Non-Invasive Treatment of Abdominal Aortic Aneurysm Clinical Trial (N-TA^{3}CT)

PROTOCOL
(Version 1.7.1)

Table of Contents

CHAPTER 1 – BACKGROUND AND SIGNIFICANCE

1.1 BACKGROUND AND SIGNIFICANCE

Scope of the Clinical Problem. Abdominal aortic aneurysms (AAAs) are a common and life-threatening condition. The natural history of AAA is one of progressive expansion. The rate of aneurysm growth and the risk of rupture are both exponentially related to aneurysm size. Aortic diameter is therefore the single best predictor of rupture risk.[1,2] Ninety-five percent of aortic aneurysms occur in the aortic segment below the renal arteries; the normal aortic diameter at this level is 2.0-2.5 cm and risk of aneurysm rupture is low until aortic diameter increases beyond 5.5 cm.[3-5] AAAs are typically asymptomatic and are often undetected until late in their natural history, when the weakened aortic wall is prone to rupture. Spontaneous rupture of an aortic aneurysm is usually fatal and is currently responsible for at least 15,000 deaths each year in the U.S.[6] This is a conservative estimate of AAA-associated deaths.

Following AAA rupture, most patients (90%) do not survive long enough to reach medical attention, and the mortality rate remains high for those who undergo emergency surgical repair.[7] Despite numerous advances in surgery, anesthesia and critical care, it is unclear that operative mortality for ruptured aneurysms has been significantly impacted. The vast majority of ruptured AAAs are in patients previously undiagnosed with aneurysm disease and thus are preventable through early detection and elective surgical repair.[8-10] Elective AAA repair is indicated for patients with AAAs at significant risk of rupture, a clinical decision currently based on aneurysm diameter greater than 5.5 cm for men.[4,5]

Efforts to limit the mortality from AAAs are dependent on early detection and elective surgical repair of the diseased aorta. Although we know of approximately 55,000 patients with AAAs that come to clinical attention each year (15,000 with ruptured AAAs and 40,000 undergoing elective AAA repair), the overall incidence of AAAs appears to be much higher than these figures would suggest.[6,11] Prospective screening studies using abdominal ultrasound indicate that AAAs occur in 4-9% of the population over the age of 65, as defined by an infrarenal aortic diameter greater than 3.0 cm.[12,13] There is also evidence that the general prevalence of AAAs is steadily increasing, both in the U.S. and other industrial nations. Even by conservative estimates, extrapolation of these figures to U.S. census data indicates that aneurysm disease currently affects at least 1.7 million individuals.[14] Moreover, the incidence of AAAs can be expected to exceed 3 million by the year 2025 because of the aging US population.

Several factors help explain the discrepancy between the known incidence of AAAs and the incomplete number of patients that are currently treated for this disease. For example, AAAs are usually asymptomatic until rupture and routine physical examination is insensitive in detecting nonruptured AAA. Thus, many aortic aneurysms are either unsuspected or simply missed, even in populations at risk. Deaths from ruptured AAAs are significantly underrepresented in statewide mortality data, as sudden death is typically attributed to other cardiopulmonary disease in the absence of autopsies, with autopsy rates below 5%.[15] Secondly, abdominal imaging studies are extremely sensitive in detecting AAAs of all sizes, such that most AAAs requiring treatment are simply found by serendipity during studies performed for unrelated reasons. Smaller AAAs identified in this fashion may not be reported since specific treatment is not carried out. Finally, up to 90% of the AAAs detected in ultrasound screening programs, which are below a threshold that warrants immediate repair at the time of initial diagnosis (i.e., less than 5.5 cm diameter).[14,16,17]

Detection of a small aneurysm raises a considerable management dilemma, in that the natural history of small asymptomatic AAAs is one of gradual expansion (at rate of 2.6-5.4 mm

per year) and eventual rupture (see Table 1 below). Because there are no proven medical interventions capable of suppressing aneurysm growth, the detection of small AAAs only leads to the need for serial measurements until repair is indicated. This "watchful waiting" approach is particularly unsettling given that aneurysms grow in an uneven and unpredictable fashion and that the biological factors influencing aneurysm expansion are incompletely understood. The absence of medical interventions by which to treat the large number of patients with small AAAs has diminished enthusiasm for ultrasound screening programs on the basis of cost. It is clear, however, that better approaches will be needed over the next decade for the evaluation and management of patients with small asymptomatic AAAs.

Defining aortic aneurysms for a clinical trial. Aortic diameter is normally distributed, changes with age and is related to gender.[3] Understanding the changes and variation in normal aortic diameter is important for making the distinction between aortic dilatation and the initiation of the aneurysmal process. The most pronounced increase in normal aortic diameter occurs during childhood and adolescence but there is also a 25 percent increase in aortic diameter between ages 25 and 75. This increase is greater for males than females, but since there is no significance difference between the sexes when data are normalized to body surface area, body mass rather than gender may be the important factor. At age 75, the normal infrarenal aortic diameter can range from 12.4 mm in a small woman to 27.6 mm in a large man. Aortic aneurysms are usually described by their greatest diameter. Several definitions of AAA relative to other segments of the aorta have been proposed, but because an infrarenal aortic diameter in excess of 29 mm is above the upper limit of normal regardless of age, sex and body surface area, a diameter in excess of 3.0 cm is the simplest and most practical definition of an aortic aneurysm. From the earliest studies of AAA size relative to rupture risk, the greatest transverse diameter has been used. The use of multichannel CT imaging now allows for volume determination within an aneurysm and there has been significant interest and controversy regarding the sensitivity of changes in volume compared to diameter. Since each aneurysm has a unique shape, there are no guidelines that define AAA by volume. All clinical decisions regarding intervention and rupture risk are based on diameter. To summarize, we have chosen the lower threshold of 3.5 cm for several reasons including: 1) aneurysms between 3.0 and 3.5 cm demonstrate significantly lower expansion rates with greater variability; 2) a 3.5 cm infrarenal aortic diameter distinguishes aneurysmal disease from any form of diffuse arteriomegaly which may have a different clinical course; 2) the expansion rates for aneurysms between 3.5 and 5.0 cm are well-defined and relatively homogeneous. The rationale for choosing an upper threshold of 5.0 cm is relatively straightforward. Our primary endpoint is growth rate over a 2 year time frame. The study protocol allows for intervention when the threshold of 5.5 cm is reached. Based on the average anticipated growth rate of 2.5 mm/year in a man enrolled with a 5.0 cm AAA, the threshold of 5.5 cm will be reached and detected at the time of the two year follow-up CT scan.

The natural history of aortic aneurysms-defining growth rates. The natural history of an untreated AAA is one of progressive expansion and eventual rupture. Retrospective studies using ultrasound or CT scan have defined mean expansion rates. Results from a number of the larger published trials are shown in the following table.

Study	AAA size	Patient number	Primary	Growth mm/yr.
PAT[18]	3.0-5.0	272*	US	2.6
UK trial[5]	4.0-5.5	321*	US	3.3
ADAM[4]	4.0-5.4	567*	CT	3.2
Schlosser[19]	3.5-5.0	92*	US	3.1

*Number of patients in control group (placebo treated or nonsurgical); PAT = Propanolol Aneurysm Trial

This includes the three largest prospective trials and recent prospective trials and demonstrate a consistent expansion rates at 2.6-3.3 mm/yr. The lower growth rate in the trial by Laupacis reflects to inclusion of small aneurysms in the 3.0-3.5 range which exhibit a growth rate of less than 2 mm/year with significant variability. This is partly related to the fact that a significant number of aortas in this size range will not progress. The lower threshold for our proposed trial is 3.5 cm. Conversely, the UK trial and ADAM trial have the highest growth rates since they included aneurysms up to 5.5 cm. We have set the upper limit of our study at 5.0 cm for men and 4.5 cm for women so that the patients can be followed for some time before reaching the surgical threshold (5.5 cm for men and 5.0 cm for women). A recent study by Schlosser et al included patients in a screening and surveillance program with aneurysms between 3.0 and 5.5. Dr. Schlosser was kind enough to provide the data set of patients between 3.5 and 5.0 and this is shown in the table. We included these data to show that growth rates have not changed over time since publication of the larger prospective trials. For the purposes of this trial, we anticipate a growth rate of 2.5 mm/yr. We believe that this is an accurate (if not conservative) estimate considering the size of aneurysms to be included and followed in this trial.

Screening for AAA. Using an approximate growth rate of 2.5 mm/yr. for an aneurysm that is 3.5 cm at the time of detection, the typical threshold for intervention (5.5) would be reached within 7 years of diagnosis (Figure 1).

The current management if a small AAA is detected consists of follow-up imaging to detect expansion, coupled with elective surgical repair when the aorta reaches 5.5 cm diameter (i.e., "watchful waiting"). Because there are no proven medical interventions capable of suppressing aneurysm growth, the detection of small AAAs leads to dilemma for the patient and physician. They are faced with a problem that requires serial observation but for which there is no therapy until repair is indicated. The rationale for waiting is bases on two important facts: 1) the risk of repair exceeds the risk of rupture for AAA less than 5.5 cm in man and less than 5.0 cm in women; 2) and because of age and comorbid disease many patients die of other causes before their AAA reaches the threshold for repair.[20] Most aneurysms detected through population screening studies are small at the time of discovery. While this fact would seem to be of benefit to the patient, the fact that only 10% of aneurysms detected by screening are above the threshold for immediate repair is the precise reason screening is not now considered cost-effective except in select groups. Because of this low incidence of aneurysms requiring immediate intervention, the cost of screening per life saved has been estimated to be as high as $78,000.[21,22] If, however, an inexpensive medical treatment (such as doxycycline) were available for the 90% of patients with aneurysms below the operative threshold, ultrasound screening would be considered highly cost effective. One time screening is now recommended only for, men over age 64 who have ever smoked. CMS now covers the cost of screening for AAA in this group at the time of their Medicare introductory physical examination.

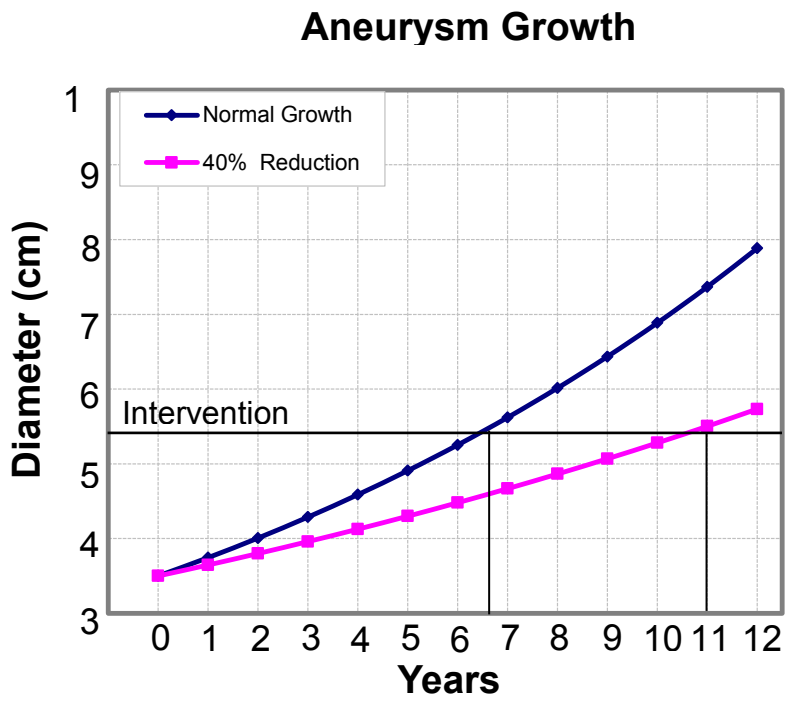


Figure 1

Factors influencing aneurysm progression. AAA have been typically detected by serendipity when abdominal or pelvic imaging studies are obtained for an unrelated problem. The mean growth rate for small AAAs (≤ 5.5 cm) is 2.5 mm/y, increasing with aneurysm diameter.(see table above) Studies of AAA expansion, and the factors associated with expansion, have been limited by sample size or a limited number of serial observations. In the UK small aneurysm trial, AAA expansion in 1,743 patients followed for up to 7 years was most strongly associated with diameter at baseline.[23] No association with growth rate was noted between age or gender. Self-reported cigarette smoking status was associated with an incrementally increased growth rate of 0.4 mm/year which persisted after adjustment for potential confounding variables. Of other potential risk factors considered in the UK SAT, including hypertension, peripheral arterial occlusive disease (PAD), total or HDL plasma cholesterol concentration and diabetes, only the presence of PAD or diabetes influenced aneurysm growth; PAD decreasing it by 0.2 mm per year for each 0.2 change in ankle brachial index (95% CI -0.03 to 0.25), and diabetes reducing the growth rate by 0.79 mm/year (95% CI 0.27 to 1.33). Based on these data, investigators calculated that screening intervals of 36, 24, 12, and 3 months for patients with AAA diameter of 35, 40, 45, and 50 mm, respectively, yield less than a 1% chance of patients unexpectedly exceeding 55 mm in diameter between examinations.[24] In clinical practice, examination intervals vary but rarely exceed more than 12 months with increasing frequency associated with progressive enlargement. Part of the reason for the more frequent studies is reassurance for both the patient and physician. Quality of life surveys indicate that diagnosis without treatment of AAA is usually associated with significant anxiety.

Although not considered in the analyses of most AAA trials, lifelong patterns of lower extremity exercise may provide some protection from AAA. Computational flow modeling studies of hemodynamic conditions in the distal aorta suggest that the decreased flow from prolonged sedentary existence may promote aneurysmal disease.[25] Indirect clinical evidence in

support of this concept include the fact that above knee traumatic amputation and chronic spinal cord injury are associated with increased AAA risk independent of other risk factors including cigarette smoking.[25,26]

Tobacco. Tobacco smoking as a specific risk factor for AAA disease prevalence, incidence and progression deserves special mention. The relative risk of AAA in individuals who have ever smoked is 2.5 times greater than the relative risk for coronary heart disease.[27] AAA is more closely associated with cigarette smoking than any other tobacco-related disease except lung cancer. [27] Nearly all (>90%) AAA patients relate a history of smoking, however, only about half of those continue to smoke at the time of diagnosis.[28] Several small studies have associated continued cigarette smoking with more rapid aneurysm expansion, Lindholt evaluated and prospectively followed 117 AAA patients. He found a positive correlation between continued smoking and the rate of expansion.[29] In the UK SAT itself, smoking and initial aneurysm size were the only two factors positively associated with aneurysm growth although they did not find a dose response between self-reported smoking habits or serum cotinine levels and aneurysm growth rate.[24] Animal studies have confirmed accelerated aneurysm growth with cigarette smoke exposure.[30] When the studies are considered together, the best evidence suggests that continued smoking is associated with a relatively small (15%) increase in growth rate that, when compounded over several years, has important implications. At the present time, recommendations for and assistance with smoking cessation is the standard of care although initial success rates are low.

β-blockers. Several animal studies have indicated that propranolol might have beneficial effects on aneurysmal disease based on both its hemodynamic properties and its biochemical effects on matrix proteins. Two clinical studies used retrospective analysis to assess the impact of β-blockers in aneurysm growth rates.[31,32] Both identified a significant inhibitory effect of β-blockers. These studies were the basis for two multicenter randomized trials testing propranolol in aneurysm patients. Propranolol did not inhibit aneurysm expansion in a trial reported by Lindholt et al.[33] These results were compromised by low compliance in the propranolol arm; only 22% of patients continued the medication for 2 years. A Canadian trial that recruited 552 patients suffered similarly from compliance problems.[18] The growth rate in the placebo group and in the propranolol group did not differ although there was a slight trend in favor of propranolol. Quality of life, assessed by the SF-36 questionnaire, showed that propranolol had a significant negative effect as one would anticipate from the low compliance rate. A randomized trial of selective beta-blockers has not been performed.

Statins. Statin therapy reduces the progression of atherosclerosis and improves clinical outcomes in cardiovascular diseases. Although effective in reducing atherogenic lipoproteins, statins also demonstrate additional biologic effects (i.e., pleiotropic effects), including reduction of high sensitivity C-reactive protein (CRP) levels, that may be relevant to the pathogenesis of AAA disease.[34] Several studies have found an association between the presence of AAA and total cholesterol.[35,36] There is, however, no clear relationship between total cholesterol and AAA expansion rate. Despite the absence of a relationship between cholesterol and growth rate, there is evidence from a number of studies suggesting that statins may influence aneurysm growth rate, presumably via these pleiotropic effects. Simvastatin therapy at 2 mg/kg/day reduces both aortic diameter and the percentage of mice with aneurysms after elastase infusion.[37] No changes in effect size were noted by repeating these experiments in hypercholesterolemic apoE-deficient mice.

In human AAA specimens explanted for organ culture, addition of cervistatin reduces tissue levels of both total and active MMP-9 in a concentration-dependent manner. Evans et al treated AAA patients with a three week pre-operative course of simvastatin and showed that MMP-9 levels in excised aneurysm tissue were decreased.[38] It is unclear, however, that these observations translate into an effect on the expansion rate of an existing aneurysm. The VA ADAM study, by far the largest prospective study with serial longitudinal observations, did not find an effect on expansion rate from lipid lowering medication.[27] Several cohort studies have found decreased aneurysm expansion in patients on statins.[19,39] Historically, these types of cohort studies overestimate this effect, a lesson learned from cohort studies of beta-blockers. Felix Schlosser provided data from their recent cohort study showing that patients with 3.5-5.0 cm AAA and taking statins grew at 2.8 mm/year which is not significantly different from growth rates in these cohorts collected before widespread use of statin.(personal communication) We hypothesize that growth in our control group will be 2.5 mm/year. These data from Schlosser demonstrate that even if statins exhibit some small inhibitory effect, growth rates in those patients taking statins are still expected to be greater than the 2.5 mm/year we have chosen as a conservative estimate of expected growth rate. If growth rates in our control groups are greater than 2.5 mm/year, the power of our study to detect 40% inhibition will increase significantly. Finally, we provide evidence in the section on preliminary data showing that doxycycline exhibits inhibitory effects on a wide array MMPs and inflammatory signaling pathways that are not or are only minimally affected by statins.

Because AAA, CAD and PVD share common risk factors including dyslipidemia, there will be clear indications for statin use in most AAA patients. Efforts to meet the National Heart, Lung, and Blood Institutes (NHLBI) increasingly stringent Adult Treatment Protocol (ATP) guidelines for LDL management has led to wider use of statins. The Women's Health Initiative has categorized AAA as a PVD equivalent, a relative indication for statins.[40] A high prevalence of statin use among AAA patients will make it impossible to perform a randomized trial of statins that could definitively determine whether statins impact aneurysm growth rate. At present, the best assessment of the statin effects on AAA expansion will be a prospective cohort study with longitudinal follow-up. The study design we have proposed will address this important question by information by correlating statin use with the precise measurements of aneurysm expansion over a two year period.

ACE inhibitors and angiotensin receptor blockers. ACE inhibitors have shown to both stimulate and inhibit MMPs depending on cell type or animal model. Losartan does not appear to have a direct effect on MMPs. A number of animal experiments using different models of aneurysmal disease have suggested an important role for the angiotensin/renin axis in aneurysm development. Captopril, but not losartan, an angiotensin receptor blocker, prevents aneurysm formation in the rat elastase model of AAA.[41] Another commonly studied aneurysm model is based on chronic infusion of angiotensin II into apo E deficient mice resulting initially in midaortic dilatation and eventual rupture.[42] Losartan prevents aneurysm formation in this model. This effect of losartan is consistent with observations in genetically engineered mice with Marfan Syndrome (MFS). Work done in these mice has suggested that the inability of mutated fibrillin to sequester TGF-β plays a role in the progression of tissue changes associated with MFS.[43] In a series of studies, TGF-β antagonism by losartan was effective in preventing progressive matrix degradation.[44] The reason for the discrepant effects of losartan -- ineffective in the elastase aneurysm model and effective in the angiotensin and Marfan models -- may relate to differences among the models. In the angiotension infusion model, initial dissection of the

upper abdominal aorta is followed by dilatation.[45] This process may have more similarities to the Marfan syndrome models where the thoracic aorta is affected. Clinical trials of losartan in MFS have recently begun enrollment.

Hackam et al recently published results of an analysis of a linked administrative data base from Ontario Canada analyzing ruptured (n=3379) and nonruptured aortic aneurysms (n=11,947) from 1992-2002.[46] ACE inhibitor use within the prior 3-12 months was less frequent among those admitted for aneurysm rupture (OR 0.82; CI 0.74-0.90). Beta-blockers, lipid lowering agents, angiotensin receptor blockers showed no relationship to rupture. In a published response to the paper, Lederle and Taylor note that among those patients who discontinued ACE inhibitors within the past 3-12 months, there is a harmful effect in favor of aneurysm rupture.[47] The case control study by Schouten et al and post hoc analysis of the UK aneurysm trial data did not find a relationship between ACE inhibitors and aneurysm expansion rates.[24,48] Most patients presenting with aneurysm rupture have large, undetected aneurysms while patients with known aneurysms typically undergo repair long before their rupture risk becomes significant. Thus, this information regarding ACE inhibitors and rupture risk might find its most practical application among the small number of patients deemed unfit for repair. The proposed trial may provide more insight into the effects of ACE inhibitors. Information regarding the use of ACE inhibitors can be correlated with aneurysm expansion rate in post hoc analysis.

Macrolides. A number of antibiotics have been proposed as a treatment for AAA with varying rationales. One line of reasoning is that AAA progression is enhanced by secondary infection within the aortic wall. Chlamydia pneumonia has been found in atherosclerotic plaque and the wall of abdominal aortic aneurysms. There was once great enthusiasm for the hypothesis that treatment of the secondary Chlamydial infection could slow progression of atherosclerosis. This has been diminished by subsequent prospective randomized trials showing no cardiovascular benefit to a year of a treatment with azithromycin in patients with stable CAD.[49] A small study by Lindholt et al. suggested that serologic evidence of a Chlamydia pneumonia infection was associated with an increased rate of aneurysm expansion.[50] This led to a randomized clinical trial in which 43 patients received a one month course of roxithromycin while 49 patients received placebo.[51,52] Patients in the treatment arm had an expansion rate at the end of the study of 1.56 mm per year compared to a rate of 2.75 mm per year in the placebo-treated group. The inhibition was greater in the first year than the second year. The study did not clarify the mechanism of effect since there was no correlation between Chlamydia titers and the ability of roxithromycin to inhibit aneurysm expansion.

Tetracyclines. The tetracycline antibiotics have been studied because of their known inhibition of matrix metalloproteinases (MMP). Petrinic et al were the first to demonstrate that doxycycline could suppress aortic wall MMP activity, elastin degradation, and aneurysm development in the elastase-induced rat model.[53] They achieved similar results using non-antimicrobial (chemically-modified) tetracyclines and nonselective hydroxamic acid derivatives as MMP inhibitors, indicating that the aneurysm-suppressing effects of doxycycline are most likely related to its activity as an MMP inhibitor.[54] Longo et al characterized a second murine aneurysm model using calcium chloride applied to the ablumenal surface to induce the aneurysm.[55] In this model, doxycycline demonstrates the same dose-dependent inhibition of aneurysm expansion.[56] The plasma doxycycline levels achieved in these animal studies were in the same range as those seen in AAA patients receiving doxycycline (100 mg bid).[57] These murine studies suggest that inhibition can still be achieved at plasma levels in the 1-2 ug/ml range.[56]

A number of studies in patients have suggested that doxycycline can inhibit MMPs in aneurysm tissue. Curci et al treated a series of patients with a three week course of doxycycline prior to open aneurysm repair.[58] Tissue levels of MMP-9 were significantly reduced by doxycycline in comparison to untreated patients. Baxter et al. in a small series of 36 patients on a six-month course of doxycycline, showed plasma MMP-9 levels decreased significantly in comparison to baseline levels.[57] This work has been followed by a small prospective randomized trial of doxycycline in which 32 patients were randomized with 17 receiving doxycycline (150 mg/day) for 3 months.[59] Chlamydia pneumonia titers were assessed but found not to be affected by doxycycline treatment. The calculated growth rate at the end of the 18 month period of observation was 1.5 mm per year in the doxycycline treated group versus 3.0 mm per year and the placebo treated group. This difference did not achieve statistical significance but the 6 and 12 month time periods did show a significant difference in favor of doxycycline treatment. Level B evidence (small randomized trials) suggests that roxithromycin or doxycycline will decrease the rate of aneurysm expansion.

Potential Medical Therapies for Treating Small AAA

Intervention	Effect on AAA growth	Level of evidence	Class of recommendation
Propranolol[18,33]	no inhibition	A	III
Macrolides[52]	inhibition	B	IIa
Tetracycline[60*,90]	Inhibition No inhibition	B A	IIa III
Statins[19,39]	inhibition	B	IIb
ACE inhibitors[24]	No inhibition	B and C	IIb
AR blockers[42]	Animal data	C	IIb

*Inhibition at 6 and 12 months following 3 months of treatment
Level A = Large randomized clinical trials. Level B = Small randomized clinical trials. Level C = Observational studies and expert opinion. Class III = Ineffective or harmful, recommended against. Class IIa = Possibly efficacious. Class IIb = Unknown efficacy.

Potential impact of pharmacotherapy on aneurysm expansion. While the natural history of AAAs is one of progressive expansion, the rate of aneurysm growth and the risk of rupture are both exponentially related to aneurysm size. Aortic diameter is therefore the single best predictor of rupture risk.[1,2] Ninety-five percent of aortic aneurysms occur in the segment below the renal arteries; the normal aortic diameter at this level is 2.0-2.5 cm and risk of aneurysm rupture is low until aortic diameter increases beyond 5.5 cm.[4,5] A small 3.5 cm AAA growing at an average rate (7%/yr.) will reach 5.5 cm in 7 years. Ideally, a drug might be found that would completely arrest growth so that repair would never be required. More realistically, drug therapy may prove to be less than 100% effective. The proposed trial is designed to test the hypothesis that doxycycline will reduce the rate of AAA expansion by 40%. This threshold was chosen for several reasons. Physicians (internists, family practitioners and surgeons) were surveyed and asked to indicate a threshold for AAA growth inhibition that they would consider clinically useful. They reported that they would prescribe such a medication only if it exhibited at least 30-50% efficacy in reducing growth. They recognized that inhibiting AAA expansion to some lesser degree would not obviate the need for intervention but simply postpone it to a time when the individual will be older and, perhaps, less fit. A 40% reduction results in a clinically significant

delay (≥ 4 yr.) in the median time it would take for a 3.5 cm AAA to reach 5.5 cm. Using the proposed study design, we have a 90% chance to detect a 40% decrease in AAA growth.

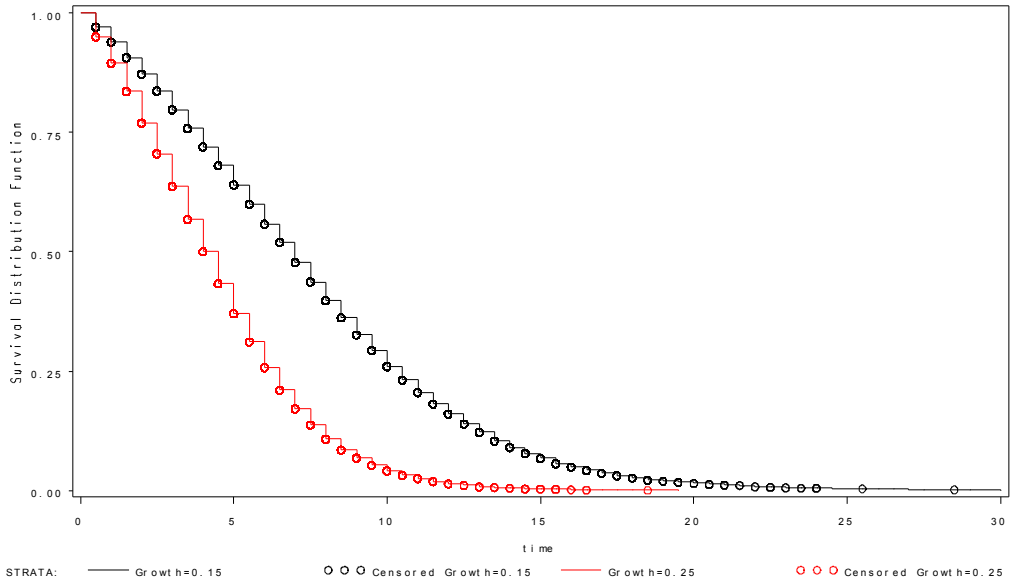
An aneurysm detected at 3.5 cm and growing at an average rate cited in earlier studies (0.3 cm/year) will reach the 5.5 cm (a recommended threshold for intervention) in 7 years (Fig.1). We have conducted a Monte Carlo simulation study to project the effect on use of surgical procedures to treat small abdominal aortic aneurysms among patients whose presenting aneurysm diameters and ages have the same distributions as those of patients screened in our clinical sites. This simulation study is based on a conservative anticipated growth rate of 2.5 mm/year and age-stratified mortality of the U.S. male population from 2006. Time to surgery is presented in Figure 2, and counts of patient outcomes out of 60,000 screened for an untreated group and a group treated with an agent that reduces growth by 40% (to 1.5 mm/year) are presented in the accompanying table.

