Study Protocol

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

August 13, 2014
September 10, 2014
September 22, 2014
April 14, 2015
March 4, 2016
November 10, 2016
September 20, 2017

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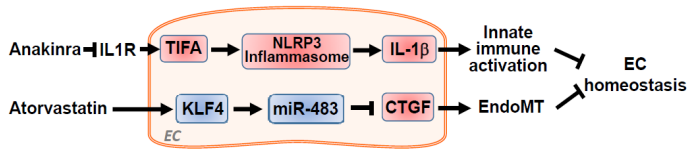
1. Introduction

As of September 7, 2017, the dose escalation study as proposed in this protocol continues to accrue subjects at 8 mg/kg/day of anakinra as was approved by the DSMB on February 10, 2017. Thus far, anakinra has been well-tolerated and safe without any serious adverse events (SAEs) related to study drug. Throughout this protocol, this study is named “Phase I/IIa anakinra study”.

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 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)

Fig 1. Molecular rationale for anakinra/atorvastatin combination therapy



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

However, 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 coronary artery abnormalities (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) Subject accrual was recently completed for this study and atorvastatin was demonstrated to be safe and well-tolerated. In this study of children at least 2 years old, the maximum dose in the study (0.75/mg/day up to 80 mg/day) was found to be the maximum tolerated dose.

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 2 years 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 name for Phase I/IIa anakinra study

Anakinra (Kineret)

2B. Product names for Combination therapy pilot study

Anakinra (Kineret)
Atorvastatin (Lipitor)

3. Chemical name

3A. Chemical name for Phase I/IIa anakinra study

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

3B. 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 Phase I/IIa anakinra study

Treatment of infants and children with coronary artery abnormalities from acute KD.

4B. Proposed indication for Combination therapy pilot study

Treatment of children with coronary artery abnormalities from acute KD.

5. Dosage form, route, and dosing regimen

5A. Dosage form, route, and dosing regimen for Phase I/IIa anakinra study

Anakinra will be administered first as an intravenous dose and then once or twice daily by subcutaneous injection following a pre-determined dose escalation protocol with three dosage levels: 4 mg/kg, 6 mg/kg, and 8 mg/kg, up to 200 mg. Once the maximum tolerated dose has been determined, the initial dose of anakinra will be given subcutaneously as with all subsequent doses.

5B. 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. Updated list of investigators at lead site of UC San Diego

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7. Study Overview

7A. Overall objective

7A1. Overall objective for Phase I/IIa anakinra study

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

7A2. Overall objective for Combination therapy pilot study

The goal of this study is to determine the safety and activity of these two drugs 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

7B1. Hypothesis for Phase I/IIa anakinra study

We postulate that anakinra will be safe in infants and children with acute KD.

7B2. Hypothesis for Combination therapy pilot study

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

7C1. Hypothesis for Phase I/IIa anakinra study

This is a Phase I/IIa, dose escalation study.

7C2. Hypothesis for Combination therapy pilot study

This is a pilot study.

7D. Study Population

7D1. Study population for Phase I/IIa anakinra study

Infants and children with acute KD who have a Z-score ≥ 2.5 of the left anterior descending (LAD) or right coronary arteries (RCA).

7D2. Study population for Combination therapy pilot study

Children with acute KD who have a Z-score ≥ 2.5 of the LAD or RCA.

7E. Study Duration

7E1. Study duration for Phase I/IIa anakinra study

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

7E2. Study duration for Combination therapy pilot study

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

7F. Primary Outcome

7F1. Primary Outcome for Phase I/IIa anakinra study

Safety of anakinra in the study population

7F2. Primary Outcome for Combination therapy pilot study

Safety of anakinra and atorvastatin in combination

7G. Secondary Outcomes

7G1. Secondary Outcomes for Phase I/IIa anakinra study

1. Pharmacokinetics of anakinra
2. Immune-monitoring pre-and post-therapy
    a. Enumeration of myeloid dendritic cell populations
    b. Enumeration of activated CD4+ and CD8+ DR+ T cells, enumeration regulatory T cells
3. Measures of inflammation pre-and post-therapy
    a. Transcript abundance changes over time by RNAseq
    b. Levels of high sensitivity (hs) C-reactive P (CRP), alpha-1 antitrypsin, TNFaR1 and R2, IFNg, IL-1 RAP, IL-1R1 and R2, MMP9, caspase-1, IL-6R, IL-18RAP, IL-12, IL-2, TGFb and IL-10
4. Echocardiographic assessment of coronary artery internal diameters expressed as Z scores pre-and post-therapy

