingham" HL/AI64753 and "Endothelial Vasomotor Function in the Framingham Heart Study" (HL60040), which examine plasma markers of inflammation and vascular function, respectively, in the Offspring Cohort of the Framingham Heart Study. To date over 3,800 subjects have been studied at Framingham. In TACT, the same approach and methodology will be used to conduct a comprehensive examination of vascular function before and during chelation therapy.

3.5 Pilot phase experiences

Drs. Lamas and Hennekens have a longstanding interest in rigorously testing the most plausible small to moderate benefits and risks of chelation therapy in cardiovascular disease in a randomized trial of sufficient size to demonstrate either a clear positive or informative null result.

Accordingly, in early 1999, collaboration was initiated with a multidisciplinary team of investigators that includes experts in alternative medicine, specifically chelation therapy as well as cardiology, epidemiology, biostatistics, pharmaco-economics, quality of life, and vascular function. When an investigator-initiated grant to NHLBI was approved but not funded in mid-2000, a pilot phase was initiated prior to re-submission. The pilot phase includes recruitment, treatment, and follow-up of 40 patients in a randomized double blind placebo controlled trial of chelation therapy in a design very similar to TACT. Randomization and infusion of blinded chelation/placebo solution has already started, so data will be available by early 2002. Additional details of our pilot trial are in Appendix 6.

4.0 RESEARCH DESIGN AND METHODS

4.1 Patient recruitment

We propose to randomize 1600 patients with a prior MI who are 50 years or older in a 2X2 factorial, double blind, placebo-controlled trial of EDTA chelation therapy and/or high-dose vitamin therapy. We are choosing patients age 50 or older to achieve a more equivalent distribution of men and women for whom CAD is the leading cause of death, and in order to more closely reflect the ACAM age distribution of patients undergoing chelation therapy. We are restricting the diagnosis of prior CAD to myocardial infarction because of the relatively high frequency of recurrent clinical events in such patients.

4.2 Patient selection - Inclusion criteria (all inclusion criteria must be present)

1. Men or women age 50 years and older.
2. Documented myocardial infarction (MI) over 3 months prior to evaluation; a TACT MI must meet the following criteria: MI is defined based on ischemic symptoms ≥30 minutes, and either
   1) cardiac serum marker elevation (CPK ≥2x upper limit of normal and CPK-MB elevated above the upper limit of the lab normal) or troponin T, or troponin I at least twice the upper limit of normal
   OR
   2) electrocardiographic changes, defined as new Q-waves of ≥0.03 sec and/or 1/3 of QRS complex in ≥2 related ECG leads
3. Hematocrit 35 to 50%.

4.3 Patient selection - Exclusion criteria (no exclusion criterion may be present)

1. Prior chelation therapy.
2. History of allergic reactions to EDTA or any other components of the chelation solution, including heparin.
3. Coronary or peripheral arterial revascularization procedure performed within the last 6 months.

4. Planned revascularization procedure in the 6 months following enrollment.
5. Congestive heart failure within 6 months prior to enrollment.
6. Poor or no venous access in the upper extremities
7. Baseline serum creatinine >2.0.
8. Baseline platelet count <100,000.
9. History of cigarette smoking within the last 3 months, as chelation is thought to lose effectiveness in active smokers due to overwhelming oxidant stress.
10. ALT or AST > 2.0 times the upper limit of normal.
11. Documented metabolic and/or mineral abnormalities including Wilson's disease, hemochromatosis, parathyroid disease, vitamin D deficiency.
12. Women of childbearing potential (expected to be a very small number given the age range and presence of CAD).
13. Any medical condition including a current diagnosis of cancer (except non-melanoma skin cancer) that will limit patient survival over the duration of the trial.

4.4 Screening evaluation

Potentially eligible patients without exclusion criteria will undergo an initial screening visit in which clinical eligibility will be confirmed and the trial protocol will be explained in detail. Patients agreeing to participate will be asked to provide informed consent. Baseline case report data will be obtained at this time including relevant history and use of all conventional and alternative therapies. Baseline laboratory tests will be performed. Baseline information on quality of life will also be obtained at this time.

4.5 Randomization

The day after the patient visit, the Coordinator will check lab results for any previously undetected exclusion criteria. If the patient is still eligible, the Coordinator will telephone the patient to notify him or her of acceptance into TACT and schedule the first infusion. The coordinator will also telephone the Duke Randomization Hotline. After a dialogue with the Interactive Voice Response System to independently verify eligibility, the patient will be randomly allocated to one of the treatment arms and assigned a study number. The Central Pharmacy will be electronically notified of the randomization, and of the schedule for the first infusion. Randomized patients participating in the Vascular Function Study will have baseline measurements of endothelial function prior to the first infusion. Details concerning collection, recording, and transmitting data are contained in Appendix 3.

4.6 Treatment regimen

Chelation therapy as recommended by ACAM and practiced in the community includes the use of high dose antioxidant vitamin and mineral supplements. The science underlying these supplements is based on the premise that vitamins and minerals are depleted during chelation and that reparative processes activated by chelation will be retarded by these depletions. Thus, any clinical benefit of chelation therapy may be due either to the effect of EDTA chelation, or high dose supplements, or both. We are proposing a full 2 X 2 factorial design that will independently test the effects of the standard, ACAM-recommended chelation solution versus placebo solution, and the effects of a high-dose supplementation, versus a low dose regimen to simply replace chelation-related losses. Thus, the present infusion protocol has been developed in collaboration with respected chelation practitioners to provide a safe but intensive course of therapy and to include the most commonly used additives in the infusions. The chelation solution[79, 80] will consist of 500ml of sterile water with the following additives: 3 grams of Na2EDTA, 2 grams of magnesium chloride, 100 mg of procaine HCL, 2500 units of heparin, 7 grams of ascorbate, 2 mEq KCl, 840 mg sodium bicarbonate, 250mg pantothenic acid. The infusion will be administered over 3 hours. ACAM has, in the past, suggested the addition of 100 mg of thiamine to the chelation solution. However, in consultation with the ACAM Liaison Committee, we have been advised that in usual present-day practice, thiamine is not administered due to concerns about allergic reactions.

4.7 Pharmacology and toxity of EDTA

EDTA is a chelating agent with affinity for divalent and trivalent metals. Its primary indication is for the treatment of hypercalcemia, and heavy-metal poisoning. It preferentially binds calcium ions, forming a stable,

soluble complex that is excreted by the kidneys. Transient reduction of serum calcium can be observed following IV infusion of EDTA. 1gm of EDTA can effectively bind approximately 120mg of calcium. Disodium EDTA is not metabolized in the body or reabsorbed by renal tubules. The EDTA molecule leaves the body intact. The half life of EDTA, in patients with intact renal function is approximately 45 minutes. After 24 hours, approximately 90% of the EDTA has been eliminated[81]. According to ACAM's protocol and safety information, absolute contraindications to the use of EDTA are severe allergy, renal dysfunction, and acute lead encephalopathy. Reported toxicities include renal dysfunction, mucocutaneous lesions, glycosuria and trace element depletion[82 83 84 85]. Febrile reactions can occur 4 to 8 hours after infusion, although these are rare. They are manifested by a rapid onset of malaise, fatigue, chills and fever, and may be associated with the development of severe myalgia, frontal headaches, anorexia and occasional nausea and vomiting. Other observed reactions include a histamine-like manifestation of symptoms, including sneezing, nasal congestion and lacrimation. All of these above-mentioned reactions are associated with high doses of EDTA, as well as with a rapid infusion rate. These have been minimized with the approved ACAM protocol of administration that will be used in TACT. Nonetheless, all reactions will be documented on the electronic data collection forms.

4.8 Explanation of additives

Magnesium reduces the local discomfort that may result from the infusion, and replaces magnesium losses during chelation. Sodium bicarbonate buffer is also added to provide a more physiologic pH and reduce any discomfort from the infusion. Procaine is added to further reduce any local discomfort. Heparin at doses too small to produce a systemic anticoagulant effect is added to reduce the risk of local phlebitis. Vitamin C is believed to work synergistically with EDTA as an antioxidant, and aids in making the solution isoosmolar. Pyridoxine is added because EDTA chelates it. Pantothenic acid is added due to its potential antioxidant activity.

4.9 Placebo Infusion

The placebo infusion will consist of a 563cc infusion of 0.9N NaCl, and 1.2% dextrose (14 ml of 50% dextrose, the ascorbate control).

4.10 Vitamin and mineral supplementation

The current totality of evidence on vitamin and mineral supplementation includes a vast quantity of basic research and epidemiologic studies as well as emerging data from randomized trials. In basic research, vitamins and minerals have been demonstrated to have a large number of plausible biological mechanisms for benefit in cardiovascular disease. Further, the majority of epidemiologic studies indicate that individuals who self-select for various vitamin and mineral supplements tend to have lowered risk of cardiovascular disease. The data from randomized trials, however, have not generally supported a clearly beneficial effect of various vitamins and minerals. Further reliable data should emerge over the next few years.

In addition to the possible benefits of vitamin and mineral supplementation alone, which requires further testing in randomized clinical trials, it has also been suggested that these agents may be necessary to demonstrate a clear benefit of chelation therapy. Thus, we propose to test the independent benefits of these supplements in a 2X2 factorial design. If both chelation therapy, as well as vitamin and mineral supplementation therapy, are beneficial, this design alone will permit the estimation of the contribution of each to the overall effect. It is also possible that vitamin and mineral supplementation will be beneficial and that the result for chelation therapy will be null. Such a result would offer a plausible explanation for the positive results of the case series that are independent of chelation therapy.

While most but not all of our ACAM collaborators believe that vitamin and mineral supplementation is necessary to demonstrate a benefit of chelation therapy, we retain flexibility about whether they must be tested as well as their precise composition.

Nonetheless, since this trial is likely to be the most definitive test of the benefits and risks of chelation therapy, it would be unfortunate if a null result were challenged by chelation practitioners because of inadequate vitamin and mineral supplementation. Our selection of the precise contents and dosages of the supplements is based on extensive discussion with ACAM and reflects clinical practice.

We propose that all patients will receive the low-dose regimen which repletes any chelation-related losses. Patients assigned to the high-dose regimen will receive all active supplements. Patients assigned to the low-dose regimen will receive matching placebos for the high-dose regimen. The low-dose regimen includes the following: manganese 15mg, chromium 50 mcg, zinc 25 mg, copper 2 mg, B6 25 mg. The high dose regimen was defined by consensus with the ACAM Liaison Committee (Section 4.27.3) and consists of the following to be taken twice daily: vitamin A (fish liver oil) 1656.67mg, vitamin A beta carotene 333.3 2500mg, vitamin D3 200mg, vitamin E succinate 135mg, vitamin C 200mg, vitamin K1 0.01mg, thiamine mononitrate granular 18.57mg, niacinamide 25mg, niacin 18.33mg, biotin 0.05mg, pyridoxine 18.33mg, pantothenic acid 88.87mg, cyanocobalamin 0.017mg, folic acid 0.133mg, calcium citrate 80mg, calcium ascorbate 3mg, iodine 0.025mg, magnesium 80mg, magnesium 3mg, copper 0.33mg, zinc 3.33mg, potassium 6.5mg, potassium aspartate 10mg, manganese 3.33mg, choline bitartrate 25mg, citrus bioflavinoid 16.67mg, chromium 0.033mg, selenium 0.033mg, molybdenum 0.025mg, vanadyl sulfate 0.033mg, boron 0.333mg, and coenzmye Q10 (ubiquinone) 50mg.

