11th International Kawasaki Disease Symposium

Novel Biomarker for Diagnosis of Kawasaki Diseases

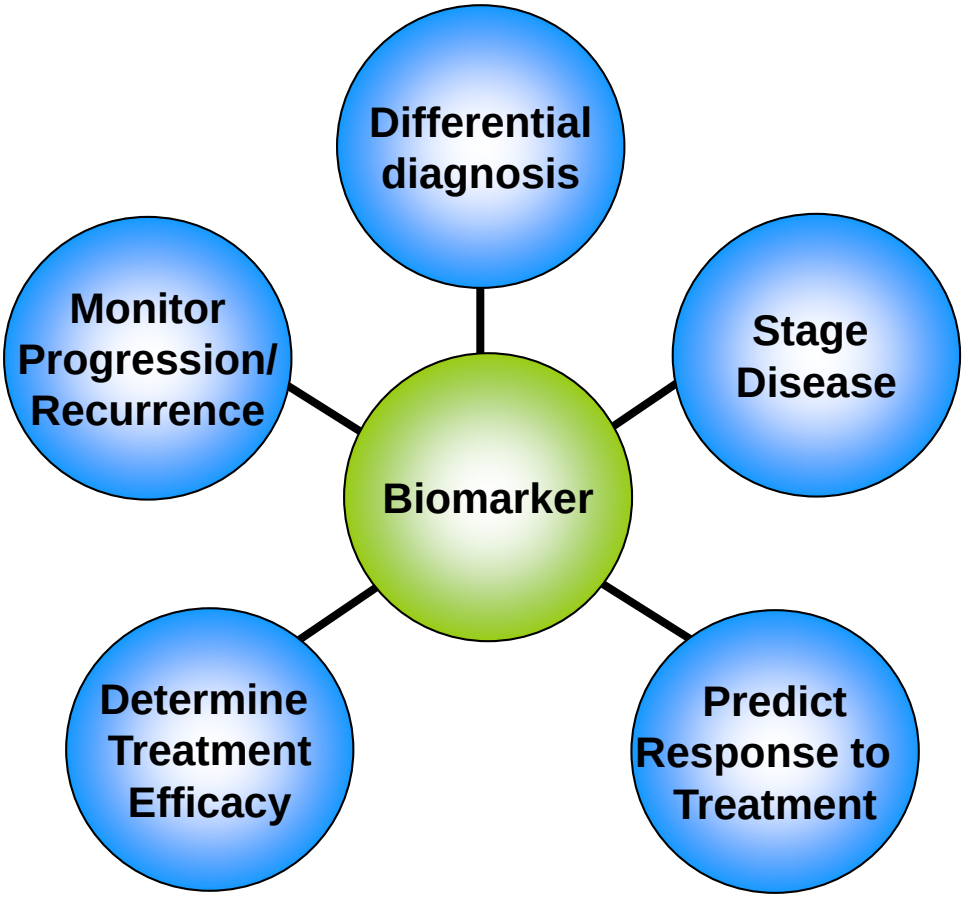
2015 Feb 4th

Tai-Ming Ko

Jer-Yuarn Wu, Yuan-Tsong Chen

- DISCLOSURES: None

Biomarkers for human disease



Sensitivity

Specificity

AUC (Area Under Curve)

- AUC=0.5, no discrimination
- 0.7≤AUC<0.8, acceptable discrimination
- 0.8 ≤ AUC< 0.9, excellent discrimination
- AUC ≥ 0.9, excellent discrimination

What "biomarker" can do for Kawasaki disease (KD)?

Diagnosis of Kawasaki disease is currently based on clinical features

Suspected KD Pts

Fever > 5d

Differential diagnosis

clinical features

recovery

IVIG (intravenous immunoglobulin)

- **Diagnostic Guidelines: ≥ 4 of the following 5 principal features**
  - **Erythema and edema of hands and feet**
  - **Polymorphous exanthema**
  - **Bilateral bulbar conjunctival injection**
  - **Erythema of lips, strawberry tongue**
  - **Cervical lymphadenopathy (≥1.5 cm in diameter)**

(*Circulation. 2011*)

Delay diagnosis of KD results in poor outcome with IVIG treatment

Suspected KD Pts

Fever > 5d

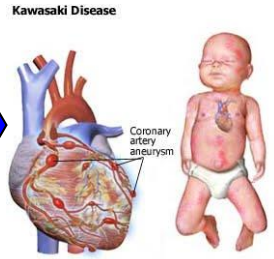
Differential diagnosis

clinical features

recovery

IVIG
(intravenous immunoglobulin)

Delay diagnosis (>10 days)



15% ~ 25% of untreated Pts

Coronary Artery Aneurysm (CAA)

1. **myocardial infarction**
2. **ischemic heart disease**
3. **sudden death**

(*Circulation. 2004*)
(*Am Fam Physician. 2006*)

Major Problems of KD diagnosis

- **Kawasaki disease is an acute, difficult-to-recognize, pediatric vasculitis that often remains undiagnosed.**
- **Kawasaki disease clinically mimics other common conditions.**
- **No diagnostic test currently exists.**

Nature Reviews Cardiology **9**, 375 (2012)

Challenge in KD diagnosis

iKD
(incomplete presentation)

15 % of KD Pts

- **Unexplained fever for ≥5 days**
- **≤ 3 of the principal features**

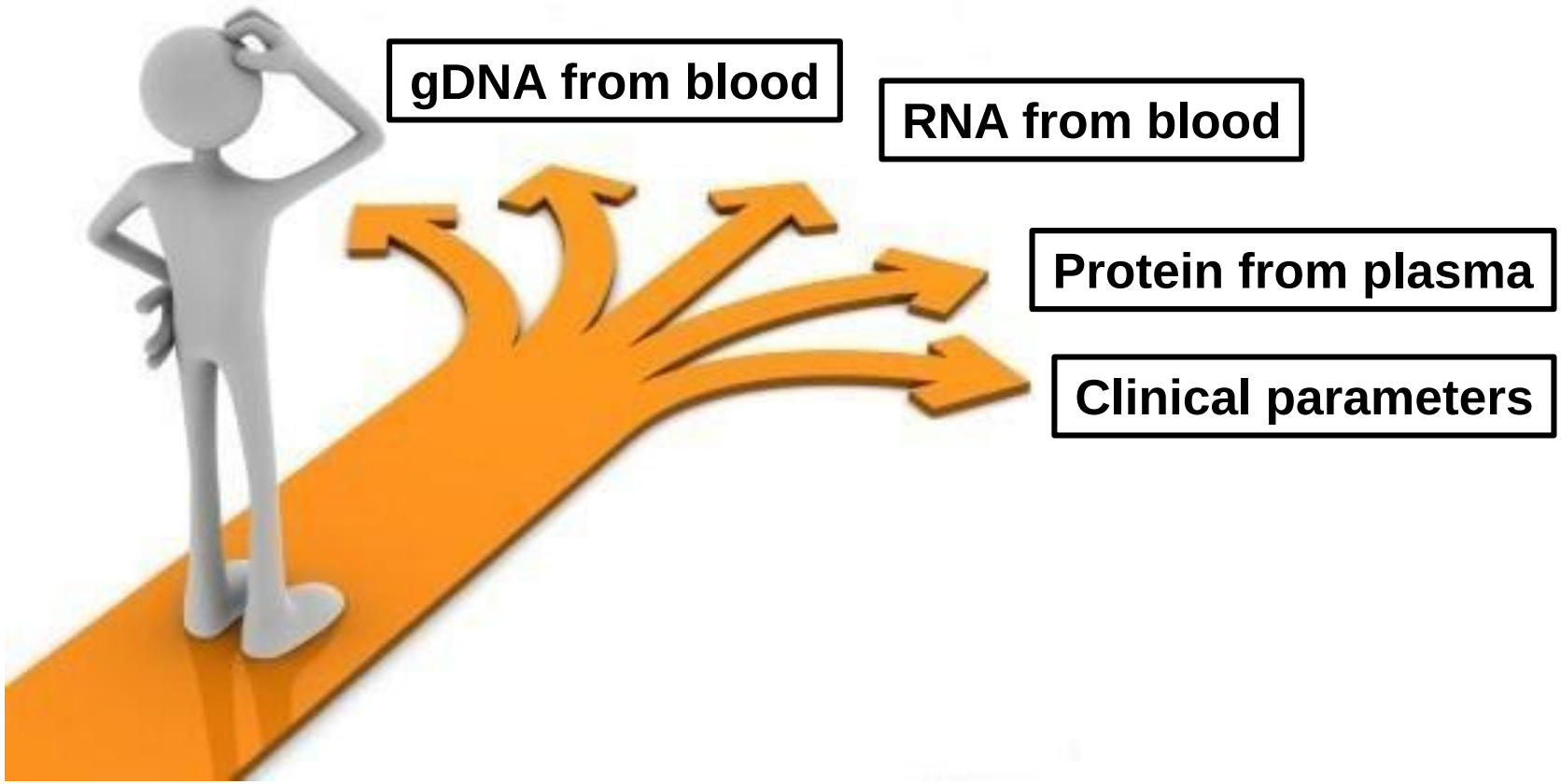
non-KD
(fever & inflammatory diseases)

