Tried, True, and New: Diagnostics in Inborn Errors of Immunity

Rebecca Marsh, MD

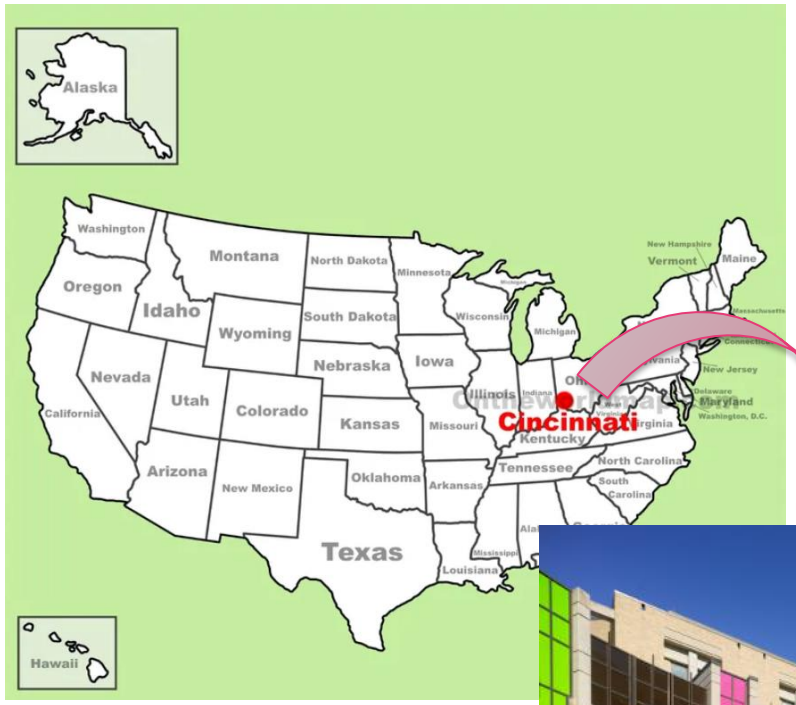
Co-Director, Diagnostic Immunology Laboratory

Cincinnati Children's Hospital

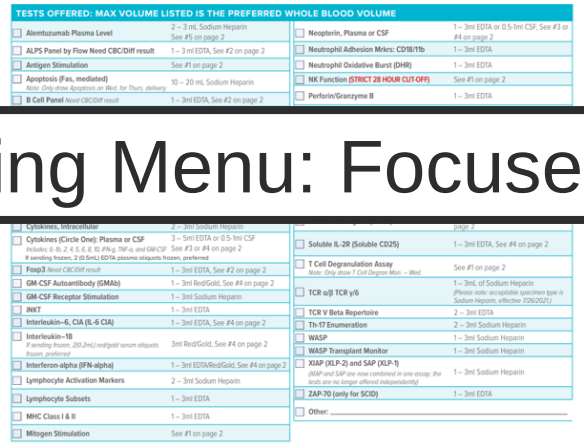
Disclosures

- Employed part-time by Pharming Healthcare, Warren NJ
- Advisory Board Work Sumitomo, Amgen, SOBI, AB2 Bio

CCHMC Diagnostic Immunology Lab



Background: DIL Current Test Offerings

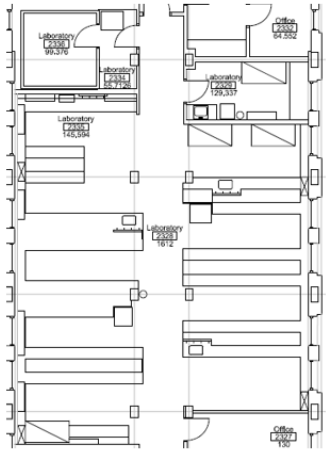


Clinical Cell Therapy Offerings
- Stem Cell Graft Phenotyping/Enumeration

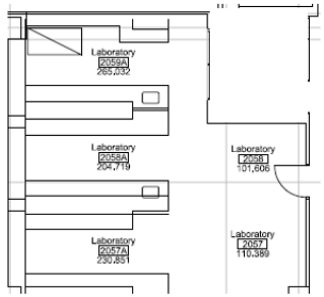
Clinical Trial Cell Therapy Offerings

Testing Menu: Focused on Immunology

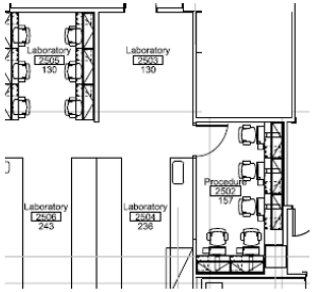
Assays used in Clinical Trials
- Alemtuzumab
- IL-18
- GM-CSF AutoAb
- GM-CSF R signaling
- CD11/CD18



Specimen Intake, Flow Cytometry, Cell Immunology



Protein Biomarker Lab (ELISA)



Test Development

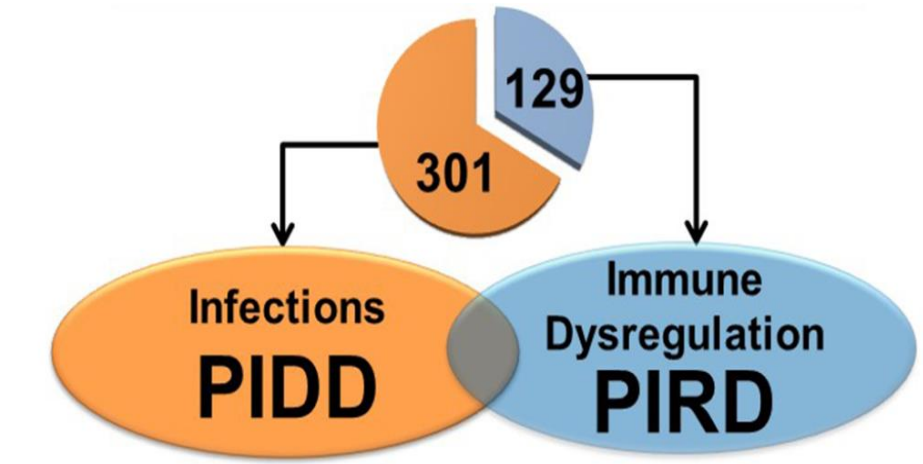
3 Directors
2 Managers
3 Technical Supervisors
~40 Staff

Objectives for Today

- Present a case-based overview of the practical applications of diagnostic testing to aid in the diagnosis and management of patients with inborn errors of immunity
- We will cover the use of various tests and platforms to:
  - Diagnose IEIs
  - Interrogate genetic variants of uncertain significance
  - Facilitate precision dosing strategies

First: What Are Inborn Errors of Immunity?

- Genetic diseases which lead to defects in 1 or more parts of the immune system and result in immune deficiency and/or immune dysregulation

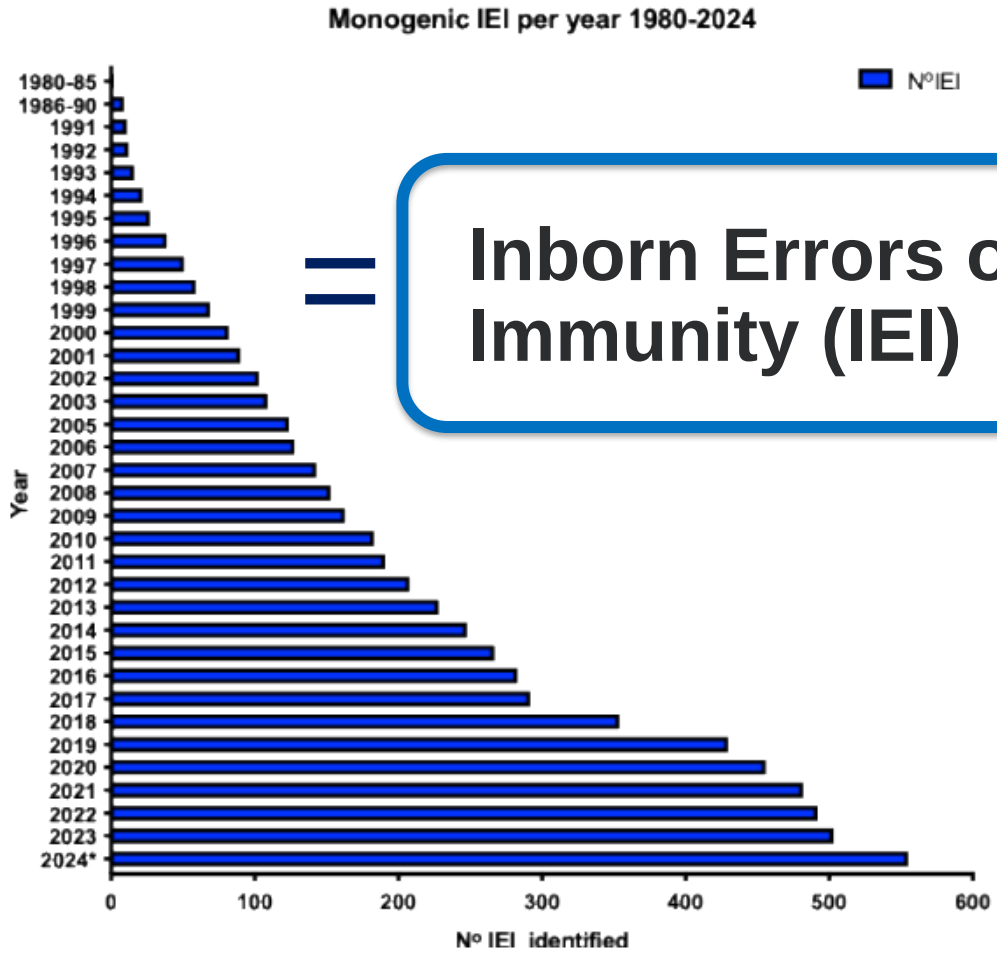


Primary Immune Deficiency Disorders (PIDD)

- Infection-dominant pathology
- Therapies focused on infection treatment or prevention
- Example: SCID

Primary Immune Regulatory Disorders (PIRD)

- Immune-mediated pathology dominant
- Therapies focused on immune modulation
- Example: IPEX



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Inborn Errors of Immunity (IEI)

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Chan AY, Torgerson TR. Primary immune regulatory disorders: a growing universe of immune dysregulation. Curr Opin Allergy Clin Immunol. 2020 Dec;20(6):582-590.
https://wp-iuis.s3.eu-west-1.amazonaws.com/app/uploads/2025/01/08170256/Fig-1.-Overview-of-IEIs-June-2024.pdf

Signs of IEI

- **Infections**

Immune Deficiency Foundation

- **Immune Dysregulation**:
  - Autoimmune Cytopenias
  - Autoimmune or Inflammatory Organ Disease (esp. GI Tract, Liver, Lung, Brain, Joints, Skin, Type 1 Diabetes)
  - Non-malignant Lymphoproliferation
  - Recurrent Fever Syndromes
  - Systemic Inflammatory Diseases
  - Hemophagocytic Lymphohistiocytosis (HLH)

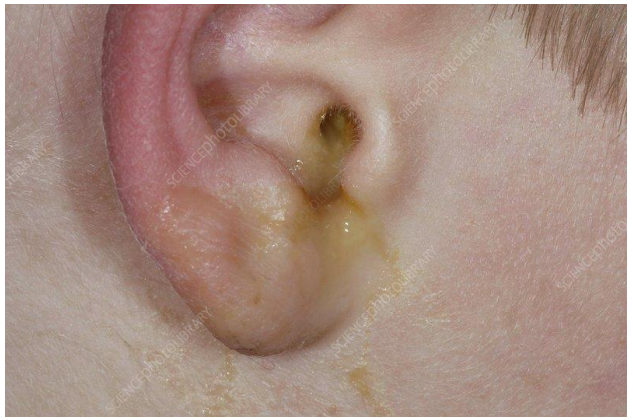
Is Genetic Testing Performed for All Patients with IEI?

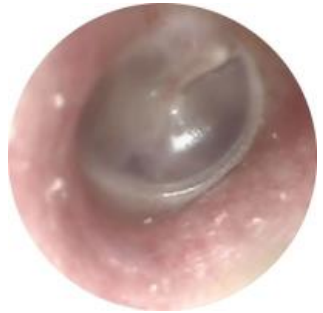
- Arguably: **YES!**
  - Most patients undergo testing with NGS panels, WES, or WGS

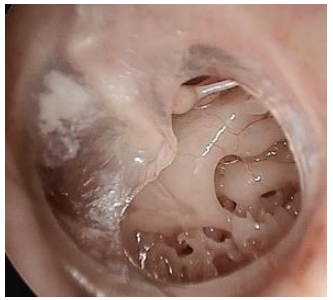
- So- do we still need other diagnostic tests?
  - **YES!**

Case 1

- 3 year old male referred for possible immunodeficiency
  - Recurrent sinus and ear infections
    - Every month; multiple rounds of antibiotics
    - Several perforations of the ear drums
    - Hearing loss requiring hearing aids
  - Poor weight gain
  - Knee effusion/arthritis
    - Swollen, stiff in morning, loosens up during the day, but after a lot of running it will hurt and grandpa has to carry him because he won't walk







Perforated eardrum with infection - Stock Image - C004/2400 - Science Photo Library

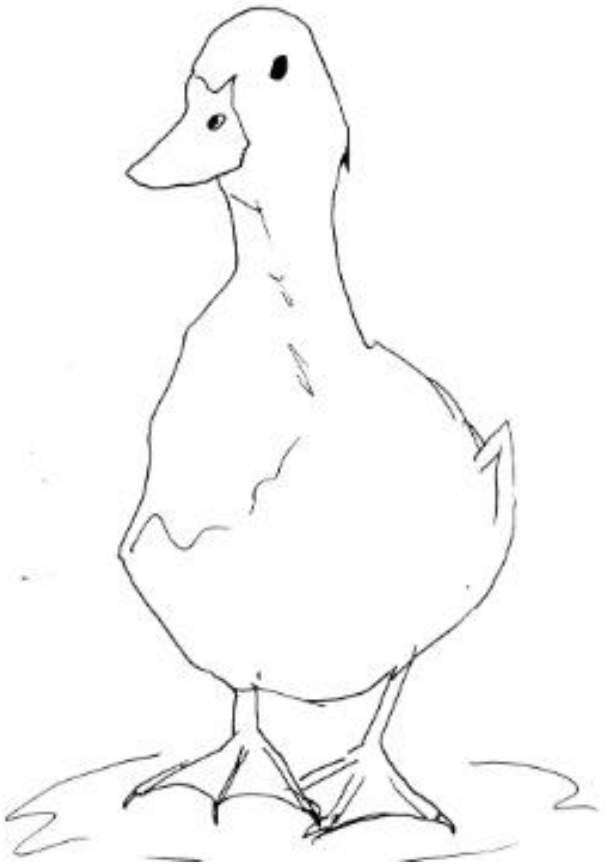
Ear Problems | ENT Specialist Surgeon Adelaide | Paul Varley ENT

Most Likely Diagnosis: XLA

- **What is X-linked agammaglobulinemia?**
  - The first immunodeficiency disease ever identified
  - Caused by pathogenic variants in the BTK gene (Bruton's tyrosine kinase) located on the X chromosome
  - B cells don't develop and patients cannot make antibodies
  - Children (boys) with this disease are prone to infections of the ears, sinuses, and lungs, also bloodstream and other organ infections

Case 1, Continued

- Genetic Panel: Normal
- What?!?
  - Now what do we do??
  - Seems like it should be XLA!

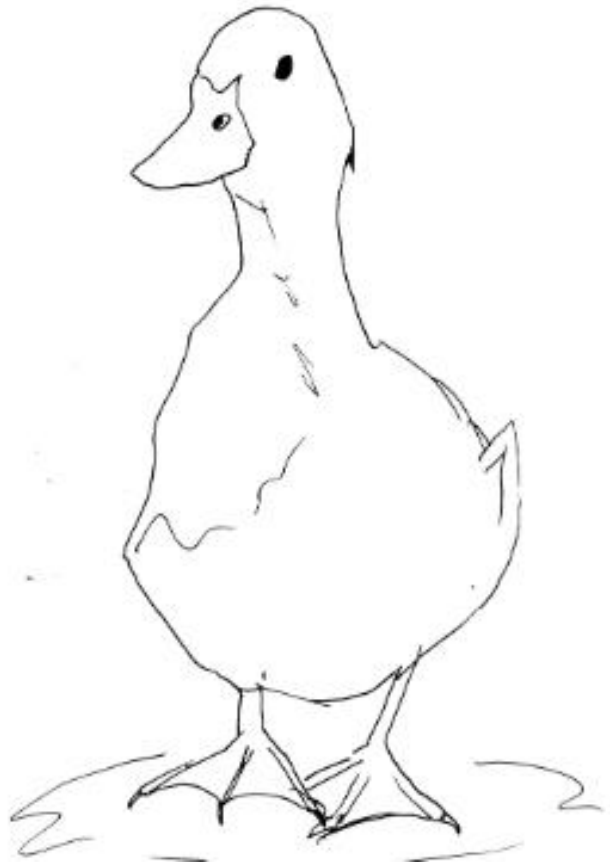


Case 1, Continued

- Initial Lab Results

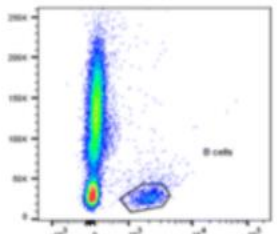
FLOW CYTOMETRY	
CD3%	95
CD4%	50
CD8%	40
CD19%	0
CD16/56%	5
CD3 ABS	2,547
CD4 ABS	1,353
CD8 ABS	1,071
CD19 ABS	0
CD16/56 ABS	130
CD4:CD8	1.2

IGA	<6.0 *
IGG	145 *
IGM	20 *

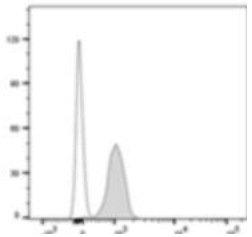


Case 1, Continued B cells

Control



CD19



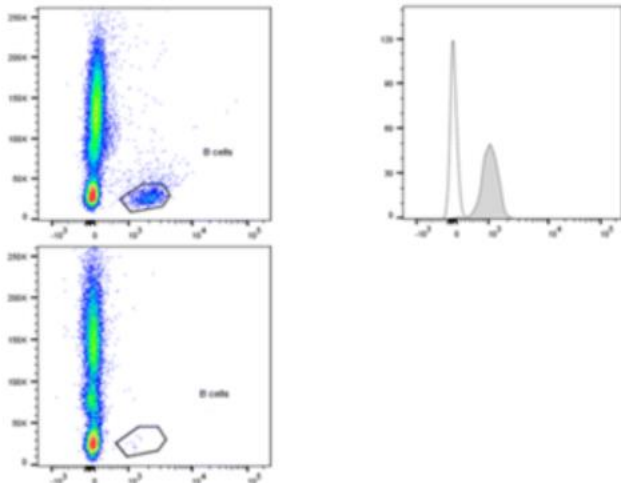
BTK

Chear CT, Nallusamy R, Chan KC, Mohd Tap R, Baharin MF, Syed Yahya SNH, Krishnan PB, Mohamad SB, Ripen AM. Atypical Presentation of Severe Fungal Necrotizing Fasciitis in a Patient with X-Linked Agammaglobulinemia. J Clin Immunol. 2021 Aug;41(6):1178-1186.

Case 1, Continued B cells

Control

Patient



CD19

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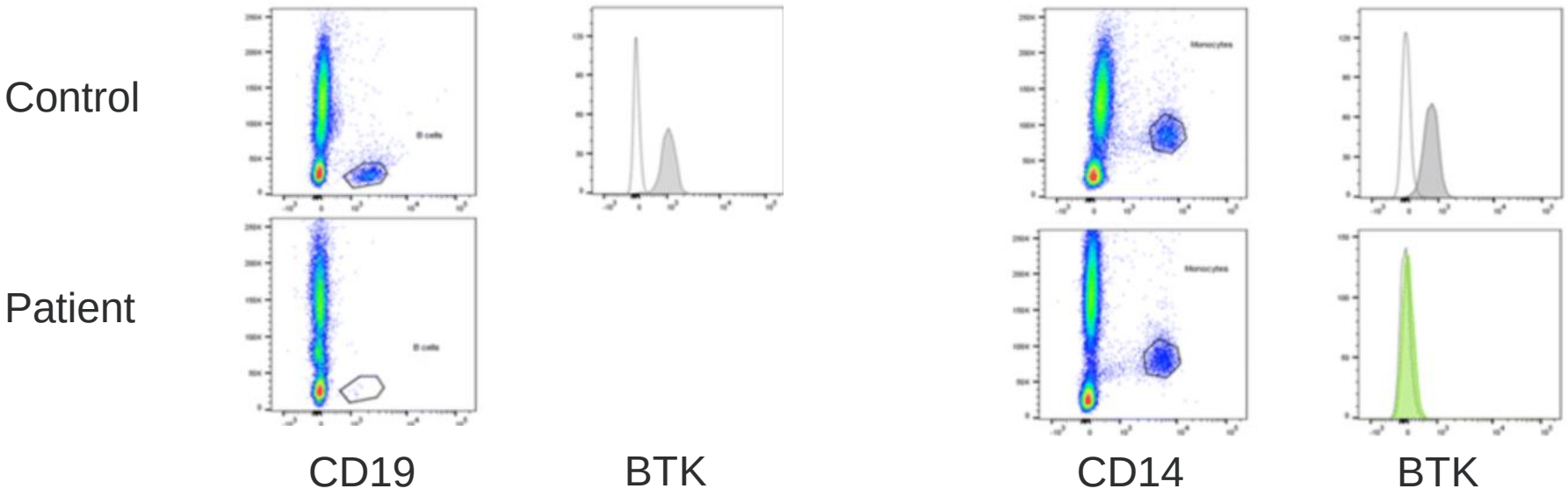
Case 1, Continued



