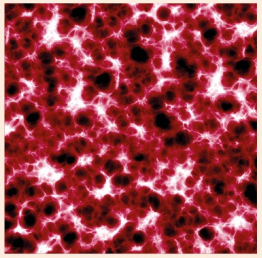
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI MEDICINA SPECIALISTICA, DIAGNOSTICA E SPERIMENTALE

POLICLINICO DI SANT'ORSOLA

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Bologna

2018... 2022
T-Cell Lymphomas: Finally vision and mission!



Genetic and biological data for targeting the PTCL-Epigenome

H.Miles Prince Peter MacCallum Cancer Centre and Epworth Healthcare, Australia

Bologna
ROYAL HOTEL CARLTON
October 25-26, 2022

President: **Pier Luigi Zinzani**
Co-President: **Michele Cavo**

Disclosures

Disclosures of H.Miles Prince

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Celgene/BMS	X					X	
Takeda/Millenium	X					X	
Merck	X					X	
Mundipaharma	X					X	

What we need to consider when considering targeting molecular (genomic/epigenetic) pathways in PTCL

- What are the key pathogenic pathways in PTCL?
- Which are the most important pathways in the various ‘cells of origin’ in the different PTCL entities
  e.g. AITL vs. ALCL etc.
- Are epigenetic-targeting drugs always targeting the epigenome?

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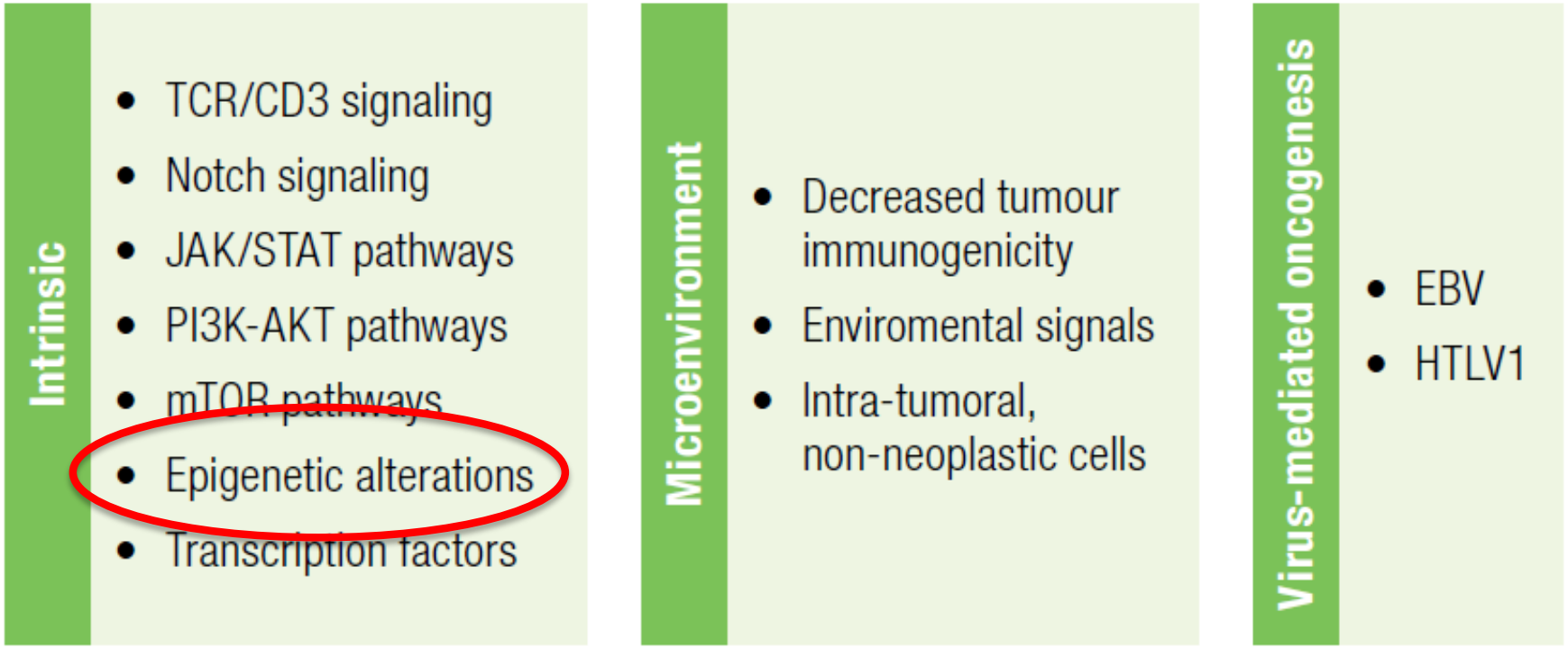
Pathogenic pathways in PTCL



Pathogenic pathways in PTCL

Intrinsic

- TCR/CD3 signaling
- Notch signaling
- JAK/STAT pathways
- PI3K-AKT pathways
- mTOR pathways
- Epigenetic alterations
- Transcription factors

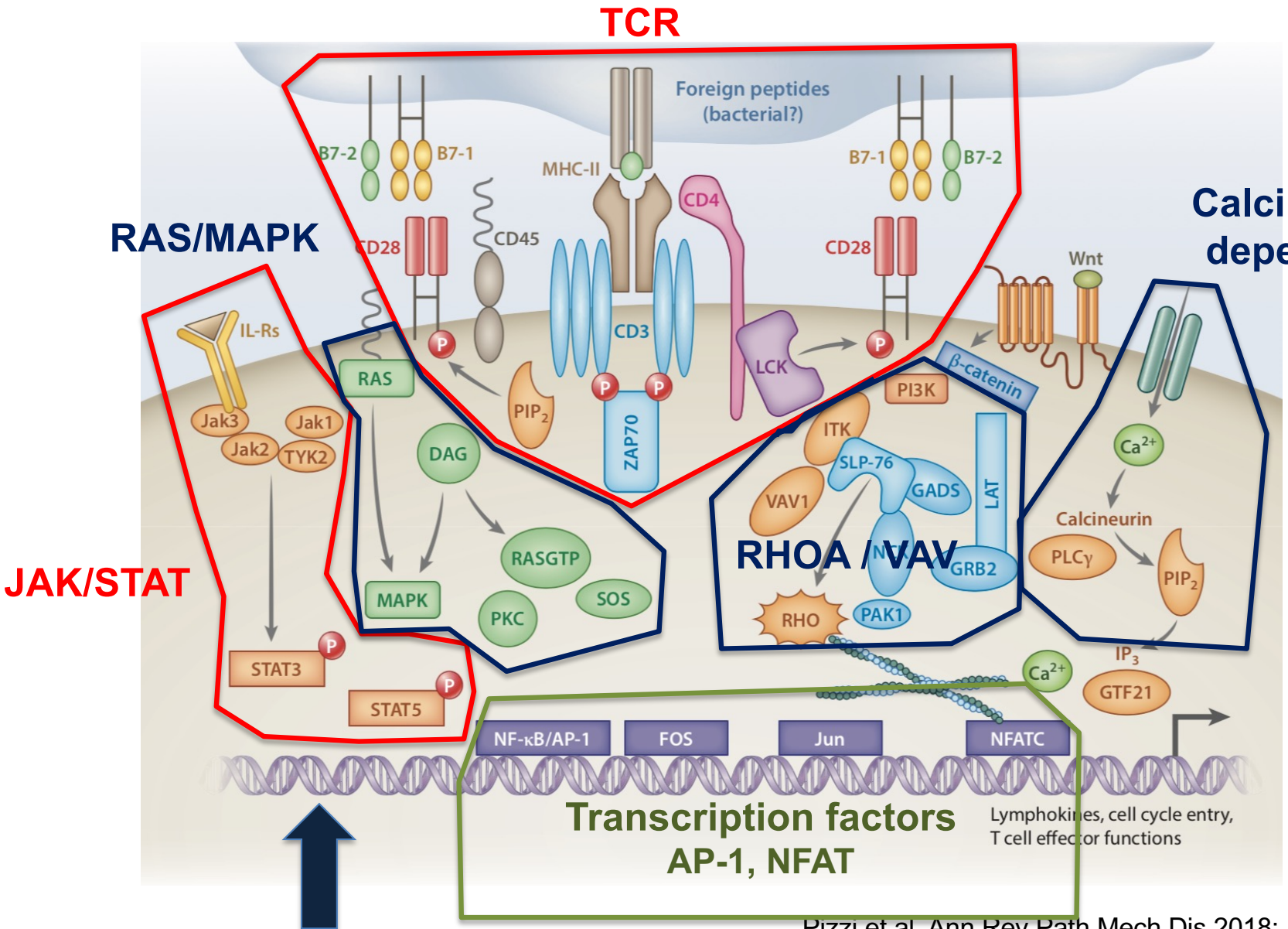
Microenvironment

- Decreased tumour immunogenicity
- Enviromental signals
- Intra-tumoral, non-neoplastic cells

Virus-mediated oncogenesis

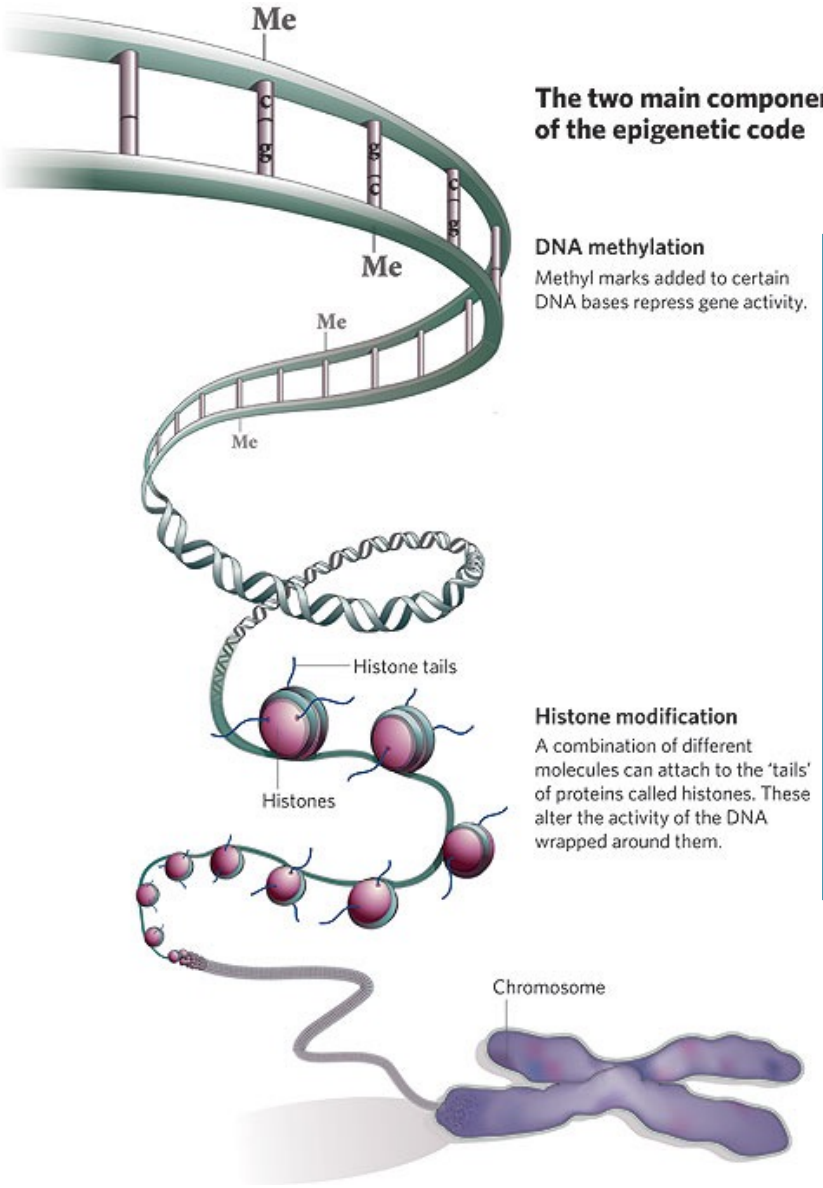
- EBV
- HTLV1

T-cell survival and proliferative signalling



EPIGENETIC LESIONS

Pizzi et al. Ann Rev Path Mech Dis 2018; 13: 293-320



The two main components of the epigenetic code

DNA methylation

Methyl marks added to certain DNA bases repress gene activity.

