

# Opportunities for Precision Medicine in Lymphoma

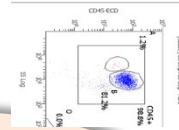
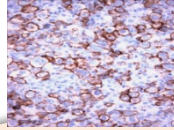
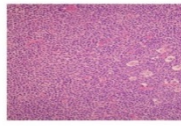
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Professor, Director of Hematopathology  
University of Pennsylvania



Penn Medicine

# Integrated evaluation of molecular abnormalities in hematologic disease



Gene rearrangements

Chromosomal translocations

Gene mutations

Epigenome

Altered gene expression

SNP array

aCGH

NGS

Mass spectrometry

New insights into pathogenesis

Next Generation Pathologist

Early Detection

Diagnostics

Prognostics

New Therapeutics

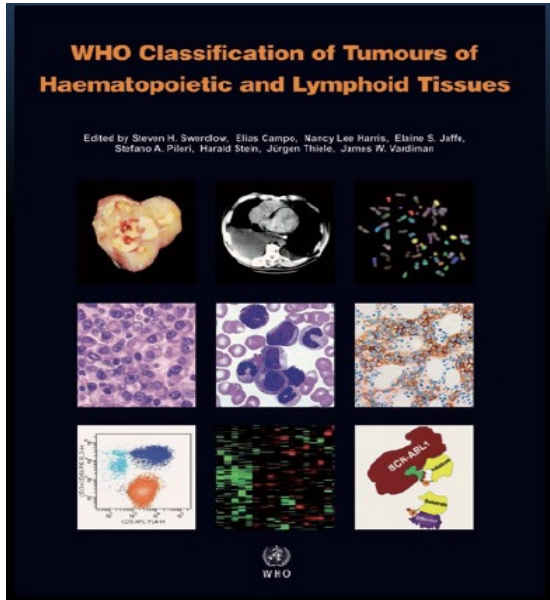
# Personalized Medicine

## Impact on Hematopathology

- Multiplexed technologies such as NGS and mass spectrometry in moving into clinical care
- Expanding arsenal of targeted and immune oncology therapies
- Assessment of diagnostic, prognostic and predictive molecular markers is becoming more standard in clinical care
- Use of blood specimen analysis for circulating cell-free DNA and RNA moving into clinical medicine

# Objectives

- Enhance understanding of the **genetic heterogeneity** of lymphoma
- Gain information about how genetic alterations in lymphomas may aid in the **diagnosis and disease monitoring**
- Better understanding of the opportunities for **tailored therapies** in patients with lymphomas



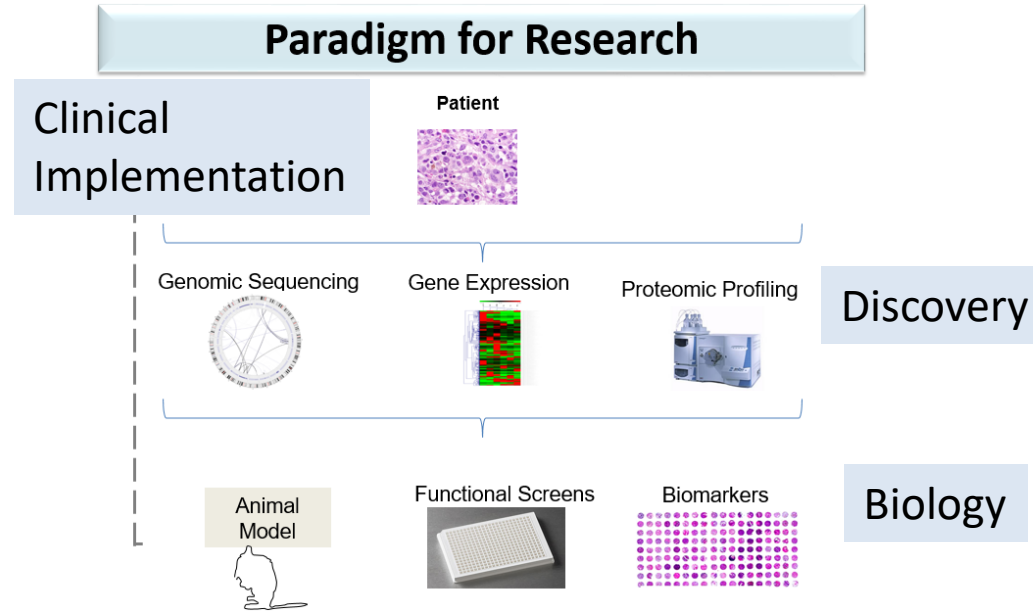
# Outline

## Splenic Lymphoma

Role of circulating tumor DNA in diagnosis and management

## Anaplastic large cell lymphoma

Genetics in diagnosis and disease monitoring

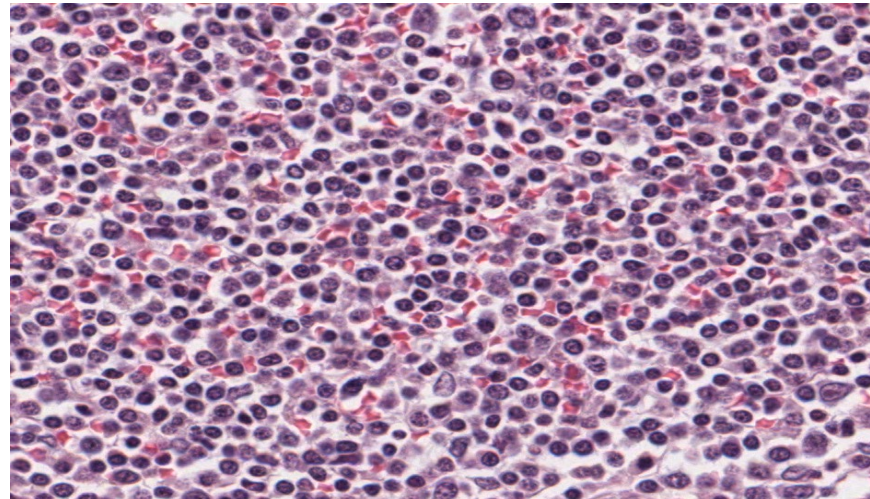
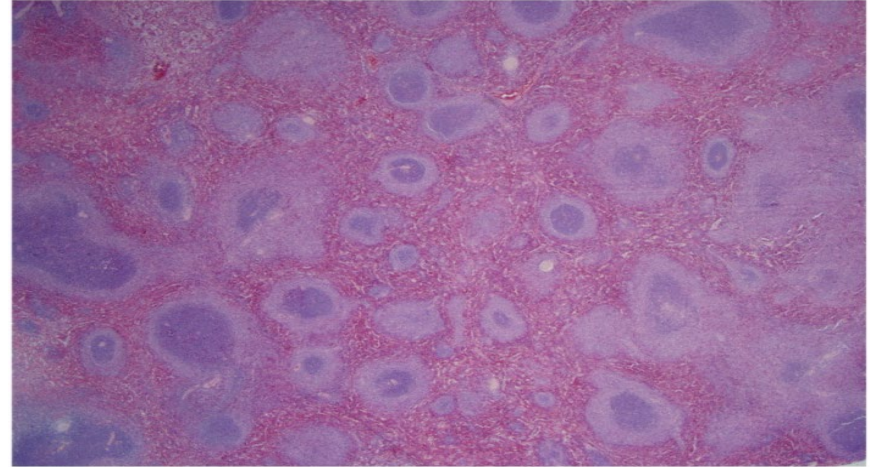


# Case 1

A 53 year old man with abdominal discomfort.

Physical examination and radiologic studies revealed an enlarged spleen which was removed.

The spleen weighed 3.55 kg with dimensions of 25 x 19.5 x 11.7 cm.

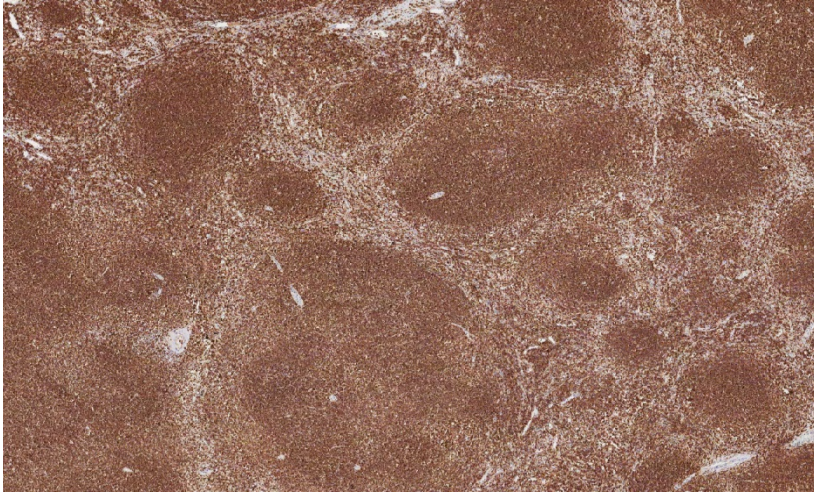


# Differential diagnosis

- Reactive
- Splenic marginal zone lymphoma
- Splenic B-cell lymphoma, unclassifiable
- Follicular lymphoma
- Mantle cell lymphoma
- Chronic lymphocytic leukemia/SLL
- Hairy cell leukemia
  
- T cell lymphoma

# Immunophenotype

CD20

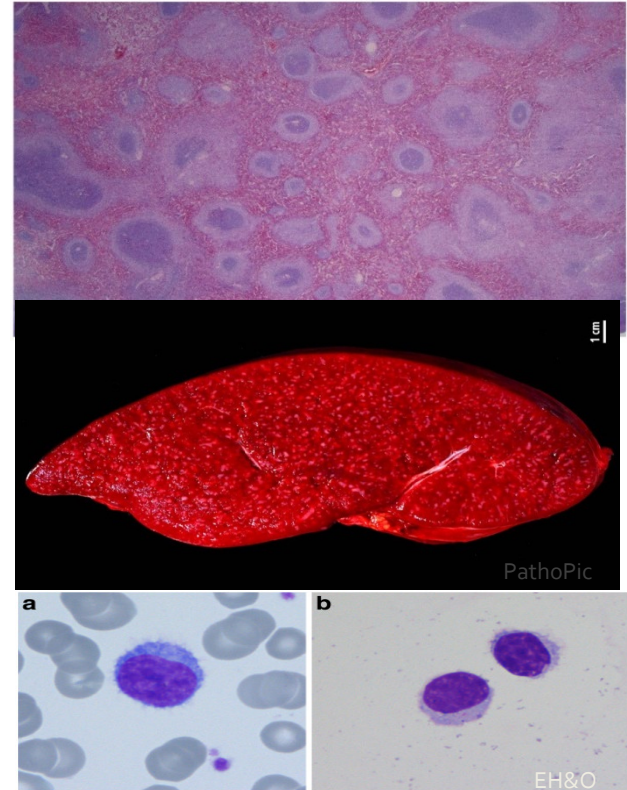


CD5 negative  
CD43 negative  
Cyclin D1 negative  
CD10 negative



# Splenic Marginal Zone Lymphoma

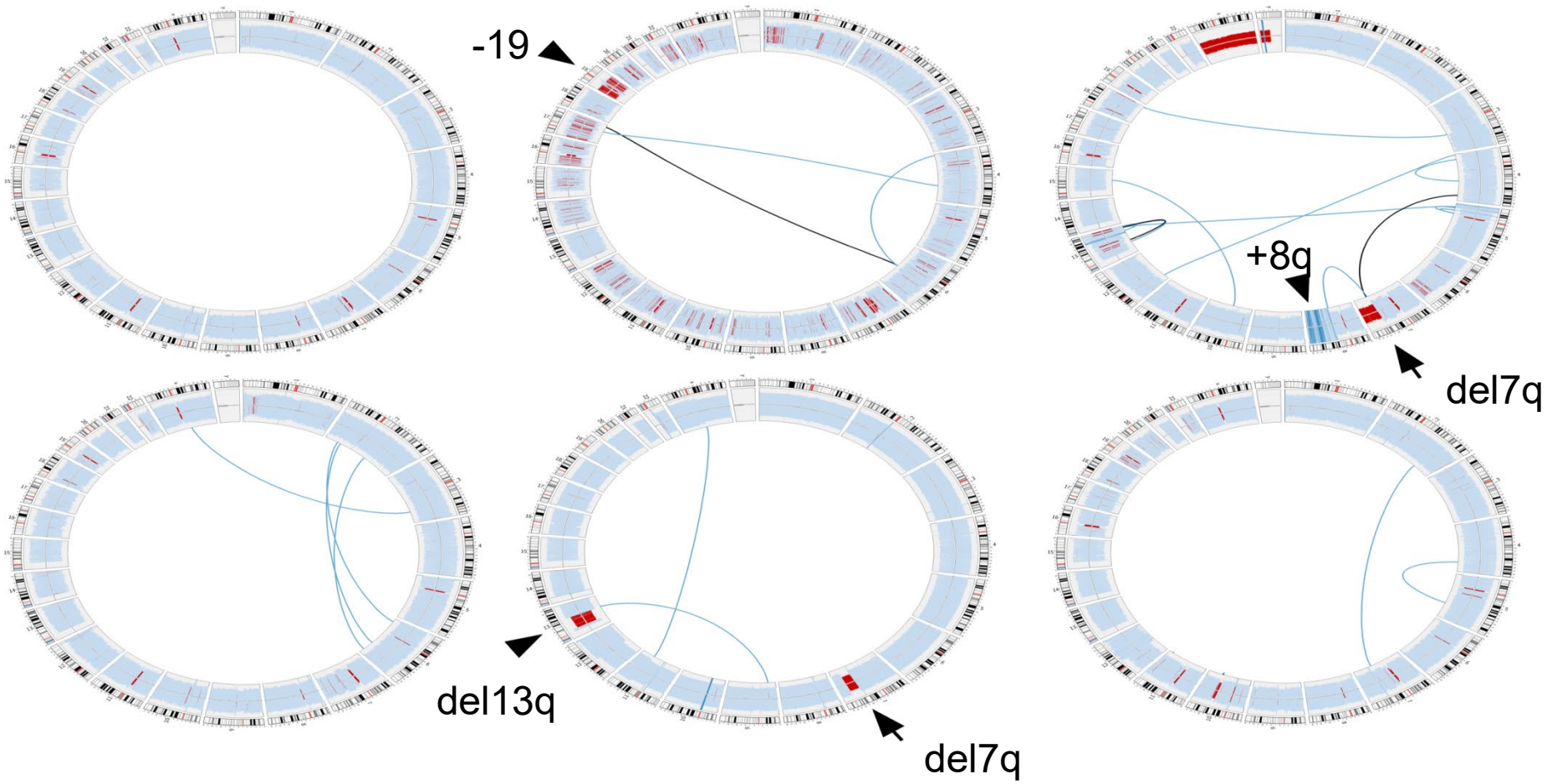
- Indolent lymphoma involving splenic white pulp, blood, and bone marrow
- Splenomegaly
- Bone marrow and PB exam leads to splenectomy for diagnosis
- Striking clinical variability dependent on tumoral load and performance status
- First-line therapies
  - splenectomy
  - anti-B-cell biologicals
- Median survival 10yr



