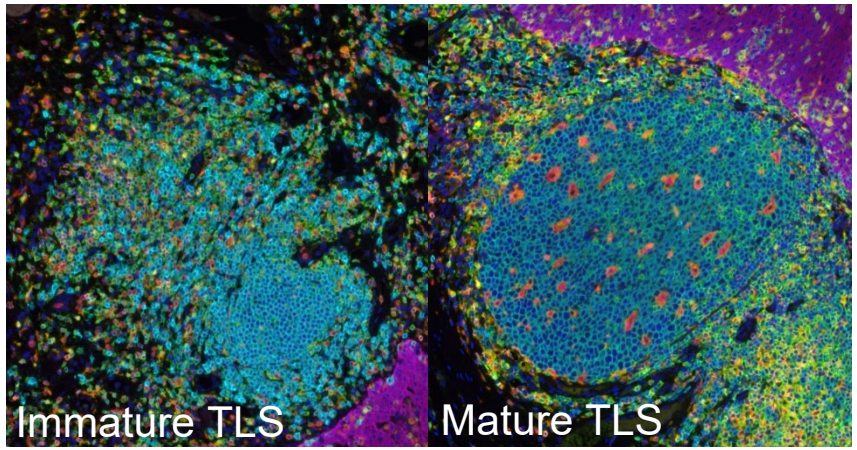
University of Pittsburgh

UPMC | HILLMAN CANCER CENTER

B cells and tertiary lymphoid structures in cancer: The knowns and the knowledge gaps



Our goal is to increase TLS formation, maturation, and function in cancer patients

Tullia C. Bruno, PhD

"T cell enthusiast, B cell convert"

UPMC Hillman Cancer Center

University of Pittsburgh

Department of Immunology

June 20, 2023

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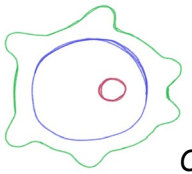
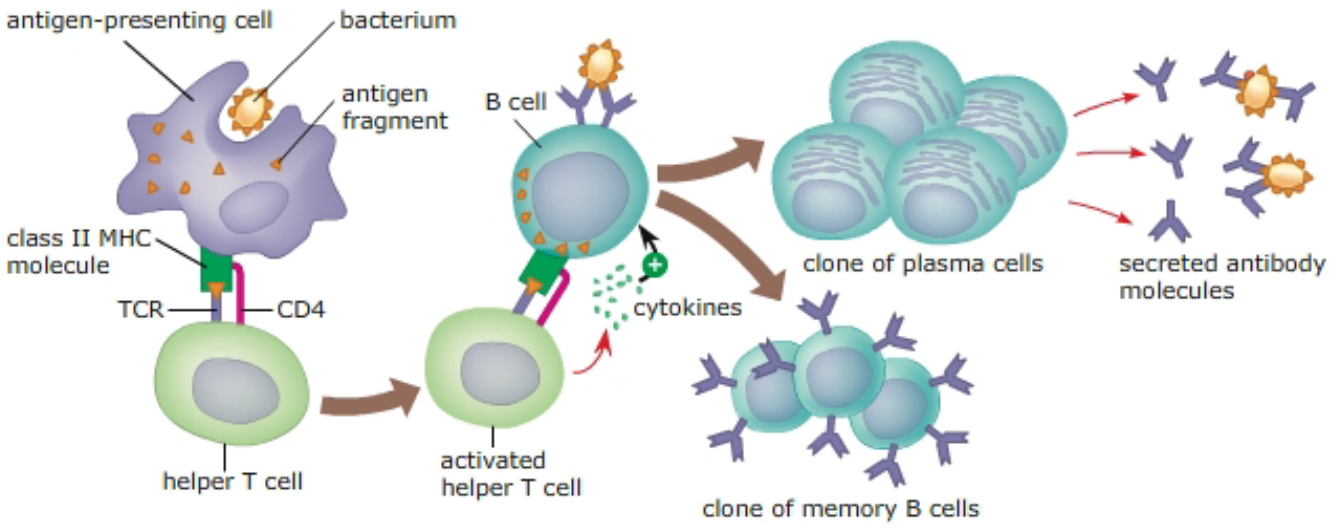


Courtesy of Alex McDonough

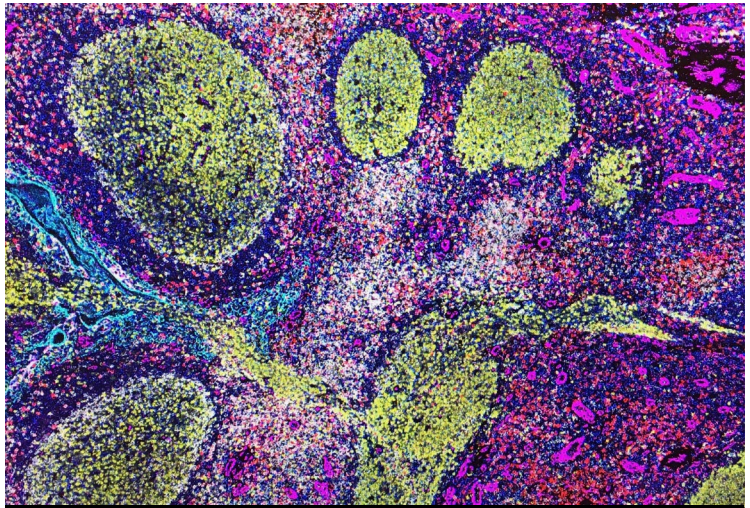
How do we generate memory (virally-specific) B cells?

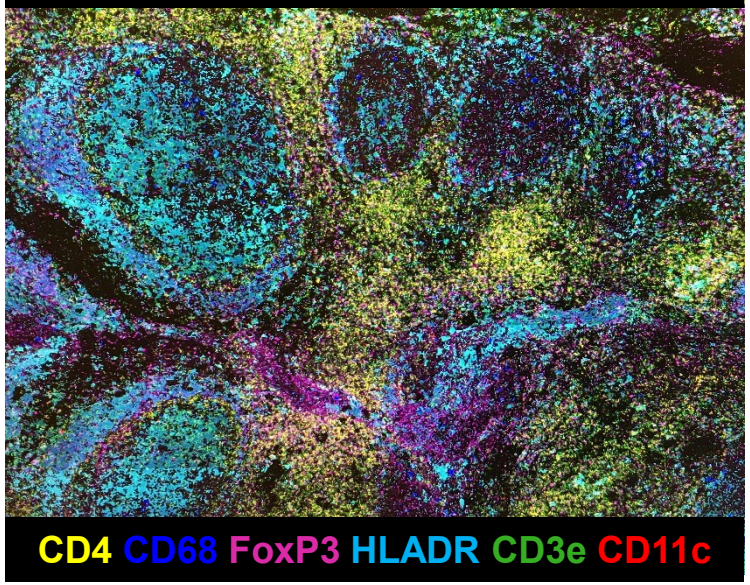


From *Biology* by Campbell and Reece © 2008 Pearson Education, Inc.

This occurs in secondary lymphoid organs such as lymph node, tonsil and spleen

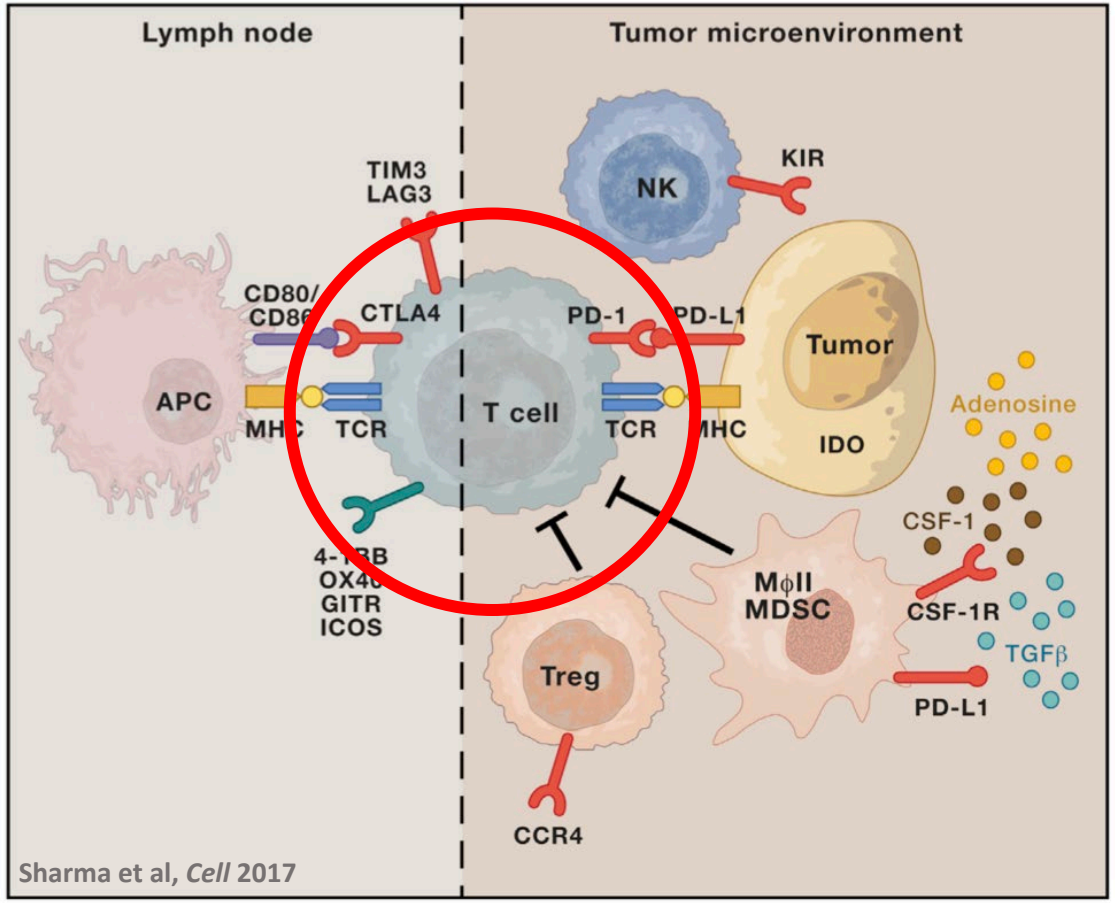
Human tonsil

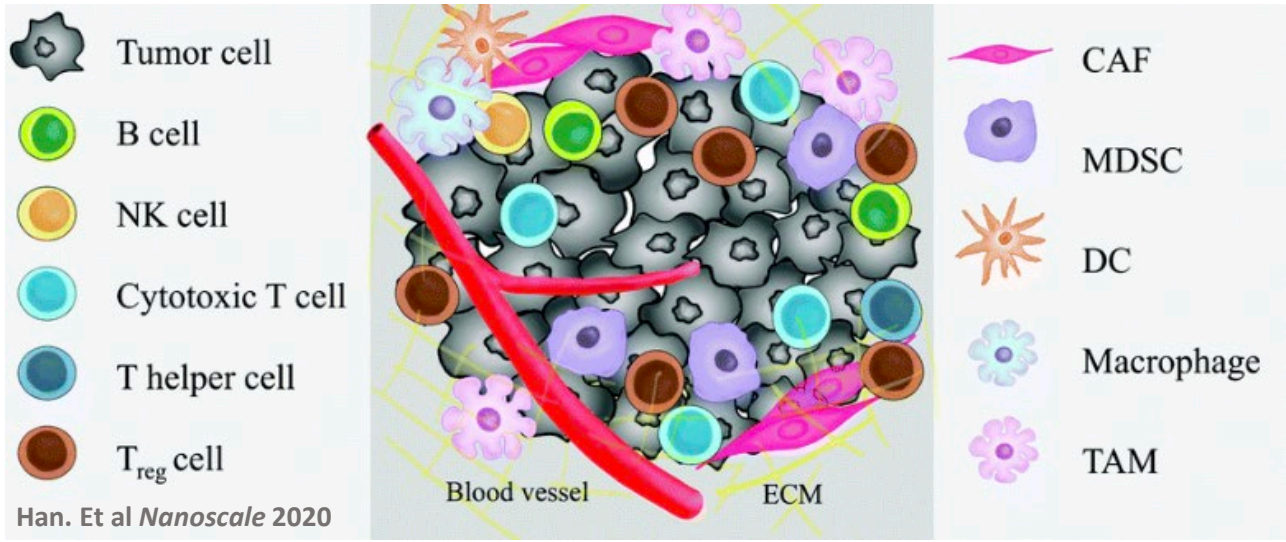




Images obtained in Bruno lab (CODEX)

MULTIPLE immune subsets in the tumor microenvironment (TME) contribute to the immune response





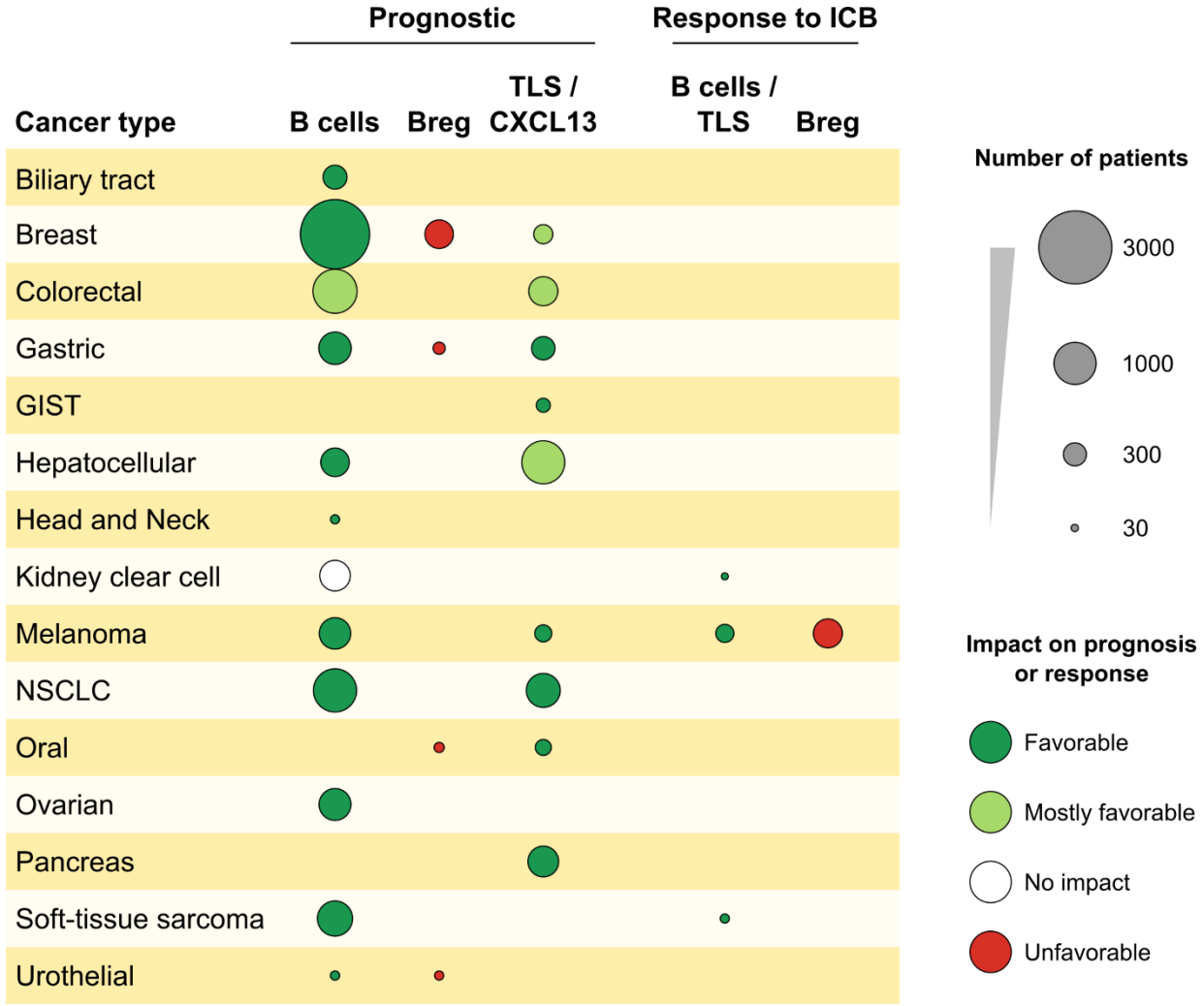
Including B cells and other components of tertiary lymphoid structures (TLS)

