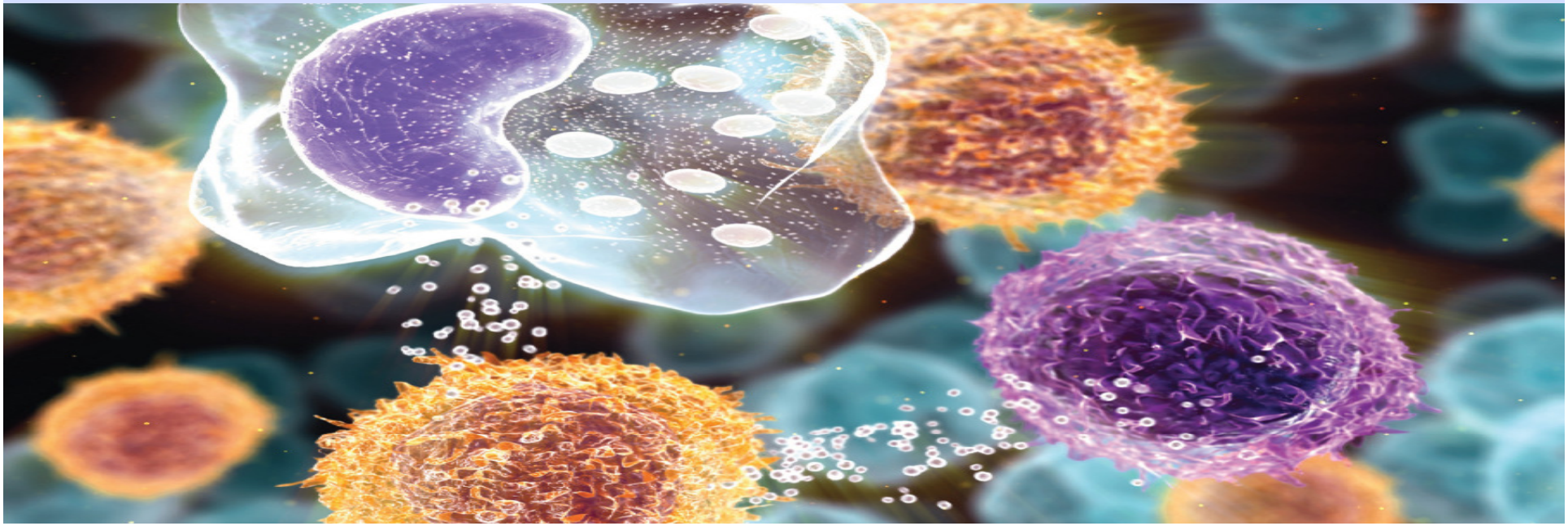
OMICS Group Conferences
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4th International Conference and Exhibition on
Immunology
September 28-30, 2015 Crowne Plaza Houston River Oaks, Houston, TX, USA

Innovative Diagnostic Approach in Primary Immunodeficiency Disorders



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Innovative Diagnostic Approach in Primary Immunodeficiency Disorders

Michelle Tseng
mtseng@oandoalpan.com
Amerimmune Immunology Laboratory
Fairfax, Virginia, United States

Impacts of Delayed Diagnosis

Contracted other infections with potentials to developing long-term diseases

John



Toni



Brooklyn Ethan



Immunodeficiency Canada; retrieved from http://immunodeficiency.ca/support/patient-support-stories/

Presentation Outline

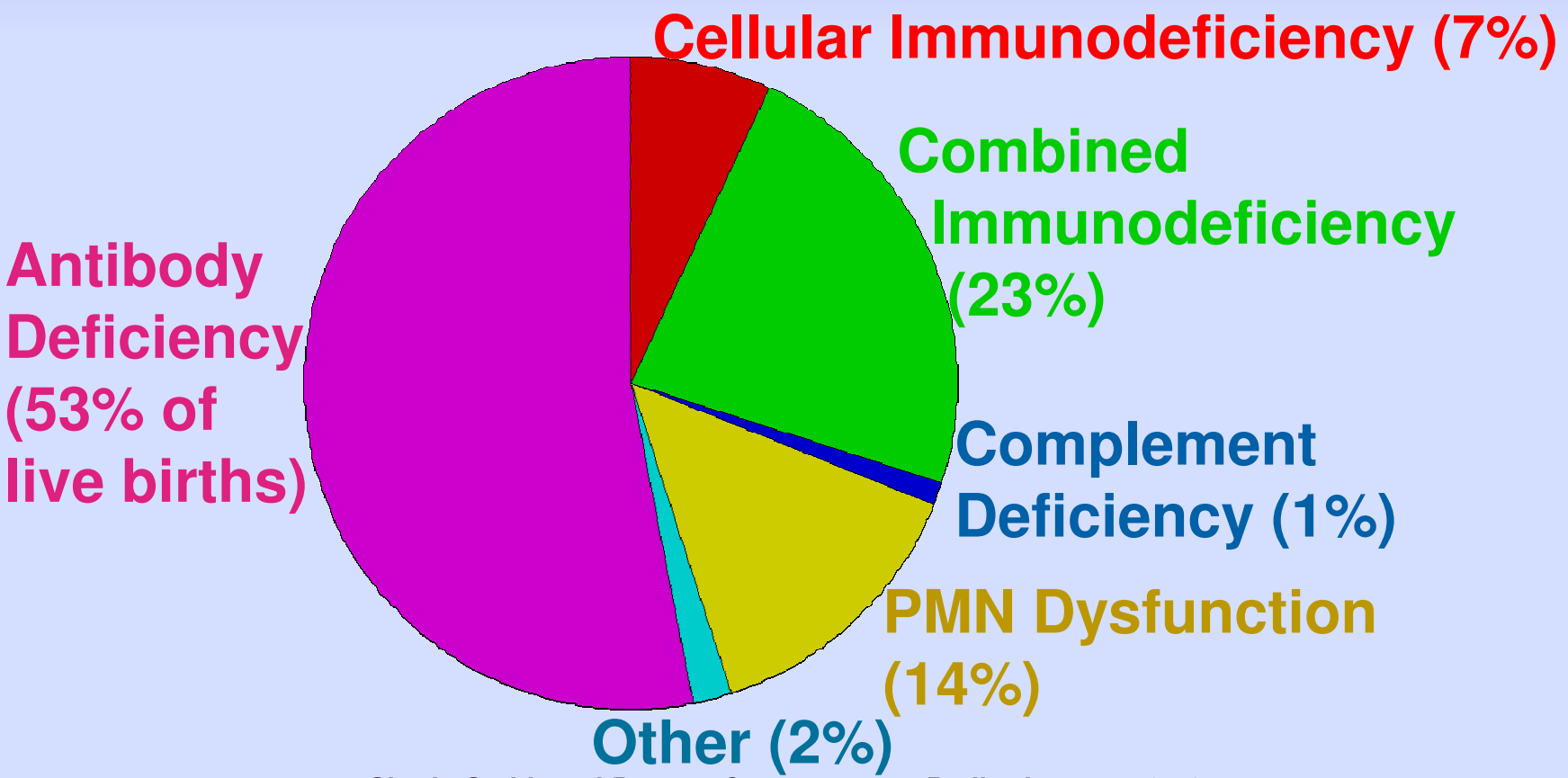
- Overview of Primary immunodeficiency disorders (PIDs), scope of immune workup, and diagnosis method
- Discuss challenges in the current methodology used in PIDs diagnosis
- Introduce the Amerimmune Curbside Consultation approach
- Summary