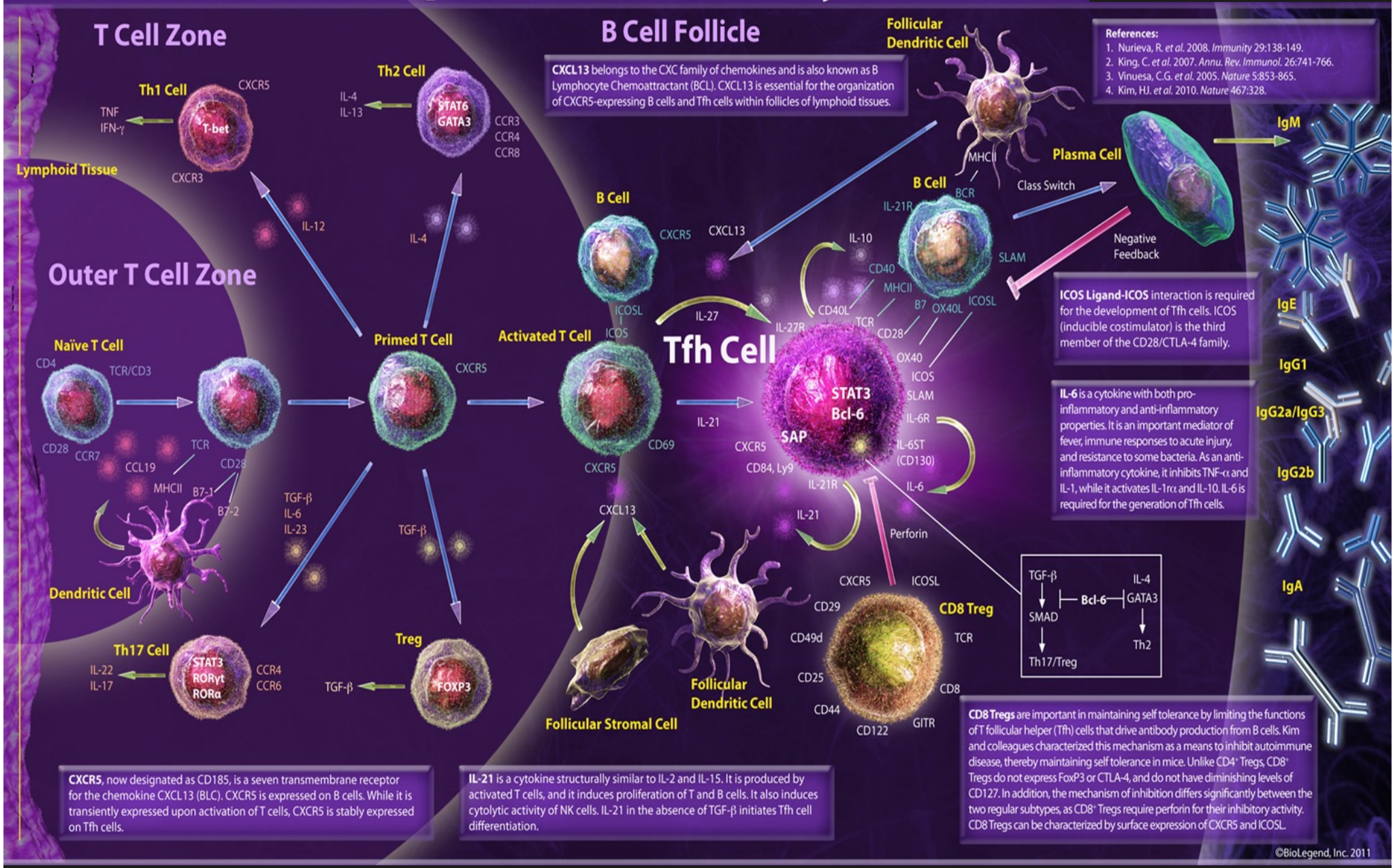
Histone modification

A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

What we need to consider when considering targeting molecular (genomic/epigenetic) pathways in PTCL

- Which are the most important pathways in the various ‘cells of origin’ in the different PTCL entities
  e.g. AITL vs. ALCL etc.

T Follicular Helper Cell Pathway



CXCL13 belongs to the CXC family of chemokines and is also known as B Lymphocyte Chemoattractant (BCL). CXCL13 is essential for the organization of CXCR5-expressing B cells and Tfh cells within follicles of lymphoid tissues.

References:

1. Nurieva, R. *et al.* 2008. *Immunity* 29:138-149.
2. King, C. *et al.* 2007. *Annu. Rev. Immunol.* 26:741-766.
3. Vinuesa, C.G. *et al.* 2005. *Nature* 5:853-865.
4. Kim, HJ. *et al.* 2010. *Nature* 467:328.

ICOS Ligand-ICOS interaction is required for the development of Tfh cells. ICOS (inducible costimulator) is the third member of the CD28/CTLA-4 family.

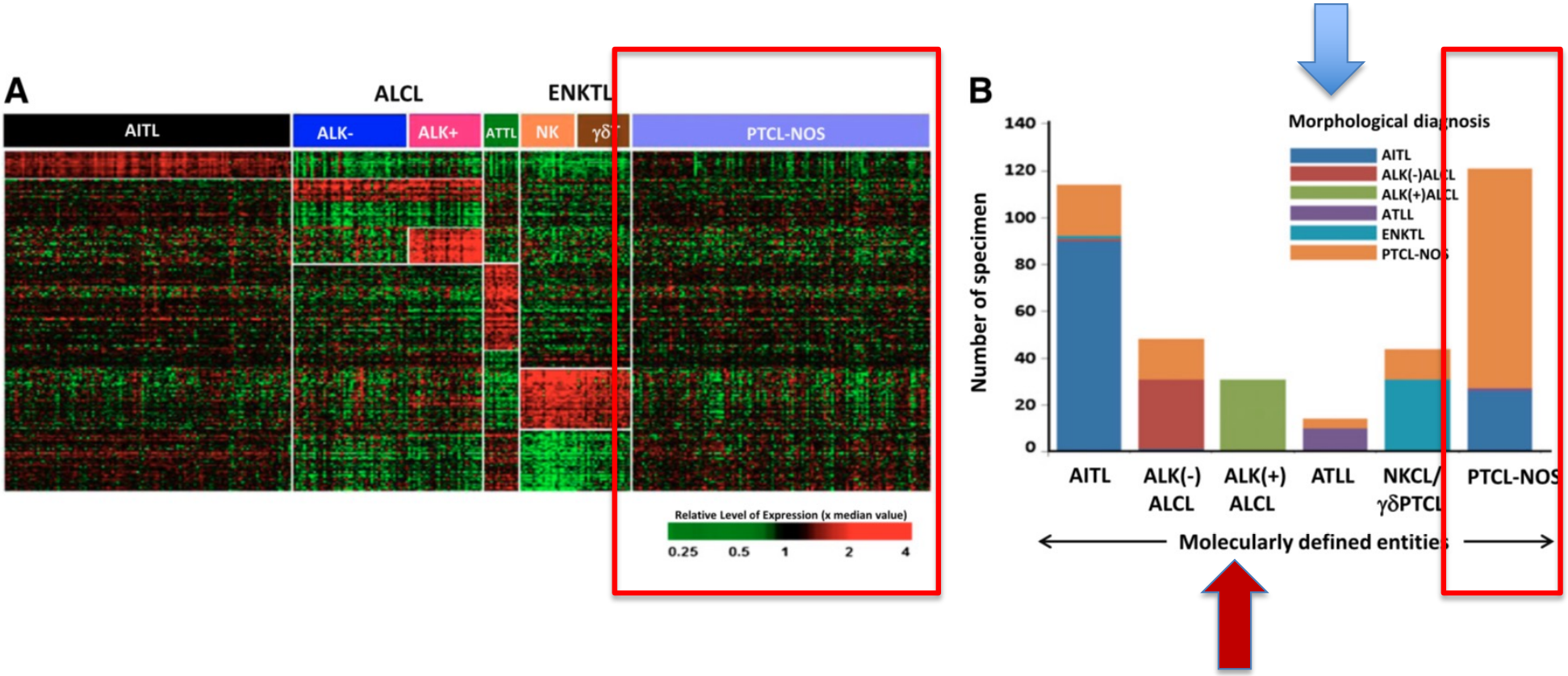
IL-6 is a cytokine with both pro-inflammatory and anti-inflammatory properties. It is an important mediator of fever, immune responses to acute injury, and resistance to some bacteria. As an anti-inflammatory cytokine, it inhibits TNF-α and IL-1, while it activates IL-1rα and IL-10. IL-6 is required for the generation of Tfh cells.

CXCR5, now designated as CD185, is a seven transmembrane receptor for the chemokine CXCL13 (BLC). CXCR5 is expressed on B cells. While it is transiently expressed upon activation of T cells, CXCR5 is stably expressed on Tfh cells.

IL-21 is a cytokine structurally similar to IL-2 and IL-15. It is produced by activated T cells, and it induces proliferation of T and B cells. It also induces cytolytic activity of NK cells. IL-21 in the absence of TGF-β initiates Tfh cell differentiation.

CD8 Tregs are important in maintaining self tolerance by limiting the functions of T follicular helper (Tfh) cells that drive antibody production from B cells. Kim and colleagues characterized this mechanism as a means to inhibit autoimmune disease, thereby maintaining self tolerance in mice. Unlike CD4+ Tregs, CD8+ Tregs do not express FoxP3 or CTLA-4, and do not have diminishing levels of CD127. In addition, the mechanism of inhibition differs significantly between the two regular subtypes, as CD8+ Tregs require perforin for their inhibitory activity. CD8 Tregs can be characterized by surface expression of CXCR5 and ICOSL.

Cell of origin – expression profiling



Iqbal et al Blood 2014; 123: 2915-23

New additions to T cell lymphoma 2016

T-cell large granular lymphocyte leukemia	• New subtypes recognized with clinicopathologic associations. • *STAT3* and *STAT5B* mutations in a subset, latter associated with more clinically aggressive disease.
Systemic EBV$^+$ T-cell lymphoma of childhood	• Name changed from lymphoproliferative disorder to lymphoma due to its fulminant clinical course and desire to clearly distinguish it from chronic active EBV infection.
Hydroa vacciniforme–like lymphoproliferative disorder	• Name changed from lymphoma to lymphoproliferative disorder due to its relationship with chronic active EBV infection and a spectrum in terms of its clinical course.
Enteropathy-associated T-cell lymphoma (EATL)	• Diagnosis only to be used for cases formerly known as type I EATL, typically associated with celiac disease.
Monomorphic epitheliotropic intestinal T-cell lymphoma	• Formerly type II EATL; segregated from type I EATL and given a new name due to its distinctive nature and lack of association with celiac disease.
Indolent T-cell lymphoproliferative disorder of the GI tract	• New indolent provisional entity with superficial monoclonal intestinal T-cell infiltrate, some cases show progression.
Lymphomatoid papulosis	• New subtypes described with similar clinical behavior but atypical histologic/immunophenotypic features.
Primary cutaneous γ δ T-cell lymphoma	• Important to exclude other cutaneous T-cell lymphomas/lymphoproliferative disorders that may also be derived from γ δ T cells such as mycosis fungoides or lymphomatoid papulosis.
Primary cutaneous acral CD8$^+$ T-cell lymphoma	• New indolent provisional entity, originally described as originating in the ear.
Primary cutaneous CD4$^+$ small/medium T-cell lymphoproliferative disorder	• No longer to be diagnosed as an overt lymphoma due to limited clinical risk, localized disease, and similarity to clonal drug reactions. • Remains a provisional entity.
Peripheral T-cell lymphoma (PTCL), NOS	• Subsets based on phenotype and molecular abnormalities being recognized that may have clinical implications but are mostly not a part of routine practice at this time.
Nodal T-cell lymphomas with T-follicular helper (TFH) phenotype	• An umbrella category created to highlight the spectrum of nodal lymphomas with a TFH phenotype including angioimmunoblastic T-cell lymphoma, follicular T-cell lymphoma, and other nodal PTCL with a TFH phenotype (specific diagnoses to be used due to clinicopathologic differences). • Overlapping recurrent molecular/cytogenetic abnormalities recognized that potentially could impact therapy.
ALK$^-$ anaplastic large-cell lymphoma	• Now a definite entity that includes cytogenetic subsets that appear to have prognostic implications (eg, 6p25 rearrangments at *IRF4/DUSP22* locus).
Breast implant–associated anaplastic large cell lymphoma	• New provisional entity distinguished from other ALK$^-$ ALCL; noninvasive disease associated with excellent outcome.