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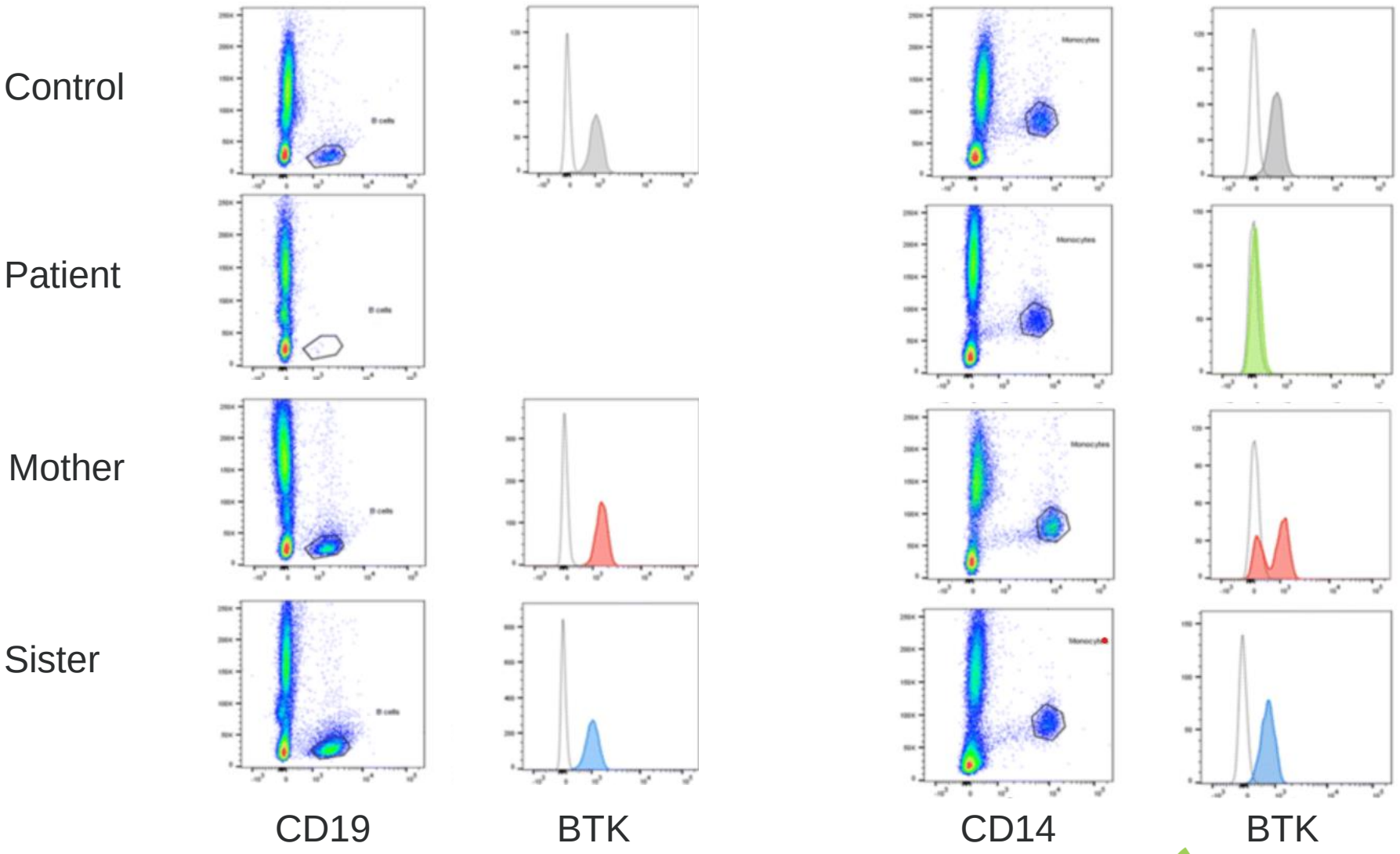

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We Went Back to Our Genetics Friends…

Deletion/Duplication Analysis

Collection Date and Time:
Laboratory Accession #
Specimen
Ordering Provider
Hospital # / Location : CCHMC
Receive Date and Time
Ethnicity : Unknown

Genes of Interest: BTK

BTK Partial Gene Hemizygous Deletion (I1-I15 and E19-E19)
x0,Xq22.1(100602644_100604750)x0

Note: the reference DNA sequence number used in this test: NM_000061.2

RESULT: Deletions detected

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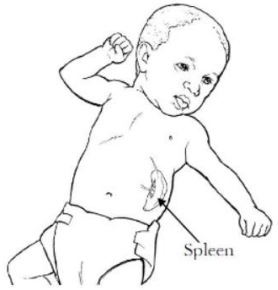
Case 2

- 6-month-old male comes to the Emergency Room
  - 5 days of fever
  - Not eating well
  - Less active
- No other symptoms, had a cold 2 weeks ago
- Exam: Has a fever, heart rate is fast, and doctors can feel an enlarged spleen



https://www.nationwidechildrens.org/family-resources-education/health-wellness-and-safety-resources/helping-hands/splenectomy

Case 2

- Labs:
  - Several blood counts are low:
    - Platelets 47
    - White blood cell count 1.9, Neutrophil count 1137
  - Liver enzymes are elevated
    - AST 296, ALT 77
    - Fibrinogen is low 137
      - (protein made by the liver that stops bleeding by helping blood clots to form)
  - Ferritin is high 2970
      - (ferritin is a blood protein that contains iron; ferritin is elevated in inflammation)

Case 2

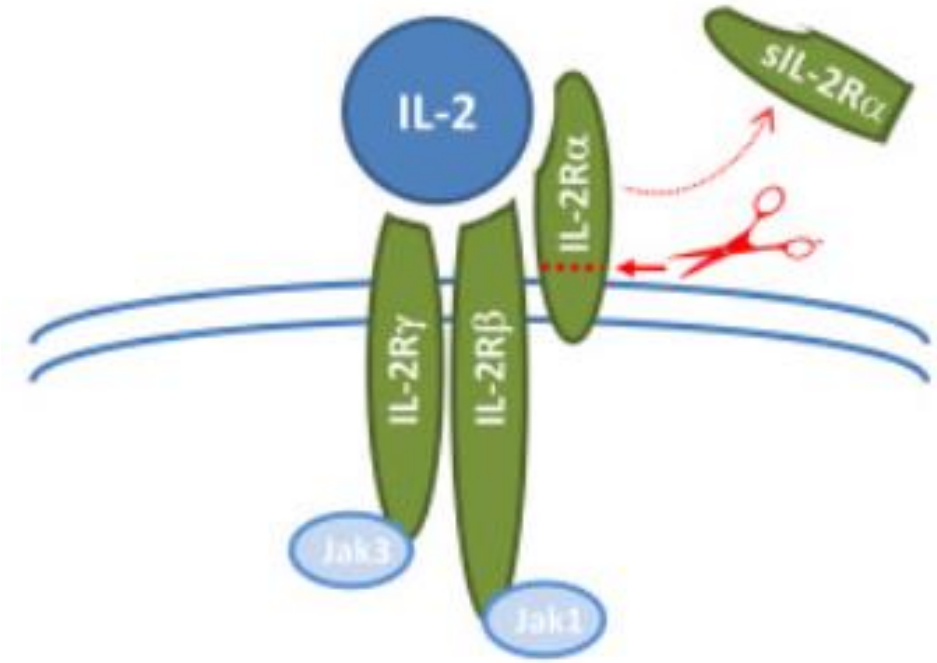
- Baby was admitted to the hospital, given IV fluids and antibiotics.
- Many tests done:
  - Bone marrow examination: no cancer
  - CT Scan did not show any concerns for cancer or infection
  - Lots of testing for infections: negative

Case 2

- Does This Patient Have Hemophagocytic Lymphohistiocytosis (HLH)?
  - HLH is a severe life-threatening syndrome of overwhelming inflammation characterized by fevers, cytopenias, splenomegaly, liver inflammation and dysfunction, seizures, and other problems

Tried & True HLH Biomarker: Soluble IL-2Rα

- Activated T cells upregulate the IL-2 receptor; the IL-2Rα is cleaved by proteases released by activated mononuclear phagocytes
- High levels indicate high levels of T cell activation
- Levels are high in HLH



The IL-2 – IL-2 receptor pathway in health and disease: The role of the soluble IL-2 receptor

Jan Damoiseaux

Tried & True HLH Biomarker: Soluble IL-2Rα

Soluble IL-2Rα 13,234

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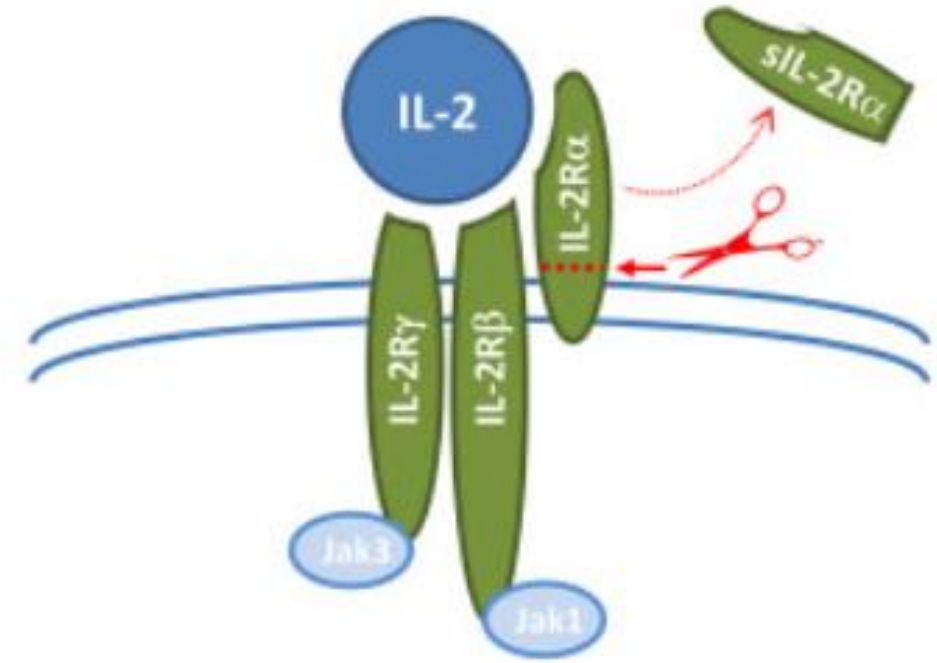

The IL-2 – IL-2 receptor pathway in health and disease: The role of the soluble IL-2 receptor

Jan Damoiseaux

Newer Biomarker: CXCL9

- CXCL9: Good marker of IFN-γ pathway activity
  - CXCL9 is a chemokine secreted by cells like monocytes, endothelial cells, and fibroblasts in response to IFN-γ which is a critical inflammatory cytokine in HLH

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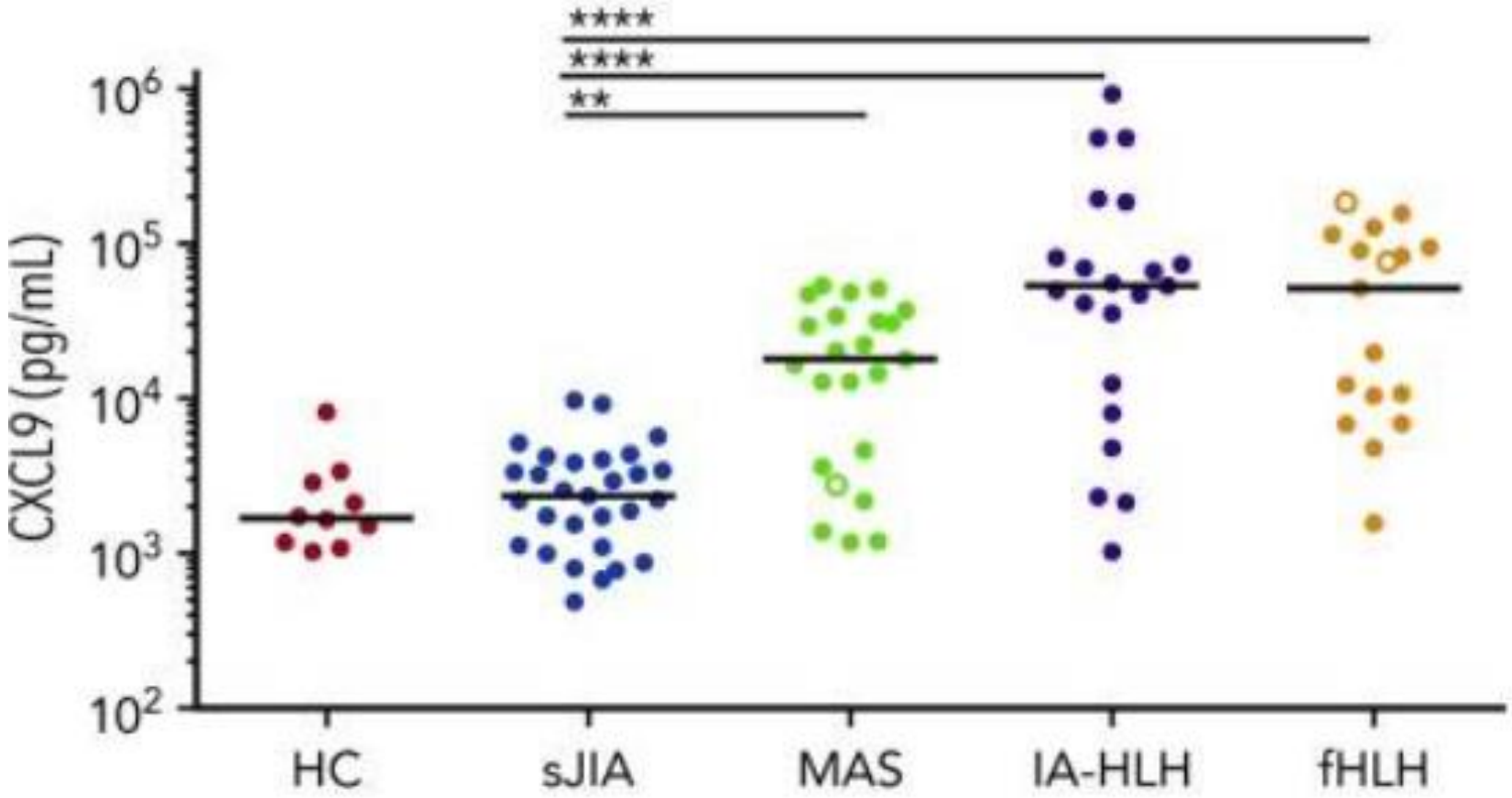
CXCL9: 5,783

CXCL9 in HLH



Weiss et al, 2018

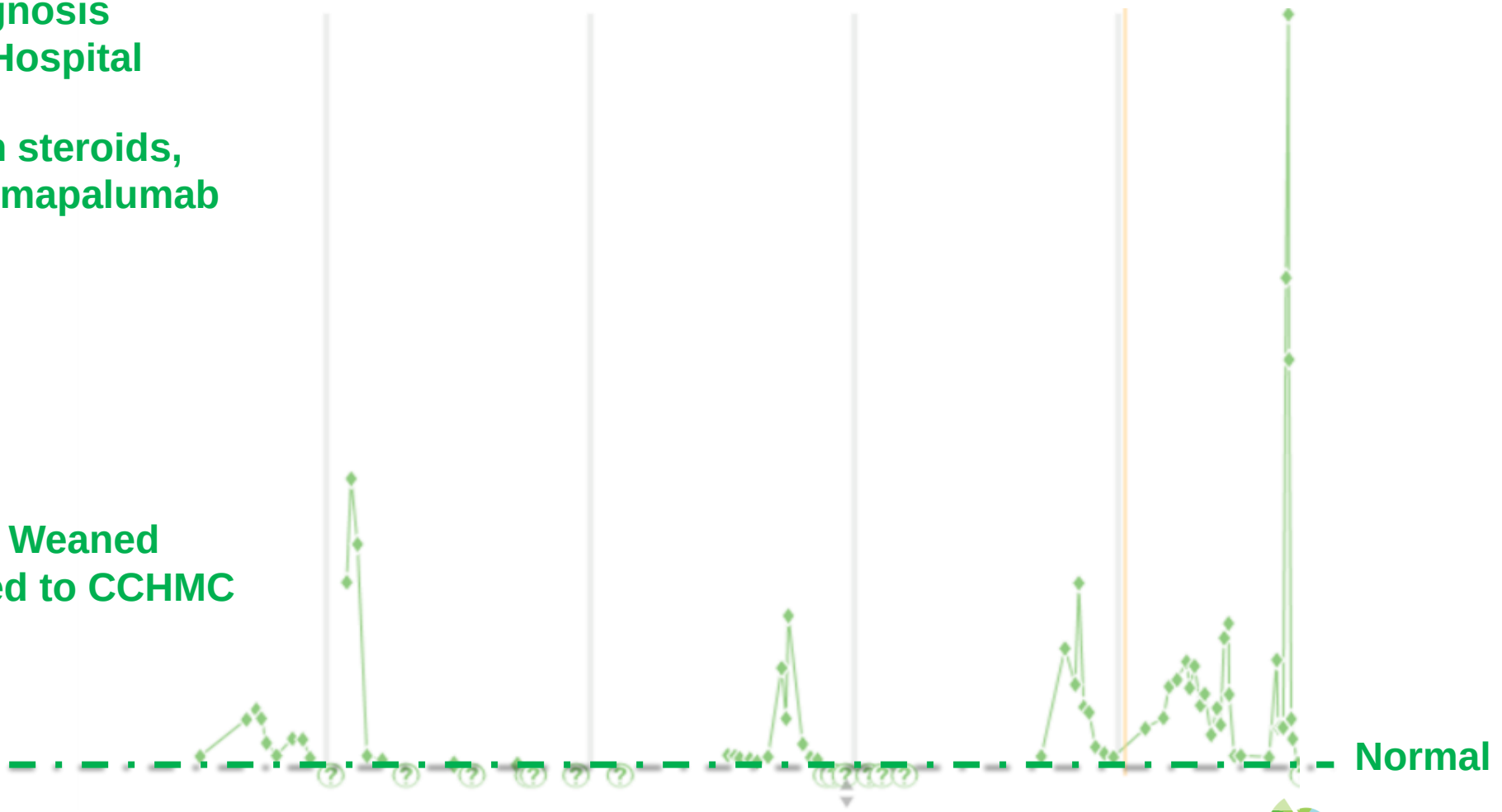
CXCL9 Over Time

4000

5783 at Diagnosis
At Outside Hospital

Treated with steroids,
Etoposde, emapalumab

Improved,
Therapies Weaned
Transferred to CCHMC



Normal

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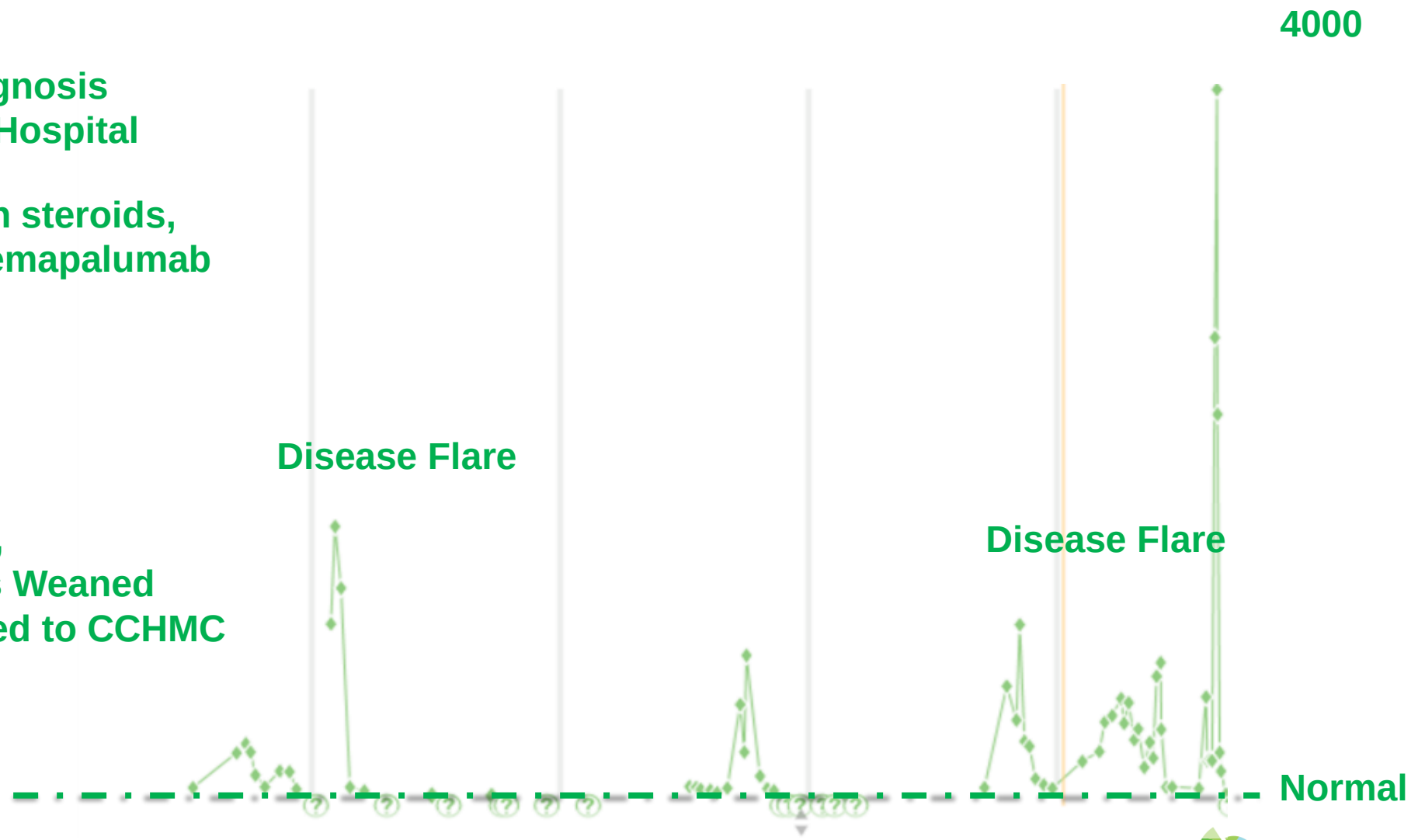
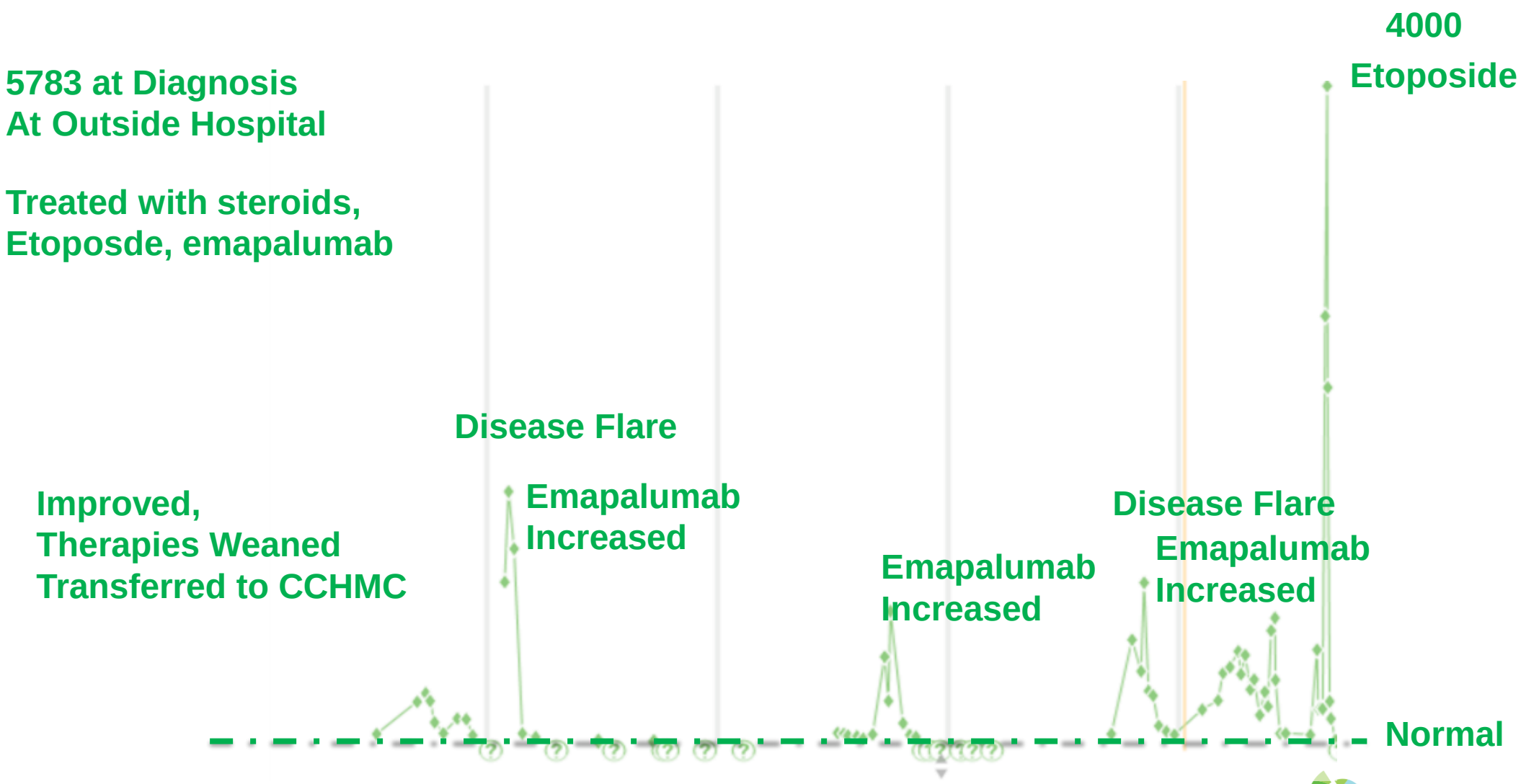
CXCL9 Over Time