Brief Overview of PIDs

- Definition of Primary immunodeficiency disorders (PIDs):
  - group of over 150 chronic immune disorders
  - caused by hereditary or genetic defects
  - not contagious; characterized by infections
  - susceptible to opportunistic infections

- Prevalence of PIDs:
  - diagnose at any age
  - affect ~ 1 in 1,200 persons in U.S.

Relative Distribution of PIDs: Categorized by Defect Type



Skoda-Smith and Barrett, Contemporary Pediatrics 17:156-165

- sIgA deficiency ranges from 1:300 to 1:100,000
- 80% of affected persons < 20 years of age
- 70% males (5:1 males in children; 1:1 in adults)

Scope of Immune Workup in PIDs Diagnosis

- 10 warning signs of PIDs (clues)
- Family medical history
  - vaccination record, infections, auto-immune disorders… etc.
- Basic and advanced laboratory tests
  - lymphocyte lineage enumeration by flow cytometry
  - biochemical tests for soluble molecule
  - cellular functional tests
  - genetic tests

Scope of Immune Workup:
10 Warning Signs of PIDs

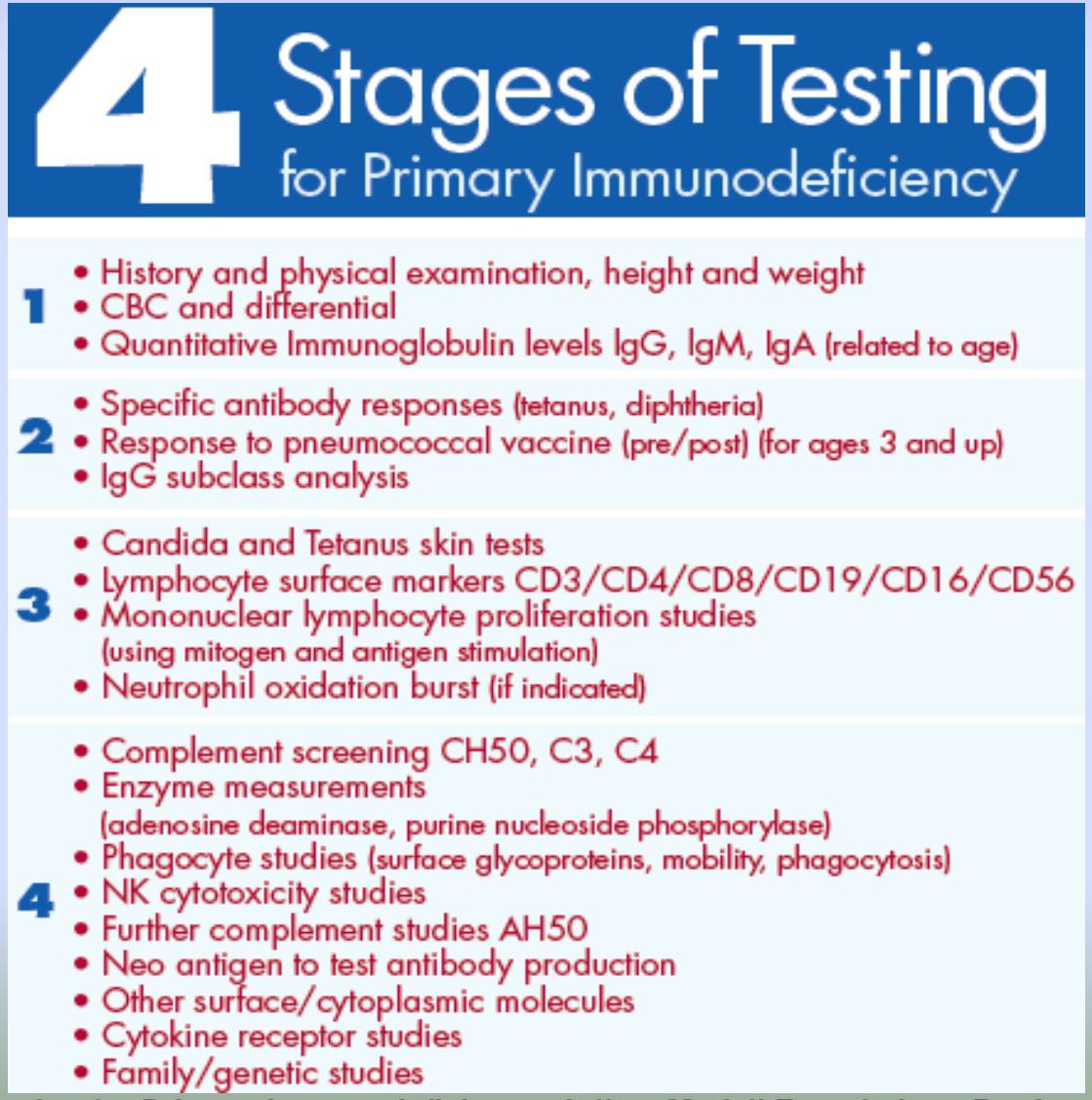


