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Calcineurin Inhibitor Treatment of Intravenous Immunoglobulin–Resistant Kawasaki Disease

Adriana H. Tremoulet, MD¹, Paige Pancoast, PharmD¹, Alessandra Franco, MD, PhD¹, Matthew Bujold, BS¹, Chisato Shimizu, MD¹, Yoshihiro Onouchi, MD², Alyson Tamamoto, BS³, Guliz Erdem, MD³, Debra Dodd, MD⁴, and Jane C. Burns, MD¹

¹Department of Pediatrics, University of California at San Diego and Rady Children's Hospital, La Jolla, CA

²Laboratory for Cardiovascular Diseases, Center for Genomic Medicine, RIKEN, Yokohama, Japan

³Department of Pediatrics, Kapiolani Medical Center, Honolulu, HI

⁴Department of Pediatrics, Monroe Carell Jr Children's Hospital at Vanderbilt, Nashville, TN

Abstract

Objective—To describe the clinical course and outcome of 10 patients with Kawasaki disease (KD) treated with a calcineurin inhibitor after failing to respond to multiple therapies.

Study design—Demographic and clinical data were prospectively collected using standardized case report forms. T-cell phenotypes were determined by flow cytometry, and KD risk alleles in *ITPKC* (rs28493229), *CASP3* (rs72689236), and *FCGR2A* (rs1801274) were genotyped.

Results—Intravenous followed by oral therapy with cyclosporine (CSA) or oral tacrolimus was well tolerated and resulted in defervescence and resolution of inflammation in all 10 patients. There were no serious adverse events, and a standardized treatment protocol was developed based on our experiences with this patient population. Analysis of T-cell phenotype by flow cytometry in 2 subjects showed a decrease in circulating activated CD8⁺ and CD4⁺ T effector memory cells after treatment with CSA. However, suppression of regulatory T-cells was not seen, suggesting targeting of specific, proinflammatory T-cell compartments by CSA.

Conclusion—Treatment of refractory KD with a calcineurin inhibitor appears to be a safe and effective approach that achieves rapid control of inflammation associated with clinical improvement.

Although the majority of patients with Kawasaki disease (KD) become afebrile following a single infusion of intravenous immunoglobulin (IVIG) in combination with aspirin, persistent or recrudescent fever occurs in 15%-25% of patients with KD, who are classified as IVIG-resistant. These patients require additional anti-inflammatory therapy, necessitating prolonged hospitalization, and are at increased risk for developing coronary aneurysms.¹⁻³

Several lines of evidence suggest that KD is a T-cell-mediated disease. Examination of limited autopsy tissues has demonstrated infiltration of the coronary arterial wall by CD8⁺ T cells.⁴ T-effector memory cells (interleukin [IL]-15r⁺ CCR7⁻) circulate during the acute illness, and cessation of inflammation is associated with expansion of the regulatory T-cell (Treg) population in peripheral blood.⁵ Studies of a mouse model that mimics the coronary

artery lesions of KD have suggested that T cells are essential for the development of coronary artery inflammation.⁶⁻⁸ One study has suggested a beneficial effect of mizoribine, an inhibitor of lymphocyte proliferation, in a *Candida albicans* KD mouse model.⁹

Susceptibility to KD is influenced by genetic variation in the transforming growth factor- β , calcineurin/nuclear factor of activated T cells (NFAT), and immunoglobulin receptor pathways. Functional single nucleotide polymorphisms (SNPs) in the *inositol 1,4,5-trisphosphate 3-kinase C* (*ITPKC*, rs28493229) gene on chromosome 19q13.2 and the *caspase-3* (*CASP3*, rs113420705; formerly rs72689236) gene on chromosome 4 influence susceptibility to KD, risk of coronary artery aneurysms, and IVIG resistance in both Japanese and US children.¹⁰⁻¹² ITPKC acts as a negative regulator of T- and B-cell activation through the calcineurin/NFAT signaling pathway, and the risk allele may result in increased signaling through this pathway, leading to cell activation. Caspase-3 promotes apoptosis, and the risk allele in the enhancer region may result in reduced caspase-3 transcription and thus decreased apoptosis and a prolonged proinflammatory state. Taken together, these data support a potential role for calcineurin inhibitors in the treatment of children with IVIG-resistant KD.¹³

In a genome-wide association study, a functional polymorphism in the *Fc fragment of IgG low-affinity IIa receptor* (*FCGR2A*) gene (rs1801274, H131R [A/G], risk allele H131 [A]) met genome-wide significance for association in 4 cohorts of patients of European and Asian descent with KD.¹⁴ FCGR2A binds IgG, leading to cell activation,¹⁵ and the A allele at the nonsynonymous polymorphic locus, H131R, increases the binding affinity to IgG2.¹⁶ Studies testing the influence of this SNP on disease severity are in progress.

