# Study Protocol

## Anakinra (Kineret®) and Atorvastatin (Lipitor®) in Infants and Children with Coronary Artery Abnormalities in Acute Kawasaki Disease

**July 25, 2022**

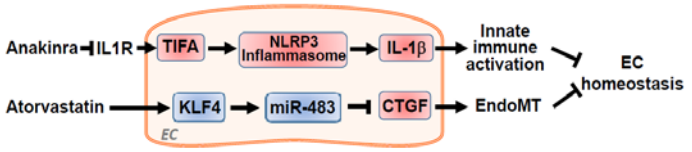
**Adriana H. Tremoulet, MD, MAS (PI)**
**Jane C. Burns (Co-investigator)**
**Sonia Jain, PhD (Biostatistician)**

## Table of Contents

# 1. Introduction

Our recent work has demonstrated that the inflammasome-dependent interleukin (IL)-1 pathway is upregulated with increased transcript abundance and levels of proteins including IL-1β and IL-1 receptor type 1 (IL-1R1) in infants and children with acute Kawasaki disease (KD).(Hoang 2014) In addition, our preliminary studies indicate that KD sera induce expression of TIFA (TNF-receptor-associated factor [TRAF]-interacting protein with a forkhead-associated [FHA] domain) in endothelial cells (ECs) (**Figure 1**). Notably, TIFA, an upstream activator of NF-κB through IL-1R signaling, has emerged as a key mediator in innate immunity and a likely participant in the activation of the NLRP3 (nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 3) inflammasome leading to IL-1β production.(Ea 2004, Huang 2012, Lin 2016). Our recent publication of a Phase I/IIa study of anakinra in KD patients with coronary artery abnormalities (CAA) demonstrated that up to 6 weeks of anakinra (up to 11 mg/kg/day) is safe and well tolerated by infants and children with acute KD. (Yang 2021) Anakinra clearance increased with illness day at diagnosis. Simulations demonstrated that more frequent intravenous (IV) dosing may result in more sustained concentrations without significantly increasing the peak concentration compared with subcutaneous (SC) dosing. Thus, both IV and SC anakinra are safe in infants and children with acute KDD and CAA.

**Fig 1.** Molecular rationale for anakinra/atorvastatin combination therapy




Upregulated during KD (red); downregulated during KD (blue)

Additional recent work by our group has demonstrated that it is not only the blocking of the IL-1 pathway that is important to prevent CAA in KD. The transformation of endothelial cells to a myofibroblast phenotype through endothelial-mesenchymal transition (EndoMT) in the coronary artery wall also contributes to aneurysm formation. (Shimizu 2013) Statins, commonly prescribed as cholesterol-lowering agents, have pleotropic effects on vascular ECs that include improving EC function, decreasing oxidative stress, and modulating innate and acquired immune responses. (Liao 2005, Oesterle 2017) Using an *in vitro* model with human ECs, we demonstrated that the krüppel-like factor 4 (KLF4)-microRNA-483 (miR-483) axis is suppressed and markers of EndoMT, including connective tissue growth factor (CTGF), are induced following incubation with sera from acute KD patients (**Figure 1**). This effect is reversed when ECs are cultured with sera from atorvastatin-treated KD patients.(He 2017) Given the pleotropic effects of statins, we conducted a Phase I/IIa, dose-escalation trial of atorvastatin for acute KD patients with early CAA (IND # 113304 held by Dr. Jane C. Burns (co-I of this proposal); NCT01431105). (Tremoulet 2015) In this study, a 6-week course of up to 0.75 mg/kg/day (max 80 mg/day) of atorvastatin was well tolerated with no serious adverse events attributable to the study drug. The areas under the curve for atorvastatin and its metabolite were larger in the study subjects compared with those reported in adults, suggesting a slower rate of metabolism in children.

These *in vitro* data, in combination with the safety of anakinra and atorvastatin in Phase I/IIa, dose-escalation studies, serve as the motivation to propose a pilot study combining anakinra at 8 mg/kg/day and atorvastatin at 0.75 mg/kg/day in children with acute KD at least 1 year old who are suffering from CAA. Throughout this protocol, this study is referred to as the “Combination therapy pilot study”.

# 2. Product name

## *2A. Product names for Combination therapy pilot study*

Anakinra (Kineret)

Atorvastatin (Lipitor)

# 3. Chemical name

## *3A. Chemical name for Combination therapy pilot study*

Recombinant-Methionyl Human Interleukin-1 Receptor Antagonist (r-metHuIL-1ra)
Atorvastatin

# 4. Proposed indication

## *4A. Proposed indication for Combination therapy pilot study*

Treatment of children with coronary artery abnormalities from acute KD.

# 5. Dosage form, route, and dosing regime

## *5A. Dosage form, route, and dosing regimen for Combination therapy pilot study*

Anakinra at 8 mg/kg/day up to a maximum of 200 mg and atorvastatin at 0.75 mg/kg/day up to a maximum of 80 mg will be administered as part of a pilot study.

# 6. List of investigators at UC San Diego

Adriana H. Tremoulet, MD, MAS (PI)
Professor
Associate Director, Kawasaki Disease Research Center
Division of Host Microbe & System Therapeutics
Department of Pediatrics
University of California, San Diego
Rady Children's Hospital San Diego

Jane C. Burns, MD
Professor
Director, Kawasaki Disease Research Center
Division of Allergy, Immunology and Rheumatology
Department of Pediatrics
University of California, San Diego
Rady Children's Hospital San Diego

Sonia Jain, PhD
Professor
Co-Director, Biostatistics Research Center
Division of Biostatistics and Bioinformatics
Department of Family and Preventive Medicine
University of California, San Diego

# 7. Study Overview

## *7A. Overall objective*

The goal of this study is to determine the safety and activity of anakinra and atorvastatin in combination in acute KD patients with coronary artery Z score ≥ 2.5. The goal will be to find a treatment regimen that could prevent or attenuate coronary artery damage in acute KD.

## *7B. Hypothesis*

We postulate that anakinra and atorvastatin in combination will be safe in children with acute KD. We also postulate that this combination therapy will reduce inflammation in children with acute KD more than using anakinra or atorvastatin alone.

## *7C. Study Type*

This is a pilot study.

## *7D. Study Population*

Children with acute KD who have a Z-score ≥ 2.5 of the LAD or RCA will be eligible for this study.

## *7E. Study Duration*

The entire study will last 5 years. Each subject will be in the study for up to 6 weeks.

## *7F. Primary Outcome*

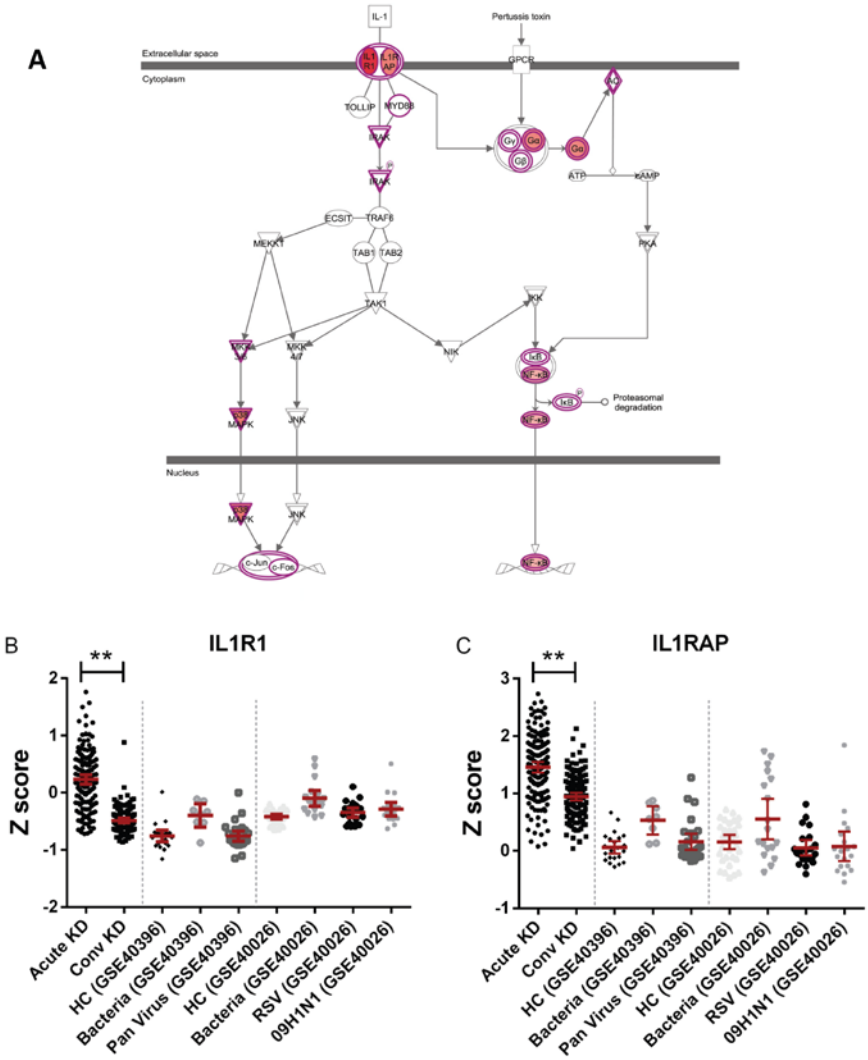
Safety of anakinra and atorvastatin in combination will be the primary outcome.