7G2. Secondary Outcomes for Combination therapy pilot study

1. Measures of inflammation
    a. Plasma levels of IL-1R accessory protein, soluble IL-1R1, IL-17, IL-6, sIL-6R, TNFαR1, IFNγ, IL-10, and MMP9

b. Transcript abundance changes over time by RNAseq of the above analytes

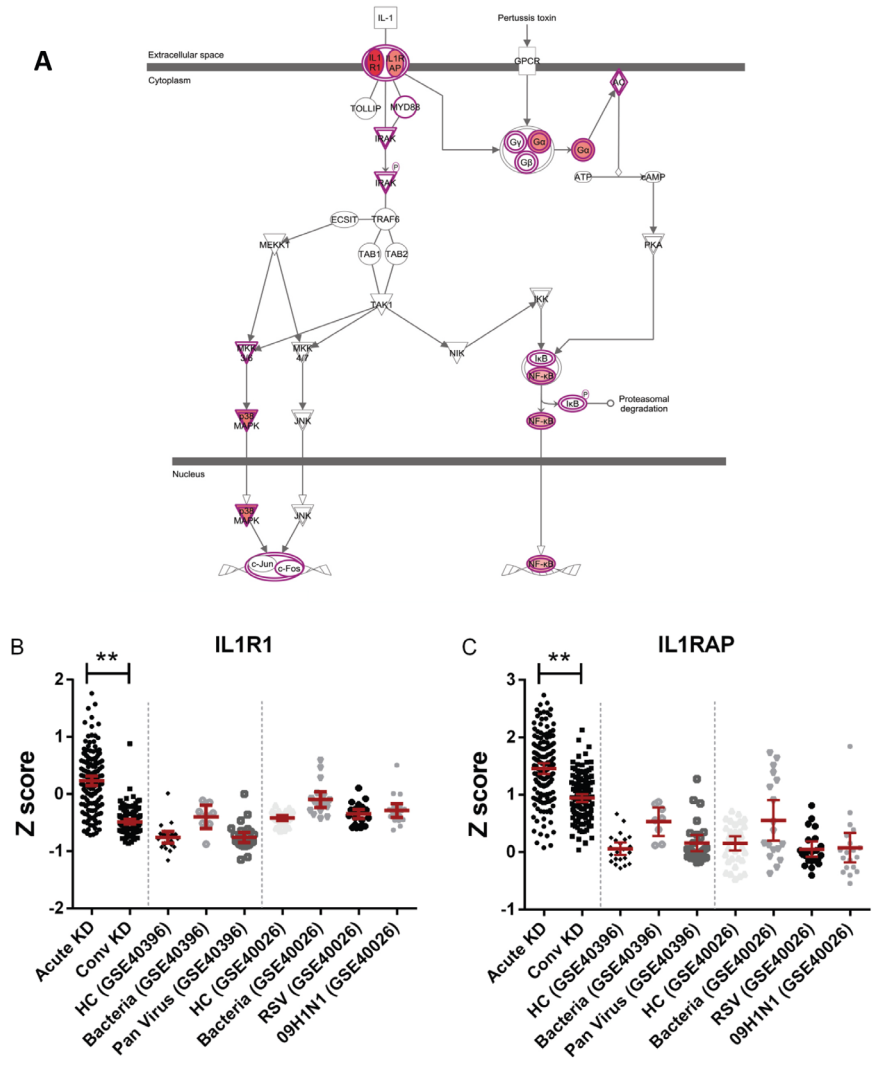
2. Endothelial cell function studies to evaluate for suppression of the innate immune response by anakinra and restoration of EC function by atorvastatin.
3. Echocardiographic assessment of coronary artery internal diameters expressed as Z scores

8. Background & Significance

8A. Significance

Kawasaki Disease (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 cared for over 85 new KD patients in 2013 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

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

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.**

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	PIK3CB	3.08E-42	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	TNFRSF1A	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	ITGAX	9.36E-37	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	MYL9	3.51E-35	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	MAPK3	8.30E-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	CXCR1	5.79E-34					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	GNAI3	1.03E-32					NFKB2	6.08E-17	ORM1	1.40E-19	SEPT4	2.00E-31
	NFKB2	6.08E-17	IL1RAP	2.49E-17	IGF1R	5.36E-32	TNFRSF1B	2.29E-22	MAP2K6	1.93E-24	NFKB2	6.08E-17	GNG10	4.99E-30							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	ITGB3	4.83E-29							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	NOD2	2.60E-07	ITGB5	8.20E-29					NFKB2	6.08E-17	IQGAP1	1.51E-28
					LY96	1.69E-25	FOS	9.98E-18	TNFRSF1B	2.29E-22					IQGAP1	1.51E-28							ARHGEF1	5.05E-28
					TIMP1	1.50E-22	IL1RAP	2.49E-17	GRB2	3.61E-22					VASP	1.23E-27							ACTA2	1.89E-27
					TNFRSF1	2.29E-22	NFKB2	6.08E-17	ORM1	1.40E-19					CYBB	2.34E-27							CYBB	2.34E-27
					FAS	1.68E-20	IL6R	2.08E-16	FOS	9.98E-18					DEFA1 (in	1.35E-26							ARPC1B	5.45E-21
					IL1RAP	2.49E-17			IL1RAP	2.49E-17					MPO	1.00E-19							RHOG	1.18E-18
					NFKB2	6.08E-17			NFKB2	6.08E-17					RHOG	1.18E-18							FOS	9.98E-18
					IL6R	2.08E-16			SERPING	1.09E-16					FOS	9.98E-18							GNG11	2.06E-17
									IL6R	2.08E-16					GNG11	2.06E-17							NFKB2	6.08E-17
									VWF	2.61E-13					CXCR2	5.94E-14							SEPT5	6.56E-13
									C4BPA	2.73E-08					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 is safe to use in young children with KD and has demonstrable anti-inflammatory activity at a well-tolerated dose. Assessments of drug activity will allow us to determine appropriate end-points for a future Phase III study to address the efficacy of anakinra in attenuating vascular wall damage and preventing aneurysms in KD patients.**

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 recent 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.

