PTCL-NOS: Gene expression profiling

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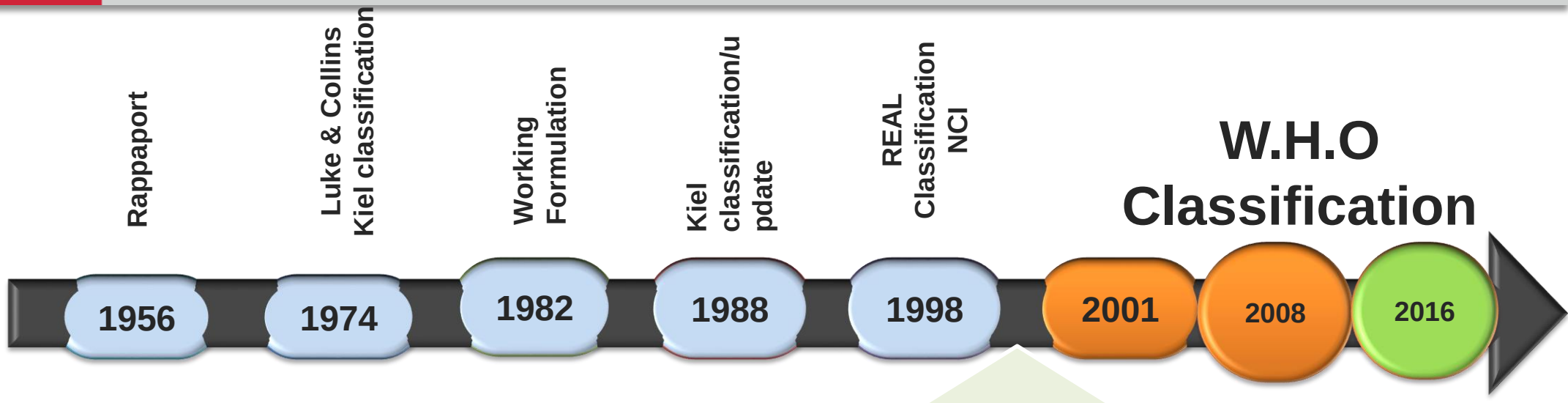
T-cell Lymphomas: we are close to the finalization”

History of Lymphoma Classification



Morphology/ Clinical

+ Immunohistochemistry

+ Cytogenetics, FISH

+ Molecular Biology

Nebraska

Classification project-1999

Transcriptomics

+ Gene Expression Profiles

Genomics

+ Next Generation Sequencing

PTCL entities

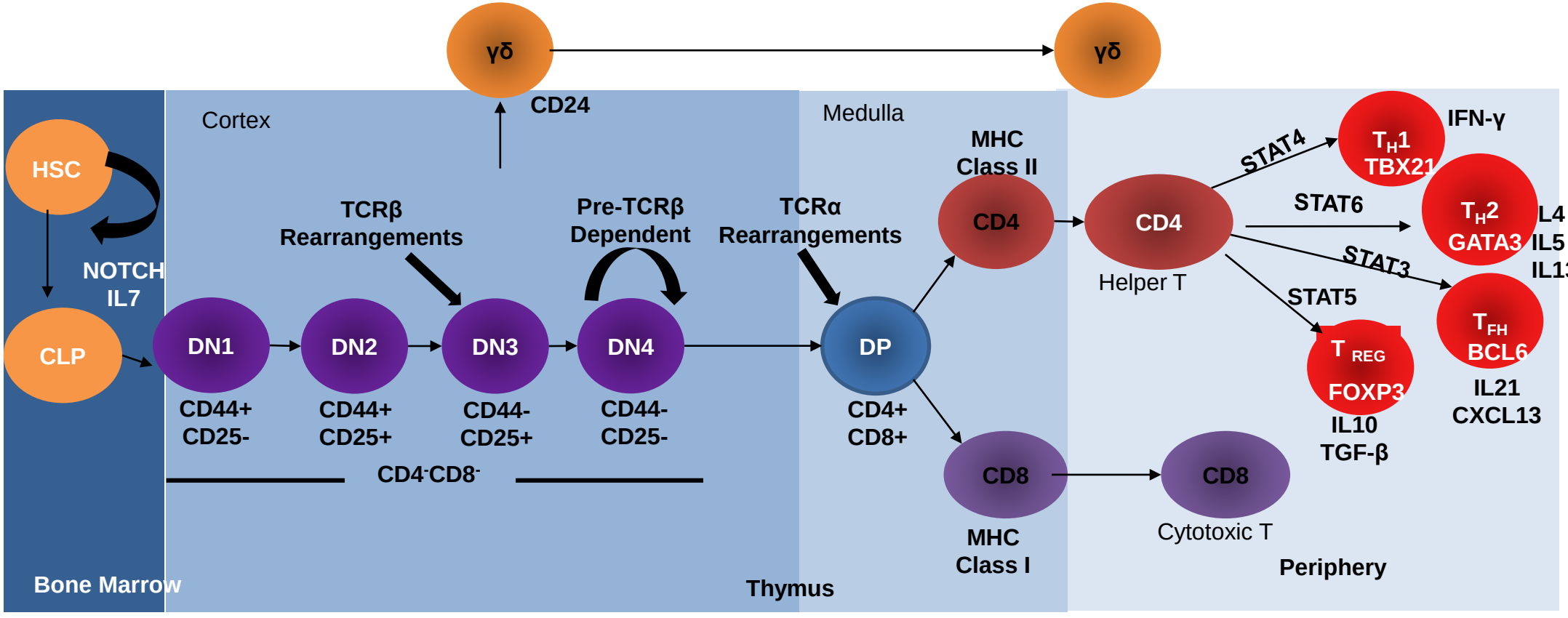
~19 ~25 ~30

Gene Signatures in PTCL

- **The genomic characteristics of each tumor through expression of a unique set of genes is known as "GENE SIGNATURE**

  - **Molecular diagnosis**
  - **Pathobiology and target characterization**
  - **Rationalize/Justify the new clinical investigations**

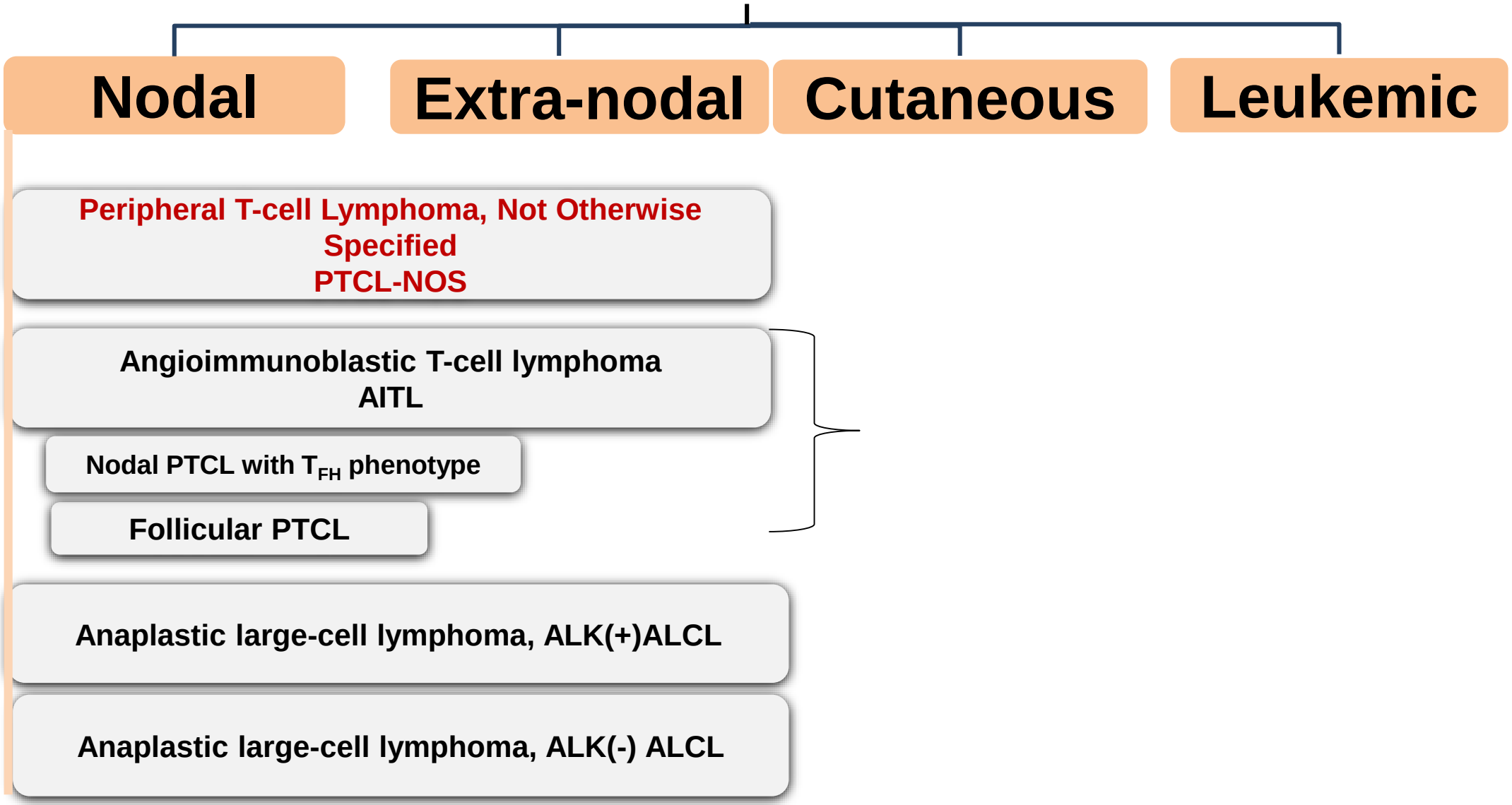
Mature T cell development and activation



HSC: Hematopoietic stem cells
CLP: common lymphoid progenitor

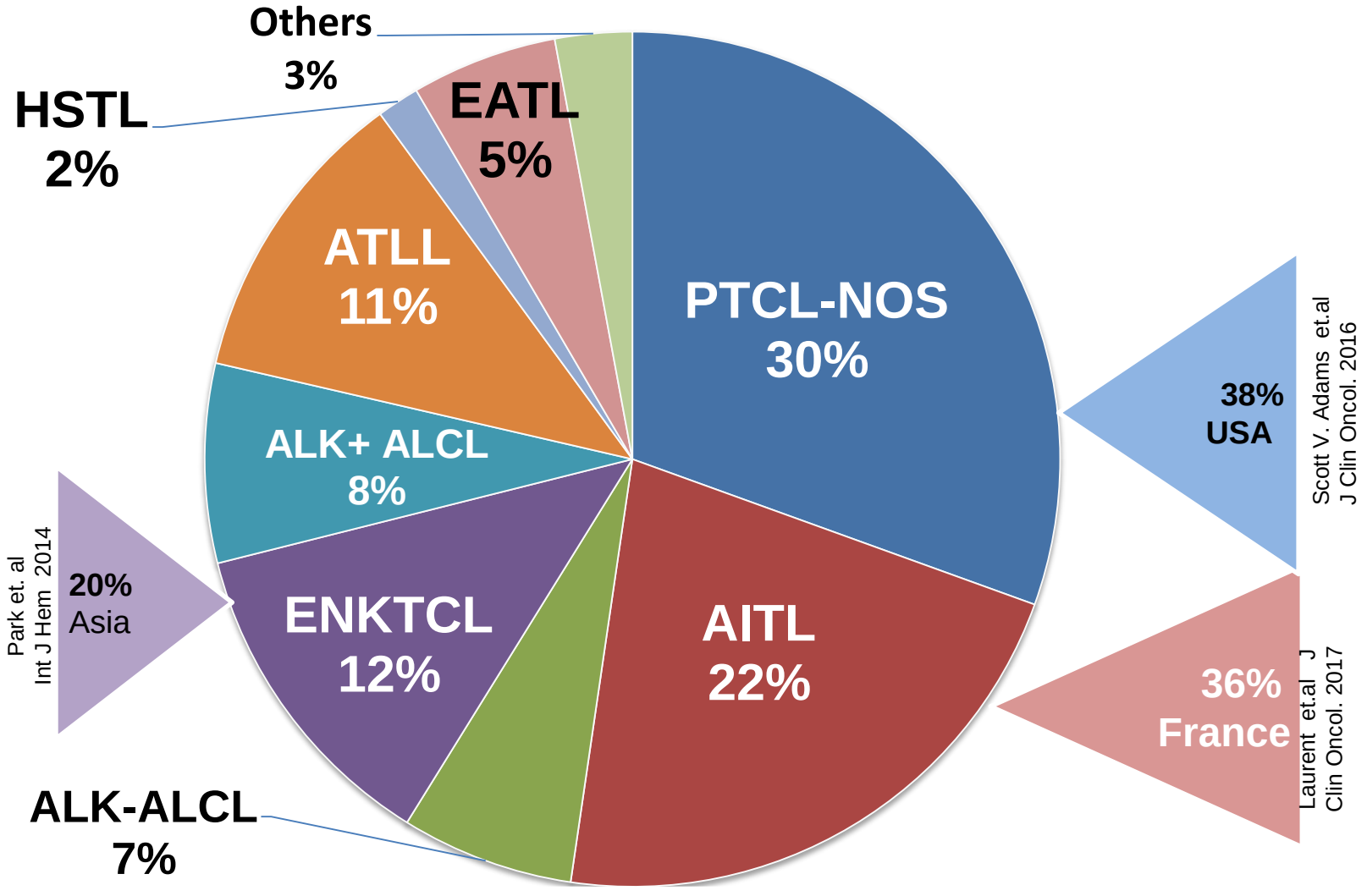
Complexity of T-cell immunobiology, numerous subsets and functional plasticity makes disease classification challenging

W.H.O. classification of mature T/NK-cell neoplasms (2016 revised version)



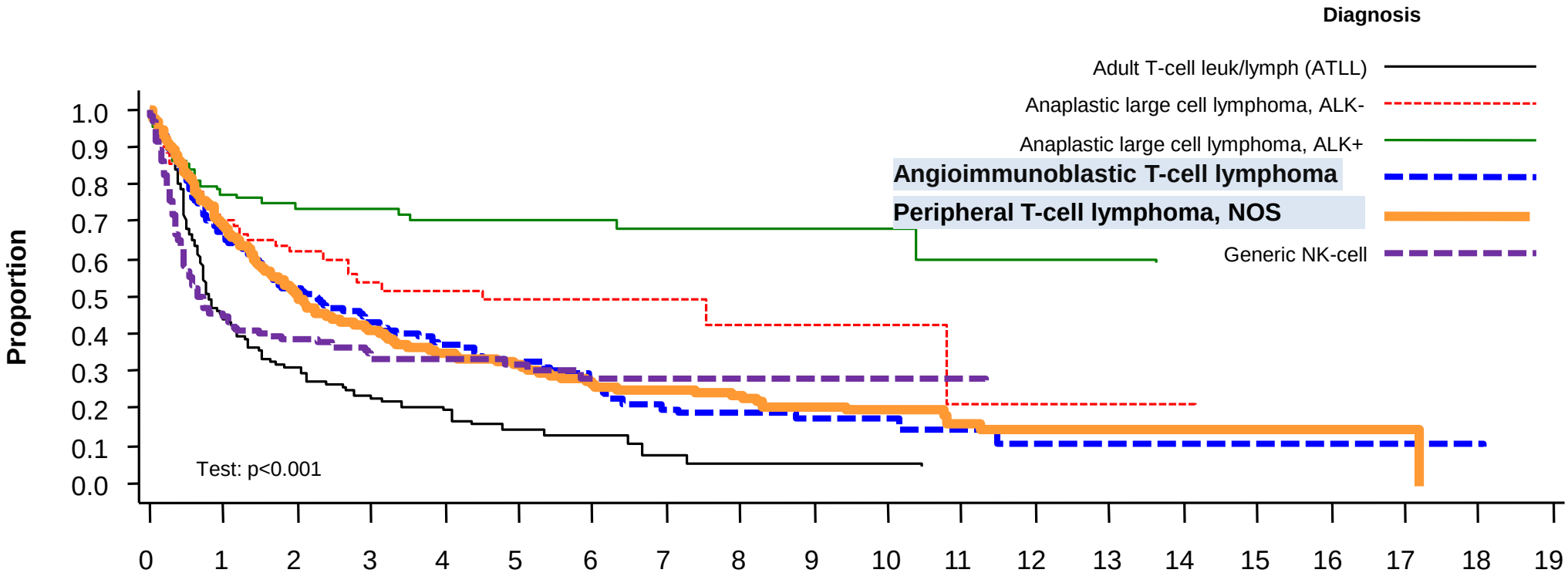
adapted from Swerdlow SH et al. Blood 2016 127:2375-2390)

Overall frequency of PTCL subtypes



Park, S. & Ko, Y.H. Int J Hematol (2014)
Scott V. Adams et.al J Clin Oncol. 2016; 34: 963–971
Vose et.al J Clin Oncol. 2008, 26:4124–4130