- **Diseases With Similar Clinical Findings**
  - **infections (eg, enterovirus, EBV)**
  - **Drug hypersensitivity reactions**
  - **Stevens-Johnson syndrome**
  - **Juvenile rheumatoid arthritis**
  - **Bacterial cervical lymphadenitis**
  - **Scarlet fever**
  - **Staphylococcal scalded skin syndrome**
  - **Toxic shock syndrome**
  - **Rocky Mountain spotted fever**
  - **Leptospirosis**
  - **Mercury hypersensitivity reaction**

(*Circulation. 2004*)
(*Nihon Rinsho. 2008*)
(*Pediatr Cardiol. 2012*)

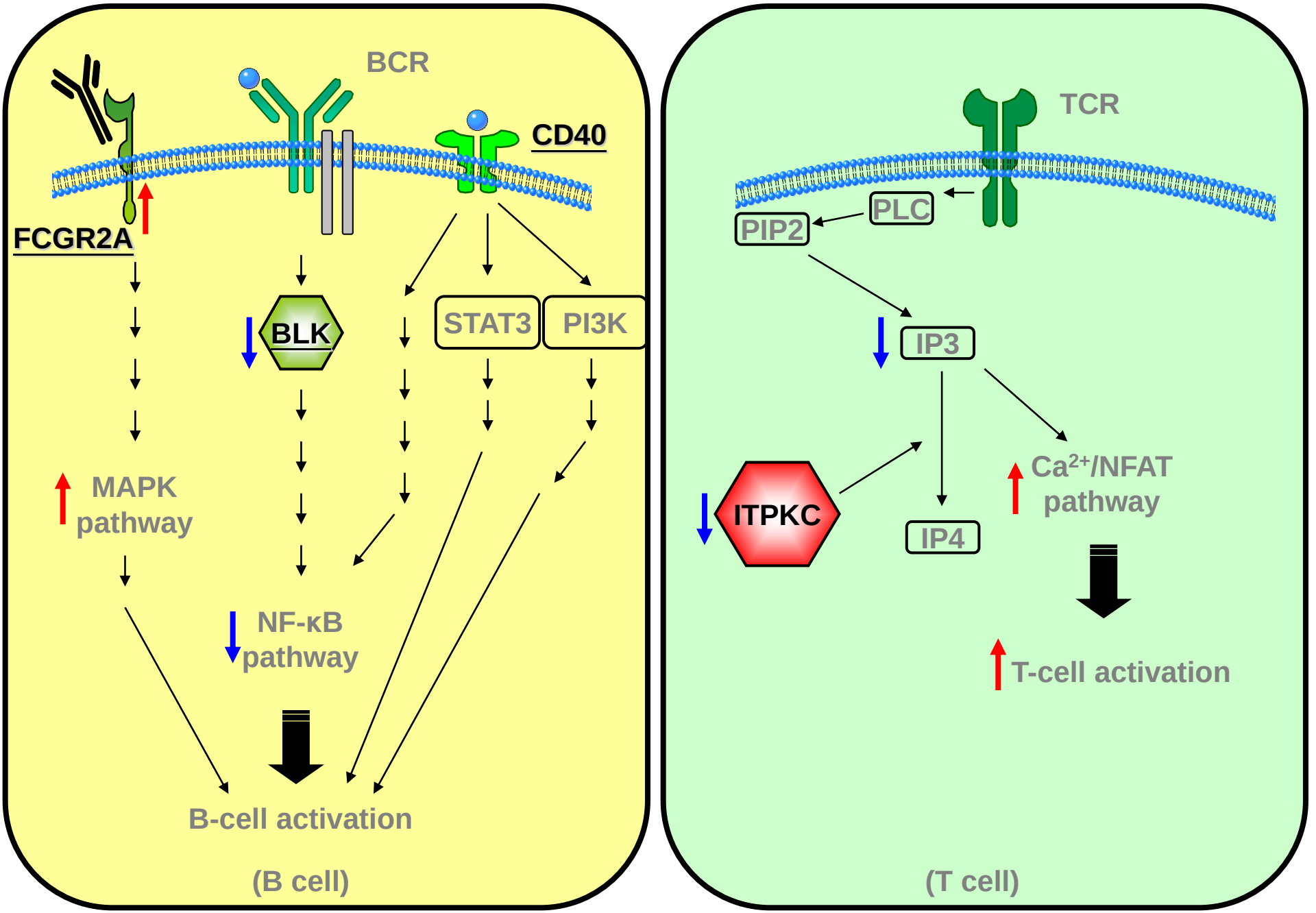
How to develop a simple diagnostic test for KD ?

What's the way to identify biomarkers for KD diagnosis?



Kawasaki disease and immune system (genetic studies)

- **ITPKC (T cell, activation pathway)**
  - Onouchi, Y., et al. *Nat Genet* **40**, 35-42 (2008).
- **COPB2 (T cells) & IGHV (B cells)**
  - Tsai, F.J., et al. *PLoS One* **6**, e16853 (2011).
- **FCGR2A (B cells, Fc fragment of IgG)**
  - Khor, C.C., et al. *Nat Genet* **43**, 1241-1246 (2011).
- **BLK (B cells, B-cell lymphocyte kinase)**
  - Onouchi Y, et al., *Nat Genet.* 2012 Mar 25;44(5):517-21.
  - Lee, YC et al. *Nat Genet. 2012 Mar 25;44(5):522-5.*
- **CD40 (T cells, interaction between B cells and T cells)**
  - Onouchi Y, et al., *Nat Genet.* 2012 Mar 25;44(5):517-21.
  - Lee, YC et al. *Nat Genet. 2012 Mar 25;44(5):522-5.*


BCR
CD40
FCGR2A
BLK
STAT3
PI3K
MAPK pathway
NF-κB pathway
B-cell activation
(B cell)
TCR
PLC
PIP2
IP3
ITPKC
IP4
Ca2+/NFAT pathway
T-cell activation
(T cell)

ROC (Receiver Operating Characteristic) curve for predictive model of KD with 6 SNPs

Table 2. Susceptibility Genes for KD Identified With Association at Genome-Wide Significance ($P<5.0\times10^{-8}$)

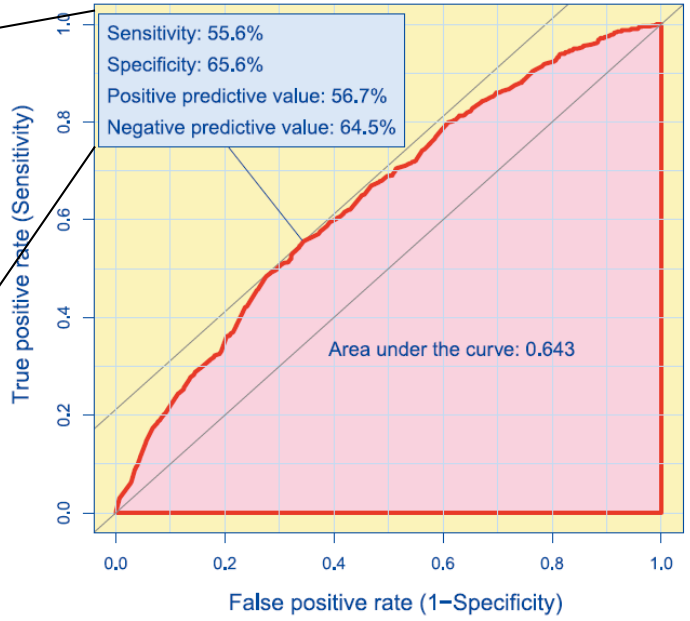
Gene	Locus	Methods	Original reports	Replication studies	
				Positive	Negative
*FCGR2A**	1q23	GWAS	42	45	–
CASP3	4q34–35	Linkage analysis – positional candidate gene study	21	25**	–
*HLA**	6p21.3	GWAS	45	–	–
*BLK**	8p23–p22	GWAS	45, 46	–	–
ITPKC	19q13.2	Linkage analysis – linkage disequilibrium mapping	20	23, 42	22, 24
*CD40**	20q12-q13.2	GWAS	45, 46	–	–

Sensitivity: 55.6%

Specificity: 65.6%

Positive predictive value: 56.7%

Negative predictive value: 64.5%



AUC =0.643

(Circ J. 2012)

Specific Aim:

Identify biomarkers that can be used to facilitate diagnosis of KD

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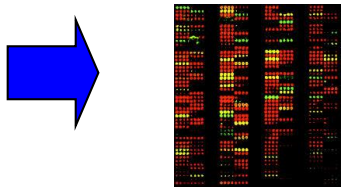
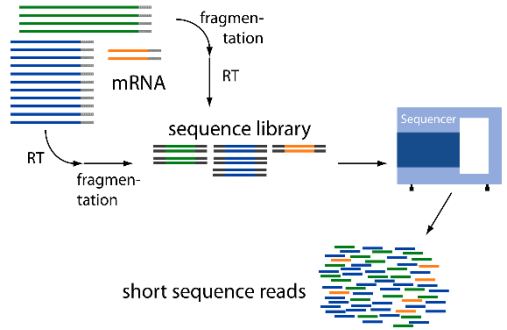
"Systematic / Unbiased / High-throughput screening" Approach:

- **Potential protein targets in blood**
  - **Protein array**



- **Gene expression profile of PBMCs**
  - **Deep sequencing of RNA**



Specific Aim:

Identify biomarkers that can be used to facilitate diagnosis of KD

Study design for biomarkers evaluation

- **Replicable phenotype: multiple stages**

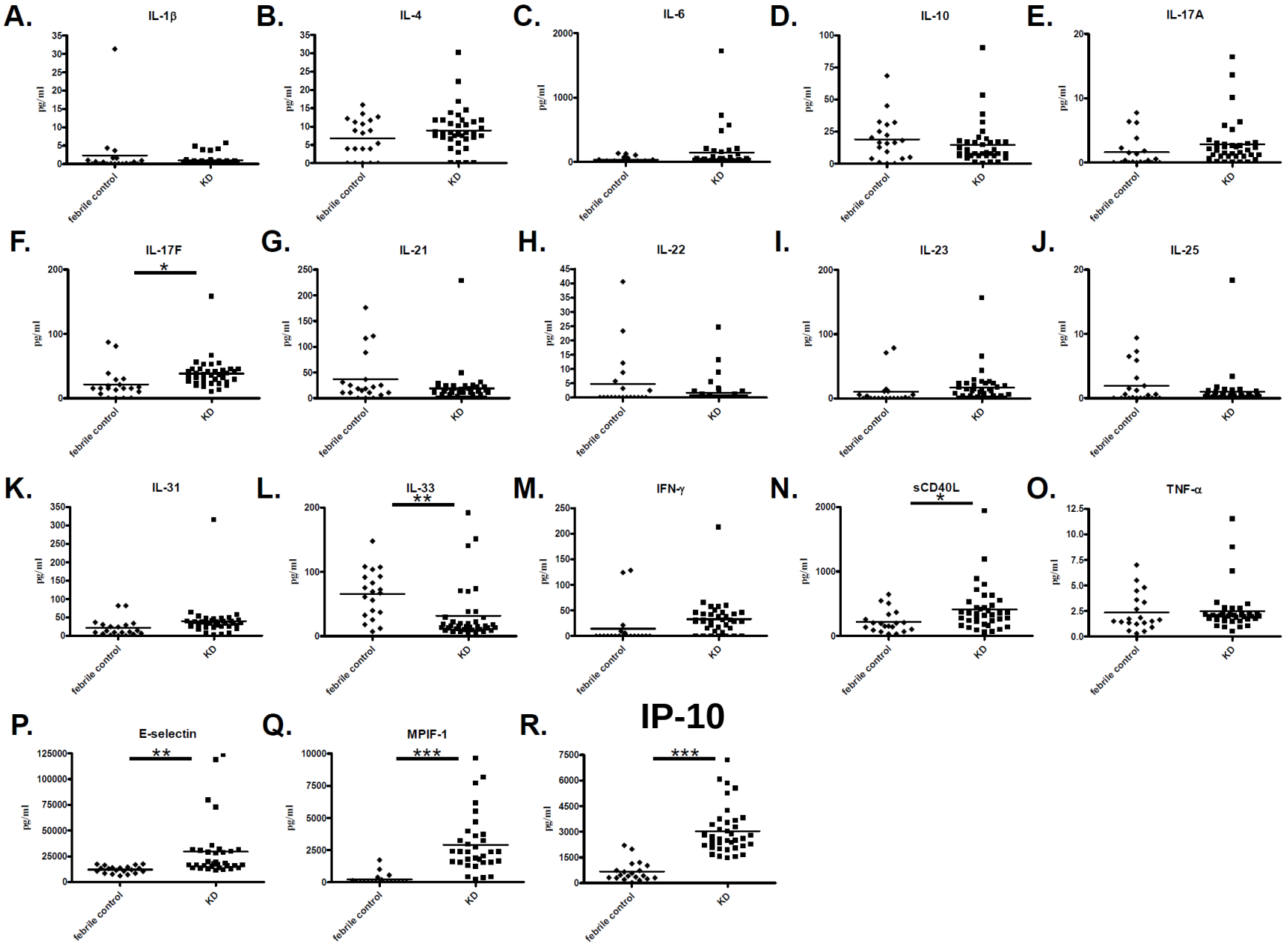
Discovery

(protein array → EIA) → **Replication** → **Blinded Validation**

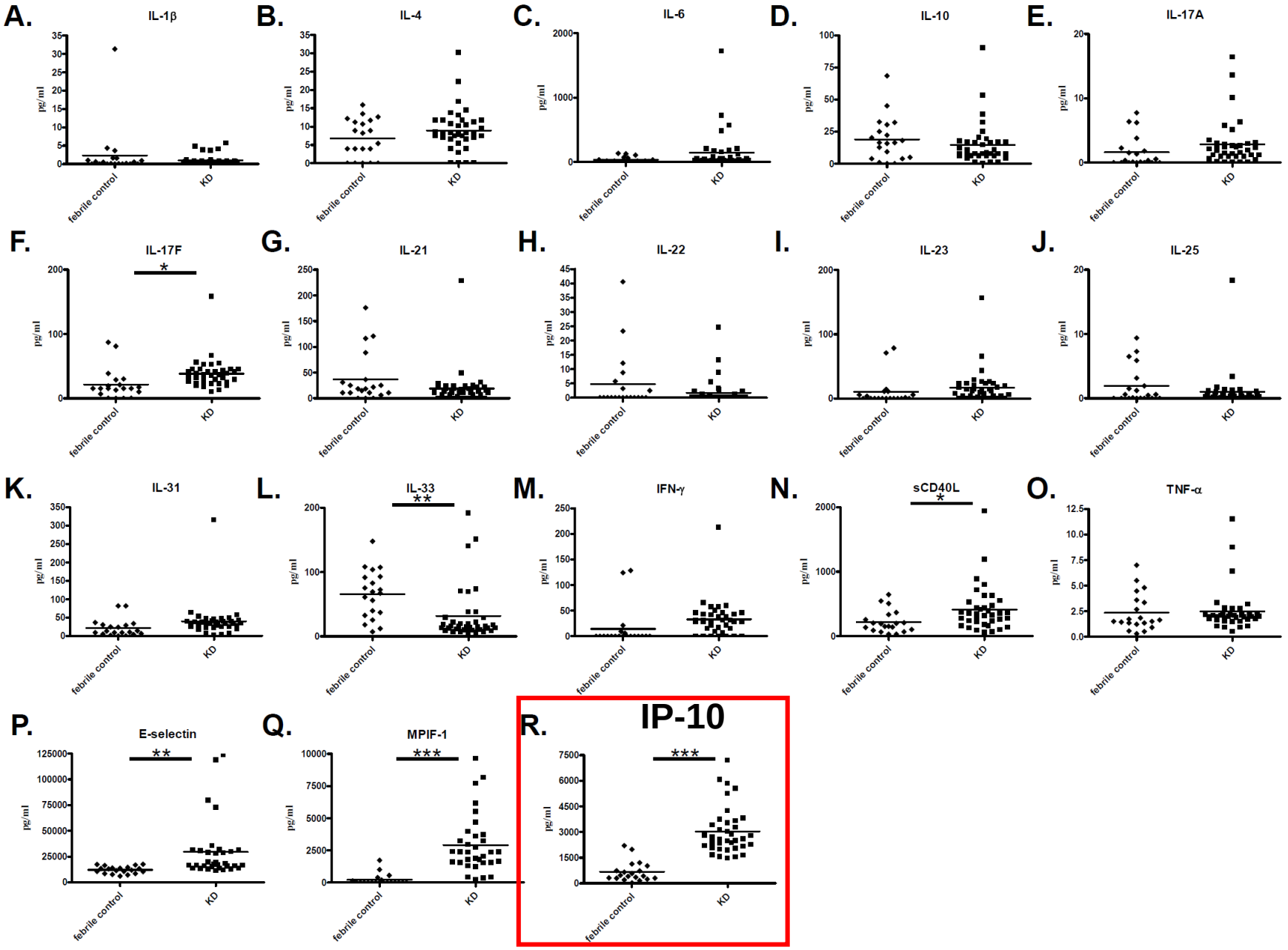
- **Suitable comparison: specified control**
  - **KD (Acute & Convalescent)**
  - **KD & Non-KD with fever only**
  - **KD & Non-KD with fever and clinical features suggestive of KD**

- **General pattern: adequate sample size**
  - **Study subjects: 214 children (100 KD, 114 non-KD)**