# Molecular genetics of splenic lymphoma

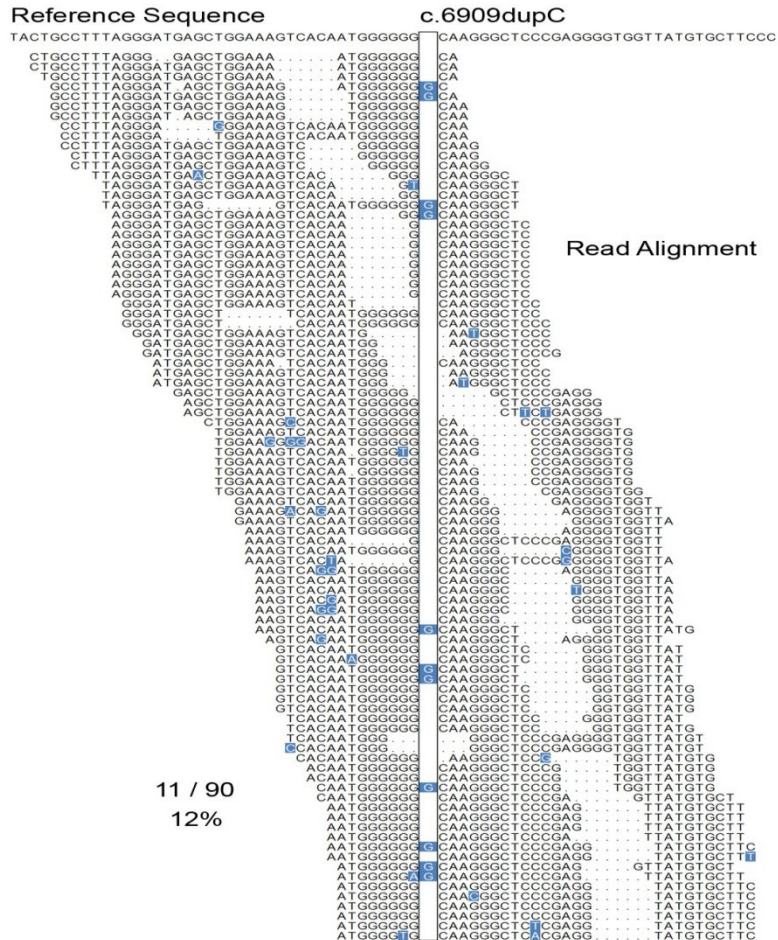
- del7q, +3/+3q [+18, +12]
- no recurrent translocation
- no known genetic etiology (circa 2012)

# SMZL Genome Complexity

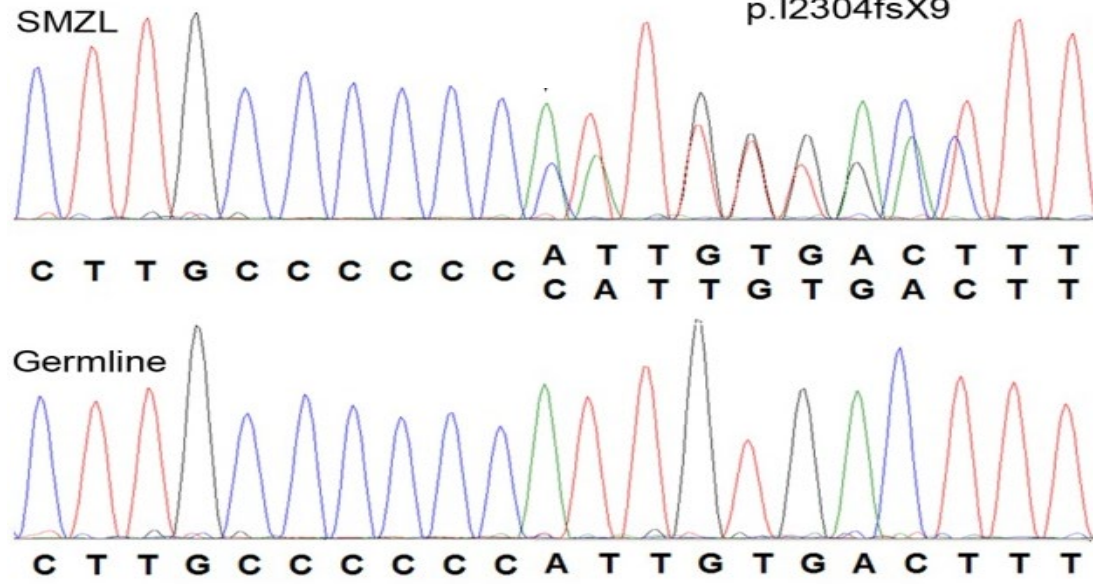


# NOTCH2

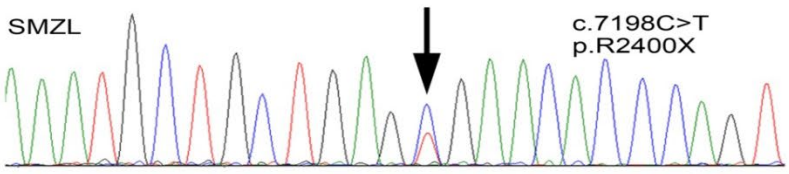
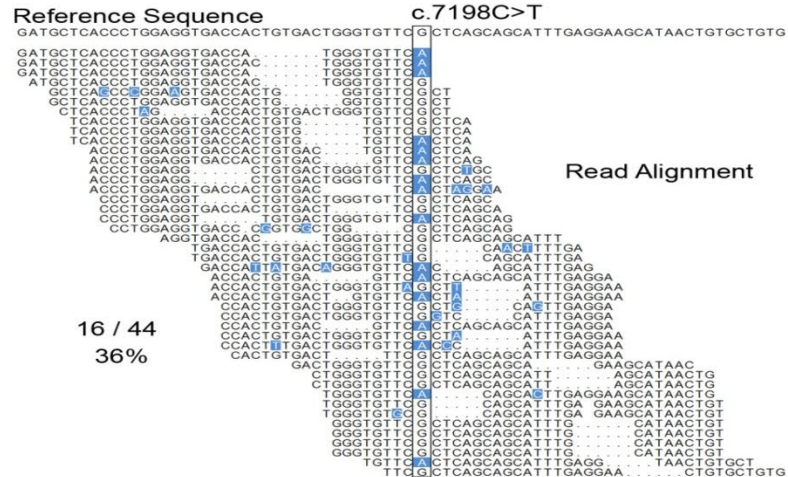
## Frameshift Mutation



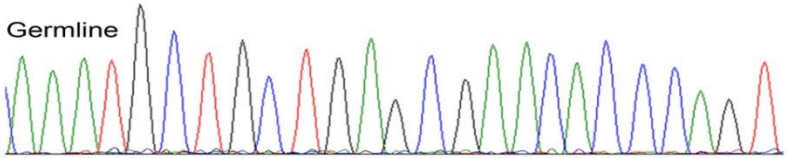
c.6909dupC  
p.I2304fsX9



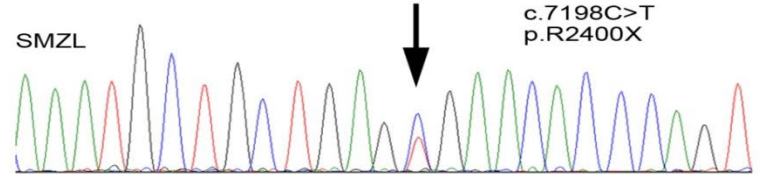
# Recurrent *NOTCH2* Nonsense Mutations



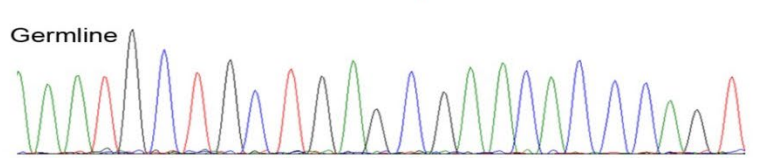
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A A A T G C T G C T G A G C T G A A C A C C C A G T

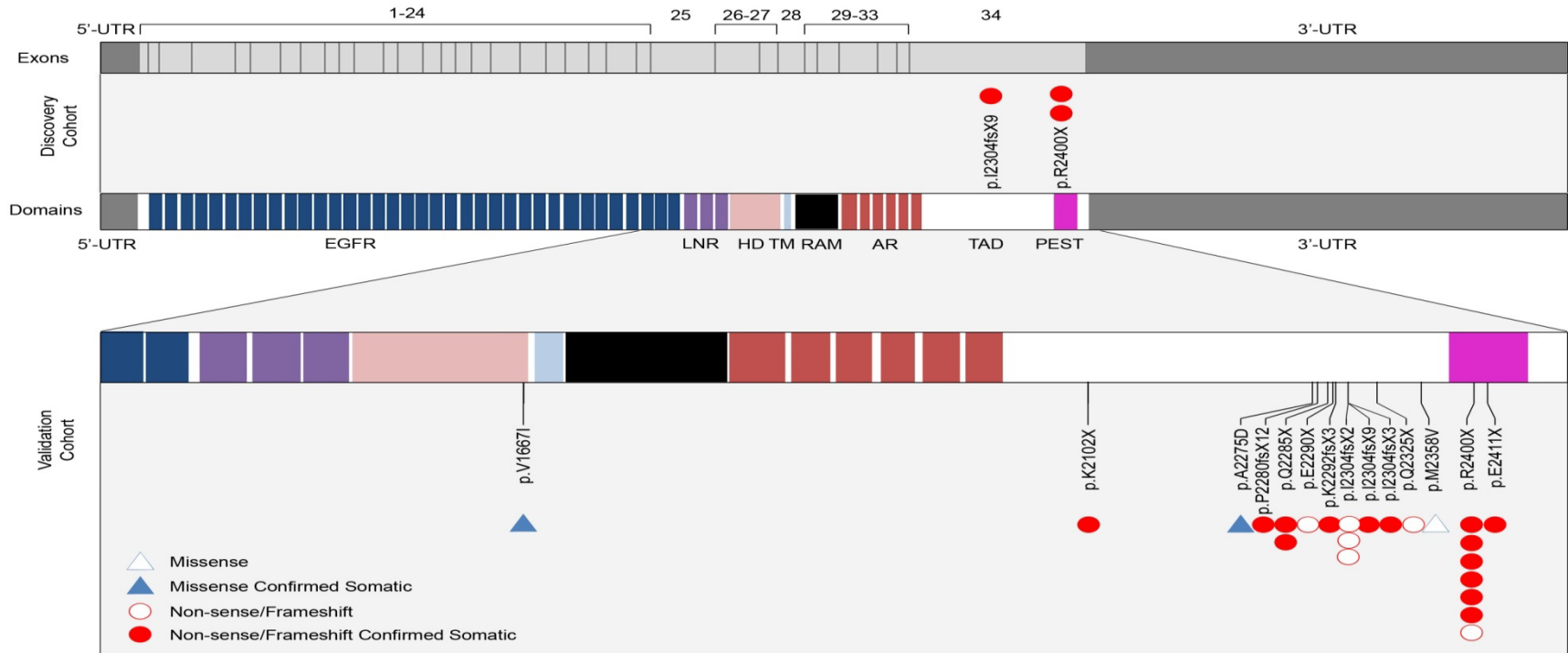


A A A T G C T G C T G A G C T G A A C A C C C A G T



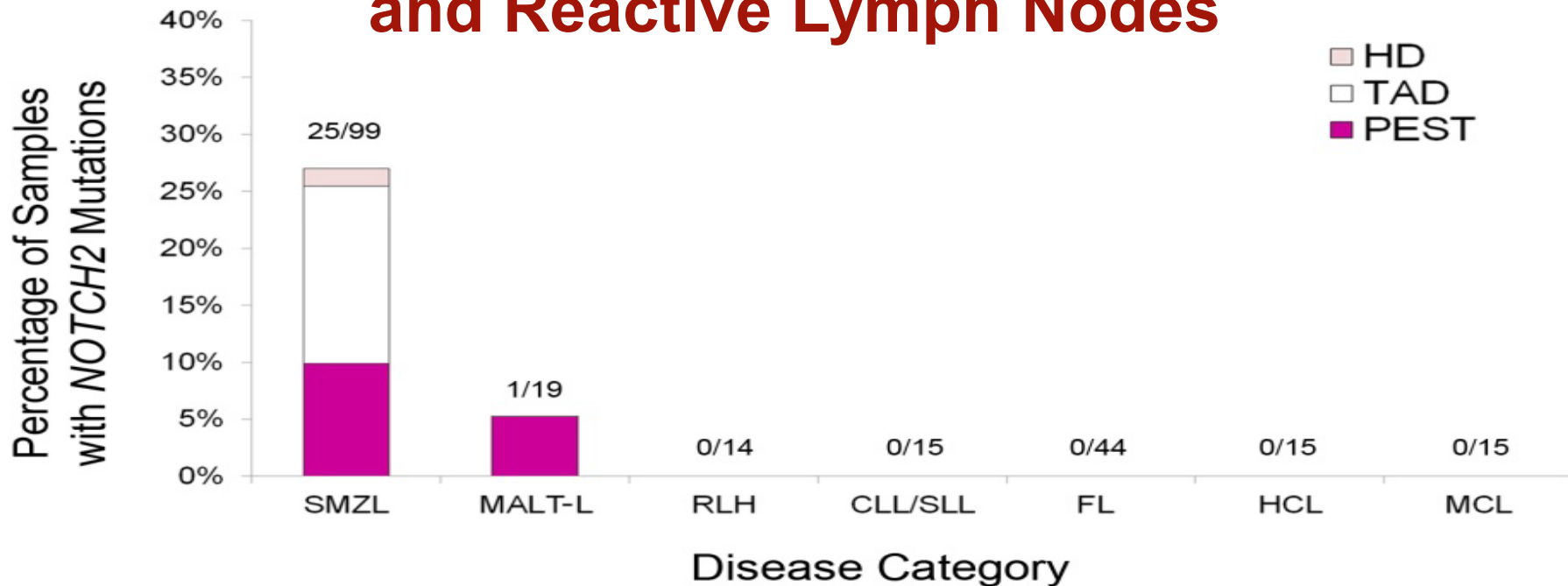
A A A T G C T G C T G A G C T G A A C A C C C A G T