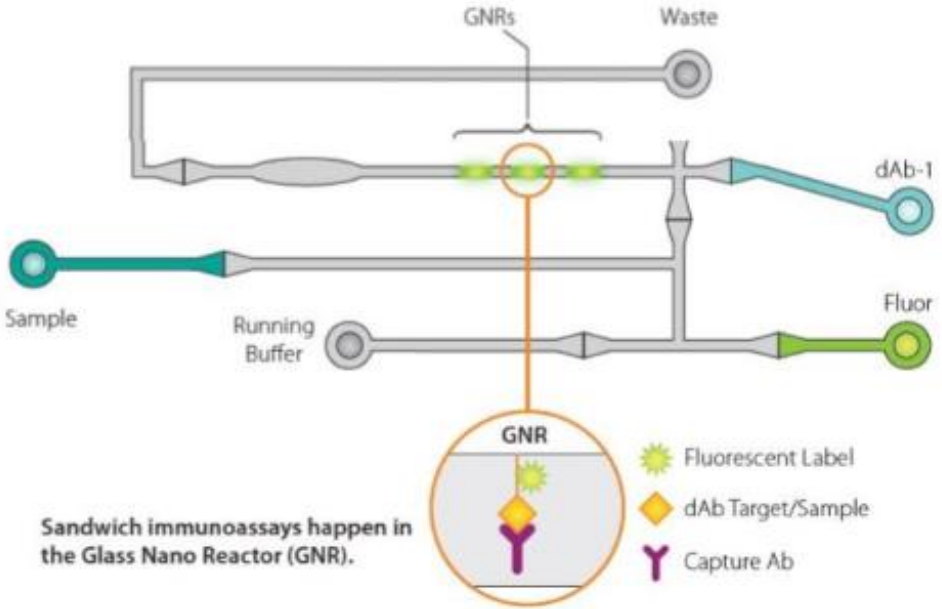


CXCL9 Over Time



Scaling Testing Capability Up in Our Small Lab: Ella Machine (Protein Simple)

1. Sample is routed through microfluidic channels.
2. Capture antibody captures target analyte.
3. Stringent wash removes unbound analyte.
4. Detection antibody migrates through microfluidic channel.
5. Stringent wash removes unbound detection antibody.
6. Scan GNRs.



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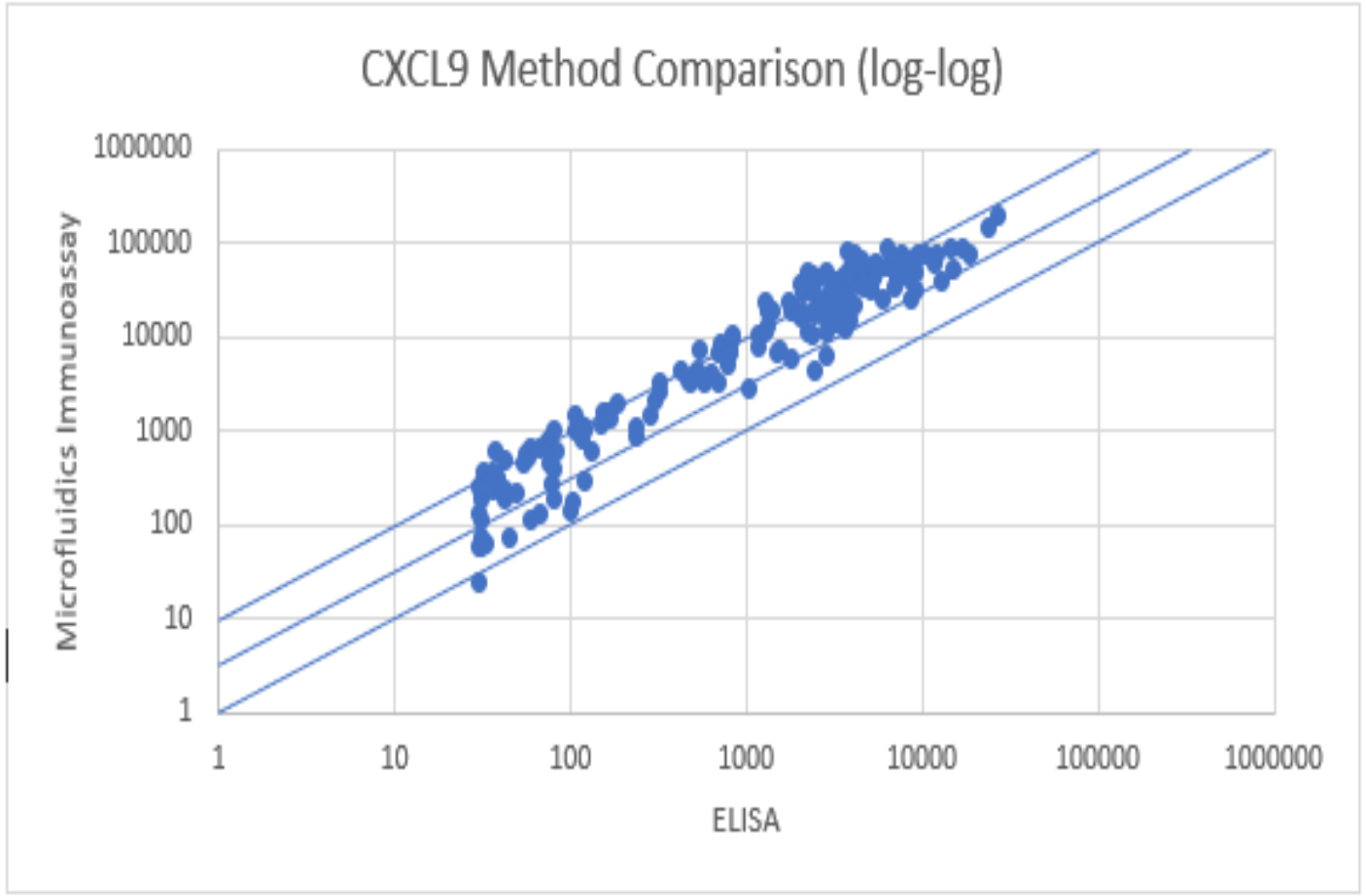
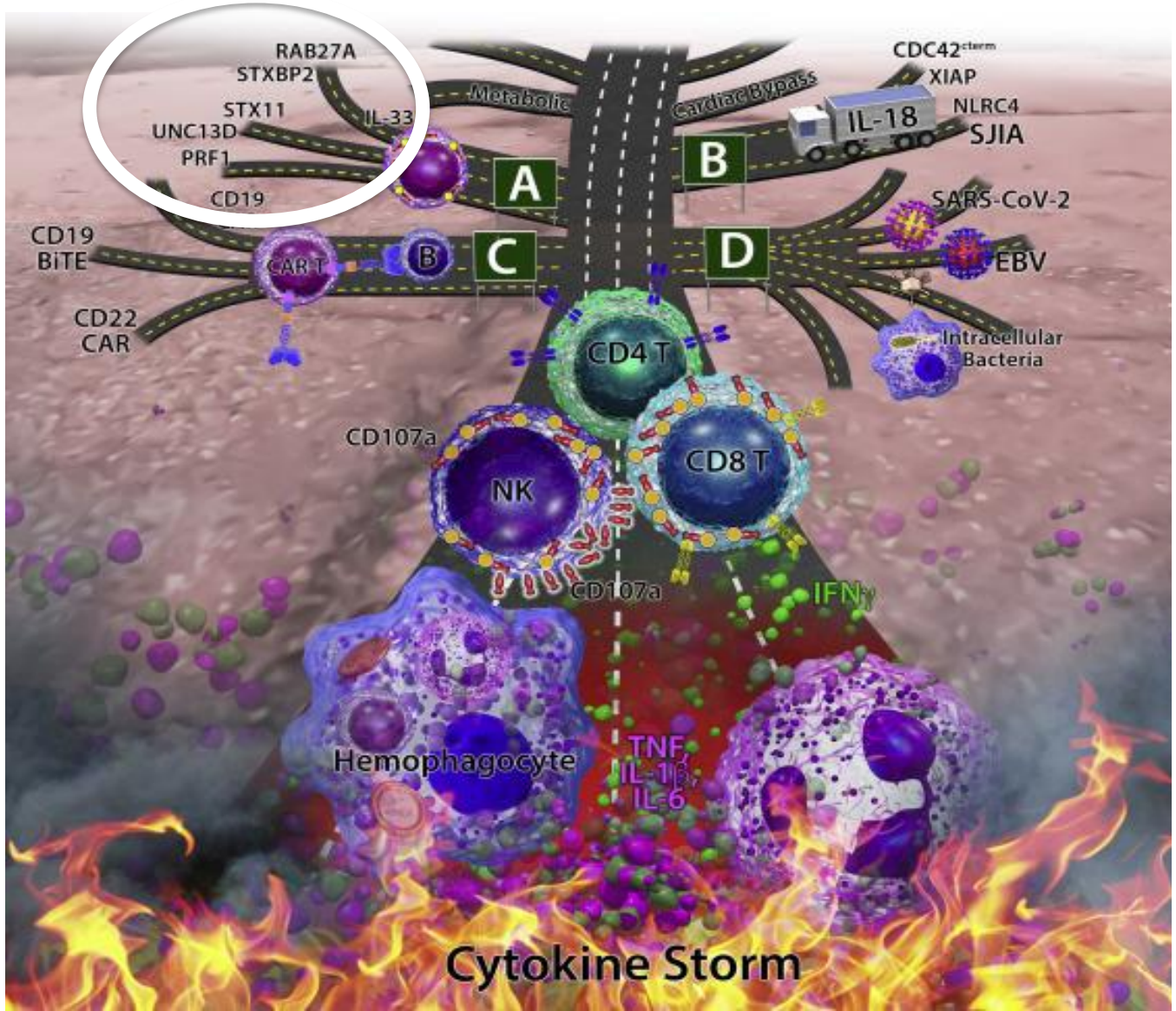
Ella Values Versus Manual ELISA



Ella: 5 to 10 fold higher results

(Normal ≤ 647pg/mL)