A study of the use of the calcineurin inhibitor cyclosporine (CSA) in IVIG-resistant Japanese patients with KD has documented the safety of this therapeutic approach.¹⁷ Here we report 10 patients with treatment-resistant KD who were successfully treated with calcineurin/NFAT inhibitors (9 with CSA and 1 with tacrolimus). We sought to better define clinically effective dosing and tapering of the calcineurin inhibitors in this population and to characterize treatment-associated side effects. In addition, we analyzed genotypes for risk alleles in T-cell activation pathways and characterized the time course of changes in T-cell subsets in 2 children with KD treated with CSA.

Methods

IVIG resistance was defined as persistent or recrudescence fever (temperature $\geq 38.0^{\circ}\text{C}$ measured rectally or orally) at least 36 hours, but not longer than 7 days, after completion of the first IVIG infusion (2 g/kg).¹ Eight subjects were treated with CSA at Rady Children's Hospital San Diego between January 1, 2007, and December 31, 2010, for persistent fever after 2 doses of IVIG. For intravenous (IV) therapy, we prescribed nonmodified CSA, and for oral therapy we prescribed a modified CSA microemulsion (Neoral; Novartis, East Hanover, New Jersey), which has been shown to have better and more consistent oral absorption than nonmicroemulsion formulations.^{18,19} Four of the 8 subjects were enrolled in an ongoing randomized, placebo-controlled clinical trial of infliximab (1 dose of 5 mg/kg) for intensification of initial therapy, and their treatment assignment remained blinded. Two additional cases received care at Kapiolani Medical Center (patient 9) and Vanderbilt University (patient 10). All patients met the American Heart Association criteria, with at least 4 days of fever and 4 of 5 clinical features.²⁰ The following clinical data were recorded: illness day at diagnosis (first day of fever = day 1 of illness), age at diagnosis, laboratory test results over the course of illness (ie, erythrocyte sedimentation rate, C-reactive protein [CRP] level, complete blood count), temperature data, medications, adverse events, echocardiography data, drug dose and serum level, and drug response. During

calcineurin inhibitor therapy, patients were monitored at baseline for serum alanine aminotransferase, blood urea nitrogen, creatinine, magnesium, calcium, potassium, and CRP and subsequently serially evaluated for serum magnesium and CRP. Patients were classified as having normal coronary arteries if the mean internal diameter of the right coronary artery or left anterior descending coronary artery, normalized for body surface area (*z*-score), was < 2.5 SD units.²¹ The maximum coronary artery *z*-score, or *z*-max, was defined as the highest *z*-score in the left anterior descending coronary artery or the right coronary artery measured by echocardiography during the first 6 weeks after onset of fever. This study protocol was reviewed and approved by the University of California San Diego's Institutional Review Board.

Eight patients in whom DNA was collected as part of a separate University of California San Diego Institutional Review Board–approved protocol were genotyped for the KD risk alleles in *ITPKC*, *CASP3*, and *FCGR2A*. Genomic DNA from whole blood or mouth wash samples was extracted as described previously.²² *ITPKC* (rs28493229) and *FCGR2A* (rs1801274) were genotyped using Taqman polymerase chain reaction assays (Applied Biosystems Assay ID: C__25932098_10 and C_9077561_20, respectively; Foster City, California), following the manufacturer's instructions. A *CASP3* (rs113420705; formerly rs72689236), custom Taqman PCR assay was designed (forward primer: GCGGATGGGTGCTATTGTGA; reverse primer: CGAGGGCGGCAGTCA; probe 1: CTCATACCTTCTACAACCG; probe 2: CATACCTTCCACAACCG) and performed as described earlier.