(*Blood*. 2016;127(20):2375-2390)

Activating mutations in genes related to TCR signaling in angioimmunoblastic and other follicular helper T-cell–derived lymphomas

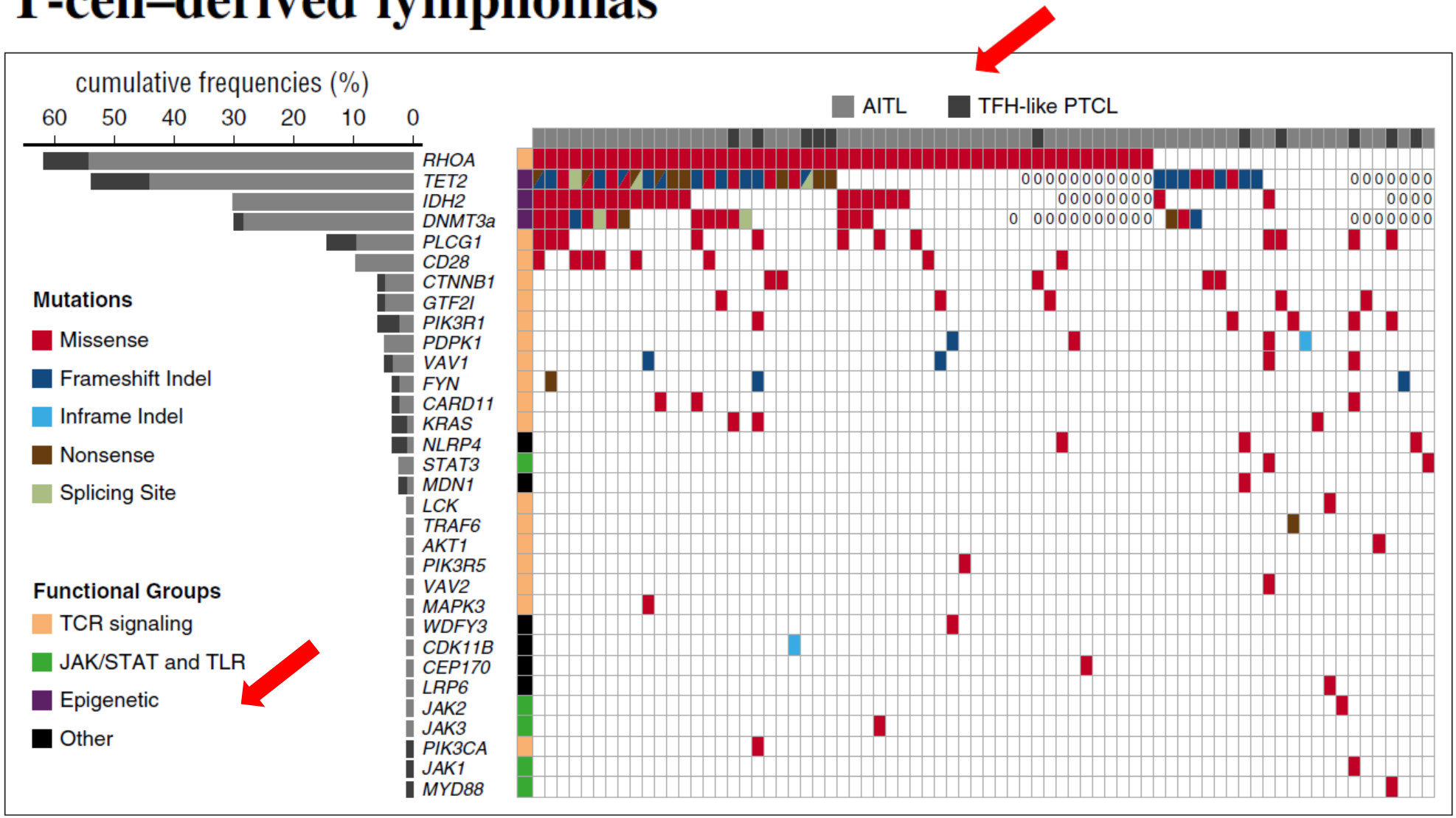
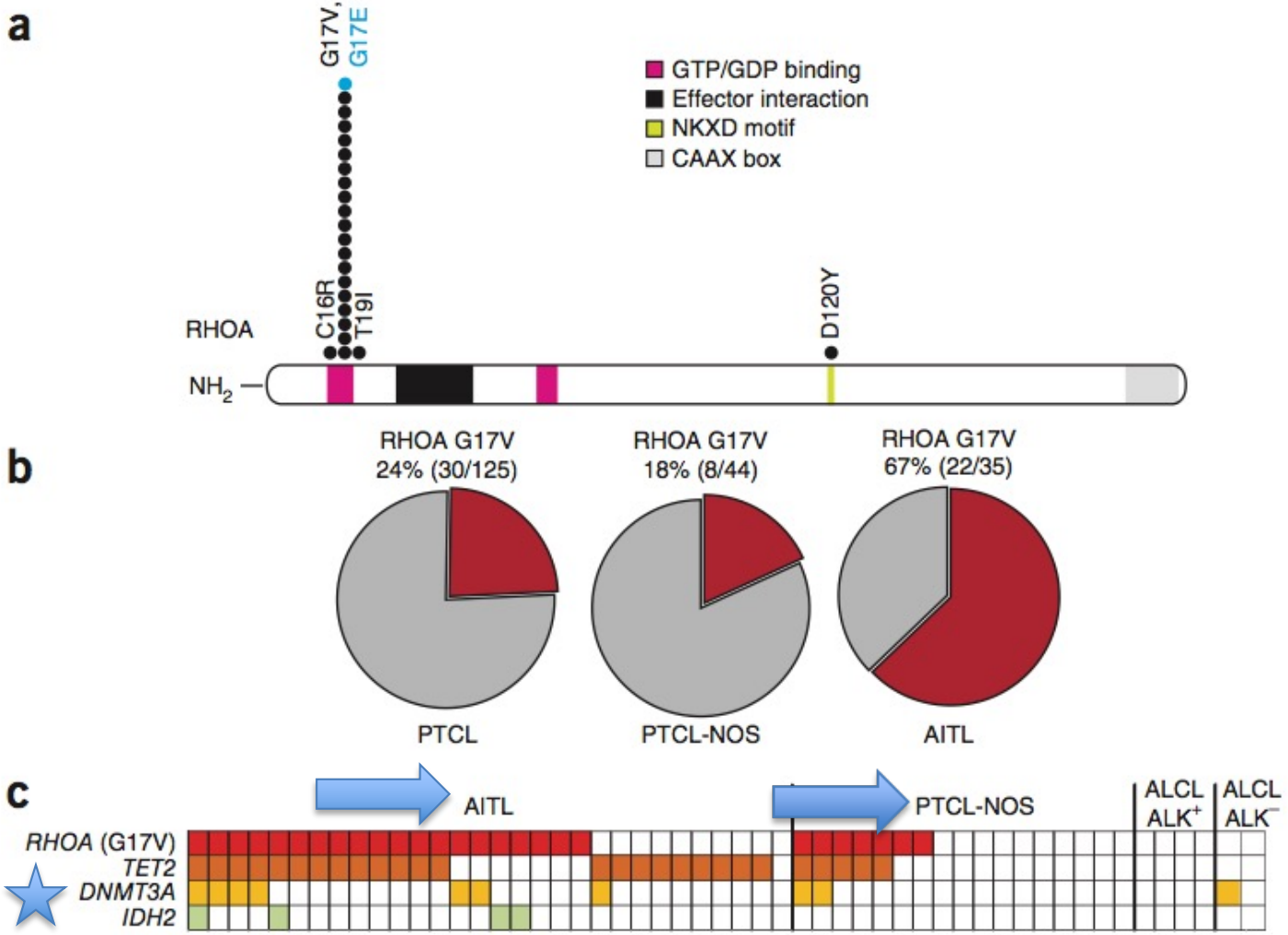


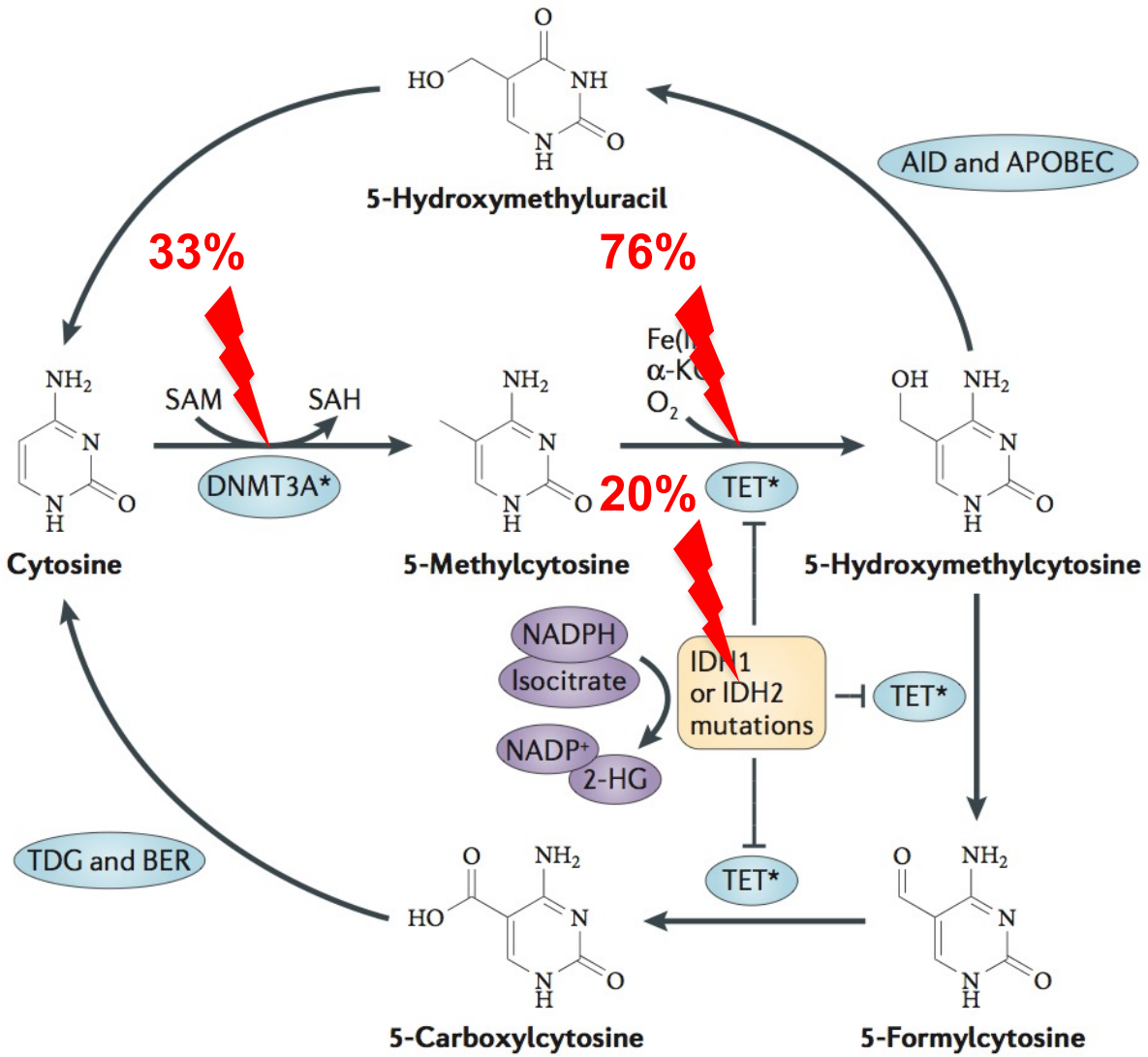
Figure 1. Mutational landscape of nodal TFH-derived lymphomas. The results of targeted deep sequencing of 69 genes in 72 AITL (light gray) and 13 TFH-like PTCL (dark gray) are presented. Ten cases (8 AITL and 2 TFH-like PTCL) with no mutations detected are not represented. *TET2, DNMT3A*, and *IDH2* mutations available for a subset of the cases reported in previous studies[8,9] are also shown. Case-mutation pairs for which data are not available are indicated by a 0. Mutated genes (rows) are arranged by decreasing order of mutation frequency. Patients (columns) are arranged from left to right based on their mutational status following gene ranking.

The Angioimmunoblastic TCL genome:
Epigenetic Modifications important



Palomero et al *Nat Genet* 2014; 46: 166-70.