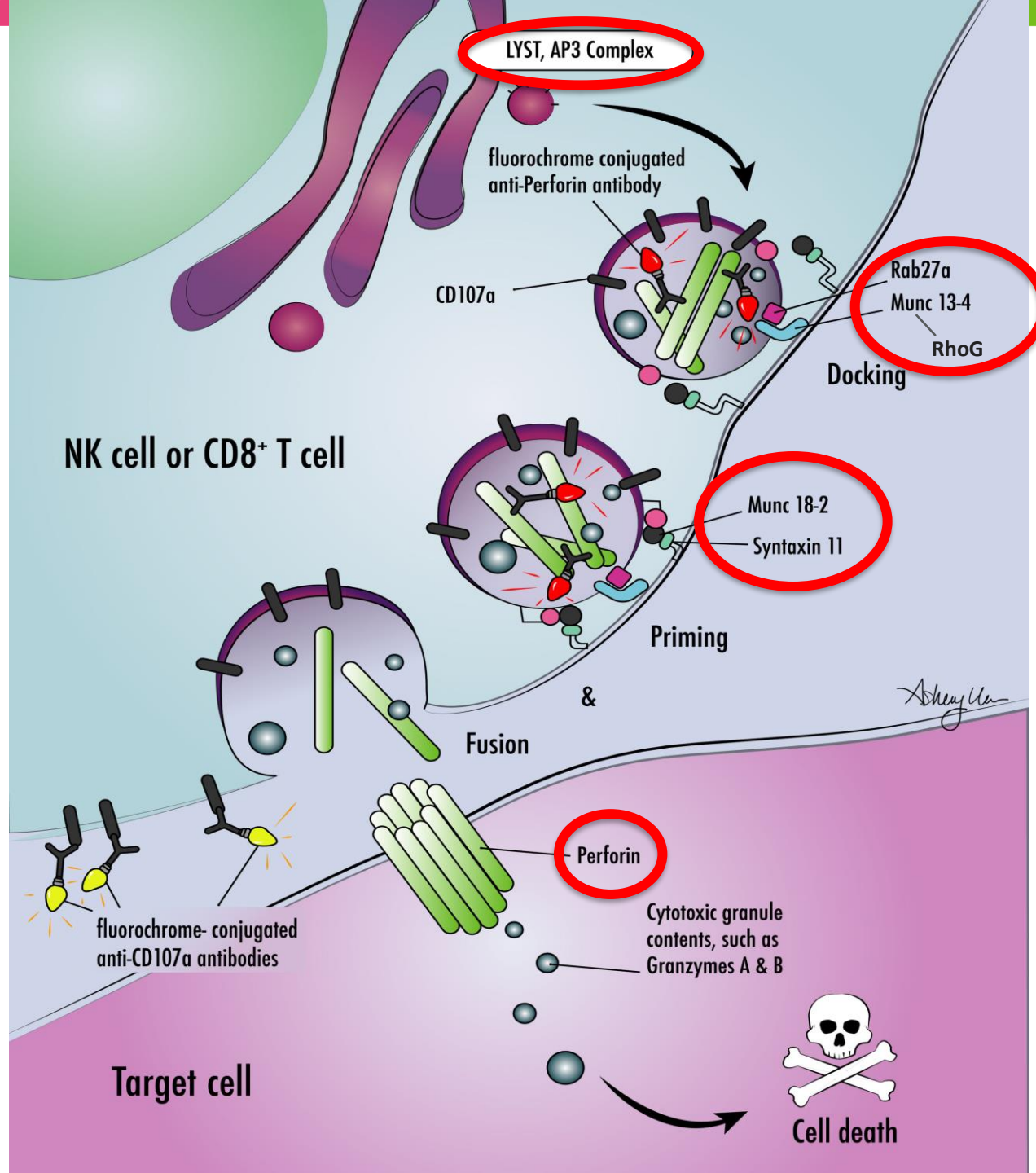
Genetic HLH Disorders

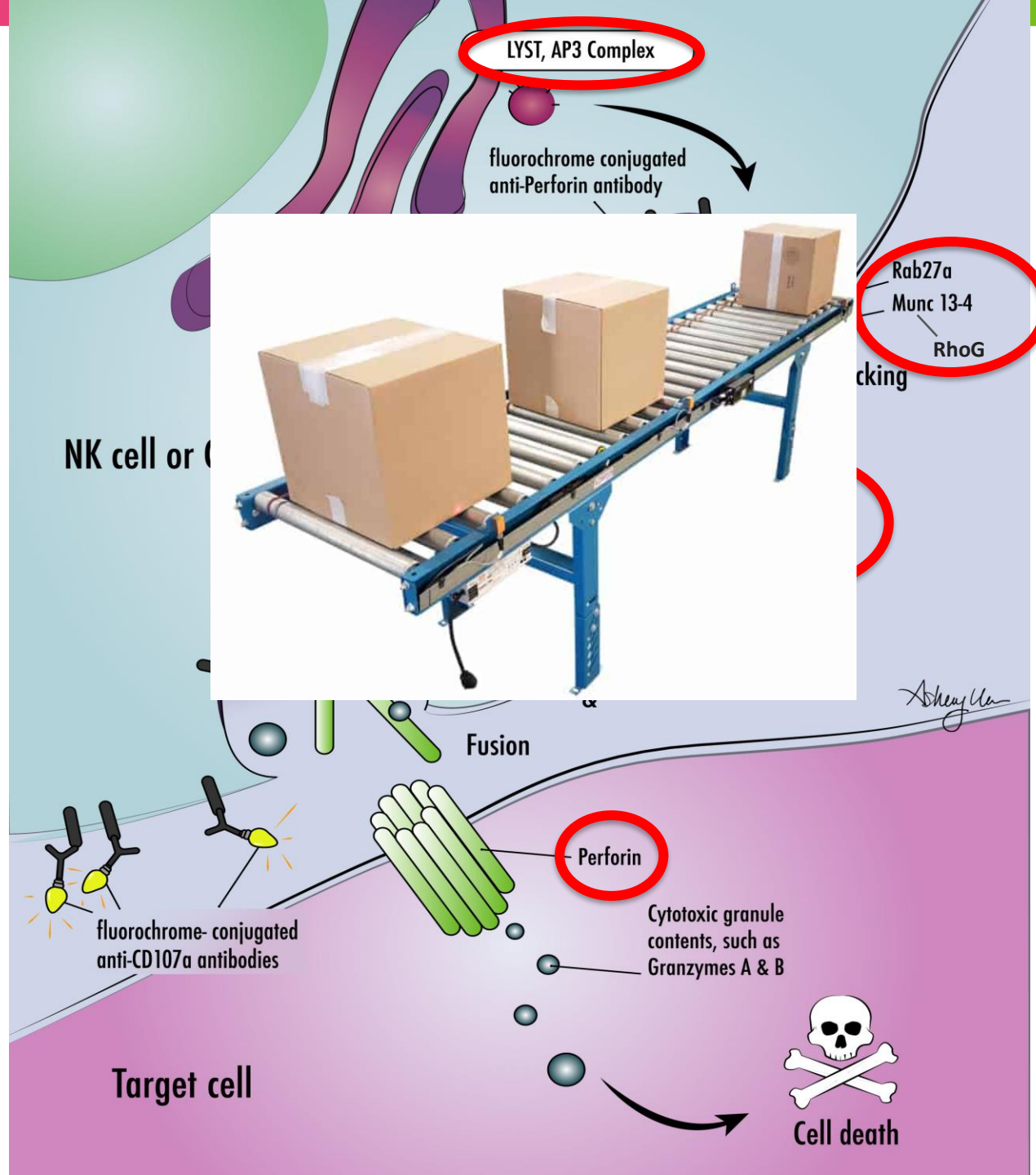


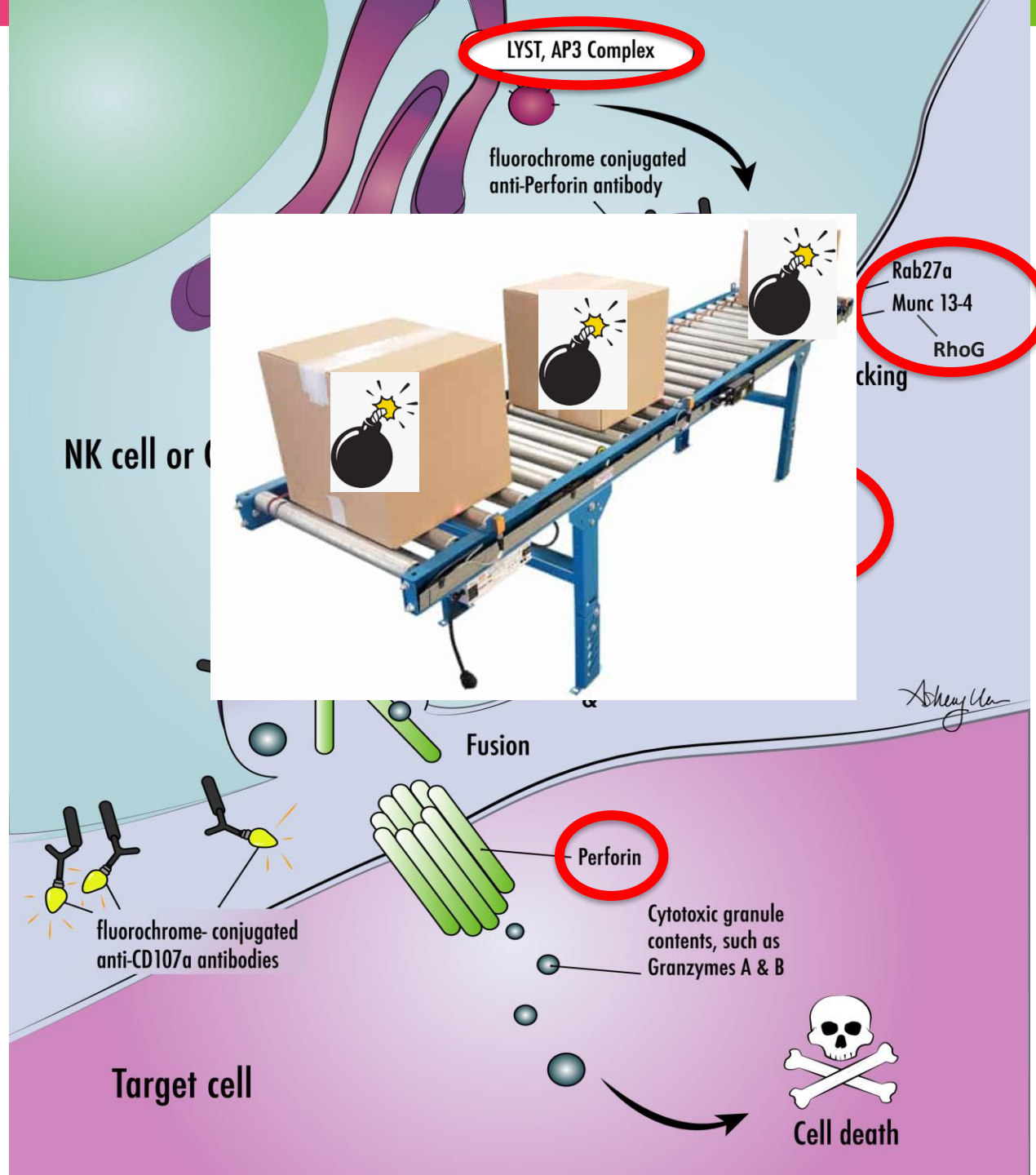
Scott Canna &
Randy Cron

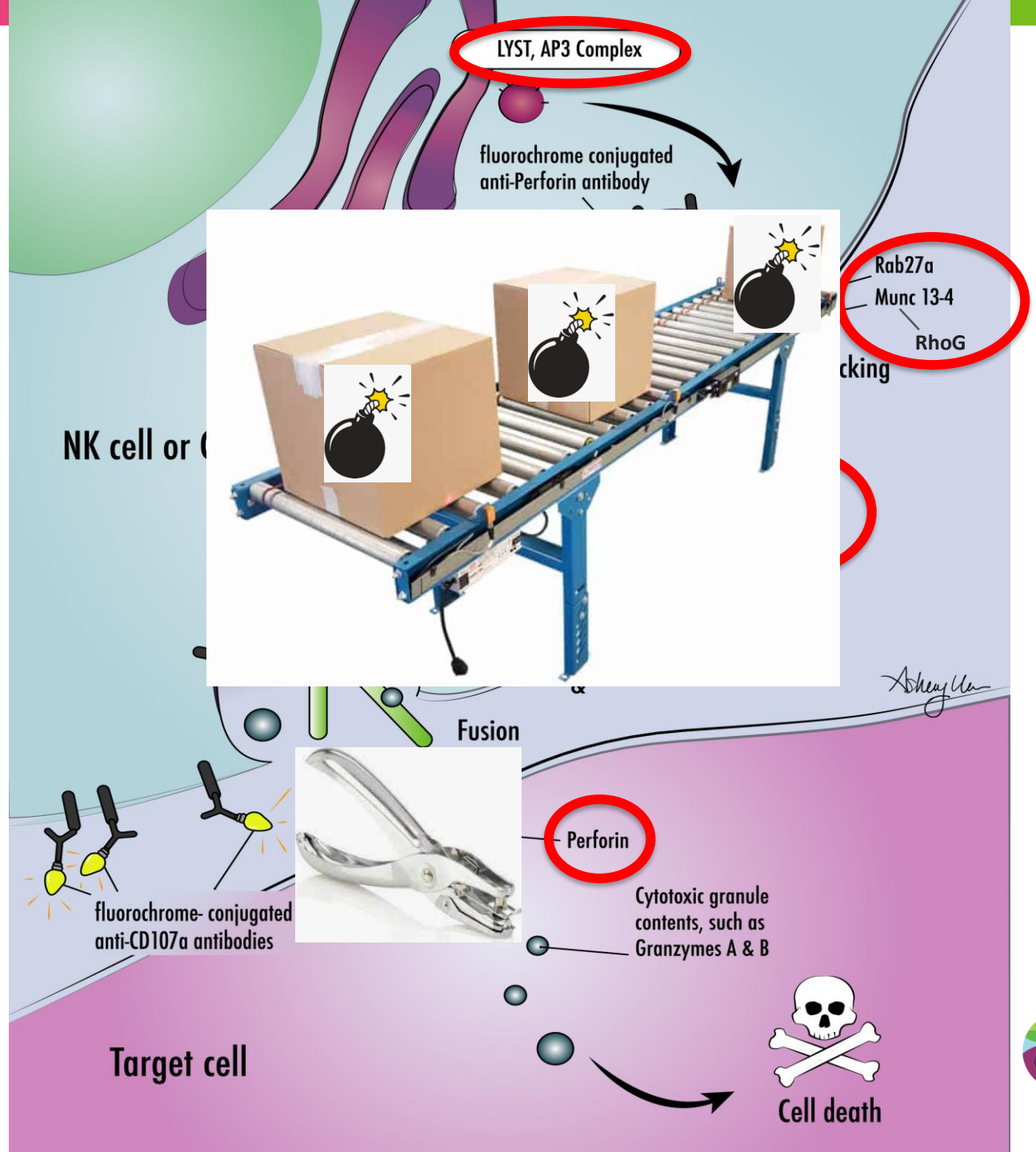
ScreeningTests for Genetic HLH

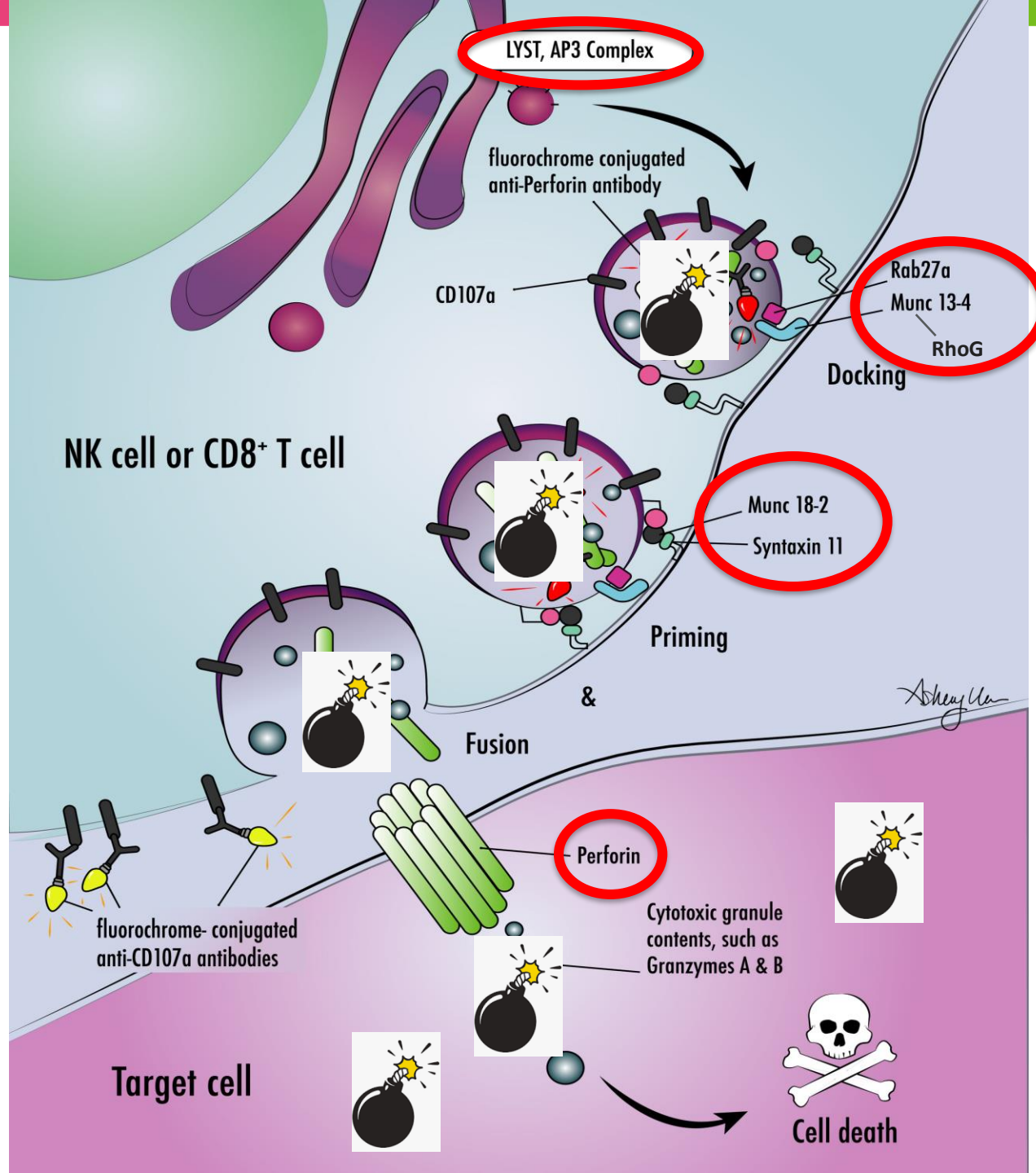
- Familial HLH
  - *PRF1*
    - Perforin Expression
  - *UNC13D, STXBP2, STX11, RAB27A*
    - CD107a
- XLP1
  - SAP Expression
- XLP2
  - IL-18
  - XIAP Expression
  - Functional Assay
- Griscelli Syndrome
  - CD107a
- Chediak Higashi
  - CD107a

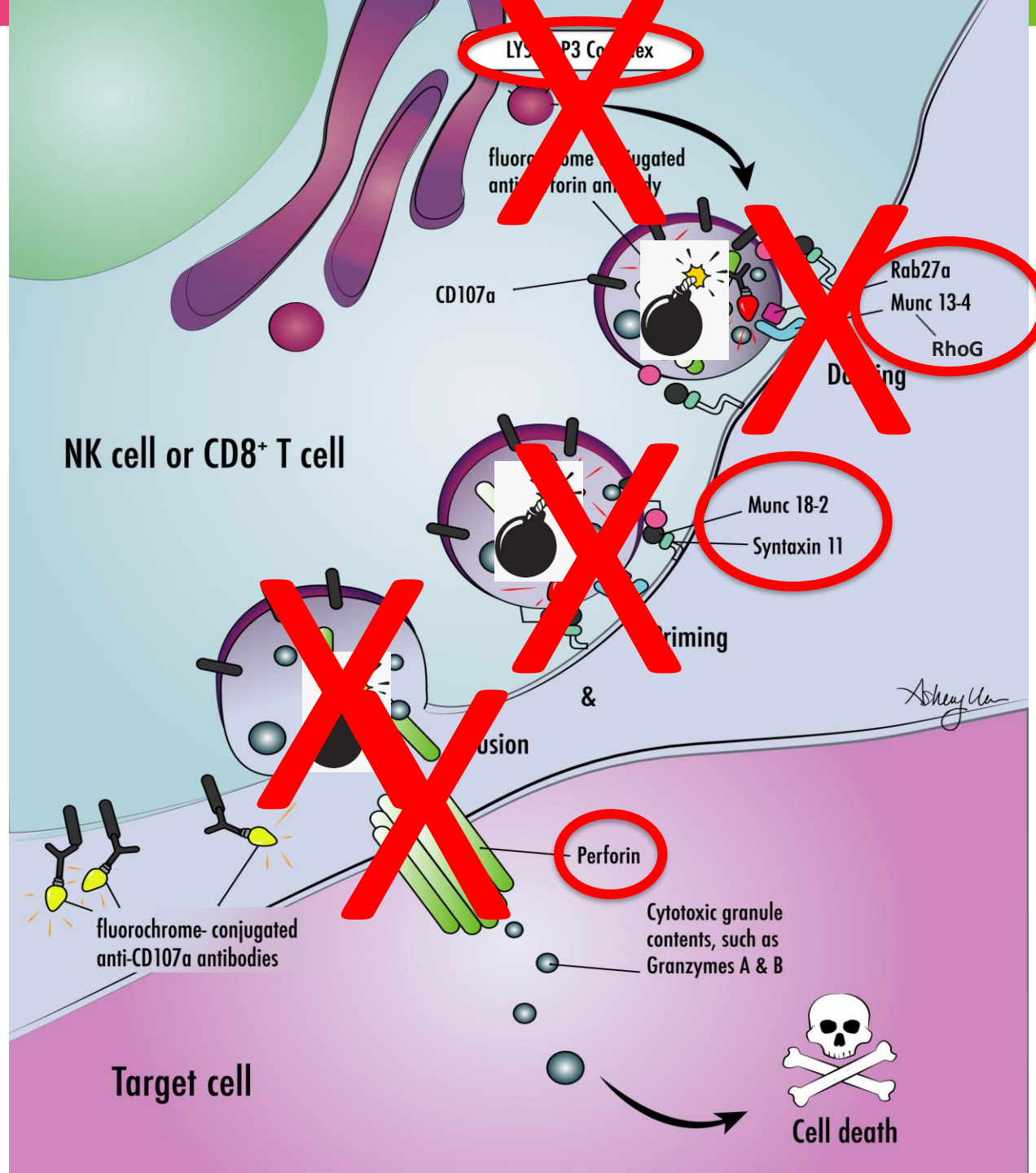








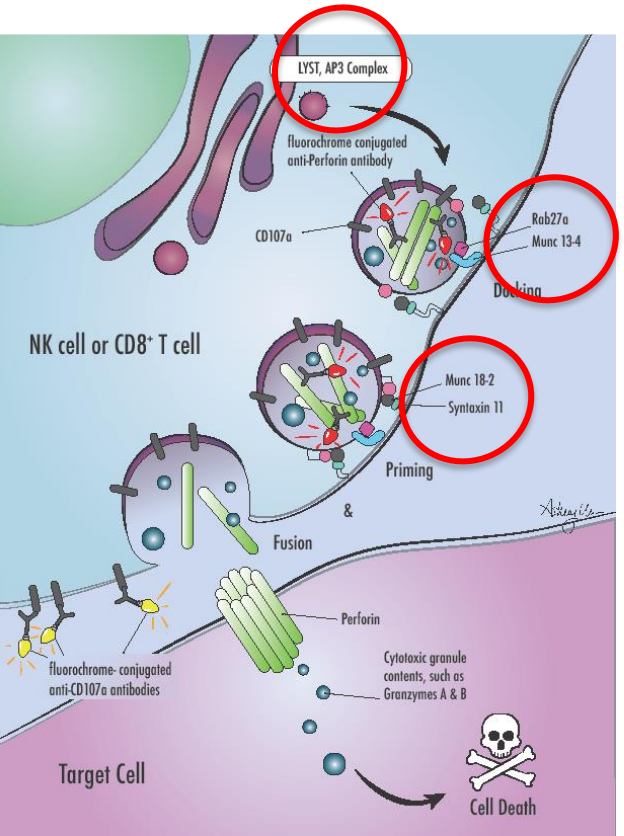


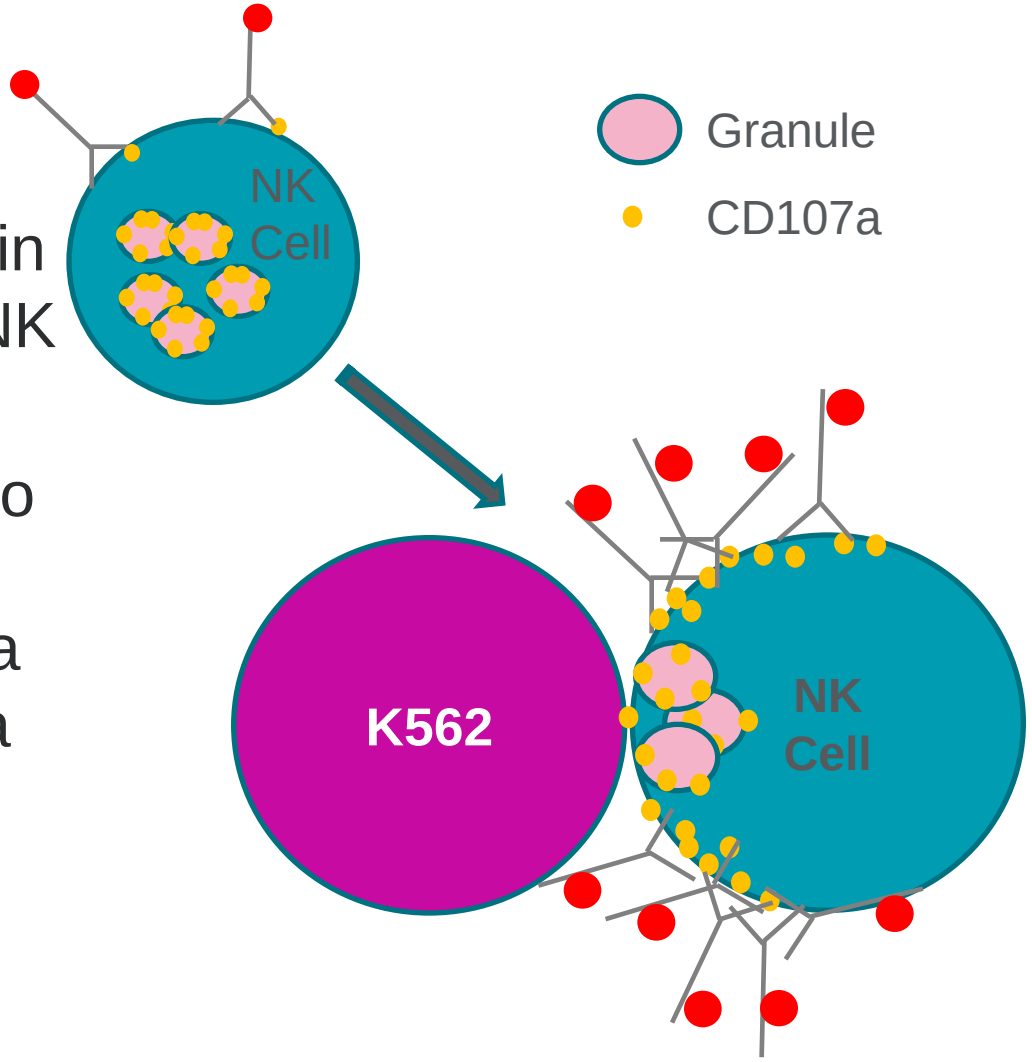


CD107a / NK Cell Degranulation

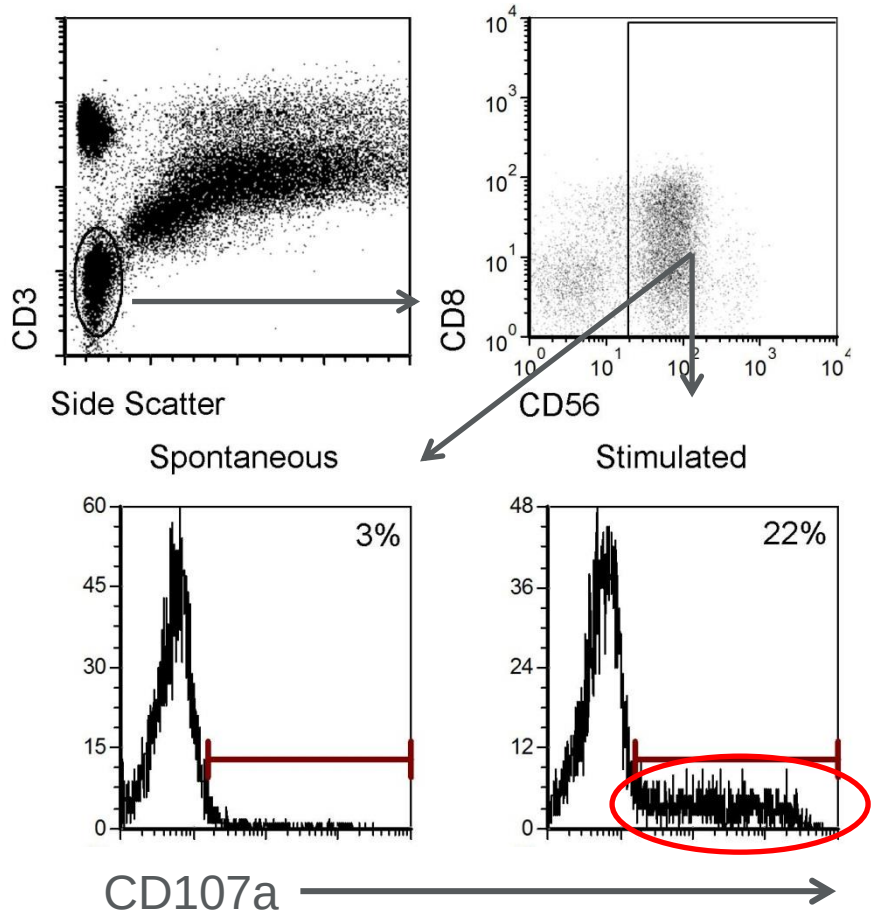
Screens for pathogenic variants in *UNC13D, STX11, STXBP2, RAB27A, LYST*

- Methods
  - CD107a (LAMP-1) is normally expressed within granule membranes of NK cells
  - NK cells are stimulated to degranulate in the presence of anti-CD107a antibodies labeled with a fluorophore
  - As granules undergo exocytosis, CD107a is transiently expressed on the cell surface