# 8. Background & Significance

## *8A. Significance*

KD, the most common cause of acquired heart disease in children in Western developed countries and Asia, is a systemic vasculitis of unknown etiology. In the United States, there are at least 5-6,000 new cases each year (Kaneko 2011). However, without a specific diagnostic test, the true burden of disease is unknown. At Rady Children's Hospital San Diego, we care for over 100 new KD patients annually and follow over 1,200 families in our outpatient KD Clinic (350 outpatient visits/year), of whom 9% developed aneurysms and 25% developed dilated coronary arteries. In Japan, the country of highest incidence (>215/100,000 children < 5 yrs.), there are more than 12,000 new cases each year and rates continue to rise (Nakamura 2010). KD causes both a myocarditis and a vasculitis that damages the coronary arteries and other medium-sized muscular arteries (Yutani 1980, Yonesaka 1989). The major sequelae of aneurysms include thrombosis, late coronary artery stenosis, myocardial ischemia, myocardial infarction, and death (Kato 1996, Gordon 2009). **Clearly, aneurysm prevention is a primary goal of treatment during the acute phase of the disease, which leads us to focus on treatment of patients with early signs of coronary artery abnormalities (CAAs) in this pilot study.**

**Figure 2.** IL1signaling pathway was the key upregulated pathway in acute KD




A) Transcripts involved in IL1 signaling pathways were more abundant in acute KD; differentially abundant transcripts between acute and convalescent KD samples are highlighted in red. B) and C) IL1R1 and IL1receptor associated protein (RAP) were differentially expressed only in acute vs. convalescent KD but not in other diseases. ** P value < 0.01

Intravenous immunoglobulin (IVIG) in combination with aspirin is the only approved therapy for KD. The major acute risk of aneurysm formation is thrombosis, which can be prevented with systemic anti-coagulation with warfarin or enoxaparin in addition to antiplatelet therapy with aspirin or clopidogrel in patients with large aneurysms (>6 mm). **However, there is no recommended therapy to halt the**

**progression of arterial wall destruction and prevent aneurysm formation beyond the initial dose of IVIG.**

### 8A1. Role of IL-1 pathway in KD and blockade by anakinra

In KD, the IL-1 pathway is upregulated, as demonstrated by increased transcript abundance by microarray and by increased levels of pathway proteins in the plasma (Maury 1988, Leung 1989, Popper 2007, Hoang 2014). (**Figure 2 and Table 1**)