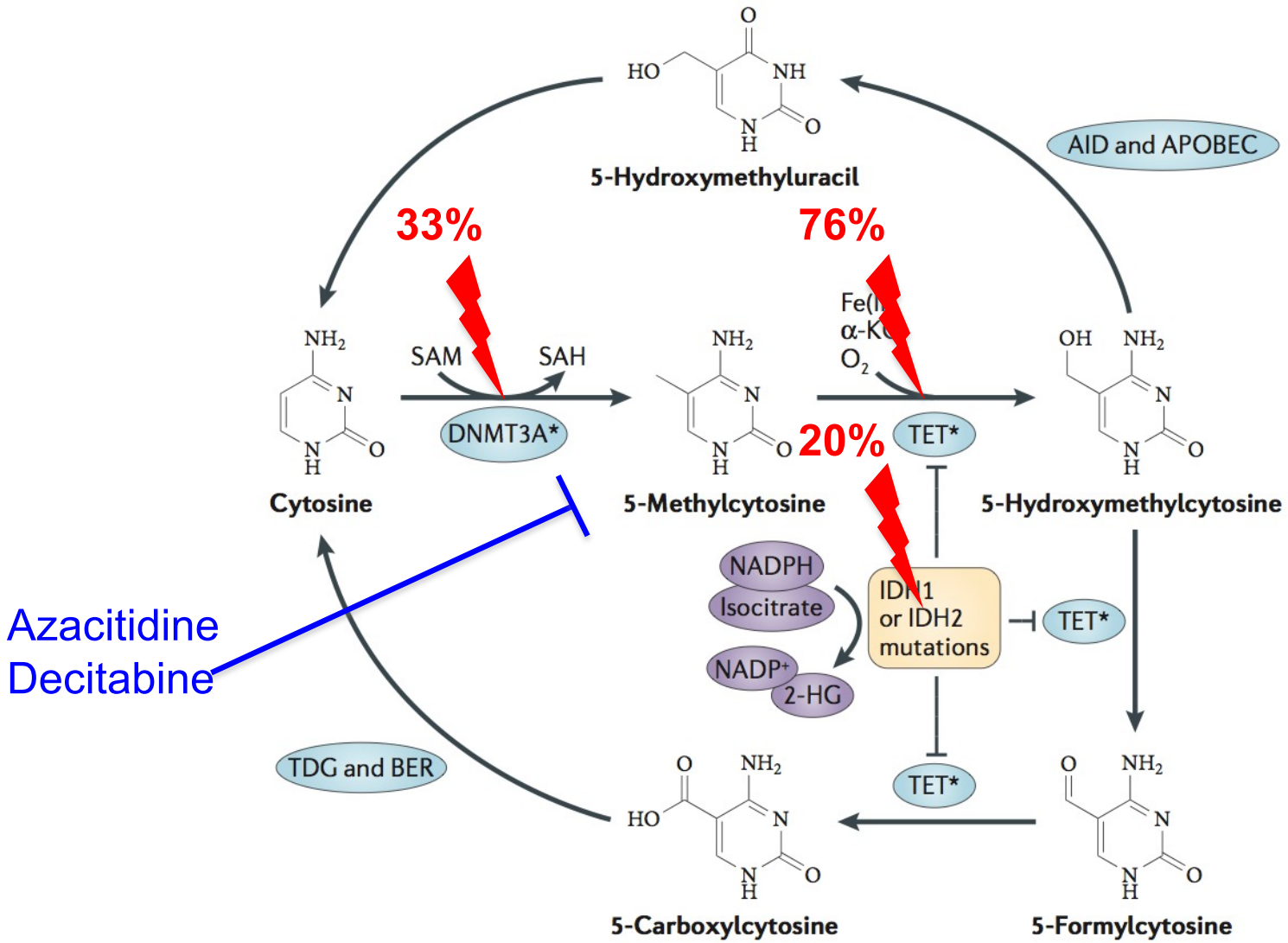
The AITL genome



Shih AH et al. *Nat Rev Cancer* 2012; 12: 599-612

Odejide et al. *Blood* 2014; 123: 1293-6

The AITL genome



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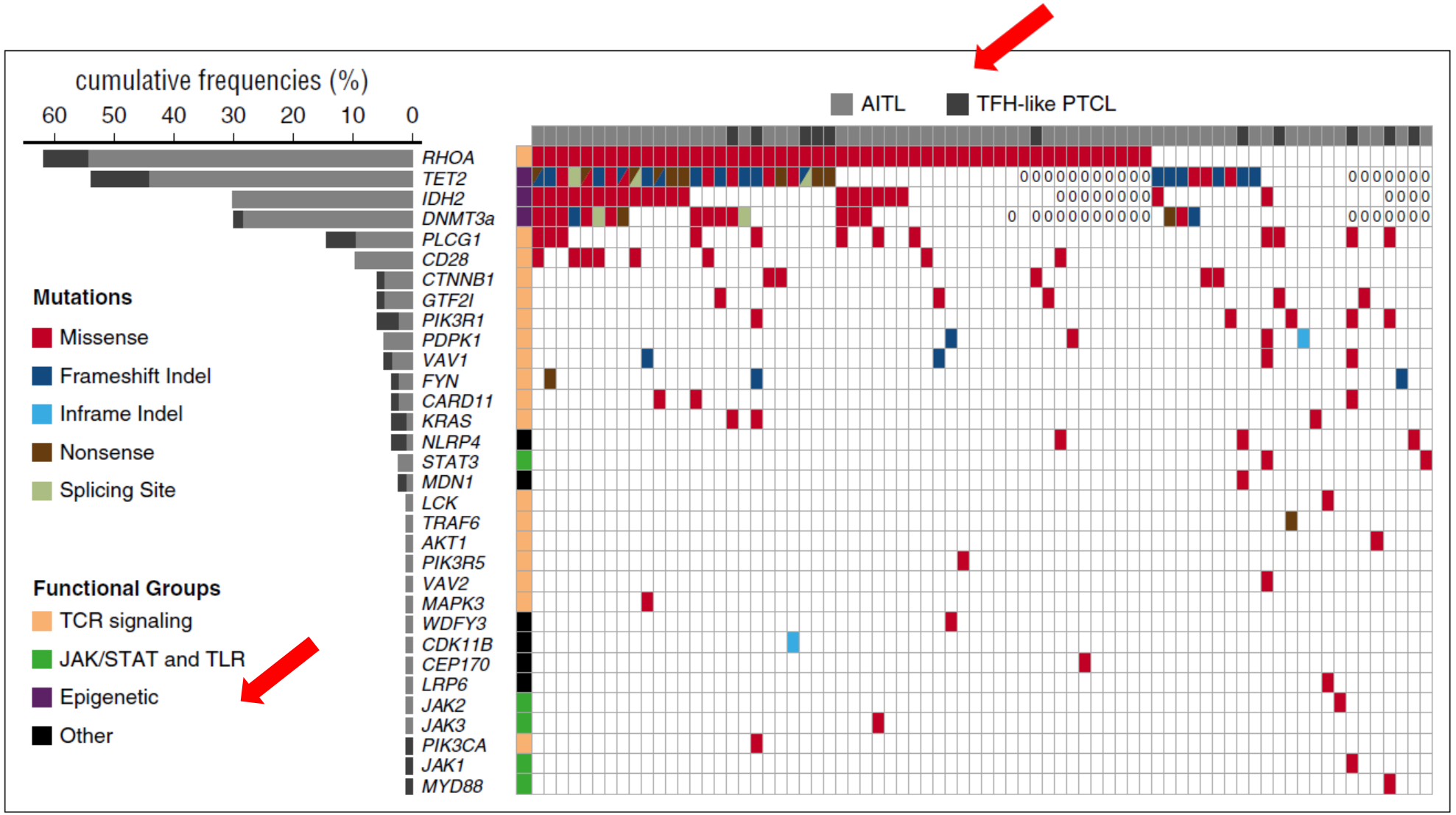
But epigenetics is not the whole story with AITL……



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But epigenetics is not the whole story with AITL……

Activating mutations in genes related to TCR signaling in angioimmunoblastic and other follicular helper T-cell–derived lymphomas