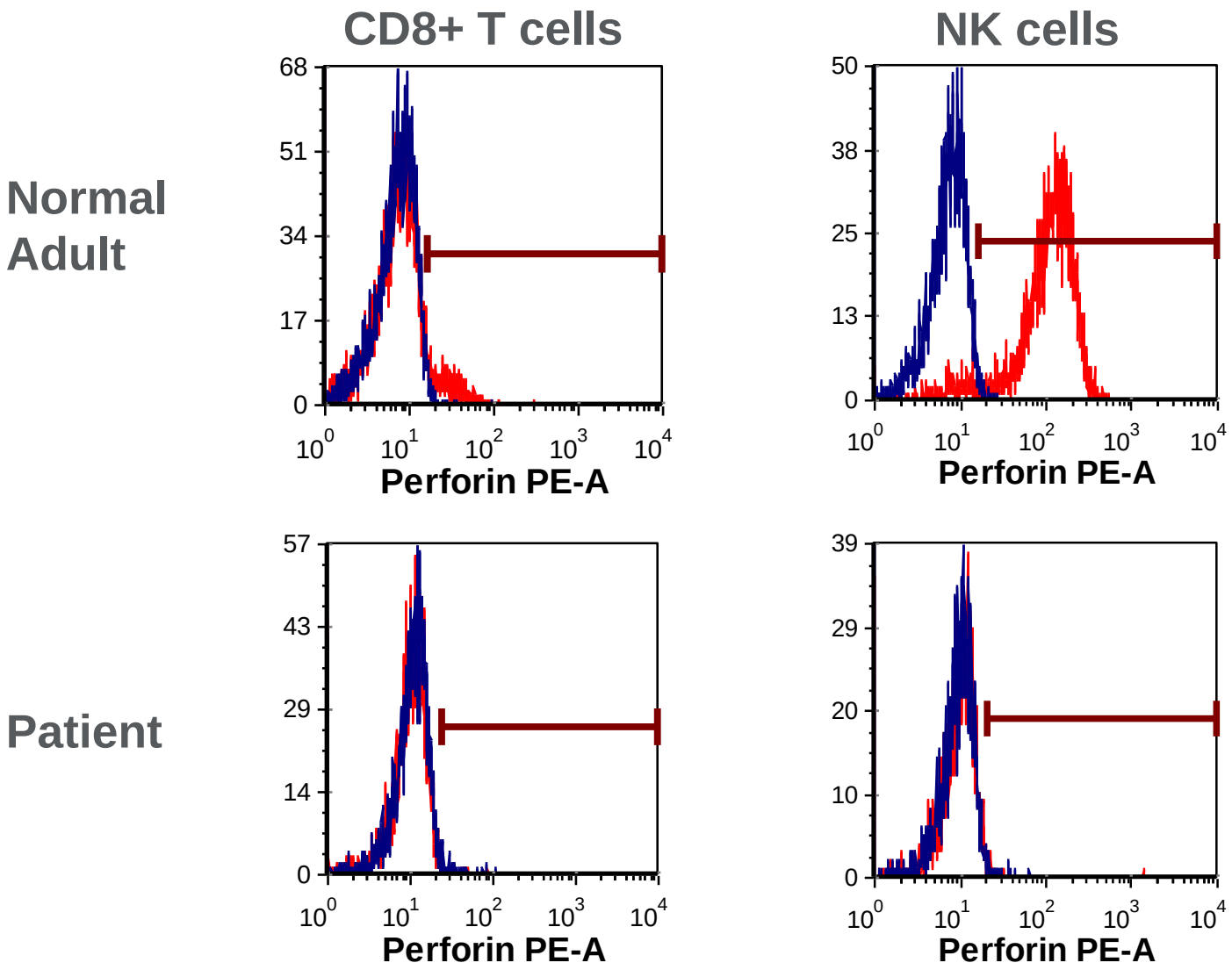




Patient CD107a: Normal

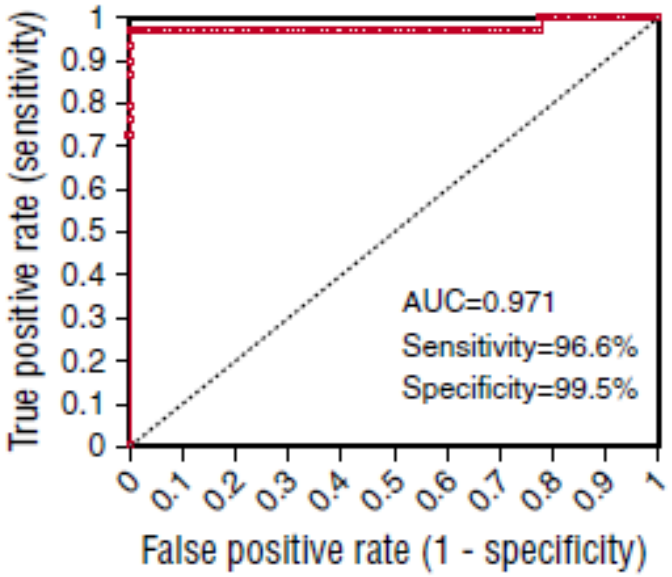


Patient Perforin Expression: Absent



Genetic HLH Screening Test Accuracy at CCHMC DIL

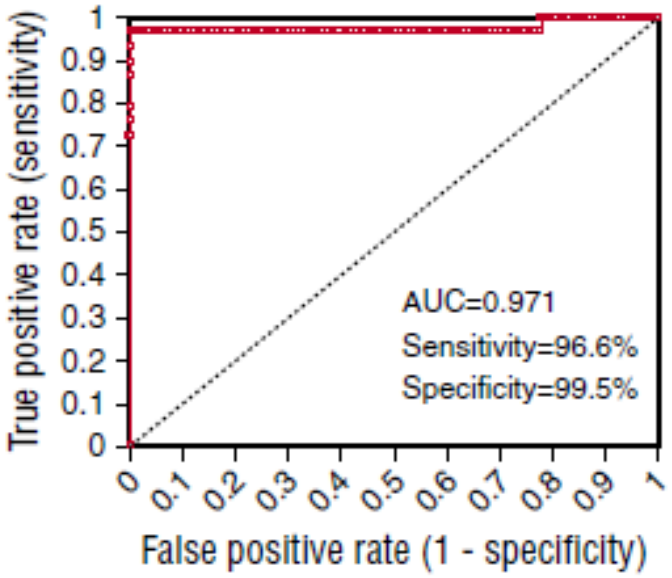
Perforin



Rubin et al, 2017

Genetic HLH Screening Test Accuracy at CCHMC DIL

Perforin



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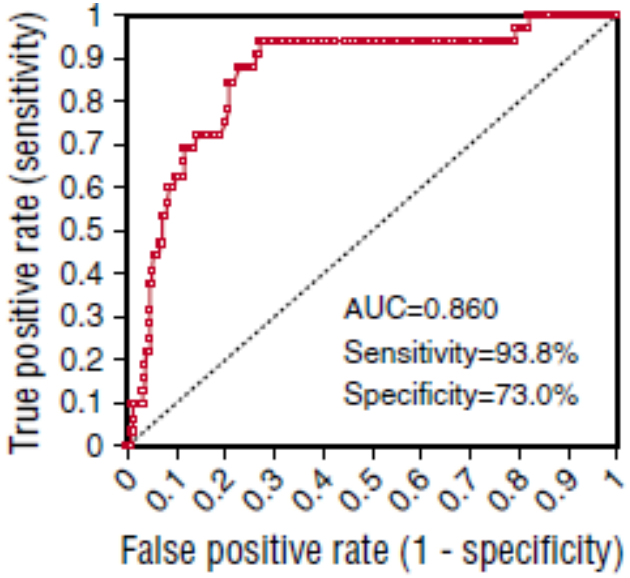
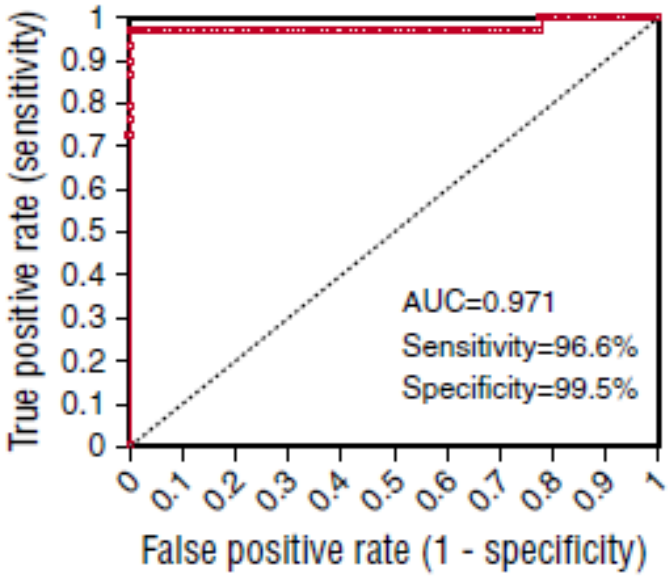


Rubin et al, 2017

Genetic HLH Screening Test Accuracy at CCHMC DIL

Perforin



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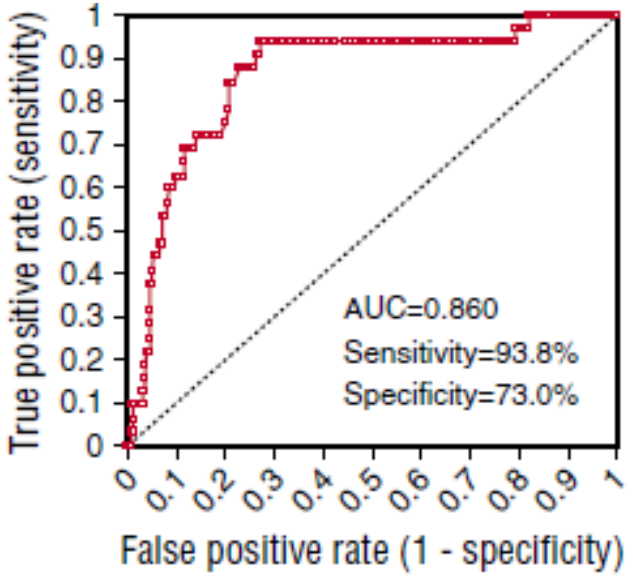
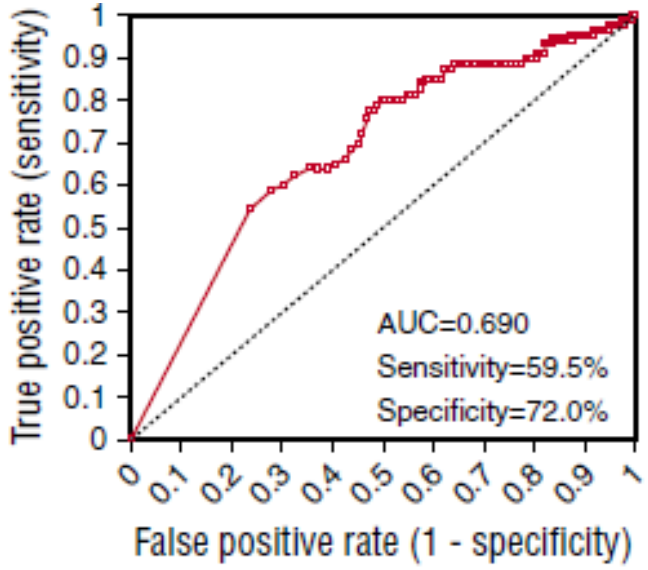


NK Function LU



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Genetic HLH Screening Test Accuracy at CCHMC DIL

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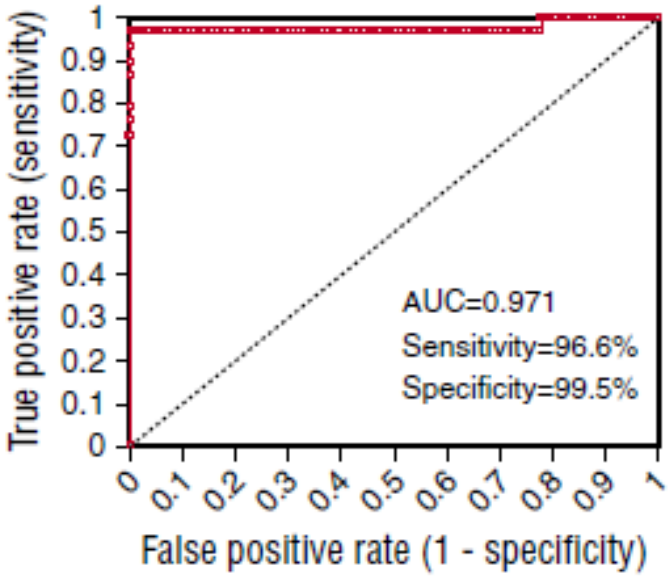

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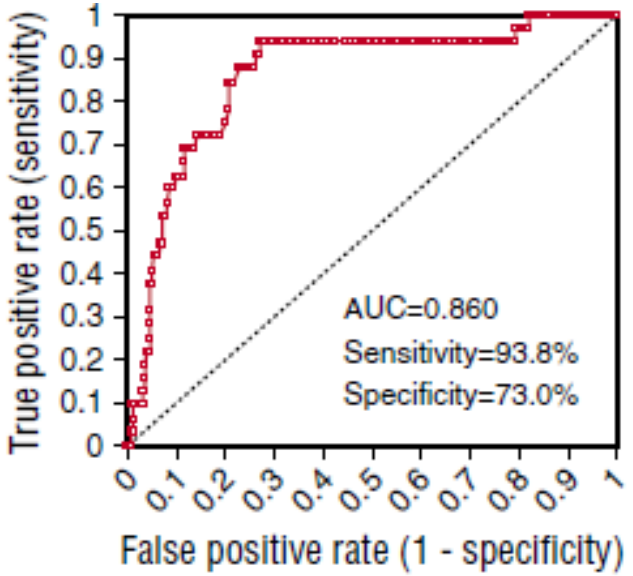

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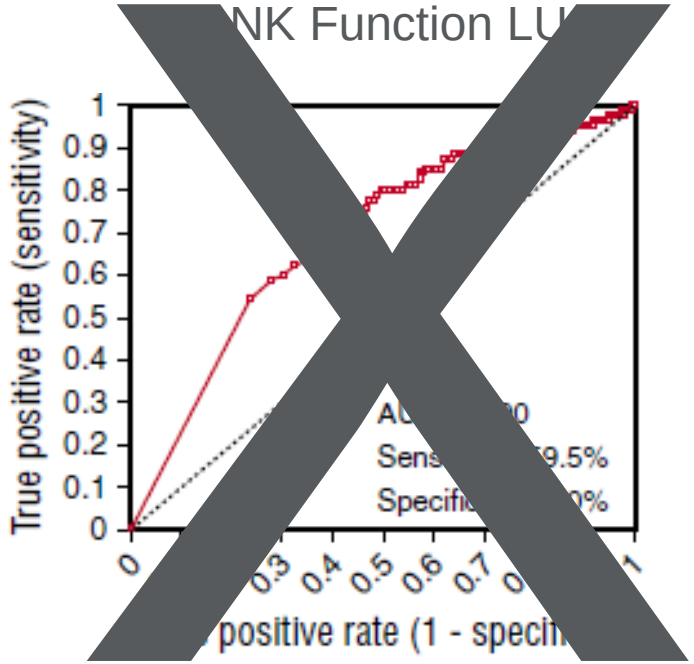

Rubin et al, 2017

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New: Macheon 48 Hour STAT HLH Panel

HLH Genetic Panel 3.0

MORE INFO

- STAT: < 48 hours (M-F)
- NGS, Inversion Assay
- Draw Tube: Purple Top
- Sample Type: EDTA Whole Blood

Hemophagocytic Lymphohistiocytosis (HLH) is a life-threatening disease where an underlying immune defect and/or triggering event initiates excessive activation of immune cells (macrophages and lymphocytes) leading to multi-organ dysfunction and failure. Treatment of HLH may vary depending on the underlying cause, including whether a genetic cause is detected. Timely diagnosis has been a major challenge, with patients having to start aggressive therapies or be admitted to the ICU before final diagnostic results are available. Note: this is the third time (3.0 name designation) we have updated the gene list to reflect the most current understanding of HLH in the scientific literature.

MACHAON DIAGNOSTICS

Case 3

- 8-year-old male referred for possible IEI
  - Juvenile Idiopathic Arthritis (JIA): diagnosed at 4 years of age, treated with Methotrexate with incomplete control of symptoms
  - Inflammatory Bowel Disease (IBD): diagnosed at age 7 years, bright red blood in the stool, flatulence and abdominal pain, treated with Humira and Methotrexate, difficult to control
  - Recurrent abscesses and boils, requiring incision and drainage on 4 occasions with cultures consistent with Methicillin-Sensitive *Staphylococcus Aureus*
  - Blood IL-18 Levels High

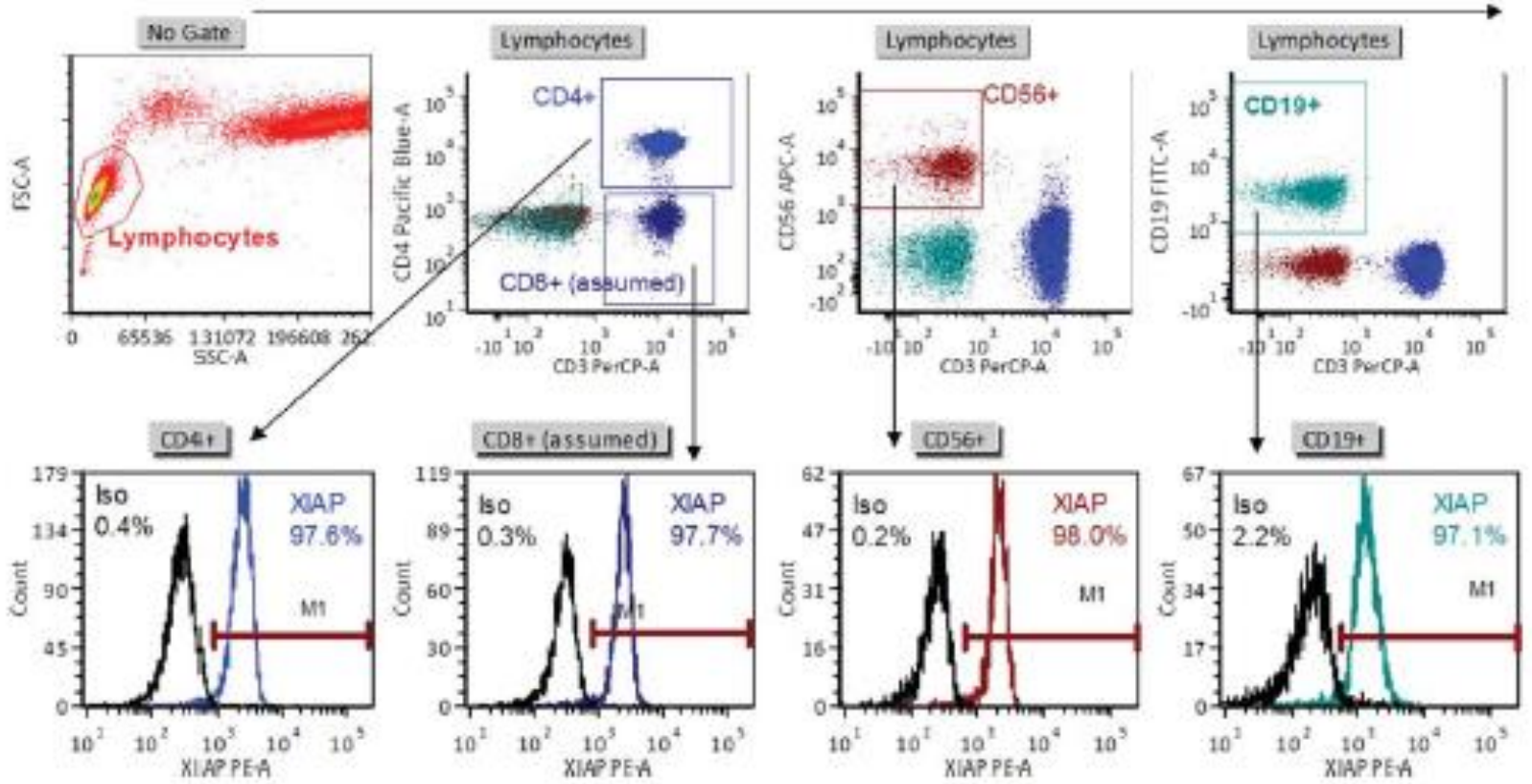
Most Likely Diagnosis: XIAP Deficiency

- **What is XIAP Deficiency?**
  - XIAP deficiency is caused by pathogenic variants in the XIAP/BIRC4 gene
  - Deficiency of XIAP causes defects in the way the NRLP3 inflammasome is regulated (also other cellular defects)
  - Patients (mostly males) are prone to developed a variety of inflammatory complications
    - Hemophagocytic lymphohistiocytosis, recurrent milder systemic hyperinflammatory episodes and cytopenias, inflammatory bowel disease, systemic juvenile idiopathic arthritis, uveitis, skin abscesses, infections, other complications

Genetic Testing

- XIAP c.632A>G(p.Glu211Gly)

XIAP: Flow Cytometry



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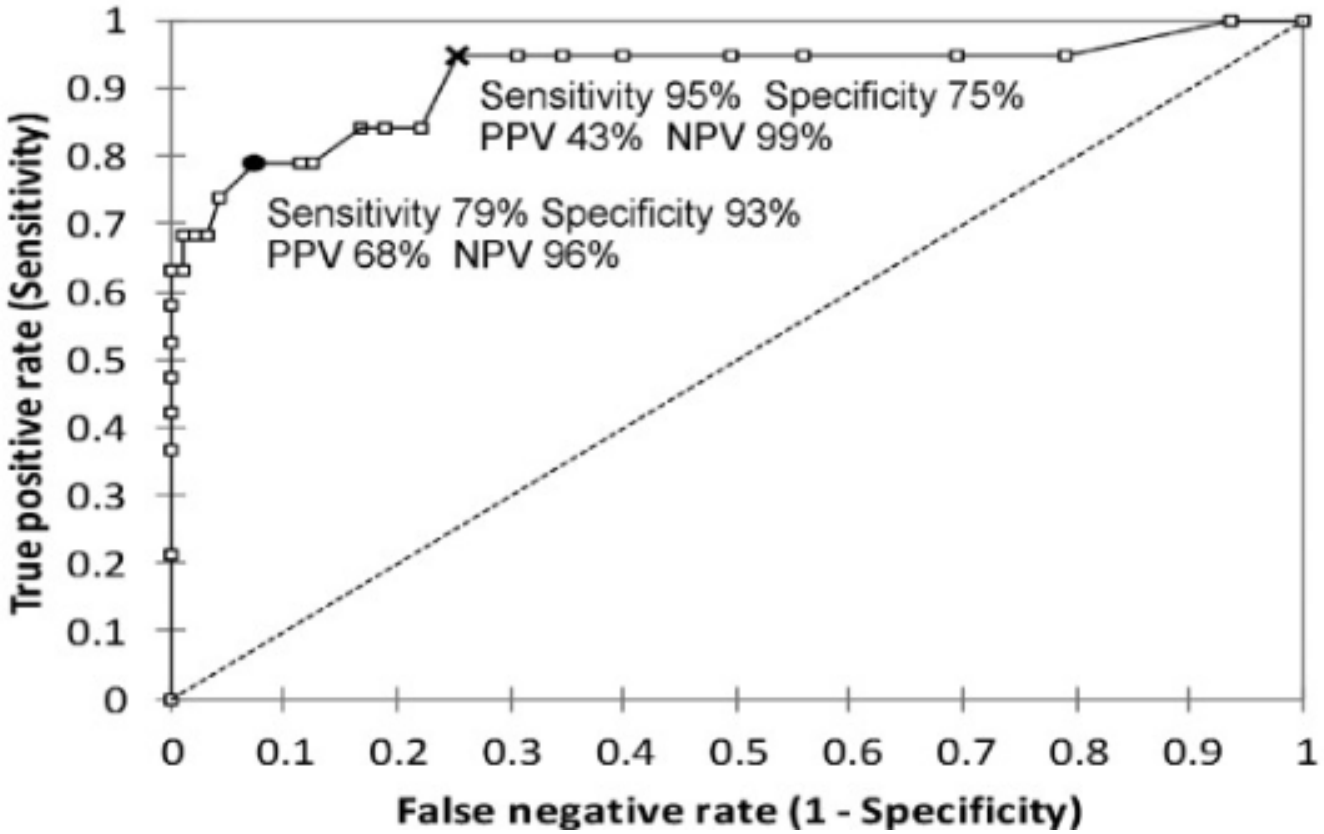
What?!?

- Now what do we do??
- Seems like it should be XIAP Deficiency!