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.

On August 1, 2017, this original Phase I/IIa dose-escalation and expansion cohort study was completed. On August 4, 2017, an annual report of the atorvastatin IND was sent to the FDA demonstrating that there had been no unexpected, adverse events in this study and that atorvastatin was safe and well-tolerated. An abstract reporting the results of the Phase I study has been accepted for poster presentation at the American Heart Association in November 2017.

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). A recent 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. Ongoing KD European clinical trial of anakinra

Sobi Pharmaceuticals, the manufacturer of Kineret® (anakinra), is currently supporting a clinical trial in Europe, treating IVIG-resistant KD patients (≥ 8 months of age) with a 14-day course of anakinra. In this trial, there will be within-subject dose escalation starting at 2mg/kg and increasing every 24h through 4 mg/kg and 6 mg/kg based on persistence of fever.

8F. Potential to effect change in clinical practice

8F1. Potential of Phase I/IIa anakinra trial to effect change in clinical practice

The current clinical practice is to give every patient the same standard therapy (IVIG 2g/kg with aspirin 30-50/kg) and to monitor for CAA. In the unfortunate child who carries the genetic risk for CAA, no intervention to modify vessel wall inflammation is currently recommended (Newburger 2004).

In a recent article entitled "Mining for therapeutic gold", Director of the NIH, Dr. Francis Collins, stated that "repurposing" therapeutics should be a strategy "to translate research into clinically useful products" (Collins 2011). Our goal is to translate our findings on the role of the IL-1 pathway in acute KD and "repurpose" anakinra to test the hypothesis that the anti-inflammatory properties of this drug will prevent or attenuate coronary artery aneurysms in infants and children with KD.

8F2. Potential of Combination therapy pilot study 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 for anakinra Phase I/IIa study

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

The safety of anakinra will be assessed by monitoring for leukopenia, clinical and laboratory signs of infection, and injection site reactions.

Specific Aim 2 will describe the pharmacokinetics of anakinra and test the hypothesis that anakinra will decrease systemic and coronary artery inflammation associated with acute KD.

Blood will be tested pre-drug administration, 48 hours after initiation of therapy and at 2 weeks and 6 weeks for markers of inflammation and enumeration of dendritic cells and T cells. Echocardiographic assessment of CAA at these time points will be compared to historical controls matched for age, sex, illness day at diagnosis, and coronary artery status on initial echo.

9B. 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 as well as total and 24-OH cholesterol levels.

Specific Aim 2 will test the hypothesis that a combination of anakinra and atorvastatin therapy will decrease systemic inflammation and restore endothelial cell dysfunction.

Cytokine levels and matrix metalloproteinase activity in sera and transcript abundance in whole blood RNA will be measured pre- and post-treatment. These sera will also be tested *in vitro* with cultured endothelial cells with read-outs for innate immune signaling pathways and endothelial to mesenchymal transition (EndoMT).

10.Timeline

Table 3. Timeline of studies

Study and Task	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Phase I/IIa anakinra study								
• Spec Aim1: Safety/ tolerability/dose escalation								
• Spec Aim 2: PK & Anti-inflammatory Studies								
• Data analysis and manuscript preparation								
Combination therapy pilot study								
• IND amendment and IRB approval								
• Spec Aim 1: Safety/tolerability								
• Spec Aim 2: Anti-inflammatory studies								
• Data analysis and manuscript preparation								

11. Study design

11A. Accrual and Study Duration

11A1. Accrual and study duration for Phase I/IIa anakinra study

We estimate enrolling 0-2 patients per month at the two research sites over the course of a year with a target enrollment of 30 patients over a 4 year period. All subjects enrolled will be in the study for up to 6 weeks during which time they will receive once or twice daily subcutaneous injections of anakinra administered by the parents at home.

11A2. 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 up to 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.

11B. Inclusion

11B1. Inclusion for Phase I/IIa anakinra study

1. Meets clinical criteria for KD according to American Heart Association guidelines (**Table 4**): Fever (T≥38°C or 100.4°C) ≥ 3 days and ≥ 2 clinical criteria with LAD or RCA z-score ≥ 2.5
2. Patient is at least 28 days old
3. Patient presents within the first 20 days after fever onset
4. Parent or legal guardian able and willing to provide informed consent
5. Able to be dosed between 20 mg (min amount on syringe) and 200 mg at 8 mg/kg/day

Dose escalation level*	Minimum subject weight to administer 20 mg	Maximum subject weight to administer 200 mg
8 mg/kg	2.5 kg	25 kg

*This table includes only the 8 mg/kg dose level as enrollment in the 4 and 6 mg/kg cohorts has been completed as of the Nov 2016 revision.
*As of the August 2017 revision, we have enrolled 2 subjects at 8mg/kg/day without any dose limiting toxicities.