T-cell phenotypes were studied at 4 time points: before initial therapy with IVIG, 36–48 hours after initiation of CSA, and in the subacute and convalescent phases of KD (2–3 weeks and 1–3 months after onset of fever, respectively). Peripheral blood mononuclear cells were isolated by standard Ficoll-Hypaque density gradient centrifugation. T cells were expanded for 48 hours with 30 U/mL of recombinant IL-2. Cells were harvested, stained with a combination of monoclonal antibodies to detect circulating effector (CD4⁺ or CD8⁺, IL-15R⁺ CCR7[−]) memory T cells and Tregs (CD4⁺/CD8⁺, CD25^{high}), and analyzed with a FACSCalibur flow cytometer (BD Biosciences, Sparks, Maryland). The following antibodies were used: anti-CD4 PerCP-Cy5.5 (clone RPA-T4, mouse IgG1k), anti-CD8 APC (clone RPA-T8, mouse IgG1k), anti-CCR7 PE (clone 3D12, rat IgG2a,k), anti-IL15Ra FITC (clone JM7A4, mouse IgG2b), anti-CD25 PE (clone BC96, mouse IgG1k) (all from eBioscience, San Diego, California), and anti-DR framework FITC (clone G41-6, mouse IgG2a,k; BD Bioscience).

Results

Of the 269 patients with KD treated within the first 10 days of illness at Rady Children's Hospital San Diego between 2007 and 2010, 8 (3%) received CSA for IVIG-resistant KD. In comparison, the annual rate of IVIG resistance at the hospital during the study period ranged from 15.0% to 23.6%. One additional child received CSA, and another received tacrolimus for treatment-resistant KD at other institutions. Patient characteristics and baseline laboratory data at the time of KD diagnosis are presented in Table I.

All 10 patients received at least 2 doses of IVIG at 2 g/kg, as well as standard aspirin therapy at 80–100 mg/kg/day (Table II). Before initiation of calcineurin inhibitor therapy, 3 patients received at least 2 doses of pulsed methylprednisolone (30 mg/kg/day IV), 4 patients received infliximab (5 mg/kg IV), and 4 others were enrolled in an ongoing Food and Drug Administration-sponsored Phase III clinical study and received IVIG plus either infliximab or placebo as part of their primary therapy for KD. Four patients (1, 2, 8, and 10)

developed a coronary artery aneurysm before initiation of calcineurin inhibitor treatment; all 4 experienced subsequent improvement in their aneurysms.

Six of the 7 patients treated with CSA for persistent or recrudescence fever, experienced rapid defervescence and clinical improvement within 24 hours of their first dose of CSA. One child (patient 1) with refractory KD developed hypertension, gastrointestinal bleeding, and pneumatosis intestinalis while receiving high-dose steroids (pulsed steroids, 30 mg/kg IV daily for 3 days, followed by 5 mg/kg/day IV for 16 days) before initiation of CSA therapy; her steroid dose was tapered once she reached a therapeutic level of CSA. Our CSA C0 (trough) target after 3 doses was 50-150 ng/mL, and our CSA C2 maximum level was 600 ng/mL. Increasing the dose of CSA in patient 3 and the dose of tacrolimus in patient 10 in response to low trough levels led to improved responses. A brisk taper over a 2-week period led to a recrudescence of arthritis in patient 4, which was easily corrected by increasing the dose, followed by a slower taper. The total duration of therapy ranged from 2 weeks to 3 months.

Adverse events included hypomagnesemia (patients 2 and 3), hirsutism (patients 4 and 8), and acute otitis media (patient 8) (Table II). After our experience with the first 3 patients, we modified our treatment protocol to include oral supplementation with a protein/magnesium complex at the initiation of CSA therapy (Table III).

Seven of the 8 patients in whom DNA was available for genotyping were found to carry at least 2 KD risk alleles (Table IV; available at www.jpeds.com).

Circulating CD4⁺ and CD8⁺ T effector memory cells (IL-15r⁺ CCR7⁻) were detectable at the time of KD diagnosis, and levels decreased within 36-48 hours of initiation of CSA therapy (Figure; available at www.jpeds.com). During the CSA taper in the subacute illness period, the percentage of CD4⁺ T effector memory cells exhibited a transient increase. CD4⁺ CD25^{high} Tregs were present at the time of initial diagnosis of KD, and levels increased during the course of CSA treatment (Figure).