<u>Goal:</u>

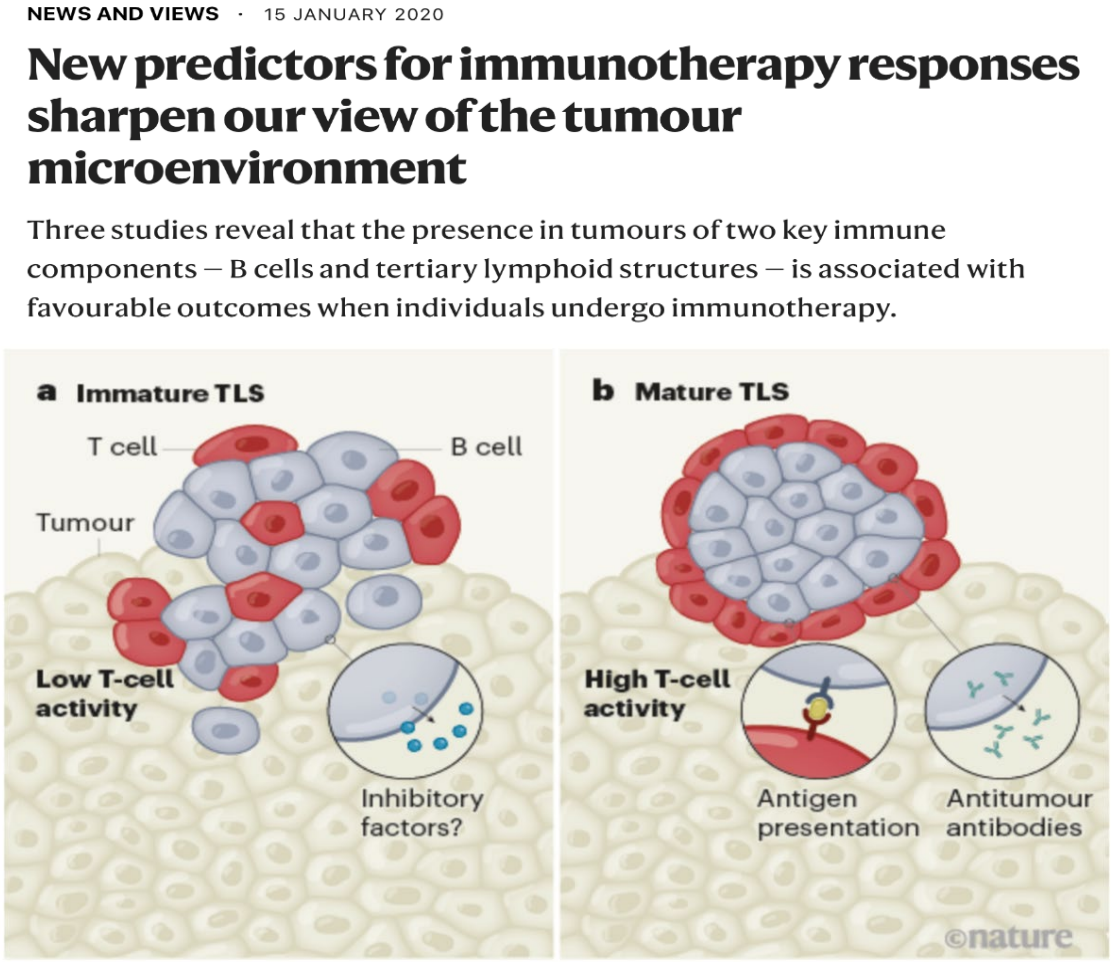
Harness the complete TME for improved immunotherapies

B cells and TLS correlate with improved prognosis and superior IO response

TLS are found in multiple human solid tumors



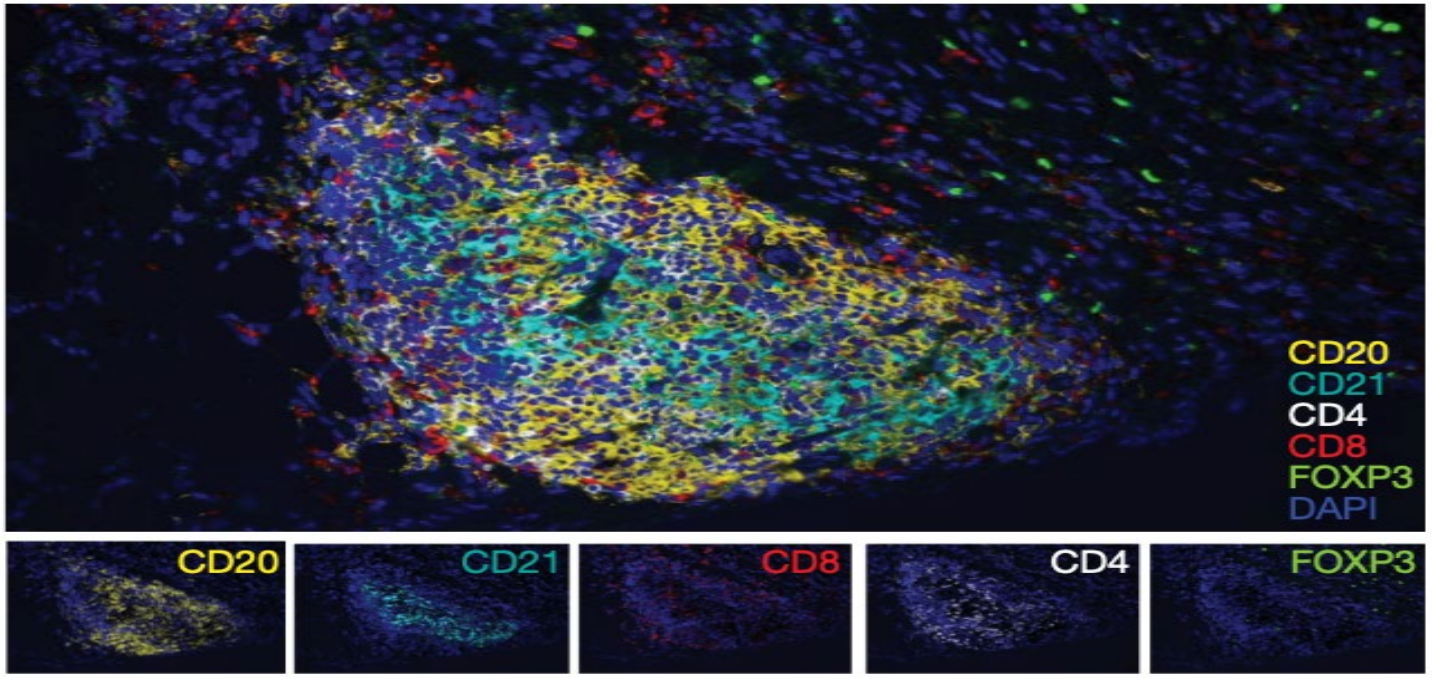
J Exp Med. 2020;218(1). doi:10.1084/jem.20200851



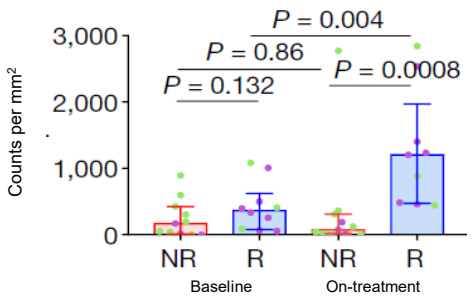
How do we maximize B cell and TLS function within the TME?

How do we think beyond the current clinical data and get to mechanism?

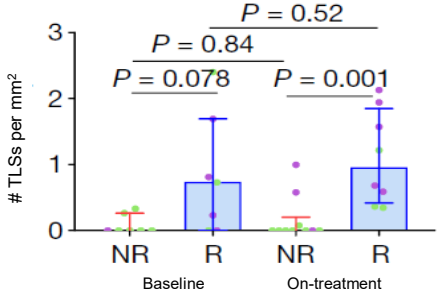
Resectable Melanoma



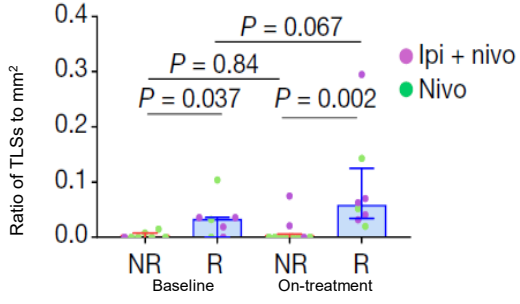
CD20 density



TLS density

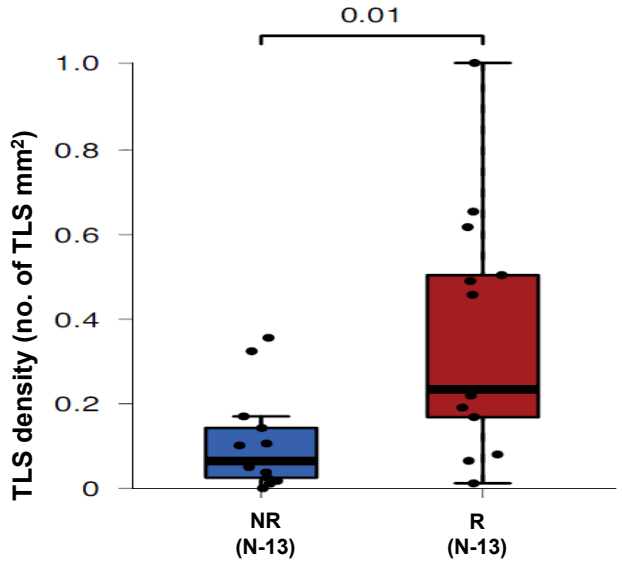


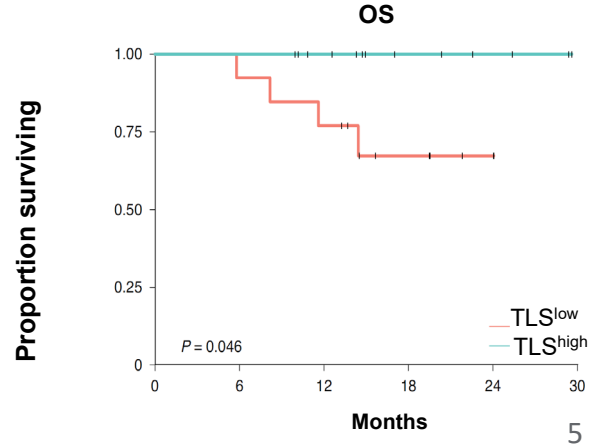
TLS/tumor area



Helmink et al. *Nature* 2020 (Wargo lab)

Resectable Urothelial Carcinoma

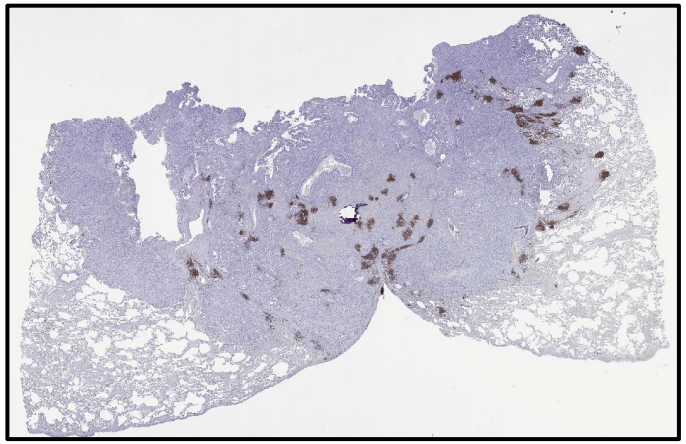




Gao J et al., *Nat Med* 2020

Current challenges and opportunities in the B cell and TLS field

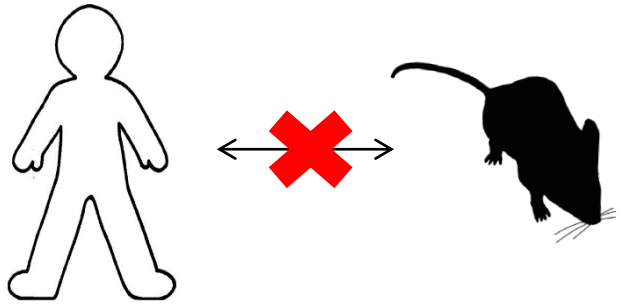
Variability of human tissue



Finding and studying TLS requires:

- State of the art spatial techniques
- Ample tissue
- Paired samples from pre and post ICI

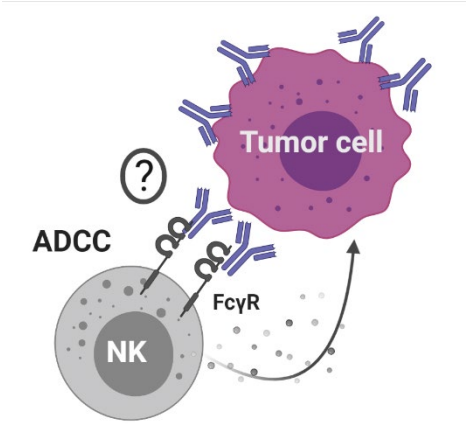
Lack of in vivo models with TLS



Most murine models <u>DO NOT</u>:

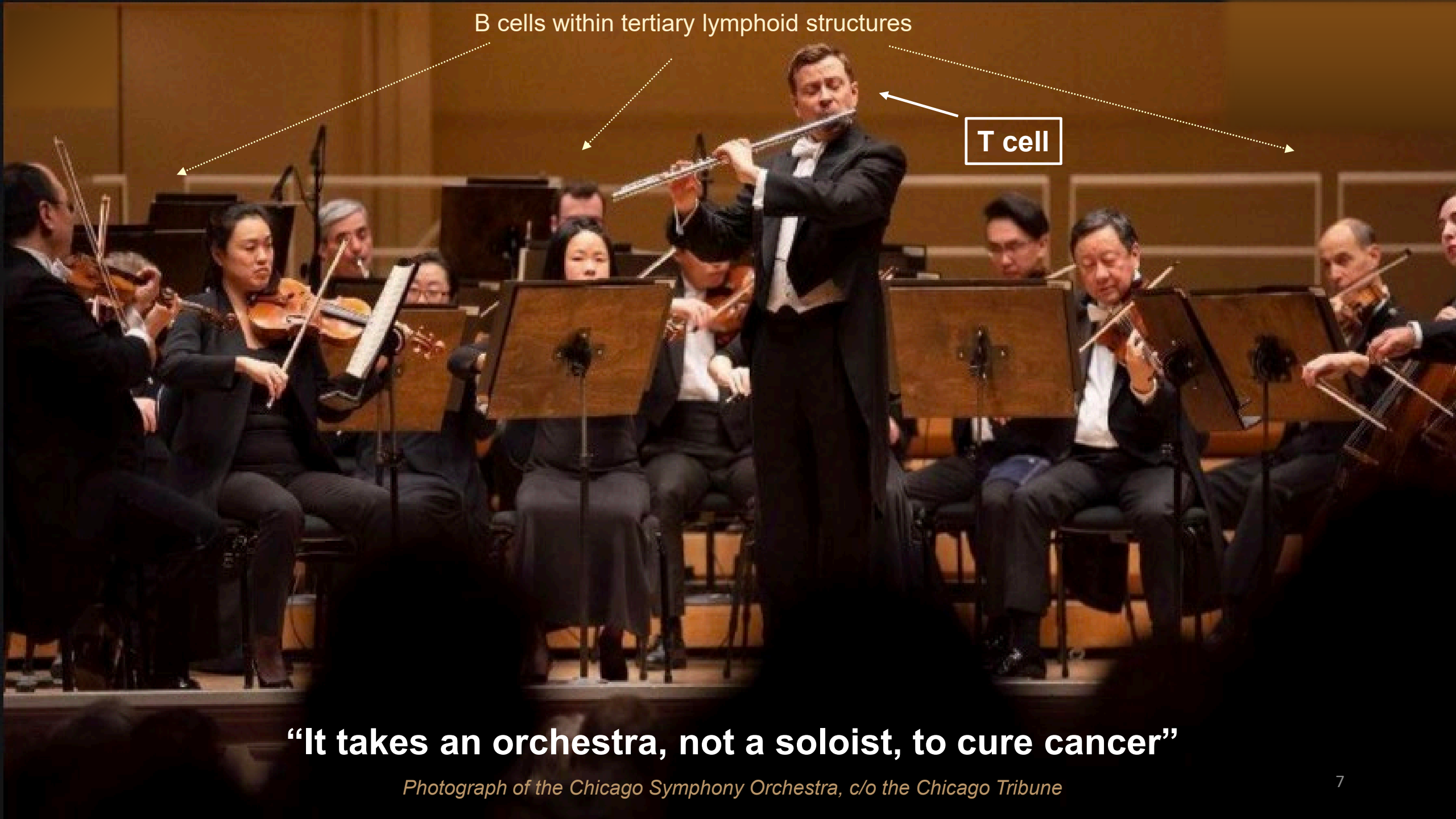
- Contain TLS
- Reflect penetrance of TLS in human tissues
- Reflect TLS composition observed in patients

Lack of tools for ab recognition



Identifying what B cells recognize is:

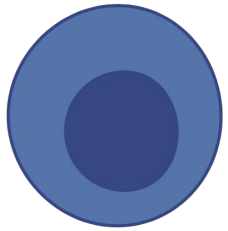
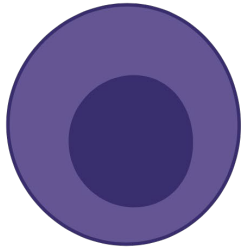
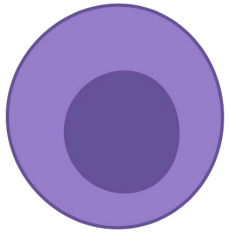
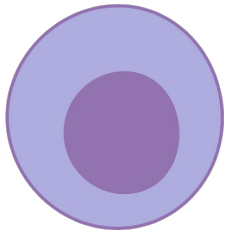
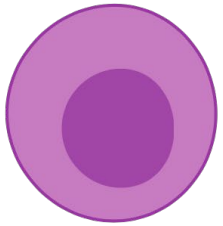
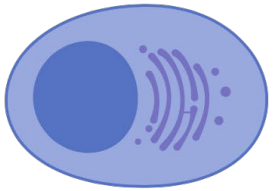
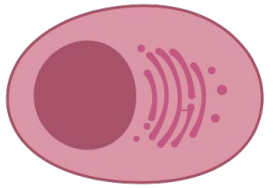
- More complex than T cells with neoantigens
- Requires high throughput pipelines



“It takes an orchestra, not a soloist, to cure cancer”