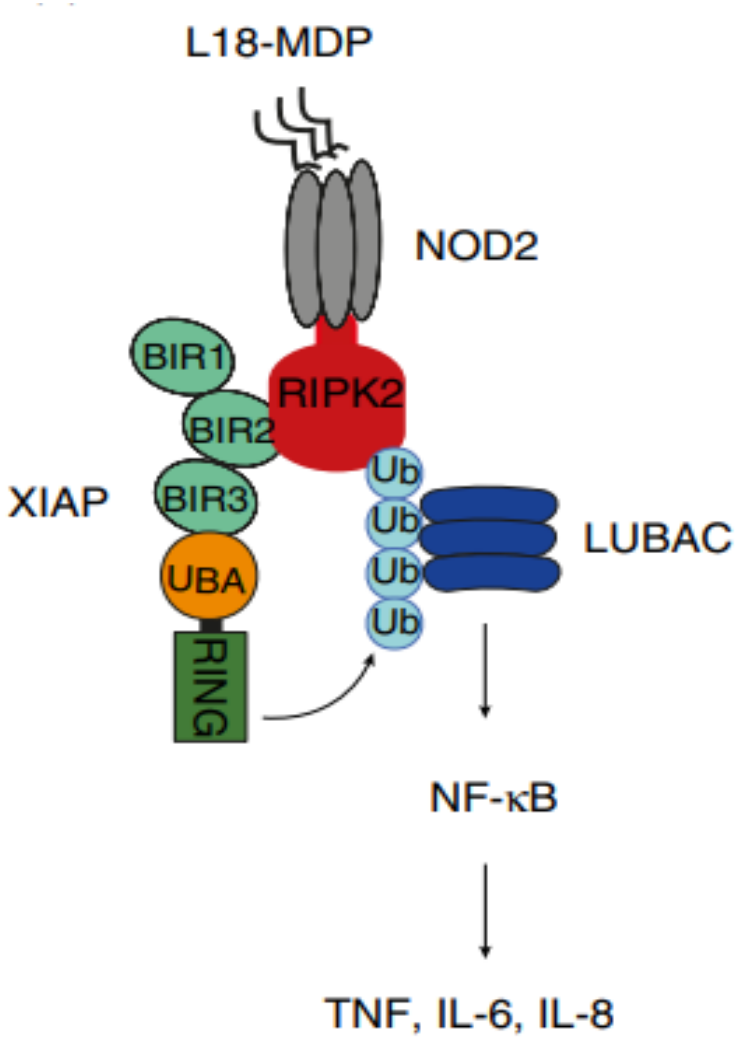
- Repeat XIAP flow cytometry was again normal.
- Postulated that the patient's XIAP gene variant results in protein expression which is stable but not functional, and that the variant is proximal to the antibody binding site (immunogen is aa. 268-426) utilized for XIAP flow analysis, thus resulting in a false negative finding.

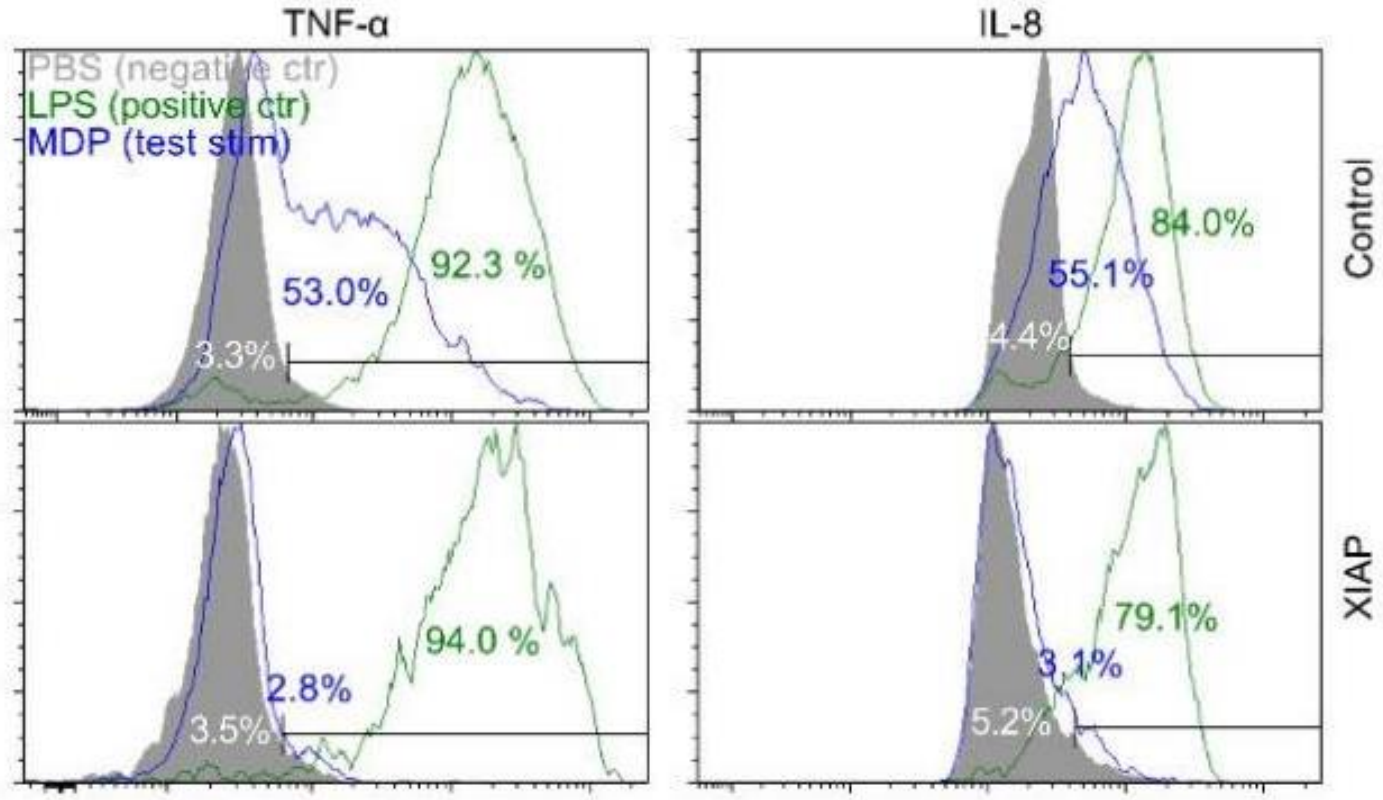
Accuracy of XIAP Protein Detection by Flow Cytometry



Gifford CE, Weingartner E, Villanueva J, Johnson J, Zhang K, Filipovich AH, Bleesing JJ, Marsh RA. Clinical flow cytometric screening of SAP and XIAP expression accurately identifies patients with SH2D1A and XIAP/BIRC4 mutations. Cytometry B Clin Cytom. 2014 Jul;86(4):263-71.

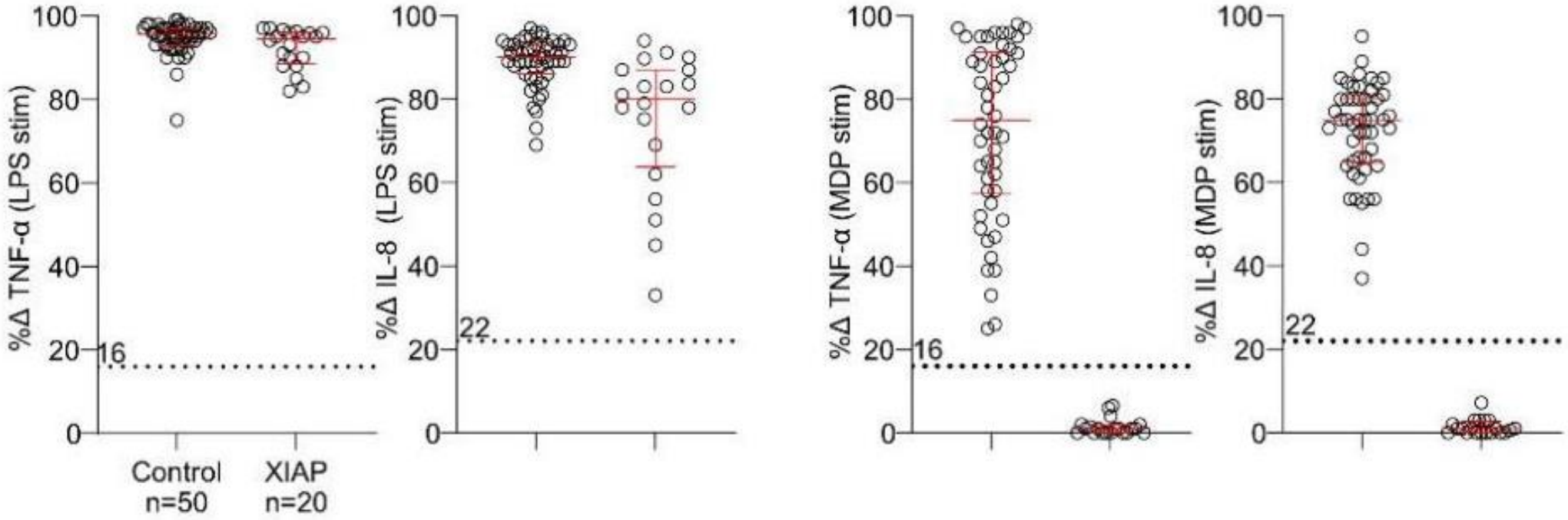
Evaluation of Function: NOD2/XIAP Signaling

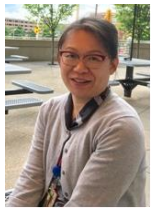




Ammann S, Elling R, Gyrd-Hansen M, Dückers G, Bredius R, Burns SO, Edgar JD, Worth A, Brandau H, Warnatz K, Zur Stadt U, Hasselblatt P, Schwarz K, Ehl S, Speckmann C. A new functional assay for the diagnosis of X-linked inhibitor of apoptosis (XIAP) deficiency. Clin Exp Immunol. 2014 Jun;176(3):394-400.

XIAP/NOD2 Functional Testing: High Sensitivity and Specificity





PNagako Stuart, PhD Sam Chiang, PhD

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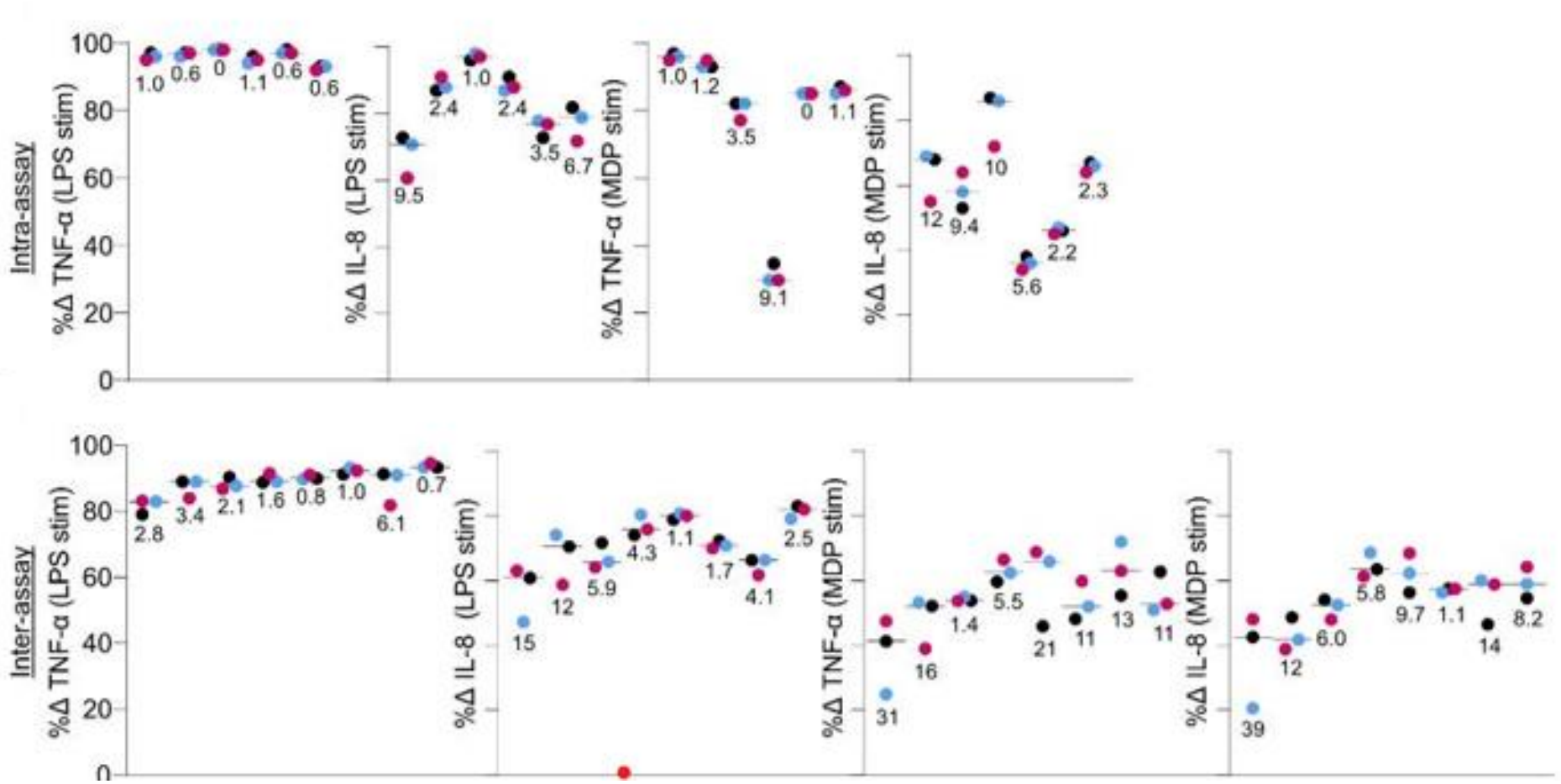
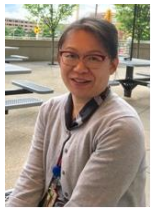
Precision Studies

Intra-Assay

Inter-Assay





Nagako Stuart, PhD Sam Chiang, PhD

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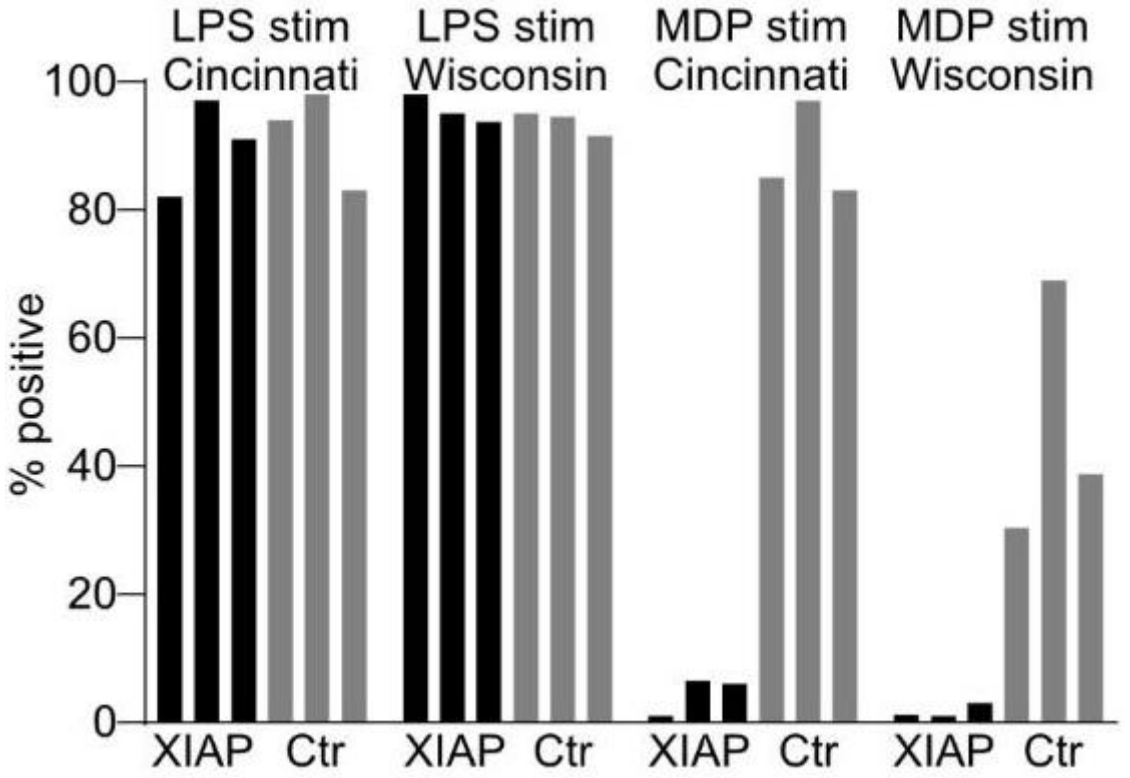
Lab-Lab Comparison: Medical College of Wisconsin



Case 4

HPI

- 14 year old male presents to pulmonology clinic with chronic cough and sputum production
- BMI < 1st percentile
  - Denies diarrhea or other GI symptoms

Family History

Patient's father died of complications of T1DM, autoimmune enteropathy and autoimmune thyroid disease

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Case 4

HPI

- 14 year old male presents to pulmonology clinic with chronic cough and sputum production
- BMI < 1st percentile
  - Denies diarrhea or other GI symptoms

Family History

Patient's father died of complications of T1DM, autoimmune enteropathy and autoimmune thyroid disease

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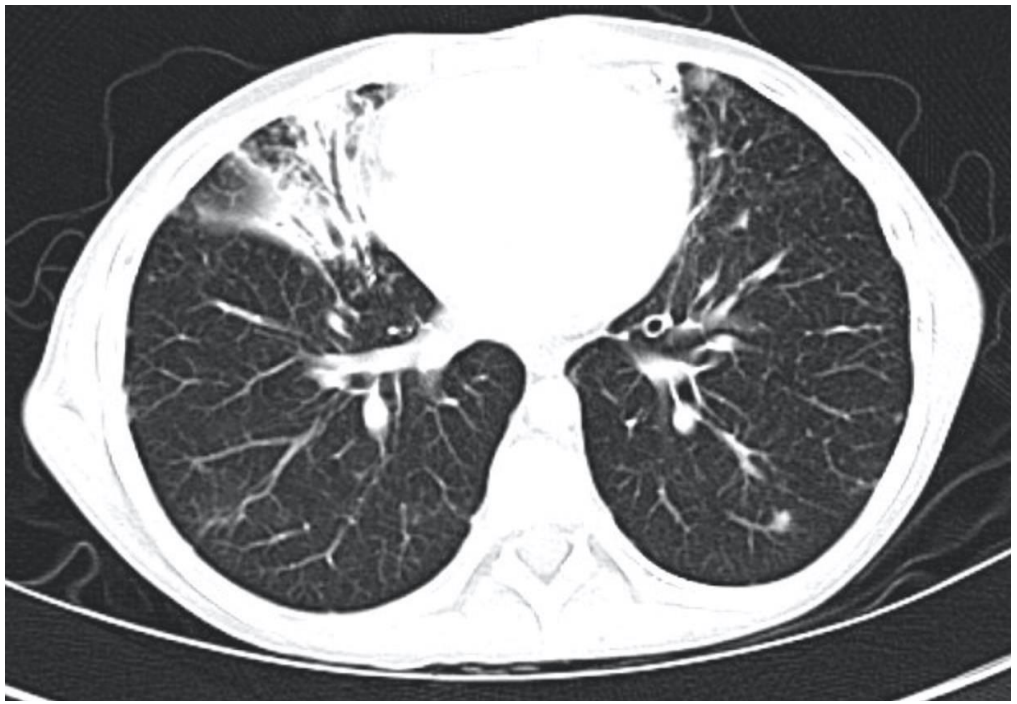
CT Scan

Bronchiectasis in the right middle lobe, lingula, left lower lobe. Scattered nodular opacities. Consolidation in right middle lobe.



Lung biopsy

Diffusely distributed non-caseating granulomas

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Laboratory Evaluation

Immunoglobulins

IgG	1,252
IgG1	965 (f)
IgG2	239 (f)
IgG3	180 (f) H
IgG4	16 (f)
IgA	<7 L
IgM	64
IgE	1

Lymphocyte subsets

CD3+, Absolute	672 L
Percent CD3+/CD4+	51
CD3+/CD4+, Absolute	515 L
Percent CD3+/CD8+	12 L
CD3+/CD8+, Absolute	122 C
Percent CD3-/CD16+ or CD56+	11
CD3-/CD16+ or CD56+, Absolute	108
Percent CD19+	21
CD19+, Absolute	209

Naïve/Memory T cell phenotyping

TEMRA CD4+CD45RA+CCR7- T cells	0.2 (f)
Naive CD4+CD45RA+CCR7+ T cells	11.1 L
Effector memory CD4+CD45RA-CCR7- T cells	18.9
Central memory CD4+CD45RA-CCR7+ T cells	69.8 H
TEMRA CD8+CD45RA+CCR7- T cells	6.3
Naive CD8+CD45RA+CCR7+ T cells	65.4
Effector memory CD8+CD45RA-CCR7- T cells	9.3 L
Central memory CD8+CD45RA-CCR7+ T cells	19.0

Naïve/Memory B cell Phenotyping

Switched memory IgD-CD27+ B cells	1.00 (f) L
Unswitched memory IgD+CD27+ B cells	4.40 L
Naive IgD+CD27- B cells	91.10 H

"CVID Panel" B cell Phenotyping

Transitional CD24hiCD38hi B cells	7.9 (f)
Plasmablasts CD24lowCD38hi	0.8 L
CD19+CD21LowCD38Low B Cells	18.3 H
Marginal zone-like CD24hiCD38low B cells	28.7

Soluble IL2R – 1228 - normal range 45-1105

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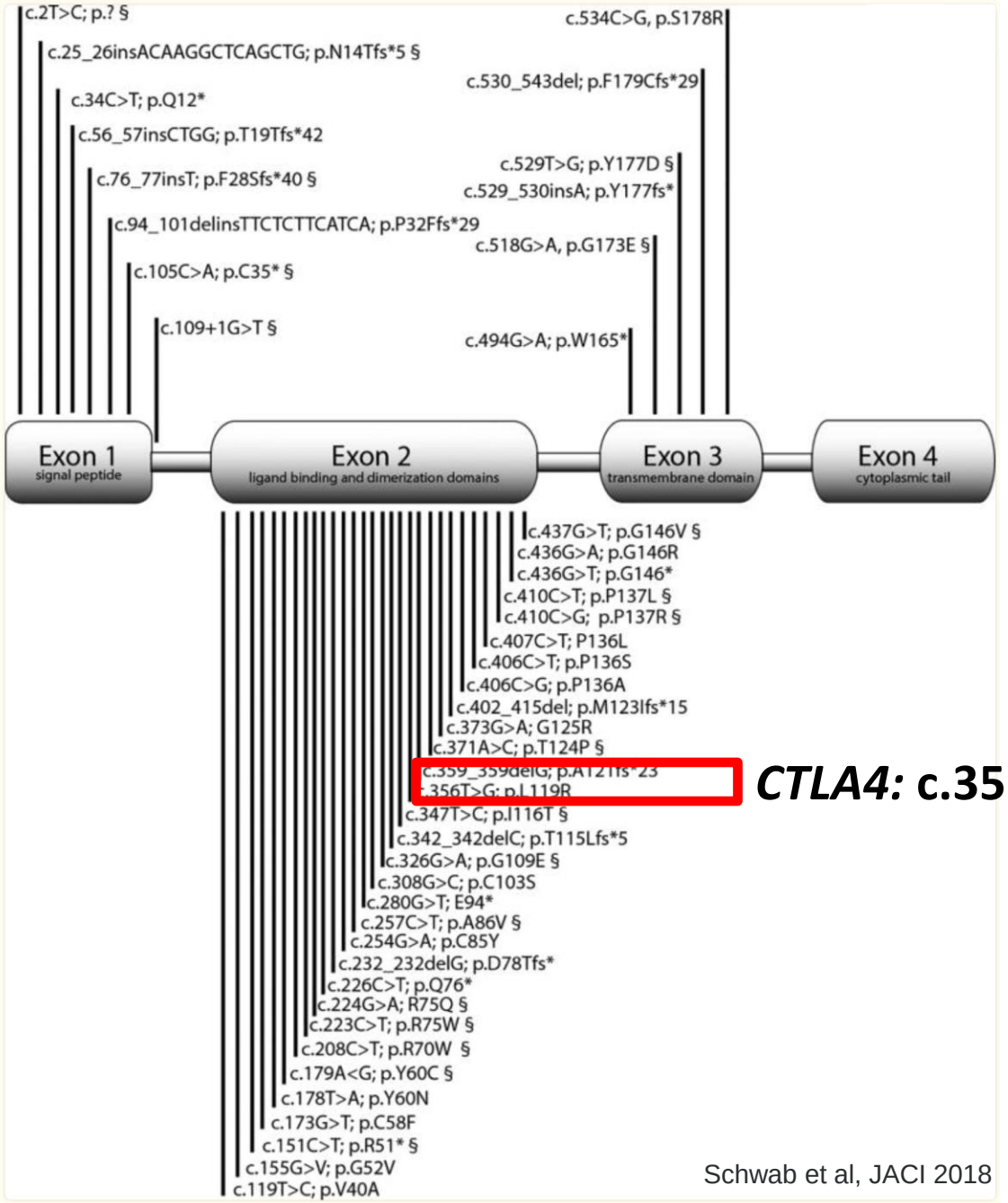
Genetic Testing