4.11 Blinding the treatment groups

Unfortunately, the chelation solution cannot be supplied mixed to the sites. Neither EDTA nor ascorbate are thought to be stable if shipped mixed with the other components of the chelation solution, nor are they thought to be stable if shipped mixed with each other only. The shipped and refrigerated pack will contain a syringe with ascorbate, one with EDTA, and a bag for intravenous infusion with all the other components mixed. EDTA in solution is clear and of approximately the same density and viscosity as water. Thus, the placebo-EDTA syringe will contain normal saline. Blinding the ascorbate syringe is more challenging. The ascorbate solution is a pale yellow color, which, upon mixing (14ml of ascorbate solution in 549ml) becomes indistinguishable from the clear saline placebo solution. In addition, ascorbate, in the concentration provided by the manufacturer, is viscous and provides resistance to transfer into the infusion bag through a 21-gauge needle. Thus, the placebo-ascorbate syringe will contain 14 ml of 50% dextrose. The investigative team has tested different concentrations of glucose and has found that the resistance to transfer through a 21-gauge needle of 50% dextrose is indistinguishable from that of the ascorbate concentration that will be used. Blinding the pale yellow color of ascorbate is likewise challenging. The syringe containing ascorbate or ascorbate-placebo will be covered in translucent yellow adhesive tape, thereby obscuring the different colors of the syringe solutions. At the time of infusion, the contents of the syringe are injected into the infusion bag. The ascorbate is so pale that there is no discernible yellow "puff" as it enters the bag, and the blind therefore is preserved. The blinded research coordinator will then administer the infusion, not knowing whether it is chelation solution, or control solution, and the double blind will be preserved.

4.12 Treatment Schedule

Our proposed plan of therapy has been derived based on consultations with ACAM representatives, published ACAM guidelines[79 80], then tempered by practical considerations to enhance patient compliance. The clinical practice recommended by ACAM is based on 30 weekly infusions. The remaining 10 infusions can then be administered over up to 20 months, to minimize the time the individual patient spends without ongoing maintenance infusions. The entire regimen can take up to 27.5 months to complete.

4.13 Concomitant surgical and medical therapies

4.13.1 Surgical therapies

Patients will be randomized only if there is no planned revascularization procedure or surgery within the next 6 months. The rationale for this exclusion criterion is based on the high rate of restenosis and graft occlusion leading to clinical events that would likely be unaffected by chelation therapy.

4.13.2 Medical therapies

All medical, and indeed, all surgical therapies will be instituted based on the judgment of the responsible clinician. The use of aspirin, beta adrenergic blocking agents, ACE inhibitors, and statins will be strongly encouraged in all patients eligible for these therapies in order ensure that background medical therapy in TACT is fully consistent with current evidence and national practice guidelines. Detailed information will be collected on the case report form covering all medical and surgical therapies patients undergo during the trial.

4.14 Overview of patient monitoring during infusion and follow up

	Screen & Randomize	Infusion intensive phase (weekly x 39)	Infusion maintenance phase (bi-monthly x 16)	Final infusion: 28 months	3 times yearly at 3 month intervals: months 28 to closeout	Yearly	Closeout
[redacted]							
Clinical History	+	At each infusion	At each infusion	+		+	+
Physical Exam	+	At each infusion	At each infusion	+		+	+
Telephone					+		
ECG	+					Including during infusion phase	+
Monitoring Labs	+	See lab schedule	See lab schedule	See lab schedule			
Inflammatory markers	+						
Economics and QOL	+	At 6 months	At 12 months		Resource use	At 24 months	
Endothelial function	+	At last weekly infusion		+			
Oxidative stress	+	At last weekly infusion		+			

Please note that patients are contacted 4 times yearly, at 3 month intervals – 3 times are by telephone, and a clinic visit is made yearly. Thus telephone visits above are 3 times yearly at 3-month intervals.

4.15 Infusion visits

Patients will receive a total of 40 infusions. The initial infusion will be preceded by the evaluation listed in sections 4.4 and 4.5. Each infusion encounter will be preceded by a brief interval history with specific emphasis on cardiac symptoms and clinical events, including hospitalizations. Vital signs will be measured before, during, and after the infusion. A brief cardiopulmonary exam will be recorded at baseline and at the end of the infusion. Symptoms occurring during the infusions will be elicited and recorded on the CRF. Scheduled labs will be drawn prior to the beginning of the infusion. Safety labs, consisting of CBC, platelet count, creatinine, glucose, and calcium will be drawn at baseline, and 9 additional times during the infusion regimen; immediately preceding infusions 2, 5, 10, 15, 20, 25, 30, 36, and 40.

4.16 Routine visits

Following the infusion phase, patients will have contact with the clinical unit 4 times yearly at 3-month intervals. Three of the contacts will be by telephone. This telephone follow up methodology is based on the follow-up schedule in place for the Occluded Artery Trial, a 3200 patient, NHLBI-sponsored study which Dr. Lamas Co-Chairs. Once yearly, and at the closeout visit, patients will be seen at the clinical unit. The yearly visits and the closeout visit will consist of an interval history designed to capture clinical events. An ECG will be recorded at baseline and yearly. The routine ECGs will be read at the clinical site. Alfonso Tolentino MD, who provided Core ECG services for a 2010-patient NIH-sponsored trial of cardiac pacing, will carry out quality control over-reads at the CCC on all ECGs that show a new MI, and on 10% of other ECGs (see Consultants).

Whenever a clinical event occurs that is a component of the primary endpoint or of the secondary clinical endpoints, the clinical unit is responsible for notifying the DCC within 24 hours of discovery via the electronic data capture system. Final, complete clinical data will be entered on the appropriate data collection form. Hardcopies of original clinical data will be sent to the DCC, including copies of medical and laboratory records and ECGs for review by the blinded Clinical Events Committee. In all cases the patient name will be masked and replaced with the TACT ID number prior to transmission to the DCC.

4.17 EQOL assessments

The philosophy of the TACT proposal is to integrate collection of economic and quality of life data into the mainstream activities of each TACT site. The medical resource consumption data required for this study will be collected on the clinical case report form. Relevant baseline economic and quality of life data will be collected by a structured interview that will be administered by the site coordinator prior to randomization. Follow-up economic status and interval medical care will be assessed at each infusion visit until the infusion regimen is finished and then at 3- month intervals as part of the standard TACT clinical follow-up process. Follow-up quality of life will be assessed by telephone at six months, one year and 2 years after enrollment by EQOL personnel at the DCRI using a structured interview format. For patients who have died or are unable to participate in the interview, a short proxy form will be used. Collection of the post-randomization follow-up quality of life data by the EQOL Coordinating Center expert patient interviewers will allow us to collect high quality data in a standardized fashion efficiently and for a relatively modest cost. An on-site interview by site coordinators is an alternative that has been used by us in other trials. Training the coordinators in proper interviewing techniques and then monitoring their performance is actually a more labor-intensive way to collect QOL based on our experience. Thus, we have proposed central telephone-based interviews for the key follow-up QOL assessments in TACT (at 6 months, 1 and 2 years after enrollment).

4.18 The Vascular Function Core Lab Evaluations in a 400-patient subset

The vascular function assessment begins with automated supine blood pressure measurements 3 to 5 times until stable. These measurements are recorded as stated below, and endothelial function assessment follows. The brachial ultrasound method to assess endothelium-dependent flow-mediated dilation has been extensively validated[2728]. A narrow gauge blood pressure cuff is positioned on the upper right arm. The sonographer uses a high resolution ultrasound probe to obtain optimal images of the brachial artery 2-15 cm above the antecubital crease and then takes great care not to move the transducer position throughout the remainder of the study. The arm cuff is inflated for 5 minutes at 200 mmHg (if systolic blood pressure is greater than 150 mmHg, the cuff is inflated 50 mmHg above systolic pressure). Following cuff release, the sonographer records Doppler flow for 15 seconds and then reverts to 2D mode and records images of the brachial artery for an additional 105 seconds (up to 2 minutes after cuff deflation). After a 10 minute rest period, images of the brachial artery are recorded before and 5 minutes after the patient takes sublingual nitroglycerin (0.4 mg) to produce endothelium-independent nitric oxide-mediated dilation of the brachial artery. The procedure is well tolerated by subjects and the level of discomfort is estimated to be equivalent to that associated with standard venipuncture.[95] This procedure will be performed at baseline (after randomization and before the first infusion), at the end of the weekly infusions (week 30), and at the end of all the infusions. In all cases, endothelial function will be assessed before the infusion scheduled for that visit, not immediately after.

4.19 Safety and other laboratory monitoring.

In TACT, the DSMB will assume primary responsibility for assessing the safety of the interventions being tested. Since data are presented to the DSMB only a few times a year, we will supplement this aggregated monitoring with procedures at each clinical site to evaluate individual patient tolerance to the treatment interventions. In the ACAM clinical experience with chelation therapy assessment of safety is conducted prior to beginning chelation therapy, at the fifth treatment, and at each fifth infusion thereafter[30]. In TACT, the safety monitoring will be enhanced so that a complete safety profile is obtained prior to infusion number 2 as well. Indices of kidney, liver, hematologic, and metabolic function will be monitored to assure patient safety. In patients whose creatinine doubles from baseline or reaches 2.5 mg/dl, whichever is lower, the following infusion will be held if the patient is in the weekly infusion phase of the trial. Labs will be re-measured prior to the next infusion being scheduled 2 weeks later. If there is any upward change in creatinine that does not reach the threshold for holding an infusion, the Central Pharmacy will adjust downward the EDTA content of the infusion according to ACAM recommendations (based on a standard equation for creatinine clearance; see Appendix 7). If there is no change in renal function, then the infusions will proceed as planned. With respect to liver function, ALT, AST, alkaline phosphatase or bilirubin > 2 times the upper limit of normal is a relative contraindication to intravenous EDTA. With regards to hematologic abnormalities, CBC and platelet counts will be monitored. With respect to the CBC, hematocrit, total white cell count and neutrophil count will be monitored. If any of these parameters falls below the lower limit of normal, the site will be notified. If there is a

fall in platelet count below 50% of the baseline platelet count, or to <60,000, infusions will stop, and the Central Pharmacy will omit heparin from subsequent infusions for that patient. The site will be notified that the platelet count is low. Infusions without heparin will resume after the platelet count has risen to within 20% of the baseline platelet count. As regards metabolic functions, a calcium below 10 mg/dL or a glucose below 50 mg/dL shall be deemed a relative contraindication to EDTA. Any of the abovementioned kidney, liver, hematologic, or metabolic abnormalities shall be tracked as adverse events and reported to the DSMB.