The additional time it would take a small 3.5 cm AAA to reach 5.5 cm is 4 years (median), for 40% inhibition of normal growth. A reduction in growth rate of 40% would have impact because, excluding patients who are found on screening to have aneurysms larger than 5.5 cm, the 40% reduction in growth would be associated with an increase in the proportion of small abdominal aortic aneurysm patients who never undergo surgical intervention from 22% (10,311 ÷ (35,929 + 10,311)) to 35% (16,176 ÷ (30,064 + 16,176)), a 59% relative increase. Establishing a 40% reduction in growth rate would clearly demonstrate the biological effect of inhibition and have a monetary value of about $0.5 billion/year in reduced surgical interventions in the U.S. where approximately 1.5 million men and 1.5 million women will reach age 65 annually over the next decade, and about 8% of men and 2% of women will have small aneurysms (19,500 surgical interventions prevented per year x $30,000/intervention = $5.85 hundred million). Growth inhibition of 50% or more as observed by Mosorin would have a larger impact because as the efficacy of inhibition increases, the percent of patients who will die of other causes before reaching 5.5 cm is further increased.[60] Also, because of comorbid cardiovascular disease and other conditions, the mortality rate from all causes in patients with AAA is likely to be larger than the rate used in our conservative projections. A 30% growth rate reduction would demonstrate a biological effect but have much less clinical impact. Again, the proposed trial is designed to give us a 90% chance to detect a 40% decrease in AAA growth rate at the 95% confidence level.

New insights into the pathobiology of aortic aneurysm. It is of interest to note that all current treatment approaches to aortic aneurysms are based upon a "mechanical" concept of the disease (i.e. segmental graft repair of the diseased aorta), yet investigations emerging over the past several years have emphasized the complex cellular and molecular nature of this disorder. These studies have indicated that with greater knowledge and a conceptual shift in focus, aortic aneurysms might also be amenable to alternative, "mechanism-based" treatment strategies. . In seeking molecular targets by which to control the progression of aneurysm growth, an accumulation of studies has led to the belief that pharmacologic strategies to inhibit matrix metalloproteinases (MMPs) are now a feasible means to suppress the progression of aneurysmal degeneration. Although the pathophysiologic events underlying the initial development of AAA are still incompletely understood, it is clear that the progression of aneurysmal degeneration involves destructive remodeling of aortic wall connective tissue. Recent studies have implicated three processes in this pathologic pattern of remodeling: chronic mononuclear inflammation, progressive destruction of structural matrix proteins associated with excessive local production of matrix-degrading proteinases, and impaired connective tissue repair.

Figure 2. Product-limit estimates from two growth rates: X axis: years

- Growth rate=0.25 cm per year
- Growth rate=0.15 cm per year
- Excludes patients whose AAA diameter≥5.5cm at screening.



Number of Surgeries that Could Be Prevented in a Cohort of 60,000 Patients
Follow-Up Interval=6 months

Stratum	Total No. of simulated patients	No. of screened patients whose diameter were larger than 5.5 cm at baseline (time of the referral)	No. of patients whose diameter grows ≥5.5 cm during follow-up	No. of patients, never operated, dying of causes other than aneurysm rupture*
Growth rate=1.5 cm per year	60,000	13,760	30,064	16,176
Growth rate=2.5 cm per year	60,000	13,760	35,929	10,311
Change	———	———	-16.3%	+56.9%

* Mortality determined according to U.S. general population age and gender rates.

Matrix-degrading Proteinases. Through numerous studies conducted over the past decade, a pathophysiologic concept has emerged that proteolytic degradation of medial elastin is responsible for weakening and dilatation of the aortic wall, and that collagen degradation is responsible for aneurysm rupture.[55,61-63] Because elastin is one of the most durable structural proteins of the extracellular matrix, the dissolution of elastic fibers requires the presence of

specific proteinases. Abundant evidence now suggests that the elastin and collagen degradation in aneurysm tissue is mediated in large part by members of the MMP family, including collagenase-1 (MMP-1), stromelysin-1 (MMP-3), the 72-kDa and 92-kDa gelatinases (MMP-2 and MMP-9, respectively), macrophage elastase (MMP-12), and collagenase-3 (MMP-13).[62-67]

MMPs constitute a large family of structurally-related metalloendopeptidases that are collectively capable of degrading all components of the extracellular matrix. They play important roles in normal tissue development and remodeling, particularly during embryonic growth, uterine involution and wound healing. Abnormal expression of MMPs also contributes to a variety of pathological processes, including osteoarthritis and rheumatoid arthritis, tumor invasion and metastasis, pulmonary emphysema and atherosclerosis.[68-72] Local regulation of MMP activities is critical to prevent widespread tissue destruction during normal remodeling and in disease. MMPs are controlled at several different levels, including transcriptional induction and suppression of MMP genes, extracellular processes required for proenzyme activation, and interactions with naturally-occurring MMP inhibitors. The activation of the proMMPs is thought to be mediated by other MMPs, serine proteases, or oxidative processes. Physiological inhibitors of MMP activity include plasma-derived alpha$_2$-macroglobulin and at least four specific tissue inhibitors of metalloproteinases (TIMPs).

Of the MMPs implicated in human aneurysm disease, MMP-2, MMP-9, and MMP-12 have attracted particular interest because they have activity against insoluble elastin fibers.[62,63,73] MMP-9 is the most abundant elastolytic proteinase produced by human AAA tissues *in vitro*, it is expressed *in situ* by aneurysm-infiltrating macrophages located at the site of tissue damage, and it is synthesized in AAA tissue in a manner correlating with increased aneurysm diameter.[55,73] Whereas MMP-2 is found in close association with the extracellular matrix in aneurysm tissues, MMP-9 is elevated in the circulating plasma of patients with AAAs.[63,74] MMP-12 is also thought to play a critical role in aneurysm development, because it is selectively expressed by macrophages within the elastic media of AAA tissue and it is specifically localized to elastin fiber fragments by immunohistochemistry.[62,66] These observations have fostered the notion that MMP-2, MMP-9 and MMP-12 provide useful biological markers of aortic aneurysm disease, and perhaps more importantly, potential targets for pharmacologic therapy.

Use of tetracyclines as MMP inhibitors. During studies on the mechanisms of periodontitis in diabetic rats, Golub and colleagues made the discovery that tetracyclines have substantial metalloproteinase-inhibiting effects.[75] In subsequent studies they suggested that this inhibition took place through a mechanism similar to that found with the endogenous tissue inhibitors of metalloproteinases.[76] Non-antibiotic chemically-modified tetracyclines (CMTs) have a similar efficacy as MMP inhibitors, demonstrating that the MMP-inhibiting property of tetracyclines is unrelated to their antimicrobial activity.[54] Tetracyclines prevent matrix degradation in many animal models of disease, and because of their safety profile, they have been successfully tested in several conditions associated with elevated MMP activity and connective tissue destruction. Furthermore, it has now been clearly demonstrated that doxycycline can effectively penetrate and suppress MMPs in the complex tissue environment of degenerative human aortic aneurysms.[58,77]

Drug	Medical Condition	Comments
Doxycycline	Atherosclerotic Carotid Plaques	Doxycycline decreases MMP expression in carotid plaque removed during endarterectomy.[78]
Minocycline	Rheumatoid Arthritis (RA) Trial 1	Minocycline improved laboratory and clinical parameters in RA.[79]
	RA Trial 2	Improved laboratory and clinical parameters in RA.[80]
	RA Trial 3	Minocycline superior to placebo in clinical parameters.[81]
	RA Trial 4	On 2 year follow-up minocycline treatment resulted in improved outcomes compared to standard therapy.[82]
Minocycline	Acute Stroke	Minocycline treatment was associated with a better stroke score days 7 and 30.[83]
Tetracycline	RA	Tetracycline and clindamycin improved outcomes in refractory RA.[84]

Why doxycycline is the best choice for a clinical trial. Following modification of chlortetracycline, which was discovered in 1944, a number of useful antibiotics including tetracycline were created. Because of the relatively short half-life of these compounds, two new compounds with increased liposolubility were created (doxycycline and minocycline). These compounds exhibited rapid intestinal absorption, longer half-life, superior tissue penetration and reduced toxicity. Doxycycline is almost completely absorbed from the upper GI tract (95%) which prevented many of the lower GI tract side effects associated with the earlier tetracyclines.[85] Since doxycycline is 80-95% protein bound and is lipid soluble, blood levels are sustained allowing it to be administered on a 12-24 hour dosing regimen. Only 30-40% of doxycycline is eliminated by renal mechanisms. The majority is eliminated by hepatic and intestinal clearance and this path of excretion is enhanced with renal failure. Thus, no accumulation occurs even when patients are anuric. All of these features of doxycycline make appealing for a trial involving elderly patients with AAA. Minocycline shares many of the favorable pharmacologic features of doxycycline including a high percentage of absorption in the proximal GI tract, a long half-life and combined GI and renal excretion.[77] In fact, the half-life of minocycline is longer than doxycycline so that it could be administered once daily. A major drawback to the chronic administration of minocycline has been identified in trial of rheumatoid arthritis where it has been found to cause greenish discoloration of the skin, a problem which is usually but not always reversible.[86]

Of the other potential candidate drugs that may impact AAA, propranolol has been eliminated by negative prospective studies. As discussed above, a majority of AAA patients (67% of eligible patients surveyed at the clinical sites for our trial) will be on statins for other reasons. Because of the proven benefits of statins in preventing cardiac events, a randomized trial of statins in AAA would be unethical since it would require withdrawal of statin therapy. Although macrolide antibiotics show promise, enthusiasm was diminished by the negative results of trials in coronary disease.[52] Furthermore, these drugs are first and second line therapy as antibiotics so that their long term use in such a trial raises concerns about promoting the development and growth of antibiotic resistance. Because of past widespread use of tetracyclines

in humans, they are relegated to the treatment of a very limited number of infections. Testing other antihypertensives such as ACE inhibitors and AARB inhibitors is extremely complex in a patient population where hypertension is common and specific indications exist for the use of each class of antihypertensive medications. And, while there are a number of promising proteinase inhibitors in development, there will be some period of time before these drugs are proven safe, especially with regard to potential for inducing fibrosis. If, however, doxycycline provides the initial proof of concept that medical intervention can slow aneurysm progression, the search for more effective agents can proceed in ernest.

Clinical evidence that doxcycline will inhibit AAA. The first randomized, placebo controlled trial using doxycycline to inhibit AAA expansion was published in October 2001.[60] A total of 32 patients were randomized with 17 receiving doxycycline (200 mg/day) for 3 months. While there was no decrease in the overall expansion during the trial, the interval analysis at 6-12 months and 12-18 months indicated significantly less expansion in the doxycycline treated patients. While an accompanying editorial pointed out the weaknesses related to the small sample size especially as this relates to the use of multiple interim analyses, this trial provides the first clinical evidence suggesting that doxycycline could inhibit aneurysm expansion. This information is particularly important in the context of the proposed clinical trial given that some of the preliminary data supporting the trial has been generated in animal models that cannot precisely recapitulate chronic human diseases. It also highlights the importance of moving forward with a large properly controlled trial. Unfortunately, many clinicians will not identify the inadequacy of such studies. In the absence of other medical options for AAA and the relatively low incidence of side-effects, the data from small trials such as this will lead to increased use of doxycycline in AAA patients without adequate proof of efficacy. With dissemination of information from these small trials, it may become increasingly difficult to get patients to consent to a trial where they have a 50% chance of receiving placebo.

Two recent articles emphasize the diverse and potentially beneficial effects of doxycycline on aneurysm tissue.[77,87] Both papers are based on data from a study of 60 patients scheduled for open abdominal aortic aneurysm repair who were randomly assigned to treatment with one of 3 daily doses of doxycycline (50, 100 or 150 mg daily) or placebo. Treatment was begun 2 weeks prior to the planned surgical procedure, with aortic tissue harvested at the time of procedure. As in prior studies, a reduction in the total MMP-9 protein was seen in the aortas taken from individuals treated with doxycycline.[58] In addition, the expression of MMP-3 and MMP-25 were significantly reduced, and there were significant increases in protease inhibitors, Cystatin C and TIMP-1, particularly at higher doses of doxycycline. In addition, there appeared to be a dose dependent decrease in the activation of MMP-8 and MMP-9 extracted from the tissue.

The neutrophil content of the aortic wall was found to be significantly reduced as well as the numbers of CD8+ T-lymphocytes, but not other inflammatory cell types. They also found significant reductions in tissue levels of Interleukin (IL)-6 and IL-8, as well as consistent effects on the upstream regulators of these interleukins including AP-1 activation and C/EBP expression. Although some effects of doxycycline appeared to be dose independent, others, particularly the effects on aortic MMP activity, appeared to be greater at the higher doses of the drug.

These new findings in human tissue further suggest that doxycycline treatment may have multiple beneficial effects on the progression of aneurysm disease. The reduction of aortic neutrophils is a particularly encouraging effect. While there has been some indirect evidence that neutrophils may play an important role in aneurysm development, several recent publications

have now clearly demonstrated that neutrophil activity has an important role in an animal model of aneurysm disease.[88,89]

These new findings support the rationale for N-TA^{3}CT based on biologic effects of doxycycline. These new dose ranging studies, combined with our prior Phase II safety studies, also provide some additional assurance that the dosing choice of 100 mg twice daily is the most appropriate to develop a maximal biologic effect with minimal side effects.

1.2 PRELIMINARY STUDIES

Preliminary studies supporting this application demonstrate that doxycycline can inhibit aneurysm development and expansion in two distinct experimental models of AAA through the inhibition of MMPs. While we understand that information obtained from such models cannot precisely recapitulate the chronic disease we see in patients, we present additional lines of evidence suggesting that this experimental work is relevant: 1) we show that serum concentrations of doxycycline that inhibit experimental aneurysm formation can be achieved in AAA patients with relatively few side-effects; 2) we demonstrate that doxycycline, at the dose proposed for this trial (200 mg/day), inhibits MMP production in human AAA tissue. In 2011, however, the Pharmaceutical Aneurysm Stabilization Trial (PHAST) was completed in the Netherlands. This randomized, placebo-controlled, clinical trial of doxycycline (100 mg daily) for the treatment of abdominal aortic aneurysms larger than 3.5 cm on ultrasound (including aneurysms larger than 5.5 cm) was registered on the Netherlands Trial Register (http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=1345). PHAST reported a small increase in the rate of growth of aneurysms associated with doxycycline but did not observe an increase in adverse aneurysm outcomes associated with doxycycline.[90] This information combined with the data cited above from the Mosorin clinical trial showing decreased AAA expansion with doxycycline, demonstrate the need for a large, well-controlled trial to definitively answer this important question.

Pilot Study: Doxycycline treatment in patients with small asymptomatic AAA. Despite the promising preliminary data suggesting that doxycycline would inhibit the growth of aortic aneurysms and the long record of safety with the clinical use of doxycycline, several critical questions had to be answered before a larger and more definitive trial could be designed. The pilot trial addressed three important questions; 1) How would high dose doxycycline be tolerated and what would the level of compliance be in the elderly AAA patient population? 2) What would the plasma concentrations of doxycycline be in patients taking high dose doxycycline and how would these levels compare to the therapeutic levels required in animal models? 3) What effect would doxycycline have on plasma MMP-9 levels? Our attempts to recapitulate human disease in acute animal models cannot be entirely successful since we cannot mimic the chronic atherosclerotic features of AAA. We have previously shown that AAA is a dynamic remodeling process. Based on one known effect of doxycycline, its ability to decrease MMP levels, we expect that it will inhibit aneurysm growth. Doxycycline has other properties such as its ability to inhibit proliferation and induce apoptosis in some cell lines. These actions may not be beneficial in a complex remodeling process such as AAA. Thus, a final goal of the pilot trial was to be sure that doxycycline was safe and did not cause an unexpected increase in aneurysm growth.

Because our primary endpoint was not aneurysm growth rate, we were more lenient with regard to initial aneurysm size and imaging modality. Among the 36 patients enrolled and followed for six months, the initial range of aneurysm diameter was 3.3 to 5.5 cm.[57] Patients were recruited from the practices of the trial investigators. All patients were placed on high-dose (100 mg p.o. b.i.d.) doxycycline for six months. Since there was no funding for the imaging

studies, we allowed that patients could be studied using CT scan or ultrasound as long as the initial and final studies were done using the same technique. IRB approval was obtained and patients were enrolled from six of the centers that will be participating in the larger trial.

Patient enrollment began with review of the imaging study and recording of greatest transverse aneurysm diameter. The compatibility of the patient's current medications with doxycycline was analyzed (Drug-reax Micromedex Health Care Series, Englewood, CO) and patients were questioned about allergies to tetracyclines. The Euroqual health survey, physical examination and history were obtained followed by a venipuncture for plasma (EDTA) MMP-9 and doxycycline levels. The plasma samples were frozen for batched analysis. The patients were given a three-month supply of medication and were told to take 100 mg b.i.d with food. They were asked to keep and return the medication that they did not take at their three-month follow up. At the first follow-up, patients were questioned about side effects, onset of new medical problems or exacerbation of existing medical problems. Blood was drawn for plasma MMP-9 and doxycycline levels. They were then given the final three months supply of medication. At six months, the Euroqual health survey, a physical examination and history were obtained followed by a venipuncture for plasma MMP-9 and doxycycline levels. The medication was counted as a measure of compliance. An imaging study (CT scan or ultrasound) was obtained. The trial was complete on June 1, 2000.

Of the 36 patients initially enrolled, three chose to discontinue the study before the end of the 6-month period of follow-up, for a retention rate of 92%. The patients who chose to withdraw cited tetracycline-induced photosensitivity (n = 1) and discoloration of teeth (n = 1) as the principal reasons for dropping out, while the third patient cited new concerns about being involved in clinical research based on an article in a weekly newsmagazine. These problems arose between months 2-3 in two of the patients and between months 3-4 in the other.

With regard to other adverse events related to doxycycline treatment, two additional patients reported one or more episodes of tetracycline-induced photosensitivity. In each case, these episodes were controlled by limiting sun exposure and did not require withdrawal from the study. While a second patient also reported mild discoloration of the teeth, she chose to continue in the trial. One patient suffered an acute myocardial infarction 2 months after starting the study, but chose to continue on doxycycline treatment through the duration of the trial. By patient self-report and assessment of medication counts at each follow-up visit, a high rate (94% of medication taken) of compliance with doxycycline treatment was observed throughout the 6-month period of the pilot study. This was confirmed by plasma doxycycline levels. The mean value of 4.62 +/-0.67 μg/ml (range 1.31-14 μg/ml) was obtained.

Patient characteristics

Number	Age	Men/Women	Caucasian	Black	Hispanic
36	69+/-1.9 yrs	30/6	35	1	0

We originally planned to enter 30 patients in the pilot study, but were inundated with requests by patients to participate. We allowed six additional patients to enroll. Investigators at several of the sites also chose to put patients on doxycycline outside the trial because of the large number of requests to participate after patients heard about the trial. This enthusiasm for enrolling in a trial reflects the anxiety of patients with AAA the fact that there are no other medical therapies available.

Plasma MMP-9 levels were measured before treatment and at the 3- and 6-month interval evaluations. The mean plasma MMP-9 level before treatment was 140.91 ± 45.42 μg/ml, a level somewhat higher than that measured in our previous analysis of patients with AAAs undergoing surgical treatment. This may reflect an even greater degree of connective tissue remodeling in small AAAs as compared to those already large enough to require surgical repair. Importantly, MMP-9 levels decreased during a short course of treatment with doxycycline, with a mean decrement of 64.1% ± 8.3% from the pre-treatment baseline. This difference did not achieve significance at three months but was significant at 6 months compared to baseline. There was no apparent correlation between plasma MMP-9 concentrations and doxycycline levels. The observations support the concept that serial measurements of plasma MMP-9 may be of use in evaluating the response of individual patients to doxycycline treatment. The effects of treatment with doxycycline on plasma MMP-9 levels are comparable to successful surgical repair and substantially greater than those seen with incomplete endovascular aneurysm exclusion. The larger trial proposed will provide much greater information regarding the relationship between doxycycline levels, MMP-9 levels and inhibition of aneurysm expansion.

Effects of doxycycline on experimental AAA. There has been longstanding interest in the feasibility of MMP inhibition as a strategy for the suppression of aneurysmal degeneration. To examine this possibility, Petrinec et al. characterized the development of AAA following elastase-induced injury to the rat aortic wall.[53] Under the proper experimental conditions, there is only minimal structural damage to the medial elastic lamellae immediately after elastase perfusion and the aorta only dilates about 30-50 percent over normal. Aortic wall structure and diameter also remain stable for several days after elastase perfusion, yet the damaged aorta begins to progressively expand thereafter, enlarging to aneurysmal proportions (> 100 percent of normal diameter) within 7 to 14 days. Importantly, the delayed onset of aortic dilatation is temporally and spatially associated with aortic wall infiltration by mononuclear phagocytes, increased local expression of elastolytic metalloproteinases (including MMP-2 and MMP-9), and pronounced destruction of the medial elastic lamellae. The aortic wall response to elastase perfusion therefore recapitulates many of the morphologic and biochemical events evident by histology and biochemical assays of human AAA, encouraging the use of this model for pathophysiologic and pharmacologic investigations.

Petrinec et al. used doxycycline to suppress aortic wall MMP activity, elastin degradation, and aneurysm development in the elastase-induced rat model.[53] Whereas treatment with doxycycline (60 mg/kg/day) reduced the extent of aortic dilatation by about 60%, this effect was subsequently shown to be dose-dependent with half-maximal suppression at clinically-achievable, but relatively high doses (6 mg/kg/day).[54] They achieved similar results using non-antimicrobial (chemically-modified) tetracyclines and nonselective hydroxamic acid derivatives as MMP inhibitors, indicating that the aneurysm-suppressing effects of doxycycline are most likely related to its activity as an MMP inhibitor.[54] These and other studies suggested that tetracyclines might have significant advantages as a clinically-applicable strategy for achieving MMP inhibition in patients with AAAs.

More recent studies have focused on whether treatment with doxycycline might also influence the growth of established aortic aneurysms. To address this question, a series of rats underwent elastase perfusion. In a subset of animals sacrificed at day 7, the mean increase in AD was 111% ± 8%, indicating that aneurysms had largely developed by this interval. The remaining animals were then provided either normal drinking water or water supplemented with

doxycycline (30 mg/kg/day) and sacrificed on day 14 or 21. Animals treated with doxycycline exhibited a marked attenuation of the late progression of aneurysmal dilatation.[53]

AAA in the mouse. To further examine if MMPs might play a functionally significant role in the development of experimental AAAs, Petrinec et al. developed and characterized an elastase-induced model of AAAs in the mouse.[53] In parallel, Longo et al. has characterized a second murine aneurysm model using calcium chloride applied to the ablumenal surface to induce the aneurysm.[55] This injury produces an inflammatory response leading to increased MMP expression, matrix degradation, and aortic dilatation. As in previous studies in the rat, doxycycline was used as a non-selective MMP inhibitor. In mice treated with doxycycline beginning the day after elastase perfusion, there was a significant reduction in mean diameter on day 14 compared to untreated controls. The incidence of AAAs in individual animals was also significantly reduced by doxycycline treatment, from 91% to 50%, associated with preservation of the elastic media.[54] Using the CaCl model, doxycycline demonstrates the same dose-dependent inhibition of aneurysm expansion.[56] The plasma doxycycline levels achieved in these animal studies (see figure below) were in the same range as those seen in AAA patients receiving high dose (100 mg bid) doxycycline (patient levels mean 4.62 ug/ml, SE 0.67, range 1.31-14). These murine studies suggest that inhibition can still be achieved at plasma levels in the 1-2 ug/ml range.

Suppression of aortic aneurysms by targeted gene disruption of MMP-9. Based on previous studies suggesting that MMP-9 might play a particularly important role in the pathophysiology of aortic aneurysms, one of the important goals of these studies was to discern if this enzyme is *required* for the development of experimental AAAs. The response to elastase perfusion was therefore examined in mice with targeted disruption of the MMP-9 gene.[62] While functional abnormalities in MMP-9 (-/-) mice have been described, these animals do not exhibit spontaneous cardiovascular abnormalities. Experiments with these animals revealed that elastase-induced aneurysmal dilatation is substantially reduced in MMP-9 (-/-) mice, with an increase in aortic diameter of only 87 ± 10% on day 14 compared to 132 ± 8% in the 129/SvEv background controls ($P < 0.05$).[17] AAAs developed in only 40% of the MMP-9 (-/-) animals compared to 94% in wild type controls ($P < 0.05$). The suppression of aneurysmal dilatation achieved by genetic deficiency in MMP-9 was similar to that achieved in wild-type mice treated with doxycycline. These studies demonstrate that expression of MMP-9 is required in the process of elastase-induced aneurysmal degeneration and that treatment with doxycycline can reduce aneurysm development to an equivalent extent in this model of AAAs. Using the CaCl murine model of AAAs, Dr. Baxter's laboratory has independently corroborated the effects of MMP-9 deficiency on aneurysm development. Their studies also demonstrated that there is some degree of aneurysm suppression in MMP-12 (-/-) mice.[66]

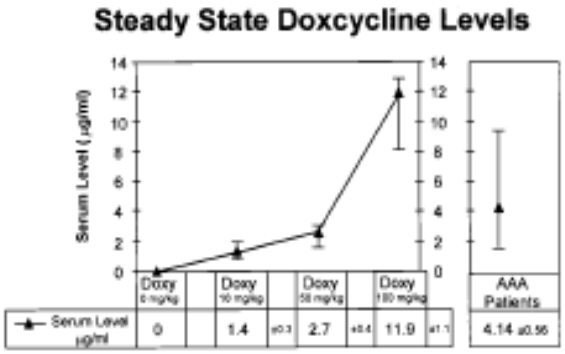


Fig. 1. Serum doxycycline concentration measurements in mice with three different oral doses of doxycycline and in control group shown in comparison with plasma doxycycline measurements in patients with AAAs.

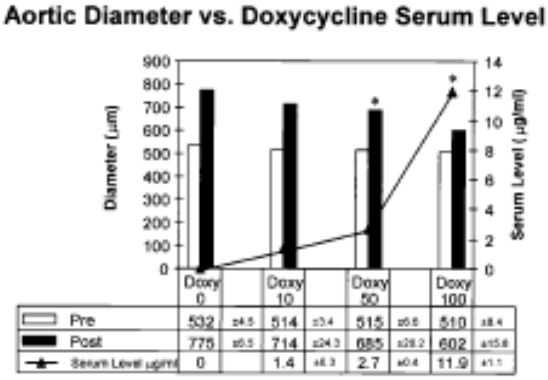


Fig. 2. Effects of different doses of oral doxycycline on aneurysm inhibition in murine model (aortic diameter on *left axis*) and relationship to serum doxycycline levels (*right axis*). Treatment groups of 50 mg/kg and 100 mg/kg showed statistically significant attenuation in aortic growth when compared with control group (0 mg/kg). *$P < .05$.