**Table 1.** Comparison of the gene pathway analysis of up-regulated pathways in each group of patients

| Pathways | TREM1 pathway | p-value | IL10 pathway | p-value | Hepatic fibrosis pathway | p-value | IL6 pathway | p-value | acute phase response pathway | p-value | Role of pattern recognition receptors pathway | p-value | IL8 pathway | p-value | Toll like receptor pathway | p-value | iNOS pathway | p-value | IL1 pathway | p-value | LXR-RXR pathway | p-value | Signaling by Rho family GTPases | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| log (p-value) | 11.05 | | 9.72 | | 7.99 | | 7.82 | | 7.76 | | 7.47 | | 7.24 | | 7.02 | | 6 | | 5.11 | | 5.07 | | 4.37 | |
| Genes | TLR5 | 2.10E-121 | MAPK14 | 3.32E-94 | MMP9 | 1.97E-98 | MAPK14 | 3.32E-94 | HP | 3.86E-124 | TLR5 | 2.10E-121 | IRAK3 | 1.04E-98 | TLR5 | 2.10E-121 | IRAK3 | 1.04E-98 | IRAK3 | 1.04E-98 | MMP9 | 1.97E-98 | LIMK2 | 2.98E-92 |
| | TLR2 | 8.63E-72 | SOCS3 | 5.50E-69 | TGFA | 3.23E-73 | SOCS3 | 5.50E-69 | MAPK14 | 3.32E-94 | NLRC4 | 1.34E-102 | MMP9 | 1.97E-98 | IRAK3 | 1.04E-98 | MAPK14 | 3.32E-94 | MAPK14 | 3.32E-94 | CETP | 2.76E-73 | MAPK1 | 3.23E-68 |
| | MAPK1 | 3.23E-68 | MAPK1 | 3.23E-68 | IFNAR1 | 2.68E-64 | MAPK1 | 3.23E-68 | OSM | 1.49E-75 | TLR2 | 8.63E-72 | LIMK2 | 2.98E-92 | MAPK14 | 3.32E-94 | MAPK1 | 3.23E-68 | MAPK1 | 3.23E-68 | IL1R2 | 4.67E-63 | GNG5 | 1.73E-66 |
| | TLR8 | 2.55E-67 | IL10RB | 8.73E-67 | IL1R2 | 4.67E-63 | IL1R2 | 4.67E-63 | SOCS3 | 5.50E-69 | C1QB | 8.06E-70 | ITGAM | 1.04E-91 | TLR2 | 8.63E-72 | MYD88 | 1.04E-56 | GNG5 | 1.73E-66 | IL1RN | 8.92E-52 | CDC42EP | 6.42E-49 |
| | CASP5 | 3.96E-62 | IL1R2 | 4.67E-63 | TIMP2 | 1.96E-62 | IL1RN | 8.92E-52 | MAPK1 | 3.23E-68 | MAPK1 | 3.23E-68 | MAPK1 | 3.23E-68 | MAPK1 | 3.23E-68 | TLR4 | 1.52E-43 | MYD88 | 1.04E-56 | IL1R1 | 1.31E-50 | CDC42 | 9.29E-49 |
| | MYD88 | 1.04E-56 | IL4R | 7.77E-56 | BAMBI | 5.59E-56 | IL1R1 | 1.31E-50 | MYD88 | 1.04E-56 | TLR8 | 2.55E-67 | GNG5 | 1.73E-66 | TLR8 | 2.55E-67 | NFKBIA | 1.33E-41 | IL1R1 | 1.31E-50 | IL1B | 5.43E-50 | SLC9A1 | 3.70E-48 |
| | IL1B | 5.43E-50 | IL1RN | 8.92E-52 | IL4R | 7.77E-56 | IL1B | 5.43E-50 | IL1RN | 8.92E-52 | MYD88 | 1.04E-56 | ARRB2 | 4.61E-48 | MYD88 | 1.04E-56 | IFNGR2 | 3.74E-36 | GNA15 | 1.22E-45 | SERPINA1 | 4.42E-44 | GNA15 | 1.22E-45 |
| | CASP1 | 9.1E-44 | IL1R1 | 1.31E-50 | IL1R1 | 1.31E-50 | TNFAIP6 | 1.73E-49 | IL1R1 | 1.31E-50 | IL1B | 5.43E-50 | GNB2 | 1.33E-45 | TLR4 | 1.52E-43 | IFNGR1 | 1.68E-33 | GNB2 | 1.33E-45 | TLR4 | 1.52E-43 | GNB2 | 1.33E-45 |
| | TLR4 | 1.52E-43 | IL1B | 5.43E-50 | IL1B | 5.43E-50 | PIK3CB | 3.08E-42 | IL1B | 5.43E-50 | RNASEL | 8.12E-47 | PDGFC | 1.87E-44 | NFKBIA | 1.33E-41 | CD14 | 1.41E-30 | NFKBIA | 1.33E-41 | IL18RAP | 1.09E-40 | PIK3CB | 3.08E-42 |
| | LAT2 | 1.91E-37 | FCGR2A | 3.57E-47 | ECE1 | 7.47E-49 | NFKBIA | 1.33E-41 | SOD2 | 1.17E-48 | C3AR1 | 2.81E-44 | PIK3CB | 3.08E-42 | TLR6 | 2.38E-32 | LY96 | 1.69E-25 | GNAQ | 5.30E-35 | ABCA1 | 8.27E-38 | RHOT1 | 1.22E-39 |
| | ITGAX | 9.36E-37 | CCR1 | 8.27E-45 | PDGFC | 1.87E-44 | IL18RAP | 1.09E-40 | SERPINA | 4.42E-44 | CASP1 | 9.1E-44 | RHOT1 | 1.22E-39 | CD14 | 1.41E-30 | JAK2 | 3.14E-25 | GNAI3 | 1.03E-32 | TNFRSF1A | 3.62E-34 | NCF2 | 3.24E-37 |
| | MAPK3 | 8.30E-35 | NFKBIA | 1.33E-41 | TLR4 | 1.52E-43 | MAPK3 | 8.30E-35 | PIK3CB | 3.08E-42 | TLR4 | 1.52E-43 | PROK1 | 4.35E-38 | LY96 | 1.69E-25 | FOS | 9.98E-18 | ADCY3 | 2.11E-30 | LDLR | 1.87E-32 | CLIP1 | 2.47E-35 |
| | STAT3 | 5.35E-34 | IL18RAP | 1.09E-40 | IL18RAP | 1.09E-40 | TNFRSF1 | 3.62E-34 | NFKBIA | 1.33E-41 | PIK3CB | 3.08E-42 | NCF2 | 3.24E-37 | MAP2K6 | 1.93E-24 | NFKB2 | 6.08E-17 | GNG10 | 4.99E-30 | CD14 | 1.41E-30 | MYLK | 2.77E-35 |
| | TLR6 | 2.38E-32 | STAT3 | 5.35E-34 | PROK1 | 4.35E-38 | STAT3 | 5.35E-34 | MAPK3 | 8.30E-35 | C1QC | 1.87E-38 | PRKCD | 8.35E-37 | FOS | 9.98E-18 | | | MAP2K6 | 1.93E-24 | LY96 | 1.69E-25 | MYL9 | 3.51E-35 |
| | TREM1 | 6.11E-26 | CD14 | 1.41E-30 | IFNGR2 | 3.74E-36 | CD14 | 1.41E-30 | TNFRSF1A | 3.62E-34 | C5AR1 | 8.24E-38 | ITGAX | 9.36E-37 | NFKB2 | 6.08E-17 | | | FOS | 9.98E-18 | S100A8 | 6.69E-24 | GNAQ | 5.30E-35 |
| | JAK2 | 3.14E-25 | FCGR2C | 1.37E-28 | MYL9 | 3.51E-35 | CEBPB | 1.72E-25 | STAT3 | 5.35E-34 | PRKCD | 8.35E-37 | MYL9 | 3.51E-35 | TLR1 | 8.07E-16 | | | GNG11 | 2.06E-17 | TNFRSF1B | 2.29E-22 | MAPK3 | 8.30E-35 |
| | GRB2 | 3.61E-22 | MAP2K6 | 1.93E-24 | TNFRSF1 | 3.62E-34 | JAK2 | 3.14E-25 | CEBPB | 1.72E-25 | MAPK3 | 8.30E-35 | MAPK3 | 8.30E-35 | | | | | IL1RAP | 2.49E-17 | RXRA | 6.14E-20 | GNAI3 | 1.03E-32 |
| | MPO | 1.00E-19 | FOS | 9.98E-18 | IFNGR1 | 1.68E-33 | MAP2K6 | 1.93E-24 | JAK2 | 3.14E-25 | TLR6 | 2.38E-32 | CXCR1 | 5.79E-34 | | | | | NFKB2 | 6.08E-17 | ORM1 | 1.40E-19 | SEPT4 | 2.00E-31 |
| | NFKB2 | 6.08E-17 | IL1RAP | 2.49E-17 | IGF1R | 5.36E-32 | TNFRSF1 | 2.29E-22 | MAP2K6 | 1.93E-24 | NFKB2 | 6.08E-17 | GNAI3 | 1.03E-32 | | | | | | | CD36 | 1.55E-18 | MAP3K11 | 3.45E-31 |
| | TLR1 | 8.07E-16 | NFKB2 | 6.08E-17 | CD14 | 1.41E-30 | GRB2 | 3.61E-22 | MAP3K5 | 3.78E-23 | TLR1 | 8.07E-16 | GNG10 | 4.99E-30 | | | | | | | IL1RAP | 2.49E-17 | GNG10 | 4.99E-30 |
| | NOD2 | 2.6E-07 | BLVRA | 4.04E-16 | ACTA2 | 1.89E-27 | MCL1 | 1.41E-21 | RBP7 | 1.36E-22 | NLRP3 | 3.77E-15 | ITGB3 | 4.83E-29 | | | | | | | NFKB2 | 6.08E-17 | IQGAP1 | 1.51E-28 |
| | | | | | LY96 | 1.69E-25 | FOS | 9.98E-18 | TNFRSF1E | 2.29E-22 | NOD2 | 2.60E-07 | ITGB5 | 8.20E-29 | | | | | | | | | ARHGEF1 | 5.05E-28 |
| | | | | | TIMP1 | 1.50E-22 | IL1RAP | 2.49E-17 | GRB2 | 3.61E-22 | | | IQGAP1 | 1.51E-28 | | | | | | | | | ACTA2 | 1.89E-27 |
| | | | | | TNFRSF1 | 2.29E-22 | NFKB2 | 6.08E-17 | ORM1 | 1.40E-19 | | | VASP | 1.23E-27 | | | | | | | | | CYBB | 2.34E-27 |
| | | | | | FAS | 1.68E-20 | IL6R | 2.08E-16 | FOS | 9.98E-18 | | | CYBB | 2.34E-27 | | | | | | | | | ARPC1B | 5.45E-21 |
| | | | | | IL1RAP | 2.49E-17 | | | IL1RAP | 2.49E-17 | | | DEFA1 (in | 1.35E-26 | | | | | | | | | RHOG | 1.18E-18 |
| | | | | | NFKB2 | 6.08E-17 | | | NFKB2 | 6.08E-17 | | | MPO | 1.00E-19 | | | | | | | | | FOS | 9.98E-18 |
| | | | | | IL6R | 2.08E-16 | | | SERPING | 1.09E-16 | | | RHOG | 1.18E-18 | | | | | | | | | GNG11 | 2.06E-17 |
| | | | | | | | | | IL6R | 2.08E-16 | | | FOS | 9.98E-18 | | | | | | | | | NFKB2 | 6.08E-17 |
| | | | | | | | | | VWF | 2.61E-13 | | | GNG11 | 2.06E-17 | | | | | | | | | SEPT5 | 6.56E-13 |
| | | | | | | | | | C4BPA | 2.73E-08 | | | CXCR2 | 5.94E-14 | | | | | | | | | | |
| | | | | | | | | | | | | | AZU1 | 1.14E-12 | | | | | | | | | | |

Most abundant transcripts for the top upregulated pathways (KD acute vs. conv., other groups vs. healthy controls) and shared among KD, viral and bacterial infection groups. Pathways are ordered by descending log (p-value). Individual probe P values are uncorrected. Yellow highlighting identifies transcripts that were significant in more than one pathway. Red box identifies transcripts in the IL-1 pathway.