# Recurrent *NOTCH2* Mutations in SMZL

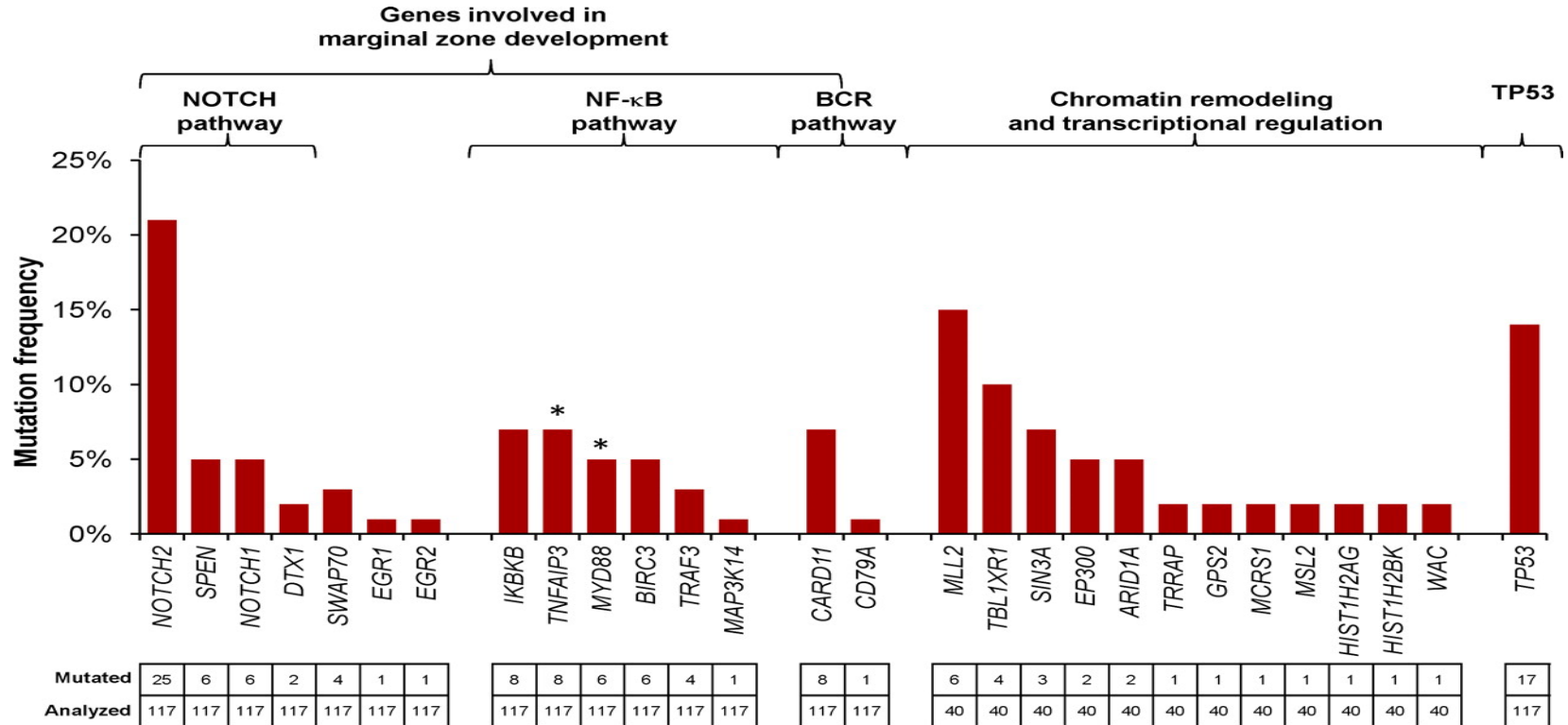


Additional 93 SMZL specimens sequenced in validation cohort.  
 22 additional cases with *NOTCH2* mutations identified

# Frequency of *NOTCH2* Mutations in Various Lymphoma Subtypes and Reactive Lymph Nodes

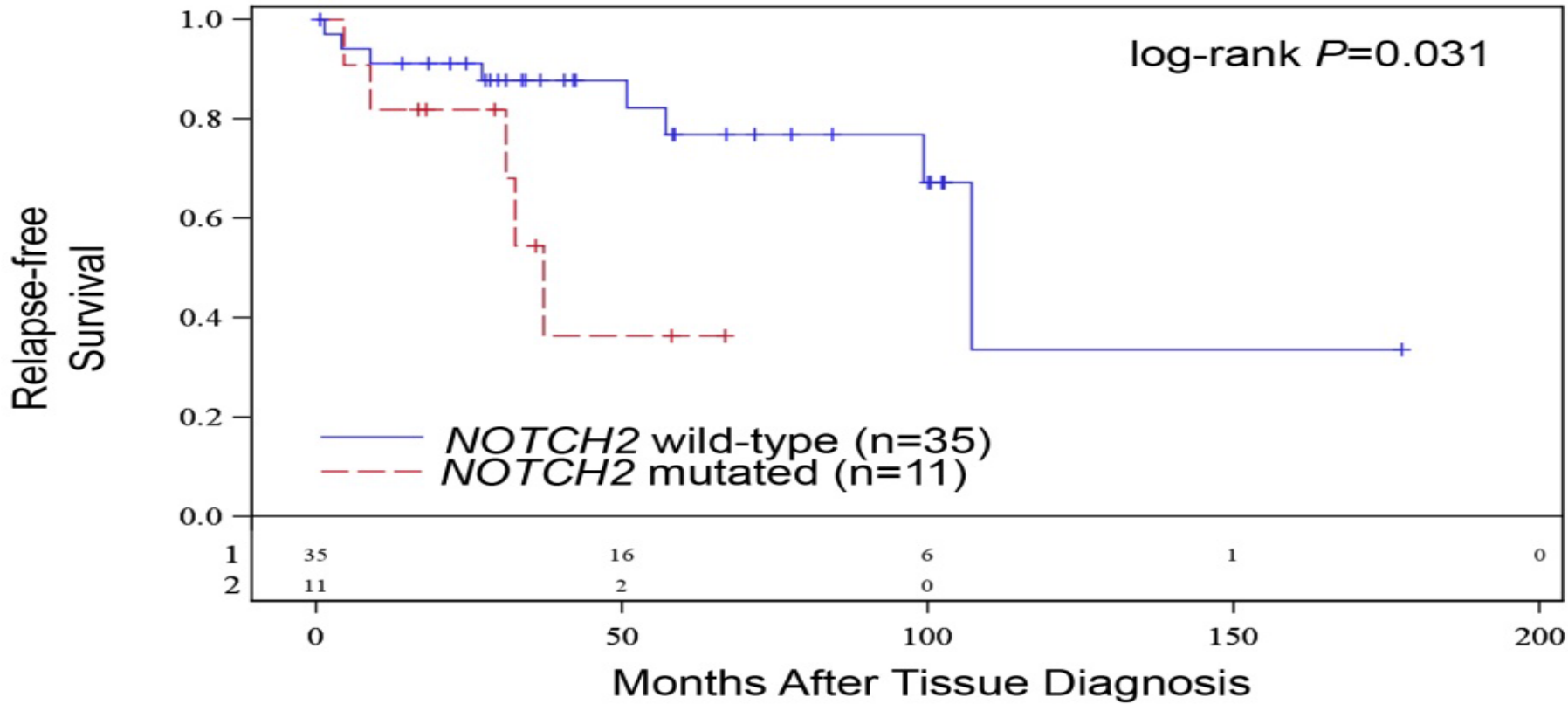


# Recurrently targeted pathways in SMZL



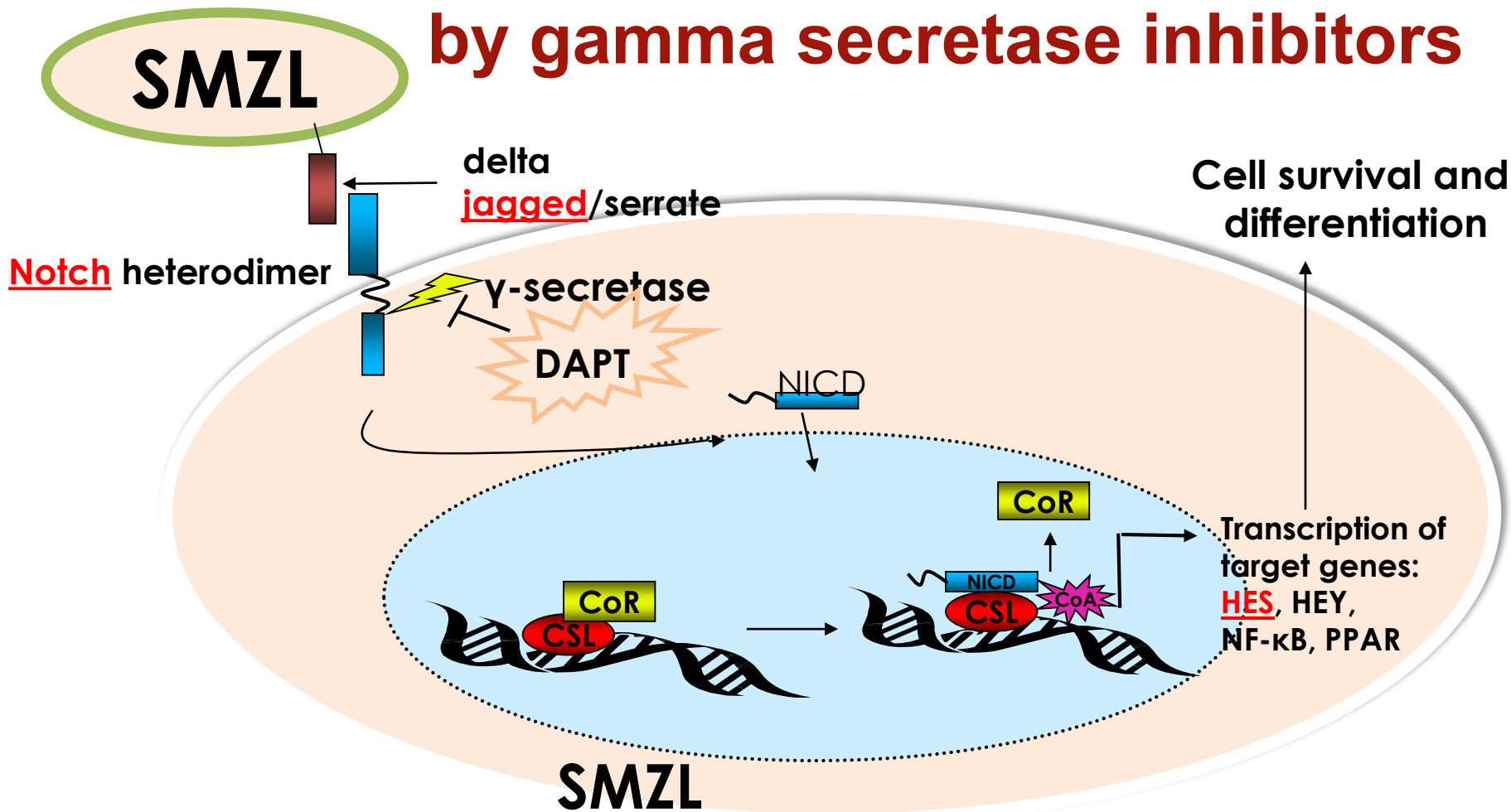


# Decreased Relapse-free Survival in *NOTCH2*-mutated SMZL



Kiel MJ, .. Lim, MS, Elenitoba-Johnson KSJ  
J Exp Med. 2012 Aug27;209(9):1553-65.

# Notch signaling can be inhibited by gamma secretase inhibitors



# Conclusions

- *NOTCH2* is recurrently mutated in SMZL
- Mutations cluster in C-terminus  
causing gain-of-function of *NOTCH2*
- *NOTCH2* mutations are specific to MZL
- *NOTCH2* mutations confer an adverse prognosis

# Genomic landscape of splenic marginal zone lymphoma

## ***KLF2* mutations**

- Identified in 40/96 (42%) SMZL
- Interferes with ability of KLF2 to suppress NF-κB activation by TLR, BCR, BAFFr and TNFR signaling
- IGHV1-2 rearrangement and 7q deletions occur in cases of *KLF2* mutation
- *KLF2* deficiency causes splenic marginal zone hyperplasia in mice

## ***MYD88* and *TP53* mutations**

in SMZL without *KLF2* mutations

***NOTCH2*, *TRAF3*, *TNFAIP3* and *CARD11* mutations** ----in SMZL with and without *KLF2* mutation

*Clipson A et al., Leukemia 2015*

*Piva R et al., Leukemia 2014*

# Prognostic significance of genetic alterations in SMZL

- *KLF2* mutations are early, clonal events enriched in patient with del(7q) and IGHV1-2\*04 BCR immunoglobulins and associated with short median time to first treatment.
- *NOTCH2* mutations and 100% germline IGHV gene identity were independent markers of short time to first treatment,
- *TP53* mutations are an independent marker of short OR.

*Parry M et al., Genetics and prognostication in splenic marginal zone lymphoma: Revelations From deep sequencing. Clin Cancer Res 2015 Haematologica 2017*

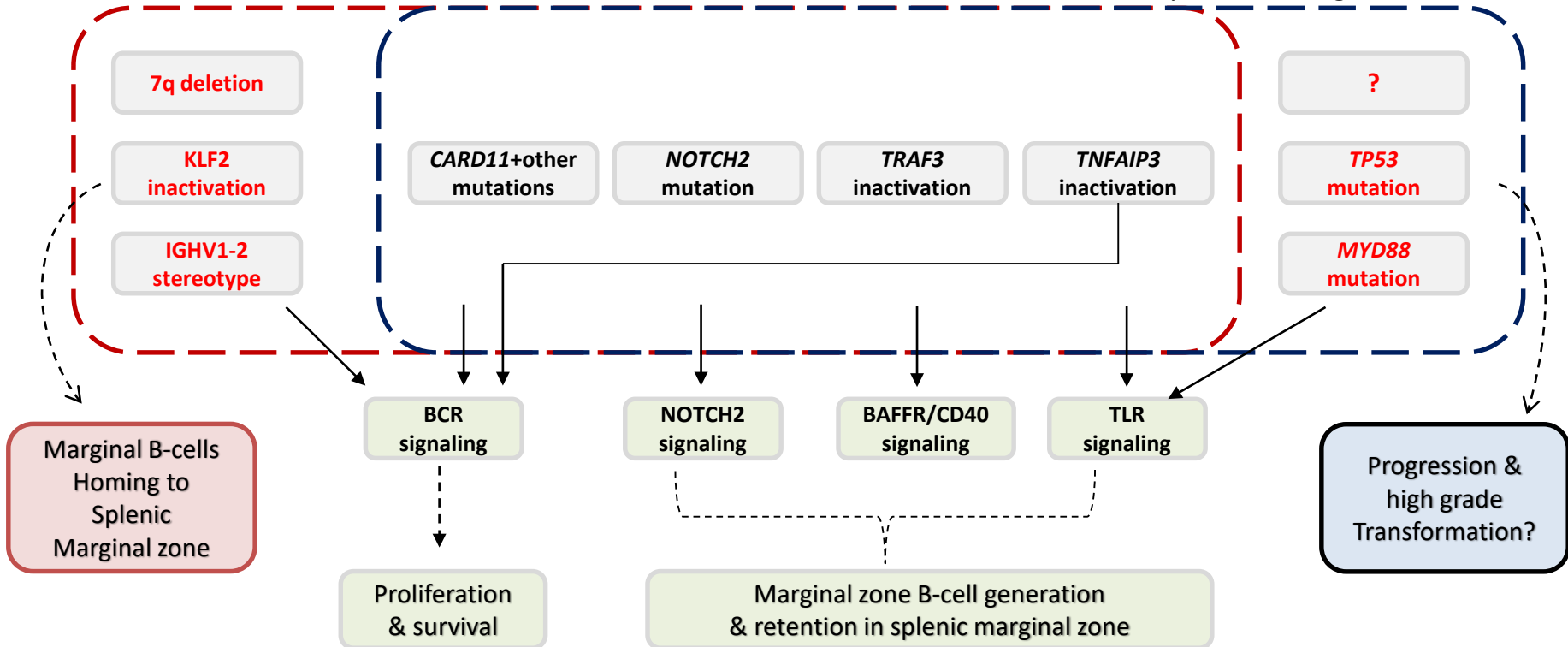
# Proposed molecular mechanisms of SMZL

## SMZL with *KLF2* mutation

T cell-independent marginal zone B-cells

## SMZL without *KLF2* mutation

T cell-independent marginal zone B-cells



# Distinct genetic features of splenic lymphoma

- Reactive
- Splenic marginal zone lymphoma (*NOTCH2, KLF2, SPEN, etc*)
- Splenic B-cell lymphoma, unclassifiable
  - Hairy cell leukemia, variant (*MAP2K1*)
  - Splenic diffuse red pulp small B cell lymphoma (*BCOR* mutations without *KLF2, TNFAIP3, MYD88* mutations)
- Follicular lymphoma (*IGH-BCL2, EZH2, CREBBP*)
- Mantle cell lymphoma (*IGH-Cyclin D1, NOTCH1*)
- Chronic lymphocytic leukemia/SLL (*NOTCH1, XPO1*)
- Hairy cell leukemia (*BRAF V600E*)
  
