

Endotyping Chronic Rhinosinusitis Using Multiplexed Imaging

Ivan Lee, M.D., Ph.D.

Allergy/Immunology Fellow, PGY V

Laboratory of Garry Nolan & Jayakar Nayak

Stanford University

08/16/2019

No
Financial
Disclosures



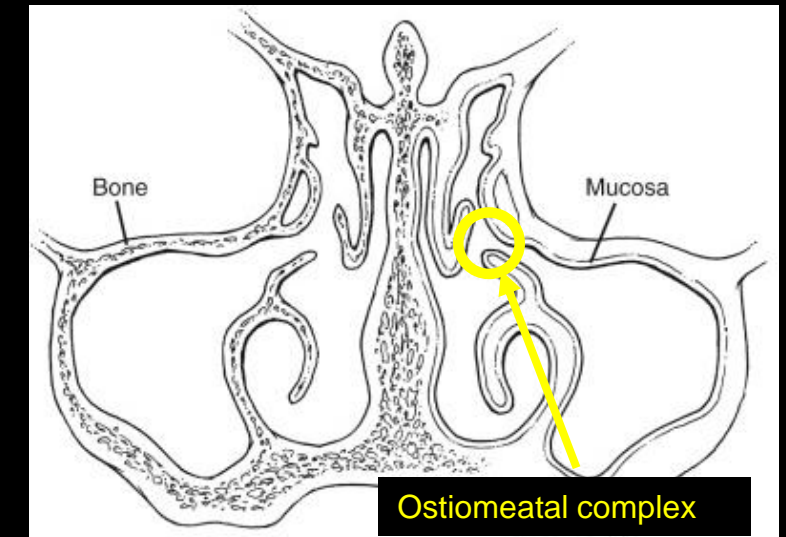
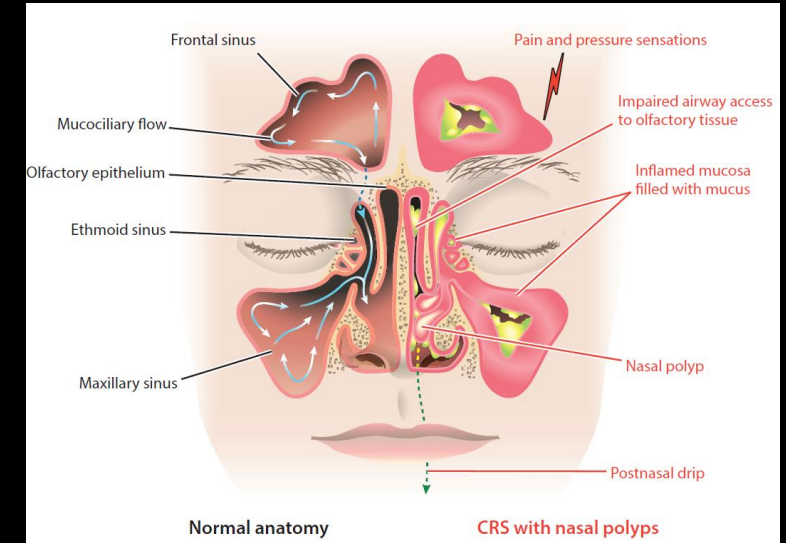
Stanford
MEDICINE

Outline

- Introduction
- Clinical/Molecular Characteristics of Chronic Rhinosinusitis
- Research Needs/Hypothesis
- Next Generation Immunophenotyping Tool
- Research Progress

Chronic Rhinosinusitis (CRS)

- Debilitating, relapsing, remitting disease of inflammation in the nose and sinuses
 - Acute rhinosinusitis = infection
 - Chronic rhinosinusitis = inflammation
- Affects ~12% of the U.S. population
- Annual cost in the U.S. ~\$8 billion
- Top 10 diagnosis associated with loss of productivity