Furthermore, in the *Lactobacillus casei* cell wall extract mouse model that develops a KD-like coronary arteritis, the IL-1 receptor antagonist, anakinra, prevented the development of coronary lesions, as did knocking out the IL-1 receptor (Lee 2012). Anakinra has been used to treat a severe KD patient who was non-responsive to IVIG treatment (Cohen 2012).Treatment with anakinra was safe and coincided with a decrease in systemic and coronary artery inflammation. **The proposed study will determine if anakinra in combination with atorvastatin is safe to use in young children with KD and has demonstrable anti-inflammatory activity at a well-tolerated dose.**

## *8B. Use and safety of anakinra in infants and children*

Anakinra is currently used in several inflammatory diseases in infants and children, including systemic juvenile idiopathic arthritis and the autoinflammatory syndromes known as cryopyrin-associated periodic syndromes (CAPS)(Hoffman 2009, Ringold 2013). In a study of 26 patients with the autoinflammatory syndrome neonatal-onset multisystem inflammatory disease (NOMID), treatment with anakinra for 36 months had a low adverse event rate, with upper respiratory infection as the most commonly reported complication (Sibley 2012). A similar study using anakinra in another CAPS, Muckle Wells syndrome, did not demonstrate any SAEs in the 12 patients treated with anakinra, of whom 5 were children (Kuemmerle-Deschner 2011). Two case reports have been published reporting the use of anakinra in children with refractory, acute KD. A 2 year old with giant aneurysms who failed therapy with IVIG and steroids and developed giant aneurysms was treated with a 6 week course of anakinra at 1 mg/kg (Cohen 2012). He suffered no adverse effects and at 6 months coronary

angiography demonstrated resolution of the aneurysms, which was unexpected in such a severe case of KD. An 11 week old with KD and giant aneurysms was refractory to three doses of IVIG, steroids and a dose of infliximab (Shafferman 2014). She was treated with anakinra initially at 6 mg/kg/day and then increased to 9 mg/kg/day. She too did not suffer any adverse effects and at an 8 month follow up visit her coronary artery status had also significantly improved.

Given that this medication is administered once daily as a subcutaneous injection, the most common adverse reaction is irritation at the injection site. **Table 2** is a summary of published and unpublished data on the use of anakinra in children <2 years old:

**Table 2.** Use of anakinra in children < 2 years old

| Ages Treated (in months) | Max Dose (mg/kg) | Diagnosis | Reference |
|---|---|---|---|
| 17 | 1.6 | CINCA | (Matsubara 2006) |
| 3 and 4 | 6 & 10 | NOMID | (Neven 2010) |
| 2,4,13 and 22 | 1-4 | DIRA | (Aksentijevich 2009) |
| 1 | 1 | DIRA | (Stenerson 2011) |
| 8 | 11 | sJIA | (Record 2011) |
| 10 and 18 | 2-4 | sJIA | (Nigrovic 2011) |
| 5 | 3 | DIRA | (Minkis 2012) |
| 10, 13, 16, 20 | 2-4 | NOMID | (Sibley 2012) |
| 18 | 8 | MVK | (Ruiz Gomez 2012) |
| 24 | 9 | KD | (Cohen 2012) |
| 6 | 2-4 | Def IL-36Ra | (Rossi-Semerano 2013) |
| 8 | 2-10 | sJIA | (Urien 2013) |
| 3 and 14 | 2-3 | MVK | (Levy 2013) |
| 6 | 2 | NOMID | (Montealegre 2014) |
| 2 | 9 | KD & MAS | (Shafferman 2014) |
| 12 and 15 | 6 & 8 | CAPS | (Hoffman 2014) |
| 18 | 15 | sJIA & MAS | (Cartwright 2014) |
| 11 | 8 | CAPS | (Jerath 2014) |
| 13** | 4 | sJIA | (Momborquette 2014) |
| 15 and 18 | 3.5 & 4 | CINCA & sJIA | (Guedes 2014) |
| 8, 10, 15, 17, and 22 | 1 to 9 | sJIA | (Punaro 2014) |
| 6, 9 and 15 | 1-3 | CAPS | (Ombrello 2014) |
| 20 | 5 | Hyper IgD Syndrome | (Ombrello 2014) |

CINCA = chronic infantile, neurological, cutaneous and arthritis; NOMID = neonatal onset multi-system inflammatory syndrome (CINCA and NOMID are the same syndrome); DIRA = deficiency of the IL-1 receptor antagonist; sJIA = systemic juvenile idiopathic arthritis; MVK = mevalonate kinase deficiency; KD = Kawasaki disease; MAS = macrophage activation syndrome; CAPS = cryopyrin associated periodic syndrome

No adverse effects noted besides mild local reaction unless otherwise noted; **coxsackie virus while also on methotrexate and steroids;

Thus, at least 39 children < 2 years of age, and as young as 2 months of age, have been treated with anakinra with doses ranging from 1 to 15 mg/kg.

Our recent publication of a Phase I/IIa study of anakinra in KD patients with coronary artery abnormalities (CAA) demonstrated that up to 6 weeks of anakinra (up to 11 mg/kg/day) is safe and well tolerated by infants and children with acute KD. (Yang 2021) Anakinra clearance increased with illness day at diagnosis. Simulations demonstrated that more frequent intravenous (IV) dosing may result in more sustained concentrations without significantly increasing the peak concentration compared with subcutaneous (SC) dosing. Thus, both IV and SC anakinra are safe in infants and children with acute KDD and CAA.

## *8C. Safety of atorvastatin in children*

The safety of atorvastatin in children as young as 2 years of age with acute KD was assessed in a Phase I/IIa dose escalation study (IND 113304 issued to Dr. Jane C. Burns, co-investigator of this study). On May 19, 2016, the DSMB for this Phase I/IIa study evaluated the data from the Phase I dose-escalation study of atorvastatin for 6 weeks in children at least 2 years with acute KD and coronary artery damage. The DSMB determined that a 6 week course of atorvastatin was safe and well-tolerated in children ≥ 2 years with acute KD. In addition, based on the review of the safety of the Phase I study, the DSMB determined 0.75 mg/kg/day of atorvastatin to be the maximum tolerated dose. An additional 10 subjects were enrolled at 0.75 mg/kg/day as part of the dose expansion cohort study and further demonstrated the safety and tolerability of this dose in acute KD. The level of the cholesterol brain metabolite, 24-OH cholesterol, was measured in both statin-treated KD patients and in those treated with standard therapy for KD. No significant difference was found between the groups in the levels of 24-OH cholesterol, further demonstrating the safety of atorvastatin in acute KD as it does not reduce the levels of 24-OH cholesterol, which are important for normal brain development. In this study, a 6-week course of up to 0.75 mg/kg/day (max 80 mg/day) of atorvastatin was well tolerated with no serious adverse events attributable to the study drug (Tremoulet et al 2019). The areas under the curve for atorvastatin and its metabolite were larger in the study subjects compared with those reported in adults, suggesting a slower rate of metabolism in children.

## *8D. Importance of the problem*

Once aneurysms have formed, there is no way to turn back the biologic clock and undo them. The transmural inflammation destroys the normal architecture and even in children who remodel the aneurysm to form a more normal lumen, the vessel is never functionally normal again. The remodeled segment cannot dilate normally during increased myocardial oxygen demand and thus serves as a functional stenosis. It has been estimated that there are currently over 24,000 young adults in the United States with a history of KD, including over 8,000 with a history of coronary artery abnormalities. Without a new therapeutic approach this number is expected to grow by 1,400 individuals each year (Gordon 2009). An important study found that over 5% of all young adults (<40 years) evaluated by cardiac catheterization for suspected myocardial ischemia have aneurysms compatible with antecedent KD (Daniels 2012).

## *8E. KD European clinical trial of anakinra*

A clinical trial in Europe, treating IVIG-resistant KD patients (≥ 8 months of age) with a 14-day course of anakinra, demonstrated that anakinra was well tolerated in the study patients and may have some efficacy in reducing fever, markers of systemic inflammation, and coronary artery dilatation in individuals with IVIG-refractory KD. (Kone-Paut et al. 2021)

## *8F. Potential to effect change in clinical practice*

Our goal is to translate our findings on the role of the IL-1 pathway and EndoMT in acute KD and “repurpose” anakinra and atorvastatin in combination to test the hypothesis that the anti-inflammatory properties of these drugs in combination will prevent or attenuate coronary artery aneurysms in children with KD.

## *8G. Paradigm shift if Specific Aims are achieved*

By performing this study, we will bring to the forefront the need for new therapies for the subset of KD patients who are genetically predisposed to develop coronary artery damage. The paradigm shift will be to intensify initial therapy for KD patients, thus acknowledging the urgency of early intervention to prevent the permanent disability associated with aneurysm formation. Furthermore, given the unique immunological expertise within our group, demonstration of the immunomodulatory effects in a cardiovascular disease could pose a novel treatment for other cardiovascular diseases.

# 9. Hypotheses and Specific Aims

## *9A. Specific Aims of Combination therapy pilot study*

***Specific Aim 1 will test the hypothesis that a 2 to 6 week course of anakinra and atorvastatin in combination will be safe and well-tolerated in children with acute KD who have a Z-score of ≥2.5 of LAD or RCA.***

While the safety of anakinra will be assessed by monitoring for leukopenia, infection and injection site reactions, the safety of atorvastatin will be assessed by monitoring for liver and muscle toxicity.