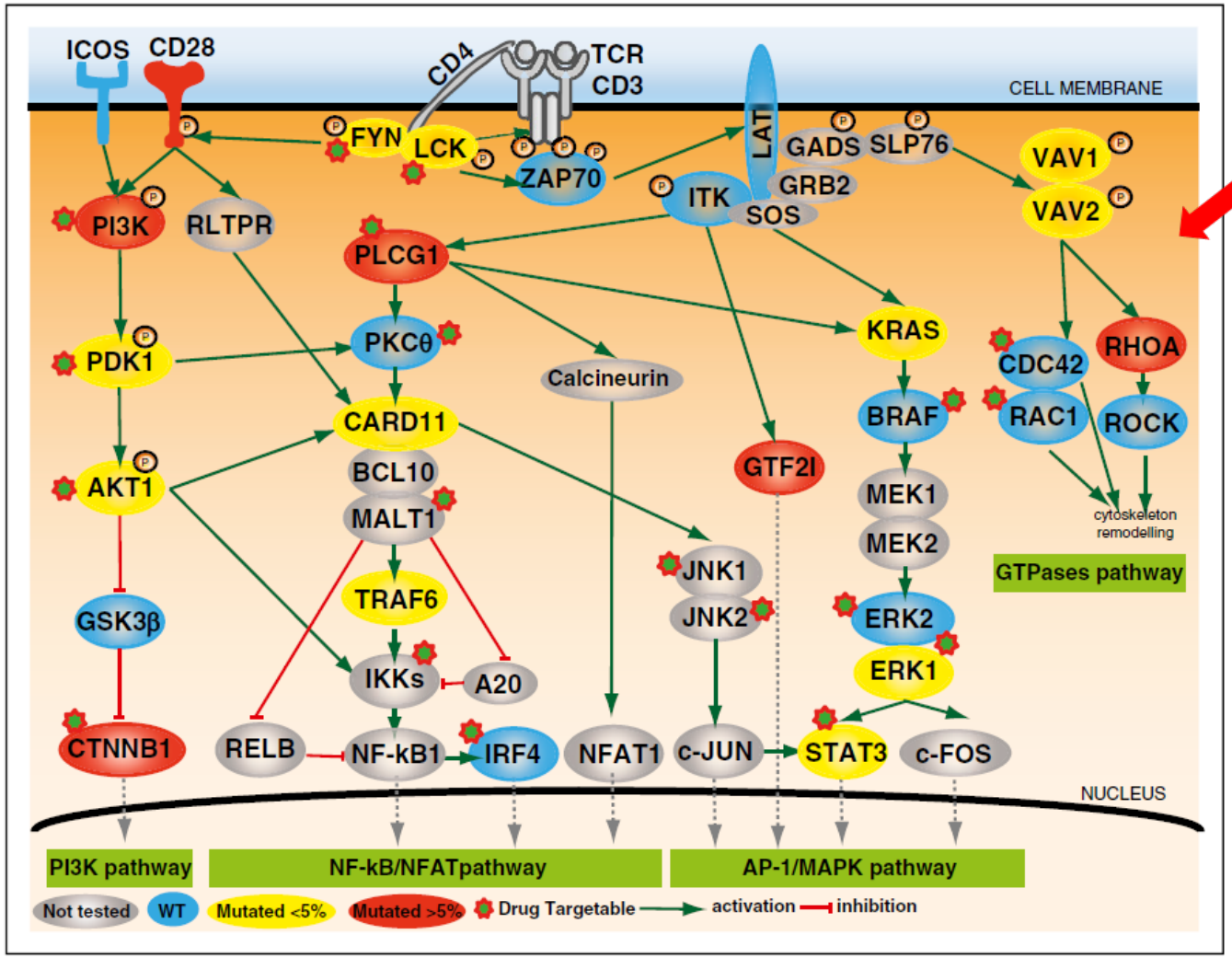
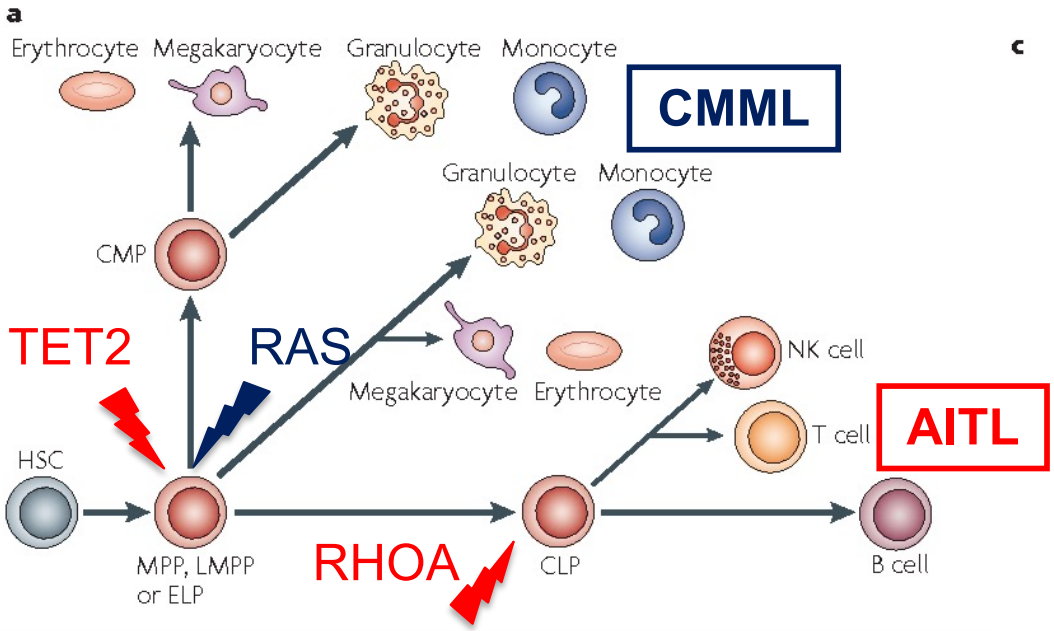
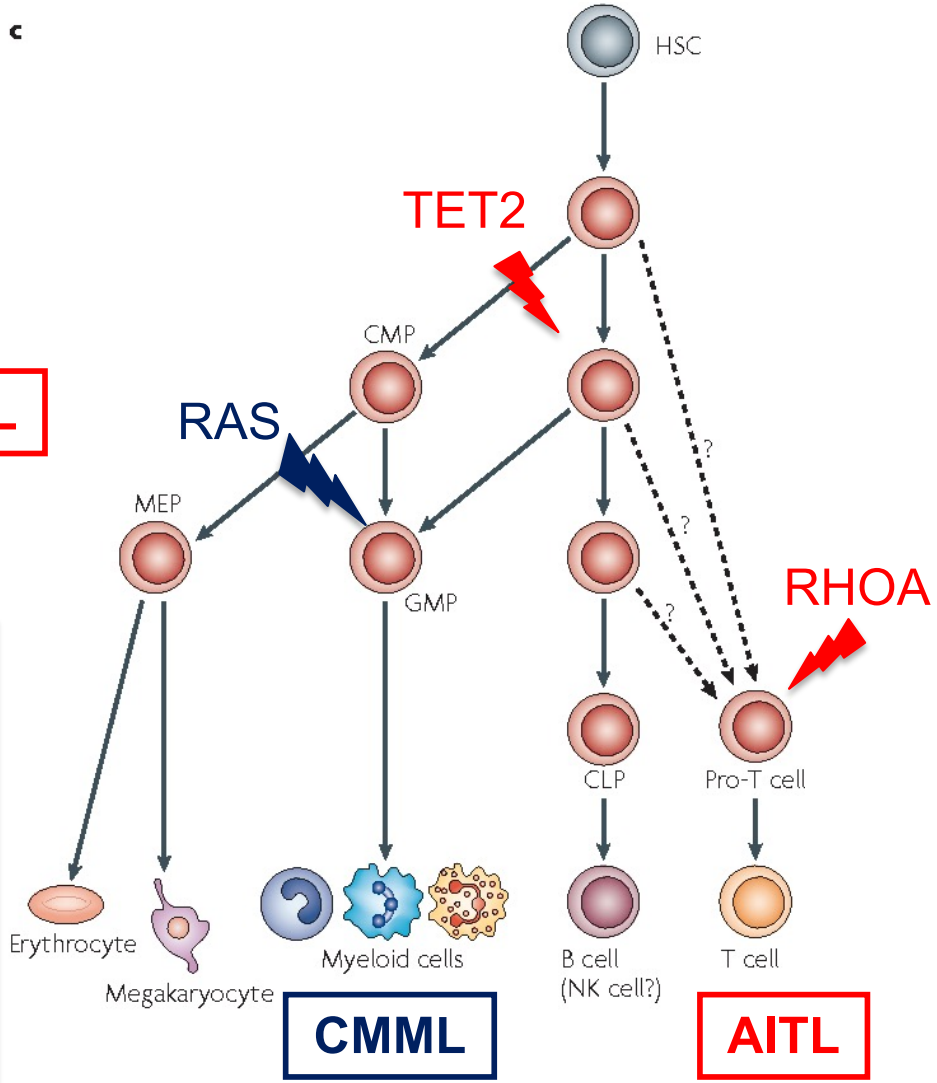


Figure 2. Mutations of TCR signaling–related genes in nodal lymphomas of TFH origin. The intracellular pathways after TCR ligation and costimulatory activation were reconstructed using the Ingenuity pathway analysis (IPA) tools, the KEGG database, and other references. Four main pathways are individualized, from left to right: (1) PI3K pathway after CD28/TCR-dependent FYN phosphorylation and ultimately resulting in CTNNB1 translocation into the nucleus; (2) NF-κB/NFAT pathway proximally initiated by ITK-dependent PLCG1 activation and resulting in NFAT1, NF-κB, and IRF4 activation; (3) AP-1/MAPK pathway that comprises ITK-dependent GTF2I activation, MALT1-induced JNKs activation, and PLCG1-GRB2/SOS–induced MAPK components activation; and (4) GTPase-dependent pathway, including RHOA, responsible for cytoskeleton remodeling upon costimulatory/TCR activation. The main positive interactions are indicated by solid green arrows, whereas inhibitory effects are indicated in red. The TCR signaling elements are depicted in yellow or red if the coding genes were mutated in <5% or ≥5% cases, respectively. The most frequently mutated genes (*PLCG1*, *CD28*, *PI3K* components, *CTNNB1*, and *GTF2I*) were part of costimulatory, NF-κB/NFAT, PI3K, and AP-1/MAPK intracellular signaling pathways. Proteins corresponding to WT genes are indicated in blue, and genes that were not sequenced are in gray. ERK1, ERK2, JNK1, JNK2, and PDK1 are protein names for *MAPK1*, *MAPK3*, *MAPK8*, *MAPK9*, and *PDPK1* genes, respectively.

And do we really understand ‘cell of origin’?





Ceredig et al *Nat Rev Immunol* 2009; 168: 293-300.

Other PTCLs where the epigenome has implications

ATLL

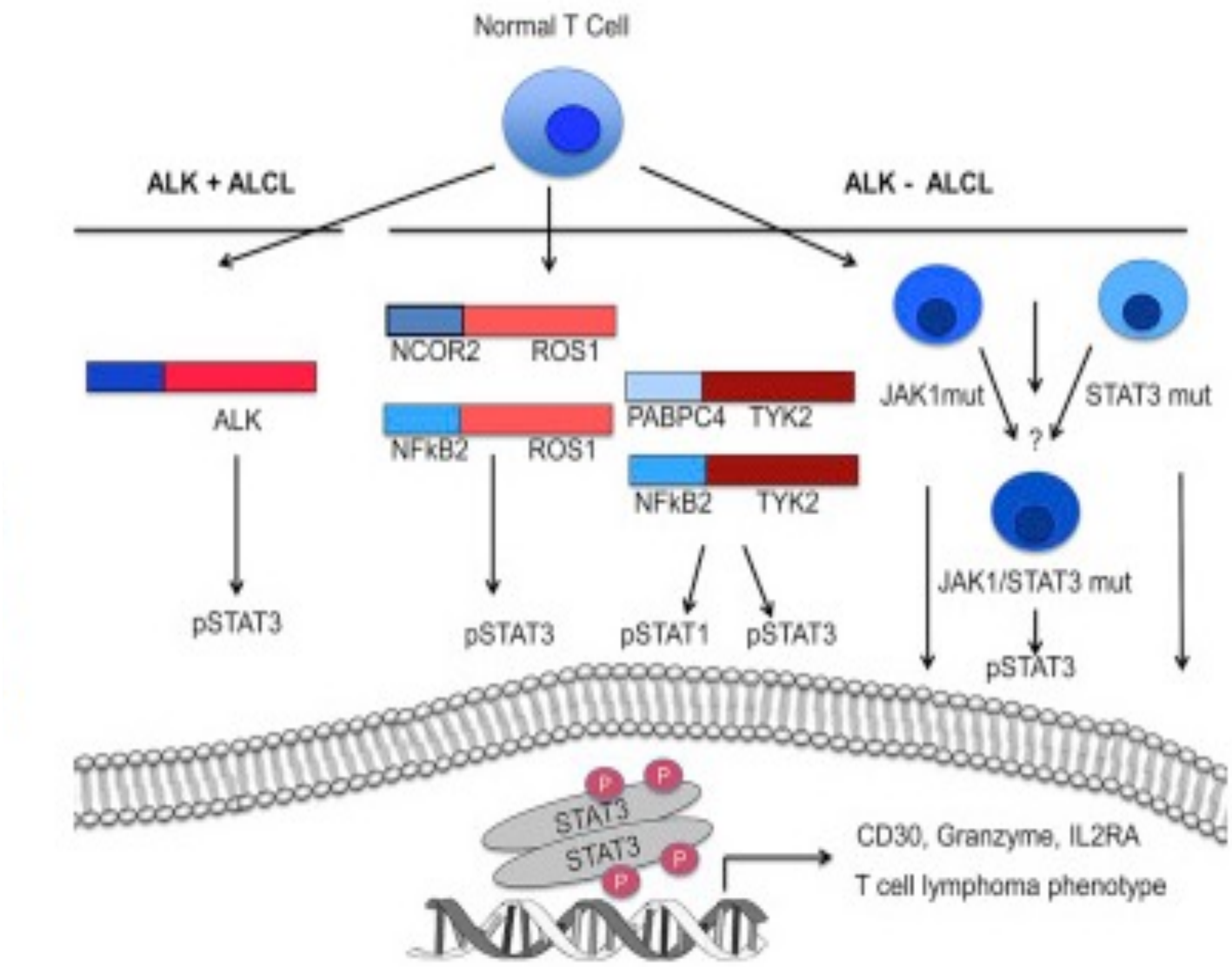
- Methylation pathway genes (*TET2*, *DNMT3*, and *IDH2*) are altered in ATLL, albeit to a far less extent than in AITL.
- Polycomb-dependent repression is enhanced in ATLL
  - by trimethylation of histone lysine 27 of H3 and affects half the genes in ATLL.
- *EZH2* and other components of the PRC2 complex are upregulated in ATLL.
- *KDM6B*, a gene that encodes a lysine-specific demethylase that specifically demethylates di- or tri methylated lysine 27 of histone H3 is considered a repressive histone mark controlling chromatin condensation. The gene is downregulated in ATLL, thus locking in the effects initiated by Tax even when Tax expression is lost during disease progression.

