Seattle Children's Hospital

ETANERCEPT IN KAWASAKI DISEASE
IND 101,223

A randomized, double blind, placebo controlled study of the effects of etanercept in subjects presenting with Kawasaki Disease

Research Study Protocol

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Study Product: Etanercept (trade name: Enbrel)

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List of Abbreviations

AE: adverse event
AHA: American Heart Association
AAP: American Academy of Pediatrics
AST: aspartate aminotransferase
ALT: alanine aminotransferase
CBC: complete blood count
CHO: Chinese hamster ovary
CHRMC: Children's Hospital and Regional Medical Center
CRC: Children's Research Institute Clinical Research Center
CRF: case report form
CRP: C-reactive protein
DMC: Data Monitoring Committee
DNA: deoxyribonucleic acid
Echo: echocardiogram
EKG: electrocardiogram
ESR: erythrocyte sedimentation rate
FDA: Federal Drug Administration
HIPAA: Health Insurance Portability and Accountability Act of 1996
Ig: immunoglobulin
IV: intravenous
IVIG: intravenous immunoglobulin
IRB: Institutional Review Board
KD: Kawasaki Disease
PCRC: Pediatric Clinical Research Center
PHI: private health information
PI: Principal Investigator
PK: pharmacokinetics
PPD: purified protein derivative
SAE: serious adverse event
TNF: tumor necrosis factor
TNFR: tumor necrosis factor receptor

Study Summary

Title	Etanercept in Kawasaki Disease
Study Phase	Clinical phase II study
Methodology	Randomized double blind placebo controlled clinical study
Study Duration	4 years
Study Centers	Children's Hospital and Regional Medical Center, Primary Children's Medical Center, Children's Hospital of Wisconsin, Sainte-Justine Hospital, Steven & Alexandra Cohen Children's Medical Center of New York, Texas Children's Hospital, Columbia University Medical Center, and Montefiore Medical Center.
Objectives	Determine if etanercept (0.8 mg/kg, maximum 50 mg) given subcutaneously reduces IVIG refractory rate and retreatment rate in the KD patient population.
Number of Subjects	196
Diagnosis and Main Inclusion Criteria	Patients aged 2 months to 20 years of age presenting with Kawasaki Disease as defined by the American Academy of Pediatrics and the American Heart Association.
Study Product, Dose, Route, Regimen	Etanercept (trade name: Enbrel) 3 weekly subcutaneous injections at 0.8 mg/kg dosage
Reference therapy	Single infusion of IVIG (2 g/kg) with aspirin

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to U.S. and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

We propose a clinical trial evaluating a novel therapy for Kawasaki Disease (Syndrome). We intend to use the data obtained in this study to apply for an Orphan Product Designation for Etanercept (brand name Enbrel). Kawasaki is classified as an autoimmune disease and disseminated vasculitis. It is characterized by fever, bilateral, nonexudative conjunctivitis, erythema of the lips and oral mucosa, changes in the extremities, rash, and cervical lymphadenopathy. Coronary artery aneurysms or ectasia develop and may lead to myocardial infarction (MI), sudden death, or ischemic heart disease. This disease is more prevalent in Japan and in children of Japanese ancestry with an annual incidence of 112 cases per 100 000 children < 5 years old. The KD incidence in the United States has been estimated from hospital discharge data [1] at approximately 4000 cases per year. Unfortunately, the data do not include many Western States such as Washington, which have large KD populations, and thus represent underestimates. Nevertheless, Holman et al estimated that 4248 hospitalizations associated with Kawasaki disease occurred in the United States in 2000 with a median age of 2 years.[2] Accordingly, KD is becoming a major health problem in the United States, but qualifies for Orphan Disease status as less than 200,000 individuals are affected. In the United States, the disease has surpassed Rheumatic Fever as the most frequent cause of acquired heart disease in children.

A combination of aspirin and intravenous gamma globulin (IVIG) is the current standard regimen for treatment of acute KD[3]. Although aspirin has important anti-inflammatory activity at high doses and antiplatelet activity at low doses, it does not alter the risk for coronary artery disease. Intravenous gamma globulin now represents the standard for treatment. IVIG is not a specific therapy and the mechanism of action in KD still requires clarification. Recent data support a hypothesis, that IVIG modulates immune response in KD through FcGamma receptors on inflammatory cells [4-7]. Some studies suggest that immune modulation by IVIG is impaired in patients with specific polymorphisms for FcGamma receptors. This hypothesis is currently under investigation in our own laboratory using a large cohort of patient DNA collected in a multi-center consortium.

Despite substantial research in the field including clinical trials, there has been no major shift in the treatment paradigm for acute Kawasaki Disease since the introduction and refinement of immune gamma globulin therapy in the 1980s [8]; and 1990s[9]. The vast majority of the research in this area focuses on epidemiology, identification of risk factors for coronary artery involvement, genetic susceptibility, refinement of current therapy such as the addition of corticosteroids, and the identification and treatment of long term sequela [10]. Recent studies report the incidence of persistent coronary artery involvement in the 15-20% range despite treatment and as high as 30% in non-responders to IVIG. [11] This growing affected population will age, and the impact of coronary artery lesions attained in childhood may be immense. Recent reports from Japan indicate that the mortality rate in males with coronary artery lesions is more than twice expected.[10]. Therefore, the primary goals of therapy should be directed at reducing coronary artery dilation in addition to treating active inflammation.

Efficacy of current treatment: The joint statement of the American Heart Association and the American Academy of Pediatrics states emphatically that "the efficacy of IVIG administered in the acute phase of Kawasaki disease in reducing the prevalence of coronary artery abnormalities is well established." [3] The first major controlled randomized trials in KD patients in the United States (1986) showed superior prevention of coronary artery disease in children with acute KD by IVIG and ASA versus ASA alone[8]. Subsequently, Newburger et al showed that a single high dose of IVIG (2g/kg) administered over 10 hours reduced the

prevalence of coronary abnormalities compared to daily infusions of 400 mg per kilogram for four consecutive days. Both groups also received ASA (100 mg per kilogram per day) for 14 days [12]. Accordingly, AHA/AAP guidelines recommend IVIG 2g/kg in a single dose with some modifications in ASA dosing. In Japan, where prevalence of KD is substantially higher than in the U.S., IVIG is usually given two separate 1g/kg doses on consecutive days [13].

Although the historical studies show that IVIG prevents coronary artery dilation [8, 12], the failure rate of this nonspecific treatment for KD remains high[14]. It is difficult to determine current IVIG efficacy, as criteria and technological advances have altered for reporting coronary artery disease (CAD), and we could not ethically perform a randomized placebo trial excluding affected patients from receiving IVIG. Discrepancies exist between rates of IVIG failure reported from Japan and the United States [13, 14]. These differences are partially due to methodology in defining coronary artery dilation. The discrepancies create challenges in interpretation and performing comparisons of clinical trials, as well as evaluation of new adjunctive therapies. For instance, Inoue et al reported that only 3.4% of patients had CAD by 1 month after receiving the standard Japanese IVIG regiment[13], while the most recent U.S. multicenter trial reported 18.9% CAD in all patients at 6 weeks, and 9% for patients without CAD at baseline[14].

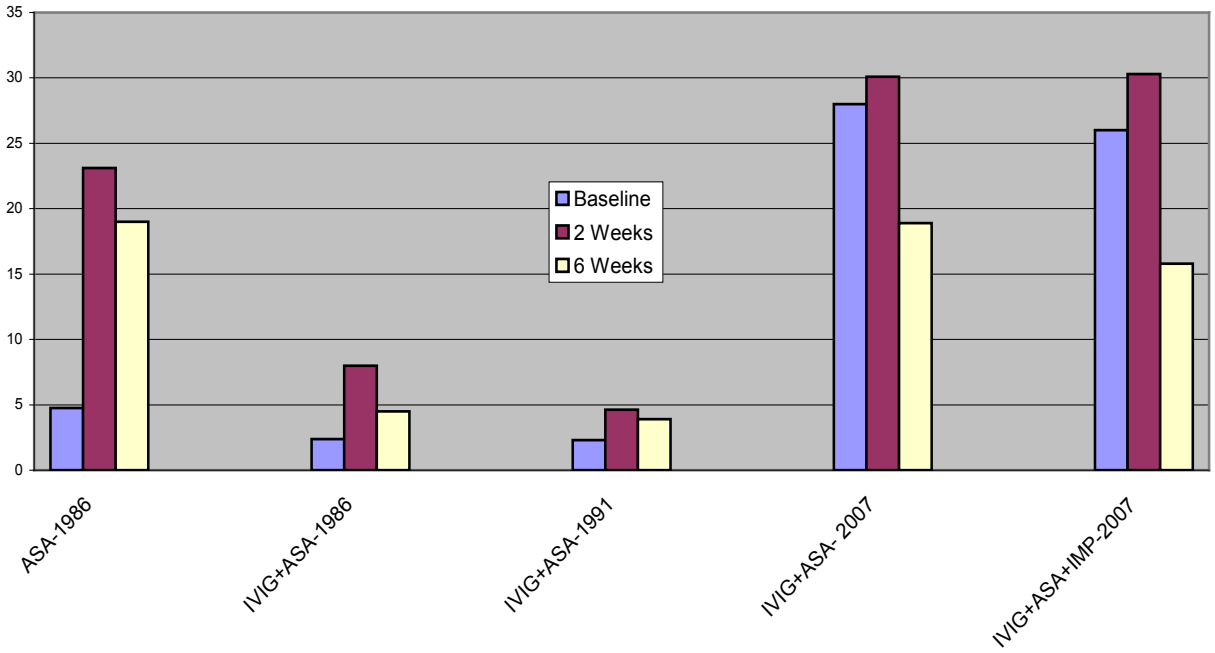


Figure 1. Percentage of patients with coronary artery disease extracted from several clinical studies performed in the U.S. Data is reported chronologically along x-axis, Newburger et al 1986[8], groups receiving ASA or IVIG + ASA; Newburger et al 1991[12], group receiving single dose IVIG 2gm/kg with ASA; Newburger et al, 2007[14], groups receiving IVIG 2gm/kg with ASA, or IVIG + ASA + pulse methyl-prednisilone. Note the discrepancy in CAD reporting between IVIG-ASA-1991 and IVIG-ASA-2007 (see text).

In figure 1, we show the incidence of coronary artery disease reported for three major multi-center clinical trials performed in the United States over a twenty year period. This figure clearly shows an increase in the reported rate of CAD in KD patients undergoing these trials between 1986 and 2007. The data suggest that changes in echocardiographic interpretation and reporting are largely responsible for the trend in the U.S. and differences with Japanese studies. The first paragraph of the 2004 AHA/AAP consensus statement, Newburger et al states: "coronary artery aneurysms or ectasia develop in ~15% to 25% of untreated children with the disease." [3] However as noted, the Pediatric Heart Network (PHN) study in New England Journal 2007 reports

18.9% of patients with CAD after standard IVIG treatment [14]. This rate for persistent CAD at 6 weeks falls within the range quoted in the 2004 consensus document for CAD in untreated patients. The recent PHN data show that 30% of all KD patients have CAD involvement by 1 week, and 9% of patients without abnormalities at baseline still develop CAD despite treatment with IVIG. Additionally, a recent retrospective review indicates that a high rate of persistent coronary artery dilation or aneurysms occurs in infants > 1 year of age despite treatment with IVIG [15].

Disappearance of fever within 48 hours generally heralds a successful response to therapy. However, Burns et al reported that 13% of 378 patients remained febrile after 48 hours [16]. From our institution Wallace et al reported 23% of patients either did not respond initially or had fever recurrence within 10 days [11]. New data from our institution suggest that the refractory rate may be somewhat higher (see section C). The published reports emphasize doubling in the relative risk of coronary artery disease in patients with initial IVIG treatment failure. Recent studies by the Pediatric Heart Network support the tenet that we have been under diagnosing CAD in the KD population [17]. However, the published multi-center study did not directly evaluate rate of CAD in patients, refractory to initial IVIG. Extrapolating from prior data relating two-fold increased risk in patients not receiving IVIG [8]suggests that CAD may occur in up to 35% in these patients at 6 weeks after initial IVIG, and supports estimates of increased risk published by Burns et al[18]and Wallace et al[11].

Physicians treating KD have recognized the limited efficacy of IVIG, and have searched for adjunctive therapies, which would lessen CAD and reduce the IVIG refractory rate. Corticosteroids are traditionally used in treatment of other types of vasculitis. An early study by Kato suggested that steroids exert a detrimental effect when used as initial treatment for KD[19]. Subsequent studies have established safety for corticosteroids during KD, but efficacy trials have shown mixed results with regard to reducing IVIG failure and CAD[13, 14]. Two recent prospective studies have been published examining these issues. Inoue et al performed a prospective non-blinded randomized study using standard IVIG and ASA with or without daily doses of IV methylprednisilone until defervescence followed by daily oral predinisolone until normalization of CRP[13]. In this multi-center Japanese study, the corticosteroid group showed a reduction in CAD early in the disease, which did not persist past 4 weeks. Initial IVIG treatment failure was reduced in the corticosteroid group. The primary end-point, CAD up to 1 month, was not determined in an unblinded fashion, and CAD was judged by Japanese Ministry of Health Criteria. The rates of CAD at all time points were therefore far less than reported in most recent studies performed in the U.S, which employ coronary artery z-scores to grade CAD[14]. This includes the recent study performed by Newburger and the Pediatric Heart Network Investigators[14]. The PHN multi-center placebo controlled and double blinded study showed that a single pulse methyl-prednisilone dose in addition to IVIG did not reduce the rate of CAD at any time-point in the disease up to 5 weeks after treatment. The rate of initial IVIG failure was also not reduced. In summary, these latter randomized studies, whether performed in a blinded design or not did not show important clinical change in CAD or alteration in IVIG resistance.