# 10. Study design

### *10A. Accrual and study duration for Combination therapy pilot study*

We estimate enrolling 0-2 patients every 6 months with a target enrollment of 10 patients over a 5-year period. All subjects enrolled will be in the study for 6 weeks during which time they will receive once or twice daily subcutaneous injections of anakinra and once daily oral administration of atorvastatin administered by the parents at home.

## *10B. Inclusion*

1. Meets clinical criteria for KD according to American Heart Association guidelines (**Table 4**): Fever (T≥38$^{o}$C or 100.4$^{o}$C) ≥ 3 days and ≥ 2 clinical criteria with LAD or RCA z-score ≥ 2.5
2. Patient at least 1yo
3. Patient presents within the first 20 days after fever onset
4. Parent or legal guardian able and willing to provide informed consent

| **Table 4. Diagnostic criteria for KD with CAA** (Adapted from American Heart Association (Newburger 2004)): |
|---|
| KD standard clinical criteria :<br>• Bilateral conjunctival injection<br>• Changes of the mucous membranes of the upper respiratory tract: injected pharynx, injected, fissured lips, strawberry tongue<br>• Changes of the peripheral extremities: peripheral edema, peripheral erythema, periungual desquamation<br>• Polymorphous rash<br>• Cervical adenopathy >1.5 cm |

## *10C. Exclusion*

1. Use of an IL-1beta antagonist within the 3 months prior to enrollment

2. Have any chronic disease, except asthma, atopic dermatitis, autism or controlled seizure disorder
3. History of hypersensitivity to anakinra
4. Personal or immediate family history of tuberculosis (TB) or TB exposure
5. Active, culture-positive bacterial infection
6. Baseline eGFR lower than 45 mL/min/1.73m$^2$ (as subjects ≥ 1 year old)
7. Use of a statin, fibrate, or niacin within the 3 months prior to enrollment
8. Screening creatine phosphokinase (CK) ≥ 3x upper limit of normal for age
9. Taking a CYP3A4 inhibitor (i.e. cyclosporine, clarithromycin or doxycycline) in the last 7 days
10. History of allergy to atorvastatin or its derivatives

## *10D. Data Collection*

1. **Demographic data**:
   a. Patient's age at KD onset, sex, self-reported ethnicity of each biologic parent
2. **Clinical data:**
   a. Physical findings confirming the KD case definition (Newburger 2004)
   b. Illness day at study entry
   c. Response to IVIG (IVIG-resistance will be defined as persistent or recrudescent fever (T≥38.0˚C rectally) ≥36 h and < 7d following the end of the IVIG infusion (2g/kg) (Tremoulet 2008).
   d. Name, dose and indication of concomitant medications taken while on study
   e. Serum creatinine at baseline prior to receiving study drug
   f. Severity and duration of infections (with any associated cultures or diagnostic testing) during study
   g. Complete blood count, CRP, hsCRP, ESR, aspartate aminotransferase (AST), and ALT at baseline, 48 hours (not ESR as IVIG recently administered), and 2 and 6 weeks
   h. CK and fasting lipid panel at baseline (after enrollment), 48 hours, and 2 and 6 weeks (if still on study drug)
   i. Echocardiographic data at baseline, 48 hours, and 2 and 6 weeks (standard of care for patients with CAA)

## *10E. Laboratory Samples*

Subjects will have the following samples collected at baseline (pre- anakinra and -atorvastatin), 48 hours and 2 and up to 6 weeks (if still on study drug) (+/- 7 days) from enrollment:
**1. RNA collection:** Whole blood RNA will be collected using PAX gene tubes. RNA will be isolated according to the manufacturer's instructions and aliquoted and stored at -80°C.
**2. Plasma and serum:** EDTA plasma and serum will be collected for measures of inflammation.

## *10F. Echocardiography Core Lab*

An echocardiogram will be performed at the following time points:

- During initial hospitalization (as soon after admission as possible)
- 48 hours after enrollment in this study
- Study week 2 (Study day 14 ± 7 days)
- Study week 6 (Study day 42 ± 7 days)

2-D transthoracic echocardiograms (2-D Echo) will be performed on all KD subjects according to a strict pre-determined protocol at standard of care time points. All echocardiographic images will be analyzed by Dr. Beth Printz, Director of Non-Invasive Cardiovascular Imaging at Rady Children's Hospital San Diego. She will be blinded to patient data and clinical status. Dr. Printz will also assure adherence to the echo protocols across all echocardiogram technicians. 2-D Echo will be performed at KD diagnosis (within 24h of IVIG infusion) and again at two and six weeks following enrollment with sedation when

clinically indicated. The internal lumen diameters of the left main (LMCA), proximal and distal LAD, circumflex, posterior descending, and proximal and distal RCA will each be measured. Dimensions of the LMCA, proximal LAD, and proximal RCA will be adjusted for body surface area and expressed in standard deviation units (Z-scores) (Manlhiot 2009). A variable "Z worst any vessel" will be created to capture the maximal Z score of the RCA or LAD at any time point, as well as the Z-score of the LMCA if an aneurysm is present there with a Z-score larger than that of the RCA or LAD. Coronary artery aneurysm will be defined as a Z-score of 2.5 or larger of the RCA or LAD(Nishiike 2006).

Additional echocardiographic data will be collected to assess aortic root dimensions and left ventricular dimensions and function, including indices of diastolic ventricular function (mitral inflow Doppler and tissue Doppler imaging), as recent reports have demonstrated abnormalities in each of these parameters following acute KD (Kato , Printz) Aortic root and ventricular dimensions and function will be expressed as body surface area (BSA)- or age-adjusted Z scores. A dilated aortic sinus diameter will be defined as a BSA-adjusted Z score ≥ 2.0 (Ravekes 2001). LV systolic dysfunction will be defined as an age-adjusted LV fractional shortening Z score < -2; LV diastolic dysfunction will be defined as an abnormal age-adjusted mitral inflow Doppler E'-wave, E'/A' ratio, or mitral deceleration time according to published normative data (Chaudhuri).

### *10G. Data Management*

Subjects will be assigned a unique study number. Demographic and clinical information including all numerical echo data parameters will be entered into an established, password-protected, database in use by our research group since 2001 (http://www-pediatrics.ucsd.edu/kawasaki) that currently contains data on over 1,200 KD subjects. The web portal uses the secure web application Research Electronic Data Capture (REDCap) (Harris 2009).

### *10H. Patient adherence:*

Remaining syringes of anakinra and tablets of atorvastatin will be counted at the 2 and 6 week visits to assess patient adherence.

## 11. Dosing Protocol

### *11A. Drug Dosing*

All subjects will be treated with IVIG (2g/kg) and aspirin (30-50 mg/kg/day divided every 6 hours; lowered to 3-5 mg/kg/day once sent home), which is the standard of care. All subject will also receive 10 mg/kg of infliximab for CAA, which is standard of care at Rady Children's Hospital San Diego. Anakinra will be administered once or twice daily subcutaneously up to 8 mg/kg/day with a maximum dose of 200 mg/day. If the daily dose of anakinra for a subject is ≤ 100mg, then the subject will receive a subcutaneous injection of anakinra once daily. If the daily dose of anakinra is greater than 100 mg (with a max dose of 200 mg daily), then the dose will be split in half with administration subcutaneously every 12 hours (i.e. a total daily dose of 150 mg will be administered as 75 mg subcutaneously every 12 hours).

Atorvastatin will be administered orally once daily at 0.75 mg/kg/day with a maximum dose of 80 mg/day.

### *11B. Duration of Study Drug*

All subjects will receive 2 weeks of combination therapy. Subjects with an echo at 2 weeks that shows either a LAD or RCA z-score ≥ 2.0 will receive an additional 4 weeks of combination therapy to complete a total course of 6 weeks. All subjects will remain on study for the full 6 weeks whether or not they are receiving anakinra and atorvastatin.

## *11C. Dose Limiting Toxicity/Adverse Drug Toxicity*

An adverse drug toxicity will be defined as any of the following at the 2 or 6 week time point:

- Serious infection qualifying as an SAE (see Section 13) and requiring intervention
- A decrease in the white blood cell count (WBC) to <1500/mm$^3$ (Grade 3 severity by NIH/NIAID) (NIH 2009)
- An anaphylactoid reaction to an injection of anakinra
- ALT or AST more than 3x the upper limit of age and sex-adjusted normal AND >50% increase over baseline (pre-IVIG)
- CK elevation > 10 times the upper limit of normal or symptoms of muscle pain due to myositis
- A decrease in total cholesterol (TC) level that is at least 10% lower than entry level AND below 100 mg/dl (~2.5th percentile for children age 2 yrs.)