11B2. Inclusion for Combination therapy pilot study

1. Meets clinical criteria for KD according to American Heart Association guidelines (**Table 4**): Fever (T≥38°C or 100.4°C) ≥ 3 days and ≥ 2 clinical criteria with LAD or RCA z-score ≥ 2.5
2. Patient at least 2yo and weighs ≤ 25 kg (weight limit based on anakinra max dose of 200 mg/day at a calculated dose of 8 mg/kg/day)
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 :

- Bilateral conjunctival injection
- Changes of the mucous membranes of the upper respiratory tract: injected pharynx, injected, fissured lips, strawberry tongue
- Changes of the peripheral extremities: peripheral edema, peripheral erythema, periungual desquamation
- Polymorphous rash
- Cervical adenopathy >1.5 cm

11C. Exclusion

11C1. Exclusion for Phase I/IIa anakinra study

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. Patient has a 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 Scr ≥ 0.50 mg/dL (28 days old to 1 year old) or an eGFR lower than 45 mL/min/1.73m^2 (≥ 1 year old)

11C2. Exclusion for Combination therapy pilot study

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

11D. Data Collection

11D1. Data collection for Phase I/IIa anakinra study

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. Complete white blood count (WBC), hsCRP, erythrocyte sedimentation (ESR), fibrinogen, and alpha-1-antitrypsin levels at baseline, 48 hours (not ESR as IVIG recently administered), 2 weeks and 6 weeks (if still on study drug) after drug administration
   f. Serum creatinine at baseline prior to receiving study drug
   g. Severity and duration of infections (with any associated cultures or diagnostic testing) during study
   h. Echocardiographic data at baseline, 48 hours, and 2 and 6 weeks (standard of care for patients with CAA)

11D2. Data collection for Combination therapy pilot study

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)

11E. Laboratory Samples

11E1. Laboratory samples for Phase I/IIa anakinra study

Subjects will have the following samples collected at baseline (pre- anakinra), 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, serum, and heparinized whole blood collection: EDTA plasma and serum will be collected for measures of inflammation. Whole blood (5 ml in large green top) in a sodium heparin tube will be collected for T cell subset and cytokine studies.

11E2. Laboratory samples for Combination therapy pilot study

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.

11F. Pharmacokinetic Samples (only collected in Phase I/IIa anakinra study)

- Dosing history (dates, times and amounts of doses)
- Height and weight on day of PK sampling
- Dates and times PK samples drawn
- PK samples around initial half of the 1st dose will be drawn during the following sample windows
    1. 1-3 hours after the dose
    2. 4-6 hours after the dose
    3. 7-9 hours after the dose, prior to administration of the second half of the dose at 12 hours
- Trough samples 48 hours, and 2 and 6 weeks after the start of the first dose

11G. Echocardiography Core Lab for Phase I/IIa anakinra study and Combination therapy pilot study

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).

11H. Data Management for Phase I/IIa anakinra study and Combination therapy pilot study

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).

11I. Patient adherence:

11I1. Patient adherence for Phase I/IIa anakinra study

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

11I2. Patient adherence for Combination therapy pilot study

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

12. Dosing Protocol

12A. Dose Levels

12A1. Dosing for Phase I/IIa anakinra study

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. In order to minimize the pain from subcutaneous injections, the first two doses of anakinra have been administered intravenously and divided every 12 hours for the dose escalation part of the study (i.e. 4 mg/kg/day is 2 mg/kg every 12 hours for two doses). As has been published in the peer-reviewed literature, we administered the dose by bolus over a 1 to 3 minute period (Opal 1997). As anakinra is formulated for intravenous administration, dilution is not necessary prior to administration. The unused material is discarded in a medical waste container after each administration. See **Appendix A** for details regarding preparation of the study drug for administration of less than 20 mg.

All subsequent doses are administered subcutaneously starting 24 hours after the first dose of the medication. This study has 3 dose levels (4 mg/kg/day, 6 mg/kg/day, and 8 mg/kg/day), with a maximum dosage of 200 mg daily (**Table 5**).

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).

Table 5. Dose Levels		
Dose Cohort	**Dose Level**	**Number of Subjects**
1	4 mg/kg/day	3-6
2	6 mg/kg/day	3-6
3	8 mg/kg/day	3-6
TOTAL		9-18

Due to anakinra's predominant renal clearance, its dose is reduced in adults with significant renal dysfunction, 30mL/min (30% of normal adults). Given immaturity in renal function of infants, no infants will be enrolled during the first 4 weeks of life. At the age of 4 weeks, the typical GFR for a term infant is approximately 1.5 mL/min/kg (Rhodin et al. Pediatr Nephrol. 2009 Jan;24(1):67-76.). This represents 40-45% of the mature adult value (allometrically scaled), or approximately 50% above the threshold for dose reduction due to renal dysfunction in adults (45 mL/min/1.73m^2). We will require that individual study participant infants have renal function significantly better than eGFR of 30 mL/min/1.73m^2. At 4 weeks of age, the original infant Schwartz equation predicts a eGFR of 35 mL/min/1.73m^2 with a serum creatinine of 0.7 mg/dL. To provide an additional safety buffer and account for more specific creatinine assay in use today, we will only enroll infants with screening Scr ≤ 0.50 mg/dL, approximately equivalent to an eGFR of 45 mL/min/1.73m^2. For children ≥ 1 year of age who may be eligible for enrollment based on weight, higher Scr will be acceptable as long as the eGFR remains at least 45 mL/min/1.73m^2. Calculations will be made with the widely accepted modified Schwartz equation (http://www.globalrph.com/specialpop.htm). As we do not expect renal abnormalities to occur with KD or because of anakinra, Scr will only be assessed at baseline.