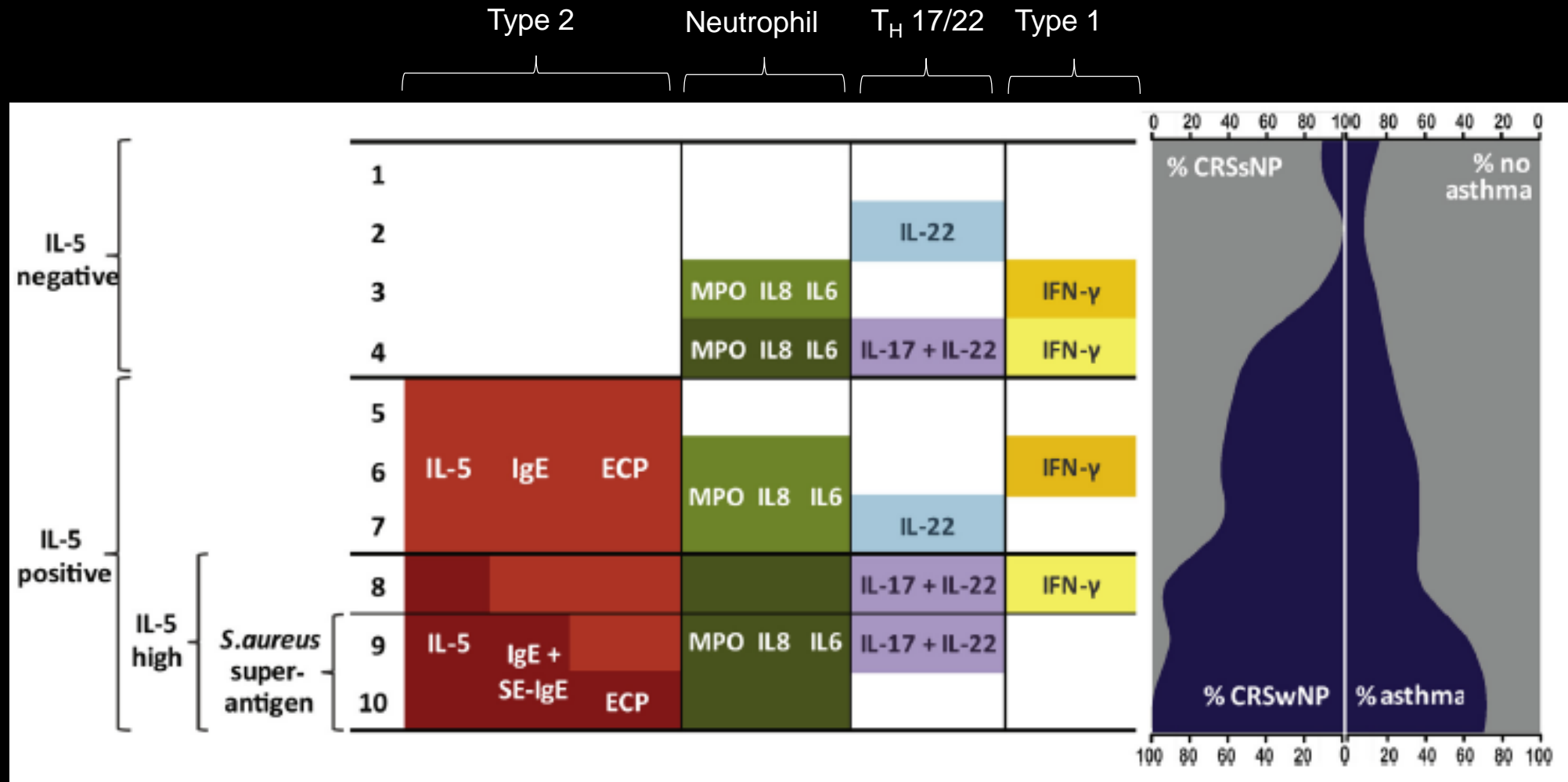
Phenotypic Classification of CRS

	CRS with Nasal Polyps (CRSwNP)	CRS w/o Nasal Polyps (CRSSNP)
Phenotype		
Prevalence	~1/3	~2/3
Asthma	Higher prevalence	Lower prevalence
Recurrence	Higher	Lower
Severity	Higher	Lower