- T cell lymphoma

# Circulating tumor DNA for lymphoma

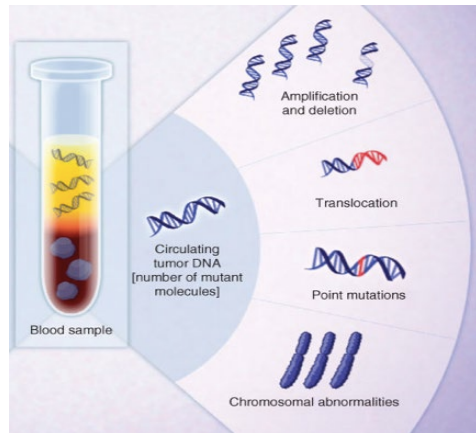
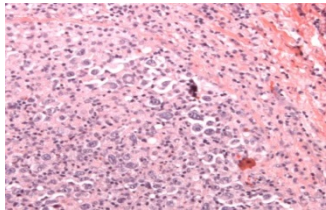
*Rossi D et al., Blood 2017*

*Scherer F et al., Science Trans Med 2016*

*Jian Y et al Genome Biology 2014*

*Roschewski M et al., Blood 2016*

*Roschewski M., Lancet Oncol 2015*

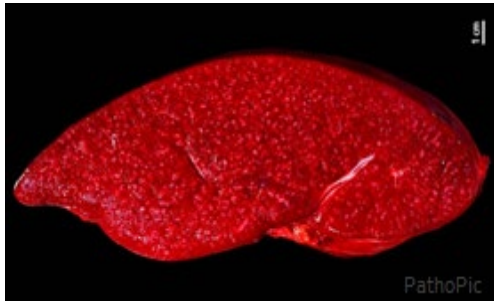


- cfDNA for MRD for DLBCL
- Retrospective and prospective studies show accurate genotyping to detect somatic mutations of allelic abundance >20%
- Non-invasive tool to track treatment-resistant clones in DLBCL



# Circulating tumor DNA for diagnosis/prognosis of splenic marginal zone lymphoma

**Current Clinical Practice**  
**Diagnosis Requires Splenectomy**

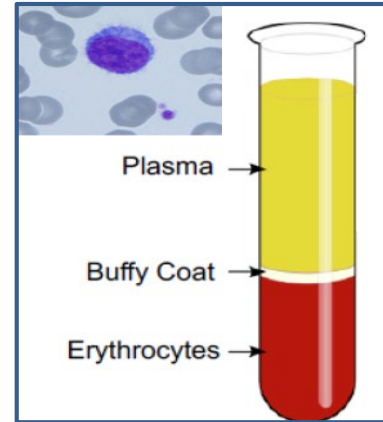


Splenic Marginal Zone Lymphoma

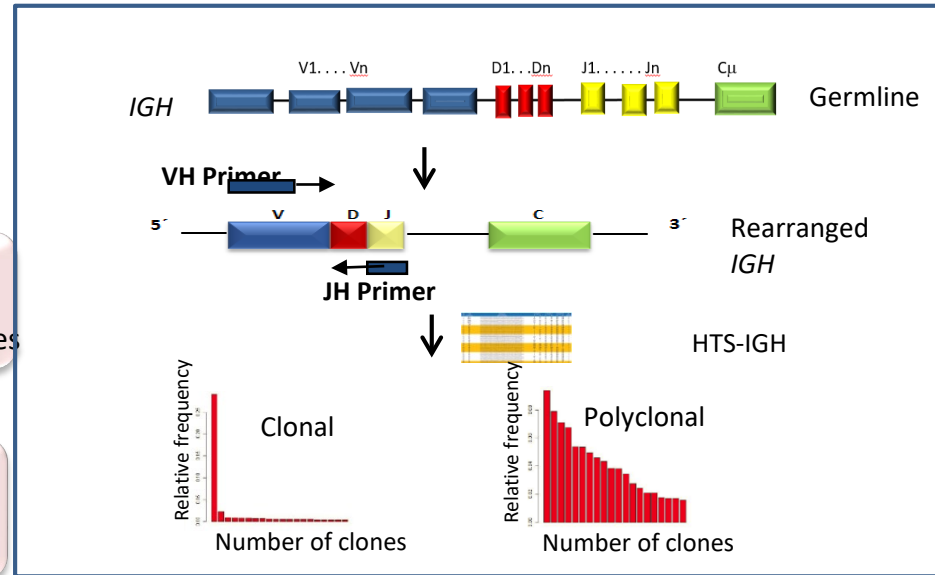
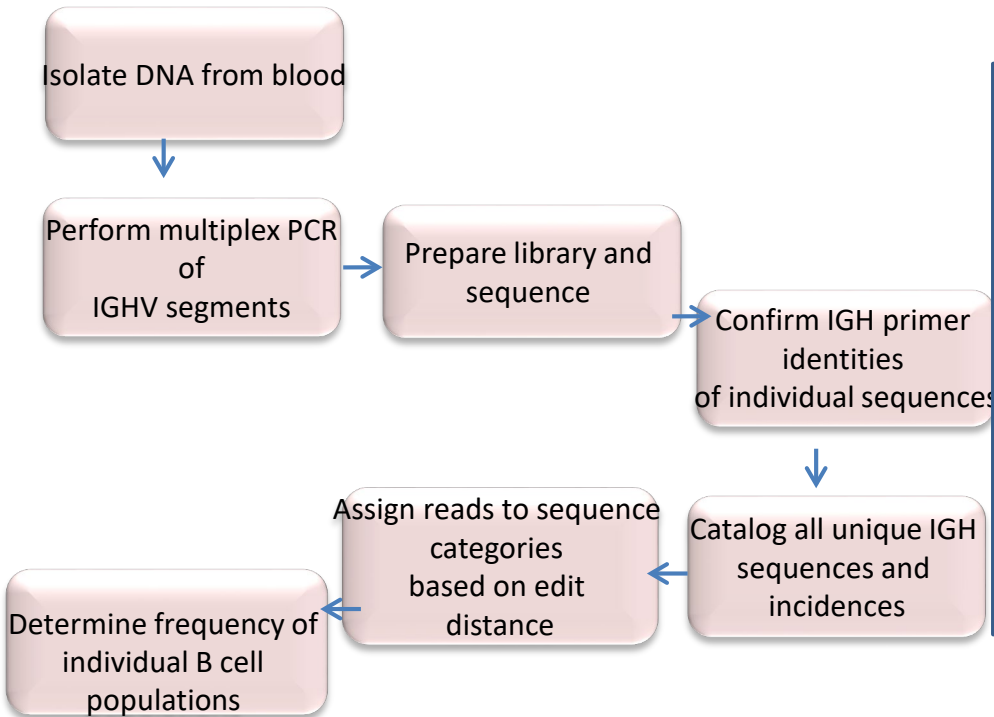


**Clinical Gap**

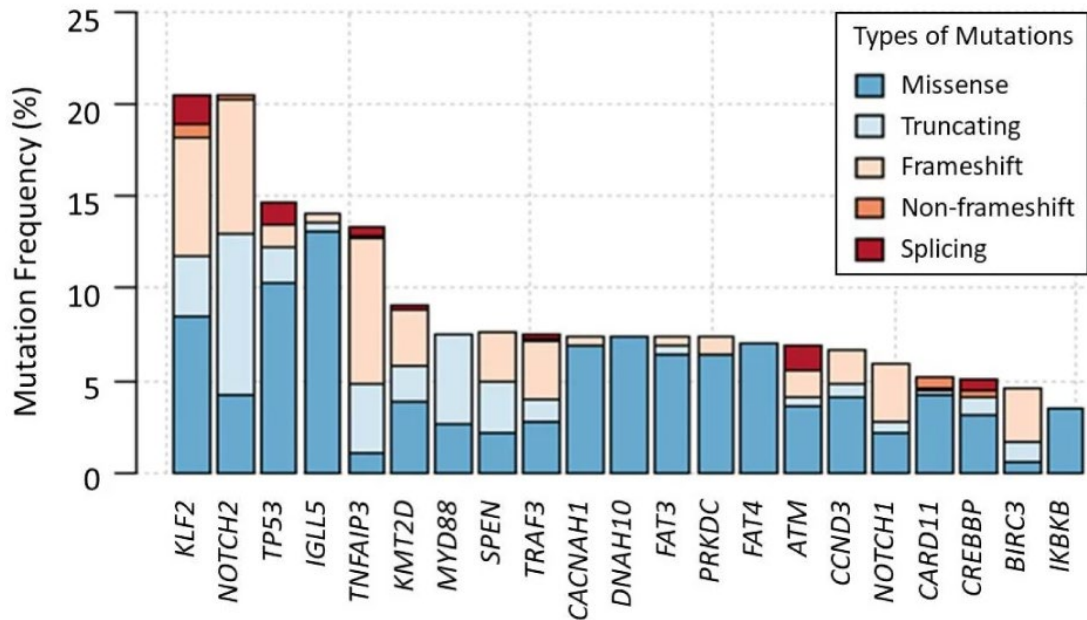
**Ideal Practice**  
**Diagnosis Made From Blood**



# High throughput sequencing of IGH genes (Ig repertoire)



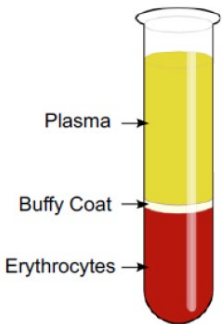
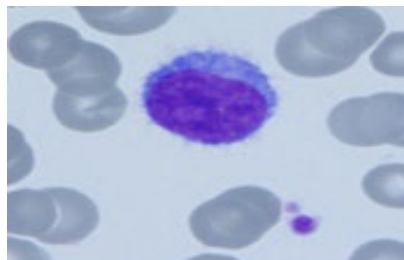
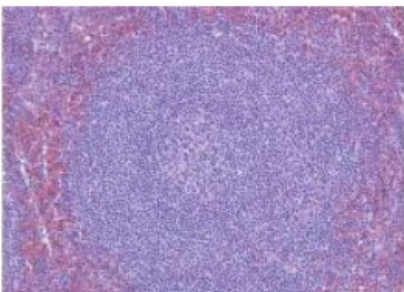
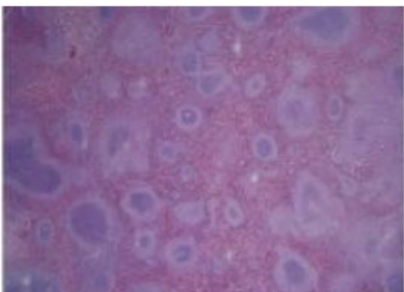
# Genes for splenic lymphoma NGS assay



ANKR30B	CCND3	CARD11	KLHL6
C2CD3	MTOR	CD79A	TBL2XR1
KLHL1	SPEN	CD79B	DNMT3A
KMT2D	SWAP70	CITA	CDKN2A
MAP3K3	TRRAP	CXCR4	TNFRSF14
MAP3K14	USH2A	EGR2	TRAF3
MAP3K5	BRAF	GNA13	BIRC3
NCOA6	EZH2	ID3	MYD88
EGR1	IDH2	KLF2	POT1
EGR2	MAP2K1	NFKBIE	XPO1
CUL1	NOTCH1	PLCG1	JAK3
CUL2	TP53	PLCG2	ATM
CUL3	NOTCH2	RP515	ARID1A
FBXO10	TET2	RRAGC	BCL2
FBXO11	SF3B1	SOC51	BCL6
IRF4	B2M	STAT3	HIST1HE
SIN3A	BTK	STAT5B	KMT2D
SIAH2	CREBBP	TCF3	MYC
IMARCA2	RHOA	TNFAIP3	PIM1
SF3B1			

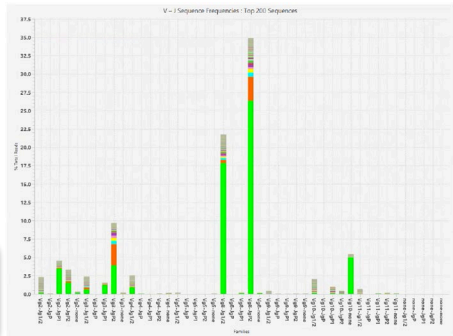
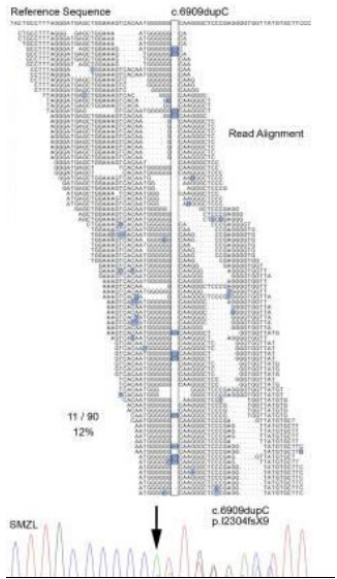
# Genomic biomarkers of splenic lymphoma

SMZL



Targeted NGS Panel

Ig-HTS



University of Pennsylvania, University of Pittsburgh, Columbia University, MD Anderson Cancer Center  
NIH R01 CA255655-01

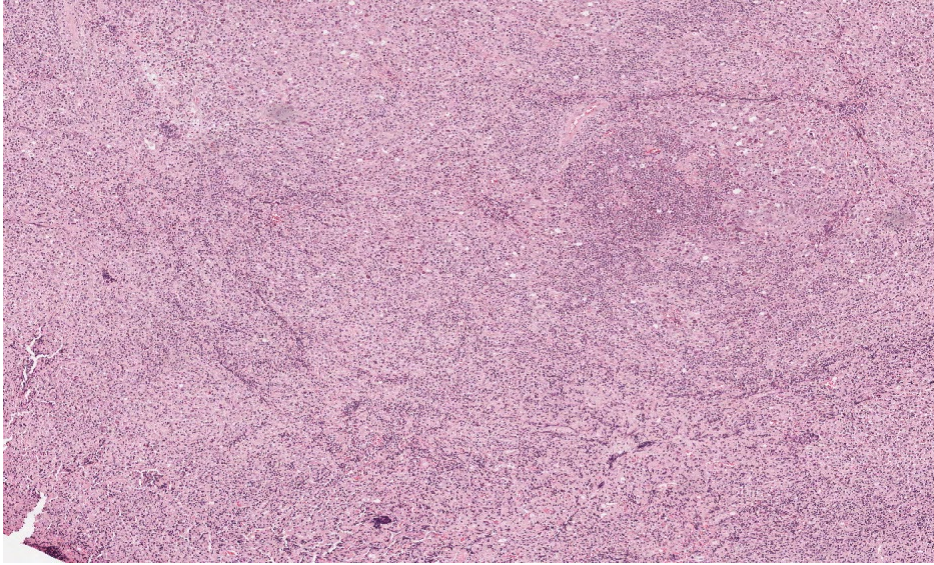
# Take home messages

- Many subtypes of B-cell lymphomas can present in the spleen
- NGS studies identified *NOTCH2*, *KLF2*, *SPEN* mutations in SMZL
- Gene mutations in NFkB pathway, chromatin remodeling are also present in SMZL
- Molecular studies may help in subclassification of other B-cell lymphomas that present in the spleen
- Circulating tumor DNA may aid in early diagnosis of splenic lymphomas