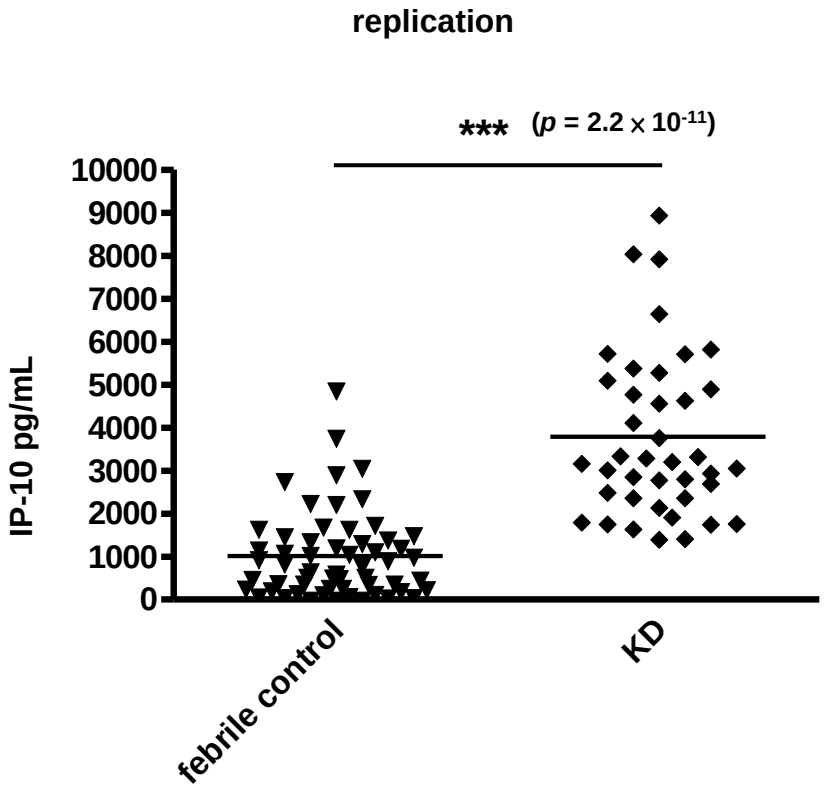
Plasma Profile: The Discovery Study



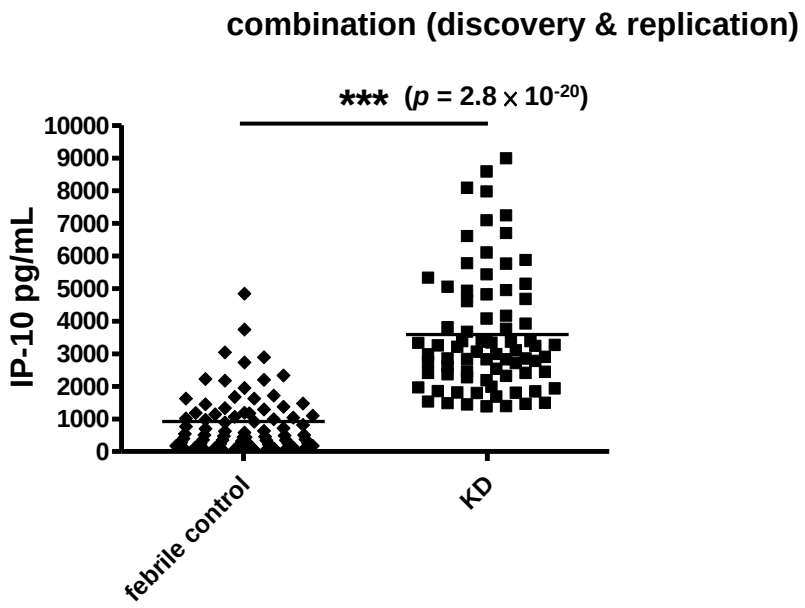
Plasma Profile: The Discovery Study



IP-10 Levels: The Replication Study

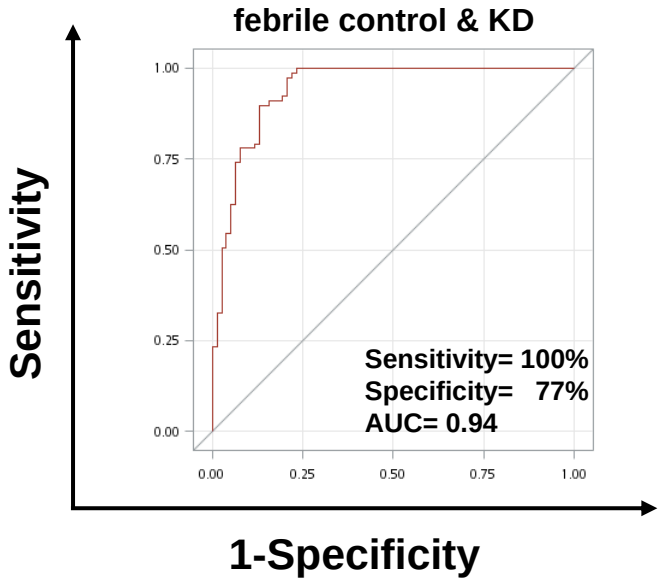


IP-10 Levels: Combined Studies (Discovery & Replication)



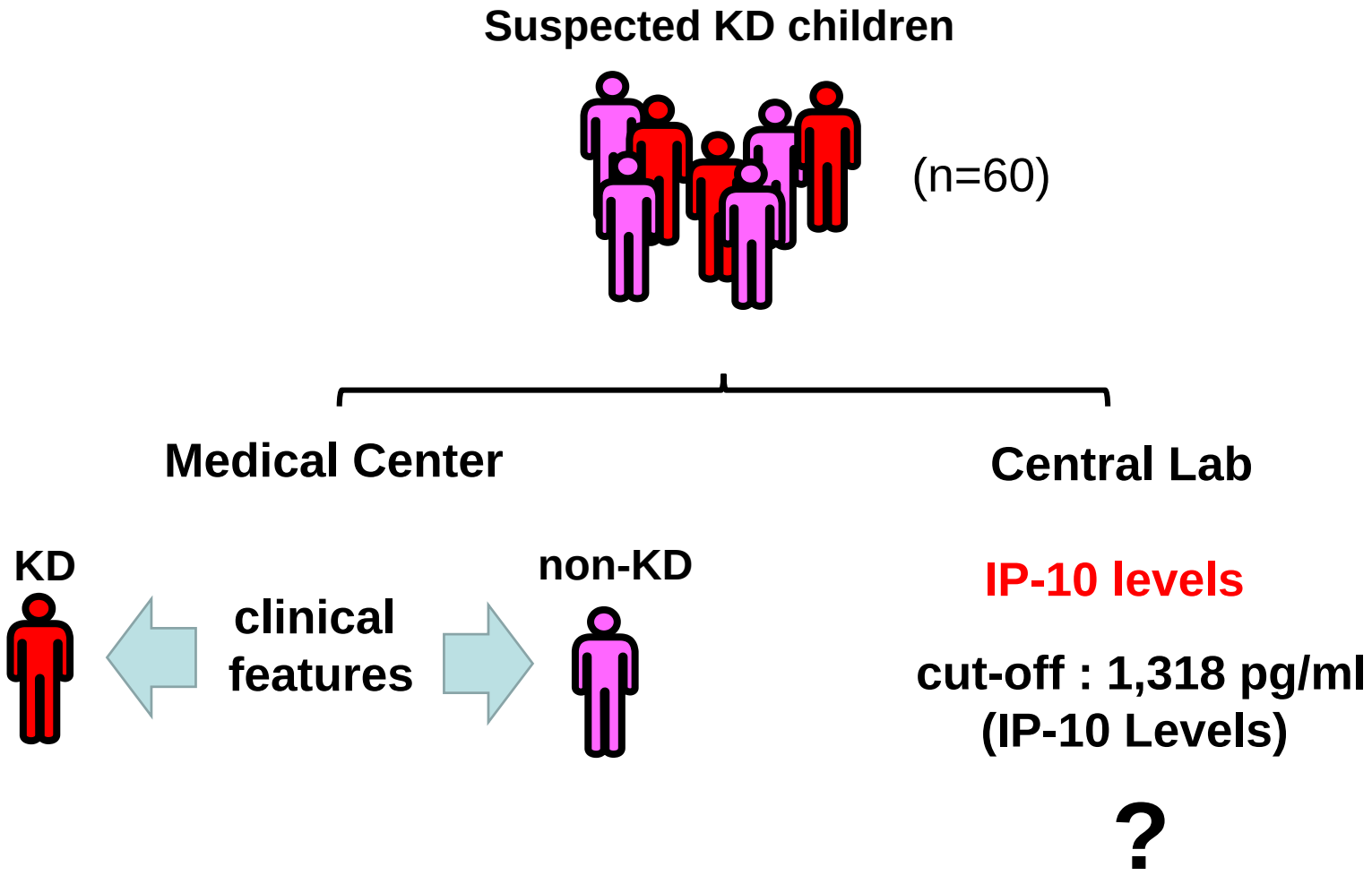
(n=77) (n=77)