Data from Inoue et al suggest that more prolonged anti-inflammatory therapy in addition to IVIG and ASA may ameliorate early CAD in acute KD[13]. It is not clear whether this amelioration is related to a reduction in the rate of IVIG refractoriness or to the prolonged nature of the treatment compared to the standard regimen.

IVIG is a non-specific therapy, and its therapeutic mechanism in KD still requires clarification. The infusions require hospitalization and adverse events are common. Infusions are interrupted for fever spikes, which is obviously problematic in febrile illness such as KD.

Replacement of IVIG will be difficult due to ethical issues caused by the putative therapeutic response. Despite development of new, specific, and more powerful anti-inflammatory agents, it is unlikely that a randomized trial between IVIG and a new therapy could be approved by an Institutional Review Board. Accordingly, the first step would be substantiation of a new therapy as an important adjunctive therapy.

The overall data support the need for development or validation of adjunctive therapy for KD that should have the following attributes:

1) therapy is specific and directed at blocking an established inflammatory pathway, known to be activated in KD
2) treatment is prolonged extending at least 2 weeks after initial IVIG treatment and through the entire phase of acute KD and into the convalescent phase
3) therapy can be validated by maintenance of steady-state drug levels throughout the treatment period
4) therapy has an excellent safety profile in children
5) therapy is cost-effective and convenient

Tumor necrosis factor-α (TNF-α) in KD

Features of Kawasaki Disease including vascular inflammation, high fever and marked elevation of the acute phase protein, CRP, implicate cytokines as key role players in the pathogenesis. Several cytokines are elevated in the acute phase of KD including TNF-α, interleukin-1 and interleukin-6[20-24]. However, TNF-α has received particular attention for several reasons. TNF-α activation of macrophages includes enhanced production and release of TNF-α receptor[25]. Patients with KD demonstrate marked elevation in serum soluble TNF-α receptor with even higher levels in patients with coronary artery ectasia, despite IVIG treatment [26]. Immunochemistry studies show that CD14 peripheral blood monocytes/macrophages during acute KD strongly expressed TNF-α in their cytoplasm[27]. Expression of TNF-α weakens in the convalescent phase, suggesting that some of the intracytoplasmic granules in these monocytes/ macrophages are secretory granules, which secrete TNF-α[27]. TNF-α promotes migration of P-selectin and production of E-selectin promoting conformational changes in endothelium, which may promote vascular dilatation and coronary aneurysm noted in KD [28].

The hypothesis that TNFα promotes coronary artery inflammation is supported by data obtained in a well established mouse model of KD, created through intraperitoneal injection of L. Casei wall extract. These mice develop coronary artery inflammation, ectasia, and aneurysms, which histopathologically resemble coronary artery lesions seen in postmortem studies from children with KD. Coronary artery inflammation and vessel wall damage are absent in wild-type mice treated with the TNF-α receptor blocker, Etanercept (8 mg/kg twice weekly times four weeks, intraperitoneal), and in TNFRI-deficient ($TNFRI^{-/-}$) mice [20]. Coronary artery inflammation and ectasia does occur in mice deficient for TNFRII, which does not bind TNF-α in mice. Therefore, TNF-α plays an important role in the pathogenesis of coronary artery inflammation in this model. In summary, KD patients show activation of TNF-α pathway, and animal models show that TNF-α antagonism abrogates coronary artery inflammation.

Pentoxifylline is a methyl-xanthine compound and phosphodiesterase inhibitor that inhibits TNF-α messenger RNA transcription. A single study showed a trend towards improved prevention of CAD when using pentoxifylline in addition to ASA and low dose IVIG, compared to ASA and low dose (IVIG 1 gm/kg total)[29]. The study had relatively few patients and was likely not empowered to detect significant differences between groups. Furthermore, the subsequent development of more specific TNF antagonists relegates this strategy to obscurity. The study does suggest that early and prolonged antagonism of the TNF-α pathway might prevent CAD in KD patients.

The TNF antagonists fall into 2 classes: monoclonal antibodies and soluble receptors[30]. Etanercept (Enbrel®, Thousands Oaks, CA) is a soluble TNF receptor, while infliximab (Remicade®, Horsham, PA) and adalimumab (Humira®, Abbott Park, ILL) are monoclonal antibodies against TNF. These agents are now widely used for the treatment of rheumatoid arthritis including juvenile onset and other inflammatory diseases. As of December 2006, more than 400,000 patients worldwide had been treated with etanercept and more than 500,000 had been

treated with infliximab [31]. As of August 31, 2004, 10,050 patients with RA had enrolled in adalimumab clinical trials worldwide[31]. Infliximab is used for inflammatory bowel disease in children, while Etanercept is FDA approved for use in JRA for children down to 2 years of age.

1.2 Investigational Agent

Etanercept, a dimeric fusion protein, consists of 2 molecules of the extracellular ligand-binding portion of human TNF receptor 2 attached to the Fc domain of human IgG1[31]. Infliximab is a chimeric IgG1 monoclonal antibody composed of human constant and murine variable regions [32], whereas adalimumab is a recombinant human IgG1 monoclonal antibody[33]. Each infliximab or adalimumab molecule can bind up to 2 TNF molecules; a single TNF homotrimer can bind up to 3 molecules of infliximab or adalimumab [34]. In contrast, etanercept binds to the interface of 2 TNF subunits in a 1:1 ratio [35]. These differences in binding avidity suggest that Etanercept may be easier to titrate according to treatment effect without inducing adverse effects or resistance. However, it is also possible that the monoclonal antibodies may be more potent and effective inhibitors of TNF. The pharmacokinetics of the drugs delivered by subcutaneous route, etanercept and adalimumab, show that blood concentration levels are subjected to less severe peaks and troughs than infliximab, delivered by intravenous infusion[36, 37].

In 2004, isolated case reports of infliximab use for refractory KD began to appear in the literature[38]. Burns et al then retrospectively reviewed the experience of clinicians in the United States, who had used inflixmab for KD patients with either persistent vasculitis or persistent or recrudescent fever > 48 hours after treatment with IVIG [39]. None of these publications discuss exactly why infliximab was chosen over other TNF inhibitors. This is curious as pharmacokinetics and safety data in younger children, was and is available for Etanercept alone[40, 41]. The retrospective analysis performed by Burns et al showed that complete response, indicated by resolution of fever, was documented in 10/16 patients[39]. Infliximab therapy was initiated late in the disease and the majority of these patients already had coronary vasculitis prior to administration of a TNF-α blocker. One patient with severe coronary artery aneurysms preceding treatment died at home. The data from this retrospective review served as background to initiate an ongoing clinical phase I trial of infliximab versus repeat IVIG infusion for refractory KS. No information regarding the progress of this study has been published.

Etanercept for the proposed study is available and will be provided free of charge by Amgen (see supporting letter).

Etanercept is FDA-approved for use in the U.S. for reducing signs and symptoms of moderate to severe adult rheumatoid arthritis, moderate to severe juvenile rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and chronic moderate to severe plaque psoriasis. It has been approved for use in patients 2 years of age and older (ages ≤ 17 yrs for juvenile rheumatoid arthritis patients only). Since its market approval in November 1998, over 400,000 patients have been estimated to have received etanercept therapy[42].

Etanercept lowers concentration of proinflammatory cytokines such as TNF-α, IL-3 and IL-6 in patients with several inflammatory diseases[43, 44]. As noted above these cytokines, and in particular, TNF-α, are implicated as proinflammatory agents in KD. Thus, Etanercept modulation of these cytokines may reduce inflammation in KD.

Potential toxicity: Randomized, double-blind, placebo-controlled trials showed that etanercept treatment had significant clinical benefit with minimal toxicity in adults with active rheumatoid arthritis that did not respond to other disease modifying drugs[45-47]. In following, The Pediatric Rheumatology Collaborative Study Group evaluated safety and efficacy in children with polyarticular juvenile rheumatoid arthritis who did not tolerate or had an inadequate response to methotrexate[48]. Patients 4 to 17 years old received 0.4 mg of etanercept per

kilogram of body weight subcutaneously twice weekly for up to three months in the initial, open-label part of a multicenter trial. Those who responded to treatment then entered a double-blind study and were randomly assigned to receive either placebo or etanercept for four months or until a flare of the disease occurred. At the end of the open-label study, 51 of the 69 patients (74 percent) had responses to etanercept treatment. In the double-blind study, 21 of the 26 patients who received placebo (81 percent) withdrew because of disease flare, as compared with 7 of the 25 patients who received etanercept (28 percent) (P=0.003). The median time to disease flare with placebo was 28 days, as compared with more than 116 days with etanercept (P<0.001).

In the open-label study, the most common adverse events were injection-site reactions (39 percent of patients), upper respiratory tract infections (35 percent), headache (20 percent), rhinitis (16 percent), abdominal pain (16 percent), vomiting (14 percent), pharyngitis (14 percent), nausea (12 percent), gastrointestinal infection (12 percent), and rash (10 percent). In the double-blind study, there were no significant differences in the frequencies of adverse events between patients who received etanercept and those who received placebo. Injection-site reactions occurred in one patient in each of the treatment groups in the double-blind study. There were no laboratory abnormalities requiring urgent treatment in the etanercept group.

In an open label extension study after 4 years of treatment [49], the rate of SAEs was 0.13 per patient-year, and the rate of serious infections was 0.04 per patient-year, in a total etanercept exposure of 225 patient-years. Serious infections included postoperative chin implant infection (n=1), wound infection (n=1), severe gastroenteritis (n=1), herpes zoster (n =1), and appendicitis (n = 1). The investigators concluded that Etanercept offered sustained benefit and an acceptable safety profile in children with JRA down to age 4 years.

Heart Failure: TNF-α antagonists have been linked to heart failure. This may be a concern as many patients with KD have myocarditis. In fact, Etanercept (Enbrel) has not been implicated in worsening of heart failure. Two Enbrel studies for chronic heart failure were terminated due to lack of efficacy, not because of an increase in mortality or adverse events. However, a study with infliximab was stopped early because of increased mortality in the treatment group. In this regard, Enbrel appears to have a superior safety profile compared to Infliximab. In their review of TNFα antagonism and heart failure, Gullestad and Aukrust [50] highlight the differences between these two drugs. The chimeric anti-TNF-α antibody (Infliximab), which is studied in patients with CHF, directly binds to the transmembrane form of TNF-α, resulting in damage of TNF-α–expressing cells including cardiomyocytes by antibody-dependent cellular toxicity, by complement-dependent cytotoxic effector mechanisms, and by induction of apoptosis [51]. This is not an issue with Etanercept, which is a fusion protein containing two portions of the TNFα receptor. Burns et al review of KD patients receiving Infliximab shows no cases of heart failure or complications in KD patients, except for one death [39]. This patient died at home after diagnosis with very large coronary artery aneurysms, and had additional risk factors including multiple treatments with methyl-prednisolone and clopidogrel (no autopsy performed). Burns et al considered heart failure concerns in their review of these patients all of whom probably have subclinical myocarditis. No adverse effect on myocardial contractility was noted. Despite the study in adults with heart failure; showing increased mortality in infliximab patients, Dr. Jane Burns has a study registered on Clinicaltrials.gov entitled “Infliximab (Remicade) for Patients With Acute Kawasaki Disease”. Subsequent to the report in the European Journal of Heart Failure regarding suspension of the Enbrel studies, the FDA and Duke Clinical Research Institute reviewed cases reported to Medwatch of heart failure after therapy with tumor necrosis factor antagonists[52]. The vast majority of these patients were elderly with risk factors for senescent cardiomyopathy or ischemic heart disease. Only 4 patients on Etanercept younger than 50 years of age reported new-onset heart failure with the youngest at 29 years and the shortest duration of drug treatment 5 months. It is unclear if the heart failure related to primary disease or drug. Safety data in the Pediatric population has been reported for Polyarticular JRA[40, 48, 49]. No case of heart failure was reported in 69 patients receiving Etanercept in the open-label trial or in the 25 enrolled in the Etanercept trial. At present, there is absolutely no data

indicating increased risk of heart failure in young patients receiving Etanercept, and certainly no data substantiating increase risk after treatment with Etanercept for a very short term course, such as two or three weeks.

In summary: the data show that Etanercept is well-tolerated with few adverse effects in a Pediatric population, and no more SAEs occur in the treatment group than the placebo group when given for several months.