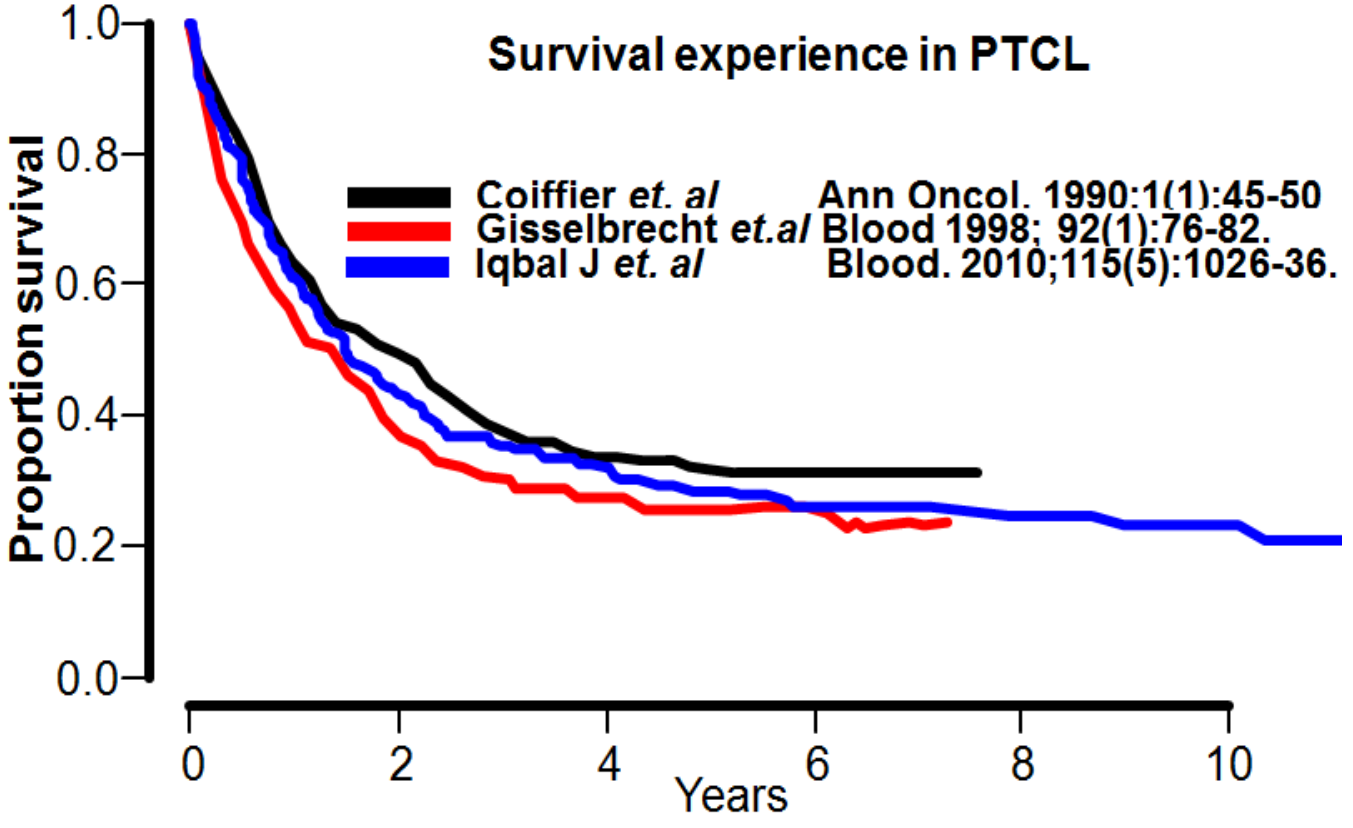
Major PTCL subtypes have inferior clinical outcome



- **Xu et.al PLOS One 2014**

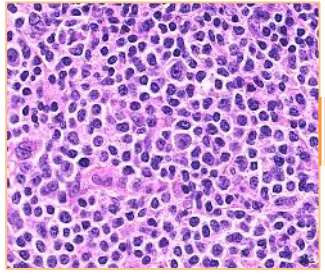
"No Survival Improvement for PTCL/AITL patients over the Past Two Decades: A Population-Based Study of 1207 Cases"

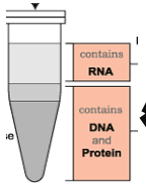
No major improvement in clinical outcome since last three decades in PTCL



OS of PTCL-NOS/AITL

High-through put technologies dissecting distinct molecular and prognostic subgroups

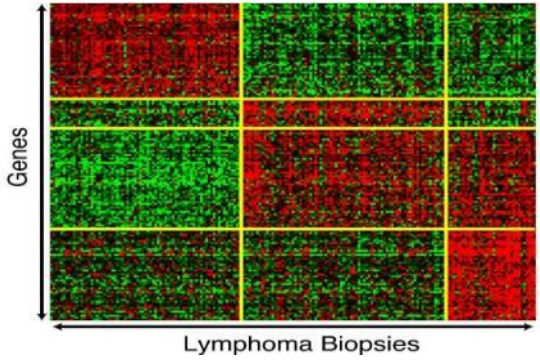


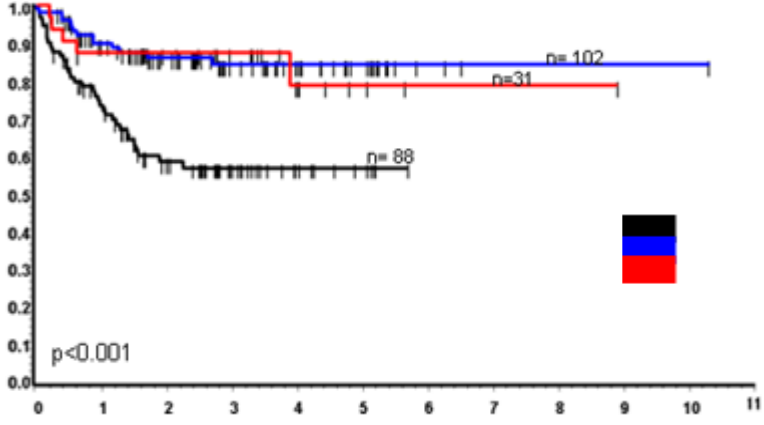


Transcriptomics

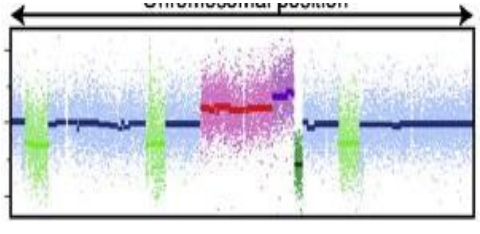
Genomics

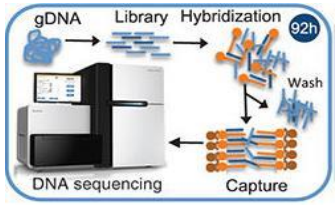
Gene expression profiling:
(mRNA expression)





Genomic DNA hybridization
(DNA copy number variations)





Genetic Evolution of molecular subgroups

Next generation sequencing

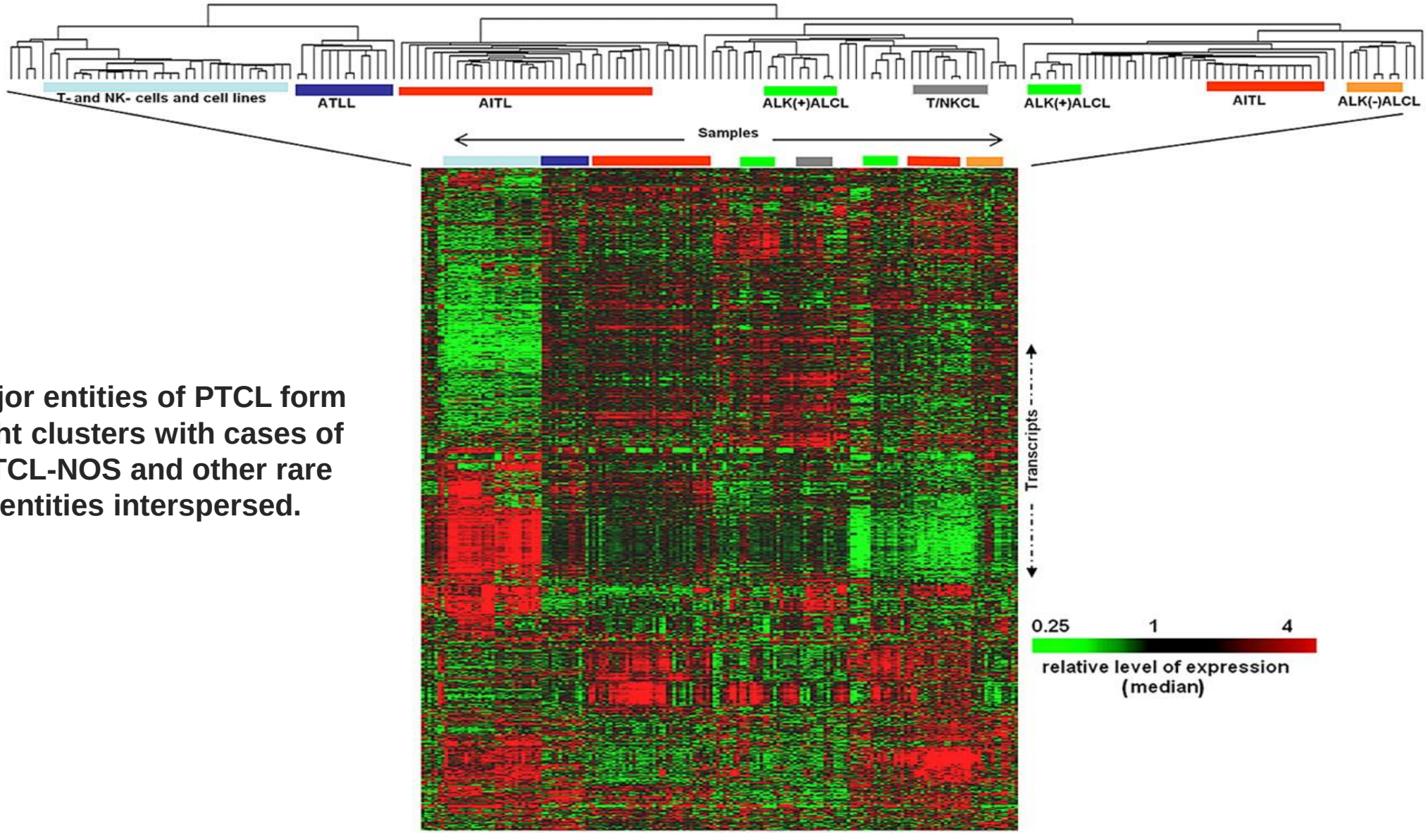
Patient-centered Research

Laboratory discoveries

Delivery to patient care /communities

Unsupervised hierarchical clustering of PTCL cases and normal T cells

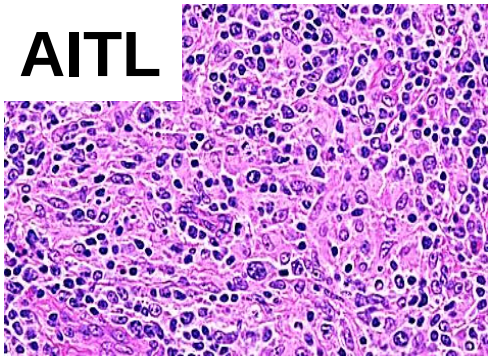
Major entities of PTCL form tight clusters with cases of PTCL-NOS and other rare entities interspersed.

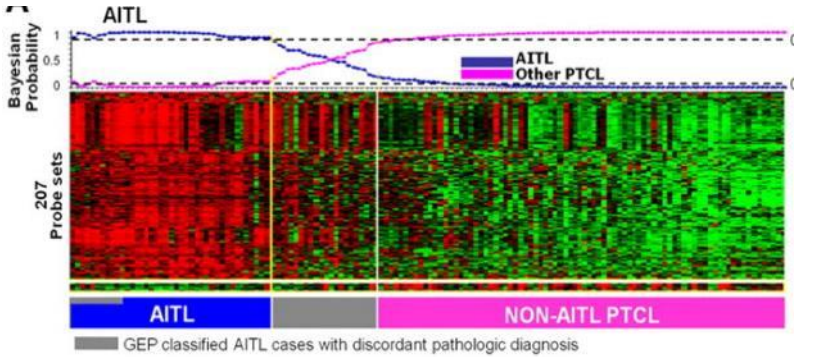


Blood 2010;115:1026-1036

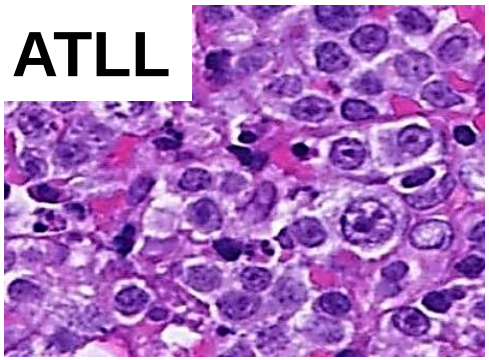
Gene expression-based molecular predictors of the major subgroups of PTCL

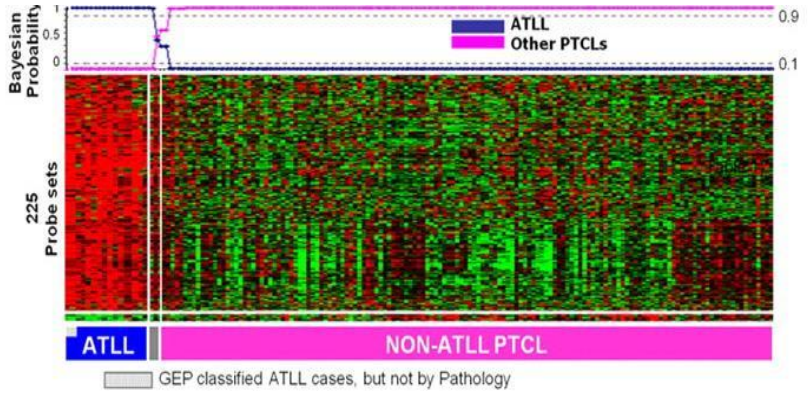
AITL



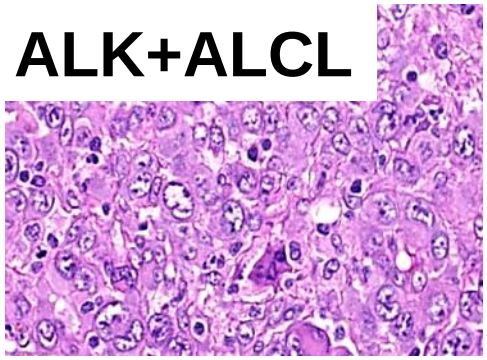


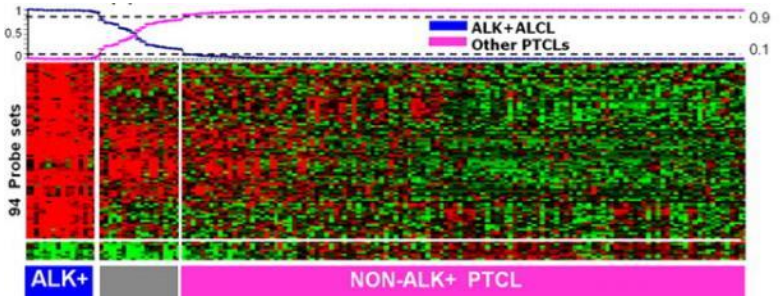
ATLL

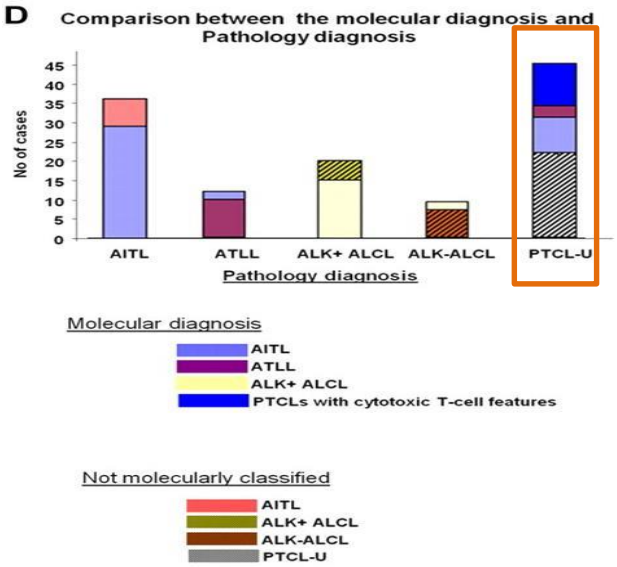




ALK+ALCL







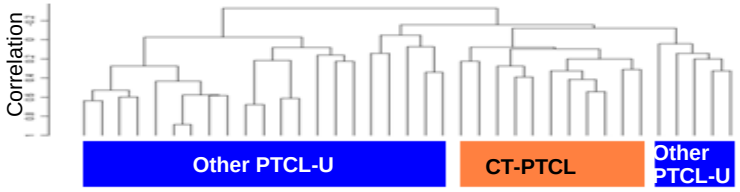
- **More than half of the PTCL-NOS cases were <u>not</u> molecularly classified**

International PTCL Project

Blood 2010;115:1026-1036

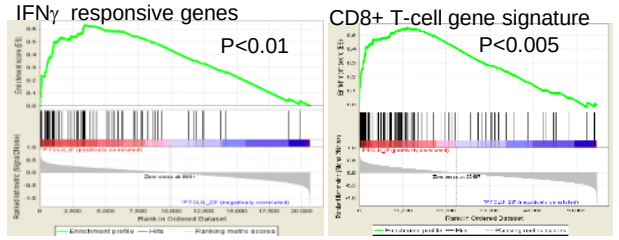
Identification of cytotoxic (αβ) PTCL group from PTCL-NOS

(A) Hierarchal clustering

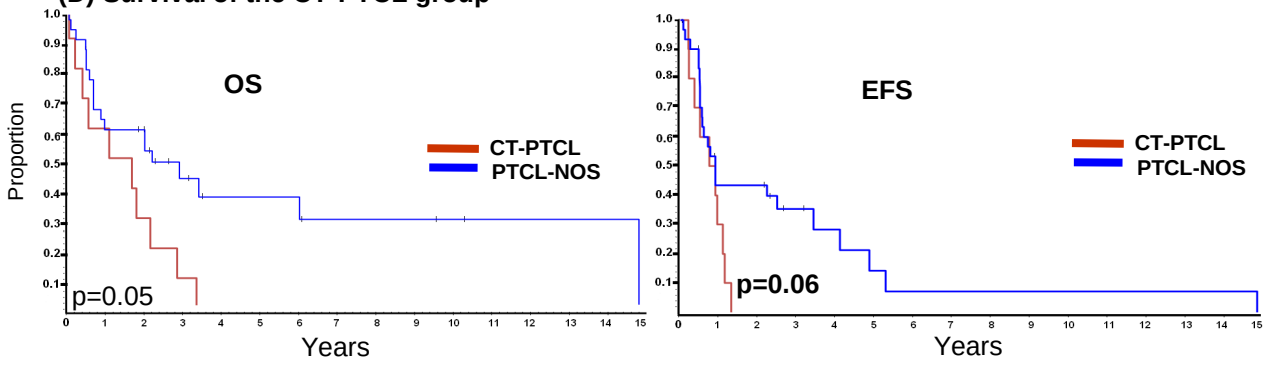


Dendrogram for clustering PTCL-NOS cases using centered correlation and complete linkage

