Gene expression profiling in peripheral T-cell lymphoma

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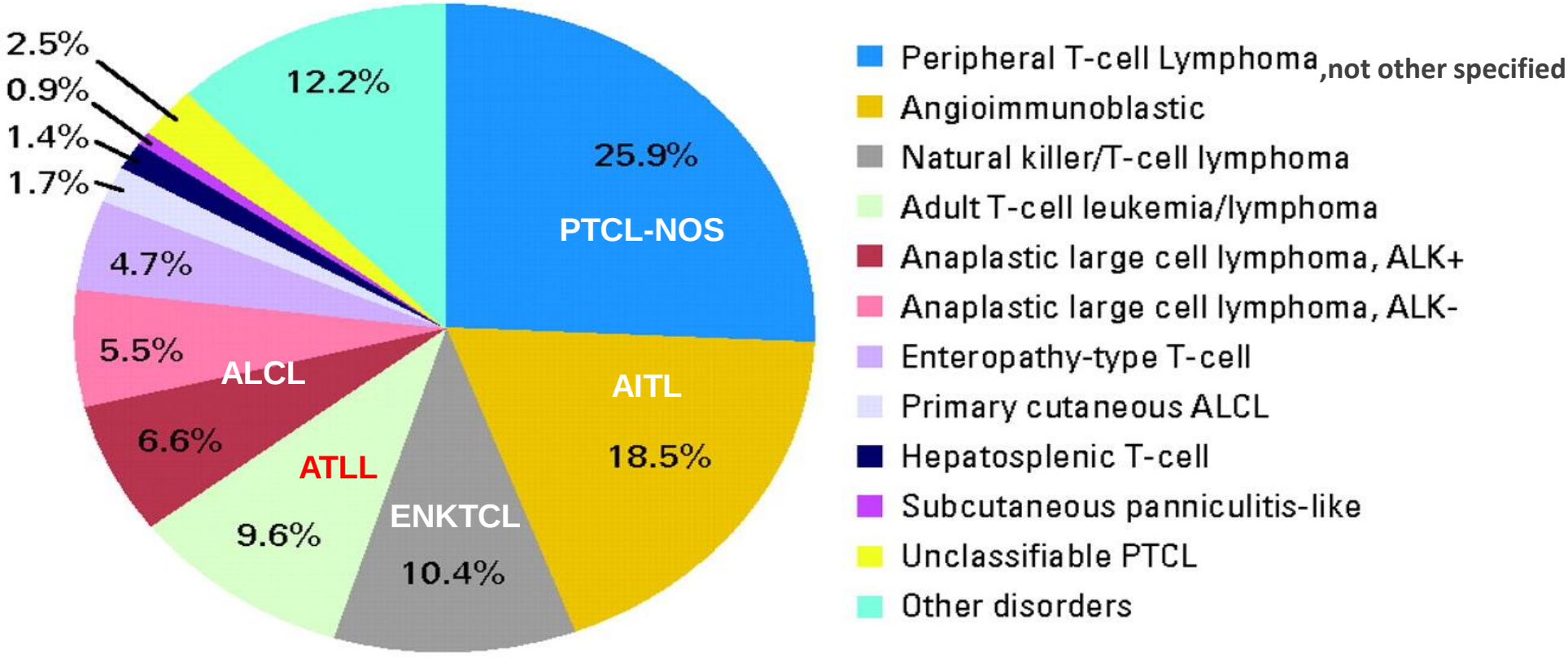
Rationale for GEP studies on PTCL

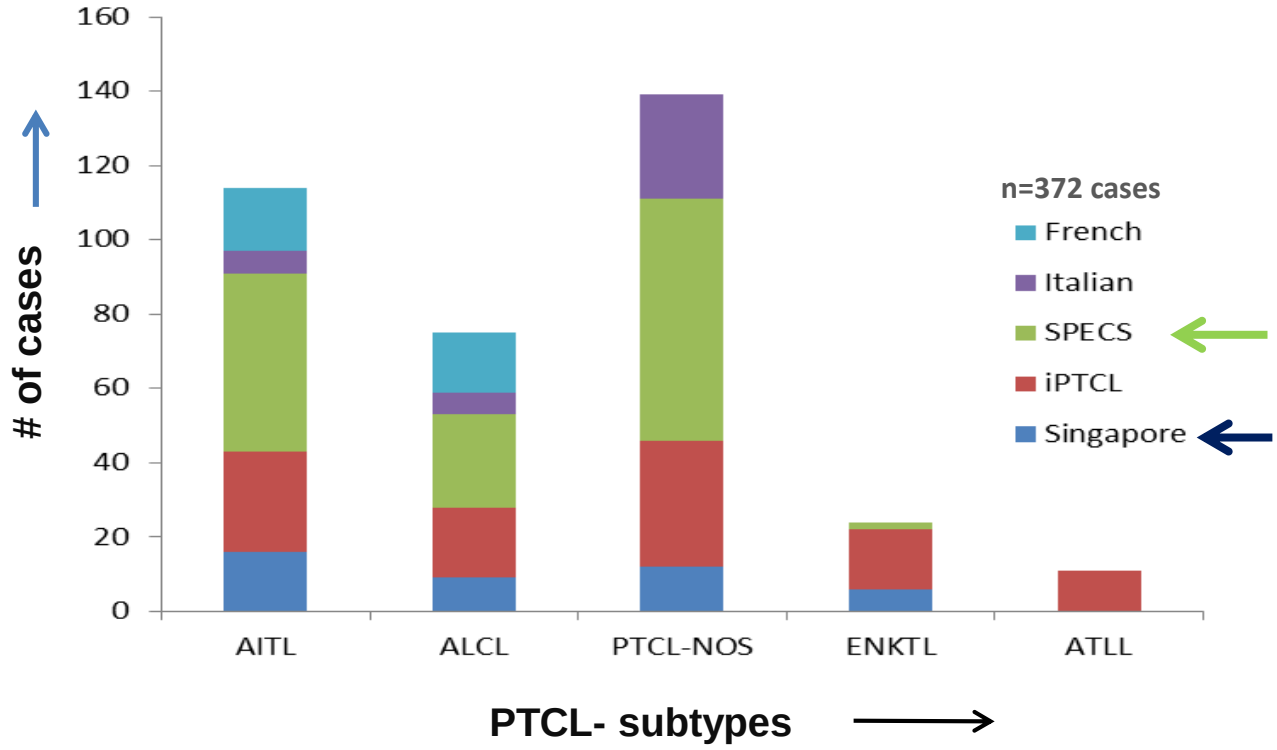
- The diagnosis of PTCL is challenging even among expert hematopathologists, compounded by the rarity and diversity of PTCLs
- May have a prominent reactive cellular infiltrate masking the neoplastic cell population
- No unique cytogenetic abnormality in the major PTCL subtypes
- Approximately 50% of the cases are categorized as PTCL, not otherwise specified (PTCL-NOS)
- Gain better understanding of the biology of PTCL including clinically significant pathways

Frequency of common subtypes of PTCL



International T-Cell Lymphoma Project, J Clin Oncol; 26:4124-4130 2008

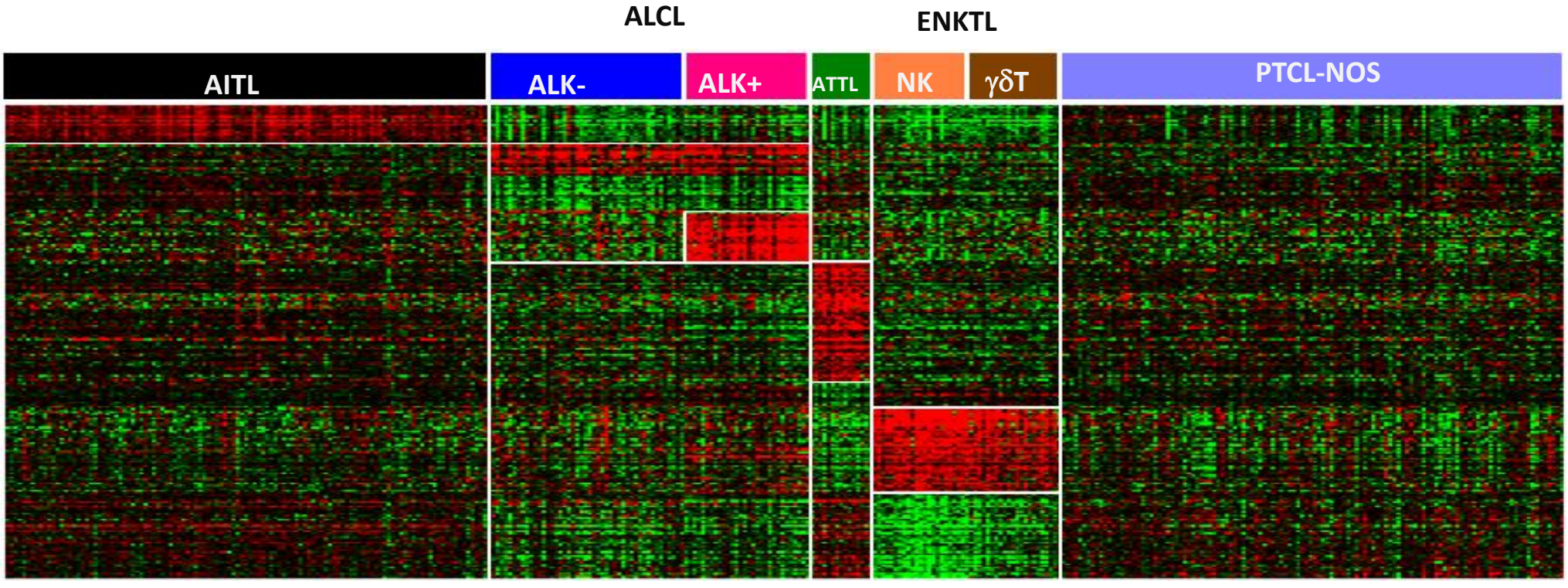
PTCL subtype and ENKTL cases with acceptable GEP