Schedule of routine monitoring laboratory examinations to be carried out on all patients; shaded cells represent safety labs.

	Screen	Inf. #1	Inf. #2	Inf. #5	Inf. #10	Inf. #15	Inf. #20	Inf. #25	Inf. #30	Inf. #36	Inf. #40
[redacted]											
Iron panel		X							X		
Lipids		X							X		
CRP/ICAM		X							X		X
Ox. Stress		X							X		X

4.20 Primary endpoint

As suggested by the RFA, the primary endpoint of this trial is a composite clinical endpoint that includes all cause mortality, myocardial infarction, stroke, hospitalization for angina and hospitalization for congestive heart failure. All randomized patients will be followed until the end of the trial. At each in-person or telephone contact, all patients will be asked about any interval hospitalizations; records for these will be obtained and forwarded to the DCC, then to the CEC. The Clinical Events Committee will adjudicate all reported non-fatal MI, non-fatal stroke, death (cardiovascular or non-cardiovascular), hospitalization for angina or hospitalization for congestive heart failure. For patients who do not attend the clinic visits, all efforts will be made to secure 100% follow-up for hospitalizations as well as the fact and cause of death. Further, at the end of the trial, in the unlikely event that there are patients for whom vital status is not obtainable, we will conduct a National Death Index Search.

- **Death.** In this population, over 90% of deaths will be cardiovascular. Nonetheless, we will ascertain both CV and non-CV causes of death since it is possible, at least in theory, that certain non-CV deaths are related to randomized treatment assignment i.e. heparin-induced thrombocytopenia. For these reasons, non-CV deaths will be included in the primary endpoint.
- **Nonfatal myocardial infarction;** a documented MI is defined based on at least 2 of the 3 following criteria 1) ischemic symptoms ≥30 minutes, 2) cardiac serum marker elevation (CPK ≥2x upper limit of normal and CPK-MB elevated above the upper limit of the lab normal) or troponin T, or troponin I at least twice the upper limit of normal and/or 3) electrocardiographic, defined as new Q-waves of ≥0.03 sec and/or 1/3 of QRS complex in ≥2 related ECG leads. If cardiac serum markers are elevated, but there were no clear ischemic symptoms, (for example an intra-operative MI), any one of the following ECG findings satisfy diagnostic criteria for an MI after randomization: new ST-T changes (ST elevation or depression), new left bundle branch block, loss of R-wave voltage ≥50% in ≥2 related leads or deep T wave inversions ≥3mm in ≥2 leads. Because revascularization procedures may result in CPK "leaks" without clear evidence of MI, post procedural MI will have a more stringent definition. This dichotomy has been used in another NHLBI-sponsored trial (OAT – Dr. Lamas Co-Chairs) which is now ongoing. The following level of enzyme elevation is required to confirm MI within 24 hours after a procedure; these must be associated with either symptoms or new ST elevation or Q waves, or both (as defined above) to meet the primary endpoint MI criteria:
  1. Post-PTCA/Stent elevation of CPK-MB (or total CPK in the absence of CPK-MB values) to ≥3x upper limits of normal and at least 3% of total CPK if both CPK and CPK-MB are available, and ≥50% increased over the value preceding the procedure.

2. Post-CABG elevation of CPK-MB ≥5 times normal (or CPK in the absence of CPK-MB), and at least 3% of total CPK if both CPK and CPK-MB are available.

- **Non-fatal stroke** is defined as a new onset neurologic deficit, consistent with a vascular distribution, that is not reversible within 24 hours and which is not due to a readily identifiable non-vascular cause (i.e., brain tumor, trauma). An event form will be completed and supporting data, including computed tomography, magnetic resonance imaging, cerebral angiography, and neurologist reports will be submitted to the DCC for eventual adjudication by the CEC.
- **Hospitalization for congestive heart failure**
  Congestive heart failure is defined as hospitalization for severe heart failure, with radiographic evidence, requiring intravenous diuretics or inotropes and unresponsive to outpatient treatment.
- **Hospitalization for angina** is defined as a hospitalization prompted by ischemic symptoms and associated with reversible or new ST shifts of 1mm or greater in 2 contiguous leads. Enzyme elevations, if present, are mild and do not meet the criteria for acute myocardial infarction.

4.21 Secondary endpoints

4.21.1 Cardiac death, or non-fatal MI or non-fatal stroke.

This composite secondary endpoint captures serious, irreversible, ischemic events. Furthermore, these are events that are less challenging to adjudicate and thus a reduction in this endpoint by the interventions being tested would be powerful evidence of efficacy.

4.21.2 Individual components of the primary endpoint

For each component of the primary endpoint, we will explore the directionality, magnitude, as well as statistical significance of any treatment effects. *A priori*, we hypothesize that any overall treatment benefit (or harm) would be reflected in a similar directionality and magnitude of the individual components of the composite primary endpoint. Any analysis of the components of the primary endpoint must be interpreted with an appreciation that the trial will not have adequate statistical power to test any individual component of the primary endpoint.

4.21.3 Coronary revascularization

We will explore the directionality, magnitude, and statistical significance of rates of percutaneous coronary intervention, coronary artery bypass surgery, or other procedures such as transmyocardial revascularization. In these analyses, the same *caveats* apply as mentioned above.

4.21.4 Safety of the interventions

As suggested in the RFA, safety monitoring will be an important part of the thorough evaluation of the treatment interventions. The following parameters will be tracked based on laboratory monitoring:

- **Kidney.** The primary measure of renal function will be serum creatinine. Specifically we will assess both the proportion of patients as well as the time to achieve a doubling of the creatinine from baseline or a level of 2.5 mg/dL, whichever is lower.
- **Liver.** With respect to liver function, both the proportion of patients as well as the time to achieve a doubling of the ALT, AST, alkaline phosphatase or bilirubin will be assessed.
- **Hematology.** With respect to hematologic parameters, the development of thrombocytopenia to below 80,000 platelets, or a 50% decrease from baseline will be tracked; and the development of either a reduction in total white cell count or neutrophils to below the normal limit will be tracked. Specifically we will assess both the proportion of patients as well as the time to development of a hematologic abnormality.

As has been the case in numerous multicenter randomized trials in which they have participated, the DCC at DCRI has developed standardized forms and protocols for adverse event monitoring and reporting. In TACT, as suggested by the RFA, all trial sites will promptly notify NCCAM and the FDA of adverse events. Dr. Lamas has already notified all institutions and investigators participating in TACT about this requirement. In addition, the PI of the DCC, Dr. Kerry Lee (or a designee) will have the responsibility to continuously monitor the accumulating data from TACT and to report any unusual occurrences immediately to the Chair of the DSMB, the NCCAM Program Officer, and the FDA. The Chair of the DSMB will decide whether to call for an immediate meeting or conference call of the entire committee, which includes the NCCAM Program Officer, as

well as whether and how to inform the Study Chair. If, as expected, there is no unusual frequency of occurrence of adverse events, then the DCC will routinely report a summary to the DSMB and NCCAM Program Officer and the FDA every 3 months.

4.21.5 Economic and Quality of Life (EQOL) overview

Analyses of quality of life and cost-effectiveness of chelation therapy is encouraged in the RFA. The specific aims of the quality of life study are: 1) comparison of health-related quality of life for the two treatment arms by intention to treat and 2) identification of factors in addition to treatment assignment that are associated with variation in quality of life outcomes. The specific aims of the economic study are: 1) measurement and comparison of cumulative total medical costs for the two treatment arms by intention-to-treat, 2) estimation of the incremental cost effectiveness of the chelation therapy arm relative to the placebo arm assessed as cost per life year added and cost per quality adjusted life year added and 3) identification of factors in addition to treatment assignment that are associated with variations in medical cost and cost effectiveness.

4.21.6 Quality of life instruments

Our approach to health-related quality of life assessment in TACT is to employ a battery of well-validated instruments including a generic core and additional scales that might be more sensitive to specific changes induced by the therapies being studied. A more detailed description of our approach is given in the EQOL Appendix 4. The generic core instrument we propose to use is the Medical Outcomes Study Short Form (SF-36) [87]. The SF-36 is composed of nine scales which can be used separately or as a set; physical function, role function-physical, role function-emotion, general health, bodily pain, social function, psychological well-being/mental health, vitality and health transitions. Each scale is scored separately and is customarily transposed to a 0 to 100 scale. We will also assess physical function using the Duke Activity Status Index (DASI)[88], a cardiac-specific measure of functional status. In addition, we will also obtain three brief supplemental measures of functional status, the Bed Days and Disability Days[89 90] questions from the National Health Interview Survey and a four-level ordinal global assessment of the effect of the patient's health on his or her ability to do activities. The presence of anginal symptoms will be assessed with the symptom scale from the Seattle Angina Questionnaire along with the New York Heart Association (NYHA) congestive heart failure class and the Canadian Cardiovascular Society Class for angina, which will be recorded on the clinical Case Report Form and collected during each follow-up telephone contact. Employment details will be obtained using an abbreviated series of questions adapted from the NHLBI Bypass Angioplasty Revascularization Investigation (BARI) Substudy of Economics and Quality of Life (SEQOL).

4.21.7 Measurement of Utilities

Patient-specific utilities will be assessed by patient interview using the EuroQoL. The EuroQoL-5D consists of two parts: a 5 dimension assessment of "your own health state today," which allows for definition of 243 discrete health states that can be mapped to previously derived population utility weights, and a self-rating (0-100) "thermometer" of current health-related quality of life.

4.21.8 Economic Data/Hospital Billing Data

Hospital bills (detailed, summary ledger and UB 92) will be collected by the EQOL Coordinating Center within 45 days of discharge from the hospital (baseline or follow-up) or as soon as possible thereafter. Cost to charge ratios (RCCs), specifically the Medicare Cost Report Worksheets C and D1 Part 2, will be obtained from each hospital where a TACT baseline or follow-up hospitalization is reported. Additional details are provided in Appendix 4.

4.21.9 Brachial artery flow-mediated endothelial function

This mechanistic substudy in a subgroup of 400 patients study focuses on endothelial function, as suggested in the RFA. Assessment of endothelial function in the brachial artery is particularly well suited to repeated examination in this trial, and has clinical relevance to endpoints. For example, a recent study indicated increased risk of cardiovascular events in hypertensive patients with impaired endothelial function in the arm, as assessed invasively.[91] Additionally, Dr. Vita recently demonstrated that assessment of endothelial function with the proposed non-invasive methodology also provides prognostic information (see attached abstract in Appendix 5). We hypothesize that either or both treatment interventions will lead to improvement in flow-

mediated dilation. If there is an improvement in flow-mediated dilation in one or both treatment groups compared to placebo and no change in nitroglycerin-mediated dilation, we will have evidence for an improvement in endothelial vasomotor function. If both flow-mediated and nitroglycerin-mediated dilation are improved, we will have evidence for improved bioactivity of nitric oxide. Such a finding might reflect an improvement in the ability of vascular smooth muscle to respond to nitric oxide, or decreased "quenching" of nitric oxide by reactive oxygen species. On the other hand, if there is no change in either parameter, we will conclude that chelation therapy has no measurable effect on endothelial vasomotor function. Important control measurements will include resting vessel diameter, baseline and hyperemic brachial artery flow, blood pressure and heart rate, and concurrent risk factors including lipoprotein profile, C-reactive protein, ICAM-1, and plasma markers of oxidative stress.