Other Classifications:

- Aspirin-exacerbated respiratory disease (AERD)
 - 8-26% of CRSwNP
 - 90% risk of recurrence within 5 years
- Allergic fungal rhinosinusitis
- Cystic fibrosis CRS

CRS is a Diverse Inflammatory Disease



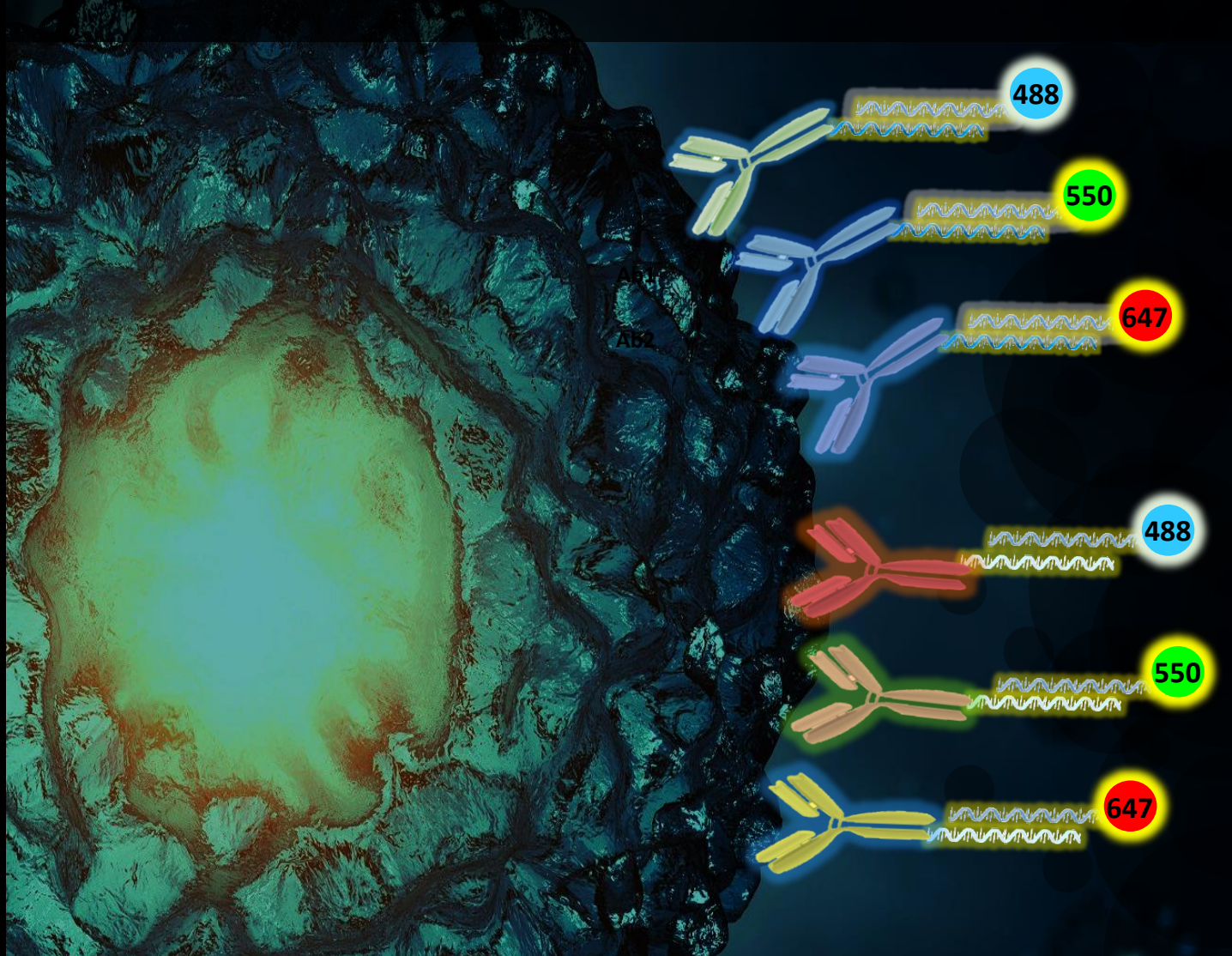
1. CRSwNP tends to be a Type 2 inflammatory disease (but not always)
2. CRSsNP tends to be a non-Type 2 inflammatory disease (but not always)