SPECS: Strategic Partnering to Evaluate Cancer Signatures
iPTCL : International peripheral T-cell lymphoma
LLMPP: Lymphoma and Leukemia Molecular Profiling Project Singapore

- **Gene expression profiling data was generated on HG U133 plus 2 arrays (Affymetrix Inc)**

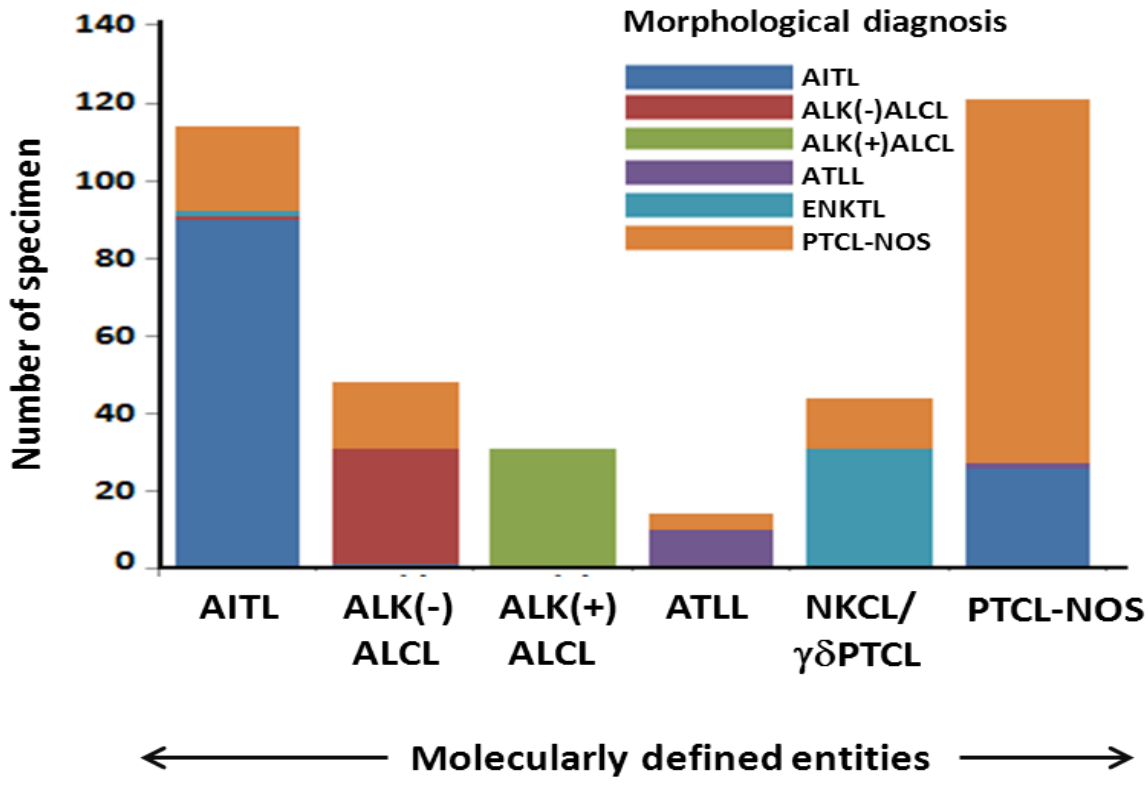
Refinement of molecular diagnostic signatures for PTCL subgroups



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

Lymphoma and Leukemia Molecular Profiling Project (LLMPP) initiative

Evaluation of pathological *vs* molecular diagnosis



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

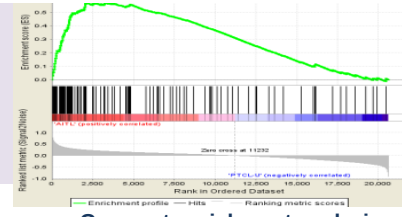
i. **AITL [14%]**
ii. **ALK(-)ALCL [11%]**
iii. **ATLL [03%]**
iv. **γδ- PTCL [09%]**

- Of 117 AITL cases 26 cases (22%) changed to PTCL-NOS.

Robust molecular signature for ALK(-)ALCL

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

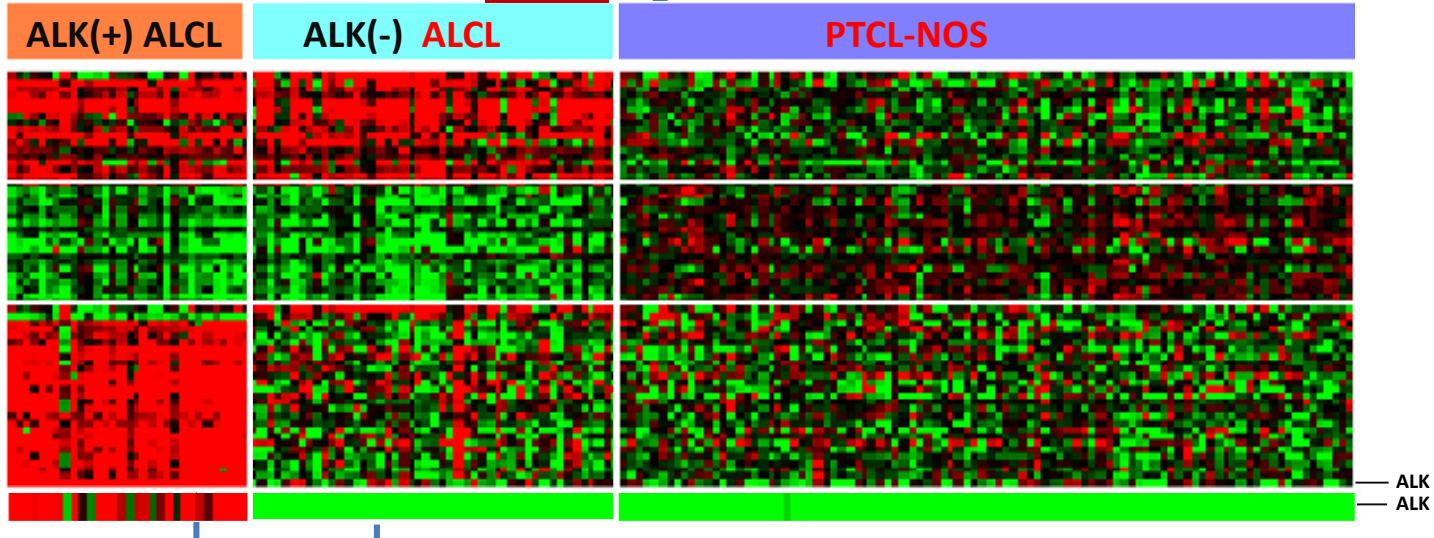
— Proliferation signature
— MYC induced gene signature
— IRF4 induced gene signature
— Absence of TCR signaling molecules



Gene set enrichment analysis

ALK(-)ALCL

Re-classified from PTCL-NOS



ALK(+) ALCL

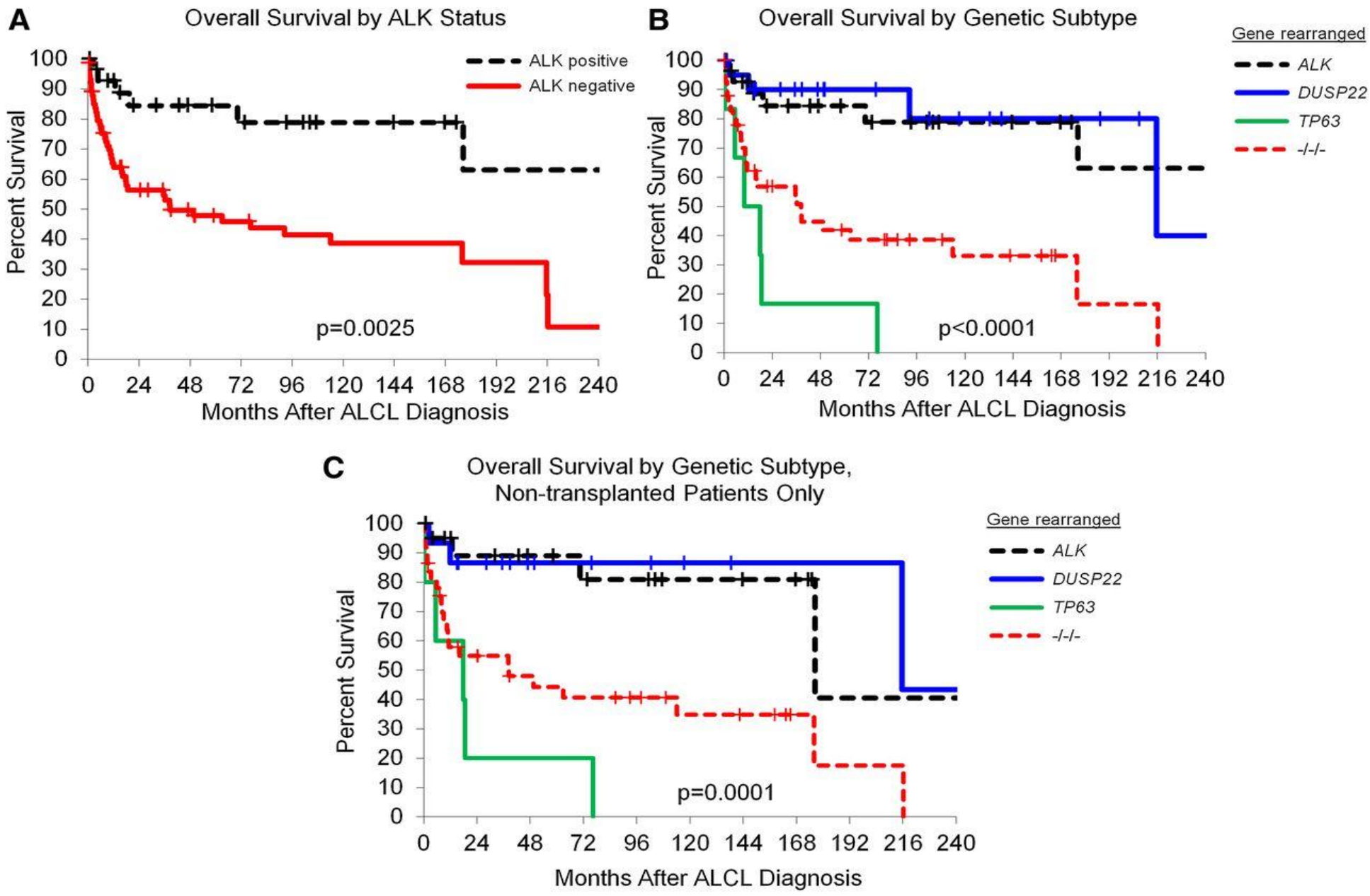
— IL10 induced signature
— STAT3 signature
— Treg gene signature
— Presence of B cells
— HIF-alpha induced gene signature