10 Warning Signs of Primary Immunodeficiency. Jeffrey Modell Foundation. Retrieved from info4pi.org.

Scope of Immune Workup in PIDs Diagnosis

- 10 warning signs of PIDs (clues)
- Family medical history
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- Basic and advanced laboratory tests
  - lymphocyte lineage enumeration by flow cytometry
  - biochemical tests for soluble molecule
  - cellular functional tests
  - genetic tests

Traditional Step-wise Stages of Immune Workup Approach



4 Stages of Testing for Primary Immunodeficiency. Jeffrey Modell Foundation. Retrieved from info4pi.org.

Challenges in Diagnosis of PIDs

- One major challenge contributed by physicians
  - lack of understanding in immune disorders
  - inadequate components of immune deficiency evaluations
  - poor interpretation of test result

- Drawbacks in utilizing the step-wise method
  - insensitive
  - inefficient

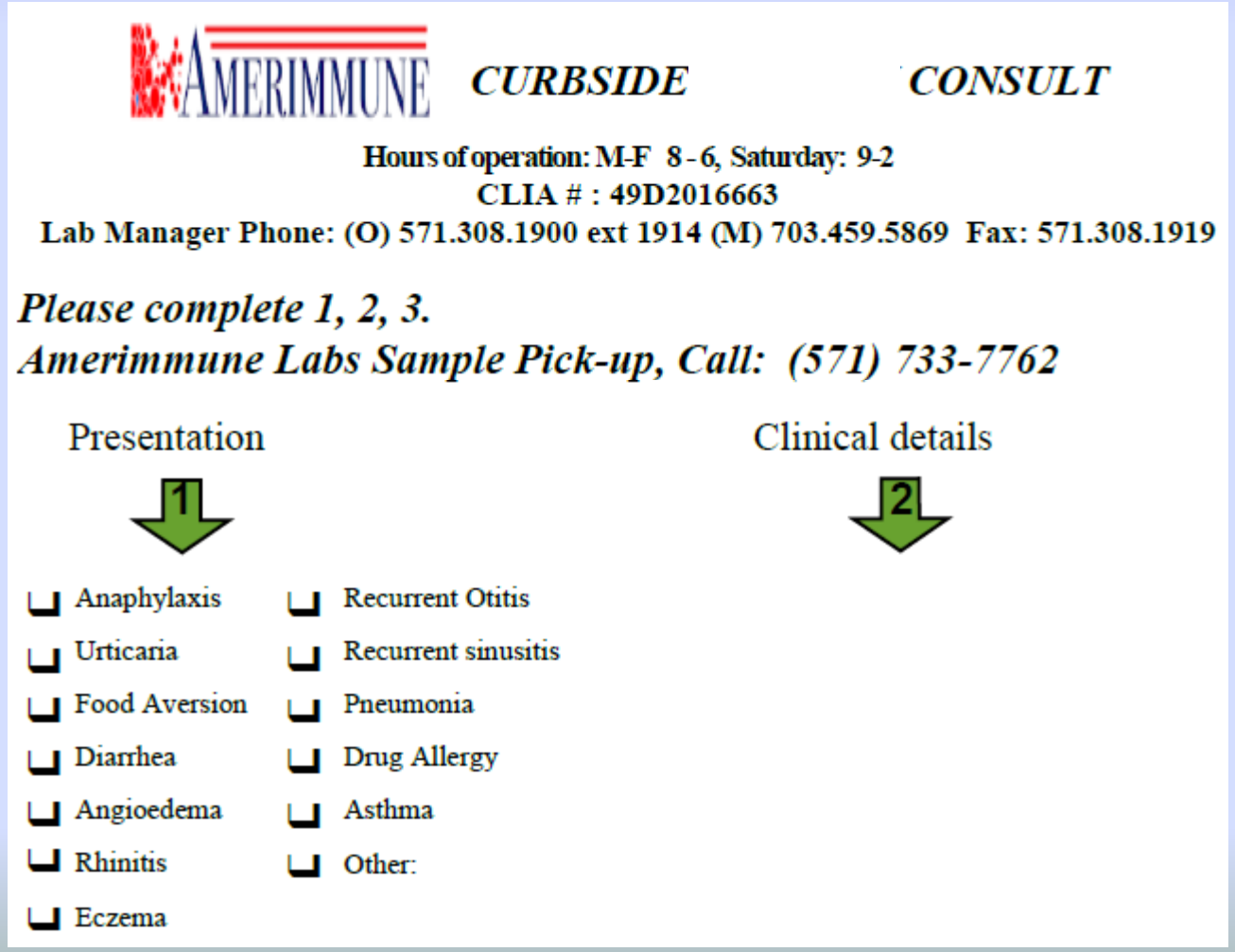
  ] Sequential immune evaluation

A Solution: Amerimmune Curbside Consultation

- Pre-set immune workup diagnostic tool
  - multi-dimensional method composed of necessary, effective immune evaluations