Research Needs

- CRS is currently a “heterogeneous” disease both at the clinical level and at the molecular level.
- Current medical therapeutics are ineffective in patients with high rates of disease recurrence.
- No biomarkers have been identified to inform the extent of surgery.

A ‘signature’ of biomarkers in quantitative or spatial combination will newly define CRS subtypes and/or disease state.

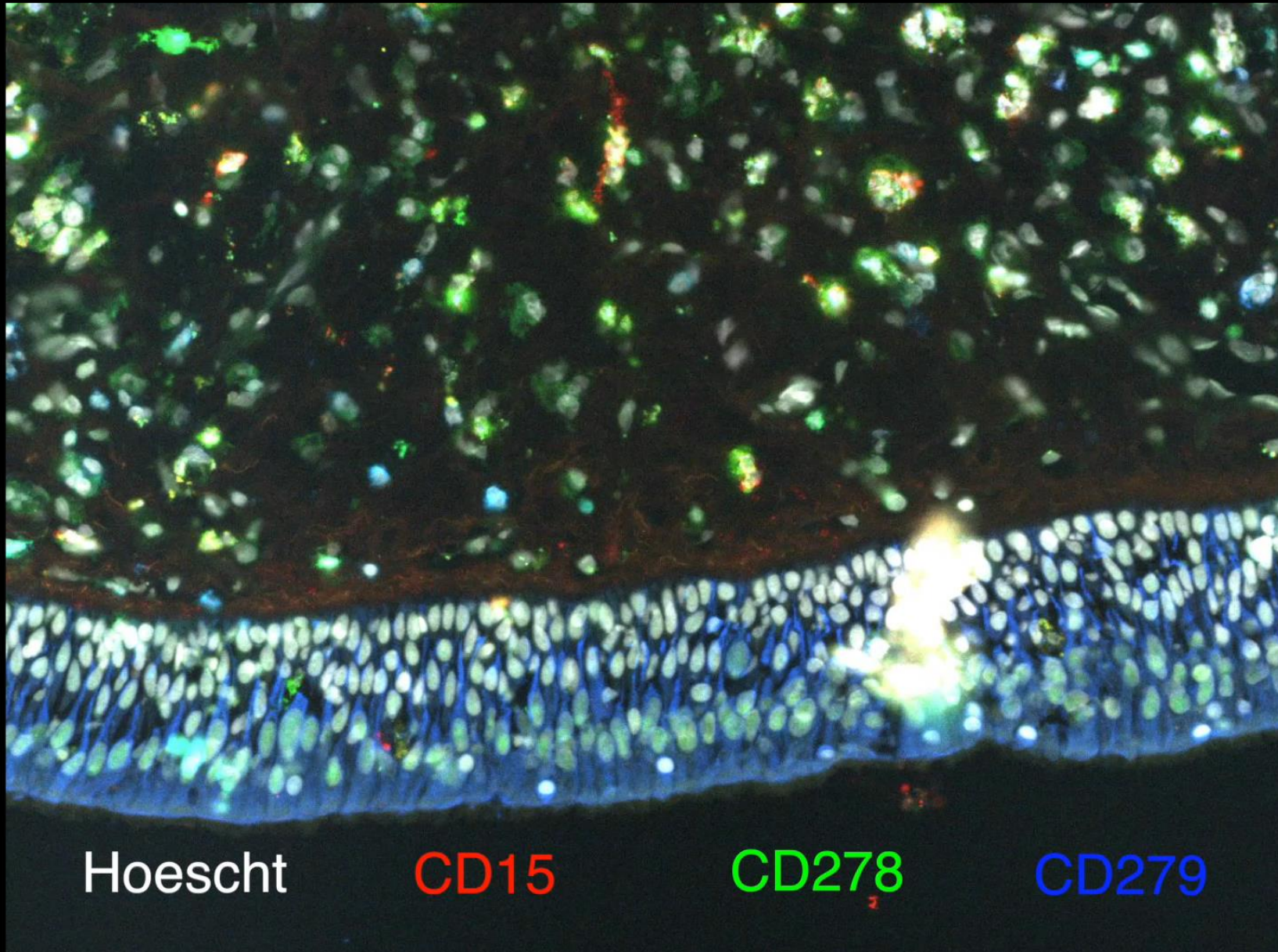
CODEX MULTIPLEXING BY REANNEALING



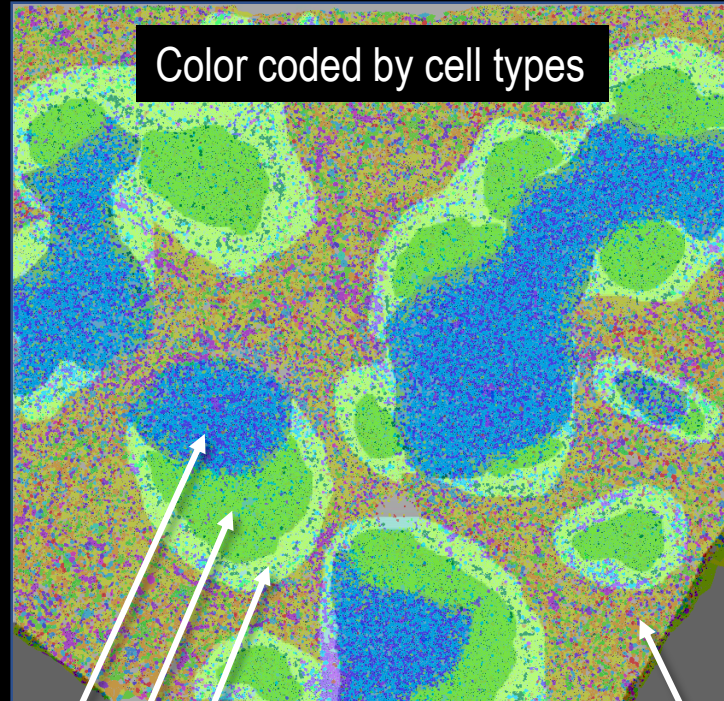
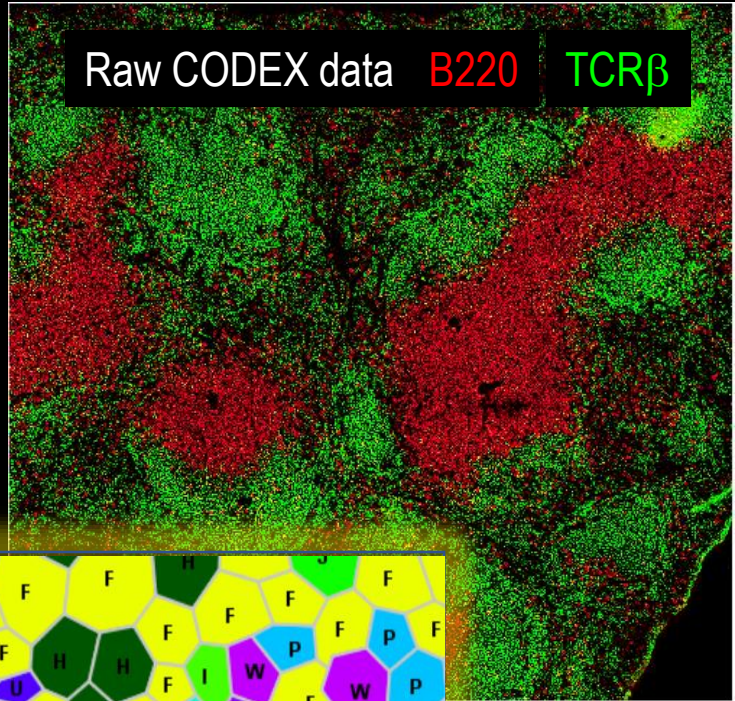
- Stain up to 50 antibodies conjugated to a single strand oligonucleotide
- Anneal anti-sense oligo-fluorophores
- Image
- Gentle Denaturation of oligos
- Repeat for all Ab/oligo sets