ALK(-)ALCL

— MYC induced gene signature
— Proliferation signature
— NF-κB target signature

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

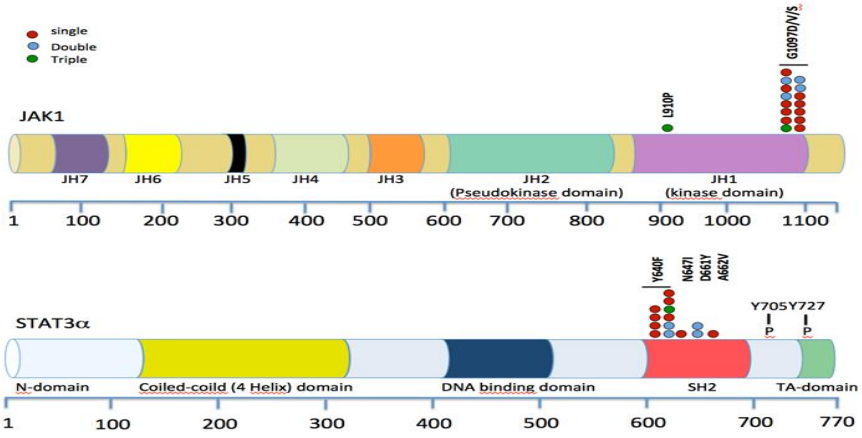
DUSP22 and TP63 rearrangements in ALK- ALCL



Parilla Castellar et al. Blood 2014,August 28, 124(9)1473-1480

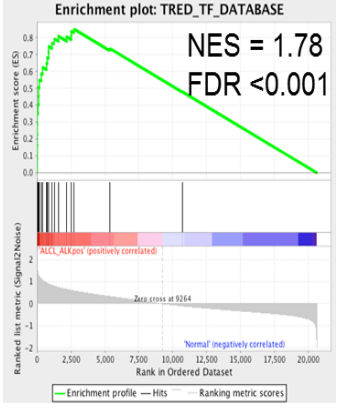
JAK/STAT3 pathway activation through mutations and fusion transcripts

JAK1 and STAT3 mutations



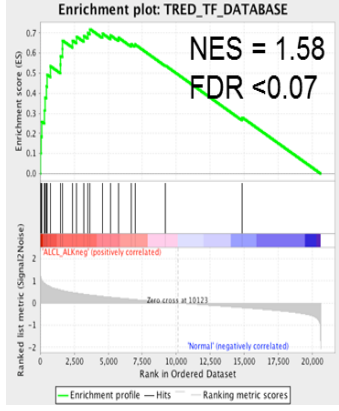
F

ALCL ALK+ vs Normal

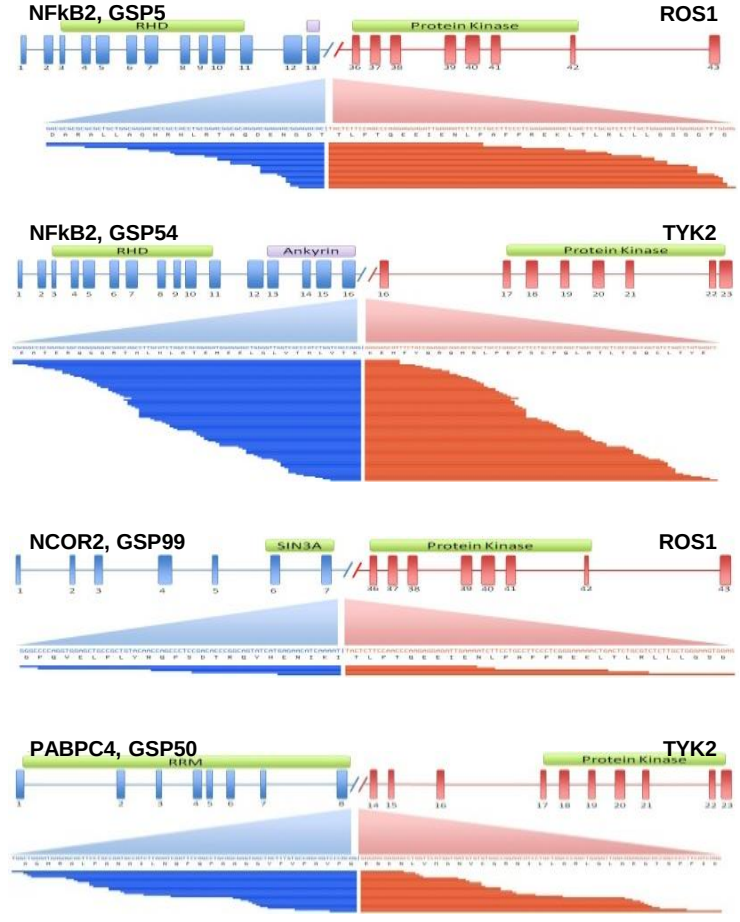


G

ALCL ALK- vs Normal

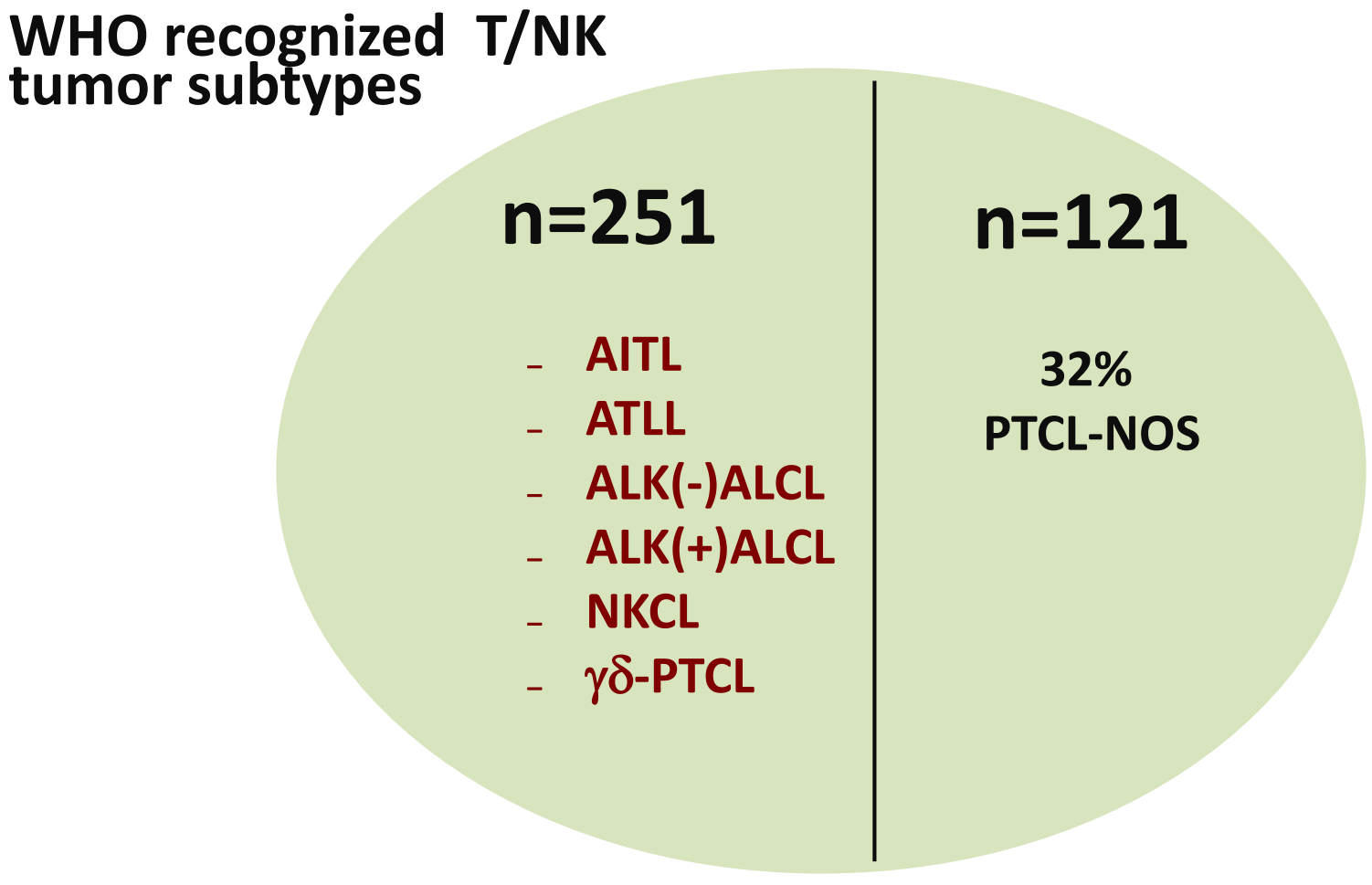


Novel fusions combining a transcription factor (NFkB2 or NCOR2) with a tyrosine kinase (ROS1 or TYK2).