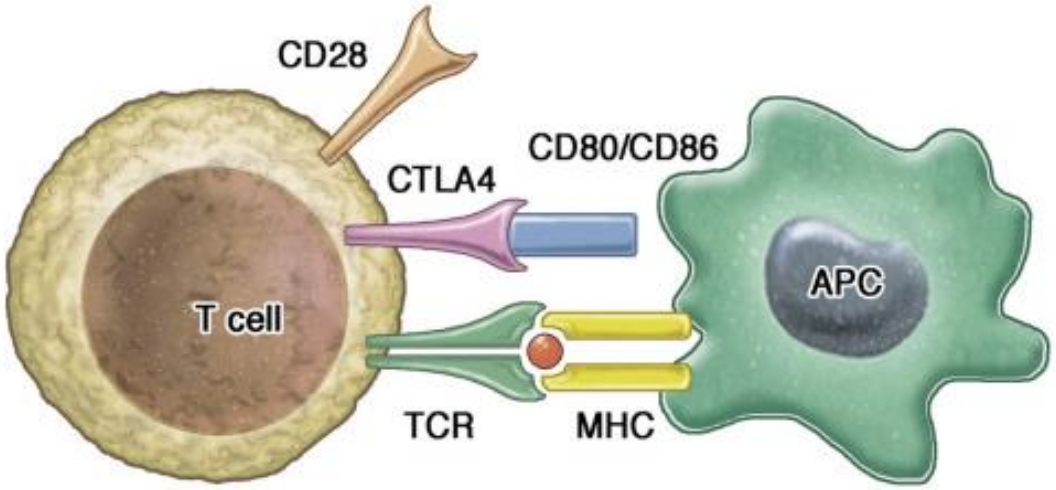


CTLA4: **c.356T>G:p.Leu119Arg**

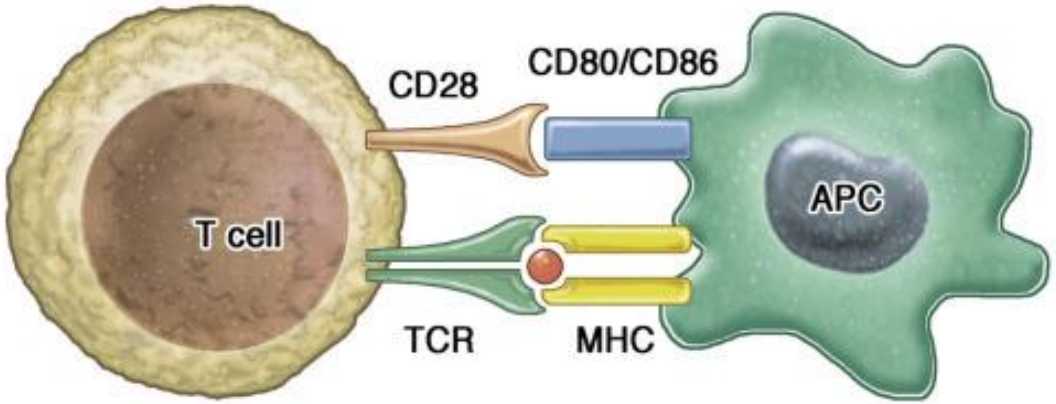
Schwab et al, JACI 2018

CTLA-4 Haploinsufficiency

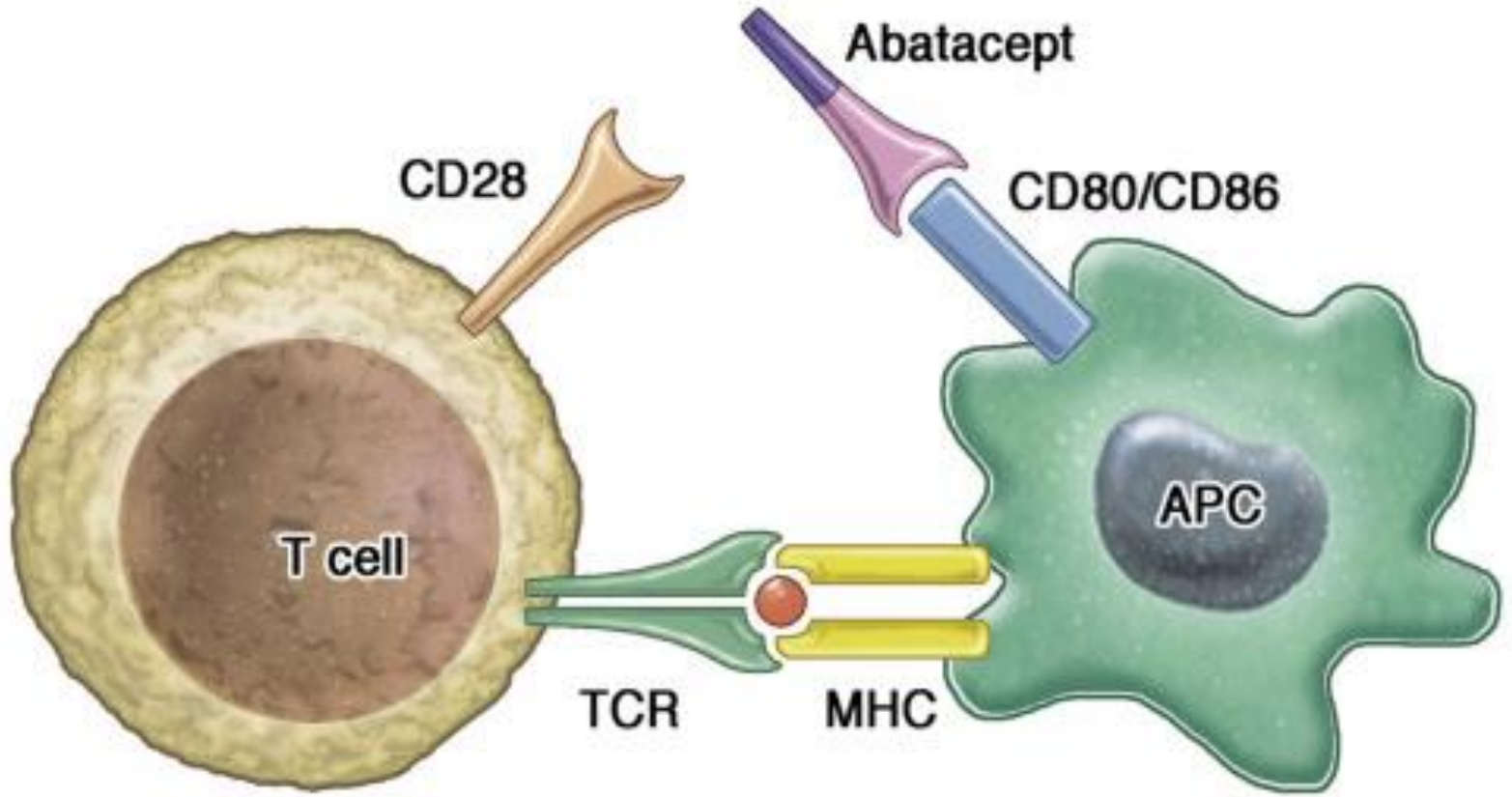
CTLA4 is a Decoy Receptor
Helps Prevent Too Much T Cell Activation



In Patients with CTLA4 Haploinsufficiency,
There is Too Much T Cell Activation



Targeted Treatment: Abatacept



Targeted Therapy with Abatacept

PFTs

	pre-treatment	6 months
FVC predicted	79% ->	91%
FEV1 predicted	69% ->	86%
FEV1/FVC predicted	87% ->	97%

Chest CT: Improvement

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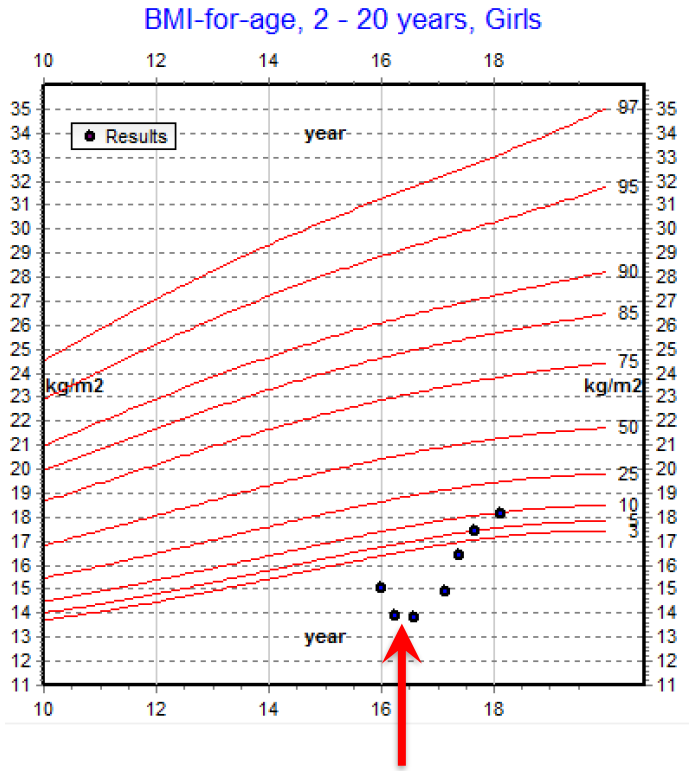
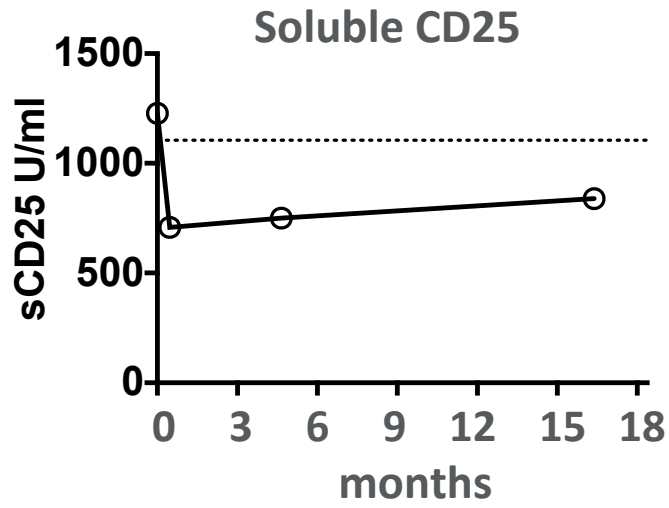
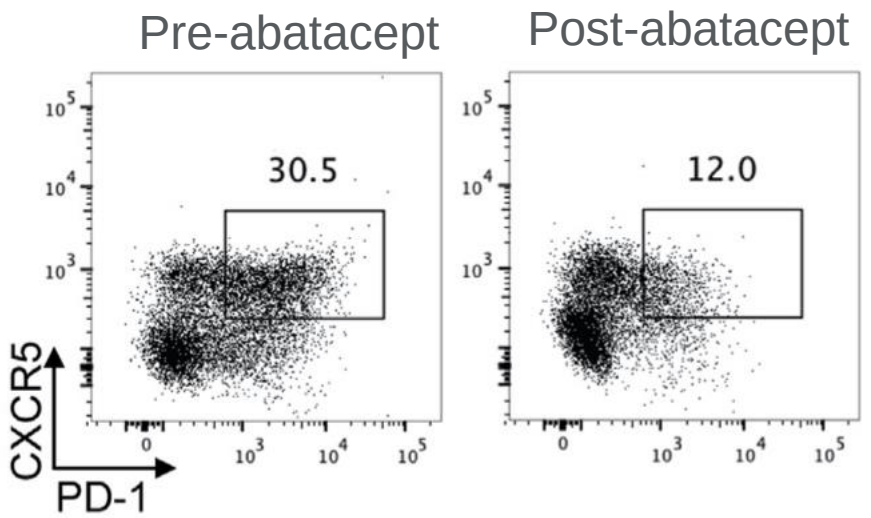


abatacept started

Found to have autoimmune enteropathy





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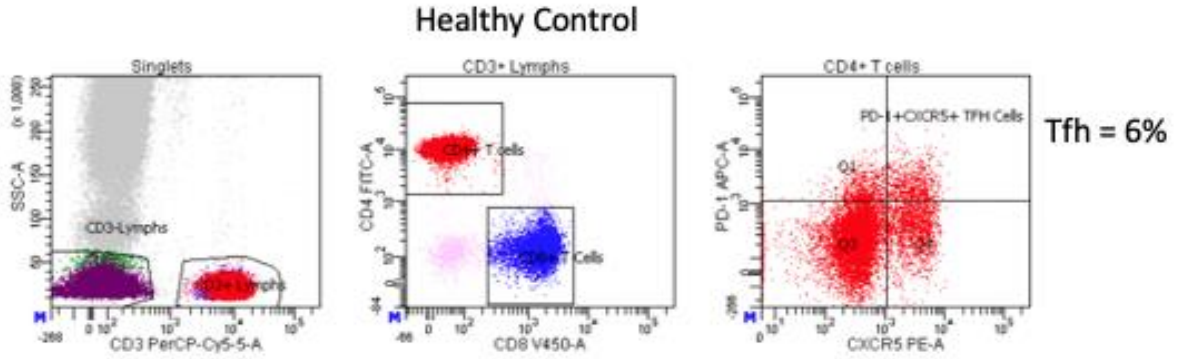
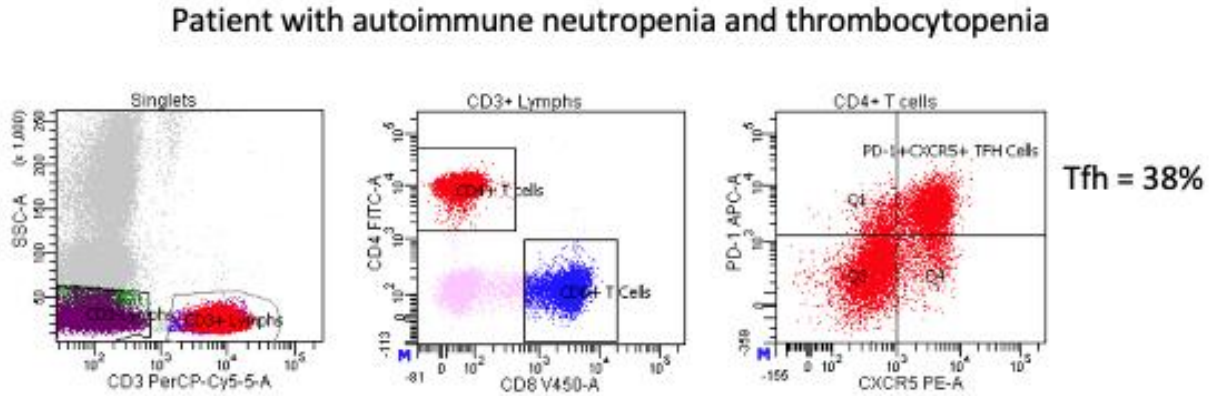
cTfh Markers

Often defined as BCL-6^{+}CXCR5^{hi}CCR7^{lo}PD-1^{+}ICOS^{+}

- **CXCR5**
  - expressed on all peripheral blood and lymph node B cells as well as on some Tfh cells.
  - Its ligand, CXCL13, is expressed constitutively on follicular HEV and **controls trafficking of cells into lymph node follicles.**
- **PD-1**
  - For antigen-experienced CD8^{+} T cells, high expression of PD-1 is associated with functional exhaustion.
  - In contrast, Tfh cells are highly functional and sensitive to antigen presented by cognate B cells.
  - **In Tfh cells, PD1 controls tissue positioning and function.**





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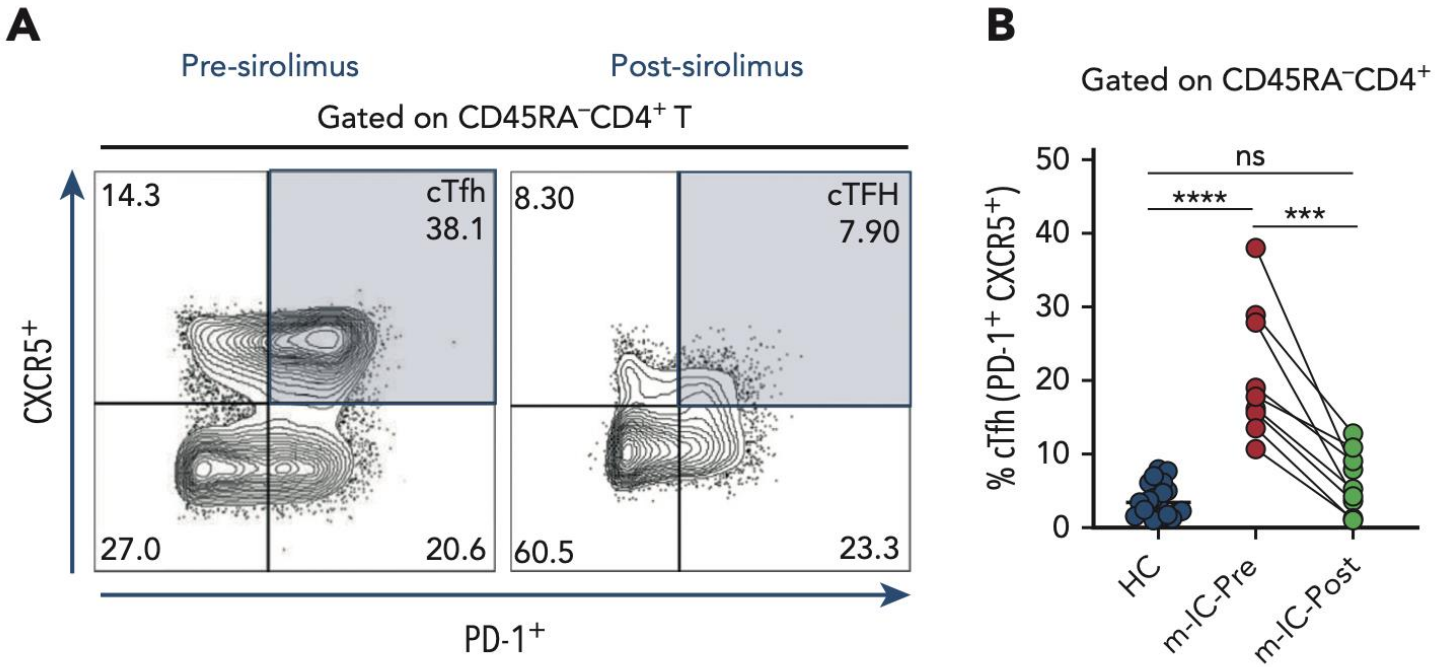
Blood 2023

IMMUNOBIOLOGY AND IMMUNOTHERAPY

mTOR inhibition attenuates cTfh cell dysregulation and chronic T-cell activation in multilineage immune cytopenias

Deepak Kumar,[1] Thinh H. Nguyen,[1] Carolyn M. Bennett,[1] Chengyu Prince,[1] Laura Lucas,[2] Sunita Park,[3] Taylor Lawrence,[2] Karin Chappelle,[2] Mariam Ishaq,[2] Edmund K. Waller,[4] Sampath Prahalad,[5] Michael Briones,[1] and Shanmuganathan Chandrakasan[1]



mTOR inhibition

- improves immune cytopenias and lymphoproliferation
- improves the dysregulated immune phenotype (cTFh in particular)

Clinical utility of cTfh enumeration becoming clear beyond characterization of patients with a small subset of monogenic disorders

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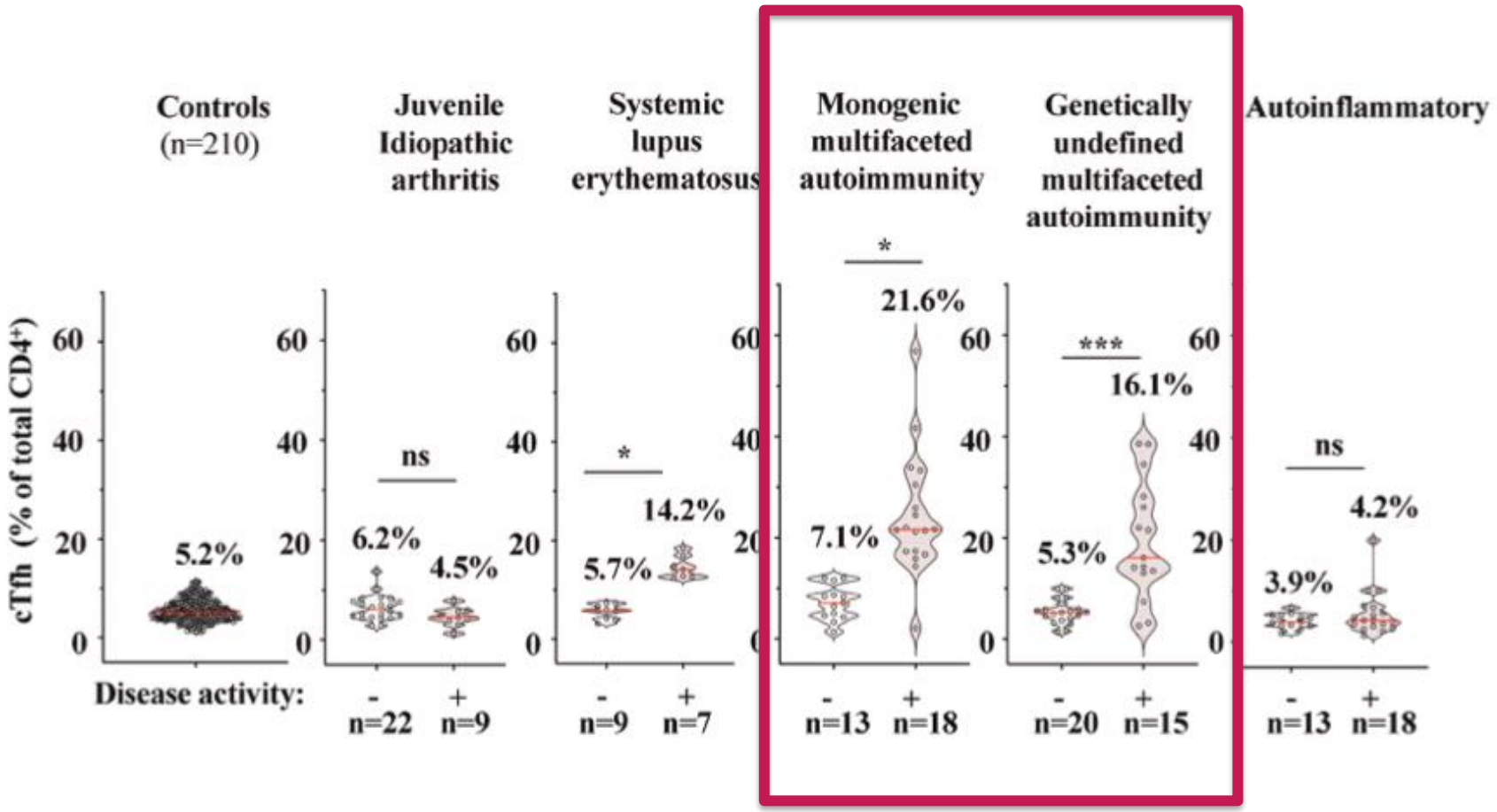
Expanded cTfh% seen in patients with 1) adaptive immune dysregulation/"Tregopathy Phenotype" *and* 2) active disease