4.21.10 Plasma markers of oxidative stress and anti-oxidant protection

We hypothesize that chelation therapy improves endothelial function by decreasing metal ion dependent formation of reactive oxygen species and lipid peroxidation in the vasculature. The measurement of oxidant stress and anti-oxidant protection is suggested in the RFA. A direct examination of this mechanism would require access to vascular tissue, which is not feasible. Most highly specific markers of lipid and protein oxidation including antibodies against oxidized LDL,[82] LDL oxidizability (lag phase), o,o'-dityrosine (a marker of protein oxidation),[83] and F_2 isoprostanes (a group of stable products of arachidonic acid oxidation) are problematic because they are expensive, labor intensive, or require sample processing on the day of collection. Recently, an enzyme-linked immunoassay has become available for measurement of one F_2 isoprostane (8-epi-$PGF_{2\alpha}$). In contrast to the original mass spectroscopic assay for F_2 isoprostanes, the availability of an ELISA makes large-scale assessment of this marker feasible. F_2 isoprostanes have been shown to be a highly specific marker of lipid peroxidation[84] and are elevated in the setting of CAD risk factors. In TACT, we will examine the effects of chelation therapy on 8-epi-$PGF_{2\alpha}$.

We will also examine plasma concentrations of ascorbic acid at baseline, and prior to an infusion taking place on an infusion day. Ascorbic acid is the primary water-soluble antioxidant in plasma and in the aqueous phase of the intercellular space, and is a potent inhibitor of lipid peroxidation. Dr. Vita recently reported that low plasma levels of ascorbic acid are associated with unstable coronary syndromes (unstable angina and acute myocardial infarction) in patients with angiographically proven coronary artery disease[85] Since the chelation therapy infusate contains 7 grams of ascorbic acid, it will be important to measure plasma levels of this antioxidant.

4.21.11 Plasma markers of endothelial activation and inflammation

As discussed earlier, there is increasing interest in the role of inflammation in the pathogenesis of cardiovascular disease events[41] as well as in endothelial dysfunction[40]. The endothelium controls vascular function by expressing a broad array of paracrine factors. Several of these markers have been shown to correlate with CAD risk. For example, the soluble form of the adhesion molecule ICAM-1 (sICAM-1) may be measured in plasma, and correlates with cardiovascular risk in the Women's Health Study[86]. These studies suggest that expression of pro-inflammatory factors by the endothelium may contribute to cardiovascular events. ICAM-1 can reliably be assessed in plasma using a commercially available ELISA kit. Although C-reactive protein is not a specific endothelial product (it is largely synthesized in the liver), there currently is great interest in this marker of inflammation because it also appears to have independent predictive value for CAD risk[87]. For these reasons, we will examine the effect of the study interventions on sICAM-1 and C-reactive protein as suggested in the RFA.

4.22 Statistical Considerations

4.22.1 Sample Size and Power Calculations

Several design factors and research objectives have been considered in developing sample size estimates for the trial. First, patient enrollment has been determined so there would be a sufficient number of endpoints to provide a high degree of confidence (power $\geq$ 90%) for detecting clinically important differences in the primary endpoint. Second, important secondary endpoints, including measures of quality-of-life, have also been considered. Third, we considered it important for the overall sample to be large enough to permit exploration of treatment effects in selected subgroups of patients where chelation therapy might be particularly advantageous, or where the question of a treatment benefit from chelation therapy is particularly relevant. The

pre-specified subgroups in the trial include those defined by age, gender, and race. Finally, the sample size has been determined to provide a reasonable level of confidence of detecting clinically important therapeutic effects even if our projections of event rates and treatment differences prove to be optimistic.

Event rates for the primary composite endpoint and other clinical outcomes were examined in a group of 7,002 patients with a history of myocardial infarction enrolled in the Duke Cardiovascular Disease Database between 1986 and 2000. These patients all underwent cardiac catheterization, but otherwise satisfied all the inclusion/exclusion criteria specified for TACT. Based on follow-up of these patients starting one month after their angiography (to avoid counting early interventional procedures based on treatment decisions made at the time of catheterization), the three-year rate for the occurrence of either death, myocardial infarction, or rehospitalization for a revascularization procedure was 28.4 %. When we also include stroke, hospitalization for heart failure, or hospitalization for angina as outcome events, the three-year rate increases to over 40%. These data cover a 14-year span during which therapeutic innovations have improved patient outcomes. Further corroboration of the validity of these estimates derive from the CARE trial, a secondary prevention trial of cholesterol reduction in post-MI patients at low risk of subsequent events, due in part to their average cholesterol levels. Thus, careful evaluation of both the Duke Cardiovascular Disease Database as well as CARE provides reassuring evidence that the 3-year incidence of the composite primary endpoint in TACT will be 35% or higher.

Based on these estimates, a sample size of 1,600 patients has been chosen for TACT to provide high power for assessing clinically important treatment differences in the primary endpoint (see Appendix 1). For comparing EDTA chelation therapy versus placebo, and high-dose vitamin/mineral supplementation versus low dose, this number of patients will provide >90% power for detecting a 25% reduction in the incidence of the primary endpoint, and 80% power for detecting a 20% reduction. Furthermore, with this number of patients, the study will have good power under even more conservative assumptions about the control group event rate.

Due to the arduous nature of the trial treatment regimen, it is expected that a few patients assigned to chelation therapy may not receive the full treatment course of 40 infusions; and a few assigned to placebo may seek and receive chelation therapy. These numbers are likely to be very small in TACT for several reasons. First, with respect to patients assigned to chelation therapy, those who enroll in this trial will be fully aware of the schedule and number of infusions, and patients unlikely to comply will be excluded prior to randomization. Second, with respect to those assigned to placebo, the time course of the infusions in this randomized double-blind placebo-controlled trial will be 7.5 months in the intensive phase and up to 20 months for the maintenance phase. Thus, it seems unlikely that a chelation patient will not comply, and it seems unlikely that a placebo patient will seek out and receive chelation.

The sample size of 1,600 patients is robust to deviations from the assumed design parameters, and provides excellent power for detecting a risk reduction of 20% in the primary clinical endpoint, an effect that is more conservative than the assumed 25%, in order to allow for attenuation of the beneficial effects due to non-compliance with therapy or other factors that would impact the underlying design assumptions. Further details of the sample size calculations are presented in Appendix 1.

4.22.2 Statistical Analysis

Statistical analysis will be performed at the Data Coordinating Center at Duke University. Although the methodologic approaches and operational details of the data analysis will be coordinated by the study biostatisticians, the major analyses of the study data will be highly collaborative among the Data Coordinating Center, the Clinical Coordinating Center, and the Steering Committee, involving both statisticians and physicians to ensure optimal interpretation of the data. All major treatment comparisons between the randomized groups in this trial will be performed according to the principle of "intention-to-treat;" that is, subjects will be analyzed (and endpoints attributed) according to the treatment arm to which patients were randomized, regardless of compliance to assigned regimen. Statistical comparisons will be performed using two-sided significance tests and 95% confidence intervals.

As in all factorial designs, the primary statistical assessments will involve a comparison of the EDTA chelation therapy arm with the placebo infusion group, and a comparison of high-dose vitamin/mineral supplementation with low-dose supplementation. The log-rank test [98], which is a special case of the more general Cox proportional hazards model[99], will be the primary analytic tool in the two-group comparisons for assessing outcome differences with respect to the primary clinical endpoint. This approach focuses on the time from trial entry until the first occurrence of any component of the composite primary endpoint, taking into account varying lengths of patient follow-up and censored observations. Using this procedure, the analysis strategy will be to first perform two-group comparisons for each treatment factor in the study design, adjusting only for the other design factor. These standard two-group comparisons will constitute the primary analyses to assess treatment differences. Kaplan-Meier survival estimates[100] based on the primary endpoint will be calculated for each treatment group to display the outcome results graphically. The significance level for each comparison with respect to the primary endpoint will be set at α=0.05. A commentary on the issues of multiple comparisons is provided in Appendix 3.

In a trial of this size, randomization is very likely to ensure an equal distribution of prognostic factors. Nonetheless, additional analyses involving covariate adjustment for prognostic factors will be performed with the Cox model. Such adjustment will be limited to a relatively small, prospectively defined set of patient characteristics that are known *a priori* to have a prognostic relationship with the clinical outcomes of interest. This adjustment will serve as a prelude to additional analyses examining differential treatment effects. The adjustment variables will include age, sex, race, infarct location (anterior versus non-anterior), time from index MI until study enrollment, history of diabetes, previous revascularization, and ejection fraction, when known.

In addition to the primary treatment comparisons indicated above, a limited number of pre-specified subgroup analyses of the primary outcome will be performed, as suggested in the RFA. Specifically, treatment comparisons will be performed within subgroups defined by age (elderly (>70) versus younger (≤70) patients); subgroups defined by gender, with special emphasis on results in women; subgroups defined by race, with emphasis on results in minority patients; and subgroups defined by MI location, time from index MI to trial enrollment, and presence/absence of diabetes. Treatment effects for the primary endpoint as characterized by the hazard ratio (with 95% confidence intervals) will be calculated and displayed for the subgroups defined by the variables listed above. The subgroup comparisons will be carefully interpreted in conjunction with the formal interaction tests described above. Indeed, many of these subgroup analyses fall within the NIH-permitted category of *"plans to conduct valid analyses of the interventions in sex/gender and racial/ethnic subgroups (without requiring high statistical power for each subgroup) when the prior studies neither support nor negate significant differences in intervention effect between subgroups"*. Secondary endpoint analyses will be performed for the individual components of the primary composite endpoint, and for other secondary clinical endpoints using the log-rank and Cox model methodology outlined above. The frequency of occurrence of adverse events in each patient group will be summarized graphically as well as with appropriate descriptive statistics. Quality of life and cost data will be analyzed by the TACT EQOL Coordinating Center in close collaboration with the Data Coordinating Center, as outlined in Appendix 4. Analyses of the mechanistic secondary endpoints involving measures of endothelial function and oxidative stress will be directed by the Data Coordinating Center. These endpoints involve continuous variables measured repeatedly over time, and several analysis strategies for analyzing these longitudinal data are specified in the statistical analysis appendix (Appendix 3) and in Appendix 5, which describe the activities of the Vascular Function Core Laboratory.