12A2. Dosing for Combination therapy pilot study

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.
Anakinra will be administered once or twice daily subcutaneously at 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.

12B. Duration of Study Drug

12B1. Duration of study drug for Phase I/IIa anakinra study

All subjects will receive 2 weeks of 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 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.

12B2. Duration of study drug for Combination therapy pilot study

All subjects will receive 2 weeks of 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 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.

12C. Dose Limiting Toxicity/Adverse Drug Toxicity

12C1. Dose limiting toxicity for Phase I/IIa anakinra study

Dose Limiting Toxicity (DLT) will be defined as any of the following at the 2 or 6 week time point:

- 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 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 a DLT or an adverse event in this study.

12C2. Adverse Drug Toxicity for Combination therapy pilot study

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. Dose Escalation for the Phase I/IIa anakinra study

The first 3 subjects in the study will be treated with 4 mg/kg/day as an IV dose of 2 mg/kg q 12 h and then as 4 mg/kg/day SQ for 2-6 weeks. If there are no dose limiting toxicities after 2-6 weeks of therapy, the safety and clinical data will be tabulated and presented to the PI, co-investigators and the Data Safety Monitoring Board (DSMB) for evaluation as to opening the next dose level (6 mg/kg) (**Tables 5 and 6**). Additional subjects will be enrolled at the highest dose if a dose limiting toxicity is not reached at 8 mg/kg/day for further study in the Phase IIa (total number of subjects is 30).

Table 6. Dose Escalation Decision Rules

Subjects with a DLT at a Given Dose	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level until maximum tolerated dose.
1 out of 3	Enter a total of 6 patients at this dose level. • If 0 of these additional patients experience a DLT, proceed to the next dose level. • If 1 of these additional patients experiences a DLT, then dose escalation is stopped (see below)
≥ 2	At least 3 additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. This lower dose level will be declared the MTD.

Probability of Dose Escalation: The following probabilities of dose escalation (**Table 7**) are computed as the sum of the binomial probabilities of the following two possible outcomes that would allow dose escalation to occur: (1) no DLT observed in the first three subjects; or (2) one DLT observed in the first three subjects, followed by no DLT observed in three additional subjects at the same dose level. For example, when the underlying DLT rate is no more than 15%, the probability of dose escalation will be at least 81% or alternatively 19% probability of early study termination. Exact 95% binomial confidence intervals will be used to describe the rate of successful dose escalation.

Table 7: The probabilities for dose escalation in Phase I					
True but unknown DLT rate	**≤0.1**	**≤0.15**	**≤0.2**	**≤0.3**	**≤0.4**
Probability of escalation	≥91%	≥81%	≥71%	≥49%	≥31%

12E. Maximum Tolerated Dose for Phase I/IIa anakinra study

The Maximum Tolerated Dose (MTD) will be defined as the highest dose of anakinra studied at which no more than one in six patients experiences a DLT during the 6 weeks of treatment. Subjects will be enrolled at the maximum tolerated dose until a total of 30 subjects have been enrolled in the study. Given the 3+3 design of the study, if there is a DLT then a total of 6 subjects will enrolled per dose level. If we reach the 8 mg/kg/day dose level, then up to a maximum of 12 subjects will be enrolled at a dose level below the MTD (4 mg/kg and 6 mg/kg). This leaves 18 subjects treated at the MTD if the third dosing level is reached. If there are no DLTs at the first two levels, then 3 subjects will be enrolled in these two drug levels (total of 6 subjects) leaving 24 subjects to be enrolled at the MTD. Thus, between 18 and 24 subjects will be enrolled at the MTD if the 3rd dose level is reached.

12F. Stopping Rules

12F1. Stopping rules for Phase I/IIa anakinra study

Individual stopping rule: A subject who experiences a DLT will discontinue anakinra immediately and will be monitored for resolution of the toxicity as medically appropriate. All subjects will be monitored for the 6 weeks from the time of enrollment or until resolution of the DLT, whichever is later.

Study stopping rule: Table 6 shows the Phase I dose escalation rules. The study will be discontinued if at the first dose (4 mg/kg/day), the number of subjects that experiences a DLT is greater than 2. Otherwise, the study will determine its MTD using the escalation rules described.

12F2. Stopping rules for Combination therapy pilot study

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 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

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.

13. Monitoring Side Effects

13A. Monitoring side effects for Phase I/IIa anakinra study

Subjects will be monitored for 6 weeks to evaluate for adverse events (AEs). The main side effect of anakinra is an infusion site reaction. The most serious adverse event would be an infection or hypersensitivity reaction. All subjects will be monitored for both AEs and SAEs. An AE 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: • Worsening anemia • Increasing platelet and lymphocyte counts • 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

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. Unanticipated problems in involving risk (UPRs) will be reported to each agency as stipulated by their reporting rules.