Other PTCLs where the epigenome has implications

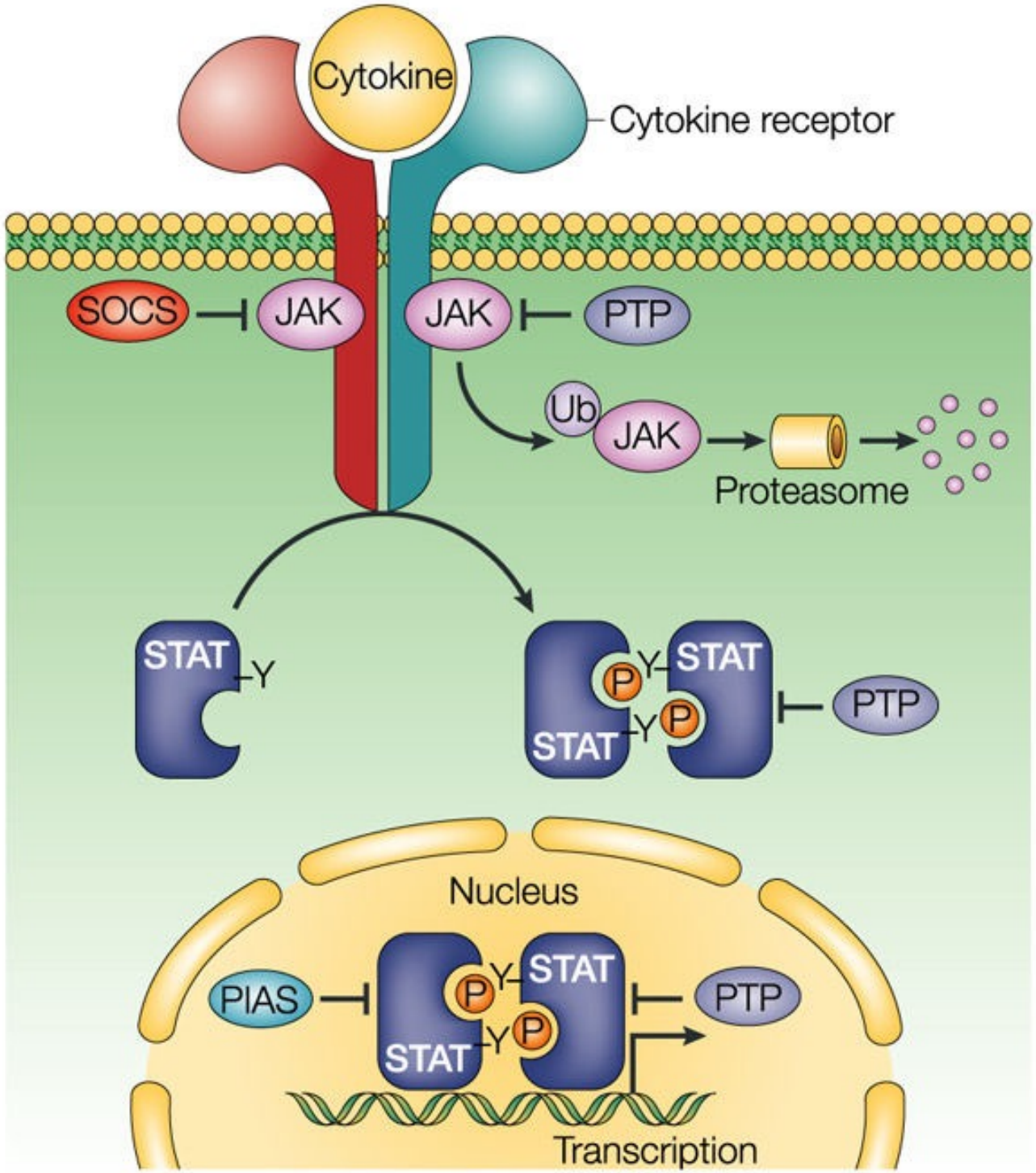
Anaplastic Large Cell Lymphoma

- *DNMT3* and *TET2* mutations have been identified in some cases albeit comparatively few compared to AITL and PTCL-TFH
- DUSP22+ ALCL associated with hypomethylation

The Jak-Stat pathway is critical for ‘most’ ALCL



JAK-STAT Pathway



ALK neg - Systemic ALCL: more detail

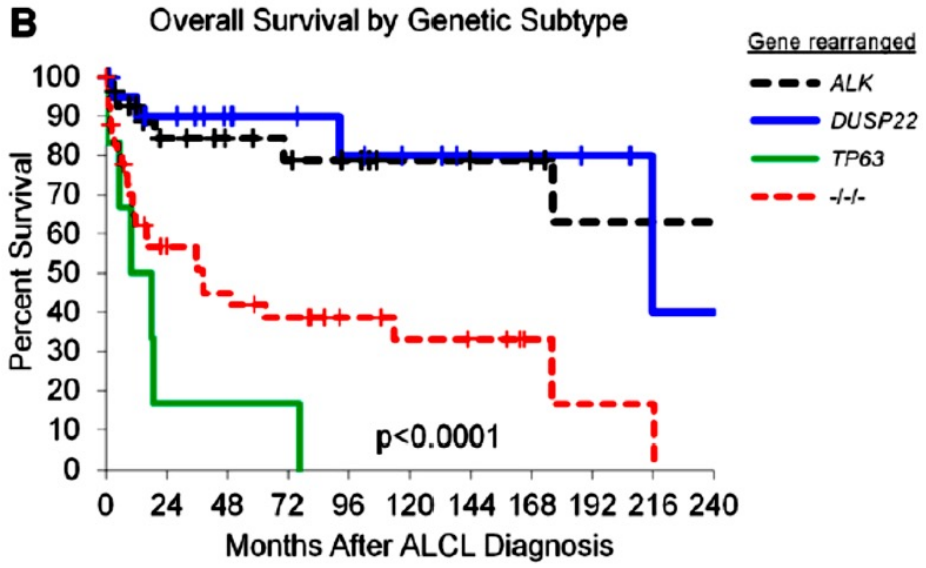
(*Blood*. 2014;124(9):1473-1480)

ALK-negative anaplastic large cell lymphoma is a genetically heterogeneous disease with widely disparate clinical outcomes

Edgardo R. Parrilla Castellar,[1] Elaine S. Jaffe,[2] Jonathan W. Said,[3] Steven H. Swerdlow,[4] Rhett P. Ketterling,[1] Ryan A. Knudson,[1] Jagmohan S. Sidhu,[5] Eric D. Hsi,[6] Shridevi Karikehalli,[7] Liuyan Jiang,[8] George Vasmatzis,[9] Sarah E. Gibson,[4] Sarah Ondrejka,[6] Alina Nicolae,[2] Karen L. Grogg,[1] Cristine Allmer,[10] Kay M. Ristow,[11] Wyndham H. Wilson,[12] William R. Macon,[1] Mark E. Law,[1] James R. Cerhan,[10] Thomas M. Habermann,[11] Stephen M. Ansell,[11] Ahmet Dogan,[1] Matthew J. Maurer,[10] and Andrew L. Feldman[1]

Key Points

- ALK-negative ALCLs have chromosomal rearrangements of *DUSP22* or *TP63* in 30% and 8% of cases, respectively.
- *DUSP22*-rearranged cases have favorable outcomes similar to ALK-positive ALCLs, whereas other genetic subtypes have inferior outcomes.



***DUSP22-IRF4* locus**

LYMPHOID NEOPLASIA

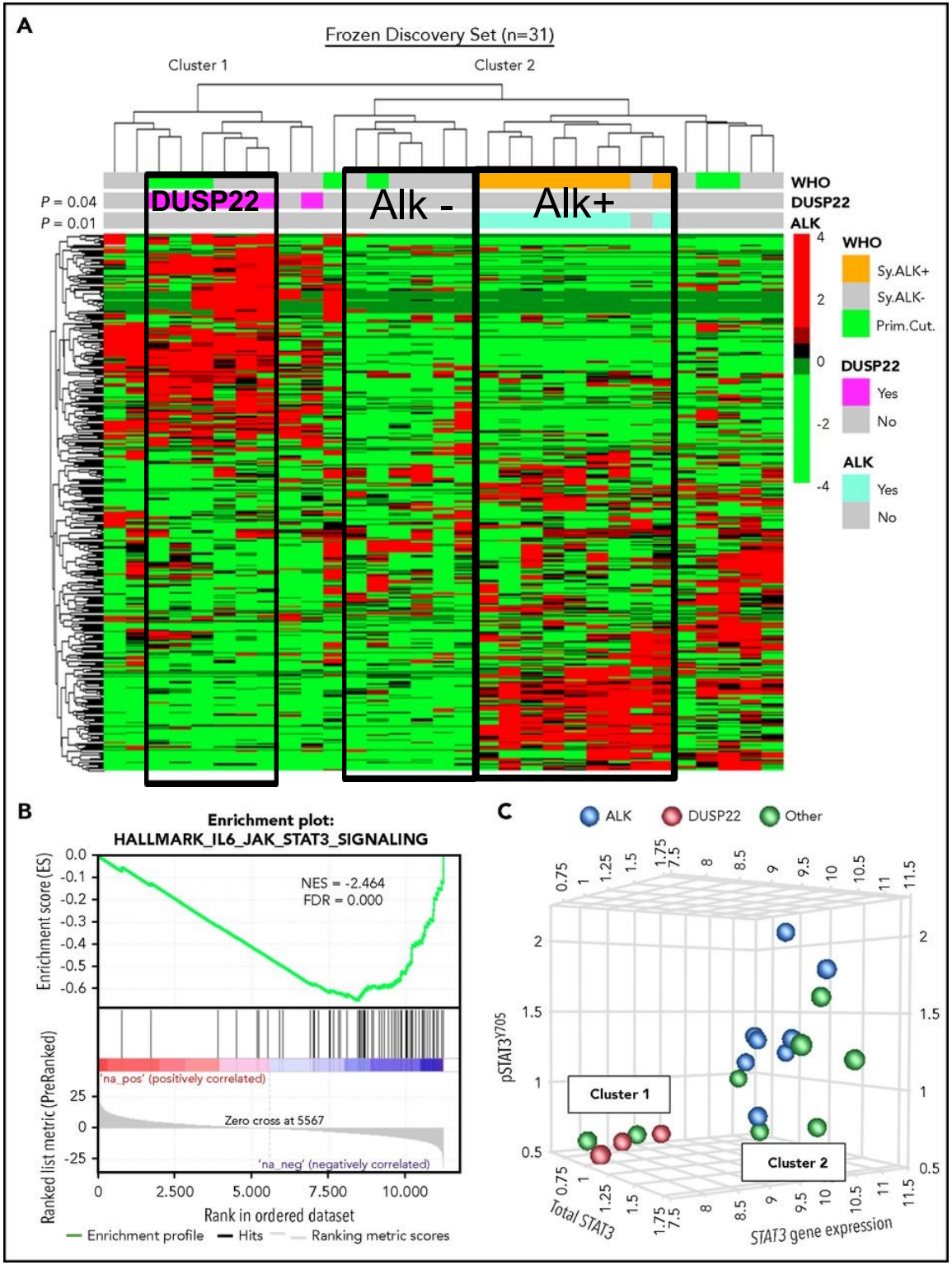
Molecular profiling reveals immunogenic cues in anaplastic large cell lymphomas with *DUSP22* rearrangements

Rebecca A. Luchtel,[1,*] Surendra Dasari,[2,*] Naoki Oishi,[1,3] Martin Bjerregård Pedersen,[4] Guangzhen Hu,[1] Karen L. Rech,[1] Rhett P. Ketterling,[1] Jagmohan Sidhu,[5] Xueju Wang,[6] Ryohei Katoh,[3] Ahmet Dogan,[1] N. Sertac Kip,[1] Julie M. Cunningham,[1] Zhifu Sun,[2] Saurabh Baheti,[2] Julie C. Porcher,[7] Jonathan W. Said,[8] Liuyan Jiang,[9] Stephen Jacques Hamilton-Dutoit,[10] Michael Boe Møller,[11] Peter Nørgaard,[12] N. Nora Bennani,[7] Wee-Joo Chng,[13-15] Gaofeng Huang,[13] Brian K. Link,[16] Fabio Facchetti,[17] James R. Cerhan,[2] Francesco d'Amore,[4] Stephen M. Ansell,[7] and Andrew L. Feldman[1]

KEY POINTS

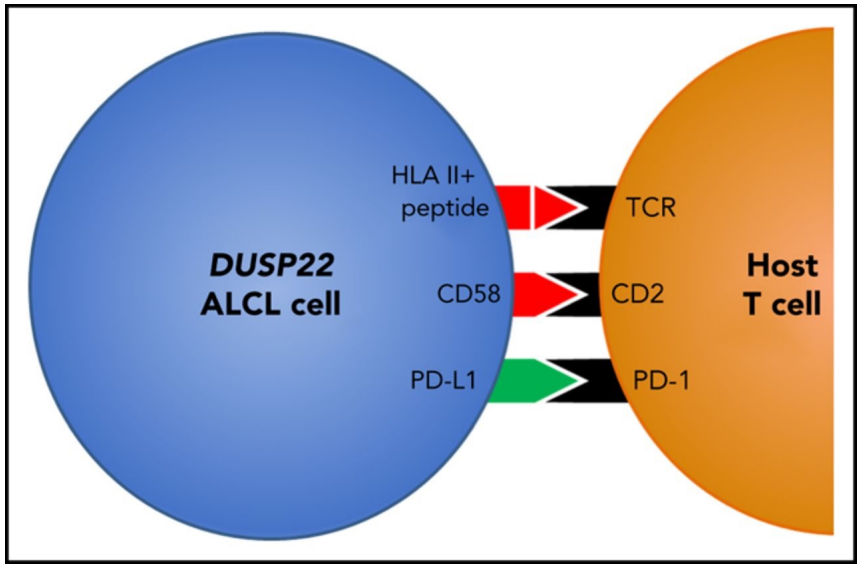
- ***DUSP22*-rearranged ALCLs belong to a distinct subset of ALCLs lacking activated STAT3.**
- ***DUSP22*-rearranged ALCLs have a unique molecular signature characterized by DNA hypomethylation and an immunogenic phenotype.**

(*Blood*. 2018;132(13):1386-1398)


A
Frozen Discovery Set (n=31)
Cluster 1
Cluster 2
P = 0.04
P = 0.01
DUSP22
Alk -
Alk+
WHO
DUSP22
ALK
4
2
0
-2
-4
WHO
Sy.ALK+
Sy.ALK-
Prim.Cut.
DUSP22
Yes
No
ALK
Yes
No
B
Enrichment plot:
HALLMARK_IL6_JAK_STAT3_SIGNALING
Enrichment score (ES)
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
NES = -2.464
FDR = 0.000
'na_pos' (positively correlated)
Zero cross at 5567
'na_neg' (negatively correlated)
Ranked list metric (PreRanked)
25
0
-25
0
2.500
5.000
7.500
10.000
Rank in ordered dataset
Enrichment profile
Hits
Ranking metric scores
C
ALK
DUSP22
Other
pSTAT3Y705
Cluster 1
Cluster 2
Total STAT3
STAT3 gene expression

The difference in the COO characteristics in *DUSP22* rearranged ALCL

- TCR signalling still occurs (unlike ALK+)
- Low STAT dependency
- Immune signature
  - High Cancer Testis Antigen
  - LFA3 high (immune)
  - Low PD-L1 – (no check point inhibition - + immune response
- DNA Hypomethylated
- Better Prognosis
- Hypothesis – should we be treating other PTCL to get a DUSP22 ‘signature’



- Demethylating agents?
- Checkpoint inhibition?