Photograph of the Chicago Symphony Orchestra, c/o the Chicago Tribune

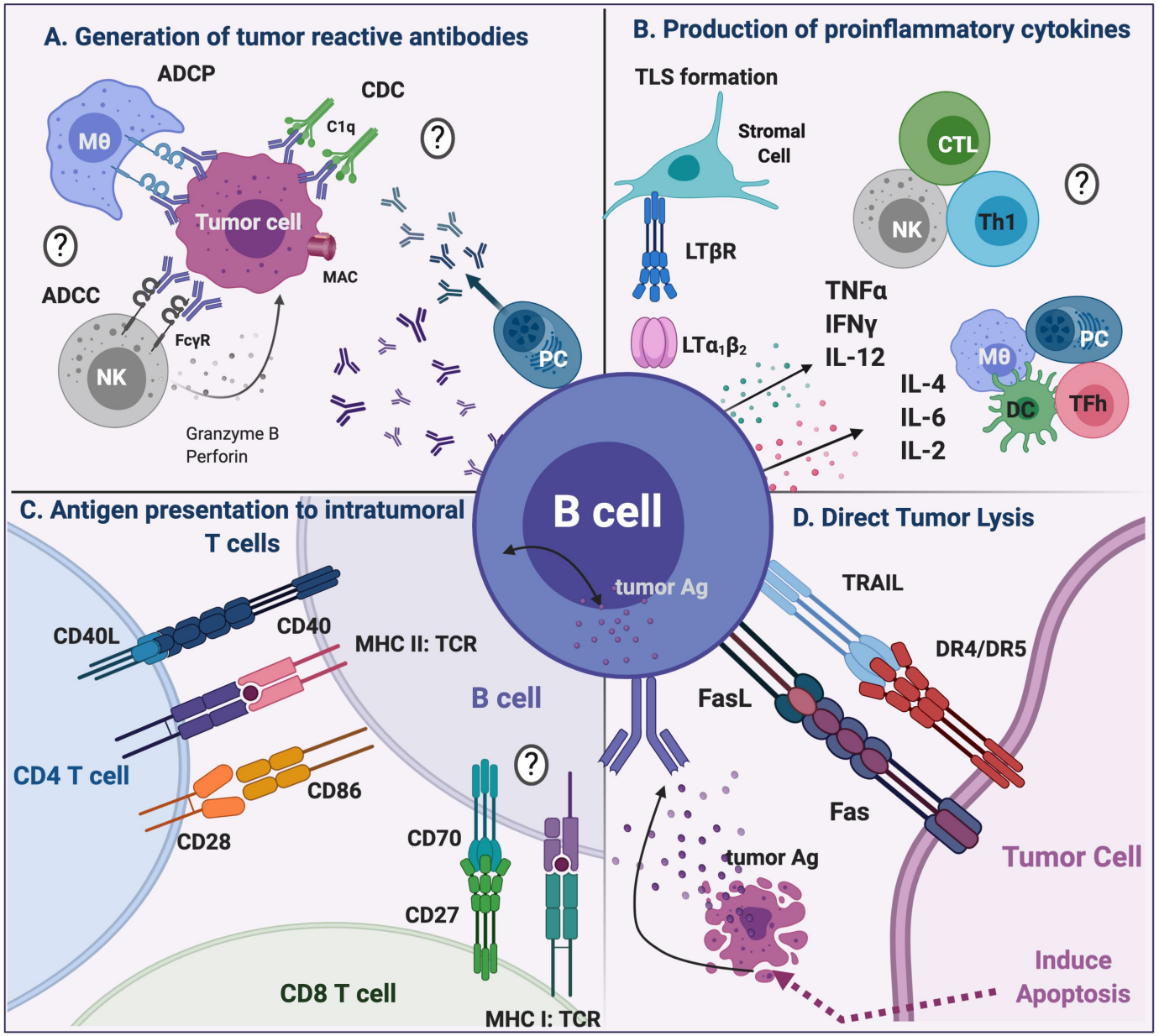
The B cell compartment is complex and diverse!

Naive	Follicular	Marginal Zone	Germinal Center	Memory	Plasmablasts	Plasma cells
						
Migration to secondary lymphoid organs	TD responses in primary follicles	Responses to blood-borne pathogens/Lipid antigens Potent activators of naïve T cells	Affinity Maturation Memory/plasma cell differentiation	Rapid response to secondary infection	Antibody secreting cells	

How do we develop an effective B cell immunotherapy?

- ✓ *Determine what B cell subpopulations are present in the TME*
- ✓ *Assess whether phenotype and function are modulated by TME*
- ✓ *Examine interactions of B cells with other cellular and non-cellular components of the TME*

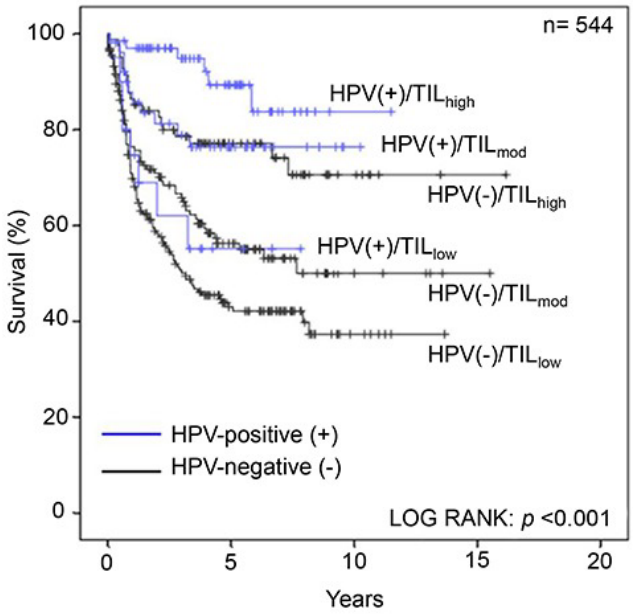
There are various functions for B cells in the context of cancer



Dr. Ayana Ruffin, book chapter update on B cells and TLS in cancer (SITC)

Current biological evidence points to B cells being impactful in the TME

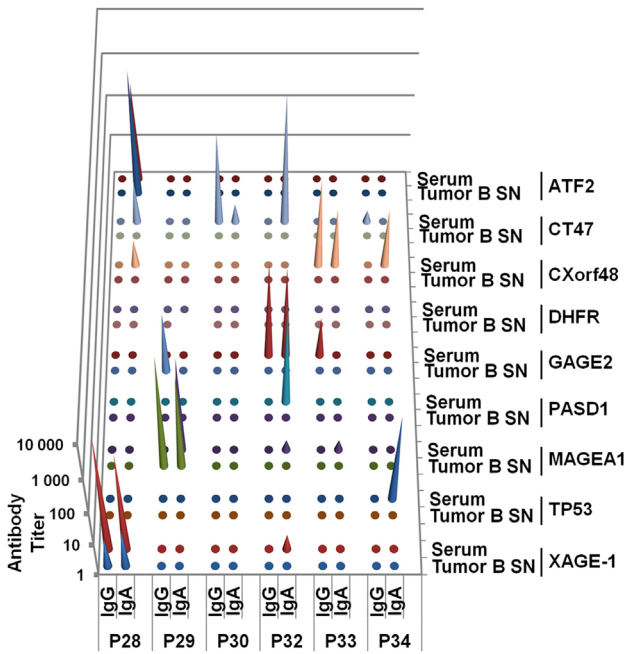
Correlate with improved survival



- ✓ Colorectal
- ✓ Lung
- ✓ Head and Neck
- ✓ Breast
- ✓ Melanoma
- ✓ Ovarian
- ✓ Prostate
- ✓ Pancreatic

Ref: Wood et al, 2016

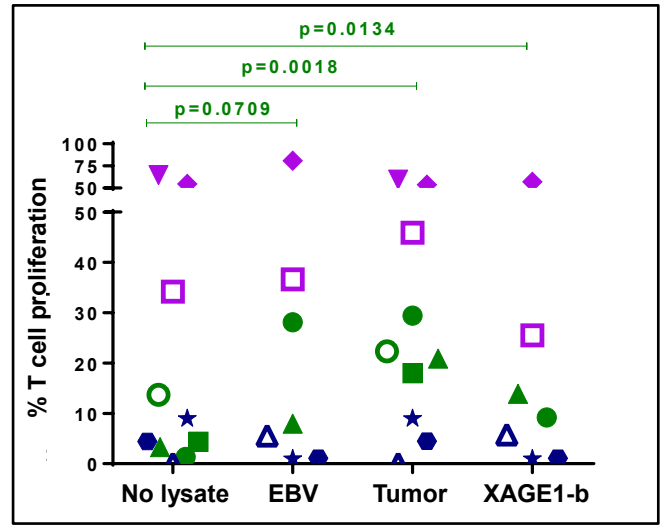
Produce tumor-reactive antibodies

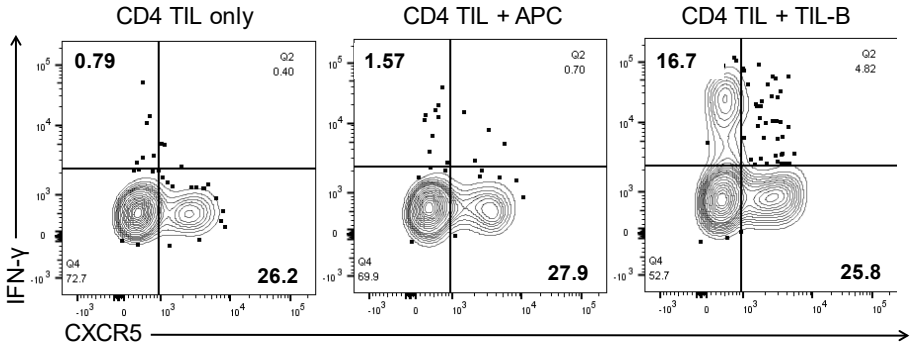


- ✓ Of note: antibodies were derived from serum

Ref: Germain et al, 2014

Present tumor antigens to T cells

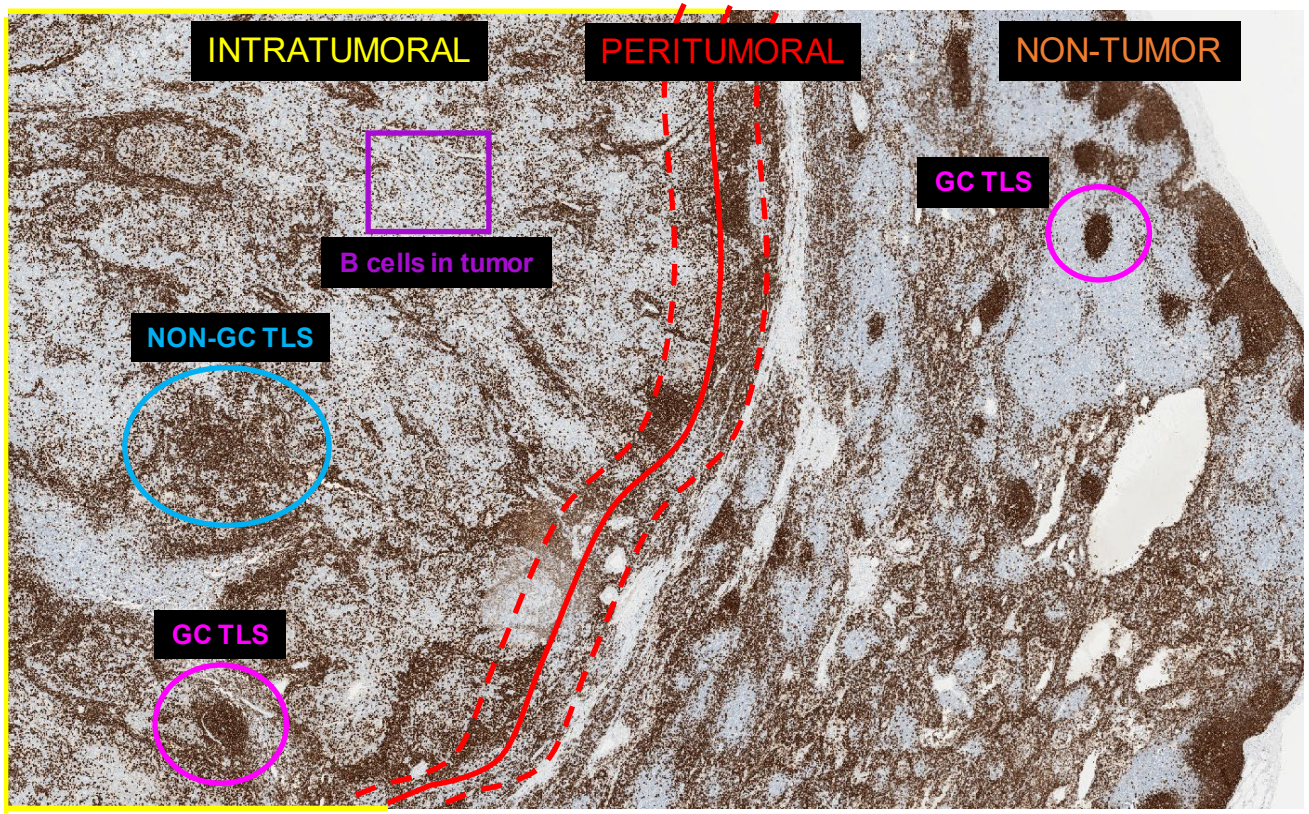


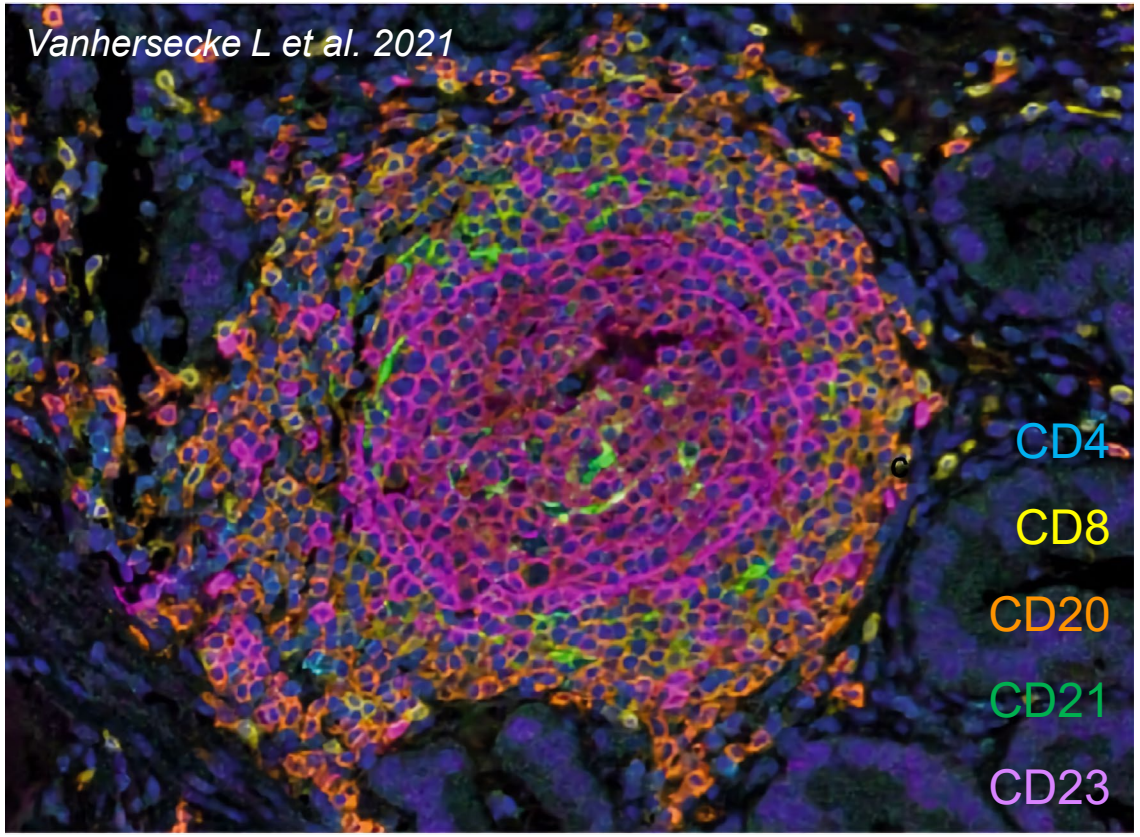


Ref: Bruno et al, CIR, 2017

Cellular neighborhoods influence anti-tumor responses in the TME

What is a tertiary lymphoid structure (TLS)?

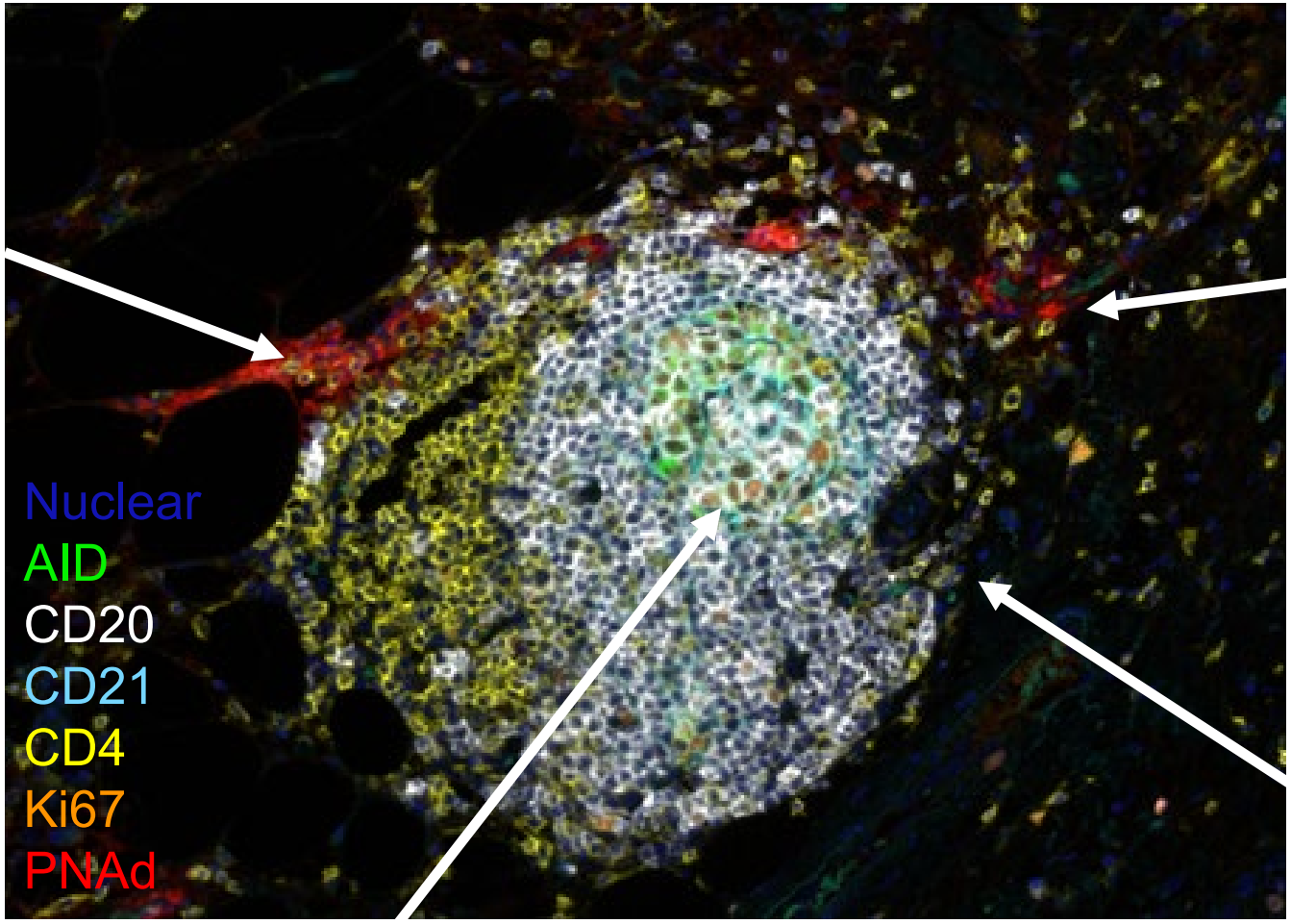




- ✓ Infiltration and segregation of CD20+ B cells and CD4+ T cells (T follicular helper lineage)
- ✓ Presence of CD21+ mature follicular dendritic cells (germinal center)
- ✓ Presence of high endothelial venules (HEV)