13B. Monitoring side effects for Combination therapy pilot study

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 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.

14. Additional Therapy for Phase I/IIa anakinra study and Combination therapy pilot study

14a. 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.

14b. 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.

15. Sample Size

15A. Sample size for Phase I/IIa anakinra study

A study population of a minimum of 18 KD patients treated with the MTD of anakinra (See Section 11E for subjects at MTD) and 18 historic matched controls from the Kawasaki Research database will have 80% power based on a two-sided, paired t-test to compare change scores from baseline between cases and controls with alpha set to 0.05 to detect a minimum difference for the change score for several transcript levels of the IL-1 family, including IL-1RAP (pooled SD of 3.2 and corresponding difference of 2.2) and IL-1R1 (pooled SD of 8 and corresponding difference of 5.6). The effect sizes are based on relevant changes that were found in the largest KD microarray study done to date (Hoang 2014).

15B. Sample size for Combination therapy pilot study

This study is not powered to show a difference in echocardiographic measurements or treatment response compared to controls. Instead, the sample size will be determined based on detectable differences in inflammatory markers and readouts from *in vitro* EC studies. A sample size of 10 KD patients treated with anakinra/atorvastatin combination therapy vs 10 matched historical controls treated with standard therapy alone will have 80% power based on a two-sided, paired t-test with alpha set to 0.05 to detect an effect size of 89% in the mean change of EC homeostasis (i.e. markers of EndoMT) and inflammasome activation (i.e. TIFA, NLRP3) using the *in vitro* EC culture system.

16. Pharmacokinetic Analysis for Phase I/IIa anakinra study

The area under the concentration-time curve (AUC) around the first dose for anakinra will be roughly estimated as the initial concentration (the concentration extrapolated back to time zero) divided by the elimination rate, if the elimination appears first-order (log scale) or zero-order (linear scale). We will also roughly estimate the AUC using the trapezoidal rule up to the last measurable concentration. The extrapolated area after the last concentration will be estimated as $C_{last/\lambda z}$, where C_{last} is the last measurable concentration and λz is the terminal slope of the curve. AUC from time 0 to infinity $AUC_{0-\infty}$ is calculated as $AUC_{0-last} + C_{last/\lambda z}$. Noncompartmental oral clearance rate (Cl/F_{NC}) is calculated as the ratio of dose to $AUC_{0-\infty}$. Apparent volume of distribution (Vd/F_{NC}) is calculated as Cl/F_{NC} over λz. Half-life ($t_{1/2}$) is calculated as 0.693/λz. Plasma anakinra trough concentrations collected in the subacute phase (weeks 2 and 6) will be compared to the 48 hour post-dose concentration collected during the acute phase (study day 2). $AUC_{0-\infty}$, $Cl/F_{1\text{-}C}$ (oral clearance from 1-compartment model), and $Vd/F_{1\text{-}C}$ (volume of distribution from 1-compartment model) will also be determined using a 1-compartment model. This second modeling approach will be used because the data are relatively sparse for an intensive PK study, which may limit our ability to estimate every parameter for some patients.

17. Immune Monitoring for Phase I/IIa anakinra study

PBMC will be separated by Ficoll Hypaque from KD subjects at three time points (pre-treatment, 2 weeks, and 6 weeks post-treatment) for staining with specific monoclonal antibodies (MoAbs) to characterize DC lineages, macrophages and their activation/maturation stage. Anti-DR (MHC class II molecules) and anti-CD86 define maturity and activation. CD11c defines the DC lineage, and is not expressed on macrophages. Antibodies anti-CD11c will be used in combination with anti-DR and anti-CD86 and other markers, well described in the literature. Briefly: to define myeloid DC: anti-CD11b, (also expressed on macrophages but negative in plasmacytoid DC and thymic DC) and anti-BDCA-1. To define immature tolerogenic myeloid DC very relevant in the immune-regulation in KD, we will look at CD14, co-expressed on macrophages but not on plasmacytoid DC, in $CD11^{c}$+ $CD11^{b}$+ myeloid DC. Macrophages will be identified by the expression of $CD11^{b}$ and CD14 in CD11c negative cells. To enumerate activated CD4+ and CD8+ T cells and regulatory T cells (Treg) we will use anti-

CD4, anti-CD8, anti-DR, anti-CD25 (Treg are $CD25^{high}$) and anti-IL-7 receptor, expressed on pro-inflammatory cells and peripherally-induced Treg but not in natural Treg.

18. Measures of Activity

18A. Measures of inflammation for Phase I/IIa anakinra study

Transcript abundance will be measured in whole blood by microarray. Levels of hsCRP, α-1-antitrypsin and fibrinogen, and the WBC and ESR will be measured in the hospital clinical laboratory. Levels of TNFaR1 and R2, IFNg, IL-1 RAP, IL-1R1 and R2, MMP9, caspase-1, IL-6R, IL-18RAP, IL-12, sIL-2R, TGFb and IL-10 will be measured by ELISA. Results of transcript abundance and measures of inflammation will be compared to database controls from the KD Research Center (>600 KD patients with available RNA and plasma samples in our biorepository) matched (1 case: 2 controls) by age (± 2 yrs. with lower limit of 2 yrs.), sex, and coronary artery Z-score.