Cost: Etanercept (Enbrel at $170/25mg-vial) is inexpensive relative to cost of IVIG (pharmacy cost = $60/gm) for the majority of KD patients.

TABLE 1. Actual pharmacy cost for IVIG versus Etanercept per patient weight.

Patient Weight (Kg)	IVIG 2 gm/kg	Enbrel – 3 (0.8 mg/kg) doses
10	$ 1200	$510
20	$ 2400	$510
30	$ 3600	$510

These calculations do not consider additional cost involved in readmission such as hospitalization days, IV infusion, etc.

1.3 Preliminary Data

Open Label Study. The Principal Investigator proposed and initiated a pilot study. The FDA waived IND for the pilot study. After scientific review of Dr. Portman's study, Amgen provided funding to support the research. Dr. Portman serves as sponsor for the investigator initiated pilot study. We performed an open label nonrandomized study evaluating safety and pharmacokinetics of Etanercept in patients aged 6 months to 5 years. The enrollment goal is 15 patients. On January 30, 2008 we enrolled the 12th patient. The first 5 patients received 0.4mg/kg etanercept subcutaneously, and patients afterward received 0.8mg/kg. Doses were given within 12 hours of completing IVIG infusion, at 7 days and at 14 days (see study schema in Section D). Demographics of patients are shown in Table 2.

TABLE 2. DEMOGRAPHICS FOR ETANERCEPT SUBJECTS

Subject	Weight (kg)	Age (months)
1	16.1	46
2	13.1	25
3	13.9	27
4	6.9	9
5	31.4	67
6	9.9	11
7	12.0	35
8	23.5	55
9	14.5	41
10	7.7	11
11	15.7	57
12	12.2	27
MEAN	**14.7**	**34.25**
STDEV	**6.8**	**19.3**

Safety: No patient required readmission after initial discharge. Two patients had mild upper respiratory infections associated with similar symptoms in family members after discharge; one of these had a single fever spike 3 weeks after IVIG treatment, which resolved quickly without treatment other than acetaminophen. One additional patient had otitis media, which required antibiotics. One patient vomited several times after eating "Chinese Food". Only one serious adverse event occurred. One patient was treated with IViG and subsequently Etanercept. Although, the patient had 4 criteria and persistent fever qualifying for the diagnosis of Kawasaki Disease as well as negative blood cultures, he developed hydrocephalus caused by

meningococcal meningitis. The patient was treated with antibiotics and recovered. The DMC and IRB agreed that this SAE was unrelated to Etanercept, but emphasized the importance of eliminating other infectious diseases, when making the diagnosis of KD.

Efficacy: This was a single arm open label design and not an efficacy study. However, we can make salient comments about these data. Unfortunately, a protocol deviation resulted in one patient receiving a second dose of IVIG for a fever spike 12 hours after completing IVIG. Clinical care guidelines recommend no second dose until 36 hours after completing IVIG, as many patients have a brief post-IVIG fever spike. Otherwise, no other patient demonstrated IVIG refractoriness. According to our institutional data (Table 3), we expected at least two and possibly three patients with IVIG refractoriness in this group. Among the 4 eligible patients refusing enrollment, one patient demonstrated recrudescent fever, was readmitted and treated with a second dose of IVIG. Ten study patients showed normalization of CRP by the 7 day visit indication suppression of acute phase proteins.

TABLE 3. CHRMC

RACE DESCRIPTION	TOTAL	SINGLE DOSE	MULTIPLE DOSE
WHITE	78	48	30
ASIAN	33	26	7
DID NOT INDICATE	16	11	5
OTHER RACE	13	9	4
SPANISH/HISPANIC	11	8	3
BLACK	10	4	6
HAWAIIAN/PAC ISLANDER	1	1	0
MALE	89	60	29
FEMALE	73	47	26
MEAN AGE AT DIAGNOSIS	3.3	3.2	3.6
TOTAL	162	107	55

Table 3 shows 162 consecutive patients with KD treated with IVIG. Ethnicity/race and demographics are reported. The overall rate of retreatment with a second dose of IVIG is 34%. Review of patient records shows that approximately 6-9 % of patients received IVIG before 36 hours after completing IVIG dose. Refractory rate according to AHA guidelines is then between 25 – 30%, as we can't be sure how many of these last patients would have had persistent fever > 36 hours after IVIG.

Coronary artery z-scores are shown for each patient in Table 4. No patient showed new coronary artery dilation or aneurysm after etanercept. Three patients showed coronary aneurysm or dilation prior to etanercept. One of these was an 11 month old infant with two large right coronary artery aneurysms at presentation, which persisted throughout the study. However, the other two patients showed resolution of CAD. This results in a 10% rate of CAD. Remember that the PHN study reported 18.9% at 5 weeks.

MAXIMAL CORONARY ARTERY Z-SCORES

	Baseline	2- week	6-week
1	2.6	2.5	2.7
2	1.7	1.2	1.2
3	0.5	-0.12	-0.12
4*	7.1	7.4	7.4
5	1	0.91	1.08
6	0.5	0.5	0.5
7	2	1.6	1.3
8	0.6	0.6	0.6
9	0.5	-0.15	-0.5
10	2.8	0.9	NA
11	0.7	0.7	NA
Mean	1.49	1.1	
STDV	1.864381244	2.011634	
	P(T<=t) two-tail	0.046	

Table 4 shows maximal z-score from each patient. Subject # 4 showed aneurysm of right proximal coronary artery. NA- patients have not yet completed protocol. Results of paired T-test two-tailed, comparing baseline z-score with 2 week z-score are shown at bottom. NA , not available as these patients have not completed protocol. Pt 12 recruited data not available.

Summary: As noted, the study design precludes any statistical analyses for efficacy. However, as a pilot study these results are promising. We demonstrate safety, pharmacokinetics consistent with previous studies performed in adults and children, and suggestion of therapeutic responses superior to standard therapy alone.

1.4 Dose Rationale

Etanercept is slowly absorbed after subcutaneous (SC) injection, and the absolute bioavailability is approximately 58% in adults[37]. Etanercept has a relatively small volume of distribution of 12 ±6 and is slowly absorbed to reach its peak serum concentration about 50 hours after injection and cleared from the body with a reported median half-life of 115 hours[37]. The recommended dosing regimen for JRA patients (4-17 years) at the time of its initial approval was 0.4 mg/kg (up to 25 mg) twice weekly by SC injection[40].

Figure 2 A. Data below is extracted from Yim et al, and shows overlay of observed and simulated data for children in JRA group receiving 0.4 mg/kg twice weekly.

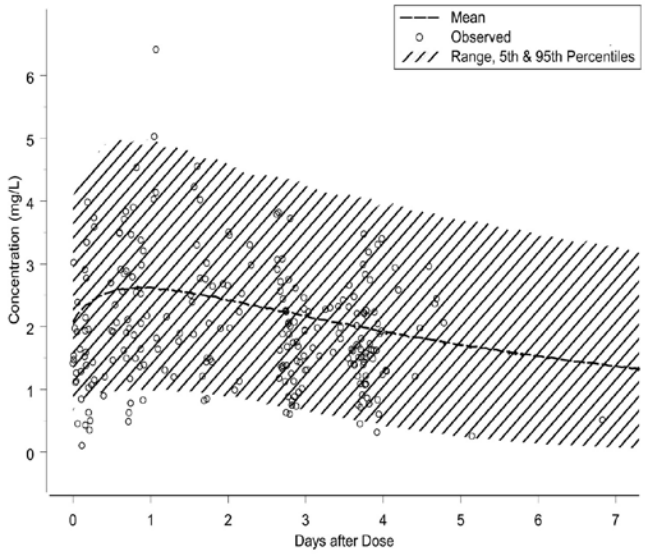
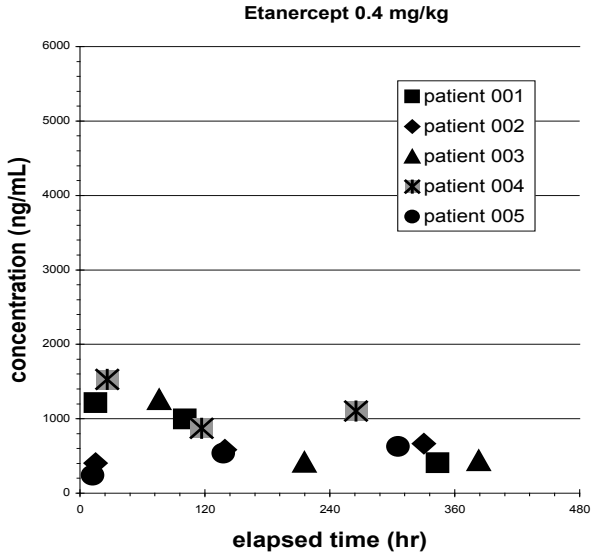


Figure 2B. Data from 5 patients in pilot study receiving 0.4 mg/kg etanercept starting immediately after IVIG infusion and then weekly x 2. 1000 ng/ml = 1 mg/L



However, based on its slow absorption and elimination and pharmacokinetic (PK) profile, a doubled dose with a half-frequency regimen (ie, 0.8 mg/kg [up to 50mg]) of a once-weekly SC injection was sought and FDA approved for patient convenience. PK simulation studies using data from juvenile rheumatoid arthritis (JRA) patients receiving 0.4 mg/kg SC twice weekly show similar steady-state levels at 1 and 8 weeks for children receiving 0.8 mg/kg[41]. After repeated subcutaneous treatment mean serum etanercept concentration was 2100 ng/ml (range 700 to 4300 ng/ml). Although eighteen JRA patients were between age 4 and 8 years, relatively few patients were under age 5 years. The data in Figures 2 A and 3A illustrate drug concentrations from the JRA population and are extracted from Yim et al [41]. These can be compared to the PK profiles for the two different drug doses used in the pilot study (data shown in Figs 2B and 3B). The pharmacokinetic profiles for 5 pilot study patients, receiving 0.4mg/kg weekly (Figure 2.B), show lower than therapeutic levels, defined by 5th to 95th %tile range in shown in 2A for JRA patients receiving twice weekly doses. Steady state concentrations after 0.8 mg/kg after 1 week (Figure 3 B) are within the simulated and therapeutic range calculated for the JRA group (3A).

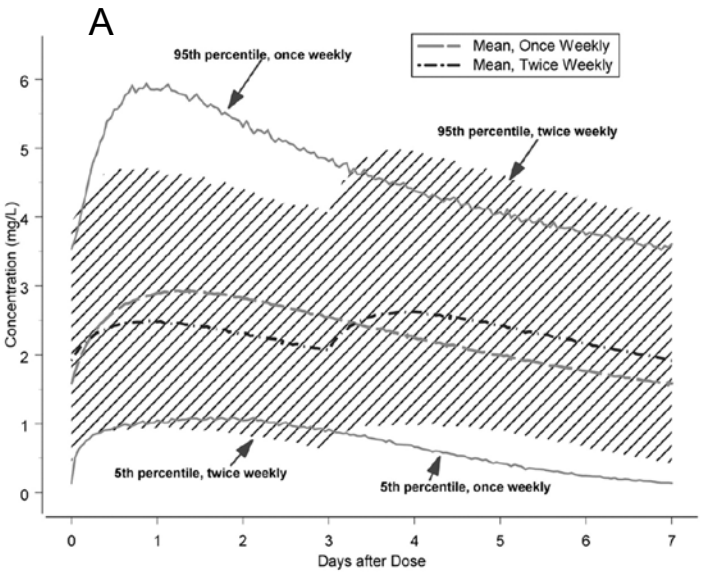


Figure 3A. The data extracted from Yim et al represents simulation for 0.8 mg/kg (once weekly) and 0.4 mg/kg (twice weekly). These simulations consider drug levels after 8 weeks of therapy,

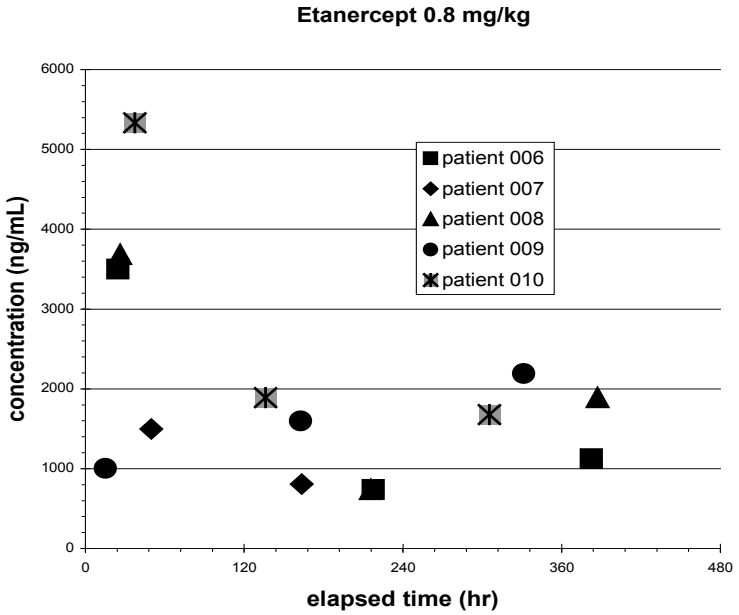


Figure 3B shows our patient data obtained in first two weeks of etanercept treatment. We were able to maintain levels in the therapeutic range even at trough within this time period.