Discussion

Although IVIG therapy in patients with KD has reduced the rate of coronary artery aneurysms from 25% in untreated patients to 5% in patients responsive to IVIG, IVIG resistance continues to be a problem worldwide and is associated with an increased risk of aneurysm formation, with reported rates as high as 15%-38% in a mixed-ethnic US population.^{1,23} In this case series, we found that IV followed by oral CSA therapy was well tolerated and resulted in rapid defervescence in most treated patients and resolution of inflammation in all treated patients. No major complications occurred, and we developed a standardized treatment regimen based on our experience with this patient population (Table III).

Various therapies, including additional IVIG, steroids, infliximab, plasmapheresis, methotrexate, cyclophosphamide, and CSA, have been used in patients with IVIG-resistant KD with varying results.^{13,24-28} All of the patients reported in previous series eventually experienced resolution of fever and markers of inflammation, but because KD is a self-limited illness, the role of these adjuvant therapies in recovery is difficult to evaluate. Of the available options for treating IVIG-resistant KD, the most common (and the one that we used first in our patient cohort) was a second dose of IVIG. To date, no prospective, randomized clinical trial of patients with IVIG-resistant KD has been conducted to determine the optimal therapeutic approach, because of limitations of sample size, statistical power, and cost. As an alternative to waiting for the development of IVIG resistance before providing additional treatment, targeted intensification of initial therapy may be the optimal

strategy to prevent aneurysms. In such countries as Japan, where a scoring system seems to effectively identify patients at increased risk for IVIG resistance, adding a calcineurin inhibitor to standard therapy could be considered.²⁹

A cohort of 28 Japanese patients treated with oral CSA (Neoral) 4-8 mg/kg/day for treatment-resistant KD was reported recently.¹⁷ Eighteen of these 28 patients (64.3%) responded with defervescence and decreased CRP within 3 days of starting CSA, and an additional 4 patients became afebrile within 4-5 days. The remaining 6 patients had persistent or recrudescence fever after at least 5 days of CSA therapy. In that study, the low rate of treatment response might have been influenced by the use of oral therapy initially, which might have been poorly absorbed, in light of the documented poor absorption of oral medications in children with acute KD.^{30,31} In addition, monitoring only trough CSA levels could have led to underdosing and delayed treatment response. Importantly, there were no serious adverse events associated with CSA treatment in these IVIG-resistant patients.

The major side effects of CSA in children include nephrotoxicity, hepatotoxicity, hypertension, electrolyte imbalance, and seizures.³² Some adverse effects occurred in our case series of 10 patients with treatment-resistant KD. Two children developed hypomagnesemia, which responded to oral supplementation. Modification of our protocol to include oral supplementation with magnesium protein complex (133 mg tablet daily) at the initiation of CSA therapy eliminated this problem. Two children developed hirsutism, which resolved after discontinuation of CSA. One child who was treated with CSA for 3 weeks for severe arthritis experienced a flare of arthritis during a rapid taper over 10 days. One child defervesced within 24 hours but developed a new fever on the sixth day of CSA therapy and was found to have otitis media, which responded promptly to a single dose of ceftriaxone.

Given the extensive use of CIs in pediatric transplantation, several studies on monitoring CSA levels in this age group have been published. Trough levels have been shown to be poorly reflective of the area under the concentration-time curve (AUC) using a 12-hour dosing interval.^{33,34} Maintaining a target AUC is critical to reduce the risk of underdosing or overdosing. However, collecting an AUC₀₋₁₂, or even an AUC₀₋₄ (which reflects the first 4 hours after dose administration and a critical period in drug absorption), requires multiple blood draws. A study in liver transplant recipients demonstrated that the concentration 2 hours postdose (C₂) correlated well with the AUC₀₋₄, and that a C₂ of 300-600 ng/mL is associated with lower rejection.³⁵ Recent data suggest that CSA C₂ concentrations, but not C₀ concentrations, reflect the effect of CSA on down-regulation of proinflammatory cytokines in liver transplant recipients.³⁶ Based on this information and after consultation with our pediatric pharmacologist and renal transplant service, we added a C₂ level to the CSA drug monitoring in our last 4 patients in the series.

Six of our patients treated with CSA initially received their calcineurin inhibitor intravenously. Two of these patients had a flare of arthritis with a rapid taper that resolved with a slower taper. Given that the goal of therapy is rapid control of inflammation and that oral absorption may be impaired in children with acute KD, IV administration likely will result in more rapid and reliable serum levels and therapeutic effects.³⁰ The most common error with IV administration of CSA leading to low or erratic drug levels is failure to use polyvinyl chloride-free tubing and to prepare the medication in a plastic rather than a glass or polyethylene container, because the drug binds to plastic.