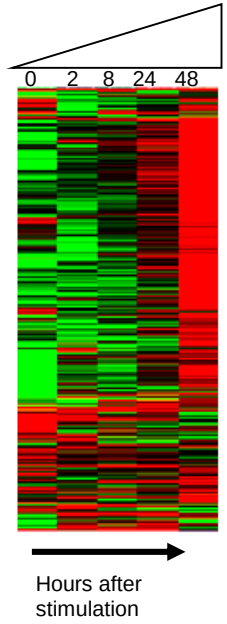
(C) GSEA analysis

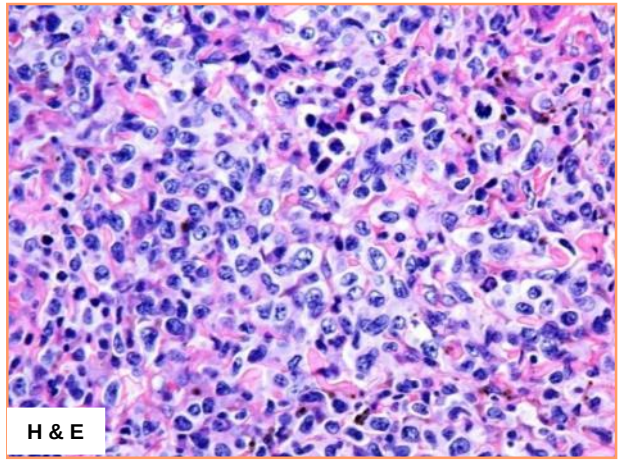


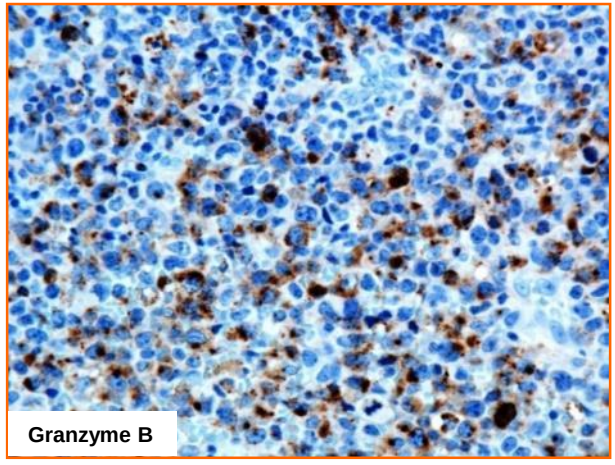
(D) Survival of the CT-PTCL group



(B) Expression of the CT-PTCL signature in normal CD8+ T-cells stimulated with anti-CD3, anti-CD28 and IL12 for various time intervals (hours)



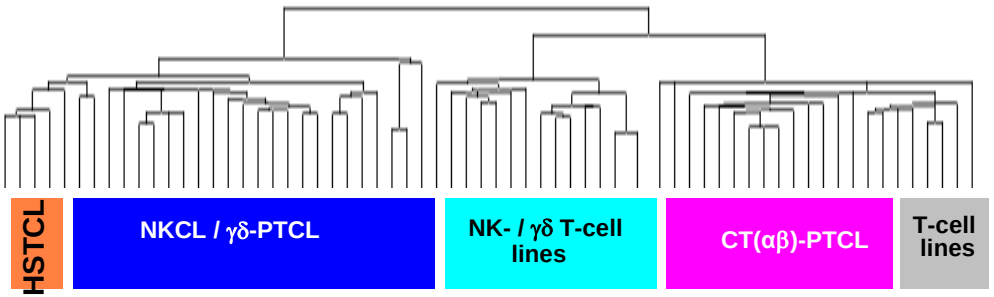


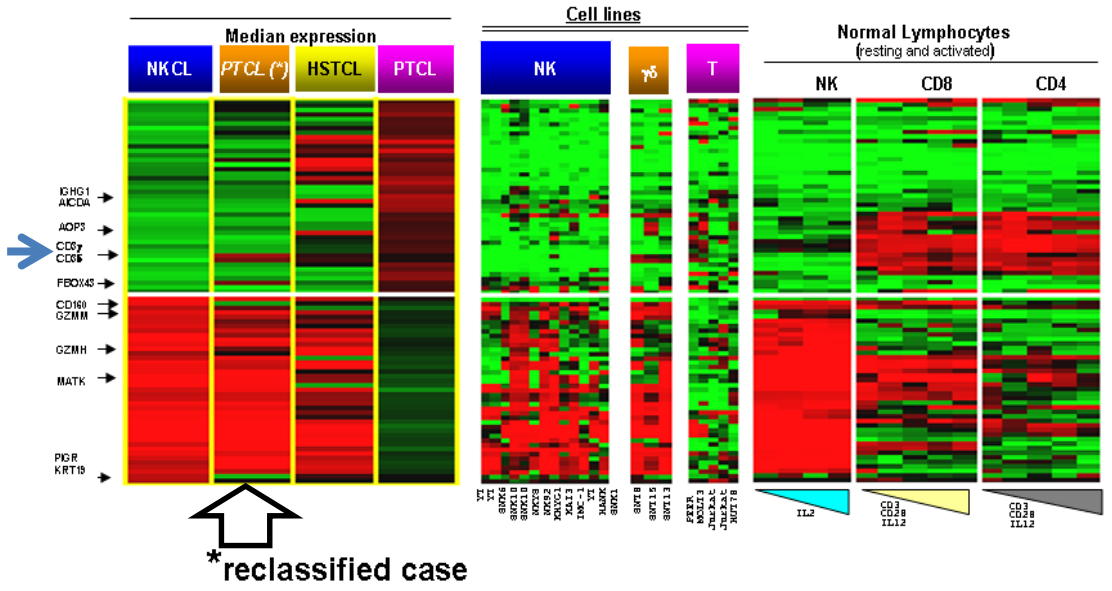


Blood 2010;115:1026-1036

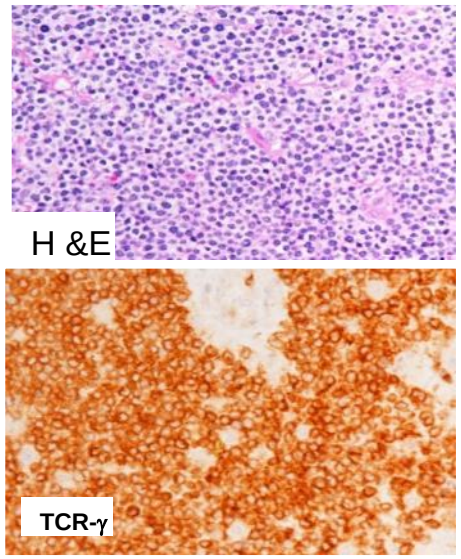
Identification of γδ-PTCL from PTCL-NOS

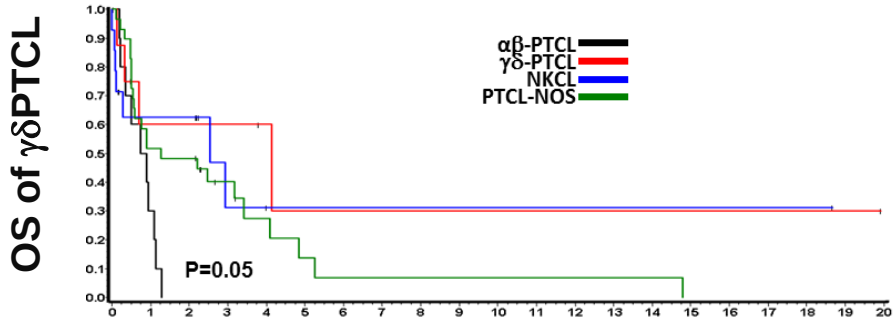
γδ-PTCL have similar gene expression signature as NKCL_but distinct from CT(αβ)- PTCL and HSTCL





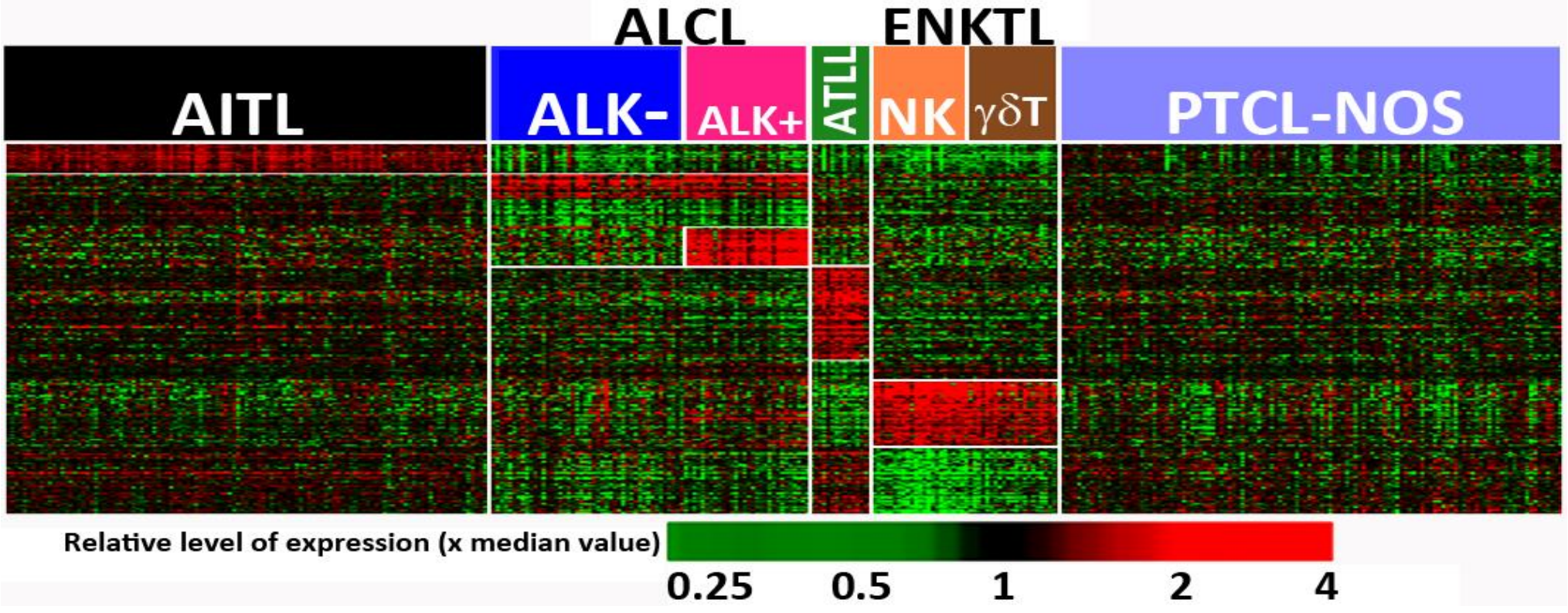
Markers	Status
CD3ε	7/7 (+)
CD2	2/4 (+)
CD5	1/7 (+)
CD7	1/1 (+)
CD8	3/5 (+)
CD4	1/6 (+)
CD56	**3/4 (+)**
TIA1	**4/4 (+)**
Granzyme B	**2/3 (+)**
TCR-beta	**5/5 (-)**
EBER-1	3/5 (-)





Leukemia. 2011 Feb;25(2):348-58.

Refinement of molecular diagnostic signatures



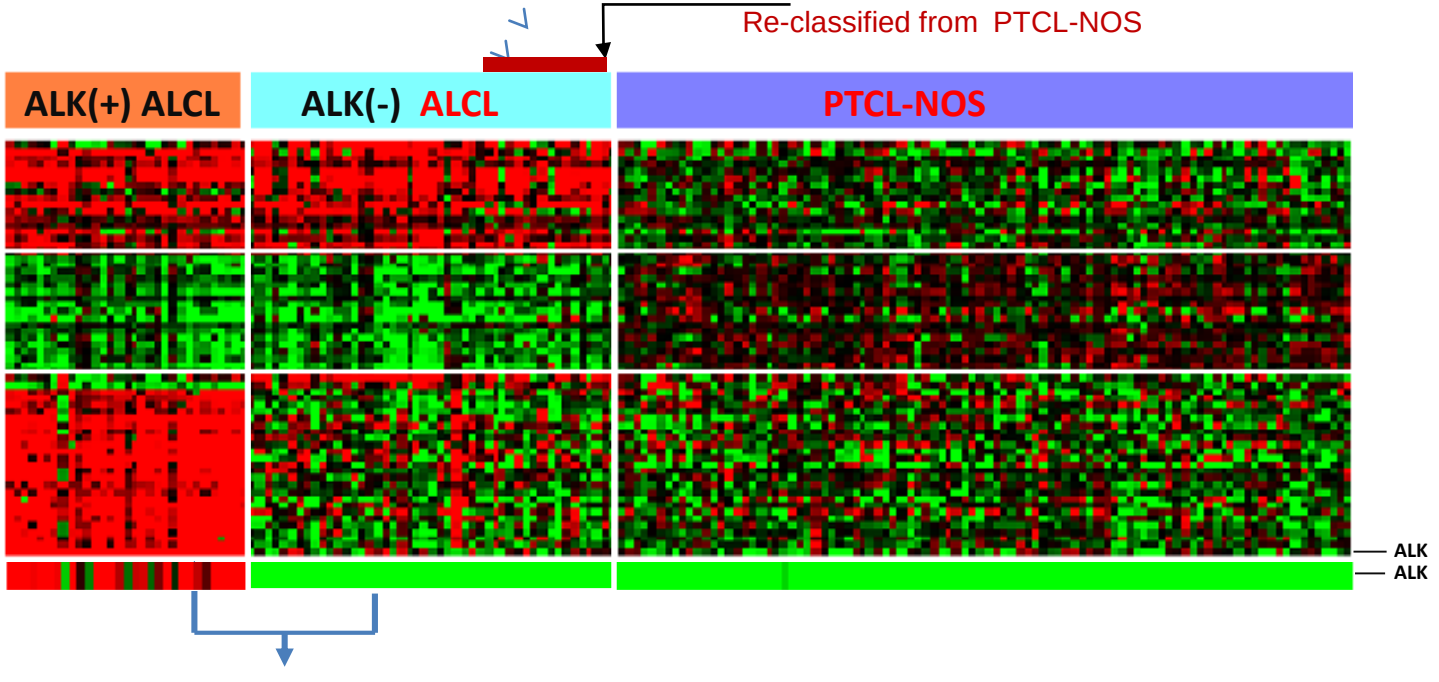
- **Unique molecular signatures were identified for major PTCL entities**

Lymphoma and Leukemia Molecular Profiling Project (LLMPP) initiative

Blood 2014

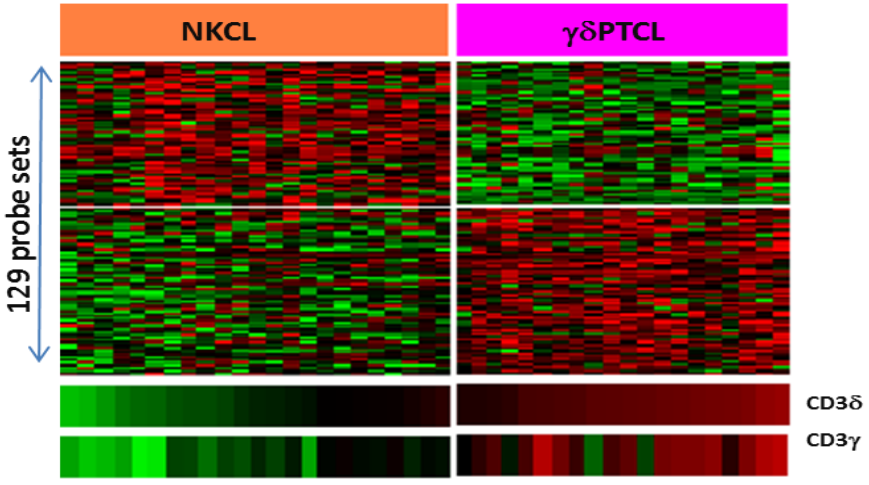
Robust molecular signature for ALK(-)ALCL

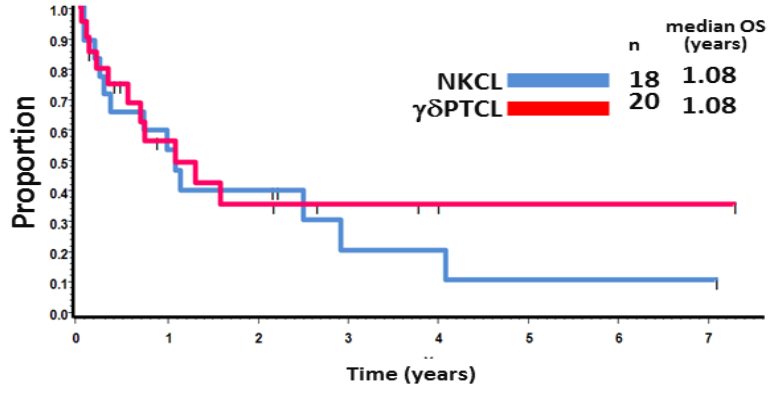
Gene signature/pathway enrichment summary in ALK(-)ALCL