- Advantages
  - physical referrals are not necessary
  - cost-effective
  - not much affected by shortages of lab facilities or immunologists
  - blend in nicely with the newly emerging specialties and health systems

A Solution: Amerimmune Curbside Consultation = Complete Evaluation



AMERIMMUNE *CURBSIDE CONSULT*

Hours of operation: M-F 8 - 6, Saturday: 9-2
CLIA # : 49D2016663
Lab Manager Phone: (O) 571.308.1900 ext 1914 (M) 703.459.5869 Fax: 571.308.1919

Please complete 1, 2, 3.
Amerimmune Labs Sample Pick-up, Call: (571) 733-7762

Presentation 1

- ☐ Anaphylaxis
- ☐ Urticaria
- ☐ Food Aversion
- ☐ Diarrhea
- ☐ Angioedema
- ☐ Rhinitis
- ☐ Eczema

Clinical details 2

- ☐ Recurrent Otitis
- ☐ Recurrent sinusitis
- ☐ Pneumonia
- ☐ Drug Allergy
- ☐ Asthma
- ☐ Other:

http://www.curbsideconsultation.com/

Curbside Consultation Approach: Immune Profiling

Amerimmune Curbside immune work-up approach:

Immune Compartment	Tests (immune cells by numbers)	Tests (Functions)
Cellular	1. CBC with differential 2. T-cell (CD3), 3. NK-cell (CD56/16), 4. αβTCR, γδ TCR, 5. CD4RO, CD8RO	***Non-specific*:** Mitogen proliferation & DHR CD25 & HLA-DR on T cells,Th17 ***Specific*:** Antigen proliferation or DTH to candida
Humoral	1. B cell (CD20/19), 2. $CD27^{+}IgG^{+}$ B cells, 3. $CD27^{+}IgM^{+}$ B cells, 4. CD21dim cells, 5. IgG+ B cells	***Specific:*** Antibody titers to tetanus, pneumococcal 14 serotype and HiB ***Non-specific:*** IgG, IgA, IgM, IgE & IgG subclasses

Amerimmune Curbside: Pilot Study Method – Comparison

- Surveyed 328 primary care providers from January, 2011 to September, 2012 in northern Virginia, U.S.
- Identified PIDs patients diagnosed in their practices
- Offered 10 warning signs & performed Curbside Consultation
  - provide patient's clinical history, pertinent immunological tests as indicated
- Laboratory results interpretation done by immunologists

Curbside Study Result: (Pre-) Cases Based on 10 Warning Signs

Distribution of percentage of patients within each specialty that had immune work up based on 10 warning signs of PIDs:

10 WARNING SIGNS OF PIDs	ENT	Pulmonary	Pediatric Cardiolog	Primary Care	Infectious Disease	Pediatric Gastroenterolog
1) >4 otitis	58	14	10	20	10	10
2) >2 serious sinus infections	68	33	24	60	59	23
3) >2 pneumonia	16	60	22	49	60	30
4) Recurrent abscess	5	18	9	19	23	4
5) Persistent thrush	10	10	11	22	20	26
6) Sepsis and deep seated infection	4	25	8	30	44	18
7) >2 months on antibiotics	17	39	20	60	79	10
8) Need for iv antibiotics	22	68	18	58	80	13
9) Failure to thrive	0	0	25	0	0	78
10) Family history of PID	17	25	30	34	21	36

Total of 9,265 patients

Curbside Study Result: (Pre-) *Low* Diagnose Sensitivity

Contribution of immune test groups to the diagnosis of PIDs:

	ENT	Pulmonary	Pediatric Cardiology	Primary Care	Infectious Disease	Pediatric Gastroenterology
Abnormal CBC or antibody titers (%)	**22**	**19**	**15**	**30**	**25**	**18**
% Distribution:						
- Low IgA	35	20	30	20	11	29
- Low IgM	10	15	5	22	22	4
- Low IgG	20	35	10	34	40	19
- Elevated IgE	38	46	8	15	29	19
- Neutropenia	20	30	10	5	15	4
- Lymphopenia	10	4	10	7	2	14
- Eosinophilia	45	36	35	21	21	35
- Monocytopenia	3	2	3	4	3	5

Curbside Study Result: (pre-) *Some* Diagnose Sensitivity

Contribution of immune test groups to the diagnosis of PIDs:

	ENT	Pulmonary	Pediatric Cardiology	Primary Care	Infectious Disease	Pediatric Gastroenterology
Abnormal humoral response (%)	**39**	**32**	**25**	**35**	**38**	**22**
% Distribution:						
- No response to PSV, Hib or tetanus	37	48	27	38	69	44
- Loss of response to PSV or Hib	63	52	73	62	31	66

Curbside Study Result: (pre-) *Varied* Diagnose Sensitivity

Contribution of immune test groups to the diagnosis of PIDs:

	ENT	Pulmonary	Pediatric Cardiology	Primary Care	Infectious Disease	Pediatric Gastroenterology
Abnormal humoral numbers (%)	**29**	**36**	**9**	**30**	**24**	**25**
% Distribution:						
- Low IgM^+CD27^+ B cell	42	32	30	20	40	44
- Low IgG^+CD27^+ B cell	25	41	55	67	39	51
- Increased IgM dim cell	5	3	1	1	0	0
- Low IgG^+ B cell	5	1	1	1	3	1
- Absent IgA^+ B cells	2	0	1	2	5	0
- Increased CD21dim	21	23	12	9	13	4
- B cell lymphopenia	23	22	14	23	35	12