Broad protease inhibitory effects of doxycycline. While it is clear that doxycycline inhibits MMP-9, there is evidence of much broader MMP inhibition. We have demonstrated that doxycycline inhibits MMP-2, another key MMP in AAA. Using gene array studies with cultured macrophages, we have recently identified spectrum of MMPs and MMP promoting pathways impacted by doxycycline.(unpublished observations)

Treatment with doxycycline reduces MMP expression and activation in human AAA tissue. To begin translating these experimental observations to patients with AAAs and to further examine the mechanisms by which doxycycline might act *in vivo*, 15 patients scheduled to undergo elective AAA repair were examined.[58] Eight of these patients were treated with oral doxycycline (100 mg bid) for one week prior to operation, while the others served as untreated but contemporaneous controls. Aneurysm tissues excised at operation were used for analysis of MMP protein (gelatin zymography and immunoblot analysis) and mRNA expression (competitive RT-PCR). The two groups were indistinguishable with respect to age, gender and AAA size, but there was a 2.5-fold reduction in extractable MMP-9 protein in the doxycycline-treated patients vs. controls ($P < 0.05$). See Figure below. It was also found that aortic wall expression of MMP-9 mRNA was reduced by 5.5-fold in the doxycycline-treated group ($P < 0.05$) (not shown). Although there was no significant difference in the amount of extractable MMP-2 protein or mRNA, there was a reduction in the activated fraction of MMP-2 in the doxycycline-treated patients (not shown). These studies indicate that even brief treatment with doxycycline can effectively reduce MMP-9 production in human aortic tissues from patients with AAAs. They also indicate that the effects of doxycycline occur through a *combination* of

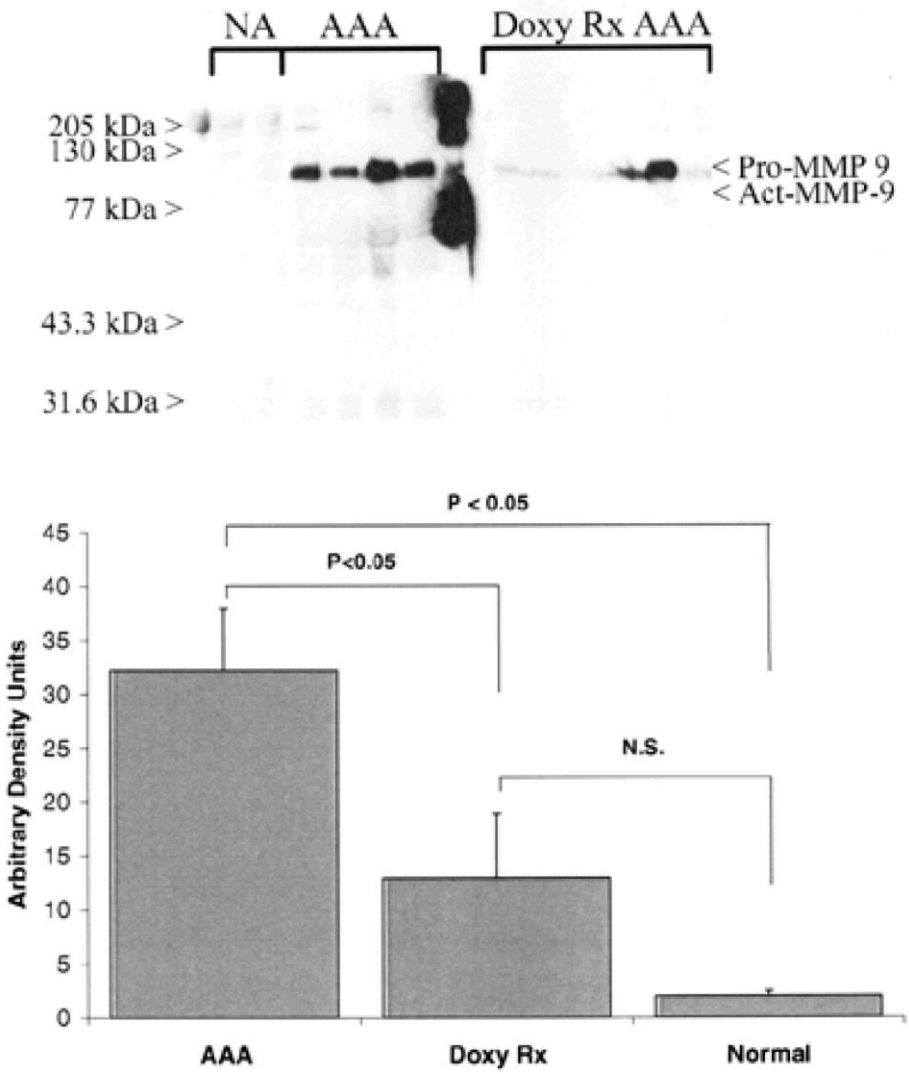
favorable mechanisms (i.e., a reduction in MMP expression and activation, as well as direct pharmacological inhibition of enzyme activities), suggesting that doxycycline might be a particularly useful agent with which to inhibit MMPs in patients with AAAs.

Plasma MMP-9 levels in patients with AAA. To assess biological events more directly involved in the enzymatic degradation of matrix proteins, McMillan et al. examined the production of MMP-9 in AAA tissue and its presence in the circulation.[74] Their initial studies involved 22 patients with AAA, 9 patients with aortoiliac occlusive disease (AOD), and 8 normal controls. Using a sensitive enzyme-linked immunosorbent assay (ELISA) to measure MMP-9 in peripheral venous blood plasma samples, they found that plasma MMP-9 was significantly higher in patients with AAA (85.66 ng/ml ± 11.64; mean ± SEM) than in AOD (25.75 ng/ml ± 4.16; $p < 0.001$) or normal controls (13.16 ng/ml ± 1.94; $p < 0.001$); the difference between AOD and normal controls was not significant. Interestingly, patients with multiple aneurysms had significantly higher plasma MMP-9 levels than patients with an isolated AAA (134.68 ng/ml ± 17.5 versus 71.03 ng/ml ± 10.7; $p < 0.04$). The production of MMP-9 was also significantly increased in organ cultures of AAA and AOD compared to normal aorta, suggesting that the diseased aortic tissue was the source of the elevated circulating MMP-9. These intriguing results provided the first evidence that circulating MMP-9 levels might provide a sensitive biomarker of aneurysm disease, a notion confirmed and extended by the preliminary studies described below. Thus, one of the aims of the proposed study will be to examine prospectively if circulating levels of MMP-9 might correlate with aneurysm growth rates or therapeutic effects in suppressing aneurysm growth. The relevant biologic samples will be obtained during the course of the study. These data will be valuable in the future as potential measures by which to monitor therapeutic efficacy during pharmacological treatment, especially if changes in MMP-9 levels precede changes in the rate of aneurysm growth.

Following the demonstration that plasma levels of MMP-9 are elevated in patients with AAAs, Hovsepian et al. sought to extend these observations.[91] Peripheral venous blood was sampled in 25 patients with AAAs (mean age 66.6 ± 3.9 yrs), 15 patients with aortoiliac occlusive disease (AOD; mean age 65.4 ± 3.9 yrs), and 5 normal healthy controls (mean age 31.2 ± 2.2 yrs). Additionally, the patients with AAA had arterial blood samples taken above and below the aneurysm. Plasma MMP-9 levels were again determined using an ELISA kit. In this study, the mean (±SE) plasma MMP-9 concentration was 36.10 ± 7.71 ng/ml in 5 normal controls, 54.71 ± 10.45 ng/ml in 15 patients with AOD (P=NS vs. normal), and 99.38 ± 17.38 ng/ml in 25 patients with AAAs (P<.05 vs. normal and AOD). With an upper limit of normal defined as 87.8 ng/ml (mean + 3 SDs), elevated levels of plasma MMP-9 were found in only 1/15 (6.7%) of patients with AOD, but in 12/25 (48%) with AAAs (P<.05). The positive predictive value of an elevated plasma MMP-9 level was 92.3%. Non-elevated plasma MMP-9 levels were an unreliable indicator of the absence of AAA, with a negative predictive value of 59.4%. Neither age, gender, nor aneurysm diameter correlated with plasma MMP-9 levels, although the highest values were observed in patients with large AAAs. The results of this study parallel those reported by McMillan et al.[74] suggest that plasma levels of MMP-9 might be a useful marker for assessing the success of medical or surgical intervention for AAA.

Plasma MMP-9 levels are decreased by successful surgical repair. Based on the hypothesis that plasma MMP-9 levels reflect the biological events occurring within the aneurysmal aorta, it would be predicted that elevated levels of plasma MMP-9 should fall considerably after aneurysm repair. A newer and less invasive technique of aneurysm repair involves placing an expandable stent graft (endografting) within an aortic aneurysm using a

femoral artery approach. Occasionally, this technique fails to exclude blood flow into the aneurysm sac (endoleak). We also considered whether the presence or absence of such a decrease in plasma MMP-9 levels might be useful as a functional biomarker if an endovascular repair were not successful. To test this possibility, plasma MMP-9 levels were measured in a series of patients scheduled to undergo elective treatment for infrarenal AAAs.[92] Eleven patients were identified who had elevated plasma MMP-9 levels prior to aneurysm repair (PreOp). Postoperative (PostOp) plasma MMP-9 levels were re-measured 3 to 10 months after open repair (OR; n = 6), endovascular repair without endoleak (ER/NoL; n = 3), or endovascular repair with endoleak (ER/wL; n = 2). Data were expressed as the mean ±SEM for each group and compared by the Student's t-test (PreOp vs. PostOp). The percent decrease in plasma MMP-9 following treatment was also determined for each patient and correlated with the success or failure of repair at 6 and 12-month follow-up intervals.



The upper panel is a Western blot showing the MMP-9 levels in aortic tissue from patients receiving doxycycline and a control group that did not receive oral doxycycline prior to operation and several normal aortas from transplant donors. The lower panel shows the densitometric quantification of these studies.[13]

Prior to aneurysm repair, the mean plasma MMP-9 concentration for all patients was 290.19 ± 45.46 ng/ml (compared to 87.8 ng/ml as the upper limit of normal in our laboratory) with no significant difference between groups (open repair, 328.05 ± 73.85 ng/ml; all endovascular repair, 244.77 ± 47.31 ng/ml; endovascular repair with no leak, 253.54 ± 76.70 ng/ml; endovascular repair with leak, 231.66 ± 66.73 ng/ml). Plasma MMP-9 levels decreased in all 11 patients following AAA repair, reaching an overall mean of 48.41 ± 19.35 ng/ml ($P < 0.001$, preoperative vs. postoperative). The mean postoperative plasma MMP-9 level was 36.99 ± 23.45 ng/ml in the 6 patients who had undergone open repair ($P < 0.01$ vs. preoperative) and 62.11 ± 33.90 ng/ml in the 5 patients who had undergone endovascular repair ($P < 0.05$ vs. preoperative). In the endovascular repair group, postoperative plasma MMP-9 levels were 7.08 ± 3.70 ng/ml for 3 patients without endoleak ($P < 0.05$ vs. preoperative) and 144.66 ± 9.69 ng/ml for 2 patients with endoleaks (P = NS vs. preoperative). These preliminary observations support the hypothesis that the aneurysm is the source of plasma MMP-9. In addition, they suggest that serial measurements of plasma MMP-9 concentrations can be used to follow the effects of medical treatment of AAA. We demonstrate below that we did observe a decrease in plasma MMP-9 levels in AAA patients taking doxycycline in 6 month pilot trial performed by the investigators involved in this proposed trial. We expect this to be an important biomarker in the trial.

Summary of background and preliminary data. It is of interest to note that all current treatment approaches to aortic aneurysms are based upon a "mechanical" concept of the disease (i.e., segmental graft repair of the diseased aorta), yet investigations emerging over the past decade have emphasized the cellular and molecular nature of this disorder. These studies have indicated that with greater knowledge and a conceptual shift in focus, aortic aneurysms might also be amenable to alternative "mechanism-based" treatment strategies. In seeking molecular targets by which to control the progression of aneurysm growth, accumulating information leads to the conclusion that pharmacologic strategies to inhibit matrix metalloproteinases (MMPs) are now a feasible means to suppress the progression of aneurysmal degeneration. We have demonstrated that doxycycline: 1) inhibits experimental aneurysm formation in a dose dependent fashion in three different animal models of AAA; 2) decreases MMP levels in human aneurysm tissue when given preoperatively; 3) decreases circulating MMP-9 levels in patients with untreated aneurysms; 4) is well-tolerated in AAA patients in doses that achieve significant inhibition of aneurysms in animal models.

The PHAST results are a warning that doxycycline treatment may be harmful instead of beneficial. We have selected the dose of 100 mg doxycycline twice a day because our pre-clinical, pharmacokinetic and pharmacodynamics data (presented above) suggest that the 100 mg daily dose used in PHAST could not achieve circulating levels of doxycycline that would be efficacious but a 100 mg twice daily dose (total 200 mg per day) could. PHAST results provide evidence that a 100 mg daily dose of doxycycline will not reduce the growth of small abdominal aortic aneurysms. In consideration of the possibility of accelerated growth of small abdominal aortic aneurysms due to doxycycline, we will be monitoring N-TA^{3}CT findings closely. We will perform repeated analyses of six-month CT scan findings anticipating a halt to study treatment if doxycycline accelerates aneurysm growth. We have planned formal futility analyses at the time of the second interim analysis for efficacy.

The use of CT scans for precise measurement of maximal transverse diameters will allow us to monitor patient safety and provide information for clinical decisions with greater certainty than has been possible in previous clinical trials (including PHAST) that were based on

ultrasound measurements. The combination of N-TA^{3}CT dose selection and measurement methods and monitoring of doxycycline and MMP-9 levels will allow us to address our clinical trial question definitively. The definitive answer to this question is in the best interest of our patients and of the public which would not be well served by a clinical trial now with a lesser dose of doxycycline, less precise measurements of abdominal aortic aneurysm maximal transverse diameters, or less information on circulating levels of doxycycline or MMP-9.

If the primary hypothesis of this study proves to be correct, and doxycycline inhibits expansion of small aneurysm, the entire approach to this common disease will be altered. Proof of the concept that AAA can be controlled medically will lead to trials designed to find more effective medications. Screening for AAA would become highly cost effective and the standard of care. The vast majority of deaths related to rupture of undiagnosed AAA would be prevented.

CHAPTER 2 – STUDY OBJECTIVES

The overall goal of the proposed study is to determine whether treatment with doxycycline 100 mg p.o., b.i.d., in patients with small abdominal aortic aneurysms (3.5 to 5.0 cm in diameter among men, 3.5 to 4.5 cm among women) is associated with reduced growth of the aneurysm in diameter compared to growth with treatment with placebo over the course of two years.

2.1 PRIMARY AIM

1. Determine if doxycycline (100 mg bid) will inhibit the increase in greatest transverse diameter of small abdominal aortic aneurysms (3.5-5.0 cm in men, 3.5-4.5 cm in women) over a 24-month period of observation in comparison to a placebo-treated control group.

2.2 SECONDARY AIMS

1. Determine if doxycycline will inhibit circulating MMP-9 levels compared to a control (placebo) group.
2. Determine if doxycycline (100 mg bid) will prevent the increase in aneurysm volume of small abdominal aortic aneurysms (3.5-5.0 cm in men, 3.5-4.5 cm in women) over a 24-month period of observation in comparison to a placebo-treated control group.
3. Determine if doxycycline (100 mg bid) will inhibit the increase in greatest transverse diameter or volume of small aortic aneurysms (3.5-5.0 cm in men, 3.5-4.5 cm in women) using CT data from all 6 month time points.
4. Determine the relationship between MMP-9 levels and interval aneurysm expansion rate.
5. Determine if doxycycline treatment will adversely affect perceived quality of life.

2.3 EXPLORATORY AIMS

1. Determine if doxycycline will reduce circulating interferon gamma levels compared to a placebo-treated group.
2. Determine the relationship between interferon gamma and aneurysm expansion rate.

2.4 ACCOMPLISHING OBJECTIVES

Pools of over nine hundred patients likely to be eligible have been identified at the 15 clinical sites. CT images obtained serially at 6 month intervals at the clinical sites will be transmitted for central reading to a specialized Imaging Core Laboratory (ICL). The blood samples collected at 6 month intervals will be shipped to the Biomarkers Core Laboratory (BCL) for MMP-9 and Interferon-γ levels. The Data Coordinating Center (DCC) will receive and process data from the clinical sites, the ICL and the BCL for analysis. A brief description of how the aims will be accomplished follows:

Primary Aim- This aim will be accomplished by quantitative assessment and comparison

of the greatest transverse diameter on CT scan obtained at baseline and at 24-month follow-up. The data will be compared by methods that account for all patients ranking them based on aneurysm diameter and informative outcomes such as aneurysm repair, aneurysm rupture and death.

Secondary Aim 1- This aim will be accomplished by quantitative assessment (μg/ml) of plasma MMP-9 levels obtained at baseline and during follow-up at six month intervals in the doxycycline and placebo treated patients. Data analysis will be accomplished with longitudinal methods for comparison of the multiple values at different time points for time by treatment interactions.

Secondary Aim 2- This aim will be accomplished by quantitative assessment and comparison of aneurysm volume on CT scan obtained at baseline and at 24-month follow-up. The data will be compared by methods that account for all patients ranking them based on aneurysm volume and other informative outcomes such as aneurysm repair, aneurysm rupture and death.

Secondary Aim 3- This aim will use CT images obtained at each six month time point rather than using only baseline and 24 month values as described for the primary aim. Analysis will be done with longitudinal methods for comparison of the multiple follow-up points for time by treatment interactions.

Specific Aim 4- This aim will use the plasma MMP-9 levels obtained at baseline and 6 month intervals and analyze these levels in relationship to change in transverse diameter during the corresponding periods. Data will be analyzed with longitudinal methods for comparison of the multiple follow-up points.

Specific Aim 5- This aim will be accomplished by comparing the results of the Medical Outcomes Study (MOS) Short Form 36 (SF-36) questionnaire assessed at baseline and during treatment comparing the doxycycline and placebo-treated groups. Data analysis will be accomplished with longitudinal methods for comparison of the multiple follow-up points for time by treatment interactions.

Exploratory Aims 1 and 2 - This aim will be accomplished by quantitative assessment (μg/ml) of plasma interferon-gamma levels obtained at baseline and during follow-up at six-month intervals in the doxycycline and placebo groups. Data analysis will be the accomplished with longitudinal methods for comparison of the multiple values at different time points for time by treatment interactions.

CHAPTER 3 – OVERVIEW OF STUDY DESIGN AND METHODS

The Non-invasive Treatment of Abdominal Aortic Aneurysm Clinical Trial (N-TA^{3}CT) is a randomized, double-blind, placebo-controlled clinical trial designed to test the hypothesis that doxycycline 100 mg p.o., b.i.d., will reduce the rate of increase of maximum transverse diameter of small (3.5-5.0 cm among men and 3.5 to 4.5 cm among women in the largest diameter) abdominal aortic aneurysms. Maximum transverse diameter will be determined by Computer Tomography (CT) scans. Men who are found on CT scan to have 3.5-5.0 cm abdominal aortic aneurysms and women who are found on CT scan to have 3.5 to 4.5 cm aortic aneurysms that do not involve the renal arteries are eligible. Patients will be randomly assigned to receive doxycycline capsules, 100 mg, p.o., b.i.d., or indistinguishable placebo capsules p.o., b.i.d. The primary outcome is abdominal aortic aneurysm maximum transverse diameter at two-year follow-up with allowance for baseline (pre-randomization) diameter. Secondary outcomes will determine if doxycycline decreases other measures of aneurysm status, MMP-9 levels in plasma and whether this decrease corresponds to inhibition of aneurysm growth.

3.1 STUDY POPULATION

3.1.1 Inclusion Criteria

The study population will include patients 55 years of age or older who are found to have small (3.5-5.0 cm among men and 3.5 to 4.5 cm among women in the largest transverse diameter) abdominal aortic aneurysms on quantitative computer tomography (CT) scans.

3.1.2 Exclusion Criteria

Patients will be excluded from the study if they are unable to give their own informed consent to participate; have symptoms related to abdominal aortic aneurysm; have other intra-abdominal vascular pathology that may require repair within 24 months (e.g., renal artery stenosis, large iliac artery aneurysms, iliac occlusive disease, aneurysmal involvement of the renal artery); have had previous abdominal aortic aneurysm repair by open surgical or endovascular technique; have an active malignancy with life expectancy less than two years; have an allergy to tetracycline; are currently or have been recently treated (previous six months) with tetracycline derivatives; they are currently taking anti-seizure medicines metabolized by pathways influenced by doxycyclines (e.g., carbamazepine, phenytoin, and barbiturates); stage II hypertension (patients whose blood pressure is persistently in the range of systolic > 160 mm Hg or diastolic > 100 mm Hg despite primary physician's best effort to achieve adequate therapy); have dialysis dependent renal failure or impending dialysis treatment for renal insufficiency; have a chronic infection requiring long-term (> 2 weeks) antibiotics, have known genetic syndromes responsible for the abdominal aortic aneurysm (e.g., Marfan's Syndrome), are under treatment with systemic immunosuppressive agents, could become pregnant, are not good candidates for clinical trial participation or are enrolled in another clinical trial.

3.2 STUDY DESIGN

We will conduct a randomized, double-blind, parallel, two-group multicenter trial (Exhibit 3-1). A schedule of study procedures and treatments is found in the Appendix.

3.3 STUDY TREATMENTS

Patients will be assigned to doxycycline 100 mg p.o., b.i.d., or matching placebo. Patients randomly assigned to doxycycline will receive bottles containing a sufficient supply of 100 mg doxycycline capsules to take one capsule twice a day until the next appointment (about 100 days). Patients randomly assigned to placebo will receive a similar appearing supply of placebo capsules.

3.4 END POINTS

3.4.1 Primary Outcome

The primary outcome is change in abdominal aortic aneurysm maximum transverse diameter on CT scan from baseline to the follow-up assessment two years after randomization as measured in the Imaging Core Laboratory (ICL).

3.4.2 Secondary Outcomes

Secondary outcomes will derive from central, ICL analyses of the CT scans performed every six months on patients and from the clinical follow-up of randomized patients, from clinical observation, local laboratory findings, study visit quality of life assessments, and from biomarker analyses to be performed in the Biomarkers Core Laboratory (e.g., changes from initial MMP-9 levels, and MMP-9 levels at 24 months of follow-up).

3.4.2.1 Clinical Outcomes of Interest

Clinical outcomes of interest related to cardiovascular disease include death, acute myocardial infarction, unstable angina, aortic aneurysm rupture, and aortic aneurysm repair. Clinical outcomes related to chronic tetracycline therapy will include gastrointestinal side effects

(e.g., nausea, vomiting and diarrhea), dermatologic side effects (e.g., rashes and photosensitivity) and occurrence of infectious diseases (e.g., C. difficile colitis).

3.4.2.2 CT Scan Outcomes

We will compare treatment groups on central, ICL assessments of infrarenal aneurysm neck length and diameter, aneurysm volume, characteristics of the neck (e.g., suitable or not for endovascular repair, neck angulation), maximum diameter at times other than two years after randomization, serial measurements of aneurysm diameter growth patterns, and wall stress.

3.4.2.3 Clinical Laboratory Studies

Liver function tests (alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase), blood urea nitrogen (BUN), creatinine and complete blood count will be assessed annually at Clinical Site laboratories, reported on study forms and compared between the two treatment groups.

3.4.2.4 Quality of Life

Quality of life will be assessed with the SF-36 administered every six months.

3.4.3 Sample Size and Data Analysis

The study will include 258 randomized patients. Approach to no more than 1600 eligible patients is anticipated to be necessary to obtain consent from 258 (16%).

The primary analysis will be performed to test the null hypothesis of no difference in growth of abdominal aortic aneurysms between the two treatment groups (doxycycline and placebo) after 24 months of follow-up as measured by CT scans analyzed in the Imaging Core Laboratory. This analysis will be performed according to the principle of intention-to-treat. In order to include patients whose 24-month abdominal CT scans are missing because of death, endovascular repair or for other reasons in the primary analysis, we will base our analysis on normal scores for percentile of rank status. We will assign worst ranks to deaths, in order of time from study entry to death, next worst ranks to rupture or endovascular repair with evidence of imminent rupture, next worst ranks to endovascular repair for reasons of aneurysm growth or other indication free of evidence of rupture, all in order of time from study entry within category, and impute from previous CT scan measurements and other patient characteristics (using standard SAS procedures) to 24 months for those few patients whose 24-month CT scans were not performed for technical reasons or are not useful for study purposes. Other patients will be assigned the remaining ranks according to the amount of change in largest, transverse diameter of the aneurysm.

A Data and Safety Monitoring Board (DSMB) will review the accumulating data for early, convincing evidence of benefit or harm. We anticipate reviews at approximately six-month intervals with the first review of primary outcome data 30 months after the first patients are randomly assigned study treatment. There will be two interim analyses of the primary outcome data and our final report for the DSMB. Interim analyses will be conducted at a one-sided alpha level of 0.0005, leaving alpha of 0.0247 for the final efficacy analysis. This adjustment of alpha level is made after the fashion of Haybittle-Peto.

Study power calculations are based on a 0.025 (one-tailed) alpha level and 90% power against the alternative hypothesis of differences in size that reflect growth less than 40% between the two treatment groups. The projected number of patients to be recruited allows for a sum of 10% of patients crossing over (percentage of patients assigned to doxycycline who do not take their study treatment plus percentage of patients assigned to placebo who start taking doxycycline) and 15% missing at random data for the 24-month CT scan assessment of maximum cross sectional aneurysm diameter.

We propose as a stopping guideline a Pocock-type boundary of Z=1.645, corresponding to a nominal one-sided 5.0% type I error rate in the direction of more aneurysm growth in subjects receiving doxycycline, at each of the reviews of six-month scan data. No adjustment of alpha or the p-value would be necessary in the primary efficacy analysis, because the study would be stopped on the basis of the analysis of six-month scans only if doxycycline were doing worse than placebo. The statistical analysis method for six-month CT scans would be the same as for 24-month CT scans, including the way any deaths or surgeries would be handled.

To take into account the multiplicity of hypotheses being tested in secondary and exploratory analyses, a p-value < 0.01 will be required to consider there to be evidence of differences present, and p<0.001 for strong evidence.

3.4.4 Time Line

The study time line includes six months for planning, training and administrative approvals (e.g., DSMB approval of the final protocol, IRB approvals of the final protocol and final contract negotiations), eighteen months for recruitment, twenty-four months minimum for follow-up, and twelve months for close-out analysis and publications (Exhibit 3-2).