KEY POINTS

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Rebecca A. Luchtel et al. Blood 2018;132:1386-1398

Other PTCLs where the epigenome has implications

Intestinal T cell lymphoma

- *SETD2* (SET domain containing 2) is the most frequently mutated gene in EATL (32% of cases)
  - The SETD2 gene encodes a histone methyltransferase that is specific for lysine-36 of H3, which has been associated with transcriptional activation.
  - Depletion of SETD2 increases the frequency of deletion mutations that arise by the alternative DNA repair process of microhomology-mediated end joining

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Hepatosplenic T cell lymphomas

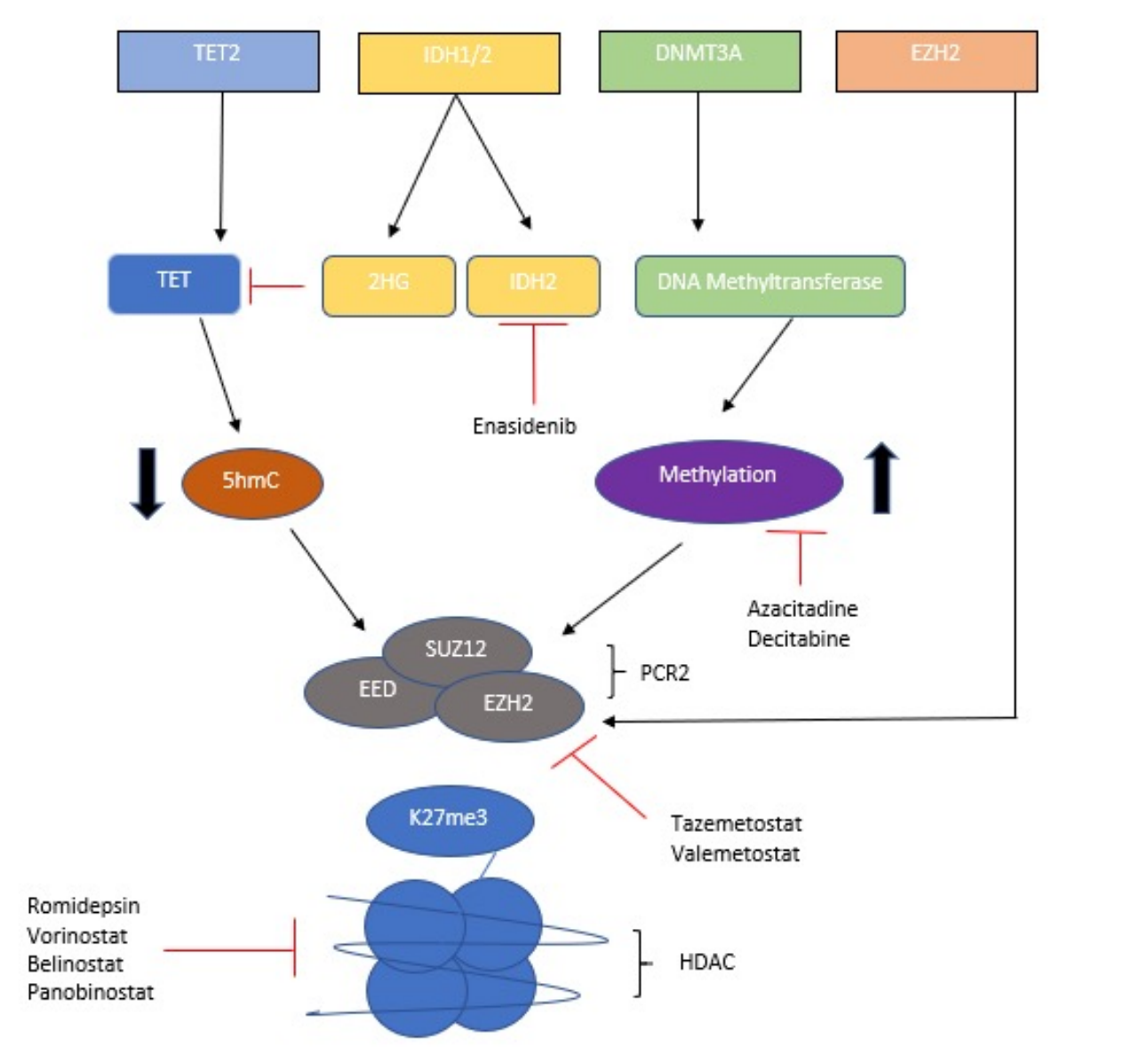
- *SETD2* and *ARID1B* in up to 62% of cases.
  - The *ARID1B* gene (AT-rich interactive domain containing protein 1B) encodes for a protein that binds to DNA helping to target SWI/SNF complexes which regulate gene expression by modulating chromatin remodelling (tumor suppressor gene).

Other PTCLs where the epigenome has implications

Mycosis Fungoides and Sezary Syndrome

- The Leiden group identified 126 recurrent genes with hypermethylation of CpG-rich promoters as candidates for transcriptional repression which may play a potential causal role in the pathogenesis of Sézary syndrome (SS).
  - methylation abnormalities are much more common in SS compared to other cancers.
  - some of the highly expressed genes identified in SS, such as those for CD158 (KIR3DL2), DNMT3, PLS3 (the T-plastin gene) , and TWIST1, have large CpG islands
- *TET* mutations are one of the most frequent early genetic abnormalities in SS
- *DNMT3A* mutations not uncommon.
- *IDH2, ARID1A/B* are well recognized but not common.

Common epigenetic targets in PTCL



TET = ten eleven translocation protein,

2HG = 2-hydroxyglutarate,

IDH2 = isocitrate dehydrogenase 2,

5hmC = 5-hydroxymethylcytosine,

EED = embryonic ectoderm development,

SUZ12 = suppressor of zeste 12,

EZH2 = enhancer of zeste 2,

PCR2 = polycomb repressive complex 2,

K27me3 = trimethylation at lysine 27 of histone 3 (aka (H3K27me3),

HDAC = histone deacetylase.

What we need to consider when considering targeting molecular pathways in PTCL

- Are epigenetic-targeting drugs always targeting the epigenome?

Phase 2 trial of romidepsin in patients with peripheral T-cell lymphoma

Richard L. Piekarz,[1] Robin Frye,[2] H. Miles Prince,[3] Mark H. Kirschbaum,[4] Jasmine Zain,[4] Steven L. Allen,[5] Elaine S. Jaffe,[2] Alexander Ling,[6] Maria Turner,[2] Cody J. Peer,[2] William D. Figg,[2] Seth M. Steinberg,[2] Sonali Smith,[7] David Joske,[8] Ian Lewis,[9] Laura Hutchins,[10] Michael Craig,[11] A. Tito Fojo,[2] John J. Wright,[1] and Susan E. Bates[2]

blood

2011 117: 5827-5834
Prepublished online February 25, 2011;
doi:10.1182/blood-2010-10-312603

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JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Results From a Pivotal, Open-Label, Phase II Study of Romidepsin in Relapsed or Refractory Peripheral T-Cell Lymphoma After Prior Systemic Therapy

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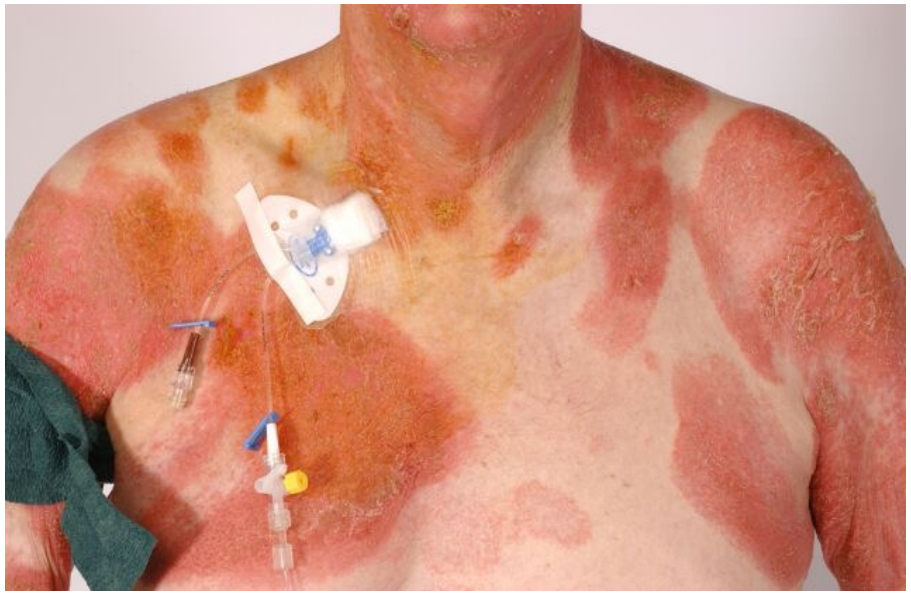
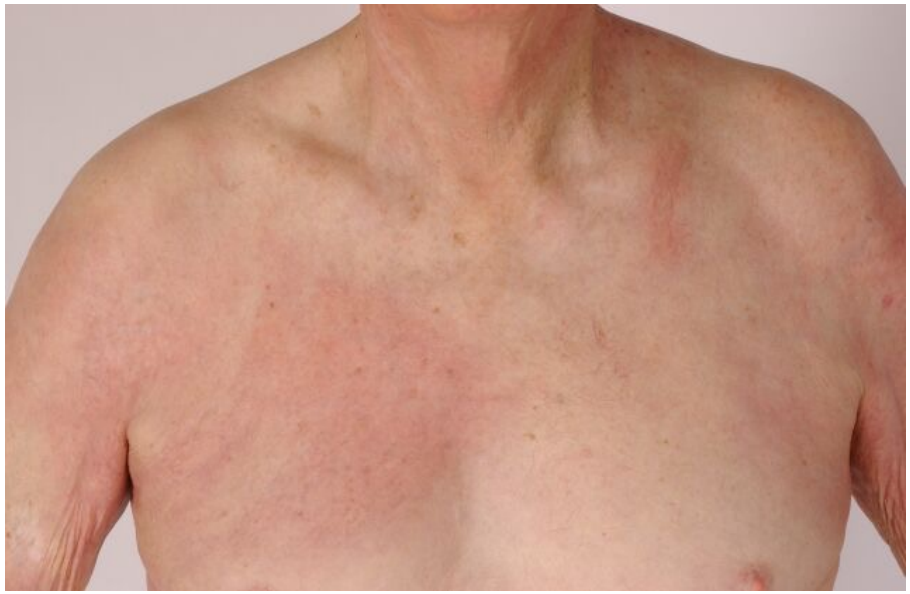
RR = 38%