These data suggest that 0.8 mg/kg weekly is adequate to maintain levels within the therapeutic range defined by the earlier JRA trials. The younger age of the KD patients does not substantially affect absorption and metabolism of etanercept. Three enrolled patients less than 2 years of age showed similar pharmacokinetic profile to all other patients and JRA patients. These patients also showed no adverse events from Etanercept.

2 Study Objectives

Kawasaki Disease (KD) or Syndrome is the leading cause of acquired heart disease in children the United States. The Center for Disease Control estimates that over 4000 U.S. children per year are diagnosed with KD. Although, KD qualifies for Orphan Disease status by NIH and FDA standards, it is a growing and important health problem for children in the U.S. and worldwide. KD is an autoimmune disease and vasculitis with a specific predilection for coronary arteries, causing aneurysm and/or ectasia. Although, the majority of patients do well, many experience long term coronary artery abnormalities. Studies in Japan where KD demonstrates exceptionally high prevalence indicate that the mortality rate in males with coronary artery lesions is more than twice expected. Treatment with intravenous immunoglobulin and aspirin currently represent the standard of care for acute Kawasaki Disease. New and emerging data show that this treatment is only partially effective for reducing the risk of coronary artery disease. Additionally, KD in many patients demonstrates resistance or refractoriness to IVIG, and requires retreatment. This refractoriness represents a primary risk factor for development of coronary artery dilation. Attempts at developing effective adjunctive therapy for IVIG and aspirin have failed. Over the past 15 -20 years, multiple investigators have provided strong evidence implicating tumor necrosis-α (TNF-α) as a primary agent for inflammation in acute KD. TNF-α antagonism with etanercept abrogates coronary artery disease in a validated mouse model of KD. Etanercept**,** a dimeric fusion protein, consists of 2 molecules of the extracellular ligand-binding portion of human TNF receptor 2 attached to the Fc domain of human IgG. Etanercept is FDA approved for multiple indications in adults and in children down to four years of age for juvenile rheumatoid arthritis (JRA). We performed a pilot study in acute

KD patients (age 6 months to 5 years). In this population, we demonstrated that etancercept (0.8 mg/kg) given subcutaneously at weekly intervals in 3 doses provides concentrations within the therapeutic range defined for JRA patients. The results of the pilot study support the safety of etanercept in children and show promise for efficacy in reducing IVIG resistance.

We will test the hypothesis that TNF-α antagonism with etanercept improves the clinical response to standard of care treatment with IVIG and aspirin therapy in KD patients between 2 months and 20 years of age. To test this hypothesis we will perform a double-blinded placebo controlled trial in 196 children with KD and obtain data in order to achieve an orphan designation for etanercept.

Primary Aim

1. Determine if etanercept (0.8 mg/kg, maximum 50 mg) given subcutaneously reduces IVIG refractory rate and retreatment rate in the KD patient population. Our goal is to randomize and complete the trial in 110 patients in the etanercept treatment group and 110 patients in the placebo group.

Secondary Aims

1. Determine if the safety profile differs between the etanercept treated group and the placebo group.
2. Determine if etanercept treatment alters the rate of coronary artery dilation and disease (CAD) defined by z-scores at 2 and 6 weeks after treatment.
3. Determine the pharmacokinetics of etanercept in KD patients

3 Research Design and Methods

We propose a placebo-controlled randomized double blind multi-center study in patients, age 2 months to 20 years, with acute Kawasaki disease. All study investigators will be blinded except for pharmacists.

3.1 Primary clinical endpoint

The primary aim of this study is to determine if Etanercept (Enbrel) 0.8 mg/kg given subcutaneously to patients with acute Kawasaki Disease will reduce the incidence of IVIG refractoriness as defined in the joint American Heart Association and American Academy of Pediatrics Endorsed Clinical Report: "Refractory Kawasaki Disease will be defined as the persistence or recrudescence of fever (≥38.0°C or 100.4°F) at least 36 hours after the end of the IVIG infusion"[3].

Rationale for primary endpoint: Refractory KD is an objective clinically important and easily measured parameter. Orphan product indications by the FDA are likely to be influenced by clinical parameters as opposed to sonographic measures. Refractoriness increases risk of coronary artery disease, number of hospitalization days and cost of treatment[11]. Several studies have unsuccessfully used coronary artery dilation (CAD) as a primary endpoint to evaluate adjunctive therapy [13, 14].Risks in using CAD are: intra-observer and inter-observer variability. Although, we will have a central echocardiographic reader, subjectivity is introduced by the sonographer actually performing the study. Coronary artery dimensions can be skewed by angle, machine settings and other parameters beyond the control of the reader. Therefore, coronary artery dilation parameters will be relegated to secondary endpoints.

3.2 Secondary Clinical Endpoints

Determine if the safety profile differs between the etanercept treated group and the placebo group.

Secondary safety endpoints will be number of adverse events, serious or otherwise within 30 days of treatment, with specific focus on serious infections, defined as "those requiring hospital admission" for treatment. The latter have been noted in patients receiving much longer courses of etanercept than proposed for this protocol, and for different diseases.

Our study will be the first to evaluate efficacy of Etanercept in reducing inflammation in an acute inflammatory disease in children, as opposed to chronic inflammatory diseases. The acute phase reactant, C-reactive protein, will be followed throughout the study as a laboratory measure of inflammation. We will also determine if etanercept modifies serum concentrations of cytokines, interleukin-3, interleukin-6, and TNF-α.

We propose the largest cohort for study of Etanercept in children performed to date.

Secondary clinical endpoints will be directed to determine if etanercept treatment alters the rate of coronary artery disease defined by z-scores at 2 and 6 weeks after treatment.

We will also determine if etanercept treatment 1) alters development of coronary artery dilation or disease in patients with normal coronaries on initial echocardiogram, 2) promotes healing of coronary artery disease, when apparent on initial echocardiogram, 3) alters treatment course by reducing total number of hospitalization days, and 4) incidence of CRP normalization by 7 days.

3.3 Dosing

Etanercept or placebo will be administered in three separate doses (0.8 mg/kg subcutaneously, maximum 50 mg) at 1 week intervals with the first dose concurrent with IVIG or within 48 hours of initiating IVIG.

Rationale for extended therapy: Current KD treatment with IVIG and ASA in the United States is based on efficacy comparison with ASA alone[8]. Subsequently, studies showed no difference in efficacy between IVIG (400 mg/kg) given in 4 separate doses on consecutive days versus a single infusion IVIG (2 gm/kg) over 10 hours[12]. Nevertheless, data across more than 2 decades show that coronary artery involvement increases during the 2 week period following treatment. Furthermore, refractoriness to IVIG can manifest up to 2 weeks after completion of IVIG. Inoue[13] et al showed some increased benefit of continuing oral steroids until inflammatory parameters such as CRP normalized. In that study, laboratory studies were performed at least twice weekly. Normalization of CRP level in the placebo group (receiving IVIG and ASA, no corticosteroid) occurred in 9.0 days (median) range 4 to 42 and mean 11.2 ± 6.2 days. These data, along with the their results showing some mild efficacy by more prolonged corticosteroid therapy, strongly support the contention that anti-inflammatory therapy for KD should be extended to near 3 weeks, provided therapy is convenient and low-risk. Accordingly, we will maintain treatment for 3 weeks to cover all patients within this vulnerable period. As our pilot data suggest that Etanercept levels are maintained at a near steady-state by week 2, we will give the final dose at 14 days ± 2 in order to maintain levels at least 3 weeks.

4 Patient Population and Sample Size

196 study participants will be recruited from the four participating medical center admissions for suspected Kawasaki Disease with IVIG administration. Seattle Children's Hospital is the coordinating site for the study. Most patients will be on the Cardiology or General Medicine Service.

4.1 Inclusion criteria:

Identification of patients with KD:
The American Academy of Pediatrics and the American Heart Association have published guidelines for epidemiological case definition. KD treatment, including IVIG and aspirin, is recommended for patients fitting these criteria. Generally, supportive laboratory data also exist including elevation of erythrocyte sedimentation rate and C reactive protein (CRP > 3.0, although usually much higher). Thrombocytosis (platelet count > 500,000) usually occurs in the second week.

- Patients in this study (age 2 months to 20 years) will fit the standard epidemiological definition of acute KD as recommended by the joint AHA/AAP guidelines or fit criteria for diagnosis of incomplete KD as described below. Patients will be enrolled only after a clinical decision to treat with IVIG has been made by the treating physician.
- Patients may be enrolled if they begin IVIG infusion within 10-13 days of onset of illness with day 1 defined as the first day of fever. A patient may be enrolled with greater than 10 days of fever upon written approval or email confirmation from the study Principal Investigator (Dr. Portman) on a case by case basis.
- Parents of subject or non-minor subject must be able and willing to give written informed consent and comply with the requirements of the study protocol and must authorize release and use of protected health information.

Patients will fit American Heart Association, American Academy of Pediatric guidelines for diagnosis of Kawasaki Disease
Fever persisting at least 5 days†
Presence of at least 4 principal features:

1) Changes in extremities
   Acute: Erythema of palms, soles; edema of hands, feet
   Subacute: Periungual peeling of fingers
2) Polymorphous exanthem
3) Bilateral bulbar conjunctival injection without exudate
4) Changes in lips and oral cavity: Erythema, lips cracking, strawberry tongue, diffuse injection of oral and pharyngeal mucosae
5) Cervical lymphadenopathy (1.5-cm diameter), usually unilateral

Patients with fever at least 5 days and 3 principal criteria can be diagnosed with Kawasaki disease when coronary artery abnormalities are detected by 2 dimensional echocardiography (see section on Echocardiography). A qualifying echocardiogram is defined as coronary artery z score $\geq$2.5 in the proximal coronary artery or left anterior descending, or aneurysm by Japanese Ministry of Health criteria.

Patients with fever at least 5 days and 2-3 principal criteria without coronary artery abnormalities can be diagnosed with Kawasaki Disease when the patient has a CRP of 3.0 mg or greater and 3 or more of the supplementary lab criteria listed below:

albumin <3.0 g/dL
anemia for age
elevation of alanine aminotransferase
platelets after 7 days >450 000/mm3
white blood cell count >15 000/mm3
urine >10 white blood cells/high-power field

† In the presence of all 5 principal criteria, Kawasaki disease diagnosis can be made on day 4 of illness.

4.2 Exclusion Criteria

1. Laboratory Criteria: Any laboratory toxicity, at the time of the screening visit or at any time during the study that in the opinion of the Investigator would preclude participation in the study or:
   a. Platelet count < 100,000/mm^3
   b. WBC count < 3,000 cells/mm^3
   c. Hemoglobin, hematocrit, or red blood cell count outside 30% of the upper or lower limits of normal for the Lab

2. General Exclusion Criteria
   a. Subject is currently enrolled in another investigational device or drug trial(s), or subject has received other investigational agent(s) within 28 days of baseline visit.
   b. Female subjects diagnosed with KD 12 years of age and older.
   c. Subjects who have known hypersensitivity to Enbrel or any of its components or who is known to have antibodies to etanercept
   d. Prior or concurrent cyclophosphamide therapy
   e. Treatment with any TNFα antagonist or steroid within 48 hours prior to initiation of IVIG
   f. Concurrent sulfasalazine therapy
   g. Active severe infections within 4 weeks before screening visit, or between the screening and baseline visits.
   h. SLE, history of multiple sclerosis, transverse myelitis, optic neuritis, or chronic seizure disorder
   i. Known HIV-positive status or known history of any other immuno-suppressing disease.
   j. Any mycobacterial disease or high risk factors for tuberculosis, such as family member with TB or taking INH. * (see below)
   k. Untreated Lyme disease.
   l. Severe comorbidities (diabetes mellitus requiring insulin, CHF of any severity, MI, CVA or TIA within 3 months of screening visit, unstable angina pectoris, uncontrolled hypertension (sitting systolic BP > 160 or diastolic BP $\geq$ 100 mm Hg), oxygen-dependent severe pulmonary disease, history of cancer within 5 years [other than resected cutaneous basal or squamous cell carcinoma or in situ cervical cancer])
   m. Exposure to hepatitis B or hepatitis C or high risk factors such as intravenous drug abuse in patient's mother, or history of jaundice (other than neonatal jaundice).* SLE, history of multiple sclerosis, transverse myelitis, optic neuritis or chronic seizure disorder.

n. Use of a live vaccine (Measles Mumps Rubella or Varicella) 30 days prior to or during this study with the exception that a patient who has received Influenza mist vaccine may be enrolled after 14d from administration of the vaccine.
o. Any condition judged by the investigator to cause this clinical trial to be detrimental to the patient
p. History of non-compliance with other therapies
q. Must not have received immunosuppressive agents for at least three months prior to enrollment.