Interim analyses will be performed at prescribed intervals (approximately every six months) for presentation to the Data and Safety Monitoring Board in monitoring the trial. Group sequential methods based on the spending function approach of Lan and DeMets[101] will be employed to control the overall Type I error rate. Monitoring boundaries similar to those proposed by O'Brien and Fleming[102] will be used, based on the flexible Lan and DeMets approach[101]. Details of the plans for interim analyses are outlined in Appendix 3.

4.22.3 Study registry

As requested in the RFA, a database or registry will be kept of all patients identified that meet enrollment criteria but are not enrolled as study participants. This ongoing registry will have the purpose of confirming that

screening activity is ongoing at clinical units, particularly in those that are under-performing; and of determining the proportion of eligible patients that are enrolled. In order to ease the burden of the site coordinator, the registry form will be provided as a screening worksheet, and mailed each month to the DCC.

4.23 Recruitment strategies

The CCC and DCC have obtained funding for an ongoing pilot trial of chelation therapy. Consequently, valuable experience has been gained on recruitment strategies for patients. A cardiology practice or practices are the most productive sites for patient recruitment. For the pilot trial, patients have been recruited from cardiology practices, and general internal medicine clinics. However, we have been surprised by the great public interest in chelation, and by the numbers of patients willing to participate in such an arduous study. For example, following an article about the pilot trial in the Miami Herald in April 2001, over 200 interested patients called the Study Chairman's office. Thus, an advertising campaign will be planned as part of the overall recruitment strategy. The pilot experience will be supplemented by analogous approaches in the alternative medicine community under guidance and support of ACAM, and by development of a public interest section on the TACT web page. All advertising will be reviewed by the CCC and approved by the local IRB.

High compliance and follow-up are crucial to the validity of the TACT results. Thus, a critical part of the recruitment and retention efforts will be the research coordinator at the clinical as well as the infusion site. Coordinators will undergo training at study meetings, and through frequent telephone sessions with the CCC and DCC, as well as through the Internet. The infusion site where chelation is administered will always be part of the DCC site visits to clinical units. In addition, prior to final approval to enroll patients, each clinical unit must specify the clinical as well as the infusion site.

4.23.1 Enrollment of women, minorities, and children

TACT will enroll a representative sample of the US population which will include at least 50% women, 12% African Americans, 8% Hispanics, 2% Asian Americans, 1% native Americans, and 2% Pacific Islanders. Dr. Lamas has a track record of enrolling a study sample representative of the US population in MOST, an NHLBI-sponsored trial of pacemaker mode selection which enrolled 2010 patients consisting of 48% women and 15% minorities, including 12% African-Americans. Thus, the Study Chairman has experience successfully recruiting a study cohort representative of the US population. TACT will not include a population of children for study. The index diagnosis, acute myocardial infarction, is too rare in children to provide any meaningful data.

4.24 Clinical Units

The clinical units will play a major collaborative role with the CCC in the recruitment, retention, and drug administration efforts in TACT. We estimate that 50 clinical units will be sufficient to enroll 1600 eligible patients over 36 months. There will be 10 Clinical Sites selected by Dr. Vita to participate in the 400-patient Vascular Function study. These sites will be selected based on their having the appropriate vascular imaging equipment, experience, and interest to participate in the Vascular Function assessments. At present, the CCC team has identified 60 interested sites listed in Appendix 6 that appear qualified to participate in TACT. The clinical units represent a mix of practice types, with 24 (40%) university or teaching hospital sites, 24 (40%) clinical practices or cardiology or freestanding research centers, and 12 (20%) chelation practices. Based on survey of patient availability and minority and gender mix, these clinical units see in aggregate, 209 eligible patients per site, per month, or over 2000 potentially eligible patients per year. Furthermore, they have a representative patient mix with 31.6% minorities of which 16.8% are African-Americans, and 10.6% are Hispanics. The 50 clinical units to start the trial will be selected based on a thorough review of their qualifications by the CCC, DCC, and the NCCAM Program Staff.

The Senior Investigator for each trial site in the consortium will be responsible for on-site clinical and scientific implementation, direction and management of the trial protocol, as well as the coordination of requirements for any adjunct studies of underlying mechanisms and surrogate markers. Senior Investigators will commit at least 10% effort to this trial. All Senior Investigators have substantial experience in the treatment and management of CAD and in the design, implementation and evaluation of clinical trials. All investigators will obtain NIH Clinical Investigators' training as offered on the NIH website. Such training will be completed and documented by the CCC prior to initiation of enrollment at each site. The organization of each Clinical Unit requires:

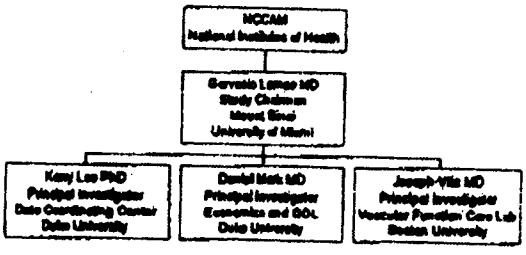
- a Senior Investigator with the above qualifications and commitment, in addition to sufficient clinical volume for recruitment of eligible patients, and training in chelation therapy. This training is already provided twice yearly by ACAM and will be included in the initiation meeting for the trial;
- if the Senior Investigator does not have personal experience with chelation therapy and does not undergo chelation training, he or she must have, as part of the Clinical Unit, a Chelation Practitioner sub-Investigator. The chelation practitioner must have formal training and certification by ACAM in chelation therapy, and must be approved for participation in TACT by the ACAM Liaison Committee;
- certificate of completion of NIH Clinical Investigator's Training, available through the NIH web site;
- a research coordinator;
- ability to draw blood and perform a standard 12-lead electrocardiogram;
- Internet access for data collection and communication with the central pharmacy;
- The CCC recognizes that the characteristics of the infusion sites will be an important determinant of patient compliance. For example, such characteristics may include clinical areas where infusions are part of daily care, and the infrastructure for administering infusions already exists, such as an ongoing chelation practice. However, prior to final approval of any Clinical Unit, the CCC will review and approve the characteristics of all infusion sites.

These requirements will lead to 3 types of Clinical Units in TACT: 1] sites with a "conventional" practitioner as Senior Investigator, and an associated chelation practitioner (such as Mount Sinai); 2] sites with a chelation practitioner Senior Investigator (such as Jefferson Medical College); and 3] sites with a conventional medicine Senior Investigator who has taken a certifying course in chelation therapy (such as the Cardiovascular Institute of Florida). As has been used successfully in previous trials in which Dr. Lamas has participated, each Clinical Unit will obtain signed consent from 10 eligible patients prior to being activated as a TACT Clinical Unit. This strategy will provide rapid enrollment of the first 600 to 700 TACT patients. Although the final roster of 50 units will be developed in collaboration with NCCAM Program Staff, Appendix 8 has regulatory and patient-base information on the 60 clinical units who have a clear capability, have expressed interest in participating in TACT, and submitted regulatory documents for consideration. Each trial site will follow the procedures required by the final protocol generated by the Steering Committee regarding study conduct and monitoring, patient management, data collection, data management, data analysis and quality control. All proposed trial sites have agreed to accept and implement the common protocol and procedures approved by the Steering Committee. Furthermore, all potential sites agree to preferentially enroll patients into TACT when competing studies are ongoing. The investigator letters of agreement, administrative letters, site surveys, and other clinical unit characteristics are contained in Appendix 8.

4.25 Overview of the Study Organization

TACT is organized similarly to other trials in which the investigative team has collaborated. There are 4 major organizational units organized under the auspices of NCCAM. These are the Clinical Coordinating Center, at the Awardee Institution, led by the Study Chairman; the Data Coordinating Center; the EQOL Coordinating Center; and the Vascular Function Core Laboratory.

Organizational Units of TACT



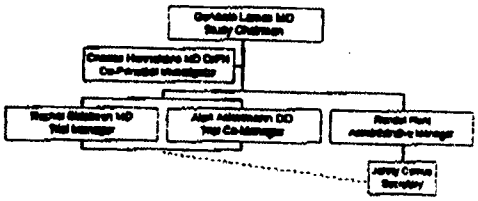
4.25.1 Clinical Coordinating Center

The Clinical Coordinating Center (CCC) and Study Chair's office is located at Mount Sinai Medical Center-Miami Heart Institute, Miami Beach, FL. Dr. Gervasio Lamas will be responsible for the scientific and administrative oversight of the trial. The CCC functions as a clinical trial center and is responsible for all aspects of conducting this trial, including protocol development and amendments, site recruitment and retention, regulatory documentation, protocol adherence, site reimbursement and leadership in data analysis, study presentations and publications. Dr. Lamas will be responsible for all sub-contracts with the other organizational units. A Co-Principal Investigator (Dr. Hennekens), a Chelation Consultant (Dr. Martin Dayton), a Trial Manager (Dr. Rachel Eidelman), a Trial Co-Manager (Dr.

Ackermann), an Administrative Manager (Randel Plant) and Study Staff will assist Dr. Lamas in coordinating TACT. As suggested by the RFA, Dr. Lamas will submit quarterly progress reports to the NCCAM Program Officer and to the DSMB. These reports will include recruitment data, indices of quality control, as well as the disposition and management of any reports of significant side effects or morbidity previously reported to him by the DSMB. In all other aspects of the trial, Dr. Lamas will remain blinded to treatment assignment for the entire duration of the trial.

Dr. Lamas will also submit annual Awardee Non-Competing Progress Reports to NCCAM. He will also, on an ongoing basis, provide any additional information required by the DSMB. Finally, Dr. Lamas will also present a mid-term and final report to the NCCAM Advisory Council. If scientific misconduct or other events that have significantly affected the quality or integrity of trial data have occurred Dr. Lamas will immediately notify the DSMB, NCCAM, the collaborating investigators, the appropriate IRBs, the FDA, and other sponsors of the affected work. In such instances Dr. Lamas will also ensure that submitted but unpublished abstracts and manuscripts will be corrected. If the affected data have already been published, Dr. Lamas will submit to NCCAM a re-analysis of the results deleting the false or otherwise unreliable data, and disclose within the text the reasons for re-analysis. Dr. Lamas will also submit the re-analyses for publication. Dr. Lamas will also notify all scientists, research laboratories and other organizations to which he has sent research materials affected by false or otherwise unreliable data.

Organization of the TACT
Clinical Coordinating Center



4.25.2 Data Coordinating Center

The Data Coordinating Center (DCC, Dr. Kerry Lee) is responsible for the treatment allocations of eligible patients, receipt and processing of all data collected by the Clinical Sites and Central Units except economic data, quality control programs, and analysis of all study data except economic and quality of life data. DCC staff will prepare data reports at specified intervals for review by an independent DSMB and will collaborate with other study investigators in the preparation of study presentations and publications. The DCC is also responsible for quality assurance of the electronic data capture system and the internet-based communications network between management and performance sites. The DCC will also monitor sites prior to site activation as well as during the course of the trial.