Combining several TLS hallmarks into a multispectral image

Infiltration and zoning of CD20⁺ B cells and CD4⁺ T cells



High endothelial venules (HEV) formation

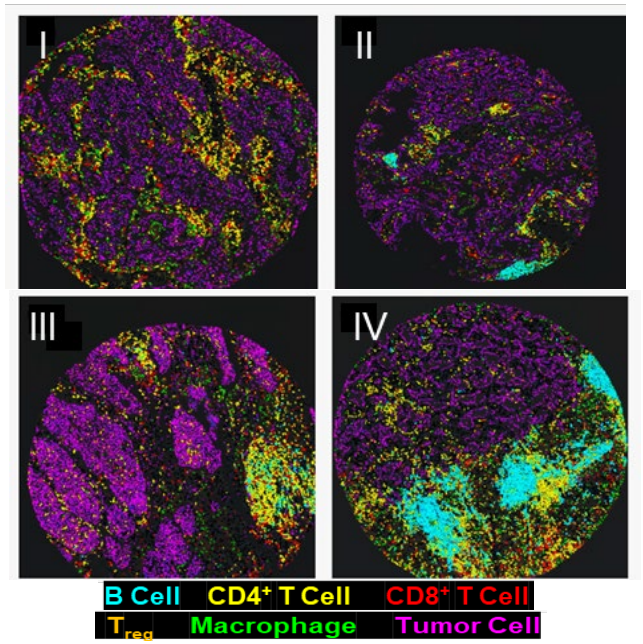
Germinal Center (GC) formation

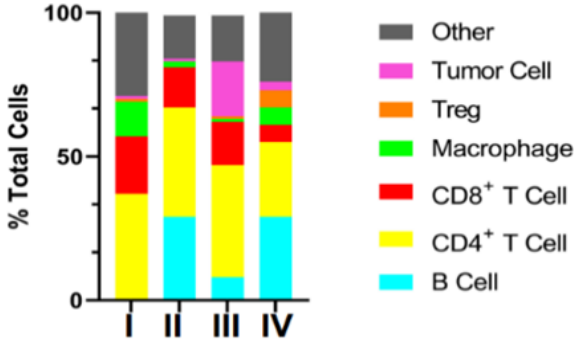
Bruno Lab

Presence of CD21⁺ Mature follicular dendritic cells

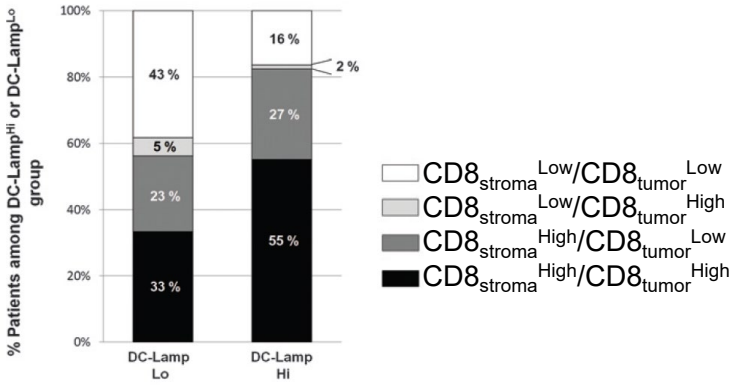
TLS are important mediators of anti-tumor immunity

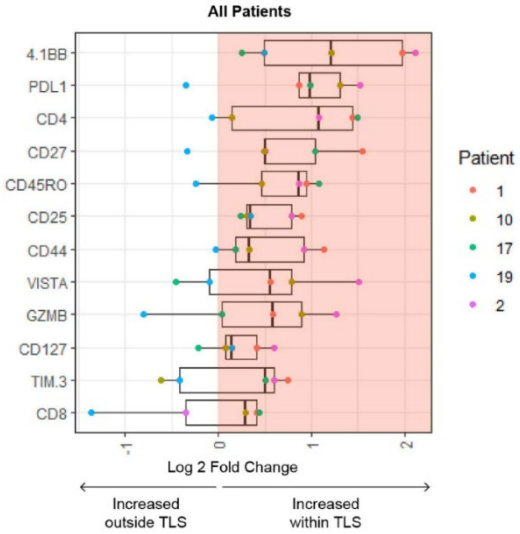
TLS composition varies





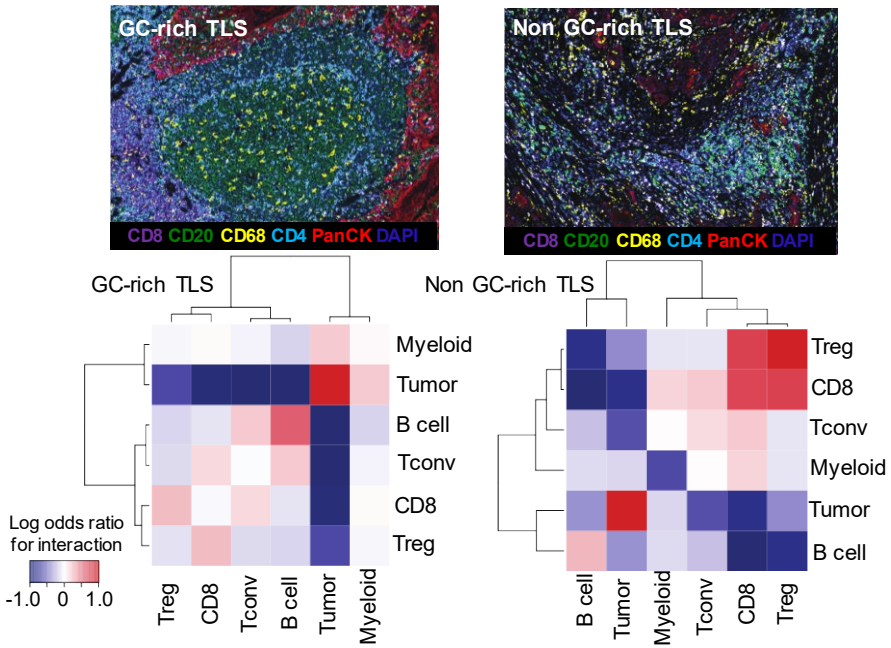
TLS correlate with CD8+ T cells





Ref: K. Ching, Goc et al, Can Res 2013, Helmink et al, Nature 2020

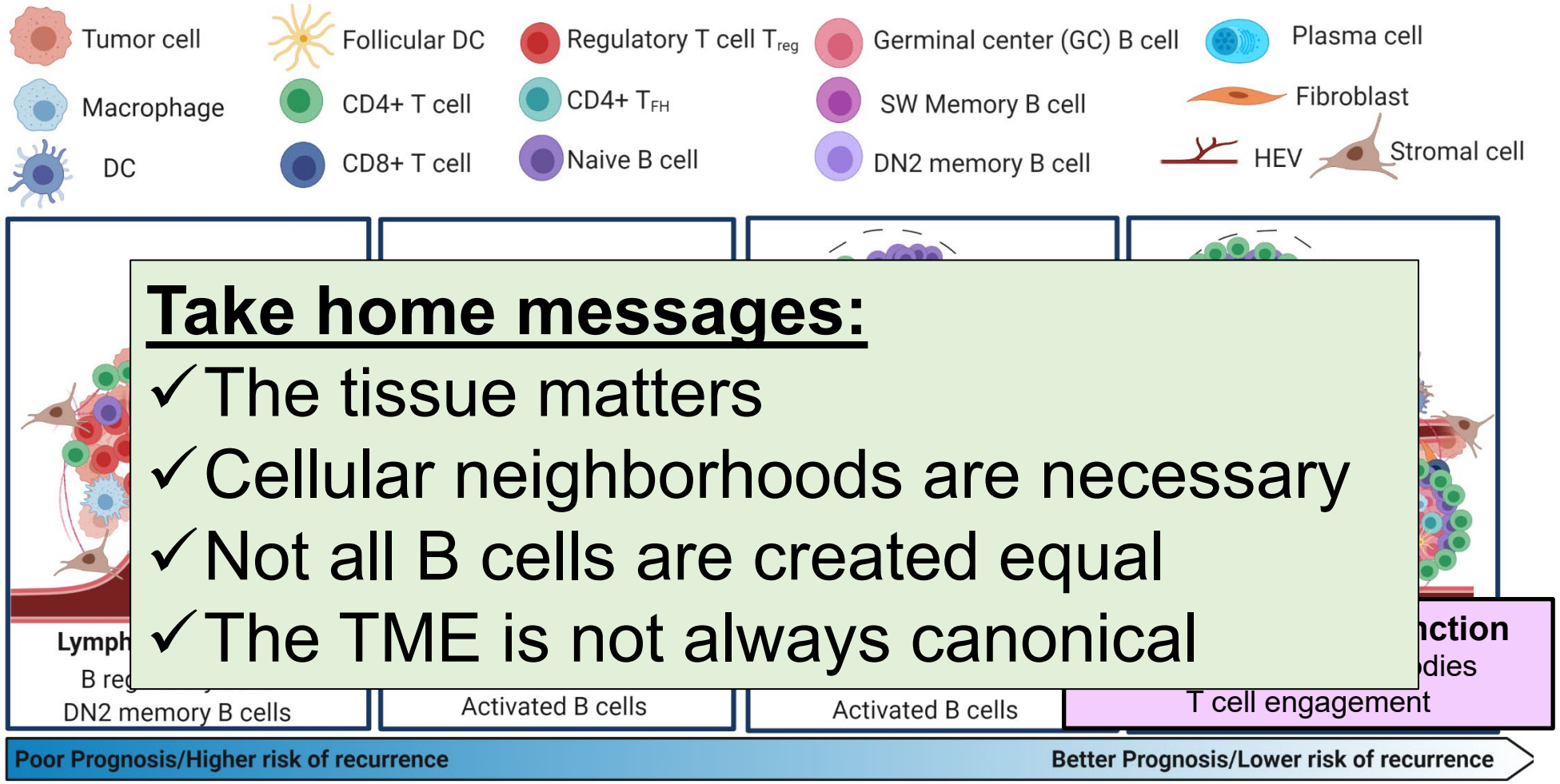
TLS maturity improves patient survival



Does a cellular neighborhood always have to be a TLS?

Ref: Ruffin et al, Nat Comm 2021

TLS are not uniform within the tumor microenvironment

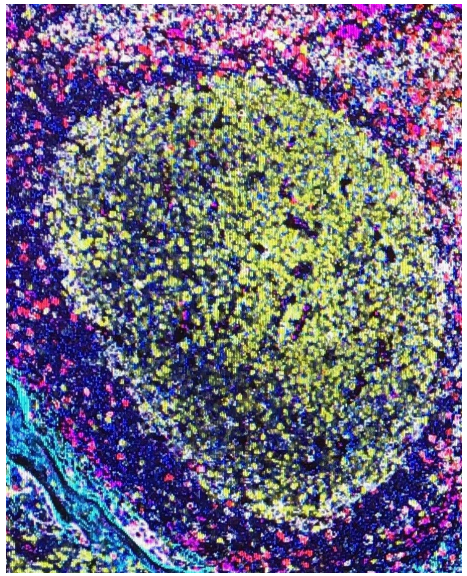


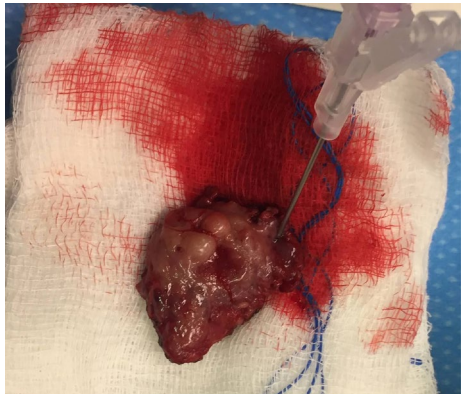
***Increased TLS frequency correlates with B and T cell responses in NSCLC patients**
(Bruno et al, 2017 CIR)

Ruffin A, Bruno T *Cancer Immunotherapy Principles and Practice 2nd edition* 2021

What are the necessary factors to create the most functionally active intratumoral TLS?

Normal Lymphoid Tissue
Tonsil





It starts with patient tissue!

- ❑ B cells, T cells, follicular dendritic cells
- ❑ TLS initiating and maintaining factors
  - ✓ CXCL13, LTB, IL21
- ❑ Immunogenic tumor antigens
  - ✓ Virus, microbiome, immunogenic cell death
- ❑ Stromal and tumor factors
  - ✓ Mesenchymal stem cells, FRCs, fibroblasts

But what is different about the TME?

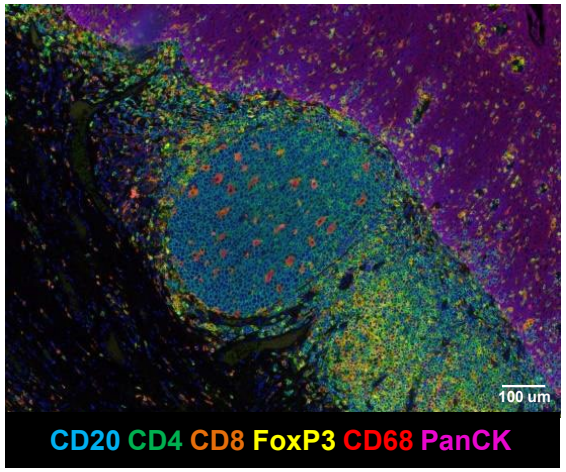
Unique and robust patient cohorts
Primary tumors and metastases

Physiologically relevant Mouse models
Mimic human TLS

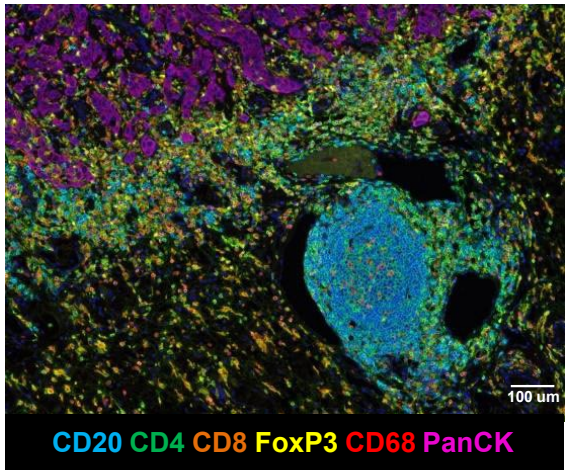
State-of-the-art techniques
Spatial transcriptomics
Multispectral Imaging
scRNAseq
Cytometry

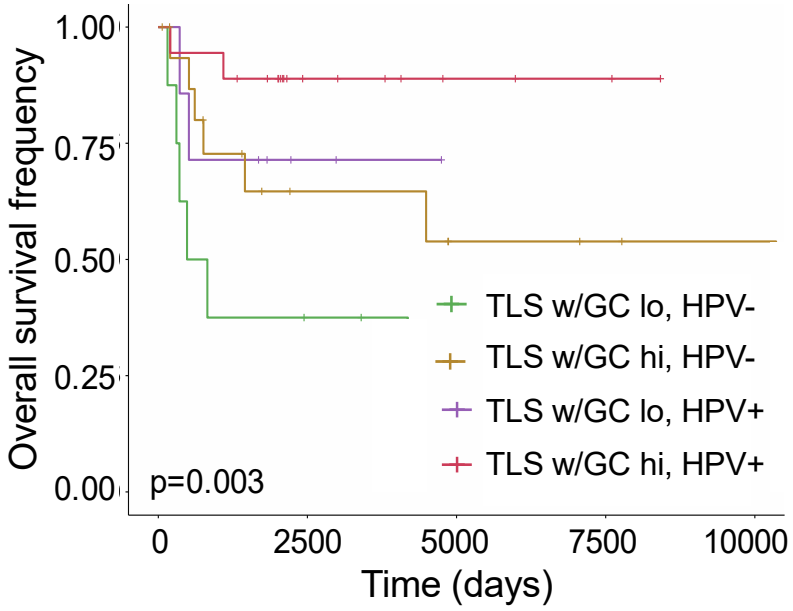
Evidence that the TME can influence TLS formation and maturation