*Note, liver enzymes, AST or ALT are frequently elevated in KD, and will not be used as an exclusion.

* PPD testing will not be performed in these patients. Reactivation of chronic granulomatous disease including tuberculosis from chronic treatment with TNFα antagonists is a valid safety concern.[31] The risk of TB reactivation for patients on etanercept therapy still requires clarification. A recent review at Cook County and Rush Hospital in Chicago showed that none of the 48 patients with positive PPDs who were treated with etanercept for average of 17 months developed active TB [53]. Additionally, Mohan et al reported the earliest reactivation occurred after 1 month of initiating etanercept with a mean of 11.5 months [54]. Use of additional immunosuppressive agents other than etanercept was a risk factor. Our cohort will receive a very short course (~ 2 weeks) of etanercept and will not receive other immunosuppressive drugs, thereby markedly reducing the already very low risk of TB in this population. For instance, the incidence of TB in King County, WA in children is 1 -2.5/100,000 or 8-12 per year. To be effective in acute Kawasaki Disease, etanercept must be given at the same time or immediately after IVIG treatment. We cannot delay while awaiting mandatory 48-72 hours for results of skin testing. The new Quantiferon gold test for TB is not yet widely available (and not in Seattle) and would still require at least 24 hours. We have considered placing TB skin testing after initial dose and discontinuing if positive. However, it is likely that TNF-α antagonism could affect the test and provide a false negative. Therefore patient exclusion will be performed based on high risk history, such as immediate family or household member with positive PPD.

4.3 SAMPLE SIZE

Calculation of sample size is based on initial IVIG refractory rate at Children's Hospital and Regional Medical Center. The last 200 patients over 4 years admitted to CHRMC 52 patients (26%) required two or more doses of IVIG. Power analyses reveal the following:

Proportion failing in the control group	Proportion failing in the experimental group given Etanercept	N **per group** needed to achieve 80% power **NOTE: TOTAL enrollment =2 x N**
0.25	0.15	270
0.25	0.10	**110**
0.25	0.05	59
0.25	0.02	42

Sample size 110 is reasonable, considering the potential population and budget. The table below that shows power for a range of effect sizes, holding the N at 110.

The table below provides the relationship between effect size and study power.

Proportion failing in the control group	Proportion failing in the experimental group given Etanercept	N per group	Statistical Power
0.25	0.08	97	80%

0.25	0.05	97	90%

The table below provides anticipated numbers of patients eligible from four U.S. and one Canadian Center, and anticipated numbers of recruited patients based on recruitment rates from prior studies. From CHMRC, estimates are based on KD patients per year and our recruitment rate in the pilot study, which enrolled patient in the narrow age range 6 months to 5 years. From Primary Children's in Utah, estimates are based on their recruitment rate and participation in the Pediatric Heart Network "Randomized trial of pulsed corticosteroid therapy for primary treatment of Kawasaki disease". From Milwaukee Children's and Cohen Children's Medical Center, estimates are based on KD admission rate and recruitment rate as adjunctive center for PHN protocols. Sainte-Justine Hospital estimates are based on high enrollment for Bristol-Myers-Squibb sponsored studies evaluating Cardiolite in Kawasaki Patients (Study # 301 (see description under Key Personnel and Sites of Investigation). Each center has clinical research structure and team permanently in place and structured.

4.4 Subject Recruitment and Screening

Patients will be recruited from admissions at participating centers for suspected KD with IVIG administration. Families of Kawasaki Disease patients will be referred to the research team by physicians involved in patients care. In general, the Cardiology service is involved with KD patient's evaluation and care immediately after admission. Standard clinical care usually includes a screening or baseline echocardiogram. Patients will be screened for study suitability by the research staff. If the family shows interest in the study, they will undergo consent procedures, which will be conducted by the research team. The study will be described verbally to the patient and family. After the introduction and description of the study, the family will be given adequate time to read and review the written consent/ assent forms. Time will be allotted for questions and additional discussion if necessary. Family friends, relatives or additional support persons (at the request of the parent/guardian) may also be present to hear and read what is being asked of the patient/family.

Patients will be enrolled only after the necessary criteria for KD diagnosis has been documented in the medical record and serious infection as the source of fever has been reasonably eliminated. Patients will be screened prior to IVIG, but will not be enrolled until IVIG orders are written.

4.5 Early Withdrawal of Subjects

4.5.1 When and How to Withdraw Subjects

Subjects may be withdrawn from the study prior to the expected completion of that subject for health, logistical, or personal reasons (i.e. serious adverse events, failure of subject to adhere to protocol requirements, subject consent withdrawal, disease progression, etc). No safety consequences are anticipated with early withdrawal from the study since etanercept is adjunctive therapy.

4.5.2 Data Collection and Follow-up for Withdrawn Subjects

Follow-up calls will be made to the patient's family upon early withdrawal from the study.

4.5.3 Emergency Blind Breaking

At the beginning of the study each site will be instructed on the method for emergency blind-breaking. Blinding codes should be broken in emergency situations only if this is required for proper treatment. The

primary investigator at the site will contact Dr. Portman to assess the necessity for un-blinding. In the unlikely event that Dr. Portman is unreachable; the primary investigator will be responsible for making the decision to un-blind the patient.

The study investigator will instruct the participating site pharmacy to break the blind to the treating physician only. The study investigators at the site as well as Dr. Portman are to remain blinded to protect the integrity of the data.

5 Study Drug

5.1 Description

Etanercept is a recombinant TNF receptor Fc Fusion protein. It is pharmacologically classified as a TNF antagonist, with the sole active ingredient being etanercept.

Etanercept powder is to be reconstituted with sterile diluent supplied by Amgen, Inc. prior to its subcutaneous injection such that the final dosage achieved is 0.8 mg/kg. Each vial of powdered etanercept contains 25 mg etanercept, 40 mg mannitol, 10 mg sucrose, and 1.2 mg tromethamine.

5.2 Treatment Regimen

Subjects will be given subcutaneous injections of 0.8 mg/kg etanercept 3 times at weekly intervals, starting at initial diagnosis.

5.3 Preparation and Administration of Study Drug

Participating Hospital's Investigational Pharmacy will prepare the study drug according to the manufacturer and research protocol's directions. Amgen will provide study drug and placebo to each site's pharmacy. Etanercept will be reconstituted aseptically with the Amgen-supplied Sterile Bacteriostatic water for Injection, USP (0.9% benzyl alcohol) so that the desired study dosage is met. Following drug preparation, the Investigational Pharmacy will dispense the study drug to the study investigators so that it may be administered to the patient.

5.4 Subject Compliance Monitoring

The study coordinator will track subject compliance with the study treatment regimen. Subjects non-compliant with the study treatment regimen will be contacted to so that conflicts which may be hindering their compliance may be resolved.

5.5 Prior and Concomitant Therapy

The following medical therapies are not permitted for study participation:

- Treatment with any TNFα antagonist or steroid within 48 hours prior to initiation of IVIG
- Current use of an investigational device or drug trial(s), or receipt of other investigational agent(s) within 28 days of baseline visit
- Prior or concurrent cyclophosphamide therapy
- Concurrent sulfasalazine therapy
- Concurrent isonicotinic acid hydrazide therapy
- Any live vaccine 30 days prior to, or during the study. Patient may be enrolled in the study 14 days after administration of Influenza mist vaccine.
- Prior (within 3 months preceding enrollment) or concurrent use of immunosuppressive agents

In addition, any medical therapies that may cause harm to the patient, as judged by the patient's physician on a case-by-case basis, will not be permitted.

IVIG (2 g/kg) and aspirin (80-100mg/kg Q6 hr until afebrile then 3-5 mg/kg/day) are standard treatment regiments for acute Kawasaki Disease, their concomitant therapy is to be expected and permitted.

5.6 Packaging

- Etanercept will be shipped by Amgen, Inc in bulk.
- Each package of etanercept will be supplied with its diluent, Sterile Bacteriostatic Water for Injection, USP (0.9% benzyl alcohol).

5.7 Receiving, Storage, Dispensing and Return

5.7.1 Receipt of Drug Supplies

Amgen, Inc will ship the study drug to the participating Hospital's Investigational Pharmacy. Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify Amgen, Inc of any damaged or unusable study treatments that were supplied to the investigator's site.

5.7.2 Storage

Etanercept is not to be used beyond the expiration date stamped on the carton, dose tray label, or diluent syringe label. The dose tray containing etanercept in powder form must be refrigerated at 2ºC to 8ºC (36ºF to 46ºF), and NOT FROZEN.

Solutions of etanercept reconstituted with Amgen-supplied Bacteriostatic Water for Injection, USP (0.9% benzyl alcohol) may be stored for up to 14 days if refrigerated at 2ºC to 8ºC (36ºF to 46ºF). Reconstituted product should be discarded after 14 days as stability and sterility cannot be assured after 14 days.

5.7.3 Dispensing of Study Drug

Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

5.7.4 Return or Destruction of Study Drug

Study drug that remains at the end of the study will be shipped back to Amgen, Inc or destroyed on site.

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

Etanercept 0.8mg/kg (maximum 50 mg) or placebo will be given within 48 hours of initiating IVIG infusion. Randomization will be performed by internal Pharmacy, unblinded (see Pharmacy procedures). Placebo format

will be: Etanercept placebo (Amgen), TMS Lyo, B20 UnLBL 2mL vial Pink Cap. Study schema and procedures are outlined below.

TIME	0*	12-84 hours #	7 ± 3 days	14 ± 3 days	44 ± 4 days
Physical Exam	x		x	x	X
Etanercept/Placebo	x		x	x	
PK	x	x	x	X	
Cytokines	x	x	x		
CBC^	x		x	x	X
CRP	x		x	x	X
ESR	x				
Echo	x			x	X
EKG	x			x	X
AST, ALT	x		x	x	
Albumin	x		x	x	X

* Time 0 or baseline is defined as time first dose of study drug is given. Time 0 or baseline labs are almost always done before the patient has consented to the study. These labs are optional unless the patient is qualifying into the study through supplementary lab data. In general, admission laboratory values used to confirm diagnosis of KD can be used for the study, provided that they were within 24 hours prior to initiating IVIG. If a time zero lab value is not drawn as part of the clinical course prior to the patient enrolling in the study, it will not be collected and will be recorded as missing data After enrollment, coordinators should try to retrieve extra serum obtained along with admission laboratory studies. This serum can be used for baseline pharmacokinetic assay, PK.

An EKG is normally done at time zero as part of clinical care. This EKG is optional, if an EKG is not performed at baseline it will not be recorded as a protocol deviation.

Parents are to record temperature after discharge daily between 4PM and 6PM for two weeks, as well as if and when they believe child has fever, and notify study coordinator if > 38.0 ° C. If patient temperature > 38° C and the investigator or treating cardiologist feels the patient needs to be readmitted, an extra study visit will occur within 12-24 hours of re-admission with PE, CBC, CRP, ESR, Echo, and ECG. This extra visit concurs with clinical guidelines for standard of care. ^CBC includes WBC with differential and platelet count. Parents are also to notify coordinator if any sign of infection. # PK, for this time point will be 48 hours (window 12 – 48 hours) after Etanercept.

We will define failure to respond to IVIG using the AHA guidelines: as persistent fever > 38.0° C extending beyond 36 hours after completion of IVIG infusion or recurrent fever > 38.0° C after 36 hours of completion of the initial IVIG infusion.

All patients with disease-related complications will receive standard care by their primary Pediatric Cardiologist and other treating physicians. This will include anticoagulation therapy when the primary Cardiologist feels it is necessary. AHA/AAP guidelines recognize the use of various types of anti-coagulation. Low dose ASA as described above represents the primary antiplatelet therapy. “Clopidogrel in combination with aspirin has been shown to be more effective than either agent alone in preventing vascular events in both coronary and cerebral territories in adults (theClopidogrel in Unstable Angina to Prevent Recurrent Events study).” However, there is no evidence in children that this is effective therapy. Many Pediatric Cardiologists use heparin infusion often

followed by Warfarin for rapidly expanding or giant coronary artery aneurysms. The current protocol does not preclude their use. We have proposed an adjunctive therapy for KD. Accordingly, we are not interfering with standard of care, and do not specify concomitant medications. All medications prescribed by primary care providers are permitted. Additionally, no live vaccine may be given for at least 11 months after receiving IVIG as recommended by the American Academy of Pediatrics.

Treatment with high dose steroids and/or infliximab during the course of the study is permitted if recommended by Rheumatology for the treatment of recurrent Kawasaki Disease.

6.1 Visit 1 - Hospital

The patient is admitted into the hospital. Patient family is referred to the research staff for study discussion and consent. The patient is screened for eligibility, study discussion is conducted, and consent is obtained. Assent will also be obtained if deemed required by IRB. (see section entitled "Protection of Human Subjects").

All patients will receive standard treatment for KD as stated in the AHA-AAP guidelines. This includes IVIG 2 gm/kg over 10 hours or longer if interruptions occur in the infusion and high dose ASA, administered at 80 to 100 mg/kg per day in 4 doses until patients have been afebrile for 48 hours. Subsequently, patients will continue low dose ASA 3-5 mg/kg per day until 6 weeks or longer at the discretion of the treating Pediatric Cardiologist.