All of the patients in this series carried at least 1 risk allele for KD, and 7 of 8 carried at least 2 risk alleles. However, the small number of patients precludes any meaningful statistical analysis. As our group has previously demonstrated, patients with KD with the *ITPKC* C allele and the *CASP3* A allele are more likely to be IVIG-resistant, but a causal relationship

has not been established.³⁷ ITPKC acts as a negative regulator of T-cell activation through the calcineurin/NFAT signaling pathway by converting inositol 1,4,5-trisphosphate to its inactive form, inositol 1,4,5-tetrasphosphate. Inositol 1,4,5-trisphosphate P3 binds to its receptor on the endoplasmic reticulum, resulting in the release of calcium into the cytoplasm. Increased calcium levels activate the calcium calmodulin-dependent phosphatase calcineurin, which dephosphorylates NFAT, which binds to the IL-2 promoter, leading to IL-2 expression and up-regulation of inflammation.³⁸ The functional SNP in *CASP3* decreases NFAT binding to the transcription factor binding site in the promoter resulting in reduced *CASP3* transcription. This in turn may reduce T-cell apoptosis, leading to a protracted proinflammatory state.³⁹ The contribution of genetic variation in *FCGR2A* to KD pathogenesis and response to treatment is still under investigation.

We have previously shown that T effector memory cells with a proinflammatory phenotype are present during the acute phase of KD, and that this population subsequently contracts while the central memory T-cell population expands.⁵ Compared with patients responsive to IVIG reported previously, our 2 patients resistant to treatment who underwent T-cell evaluation had a higher percentage of circulating CD4⁺ T effector memory cells at the time of initial diagnosis (pre-IVIG therapy, 2.3% and 2.5% vs 0.2%, median of 6 patients responsive to IVIG).⁵ Our results also demonstrate that within 2 days of CSA treatment, the CD4⁺ T and CD8⁺ effector memory cell population contracted but then rebounded as the CSA was tapered, suggesting that CSA selectively inhibits the proinflammatory T-cell compartments. CD4⁺ CD25^{high} Tregs were also present during the acute phase of the illness and expanded during treatment with CSA. Given that KD is a self-limited disease, and that Tregs are thought to play a key role in controlling the inflammation of acute KD, preserving the Treg compartment may be important for recovery.

In the absence of data from prospective clinical trials, we translated our knowledge of KD genetics to create experience-based guidelines for the management of patients with highly resistant KD. Limitations of this study include its observational nature, the small sample size, and limited data on genotype and T-cell phenotype. In addition, the protocol evolved in response to clinical issues, resulting in only 3 patients treated with the final version of our protocol. Only 1 patient was treated with tacrolimus, so no specific guidelines were developed. The choice of calcineurin inhibitor for patients with IVIG-resistant KD likely will be guided by physician and institutional experience with these agents for the treatment of other conditions. Given the small numbers of patients with highly resistant KD, a prospective clinical trial would be challenging to design and execute.

CSA given intravenously until inflammation subsides, followed by oral therapy, provides a safe and effective treatment for patients with highly resistant KD that allows outpatient management and was associated with a good outcome in this small series of patients.

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Glossary

AUC	Area under the concentration-time curve
CRP	C-reactive protein
CSA	Cyclosporine
IL	Interleukin
IV	Intravenous
IVIG	Intravenous immunoglobulin
KD	Kawasaki disease
NFAT	Nuclear factor of activated T cell
SNP	Single nucleotide polymorphism
Treg	Regulatory T cell

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Figure.

Flow cytometry analysis of circulating T cells obtained at diagnosis (pre-IVIG, acute), post-CSA (36-48 hours after initiation of CSA), subacute (2-3 weeks), and convalescent (1-3 months) time points. Cell populations were defined as CD4⁺ T effector memory (Tem) cells (IL-15r⁺ CCR7⁻), CD8⁺ Tem cells (IL-15r⁺ CCR7⁻), and CD4⁺ CD25^{high} Treg cells. Illness day 1 refers to the first day of fever. *Tem*, T effector memory cells; *WBC*, white blood cell.