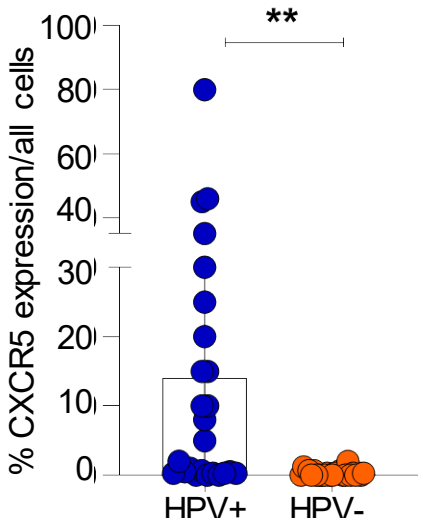
HPV+ Tumor BOT



HPV- Tumor Larynx

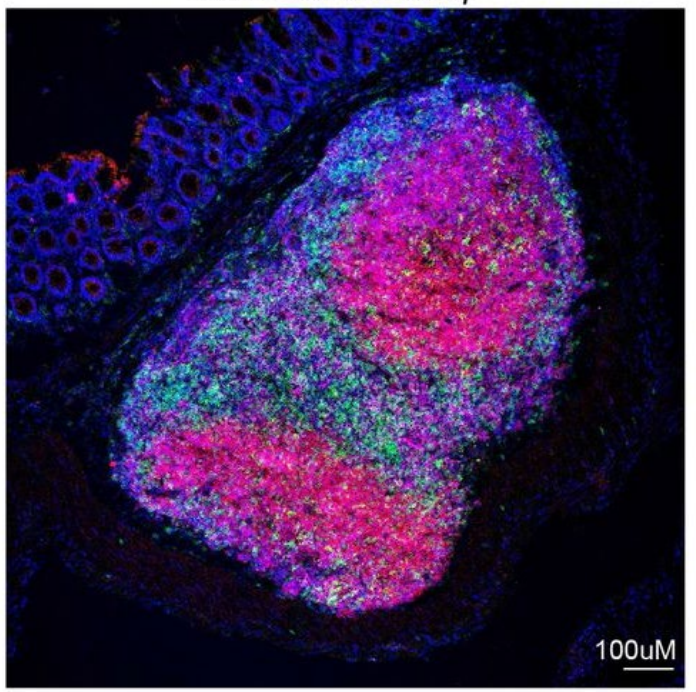


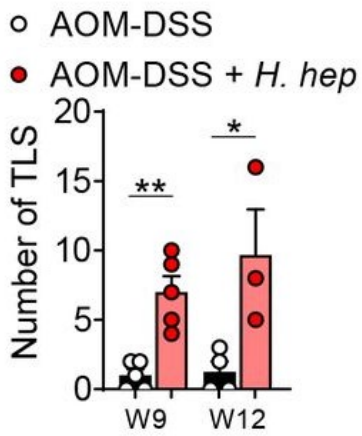




Ruffin, Cillo et al (Nat Comm 2021)

AOM-DSS + *Hhep*

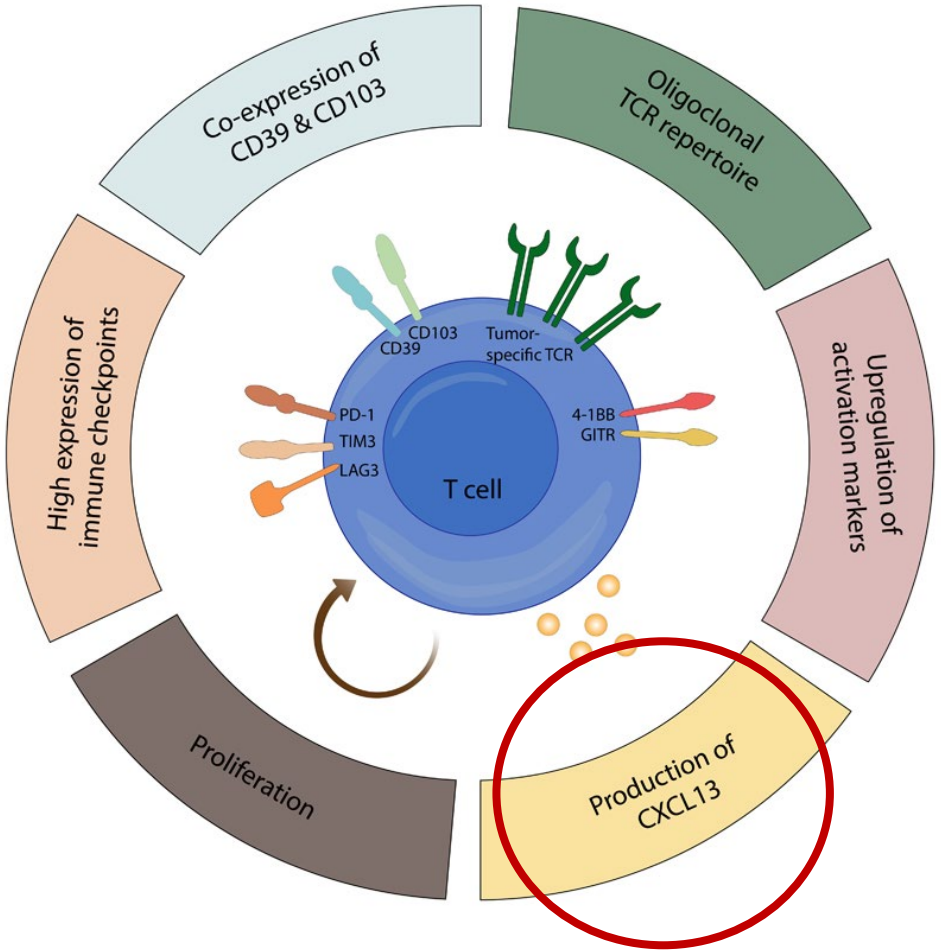


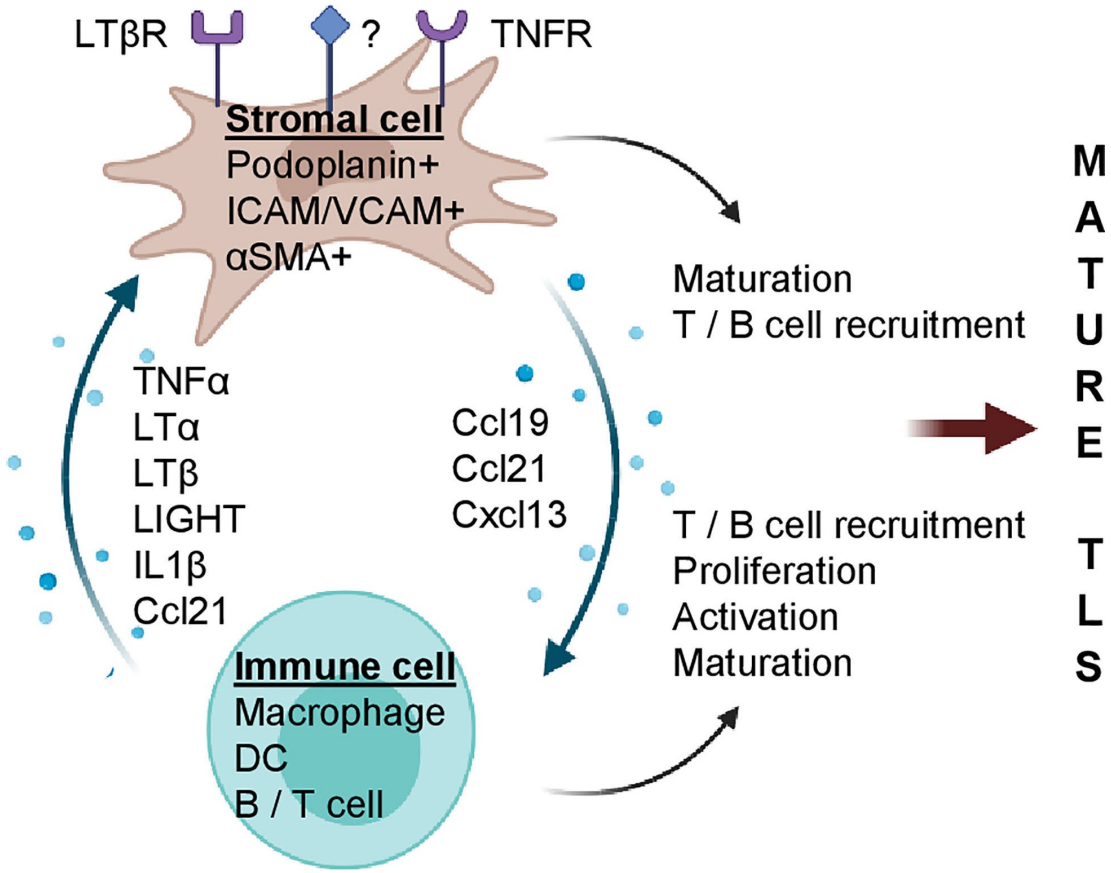


H. Hep specific CD4+ T follicular helper cells reside within TLS

Overacre-Delgoffe et al (Immunity 2021)

Tumor-specific CD8+ T cells and other immune cells participate in TLS formation





Reminder: TME is not canonical!

TFH, Treg, and tumor-specific CD8+ T cells can produce CXCL13

CD8 + T cell states in human cancer: insights from single-cell analysis

Anne M van der Leun [1], Daniela S Thommen [1], Ton N Schumacher [2]

Johansson-Percival et al (Front Immunol 2021)

Lymphotoxin signaling is an important TLS inducer

Redefining the Role of Lymphotoxin Beta Receptor in the Maintenance of Lymphoid Organs and Immune Cell Homeostasis in Adulthood

Yajun Shou[1,2], Ekaterina Koroleva[1], Cody M. Spencer[3†], Sergey A. Shein[1], Anna A. Korchagina[1], Kizil A. Yusoof[1], Raksha Parthasarathy[1], Elizabeth A. Leadbetter[1], Armen N. Akopian[4], Amanda R. Muñoz[1‡] and Alexei V. Tumanov[1‡]*

[1] Department of Microbiology, Immunology and Molecular Genetics, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States, [2] Department of Gastroenterology, Second Xiangya Hospital of Central South University, Changsha, China, [3] Trudeau Institute, Saranac Lake, NY, United States, [4] Department of Endodontics, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States

Abnormal Development of Peripheral Lymphoid Organs in Mice Deficient in Lymphotoxin

PIETRO DE TOGNI, JOSEPH GOELLNER, NANCY H. RUDDLE, PHILIP R. STREETER, ANDREA FICK, SANJEEV MARIATHASAN, STACY C. SMITH, REBECCA CARLSON, LAURIE P. SHORNICK, JENA STRAUSS-SCHOENBERGER, JOHN H. RUSSELL, ROBERT KARR, AND DAVID D. CHAPLIN fewer Authors Info & Affiliations

Lymphotoxin signalling in tertiary lymphoid structures and immunotherapy

Haidong Tang[1], Mingzhao Zhu[2], Jian Qiao[1] and Yang-Xin Fu[1,2]

Tertiary lymphoid structures (TLS) often develop at sites of persistent inflammation, including cancers and autoimmune diseases. In most cases, the presence of TLS correlates with active immune responses. Because of their proximity to pathological loci, TLS are an intriguing target for the manipulation of immune responses. For several years, it has become clear that lymphotoxin (LT) signalling plays critical roles in lymphoid tissue organogenesis and maintenance. In the current review, we will discuss the role of LT signalling in the development of TLS. With a focus on cancers and autoimmune diseases, we will highlight the correlations between TLS and disease progression. We will also discuss the current efforts and potential directions for manipulating TLS for immunotherapies.

Cellular & Molecular Immunology (2017) **14,** 809–818; doi:10.1038/cmi.2017.13; published online 17 April 2017

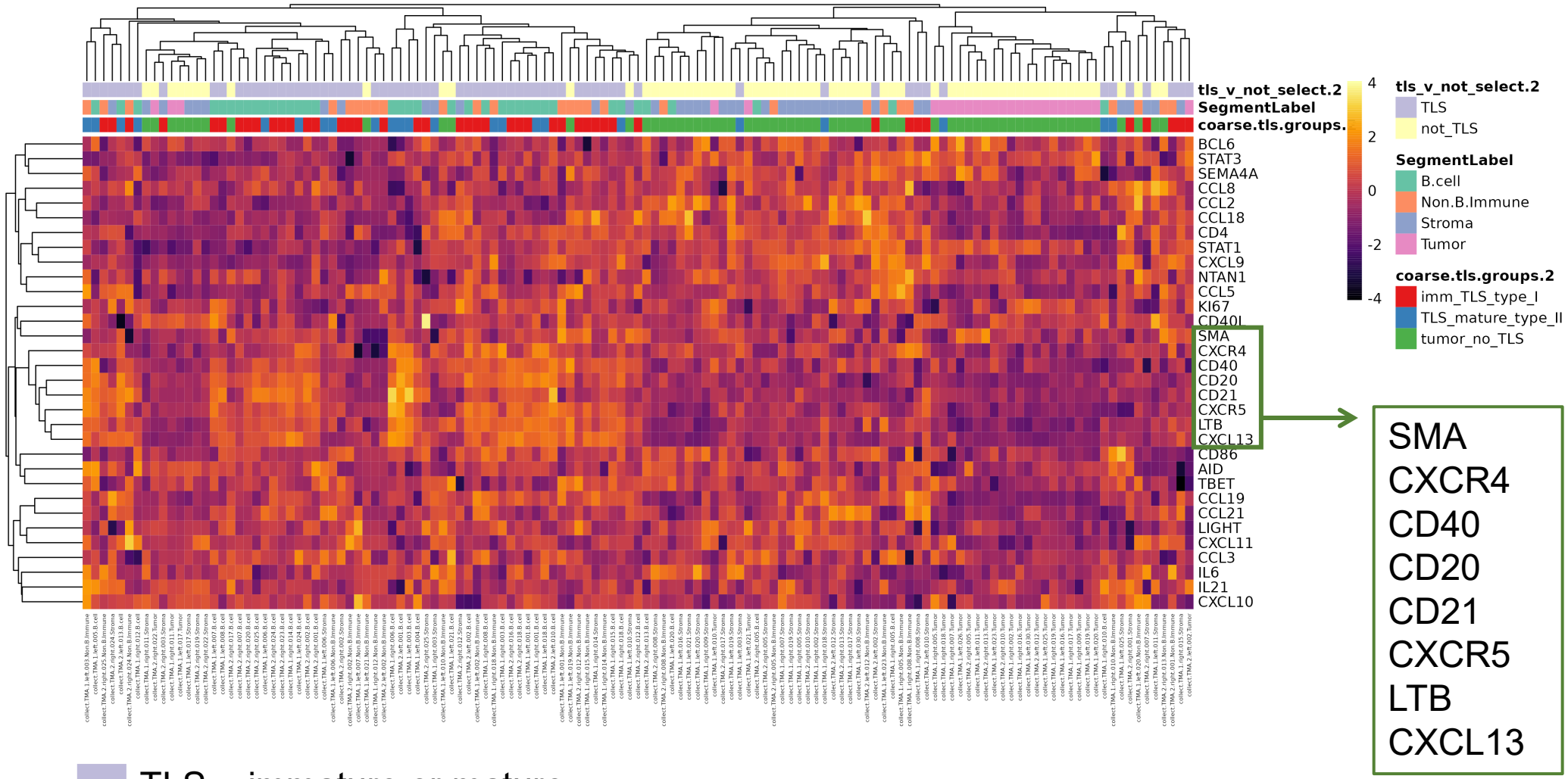
Lymphotoxin β receptor signaling is required for inflammatory lymphangiogenesis in the thyroid

Glaucia C. Furtado*, Tatjana Marinkovic*, Andrea P. Martin*, Alexandre Garin*, Benjamin Hoch†, Wolfgang Hubner‡, Benjamin K. Chen*‡, Eric Genden*§, Mihaela Skobe*¶, and Sergio A. Lira*‖

*Immunology Insitute and Departments of †Pathology, ‡Pharmacology and Biological Chemistry, §Otolaryngology, and ¶Oncological Sciences, Mount Sinai School of Medicine, New York, NY 10029-6574

Edited by Nancy Ruddle, Yale University School of Medicine, New Haven, CT, and accepted by the Editorial Board January 22, 2007 (received for review August 3, 2006)

Spatial transcriptomics of a lung cancer TMA reveals expression of TLS factors



SMA
CXCR4
CD40
CD20
CD21
CXCR5
LTB
CXCL13

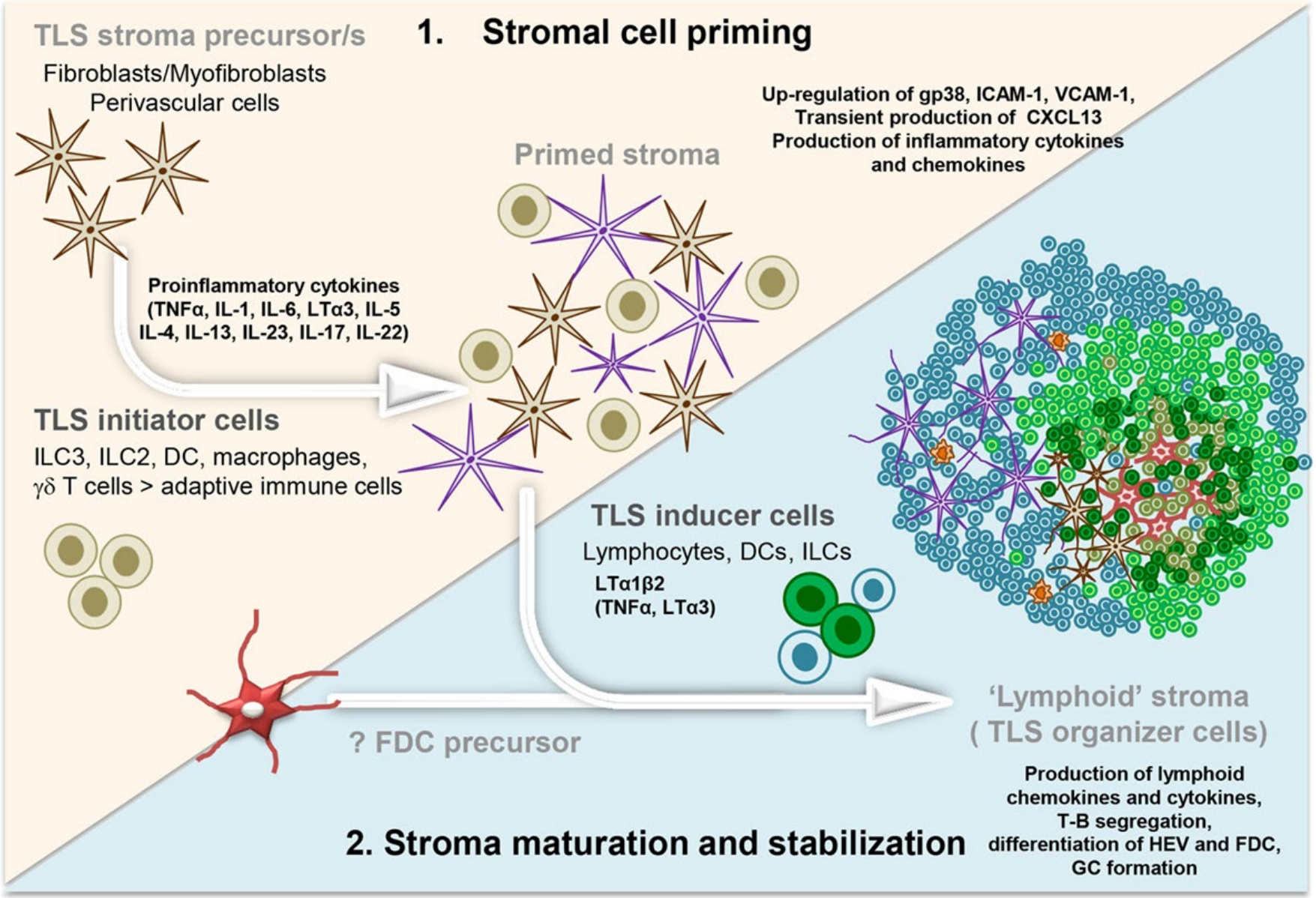
TLS = immature or mature

Not TLS = Tumor no TLS or TLS Adjacent

CONFIDENTIAL: DO NOT PHOTOGRAPH OR POST!