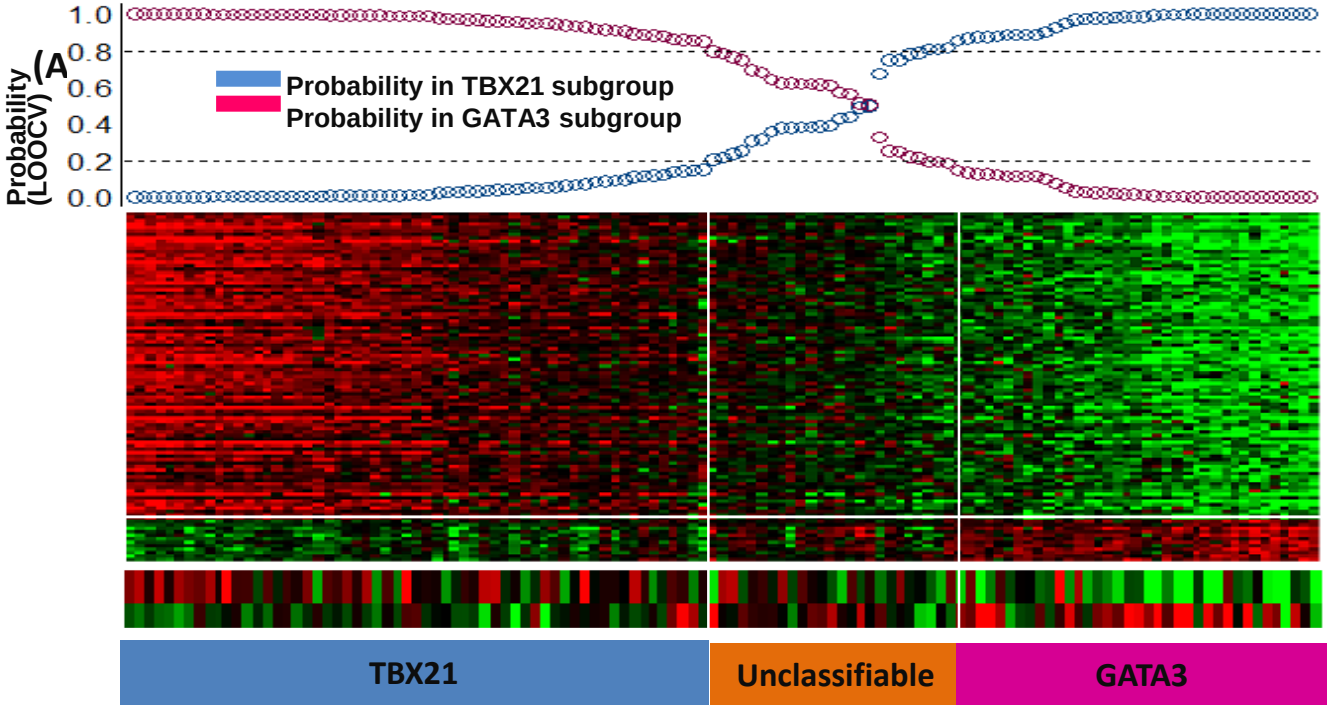


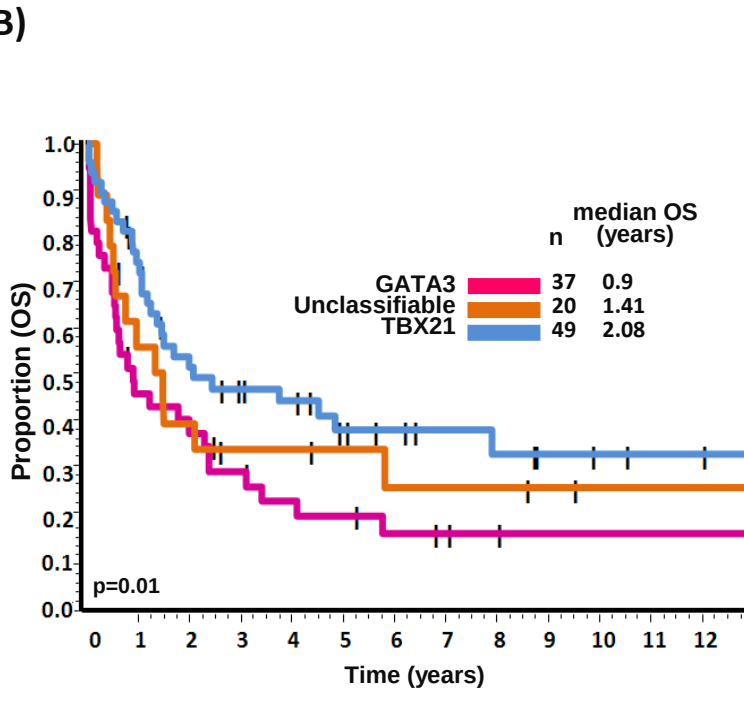
Crescenzo and Abate et.al Cancer Cell 2015

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

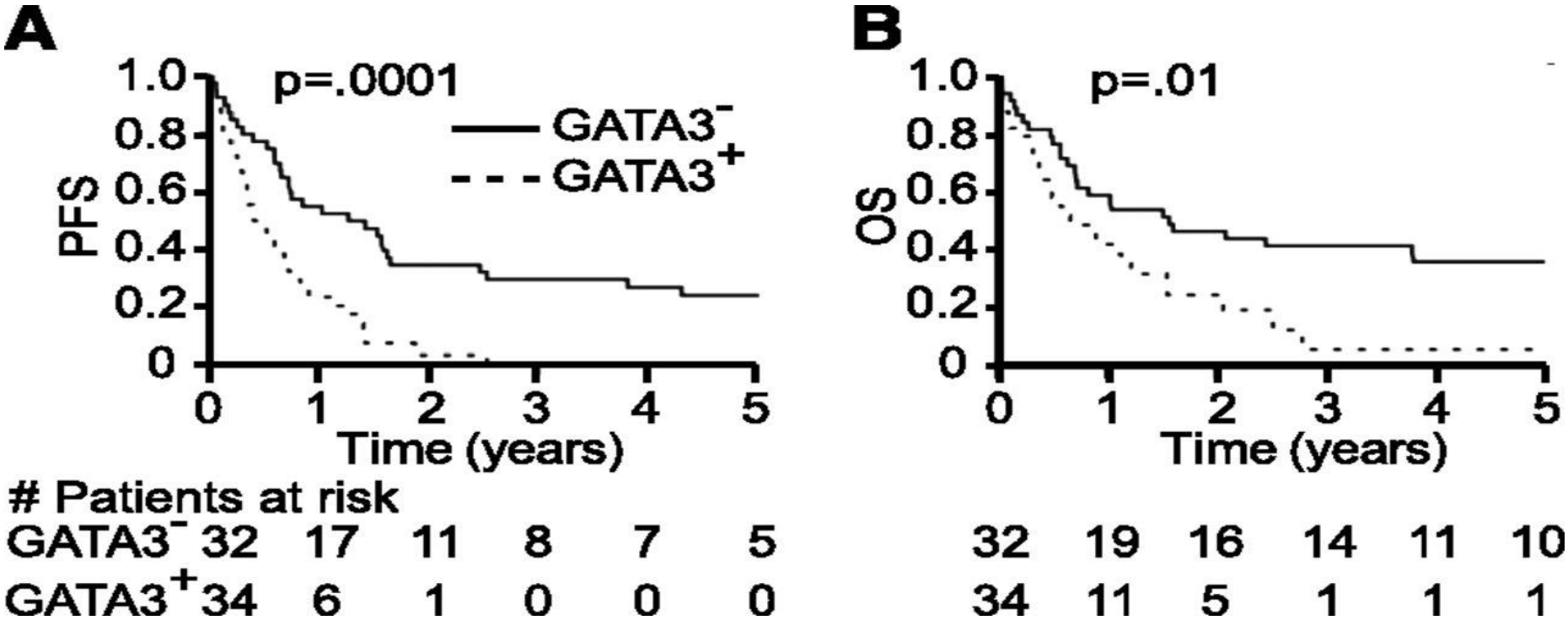


PTCL-NOS can be further divided two major subgroups



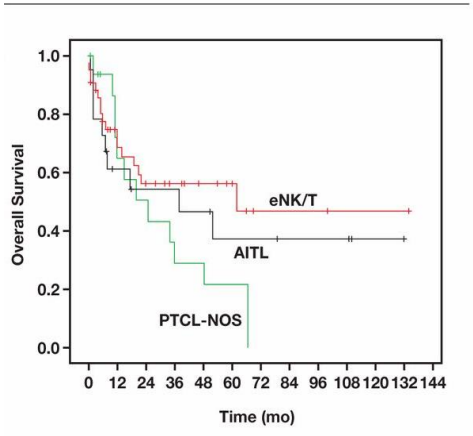


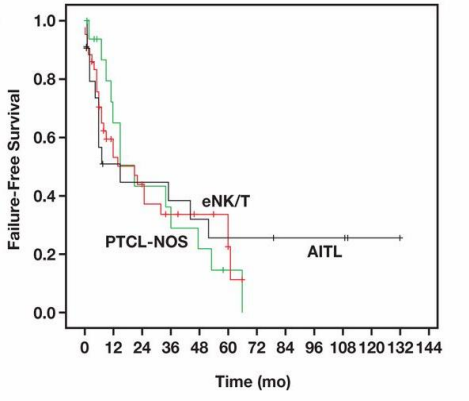
GATA3 expression identifies a subset of PTCL, NOS with inferior survival.