Curbside Study Result: (Pre-) *Better* Diagnose Sensitivity

Contribution of immune test groups to the diagnosis of PIDs (%):

	ENT	Pulmonary	Pediatric Cardiology	Primary Care	Infectious Disease	Pediatric Gastroenterology
Abnormal cellular response (%)	**10**	**13**	**51**	**5**	**13**	**35**
% Distribution:						
- Increase in αβ T cells	1	5	2	0	10	0
- Increase in γδ T cells	5	8	12	8	11	12
- CD8 lymphopenia	0	1	0	1	3	0
- CD4 lymphopenia	2	0	5	0	5	3
- CD8 lymphocytosis	5	5	5	3	3	0
- CD4 lymphocytosis	10	5	20	8	10	10
- Increased CD25	2	3	10	3	15	13
- Low Th17	20	15	5	14	10	8
- Increase in HLA-DR	60	66	53	71	48	66
- Low NKT cells	20	13	33	8	9	11
- Low NK cells	18	19	12	11	21	9
- Low CD4 memory cel	33	33	3	11	22	12
- Low CD8 memory cel	14	22	4	4	14	7

Curbside Study Result: (post-) *Improved* Diagnosis Sensitivity

Prevalence of PIDs before and after Curbside Consultation:

	ENT	Pulmonary	Pediatric Cardiology	Primary Care	Infectious Disease	Pediatric Gastroenterology	Pediatrics
	Total Number						
Participating Physicians	65	41	16	77	29	18	82
Patients in practice	250,333	135,333	60,344	350,455	70,455	10,644	288,000
PIDs in 10,000 before Curbside consultation	**4**	**4**	**2**	**3**	**21**	**2**	**2.4**
Patients who had immune workup based on 10 warning signs	1937	1469	831	1503	795	275	2455
PIDs identified	723 (37%)	696 (47%)	255 (31%)	588 (39%)	379 (48%)	157 (57%)	1091 (44%)
PIDs in 10,000 after Curbside consultation	**29**	**51**	**42**	**17**	**53**	**147**	**38**
P value (pre vs post curb prevalence)	<0.0001	<0.00001	<0.0001	<0.0001	<0.00001	<0.00001	<0.00001

Curbside Study Result: (post-) Type of PIDs Diagnosed

Distribution PIDs type diagnosed (%):

	ENT	Pulmonary	Pediatric Cardiology	Primary Care	Infectious Disease	Pediatric Gastroenterology
Predominantly Humoral Immune Deficiency	**58**	**41**	**14**	**35**	**28**	**22**
Cellular Immune Deficiency	4	6	2	3	10	12
Combined immune deficiency	2	3	1	5	9	2
Combined immune deficiency with associated or syndromic	11	7	21	6	12	12
Innate immune system defects	4	15	2	9	14	11
Phagocyte function and or number defect	1	3	0	2	11	10
Auto-inflammatory disorders	3	5	12	19	7	15
Autoimmune disorders	12	11	35	17	7	8
Un-classified immune defect	5	9	13	4	1	8

Curbside Study Overall Result: *Significant* Improved Diagnosis

- 9,265 total patients involved in over 2-year in northern VA
- Increased PIDs prevalence from 5.3 to 33 per 100,000 ($p<0.001$) before and after consultation
- Revealed higher prevalence & incidence of PIDs
- Observed significant change in case numbers of PIDs diagnose in practices include ENT, pulmonary, and pediatric gastroenterology

Summary

- Challenges in the step-wise immune workup method
- Our data showed the need for complete assessment
- Pre-set Curbside Consultation diagnostic tool significantly impacts:
  - narrows gap in identifying PIDs patients
  - provides efficient and cost-effective solution
  - improves diagnose accuracy, and shortens delays
  - solves the problems of inadequate regulated, lab facilities and shortage of immunologists
  - meets the needs of other medical specialties, and advances patient-care in this field

Acknowledgment

Amerimmune Lab:
Matthew Plassmeyer
Gerald Marti
Raavi Gupta
Stacie Anderson
Mark Ryherd
Ishmael Mourning
Soren Sonder
Yuliya Kleschenko
Connor Alexander
Ines Eugenio-Fernandez
Alice Agyeman

Immunology Clinic:
Oral Alpan
Laura Noonan
Denise Loizou
Amer Khojah

Thank You !!



Amerimmune Lab Services

Services	Tests		Diseases or Therapeutics
Diagnostics	**1st Tier:** 1. Lymph subset 2. Lymph monitor 3. B cell Maturation 4. Eosinophil 5. Memory T subsets 6. DCs 7. IPF	**2nd Tier:** Functional assays	Primary immunodeficiency disorders (PIDs), Asthma, Rheumatoid, IBD
Pre-clinical & clinical trials	Flow Cytometry, ELISA		All therapeutics
Clinical Research	Flow Cytometry, ELISA, Gene sequencing… etc.		PIDs, Asthma, Rheumatoid, IBD

Amerimmune Immunology Lab at
http://www.amerimmune.com/
Amerimmune Curbside Consultation at
http://www.curbsideconsultation.com/
Clinical Diagnostics & Clinical Trials
11212 Waples Mill Road, Suite 100,
Fairfax, VA 22031