N-TA^{3}CT Design
Exhibit 3-1

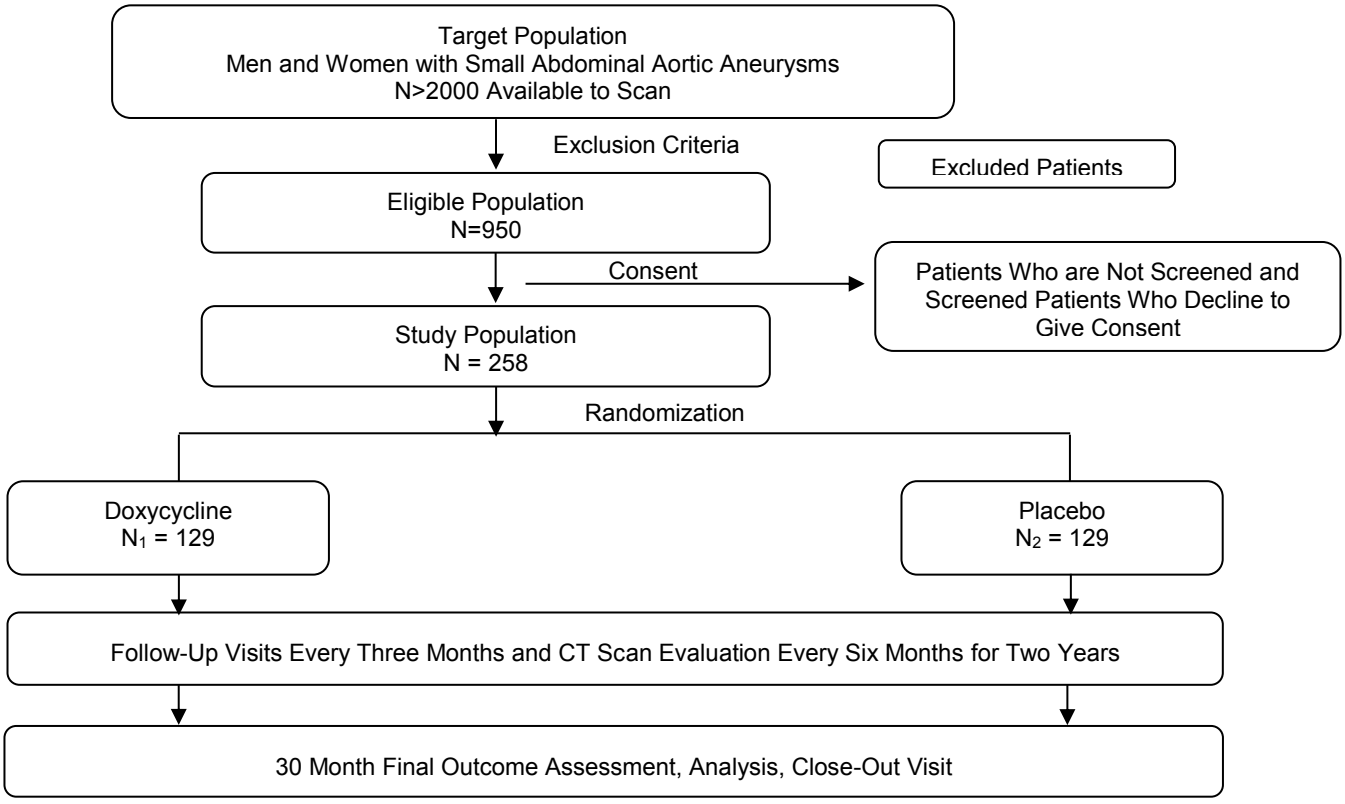
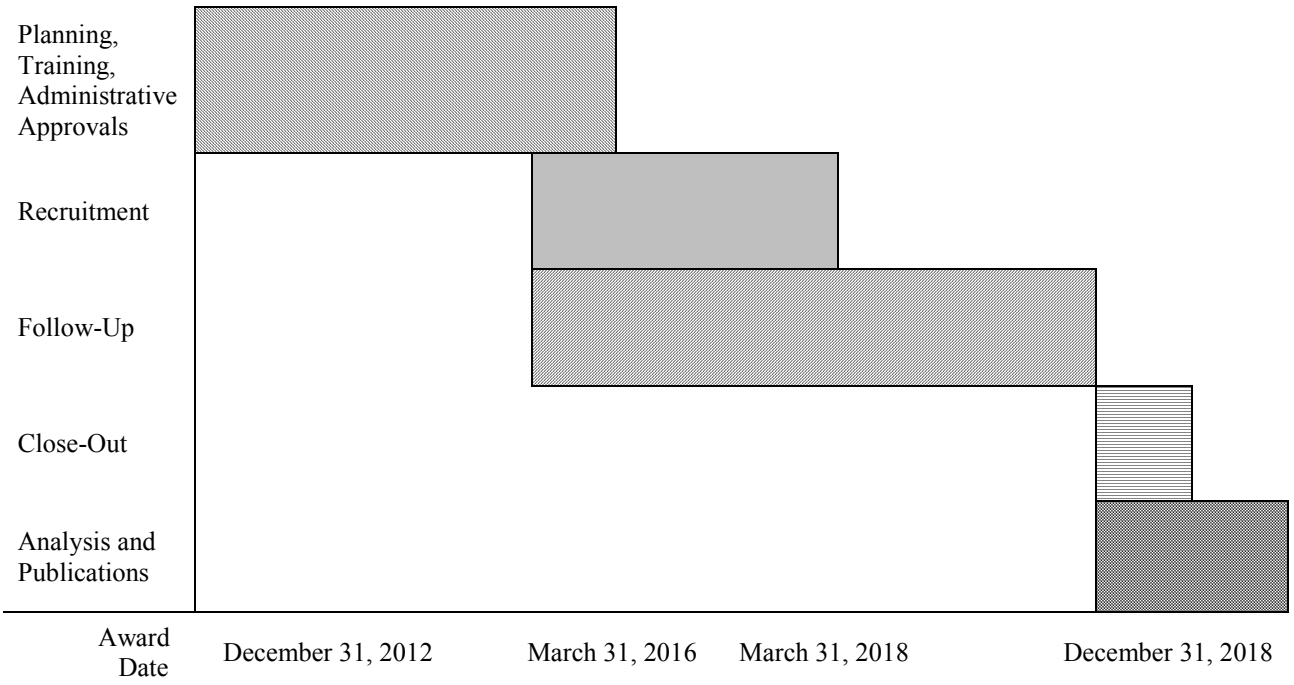


Exhibit 3-2
N-TA^{3}CT Study Timeline



CHAPTER 4 – PATIENT ELIGIBILITY, ORIENTATION AND RETENTION

4.1 INCLUSION CRITERIA

1. Patients 55 years of age or older, women post-surgical menopause or at least two years since last menses if natural menopause.
2. CT scan documented infrarenal abdominal aortic aneurysm with maximum transverse diameter larger than 35 mm and no greater than 50 mm, in men, and larger than 35 mm and no greater than 45 mm in women.

4.2 EXCLUSION CRITERIA

1. Prior repair of the abdominal aortic aneurysm.
2. Renal artery involvement or suprarenal extension of the aneurysm.
3. Documented failure of the aneurysm to increase in size over the two years prior to enrollment if the aneurysm is < 4.0 cm in maximal transverse diameter.
4. Iliac artery aneurysm > 2.9 cm in diameter.
5. Iliac artery occlusive disease planned for repair.
6. Renal artery stenosis with planned open repair.
7. Known thoracic aortic aneurysm > 3.5 cm, for aneurysms that are saccular or observed to be expanding at a rate greater than ordinary and > 4.0 cm, for aneurysms that are not saccular and are expanding at accustomed rates.
8. Known connective tissue disease (e.g., collagen vascular disorder), heritable or genetic syndrome (e.g., Marfan Syndrome, Ehlers-Danlos Syndrome) underlying the abdominal aortic aneurysm.
9. Stage II hypertension (patients whose blood pressure is persistently in the range of systolic > 160 mm Hg or diastolic > 100 mm Hg despite personal physician's best effort to achieve adequate therapy).
10. Creatinine > 2.0 g/dL or creatinine clearance < 30 ml/min.
11. Allergy or intolerance of tetracyclines.
12. Use of tetracyclines within past six months.
13. Taking anti-epileptic pharmaceutical agents (e.g., carbamazepine, diphenylhydantoin).
14. Current or planned treatment with chemotherapy or radiation therapy for cancer other than squamous cell cancer of the skin.
15. Current or planned treatment with systemic immunosuppressive agents (e.g., prednisone, azathioprine, methotrexate, cyclosporine for autoimmune disease or following transplantation of bone marrow, heart, liver, lung or other solid organ).
16. Chronic infection managed with long-term antibiotics, frequent courses of antibiotic therapy or self-administration of antibiotic therapy.
17. Personal physician/surgeon is not willing to follow the protocol.
18. Prognosis less than two-year survival or other reason the Clinical Site director believes the patient is not a suitable candidate for N-TA^{3}CT (e.g., history of repeatedly missing follow-up appointments or regular residence outside of the U.S.)
19. Enrollment in another, concurrent clinical trial study.
20. Refusal or inability of patient to provide written informed consent.

4.3 INFORMED CONSENT

Informed consent will be obtained by properly trained (in human subjects' research and N-TA^{3}CT Protocol) clinical site investigators (e.g., vascular surgeons), residents, fellows or

Clinical Site Coordinators. The patient must be competent and freely willing to give informed consent to participate in N-TA^{3}CT. The patient will be told about the purpose of the study, the treatments – doxycycline or placebo – follow-up every three months including CT scan follow-up every six months for two years. In patients who sign informed consent, a progress note will be written that identifies the patient as willing to participate in the trial.

No Clinical Site will begin enrolling patients before its consent form is acceptable to and is on file at the Clinical Coordinating Center and the Data Coordinating Center. The exact language used on a Clinical Site's consent form may vary from institution to institution, but the text must be comprehensible to persons with an 8th grade reading level, and no form will be considered as having been given final approval until it has been reviewed at the Clinical Coordinating Center. Items relevant to the presentation of each consent form are listed below:

1. The purpose of the study is to determine whether doxycycline administration can decrease the rate of growth of small abdominal aortic aneurysms. The effect of doxycycline may be to decrease the activity of an enzyme which weakens the strength of the wall of the aorta. There is a 50-50 chance of getting drug or placebo ("look-alikes").
2. The extent of patient involvement is one out-patient visit every three months for up to two years and if feasible one more visit at two and a half years. Selected (9- , 15- , 21-month) visits may be conducted by telephone if the patient prefers and the clinical site chooses to exercise this option. The patient should not join unless he/she is prepared to continue for two years. No more than three tablespoons of blood (45 ml) will be taken twice a year; over the course of the entire study, no more than 13 tablespoons (195 ml maximum) of blood will be collected. Each clinic visit will take about half an hour, and two times a year a member of the clinical site staff will administer a questionnaire concerning the quality of the patient's life and assure that the patient receives a CT scan for clinical and research follow-up of the aneurysm size.
3. The potential benefit is that aneurysm growth may be reduced. It may take some months for a beneficial effect to be noticed, if a beneficial effect exists. The inherent variability in rate of aneurysm growth may increase or decrease independent of any treatment effect. There is a small risk of aneurysm rupture which is not thought to be altered by treatment.
4. The potential risks of treatment are primarily photo sensitivity, darkening of skin (especially scars) or eyes, headache or G.I. disturbance (nausea, vomiting, diarrhea). Rarely the treatment may interact with medicine the patient is given for other conditions or result in changes to the bacteria naturally in the patient's body that can result in serious infection (e.g., C. difficile colitis), dermatological abnormalities (including skin rash), and liver or kidney dysfunction. There may be other risks, but the drug is not new, and they should be very unlikely to occur.
5. The CT scans required involve radiation exposure which might increase the risk of developing a fatal cancer. Patients could receive a maximum of six CT scans while in the study. The risk increment is small (< 1/1000; for example from a lifetime risk of 23.1% to 23.2% of fatal cancer), and is not entirely due to research because imaging studies are recommended at six-month intervals. Bi-annual imaging is routine. Some doctors use CT scans; others use ultrasound or some combination of the two modalities. The patient should be aware of the small risk associated with the CT scans.
6. There is also a risk of teratogenesis. Women who could become pregnant should not join the study. Natural or surgical menopause is a requirement for eligibility.

7. Compliance is very important if the study is to be a success; blood samples will be collected at the routine appointments and tested to see if the study drug in the capsules is getting into the blood system at the expected levels.
8. Patients will be reimbursed at least $40/visit and additional reimbursement for allowable, documented travel costs (e.g., mileage in excess of that accommodated by $40 for the visit).
9. If patients become ill, because of the abdominal aortic aneurysm or some other illness, the study will not pay for medical care. Patients and their insurance will be responsible for this care.
10. By signing the consent, the patient gives the N-TA^{3}CT investigators permission to get records from any medical facility attended during the study. The study records will be kept confidential; patients will not be identified by name; but, data may be shared with the National Institute on Aging (NIA)/National Institutes of Health (NIH).
11. The alternative to participating in the study is for the patient's medical care to continue as before. Doxycycline may be added to usual care, but because of uncertainty about whether it will work, it is not used routinely to treat aneurysms. Patients may withdraw from the study at any time, without prejudice.
12. By signing the consent, the patient acknowledges that he understands what he/she has been told, and that questions regarding the study have been answered.
13. In accord with local institutional requirements, means for seeking more information about patient protection and redress from injury due to the study, must be spelled out.
14. This protocol has been approved by the local Institutional Review Board (IRB).

4.4 IDENTIFICATION OF POTENTIAL PATIENTS

At the beginning of the study, each lead investigator at a Clinical Site will be personally contacted by the Clinical Site Coordinator. Permission will be sought from each clinical site investigator to recruit patients who have a small, abdominal aortic aneurysm. Each site will maintain a list of clinical colleagues not wishing their patients to participate in the trial. Patients of these surgeons will not be approached for recruitment.

Clinical Site Coordinators and other clinical site staff will identify patients eligible for N-TA^{3}CT by review of CT scan findings and medical records of vascular surgery practices affiliated with their clinical site. Once a potentially eligible patient is identified, the Clinical Site director, Clinical Site coordinator or other clinical site staff will contact the patient, preferably at the time of a return visit for routine abdominal aortic aneurysm follow-up, after having assured that the patient's physician/surgeon is in agreement with approaching the patient.

4.5 PATIENT COMPETENCE TO GIVE INFORMED CONSENT

The individual obtaining informed consent for the patient for N-TA^{3}CT will explain the study to the patient and after providing the explanation ask a brief series of questions about the study (e.g., purpose, treatments, follow-up plans, method to communicate concerns about the conduct of the study or request withdrawal). If the patient answers these questions correctly, the patient will be accepted as competent to give informed consent. The individual obtaining informed consent will keep notes of the patient's answers to the questions on an Evaluation of Competence to Give Informed Consent worksheet kept with the informed consent in the patient's study file. A draft Evaluation of Competence to Give Informed Consent is included in Exhibit 4-1.

4.6 PROCEEDING FROM INFORMED CONSENT TO RANDOMIZATION

Patients who give written informed consent to enroll in N-TA^{3}CT may proceed to random treatment assignment after satisfactory completion of baseline evaluations (including review of pre-randomization CT scan in the Imaging Core Laboratory) to assure eligibility and collection of pre-randomization specimens (blood, plasma and serum) for later shipment to the Biomarkers Core Laboratory.

Exhibit 4-1

NON-INVASIVE TREATMENT OF ABDOMINAL AORTIC ANEURYSM

(Consent Evaluation Form)

Patient Identification Number ___ ___ ___ - ___ ___ ___ Letter Code ___ ___ ___

Name _____

To evaluate a patient's competency to give informed consent, start by making a subjective judgment regarding item 1 below. If a patient is alert and able to communicate answer question 2. If the patient is responsible for consent of medical care, ask the patient questions 3-6. Record each response. The evaluator may select the language to use in asking the questions in order to help the patient understand them.

Items:

1. Is the patient alert and able to communicate with the examiner?
   _____ yes _____ no
   If NO, patient will not be allowed to give consent.

2. Is the patient responsible for consent for his or her own medical care?
   _____ yes _____ no
   If NO, the patient will not be allowed to give consent.
3. Ask the patient to briefly describe the study treatments.
4. Ask the patient to describe the follow-up imaging studies ("pictures of the artery bulge")
5. Ask the patient to explain what he/she would do if he/she decides that he/she no longer wishes to participate in the study.
6. Ask the patient to name a potential risk of participation in the study.

Signatures:

I certify that the above patient is alert, able to communicate and able to give acceptable answers to items 3, 4, 5, and 6 above.

_____ _____

Evaluator Date Witness Date

ACCEPTED RESPONSE TYPES

1. Yes
2. Yes
3. Two treatments, one with a drug (doxycycline) and one with an inactive, look alike (placebo).
4. X-ray pictures (CT scan) every six months.
5. Tell the study nurse, study doctor, personal doctor, or a medical center/hospital official that he/she does not wish to participate.
6. Complications of placebo or doxycycline (skin rash, dizziness, change in eye sight, nausea) or loss of privacy.

CHAPTER 5 – METHOD OF RANDOMIZATION

5.1 ELIGIBILITY ASSESSMENT

At the time an eligible patient provides informed consent, Clinical Site staff will complete all sections of the pre-randomization forms, collect pre-randomization blood, plasma and serum specimens, and submit the qualifying CT scan to the Imaging Core Laboratory by overnight courier. Within two working days of receipt of the CT scan by the Imaging Core Laboratory, a confirmation or denial of patient's eligibility according to CT scan criteria will be sent to the Clinical Site and DCC. If confirmed, the Clinical Site Coordinator must contact the patient's vascular specialist prior to randomization. If the vascular specialist confirms his/her willingness to follow the protocol and the patient meets all eligibility criteria, the Clinical Site Coordinator will complete randomization to obtain the patient's assigned study treatment kit number.

5.2 RANDOM TREATMENT ALLOCATION PROCEDURES

The DCC staff will prepare randomization schedules for each Clinical Site participating in N-TA^{3}CT. The program for generating randomization schedules will have the following characteristics: 1. There will be two randomization strata – one for men and one for women – at each clinical site; 2. Treatments are assigned in random order within blocks sizes two, four, six or eight with equal numbers of patients assigned to the doxycycline or placebo within each block. 3. Block sizes (two to eight patients per block) are randomly selected with the probability of each block size specified by DCC staff.

The DCC staff will maintain a web-based randomization system for Clinical Site staff to request treatment allocations as eligible patients are identified. The randomization system is accessible only to study personnel who enter the password for the Clinical Site and his/her assigned personal identification number (PIN). An individual can request a treatment allocation after he/she has passed training in the use of the system. Questions include specification of gender, confirmation that the patient meets all inclusion criteria and has no exclusion criteria and that the patient has given informed consent for enrollment. Depending on the answers to these items, the next available treatment allocation is issued. The date and time of the completion of the treatment assignment is the time of study entry for each patient.

5.3 CLINICAL SITE RANDOMIZATION START-UP

Each clinical site will be randomizing patients one by one during the course of the clinical trial. To assure that no clinical site randomizes too few patients to be included in data analyses, randomization of the patients into the study at any given site can begin once clinical site staff have supplied the Data Coordinating Center with a list (without direct identifiers) of 16 eligible, pre-screened patients based on clinical practice records.

CHAPTER 6 – STUDY TREATMENTS

6.1 INTRODUCTION

For patients enrolled in N-TA^{3}CT dosage will be fixed at one capsule p.o., b.i.d. (either 100 mg doxycycline or placebo). Capsules should be taken in the morning (e.g., after brushing teeth, with morning coffee) and in the evening (e.g., after dinner). These capsules will be provided to the study patients in individually marked study treatment kits containing supplies of doxycycline or placebo according to the patient's random assignment.

The Treatments Distribution Center (Catalent) coordinator will ship study treatment kits to the Clinical Sites as directed by the Data Coordinating Center. Clinical Sites will acknowledge receipt of medication kits by means of a form sent to the Data Coordinating Center.

6.2 TREATMENT PREPARATION

Doxycycline 100 mg capsules will be over-encapsulated at Catalent. Placebo (Starch 1500) will be packed in identically appearing, over-encapsulated capsules. The capsules will all be the same size, and will contain a white powder. The Treatments Distribution Center will count out a three-month supply of each type of capsule in numbered kits, bottle them in child-proof containers, and label them with a unique, study assigned number and instructions including the "Investigational Drug" warning, "Non-invasive Treatment of Abdominal Aortic Aneurysm Clinical Trial", instructions on how to take the capsules, and an "emergency call" telephone number. Study treatments will be shipped in three-month batches, in sufficient time to assure that the Clinical Sites will receive the bottles before the patient's next scheduled visit. Inventory records for drug and placebo will be kept by Treatments Distribution Center staff.

6.3 BLINDING

In the Clinical Sites, Clinical Coordinating Center, Imaging Core Laboratory and Biomarkers Core Laboratory, the patients, directors (i.e., Clinical Site lead investigator), coordinators, and other study staff will be blinded to treatment assignments. Staff of the Treatments Distribution Center and Data Coordinating Center will have access to individual patient treatment assignment on a "need-to-know" basis. The Treatments Distribution Center and Data Coordinating Center will maintain records of each patient's treatment assignment.

Plans have been made to prevent unblinding of patient treatments. Despite these precautions, if the Clinical Site director thinks he/she inadvertently has become unblinded, patient contact must be carefully managed to avoid any comment to the patient or coordinator regarding unblinding.

The Clinical Site directors will assert at the outset the intention to avoid seeking information that may unblind them with regard to individual patient's treatment assignments. Clinical Site coordinators will conduct patient follow-up visits and process and maintain files of study documents blind to treatment assignment. Discussions among Clinical Site staff or with patients regarding possible patient treatment assignment are inappropriate. As long as official unblinding has not been done and the patient notified, the Clinical Site coordinator must avoid seeking any information that may unblind him/her.

6.4 EMERGENCY UNBLINDING

Every patient will be given an identification card describing his participation in the study, listing emergency study telephone numbers (e.g., the Clinical Coordinating Center telephone number, Treatments Distribution Center telephone number, the Clinical Site director's telephone number, and the Data Coordinating Center telephone number).

In an emergency, arrangements will be made so that the patient's medication can be disclosed to the Clinical Site director (or a party designated to share this responsibility) after consultation between the Clinical Site director and a Clinical Coordinating Center or other N-TA^{3}CT leadership physician (one of whom will always be available). Reasons for unblinding are limited, are based on clinical grounds, and unblinding must be initiated by the Clinical Site director. Reasons for unblinding include accidental ingestion of study medications by another person and clinical conditions in which management might be changed if the nature of the study drug were known.

If a patient's therapy is unblinded, the Clinical Coordinating Center must send a report on the reasons for unblinding to the Data Coordinating Center. The nature of the patient's medication will be withheld from the Clinical Site coordinator.

6.5 TREATMENT INTERRUPTIONS

There may be instances of treatment interruption either related to medical conditions (e.g., acute, intercurrent illnesses such as an infection when it may be advisable to interrupt study therapy without unblinding) or for other reasons (e.g., study treatments lost in a robbery). Non-emergent interruptions for medical conditions should be allowed only with the advice of the Clinical Site director and not at the discretion of local medical doctors. The Clinical Site director is responsible for notifying the Data Coordinating Center of treatment interruptions.

6.6 ASSESSMENT OF COMPLIANCE

At approximate six-month intervals each patient's plasma samples will be assayed for doxycycline at the Biomarkers Core Laboratory.

Capsule counts will be done at each regular follow-up visit. If capsule counts are not consistent with regular compliance, Clinical Site staff will discuss the nature of any difficulties with the patient. The importance of compliance will be emphasized for all patients.

Even if patients are repeatedly considered to be non-compliant, they will continue to be followed and will continue to receive study reimbursements (e.g., for travel and parking).

6.7 MISSED VISITS AND DROP OUTS

Each regularly scheduled clinic visit missed by a patient will be reported to the Data Coordinating Center. Patients who do not wish to continue attending clinic visits in N-TA^{3}CT will continue to be followed as much as possible for CT scan evaluations, identifiable events and vital status.

6.8 DURATION OF STUDY TREATMENT

The goal of the study will be to maintain all patients on protocol for two years.

CHAPTER 7 – CONCOMITANT CARE

7.1 INTRODUCTION

Except for study treatments and CT scan monitoring, care of study patients will be determined by their personal (referring) physicians. Personal physicians will be blind to study treatment and will be advised to select concomitant care to be appropriate whether the patient is receiving doxycycline or placebo. Cooperation of treating physicians and house officers or hospitalists will be solicited as much as possible to enhance adherence to study protocol at the same time as assuring best practices and standard of care treatment for N-TA^{3}CT patients. Patients will be issued study identification cards requesting treating physicians and house officers/hospitalists to contact the patient's N-TA^{3}CT Clinical Site director with questions concerning N-TA^{3}CT patient treatment.

7.2 HOSPITAL ADMISSIONS

During hospital admissions for any cause, the patient's care must be directed to address the patient's main, acute medical condition. If a patient is admitted to a hospital, study drug is to be continued if possible. If a patient forgets to bring medication with him when admitted, a family member or friend should bring it to the hospital as soon as possible. Exact arrangements used to dispense a study drug to an inpatient may differ between Clinical Sites; such arrangements must be explored before recruitment begins, and the Clinical Coordinating Center is available for assistance in such administrative matters. For Clinical Sites which may have patients admitted to one of several hospitals, such arrangements should be made, in advance, for all likely possibilities.

7.3 BLOOD PRESSURE AND LIPID CONTROL

7.3.1 Anti-Hypertensives

At the time or randomization, N-TA^{3}CT patients must have normal blood pressure or be undertaking reasonable treatment for Stage I hypertension (e.g., on one or more appropriate anti-hypertensive agents with BP ≤ 160/100 mm Hg). Because of the prevalence of high blood pressure among patients who have abdominal aortic aneurysms, it is important that blood pressure eligibility criteria not exclude patients who would receive doxycycline if it proved efficacious. All patients must receive appropriate standard of care therapy. Stage II hypertension is not considered to be adequately managed for participation in this clinical trial. Patients with Stage II hypertension can be evaluated for enrollment if their blood pressures can be brought under control. Personal physicians will be encouraged to maintain adequate control of their patients' blood pressures, adding or changing medication to restore control when blood pressures become elevated, or undesirable side effects appear, in accordance with the reports of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure. Letters reporting blood pressure findings will be sent to personal physicians for every patient who is found to have high blood pressure (BP > 120/80) on study follow-up visits for use in patient management. Control of anti-hypertensive therapy, as other clinically indicated therapies, will remain with the patient's personal physician.

7.3.2 HMG-CoA Reductase Inhibitors ("Statins") and Other Lipid Lowering Agents

The American Heart Association and American College of Cardiology recommend that abdominal aortic aneurysm be considered equivalent to atherosclerotic cardiovascular disease (ASCVD) based upon historical studies showing that cardiac mortality is not an uncommon cause of death in abdominal aortic aneurysm patients. In order to achieve lipid level treatment goals, the preferred treatment for LDL-cholesterol reduction being HMG-CoA reductase inhibitors ("statins"). Personal physicians will be encouraged to establish adequate control of their patients' lipid levels making use of prescription for diet, exercise and lipid-lowering agents (statins if well tolerated or other agents, such as niacin, if statins are not well tolerated) as appropriate for their patients, prior to randomization. Clinical Site directors will encourage study patients' personal physicians to maintain adequate control of their patients' lipid levels, adding or changing lipid-lowering medication(s) only to restore control when lipid levels become elevated or undesirable side effects appear, in accordance with National Cholesterol Education Program guidelines.

7.4 TREATMENTS WHICH MAY INTERACT WITH DOXYCYCLINE

7.4.1 Anti-Infectives

Personal physicians will be encouraged to select anti-infectives, should they become necessary for patient management (e.g., to treat an intervening pneumonia or urinary tract infection) as if the patient had been taking doxycycline – i.e., to select bacteriocidal non-tetracycline antibiotics as much as possible.

7.4.2 Wafarin

Personal physicians will be advised that if they plan to initiate warfarin therapy for study patients there is a possibility that the study treatment (which is being maintained at a fixed dose) may influence the dose of warfarin used to achieve desired international normalized ratios (INRs). Thus, INR levels should be monitored closely until the personal physician has established that a stable therapeutic dose of warfarin has been achieved.

7.5 SMOKING AND OTHER TOBACCO USE CESSATION

Clinical Site directors will advise study patients' physicians that avoidance of tobacco use is an important part of management of abdominal aortic aneurysms and encourage the personal physicians' efforts to achieve smoking and other tobacco use cessation in their patients.

7.6 SURGERY

7.6.1 Introduction

With the exception of retroperitoneal procedures in the central body area, there are no study-specific recommendations concerning the performance of surgery.

7.6.2 Retroperitoneal Surgery

Because of the potential risk of aneurysm rupture consequent to retroperitoneal surgical procedures, the attending vascular interventionalist may elect to repair the aneurysm before retroperitoneal surgery using an endograft or at the time of the retroperitoneal procedure.

CHAPTER 8 – FOLLOW-UP PROCEDURES

8.1 INTRODUCTION

Patients will be scheduled for follow-up visits every three months. At the time of each scheduled visit, a medical review is conducted including ascertainment of clinical outcomes, study treatment compliance and adverse effects.

At semi-annual follow-up visits, outcome CT scans will be collected, specimens for Biomarkers Core Laboratory assay and storage will be collected, and study forms evaluating medical review and quality of life will be administered. At annual visits blood specimens will be collected for local evaluation of CBC, liver function and renal function. The results will be included on study forms.

8.2 FOLLOW-UP VISITS

At the three-month follow-up visits, a medical review will be conducted including ascertainment of possible adverse effects, current therapies, and collection of the previous study medication and capsule count. The prescribed study medication for the following three months is dispensed.

In addition to all three-month follow-up visit procedures, at the six-month visits and at the final follow-up assessment the patient will complete an SF-36 for an assessment of health-related quality of life, plasma and serum will be collected and prepared for shipment to the Biomarkers Core Laboratory, and a CT scan of the abdomen will be performed, anonymized and sent to the Imaging Core Laboratory.

When a patient requests that a follow-up be conducted by telephone the following applies.