18B. Measures of inflammation and endothelial cell function for Combination therapy pilot study

We will measure plasma levels of IL-1R accessory protein and soluble IL-1R1 from the study subjects and controls as surrogates for IL-1α and IL-1β, which are not stable in patient plasma. To further assess inflammation, we will also measure the following pro-inflammatory cytokines in plasma: IL-17, IL-6, sIL-6R, TNFαR1, IFNγ, as well as the anti-inflammatory cytokine IL-10. To assess the change in MMP9 activity that could be a direct effect of atorvastatin treatment,(Koh 2002, Massaro 2010) we will measure plasma MMP9 levels. Transcript levels in whole blood RNA (PAXgene® tubes) of all of the above analytes will be measured using a combination of TaqMan 5'-nuclease Gene Expression Assays (Applied Biosystems) and the Inflammatory cytokine array (Qiagen). Relative abundance of the target transcripts will be normalized to the expression level of TAF1B, which has been validated as a housekeeping gene that is not upregulated in acute KD.(Popper 2007) Time points for all of the above studies will be baseline, 48 hr, and 2 wk post-treatment. Three different control groups with samples collected at similar time points will be matched from our KD biorepository at UCSD (>600 subjects): standard treatment alone (n=20), standard treatment+atorvastatin (n=10), and standard treatment+anakinra (n=10). Banked samples, including those from Phase I trials of both anakinra and atorvastatin, will allow us to match study patients on potentially important variables including age, sex, illness day at diagnosis, and baseline coronary artery damage as measured by echocardiogram.

Subject and control sera will be compared for suppression of the innate immune response by anakinra and restoration of EC function by atorvastatin. IL-1-TIFA-NLRP3 activation as well as levels of KLF4, miR-483, and EndoMT marker genes including CTGF in cultured human aortic ECs and mouse lung ECs with subject and control sera from baseline, 48 hr, and 2 wk post-treatment will be measured. Functional assays will also be performed including NO bioavailability and EC permeability. Induction of EndoMT will be compared using paired sera from pre-treatment and 2 wk time points while induction of an EC inflammatory response will be compared using pre-treatment and 48 hr post-treatment paired sera. These readouts of innate immune activation and EC function will be correlated with clinical data.

19. Statistical Analyses

19A. Statistical analysis of safety for Phase I/IIa anakinra study and Combination therapy pilot study

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. Refer to Section 12E for the operating characteristics of the Phase I dose escalation for the Phase I/IIa anakinra study.

19B. Statistical analysis of inflammatory studies

19B1. Statistical analysis of inflammation studies for Phase I/IIa anakinra study

The objective of this matched case-control study is to determine the activity profile of anakinra in the treatment of KD subjects with CAA. Measures of inflammation will be compared to that of matched controls via paired statistical tests (e.g. McNemar's test for categorical outcomes or the paired t-test for continuous outcomes). Non-parametric alternatives will be considered only if distributional assumptions are violated.

Control Group: Controls will be chosen from the >600 subjects with data in our web-based data repository in REDCap. Controls will be matched to study patients based on age, sex, Illness Day at diagnosis, and baseline Z score.

Analysis: Each marker will be initially summarized descriptively and analyzed individually. Separate multivariable mixed effects regression modeling for clustered data (to account for the case-control paired nature of samples) will be used to characterize the markers of anakinra-treated subjects versus matched controls to adjust for any known confounders and additional time points (baseline, 48 hours, 2 weeks, and 6 weeks). In addition, any covariate at the individual level that is simultaneously unbalanced and associated with the outcome will be included in the model. Covariates of interest include demographic variables (e.g., ethnicity, Illness Day at diagnosis, response to IVIG therapy). Permutation tests may be implemented to verify that valid p-values will be obtained even if model assumptions are not correct. Note that the mixed model seamlessly accommodates different times of measurement (e.g. 2-week and 6-week time points) as well as missed measurements and data from subjects who are lost to follow-up. A corresponding Generalized Estimating Equations (GEE) model will be used as the sensitivity analysis method. An analogous secondary model including all subjects in this study will also be considered to examine the dose versus activity relationship. In this model, dosage will be included as a covariate to examine any dosage trend in activity.

The above approach will be repeated for the other markers. That is, multi-level mixed effects regression models analogous to the above methodology will be applied for other markers: Levels of high sensitivity (hs) CRP, alpha-1 antitrypsin, TNFaR1 and R2, IFNg, IL-1 RAP, IL-1R1 and R2, MMP9, caspase-1, IL-6R, IL-18RAP, IL-12, IL-2, TGFb and IL-10.