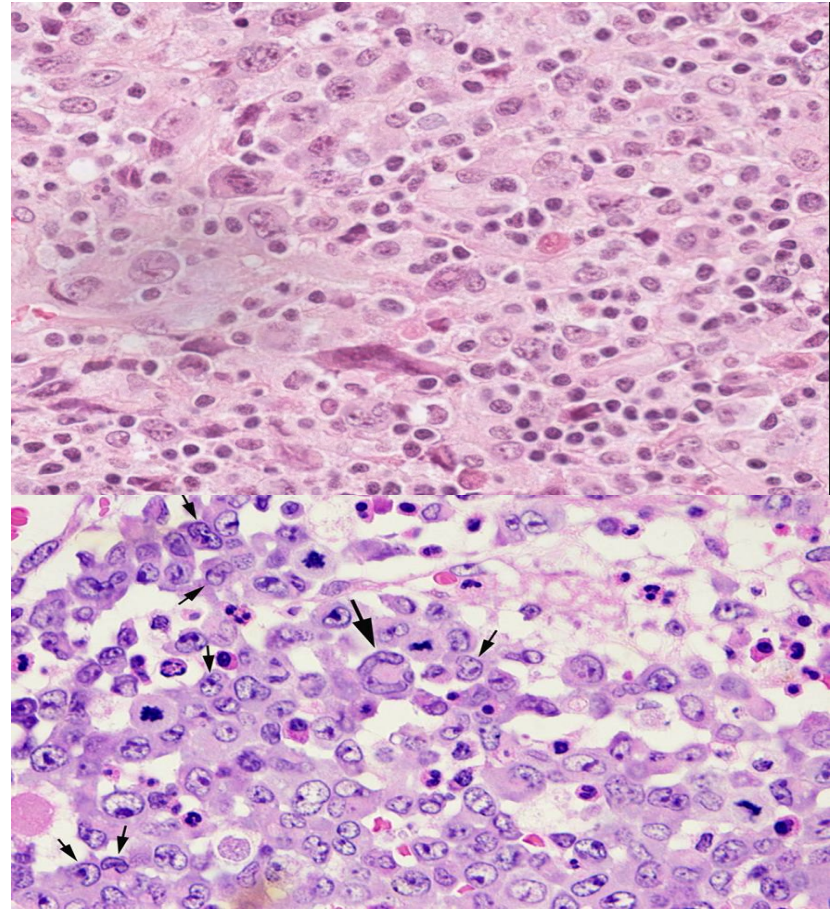
# Greetings from the City of Brotherly Love



# Case 2



- A 25 year old male presented with a one year history of diffuse lymphadenopathy of cervical, axillary, abdominal regions. He complained of fevers, weight loss, night sweats.
- An excisional biopsy of the cervical lymph node was performed.



# Differential diagnostic considerations

## Hematopoietic

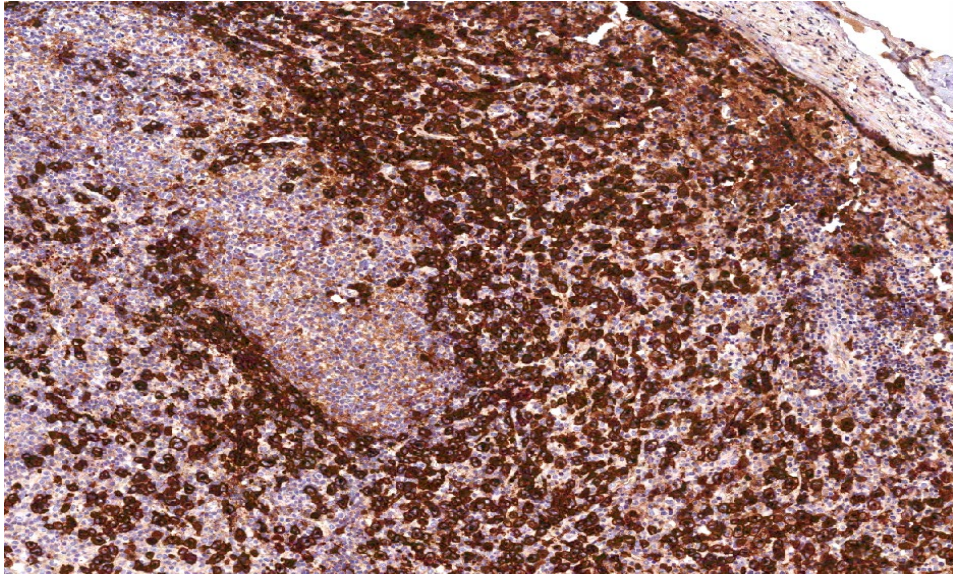
- Non-Hodgkin lymphoma
  - diffuse large B-cell lymphomas with anaplastic features,
  - anaplastic large cell lymphoma (ALK-positive and ALK-negative)
- Peripheral T cell lymphomas, NOS
- Extramedullary myeloid tumors,
- Hodgkin lymphoma
- Anaplastic myeloma

## Non-hematopoietic

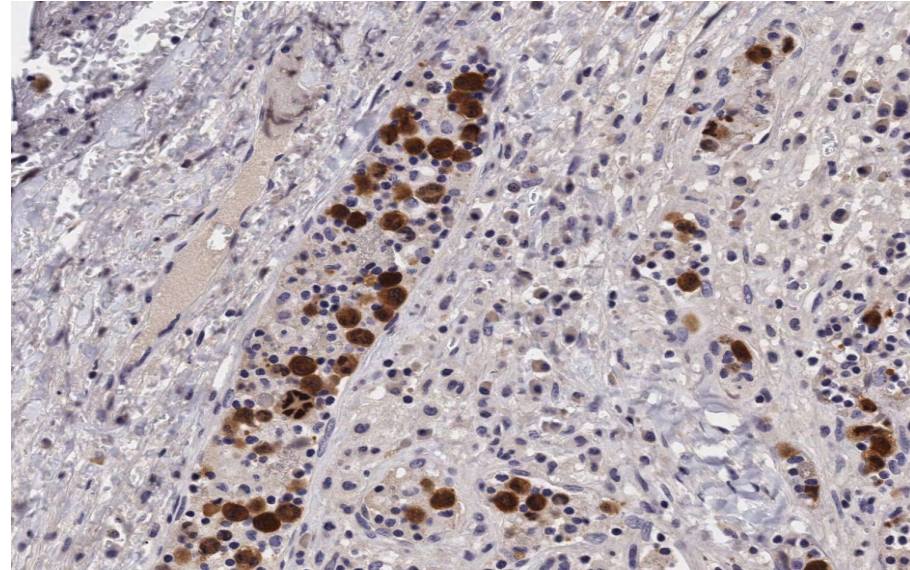
- Melanoma
- Carcinoma (anaplastic variants)



# Immunophenotype



CD30



ALK1

# Other tumors that express ALK protein

## Neoplasms

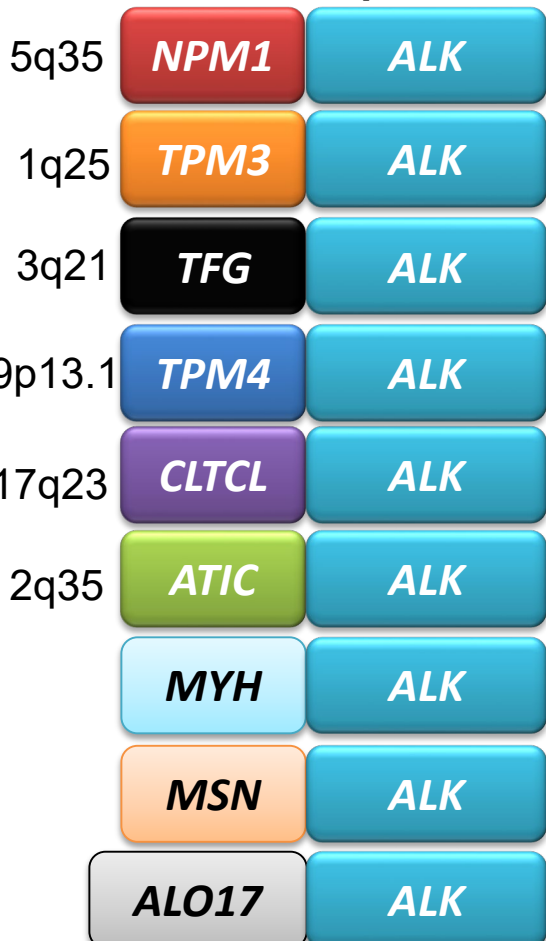
- **Lymphomas**
- **Soft tissue tumors**
- **Carcinomas**
- **Neuroblastomas**

## Mechanisms

- **Chromosomal translocations**
- **Gene amplifications**
- **Kinase activating mutations**
- **Overexpression**

# ALK in human cancer

## ALCL, ALK positive



75%

15%

~10%

## Inflammatory myofibroblastic tumors



## Non-small-cell lung cancer



## Diffuse large B-cell lymphoma

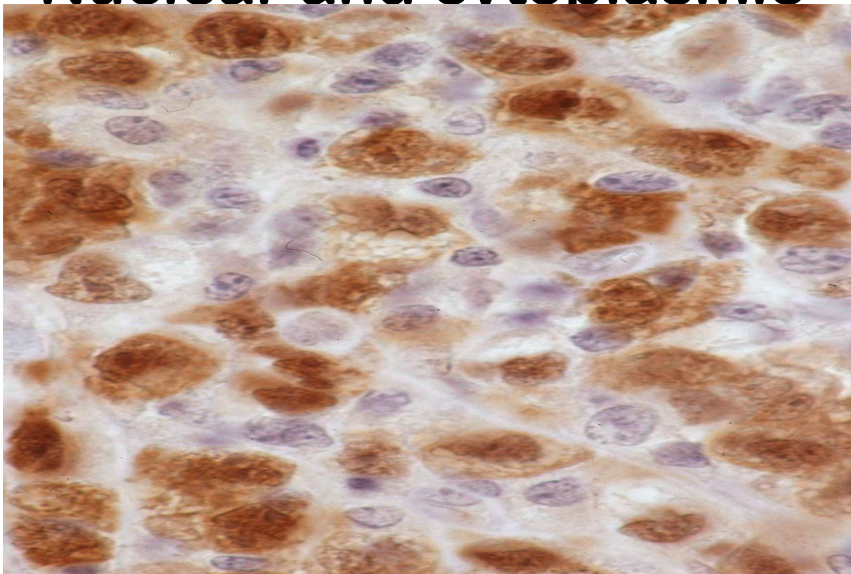


## Neuroblastoma

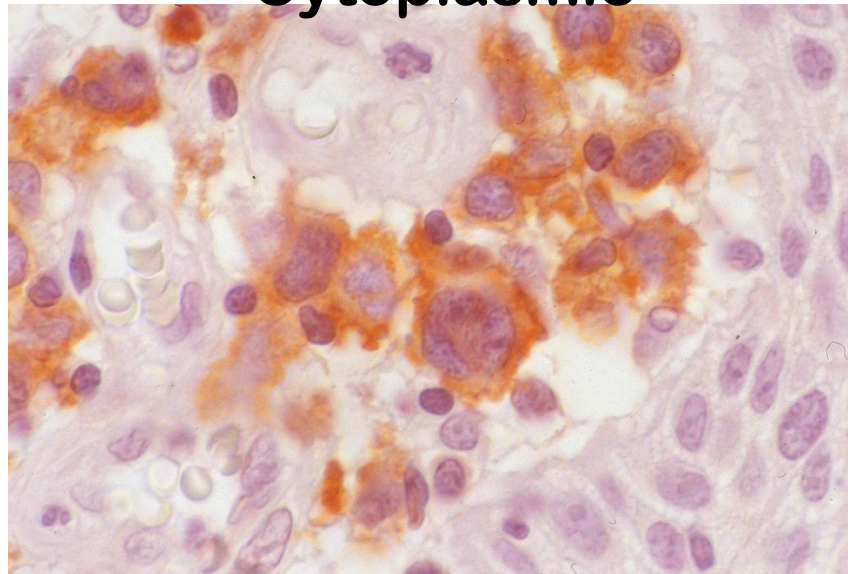


# Intracellular localization of ALK expression is dependent on the partner gene

**Nuclear and cytoplasmic**



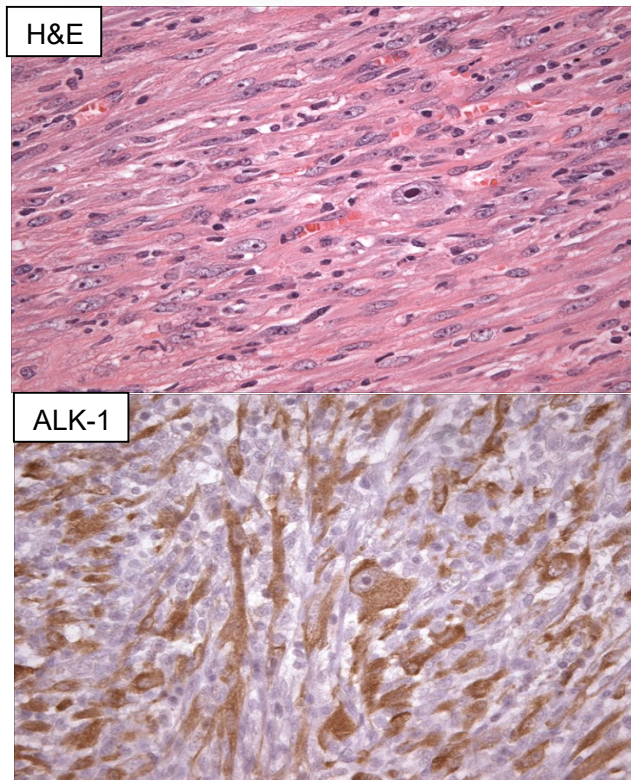
**Cytoplasmic**



# ALK translocations inflammatory myofibroblastic tumor

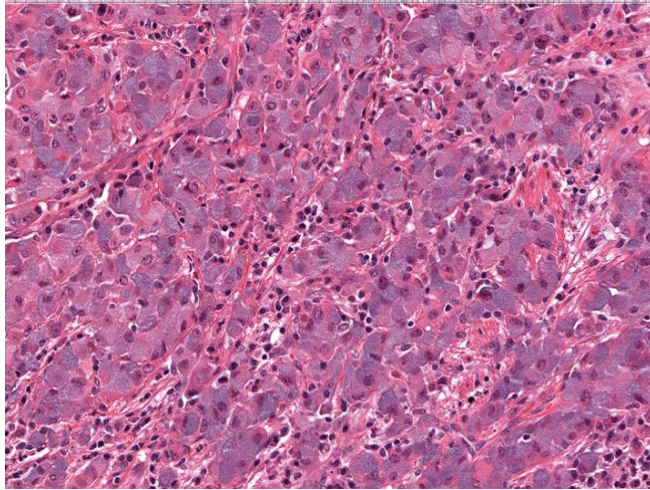


TPM3	ALK
RanBP2	ALK
ATIC	ALK
CLTCL	ALK
TPM4	ALK
CARS	ALK
SEC31L1	ALK



*Elenitoba-Johnson K et al Proteomic identification of oncogenic chromosomal translocation partners encoding chimeric anaplastic lymphoma kinase fusion proteins. Proc Natl Acad Sci 2006*