A low absolute neutrophil count (ANC) is considered part of the natural course of KD and usually occurs 2 weeks after the initial illness (Tremoulet 2011). Given this, a low ANC will not be considered an adverse drug toxicity or an adverse event in this study.

## *12D. Stopping Rules*

**Individual stopping rules:**
A subject who experiences any of the following adverse drug toxicities will discontinue anakinra immediately:

- Serious infection qualifying as an SAE (see Section 12) and requiring intervention
- A decrease in the white blood cell count (WBC) to <1500/mm$^3$ (Grade 3 severity by NIH/NIAID) (NIH 2009)
- An anaphylactoid reaction to an injection of anakinra

A subject who experiences any of the following adverse drug toxicities will discontinue atorvastatin immediately:

- ALT or AST more than 3x the upper limit of age and sex-adjusted normal AND >50% increase over baseline (pre-IVIG)
- CK elevation > 10 times the upper limit of normal or symptoms of muscle pain due to myositis
- A decrease in total cholesterol (TC) level that is at least 10% lower than entry level AND below 100 mg/dl (~2.5th percentile for children age 2 yrs.)

Any subject who experiences an adverse drug toxicity that warrants discontinuation of a study drug will be monitored for resolution of the toxicity as medically appropriate. All subjects will be monitored for 6 weeks from the time of enrollment or until resolution of the adverse drug toxicity, whichever is later.

**Study stopping rule:** The study will be discontinued if three subjects experience adverse drug toxicities.

# 12. Monitoring Side Effects

Subjects will be monitored for 6 weeks to evaluate for AEs. The main side effect of anakinra is an infusion site reaction. The most serious adverse event would be an infection or hypersensitivity reaction. See **Table 8** for definition of AEs associated with anakinra and **Table 9** for grading severity of a decrease in WBC. Subjects will also be monitored for AEs associated with atorvastatin (**Table 10**).

All subjects will be monitored for both AEs and SAEs. An adverse event is any unfavorable or unintended change in a sign (i.e. abnormal laboratory), symptom or disease temporally associated with the study treatment, whether or not it is considered to be related to the study product.

| **Table 8:** Definition of Adverse Events Associated with Anakinra | |
|---|---|
| **Adverse Event** | **NOT an Adverse Event (Expected with KD)** |
| Infusion site reaction | Atopic dermatitis or psoriasis |
| Decrease in WBC as per Table 9 | Eosinophilia (<35%) or ANC (>500) |
| Any infection | Worsening coronary artery dilatation or aneurysm compared to baseline |
| | Laboratory values compared to baseline:<br>• Worsening anemia<br>• Increasing platelet and lymphocyte counts<br>• Increasing ESR |

| **Table 9:** Grading Severity of Decrease in WBC Adverse Event (NIH 2009) | | | |
|---|---|---|---|
| **Grade 1 Mild** | **Grade 2 Moderate** | **Grade 3 Severe** | **Grade 4 Potentially Life Threatening** |
| 2,000 – 2,500/$mm^3$ | 1,500 – 1,999/$mm^3$ | 1,000 – 1,499/ $mm^3$ | < 1,000/$mm^3$ |

| **Table 10:** Definition of Adverse Events Associated with Atorvastatin |
|---|
| ALT or AST more than 3x the upper limit of age and sex-adjusted normal AND >50% increase over baseline (pre-IVIG) |
| CK elevation > 10 times the upper limit of normal or symptoms of muscle pain due to myositis |
| A decrease in total cholesterol (TC) level that is at least 10% lower than entry level AND below 100 mg/dl (~2.5th percentile for children age 2 yrs.) |

A SAE is defined as any event which:

1. Is fatal; or
2. Is life-threatening (the patient was, in the view of the Principal Investigator, in immediate danger of death from the event as it occurred); or
3. Requires hospital admission or prolongs hospitalization; or
4. Is persistent or causing significant disability; or
5. Required medical intervention, such as major surgery, to prevent a serious outcome; or
6. The Clinical Site Principal Investigator considers it to be a serious adverse event.

SAEs for this trial include, but are not limited to, anaphylaxis, arrhythmia, cardiac arrest, cardiogenic shock, death, hearing loss, hepatitis, hypertension, hypotension, myocardial infarction, renal failure, seizures, sepsis (confirmed), or shock.

Any SAE, independent of causality, which occur in a subject in the Trial, assigned to receive Product will be reported to Sobi. Reports shall be sent to the Sobi Drug Safety Department by email (drugsafety@sobi.com), or by fax (+46 8 697 32 30), within 24 hours of first awareness. Similar reporting will occur with the FDA and IRBs as stipulated by their reporting rules.

# 13. Additional Therapy

## *13a. Management of Treatment-resistant Subjects*

Treatment-resistance will be defined as persistent or recrudescent fever (T≥38.0˚C rectally) ≥36 hours and <7 days following end of IVIG infusion. Subjects who meet criteria for treatment-resistance will be treated at the Center PI's discretion.

## *13b. Additional Therapy for Coronary Artery Abnormalities*

Additional therapy for coronary artery abnormalities will be at the Center PI's discretion. A Phase III, double-blind, randomized controlled trial of the addition of infliximab to primary treatment with IVIG demonstrated that early treatment of infants and children with acute KD with infliximab plus IVIG had a more rapid decrease in inflammation, fewer days of fever, and a more rapid decrease in the internal diameter of a dilated coronary artery compared to IVIG therapy alone (Tremoulet 2014). Thus, subjects in this study will be eligible to receive infliximab at the discretion of the Center PI.

# 14. Sample Size

This study is not powered to show a difference in echocardiographic measurements or treatment response compared to controls.

# 15. Statistical Analyses

## *15A. Statistical analysis of safety*

Descriptive statistics will be calculated for demographic and baseline characteristics, variables related to biologic activity, immunologic variables, PK variables, and safety data. The study population will be described using summary descriptive statistics such as mean, median, standard deviation, and range for continuous variables and frequencies for categorical variables. All events (AEs, SAEs, and UPRs) will be recorded, documenting the course, outcome, severity, and relationship to the study treatment. Incidence rates of events and the proportion of subjects prematurely withdrawn from the study due to events will be compiled. Analyses will be performed for all patients who have received at least one dose of study treatment. Deviation from the treatment plan will be recorded in the case report forms. The percentage of subjects failing to complete the study or discontinuing prematurely (as well as the times and reasons for discontinuation) will be reported.

# 16. Data and Safety Monitoring Plan

As this is a study that involves children and the investigators must protect this vulnerable population, a DSMP has been established. To assure the safety of subjects participating in this trial, Drs. Burns and Tremoulet will meet after enrollment of every two subjects to discuss progress of the trial and possible safety issues. Safety and clinical data will be tabulated and presented by the biostatistician to the investigators after all subjects have been enrolled. An earlier meeting can be convened by Dr. Tremoulet, Dr. Burns or Dr. Jain if safety questions or other unanticipated problems arise.

The UCSD IRB, FDA, Pfizer and Sobi Pharmaceuticals will be immediately notified (within 48h) of any UPRs. For anticipated SAEs and AEs, the UCSD IRB will receive a report as per their reporting guidelines.

# **Appendix A:** Preparation of Anakinra (Kineret®) in the Hospital for Doses < 20 mg

**The following protocol is completed using a single dose, pre-filled syringe of commercially available Kineret® (100 mg/0.67ml syringe) by a pharmacist or pharmacy technician who is gowned and gloved, and is wearing a cap, face mask, and shoe covers.**

1. Aseptically transfer the anakinra directly into a sterile one ml syringe in an ISO Class 5 IV hood located in the hospital inpatient pharmacy IV Class 10,000 clean room.