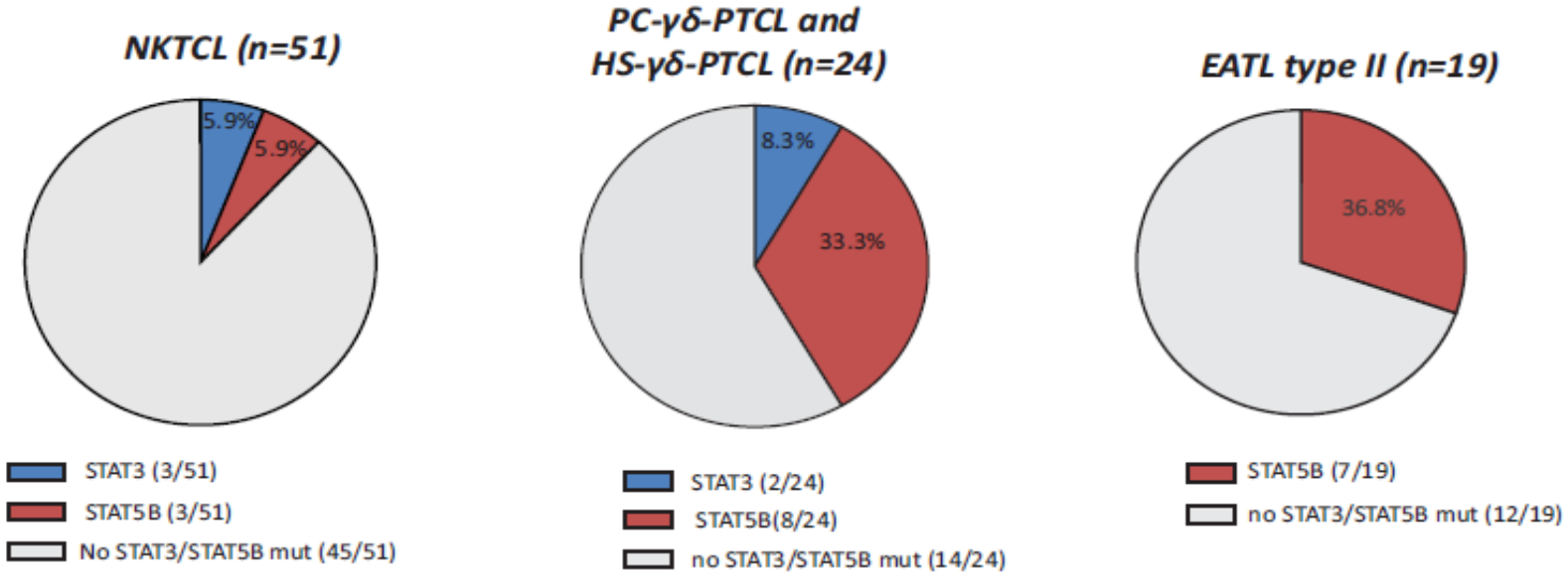


- ALK(-) ALCL is molecularly distinct from PTCL-NOS and ALK(+)ALCL

STAT3 and STAT5B mutations identified in NK or γδ-T cell derived lymphomas





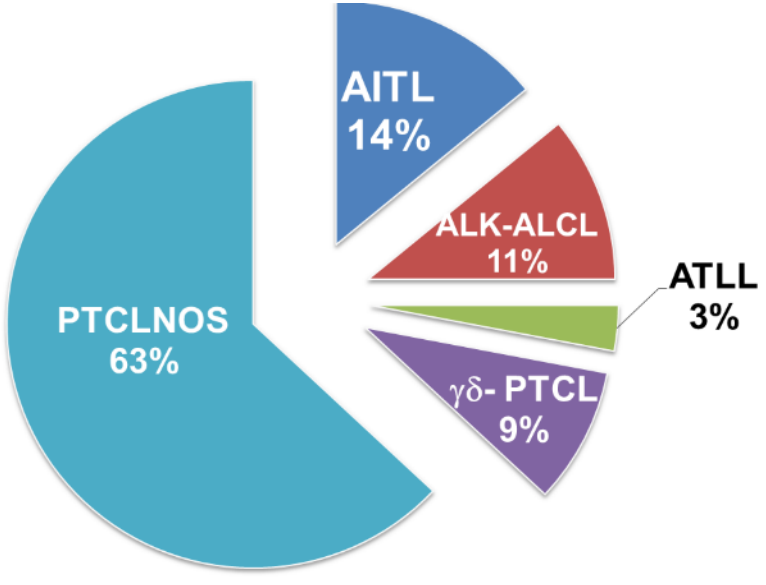


-Stat3 and Stat5B are often mutated at the SH2 domain in NK and γδ-T cell lymphomas
-*In vitro* data analysis showed sensitivity of this mutations to JAK1/2 inhibition

Nat Commun. 2015 Jan 14;6:6025.

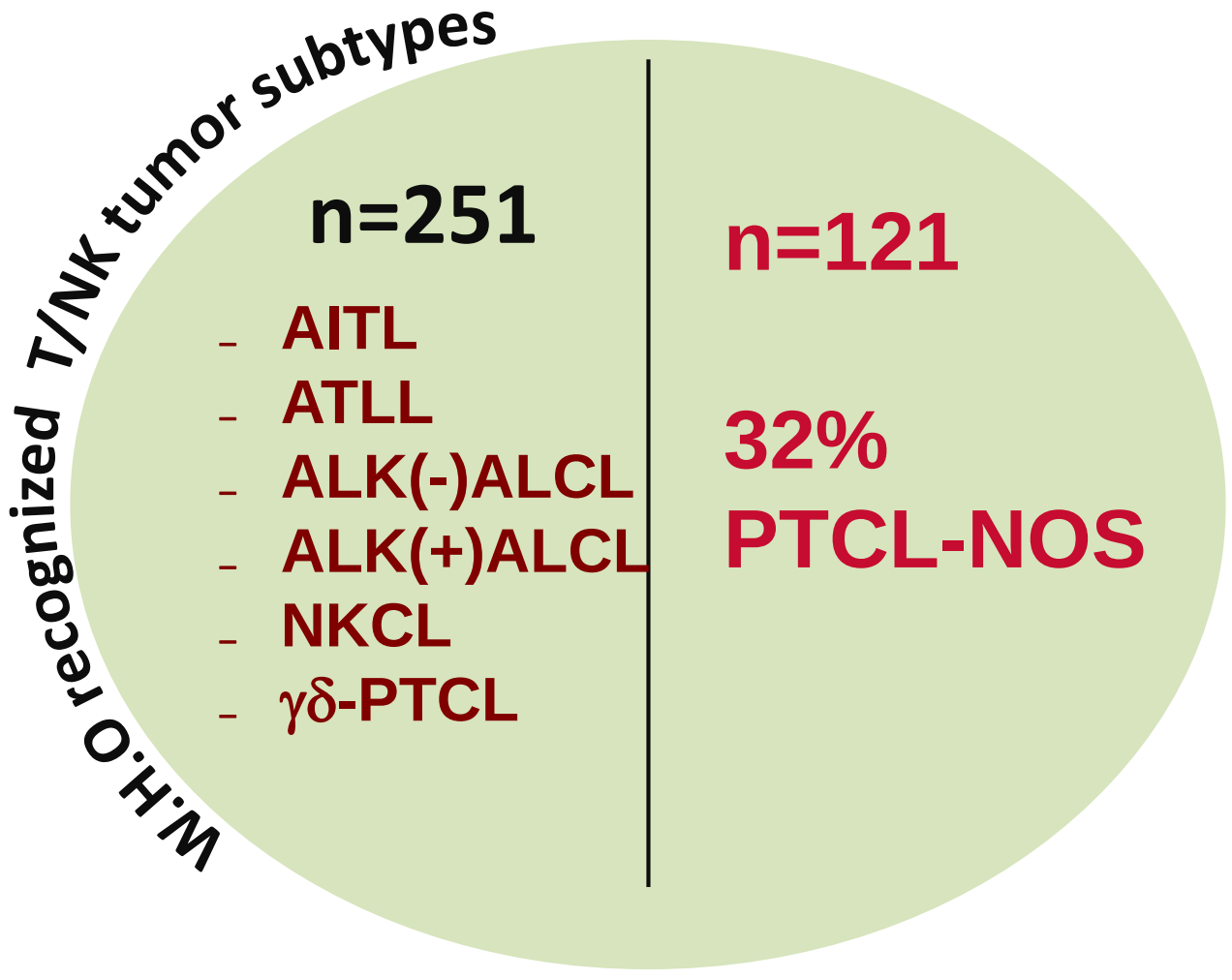
Evaluation of pathological *vs* molecular diagnosis

of 152 PTCL-NOS cases, a subset of cases were classified into unique PTCL entities

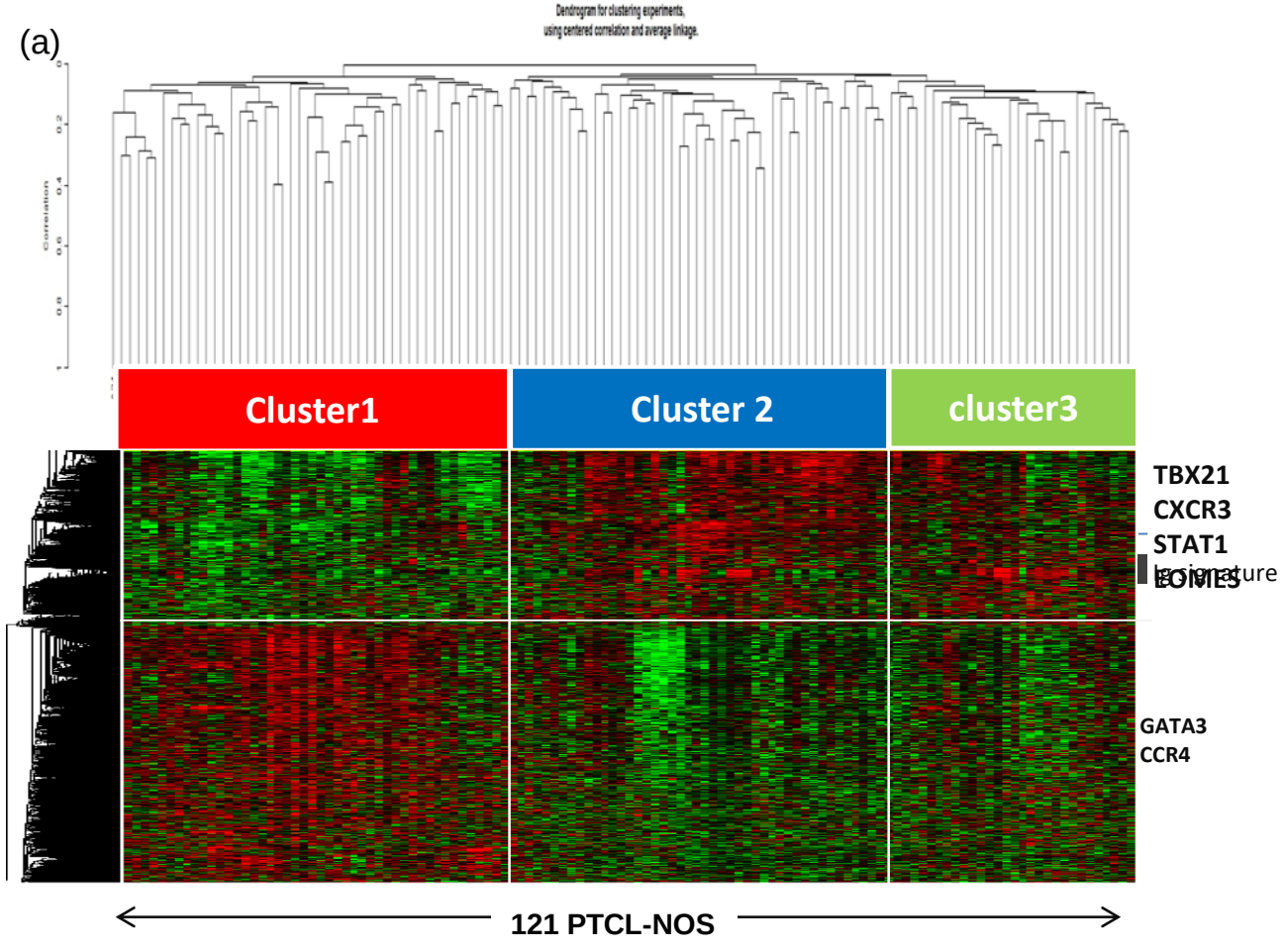


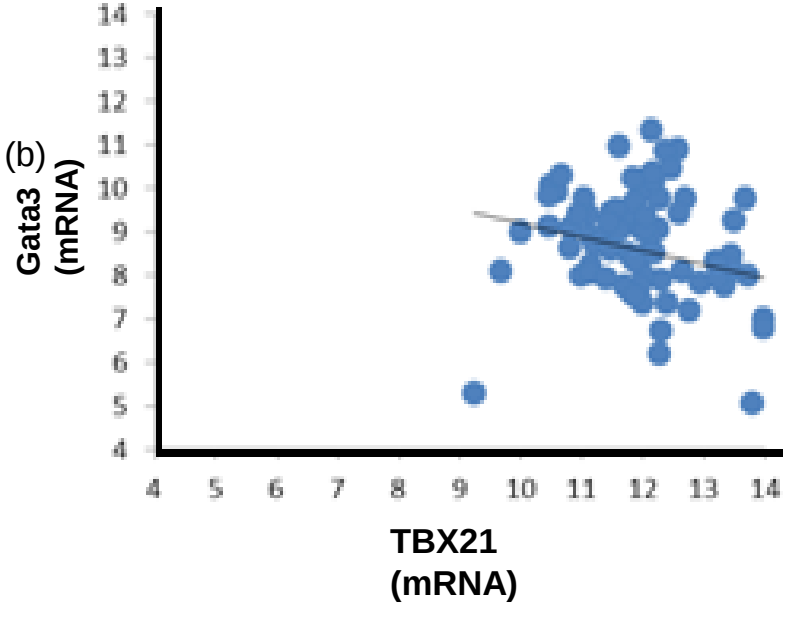
Blood. 2014 May 8;123(19):2915-23.

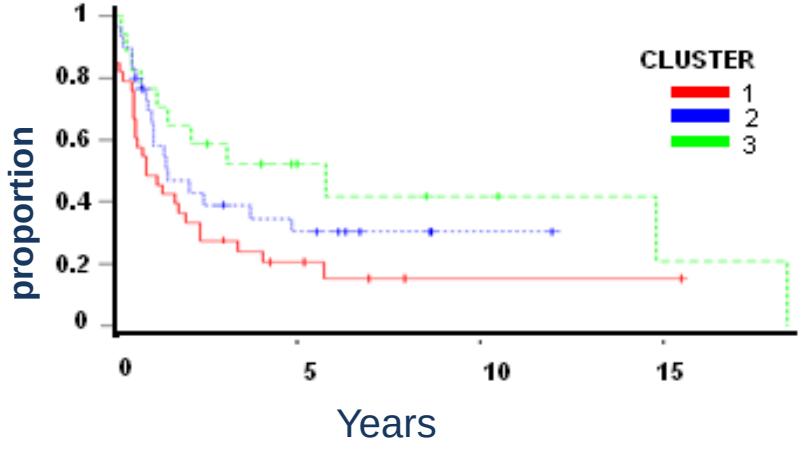
One-third of PTCL-NOS cases were not molecularly classified into WHO recognized PTCL entities



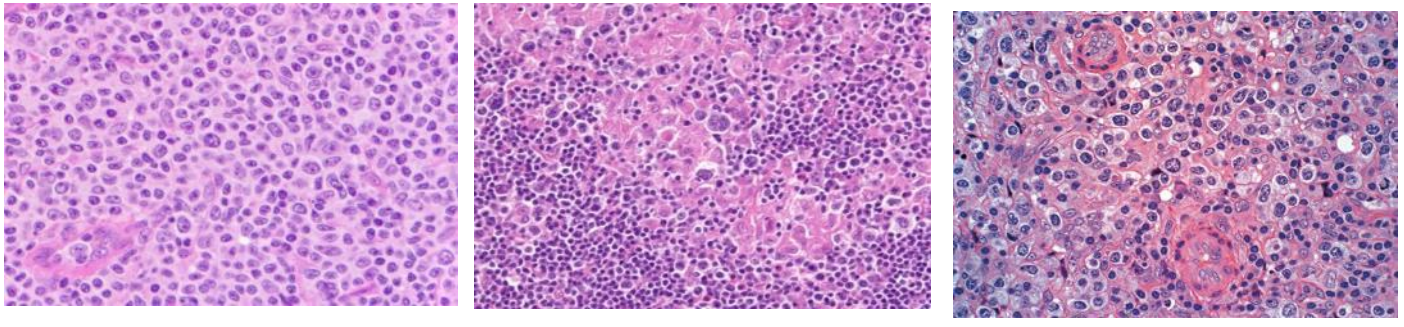
Unsupervised clustering of PTCL-NOS showed at least 3 major clusters



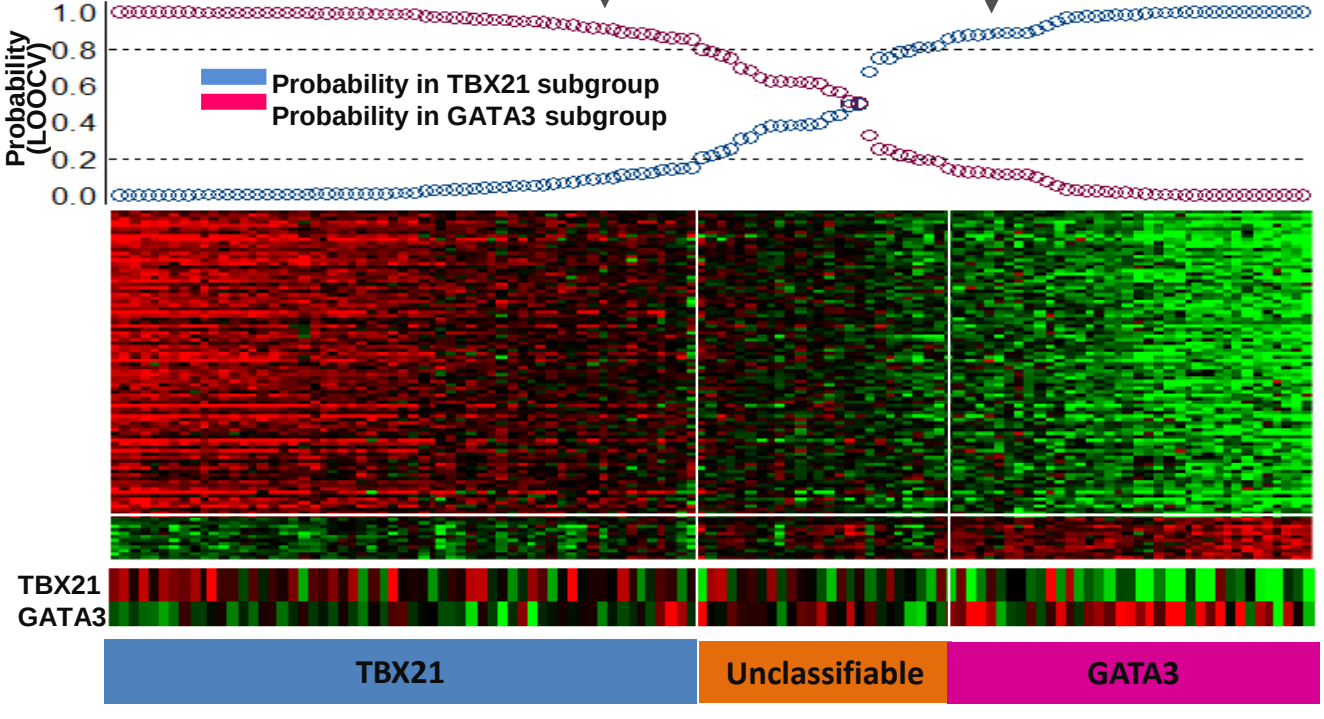




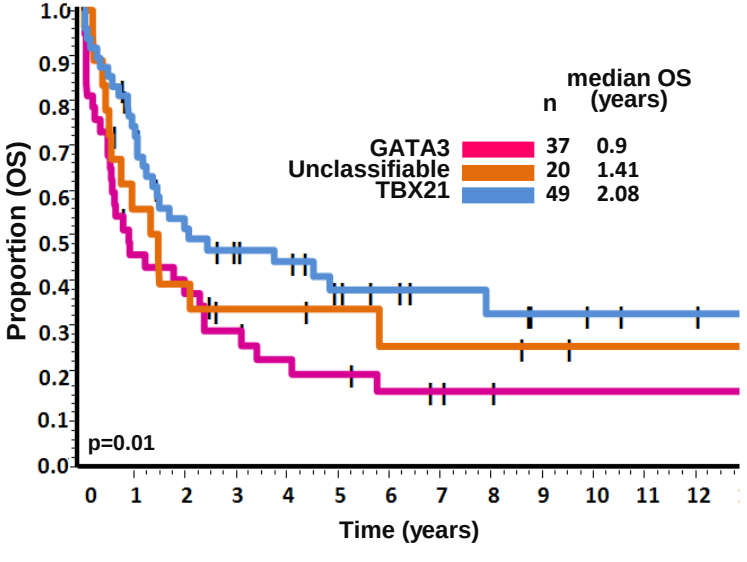
PTCL-NOS can be further divided two major subgroups