# ALK translocations in non small cell lung cancer



5% of adenocarcinomas

*EML4*

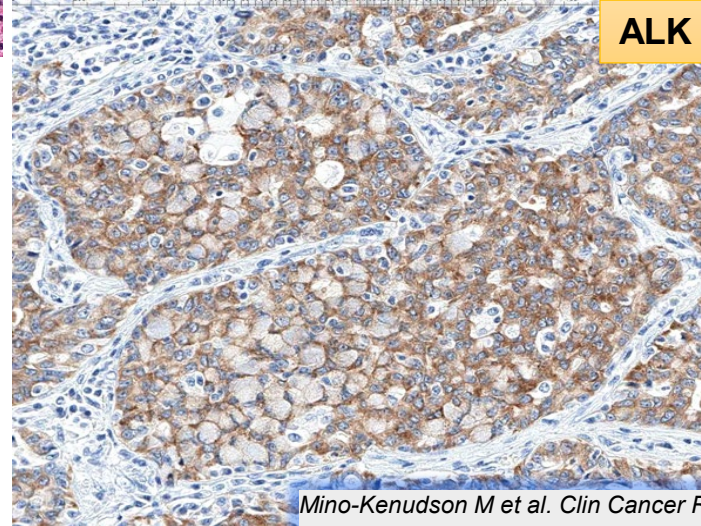
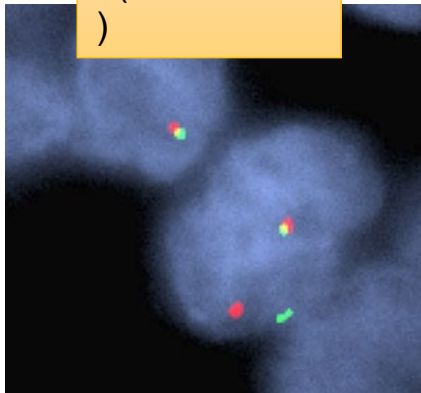
*ALK*

*KIF5B*

*ALK*

Soda et al., 2007, Nature;  
448:561-566.

(*EML4/ALK*  
)



# Anaplastic large cell lymphoma, ALK positive

## Remains a diagnostic challenge:

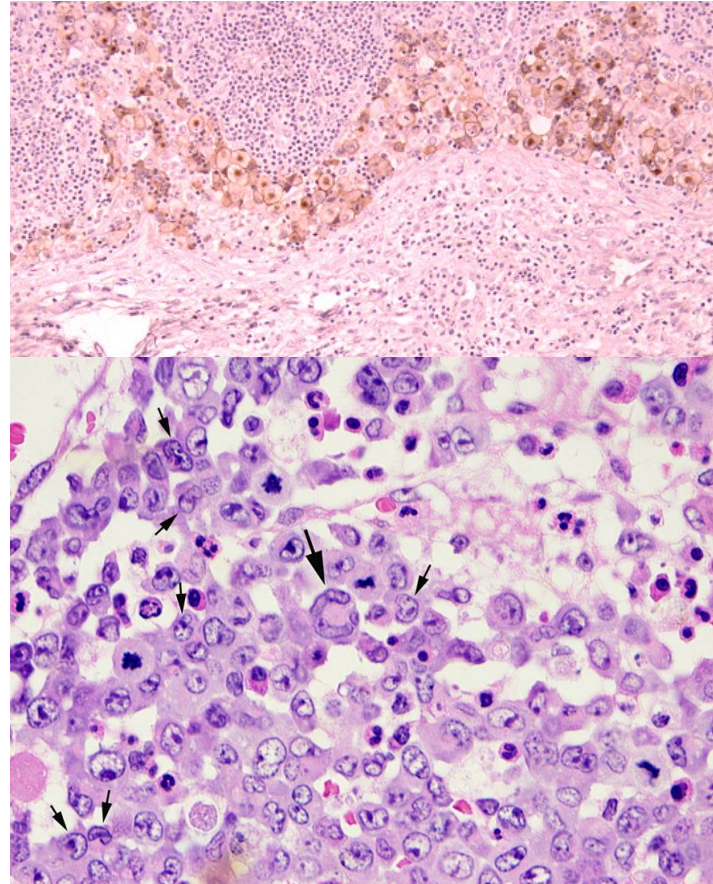
- Exhibits broad spectrum of morphologic features

- Downregulates many T cell antigens

- Aberrantly expresses proteins associated with other cancers  
(EMA, CTK, SOX2, CD13, CD68, MPO)

# Morphologic features of ALCL

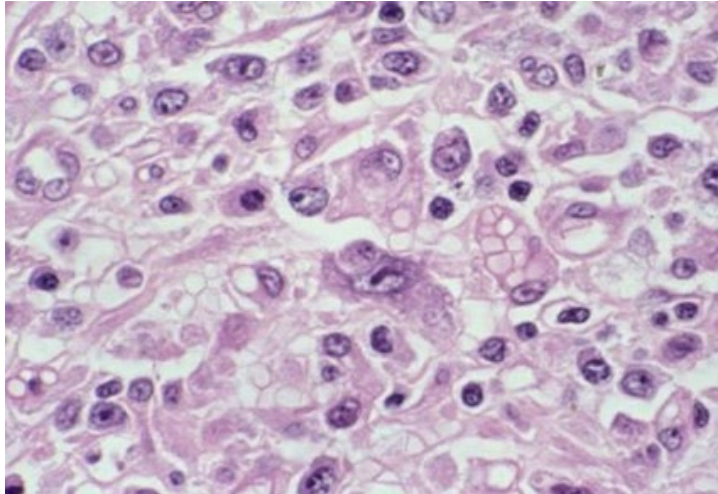
- Sinusoidal growth pattern
- Hallmark cells
- Nuclei may be polylobated with wreath-like pattern
- Abundant cytoplasm
- Vesicular chromatin
- Variably prominent nucleoli



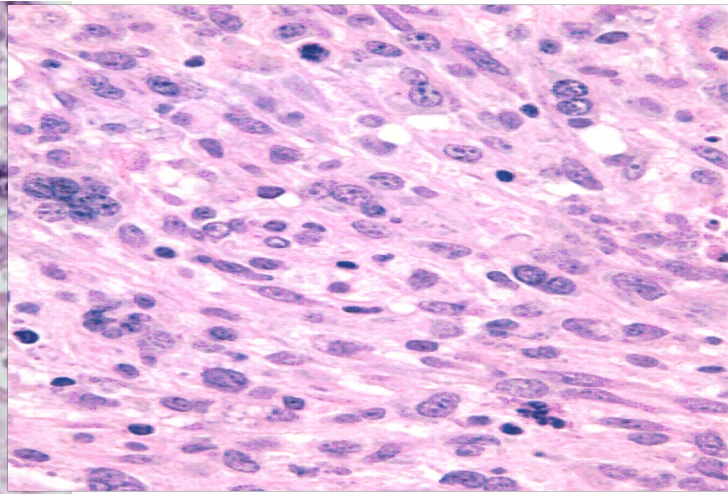


# Broad spectrum of cytologic and histologic features

**Lymphohistiocytic variant**

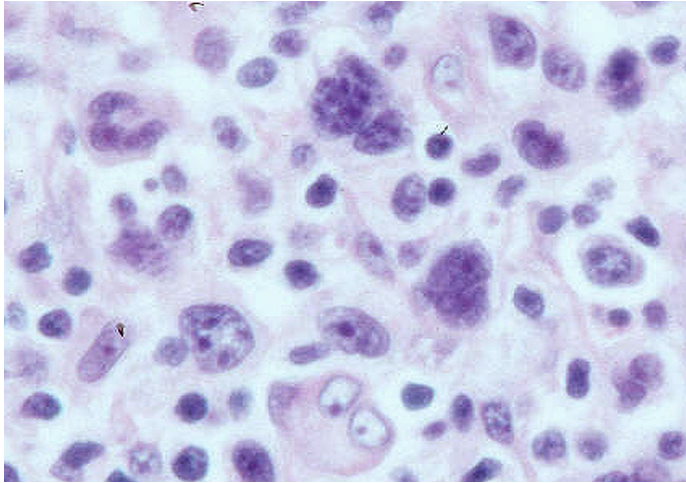


**Sarcomatoid variant**

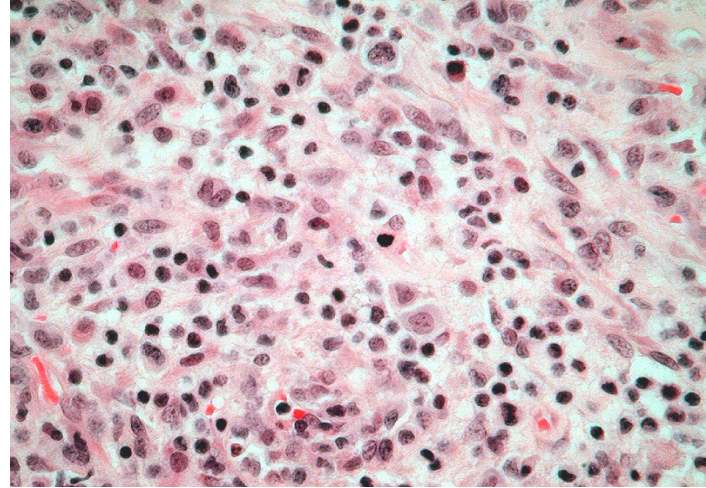


# Broad spectrum of histologic and cytologic features

**Giant cell variant**



**Small cell variant**



*Kinney MC et al., A small-cell predominant variant of primary Ki-1 (CD30)+ T cell lymphoma. Am J Surg Pathol 1993*

# Immunophenotype

Antibody	Results
Pan cytokeratin	Negative
S-100	Negative
CD45	Negative
CD20	Negative
CD3	Negative
CD2	Negative
CD4	Negative
CD7	Negative
CD8	Negative
CD30	Positive +++
ALK-1	Positive +++ N/C

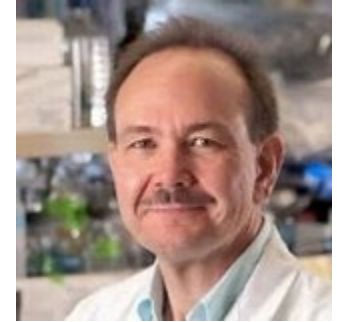


## Differential Diagnosis

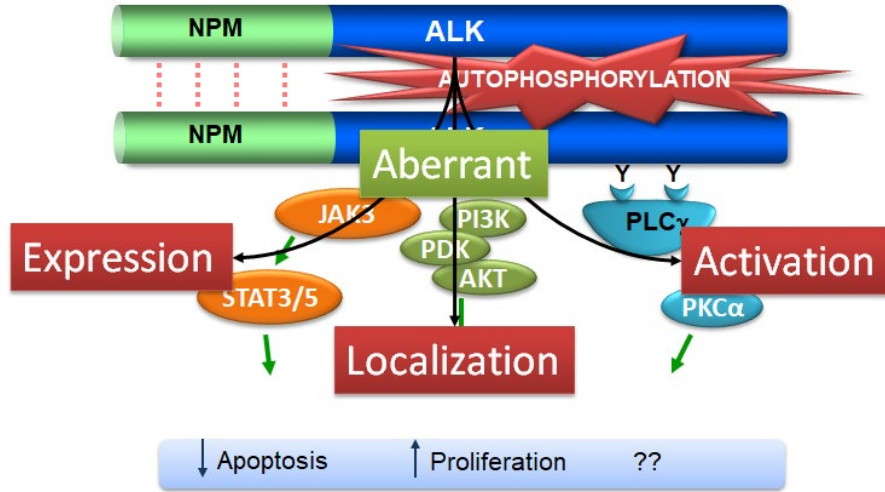
**CD30 positive neoplasms**

**ALK-1 positive neoplasms**

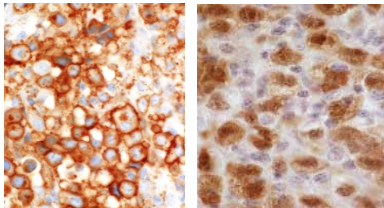
# NPM-ALK is an oncogenic tyrosine kinase



Stephen Manis, MD



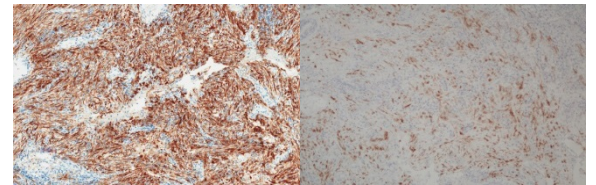
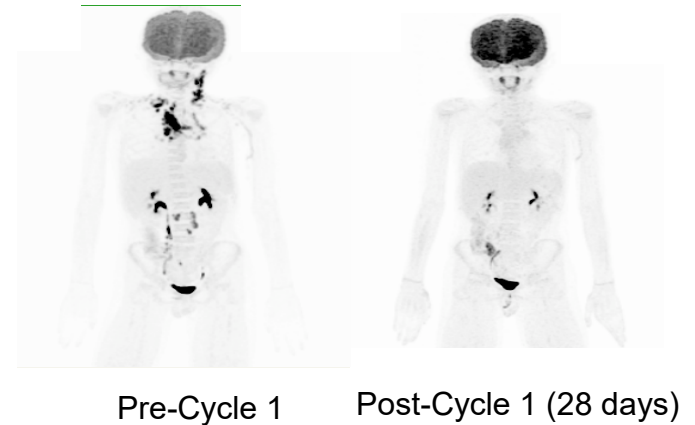
CD30 ALK



- Rare orphan disease
- Lack of interest from Pharma
- Until 2009

# ALK is a therapeutic target for ALCL

Phase 1/2 study of PF-2341066, oral small molecule inhibitor of ALK and C-MET in children with relapsed/refractory solid tumors and anaplastic large cell lymphoma  
ADV0912 *Children's Oncology Group*

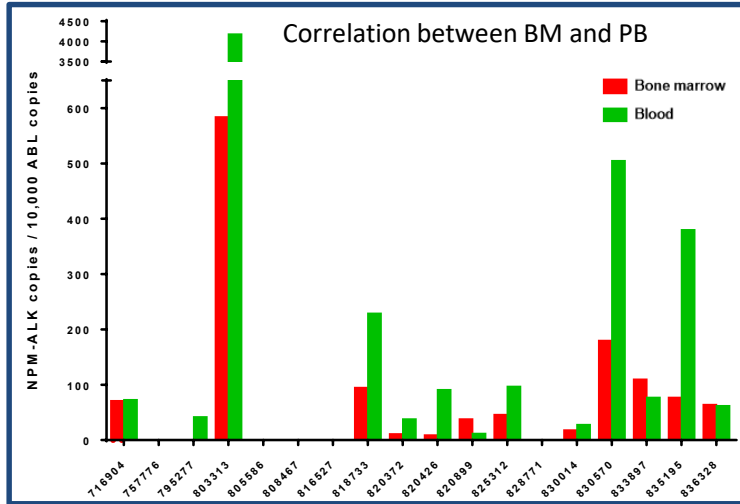


YP Mossé , MS Lim, SD. Voss , K Ruffner , J Laliberte , D Rolland, FM Balis , JM Maris, BJ Weigel , AM Ingle, C Ahern, PC Adamson, and SM Blaney *Lancet Oncology* 2013 May;14(6):472-80.