2. The prepared dose syringe is then capped and labeled and given a 4-hour expiration under 2-8C refrigerated.

# References:


Aksentijevich, I., S. L. Masters, P. J. Ferguson, P. Dancey, J. Frenkel, A. van Royen-Kerkhoff, R. Laxer, U. Tedgard, E. W. Cowen, T. H. Pham, M. Booty, J. D. Estes, N. G. Sandler, N. Plass, D. L. Stone, M. L. Turner, S. Hill, J. A. Butman, R. Schneider, P. Babyn, H. I. El-Shanti, E. Pope, K. Barron, X. Bing, A. Laurence, C. C. Lee, D. Chapelle, G. I. Clarke, K. Ohson, M. Nicholson, M. Gadina, B. Yang, B. D. Korman, P. K. Gregersen, P. M. van Hagen, A. E. Hak, M. Huizing, P. Rahman, D. C. Douek, E. F. Remmers, D. L. Kastner and R. Goldbach-Mansky (2009). "An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist." N Engl J Med **360**(23): 2426-2437.PMCID: 2876877.

Cartwright, V. (2014). Personal communication regarding treatment with anakinra.
Chaudhuri, K., T. Singh Ahluwalia, S. Singh, G. Binepal and M. Khullar "Polymorphism in the promoter of the CCL5 gene (CCL5G-403A) in a cohort of North Indian children with Kawasaki disease. A preliminary study." Clin Exp Rheumatol.

Cohen, S., C. E. Tacke, B. Straver, N. Meijer, I. M. Kuipers and T. W. Kuijpers (2012). "A child with severe relapsing Kawasaki disease rescued by IL-1 receptor blockade and extracorporeal membrane oxygenation." Ann Rheum Dis **71**(12): 2059-2061.

Daniels, L., M. Tjajadi, H. Walford, D. Fick, H. Phan, P. Linz, K. Nayak, A. Kahn, J. Burns and J. Gordon (2012). "Prevalence of Kawasaki Disease in Young Adults with Suspected Myocardial Ischemia." Circulation **125**(20): 2447-2453.

Ea, C. K., L. Sun, J. Inoue and Z. J. Chen (2004). "TIFA activates IkappaB kinase (IKK) by promoting oligomerization and ubiquitination of TRAF6." Proc Natl Acad Sci U S A **101**(43): 15318-15323.PMCID: PMC524439.

Gordon, J. B., A. M. Kahn and J. C. Burns (2009). "When children with kawasaki disease grow up myocardial and vascular complications in adulthood." Journal of the American College of Cardiology **54**(21): 1911-1920.PMCID: 2870533.

Gordon, J. B., A. M. Kahn and J. C. Burns (2009). "When children with kawasaki disease grow up myocardial and vascular complications in adulthood." J Am Coll Cardiol **54**(21): 1911-1920.

Guedes, M. (2014). "Personal communication regarding treatment with anakinra."

Harris, P. A., R. Taylor, R. Thielke, J. Payne, N. Gonzalez and J. G. Conde (2009). "Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support." J Biomed Inform **42**(2): 377-381.PMCID: 2700030.

He, M., Z. Chen, M. Martin, J. Zhang, P. Sangwung, B. Woo, A. H. Tremoulet, C. Shimizu, M. K. Jain, J. C. Burns and J. Y. Shyy (2017). "miR-483 Targeting of CTGF Suppresses Endothelial-to-Mesenchymal Transition: Therapeutic Implications in Kawasaki Disease." Circ Res **120**(2): 354-365.

Hoang, L. T., C. Shimizu, L. Ling, A. N. Naim, C. C. Khor, A. H. Tremoulet, V. Wright, M. Levin, M. L. Hibberd and J. C. Burns (2014). "Global gene expression profiling identifies new therapeutic targets in acute Kawasaki disease." Genome Med **6**(11): 541.PMCID: 4279699.

Hoffman, H. M. (2009). "Therapy of autoinflammatory syndromes." The Journal of allergy and clinical immunology **124**(6): 1129-1138; quiz 1139-1140.

Hoffman, H. M. (2014). Personal communication regarding treatment with anakinra.
Huang, C. C., J. H. Weng, T. Y. Wei, P. Y. Wu, P. H. Hsu, Y. H. Chen, S. C. Wang, D. Qin, C. C. Hung, S. T. Chen, A. H. Wang, J. Y. Shyy and M. D. Tsai (2012). "Intermolecular binding between TIFA-FHA and TIFA-pT mediates tumor necrosis factor alpha stimulation and NF-kappaB activation." Mol Cell Biol **32**(14): 2664-2673.PMCID: PMC3416184.

Jerath, R. (2014). "Personal communication regarding treatment with anakinra."

Kaneko, K., K. Yoshimura, A. Ohashi, T. Kimata, T. Shimo and S. Tsuji (2011). "Prediction of the risk of coronary arterial lesions in Kawasaki disease by brain natriuretic peptide." Pediatr Cardiol **32**(8): 1106-1109.

Kato, H., T. Sugimura, T. Akagi, N. Sato, K. Hashino, Y. Maeno, T. Kazue, G. Eto and R. Yamakawa (1996). "Long-term consequences of Kawasaki disease. A 10- to 21-year follow-up study of 594 patients." Circulation **94**(6): 1379-1385.

Kato, T., A. Numaguchi, H. Ando, M. Yasui, Y. Kishimoto, K. Yasuda, D. Fukumi and T. Yasuda "Coronary arterial ectasia in a 2-year-old boy showing two symptoms of Kawasaki disease without manifesting fever." Rheumatol Int.

Kuemmerle-Deschner, J. B., P. N. Tyrrell, I. Koetter, H. Wittkowski, A. Bialkowski, N. Tzaribachev, P. Lohse, A. Koitchev, C. Deuter, D. Foell and S. M. Benseler (2011). "Efficacy and safety of anakinra therapy in pediatric and adult patients with the autoinflammatory Muckle-Wells syndrome." Arthritis Rheum **63**(3): 840-849.

Lee, Y., D. J. Schulte, K. Shimada, S. Chen, T. R. Crother, N. Chiba, M. C. Fishbein, T. J. Lehman and M. Arditi (2012). "Interleukin-1beta is crucial for the induction of coronary artery inflammation in a mouse model of Kawasaki disease." Circulation **125**(12): 1542-1550.PMCID: 3337219.

Leung, D. Y., R. S. Cotran, E. Kurt-Jones, J. C. Burns, J. W. Newburger and J. S. Pober (1989). "Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease." Lancet **2**(8675): 1298-1302.

Levy, M., A. Arion, D. Berrebi, L. Cuisset, C. Jeanne-Pasquier, B. Bader-Meunier and C. Jung (2013). "Severe early-onset colitis revealing mevalonate kinase deficiency." Pediatrics **132**(3): e779-783.

Liao, J. K. and U. Laufs (2005). "Pleiotropic effects of statins." Annu Rev Pharmacol Toxicol **45**: 89-118.PMCID: PMC2694580.

Lin, T. Y., T. W. Wei, S. Li, S. C. Wang, M. He, M. Martin, J. Zhang, T. P. Shentu, H. Xiao, J. Kang, K. C. Wang, Z. Chen, S. Chien, M. D. Tsai and J. Y. Shyy (2016). "TIFA as a crucial mediator for NLRP3 inflammasome." Proc Natl Acad Sci U S A **113**(52): 15078-15083.PMCID: PMC5206521.

Manlhiot, C., K. Millar, F. Golding and B. W. McCrindle (2009). "Improved Classification of Coronary Artery Abnormalities Based Only on Coronary Artery z-Scores After Kawasaki Disease." Pediatr Cardiol.

Matsubara, T., M. Hasegawa, M. Shiraishi, H. M. Hoffman, T. Ichiyama, T. Tanaka, H. Ueda, T. Ishihara and S. Furukawa (2006). "A severe case of chronic infantile neurologic, cutaneous, articular syndrome treated with biologic agents." Arthritis and Rheumatism **54**(7): 2314-2320.

Maury, C. P., E. Salo and P. Pelkonen (1988). "Circulating interleukin-1 beta in patients with Kawasaki disease." N Engl J Med **319**(25): 1670-1671.

Minkis, K., I. Aksentijevich, R. Goldbach-Mansky, C. Magro, R. Scott, J. G. Davis, N. Sardana and R. Herzog (2012). "Interleukin 1 receptor antagonist deficiency presenting as infantile pustulosis mimicking infantile pustular psoriasis." Archives of Dermatology **148**(6): 747-752.PMCID: 3474848.

Momborquette, J. L. (2014). "Personal communication regarding treatment with anakinra."

Montealegre, G. (2014). Personal communication regarding treatment with anakinra.
Nakamura, Y., M. Yashiro, R. Uehara, A. Sadakane, I. Chihara, Y. Aoyama, K. Kotani and H. Yanagawa (2010). "Epidemiologic Features of Kawasaki Disease in Japan: Results of the 2007-2008 Nationwide Survey." J Epidemiol.