AUC=0.94

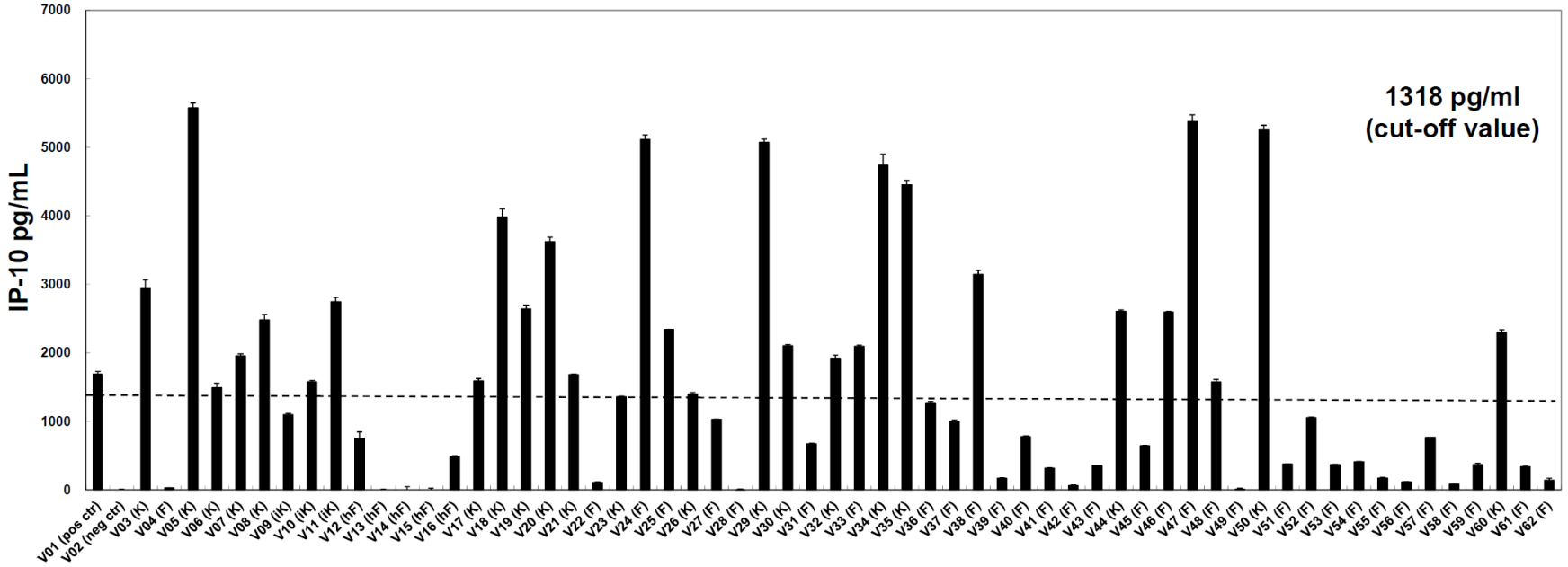


cut-off : 1,318 pg/ml (IP-10 Levels)

IP-10 Levels: Blinded Validation Study



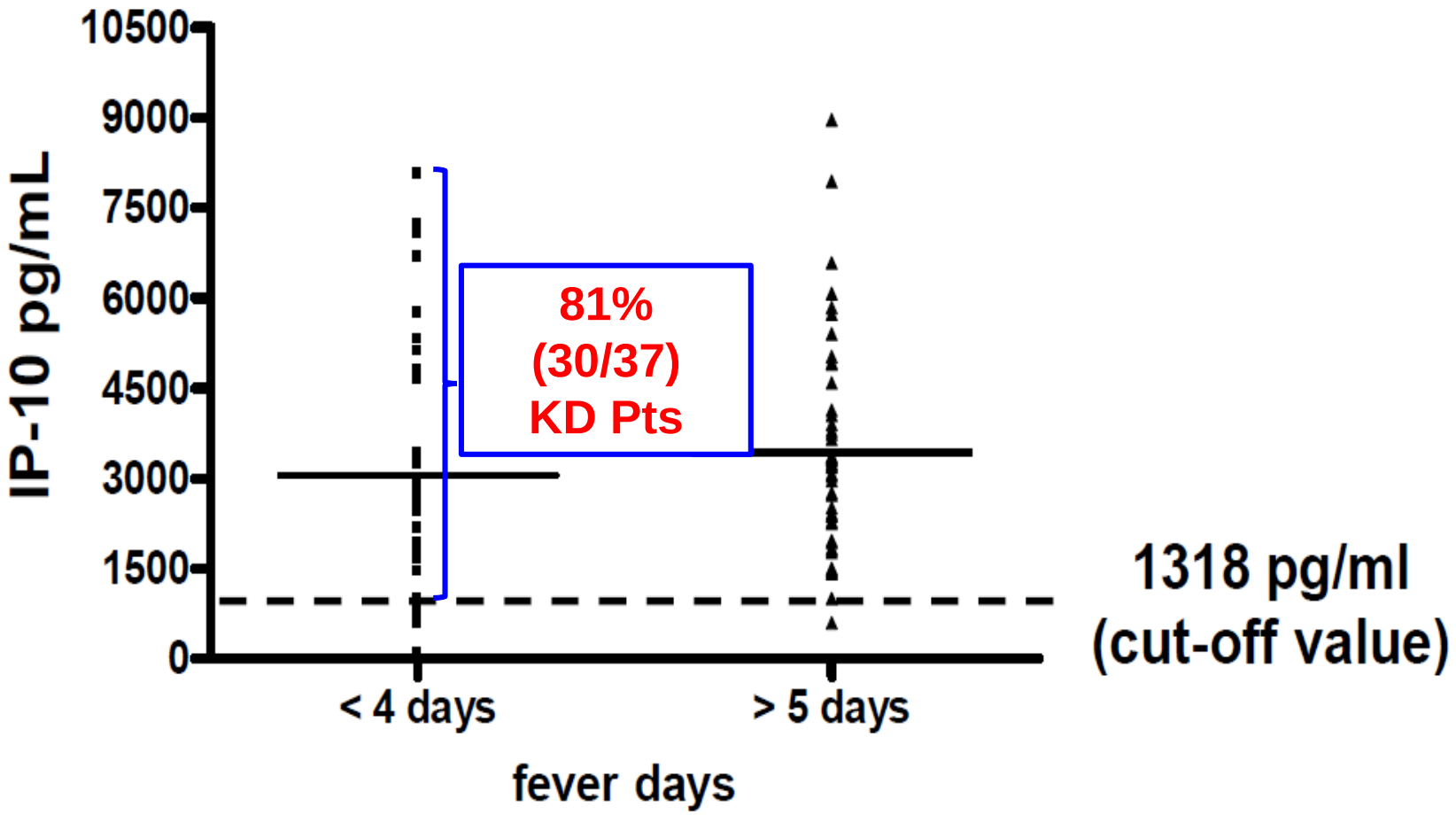
IP-10 Levels: Blinded Validation Study



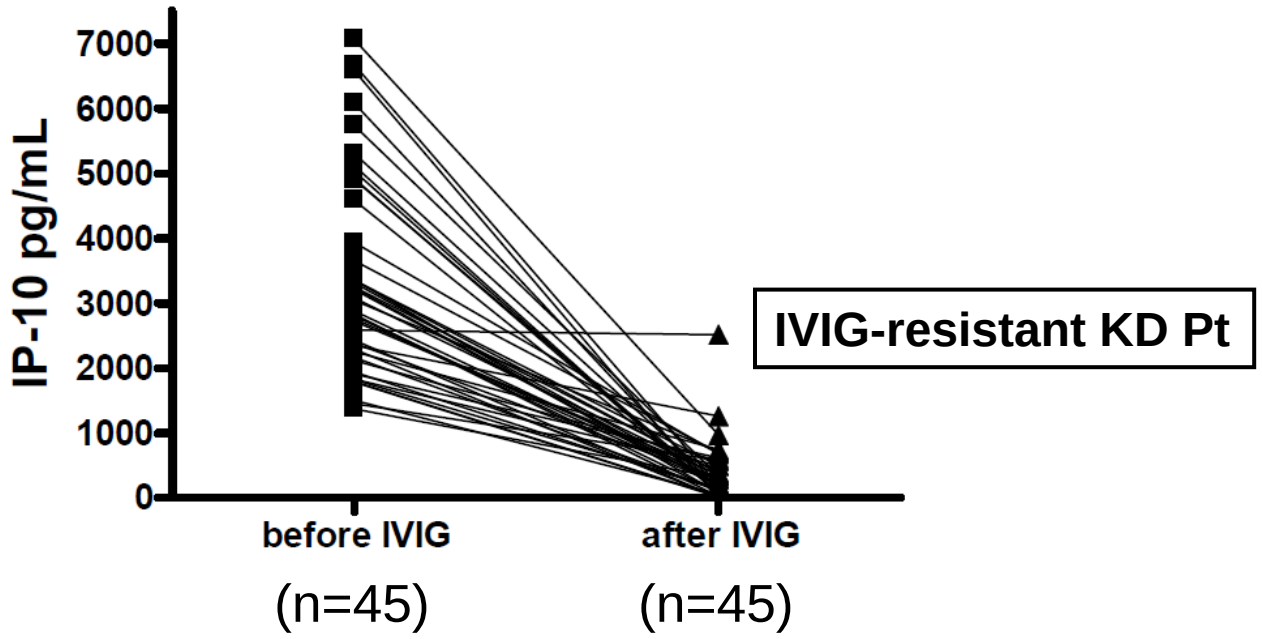
(n=60)

Sensitivity, 96% [22/23]
Specificity, 81% [30/37]