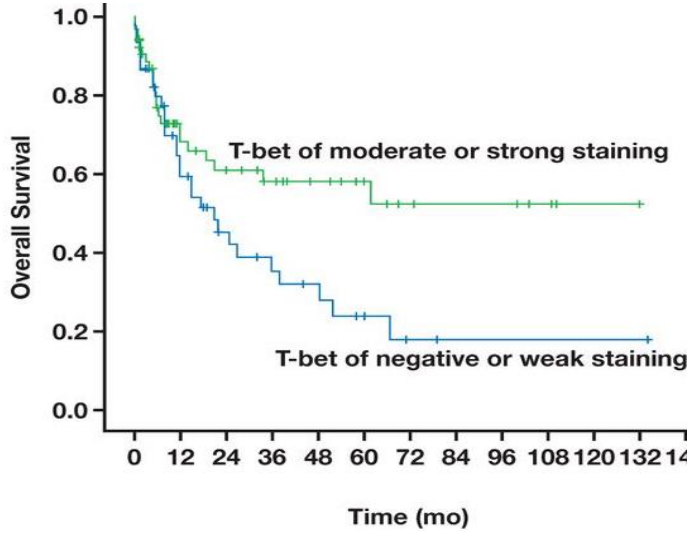


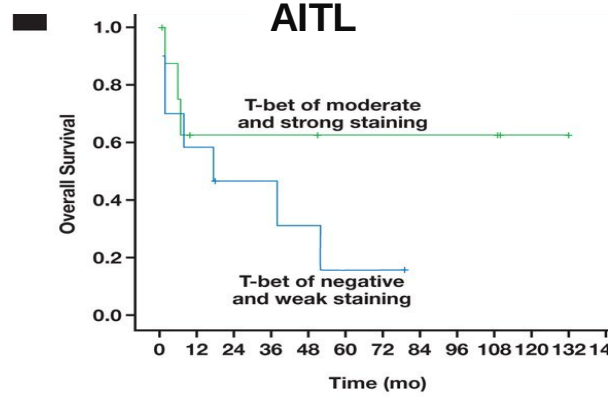
Tianjiao Wang et al. Blood 2014;123:3007-3015

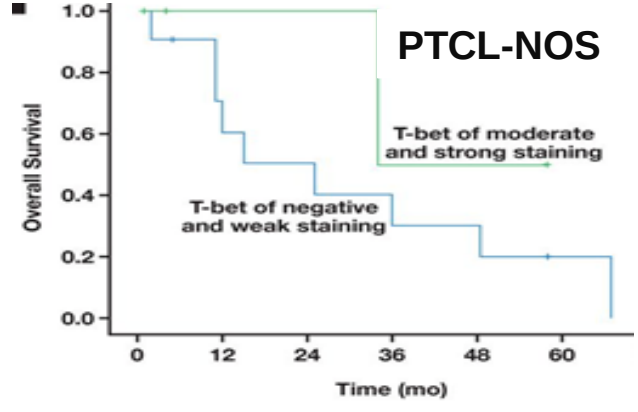
TBX21 expression by Immunohistochemistry is Predictive of survival in PTCL







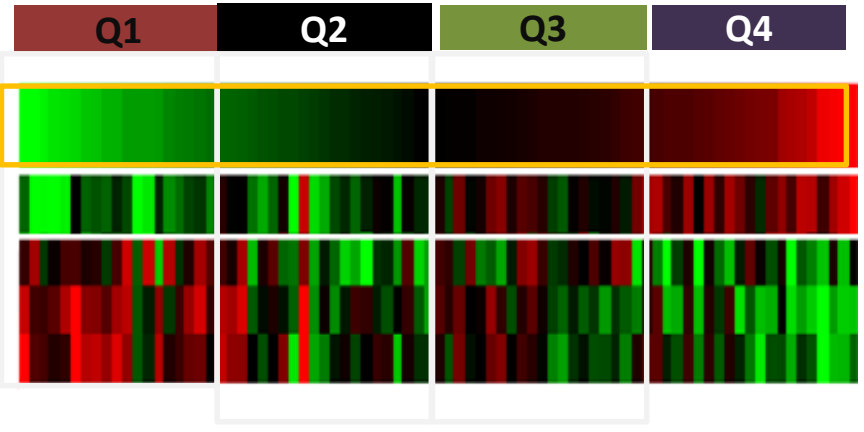


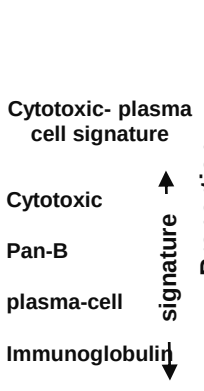


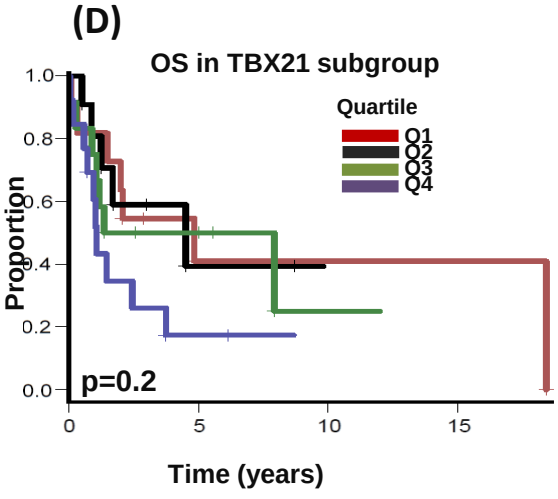
T-bet expression in Chinese PTCL cohort (n=142)

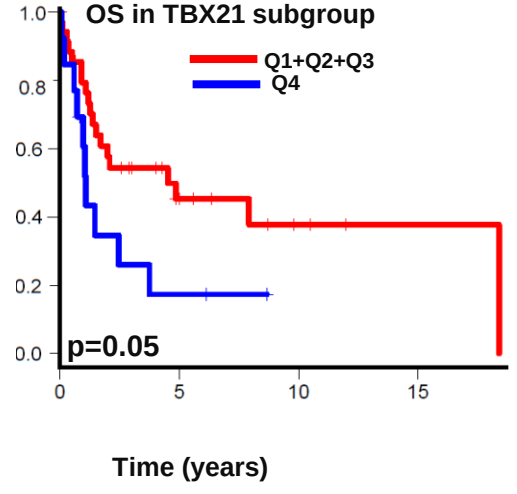
Ren YL *et.al* Am J Clin Pathol. 2012 Sep;138(3):435-47

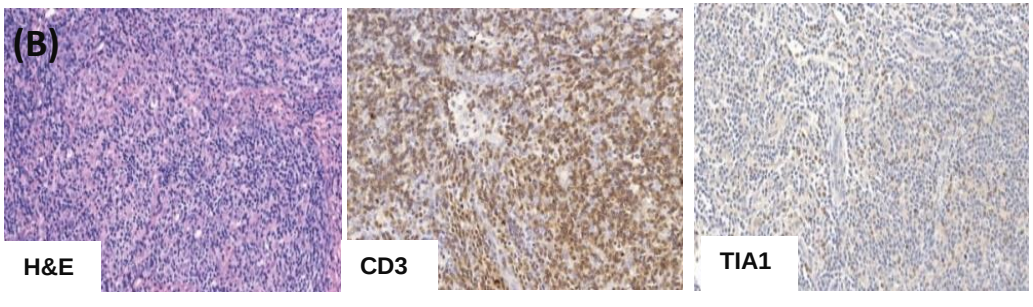
Tumor microenvironment significantly influences the prognosis in TBX21 subgroup of PTCL-NOS