<u>Telephone Follow-up Visits</u>

At the discretion of clinical site investigators and coordinators, patients who cannot without undue burden travel to the N-TA^{3}CT clinical sites may conduct visits which do not coincide with an N-TA^{3}CT required CT scan or specimen collection with a study team member via telephone. The only exception to this approach would be the three-month visit for which a clinic visit is mandatory for thorough assessment of subject's tolerance and compliance with study treatment. Telephone contact will include:

1. Identify yourself and state that you are recording form data portions of the conversation for quality control purposes.
2. Verification of subject identity.
3. Review of all required data fields on Subject Follow up form.
4. Review of all current medications.

5. Assessment of subject's compliance with taking medication by self-report.
6. Review of proper dosing, side effects, documentation of missed doses on study diary.
7. Instruct subject new study drug will be sent via mail or overnight courier. Upon receipt of new study drug subject is to stop taking medication from existing supply and begin taking from new bottle. Encourage subject to tape old bottle closed and to set aside. Remind subject not to destroy any study bottles or the box in which they came. All will need to be returned at time of next clinic visit. It is recommended similar instructions be included with the shipment of new drug.
8. Recording of the telephone contact with a device approved by the study leadership (e.g., Olympus VN-3100PC).

Completion of Case Report Forms:

1. Follow Up form: Complete with information obtained from telephone call.
2. Vital Signs: Mark as not done.
3. Concomitant Medications: Complete with information obtained from telephone call.
4. Drug Adherence: Complete Item #4 with information provided by subject. The remainder of the form is to be completed at next clinic visit when study bottles are returned.
5. Drug Dispensing: Complete at time new study treatment kit is assigned and dispensed. Do not record date sent to subject or date subject receives. Keep tracking record for pick-up and delivery of study treatment kit with source documents.

8.2.1 CT Scan Acquisition at Enrollment and Follow-up

The standardized acquisition protocol for computed tomography (CT) examination of the abdominal aorta and iliac arteries in N-TA^{3}CT is as follows for multidetector, helical CT:

1) Narrow Field-of-View centered on aorta, < 36 cm encouraged.
2) Tube current and energy to be set to best clinical standards for image quality.
3) Obtain CT scout without contrast.
4) Scan from top of diaphragm to symphysis pubis.
5) Section thickness less than or equal to 2.5 mm.
6) Reconstruction overlap minimum 50%, i.e., 1.25 mm intervals, 2.5 mm thickness.

If requested by ordering physician (suggested but not required by study protocol), infuse nonionic contrast, 300 mg iodine per/ml, total volume of 150 ml through arm vein at a rate of 3 ml per second. Delay individualized with test dose program. Each participating clinical site may have different CT contrast dosage protocols and CT scanner automatic exposure control systems. These individual measures can be taken to reduce radiation exposure and contrast dosage providing there is preservation of diagnostic-quality images. Coach breath holding instructions. Scan 2.5 mm section thickness from top of diaphragm to symphysis pubis (about 300 mm).

Specific performance standards for different equipment (e.g., Siemens 64 slice CT Scanner) will be provided in the Imaging Core Laboratory's Procedures Manual.

CT Scans should be transferred from clinical sites to the Imaging Core Laboratory in DICOM format on CD. Each CD should contain one study, on one patient only and be sent to the Imaging Core Laboratory by traceable Federal Express (FedEx) or United Parcel Service (UPS) courier.

The scan recorded in the CD should be de-identified (e.g., no patient name, address, medical record number or birth date appears on the headers or images) so that only the study assigned patient ID number (PID) and Alphabetic Code (Letcode) appears with the images. The PID/Letcode and specific visit (baseline, 6-month, 12-month, 18-month, etc.) should be in the electronic header and recorded manually (e.g., with a label provided by the Imaging Core

Laboratory) on the non-recording side of the CD. The study to be recorded on the scan should be the full dataset of axial series, without contrast required and with contrast encouraged for best practices but not required because contrast may be contraindicated in particular patients for clinical reasons. Only physicians and surgeons at the clinical site are able to judge whether a contrast study is acceptable for any particular patient.

8.2.2 Biomarker Blood Specimen Collection at Enrollment and Follow-up

Clinical sites must develop a brief but specific plan for the procurement, initial processing and temporary storage of peripheral blood samples from all enrolled patients during the study. Each site will ship specimens to the Biomarkers Core Laboratory. Blood will be collected in plasma collection tubes which contain lithium heparin and serum collection tubes. Samples will be processed into sterile 0.25 ml aliquots of serum, plasma with timely transfer of specimen on ice between collection and processing areas, if necessary. Short-term storage of the sample aliquots in -70 C freezers (or colder) is required at the clinical sites. Shippers of the materials must have taken the appropriate training for the shipment of DOT/IATA Biological Substances Category B and Dry Ice. The Biomarkers Core Laboratory will provide shipping labels and labels for each aliquot to the clinical sites. Batch shipment of the specimens packed in dry ice will be made at three-month intervals. The specimens will be maintained frozen at the Biomarkers Core Laboratory, and some aliquots analyzed in batches per this protocol and the remainder will be stored for future studies.

Subjects enrolled in the study may, optionally, participate in the banking of genetic information at the Biomarkers Core Laboratory of the study. Each enrolled subject will be offered this opportunity. After a detailed discussion of all of the risks, including the unique privacy risks of genetic material, subjects may provide consent for the collection and storage of their genetic information. At one of the study visits, the subject will have whole blood drawn (approximately 10 ml) which will be frozen as described above for transport to the Biomarkers Core Laboratory. The samples will be processed for DNA at the Biomarkers Core Laboratory, and this material will be placed into aliquots. There are no specific studies planned to evaluate the genetic information in this protocol, however this material will be maintained with the plasma and serum samples at the Biomarkers Core Laboratory and will be made available for future studies.

8.2.3 Ascertainment of Adverse Events

All medical contacts indicating possible serious or unexpected adverse events (SAEs), whether treated on an out-patient or in-patient basis, will be reviewed by the Management Committee. These SAEs will be classified by an independent committee of vascular surgeons who are in institutions that are not in the N-TA^{3}CT clinical consortium, blind to study treatment, the N-TA^{3}CT Event Classification Committee. Their classifications of aneurysm rupture, repair of aneurysm indicated by impending rupture and repair of aneurysm for reasons of size as well as other less severe indication will be used in the primary outcome analysis. SAEs will be reported to the Data and Safety Monitoring Board.

8.3 PATIENT MANAGEMENT DURING MEDICAL CONTACTS

Each patient will be given an identification card to be carried at all times and to be presented at any medical contact, stating that he/she is enrolled in the N-TA^{3}CT, that his/her medication must be continued if at all possible, that endograft devices etc. should be deployed only in accordance with N-TA^{3}CT protocol, that N-TA^{3}CT will require records of medical contacts, and that the N-TA^{3}CT Clinical Site director and Clinical Coordinating Center are to be contacted in case of need to know the study treatment assignment.

Medical staff at N-TA^{3}CT Clinical Sites should be aware of study requirements for the management of patients presenting with abdominal aortic aneurysm or its complications, and the requirements of documenting all medical contacts. The patient should be maintained on study medication if hospitalized. N-TA^{3}CT Clinical Site directors will avoid seeking information which may unblind them with respect to the N-TA^{3}CT treatment assignment.

8.4 SECONDARY ASSESSMENTS

8.4.1 Psychosocial Evaluation

Data from the Medical Outcomes Study Form SF-36 will be used in secondary analyses. Questionnaires will be administered prior to enrollment and every six months during the study and at the close-out visit.

8.5 RETENTION

Maintaining participation post-randomization is especially relevant in studies with older adults who may be lost to follow-up due to changes in living situation, acute illness, cognitive changes, or death. At the time of enrollment, the participant will be asked to provide contact information (names, addresses, phone numbers) for two people that know them well and who would likely know the whereabouts of the participant if s/he could not be contacted for a follow-up assessment.

8.5.1 Retention Promotion Efforts

At the time of randomization, participants will receive clear, easy-to-follow, written instructions about the schedule of follow-up assessments. Reviewing these instructions with the participant prior to departure will be a priority. If demonstrated compliance problems exist, study staff will review the importance of the visits and the remainder of the study schedule. Involving the participant's spouse or other family members in these reviews can be useful. Attempts wil be made to maintain continuity of follow-up care, so that, whenever possible, the same staff member contacts/sees the participant throughout the study. Every attempt will be made to make all contact with the participant pleasant. Minimizing waiting time, reimbursement for transportation costs and comfortable waiting room facilities makes the visit more pleasant, thereby enhancing participant retention at follow-up assessments.

Clinical sites will be advised to keep detailed records of rescheduled or broken study assessment appointments for each participant. Participant retention will be monitored, and efforts will be made to identify those participants who need support and encouragement. Summary reports of such difficulties help to identify problems. Critical review of such problems may offer potential solutions.

The following procedures will be implemented to prevent missed follow-up:

- Sending out pre-visit reminders (e.g., postcards and phone calls) for upcoming assessment visits.
- Participants will be called the night before a follow-up assessment to remind them of the visit and the time for transportation pick-up, if appropriate.
- Clinical sites will track attendance so that staff will be immediately alerted to a missed intervention or assessment visit.
- Immediately contacting participants (or significant others) when they miss a visit.
- Rescheduling the visit within the same window, if possible. Follow-up assessments that fall outside of the target window remain important and will be used in all analyses. Whenever an intervention visit or follow-up assessment is missed, it will be recorded as such on the appropriate form with a corresponding reason. This will also be documented in the Tracking Database.

Some randomized participants may not actively participate. Regardless of the reason(s), these participants will be followed until the end of the study, and study staff attempts to make contact every four weeks after randomization. These contacts are intended to remind the participant that they are welcome to rejoin the study at any time. Considerable effort will be expended to collect main outcome data at each of the follow-up assessments.

The following strategies will be used to promote adherence to the protocol, in terms of intervention adherence and follow-up assessment attendance.

- Participant-staff relationship A key element contributing to participants' continued commitment to the trial will involve fostering positive, respectful relationships between study participants and individual members of the staff.
- Continuity The number of study staff making contact with a participant will be controlled. In general, the same study staff contacting participants to schedule the baseline and follow-up assessments will also conduct the study visits. This will ensure consistency of study staff contact across participants.
- Clinic Environment A clinic environment that is warm and pleasant and oriented to the comfort of the participant will be maintained.
- Participant-staff communications Good and consistent communication will be essential. Instructions will be clear and interactions will be friendly and individualized. The participant will be reminded of the benefits of study participation.
- Convenience and accessibility An easily accessible location at the clinical sites, availability of transportation, and convenient hours all serve to facilitate study adherence. Clinical sites will take steps to ensure that follow-up assessment attendance is not compromised by lack of transportation, unsuitable hours of operation, or any similar circumstance. If necessary, transportation will be provided for participants or reimbursed for parking.
- Time in clinic will be kept to a minimum, consistent with maintaining quality. If waiting is necessary, the situation will be explained to the participant. However, participants will not be rushed or made to feel unwelcome. Clinical site staff will be trained to take time to visit with participants. Clinical sites may provide juice and snacks during the study assessment visits.
- Reminder phone calls will be placed the night before a visit to enhance attendance for assessment.

8.5.2 Monitoring Recruitment and Retention

Retention will be monitored through completion rates of follow-up assessment visits. Regular reports will be available to clinical sites and the Clinical Coordinating Center. Members of the Clinical Coordinating Center maintain regular phone contact with clinic staff to review retention.

8.6 FINAL FOLLOW-UP ASSESSMENT AND CLOSE-OUT

Patients who complete a two-year follow-up visit after March 29, 2016, and before May 1, 2018, will discontinue study medication at that time. For patients who have completed at least 24 months of therapy and had a two-year follow-up visit before the time of approval of Protocol Version 1.6.0, the patient will cease medication on the date of the next scheduled visit. There will be a final follow-up assessment in the 30-month window for those who completed two years of follow-up before May 1, 2018; those who complete drug treatment after 27 months will complete a final follow-up assessment between 3 and 6 months after discontinuation of therapy. No study visits are necessary between therapy discontinuation and final follow-up assessment.

Patients randomized after May 1, 2016, but before October 29, 2016, will have their 24-month visits completed on or before July 31, 2018, and, in lieu of their 30-month follow-up, final assessment visits approximately 30 days after their 24-month visits to check for any adverse events after discontinuation of study drug. This final assessment may be done by telephone. Patients randomized after October 29, 2016, will have their final on-study visit at their 18-month follow-up, to be scheduled no later than July 31, 2018. They will discontinue study drug at this visit. Their final assessment contact will be conducted approximately 30 days after their 18-month visits to check for any adverse events after discontinuation of study drug. Their primary outcome will be imputed as described in Section 11.3. To summarize, study treatment, collection of aneurysm measurements and biomarkers will be completed within the follow-up windows for July 31, 2018; final assessments for safety will be completed no later than September 1, 2018.

After the conclusion of study follow-up for all patients, a close-out visit will be offered to each patient. When notified of study close-out, each site should arrange close-out visits for enrolled patients within a relatively short period of time. Every effort will be made to schedule enrolled patients for the close-out visit. Vital status will be ascertained on patients lost to follow-up. At the close-out visit, patients will be informed of the primary results of the study and the recommendations of the N-TA^{3}CT investigators. They will be informed of their individual study treatment assignments.

CHAPTER 9 – STUDY END POINTS

9.1 INTRODUCTION

The primary objective of the Non-Invasive Treatment of Abdominal Aortic Aneurysm Clinical Trial (N-TA^{3}CT) is to determine the safety and efficacy of doxycycline (100 mg p.o. b.i.d.) in the prevention of growth of small abdominal aortic aneurysms. The primary analysis in this study will compare the change from baseline in size of abdominal aortic aneurysms at two years from randomization, using a normal-score transformation, between patients randomly assigned to doxycycline and those assigned to placebo with adjustment (co-variance) for size of aneurysm at study entry.

Follow-up CT scans will be obtained every six months regardless of compliance with assignment study treatment. The analysis of the primary study outcome will be conducted on an "intention to treat" basis with two-sided statistical tests comparing the outcome between groups of patients defined at study entry by random assignment to the doxycycline or placebo groups.

9.2 PRIMARY END POINT

For the purpose of defining the primary study outcome, the size of the abdominal aortic aneurysm will be defined as the maximum transverse diameter (adventitia to adventitia) measured on a quantitative CT-scan in the orthogonal plane. The measurements to be used in the primary end point assessment will be taken from CT scans obtained at baseline (study entry) and two years after random treatment assignment. A normal score will be assigned to each baseline and two-year follow-up measurement for analysis. If a two-year follow-up measurement is not available, the primary outcome will be defined as described in the following section.

9.3 STATISTICAL ANALYSIS OF THE PRIMARY END POINT

An attempt will be made to measure for each patient the maximum transverse diameter of the patient's abdominal aortic aneurysm two years after random treatment assignment. It is anticipated that during two years of follow-up a small percentage of the patients may die or be operated on for repair (endovascular or open) due to growth of the aneurysm beyond clinically tolerable limits (5.5 cm for men and 5.0 cm for women) or be operated on for symptoms or signs

including pain or signs of rupture or in less urgent circumstances. These patients' two-year aneurysm diameters will be missing for cause and will be accommodated in the primary outcome analysis by classification (by the N-TA^{3}CT Event Classification Committee) and rank order methods.

A committee of vascular interventionalists not active in study clinical sites and blind to treatment assignments will review relevant medical records for all deaths and abdominal aortic aneurysm repairs to classify outcomes as related or not related to rupture of aneurysm.

Patients' outcomes will be ranked at two years following randomization as follows: patients who die will be ranked worst, in order of number of days from randomization to death; patients who experience rupture of the abdominal aortic aneurysm will receive the next worst ranks, in order of number of days from randomization; patients who undergo open or endovascular repair without evidence of rupture will receive the next worst ranks, in order of days from randomization to repair; patients with follow-up CT scans will be given the remaining ranks from worst (largest diameter) to best (smallest diameter).

The primary analysis will follow the intention-to-treat principle; that is, all randomized patients will be included, assigned to treatment according to the treatment received. In the event a patient does not complete two years of CT scan follow-up for cause(s) other than death, open repair or endovascular repair (e.g., withdrawal of consent or loss to follow-up, expected to be rare events), the patient's previous CT scans and other characteristics will be used for the imputation of a measurement of the abdominal aortic aneurysm diameter to be included in the primary analysis.[93] The ranks for patients assigned to doxycycline will be compared to the ranks of patients assigned to placebo using normal scores.[94] If N is the total number of randomized patients, a rank of k will be assigned the value corresponding to k/(N+1) in a standard normal distribution; for example, a rank of 100 out of 248 patients will receive the normal score corresponding to 0.4016, or -0.2492. The change in normal score from baseline -- i.e., the two-year normal score for patients randomized to doxycycline and placebo minus the baseline normal score -- will be compared using a linear regression model (analysis of covariance -- ANCOVA), with variables for the randomized treatment assignment (0 for placebo, 1 for doxycycline), the normal score for baseline diameter, and gender in the model.

9.4 SECONDARY AND INTERMEDIATE OUTCOMES

Secondary and intermediate outcomes will include a variety of clinical findings and quality of life assessments, Biomarker Core Laboratory findings and Imaging Core Laboratory findings.

9.4.1 Clinical Secondary and Intermediate Outcomes

The clinical components of the primary outcome, death, open repair and closed repairs will be ascertained and compared between the treatment groups. Other clinical outcomes that will be compared between the treatment groups will include myocardial infarction, cerebrovascular accident (stroke), peripheral arterial thromboembolic events, venous thromboembolic events, and infections (urinary tract infections, pneumonia, gastrointestinal infection, etc.).

The frequency of adverse effects of doxycycline (e.g., clinical presentations such as photosensitivity rash, gastrointestinal symptoms and clinical biochemistry laboratory findings such as abnormalities of liver function tests or renal function tests) will be ascertained at each clinic visit and compared between treatment groups.

Health-related Quality of Life will be assessed every six months with the Medical Outcomes Study (MOS) Short Form-36 (SF-36) and compared between treatment groups and according to patient characteristics.

9.4.2 Biomarker Secondary and Intermediate Outcomes

Blood specimens collected for analysis in the Biomarker Core Laboratory will be used to assess circulating levels of MMP-9, IFN-γ and high sensitivity (hs) C-reactive protein (CRP) with comparisons between the treatment groups and according to baseline characteristics as well as cotinine and doxycycline levels for assessment of their relationship to aneurysm growth. Doxycycline levels will also be used to evaluate protocol adherence. Additional specimen analyses may be planned in ancillary studies with independent support.

9.4.3 CT-Scan Secondary and Intermediate Outcomes

CT Scans collected every six months will be analyzed in the Imaging Core Laboratory to make comparisons between the treatment groups of characteristics of abdominal aortic aneurysm dimensions assessed after randomization, other than the primary outcome, with adjustment for baseline characteristics. Secondary outcomes measured on two-year CT Scans and on other study CT Scans will include aneurysm volume, characteristics of the aneurysm neck, and wall stress.

CHAPTER 10 – DATA MANAGEMENT

10.1 INTRODUCTION

N-TA^{3}CT data management occurs in two phases – pre-randomization and post-randomization. The purpose of data management procedures is to establish in as timely a manner as feasible (as close to "real time" as practical) the set of information necessary to assess the status of every patient with regard to treatment assignment (randomization); treatment supply; follow-up visit completion; blood specimen collection, processing, shipment, receipt, analysis and storage; CT scan collection, processing, shipment, receipt, analysis and storage; and, clinical observations and inquiries. This timely and accurate set of information is necessary for monitoring study performance and progress and well as to perform data analyses for the assessment of study findings.

10.2 FORM DATA

The form data will include information collected pre-randomization to establish patient eligibility, assign a study treatment and assign a numeric identification (PID) and alphabetic (Letcode) that will be used to track the patient's study treatments, follow-up visits, clinical data, CT Scans and blood specimens throughout the study. Form data will include a record of all clinical information, whether or not blood specimens were collected (and for collected specimens their label numbers), and whether or not CT Scans were performed for each follow-up visit. Clinical Site staff will store all source documents in locked file cabinets.

Clinical data will be entered by the site staff using a secure, Web-based 21CRF11-compliant electronic data capture system (eDC). Data Coordinating Center (DCC) staff will train and test proposed clinical site staff in the basic conduct of N-TA^{3}CT and in the use of the Web systems. Each clinical site staff member who successfully completes training and passes the DCC certification test will be accorded a unique Staff ID for access to the website. The staff members will be responsible for selecting their passwords adhering to requirements for password strength. Clinical site staff may use any computer (lap-top or desktop) allowed by their medical centers for entry of study data. They have permission for entry of their patients' data based on their Staff ID. Data Coordinating Center staff will train clinical site staff to keep their passwords confidential. Passwords must be changed on a regular schedule to enhance confidentiality. The

important point for scientific integrity is that clinical site staff understand that they have responsibility for data entered under their Staff ID passwords.

Clinical site staff must maintain a hard copy of each study form entered as a source document, on site. They may complete forms manually and then key in the data, or may enter data on the website and then print out the form entered, check the print out for accuracy and then sign the printed form.

The eDC system will be programmed with extensive data edits including range and value checks, checks to make sure that all required fields are completed and logical consistency both within form page and across pages. Data edits will be responded to, tracked and closed within the eDC system. Further data edits based on monitoring or hand review of the data will be entered into the eDC system by DCC and CCC staff and similarly responded to, tracked and closed.

10.3 CT SCANS

As described in Chapter 8, clinical site staff will transmit CT scans in DICOM format on CD's to the Imaging Core Laboratory.

Imaging Core Laboratory staff will assess the abdominal aortic aneurysm measurements in duplicate (one reader, two readings) and create a database of each patient's CT scan measurements. This database will include dates of CT scan receipt, initial review (if any) and definitive evaluation.

Upon receipt of a pre-randomization CT scan from a clinical site, the Imaging Core Laboratory will notify the clinical site (with copy to the Data Coordinating Center) whether the abdominal aortic aneurysm meets eligibility criteria for N-TA^{3}CT (e.g., no renal artery or iliac artery involvement, maximum transverse diameter greater than 3.5 cm and less than 4.5 cm for women or 3.5 cm and 5.0 cm for men) within two working days of receipt.

The Imaging Core Laboratory will enter pre-randomization screening data, quality assessment data and CT scan measurements from both the qualifying CT scan and follow-up scans directly into a secure Web application linked with the randomization application. Measurement and quality assessment data will be merged with forms data as needed for reporting.

10.4 BIOMARKER SPECIMENS

As described in Chapter 8, clinical site staff will ship frozen blood, plasma and serum specimens to the Biomarkers Core Laboratory.

Biomarkers Core Laboratory staff will inventory specimens according to unique label numbers on each vial received. The vials will be numbered uniquely with freezer proof labels designed by the Biomarkers Core Laboratory where there will be no other patient-specific information in the course of the study. Specimen label number and PID/Letcode will be linkable through the Data Coordinating Center or Clinical Site of origin, only. A system of replicate labeling and processing will be used for blinded replicate quality control.

Biomarker Core Laboratory staff will assay specimens for doxycycline, cotinine, MMP-9, IFN-γ and hs-CRP on the direction of the Data Coordinating Center and create a data file of doxycycline, MMP-9, IFN-γ, hs-CRP levels for each label number analyzed. At three-month intervals, on days negotiated by the Data Coordinating Center and the Biomarkers Core Laboratory, Biomarker Core Laboratory staff will transfer doxycycline, MMP-9, IFN-γ, hs-CRP and cotinine data according to label number to the Data Coordinating Center for integration into the main study database, monitoring of specimen collection, processing, shipment and evaluation and reporting of interim results to the Data and Safety Monitoring Board.

10.5 QUALITY CONTROL

The quality of the proposed trial is dependent upon 1) achieving the projected recruitment and randomization rates, 2) the complete and accurate collection of data, and 3) adherence to the protocol. The investigators recognize that effective quality control procedures are of particular importance in a multi-center study. Therefore, we have developed quality control procedures with significant central oversight and designed a governance structure specifically to support community-based sites.

10.5.1 Training of Study Personnel

The Clinical Site directors and Coordinators are certified in procedures of key importance to the study prior to initiation of recruitment. Initial training for certification is at the first of the annual collaborator's meetings and on site initiation visits. The initial one-day coordinator's meeting will include a thorough review of recruitment procedures; obtaining informed consent; randomization; protocol adherence; and data collection and reporting procedures including use of the data entry system. Training methods include a presentation on the Procedures Manual; question and answer sessions; and hands-on experience using the data collection forms, the randomization system and the data entry system. Participants will be provided with sample charts and asked to complete data entry forms. Recommended procedures include reviewing completed forms for missing information and checking for consistency of PID and Letcode between forms. In addition, we will describe the pilot and related study experiences with specific reference to identified problems and solutions, effective mechanisms for maximizing patient recruitment, and means of insuring protocol adherence.

Certification requires passing an examination on the protocol and demonstrated proficiency in required tasks (e.g., completing study forms, using the randomization and data entry systems.) The DCC staff will work with CCC staff to prepare specific certification procedures. Recertification occurs in association with site visits and is based on the level of proficiency demonstrated in the on-going performance of study activities (e.g., a form failing edit rate less than 10%), and demonstrated expertise. Associate Clinical Site coordinators will be trained by the Clinical Site coordinator and Clinical Site director using materials developed by the CCC and DCC. Small secondary training sessions will be held at the CCC or DCC for Clinical Site staff in need of assistance to improve performance, who are from Clinical Sites joining the consortium after the training meeting or who are replacing previously trained staff under circumstances that do not permit adequate training at the Clinical Site alone (i.e., abrupt departure of certified staff).

Each Clinical Site will be visited annually for the purpose of assessing quality and providing additional training. The site visit team will include a representative of the DCC, ICL, and CCC. During these site visits, we will provide additional review of the study protocol and procedures. We will also review any problems identified by the DCC quality monitoring procedures and/or during the site visit and recommend possible remedial actions. In addition, the Clinical Site Coordinators will participate in conference calls with the CCC Lead Coordinator every month.

Collaborators' meetings will be held annually for all Clinical Site directors and coordinators. These meetings will include a review of study procedures, discussion of any problems and solutions, and an update on the status of the trial. It will also provide a forum for open discussion among the collaborators and generate continued enthusiasm for participation in the trial.

10.6 QUALITY MONITORING

10.6.1 Data Collection

The DCC has primary responsibility for monitoring data collection, providing protocol adherence review, and initial editing of the reported data. All forms entered via the data entry system will be evaluated by a central edit program designed to detect out-of-range items, inconsistent sets of items and combinations of items that are incomplete. Results of the edit will be included in the DSMB Reports.

In addition to these standard editing procedures, source documents of the first two randomized patients after the start of the study (or at the time the Clinical Site Coordinator changes) from each site and a random sample of 10% of patient source documents will be carefully reviewed by the CCC Lead Coordinator to insure the accuracy of the data entry process. The DCC will generate a list of the study identification numbers of the patients whose documents will be reviewed. This will be sent to the Clinical Site coordinator at the Clinical Site who will then copy the documents and forward to the CCC. The DCC will also forward the list of identification numbers along with a computer printout of the data items to the CCC. CCC staff will compare the data entered with the data in the source documents and record whether or not each data point was verified. This review will be designed to identify problems in recording data, data entry, data processing, as well as protocol adherence.

10.6.2 Recruitment and Randomization Rates

The DCC will carefully monitor recruitment, minority representation, and randomization rates, and reports will be prepared and distributed weekly for N-TA^{3}CT leadership (Management Committee) conference calls and will be sent in monthly reports to each site.

If recruitment is low for a site, we will review with the Clinical Site coordinator and Clinical Site director how recruitment is organized and implemented. If the CCC or DCC staff are unable to solve the problem, then study leadership will seek assistance from Clinical Site coordinators with excellent recruitment rates. If these steps do not resolve the problem, study leadership will visit the site. Besides meeting with all the key personnel, study leadership will walk through recruitment procedures.

It may be helpful for the Study Chairman to present rounds for surgical and/or medical departments. This would provide the opportunity to answer questions and to generate interest in the trial. If the problem is that one of the opinion leaders at the hospital is not supportive of the study, the Study Chairman will communicate directly with Clinical Site opinion leaders as an educational tool to help them comprehend the nature and importance of the clinical trial.