CO-Detection by indEXing (CODEX)

CODEX CRS Tissue Section

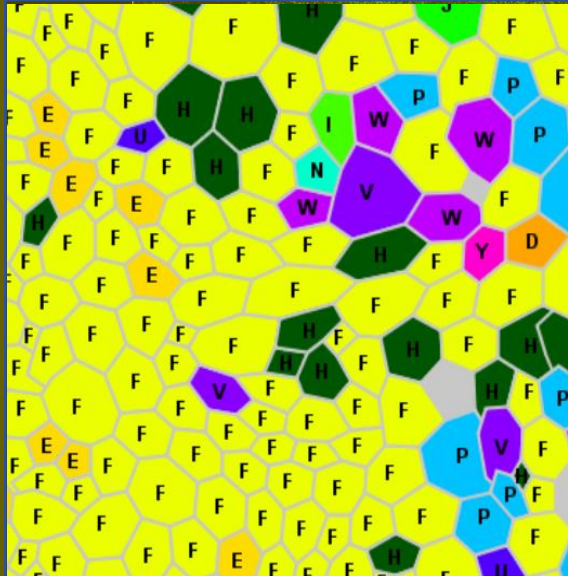


AUTOMATED ANNOTATION OF TISSUE



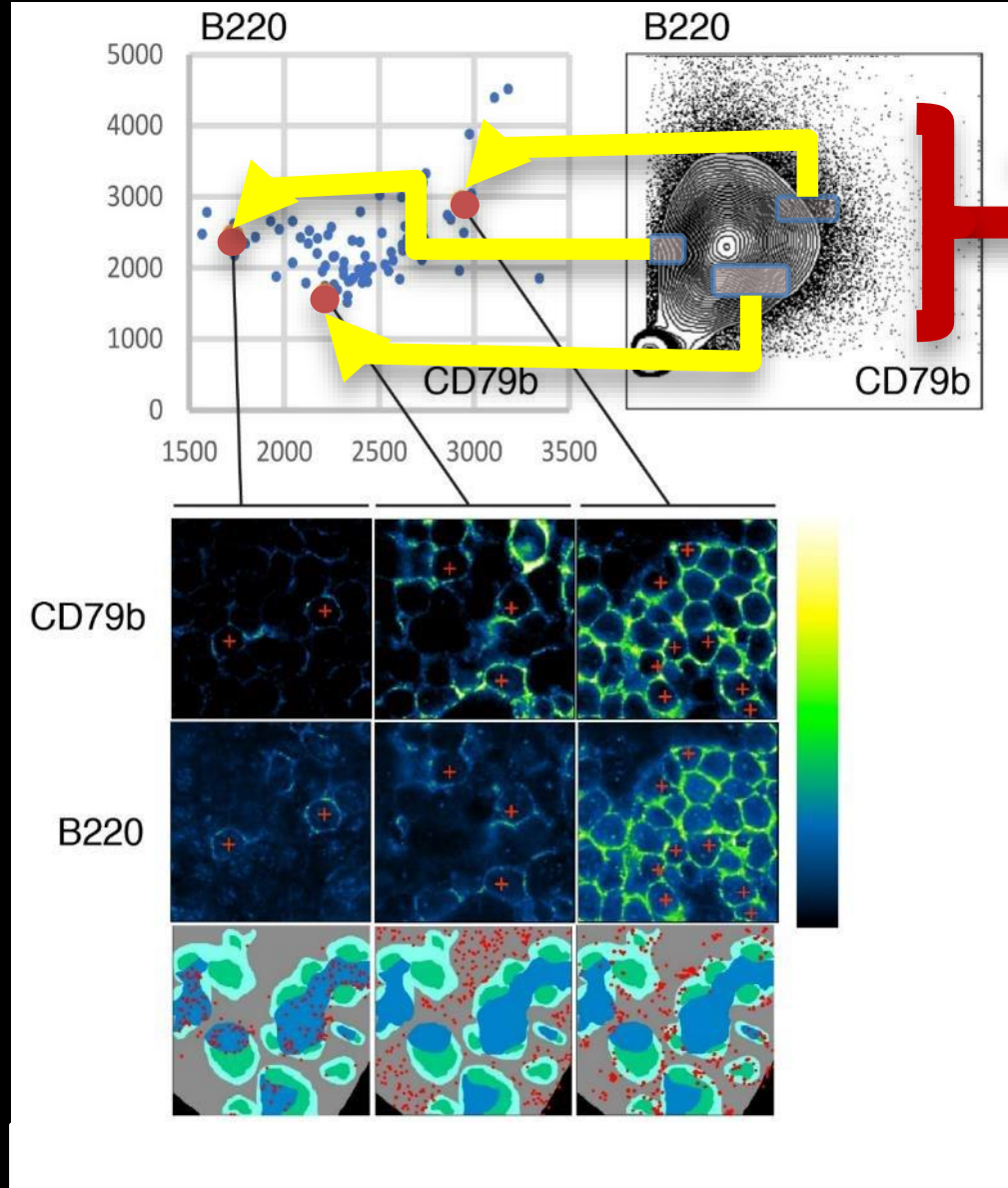
PALS (T cells)
B-cells follicle
Marginal zone