(A)

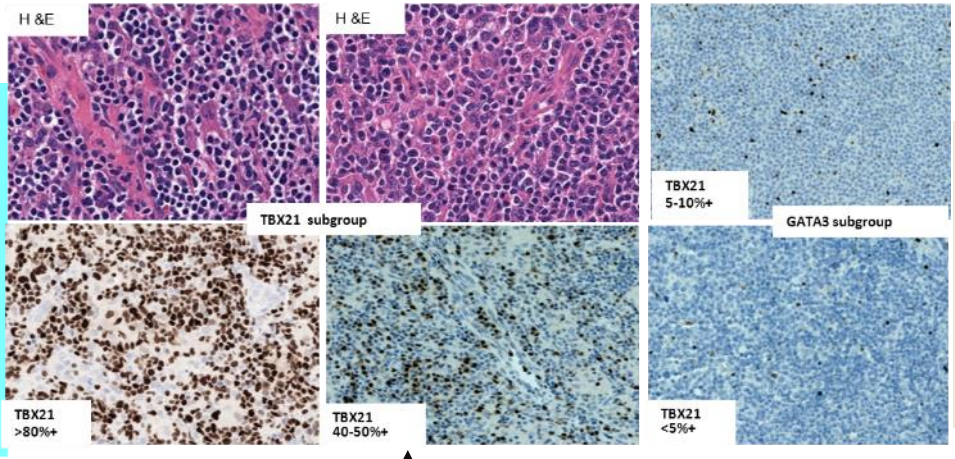


(B)

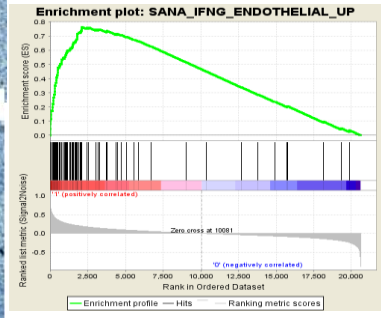


GEP identified distinct oncogenic pathways

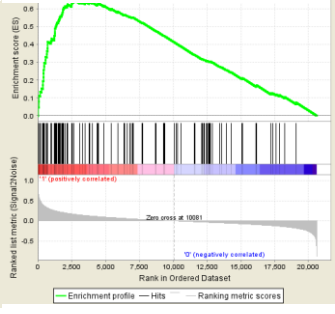
TBX21



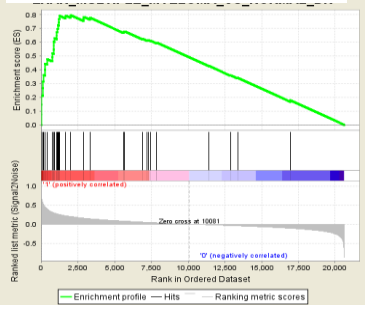
INF-γ gene signature



NF-κB target gene signature

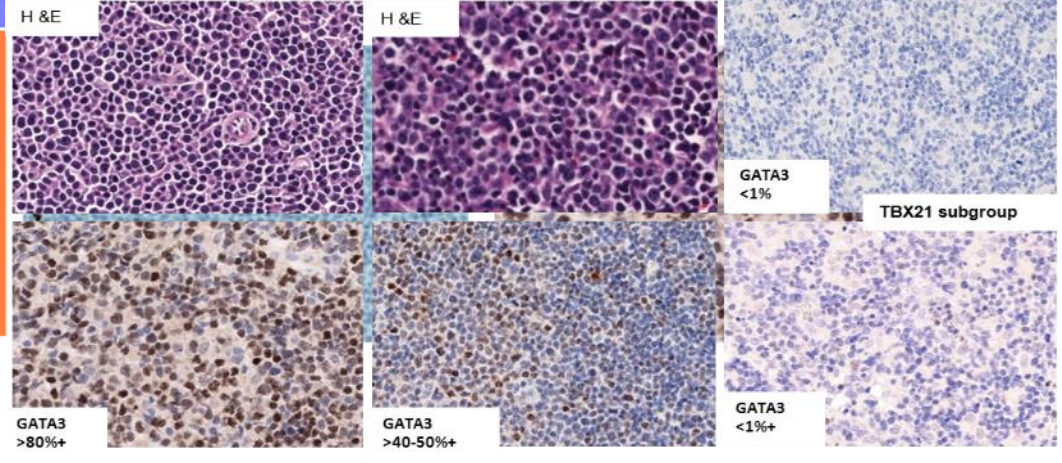


Plasma cell gene signature

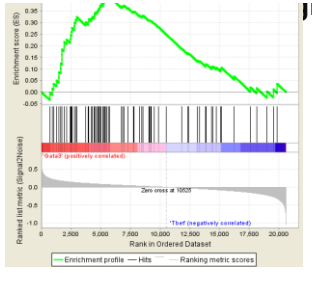


IHC validation

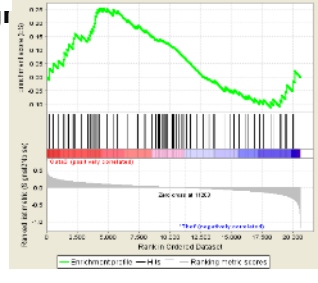
GATA3



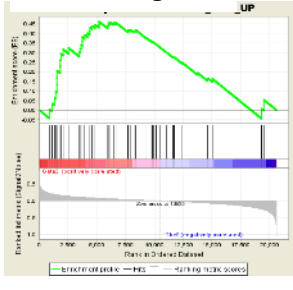
Proliferation



atenin oncogenic



MYC targets



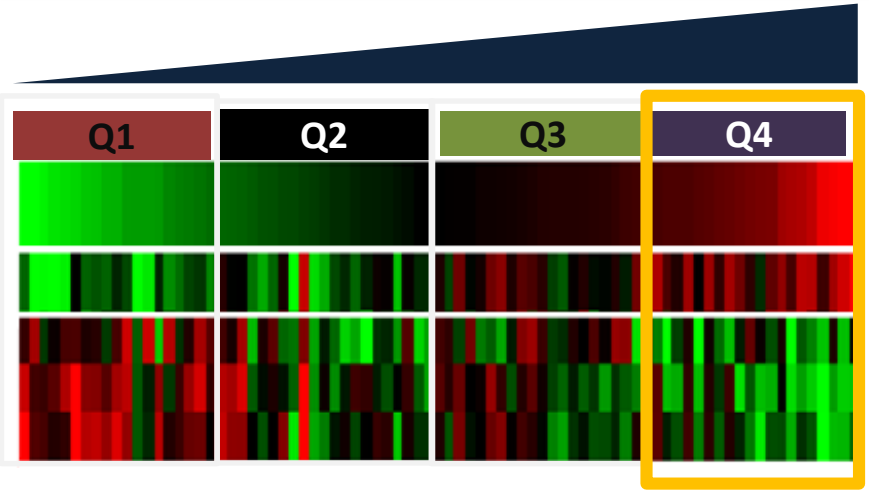
PI3Kinase regulated

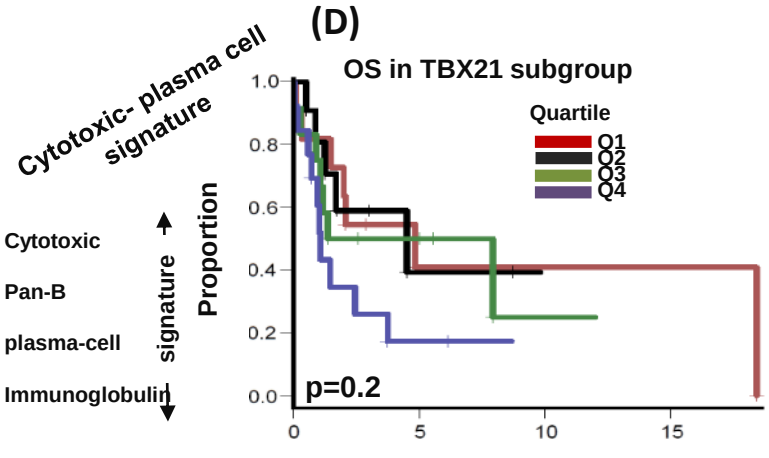
Myc induced gene targets (up)

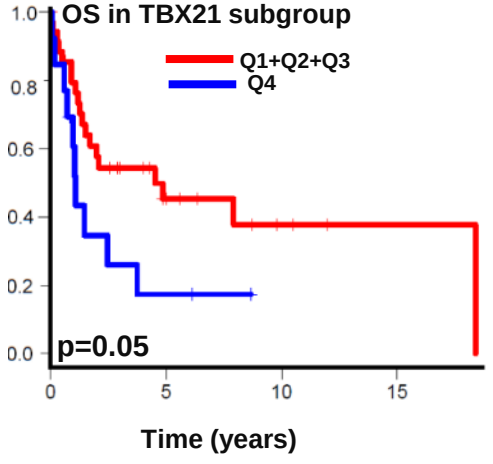
Ribosomal transcripts (mTOR)

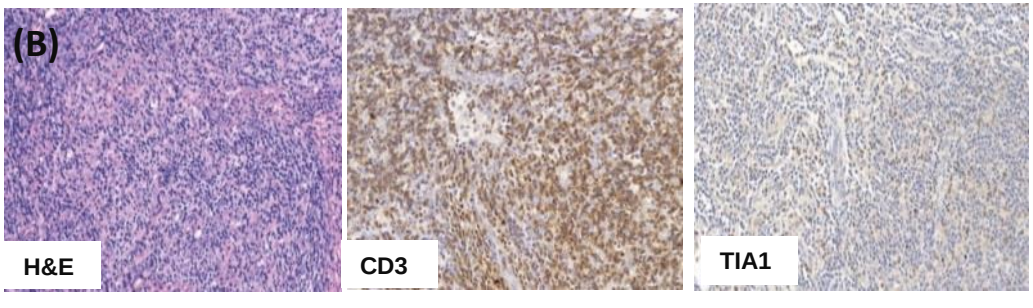
Proliferation related

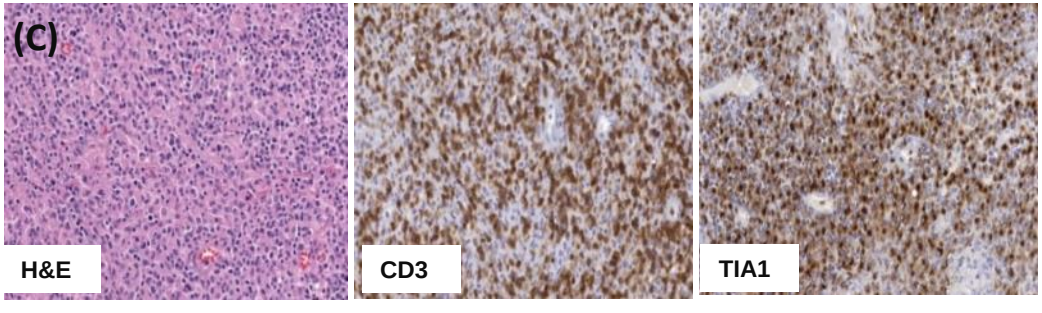
Tumor microenvironment significantly influences the prognosis in PTCL-TBX21 subgroup