The blood for the baseline measurements can be drawn with the IV start or from the IV line. Labs obtained upon hospital admission will be used for baseline data. The PK blood sample will be processed and stored for later shipping by the lab or study personnel, while the other blood samples shown in study schema are sent to the central hospital laboratory for processing and results. An Echo and EKG are performed. A nurse administers a subcutaneous injection of study drug or equivalent volume of placebo at the appropriate dosage 0.8 mg/kg (maximum 50 mg). Etanercept must be administered within 48 hours after initiating IVIG therapy, and as early as possible. Etanercept should not be given prior to starting IVIG infusion. After discharge, the parents of the patient are to record daily temperatures between 4PM and 6PM for the next two weeks in a log provided by the nurse coordinator. Thermometer will be given to family if not already available or provided by hospital and coordinator will instruct patients regarding the proper procedure for taking and recording temperature. If the parents believe that their child has a fever, they are to record the child's temperature regardless of the time of day and notify the study coordinator if temperature is > 38.0ºC. If the study coordinator is unavailable the family should call the on-call cardiologist. If the child is readmitted, an extra study visit will occur within 12-24 hours of the patient being readmitted, and a physical exam, CBC, CRP, ESR, ECG, and Echo will be completed. Parents are to notify the study coordinator if they find any signs of infection in the patient.

6.2 Visit 2 (12-84 hours) - Hospital

This visit is optional, and would be timed within 12-84 hours of etanercept administration. If the intravenous line is still in place, or a blood draw is clinically indicated within this window, approximately 4 cc of blood is drawn for the PK test. A numbing agent may be used to decrease the discomfort associated with phlebotomy. The blood is sent to the laboratory for the necessary blood work. Patients are generally observed for 24 to 48 hours after completion of IVIG dose prior to discharge. Study investigators will observe patients through that period in the hospital. Observation will continue if patient hospitalization extends beyond that period for complications of disease or therapy until the patient is discharged.

6.3 Visit 3 (Day 7 ± 72 hours)- Clinic

A physical assessment and a blood draw (approximately 7 cc) are required. A nurse may perform an abbreviated physical exam as part of the nursing assessment, since a physician investigator is not required to be present at this visit. A numbing agent may be used to decrease the discomfort associated with phlebotomy and/or study drug administration. The blood sample is sent to the laboratory for the tests listed in the study schema, while study personnel will process the PK sample and store for later shipping. A nurse administers a subcutaneous injection of study medication at the appropriate dosage.

6.4 Visit 4 (Day 14 ± 72 hours)- Clinic

A physical exam and a blood draw (approximately 7 cc) are required. A numbing agent may be used to decrease the discomfort associated with phlebotomy or study drug administration. The blood sample is sent to the laboratory for the tests listed in the schema while study personnel will process the PK sample and store for later shipping. An Echo and EKG are performed. A nurse administers a subcutaneous injection of study drug at the appropriate dosage.

6.5 Visit 5 (Day 44 ± 4 days)- Clinic

A physical exam and a blood draw (approximately 7 cc) are required. . The blood sample is sent to the laboratory for the tests listed in schema. An Echo/ EKG is performed. No study drug is administered.

6.6 Treatment for refractoriness to IVIG.

All patients who fit the requirements for refractoriness to IVIG (see section 3.1) will receive standard clinical treatment as determined by the attending physician. Standard treatment is usually a second dose of IVIG, and is defined in the American Heart Association guidelines. Retreatment with IVIG will not interrupt the study and study medication should still be administered.

6.6 Follow-up Echocardiogram Surveillance

Follow up data will be collected for patients who had a positive echo (z score $\geq$ 2.5 or aneurysm) at visit 5 for 14 months following their diagnosis or until their echo normalizes. De-identified visit notes and echocardiogram and angiogram reports that fall within this window should be sent to the study sponsor.

6.7 Echocardiography

In order to maintain consistency with other U.S clinical trials in KD[17], coronary artery dimensions will be normalized for body surface area as z scores (SDs from a predicted normal mean). Coronary artery Z-scores are based on nonlinear regression equations derived from a normal nonfebrile population, comprised of 221 healthy children aged 0 to 18 years seen in the noninvasive laboratory at Boston Children's Hospital for echocardiographic evaluation during the years 1987 to 2000 [55]. These patients had no evidence of structural or functional heart disease. Z-scores algorithms are available and will be determined for the left main coronary artery, proximal left anterior descending, and proximal right coronary artery

$$LMCA=0.31747 \cdot (BSA^{0.36008})–0.02887, SD=0.03040+(0.01514 \cdot BSA)$$

$$pLAD=0.26108 \cdot (BSA^{0.37893})–0.02852, SD=0.01465+(0.01996 \cdot BSA)$$

$$pRCA=0.26117 \cdot (BSA^{0.39992})–0.02756, SD=0.02407+(0.01597 \cdot BSA)$$

where LMCA indicates left main coronary artery, in centimeters; pLAD, proximal anterior descending coronary artery, in centimeters; pRCA, proximal right coronary artery, in centimeters; and BSA, body surface area, in meters squared calculated by the Haycock method [55].

In order to limit interobserver variability, a central Echocardiography reader will adjudicate these interpretations. Presence of coronary artery aneurysms will be determined using Japanese Ministry of Health Criteria: aneurysm diameter ≥ 1.5 times the diameter of the vessel immediately proximal. All centers will forward digital echocardiograms to the Seattle Center for central reading by a single observer. Dr. Brian Soriano is a Boston trained Pediatric Cardiologist and spent an extra year in an imaging fellowship, prior to taking a faculty position at CHRMC-UW. He is intimately familiar with procedures used to develop z-scores from the cohort of patients at Boston Children's and will provide consistency for the echo determinations.

6.8 Pharmacy Procedures
The pharmacist and pharmacy technical staff will be not be blinded. The study statistician will provide each site pharmacy with randomization table. Pharmacists will supply each patient with drug or placebo, both provided by Amgen in separate labeled vials. Pharmacists will draw dose in syringes and dispense to nursing for administration. Drug accountability will be monitored by the CRO as described below. Study randomization codes and links to patients will be kept in secure records in pharmacy. All other study personnel involved in administration of drug will be blinded.

6.9 IVIG Retreatment
As noted in section 3.1: The primary aim of this study is to determine if Etanercept (Enbrel) 0.8 mg/kg given subcutaneously to patients with acute Kawasaki Disease will reduce the incidence of IVIG refractoriness as defined in the joint American Heart Association and American Academy of Pediatrics Endorsed Clinical Report: "Refractory Kawasaki Disease will be defined as the persistence or recrudescence of fever (≥38.0°C or 100.4°F) at least 36 hours after the end of the IVIG infusion"[3]. Persistent or recrudescent fever is the sole criteria for determining the need for retreatment. The clinical decision can be supported by persistent symptoms and persistent elevation or rebound of inflammatory parameters such as CRP. The protocol requires waiting at least 36 hours after completing the first IVIG dose before initiating the second dose.

7 Statistical Design and Analysis

The primary outcome variable is the Y/N determination of whether the subject is refractory to IVIG treatment, defined as persistence of fever >38° C beyond 36 hours from completion of IVIG treatment, or recurrence of fever within 10 days, and clinical decision to retreat with IVIG. Chi-square testing will be used to compare the proportions of subjects classified as refractory to IVIG treatment between the group randomized to receive Etanercept as an adjunctive therapy and the group randomized to receive placebo. If necessary (due to low cell counts), a Fisher's Exact test can be substituted. Stratified versions of the chi-square and Fisher's tests are available in StatXact (Cytel Incorporated) to allow stratification by center.

Secondary outcome variables that are measured on a continuous scale include the coronary artery diameters obtained on the LMCA, pLAD and pRCA and the corresponding Z-scores (normalized for the patient's BSA) at each of those locations. Z-scores will be summarized at each time point by treatment group and the within-subject changes in Z-score relative to baseline will be summarized for the post-treatment time points (week 1 and week 5) as well. The within-subject changes in actual artery diameter will also be summarized at week 1 and week 5. Summary statistics will include the mean, SD and selected percentiles.

Change values will be summarized both overall for the treatment group as a whole and then stratified by whether or not the subject has evidence of dilation at baseline (Z-score ≥2.5). A Generalized Estimating Equation (GEE) model will be used to compare change values between the Etanercept and placebo groups. The GEE model is needed to accommodate adjustment for the correlation between observations obtained on the same individual at 1 and 5 weeks. Since the addition of Etanercept may have a differing magnitude of impact on change in artery diameter depending on whether the artery was dilated at baseline, terms for dilation status at baseline and the interaction between dilation and treatment group will be included in the model.

Another secondary outcome is the presence of aneurysm as defined by the Japanese Health Ministry criteria (see Echo criteria above). The pre- and post-treatment proportions of subjects in each group having an aneurysm detected will be calculated along with the exact binomial 95% confidence interval. Comparison of post-treatment aneurysm proportions will be done via a stratified Chi-square test, with strata for presence/absence of aneurysm at baseline.

Adverse events will be tabulated for each treatment group by severity, relationship to study drug and body system affected (according to the NCI CTC grading and MEDDRA classification system). The outcome of any SAE episodes that occur will be summarized. Adverse event rates will be compared between treatment groups using Poisson or negative binomial regression modeling. The proportions of subjects experiencing an adverse event of any type and the proportions experiencing an SAE will be compared using chi-square or Fisher's Exact testing.

Pharmacokinetic analyses: PK analyses will be performed after completion of the study and unblinding, so as not to unblind study investigators. Samples from patients receiving etanercept will be forwarded to the Seattle site for storage and then later submitted to Amgen for determination of serum drug concentrations. Peak and trough analyses will be performed. Drug clearance, volume of distribution and ½ life will be determined using etanercept modeling and algorithms [41] and loaded on SAAM2 software available at the University of Washington.

CRP and other standard laboratory studies will be performed in the clinical laboratory of each participating hospital. Serum cytokines, IL-3, IL-6, and TNFα will be performed in Center of Developmental Therapeutics at Children's Hospital Research Institute in Seattle. Assay will be performed on Luminex-200 multiplex system, which resides in center.

8 Safety and Adverse Events

8. 1 Definitions

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal

- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as non-serious adverse events.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator. (Note: This study population includes a notable proportion of patients requiring hospital readmissions due to refractory IVIG therapy. A clear definition of a *serious adverse event* relating to readmissions will be determined at the Organizational Meeting with the Data Monitoring Committee.)

Expected Adverse Events

The following AEs are expected with Kawasaki Disease and IVIG:

Fever, chills, sore throat, chest tightness, rash, redness of the eyes, vomiting, diarrhea, headache, nausea, irritability, meningismus, anaphylaxis or hypersensitivity, hypotension, rash, skin peeling or desquamation, extremity swelling, coronary arteritis, coronary aneurysms, myocardial infarction, myocarditis, mitral regurgitation, pericarditis, hydropic gall bladder, joint swelling or pain, lymphadenopathy, arrhythmias, blood clots.

Lab abnormalities; elevated CRP and ESR; thrombocytosis, hypoalbunemia, anemia, pyuria, elevation of liver transaminases.

Additionally, localized redness and swelling at injection site are expected AEs for etanercept. A full list of AEs is included in the Etanercept package insert.

Serious Adverse Events expected are readmission due to recurrent or persistent fever.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

Table 3 Adverse Event (Experience) Reporting Summary

	DMC/DSMB	IRB*	FDA	Investigator(s)/Sponsor
I. RELATED, UNEXPECTED		Requirements may vary according to institution		

Fatal or Life-Threatening	Telephone/email within 24 hours Written notification within 3 working days of telephone report	Written notification within 3 calendar days Follow up reports as soon as information becomes available	Telephone or fax within 7 calendar days Written notification within 15 calendar days using FDA form 3500A per 21CFR312.32	Telephone/email within 24 hours Written notification within 3 working days of telephone report
Serious/severe (including disability)	Written notification within 7 calendar days	Written notification within 15 calendar days.	Written notification within 15 calendar days using FDA form 3500A per 21CFR312.32	Telephone/email within 24 hours Written notification within 3 working days of telephone report
Mild/moderate	As per protocol	Quarterly	Annual report per 21CFR312.33	As per protocol
II. RELATED, EXPECTED				
Fatal, Life-Threatening	Telephone/email within 24 hours Written notification within 3 working days of telephone report	Written notification within 3 calendar days Follow up reports as soon as information becomes available	Telephone or fax within 7 calendar days Written notification within 15 calendar days using FDA form 3500A per 21CFR312.32	Written notification within 7 days
Serious/severe (including disability)	Written notification within 7 calendar days	IRB status report (annual)	Written notification within 15 calendar days using FDA form 3500A per 21CFR312.32	Written notification within 7 calendar days
Mild/moderate	As per protocol	IRB status report (annual)	Annual Report per 21CFR312.33	As per protocol
II. NOT-RELATED				
Fatal, Life-Threatening	Written notification within 10 days	IRB status report (annual)	Annual Report per 21CFR312.33	Written notification within 10 days

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and

is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

Table 3 summarizes regulatory guidelines for reporting adverse events.