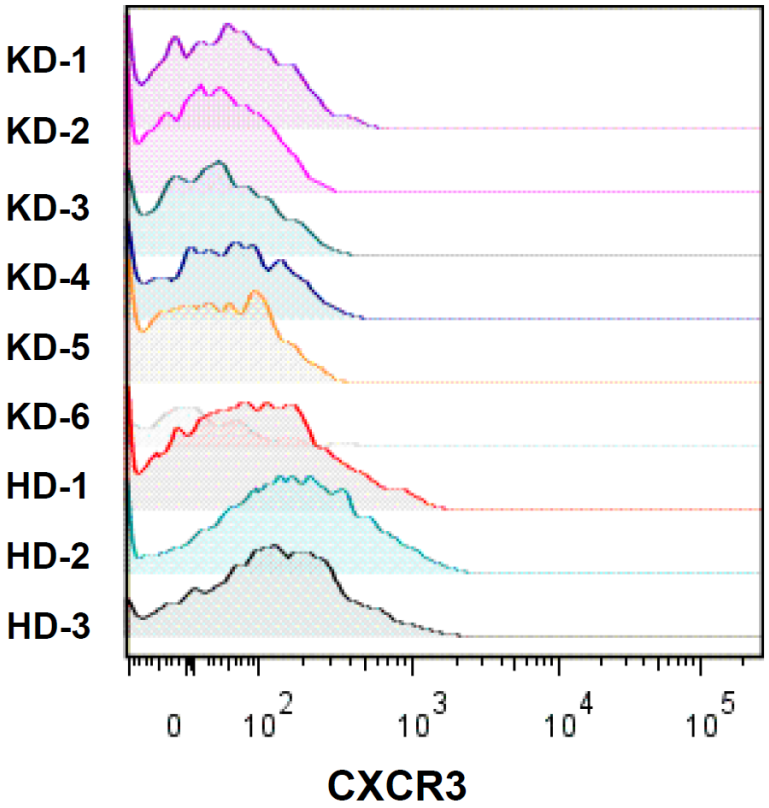
Increase of IP-10 levels in the early stage of KD (< 4days)

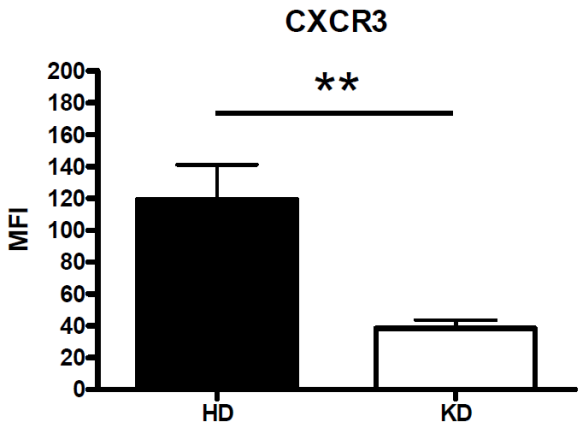


IP-10 Levels in KD in Relation to IVIG Treatment

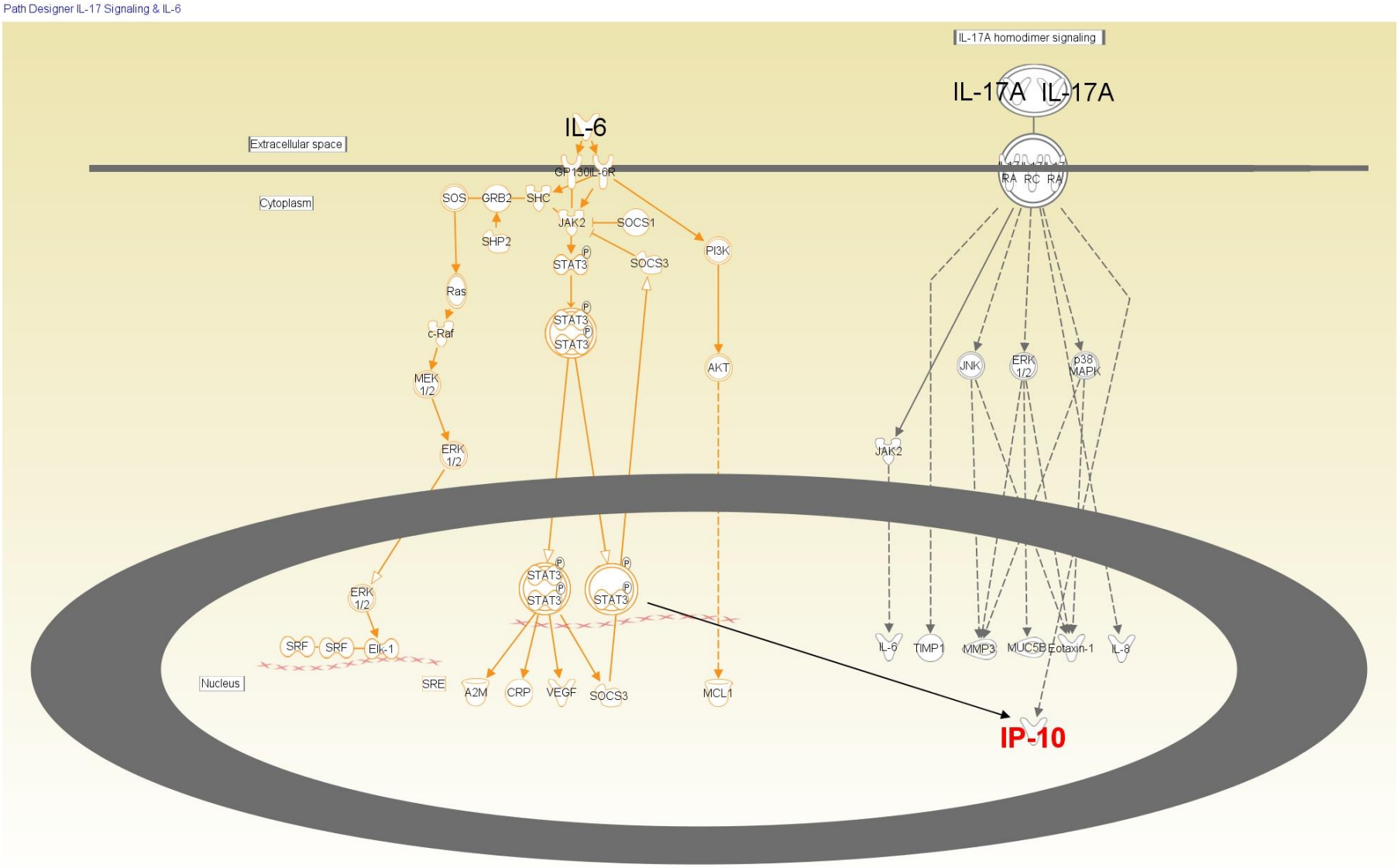


Cell Surface Chemokine Receptor CXCR3 in T Cells of Patients with Acute KD





A potential upstream signaling pathway for IP-10



Summary

- **During the discovery phase, the expression of IL-17F, sCD40L, E-selectin, MPIF-1, and IP-10 were upregulated during the acute phase in KD patients compared to that in the controls.**
- **ROC analysis of the combined discovery and replication data [n(KD)=77, n(control)=77] showed that the IP-10 level had high AUC values (0.94; sensitivity, 100%; and specificity, 77%).**
- **With 1,318 pg/mL as the optimal cut-off, the blinded validation study confirmed that the IP-10 levels were a good predictor of KD.**
- **With IVIG treatment, the IP-10 levels returned to normal.**
- **The downstream receptor of IP-10, CXCR3, was activated in the T cells of acute KD patients.**

Improve differential diagnosis of KD by IP-10

Suspected KD Pts

Fever > 5d

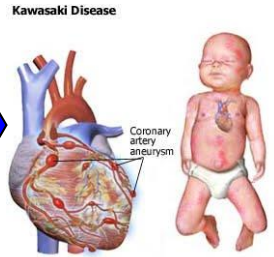
Differential diagnosis

Clinical Features + IP-10 Levels

recovery

IVIG (intravenous immunoglobulin)

Delay diagnosis (>10 days)



15% ~ 25% of untreated Pts

Coronary Artery Aneurysm (CAA)