4.25.3 Economic and Quality of Life Coordinating Center

In collaboration with the Clinical Coordinating Center and the Data Coordinating Center, the Economics and Quality of Life Coordinating Center will perform the following major functions: 1) obtain baseline economic status and quality of life data from all patients enrolled at each participating study site at the time of randomization; 2) assess detailed QOL data at 6 months, 1 year and 2 years after enrollment; 3) assess angina and symptomatic status every 3 months during study follow-up; 4) identify all major medical encounters that occur during follow-up and collect detailed health care resource consumption data and cost data for each; 5) compare cost and quality of life outcomes for the two treatment arms in any pair of treatment comparisons according to intention-to-treat; 6) estimate the incremental cost effectiveness ratio for the experimental arm and perform extensive sensitivity analyses.

4.25.4 Vascular Function Core Laboratory

Joseph Vita MD leads the Vascular Function Core Laboratory at Boston University School of Medicine. Dr. Vita will be responsible for leading the 400-patient mechanistic study to assess the effect of the test interventions on various aspects of vascular function as covered above. As such, he will be responsible for training sites, assessing equipment needs, ongoing quality assurance of data, primary data analysis, and transmission of data to the DCC for final analysis.

4.25.5 The Central Pharmacy

The Central Pharmacy is directed by Eric M. Alvarez PharmD, President of Quantum Healthcare Consultants, Inc. who has a 15-year ongoing and successful collaboration with Accucare Pharmacy. Dr. Alvarez has vast experience in the organization and delivery of pharmacy services with particular emphasis on parenteral solutions. Dr. Alvarez is past President of the Florida Pharmacy Association, and has a longstanding interest in alternative medicine. Our collaboration with Dr. Alvarez and his colleagues began in 1999, when we began to design a pilot trial of chelation therapy prior to the publication of the RFA. At that time, it became clear that effective blinding was possible, and cost was not prohibitive, only if a Central Pharmacy was developed. Thus, our Central Pharmacy has already developed the methodology for blinding the intravenous therapy, and has already received regulatory approval for TACT from the State of Florida. Our collaborative experience with the ongoing pilot trial has led to development of a team that will:

- using sterile procedures will accurately mix 64,000 bags of trial solution, of which 30,000 will be the multi-component chelation solution, and 32,000 will be placebo;
- preserve the blind;
- deliver refrigerated study solution to clinical units within 48 hours after ordering;
- communicate on a real-time basis (internet-based) with sites, CCC, and DCC;
- change dosing immediately based on safety labs and standing orders;
- suspend shipment and notify DCC and CCC when safety labs are not received, or have reached predefined alert values;
- compound and deliver blinded vitamins and supplements or their identical placebos;
- perform all of the above at a reasonable cost.

In the Pilot Trial Trial, the Central Pharmacy is mixing and delivering blinded bags of chelation solution to Clinical Units. Based on the performance of this Central Pharmacy in the pilot trial, we are confident that the large and complex number and nature of the procedures planned for TACT will also be conducted in the highest quality manner. Additional details on the Central Pharmacy are in Appendix 7.

4.25.6 The Central Laboratory

Since we had proposed a pilot trial of chelation therapy far in advance of the publication of this RFA, we had recognized the need for a central laboratory of national eminence. Accordingly, we approached Quest Diagnostics, ICON, and Physicians Reference Laboratory. As a result of numerous interactions with each of these central laboratories, it became clear that as regards fulfilling the needs of a trial of chelation therapy, Quest Diagnostics offered both the highest quality and most cost-effective services. In TACT, patients will have routine blood drawing 11 times during the course of the trial. Clinical Units will have blood pickup, and results will be available within 24 hours both by fax and Internet to the Clinical Unit, and via the Internet to the Central Pharmacy, DCC, and CCC.

4.26 Internet-based communications between management units and clinical sites

In our planning for the pilot trial, it became clear that accurate and rapid communication about the infusion data was essential. In order to maximize accuracy and speed of communications concerning infusion data between the CCC, DCC, the Central Pharmacy, and the Clinical Units, an enhanced Internet-based data collection will be used in TACT, as is being used in the pilot trial. Therefore, Internet access is a requirement at patient-care and infusion sites. The minimal system requirements are Internet Explorer version 5.0 or 5.5. Sites will be trained and provided with Internet Explorer. TACT has unique needs for rapid Internet-based communication in order to properly schedule preparation of infusions, delivery, and administration. Additionally, adjustments in the content of EDTA and heparin in the infusion are based on laboratory studies and must be made prior to preparation of the next scheduled infusion. The specific details of the algorithms used to communicate treatment assignment to the central pharmacy, shipment and infusion dates, and dose changes or infusion cessation are contained in Appendix 7.

4.27 Major Study Committees

4.27.1 The Steering Committee

A Steering Committee will be established to serve as the main governing body of the trial. The Steering Committee will be composed of the NCCAM Program Officer, the NHLBI Scientific Adviser, the cooperative agreement Principal Investigator (Dr. Lamas), the Study Co-Principal Investigator (Dr. Hennekens), the Data Coordinating Center Principal Investigator (Dr. Lee), the EQOL Principal Investigator (Dr. Mark), the Trial

Manager (Dr. Eidelman), the Trial Co-Manager (Dr. Ackermann), and up to five trial site Senior Investigators. The initial Steering Committee Senior Investigators members will be nominated by the Study Chairman and elected by the Steering Committee as follows: 3 for a term of 6 months, and 2 for a term of 1 year. Thereafter all Senior Investigator members will serve 1-year terms. Senior Investigator members of the Steering Committee will be selected based on their scientific expertise and their contributions to the trial. At the first Steering Committee meeting, the RFA suggests that the Chairperson will be selected by the Steering Committee from members other than the PI or NIH staff. While we certainly will comply with this requirement, we also believe that that no one else connected with this proposal has more leadership capability and experience as well as expertise in the design, conduct, analysis, and interpretation of randomized clinical trials in the treatment and prevention of cardiovascular disease than the trial Co-PI, Dr. Hennekens. In fact, as suggested in the RFA, if Dr. Lamas were unable to continue his role, Dr. Hennekens would assume the role of Study Chairman. With respect to the Steering Committee, outside ad hoc consultants may be added as appropriate and needed. All major scientific decisions will be determined by the Steering Committee, with the Study Principal Investigator (Dr. Lamas), the Co-Principal Investigator (Dr. Hennekens), the Data Coordinating Center Principal Investigator (Dr. Lee), the EQOL Principal Investigator (Dr. Mark), the Trial Manager (Dr. Eidelman), the Trial Co-Manager (Dr. Ackermann), Steering Committee Chair, Senior Investigators, the NCCAM Program Officer, the NHLBI Scientific Adviser and the Trial Manager having one vote each. The first meeting will be convened by the NCCAM within two months of the award. The Committee will meet at least once more during the first 12 months of the study and annually thereafter. This Committee will have primary responsibility for finalizing the trial protocol, and approving the design and implementation of all adjunct studies, facilitating the conduct and monitoring of the clinical trial and adjunct studies, analyzing and interpreting study data, reporting study results, and setting guidelines for authorships. Each Steering Committee member (or their surrogate) will be expected to participate in all other Steering Committee activities, e.g., conference calls, special subcommittees, as may be necessary. With regards to the duties and responsibilities of the Steering Committee, the applicant agrees that these will be identical to those suggested in the RFA. Thus, they are not repeated here.

4.27.2 Executive Committee

The Executive Committee, composed of the Steering Committee Chair, the TACT Principal Investigator, the PI of the DCC, the NCCAM Program Officer, and an NHLBI Scientific Adviser will make recommendations to the Steering Committee regarding study conduct as suggested by the RFA. Dr. Lamas will serve as chair of the Executive Committee. The Executive Committee will meet to monitor study progress and to review non-endpoint data. Executive Committee meetings will be scheduled for the day prior to Steering Committee meetings. Dr. Lamas and Dr. Lee will update the recruitment progress of each center and of the whole trial bimonthly for the Executive Committee. Other reports for the Executive Committee may be requested of the Steering Committee as needed. In any votes of the Executive Committee, each member will have a single vote.

4.27.3 The Clinical Events Committee

The results of this study, whether positive or negative, are likely to be controversial. For this reason, a blinded and independent committee will review abstracted clinical data to determine whether clinical endpoints and major events have occurred. At least 1 member of or consultant to this committee will have experience in chelation therapy. All criteria and definitions will be pre- specified in detail in the manual of operations, to be developed in collaboration with NCCAM, as specified in the RFA. Marc A. Pfeffer MD PhD at Brigham and Women's Hospital has agreed to lead the adjudication of events. As required by the RFA, a letter of agreement to accept this responsibility is found in Appendix 8.

4.27.4 Operations Committee

The Operations Committee will include the Study Chairman, an NIH representative, the DCC Principal Investigator, DCC data manager, Trial Manager, Trial Co-Manager, CCC Chelation Consultant, and the EQOL Principal Investigator. This committee will have weekly conference calls to review trial enrollment, conduct of the protocol, feasibility (patient burden, site burden, cost), scientific merit and issues raised by the site and core laboratories. Dr. Vita will join the conference once monthly. Such calls will ensure smooth day-to-day operations of the trial and help to identify issues that need to be brought before the Steering Committee.

4.27.5 Liaison Committee to the American College for Advancement in Medicine
This standing Committee has as its charge to keep the chelation community informed as to the progress of TACT, and to bring to the TACT investigators any new advances that may be applicable to the conduct of the trial. Additionally, the Committee assists the Study Chairman in integrating complementary, alternative, and traditional medicine for the benefit of TACT. The Liaison Committee already has been instrumental in identifying qualified practitioners who are interested in performing infusions in a double-blind placebo-controlled manner, and in developing the TACT treatment chelation, vitamin, and mineral protocol. This Committee is chaired by Dr. Ron Hoffman, the President Pro Tem of ACAM, and includes Dr. Lamas as well as other prominent experts in chelation therapy, namely: L. Terry Chappell MD, Elmer Cranton MD, Martin Dayton MD DO, Jeanne Drisko MD (who is also a co-investigator from the University of Kansas), Michael Janson MD, Alan Magaziner MD, Ralph Miranda MD, and Michael Schacter MD. A letter of agreement from the President Pro Tem of ACAM is in Appendix 8.

4.27.6 Data and Safety Monitoring Board
The Director of NCCAM will appoint an independent Data and Safety Monitoring Board, with input from the Study Chairman, as suggested in the RFA. The DSMB will meet at least twice a year. DSMB meetings will be open only to designated NCCAM staff and other individuals who have been approved to have access to unblinded data. The applicants will adhere to and comply with the lines of authority suggested in the RFA. Thus, these responsibilities are not repeated here. The DSMB will serve in an advisory role to NCCAM and to the Study Chairman. Any recommendations for alteration or termination for part or all of the trial shall be based on consideration of the accumulating data in the context of totality of evidence. Specific statistical monitoring guidelines for safety and efficacy concerning the primary and secondary endpoints are found in Appendix 3.