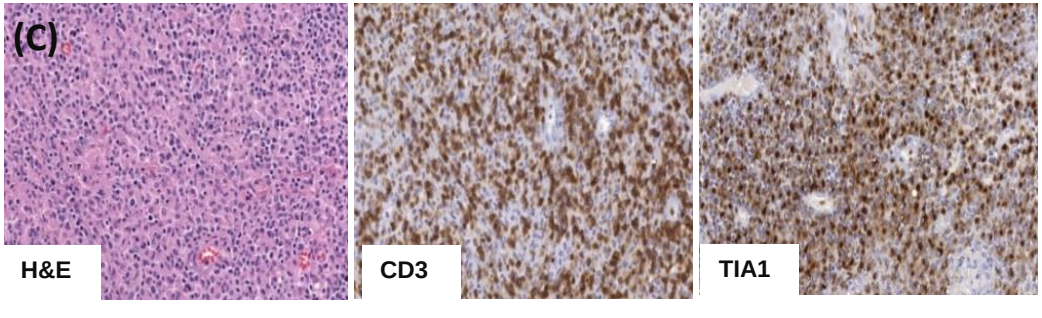




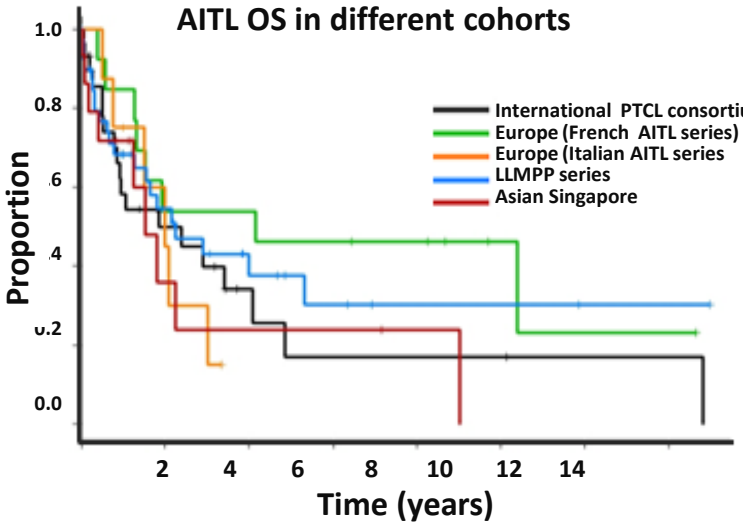








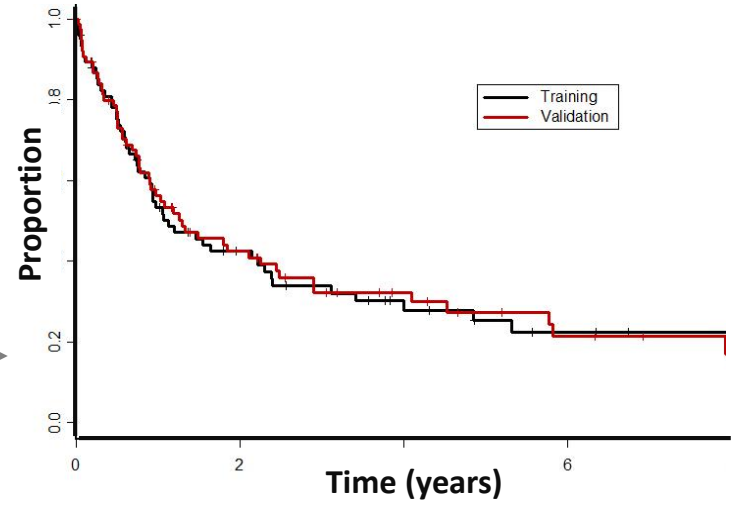
Survival model creation in AITL



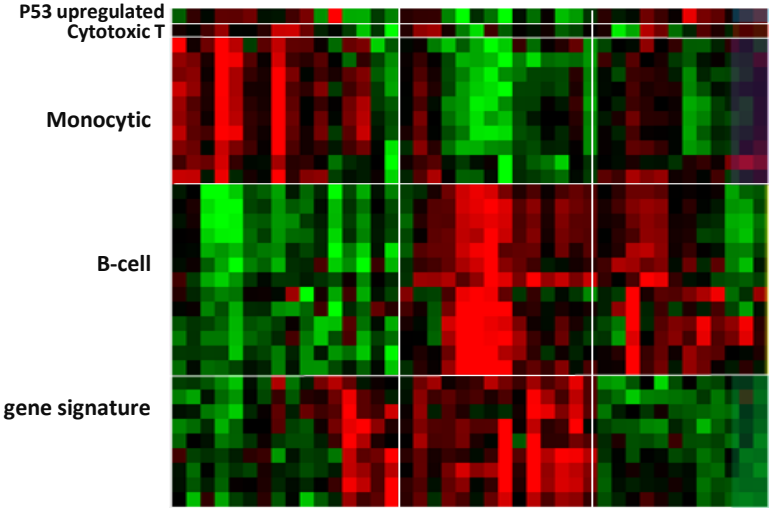
Samples divided into Training and validation set

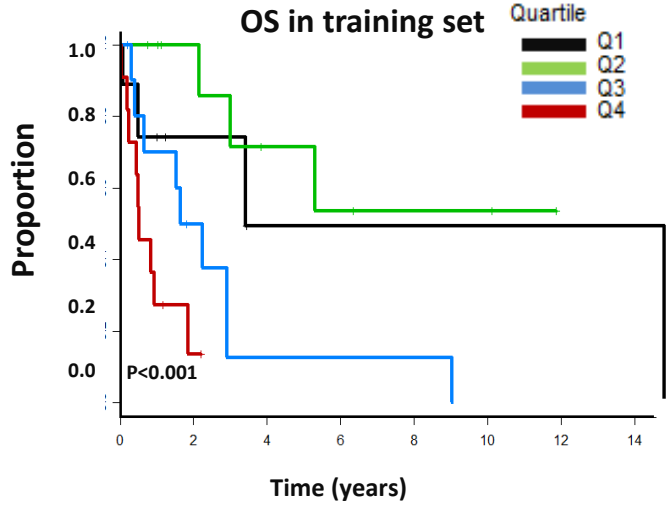
Training	41
Validation	42

Cases divided to minimize OS bias



Survival model Creation

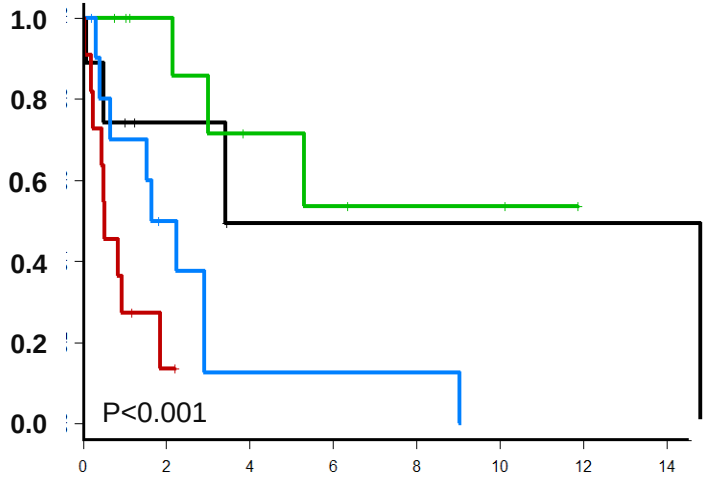




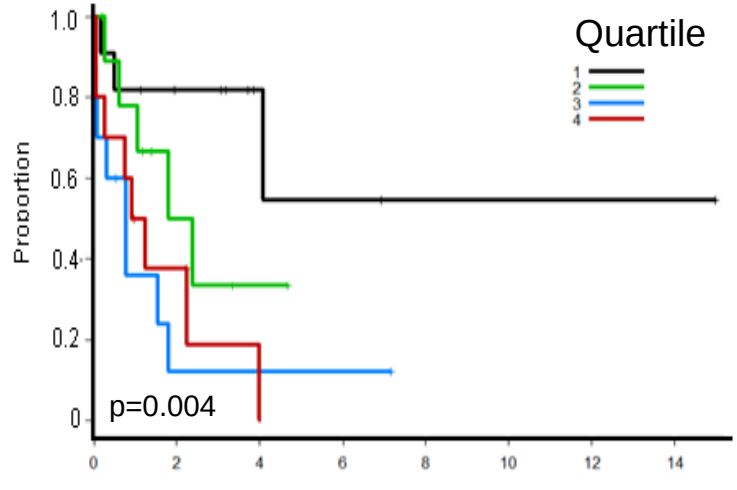
Survival prediction on AITL: role of tumor microenvironment

Signature Cluster	Effect of high expression	Training p-value	Validation p-value
p53 upregulated signature	Poor prognosis	0.001	0.014
Cytotoxic signature	Poor prognosis	0.005	0.046
Monocytic/dendritic signature	Poor prognosis	0.011	0.010
B- cell signature	Good prognosis	0.002	0.017

Training set

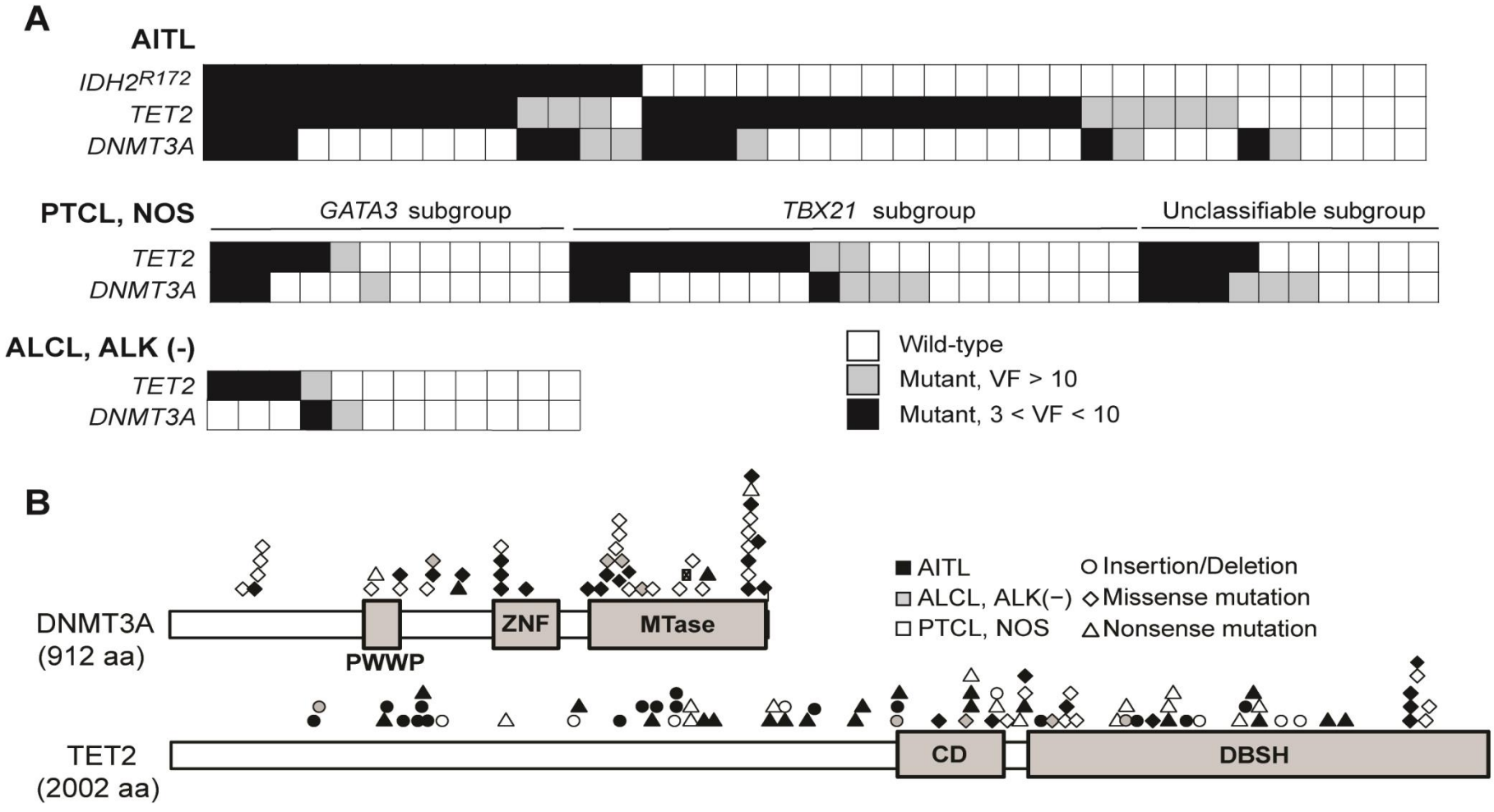


Validation set



- **Tumor microenvironment significantly influences the prognosis in AITL**
- **Role of macrophages (M1) vs (M2) and dendritic cells are being investigated**