10.6.3 Response to Protocol Violations

Major protocol violations are those that undermine the fundamental premise of the study (such as failure to administer the assigned treatment) or jeopardize patient safety (such as inappropriate administration of contrast in CT scan performance). The initial response to a major protocol violation will be remedial efforts such as conference calls, site visits and development of procedures to prevent the violation from reoccurring. However, if remedial efforts fail to address the problem (e.g., failure to maintain a treatment crossover rate < 10%), randomization function for that site will be suspended pending review by the study leadership, DSMB, and NIA. This review could result in either more aggressive remedial efforts or termination of the Clinical Site.

10.6.4 Response to Lapses in Clinical Site Performance

Lapses in Clinical Site performance include the failure to obtain the required CT scans or blood specimens as outlined in the protocol, failure to complete and submit the required forms, and failure to adhere to the assigned treatment. Both the DCC data editing process and the more thorough review of a subset of charts will be used to identify lapses in performance. These will

be discussed with the Clinical Site coordinators during conference calls with the CCC every month and reported in the DSMB.

Minor lapses in Clinical Site performance are those that impede progress of the study such as not submitting data in a timely fashion (form delinquencies). Clinical sites will receive reduced payments if there are cases whose forms are intolerably delinquent or until those form delinquencies are corrected.

CHAPTER 11 – DATA ANALYSIS

11.1 INTRODUCTION

The primary analysis will be a linear regression analysis (analysis of covariance -- ANCOVA) to test the hypothesis of no difference in the mean change from baseline in abdominal aortic aneurysm maximum transverse diameter at two years after randomization, allowing for maximum transverse diameter at the time of randomization (i.e., comparing growth in maximum transverse diameter), between patients randomly assigned to doxycycline 100 mg bid and patients assigned to placebo (see Sections 9.3 and 11.3). The analysis will be done on normal scores derived from the ranks of the diameters at baseline and at two years after randomization. Quantiles (2.5, 25, 50, 75, 97.5 percentiles) will be reported for baseline and two-year measurements. The primary analysis will be performed in accordance with the principle of intention-to-treat.

11.2 INTERIM MONITORING

A Data and Safety Monitoring Board (DSMB) will review the accumulating data for early, convincing evidence of benefit or harm. We anticipate DSMB reports at approximately six-month intervals over the period of patient recruitment and follow-up, at least five interim reports followed by a final analysis after the conclusion of data collection. Since the first follow-up CT scan will not be performed any earlier than six months after the start of recruitment, we anticipate at least five interim reports on CT scan findings prepared for the DSMB and one final analysis. To maintain the overall Type I error rate (α) at 0.025 (one-sided), while performing interim monitoring for the primary outcome, we propose to use a simple, Haybittle-Peto type [95,96] (adding a small adjustment to the final alpha level) repeated analysis adjustment for efficacy. We will perform the first interim analysis when 1/3rd of the primary outcome data are available and the second when 2/3^{rds} are available. With this approach, we will require stringent evidence of treatment efficacy. The final efficacy analysis will be performed at close to the overall α-level planned for the study (one-sided 0.025). The critical p-value proposed for each of the two efficacy interim analyses is one-sided 0.0005 (corresponding to t=3.291), which allows a final analysis at a critical p value of 0.0247 (corresponding to t=1.965) in an approach similar to that proposed by Haybittle-Peto. [95,96]

The above approach will be used in monitoring for possible early termination of the trial because of a beneficial effect of doxycycline. The DSMB will assess the interim data for possible early stopping for harm. Also, we propose that the trial could be stopped early if there is evidence of futility at the second interim analysis such that the conditional probability of rejecting the null hypothesis given data observed to the time of the analysis and the assumption that the design alternative hypothesis is correct is less than 20%.[97]

We propose as a stopping guideline a Pocock-type boundary of Z=1.645, corresponding to a nominal one-sided 5.0% type I error rate in the direction of more aneurysm growth in subjects receiving doxycycline, at each of the reviews of six-month scan data. No adjustment of alpha or the p-value would be necessary in the primary efficacy analysis, because the study would be stopped on the basis of the analysis of six-month scans only if doxycycline were doing

worse than placebo. The statistical analysis method for six-month CT scans would be the same as for 24-month CT scans, including the way any deaths or surgeries would be handled.

Before making a recommendation to terminate the trial, either for a beneficial effect, futility, or to discontinue study treatment, the DSMB will consider the interim analysis results along with other relevant factors. These factors will include enrollment and study conduct, the numbers and distributions of deaths and surgeries for aneurysm repair, the frequencies and distributions of other secondary outcomes described in Section 9.4, and findings from other relevant studies.

11.3 PRIMARY ANALYSIS

A variety of analytic methods will be used for primary, secondary and other analyses (Table 1). The primary study outcome will be the change from baseline of abdominal aortic aneurysm maximum transverse diameter measured two years from randomization. This outcome will be compared according to assigned treatment (consistent with the principle of intention-to-treat), using linear regression analysis (ANCOVA)[98], with baseline abdominal aortic aneurysm maximal transverse diameter and gender, as well as an indicator of treatment assignment, in the model. The test for differences between treatments in the primary outcome will be conducted at an overall one-sided α-level of 0.025. (The significance level could also be given as two-sided 0.05; stating it as one-sided 0.025 emphasizes the hypothesis that the investigators wish to "prove" statistically, and it also corresponds to the boundaries for interim monitoring for a beneficial effect of doxycycline.)

Prior to performing the primary outcome analysis, an interaction term for the randomization stratum (gender) with treatment assignment will be assessed in the ANCOVA model. If there is significant (at two-sided α=0.05) evidence of differences in the effect of treatment according to gender, other than a small quantitative difference between effects that are in the same direction for each gender, the analysis will be performed separately for men and for women. This gender-treatment interaction is of concern because the pathophysiology of abdominal aortic aneurysm in women could differ from that in men by more than just a simple shift in normal aortic diameter, and it would be important to know if a large treatment effect among men results in an overall treatment effect in the study but does not extend to women.

If an interaction between treatment and gender exists, the assumptions of the ANCOVA test of overall, homogeneous, treatment effect will not be met and an overall estimate of the size differential will not make sense. In the event of a treatment by gender interaction being observed, we will review study results by gender to determine the nature of the differences in treatment effects. If there is no interaction, the ANCOVA will be used to estimate the overall treatment effects across all clinical sites and both strata (genders). The primary analysis will be performed on the normal scores corresponding to percentile rank of each patient at baseline and follow-up.[94,99-101] At baseline all scores will be based on rank of abdominal aortic aneurysm maximum transverse diameter; information that must be available for the patient to be enrolled and will be centrally checked for eligibility. At two-year follow-up, rank will be assigned for patients who did not undergo two-year CT scan according to survival status, classification of patient condition at the time of surgical intervention (evidence of rupture, or symptoms or signs of imminent rupture versus undergoing repair for reason of maximal transverse diameter exceeding 5.5 cm for men or 5.0 cm for women or other less urgent indication). Multiple imputation methods available in SAS will be used for those (presumed to be few) patients who do not, for unanticipated reasons, complete a CT scan at two years (e.g., because of a missed appointment). Two-year CT

scans missing for pre-specified causes such as death, rupture, surgical intervention will be assigned ranks as described above.

The model for the primary outcome ANCOVA after removal of unimportant, interaction term(s) would be:

$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon$, where

Y = the change in normal score from baseline, assessed after two-year follow-up,

α = the study average change in normal score

β_1 = the treatment effect coefficient where

$X_1 = \{1 = \text{doxycycline or } 0 = \text{placebo}\}$

β_2 = the baseline normal score coefficient where

X_2 = the baseline normal score

β_3 = the randomization stratum (gender) coefficient where

$X_3 = \{1 = \text{female or } 0 = \text{male}\}$

ε = error.

The interaction term to be tested (and dropped from the model if unimportant) would be $\beta_4 X_1 X_3$ where β_4 is the treatment by gender interaction coefficient.

11.4 SUBGROUP ANALYSES OTHER SECONDARY OUTCOMES AND ANALYSES

Linear regression models will be used to explore age, statin use status (user versus non-user), race/minority status and other factors for effects on growth of abdominal aortic aneurysms. Secondary analyses will be conducted to assess the effect of doxycycline in subgroups of interest, for example subgroups defined by clinical factors.

To take account of the multiplicity of hypotheses being considered in secondary analyses, a p-value < 0.01 will be required to consider that some evidence of differences is present, and $p<0.001$ for strong evidence. Secondary outcomes are analyzed with more stringent alpha-levels to allow for multiple comparisons; a stringent alpha level reduces the likelihood of Type I error to some extent. This adjustment does not involve the primary outcome comparison or interim analysis plan.

Secondary outcomes to be considered include the occurrence of clinical events such as death, the occurrence of myocardial infarction or death and thromboembolism, measures of health related quality of life, high sensitivity C-reactive protein, MMP-9 levels, Interferon γ (IFN-γ) levels, other measurements made on CT scans (at two-years or other six-month time points) and subgroup analyses of the primary outcome.

With the data we plan to collect on 129 patients assigned to doxycycline and 129 patients assigned to placebo, we will have an opportunity to evaluate prognostic indicators that have not been adequately considered previously for abdominal aortic aneurysm outcomes. Among the characteristics that we evaluate are: gender, age, race/ethnicity, co-morbid states, baseline imaging characteristics of the abdominal aortic aneurysm, concomitant medications, and baseline MMP-9, high sensitivity C-reactive protein levels, etc.

Binary outcomes will be considered as present/absent variables, and the effect of treatment on these outcomes will be analyzed initially with a comparison of two proportions and a chi-square test.[101] Additional analyses, taking account of other patient characteristics, will be performed using logistic regression.[93,103,104] Outcomes in multiple categories (e.g., death, non-fatal myocardial infarction, acute coronary syndrome, no coronary artery disease) will be analyzed in 2 x k contingency tables using chi-square tests with more than one degree of freedom.[102] Time to death will be analyzed with log rank statistics.[105]

Losses-to-follow-up are expected to be few for vital status and surgical intervention status, based on our pilot experience and the required follow-up of abdominal aortic aneurysm patients for their clinical management. Missing two-year follow-up CT scans are expected to be less than 10%. Death and surgical intervention are accommodated in the proposed primary intention-to-treat analysis by rank order and normal scores methods, but if these outcomes are unrelated to treatment, they represent noise, lost opportunities to measure treatment effect on aneurysm size. CT scan data will be imputed using standard SAS procedures for participants who are missing two-year CT scans for reasons other than death or surgical intervention.[93] Sensitivity analyses will be performed to assess the impact of loss-to-follow-up. These will include, for example, assuming that all patients lost to follow-up have good outcomes, (e.g., no aneurysm growth) and assuming that all patients lost to follow-up have bad outcomes (e.g., maximal aneurysm growth). Allowance for losses of opportunity to measure treatment effects has been made in planning the number of patients to be enrolled in the study (See Section 11.5).

ANCOVA models will be fitted to assess associations between patient characteristics and abdominal aortic aneurysm growth (i.e., blood pressure, use of aspirin, use of beta-blockers).[106] GEE models will be used for assessments based on all CT scans collected.[107,108] Cotinine levels will be treated as a time dependent covariate in the GEE models.[107,108]

Protocol treatment adherence will be assessed according to capsule counts and doxycycline levels. Capsule counts will be analyzed for comparability of adherence between treatment groups and to assess the success of study performance against an absolute expectation of individualized patients achieving 80% or better adherence. Similarly, doxycycline levels will be assessed by comparing the distributions at the six-month visits against the blood levels known to be efficacious in animal models and anticipated from the proposed regimen. If there is failure to achieve anticipated levels, protocol revision to a new regimen (e.g., increased dose) could be considered. Cotinine levels will be used for objective confirmation of patient reports on smoking and use of nicotine patches. That correspondence within patient may bear on general adherence and accuracy of reporting.

11.5 STATISTICAL POWER

In this section we consider sample size and power in two stages. First we determine the sample size required for 90% power to find a significant difference between doxycycline and placebo with respect to the change in abdominal aorta diameter from baseline to two years after randomization, at a one-sided 0.025 significance level, when the treatments are compared using a linear regression model (ANCOVA) for change from baseline in normal scores corresponding to abdominal aorta diameter, with adjustment for the normal score at baseline. Then we show that the power is still high (> 80%) after allowance for deaths and surgical interventions.

The number of patients to be enrolled in N-TA^{3}CT was planned based on information from three databases – a research database, kindly provided as a privileged communication by Frank Lederle, M.D., the pilot study conducted by B. Timothy Baxter, M.D., and colleagues, and a reconstruction of the database from which results were published by Mosorin and colleagues in their pilot clinical trial of six months of doxycycline therapy (compared to placebo) for effect on abdominal aortic aneurysm diameter.[57,60] We have compared the aneurysms' average annual growth rate in these databases to published data from other studies of small abdominal aortic aneurysm and found them to be consistent. The three databases we analyzed establish an annual aneurysm growth rate of 2.5 mm/year as a reasonable lower bound for placebo.

Statistical power with our proposed method of analysis (ANCOVA) depends upon the correlation (r) of baseline measurements with follow-up measurements, the between-subject

variability (standard deviation, SD) of the measurement to be made, specified alpha-level (0.05, two-tailed) and the alternative hypothesis specified. We have specified the alternative hypothesis as a 40% reduction in aneurysm diameter growth rate (from 2.5 mm/year to 1.5 mm/year) because it is a difference in growth rate likely to be clinically important for our patients (prolonging growth to a size indicating intervention to repair by a median of four years, and close to but smaller than that observed by Mosorin (50% reduction).[60]

We found correlations of r= 0.88 for 12 placebo patients at 18 months and r=0.94 for doxycycline patients (14) in Mosorin, r=0.94 for measurements one year apart in Lederle (664 patients) and r=0.88 for all (31) treated patients in Baxter's data. We expand this range of observations to a range of r=0.80 to r=0.96 for consideration in power calculations. We found standard deviations of abdominal aortic aneurysm diameter ranging from 4.6 mm in one of Lederle's treatment groups at baseline and 6.2 mm in the other (average 5.4 mm), 5.0 mm and 5.8 mm on follow-up (average 5.4 mm), to 5.8 mm at baseline and 7.5 mm at follow-up in Baxter's data, and 7.5 mm among Mosorin's placebo patients and 6.8 mm among the Mosorin doxycycline patients at baseline (average 7.2 mm), 9.6 mm and 7.6 mm (average 8.6 mm) on follow-up respectively. We used these data to set a 5.0 mm to 9.0 mm range of standard deviations of interest. In Lederle's data there were 18 patients whose measurements were made at a two-year interval; r=0.82 and SD=4.3 mm at first measurement and 6.9 mm on the second. It seems reasonable (and conservative) to predicate initial (effective) sample size on a two-year correlation of 0.82 and a standard deviation of measurement of 7.0 mm and assess the sensitivity of the statistical power of our proposed clinical trial in the ranges of r=0.80 to 0.96 and SD=5.0 mm to 9.0 mm.

Data from published studies using doxycycline in patients who have small abdominal aortic aneurysms may be summarized as follows:

				Aneurysm Size			
				Control		**Doxycycline**	
Study	**N**	**Doxycycline Daily Dose**	**Imaging Modality**	**Initial**	**Final**	**Initial**	**Final**
Mosorin et al[60]	32	150 mg	Ultrasound	35 mm (31.0-40.0)	39 mm (30.5-44.0)	31 mm (27.5-38.5)	33 mm (27.0-38.5)
Baxter et al[57]	36	200 mg	CT Scan and Ultrasound	NA	NA	41.0 ± 0.9	42.7 ± 1.3

Using r=0.82 and SD=7.0 mm (corresponding to SD=4.2 mm for change in diameter), specifying desired statistical power to be 0.90, with a basic t-test formula and reduction of group size ($n_1=n_2$) for the efficiency of ANCOVA compared to t-test[109], we arrive at treatment group sizes of 85 patients each. On consideration of the range of correlations and standard deviations in our planning data sets, we find that the statistical power of ANCOVA for 85 patients assigned to each treatment group and a two-group comparison will be as in the table below.

Statistical Power of a Two Group Comparison at Two-Year Follow-Up

	Standard Deviation of Dependent Variable		
	5.0 mm	7.0 mm	9.0 mm
0.80	0.99	0.87	0.67

Correlation of pre- and post-treatment measurements	0.88	>0.99	0.97	0.86
	0.96	>0.99	>0.99	>0.99

We will have over 80% power in the event that our projected baseline to two-year follow-up correlation in abdominal aortic aneurysm maximal transverse diameter is modestly optimistic (i.e., really should have been 0.80 instead of 0.82) or our projected variability (standard deviation) of maximal transverse diameter was too optimistic (i.e., really should have been 9.0 mm instead of 7.0 mm), protecting study power against possible mistaken design assumptions. In the unlikely event that both assumptions are mistaken, we will still have close to 70% power.

These power calculations are based on a t-test and ANCOVA on the CT scan aneurysm diameter measurements. However, we plan to use ANCOVA on normal scores. Since the power of the normal scores test is asymptotically equivalent to the power of the t-test if the aneurysm diameter measurements are normally distributed and is likely to be higher than the power of the t-test if the measurements are not normally distributed[94], the power calculations should give a good approximation to the power of the normal scores test.

The above calculations do not allow for the possibility of death, rupture of the abdominal aortic aneurysm, or open or endovascular repair without evidence of rupture in some patients within two years after randomization. If we assume one of these events will occur in 10% of randomized patients, then interpolation in Table 2 of McMahon and Harrell[99] with survival = 90% indicates that 90% power for a rank test on aneurysm diameter corresponds to power of > 80% for a "worst-rank" test on a combined outcome of aneurysm diameter and the events noted above. (We actually expect these events to occur in less than 10% of patients, but in order to be conservative we have assumed 10% in the power calculation.)

Since a normal scores test is as powerful as or more powerful than the corresponding rank test, and thus have the same power asymptotically[94], power of > 80% also applies to our primary analysis, ANCOVA on normal scores.

We also want to allow for the possibility that 10% of patients in each treatment group will not adhere to assigned study treatment (e.g., patients assigned to doxycycline do not take their medication because of gastrointestinal discomfort or skin rash, etc., and patients assigned to placebo end up taking doxycycline for an indication like periodontitis or rosacea) and for the possibility that two-year CT scans will be missing for 15% of patients for reasons unrelated to outcome and treatment effect (i.e., noise in a system with signal we are trying to detect).

Taking into account all the above possibilities – death, aneurysm rupture, or open or endovascular repair without evidence of rupture; nonadherence; and missing scans for reasons unrelated to outcome or treatment effect – the number of patients to be randomized to each treatment group becomes $85 \div (1\text{-}0.1)^2 \div (1\text{-}0.15) = 124$ patients.[110] We have been awarded a supplement to enroll 10 additional women to increase the statistical power and precision of our gender-based analyses. Thus, our recruitment goal is 258 patients (129 in each treatment group), a number that should give us high power to find a statistically significant effect if the true effect is a 40% reduction in the mean increase in aneurysm diameter from 2.5 mm/year to 1.5 mm/year, if our well documented design assumptions (correlation and standard deviation) are approximately correct for the population we enroll. If our assumptions are absolutely correct, adherence perfect and there are no patients who do not undergo CT scan of the abdomen at two-year follow-up, we will have a very well powered study (~ 90% or more).

With 129 patients in each treatment group, setting alpha levels at the more stringent 0.01 for some evidence in secondary outcome analyses we would find statistical significance for between-treatment group differences of approximately 0.5 standard deviation (about the same as observed in other studies[10,91]) with 90% power using ordinary Student's t-tests. For clinical events that occur with a frequency of 15% we would have 80% power to find significance for reductions to 5%. Statistical power would not be appreciable for reductions in events occurring with lower frequency; lesser effect sizes would be detectable for more frequently occurring events.

Table 5: Analysis Methods for Performing Primary, Secondary and Other Analyses

Analyses	Method
1. Primary End Point Analyses	
a) Abdominal aortic aneurysm diameter	ANCOVA of change from baseline in normal scores of ranked outcomes, allowing for baseline rank and randomization stratum (gender)
b) Gender interaction with treatment	ANCOVA
2. Secondary Analyses (Treatment Group Comparisons)	
a) Baseline characteristics in each treatment group	Descriptive statistics (means, standard deviations, percentages)
b) Baseline characteristic and duration of treatment interactions with treatment effect	ANCOVA of ranked outcomes, allowing for baseline rank, randomization stratum (gender) and including interaction term(s) for the baseline characteristic(s) of interest with treatment assignment
c) Adherence	Chi-square tests with one degree of freedom, T-tests
d) Abdominal aortic aneurysm imaging (CT scan) findings on repeated studies	Multiple logistic regression, Generalized Estimating Equations (GEE) and Mixed Models
e) Clinical events such as death, aneurysm rupture, aneurysm repair, myocardial infarction, stroke, acute coronary syndrome, in univariate or combinations and the interaction of treatment effect with duration of treatment	Log Rank Tests and Cox Proportional Hazards Regression. Comparison of two proportions using a chi-square test with one degree of freedom
f) Biomarkers (MMP-9, IFN-γ)	T-tests, ANCOVA, GEE, and Mixed Models
g) SF-36	T-tests, GEE
3. Other Analyses	
a) Other characteristics' (including Statins and Biomarkers) effect on aneurysm growth	ANCOVA, GEE

b) Patient characteristics association with clinical events	Log Rank Tests and Cox Proportional Hazards Tests. Chi-square tests with one degree of freedom, and with more than one degree of freedom

CHAPTER 12 – STUDY ORGANIZATION

12.1 PARTICIPATING UNITS

The participating units in N-TA^{3}CT integrate with the N-TA^{3}CT administrative structure to form the study organization (See Exhibits 12-1 and 12-2).

12.1.1 Clinical Site

At each collaborating hospital, a Clinical Site director and Clinical Site coordinator will be identified. These two people will work closely together to assure successful performance of the trial. Clinical Sites are expected to enroll an average of just over 16 patients each (16 Clinical Sites x 16.125 patients/Clinical Site = 258 patients). Eligible patients seen over a three month period in each Clinical Site participating in N-TA^{3}CT planning are presented in Exhibit 12-1. Clinical Sites in the N-TA^{3}CT organization are subject to change according to the feasibility of study conduct at each site.

12.1.1.1 Clinical Site Director

The responsibilities of the Clinical Site director include: 1) To insure that all vascular surgery staff involved with the care of abdominal aortic aneurysm patients are well informed about the trial; 2) To insure that all patients with abdominal aortic aneurysm are routinely considered for the trial; 3) To insure that the treatment assignment is followed; 4) To communicate with Clinical Coordinating Center staff and Data Coordinating Center staff any problems or concerns related to the study; 5) To assist the Clinical Site Coordinator as necessary; 6) To ensure that all guidelines governing clinical research are adhered to.

12.1.1.2 Clinical Site Coordinator

The responsibilities of the Clinical Site Coordinator include: 1) To identify all abdominal aortic aneurysm patients for the trial; 2) To obtain informed consent from the patient if not obtained by the Clinical Site lead investigator or other investigators, fellow or resident; 3) To enroll the patient in the study by telephoning the randomization service; 4) To inform the surgeons and nurses caring for the patient of the patient's randomization in N-TA^{3}CT; 5) To complete data collection forms and process data edit queries; 6) To insure compliance with the study Protocol; 7) To e-mail or fax data forms to the Data Coordinating Center and arrange for copying and mailing of medical records to the Clinical Coordinating Center for quality assurance; 8) To insure the clinical site office and pharmacy are stocked with trial materials; 9) To participate in telephone calls with the Clinical Coordinating Center head nurse; 10) To train assistant site coordination and other staff at the Clinical Site as needed; 11) To maintain all Clinical Site study materials; and 12) To adhere to all guidelines governing clinical research.

12.1.2 Clinical Coordinating Center

The University of Nebraska Medical Center, Division of Vascular Surgery will be responsible for clinical coordination of the trial. The responsibilities of the Clinical Coordinating Center are to: 1) Provide administrative and fiscal support for the Clinical Sites; 2) Provide technical, patient assessment and Protocol adherence advice to Clinical Site staff; 3) Assist Clinical Sites to correct problems with recruitment, Protocol adherence and data collection; 4) Participate in site visits; 5) Provide advice about any aspect of the trial; 6) Direct performance of the Treatments Distribution Center; 7) Direct performance of the Biomarkers Core Laboratory; and 8) Lead presentation and publication of study results for the scientific and lay press.

12.1.3 Data Coordinating Center

The Department of Epidemiology and Preventive Medicine, University of Maryland School of Maryland, Baltimore, Maryland, will be responsible for data coordination of the trial. The responsibilities of the Data Coordinating Center are to: 1) Provide all study materials to centers; 2) Provide a 24 hour randomization service; 3) Receive data collection instruments and to verify them for completeness, retrieving any missing data from the centers; 4) Enter data into a computer database and conduct routine data edits; 5) Monitor performance to detect problems with recruitment, Protocol adherence and data collection; 6) Participate in site visits; 7) Provide advice about any aspect of the trial; and 8) Perform interim and final analyses.

12.1.4 Imaging Core Laboratory

The Imaging Core Laboratory will be responsible for the standardized, quantitative and qualitative assessments of CT scans in N-TA^{3}CT. Using labels provided by the DCC and Clinical Site programming to delete identifiers in the electronic file to obscure patient identification, Clinical Sites will send CT scan CDs to the Imaging Core Laboratory. Imaging Core Laboratory staff will send electronic files of CT scan assessments to the DCC. The DCC will direct a limited number of blinded CT scan resubmissions to the Imaging Core Laboratory for quality control. Imaging Core Laboratory CT scan measurements will be used for the primary outcome assessments in N-TA^{3}CT. The Imaging Core Laboratory will provide initial, prompt review of each qualifying CT scan to the clinical site and to the Data Coordinating Center to confirm or deny each potential study patient's eligibility on CT scan criteria, and will review (post hoc) all CT scans considered in the clinical sites to indicate vascular repair.

12.1.5 Biomarkers Core Laboratory

The Biomarkers Core Laboratory will be responsible for measurement of doxycycline levels, high sensitivity C-reactive protein levels and MMP-9 activity as well as preservation of specimens for future research. Specimens will be processed every 6 months and results will be forwarded to the Data Coordinating Center for incorporation into the trial database. The doxycycline results will be provided to the Data Coordinating Center for a check on protocol adherence. MMP-9 results will be provided to the Data Coordinating Center for incorporation into the study database.

12.1.6 Study Chairman

Study Chairman responsibilities will include: 1) To provide overall organization of the trial; 2) To serve as Chair, Steering Committee; 3) To administer the Treatments Distribution Center; 4) To work with the Clinical Sites and Data Coordinating Center to maximize collaboration and to arrange study meetings; 5) To provide collaborators with information about the progress of the trial; 6) To participate in visits to the Clinical Sites to assess quality and assist with problems; 7) To take a leadership role in defining the analysis of study data; and 8) To prepare the manuscripts describing the design and results of the study.

12.2 ADMINISTRATIVE STRUCTURE

12.2.1 Steering Committee

A Steering Committee will be responsible for overseeing the study. This committee will make major organizational and policy decisions. The committee will include the Study Chairman, Core Laboratory Directors, representatives of the Clinical Coordinating Center and the Data Coordinating Center, the Clinical Site directors, and two coordinators representing the Clinical Sites to be selected by the other Clinical Site Coordinators and the Clinical Site directors. The NIA project officer will be invited to participate. This group will have quarterly

conference calls and meet yearly prior to the annual Clinical Site directors and coordinators meeting.

12.2.2 Management Committee

A smaller committee involved with the day to day running of the study will have conference calls every two weeks to discuss the progress of the trial (including recruitment and protocol violations). This group will be composed of the Study Chairman, Director of the Data Coordinating Center and the Data Coordinating Center coordinator, Director of the Biomarkers Core Laboratory, Director of the Imaging Core Laboratory, and the Clinical Coordinating Center head nurse coordinator.

12.2.3 Publications and Ancillary Studies Committee

A Publications and Ancillary Studies Committee will be formed from the study leadership (Data Coordinating Center, Imaging Core Laboratory, Biomarkers Core Laboratory, and Clinical Coordinating Center) and leading Clinical Site directors. This committee will review all proposals for and final versions of research abstracts, presentations, and manuscripts to be submitted to journals and national meetings. The committee will also review proposals for ancillary studies. Since the success of the trial depends entirely upon the collaboration of the doctors and nurses in the participating centers, credit will be assigned to them (as the "N-TA^{3}CT Research Group") in the authorship of reports from the study. Each Clinical Site director and Clinical Site coordinator will be named personally in an appendix to the main report.