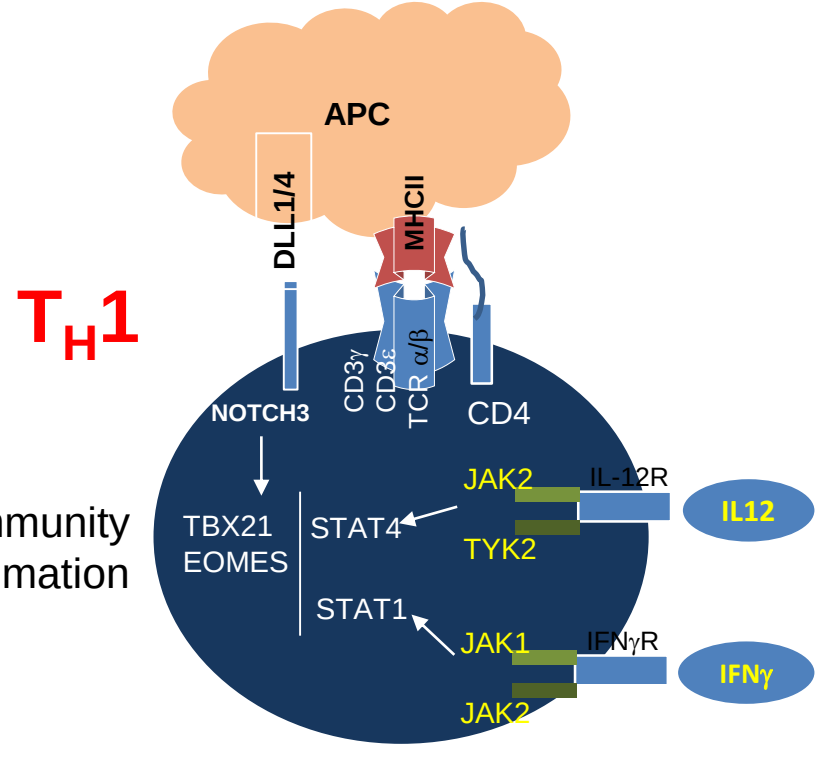




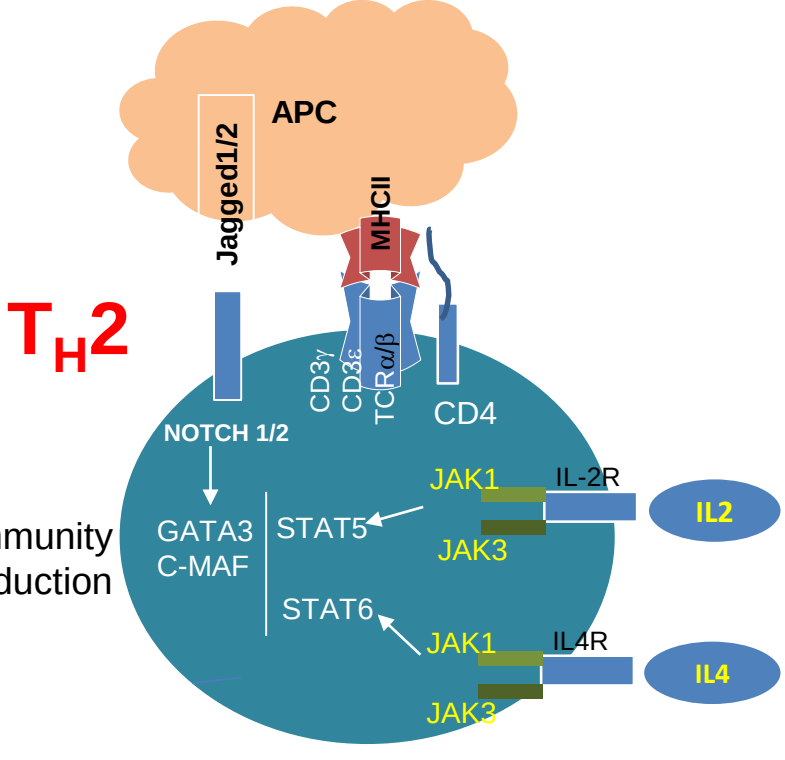




$T_H1/2$ differentiation ProgramSchematic

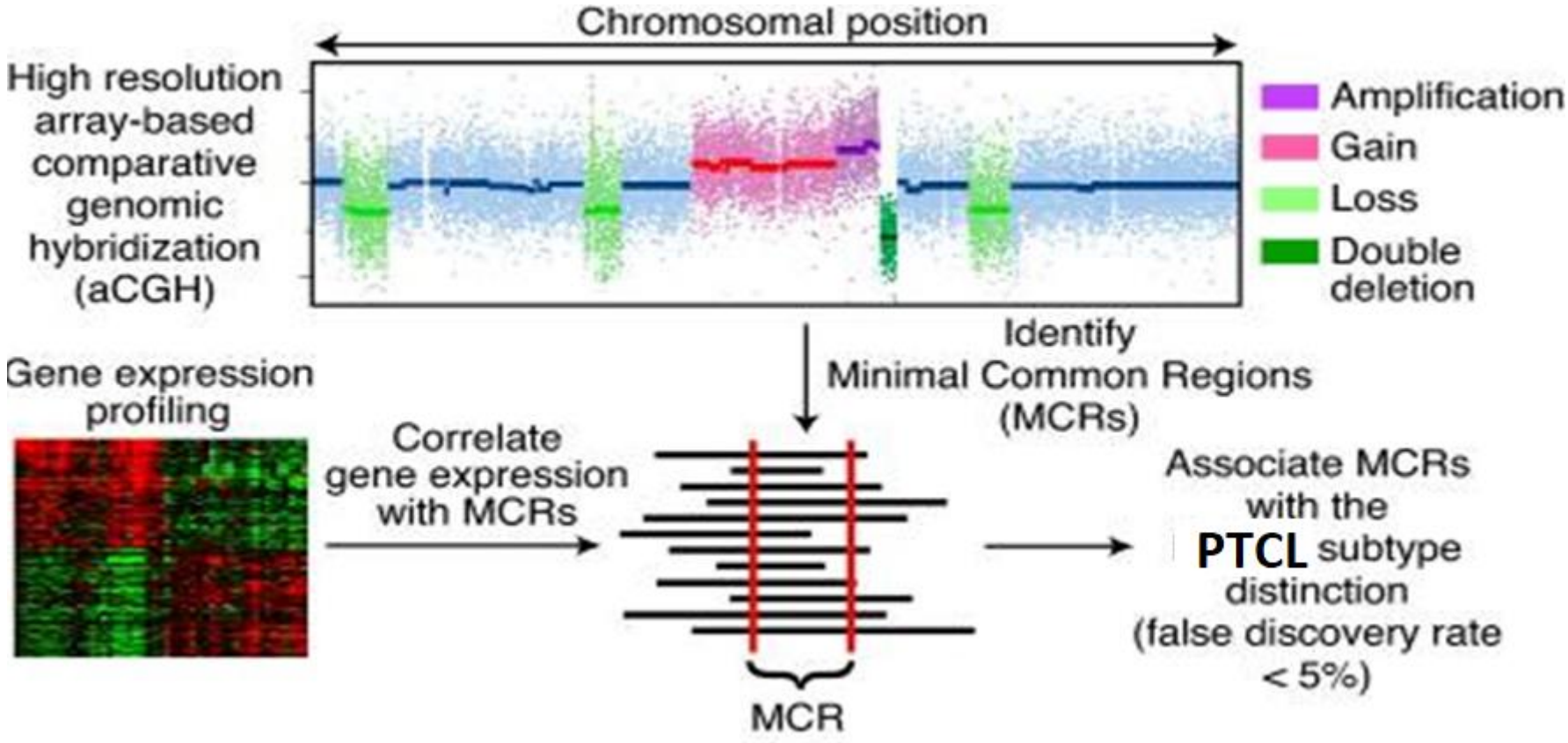


Cellular immunity
Inflammation



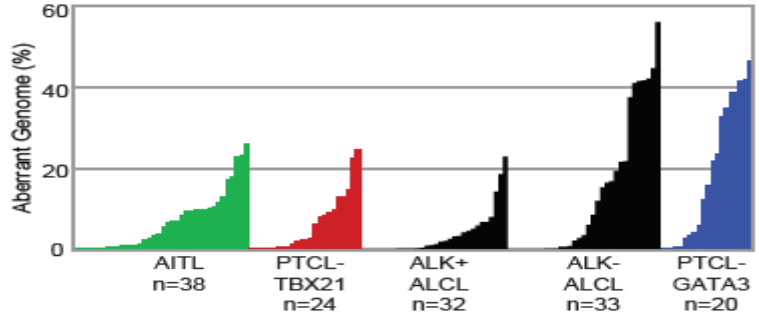
Humoral immunity
Abs production

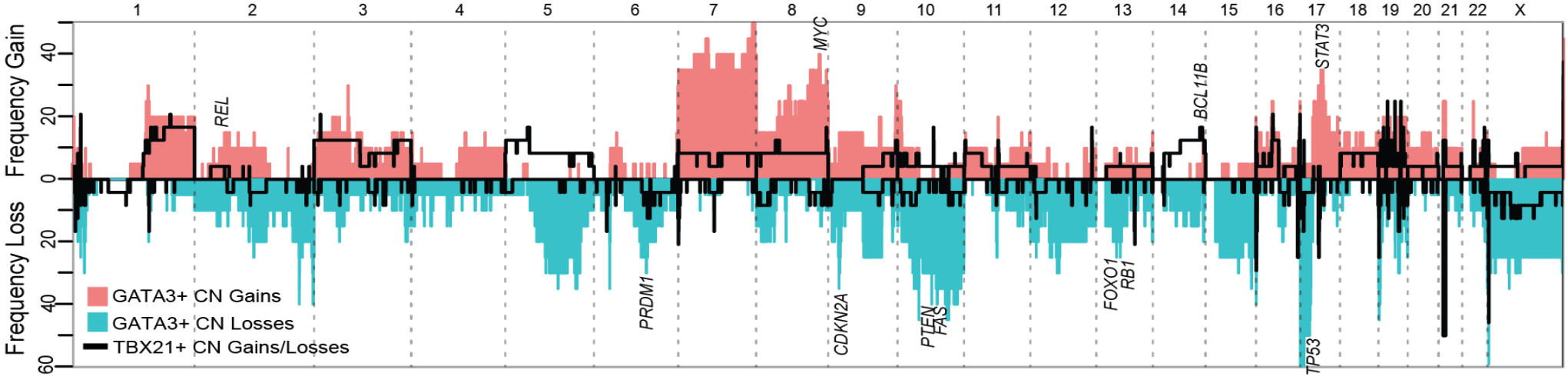
Schematic of the Gene Expression and Dosage Integration algorithm

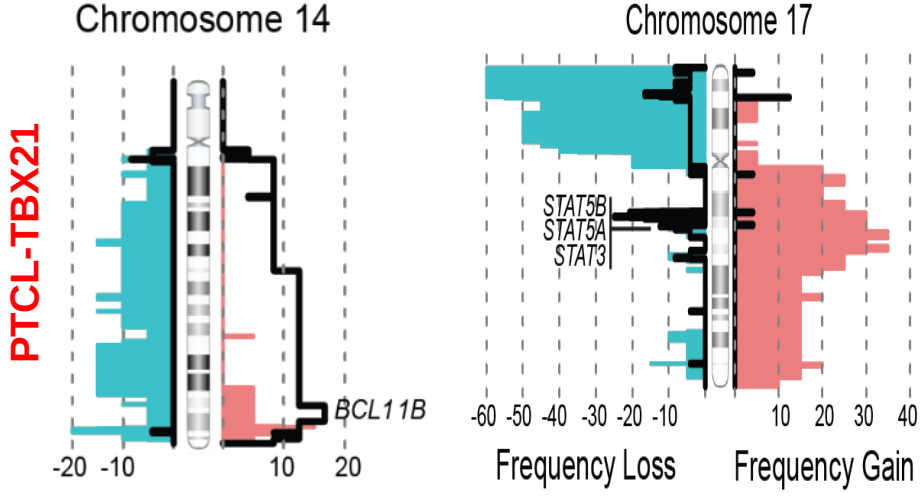


Proc Natl Acad Sci U S A. 2008 Sep 9; 105(36): 13520–13525.

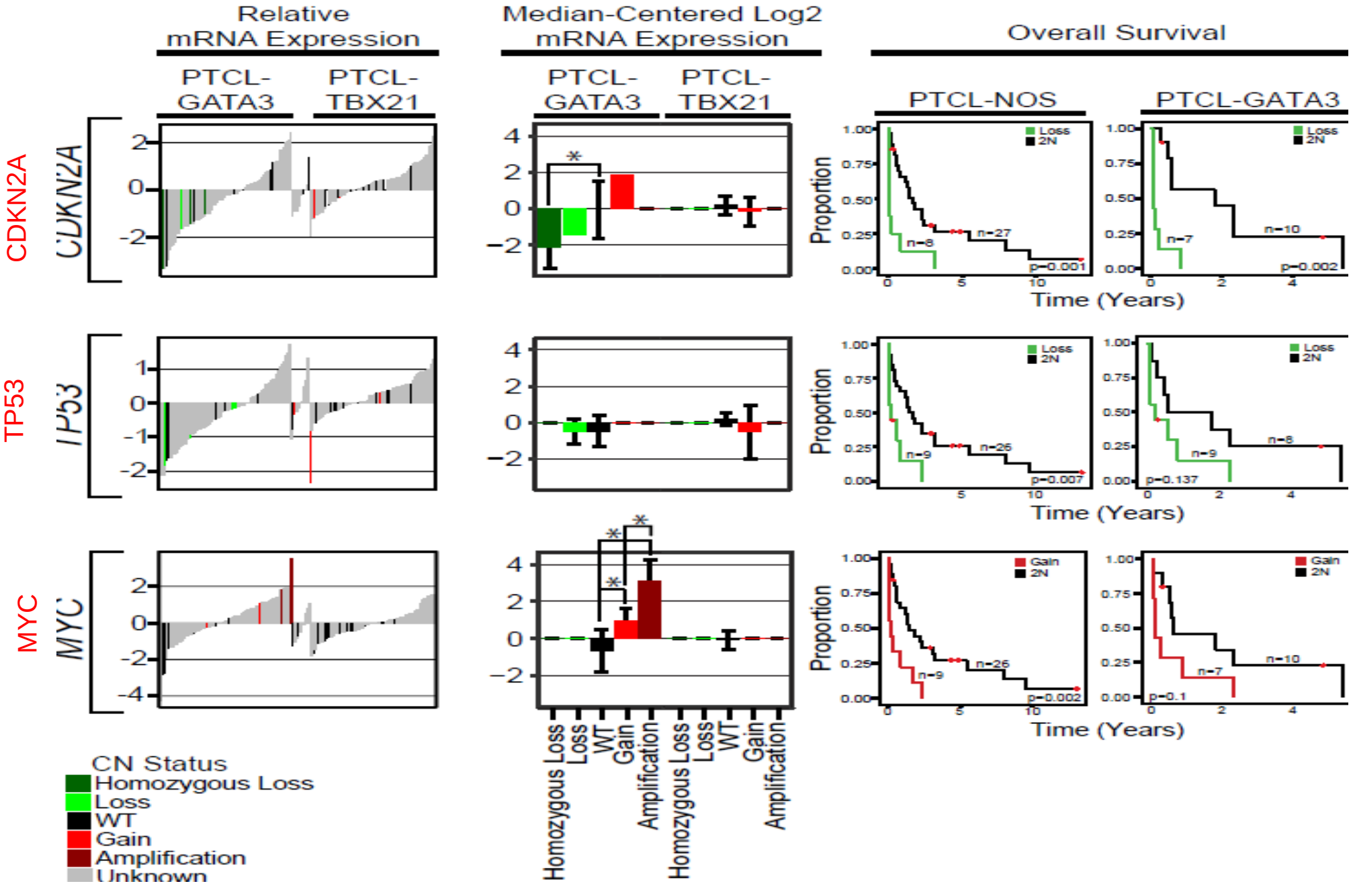
Major Subgroups within PTCL-NOS characterized by distinct genomic abnormalities



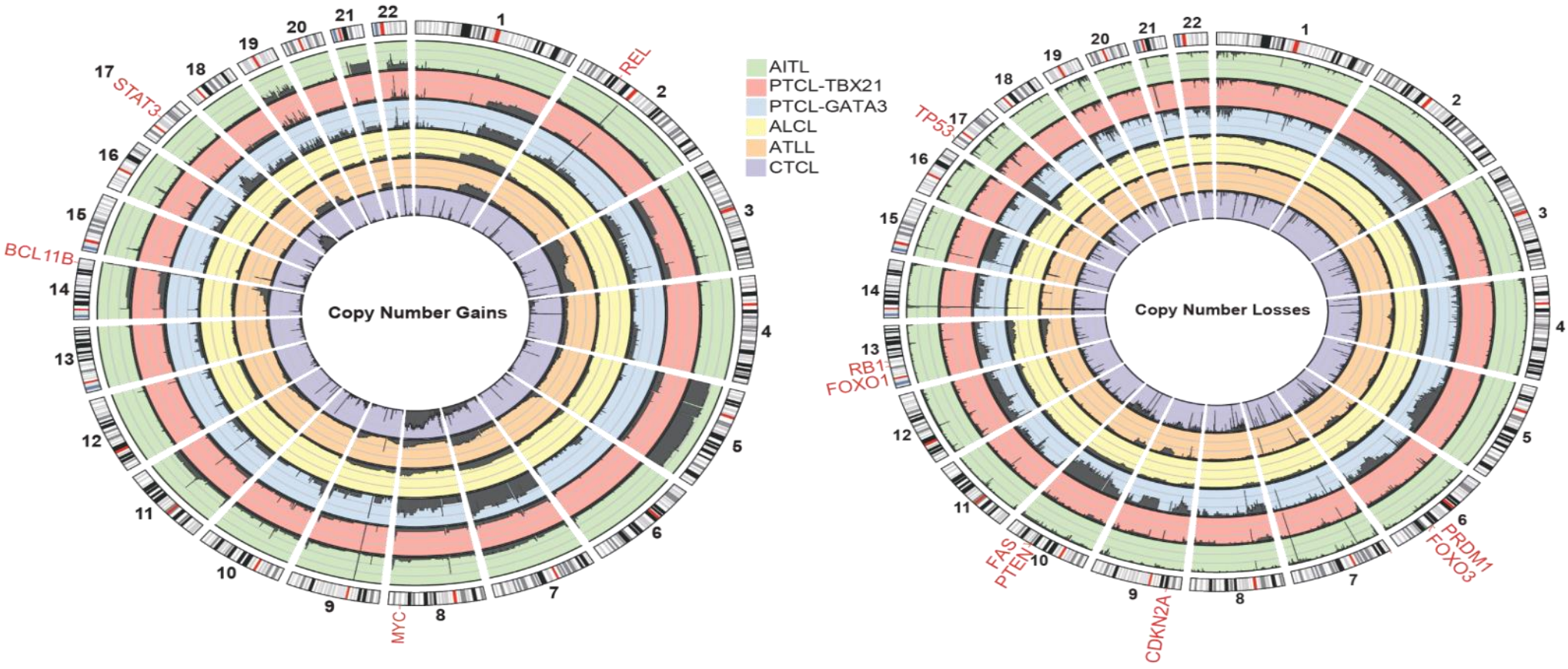


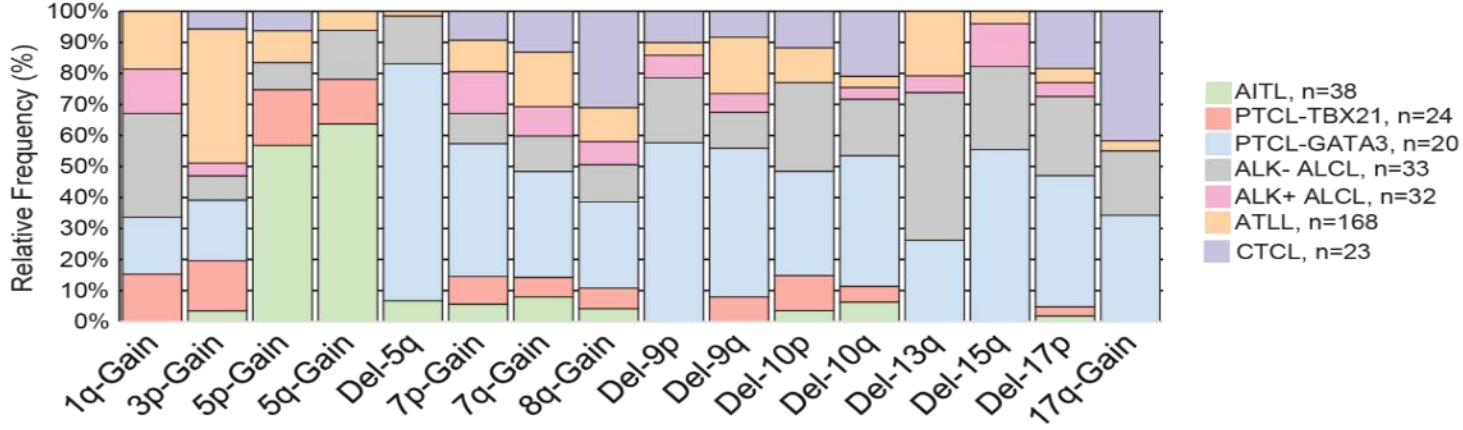


CN Abnormalities Associating with Overall Survival

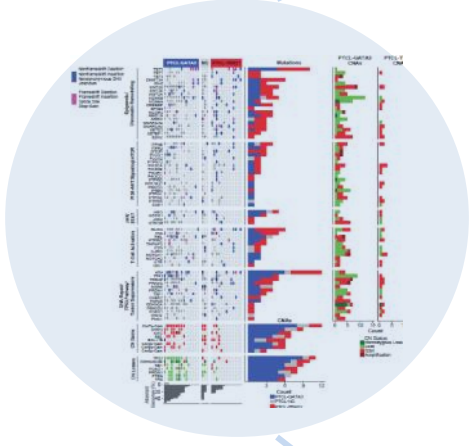


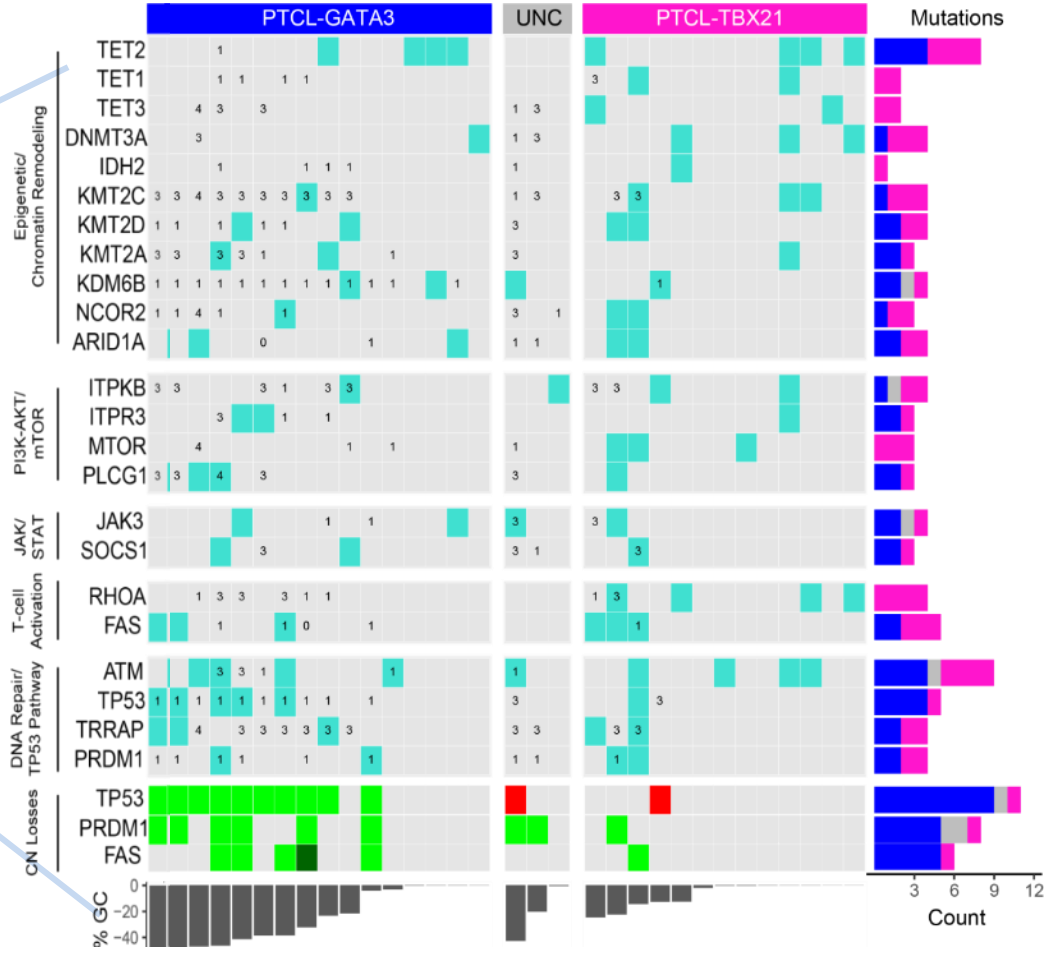
Novel PTCL subgroups have distinct Chromosomal Abnormalities