Phenotypes

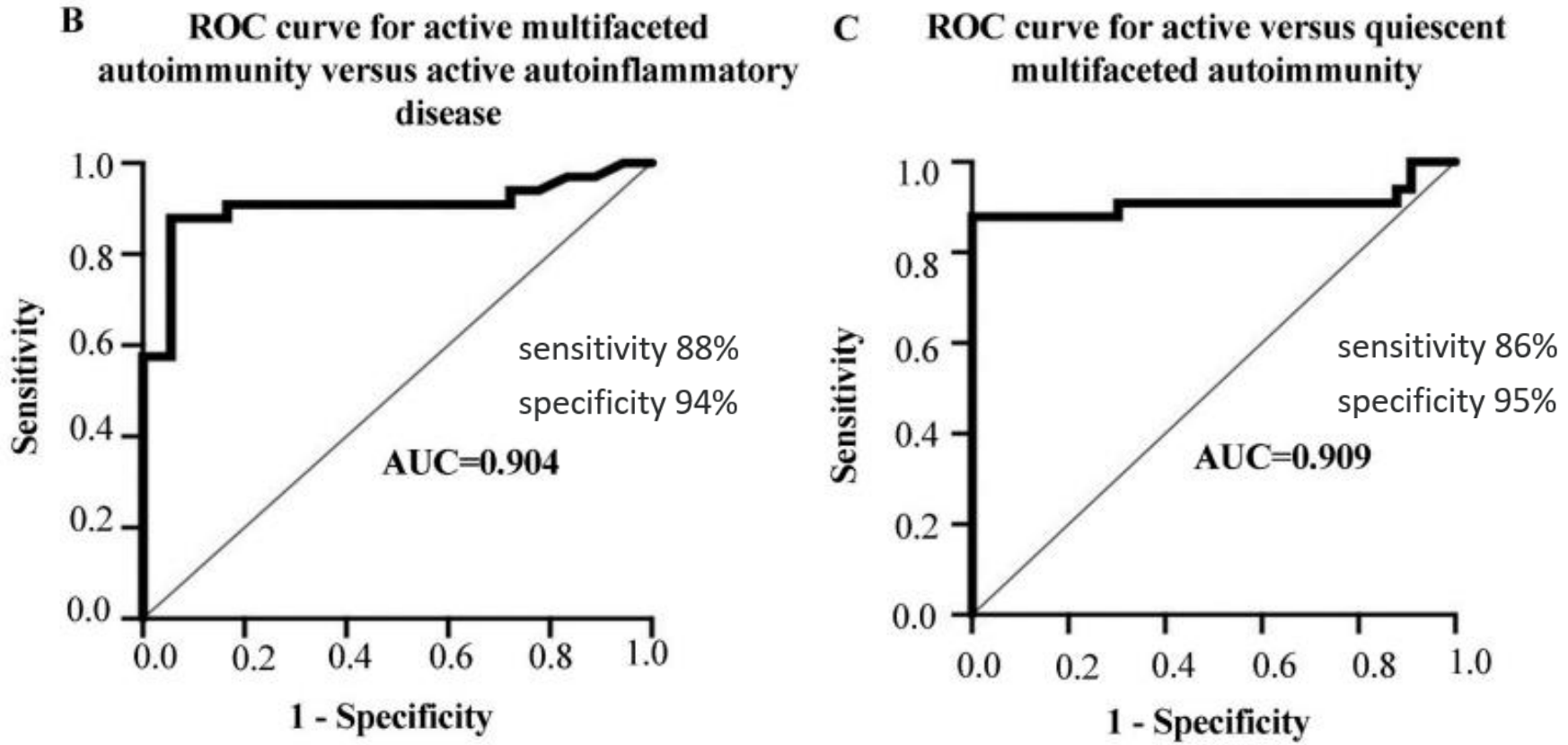
AIHA
ITP
Evans syndrome
Immune-mediated hepatitis
CNS autoimmunity
Immune-mediated GI disease
ILD

Genes

Tregopathy	**Autoinflammatory**
LRBA, 6	*RIPK1*, 1
CTLA4, 6	NLRP3, 2
FAS, 1	RNASE2HA , 1
STAT3 GOF, 2	RNASEH2B, 1
PIK3CD, 1	TNFAIP3, 1
NFKB1, 1	ADA2, 1
FOXP3, 1	STING, 1
KMT2D, 1	
SOCS1, 1	
WAS, 1	

Labere et al, Journal of Autoimmunity, 2023

Sensitivity/Specificity



Labere et al, Journal of Autoimmunity, 2023

Last Case

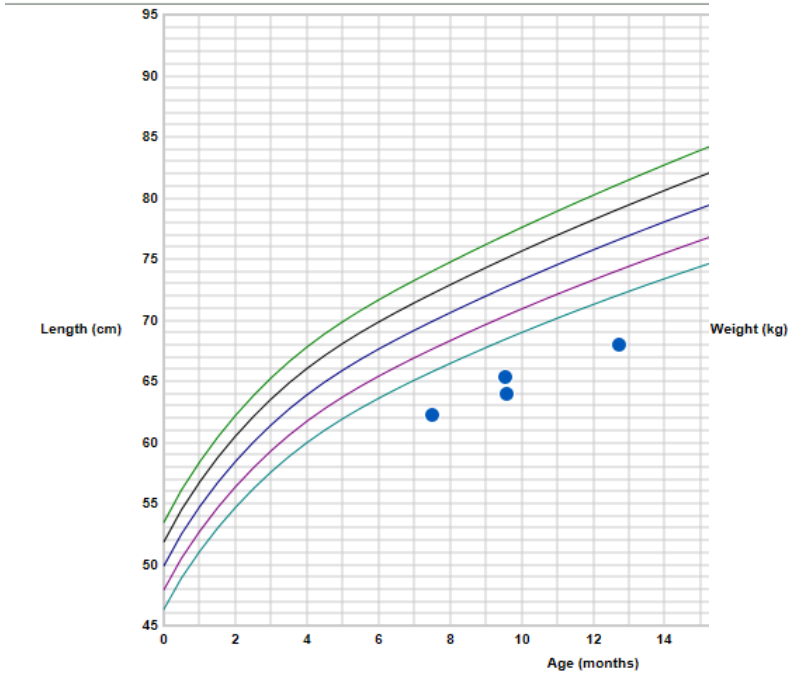
- 7-month-old, full term male
- First presented age 2 months of age with finger swelling that was concerning for trauma or osteomyelitis, and fevers
- Patient developed necrosis of toes. Rheumatology was consulted due to concern for vasculitis.
- Steroids and anakinra were started, and the toes were treated with topical nitroglycerin and then sildenafil po.
- He improved and was discharged several weeks later.

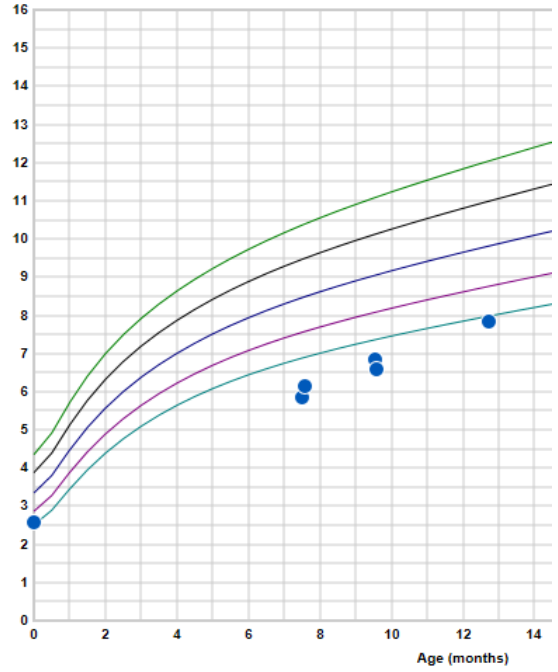
Last Case

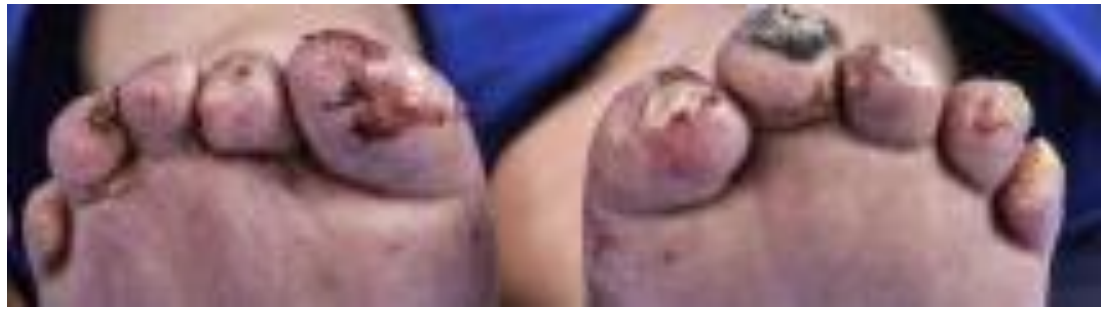
- Seen several times outpatient for blistering rash on the cheeks, feet.
- Readmitted age 3 ½ months with fevers.
- Readmitted age 5 months for fevers and pneumonia requiring oxygen.

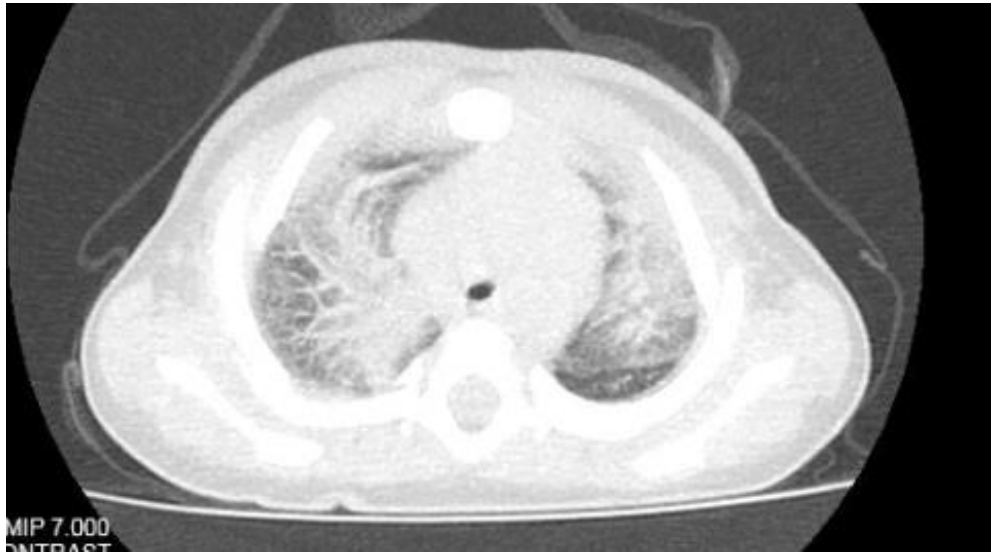
- Still with chronic fevers and autoamputation of fingers/toes

Exam









Private Information

Genetic Results

- TMEM173 Allele 1: c.439G>C(p.Val147Leu)
  Allele 2: No mutation identified
  - Previously reported in a patient with STING-associated vasculopathy with onset in infancy (SAVI)
  - STING= stimulator of type I IFN gene

Type 1 Interferonopathy RNA Signature Panel

- Raphaela Goldbach-Mansky pioneered an RNA signature panel of Type 1 Interferonopathies
- We have adapted this using the Nanostring nCounter platform as it can be multiplexed and directly quantifies the number of RNA transcripts without need for prior reverse transcription and amplification, avoiding bias

CXCL10	IFI27	IFIT3	OAS1	RTP4
DDX60	IFI44	IFIT5	OAS2	SIGLEC1
EPSTI1	IFI44L	ISG15	OAS3	SOCS1
GBP1	IFI6	LAMP3	OASL	SPATS2L
HERC5	IFIT1	LY6E	PLSCR1	USP18
HERC6	IFIT2	MX1	RSAD2	

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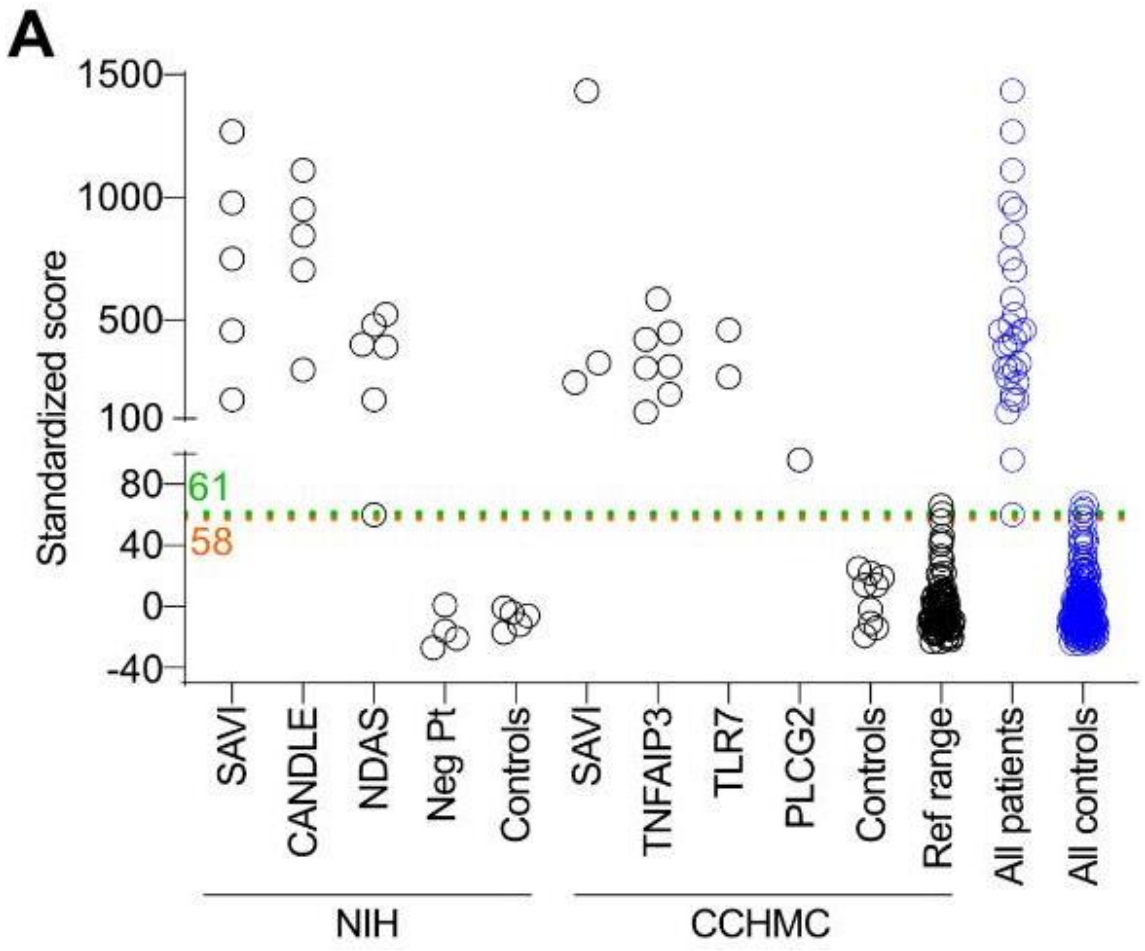
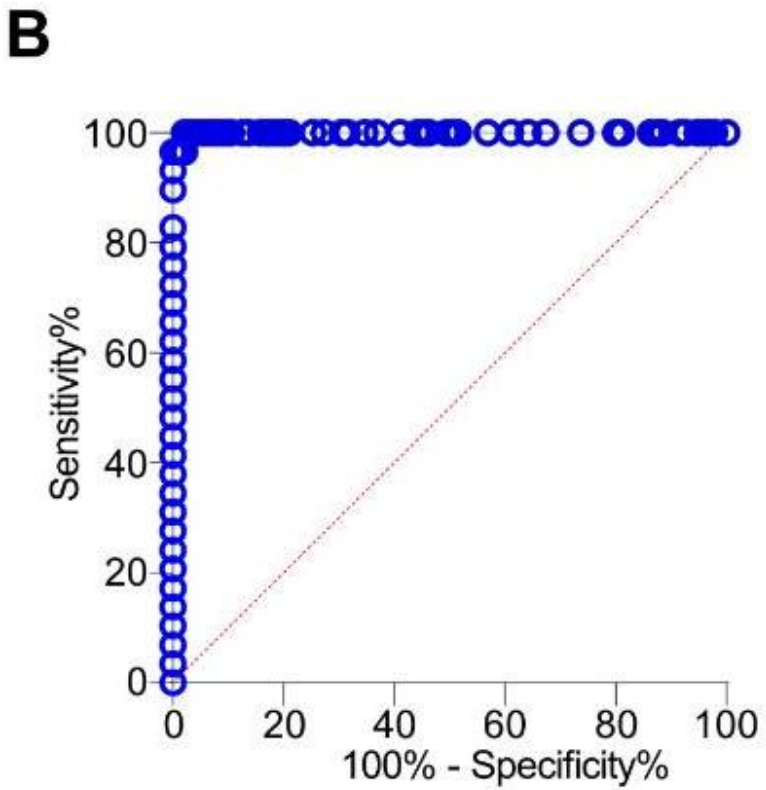
Type 1 Interferonopathy RNA Signature Panel





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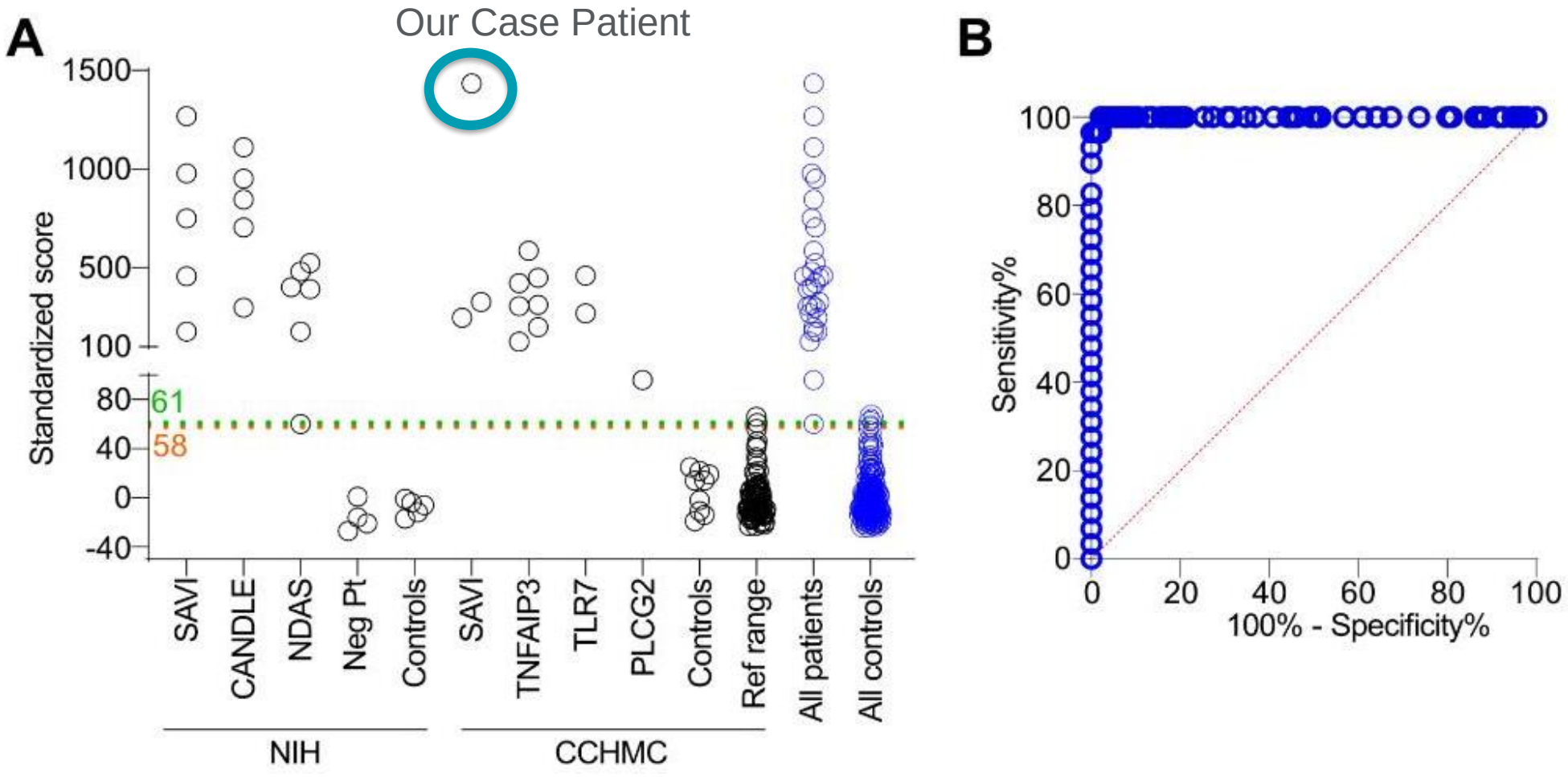
Type 1 Interferonopathy RNA Signature Panel



Private Information

Conclusion

- There are variety of tried, true, and new immunology lab diagnostics that can be used to improve the diagnosis, monitoring, and care of patients with inborn errors of immunity

Thank you

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