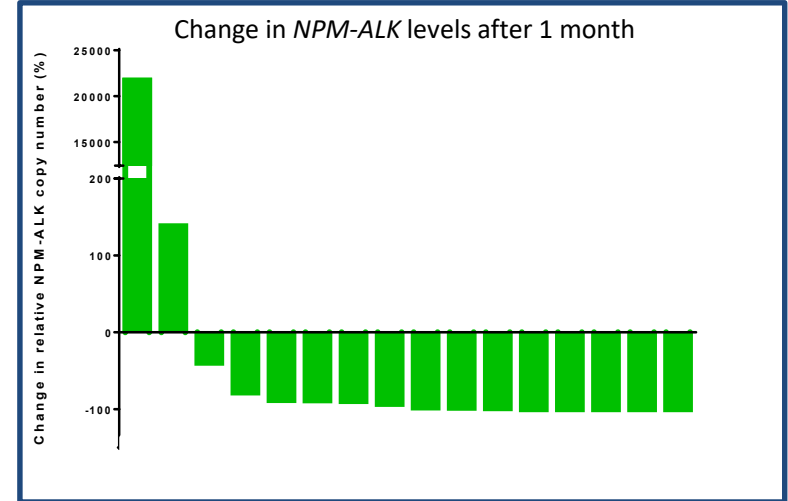
Mossé YP, Voss SD, Lim MS, Rolland D, Minard CG, Fox E, Blaney SM, Weigel BJ. Targeting ALK with Crizotinib in Pediatric Anaplastic Large Cell Lymphoma: A Children's Oncology Group Study (ADV0912). *J Clin Oncol* 2017 Aug 8

# Cell free RNA (NPM-ALK) is detectable in blood

## Correlation between bone marrow blood



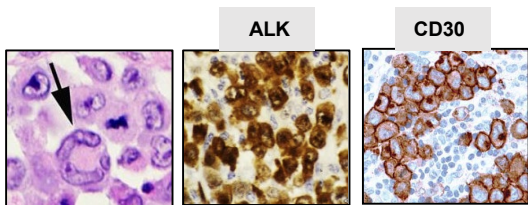
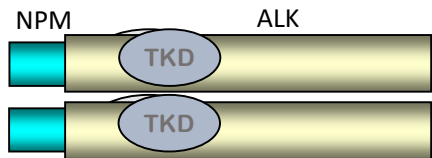
## Decreases with crizotinib treatment



Mossé YP, Voss SD, Lim MS, Rolland D, Minard CG, Fox E, Blaney SM, Weigel BJ. Targeting ALK with Crizotinib in Pediatric Anaplastic Large Cell Lymphoma: A Children's Oncology Group Study (ADVL0912). *J Clin Oncol* 2017 Aug 8

# Plasma cell free *NPM-ALK* is predictive of EFS

Pediatric ALK+ ALCL

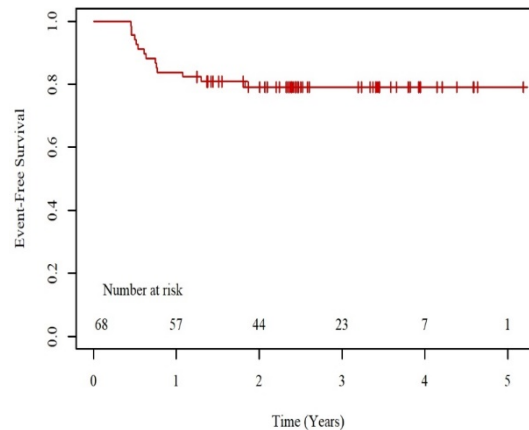


Children's Oncology Group  
ANHL12P1

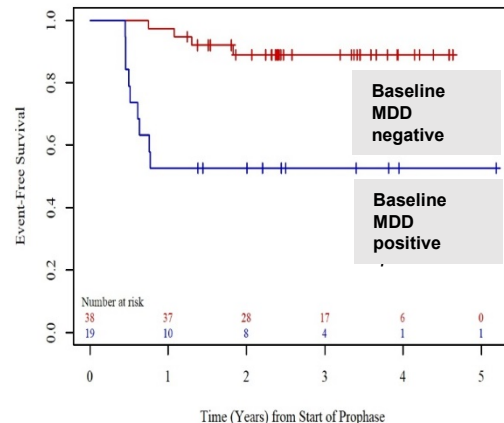
Brentuximab  
Vedotin +  
Chemotherapy

Event Free Survival (2-year EFS 79.1%, 95% CI: 67.2% to 87.1%)  
Low E et al., *Blood* In press

2-year EFS 79.1%, 95% CI: 67.2% to 87.1%



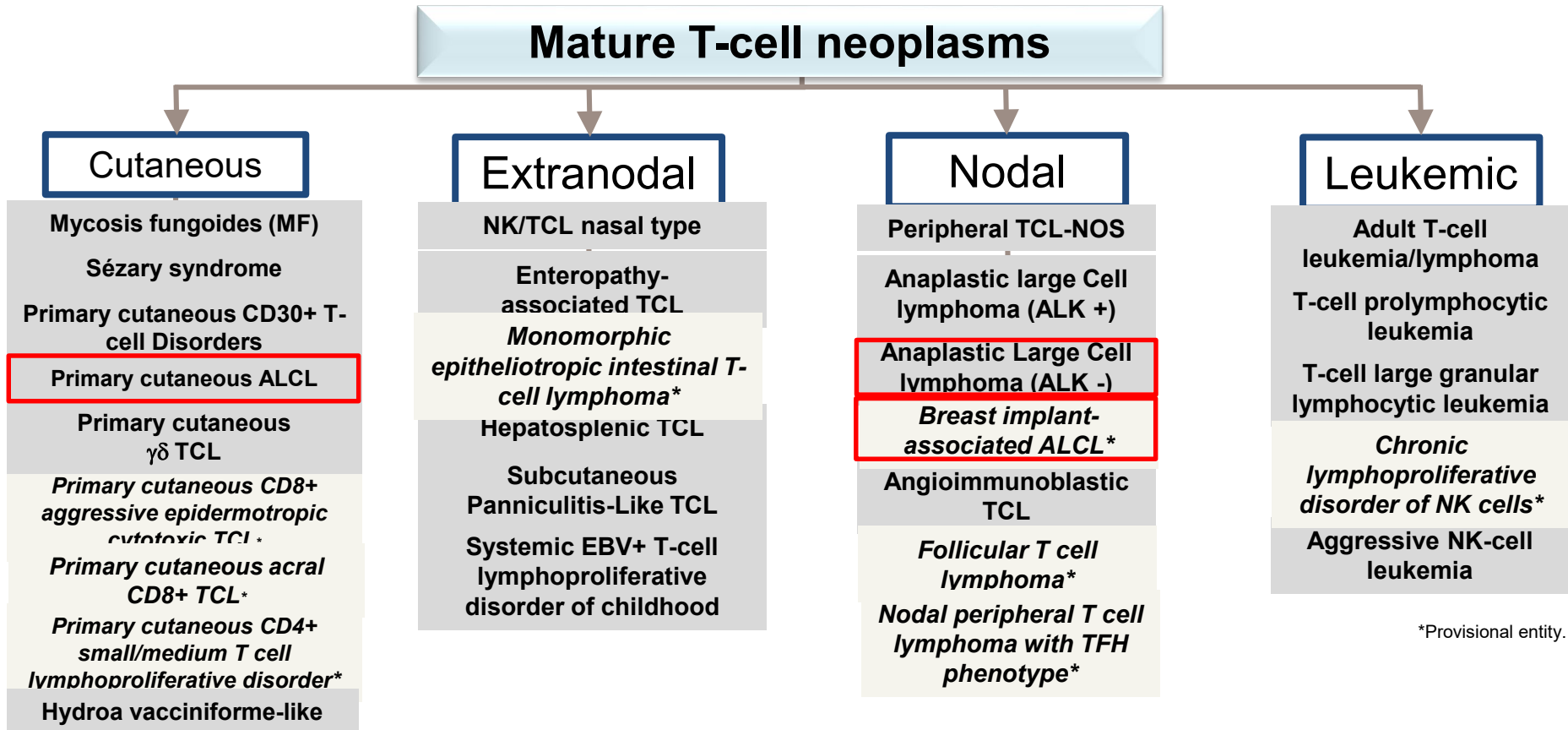
2-year EFS based on MDD (*NPM-ALK* transcript)



# **UPDATE ON GENETICS OF ANAPLASTIC LARGE CELL LYMPHOMA**



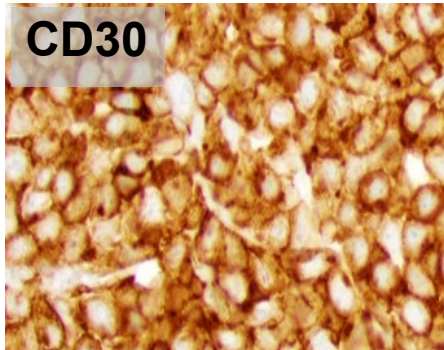
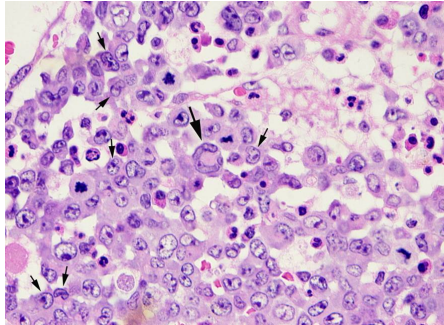
# 2017 World Health Organization



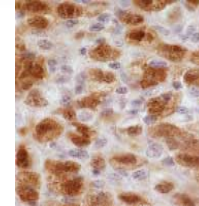
\*Provisional entity.

# Multiple mechanisms of STAT3/5 activation in ALCLs (ALK+ and ALK-)

T-cell lymphoma with  
pleomorphic features and  
strong expression of CD30



ALCL, ALK positive

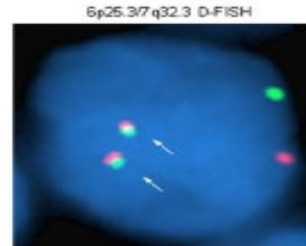


**t(2;5) *NPM-ALK***  
**1994**

ALCL, ALK negative

Many subtypes?

# *DUSP22* rearrangements in ALK- ALCL t(6;7)(p25.3;q32.3)



- Nodal ALK- ALCL (10%)
  - Primary cutaneous ALCL (25%) and rare cases of LyP
  - Downregulation of *DUSP22* (dual-specificity phosphatase that inhibits TCR signaling and growth)
- Absence of STAT3 activation
- DNA hypomethylation leading to enhanced immunogenicity (low PD-L1, high CD58, HLA class II)

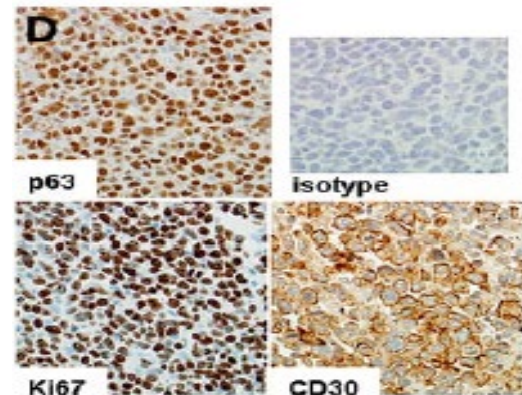
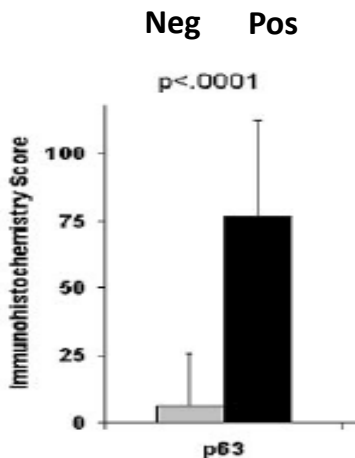
*Feldman et al Blood 2011*

*Karai et al Am J Surg Pathol 2013*

*Luchtel RA et al Blood 2018*

# TP63 rearrangements in ALK- ALCL

- TP63 rearrangements
- Not specific to histology
- P63 IHC identified but not 100% specific



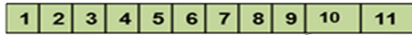


# NPM1

- Nucleophosmin 1
- Multiple functions particularly in nucleolus associating with ribonucleolar proteins



*NPM1*

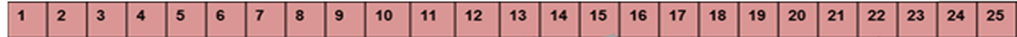


# TYK2

- Non-receptor tyrosine protein kinase
- First member of JAK family
- Signal transduction by interferons and interleukins