Table 1

Characteristics of patients with treatment-resistant KD at the time of diagnosis and before initial IVIG

Patient	Age, months	Sex	Ethnicity	Illness day at diagnosis*	ESR, mm/hour	CRP, mg/dL
1	7.6	Female	Filipino	4	77	21.3
2	131.6	Female	African American	6	1 [†]	38.2
3	27.6	Male	Hispanic	3	140	31.3
4	48.6	Male	European descent	6	37	3.4
5	25.9	Female	Hispanic	5	45	8.3
6	14.8	Female	African American	6	NA	5.3
7	46.1	Male	European descent	4	29	6.1
8	17.1	Female	Chinese	6	61	25.5
9	13.0	Female	Japanese/European descent	3	38	3.7
10	2.0	Male	European descent	7	10	5.8

ESR, erythrocyte sedimentation rate; NA, not available.

* Illness day 1 refers to the first day of fever.

[†]Low ESR associated with consumptive coagulopathy marked by elevated d-dimer and low fibrinogen (2000 ng/mL; 586 mg/dL).

Table II
Therapeutic interventions, treatment response, and outcomes of patients with KD treated with a calcineurin inhibitor

Patient	Clinical indication for calcineurin inhibitor	Anti-inflammatory therapy before calcineurin inhibitor*	Initial calcineurin inhibitor dose, mg/kg/day, [†] route	Illness day at start of calcineurin inhibitor therapy	Hospital days after start of calcineurin inhibitor therapy	CRP (mg/dL) 24 hours before/48 hours after calcineurin inhibitor therapy [‡]	New adverse events during calcineurin inhibitor therapy	Z-max [§]	Duration of calcineurin inhibitor therapy	Clinical response to calcineurin inhibitor
1	Steroid intolerance	IVIG × 3; methylprednisolone	3.0 IV	22	11	3.4/1.9	None	21.9 (LAD aneurysm)	40 days	Able to taper steroids
2	Persistent fever	IVIG × 2; infliximab; methylprednisolone	4.1 IV	8	7	14.5/7.3	Magnesium 1.3 mg/dL [¶]	3.4 (RCA aneurysm)	19 days	Afebrile within 24 hours
3	Persistent fever	IVIG × 2; infliximab	4.1 IV	6	8	7.7/4.9	Magnesium 1.3 mg/dL [¶]	1.8	27 days	Persistent fever with low CSA trough; afebrile within 72 hours of higher dose
4	Persistent rash and arthritis	Study drug; IVIG × 2	10.1 oral	25	NA	<0.5/<0.5	Hirsutism	0.6	39 days	Resolution of arthritis within 48 hours; flare with rapid taper, resolution with slower taper
5	Recurrent fever	Study drug; IVIG × 2	11.7 oral	9	2	0.9/0.6	None	1.8	37 days	Afebrile within 24 hours
6	Recurrent fever; arthritis	IVIG × 2	9.9 oral	11	2	0.9/0.7	None	0.9	76 days	Afebrile within 24 hours; flare of arthritis with rapid taper; resolution with slower taper

Patient	Clinical indication for calcineurin inhibitor	Anti-inflammatory therapy before calcineurin inhibitor [*]	Initial calcineurin inhibitor dose, mg/kg/day, route	Illness day at start of calcineurin inhibitor therapy	Hospital days after start of calcineurin inhibitor therapy	CRP (mg/dL) 24 hours before/48 hours after calcineurin inhibitor therapy [‡]	New adverse events during calcineurin inhibitor therapy	Z-max [§]	Duration of calcineurin inhibitor therapy	Clinical response to calcineurin inhibitor
7	Recurrent fever	Study drug; IVIG × 2	3.1 IV	9	5	2.5/1.4	None	0.9	31 days	Afebrile within 24 hours
8	Recurrent fever	Study drug; IVIG × 2	3.1 IV	11	5	22.5/15.1	Hirsutism and otitis media	5.5 (LAD aneurysm)	23 days	Afebrile within 24 hours
9	Persistent fever and rising inflammatory indices	IVIG × 2; infliximab	5.1 IV	8	4	6.5/3.7	None	1.9	35 days	Afebrile within 24 hours
10	Progressive coronary aneurysms, persistent fever, and rising inflammatory indices	IVIG × 2; infliximab; methylprednisolone	Tacrolimus 0.07 oral	31	9	34.2/16.4	None	15.2 (RCA aneurysm)	84 days	Afebrile within 24 hours; CRP rose with low tacrolimus levels, requiring increased dose

LAD, left anterior descending coronary artery; RCA, right coronary artery.