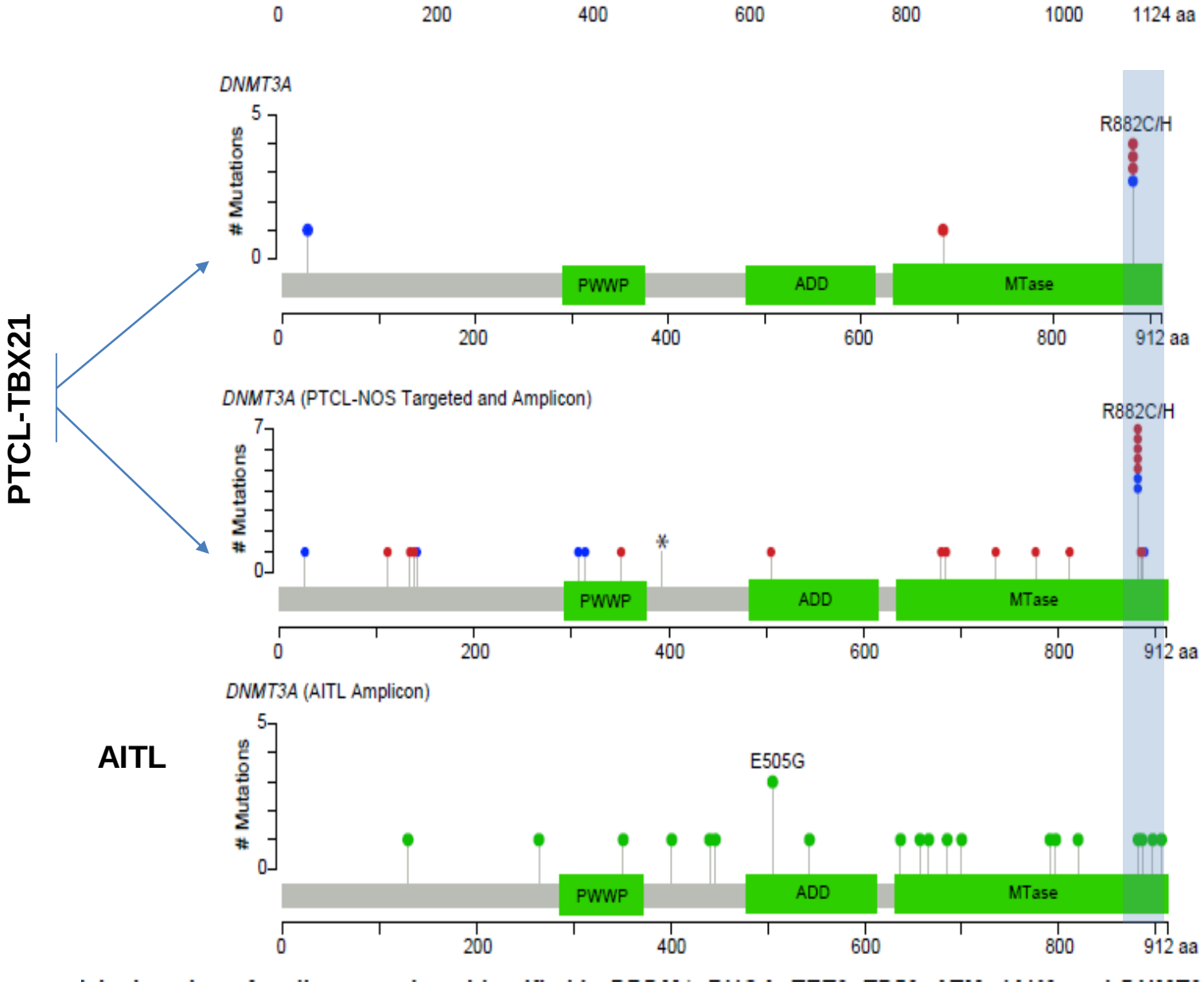
Unique Mutation Profiles in molecular subgroups



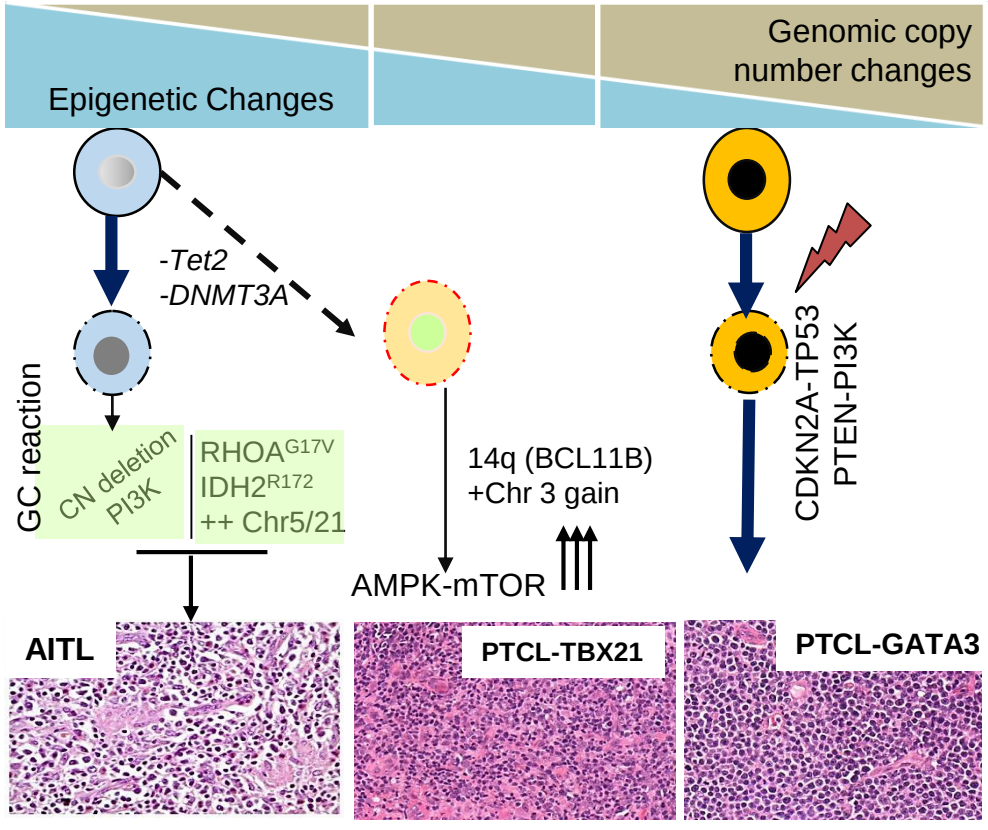


CN Status
- Homozygous Loss
- Loss
- Gain

Distinct spectrum of DNMT3A mutations



Summary



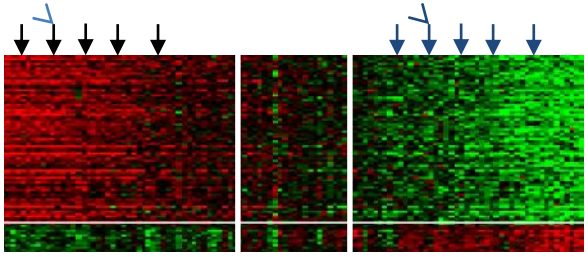
The complexity of PTCL can finally be addressed with the integration of global genomic analyses, which demonstrated that molecularly defined subgroups of PTCL have diverse genetic features and arise by distinct genetic pathways.

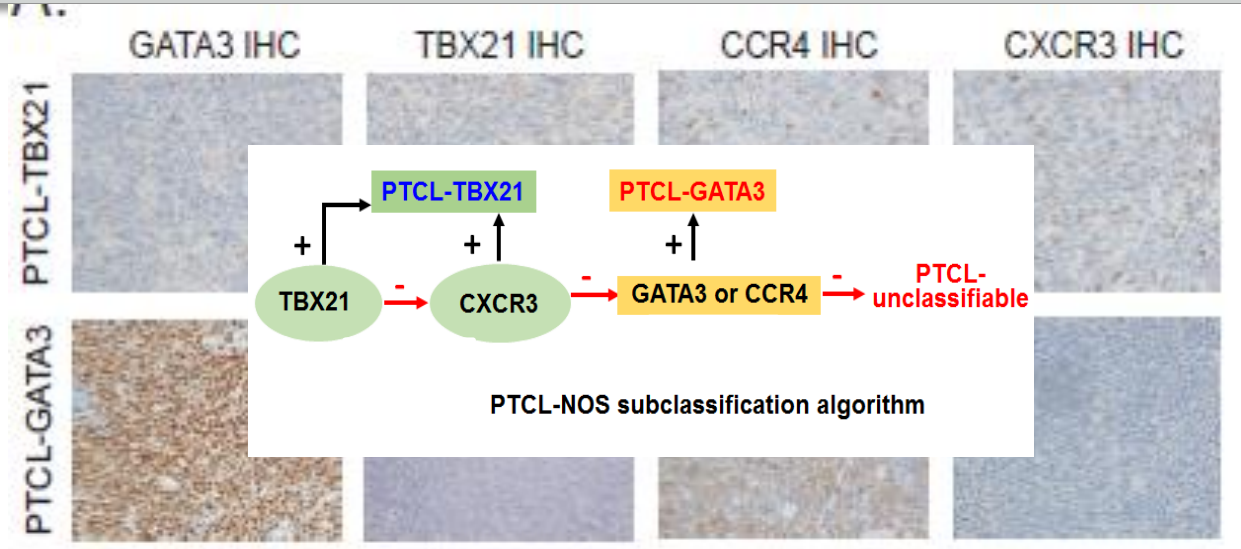
Translate Gene Signatures into clinical settings applicable to FFPE

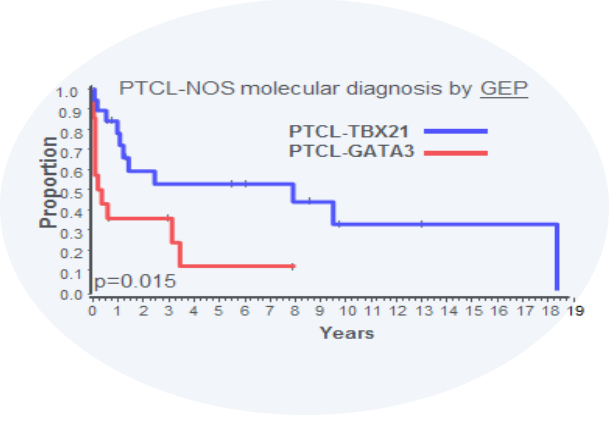
- **Immunohistochemistry based algorithm**
- **Quantitative mRNA based assay**