Measures of transcript abundance will also be analyzed via microarray analysis. Data will be pre-processed first and then microarray analysis procedures will be used to measure abundance. The R software (version 3.0.2) and Bioconductor package in R will be used for the analysis. For coronary outcomes as assessed by echocardiography, we will compare the delta Z scores (baseline minus 2-weeks and 6-weeks) between anakinra subjects and matched controls. A GEE model will be used as the sensitivity analysis method. Since this is a safety and early efficacy study, no adjustments for multiple comparisons will be made for biomarker analyses. P-values less than 0.05 will be considered statistically significant. All statistical analyses will be performed in R version 3.2.0 (www.**r**-project.org/*)*. Similarly, analyses will be repeated on all subjects to characterize any dose-activity relationship, adjusting for dosage as a covariate in the model.

To determine the "best" or "set of best" markers that have the greatest improvement from baseline in anakinra-treated patients, ROC and AUROC analysis will be considered. In the event that there is

high multi-collinearity amongst the markers, we will adopt the “glmpath” approach, which is based on Least Angle Regression (LARS) methods to select the best biomarkers (Park 2007).

Missing data assumptions: The proposed mixed-effects regression model is consistent under the assumption that the outcome data are “missing at random” (MAR), that is, mixed models with a random effect assume only that the probability that an outcome is missing depends on observed outcomes. GEE models, which are proposed as a sensitivity analysis, assume that the outcome data are “missing completely at random”, that is, that the probability that an outcome is missing does not depend on any previous, present or succeeding value of the outcome. This will enable us to assess the impact of the missing data mechanism on statistical inferences drawn from these models.

19B2. Statistical analysis of inflammation and endothelial cell studies for Combination therapy pilot study

The objective of this matched case-control study is to determine the activity profile of the combination of anakinra and atorvastatin in the treatment of KD subjects with CAA. Measures of inflammation and endothelial cell function will be compared to that of matched controls via paired statistical tests (e.g. McNemar’s test for categorical outcomes or the paired t-test for continuous outcomes). Non-parametric alternatives will be considered only if distributional assumptions are violated.

Control Group: Controls will be chosen from the >600 subjects with data in our web-based data repository in REDCap. Controls will be matched to study patients based on age, sex, illness day at diagnosis, and baseline Z score.

Analysis: Each marker will be initially summarized descriptively and analyzed individually. Separate multivariable mixed effects regression modeling for clustered data (to account for the case-control paired nature of samples) will be used to characterize the markers of treated subjects versus matched controls to adjust for any known confounders and additional time points (baseline, 48 hours, 2 weeks, and 6 weeks). In addition, any covariate at the individual level that is simultaneously unbalanced and associated with the outcome will be included in the model. Covariates of interest include demographic variables (e.g., ethnicity, Illness Day at diagnosis, response to IVIG therapy). Permutation tests may be implemented to verify that valid p-values will be obtained even if model assumptions are not correct. Note that the mixed model seamlessly accommodates different times of measurement (e.g. 48h and 2-week time points) as well as missed measurements and data from subjects who are lost to follow-up. A corresponding Generalized Estimating Equations (GEE) model will be used as the sensitivity analysis method. To determine the “best” or “set of best” markers that have the greatest improvement from baseline in anakinra/atorvastatin-treated patients, ROC and AUROC analysis will be considered to choose the best of set markers for a future clinical trial. In the event that there is high multicollinearity amongst the markers, we will adopt the “glmpath” approach, which is based on Least Angle Regression (LARS) methods to select the best biomarkers.(Park 2007)

Measures of transcript abundance will also be analyzed via RNAseq. The R software (version 3.0.2) and Bioconductor package in R will be used for the analysis. For coronary outcomes as assessed by echocardiography, we will compare the delta Z scores (baseline minus 2-weeks and 6-weeks) between anakinra subjects and matched controls. A GEE model will be used as the sensitivity analysis method. Since this is a pilot study, no adjustments for multiple comparisons will be made for biomarker analyses. P-values less than 0.05 will be considered statistically significant. All statistical analyses will be performed in R version 3.2.0 (www.**r**-project.org/*)*.

Missing data assumptions: The proposed mixed-effects regression model is consistent under the assumption that the outcome data are “missing at random” (MAR), that is, mixed models with a random effect assume only that the probability that an outcome is missing depends on observed outcomes. GEE models, which are proposed as a sensitivity analysis, assume that the outcome data are “missing completely at random”, that is, that the probability that an outcome is missing does not

depend on any previous, present or succeeding value of the outcome. This will enable us to assess the impact of the missing data mechanism on statistical inferences drawn from these models.

20. Data and Safety Monitoring

20A. Data and Safety Monitoring Board (DSMB) for Phase I/IIa anakinra study

As a first step in assuring the safety of subjects participating in this trial, monthly conference calls with the study nurse, PI, and co-investigators will be conducted to discuss progress of the trial and possible safety issues. Safety and clinical data will be tabulated and presented to the PI and co-investigators by the study biostatistician for discussion prior to opening the next dose level. The DSMB, a panel consisting of outside experts, including one clinician with expertise in KD, a pharmacologist, and a biostatistician, in collaboration with the Study Investigators, will pre-specify a plan for evaluating the data on a periodic basis and will set up pre-specified stopping rules for safety.

20B. Data and Safety Monitoring Plan (DSMP) for Combination therapy pilot study

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 2 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 five subjects have been enrolled and at the end of the study. 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.

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