8.3 Reporting of Serious Adverse Events

8.3.1 DMC Notification by Investigator

Serious adverse events will be reported to Dr. Portman within 24 hours of when investigators are first aware of SAE by telephone and/or fax. Dr. Portman will forward SAE data to the Data Monitoring Committee within 24 hours. Dr. Portman will oversee that the data and safety letter are sent to the appropriate entities within an appropriate time frame.

8.3.2 IRB Notification by Investigator

Reports of all serious adverse events (including follow-up information) must be submitted to the Institutional Review Board (IRB). The timeline of reporting procedures varies by the anticipation level and the severity of the adverse event [see Table]. Copies of each report and documentation of IRB notification and receipt will be kept in the Investigator's binder.

8.4 Stopping Rules

The DMC shall review clinical and laboratory data provided by the Principal Investigator to guide their recommendations regarding termination or continuation of the trial. If the evidence indicates the experimental intervention has an unfavorable benefit-to-risk-profile, the DMC shall recommend termination of the trial.

8.5 Safety Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Independent Data Monitoring Committee

The Data Monitoring Committee (DMC) will be composed of a pediatric rheumatologist, and a pediatric cardiologist to oversee the study. Safety data of the 196 patients with regards to adverse events, specifically focusing on infections, will be closely monitored in these patients

- *The DMC will meet at least once every 6 months via telephone conference.*
- *The safety data will be supplied to the DMC by the principal investigators team. .*
- *The DMC will prepare minutes to record the summary of its various meetings.*
- *The DMC will appropriately report its findings and/or recommendations to the PI and regulatory bodies.*

The Data Safety Plan and Data Monitoring Committee (DMC)

The data safety plan for this study is also included in detail in the Human Subject section. The DMC was established and chartered in order to monitor the progress of the pilot study. The DMC will continue this

function with amendments and additional support personnel for the larger controlled study proposed in this application.

PRIMARY RESPONSIBILITIES OF THE DMC
The DMC will be responsible for safeguarding the interests of trial participants, assessing the safety of the interventions during the trial, and for monitoring the overall conduct of the clinical trial. The DMC will provide recommendations about stopping or continuing the trial. To contribute to enhancing the integrity of the trial, the DMC may also formulate recommendations relating to the selection/recruitment/retention of participants, their management, improving adherence to protocol-specified regimens and retention of participants, and the procedures for data management and quality control.

The DMC will be advisory to the Principal Investigator. The Principal Investigator will be responsible for promptly reviewing the DMC recommendations, to decide whether to continue or terminate the trial, and to determine whether amendments to the protocol or changes in study conduct are required. The Principal Investigator will be required to forward all DMC recommendations to the IRB.

Members
The DMC will be an independent multidisciplinary group consisting of clinicians, whom collectively have experience in the management of patients with Kawasaki Disease and in the conduct and monitoring of clinical trials. Generally, three pediatric subspecialties deal with KD. These are cardiology, rheumatology, and infectious disease. Each of these subspecialties will be represented on the DMC.

DMC Chair: Mark Lewin, MD, Dr. Lewin is a senior pediatric cardiologist at CHRMC, division chief, and Associate Professor of Pediatrics. He has experience in serving on DMC and as a clinical investigator in pediatric cardiology. Dr. Lewin is currently DMC chair for the pilot trial, which is near completion. To lend continuity to the DMC he will continue as DMC chair for the proposed controlled randomized trial. Unlike, the pilot trial the proposed protocol will be multi-center. Therefore, we are engaging consultants outside of the Seattle Center. Dr. Carlos Rose, Nemours- Division of Pediatric Rheumatology, duPont Children's Hospital, Wilmington, DE has agreed to serve as consultant. We will identify an infectious disease expert as well.

Do Petersen will serve as bio-statistical consultant to the DMC. He will perform interim statistical analyses and present the results to the DMC.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

The Permission to Use, Create and Share Health Information for Research (aka HIPAA Authorization) furnished by the parents of the study participants is valid until January 31, 2017 [Attachment 2].

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required. In such an instance, it is the responsibility of the Principal Investigator to determine when these documents no longer need to be retained, abiding by regulatory guidelines that pertain to protected health information. Study data will be retained for a minimum of 5 years.

10 Compliance and Monitoring

IND # 101,223 has been assigned by the FDA. Additionally, we currently are submitting a Clinical Trial Application to Health Canada for our Canadian site. We will subcontract with an independent clinical research organization (CRO) to perform monitoring for our study. The CRO, DP Clinical, has considerable experience in monitoring the conduct of clinical trials in children. They have worked in collaboration with Dr. Portman and monitored the Triostat in Children during Cardiopulmonary Bypass Trial, funded by the OPD program, and now completed with recruitment and within the data analysis stage. The study will be performed under the Guidelines of Good Clinical Practices as outlined in FDA document "Guidance for Industry E6 Good Clinical Practice: Consolidated Guidance".

Dr. Portman as PI and holder of the IND will serve as sponsor. Division of responsibilities between the PI and the CRO are included in the table below. X = responsible; NR –not responsible, SIV – site initiation visit, IMV, interim monitoring visit, COV- close out visit.

Monitoring	**PI**	**CRO**
1. Qualify sites	X	NR
2. Provide Monitoring services (SIV, IMV, COV) at US and Canadian sites; 220 patients; First IMV after enrollment of 2 patients; subsequent visits after enrollment of 5 patients at each site.	NR	X
3. Provide monitoring reports and follow up letters to sites after each visit	NR	X
4. Final review and approval of monitoring reports	NR	X
5. Conduct drug accountability at site during IMVs (see below)	NR	X
6. Review SAEs during IMVs	NR	X
Regulatory		
1. Collect required essential documents including CRFs from each site	NR	X
2. Review and approve essential documents	NR	X
3. Provide essential regulatory documents (e.g. CV of PI, FDA1572 Form, IRB approval) to Dr. Portman for submission to regulatory agency (FDA)	NR	X
4. Submit essential documents to regulatory agency	X	NR
5. Track regulatory documents for all sites	NR	X
6. Assist sites with regulatory submission to IRBs	NR	X
7. Submit SAEs to regulatory agency	X	NR
8. Prepare IND safety reports for submission to sites, DMC, and regulatory agency	X	NR
9. Track IND safety reports	x	NR

Monitors will be blinded. Therefore, they cannot perform on-site drug accountability monitoring. At IMVs they will collect drug accountability records in sealed envelopes and forward them to un-blinded monitor in same CRO for review.

10.1 Data Management

Children's Hospital and Regional Medical Center will serve as the central site and data repository. The CRO will oversee completion of clinical research forms (CRFs) and transfer original CRFs to CHRMC for input into the central data base. Dr. Portman's staff will generate final queries to each site. Additionally, CHRMC will serve as the central site for echocardiographic reading and interpretation (see Echocardiography). Dr. Portman has previously developed an Access data-base during the currently funded FDA OPD program "Triostat in Children During Cardiopulmonary Bypass". This data-base will be amended to accept input for Kawasaki Disease Data. The Access data-base requires double entry to avoid input errors. Data will be stripped of identifiers (according to HIPAA) prior to transfer. Each patient will be assigned a study number with code to identifiers kept securely at each individual site.

The database will undergo intermittent internal audit to ascertain that CRF data are correctly inputted. The auditor, not yet assigned, will be a different individual than Data-base manager.

10.2 Conflicts of Interest

The DMC membership has been restricted to individuals free of apparent significant conflicts of interest. The source of these conflicts may be financial, scientific or regulatory in nature. Thus, neither study investigators nor individuals employed by the sponsor, nor individuals who might have regulatory responsibilities for the trial products, are members of the DMC.

The DMC members should not own stock in the companies having products being evaluated by the clinical trial. The DMC members will disclose to fellow members any consulting agreements or financial interests they have with the sponsor of the trial or with other sponsors having products that are being evaluated or having products that are competitive with those being evaluated in the trial. The DMC will be responsible for deciding whether these consulting agreements or financial interests materially impact their objectivity.

The DMC members will be responsible for advising fellow members of any changes in these consulting agreements and financial interests that occur during the course of the trial. Any DMC members who develop significant conflicts of interest during the course of the trial should resign from the DMC.

DMC membership is to be for the duration of the clinical trial. If any members leave the DMC during the course of the trial, the Principal Investigator will promptly appoint their replacements.

10.3 Timing And Purpose Of The DMC Meetings

Organizational Meeting
The initial meeting of the DMC will be an Organizational Meeting. It will be held during the final stages of protocol development, to provide advisory review of scientific and ethical issues relating to study design and conduct, to discuss the standard operating procedures for the role and functioning of the DMC, and to discuss the format and content of the Session Reports that will be used to present trial results at future DMC meetings. The Organizational Meetings will be attended by the DMC and the lead trial investigators. The DMC will be provided the drafts of the clinical trial protocol, the DMC Charter, and the current version of the case report forms. The DMC will also receive the initial draft of the Session Reports.

Interim Safety/Trial Integrity Reviews
One or more 'Safety/Trial Integrity Reviews' will be held during the progress of the study to review safety information and to review factors relating to quality of trial conduct. Unless circumstances necessitate otherwise, these meetings will be held by phone and be attended by all members of the DMC. The DMC will meet at least every 6 months.

10.4 Procedures To Ensure Confidentiality & Proper Communication
To enhance the integrity and credibility of the trial, procedures will be implemented to ensure the DMC has access to evolving information from the clinical trial regarding results of safety data. The study's Principal Investigator will be provided immediate access on an ongoing basis to patient-specific information on serious adverse events (AEs) to satisfy the standard requirement for prompt reporting to the regulatory authorities.

At the same time, procedures will be implemented to ensure proper communication is achieved between the DMC and the trial investigators. To provide a form for exchange of information among various parties who share responsibility for the successful conduct of the trial, a format for Open Sessions and Closed Sessions will be implemented. The intent of the format is to enable the DMC to provide opportunities for interaction between the DMC and others who have valuable insights into trial-related issues.

Closed Sessions

Sessions involving only DMC membership (called Closed Sessions) will be held to allow discussion of confidential data from the clinical trial. The DMC will have full disclosure of the study's safety data to achieve its mission of safeguarding the interest of participating patients.

At a final Closed Session, the DMC will develop a consensus on its list of recommendations.

Open Sessions
In order to allow the DMC to have adequate access to information provided by the study investigators, or by members of the regulatory authorities, a joint session between these individuals and DMC members (called an Open Session) will be held between the Closed Sessions. This session gives the DMC an opportunity to query these individuals about issues that have arisen during their review in the initial Closed Session. With this format, important interactions are facilitated through which problems affecting trial integrity can be identified and resolved. These individuals will either be present at the DMC meeting or be provided a telephone link.

Session Reports
For each DMC meeting, Session Reports will be provided (see Section 7 for outlines of the content of these reports). Session Reports, available to all who attend the DMC meeting, will include data on recruitment and baseline characteristics, study endpoints, eligibility violations, completeness of follow-up, and compliance. The study coordinator will prepare these Session Reports.
The Session Reports should provide information that is accurate, with follow-up that is complete to within two months of the date of the DMC meetings. The Reports should be provided to DMC members approximately three days prior to the date of the meeting.

Minutes of the DMC Meeting
The DMC will prepare minutes of their meetings. Two sets will be prepared: the Open Minutes and the Closed Minutes.

The Open Minutes will describe the proceedings in the Open Session of the DMC meeting, and will summarize all recommendations by the DMC. These minutes will be circulated immediately to the Principal Investigator.

The Closed Minutes will describe the proceedings from all sessions of the DMC meeting, including the listing of recommendations by the Committee. Copies of the Closed Minutes will be archived by the DMC chair for distribution to the Principal Investigator and regulatory authorities at the time of study closure.

The sponsors will provide a complete collection of Open and Closed Minutes to regulatory authorities at the time of new drug application and biologic licensing application.

Recommendations to the Principal Investigator
At each meeting of the DMC during the conduct of the trial, the DMC will make a recommendation to the Principal Investigator to continue or to terminate the trial. This recommendation will be based primarily on safety considerations.

The Principal Investigator is jointly responsible with the DMC for safeguarding the interests of participating patients and for the conduct of the trial. Recommendations to amend the protocol or conduct of the study made by the DMC will be considered and accepted or rejected by the Principal Investigator. The Principal Investigator will be responsible for deciding whether to continue or to stop the trial based on the DMC recommendations.

The DMC will be notified of all changes to the protocol or to study conduct. The DMC concurrence will be sought on all substantive recommendations or changes to the protocol or study conduct prior to their implementation.

10.5 Data Monitoring Guidelines

The DMC shall review clinical and laboratory data provided by the Principal Investigator to guide their recommendations regarding termination or continuation of the trial. If the evidence indicates the experimental intervention has an unfavorable benefit-to-risk-profile, the DMC shall recommend termination of the trial. The Principal Investigator will promptly report all unanticipated adverse events to the DMC for their review. The DMC will evaluate data separately for patients under 2 years of age to ensure the safety of etanercept in children in this age group.