A big thanks to Brian Isett from Sunny Bao's research group!

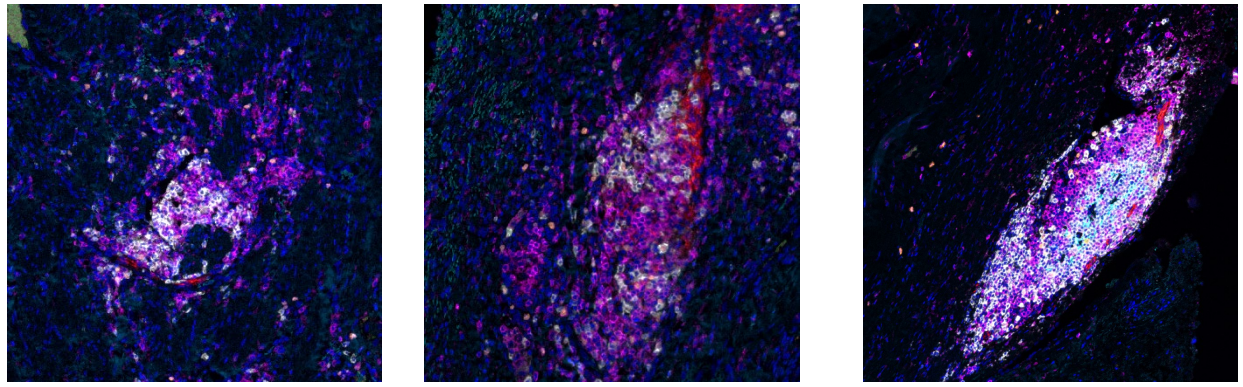
The stroma is complex, diverse, and essential for TLS formation



Barone et al (Front Immunol 2016)

Immature and mature TLS are distinct in HGSOC

***Tissue site dictates TLS formation, contexture, maturity, and activity**



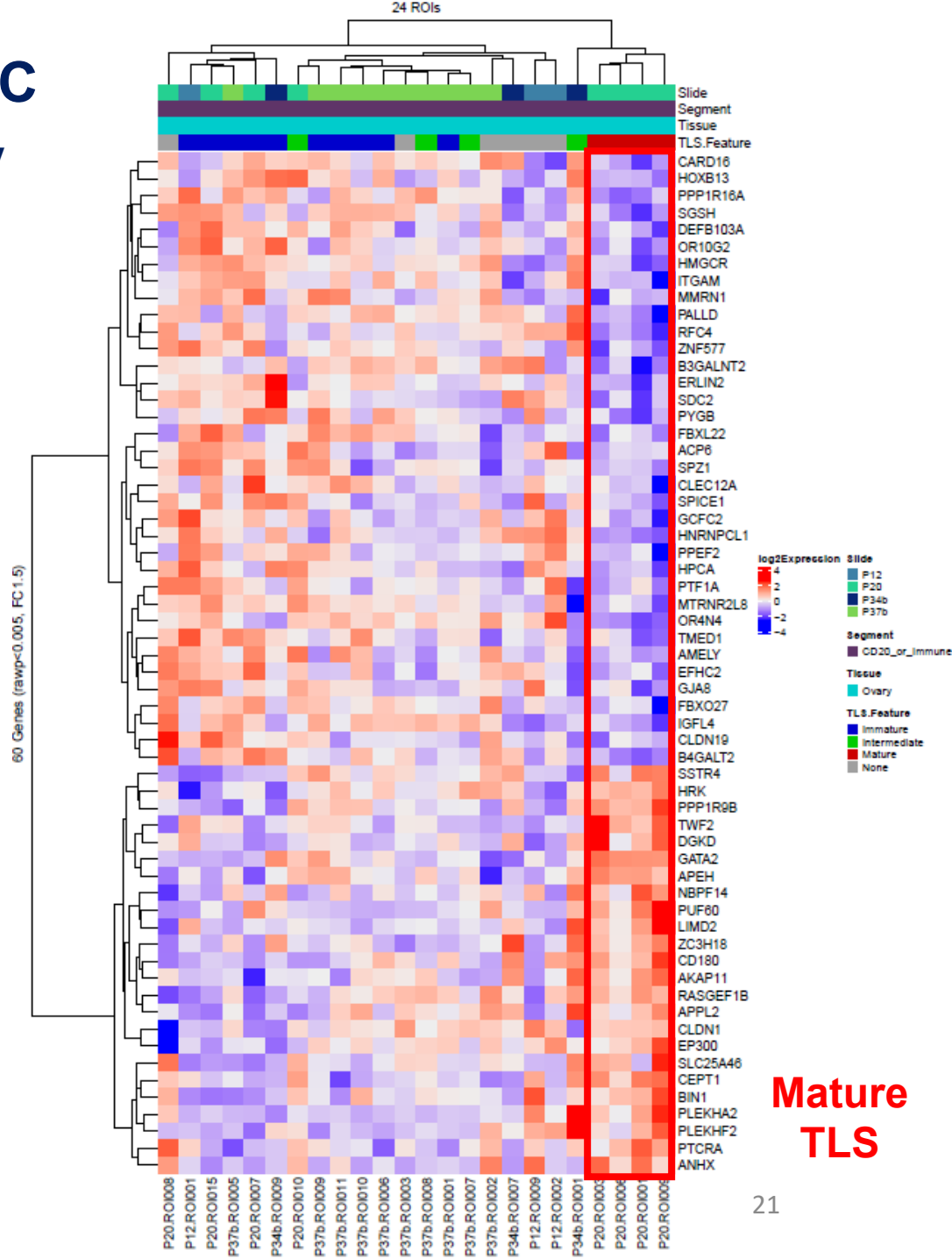
Increasing TLS maturation

Genes associated with TLS maturation

LIMD2: focal adhesion, motility

TWF2: actin cytoskeletal interaction

PLEKHA2: fibronectin, cell matrix adhesion



Mature TLS

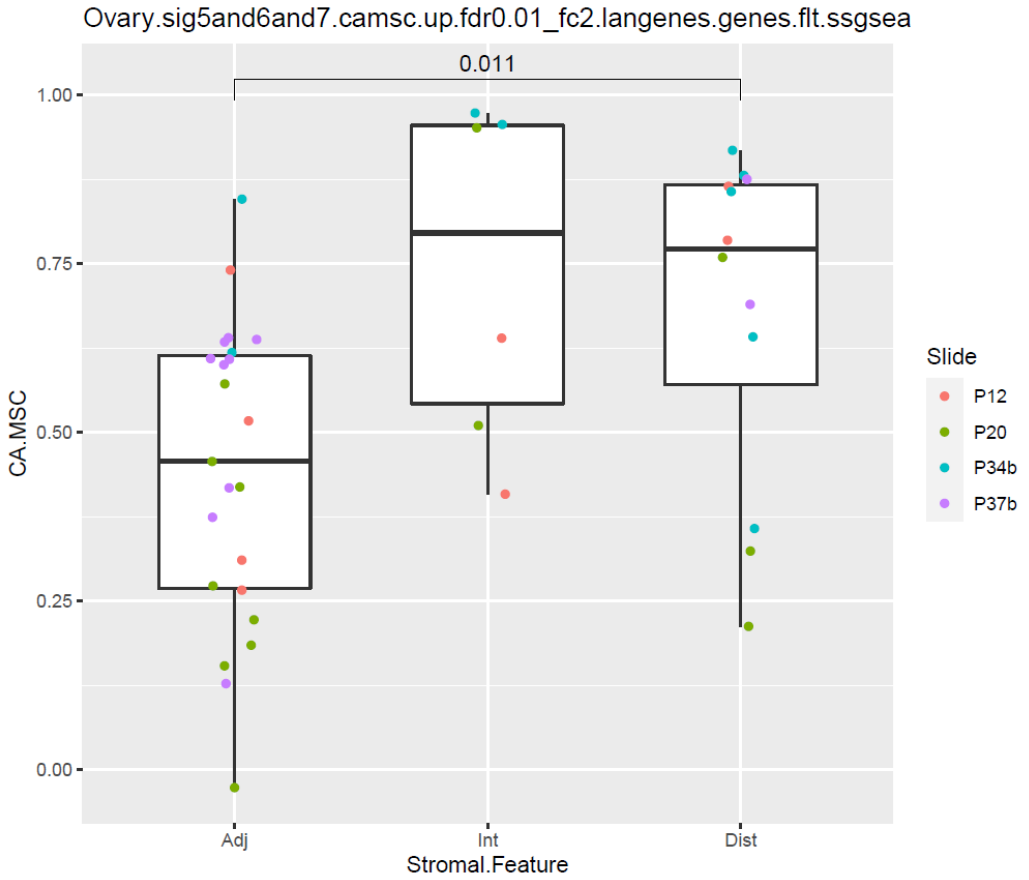
CONFIDENTIAL: DO NOT PHOTOGRAPH OR POST!

The stroma is important!
Cancer educated mesenchymal stem cells (CA-MSCs) regulate immunity

Lan Coffman, MD PhD

Bone marrow-derived MSCs (BM-MSCs)

- Migrate to inflamed areas (TME)
- Tumor Suppressive
- Precursors of Fibroblastic Reticular Cells (FRC)
    - TNF-α and LTα1β

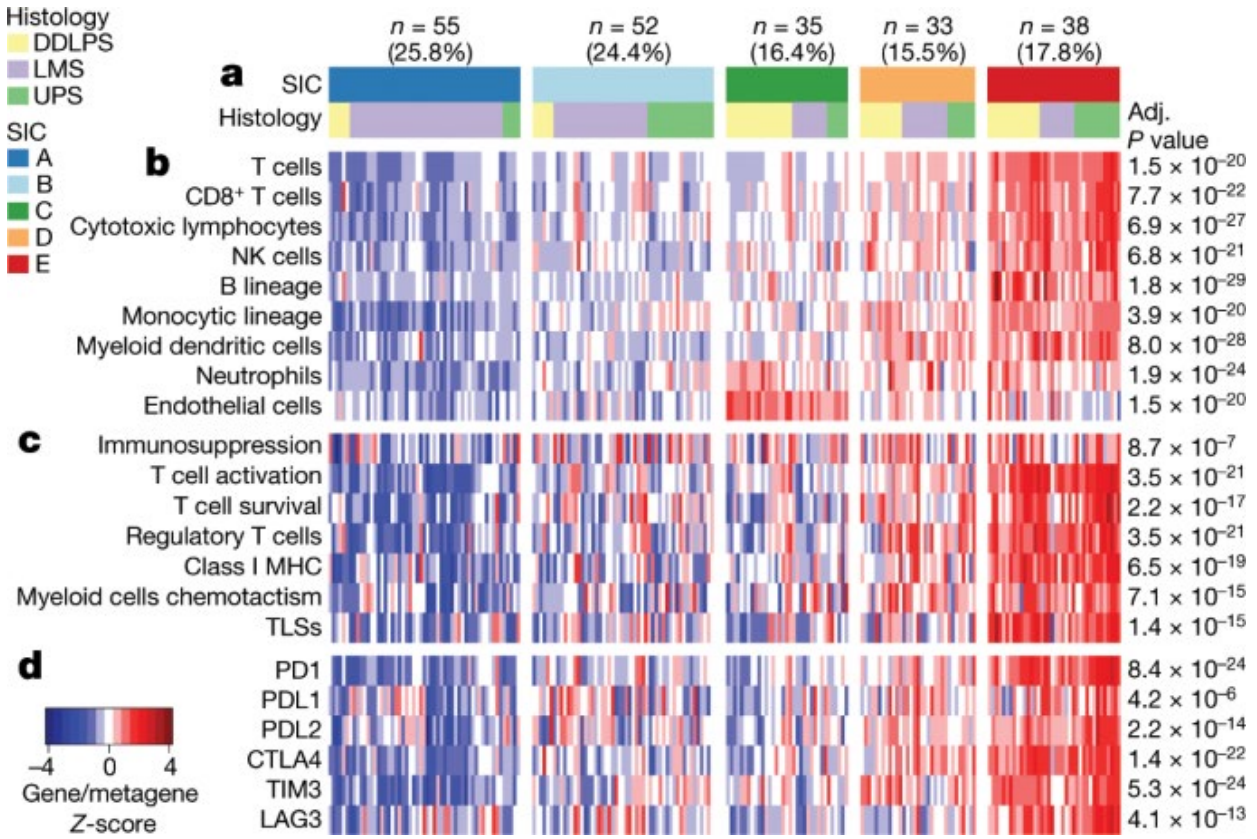


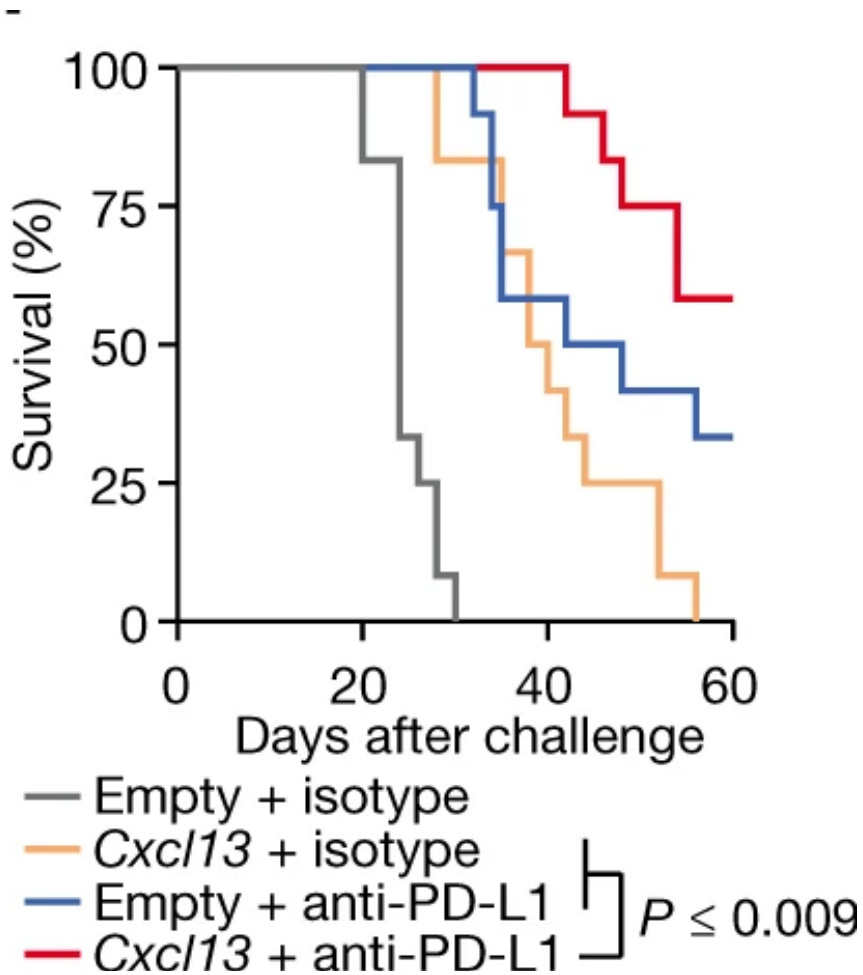
Cancer-associated MSCs (CA-MSCs)

- Reprogrammed in TME
    - Express **WT1**
- Promote tumor initiation, metastasis/chemo resistance
- A transcriptionally and epigenetically distinct population
- Immunosuppressive

Cancer reprogramming of MSCs strongly impacts TME and prognosis

A TLS biomarker is going to be essential for new clinical studies and improved IO therapies





Petitprez et al (Nature 2020)

Ng et al (Nature 2023)

Do we always need a germinal center for effective B cell responses?