1. **myocardial infarction**
2. **ischemic heart disease**
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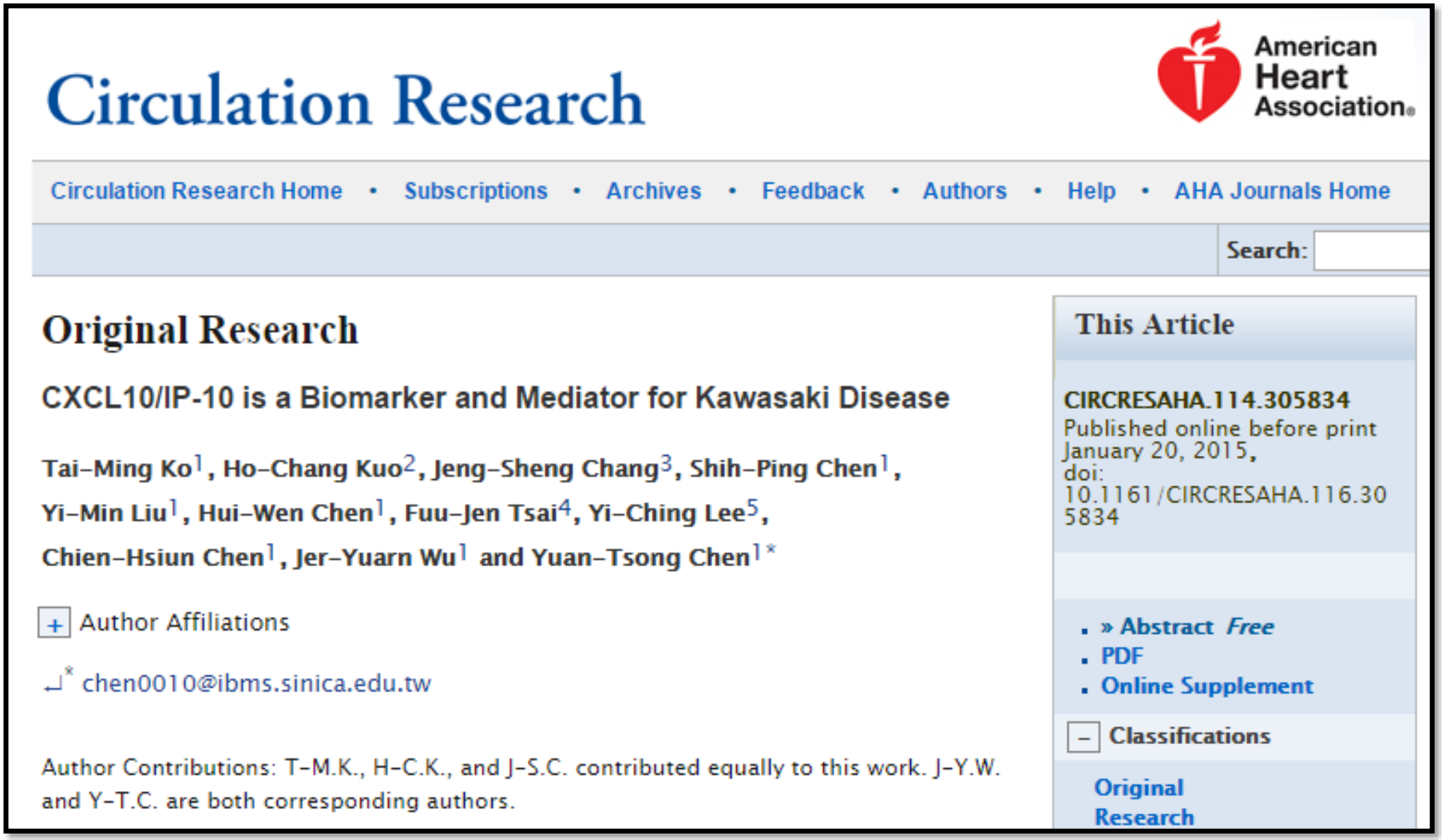
(*Circulation. 2004*)
(*Am Fam Physician. 2006*)

Reference



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Original Research

CXCL10/IP-10 is a Biomarker and Mediator for Kawasaki Disease

Tai-Ming Ko[1], Ho-Chang Kuo[2], Jeng-Sheng Chang[3], Shih-Ping Chen[1], Yi-Min Liu[1], Hui-Wen Chen[1], Fuu-Jen Tsai[4], Yi-Ching Lee[5], Chien-Hsiun Chen[1], Jer-Yuarn Wu[1] and Yuan-Tsong Chen[1*]

+ Author Affiliations

* chen0010@ibms.sinica.edu.tw

Author Contributions: T-M.K., H-C.K., and J-S.C. contributed equally to this work. J-Y.W. and Y-T.C. are both corresponding authors.

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- » Abstract *Free*
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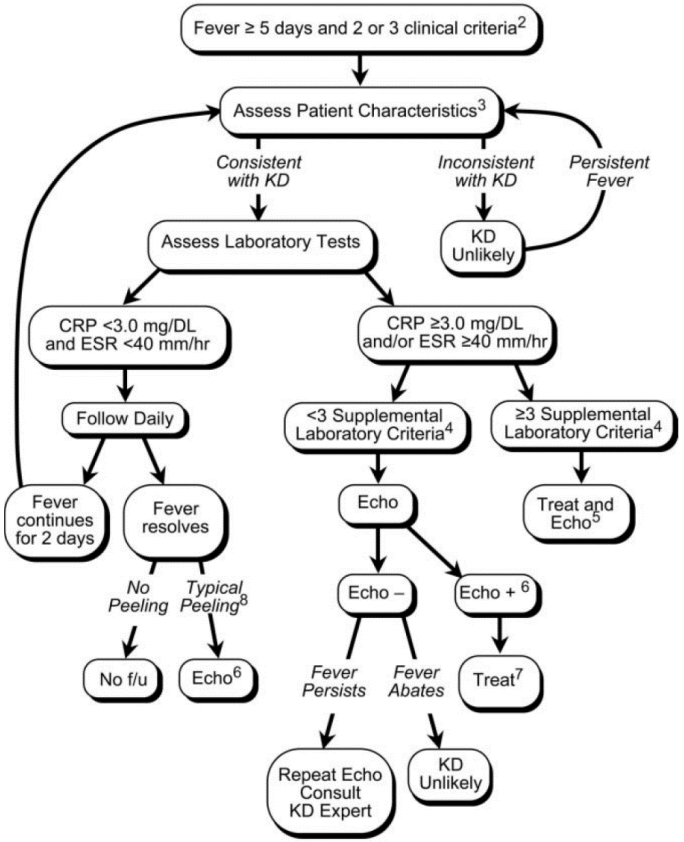
– Classifications

Original Research

(Circulation Research 2015)

Differential diagnosis algorism of iKD based on IP-10 Levels and other parameters

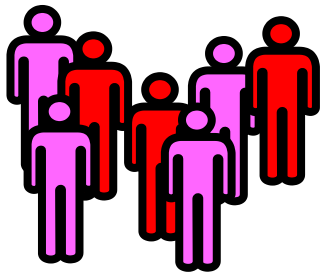
diagnosis of iKD by clinical features



(*Circulation. 2004*)

diagnosis of iKD by lab parameters

Suspected KD children



KD/iKD

non-KD

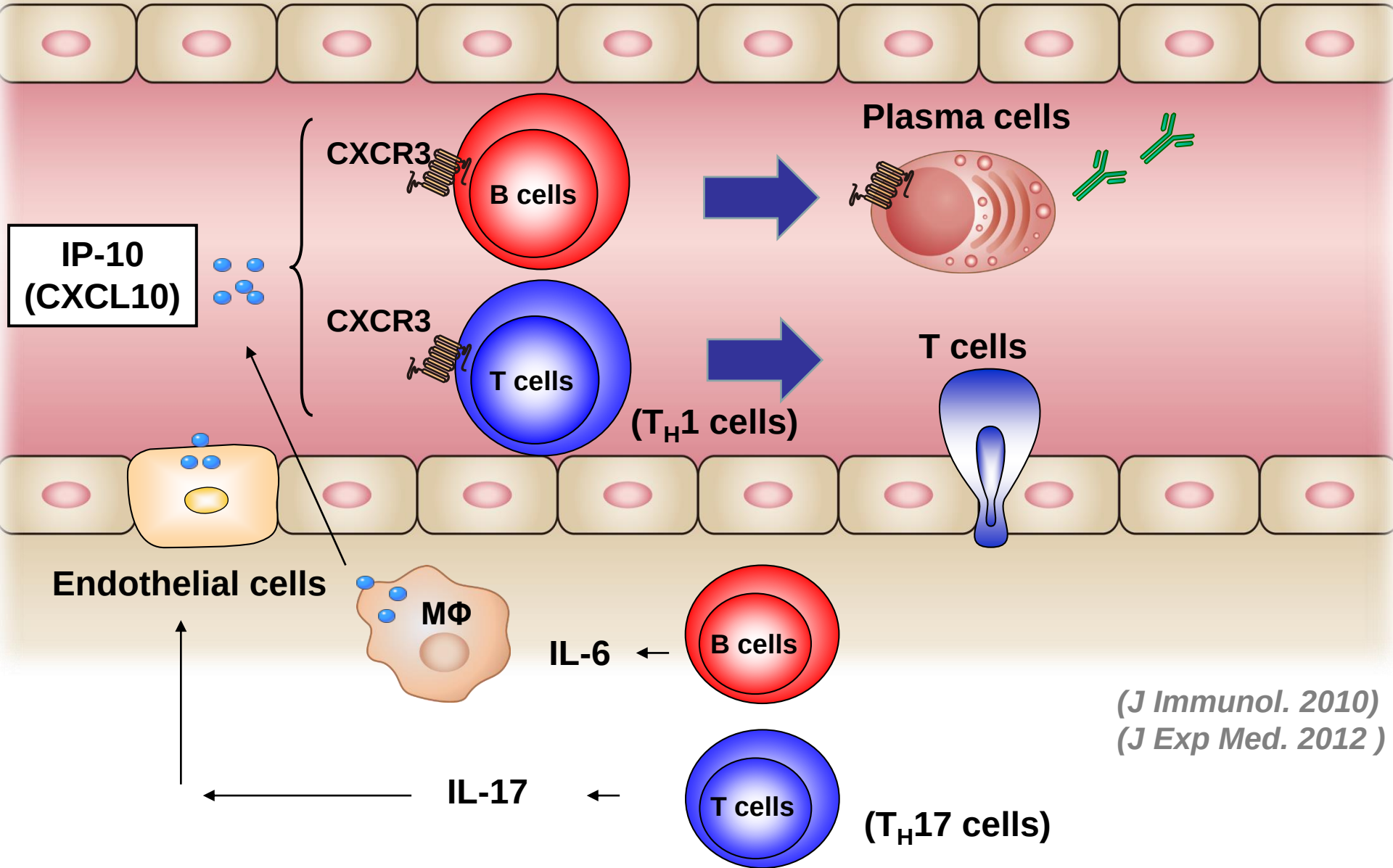
differential diagnosis algorism

Plasma IP-10 levels
Urine proteomics
RNA expression profile
DNA Genotyping
other parameter (eg., ESR)