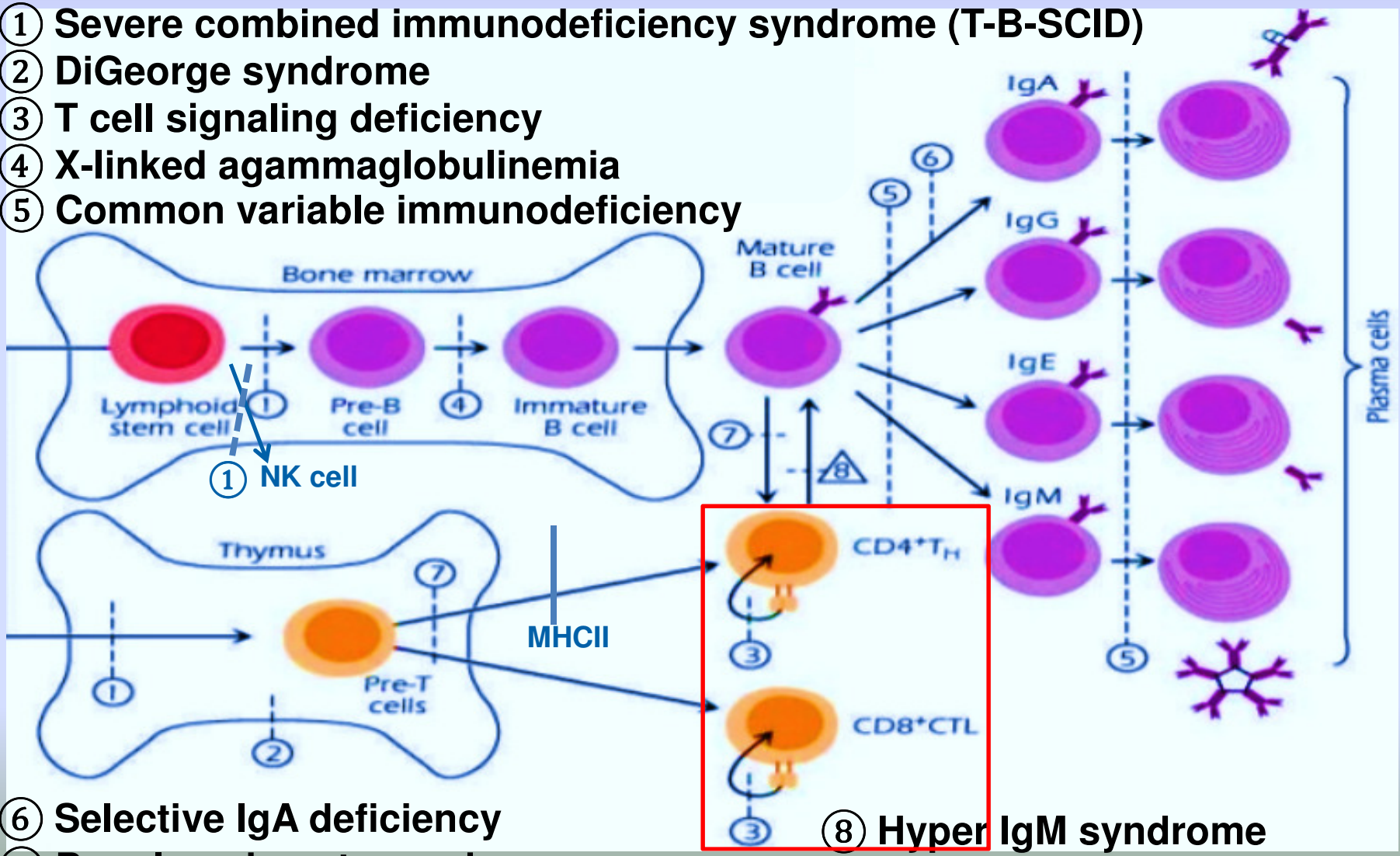
Thank You !!

Consultations & inquiries send to
Michelle Tseng at mtseng@oandoalpan.com
Matt Plassmeyer at
mplassmeyer@oandoalpan.com

Supplemental Slides

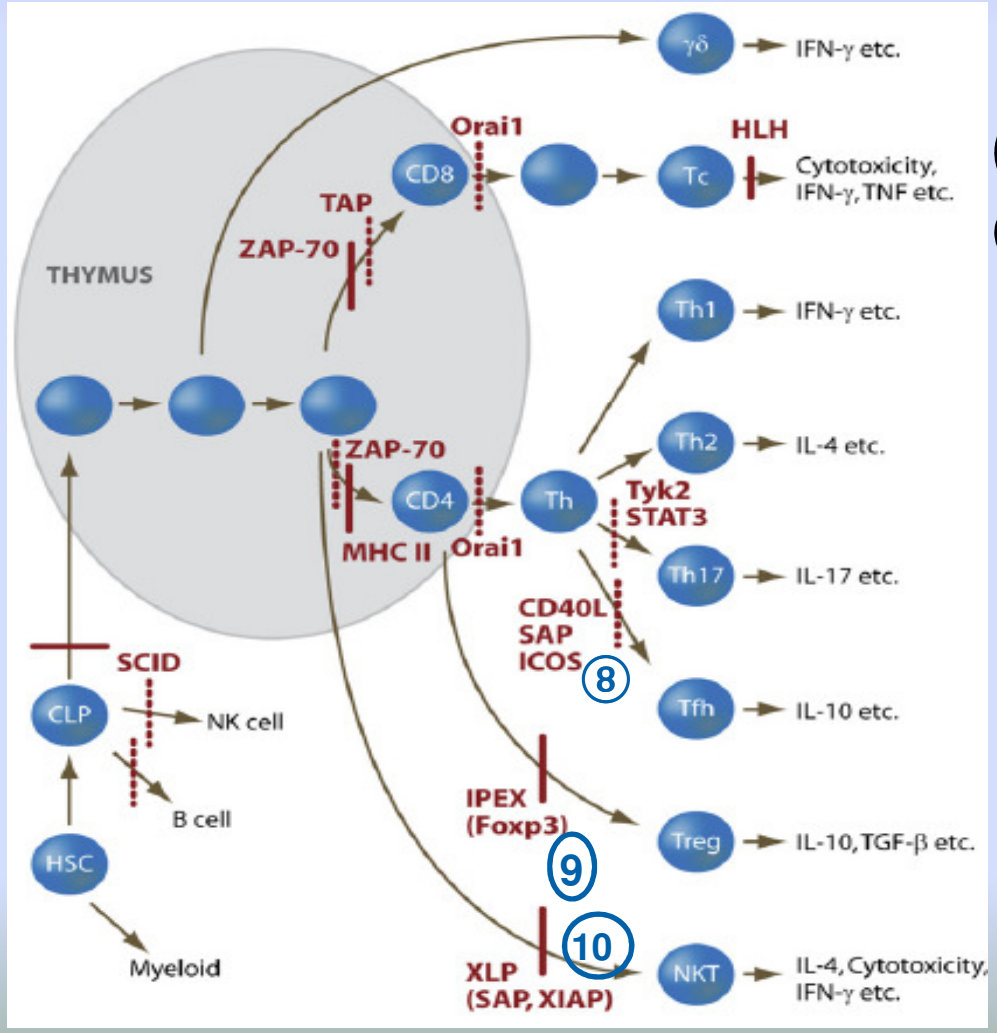
Immune Cell Development & PIDs: Occurs in Any Defective Step