Red pulp

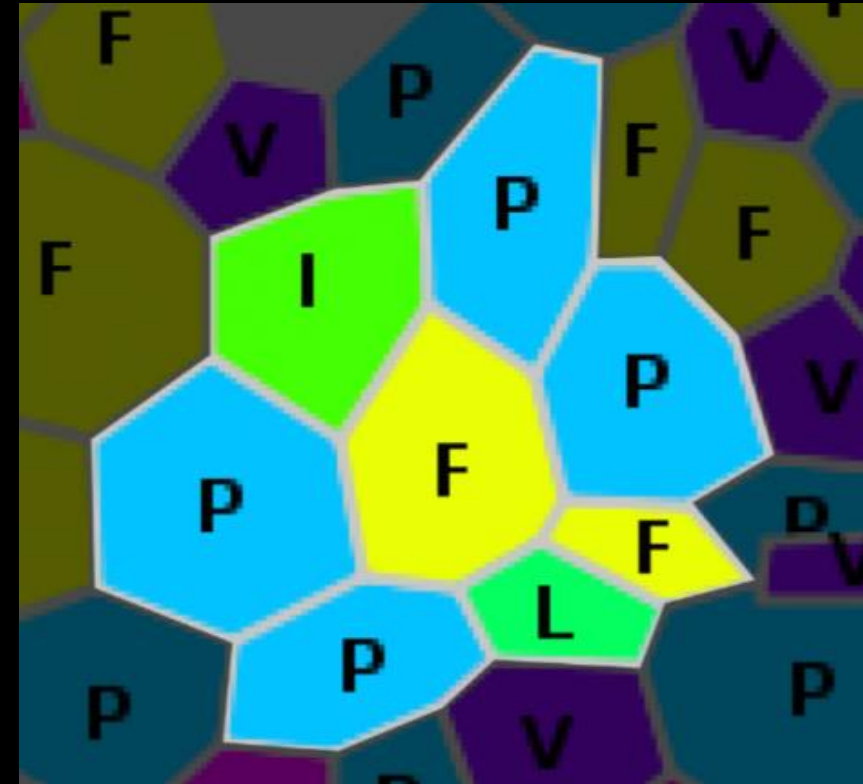


A	9587, NK-cells
B	9590, CD4+ CD8- cDC
C	9591, B220 pos DN Tcells
D	9595, F4/80+ CD16/32+ CD106pos macrophages
E	9597, follicular dendritic cells
F	9600, B-cells
G	9601, capsule
H	9602, marginal zone macrophages
I	9607, erythroblasts
J	9608, CD106+ CD16/32+ CD31+ stroma
K	9609, granulocytes
L	9611, CD8+ CD4- cDC
M	9614, CD106- Ly6C+ CD16/32- CD31+
N	9615, megakaryocytes
O	9617, CD106- Ly6C+ CD16/32+
P	9619, CD4 T cells
Q	9626, plasma cells
R	9628, CD4hi only
S	9629, Vasculature
T	9632, CD3 only
U	9635, CD106+ CD16/32+ CD31- stroma
V	9637, CD8 T cells
W	9638, ERTR7-hi stroma
X	9639, CD106+ CD16/32- Ly6C+ CD31+ stroma
Y	9643, CD4+ CD8+ CD16/32+ cDC

MARKER EXPRESSION DEFINES THE NICHE



● Expression levels of proteins relates to
Neighbors
Tissue locale



CELL, August 2018

Future Directions

- Correlate CRS inflammatory niche with clinical characteristics (severity scoring, recurrence, comorbidities, medications, etc).
- Endotype CRS in East Asia and compare/contrast with CRS in the U.S.
- Develop an algorithm to determine CRS subtypes and optimal therapy.