10.6 Content Of The DMC's Session Reports

An Outline of Session Reports:

- One-page outline of the study design, possibly with a schema
- Summary of Session Report data presented at prior DMC meetings
- Major protocol changes
- Information on patient screening
- Study accrual by month
- Eligibility violations
- Baseline characteristics
  Demographics
  Laboratory values and other measurements
  Previous treatment usage and other similar information
- Analyses of primary and secondary endpoints
- Analyses of adverse events and overall safety data
- Adherence to medication schedule
- Discontinuation of medications
- Attendance at scheduled visits
- Reporting delays for key events
- Length of follow-up data available
- Participant treatment and study status

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to the Children's Research Institute's IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment 1 for a copy of the Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the

subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This study is financed through a FDA grant and support from Amgen

Relationship with Amgen: As noted previously Dr. Portman initially approached Amgen to support the pilot trial. Dr. Portman remains the Principal Investigator and sponsor of the pilot study and will serve as sponsor for the proposed trial. Amgen will supply drug, support for performance of pharmacokinetic analyses, and ancillary funds as described in the budget justification. There are no reporting requirements to Amgen. However as the manufacturer is an important component of safety monitoring, Dr. Portman will send Amgen copies of any safety data sent to the FDA. All data will be de-identified.

12.2 Subject Stipends or Payments

Study subjects will not receive payments for study participation. Travel costs will be reimbursed for visit 3 (7 day visit) if participant would otherwise not be able to travel to Hospital.

13 References

1. Belay ED, Holman RC, Clarke MJ, et al. The incidence of Kawasaki syndrome in West Coast health maintenance organizations. *Pediatr Infect Dis J.* Sep 2000;19(9):828-832.

2. Holman RC, Shahriari A, Effler PV, et al. Kawasaki syndrome hospitalizations among children in Hawaii and Connecticut. *Arch Pediatr Adolesc Med.* Aug 2000;154(8):804-808.

3. Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Pediatrics.* Dec 2004;114(6):1708-1733.

4. Samuelsson A, Towers TL, Ravetch JV. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science.* Jan 19 2001;291(5503):484-486.

5. Biezeveld M, Geissler J, Merkus M, et al. The involvement of Fc gamma receptor gene polymorphisms in Kawasaki disease. *Clin Exp Immunol.* Jan 2007;147(1):106-111.

6. Park-Min KH, Serbina NV, Yang W, et al. FcgammaRIII-dependent inhibition of interferon-gamma responses mediates suppressive effects of intravenous immune globulin. *Immunity.* Jan 2007;26(1):67-78.

7. Karassa FB, Trikalinos TA, Ioannidis JP. The role of FcgammaRIIA and IIIA polymorphisms in autoimmune diseases. *Biomed Pharmacother.* Jun 2004;58(5):286-291.

8. Newburger JW, Takahashi M, Burns JC, et al. The treatment of Kawasaki syndrome with intravenous gamma globulin. *N Engl J Med.* Aug 7 1986;315(6):341-347.

9. Burns JC, Mason WH, Glode MP, et al. Clinical and epidemiologic characteristics of patients referred for evaluation of possible Kawasaki disease. United States Multicenter Kawasaki Disease Study Group. *J Pediatr.* May 1991;118(5):680-686.

10. Sundel RP, Baker AL, Fulton DR, et al. Corticosteroids in the initial treatment of Kawasaki disease: report of a randomized trial. *J Pediatr.* Jun 2003;142(6):611-616.

11. Wallace CA, French JW, Kahn SJ, et al. Initial intravenous gammaglobulin treatment failure in Kawasaki disease. *Pediatrics.* Jun 2000;105(6):E78.

12. Newburger JW, Takahashi M, Beiser AS, et al. A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. *N Engl J Med.* Jun 6 1991;324(23):1633-1639.

13. Inoue Y, Okada Y, Shinohara M, et al. A multicenter prospective randomized trial of corticosteroids in primary therapy for Kawasaki disease: clinical course and coronary artery outcome. *J Pediatr.* Sep 2006;149(3):336-341.

14. Newburger JW, Sleeper LA, McCrindle BW, et al. Randomized trial of pulsed corticosteroid therapy for primary treatment of Kawasaki disease. *N Engl J Med.* Feb 15 2007;356(7):663-675.

15. Moreno N, Mendez-Echevarria A, de Inocencio J, et al. Coronary Involvement in Infants with Kawasaki Disease Treated with Intravenous gamma-Globulin. *Pediatr Cardiol.* Jan 2008;29(1):31-35.

16. Burns JC, Capparelli EV, Brown JA, et al. Intravenous gamma-globulin treatment and retreatment in Kawasaki disease. US/Canadian Kawasaki Syndrome Study Group. *Pediatr Infect Dis J.* 1998;17(12):1144-1148.

17. McCrindle BW, Li JS, Minich LL, et al. Coronary artery involvement in children with Kawasaki disease: risk factors from analysis of serial normalized measurements. *Circulation.* Jul 10 2007;116(2):174-179.

18. Burns JC, Capparelli EV, Brown JA, et al. Intravenous gamma-globulin treatment and retreatment in Kawasaki disease. US/Canadian Kawasaki Syndrome Study Group. *Pediatr Infect Dis J.* Dec 1998;17(12):1144-1148.
19. Kato H, Koike S, Yokoyama T. Kawasaki disease: effect of treatment on coronary artery involvement. *Pediatrics.* Feb 1979;63(2):175-179.
20. Hui-Yuen JS, Duong TT, Yeung RS. TNF-alpha is necessary for induction of coronary artery inflammation and aneurysm formation in an animal model of Kawasaki disease. *J Immunol.* May 15 2006;176(10):6294-6301.
21. Ichiyama T, Yoshitomi T, Nishikawa M, et al. NF-kappaB activation in peripheral blood monocytes/macrophages and T cells during acute Kawasaki disease. *Clin Immunol.* Jun 2001;99(3):373-377.
22. Matsubara T, Ichiyama T, Furukawa S. Immunological profile of peripheral blood lymphocytes and monocytes/macrophages in Kawasaki disease. *Clin Exp Immunol.* Sep 2005;141(3):381-387.
23. Gupta M, Noel GJ, Schaefer M, et al. Cytokine modulation with immune gamma-globulin in peripheral blood of normal children and its implications in Kawasaki disease treatment. *J Clin Immunol.* May 2001;21(3):193-199.
24. Kaneko K, Savage CO, Pottinger BE, et al. Antiendothelial cell antibodies can be cytotoxic to endothelial cells without cytokine pre-stimulation and correlate with ELISA antibody measurement in Kawasaki disease. *Clin Exp Immunol.* Nov 1994;98(2):264-269.
25. Lang BA, Silverman ED, Laxer RM, et al. Serum-soluble interleukin-2 receptor levels in Kawasaki disease. *J Pediatr.* Apr 1990;116(4):592-596.
26. Furukawa S, Matsubara T, Umezawa Y, et al. Serum levels of p60 soluble tumor necrosis factor receptor during acute Kawasaki disease. *J Pediatr.* May 1994;124(5 Pt 1):721-725.
27. Koga M, Ishihara T, Takahashi M, et al. Activation of peripheral blood monocytes and macrophages in Kawasaki disease: ultrastructural and immunocytochemical investigation. *Pathol Int.* Jul 1998;48(7):512-517.
28. Kim DS, Lee KY. Serum soluble E-selectin levels in Kawasaki disease. *Scand J Rheumatol.* 1994;23(5):283-286.
29. Furukawa S, Matsubara T, Umezawa Y, et al. Pentoxifylline and intravenous gamma globulin combination therapy for acute Kawasaki disease. *Eur J Pediatr.* Sep 1994;153(9):663-667.
30. Tracey D, Klareskog L, Sasso EH, et al. Tumor necrosis factor antagonist mechanisms of action: A comprehensive review. *Pharmacol Ther.* Oct 26 2007.
31. Furst DE, Wallis R, Broder M, et al. Tumor Necrosis Factor Antagonists: Different Kinetics and/or Mechanisms of Action May Explain Differences in the Risk for Developing Granulomatous Infection. *Seminars in Arthritis and Rheumatism.* 2006;36(3):159-167.
32. Centocor. Infliximab (Remicade®) Prescribing Information. *http://www.remicade.com/pdf/HCP_PPI.pdf. Accessed January 25, 2008.*
33. Abbott Laboratories, Humira™(adalimumab)Prescribing Information, Package Insert *http://www.rxabbott.com/pdf/humira.pdf. Accessed Jan 25, 2008*
2003.
34. Scallon B, Cai A, Solowski N, et al. Binding and functional comparisons of two types of tumor necrosis factor antagonists. *J Pharmacol Exp Ther.* May 2002;301(2):418-426.
35. Santora LC, Kaymakcalan Z, Sakorafas P, et al. Characterization of Noncovalent Complexes of Recombinant Human Monoclonal Antibody and Antigen Using Cation Exchange, Size Exclusion Chromatography, and BIAcore. *Analytical Biochemistry.* 2001;299(2):119-129.
36. Nestorov I. Clinical pharmacokinetics of TNF antagonists: how do they differ? *Semin Arthritis Rheum.* Apr 2005;34(5 Suppl1):12-18.

37. Nestorov I. Clinical pharmacokinetics of tumor necrosis factor antagonists. *J Rheumatol Suppl.* Mar 2005;74:13-18.
38. Weiss JE, Eberhard BA, Chowdhury D, et al. Infliximab as a novel therapy for refractory Kawasaki disease. *J Rheumatol.* Apr 2004;31(4):808-810.
39. Burns JC, Mason WH, Hauger SB, et al. Infliximab treatment for refractory Kawasaki syndrome. *J Pediatr.* May 2005;146(5):662-667.
40. Lovell DJ, Giannini EH, Reiff A, et al. Long-term efficacy and safety of etanercept in children with polyarticular-course juvenile rheumatoid arthritis: interim results from an ongoing multicenter, open-label, extended-treatment trial. *Arthritis Rheum.* Jan 2003;48(1):218-226.
41. Yim DS, Zhou H, Buckwalter M, et al. Population pharmacokinetic analysis and simulation of the time-concentration profile of etanercept in pediatric patients with juvenile rheumatoid arthritis. *J Clin Pharmacol.* Mar 2005;45(3):246-256.
42. Wyeth Pharmaceuticals and Amgen Inc, Etanercept (Enbrel®) Prescribing Information, Package Insert http://www.enbrel.com/prescribing-information.jsp *Accessed January, 2008*

43. Gottlieb AB, Chamian F, Masud S, et al. TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. *J Immunol.* Aug 15 2005;175(4):2721-2729.
44. Schotte H, Schluter B, Willeke P, et al. Long-term treatment with etanercept significantly reduces the number of proinflammatory cytokine-secreting peripheral blood mononuclear cells in patients with rheumatoid arthritis. *Rheumatology (Oxford).* Aug 2004;43(8):960-964.
45. Moreland LW, Cohen SB, Baumgartner SW, et al. Long-term safety and efficacy of etanercept in patients with rheumatoid arthritis. *J Rheumatol.* Jun 2001;28(6):1238-1244.
46. Moreland LW, Schiff MH, Baumgartner SW, et al. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. *Ann Intern Med.* Mar 16 1999;130(6):478-486.
47. Weinblatt ME, Kremer JM, Bankhurst AD, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med.* Jan 28 1999;340(4):253-259.
48. Lovell DJ, Giannini EH, Reiff A, et al. Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. *N Engl J Med.* Mar 16 2000;342(11):763-769.
49. Lovell DJ, Reiff A, Jones OY, et al. Long-term safety and efficacy of etanercept in children with polyarticular-course juvenile rheumatoid arthritis. *Arthritis Rheum.* Jun 2006;54(6):1987-1994.
50. Gullestad L, Aukrust P. Review of trials in chronic heart failure showing broad-spectrum anti-inflammatory approaches. *Am J Cardiol.* Jun 6 2005;95(11A):17C-23C; discussion 38C-40C.
51. Lugering A, Schmidt M, Lugering N, et al. Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. *Gastroenterology.* Nov 2001;121(5):1145-1157.
52. Kwon HJ, Cote TR, Cuffe MS, et al. Case reports of heart failure after therapy with a tumor necrosis factor antagonist. *Ann Intern Med.* May 20 2003;138(10):807-811.
53. Manadan AM, Joyce K, Sequeira W, et al. Etanercept therapy in patients with a positive tuberculin skin test. *Clin Exp Rheumatol.* Sep-Oct 2007;25(5):743-745.
54. Mohan AK, Cote TR, Block JA, et al. Tuberculosis following the use of etanercept, a tumor necrosis factor inhibitor. *Clin Infect Dis.* Aug 1 2004;39(3):295-299.
55. Sluysmans T, Colan SD. Theoretical and empirical derivation of cardiovascular allometric relationships in children. *J Appl Physiol.* Aug 2005;99(2):445-457.