① Severe combined immunodeficiency syndrome (T-B-SCID)
② DiGeorge syndrome
③ T cell signaling deficiency
④ X-linked agammaglobulinemia
⑤ Common variable immunodeficiency



⑥ Selective IgA deficiency
⑦ Bare lymphocyte syndrome
⑧ Hyper IgM syndrome

Immune Cell Development & PIDs: Occurs in Any Defective Step



⑧ **Hyper IgM syndrome**

⑨ **IPEX**

⑩ **XLP**

Curbside Consultation Form

AMERIMMUNE

CURBSIDE ALLERGY CONSULT

Hours of operation: M-F 8-6, Saturday: 9-2
CLIA # : 49D2016663
Lab Manager Phone: (O) 571.308.1900 ext 1914 (M) 703.459.5869 Fax: 571.308.1919

Please complete 1, 2, 3.
Amerimmune Labs Sample Pick-up, Call: (571) 733-7762

Presentation (1)

- ☐ Anaphylaxis
- ☐ Urticaria
- ☐ Food Aversion
- ☐ Diarrhea
- ☐ Angioedema
- ☐ Rhinitis
- ☐ Eczema
- ☐ Recurrent Otitis
- ☐ Recurrent sinusitis
- ☐ Pneumonia
- ☐ Drug Allergy
- ☐ Asthma
- ☐ Other:

Clinical details (2)

3 Labs (circle or add tests)

Amerimmune Lab (One 3ml Purple Top)

Lymphocyte Study - Lymphopenia, neutropenia, recurrent infections.

Eosinophil Study - asthma, food allergies, EoE, urticaria

Neutrophil Study - recurrent infections and or abscess.

4

Please provide Patient Demographics and Insurance

PATIENT INFORMATION

Patient Name: _____ DOB: _____

☐ Male ☐ Female Ethnicity: ☐ Caucasian ☐ African American ☐ Other: _____

PHYSICIAN INFORMATION

Name: _____ Signature: _____

Phone: _____ Fax: _____

BLOOD DRAW INFORMATION

Date Collected: _____ Time Collected: _____ ☐ AM ☐ PM

Supporting Labs (Reference lab)

CBC with diff,
IgG, A, M and E,
IgG subclasses
Pneumococ. titers-14 serotype
HiB titers.
Tryptase
C3, C4
CRP
Sed Rate
Immature platelet fraction
Reticulocytes
C1q
Other:

Immune Workup – Lab Test Cost

Test	Test Number	CPT Codes	TAT	Price
ALPS Panel	3	88184, 88185x10, 88188	2 days	$596.00
Antigen Stimulation	4	86353x2	2 weeks	$658.00
Apoptosis	5	88184, 88185x2, 88187, 88230,	3 weeks	$1,544.00
B Cell Panel	7	88184, 88185x11, 88188	2 days	$518.00
Baff, plasma levels	6	83520	2 weeks	$443.00
CD40L (CD154)	10	88184, 88185x5, 88187	2 days	$819.00
CD45RA/RO	11	88184, 88185x8, 88188	1 day	$589.00
CD52 Expression	12	88184, 88185x6, 88187	2 days	$565.00
CD64 (Leuko64)	13	88184	1 day	$143.00
CD107a Mobilization	8	88184, 88185x3, 88187, 86352	3 days	$583.00
CD127/CD132	9	88184, 88185x8, 88188	2 days	$431.00
CTL Function	15	86352	2 weeks	$697.00
Cytokines, Intracellular	20	88184, 88185x6, 88187	2 days	$1109.00
Cytokines, Plasma/CSF	30 / 5863724	83520x10	2 weeks	$2,239.00
EBV Immortalization	1129	88230, 88240	No results	$426.00
Foxp3	18	88184, 88185x3, 88187	2 days	$610.00
IL-18	1210	83520	2 weeks	$547.00
iNKT	19	88184, 88185x2, 88187	2 days	$421.00
Lymph Activation Markers	1	88184, 88185x8, 88188	1 day	$822.00
Lymph Subsets	22	86355, 86357, 86359, 86360	1 day	$422.00
MHC Class I & II	23	88184, 88185x5, 88187	2 days	$496.00
Mitogen Stimulation	24	86353x3	7 days	$987.00
Neopterin	26	83520	7 days	$362.00
Neutrophil Adhesion Markers	2	88184, 88185, 88187	1 day	$168.00
Neutrophil Oxidative Burst	27	86352	1 day	$161.00
NK Function	25	86352, 86357	3 days	$558.00
Perforin/Granzyme B	28	88184, 88185x4, 88187	2 days	$602.00
PNH Screen (CD59/FLAER)	31	88184, 88185x8, 88188	2 days	$335.00
pSTAT5	32	88184, 88185, 88187, 86352	2 days	$372.00
SAP (XLP1)	33	88184, 88185x3, 88187	2 days	$609.00
Soluble CD163	34	83520	2 weeks	$758.00
Soluble Fas-Ligand (sFasL)	8154618	83520	2 weeks	$303.00
Soluble IL-2R	35	83520	2 days	$309.00
TCR ab/gd	36	88184, 88185x4, 88187	1 day	$556.00
TCR Vbeta	37	88184, 88185x23, 88189	2 days	$995.00
Th17 Enumeration	2025	88184, 88185x4, 88187	2 days	$611.00