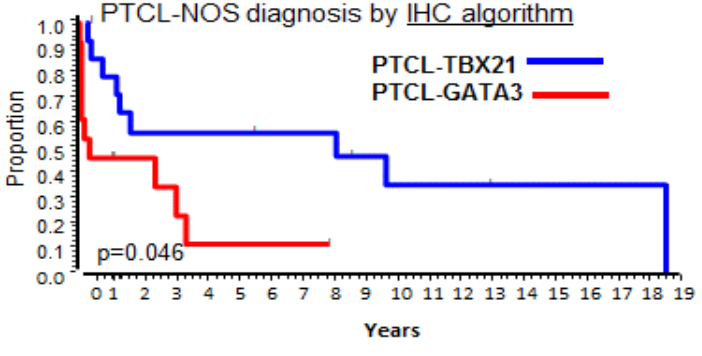
Translation using Immunohistochemistry

PTCL-NOS subgroups







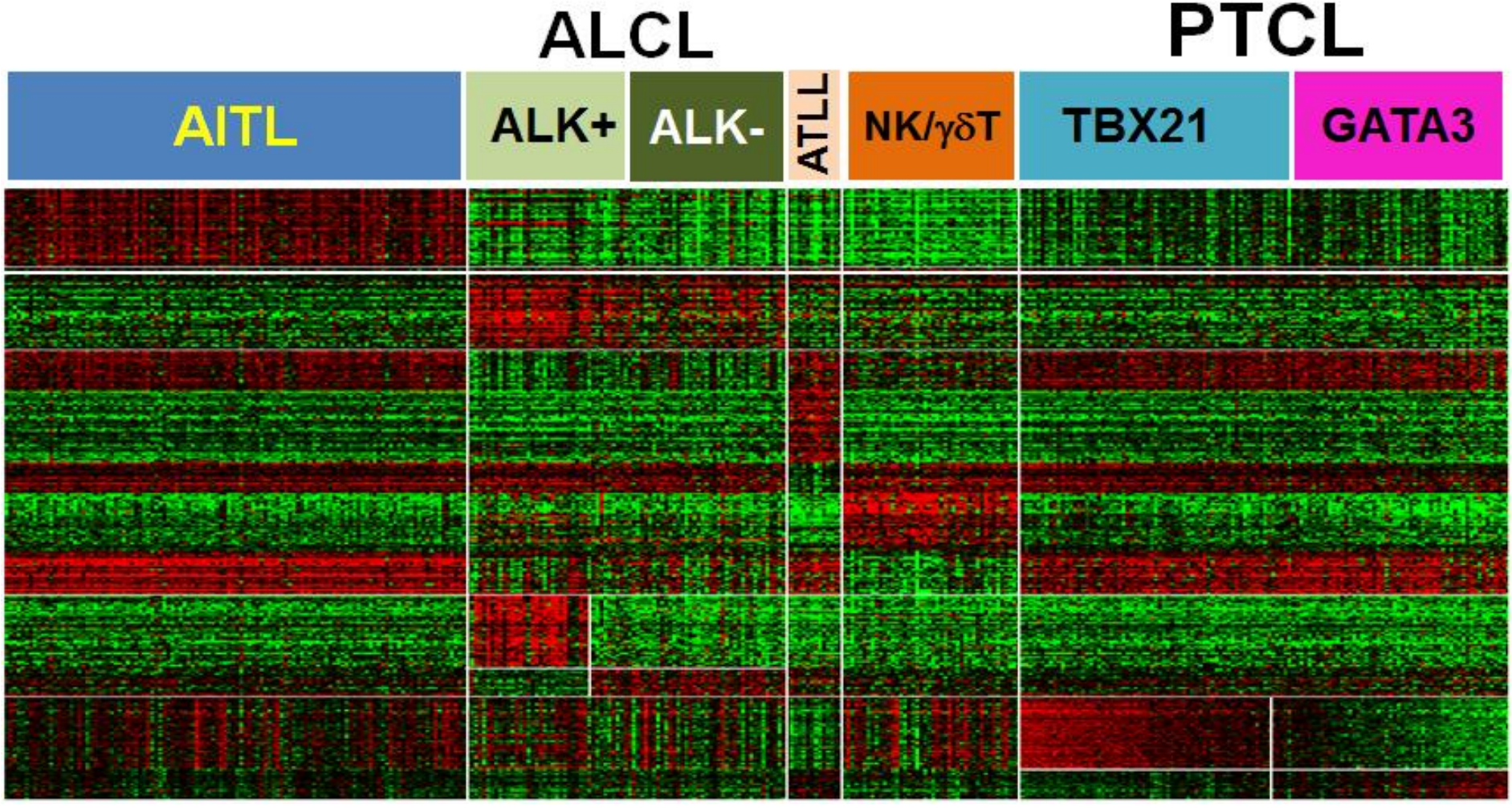


Validation Cohort
n=150 PTCL-NOS

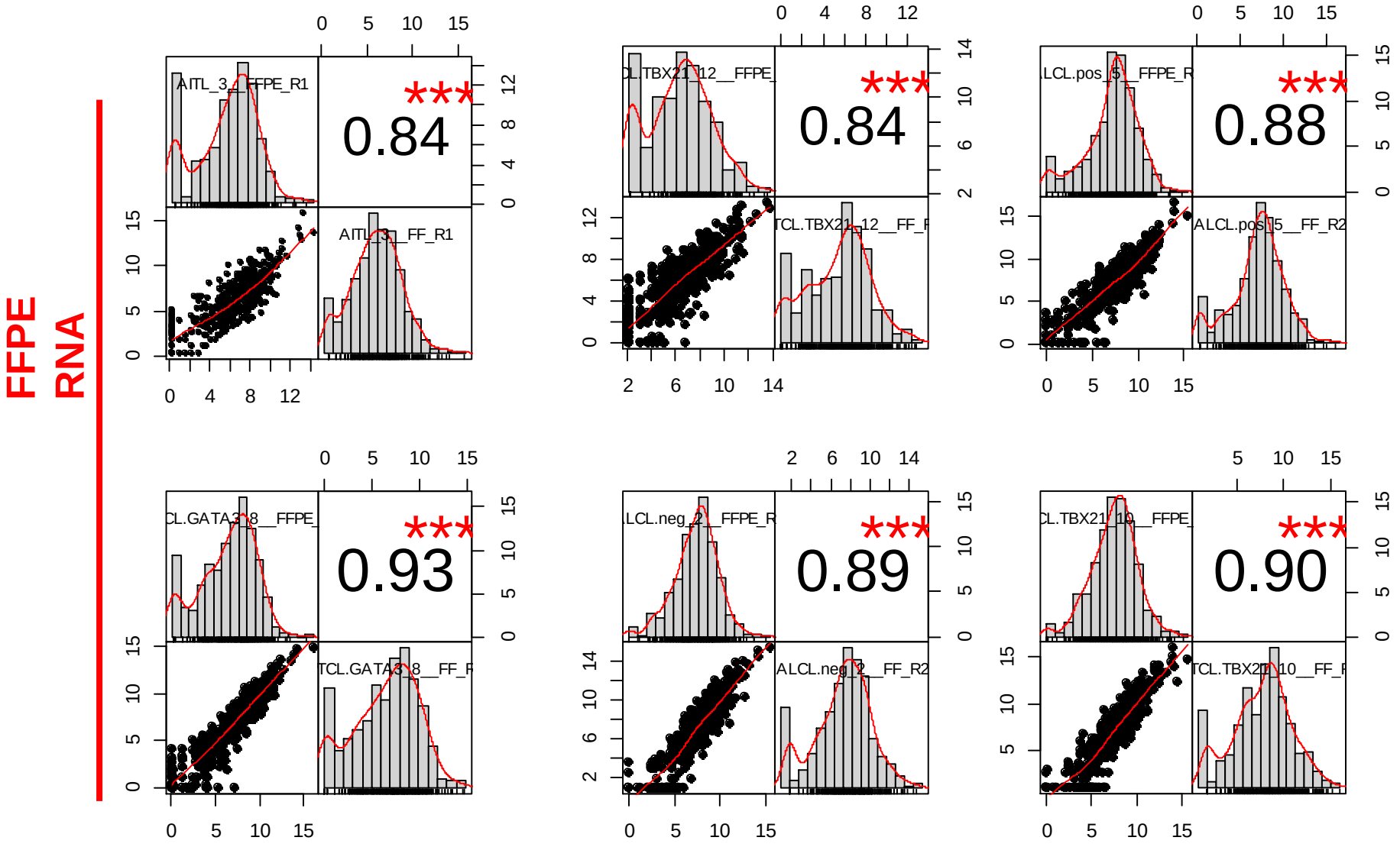
Amador et. al, USCAP 2018 Unpublished

Translating gene signatures for NanoString platform

Refinement of gene signature algorithm

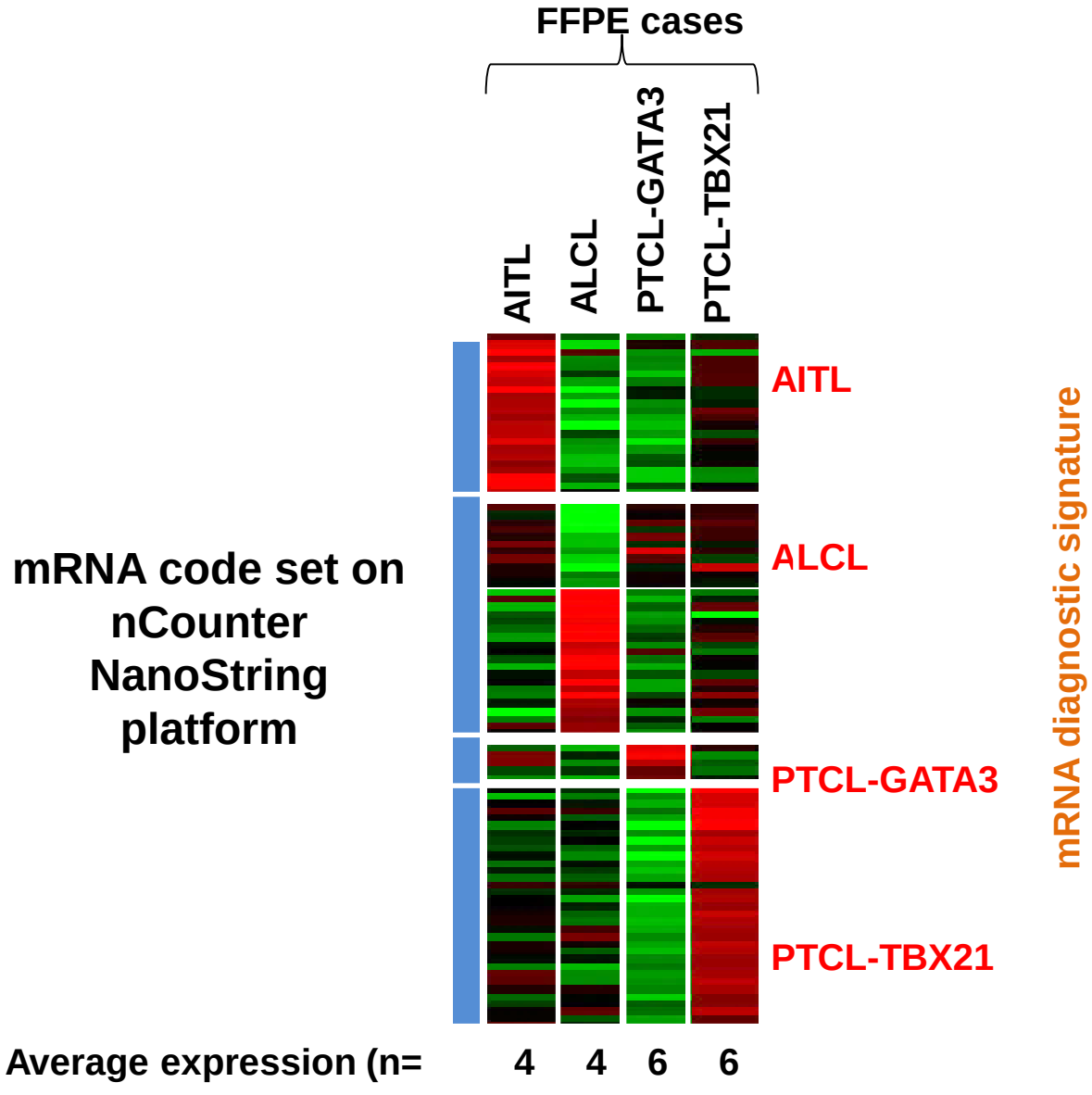


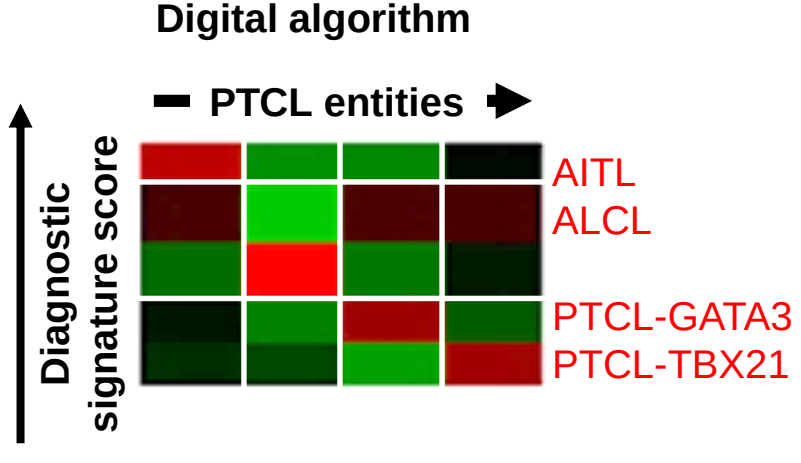
Correlation of gene signatures between Fresh frozen RNA and corresponding FFPE RNA on nCounter platform



100ng of RNA was run for all FF samples

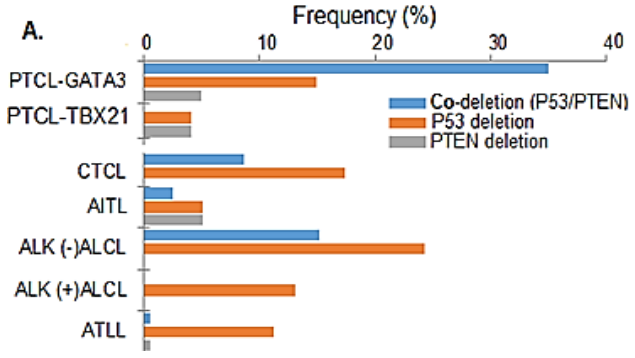
Molecular diagnostic algorithm for FFPE PTCL entities



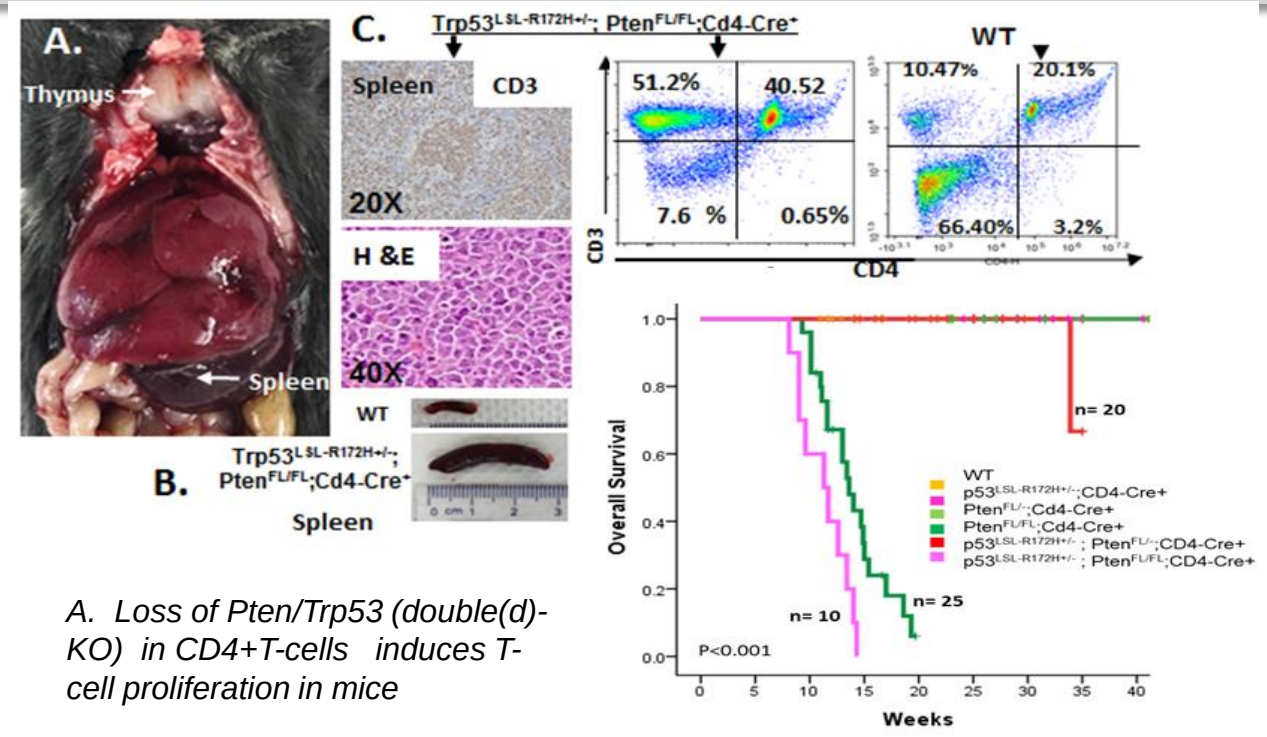


Genetically faithful model of PTCL-GATA3

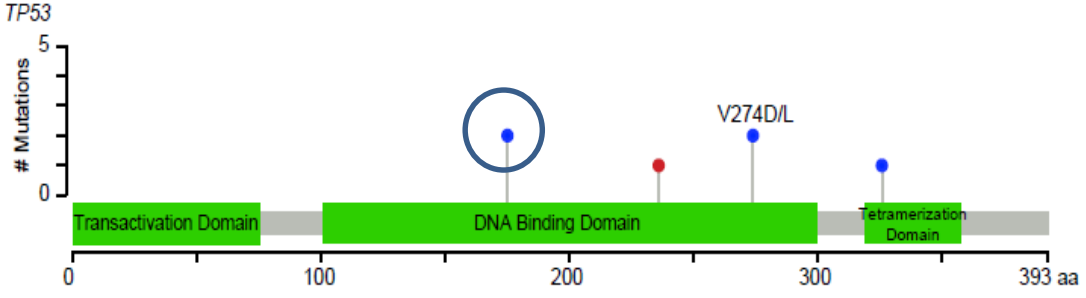
CNA analysis in PTCL entities



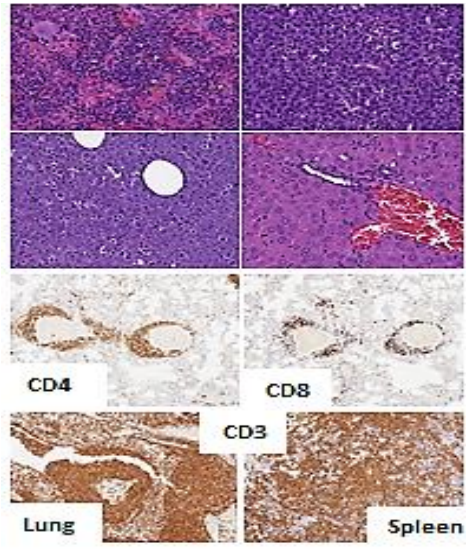
Generating Murine models



A. Loss of Pten/Trp53 (double(d)-KO) in CD4+T-cells induces T-cell proliferation in mice

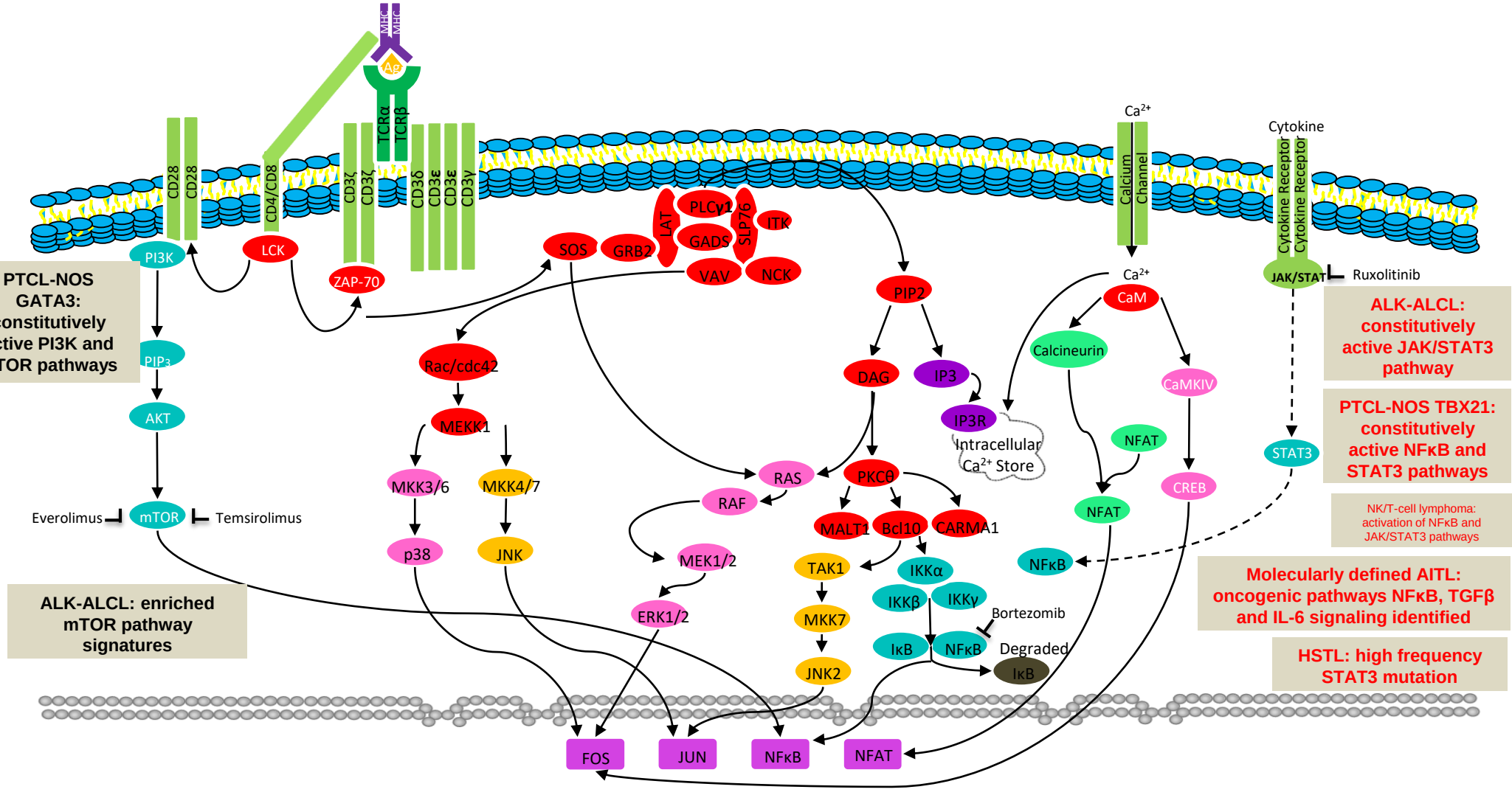


Developing PDX models of PTCL-NOS



H&E of different organs of a mouse implanted with a primary PTCL-NOS

Integrating new genomic information for targeted therapy in PCTL



Blood reviews, 2016

Acknowledgements

Alyssa Bouska
Tayla Heavican
Waseem Lone
Jiayu Yu
Catalina Amador
Keenan Hartet
Sneha R.

Fu Laboratory
Kai Fu
Andy Bi

Chan Laboratory
Wing C. Chan
Timothy McKeithan
Yuping Liu

Giorgio Inghirami

Weill Cornell Medicine/
Cornell University

Pileri Laboratory
Stefano Pileri
Maria Antonella Laginestra

National Cancer Centre Singapore
Maarja-Liisa Nairismägi
Soon Thye Lim
Choon Kiat Ong

ACKNOWLEDGEMENT

Nebraska Medical Center

- **Catalina Amador**
- **Timothy Greiner**
- **Kai Fu**
- **Julia Vose**
- **James Armitage**
- **Martin Bast**
- **Lynette Smith**

-City of Hope Medical Center

—John Chan
—Dennis Weisenburger
—Timothy Mckeithan

- **-NCI-NIH**
  - — Lou Staudt
  - — George Wright
  - — Elaine S. Jaffe

- **LLMPP consortium members**
- **International PTCL Project**

Funding Support

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— Eppley cancer Center Support
— Nebraska Medical Center Research support