- Provide individualized care based on the unique clinical and molecular profile of the patient.

Acknowledgments

Nolan Lab

- Garry Nolan
- Tung Hung Su
- Guojun Han
- Pauline Chu
- Nikolay Samusik
- Sarah Black
- Shigemi Kinoshita
- Dave McIlwain

Stanford Allergy

- Sean McGhee
- Anne Liu
- Chitra Dinakar

Funding

- Thrasher Research Fund Early Career Award (2019-)
- Stanford Maternal and Child Health Research Institute (MCHRI) Clinical Trainee Support (2019-)
- Stanford Translational Research and Applied Medicine (TRAM) Pilot Grant (2018-)
- Stanford Pediatrics Physician-Scientist Track Development Grant (2017-2018)
- Nolan Lab Funding
- Nayak Lab Funding

Nayak Lab/Tissue Team



Jayakar Nayak



Tsuguhisa Nakayama



Nicole Borchard



Phil Gall



Sachi Dholakia



David Zarabanda



Yasuyuki Noyama

Stanford Sinus Center



Location:
801 Welch Rd.
Stanford, CA 94305-5739
(650) 723 - 5281



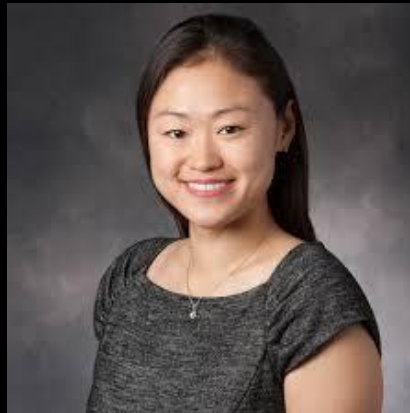
Jayakar Nayak



Peter Hwang



Zara Patel



Jennifer Lee



Carol Yan



Matt Tyler

International Team



東京慈恵会医科大学



Thank you!

- Questions/suggestions?

Polyps have a variable extent of inflammation