IDH2 mutation is specifically associated with molecularly defined AITL cases



RhoA mutation map in PTCL

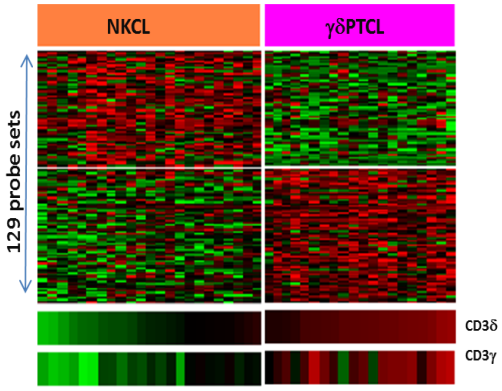
- AITL
  * G17E case includes an S26R mutation in same clone
  ^ K18M major clone has G17V minor clone
- PTCL, NOS
- ALCL, ALK-

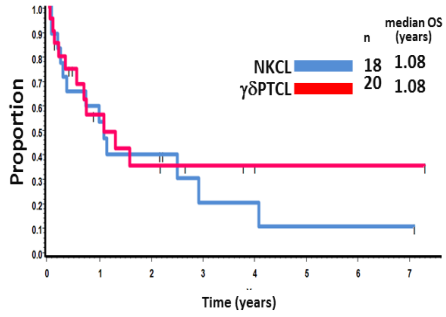
- GTP/Mg^{++}-binding “boxes”
- PKN/PRK1 binding site
- Effector region “Switch I”

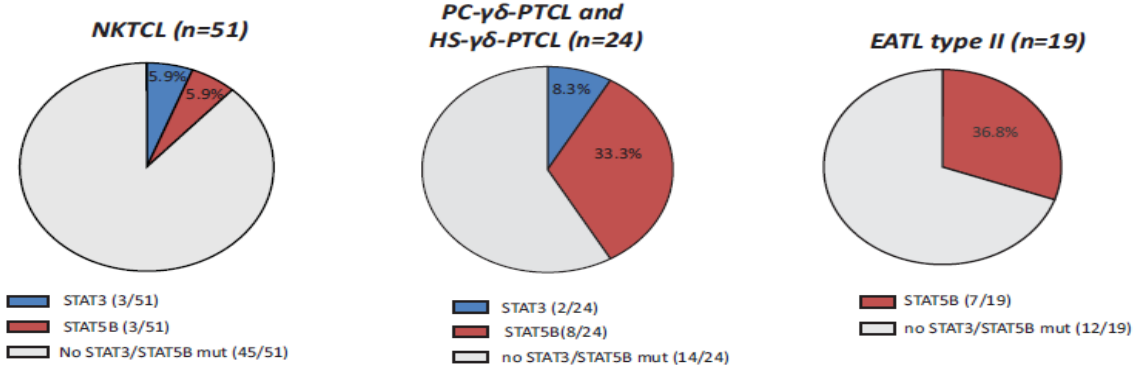
number (% of screened)

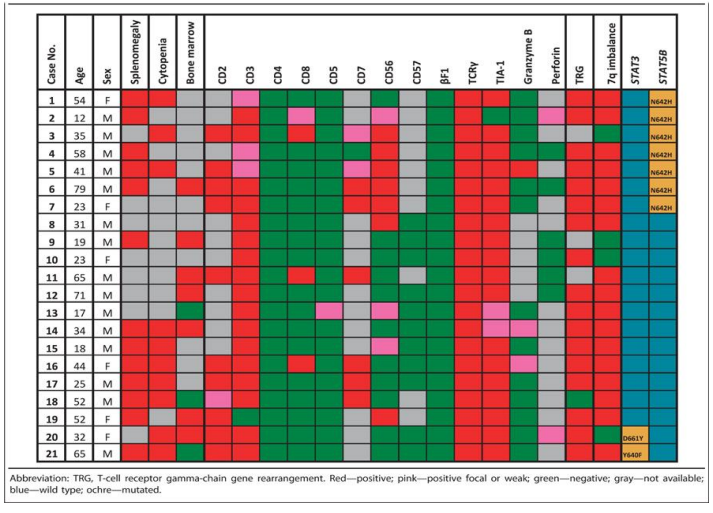
		G17	S26	Other
AITL (n=39)°		**28 (71.8)***	**0***	**1 (2.6)^**
PTCL, NOS (n=41)	**Tbx21 (n=19)**	**5 (26.3)**	**1 (5.3)**	**0**
	Gata3 (n=12)	**2 (18.2)**	**0**	**0**
	Unclassified (n=10)°	**3 (33.3)**	**0**	**0**
ALCL, ALK- (n=12)		**0**	**0**	**1 (8.3)**

STAT3 and 5B mutations identified in NK or γδ-T cell derived lymphomas



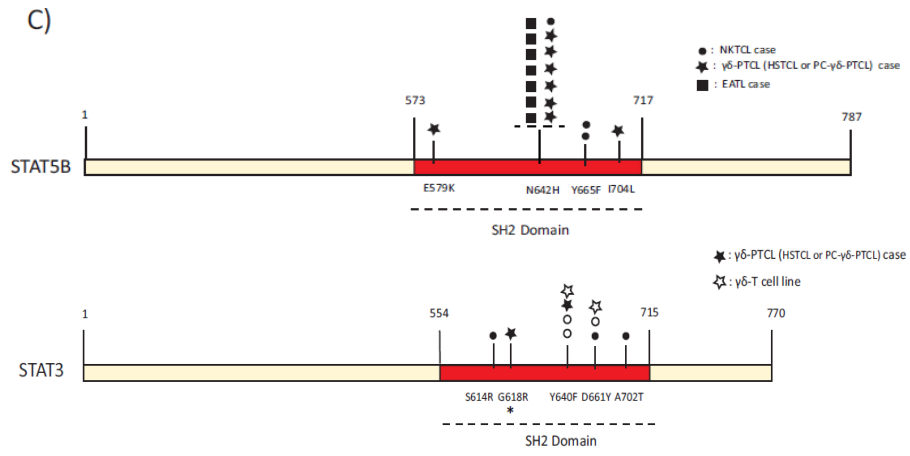


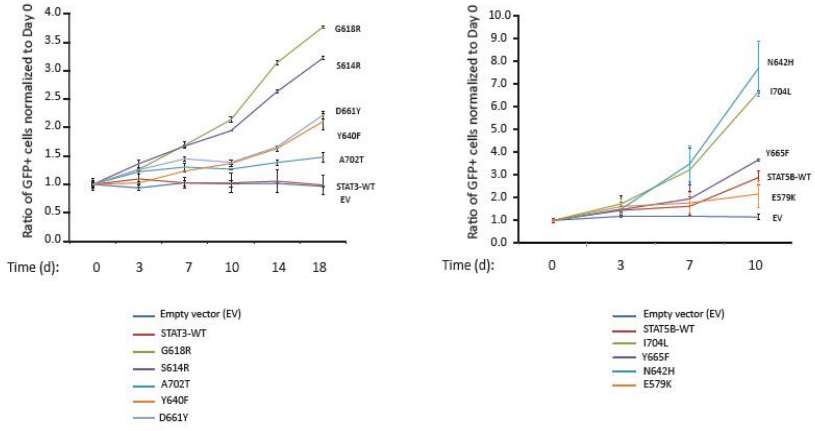


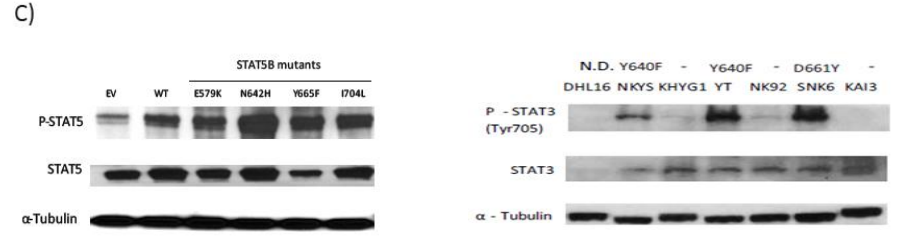


Case No.	Age	Sex	Splenomegaly	Cytopenia	Bone marrow	CD2	CD3	CD4	CD8	CD5	CD7	CD56	CD57	βF1	TCRγ	TIA-1	Granzyme B	Perforin	TRG	7q imbalance	STAT3	STAT5B
1	54	F																				N642H
2	12	M																				N642H
3	35	M																				N642H
4	58	M																				N642H
5	41	M																				N642H
6	79	M																				N642H
7	23	F																				N642H
8	31	M																				
9	19	M																				
10	23	F																				
11	65	M																				
12	71	M																				
13	17	M																				
14	34	M																				
15	18	M																				
16	44	F																				
17	25	M																				
18	52	M																				
19	52	F																				
20	32	F																				D661Y
21	65	M																				Y640F