Neven, B., I. Marvillet, C. Terrada, A. Ferster, N. Boddaert, V. Couloignier, G. Pinto, A. Pagnier, C. Bodemer, B. Bodaghi, M. Tardieu, A. M. Prieur and P. Quartier (2010). "Long-term efficacy of the interleukin-1 receptor antagonist anakinra in ten patients with neonatal-onset multisystem inflammatory disease/chronic infantile neurologic, cutaneous, articular syndrome." Arthritis and Rheumatism **62**(1): 258-267.

Newburger, J. W., M. Takahashi, M. A. Gerber, M. H. Gewitz, L. Y. Tani, J. C. Burns, S. T. Shulman, A. F. Bolger, P. Ferrieri, R. S. Baltimore, W. R. Wilson, L. M. Baddour, M. E. Levison, T. J. Pallasch, D. A. Falace and K. A. Taubert (2004). "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." Circulation **110**(17): 2747-2771.

Nigrovic, P. A., M. Mannion, F. H. Prince, A. Zeft, C. E. Rabinovich, M. A. van Rossum, E. Cortis, M. Pardeo, P. M. Miettunen, G. Janow, J. Birmingham, A. Eggebeen, E. Janssen, A. I. Shulman, M. B. Son, S. Hong, K. Jones, N. T. Ilowite, R. Q. Cron and G. C. Higgins (2011). "Anakinra as first-line disease-modifying therapy in systemic juvenile idiopathic arthritis: report of forty-six patients from an international multicenter series." Arthritis and Rheumatism **63**(2): 545-555.

NIH and DAIDS. (2009). "Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events." from http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/daidsaegradingtable.pdf.
Nishiike, S., M. Nagai, A. Nakagawa, M. Konishi, Y. Sakata, T. Aihara and T. Harada (2006). "Laryngeal tuberculosis following laryngeal carcinoma." J Laryngol Otol **120**(2): 151-153.

Oesterle, A., U. Laufs and J. K. Liao (2017). "Pleiotropic Effects of Statins on the Cardiovascular System." Circ Res **120**(1): 229-243.

Ombrello, A. (2014). "Personal communication regarding treatment with anakinra."

Popper, S. J., C. Shimizu, H. Shike, J. T. Kanegaye, J. W. Newburger, R. P. Sundel, P. O. Brown, J. C. Burns and D. A. Relman (2007). "Gene-expression patterns reveal underlying biological processes in Kawasaki disease." Genome biology **8**(12): R261.PMCID: 2246263.

Printz, B. F., L. A. Sleeper, J. W. Newburger, L. L. Minich, T. Bradley, M. S. Cohen, D. Frank, J. S. Li, R. Margossian, G. Shirali, M. Takahashi and S. D. Colan "Noncoronary cardiac abnormalities are associated with coronary artery dilation and with laboratory inflammatory markers in acute kawasaki disease." J Am Coll Cardiol **57**(1): 86-92.

Punaro, M. (2014). "Personal communication regarding treatment with anakinra."

Ravekes, W. J., S. D. Colan, K. Gauvreau, A. L. Baker, R. P. Sundel, M. E. van der Velde, D. R. Fulton and J. W. Newburger (2001). "Aortic root dilation in Kawasaki disease." Am J Cardiol **87**(7): 919-922.

Record, J. L., T. Beukelman and R. Q. Cron (2011). "Combination therapy of abatacept and anakinra in children with refractory systemic juvenile idiopathic arthritis: a retrospective case series." Journal of Rheumatology **38**(1): 180-181.

Ringold, S., P. F. Weiss, T. Beukelman, E. M. DeWitt, N. T. Ilowite, Y. Kimura, R. M. Laxer, D. J. Lovell, P. A. Nigrovic, A. B. Robinson and R. K. Vehe (2013). "2013 update of the 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: recommendations for the medical therapy of children with systemic juvenile idiopathic arthritis and tuberculosis screening among children receiving biologic medications." Arthritis Rheum **65**(10): 2499-2512.

Rossi-Semerano, L., M. Piram, C. Chiaverini, D. De Ricaud, A. Smahi and I. Kone-Paut (2013). "First clinical description of an infant with interleukin-36-receptor antagonist deficiency successfully treated with anakinra." Pediatrics **132**(4): e1043-1047.

Ruiz Gomez, A., M. L. Couce, J. Garcia-Villoria, A. Torres, A. Bana Souto, J. Yague, M. A. Vilaseca, A. Ribes and J. I. Arostegui (2012). "Clinical, genetic, and therapeutic diversity in 2 patients with severe mevalonate kinase deficiency." Pediatrics **129**(2): e535-539.

Shafferman, A., J. D. Birmingham and R. Q. Cron (2014). "High dose anakinra for treatment of severe neonatal Kawasaki disease: a case report." Pediatr Rheumatol Online J **12**: 26.PMCID: 4103976.

Shimizu, C., T. Oharaseki, K. Takahashi, A. Kottek, A. Franco and J. C. Burns (2013). "The role of TGF-beta and myofibroblasts in the arteritis of Kawasaki disease." Human Pathology **44**(2): 189-198.PMCID: 3518690.

Sibley, C. H., N. Plass, J. Snow, E. A. Wiggs, C. C. Brewer, K. A. King, C. Zalewski, H. J. Kim, R. Bishop, S. Hill, S. M. Paul, P. Kicker, Z. Phillips, J. G. Dolan, B. Widemann, N. Jayaprakash, F. Pucino, D. L. Stone, D. Chapelle, C. Snyder, J. A. Butman, R. Wesley and R. Goldbach-Mansky (2012). "Sustained response and prevention of damage progression in patients with neonatal-onset multisystem inflammatory disease treated with anakinra: a cohort study to determine three- and five-year outcomes." Arthritis Rheum **64**(7): 2375-2386.PMCID: 3474541.

Stenerson, M., K. Dufendach, I. Aksentijevich, J. Brady, J. Austin and A. M. Reed (2011). "The first reported case of compound heterozygous IL1RN mutations causing deficiency of the interleukin-1 receptor antagonist." Arthritis and Rheumatism **63**(12): 4018-4022.

Tremoulet, A. H., B. M. Best, S. Song, S. Wang, E. Corinaldesi, J. R. Eichenfield, D. D. Martin, J. W. Newburger and J. C. Burns (2008). "Resistance to intravenous immunoglobulin in children with Kawasaki disease." J Pediatr **153**(1): 117-121.

Tremoulet, A. H., S. Jain and J. C. Burns (2015). "Evaluating a novel treatment for coronary artery inflammation in acute Kawasaki disease: A Phase I/IIa trial of atorvastatin." Expert Opin Orphan Drugs **3**(9): 967-970.PMCID: PMC4979608.

Tremoulet, A. H., S. Jain, D. Chandrasekar, X. Sun, Y. Sato and J. C. Burns (2011). "Evolution of laboratory values in patients with Kawasaki disease." The Pediatric Infectious Disease Journal **30**(12): 1022-1026.PMCID: 3222731.

Tremoulet, A. H., S. Jain, P. Jaggi, S. Jimenez-Fernandez, J. M. Pancheri, X. Sun, J. T. Kanegaye, J. P. Kovalchin, B. F. Printz, O. Ramilo and J. C. Burns (2014). "Infliximab for intensification of primary therapy for Kawasaki disease: a phase 3 randomised, double-blind, placebo-controlled trial." Lancet **383**(9930): 1731-1738.

Urien, S., C. Bardin, B. Bader-Meunier, R. Mouy, S. Compeyrot-Lacassagne, F. Foissac, B. Florkin, C. Wouters, B. Neven, J. M. Treluyer and P. Quartier (2013). "Anakinra pharmacokinetics in children and adolescents with systemic-onset juvenile idiopathic arthritis and autoinflammatory syndromes." BMC Pharmacol Toxicol **14**(1): 40.PMCID: 3750485.

Yonesaka, S., T. Nakada, Y. Sunagawa, K. Tomimoto, S. Naka, T. Takahashi, T. Matubara and I. Sekigami (1989). "Endomyocardial biopsy in children with Kawasaki disease." Acta Paediatr Jpn **31**(6): 706-711.

Yutani, C., K. Okano, T. Kamiya, K. Oguchi, T. Kozuka, M. Ota and S. Onishi (1980). "Histopathological study on right endomyocardial biopsy of Kawasaki disease." Br Heart J **43**(5): 589-592.