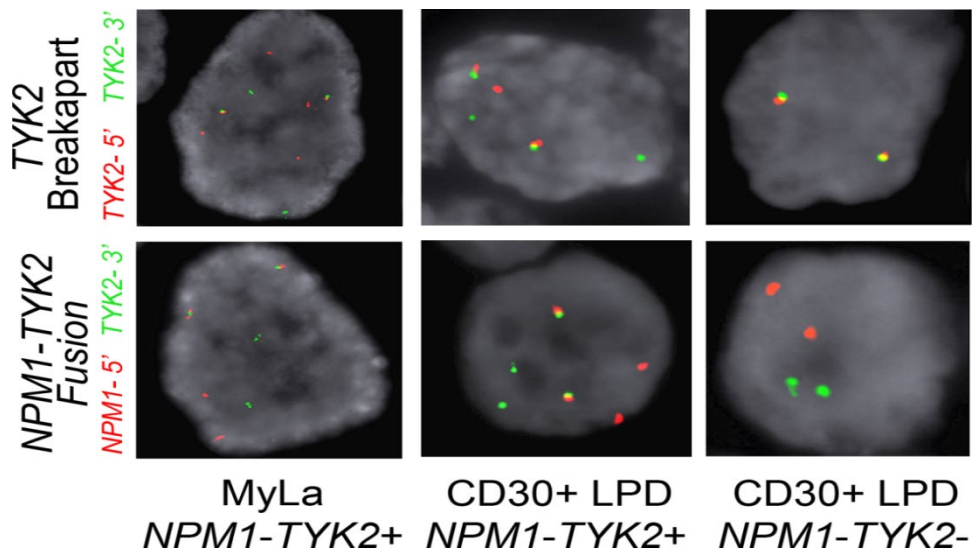
*TYK2*



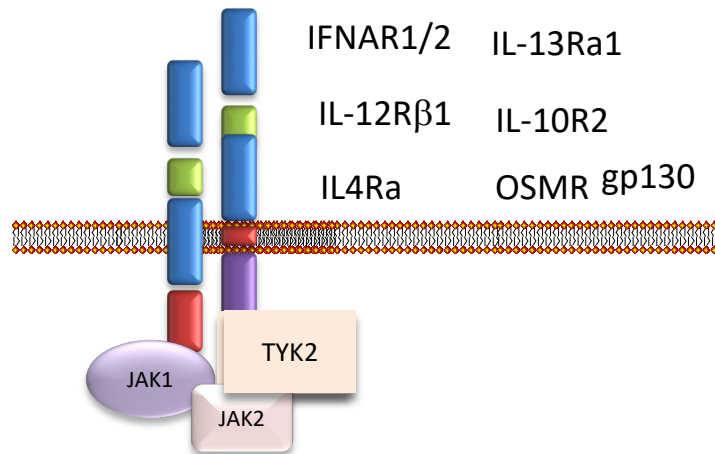
*NPM1/TYK2*



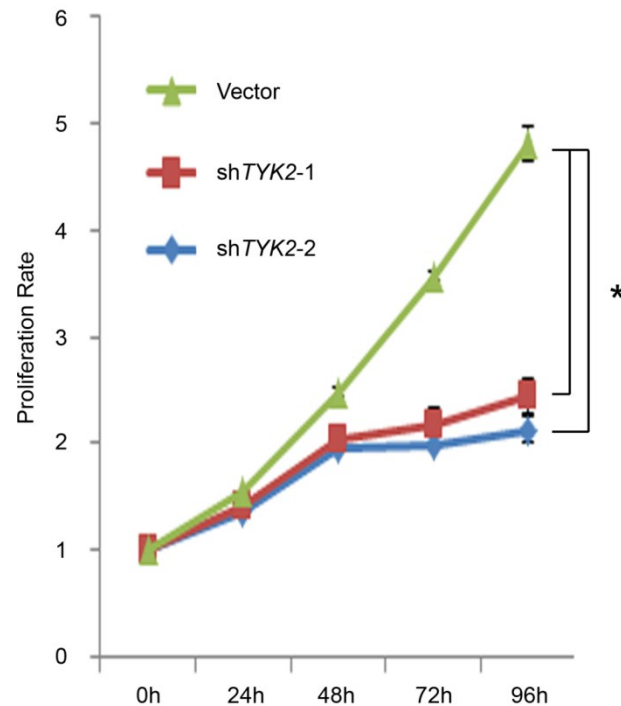
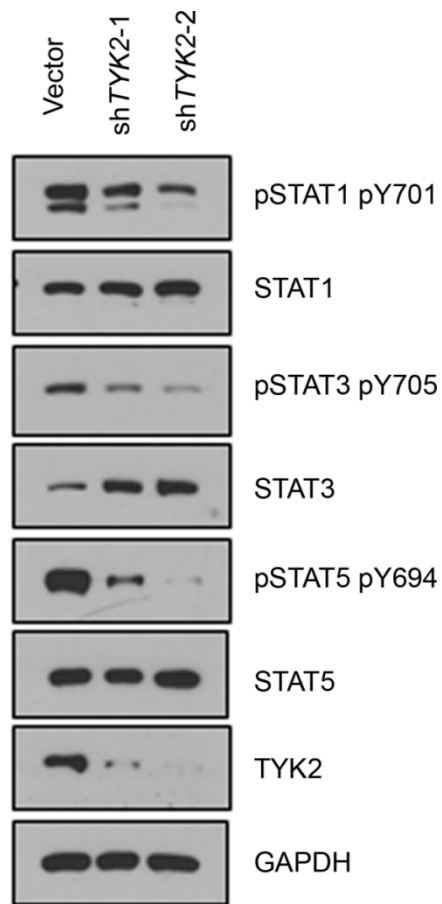
# Diagnostic and therapeutic implications *TYK2* translocation



Signaling of immunoregulatory cytokine



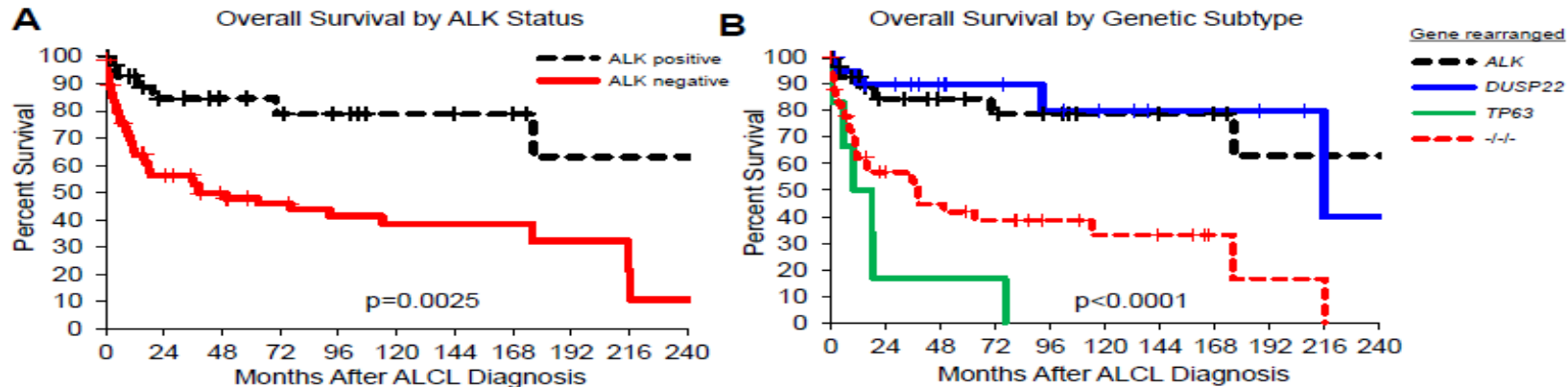
# Knockdown of *NPM-TYK2* decreases cell proliferation





# Rearrangements in ALK- ALCL

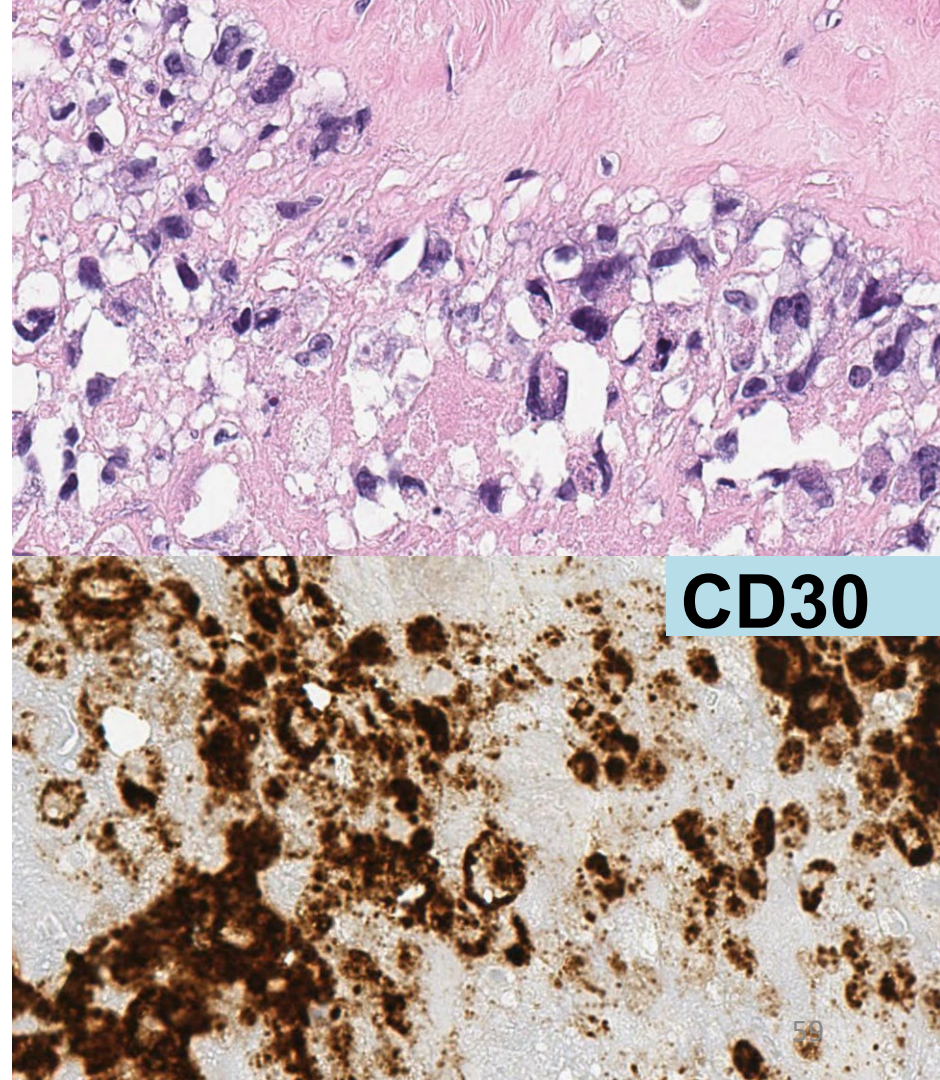
- 22/73 (30%) *DUSP22* translocated
- 6/73 (8%) *TP63* translocated
- Mutually exclusive
- 45 were ALK/*DUSP22*/*TP63* triple negative



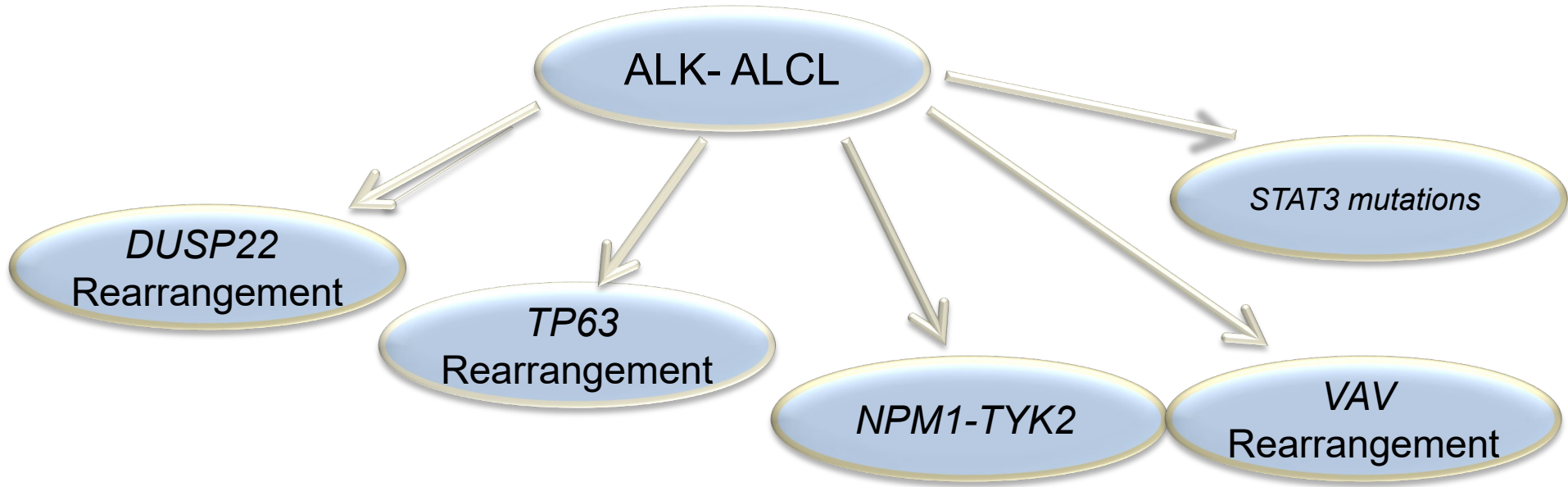


# Breast implant-associated ALCL

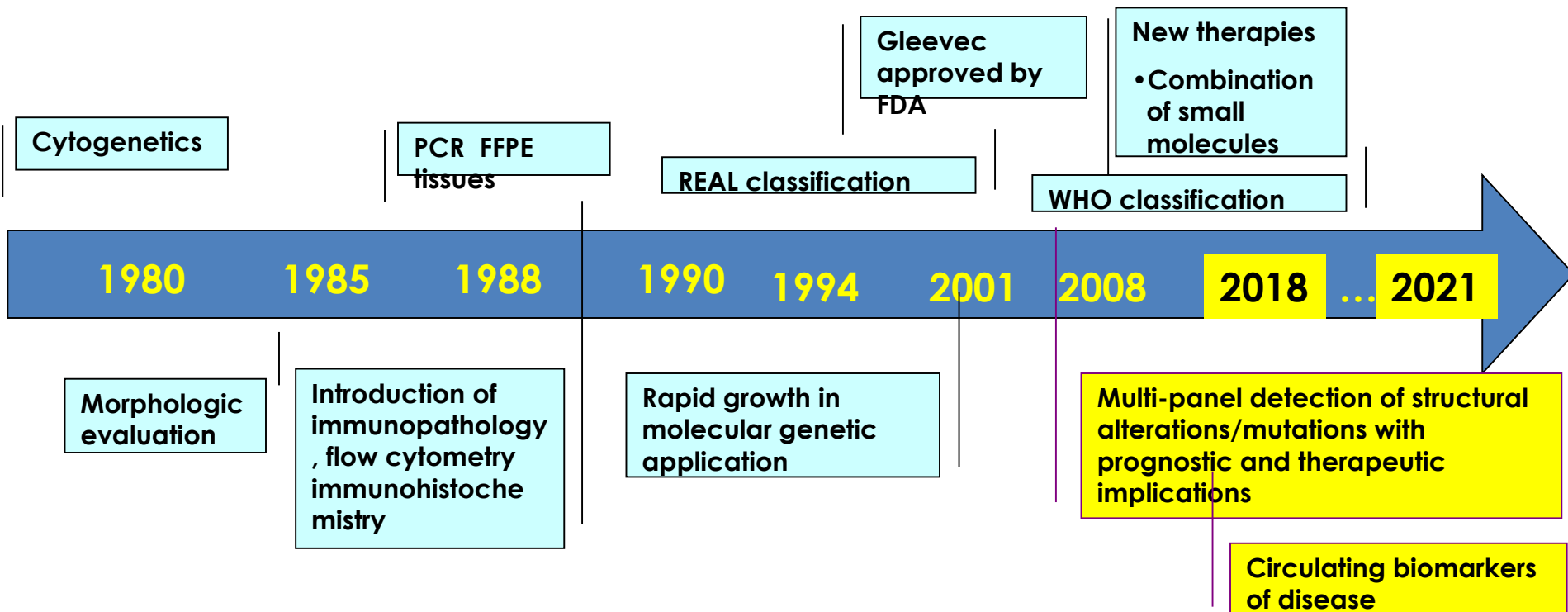
- Provisional entity in 2017 WHO
- Very rare
- Often associated with a seroma surrounding implant
- Strong CD30 positivity, negative for ALK
- Typically patients have good outcomes with capsulectomy, but if invasion of breast or formation of a tumorous mass, systemic therapy may be needed
- *STAT3* mutations



# Prognostic and Therapeutic Importance of Subclassification of ALK- ALCL



# Evolution of Molecular Diagnostics in Hematopathology



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Santiago Schnell PhD

CHILDREN'S  
ONCOLOGY  
GROUP

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cancer experts

QUESTIONS?

- 
- p.E2411X
  - p.R2400X
  - p.M2358V
  - p.Q2325X
  - p.I2304fsX3
  - p.I2304fsX0
  - p.I2304fsX2
  - p.K2292fsX3
  - p.E2290X
  - p.Q2285X
  - p.T2280fsX12
  - p.A2275D
  - p.K2102X
  - p.V1667I