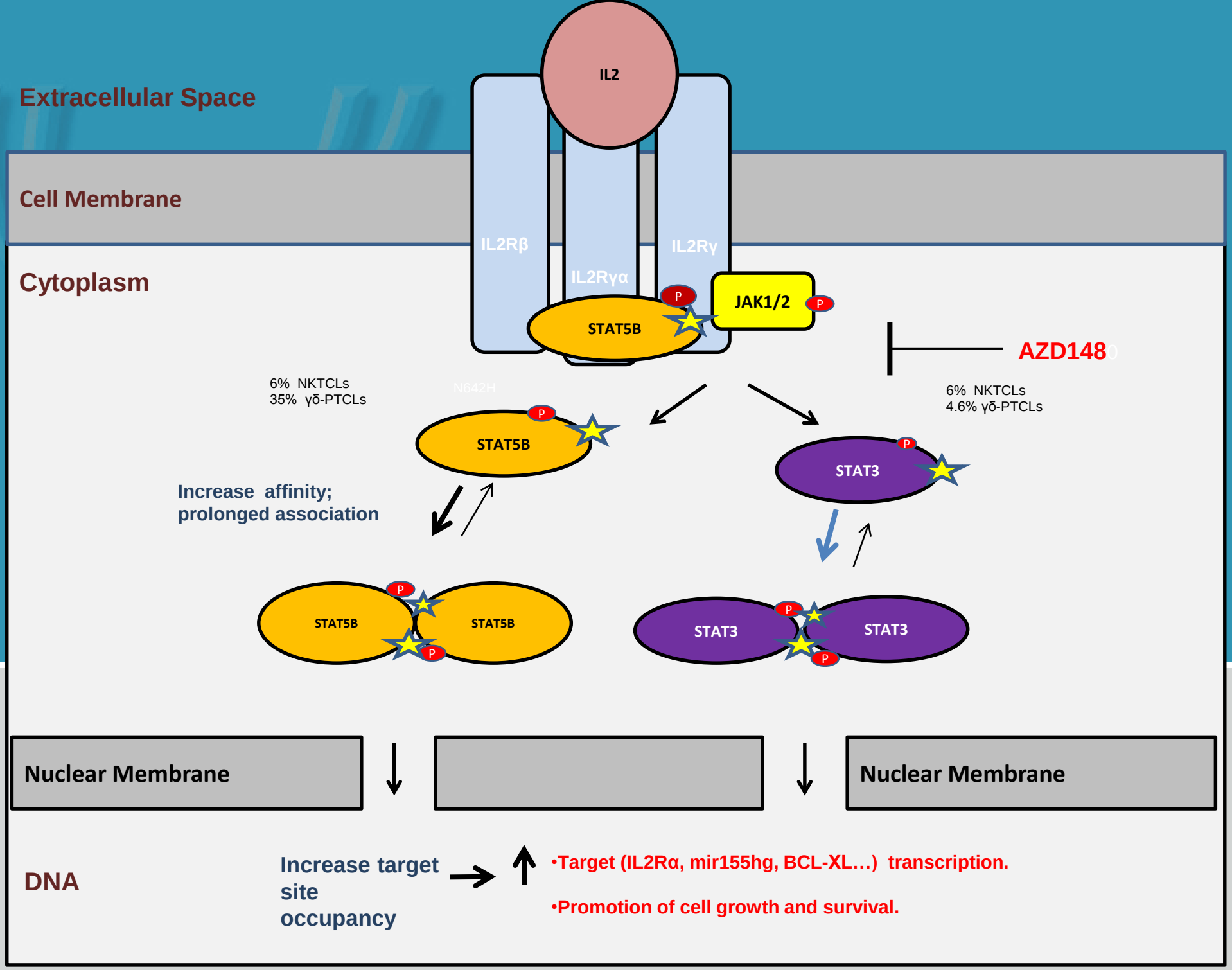
Abbreviation: TRG, T-cell receptor gamma-chain gene rearrangement. Red—positive; pink—positive focal or weak; green—negative; gray—not available; blue—wild type; ochre—mutated.



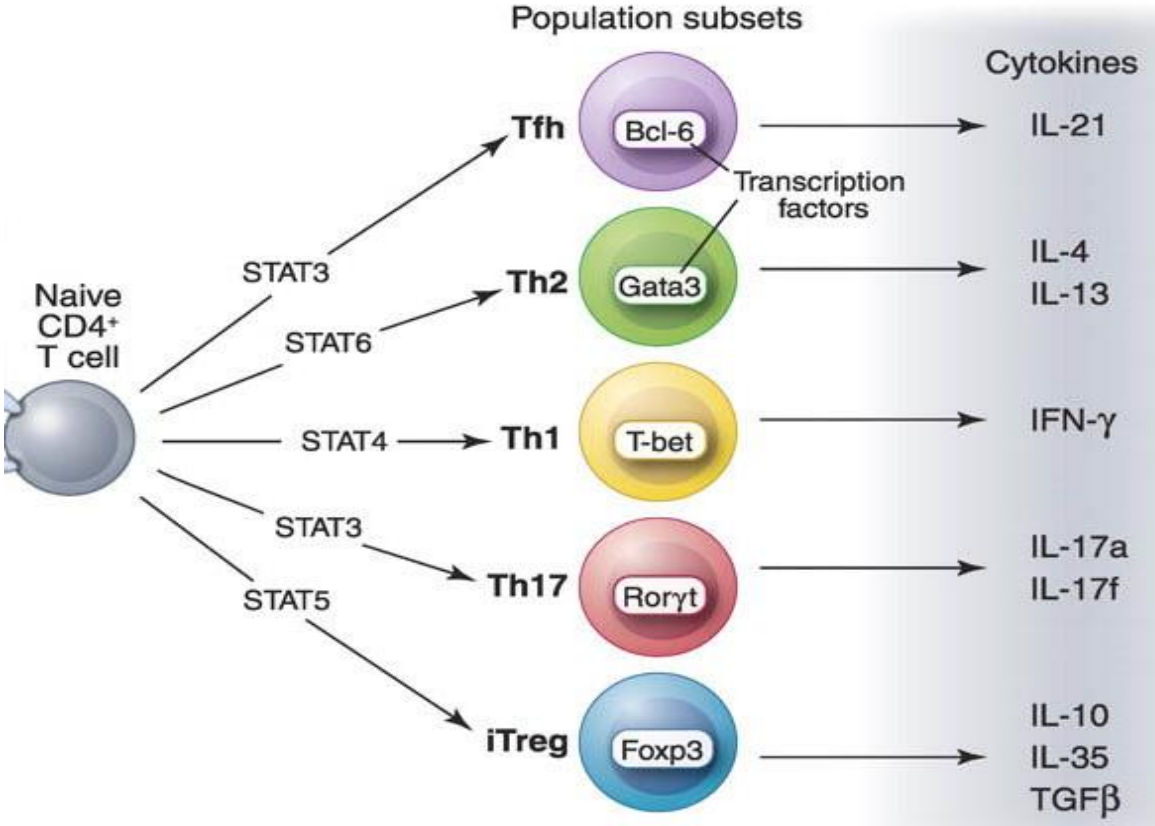




Kucuk C Nat Commun. 2015 Jan 14;6:6025.


Extracellular Space
IL2
Cell Membrane
IL2Rβ
IL2Rγα
IL2Rγ
Cytoplasm
JAK1/2
P
STAT5B
AZD1480
6% NKTCLs
35% γδ-PTCLs
N642H
6% NKTCLs
4.6% γδ-PTCLs
STAT5B
STAT3
Increase affinity;
prolonged association
STAT5B
STAT5B
STAT3
STAT3
Nuclear Membrane
Nuclear Membrane
DNA
Increase target site occupancy
•Target (IL2Rα, mir155hg, BCL-XL…) transcription.
•Promotion of cell growth and survival.

Cell-of-origin for PTCL subgroups
Can that be assigned ?



AITL

PTCL-GATA3/Th2 subgroup

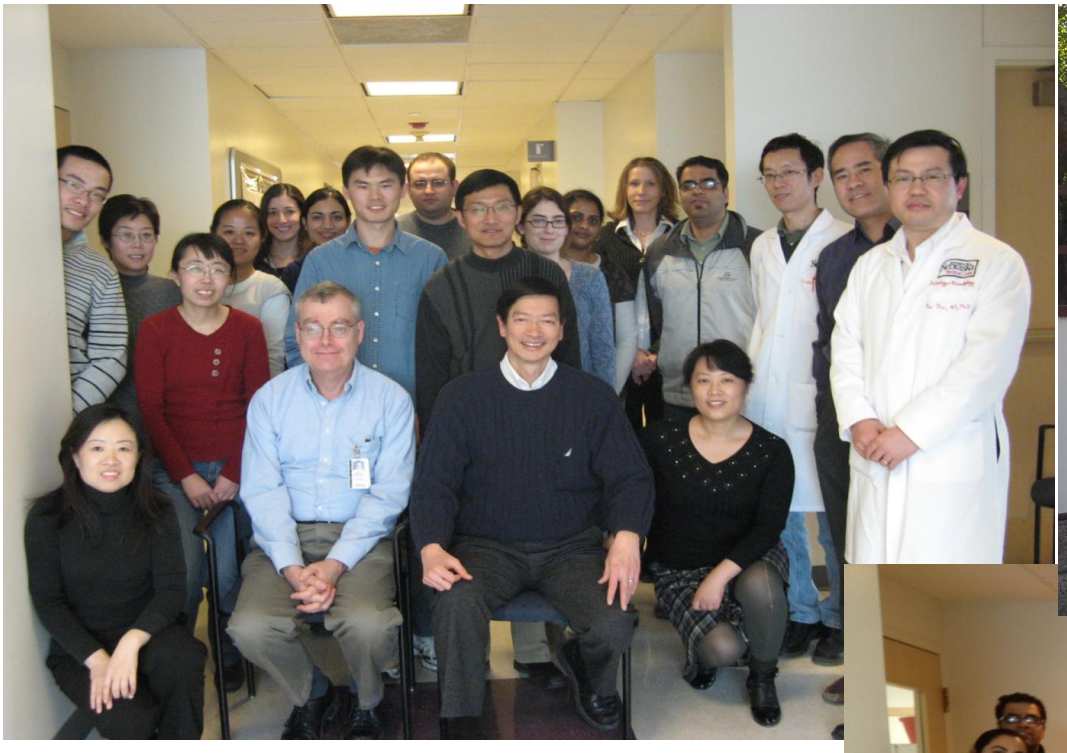
PTCL-NOS/Th1 subgroup

ALCL (ALK+)
ALCL (ALK-)?

ATLL

γδT ⟶ γδPTCL

Laurence de Leval, and Philippe Gaulard Blood 2014;123:2909-2910



I-PTCL
LLMPP
Nordic and ACT
Sichuan University
Singapore
Hong Kong

Tim McKeithan
Javeed Iqbal
Alyssa Bouska
Can Kucuk
Xiaozhou Hu
Bei Jiang
Weiwei Zhang
Chao Wang
Joe Rohr
Cindy Lachel
Yuping Li
Xinhua Wang

Andrew Canonn
Zhongfeng Liu
Shaungping Guo
Karen Deffenbacher
Cuiling Liu

Kai Fu
Tim Greiner
Dennis Weisenburger
Julie Vose

Iqbal Lab