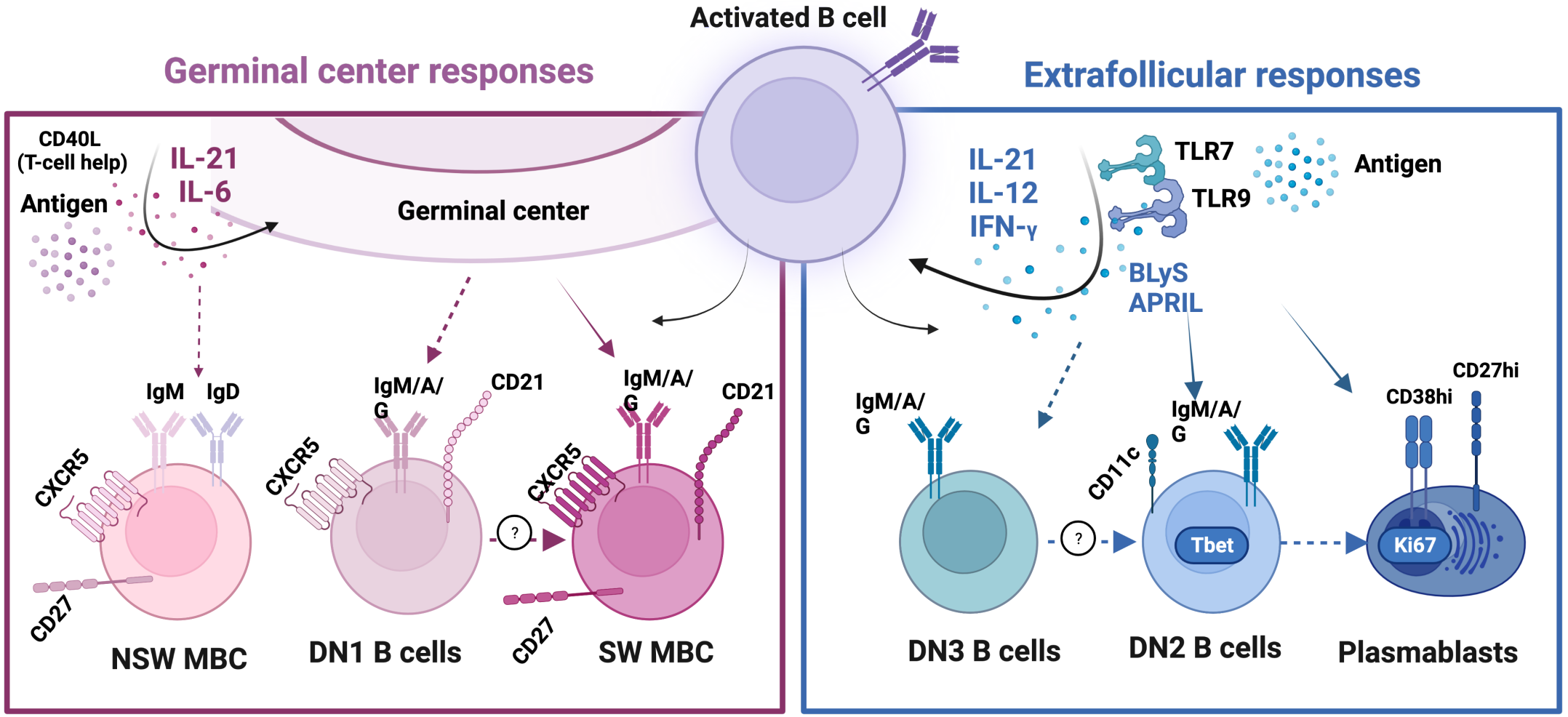
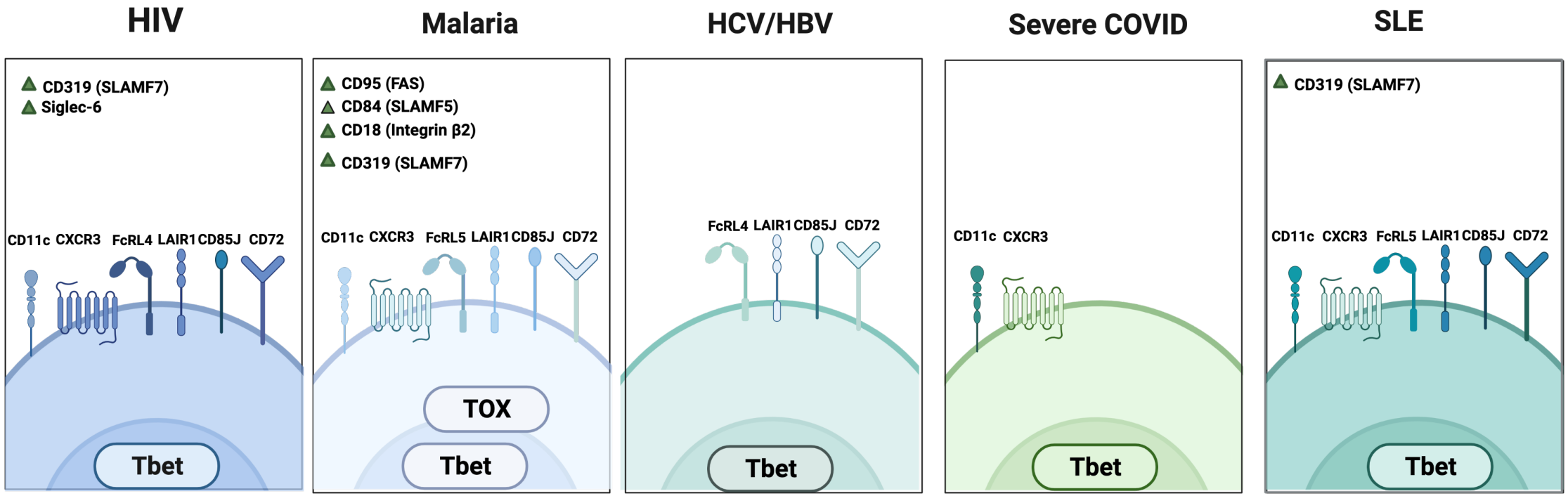


Figure courtesy of Dr. Ayana Ruffin

Elsner et al 2020, Immunity
Woodruff et al, 2020 Nature Immun
Jenks et al 2018, Immunity

Accumulation of EF associated memory B cells is a prominent feature of chronic infection and autoimmune disorders



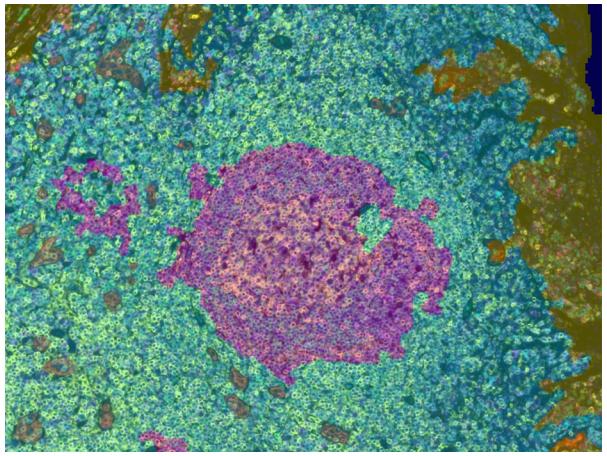
Created with Biorender.com

Jenks et al 2018 *Immunity*
Portgual et al 2017 *Cellular Immunology*

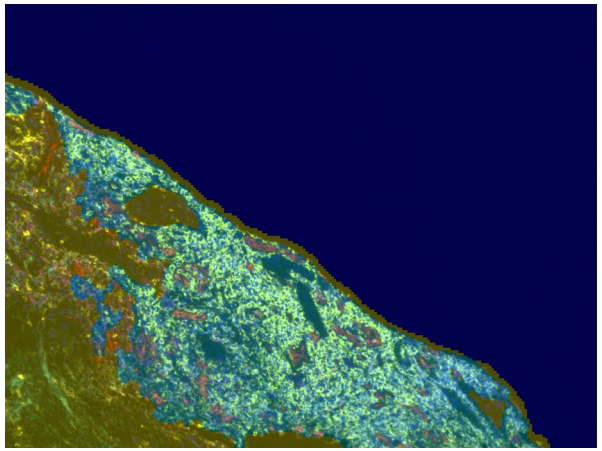
Austin et al, 2019 *Sci Transl Med*
Woodruff et al, 2020 *Nature Immunology*
Kardava et al, 2011 *J clin Invest*

Cellular neighborhoods can dictate memory B cell function

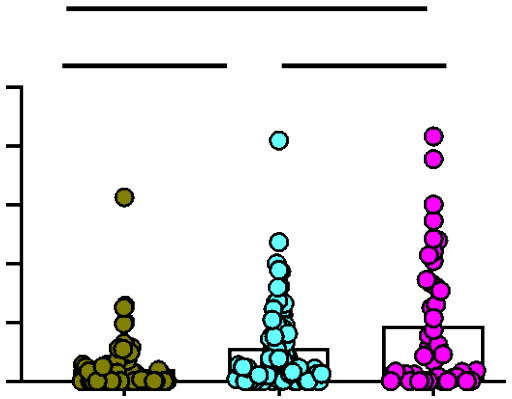
HPV+ BOT(TLS w/GC)

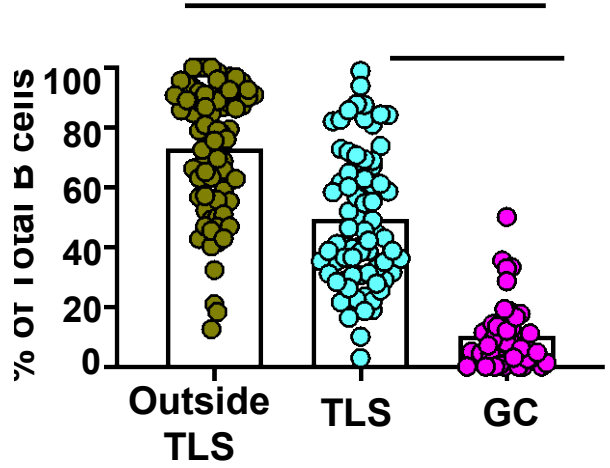


HPV- Tongue (TLS w/o GC)



TLS GC Outside TLS





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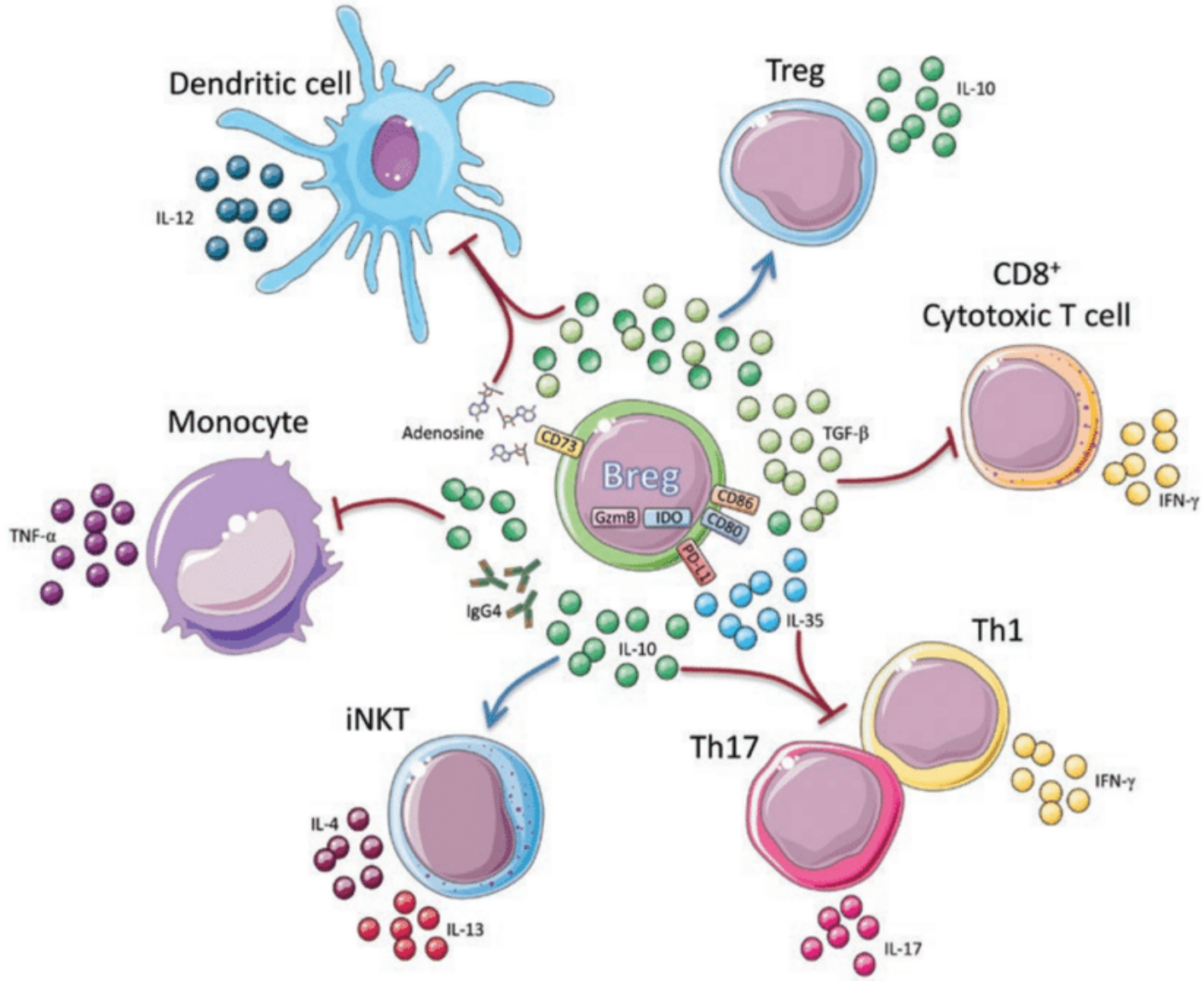
Ruffin et al, IN REVISION

Location of memory B cell subsets associates with function

	Ex vivo function					
	BCR signaling	Antibody production	Isotype Switching	Differentiation	Glucose Avidity	Mitochondria integrity
SW	+++	++	+++	+++	-	+++
DN3	-	-	+++	+	+++	-/+

Elsner et al 2020, Immunity
Woodruff et al, 2020 Nature Immun
Jenks et al 2018, Immunity

The proposed function of B regulatory cells



Maravillas-Montero JL, Acevedo-Ochoa E. Human B Regulatory Cells: The New Players in Autoimmune Disease. Rev Invest Clin. 2017 Sep-Oct;69(5):243-246. doi: 10.24875/ric.17002266. PMID: 29077695.

Table 1
Regulatory B cell description in the human setting

Phenotype	Activation	Action	Effect	Author
$CD19^{+}CD24^{hi}CD38^{hi}$	CD40L transfected cells	Soluble: IL-10 Contact: CD80 and CD86	Inhibition of INF-γ and TNFα $CD4^{+}$ T cell secretion	Blair et al. (2010) [4]
$CD27^{+}$ or $CD24^{hi}$	CpG-B and anti-Ig	Soluble: IL-10	Inhibition of effector T cell proliferation	Bouaziz et al. (2010) [3]
$CD27^{+}CD24^{hi}$	CpG-B and recombinant CD40L	Soluble: IL-10	Inhibition of TNFα monocyte secretion	Iwata et al. (2010) [5]

Confirmation of IL10 production is important but can be difficult to detect!

de Masson A, Le Buanec H, Bouaziz JD. Purification and immunophenotypic characterization of human B cells with regulatory functions. Methods Mol Biol. 2014;1190:45-52. doi: 10.1007/978-1-4939-1161-5_4. PMID: 25015272.

Further complexities with human Bregs

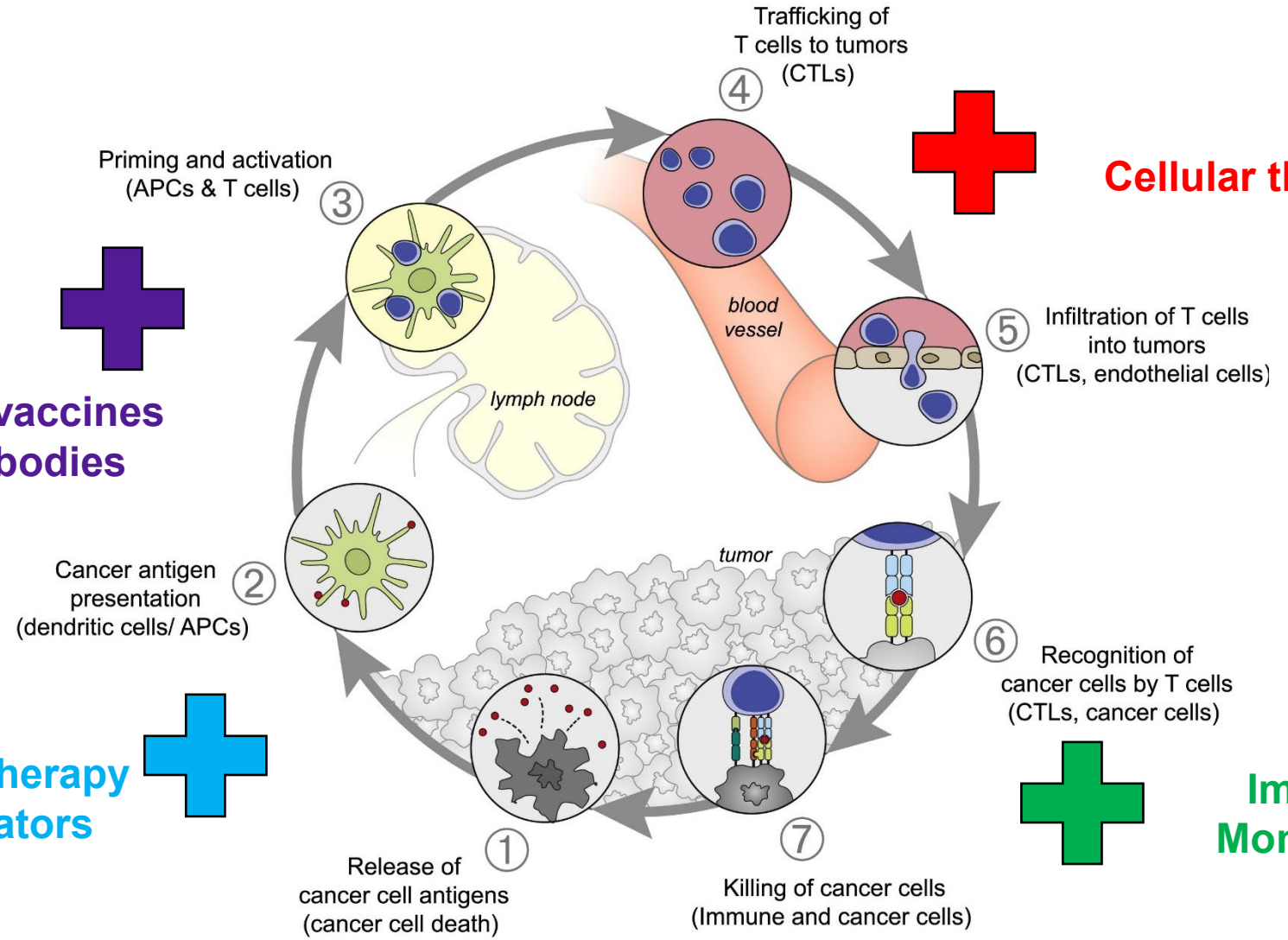
Table 1: Human regulatory B cells in health and disease: therapeutic potential

Table 1. Mechanisms of human Breg–mediated suppression

Subtype	Phenotype	Mechanism of suppression	Target of suppression	References
Immature B cells	$CD24^{hi}CD38^{hi}$	IL-10, PD-L1, CD80, CD86, CD1d	$CD4^+$ T cells, $CD8^+$ T cells, pDCs, iNKT cells	4, 18, 21, 59
B10 cells	$CD24^{hi}CD27^+$	IL-10	Monocytes	13
$GZMB^+$ B cells	$CD38^+CD1d^+IgM^+CD147^+$	GZMB, IL-10, IDO	$CD4^+$ T cells	14
Br1 cells	$CD25^{hi}CD71^{hi}CD73^{lo}$	IL-10, IgG4	$CD4^+$ T cells	19
Plasmablasts	$CD27^{int}CD38^{hi}$	IL-10	–	15
–	$CD39^+CD73^+$	Adenosine	$CD4^+$ T cells, $CD8^+$ T cells	22
iBregs	–	TGF-β, IDO	$CD4^+$ T cells	20
–	$CD19^+TIM1^+$	IL-10	$CD4^+$ T cells, $CD8^+$ T cells	16

J Clin Invest DOI: 10.1172/JCI85113

Cancer immunotherapies aim to reinitiate the cancer immunity cycle



Cellular therapies

Cancer treatment vaccines
Monoclonal antibodies

Oncolytic virus therapy
immunomodulators

Immunomodulators
Monoclonal antibodies

How would a B cell immunotherapy fit into the current treatment model?