12.2.4 Data and Safety Monitoring Board

Members of the Data Safety and Monitoring Board (DSMB) will be appointed by and report to the National Institute on Aging (NIA) director. They will monitor accruing data in order to confirm that the patients in the trial are being cared for safely. The DSMB will be responsible for:

1. Reviewing and analyzing the progress of the study.
2. Approving amendments to the trial Protocol, if warranted.
3. Monitoring the safety of the study treatments.
4. Reviewing data quality.
5. Reviewing interim analyses and recommending early stopping or continuation of the trial.

The DCC and the Management Committee provide input to this committee as requested. The DSMB will review study data reports every six months and primary end point analysis every six months after the first patients complete two years of follow-up. The DSMB may convene in face-to-face meetings or on conference calls.

12.2.5 Role of DCC Staff

At least one DCC staff member will be assigned to each study committee and subcommittee to participate in all meetings and conference calls. The DCC director will serve as Executive Secretary of the Steering Committee; Management Committee; Publications and Ancillary Studies Committee; Event Classification Committee; and will work with the Chairperson of each committee to draft the agenda, summary notes, and lists of action items for each meeting or conference telephone call.

The Executive Secretary of the Publications and Ancillary Studies Committee will supervise the development and implementation of a system to track preparation and review of study manuscripts and abstracts on main results, databank studies and ancillary studies to disseminate the design, methods and results of this trial.

12.2.6 Data Coordinating Center Interface with Clinical Coordinating Center

Conference calls, facsimile transmission, e-mail and courier will be used to maintain frequent and regular communications between the Clinical Coordinating Center and the Data Coordinating Center. The investigators will discuss study activities in person or by telephone every two weeks during the Management Committee conference calls. These calls serve to review study progress and Protocol adherence at the participating Clinical Sites, review committee activities, and plan study meetings. In the first months of N-TA^{3}CT, CCC and DCC staff will develop training sessions for Clinical Site staff, and in the last months they will lead manuscript preparation efforts. Documents and materials for training and draft manuscripts will be reviewed with the Management Committee as will plans for long-term archival of study data.

12.2.7 Data Coordinating Center Role in Training of Study Personnel

Prior to the start of recruitment and in conjunction with the training session planned by the Clinical Coordinating Center, DCC staff will organize training for Clinical Site staff to review enrollment of patients using the Interactive Web-based Randomization System (IWRS), data collection procedures, submitting forms by electronically to the DCC, and responding to edit queries. DCC staff will monitor each Clinical Site to assure that staff at each site have completed the required training, and that all other steps necessary to begin participant recruitment are completed. Clinical Site staff who have completed training procedures in Clinical Sites that are approved to participate in N-TA^{3}CT will be given certification numbers that will be used to track the individual responsible for each data item and activity in N-TA^{3}CT.

12.3 STUDY TIME LINE

The study will last five years and be broken into the following phases. 1) Planning and Organization Phase 1 (months 1-4): Finalize the Manual of Operations and data collection instruments. Communicate with the Clinical Site directors at each study site. Obtain IRB approval at clinical sites with assistance from CCC staff. Develop computer software for randomization and data entry. This time period is feasible because we a) are implementing the protocol that was developed under the planning grant, b) the forms we will be using have been extensively tested, and c) staff from the CCC will assist Clinical Site staff in completing IRB applications. The development of computer software and manual of operations will be addressed quickly at the beginning of the study. 2) Planning and Organization Phase 2 (months 5-6): Obtain approval of Study Protocol from the DSMB. Communicate final study protocol and procedures. Plan and schedule training. Hire Clinical Site Coordinators. Hold annual Collaborators' Meeting for Clinical Site directors and coordinators. Distribute final forms. 3) Recruitment and Follow-up Phase (second half of Year 1, Years 2, 3, and 4): Initiate patient enrollment and continue for 1.5 years or until patient recruitment is completed or the study is stopped early. Hold annual Collaborators' Meetings. Visit Clinical Sites once during the first year of patient recruitment and annually thereafter. CCC prepares and distributes quarterly newsletters. Bi-annual meetings (either in person or via conference call) of the Data and Safety Monitoring Board. 4) Close-out and Analysis Phase (Last Year): Complete follow-up and data cleaning. Perform data analysis. Discuss results at collaborators meeting. Prepare manuscripts and final reports.

Exhibit 12-1

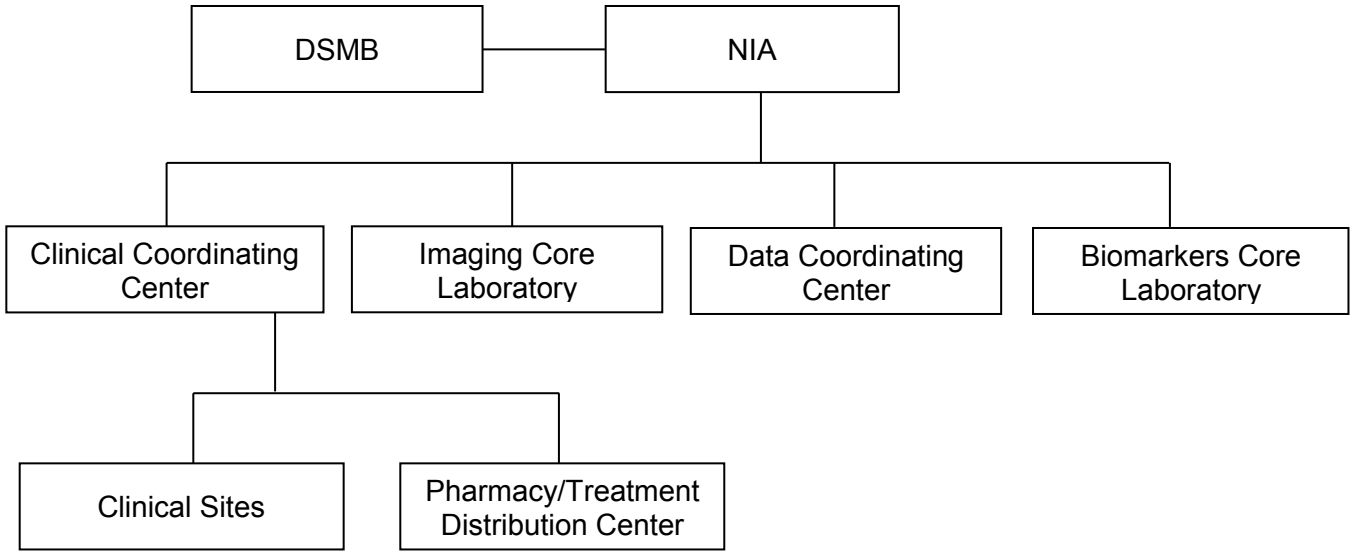
Clinical Sites Participating in N-TA^{3}CT Planning*	Aneurysm Patients Without Repair	Meeting Permanent Eligibility Criteria
University of Nebraska Medical Center	26	17
Northwestern University	35	18

Washington University School of Medicine	48	22
Emory University School of Medicine	24	15
Geisinger Medical Center	143	70
New York Weill Cornell Medical Center	28	21
University of Utah Health Sciences Center	31	15
University of Arizona Health Sciences Center	25	11
The University of Michigan Health System	102	35
University of Cincinnati School of Medicine	23	16
Oregon Health Sciences University	19	9
Cleveland Clinic Lerner College of Medicine	171	89
University of Maryland School of Medicine	19	16
University of South Florida Health South	12	8
Baptist Hospital of Miami	24	9
Total	730	371

*Other Clinical Sites Involved Include The University Of Virginia and The Beth Israel/Deaconess Medical Center (Boston, Massachusetts)

Exhibit 12-2

N-TA^{3}CT Clinical Trial Organization



CHAPTER 13 – POLICY MATTERS

13.1 PUBLICATION POLICY

13.1.1 General Statement of Editorial Policy

It is anticipated that the Doxycycline Treatment of Abdominal Aortic Aneurysm Clinical Trial (N-TA^{3}CT) will generate considerable new data relative to patients with aortic aneurysms. The Steering Committee fosters and guides development of scientific reports originating from data obtained in the project. The scientific integrity of the project requires that all data from all Clinical Sites be analyzed study wide and reported as such. Thus, an individual center is

expected not to report and publish data collected from its center alone. Development of sub studies or data bank studies dealing with specific analyses are encouraged. All presentations and publications of any data collected by the N-TA^{3}CT Research Group are expected to protect the integrity of the main objectives of the overall project. Major findings are not presented prior to release of "mainline" results of the study by agreement of all N-TA^{3}CT Clinical Site directors. The Steering Committee determines the timing of presentation of mainline results (including papers on design and methods) and designation of the meetings at which they might be presented.

Publications are grouped into five general categories (see Section 13.1.2). Topics for consideration to be developed into publications are generated from questions or hypotheses that are submitted to the Steering Committee by investigators, study coordinators and other study-related staff. A writing group with a designated Chairperson is selected for each topic.

The Publications and Ancillary Studies Committee has primary responsibility for reviewing and approving all abstracts and all manuscripts on mainline findings, special laboratory studies, data bank or ancillary studies submitted for presentation or publication. Abstracts and manuscripts are also reviewed by the NHLBI according to existing procedures.

Investigators at all N-TA^{3}CT Clinical Sites, the Clinical Coordinating Center (CCC), the Biomarkers Core Laboratory (BCL), the Imaging Core Laboratory (ICL), and the Data Coordinating Center (DCC) have equal status with regard to developing proposals, participating in such studies as approved by the Steering Committee, and collaborating in the development and publication of research papers based on study material. With the approval of the Principal Investigator, study coordinators and other staff at these centers are encouraged to participate in this process. The Management Committee has developed standards for regular evaluation of the submission and completion of these protocols. The Management Committee determines priorities for analyses among data bank study proposals which have been approved.

N-TA^{3}CT Investigators at Clinical Sites, the BCL, the ICL, the CCC or DCC proposing studies that require the collaboration of CCC, BCL, ICL, or DCC (e.g., specimen, image or data analysis) contact the appropriate individuals prior to submission of a given proposal. The appropriate staff in the CCC and DCC participate in drafting the proposal, indicate willingness to participate, and identify sources of funding to support the level of effort required for the project.

The CCC, BCL, ICL, and DCC Investigators are consulted in the development and analysis of protocols that require review of accumulated data or data on file at the CCC, BCL, ICL, or DCC. The members of the CCC, BCL, ICL, and DCC collaborate in designing and carrying out all N-TA^{3}CT research.

13.1.2 Types of Research

Research and the resulting presentations and publications are grouped into the following categories:

1. Design paper(s) and reports on methods.
2. Mainline findings.
3. Data bank studies.
4. Ancillary studies.
5. Independent studies.

Distinctions among these types of studies are given in Section 13.2. If possible, analysis of data may be conducted prior to the end of the N-TA^{3}CT investigation and is strongly encouraged, so that the maximum information can be published from this study and so that the methods for evaluating and analyzing study data may be refined in preparation for later analyses.

13.1.3 Authorship

The first publication(s) pertaining to the fundamental goals of N-TA^{3}CT involving patients enrolled in the study will list the members of the writing team as the authors with "the N-TA^{3}CT Research Group" as the last author. An appendix listing the Principal Investigator and Co-Investigators will be included at the end of the manuscript's text. It is intended that there will be more than one publication concerning the mainline goals; all publications will list the writing team as the authors on behalf of the N-TA^{3}CT Investigators.

13.1.4 Purpose of Procedural Guidelines

The procedures adopted by the investigators for use of study data are intended to protect the interests of all participants in the study, to assure that study data conform to the requirements of study design and are accurately presented, that authorship is appropriately acknowledged, that the text of each publication is well-written, to ensure that all investigators are aware of ongoing analysis projects, to avoid duplication of analysis projects and to ensure that publication or presentation of study data does not occur without the knowledge and approval of the Publications and Ancillary Studies Committee and the Steering Committee.

13.2 DESIGN AND METHODS REPORTS, MAINLINE FINDINGS, DATA BANK, ANCILLARY, AND INDEPENDENT STUDIES

13.2.1 Design Papers and Reports on Methodology

Manuscripts concerning the overall design, protocol, procedures, or organizational structure of the study that do not involve mainline findings or data collected on study patients may be published prior to the end of the study. Such preliminary publications will be developed and reviewed according to the same guidelines used for other reports of mainline findings.

Many public presentations about N-TA^{3}CT that do not involve protocol data, special laboratory studies, data bank or ancillary study data (e.g., grand rounds talks concerning the study's general design and objectives) do not require formal preliminary review and approval by the Publications and Ancillary Studies Committee. However, if there is any doubt, investigators are asked to first consult with the Publications Committee indicating their intention to present the material, in order to avoid the premature release of study data or the inappropriate publication of confidential information.

13.2.2 Reports of Mainline Findings

A report on mainline findings is one addressing the fundamental goals of N-TA^{3}CT or that involves protocol data -- such as changes in aneurysm size or MMP-9 levels over time in study patients -- which cannot be released prior to the end of the study. These studies will summarize the findings based on the entire study population and will be written at the conclusion of the project. These reports must be reviewed and approved by the Publications and Ancillary Studies Committee and ratified by the Steering Committee.

13.2.3 Data Bank Studies

A data bank study uses data or specimens (including banked specimens) which are routinely collected on patients who are recruited and/or enrolled in the N-TA^{3}CT. Analysis of these data are used to answer specific scientific questions. Data used in this research are not directly related to the fundamental goals of the study. Data bank studies must be approved by the Management Committee and ratified by the Steering Committee. All presentations or publications of data bank studies are to be reviewed following the procedures outlined in Section 14.4.

13.2.4 Ancillary Studies

An ancillary study uses supplementary data that are collected on patients who are recruited and/or enrolled in N-TA^{3}CT, over and above the data collection required by the protocol. Such studies are restricted to consideration of a specific test technique or involve only the supplemental data collected on study cases and controls. Ancillary studies are reviewed and approved by the Management Committee and ratified by the Steering Committee prior to initiation to ensure that they do not conflict with the main protocol. Review by the Publications and Ancillary Studies Committee is required for presentation or publication of an ancillary study.

13.2.5 Independent Studies

Independent studies of concern to N-TA^{3}CT are studies conducted in potential patients who are not enrolled in the study, but data are collected at a Clinical Site. These data are not transmitted to the N-TA^{3}CT Clinical Coordinating Center or Data Coordinating Center.

It is understood that each Clinical Site has the right to conduct studies which are independent of N-TA^{3}CT in patients with who do not meet criteria for enrollment into the study. Independent studies of patients who meet eligibility criteria but are not enrolled in N-TA^{3}CT must be reviewed by the Management Committee. Study investigators agree not to conduct independent studies which would compete with or have a detrimental effect on the conduct of N-TA^{3}CT during the period of recruitment and follow-up.

13.3 GUIDELINES FOR PREPARATION OF PROPOSALS FOR DATA BANK AND ANCILLARY STUDIES

13.3.1 Data Bank and Ancillary Studies

Each proposal for an ancillary or data bank study should contain a brief description of the objectives, methods, analysis plans, significance of the study, and proposed collaborators. Full details should be given concerning any procedures to be carried out, such as cytokine evaluations, exercise tests or psychological testing, etc. Mention should be made of any substances to be injected or otherwise administered to the patients. Any observations to be made or procedures to be carried out on patients or on banked specimens outside of the Clinical Site should be described. Mention should be made of the extent to which the data bank or ancillary study requires extra clinic visits or prolongs the usual clinic visits. Information should be given concerning the extent to which the ancillary study requires specimens in addition to those presently required by the protocol. If blood specimens are to be obtained from the patients or banked specimens are required, mention should be made of the number of specimens as well as a description of all procedures to be carried out on these specimens.

13.4 PROCEDURES FOR INITIATION AND APPROVAL OF STUDIES

13.4.1 Reports on Mainline Findings

Reports on mainline findings from N-TA^{3}CT generally involve the collaboration of many investigators. Proposals for these reports are introduced and developed by any N-TA^{3}CT Investigator or staff member with the approval of the Clinical Site director. These reports are reviewed and approved by the Management Committee and ratified by the Steering Committee.

13.4.1.1 Submission of Proposals

Two copies of each proposal should be submitted to the Data Coordinating Center for inventory and transmission to the Management Committee. The Director of the DCC notifies the Investigator when the project is approved, disapproved or whether additional information is needed before a decision can be made.

13.4.1.2 Preparation of Mainline Reports

After approval of a proposed topic for a mainline report, members are elected or invited to serve on an ad hoc Writing Subcommittee and a Chairperson is chosen. These investigators work with the CCC and DCC staff to conduct the data analysis needed to investigate the question at hand and prepare a manuscript based on these findings. Every effort is made by the Subcommittee to consider and incorporate in this manuscript the comments and suggestions from the Steering Committee. Often the Subcommittee members meet with staff from the CCC, DCC or other Clinical Sites for development of these papers.

13.4.1.3 Review and Approval of Abstracts and Manuscripts Prior to Presentation and Publication

Every study manuscript considered suitable for publication is submitted by the Chairperson of the Writing Subcommittee to the DCC for distribution to the Publications Committee. The Chair of the Publications and Ancillary Studies Committee is responsible for arranging and implementing review according to the following procedures.

1. The manuscript is forwarded promptly to at least two reviewers selected from the members of the Steering Committee or their associates, with the request to respond within two weeks with a detailed critical review of the manuscript. Outside reviewers are selected when appropriate.
2. Reviews are forwarded to all members of the ad hoc Writing Subcommittee with a request for appropriate revision and response.
3. The ad hoc Writing Subcommittee is expected to respond to the review in a reasonable period of time, forwarding to the CCC the revised manuscript and a letter commenting in detail on the points raised by the reviewers; DCC staff will distribute these materials to the Publications and Ancillary Studies Committee.
4. After review by the Publications and Ancillary Studies Committee, the DCC staff return the manuscript to the ad hoc Writing Subcommittee with final comments or suggested changes.
5. If acceptable to the study leadership (Management Committee), the completed manuscript is submitted by the Chairperson of the Writing Subcommittee to the appropriate journal. A copy of the transmittal letter and copy of the manuscript are submitted to the DCC for distribution to the Steering Committee.

13.4.2 Data Bank Studies

13.4.2.1 Submission of Proposals

Data bank studies must be approved by the Management Committee and ratified by the Steering Committee. Before beginning a data bank project, a proposal initiated by one or more of the Investigators and/or their associates is submitted to the DCC for inventory and distribution to the Management Committee for consideration. The Director of the DCC notifies the Investigator when the project is approved, disapproved or additional information is needed before a decision can be made.

13.4.2.2 Conduct of Data Bank Studies

After approval is given by the Steering Committee, the Investigators (on the data bank project) work with the CCC and DCC staff to conduct the data analysis.

13.4.2.3 Priorities for Work

Because of the routine work load at the CCC and DCC, it is necessary to establish priorities for data processing and analysis. Therefore, the DCC staff conduct analyses on data

bank studies in the order in which they have been approved or, as necessary, seek guidance from the Management Committee for determining priorities for analysis.

13.4.2.4 Authorship

After a data bank study proposal is approved by the Steering Committee, its research and development are the responsibility of the identified investigators on the project. Authorship decisions on data bank studies take into account the unique cooperative effort that has produced the results. For clinical papers in particular, individuals from Clinical Sites, CCC, DCC and NIA staff have the opportunity to join writing teams when their contributions are appropriate. On the other hand, there will be papers of more limited scope which probably do not warrant a large number of authors. The following mechanism is utilized to determine authorship:

1. The lead author proposes a list of co-authors, based on the above guidelines.
2. The Management Committee reviews and approves, or makes recommendations regarding alterations in the proposed list of authors.

The names of these investigators is followed by the designation "and N-TA^{3}CT Research Group" on the byline.

13.4.2.5 Review and Approval of Abstracts and Manuscripts Prior to Presentation or Publication

The Publications and Ancillary Studies Committee reviews all data bank study abstracts and manuscripts prior to submission for presentation and publication. Recommendations are forwarded to the Management Committee for review and final decision. All abstracts must be received by the Publications and Ancillary Studies Committee members, all co-authors, DCC, and CCC at least two weeks prior to the submission deadline. Manuscripts prepared based on data bank studies must be submitted to the DCC at least one month (30 days) before the scheduled submission date. After review, the Publications Committee makes recommendations to the Management Committee in consultation with the DCC. The Director of the DCC notifies the authors and Steering Committee of the decision within one month of the receipt of a manuscript, within one week for abstracts. The approved manuscript or abstract is then submitted.

13.4.3 Ancillary Studies

13.4.3.1 Submission of Proposals

Ancillary study proposals are reviewed by the Management Committee and are ratified by the Steering Committee to ensure that the proposed study does not conflict with the primary goals of N-TA^{3}CT.

Two copies of each proposal are submitted to the Data Coordinating Center for inventory and transmission to the Management Committee. The Director of the DCC notifies the Investigator when the project is approved, disapproved or additional information is needed before a decision can be made.

Abstracts and manuscripts are to be submitted for review prior to submission.

13.4.4 Independent Studies

Results of independent studies which are approved as acceptable by the Management Committee may be published or presented at the discretion of investigators initiating the independent study.

13.5 RELEASE OF N-TA^{3}CT DATA OR SPECIMENS TO NON- N-TA^{3}CT INVESTIGATORS

Requests for study results, study data, or banked specimens may be submitted by investigators who are not participating in N-TA^{3}CT during the course of this investigation. These

requests will arise primarily from colleagues and researchers who are interested in abdominal aortic aneurysm. Each request should be submitted in writing and provide the same information as required for study data bank and ancillary studies submitted by N-TA^{3}CT Investigators. The Management Committee reviews each request and the following principles are addressed in determining the disposition of each request.

1. Overlap with the study major goals or approved data bank and ancillary studies.
2. The scientific importance of the request.
3. The efforts and costs of providing the information.
4. The willingness of the individuals submitting the request to accept the limitations, as specified by the N-TA^{3}CT Management Committee, on the uses that can be made of the data and data analysis.

At least one month prior to the end of funding, the Data Coordinating Center staff will prepare data files and appropriate documentation for submission to the NIA Project Office. These files will not include personal identifiers of patients. The release of these data tapes will be based on the NIH Policy on Release of Data from Large Scale NIH Sponsored Studies.

13.6 CONFLICT OF INTEREST POLICY

13.6.1 General Principles

The N-TA^{3}CT investigators have agreed to a policy on conflict of interest which has few specific restrictions, but a broad indication for disclosure of potential conflicts of interest. The N-TA^{3}CT investigators wish to endorse the spirit and content of the 21st Bethesda Conference: Ethics in Cardiovascular Medicine dealing with these issues, and seek to make this policy consistent with the record of that conference.

To address actual or perceived conflict of interest in N-TA^{3}CT, the participating investigators voluntarily agree to abide by the guidelines described in the policy statement developed for N-TA^{3}CT. See Exhibit 14-1 for a copy of the Conflict of Interest Statement.

13.6.2 Individuals to be Governed by These Guidelines

Members of the N-TA^{3}CT Study Group who will be governed by these guidelines include the Study Chairman, the director at each Clinical Site, key personnel in the Clinical Coordinating Center, Biomarkers Core Laboratory, Imaging Core Laboratory, and Data Coordinating Center. Co-Investigators and other staff who have major responsibility for enrollment, recruitment, follow-up or collection of data for N-TA^{3}CT at Clinical Sites will also be governed by these guidelines. The Principal Investigator for each N-TA^{3}CT Center or Core Laboratory will submit a list of individuals who will be governed by these guidelines at the beginning of the study. The leaders of each participating unit will review the guidelines with all appropriate staff prior to the start of patient recruitment and at least annually thereafter.

13.6.3 Time Period of the Policy

The guidelines set forth in this policy commence at the start of patient recruitment and will terminate at the time of initial public presentation or publication of the principal results. Investigators not privy to end point data who discontinue participation in the study during recruitment will be subject to these guidelines until their departure from the study.

13.6.4 Financial Guidelines

1. The investigators agree not to own, buy or sell stock or stock options during the aforementioned time period in any of the pharmaceutical companies or related medical equipment companies with products used in this study. In addition, the investigators agree not to have retainer-type consultant positions or positions of decision making

responsibilities (e.g., board of directors, policy advisory committee), hold patents or partnerships with these companies for the time period defined above.

2. The Clinical Coordinating Center will maintain conflict of interest statements updated annually from each investigator.

Activities not explicitly prohibited, but to be reported annually with information maintained by the Clinical Coordinating Center include:

1. Participation of investigators in authorized educational activities (e.g., FDA approved workshops for training in use of devices) that are supported by the companies defined above. Note: general speaker bureaus or lecture/ continuing medical education activities sponsored by these companies are not allowed.
2. Participation of investigators in other research projects supported by the companies defined above.

Financial interests in the companies defined above, over which the investigators has no control, such as mutual funds or blind trusts are not restricted under these policies.

13.6.5 Reporting of Financial Disclosures and Other Activities

The investigators agree to update their financial disclosures and related activities as described above on an annual basis and submit these data to the Clinical Coordinating Center for storage. The Clinical Coordinating Center staff maintain the confidentiality of these records and prepare any reports indicating a potential conflict of interest for review by the Management Committee. In the case of actual or perceived conflict of interest, the Study Chairman brings it to the attention of the Management Committee, NIA Project Office, and the Data and Safety Monitoring Board.

13.6.6 Review of Policy Statement

The investigators agree to review these guidelines on an annual basis and take any additional steps to insure that the scientific integrity of the study remains intact.

13.6.7 Relationship to Institutional Policies on Conflict of Interest

Since existing policies on conflict of interest vary among participating institutions, in addition to the above policy, it is expected that investigators comply with the policies on conflict of interest which exist within their individual participating institutions (medical schools and hospitals). This is the responsibility of each individual investigator.

13.7 HUMAN SUBJECTS TRAINING

All N-TA^{3}CT investigators and staff who have any contact with N-TA^{3}CT patients, with health care providers treating N-TA^{3}CT patients, with individual patients' N-TA^{3}CT data or specimens must complete approved training in human subjects research and provide documentation of current training to the Clinical Coordinating Center annually.

EXHIBIT 13-1 – Conflict of Interest Statement for N-TA^{3}CT Investigators

CONFIDENTIAL

Except as noted below:

- I am not a part-time, full-time, paid or unpaid employee of any organizations: (a) whose products or services will be used or tested in the study under review, or (b) whose products or services would be directly and predictably affected in a major way by the outcome of the study;
- I am not an officer, member, owner, trustee, or director of such organizations;
- I do not have any financial interests or assets in any organizations meeting the above criteria, nor do my spouse, dependent children, nor organizations with which I am connected.

PLEASE COMPLETE THE APPROPRIATE BOX BELOW

☐ NO RELEVANT INTERESTS OR ACTIVITIES.

☐ EXCEPTIONS ARE NOTED IN THE ATTACHED LETTER.

I will notify the Clinical Coordinating Center Principal Investigator promptly if:

- A change occurs in any of the above during the tenure of my responsibilities, or
- I discover that an organization with which I have a relationship meets the criteria for a conflict of interest.

I am aware of my responsibilities for maintaining the confidentiality of any non-public information that I receive or become aware of through this activity, and for avoiding using such information for my personal benefit, the benefit of my associates, or the benefit of organizations with which I am connected or with which I have a financial involvement.