^{*} IVIG 2 g/kg and methylprednisolone 30 mg/kg/day for 3 days, followed by a dose ranging from 2-5 mg/kg/day; infliximab at 5 mg/kg for 1 dose. "Study drug" means that the patient received either placebo or infliximab (5 mg/kg) as part of a Phase III, multicenter, double-blind, placebo controlled trial to assess the efficacy of infliximab as additional first-line therapy in KD; all patients also received aspirin at 80-100 mg/kg/day.

[‡] Treated with CSA unless stated otherwise.

[‡] Pre-calcineurin inhibitor treatment studies were obtained no more than 24 hours before calcineurin inhibitor infusion; post-calcineurin inhibitor treatment studies were obtained within 36-48 hours after the first calcineurin inhibitor infusion.

[§] Z-max, highest z-score of the LAD or RCA measured by echocardiography within the first 6 weeks of illness.²¹

[¶] Normal range: 1.8-2.3 mg/dL.

^{//} CI therapy started as an outpatient.

Table III**Protocol for CSA therapy in patients with highly resistant KD**

Baseline laboratory studies	Alanine aminotransferase, blood urea nitrogen, creatinine, magnesium, calcium, potassium, and CRP
Dose [*]	Start with CSA IV (variable absorption of oral medications in acute KD): 3 mg/kg/day divided every 12 hours. Switch to Neoral once afebrile >24 hours. Neoral (10 mg/mL liquid formulation) oral: 10 mg/kg/day divided every 12 hours.
Administration instructions [†]	IV: Use PVC-free tubing; medication must be in glass or polyethylene containers. Oral: Administer at the same time each day.
Monitoring	CSA trough level (C0): <ul style="list-style-type: none"> • Draw before third IV or oral dose • Goal: 50-150 ng/mL CSA 2-hour level (C2): <ul style="list-style-type: none"> • Draw after third IV or oral dose • Goal: 300-600 ng/mL Magnesium: <ul style="list-style-type: none"> • Monitor every third day during hospitalization. • Consider supplementation at time of drug initiation (either magnesium gluconate or magnesium-protein complex). CRP: <ul style="list-style-type: none"> • Monitor every 1-2 days during hospitalization.
Taper of Neoral	Start taper once patient is afebrile, clinically improving, and CRP < 1.0 mg/dL or after 2 weeks of therapy, whichever is longer. Taper by 10% every 3 days over 1 month.

^{*} Dose adjustment is required in both renal and hepatic impairment, which are rare in KD.

[†] IV formulation must be protected from light. Neoral oral solution is not light-sensitive; absorption is best when administered 1 hour before a meal, but this if not possible, it should be given the same time each day to ensure uniform daily absorption; avoid grapefruit juice, which inhibits CSA metabolism.

Table IV

Genotype of patients with highly resistant KD

Patient	Ethnicity	<i>ITPKC</i> (rs28493229) (risk allele: C)	<i>CASP3</i> (rs72689236) (risk allele: A)	<i>FCGR2A</i> (rs1801274) (risk allele: A)
2	African American	GG (0.97) *	<u>AA</u> (0.26)	<u>AG</u> (0.50)
3	Hispanic	GG (0.73)	<u>AG</u> (0.44)	GG (0.22)
4	European descent	<u>GC</u> (0.21)	<u>AA</u> (0.55)	<u>AG</u> (0.48)
5	Hispanic	<u>GC</u> (0.25)	<u>AG</u> (0.44)	GG (0.22)
6	African American	<u>GC</u> (0.03)	<u>AG</u> (0.44)	<u>AG</u> (0.50)
7	European descent	GG (0.77)	<u>AA</u> (0.55)	<u>AG</u> (0.48)
8	Chinese	GG (0.84)	<u>AG</u> (0.43)	<u>AG</u> (0.44)
10	European descent	GG (0.77)	<u>AA</u> (0.55)	<u>AA</u> (0.26)

Risk allele is underlined. DNA data were not available for patients 1 and 9.

* Percentage of the healthy reference population (ethnically matched) carrying this genotype.⁴⁰