4.26.6 Publications, Presentations and Ancillary Studies (PPAS) Committee
This Committee will review all proposals for data analysis, as well as research abstracts, presentations and manuscripts before submission. The committee will also review proposals for ancillary studies. This Committee will be Chaired by Dr. Hennekens, co-PI of TACT, and will include Dr. Kerry Lee, PI of the DCC, and Dr. Ron Hoffman, President of ACAM, as well as representatives from NCCAM and NHLBI. A letter of agreement to perform this function is contained in the Consultant section of the application.

4.27 Timeline
As specified in the RFA, TACT will consist of four phases: 1) an initial 6-month phase during which the protocol is finalized (e.g., study procedure and Manual of Operations, data collection manuals and Internet data collection training for sites, data management, training, establishment of the Data and Safety Monitoring Board, etc.) by the trial Steering Committee (see Steering Committee above); existing sites are approved by the Steering Committee and NCCAM; and new sites are recruited as necessary by the CCC and DCC; 2) a 36-month recruitment period during which patients are recruited, treated, and followed; 3) a 12-month period of intervention and follow-up alone; and 4) a 6-month period of data analysis and dissemination.

	Month 1 to 6	Month 7 to 41	Month 42 to 54	Month 55 to 60
Site recruitment	■			
Finalize protocol	■			
Recruitment		■		
Intervention		■	■	
Follow-up		■	■	
Data analysis and dissemination				■

HUMAN SUBJECTS RESEARCH

1.0 Risks to the subjects.

1.1 Human subjects involvement and characteristics.

Prior to any testing, the investigator will introduce him/herself to the patient, explain the trial in detail, give the patient opportunity to ask questions, and have willing patients sign informed consent to participate in the trial. After patients have given informed consent, they will be required to give information about their state of health, answer questions about quality of life, and undergo a physical exam and blood drawing in order to assess eligibility. Patients that are enrolled in the 10 sites that will be performing endothelial function testing will have this test performed. The trial itself requires participation from 2 to 4 years. During this involvement, patients will receive 40 infusions of chelation solution or placebo, and will take up to 8 vitamin or vitamin placebo pills daily. Once yearly patients will have an ECG as part of a visit. In addition to the infusion visits, patients will have blood drawn 11 times during the course of the study. Each blood draw will be for less than 15 ml (1 Tb) of blood. The 400 patients in the endothelial function study will also have a spot urine test collected 3 times during the course of the study.

The detailed entry criteria are described in the Sections 4.2 and 4.3. The total number of patients to be recruited will be 1600 in 50 clinical sites in the US, of whom 400 will have additional endothelial function testing. Participants will be post-MI patients age 50 years or older, with fairly normal renal function (creatinine >2.0 are excluded), and no heart failure within the last 6 months. Patients with hematologic, metabolic, or hepatic abnormalities will be excluded. A special effort will be made to recruit minority patients and women, as described in Section 4.23.1. Children will not be studied, since MI is vanishingly rare in children. We are choosing patients age 50 or older to achieve a more equivalent distribution of men and women for whom CAD is the leading cause of death, and in order to more closely reflect the age distribution of patients undergoing chelation therapy. We are restricting the diagnosis of prior CAD to myocardial infarction because of the relatively high frequency of recurrent clinical events in such patients.

1.2 Sources of Materials

Hospital records, clinical history, physical exams, blood specimens, urine specimens (in 400 patients) and data collection forms will be the sources of research material obtained from participating patients. Routine safety labs drawn 11 times during the trial will be performed for the trial, and will be made available to the patients or their physician upon the patient's request. Studies such as endothelial function testing, and markers of oxidative stress and inflammation will be obtained solely for the purposes of research and be unavailable to the research participant. Blood will be obtained from research patients for the measurement of inflammatory markers and blood and urine for the measurement of oxidative stress.

1.3 Potential Risks

The potential risks to the patient include risks attendant to the infusion of EDTA. These risks are principally those related to renal failure, hematologic abnormalities, hepatic abnormalities, and those related to deficiencies of minerals that are chelated and removed by the EDTA itself. Rarely, hypoglycemia may occur. Any of the above mentioned kidney, liver, hematologic, or metabolic abnormalities shall be tracked as adverse events and reported to the DSMB. These safety considerations may lead to our suspending infusions on some patients; however, based on the clinical experience of ACAM, we expect this number to be very small. Other risks have to do with local discomfort at the site of the intravenous infusion and the attendant local risks of ecchymoses, hematoma formation, phlebitis, and venous sclerosis. Other risks are unknown, both of the infusion as well as the oral vitamin supplements. Alternatives for the patient rejecting initial or subsequent participation includes counseling regarding accepted and proven secondary prevention strategies. Patients will be told that rejecting initial or subsequent infusion will not prejudice their care in any way.

2.0 Adequacy of protection against risk

2.1 Recruitment and Informed Consent

Research subjects will be recruited from clinical cardiology practices, and from responses to investigators' public appearances or advertisements. Prior to initiating the process of obtaining consent, Sites will need to obtain an FWA number for their site, if one is not already present. Furthermore, SIs will certify to the CCC that NIH Clinical Investigator's training has been completed and provide a certificate number. Following careful

review of the consent form by the Trial Manager and the Study Chairman at the CCC, and approval by the local institution, will a site be considered eligible for enrolling patients. Since patients become eligible to participate in TACT 3 months after their myocardial infarction, patients will never be approached for study participation in the acute MI setting. Consent will be obtained by the clinical center SI or by a sub-investigator. The process of obtaining consent involves a full explanation of the study in the patient's native language, as well as risks, benefits, and alternative procedures. Patients will sign a consent form that has been approved by a local IRB. Whenever possible, non-English-speaking patients will sign a certified translation of the consent form in their native language.

3.0 Protection Against Risk

The procedures in place to minimize risks include screening the medical record to identify exclusion criteria, performing screening laboratory tests and additional studies of renal function during the infusion protocol, carefully selecting an experienced infusion site, and preserving patient confidentiality. Indeed, the greatest risk is from toxicity related to an overly vigorous chelation regimen. We have specifically enhanced the monitoring regimen so as to enhance patient safety. Thus, there are extensive procedures in place to protect against the potential risk of chelation therapy.

The chelation protocol used at present has been published by ACAM, the national society that represents the most chelation practitioners, is in use in hundreds of thousands of infusions yearly, and is thought to be safe. However, as suggested by the RFA, assessment of safety is an important secondary endpoint of the trial. In the ACAM clinical experience with chelation therapy, assessment of safety is conducted prior to beginning chelation therapy, at the fifth treatment, and at each fifth infusion thereafter. In TACT, the safety monitoring will be enhanced so that a complete safety profile is obtained prior to infusion number 2, prior to infusion 5, and then approximately every fifth infusion. This is approximately twice as often as routine standard-of-care. Indices of kidney, liver, hematologic, and metabolic function will be monitored to assure patient safety. In patients whose creatinine doubles from baseline or reaches 2.5 mg/dl, whichever is lower, the next infusion will be skipped if the patient is in the weekly infusion phase of the trial, and labs re-measured prior to the next infusion being scheduled 2 weeks later. If there is any upward change in creatinine that does not reach the threshold for holding an infusion, the Central Pharmacy will adjust downward the EDTA content of the infusion according to ACAM recommendations (based on a standard equation for creatinine clearance; see Appendix). If there is no change in renal function, then the infusions will proceed as planned. With respect to liver function, ALT, AST, alkaline phosphatase or bilirubin > 2 times the upper limit of normal is a relative contraindication to intravenous EDTA. With regards to hematologic abnormalities, CBC and platelet counts will be monitored. With respect to the CBC, hematocrit, total white cell count and neutrophil count will be monitored. If any of these parameters falls below the lower limit of normal, the site will be notified. If there is a fall in platelet count below 50% of the baseline platelet count, or to <60,000, infusions will stop, and the Central Pharmacy will omit heparin from subsequent infusions for that patient. The site will be notified that the platelet count is low. Infusions without heparin will resume after the platelet count has risen to within 20% of the baseline platelet count. As regards to metabolic functions, a calcium below 10 mg/dL or a glucose below 50 mg/dL shall be deemed a relative contraindication to EDTA. Confidentiality will be maintained to the best of the ability of the study staff. When case report forms are filled out by the local clinical unit and mailed to the DCC, patients will be identified by study number, not by name. The same precaution will be true for the blood and imaging studies. The telephone calls from the EQOL Coordinating Center, of necessity, require that study staff outside the clinical unit know patient identifying characteristics. However, the EQOL CC will destroy all identifying material at the end of the study. These efforts will serve to ensure patient confidentiality. Data will be monitored to ensure patient safety. A DSMB will be constituted and will meet to review the protocol prior to the study, and monitor the study and the collection of data subsequent to the initiation of TACT. The DSMB will remain independent, as it will be selected by the NCCAM.

4.0 Potential Benefits of the Proposed Research to the Subjects and Others

At present, many patients seek out chelation therapy and high dose vitamin and mineral supplements without clear evidence that it will be beneficial. We believe there is sufficient belief to justify exposing half the patients at random to this therapy, as well as sufficient doubt to justify withholding therapy from the other half. Thus, the risks this protocol presents to the subject are reasonable in relation to the expected benefit. The chelation protocol used has been in use for years and is found to be quite safe by chelation practitioners. EDTA is

approved for particular chelation therapies, as discussed earlier, and it carries similar inherent benefit to risk ratios as practiced for CAD. Thus, there is enough evidence of benefit and safety to risk exposure to the therapy, and enough question about efficacy to risk not exposing patients to the therapy. Finally, if this alternative medical practice should prove beneficial, all patients with coronary disease would benefit by adding this treatment to the anti-atherosclerotic clinical armamentarium.

5.0 Importance of the Knowledge to be Gained

Chelation therapy and high dose supplements are ongoing clinical strategies that are practiced without the benefit of a clear evidence base. Although over 800,000 doses of chelation therapy were administered in 1997, this is a comparatively small number for such a prevalent disease as CAD. If the present trial shows that either of the test interventions is beneficial, then there would be a marked increase in the use of these therapies, resulting in improved outcomes for CAD patients. Should there be a null result, this would be a basis upon which rational policies about chelation and vitamin therapy could be based.

6.0 Inclusion of women

In accordance with NIH policy that women should be included in all NIH-supported biomedical research, the TACT investigators have committed to enroll a population that is representative of the US gender distribution as a whole (50% women). The Study Chairman and the PI of the DCC have recently completed an NIH-supported trial of pacemaker mode selection that had nearly equal proportions of women (48%) and men. Thus, the investigative leadership starts with a successful track record in meeting this scientific and policy goal. The following steps have been taken and will be taken to assure and equal gender representation:

- we are selecting patients for study that are age 50 or older. This has the corollary benefit of achieving a more equivalent distribution of men and women for whom CAD is the leading cause of death;
- we will set goals for each site and for the study as a whole for enrollment of women. Sites that do not meet these goals will be called by the Study Chairman and advised on how to improve their enrollment of women, based on the Study Chairman's experience in enrolling a representative sample of women;
- sites meeting recruitment goals for women will be recognized at study meetings and in the study newsletters;
- we will instruct clinical sites to focus recruitment activities in areas with a high density of women patients, such as posters in gynecologists offices, at breast cancer screenings, and church groups, for example;
- the CCC will review all study-related advertising. As part of this review, the CCC will encourage sites not meeting their recruitment goals for women to target advertising to women-friendly placements.