_____	_____	_____
Investigator (type name)	Signature	Date

References

1. Szilagyi DE, Smith RF, DeRusso FJ, Elliott JP, and Sherrin FW. Contribution of abdominal aortic aneurysmectomy to prolongation of life. Ann Surg 1966;164:678-699.
2. Lederle,FA, Johnson GR, Wilson SE, Ballard DJ, Jordan WD, Jr, Blebea J, Littooy FN, Freischlag JA, Bandyk D, Rapp JH, et al. Rupture rate of large abdominal aortic aneurysms in patients refusing or unfit for elective repair. JAMA 2002;287:2968-2972.
3. Lederle FA, Johnson GR, Wilson SE, Gordon IL, Chute EP, Littooy FN, Krupski WC, Bandyk D, Barone GW, Graham LM, et al. Relationship of age, gender, race, and body size to infrarenal aortic diameter. The Aneurysm Detection and Management (ADAM) Veterans Affairs Cooperative Study Investigators. J Vasc Surg 1997;26:595-601.
4. Lederle FA, Wilson SE, Johnson GR, Reinke DB, Littooy FN, Acher CW, Ballard DJ, Messina LM, Gordon IL, Chute EP, et al. Immediate repair compared with surveillance of small abdominal aortic aneurysms. N Engl J Med 2002;346:1437-1444.
5. Mortality results for randomised controlled trial of early elective surgery or ultrasonographic surveillance for small abdominal aortic aneurysms. The UK Small Aneurysm Trial Participants. Lancet 1998; 352:1649-1655.
6. Ernst CB. Current therapy for infrarenal aortic aneurysms. N Engl J Med 1997; 336:59-60.
7. Bengtsson H, Bergqvist D, Ekberg O, and Ranstam J. Expansion pattern and risk of rupture of abdominal aortic aneurysms that were not operated on. Eur J Surg 1993;159:461-467.
8. Scott RA, Wilson NM, Ashton HA, and Kay DN. Influence of screening on the incidence of ruptured abdominal aortic aneurysm: 5-year results of a randomized controlled study. Br J Surg 1995;82:1066-1070.
9. Ashton HA, Buxton MJ, Day NE, Kim LG, Marteau TM, Scott RA, Thompson SG, and Walker NM. The Multicentre Aneurysm Screening Study (MASS) into the effect of abdominal aortic aneurysm screening on mortality in men: a randomised controlled trial. Lancet 2002;360:1531-1539.
10. Lindholt JS, Juul S, Fasting H, and Henneberg EW. Hospital costs and benefits of screening for abdominal aortic aneurysms. Results from a randomised population screening trial. Eur J Vasc Endovasc Surg 2002;23:55-60.
11. Cowan J, Dimick J, Henke P, Rectenwald J, Stanley J, and Upchurch G. Epidemiology of Aortic Aneurysm Repair in the United States from 1993 to 2003. Annals of the New York Academy of Sciences 2006;1085:1-10.
12. Alcorn HG, Wolfson SK, Jr, Sutton-Tyrrell K, Kuller LH, and O'Leary D. Risk factors for abdominal aortic aneurysms in older adults enrolled in The Cardiovascular Health Study. Arterioscler Thromb Vasc Biol 1996;16:963-970.
13. Lederle FA, Johnson GR, Wilson SE, Chute EP, Littooy FN, Bandyk D, Krupski WC, Barone GW, Acher CW, and Ballard DJ. Prevalence and associations of abdominal aortic aneurysm detected through screening. Aneurysm Detection and Management (ADAM) Veterans Affairs Cooperative Study Group. Ann Intern Med 1997;126:441-449.
14. US Census Bureau-American FactFinder. The US Census Bureau.
15. Burton EC. The autopsy: a professional responsibility in assuring quality of care. Am J Med Qual 2002;17:56-60.
16. Lindholt JS, Juul S, Fasting H, and Henneberg EW. Screening for abdominal aortic aneurysms: single centre randomised controlled trial. Bmj 2005;330:750.
17. Lederle FA. Ultrasonographic screening for abdominal aortic aneurysms. Ann Intern Med 2003;139:516-522.

18. Propranolol for small abdominal aortic aneurysms: results of a randomized trial. J Vasc Surg 2002; 35:72-79.
19. Schlosser FJ, Tangelder MJ, Verhagen HJ, van der Heijden GJ, Muhs BE, van der Graaf Y, and Moll FL. Growth predictors and prognosis of small abdominal aortic aneurysms. J Vasc Surg 2008;47:1127-1133.
20. Darling RC, Messina CR, Brewster DC, and Ottinger LW. Autopsy study of unoperated abdominal aortic aneurysms. The case for early resection. Circulation 1977;56:II161-164.
21. Wilmink AB, Quick CR, Hubbard CS., and Day NE. Effectiveness and cost of screening for abdominal aortic aneurysm: results of a population screening program. J Vasc Surg 2003;38:72-77.
22. Lindholt JS, Juul S, Fasting H, and Henneberg EW. Cost-effectiveness analysis of screening for abdominal aortic aneurysms based on five year results from a randomised hospital based mass screening trial. Eur J Vasc Endovasc Surg 2006;32:9-15.
23. Long-term outcomes of immediate repair compared with surveillance of small abdominal aortic aneurysms. N Engl J Med 2002; 346:1445-1452.
24. Brady AR, Thompson SG, Fowkes FG, Greenhalgh RM, and Powell JT. Abdominal aortic aneurysm expansion: risk factors and time intervals for surveillance. Circulation 2004;110:16-21.
25. Yeung JJ, Kim HJ, Abbruzzese TA, Vignon-Clementel IE, Draney-Blomme MT, Yeung KK, Perkash I, Herfkens RJ, Taylor CA, and Dalman RL. Aortoiliac hemodynamic and morphologic adaptation to chronic spinal cord injury. J Vasc Surg 2006;44:1254-1265.
26. Vollmar JF, Paes E, Pauschinger P, Henze E, and Friesch A. Aortic aneurysms as late sequelae of above-knee amputation. Lancet 1989;2:834-835.
27. Lederle FA, Nelson DB, and Joseph AM. Smokers' relative risk for aortic aneurysm compared with other smoking-related diseases: a systematic review. J Vasc Surg 2003;38:329-334.
28. Powell JT, Worrell P, MacSweeney ST, Franks PJ, and Greenhalgh RM. Smoking as a risk factor for abdominal aortic aneurysm. Ann N Y Acad Sci 1996;800:246-248.
29. Lindholt JS, Heegaard NH, Vammen S, Fasting H, Henneberg EW, and Heickendorff L. Smoking, but not lipids, lipoprotein(a) and antibodies against oxidised LDL, is correlated to the expansion of abdominal aortic aneurysms. Eur J Vasc Endovasc Surg 2001; 21:51-56.
30. Bergoeing MP, Arif B, Hackmann AE, Ennis TL, Thompson RW, and Curci JA. Cigarette smoking increases aortic dilatation without affecting matrix metalloproteinase-9 and -12 expression in a modified mouse model of aneurysm formation. J Vasc Surg 2007;45:1217-1227.
31. Gadowski GR, Pilcher DB, and Ricci MA. Abdominal aortic aneurysm expansion rate: effect of size and beta-adrenergic blockade. J Vasc Surg 1994;19:727-731.
32. Leach SD, Toole AL, Stern H, DeNatale RW, and Tilson MD. Effect of beta-adrenergic blockade on the growth rate of abdominal aortic aneurysms. Arch Surg 1988;123:606-609.
33. Lindholt, JS, Henneberg EW, Juul S, and Fasting H. Impaired results of a randomised double blinded clinical trial of propranolol versus placebo on the expansion rate of small abdominal aortic aneurysms. Int Angiol 1999;18:52-57.
34. Gotto AM, Jr. Role of C-reactive protein in coronary risk reduction: focus on primary prevention. Am J Cardiol 2007;99:718-725.

35. Lederle FA, Kane RL, MacDonald R, and Wilt TJ. Systematic review: repair of unruptured abdominal aortic aneurysm. Ann Intern Med 2007;146:735-741.
36. Iribarren C, Darvinian JA, Go AS, Fireman BH, Lee CD, and Grey DP. Traditional and novel risk factors for clinically diagnosed abdominal aortic aneurysm: The Kaiser multiphasic health checkup cohort study. . Ann Epidemiology 2007; E pub ahead of print.
37. Steinmetz EF, Buckley C, Shames ML, Ennis TL, Vanvickle-Chavez SJ, Mao D, Goeddel LA, Hawkins CJ, and Thompson RW. Treatment with simvastatin suppresses the development of experimental abdominal aortic aneurysms in normal and hypercholesterolemic mice. Ann Surg 2005;241:92-101.
38. Evans J, Powell JT, Schwalbe E, Loftus IM, and Thompson MM Simvastatin Attenuates the Activity of Matrix Metalloprotease-9 in Aneurysmal Aortic Tissue. Eur J Vasc Endovasc Surg. 2007.
39. Sukhija R, Aronow WS, Sandhu R, Kakar P, and Babu S. Mortality and size of abdominal aortic aneurysm at long-term follow-up of patients not treated surgically and treated with and without statins. Am J Cardiol 2006;97:279-280.
40. Lederle FA, Larson JC, Margolis KL, Allison MA, Freiberg MS, Cochrane BB, Graettinger WF, and Curb JD. Abdominal aortic aneurysm events in the women's health initiative: cohort study. Bmj 2008;337:a1724.
41. Liao S, Miralles M, Kelley BJ, Curci JA, Borhani M, and Thompson RW. Suppression of experimental abdominal aortic aneurysms in the rat by treatment with angiotensin-converting enzyme inhibitors. J Vasc Surg 2001;33:1057-1064.
42. Daugherty A, Manning MW, and Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. J Clin Invest 2000;105:1605-1612.
43. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, Ramirez F, Sakai LY, and Dietz HC. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. Nat Genet 2003;33:407-411.
44. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, Myers L, Klein EC, Liu G, Calvi C, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 2006;312:117-121.
45. Daugherty A, and Cassis LA. Mechanisms of abdominal aortic aneurysm formation. Curr Atheroscler Rep 2002;4:222-227.
46. Hackam DG, Thiruchelvam D, and Redelmeier DA. Angiotensin-converting enzyme inhibitors and aortic rupture: a population-based case-control study. Lancet 2006;368:659-665.
47. Lederle FA, and Taylor BC. ACE inhibitors and aortic rupture. Lancet 2006;368:1571; author reply 1572.
48. Schouten O, van Laanen JH, Boersma E, Vidakovic R, Feringa HH, Dunkelgrun M, Bax JJ, Koning J, van Urk H, and Poldermans D. Statins are associated with a reduced infrarenal abdominal aortic aneurysm growth. Eur J Vasc Endovasc Surg 2006;32:21-26.
49. Burkhardt U, Zahn R, Hoffler U, Siegler KE, Frilling B, Weber M, Gottwik M, Wehr M, Seidel F, Rosocha S, et al. Antibody levels against Chlamydia pneumoniae and outcome of roxithromycin therapy in patients with acute myocardial infarction. Results from a sub-study of the randomised Antibiotic Therapy in Acute Myocardial Infarction (ANTIBIO) trial. Z Kardiol 2004;93:671-678.

50. Lindholt JS, Ashton HA, and Scott RA. Indicators of infection with Chlamydia pneumoniae are associated with expansion of abdominal aortic aneurysms. J Vasc Surg 2001;34:212-215.
51. Lindholt JS, Stovring J, Andersen PL, Henneberg EW, and Ostergaard L. A review of macrolide treatment of atherosclerosis and abdominal aortic aneurysms. Curr Drug Targets Infect Disord 2003;3:55-63.
52. Vammen S, Lindholt JS, Ostergaard L, Fasting H, and Henneberg EW. Randomized double-blind controlled trial of roxithromycin for prevention of abdominal aortic aneurysm expansion. Br J Surg 2001;88:1066-1072.
53. Petrinec D, Liao S, Holmes DR, Reilly JM, Parks WC, and Thompson RW. Doxycycline inhibition of aneurysmal degeneration in an elastase-induced rat model of abdominal aortic aneurysm: preservation of aortic elastin associated with suppressed production of 92 kD gelatinase. J Vasc Surg 1996;23:336-346.
54. Curci JA, Petrinec D, Liao S, Golub LM, and Thompson RW. Pharmacologic suppression of experimental abdominal aortic aneurysms: acomparison of doxycycline and four chemically modified tetracyclines. J Vasc Surg 1998;28:1082-1093.
55. Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, and Baxter BT. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. J Clin Invest 2002;110:625-632.
56. Prall AK, Longo GM, Mayhan WG, Waltke EA, Fleckten B, Thompson RW, and Baxter BT. Doxycycline in patients with abdominal aortic aneurysms and in mice: comparison of serum levels and effect on aneurysm growth in mice. J Vasc Surg 2002;35:923-929.
57. Baxter BT, Pearce WH, Waltke EA, Littooy FN, Hallett JW, Jr, Kent KC, Upchurch GR, Jr, Chaikof EL, Mills JL, Fleckten B, et al. Prolonged administration of doxycycline in patients with small asymptomatic abdominal aortic aneurysms: report of a prospective (Phase II) multicenter study. J Vasc Surg 2002;36:1-12.
58. Curci JA, Mao D, Bohner DG, Allen BT, Rubin BG, Reilly JM, Sicard GA, and Thompson, R.W. Preoperative treatment with doxycycline reduces aortic wall expression and activation of matrix metalloproteinases in patients with abdominal aortic aneurysms. J Vasc Surg 2000;31:325-342.
59. Morrison CJ, Butler GS, Bigg HF, Roberts CR, Soloway PD, and Overall CM. Cellular activation of MMP-2 (gelatinase A) by MT2-MMP occurs via a TIMP-2-independent pathway. J Biol Chem 2001;276:47402-47410.
60. Mosorin M, Juvonen J, Biancari F, Satta J, Surcel HM, Leinonen M, Saikku P, and Juvonen T. Use of doxycycline to decrease the growth rate of abdominal aortic aneurysms: a randomized, double-blind, placebo-controlled pilot study. J Vasc Surg 2001;34:606-610.
61. Dobrin PB, and Mrkvicka R. Failure of elastin or collagen as possible critical connective tissue alterations underlying aneurysmal dilatation. Cardiovasc Surg 1994;2:484-488.
62. Curci JA, Liao S, Huffman MD, Shapiro SD, and Thompson RW. Expression and localization of macrophage elastase (matrix metalloproteinase-12) in abdominal aortic aneurysms. J Clin Invest 1998;102:1900-1910.
63. Davis V, Persidskaia R, Baca-Regen L, Itoh Y, Nagase H, Persidsky Y, Ghorpade A, and Baxter BT. Matrix metalloproteinase-2 production and its binding to the matrix are increased in abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol 1998;18:1625-1633.

64. Webster MW, McAuley CE, Steed DL, Miller DD, and Evans CH. Collagen stability and collagenolytic activity in the normal and aneurysmal human abdominal aorta. Am J Surg 1991;161:635-638.
65. Carrell TW, Burnand KG, Wells, GM, Clements JM, and Smith A. Stromelysin-1 (matrix metalloproteinase-3) and tissue inhibitor of metalloproteinase-3 are overexpressed in the wall of abdominal aortic aneurysms. Circulation 2002;105:477-482.
66. Longo GM, Buda SJ, Fiotta N, Xiong W, Griener T, Shapiro S, and Baxter BT. MMP-12 has a role in abdominal aortic aneurysms in mice. Surgery 2005;137:457-462.
67. Cho BS, Roelofs KJ, Ford JW, Henke PK, and Upchurch GR, Jr. Decreased collagen and increased matrix metalloproteinase-13 in experimental abdominal aortic aneurysms in males compared with females. Surgery 2010;147:258-267.
68. van den Berg WB. Osteoarthritis year 2010 in review: pathomechanisms. Osteoarthritis Cartilage 2011;19:338-341.
69. Kontogiorgis CA, Papaioannou P, and Hadjipavlou-Litina DJ. Matrix metalloproteinase inhibitors: a review on pharmacophore mapping and (Q)SARs results. Curr Med Chem 2005; 12:339-355.
70. Wang X, Li KF, Adams E, and Van Schepdael A. Matrix metalloproteinase inhibitors: a review on bioanalytical methods, pharmacokinetics and metabolism. Curr Drug Metab 2011; 12:395-410.
71. Sampsonas F, Karkoulias K, Kaparianos A, and Spiropoulos K. Genetics of chronic obstructive pulmonary disease, beyond a1-antitrypsin deficiency. Curr Med Chem 2006; 13:2857-2873.
72. Ketelhuth DF, and Back M. The role of matrix metalloproteinases in atherothrombosis. Curr Atheroscler Rep 2011; 13:162-169.
73. Pyo R. Lee JK, Shipley JM, Curci JA, Mao D, Ziporin SJ, Ennis TL, Shapiro SD, Senior RM, and Thompson RW. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. J Clin Invest 2000; 105:1641-1649.
74. McMillan WD, Tamarina NA. Cipollone M, Johnson DA, Parker MA, and Pearce WH. Size matters: the relationship between MMP-9 expression and aortic diameter. Circulation 1997; 96:2228-2232.
75. Golub LM, Goodson JM, Lee HM, Vidal AM, McNamara TF, and Ramamurthy NS. Tetracyclines inhibit tissue collagenases. Effects of ingested low-dose and local delivery systems. J Periodontol 1985; 56:93-97.
76. Golub LM. Ramamurthy NS, McNamara TF, Greenwald RA, and Rifkin BR. Tetracyclines inhibit connective tissue breakdown: new therapeutic implications for an old family of drugs. Crit Rev Oral Biol Med 1991; 2:297-321.
77. Abdul-Hussien H, Hanemaaijer R, Verheijen JH, van Bockel JH, Geelkerken RH, and Lindeman JH. Doxycycline therapy for abdominal aneurysm: Improved proteolytic balance through reduced neutrophil content. J Vasc Surg 2009; 49:741-749.
78. Axisa B, Loftus IM, Naylor AR, Goodall S, Jones L, Bell PR, Thompson MM. Prospective, randomized, double-blind trial investigating the effect of doxycycline on matrix metalloproteinase expression within atherosclerotic carotid plaques. Stroke 2002; 33(12):2858-64.

79. Kloppenburg M, Breedveld FC, Terwiel JP, Mallee C, Dijkmans BA. Minocycline in active rheumatoid arthritis. A double-blind, placebo-controlled trial. Arthritis Rheum 1994; 37(5):629-36.
80. Tilley BC, Alarcón GS, Heyse SP, Trentham DE, Neuner R, Kaplan DA, Clegg DO, Leisen JC, Buckley L, Cooper SM, Duncan H, Pillemer SR, Tuttleman M, Fowler SE. Minocycline in rheumatoid arthritis. A 48-week, double-blind, placebo-controlled trial. MIRA Trial Group. Ann Intern Med 1995; 122(2):81-9.
81. O'Dell JR, Blakely KW, Mallek JA, Eckhoff PJ, Leff RD, Wees SJ, Sems KM, Fernandez AM, Palmer WR, Klassen LW, Paulsen GA, Haire CE, Moore GF. Treatment of early seropositive rheumatoid arthritis: a two-year, double-blind comparison of minocycline and hydroxychloroquine. Arthritis Rheum 2001; 44(10):2235-41.
82. O'Dell JR, Haire CE, Palmer W, Drymalski W, Wees S, Blakely K, Churchill M, Eckhoff PJ, Weaver A, Doud D, Erikson N, Dietz F, Olson R, Maloley P, Klassen LW, Moore GF. Treatment of early rheumatoid arthritis with minocycline or placebo: results of a randomized, double-blind, placebo-controlled trial. Arthritis Rheum 1997; 40(5):842-8.
83. Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A, Anca-Hershkowitz M, Sadeh M. Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. Neurology 2007; 69(14):1404-10.
84. Gompels LL, Smith A, Charles PJ, Rogers W, Soon-Shiong J, Mitchell A, Doré C, Taylor PW, Mackworth-Young CG. Single-blind randomized trial of combination antibiotic therapy in rheumatoid arthritis. J Rheumatol 2006; 33(2):224-7.
85. Barza M, and Schiefe RT. Antimicrobial spectrum, pharmacology and therapeutic use of antibiotics. Part 1: tetracyclines. Am J Hosp Pharm 1977; 34:49-57.
86. Geria AN, Tajirian AL, Kihiczak G, and Schwartz RA. Minocycline-induced skin pigmentation: an update. Acta Dermatovenerol Croat 2009; 17:123-126.
87. Abdul-Hussien H, Hanemaaijer R, Kleemann R, Verhaaren BF, van Bockel JH, and Lindeman JH. The pathophysiology of abdominal aortic aneurysm growth: corresponding and discordant inflammatory and proteolytic processes in abdominal aortic and popliteal artery aneurysms. J Vasc Surg 2010; 51:1479-1487.
88. Ramos-Mozo P, Madrigal-Matute J, Martinez-Pinna R, Blanco-Colio LM, Antonio Lopez J, Camafeita E, Meilhac O, Michel JB, Aparicio C, de Ceniga MV, et al. Proteomic Analysis of Polymorphonuclear Neutrophils Identifies Catalase as a Novel Biomarker of Abdominal Aortic Aneurysm. Arterioscler Thromb Vasc Biol 2011.
89. Eliason JL, Hannawa KK, Ailawadi G, Sinha I, Ford JW, Deogracias MP, Roelofs KJ, Woodrum DT, Ennis TL, Henke PK, et al. Neutrophil depletion inhibits experimental abdominal aortic aneurysm formation. Circulation 2005; 112:232-240.
90. Meijer CA, Stijnen T, Wasser M, Hamming JF, Hajo van Bockel J, Lindeman J., for the Pharmaceutical Aneurysm Stabilisation Trial Study Group. Doxycycline for Stabilisation of Abdominal Aortic Aneurysms: A Randomized Trial. Annals of Internal Medicine 2013; 159:815-828.
91. Hovsepian DM, Ziporin SJ, Sakurai MK, Lee JK, Curci JA, and Thompson RW. Elevated plasma levels of matrix metalloproteinase-9 in patients with abdominal aortic aneurysms: a circulating marker of degenerative aneurysm disease. J Vasc Interv Radiol 2000; 11:1345-1352.

92. Hackmann AE, Rubin BG, Sanchez LA, Geraghty PA, Thompson RW, and Curci JA. A randomized, placebo-controlled trial of doxycycline after endoluminal aneurysm repair. J Vasc Surg 2008; 48:519-526; discussion 526.
93. Rubin DB. Multiple Imputations after 18+ years. Journal of the American Statistical Association 1996; 91:473-489.
94. Conover WJ. Practical Nonparametric Statistics, 3rd edition. New York: Wiley, 1999:396 ff.
95. Haybittle JL. "Repeated assessments of results in clinical trials of cancer treatment". Brit. J. Radiol 1971; 44 (526): 793–797. doi:10.1259/0007-1285-44-526-793.
96. Peto R, Pike MC, Armitage P, et al. "Design and analysis of randomized clinical trials requiring prolonged observation of each patient. I. Introduction and design". Brit. J. Cancer 1976; 34 (6): 585–612. doi:10.1038/bjc.1976.220. PMC 2025229. PMID 795448. http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pmcentrez&artid=2025229.
97. Futility Analysis by Jon Nicholl presented in 2008 in the RSS/NIHR MTA/MRC Workshop on Stopping Trials for Futility (http://www.sheffield.ac.uk/polopoly_fs/ 1.44156!/file/Futility-meeting-Nicholl2.ppt)
98. Snedecor GW and Cochran WG. Analysis of covariance. Statistical Methods, 6th edition. Ames, IA: Iowa State University Press, 1967:419-44.
99. McMahon RP and Harrell FE, Jr. Power Calculation for Clinical Trials when the Outcome is a Composite Ranking of Survival and a Nonfatal Outcome. Controlled Clinical Trials 2000; 21:305-312.
100. Charache SC, Terrin ML, and Moore RD et al. Effect of Hydroxyurea on the Frequency of Painful Crises in Sickle Cell Anemia. New England Journal of Medicine 1995; 332:1317-1322.
101. Charache SC, Terrin ML, Moore RD, Dover GJ, McMahon RP, Barton FB, Waclawiw, M, Eckert SV, and Investigators of the Multicenter Study of Hydroxyurea. Design of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Controlled Clinical Trials 1995; 432-446.
102. Fleiss JL. Statistical Methods for Rates and Proportions, 2nd edition. New York: Wiley, 1981:19-32, 56-82, 165-173.
103. Hosmer DW and Lemeshow S. Applied Logistic Regression. New York: John Wiley & Sons, 1989:25-37, 106-118.
104. Agresti A. Logit Models. Analysis of Ordinal Categorical Data. New York: Wiley, 1984:104-137.
105. Kalbfleisch JD and Prentice RL. The Statistical Analysis of Failure Time Data. New York: Wiley, 1980:122-127.
106. Snedecor JW and Cochran WC. Statistical Methods, 6th edition. Ames, IA: Iowa State University Press, 1967:91-116.
107. Zeger SL, Liang KY and Albert PS. Modifier longitudinal data: a generalized estimating equation approach. Biometrics 1988; 44:1049-1060.
108. Schluchter MD. Methods for the analysis of informatively censored longitudinal data. Statistics in Medicine 1992; 11:1861-1870.
109. Frison L and Pocock SJ. Repeating Measures in Clinical Trials: Analysis using Mean Summary Statistics and its Implications for Design. Statistics in Medicine 1992; 1685-1704.

110. Rosner B. Fundamentals of Biostatistics, 6th edition. Belmont, CA: Thomson Brooks/Cole, 2006:421-423.

Appendix – N-TA^{3}CT Procedures and Treatments

Study Procedures/Treatments	Study Visit Baseline	3 Months ± 45 days	6 Months ± 45 days	9 Months ± 45 days	1 Year ± 45 days	15 Months ± 45 days	18 Months ± 45 days	21 Months ± 45 days	2 Years ± 45 days‡	30 Months ± 45 days
Screen for Inclusion/Exclusion	X									
Consent, Questions, Signature	X									
Randomization	X									
Vital Signs	X	X	X	X	X	X	X	X	X	
Medical Review	X	X	X	X	X	X	X	X	X	X
Death		X	X	X	X	X	X	X	X	X
Compliance		X	X	X	X	X	X	X	X	
AE's		X	X	X	X	X	X	X	X	X
Current Therapies		X	X	X	X	X	X	X	X	X
SF-36	X		X		X		X		X	X
CT Scan Performed	X		X		X		X		X	X****
Send CT Scan To Imaging Laboratory	X		X		X		X		X	X
Biomarkers Core Lab Assays*										
Doxycycline Levels	X		X		X		X		X	X
hsCRP's	X		X		X		X		X	X
MMP-9	X		X		X		X		X	X
Cotinine Levels	X		X		X		X		X	X
Interferon Gamma	X		X		X		X		X	X
DNA**			X							
Local Labs (standard of care)***										
Complete Blood Count***	X				X				X	
Liver Function Tests*** (alanine aminotransferase; aspartate aminotransferase)	X				X				X	
Blood Urea Nitrogen***	X				X				X	
Creatinine***	X				X				X	
Lipid Panel***	X				X				X	
Dispense Emergency Card	X									
Dispense Study Drug	X	X	X	X	X	X	X	X		
Collect Study Drug		X	X	X	X	X	X	X	X	
Dispense Diary	X	X	X	X	X	X	X	X	X	

* At Baseline and Year 2 visits draw 2 plasma (green) and 2 serum (red) kits. All other visits only require 1 plasma (green) and 1 serum (red) kit.
** DNA should be drawn at the 6-month visit. If it is missed it can be drawn at the 1 year or 18 month visit.
*** These tests may be collected and data entered any time within one year prior to the indicated due date. Please see text on next page for clarification of events.
**** CT scan if available.
‡ With the elimination of 27-month visits the window for the 2-year visit is extended forward to +90 days and for the 30-month visit backward to -90 days to maintain contiguity.

Standard of care monitoring for patients who are treated for abdominal aortic aneurysm or coronary artery disease or peripheral arterial disease or who are treated with or may be treated with these medications may include an annual complete blood count and analyses of circulating blood urea nitrogen (BUN), creatinine, liver enzyme and lipid levels.

Because personal physicians may be ordering the routine laboratory analyses independently of our clinical trial and to avoid the possibility of coverage refusals, recover the most recent complete blood count, BUN, creatinine, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) and lipid levels that have been collected within one year. If lipid analyses have not been performed, the coordinators may let the lipid analyses go, and if a complete blood count has been performed but no white blood cell differential (neutrophil %, lymphocyte %, eosinophil % and basophil %) the differential cell count can be let go; they are of interest and may be data entered but are not essential for safety monitoring by investigators who are not taking responsibility for the patients' management outside of N-TA^{3}CT.

The complete blood count (CBC), BUN, creatinine and ALT or AST levels are necessary for our safety monitoring. If they have not been collected within a year prior to expectation, collect blood for these specific tests. Obtain a differential white blood cell count along with the CBC. These blood tests may be entered anytime within the one-year window.