$1,972

Amerimmune Cellular & Humoral Immune Lab Tests Cost

Cost <65%

Immune Compartment	Tests	Function
Cellular	1. CBC with differential 2. T-cell (CD3), 3. NK-cell (CD56/16), 4. αβTCR, γδ TCR 5. CD4RO, CD8RO	*Non-specific*: Mitogen proliferation & DHR CD25 & HLA-DR on T cells, Th17 *Specific*: antigen proliferation or DTH to candida
Humoral	1. B cell (CD20/19), 2. $CD27^+$ IgG^+ B cells, 3. $CD27^+IgM^+$ B cells. 4. CD21dim cells 5. IgG+ B cells	*Specific:* antibody titers to tetanus, pneumococcal 14 serotype and HiB *Non-specific:* IgG, IgA, IgM, IgE & IgG subclasses

Immune Workup in PIDs Diagnosis

History of PIDs Diagnosis

1st case – Ataxia telangiectasia

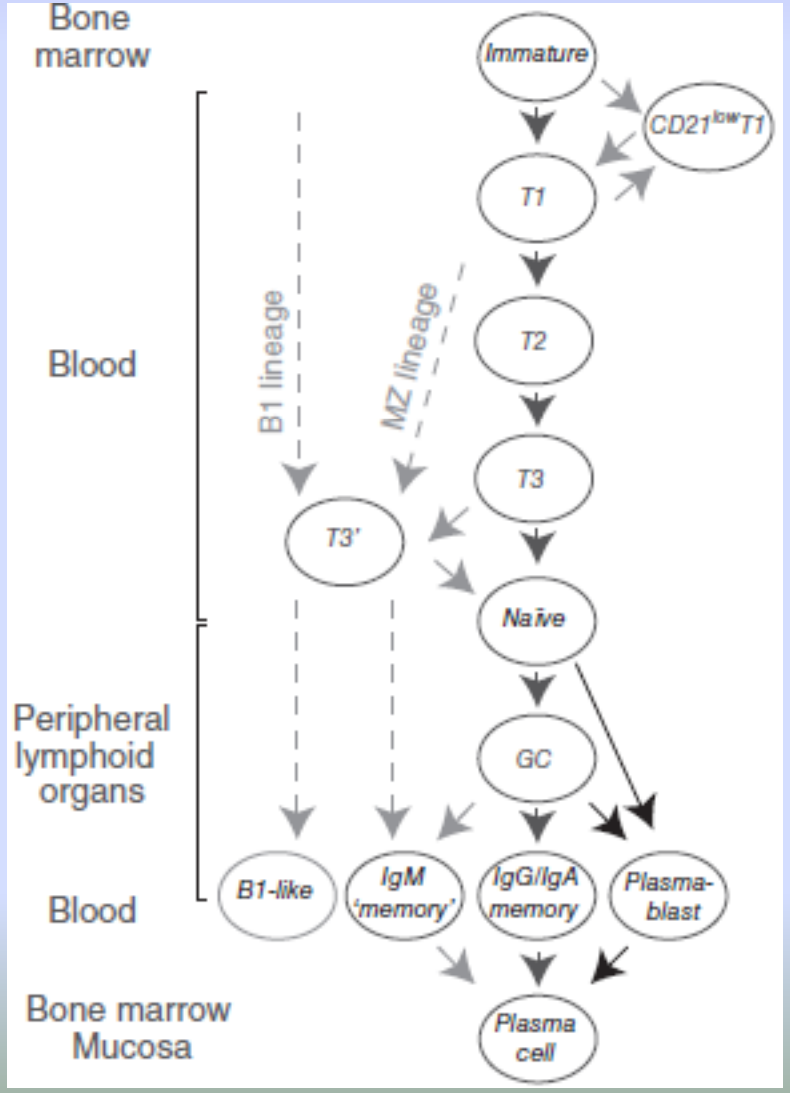


Shearer, W.T. and Fischer, A. J. Allergy Clin. Immunol., Vol. 117, No.4

A Solution: Curbside Consultation

- Pre-set immune diagnostic tool
  - "curbside", same-day pick up specimen from healthcare facilities to Amerimmune lab
  - new quantitative and qualitative "hybrid" approach for immune workup
  - solve the problems of inadequate regulated, advanced lab facilities and shortage of immunologists
  - meets the needs of other medical specialties, improves social problem of health status, and advances patient-care in this field

B/T Cell Development & PIDs



B Cell Development