According to ACAM experts, the safety of EDTA chelation during pregnancy is not established. Therefore, we will exclude women of childbearing potential. Given the age required for entry (50 years or greater) and the diagnosis of MI, we expect the number of women excluded to be extremely small.

7.0 Inclusion of minorities

NIH policy requires that minorities be included in all NIH-supported biomedical research, so as to recruit a sample of patients representative of the US population. The TACT investigators have committed to enroll a population that is representative of the US population as a whole. The Study Chairman and the PI of the DCC have recently completed an NIH-supported trial of pacemaker mode selection that had 15% minorities including 12% African-Americans. Thus, the investigative leadership starts with a successful track record in meeting this scientific and policy goal. The following steps have been taken and will be taken to assure a representative patient sample:

- we will set goals for each site and for the study as a whole for minority enrollment. Sites that do not meet these goals will be called by the Study Chairman and advised on how to improve their enrollment of minorities, based on the Study Chairman's experience in enrolling a representative sample;
- sites meeting recruitment goals for minorities will be recognized at study meetings and in the study newsletters;
- we will instruct clinical sites to focus recruitment activities in areas with a high density of minority patients;

- the CCC will review all study-related advertising. As part of this review, the CCC will encourage sites not meeting their recruitment goals for minorities to target advertising to minority-friendly placements.

The CCC believes that the same strategies that were so effective in MOST will be equally or more effective in TACT in enrolling a sample representative of the US population as a whole, both regarding women, and minorities.

8.0 Subject selection criteria and rationale
Participants will be post-MI patients age 50 years or older, with fairly normal renal function (creatinine >2.0 are excluded), and no heart failure within the last 6 months. Patients with hematologic, metabolic, or hepatic abnormalities will be excluded. A special effort will be made to recruit minority patients and women, as described in Section 4.23.1. Children will not be studied, since MI is vanishingly rare in children. We are choosing patients age 50 or older to achieve a more equivalent distribution of men and women for whom CAD is the leading cause of death, and in order to more closely reflect the age distribution of patients undergoing chelation therapy. We are restricting the diagnosis of prior CAD to myocardial infarction because of the relatively high frequency of recurrent clinical events in such patients.

9.0 Exclusion of sex/gender or racial/ethnic group
No sex/gender or racial/ethnic group will be excluded from the trial.

10.0 Enrollment
The proposed dates of enrollment are 9/1/2002 to 8/31/2006.

11.0 Outreach efforts to recruit women and minorities as research patients
The following steps have been taken and will be taken to assure and equal gender representation:

- we are selecting patients for study that are age 50 or older. This has the corollary benefit of achieving a more equivalent distribution of men and women for whom CAD is the leading cause of death;
- we will set goals for each site and for the study as a whole for enrollment of women. Sites that do not meet these goals will be called by the Study Chairman and advised on how to improve their enrollment of women, based on the Study Chairman's experience in enrolling a representative sample of women;
- sites meeting recruitment goals for women will be recognized at study meetings and in the study newsletters;
- we will instruct clinical sites to focus recruitment activities in areas with a high density of women patients, such as posters in gynecologists offices, at breast cancer screenings, and church groups, for example;
- the CCC will review all study-related advertising. As part of this review, the CCC will encourage sites not meeting their recruitment goals for women to target advertising to women-friendly placements.

The following steps have been taken and will be taken to assure a representative patient sample:

- we will set goals for each site and for the study as a whole for minority enrollment. Sites that do not meet these goals will be called by the Study Chairman and advised on how to improve their enrollment of minorities, based on the Study Chairman's experience in enrolling a representative sample;
- sites meeting recruitment goals for minorities will be recognized at study meetings and in the study newsletters;
- we will instruct clinical sites to focus recruitment activities in areas with a high density of minority patients;
- the CCC will review all study-related advertising. As part of this review, the CCC will encourage sites not meeting their recruitment goals for minorities to target advertising to minority-friendly placements.

12.0 Proposed sample composition

	American Indian/ Alaskan Native (0.5%)	Asian/ Pacific Islander (3%)	Black, not of Hispanic Origin (9%)	Hispanic (6.5%)	White, not of Hispanic Origin (81%)	Total
Females 50%	4	24	72	52	648	800
Males 50%	4	24	72	52	648	800
Total	8	48	144	104	1296	1600

The composition of the study population has been calculated based on the 2000 census. The investigative team believes this to be an achievable goal based on our track record in a prior even larger NIH trial.

13.0 Inclusion of Children

Children will not be included in the present study. Myocardial infarction due to atherosclerotic coronary heart disease is vanishingly rare in children.

14.0 Data and Safety Monitoring Plan

The Director of NCCAM will appoint an independent Data and Safety Monitoring Board, with input from the Study Chairman, as suggested in the RFA. The DSMB will meet at least twice a year. DSMB meetings will be open only to designated NCCAM staff and other individuals who have been approved to have access to unblinded data. The applicants will adhere to and comply with the lines of authority suggested in the RFA. Thus, these responsibilities are not repeated here. The DSMB will serve in an advisory role to NCCAM and to the Study Chairman. Any recommendations for alteration or termination for part or all of the trial shall be based on consideration of the accumulating data in the context of totality of evidence. Specific statistical monitoring guidelines for safety and efficacy concerning the primary and secondary endpoints are found in the DCC Appendix.

It is anticipated that the DSMB will meet at approximately 6-month intervals to review the accumulating data (with one of the two meetings per year conducted as a conference call). Prior to each meeting of the DSMB, the Data Coordinating Center will conduct the desired statistical analyses and prepare a summary report that will be carefully reviewed by the DSMB. The extracted data files and analysis programs for each DSMB report will be archived and maintained at the Data Coordinating Center for the life of the study. Reports will be presented describing the progress of patient enrollment, the rates of compliance with therapy, and the frequency of protocol violations.

These interim safety and efficacy reports introduce well-recognized statistical problems related to the multiplicity of statistical tests performed on an accumulating set of data. As a solution to the problem of repeated tests, we propose to adopt for use in TACT a group sequential method similar to that proposed by O'Brien and Fleming as a guide in interpreting interim analyses. This procedure requires large critical values early in the study, but relaxes (i.e., decreases) the critical value as the trial progresses. Because of the conservatism early in the trial, the critical value at the final analysis is near the "nominal" critical value. Hence the sample size requirements with this group sequential procedure remain essentially the same as the conventional fixed sample size estimate. The actual method for this interim monitoring that will be employed in TACT is the general approach to group sequential testing developed by Lan and DeMets for which neither the number of looks nor the increments between looks must be pre-specified. Rather, the Lan-DeMets approach only requires specification of the rate at which the Type I error (which in this trial will be chosen to be α=0.05) will be "spent". This procedure allows "spending" a little of α at each interim analysis in such a way that at the end of the study, the total Type I error does not exceed 0.05. One such spending function generates boundaries that are nearly identical to the O'Brien-Fleming boundaries. It is this approach that we propose to use in TACT, namely two-sided O'Brien-Fleming type boundaries generated using the flexible Lan-DeMets approach to group sequential testing. Since the number of looks and the increments between looks need not be prespecified, it allows considerable flexibility in the monitoring process for accommodating additional comparative examinations of the data in response to concerns of the DSMB that may arise during the course

of the trial. Assuming that the DSMB will conduct its first formal data review in the latter half of the first year of recruitment, and then continue those reviews approximately every 6 months thereafter through the patient recruitment period (3 years) and the follow-up phase (1 year), there will be approximately 7-8 reviews of the data. With 8 interim analyses approximately equally spaced in time, the Lan and DeMets "spending function" that approximates the O'Brien-Fleming stopping boundaries involves a very stringent alpha level (0.00001) for declaring significance at the first interim analysis. At the subsequent interim analyses, the required significance levels will be somewhat less stringent. The requirements for significance at each interim analysis, depending on exactly when the analysis occurs, can be computed with the Lan-DeMets methodology, for which we have suitable computer software. The final analysis can be undertaken with a significance level of approximately 0.04, relatively close to the nominal 0.05 level.

The analytic approach that will be used at the interim analyses for assessing treatment differences will be the time-to-event analysis methods described above, except that interpretation of statistical significance associated with treatment comparisons of the key study endpoint will be guided using the group sequential stopping boundaries outlined above. The appropriateness of using the log-rank test (or equivalently the Cox model) in the group sequential framework has previously been well established. For each of these interim analyses, the critical value of the test statistic and the corresponding p-value required for significance in that particular analysis will be presented so that significance can be assessed precisely. If significantly large and important treatment differences are observed at any of the interim analyses, the Data and Safety Monitoring Board may recommend that randomization of patients be stopped, or that the design and conduct of the trial be appropriately modified. Judgment concerning the continuation or termination of the study will involve not only the degree of statistical significance observed at the interim analysis, but also the likelihood of achieving significance should enrollment continue to the originally projected sample size. As an aid in this latter assessment, the Data Coordinating Center will supplement the group sequential analyses outlined above with calculations of conditional power based on the method of stochastic curtailment. This procedure evaluates the conditional probability that a particular statistical comparison will be significant (or not significant) at the end of the trial at the α level used in the design, given the hypothesized treatment difference and the data obtained to date. Conditional power for the primary composite clinical endpoint will be computed and provided to the DSMB as part of the interim study reports.

The approach to interim monitoring outlined above will be carried out in parallel for the assessment of both treatment factors in the study design. Since the primary endpoint is a composite of death and several non-fatal outcomes, it will also be important to monitor the mortality component of this endpoint as part of the safety monitoring of the trial. Thus mortality rates and associated confidence intervals for each arm in the study design will also be monitored at the interim reviews to ensure that the safety of patients enrolled in the trial is not compromised. A summary of the incidence of other serious adverse events will also be regularly reviewed by the DSMB.

In TACT, the DSMB will have a particularly valuable role to the Steering Committee and NCCAM if there emerges any statistically extreme benefit or harm. The DSMB will need to put any such interim data in proper perspective. If protocol modifications are warranted, close consultation among the Steering Committee, the DSMB, and the NCCAM staff will be needed. A separate DSMB charter that outlines in detail the operating guidelines for the committee and the protocol for evaluation of data will be developed prior to the start of patient randomization and agreed upon in the initial meeting of the DSMB. Minutes of all DSMB meetings will be prepared by the Data Coordinating Center and promptly distributed to the committee members.

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