Chen et al 2013 Immunity

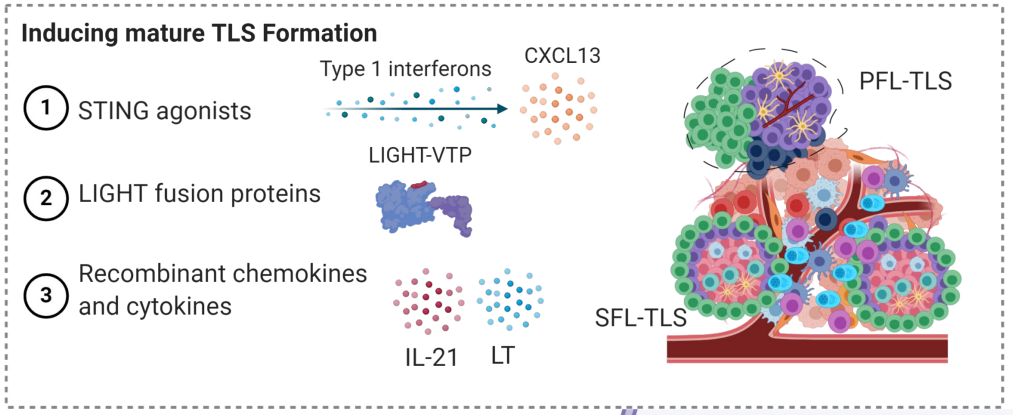
Targeting B cells and TLS for cancer immunotherapy

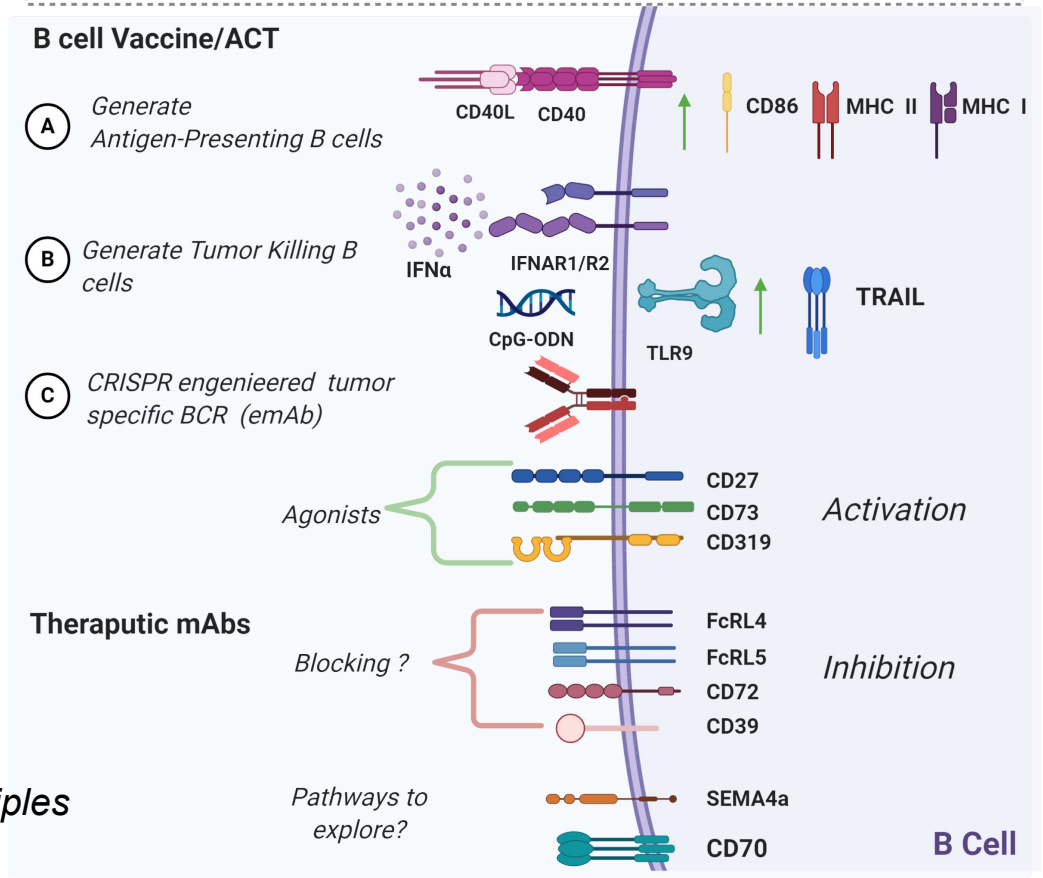
Oncolytic virus therapy immunomodulators

Adoptive cell therapy

Cancer treatment vaccines TLR agonists

Monoclonal antibodies (Agonistic/blocking)

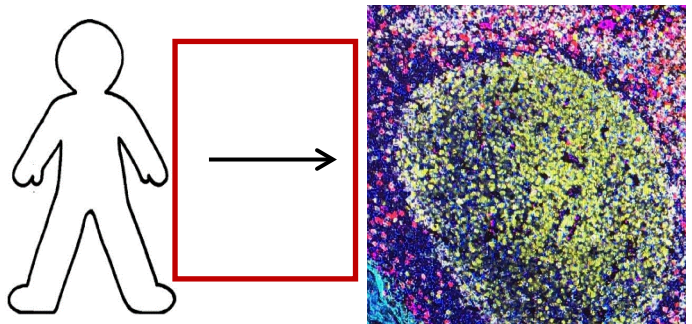




Ruffin A, Bruno T *Cancer Immunotherapy Principles and Practice 2nd edition* 2021

Take home messages and clinical translation

- ✓ B cells are important humoral mediators of adaptive immunity
- ✓ B cell function is dictated by cellular neighborhoods
- ✓ TLS are complex immunological hubs that are correlated with improved survival and IO response
- ✓ TLS frequency and composition is varied depending on the TME
- ✓ Spatial transcriptomics is key for revealing differences in TLS formation, maturation, and activity
- ✓ TLS initiating and TLS-maintaining factors are decreased in tumor TLS
- ✓ TLS formation and maturation is regulated by the stroma
- ✓ B-T cell crosstalk and function is dictated by TLS formation



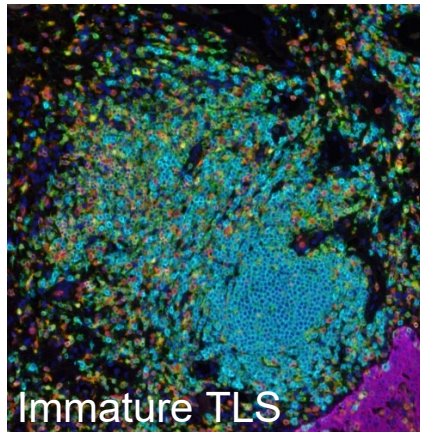
Tumor-stroma-immune-microbiome

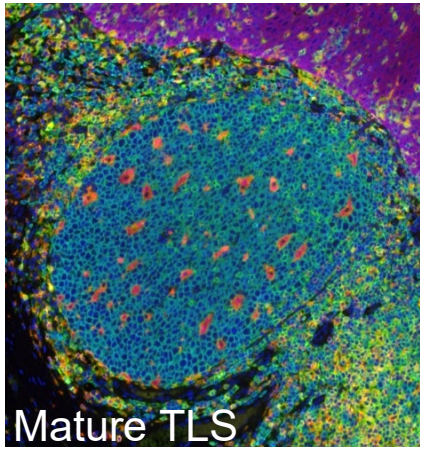
Mature TLS

Increased CD8+ T cells

Feedback loop from CD8+T cells via CXCL13

Anti-PD1 blockade for enhanced tumor killing





University of Pittsburgh

Immunology Department

Acknowledgements

UPMC | HILLMAN CANCER CENTER

The Bruno Lab

Ayana Ruffin
Sheryl Kunning
Ian MacFawn
Hye Mi Kim
Asia Williams
Noor Nader
Grant Magnon
Jennifer Lue
Allie Casey
Medard Kaiza
Rufiaat Rashid
Zel Zhang
Hannah Bumgarner
Alex McDonough
Caleb Lampenfeld
Mia Liu
Xiang Li
Anjali Rohatgi

Lung Cancer
Laura Stabile
Tim Burns
Dave Wilson
James DeGregori (Colorado)

Ovarian Cancer
Lan Coffman

HNSCC SPORE
Robert Ferris
Heath Skinner
Amy Cuda
Carly Reeder

Dario Vignali Lab
Anthony Cillo

Team TLS
Jen Wargo
Tina Cascone
Kevin McBride
Nik Joshi
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UColorado HIMSR

Dept of Otolaryngology
Seungwon (Steve) Kim
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MEL SPORE
John Kirkwood
Hassane Zarour

Normal tissues
Alison Morris
John Sembrat
Bill Janssen (NJH-Colorado)

Cancer Bioinformatics
Riyue Bao
Raj Acharya
Brian Isett

HCC CF
Mike Meyer
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AACR-Johnson and Johnson
DoD Lung Cancer Research Program
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Mark Foundation Endeavor Award
DoD Melanoma Research Program
U Pitt Skin SPORE
U Pitt HNSCC SPORE
Sy Holzer Immunotherapy Award
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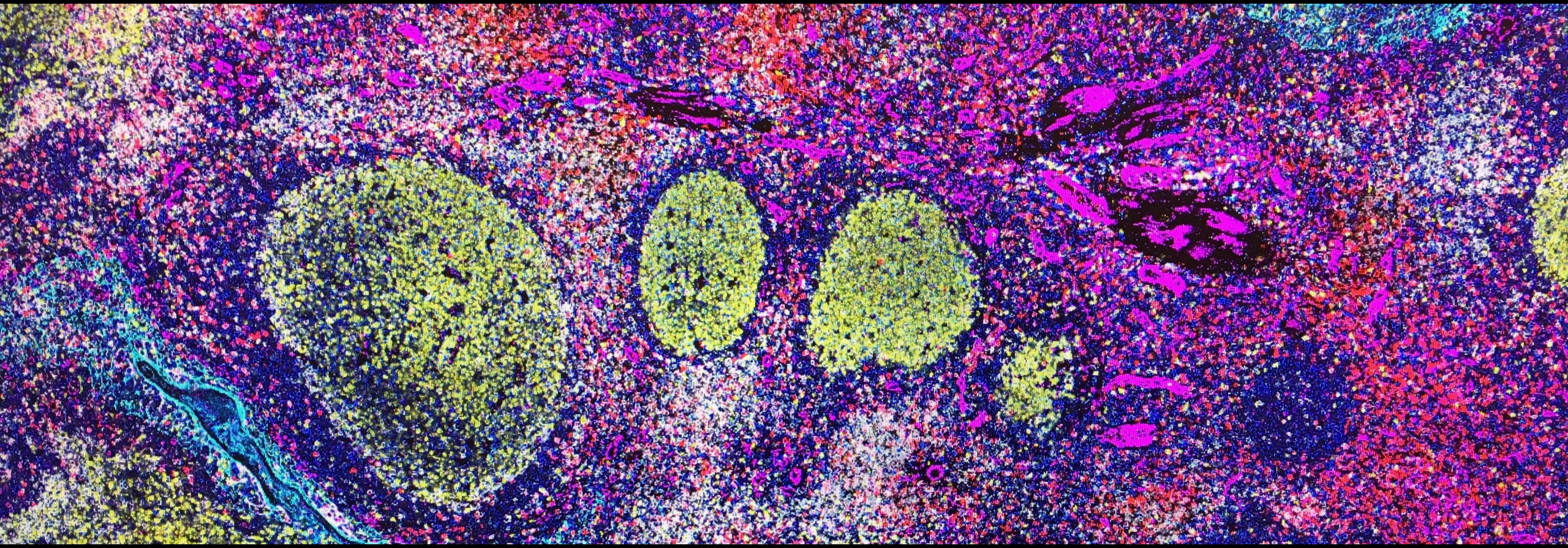
Patients and their families!

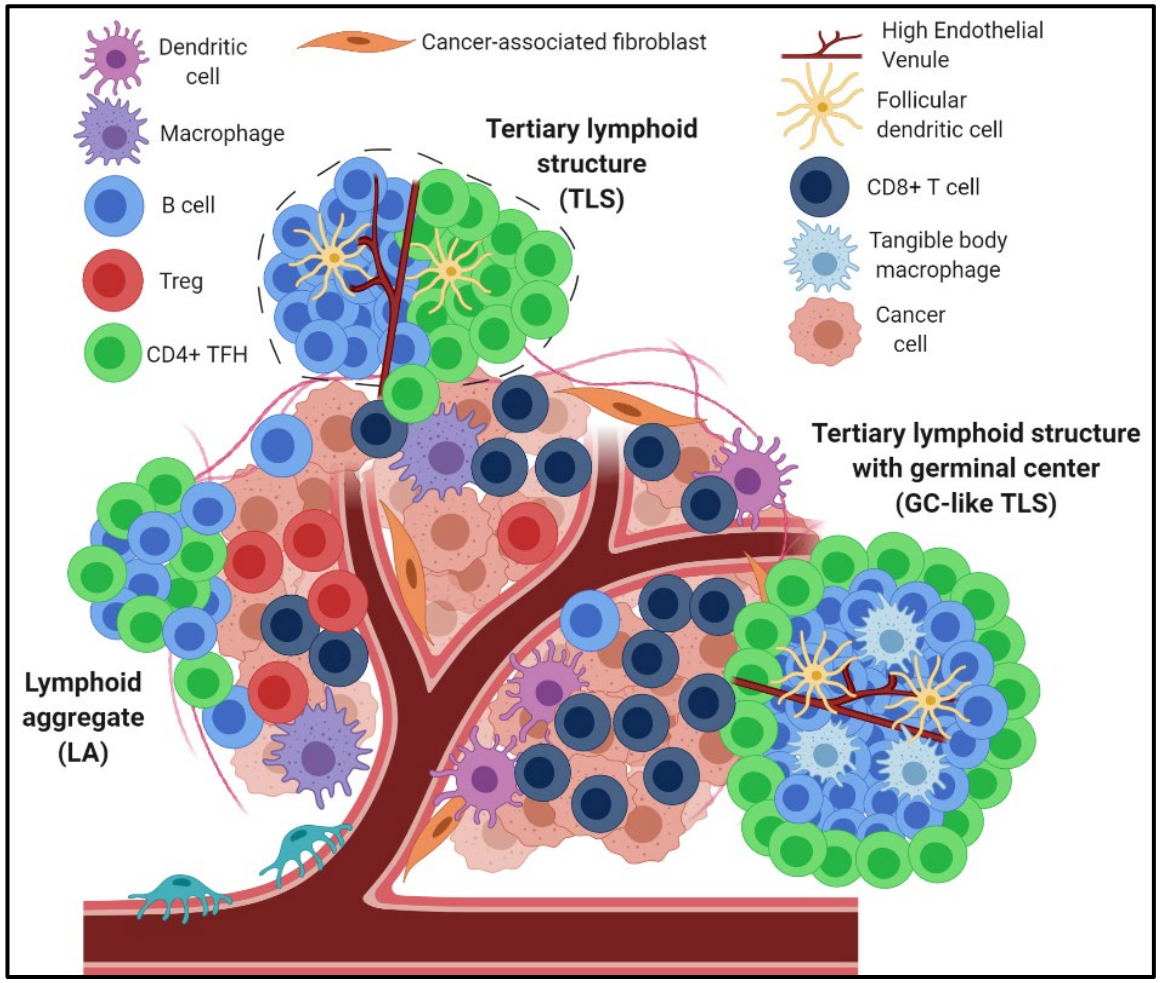
Thank you for your attention!

Interested in B cells and TLS? Join our BC^3 consortium!



Or come to our IN-PERSON Keystone—October 2023

Despite all these data, there are still key unanswered questions!



Key questions:

- By understanding the function of B cells within TLS, can we implement a novel, B cell-focused immunotherapy?
- What is the specificity of B cells in the TME?
- How do we increase tumor-specific antibody production in cancer patients?
- Does ICI modulate B cell and TLS function?
- How do B cells and TLS improve ICI?