With IVIG

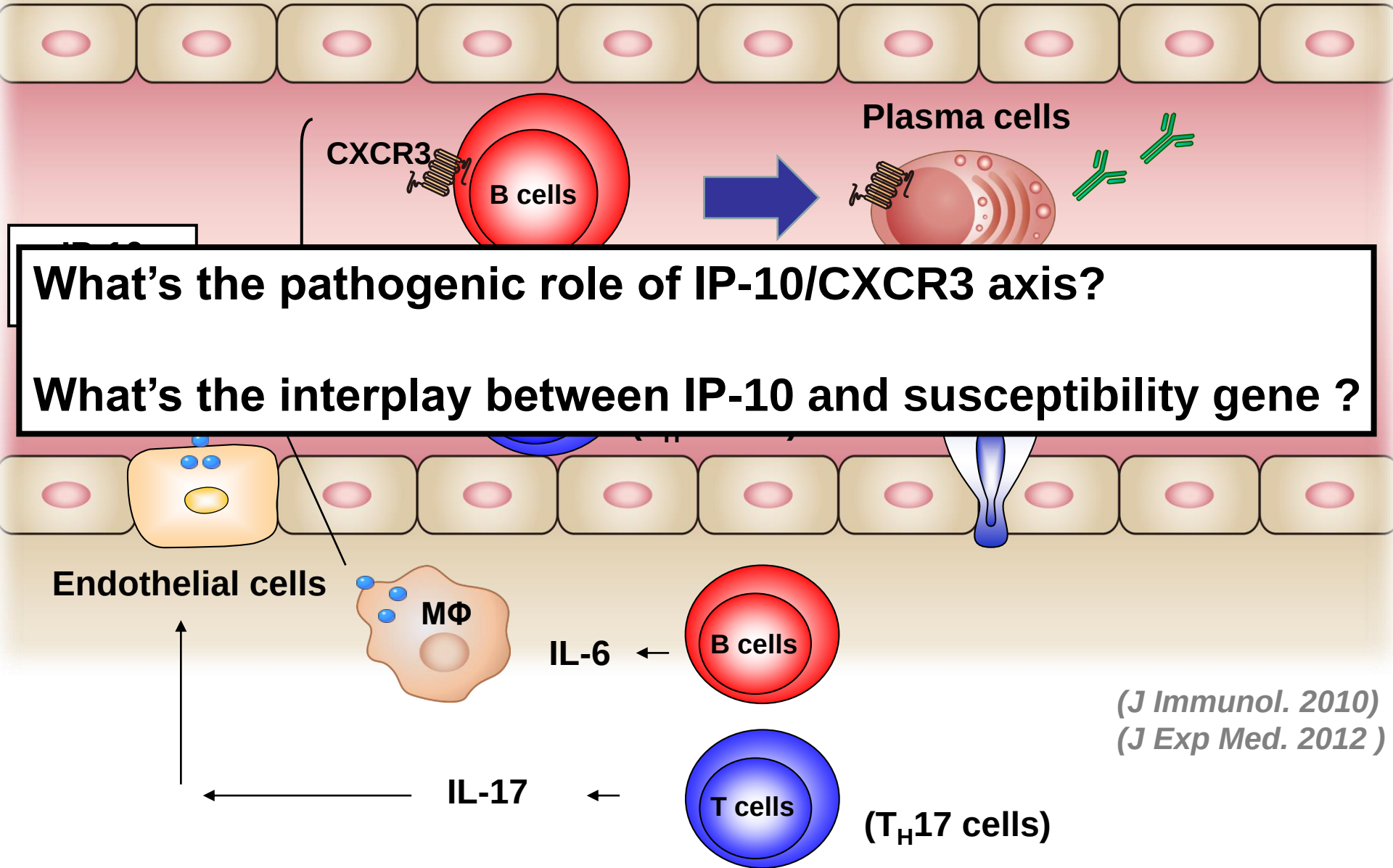
Without IVIG

Hypothetical model: IP-10/CXCR3 axis in KD



(J Immunol. 2010)
(J Exp Med. 2012)

Hypothetical model: IP-10/CXCR3 axis in KD



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- **Fuu-Jen Tsai**

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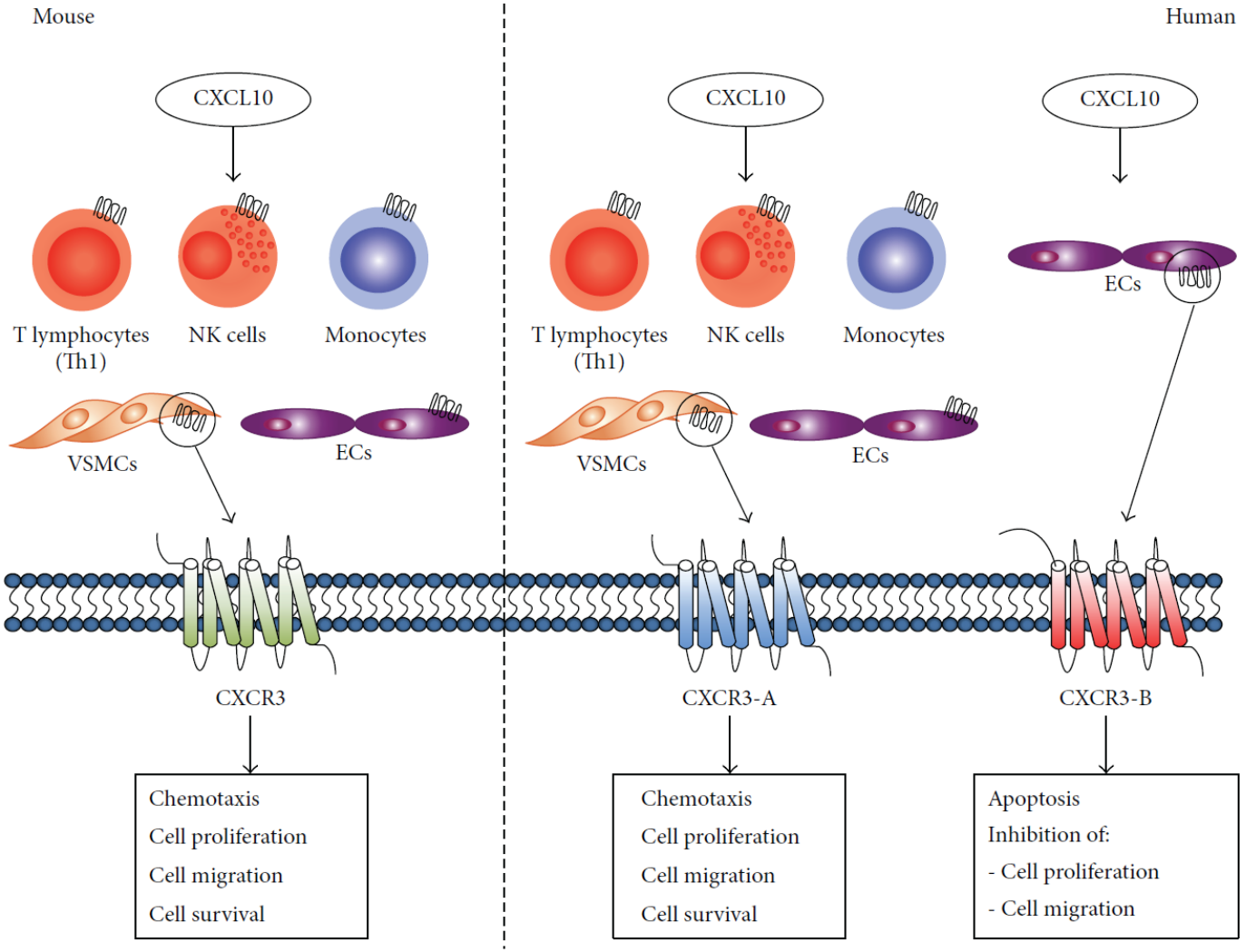
Thank you

The Multifaceted Functions of CXCL10 in Cardiovascular Disease



(BioMed Research International, 2014)