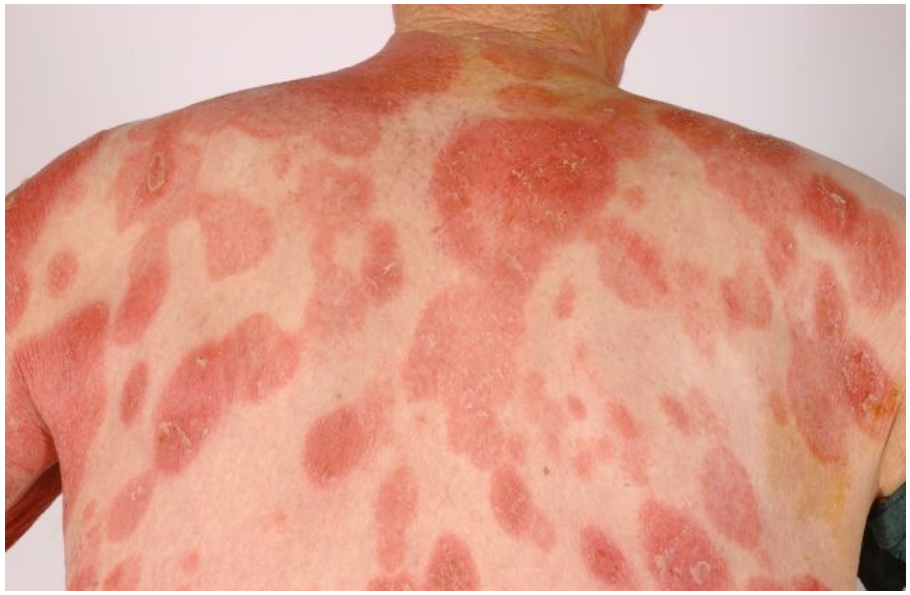
Romidepsin in CTCL

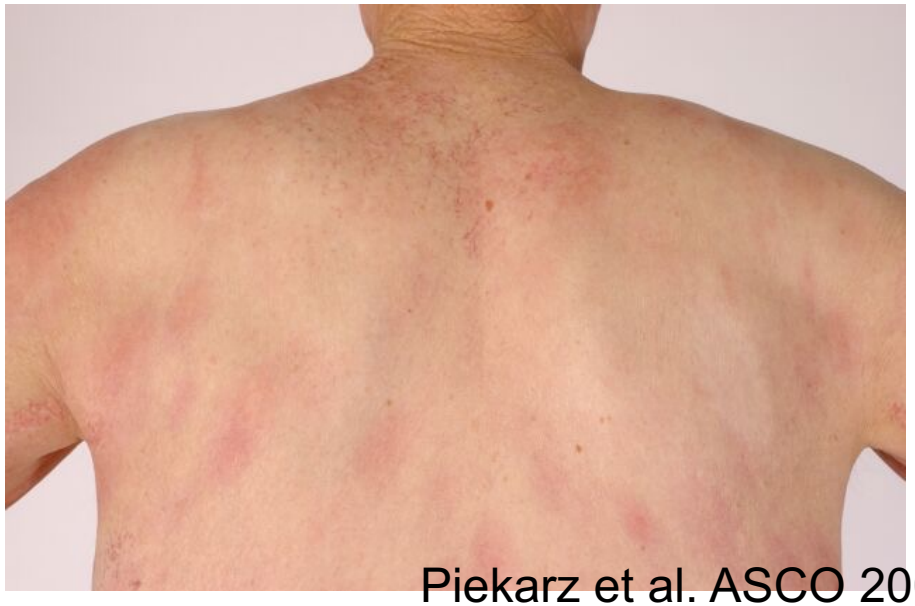
Jan 04



Feb 04

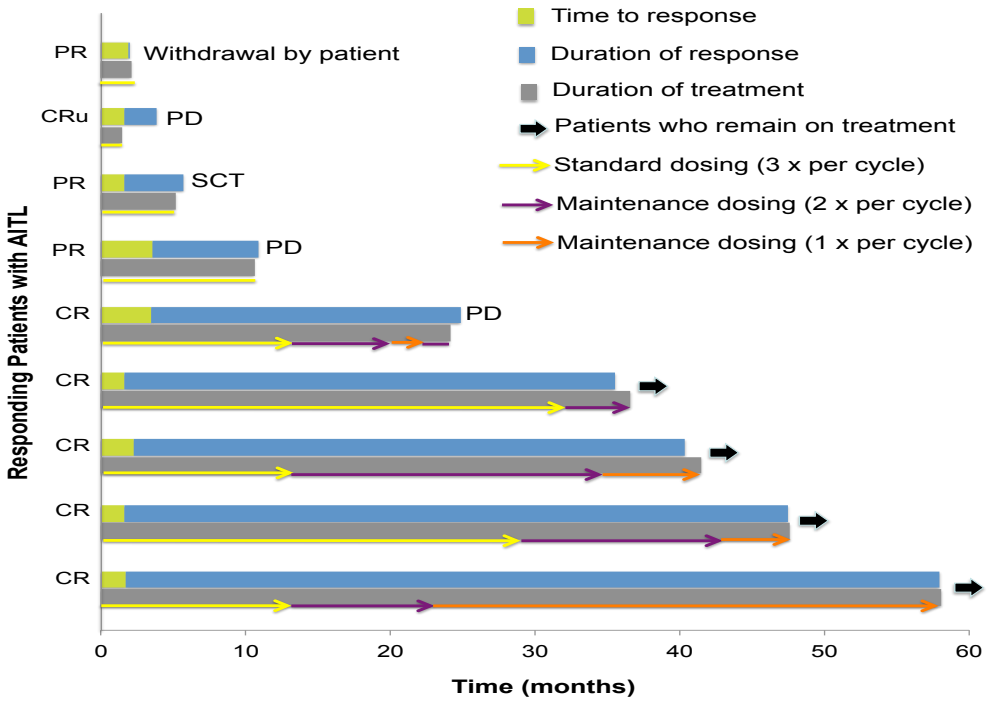






Piekarz et al. ASCO 2007

Romidepsin in AITL



- **The ORR 33% (9/27)**
- **6 of 9 responders achieved CR/CRu**
- **5 of 9 long-term response (≥ 12 m)**
- **All 5 received maintenance romidepsin.**

Pro B. J Hematol&Oncol 2014

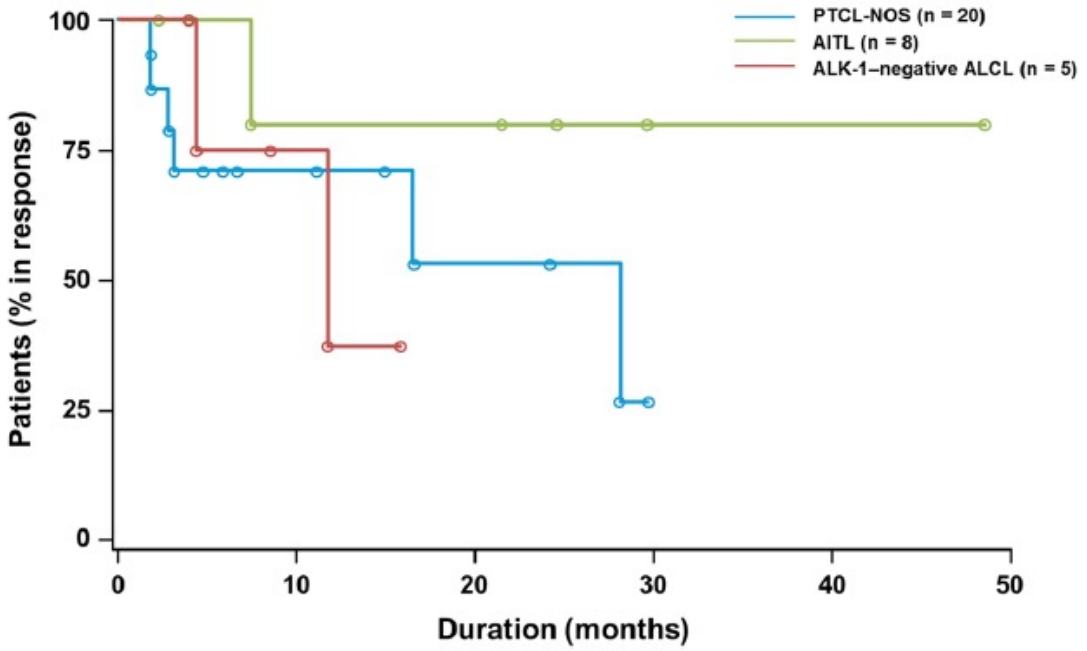
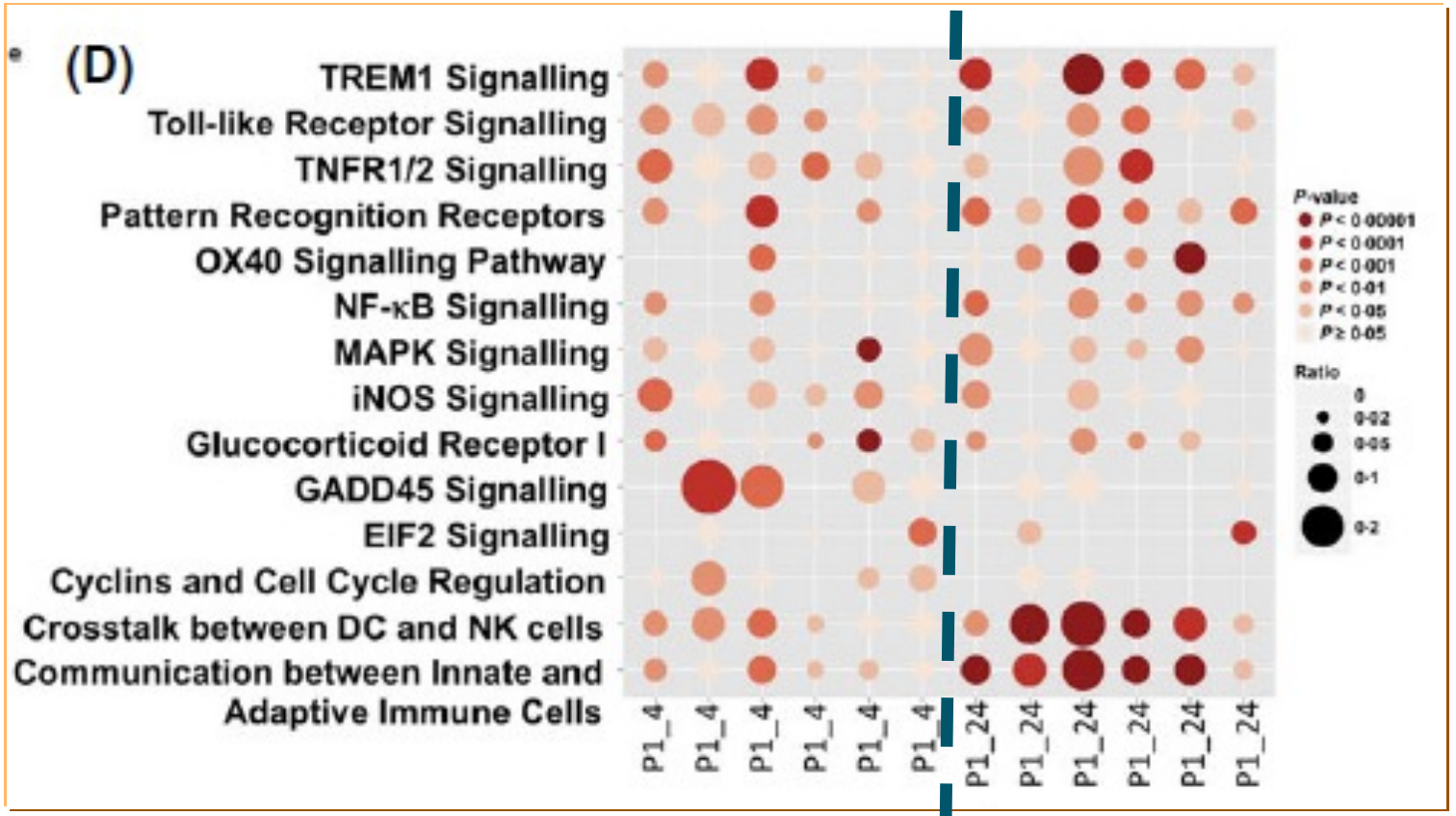


Figure 1 Durations of response for the 3 most common subtypes of PTCL in patients who achieved a response (CR or PR). ◦ Indicates a censored patient.

Biomarkers?

Romidepsin in peripheral and cutaneous T-cell lymphoma: mechanistic implications from clinical and correlative data



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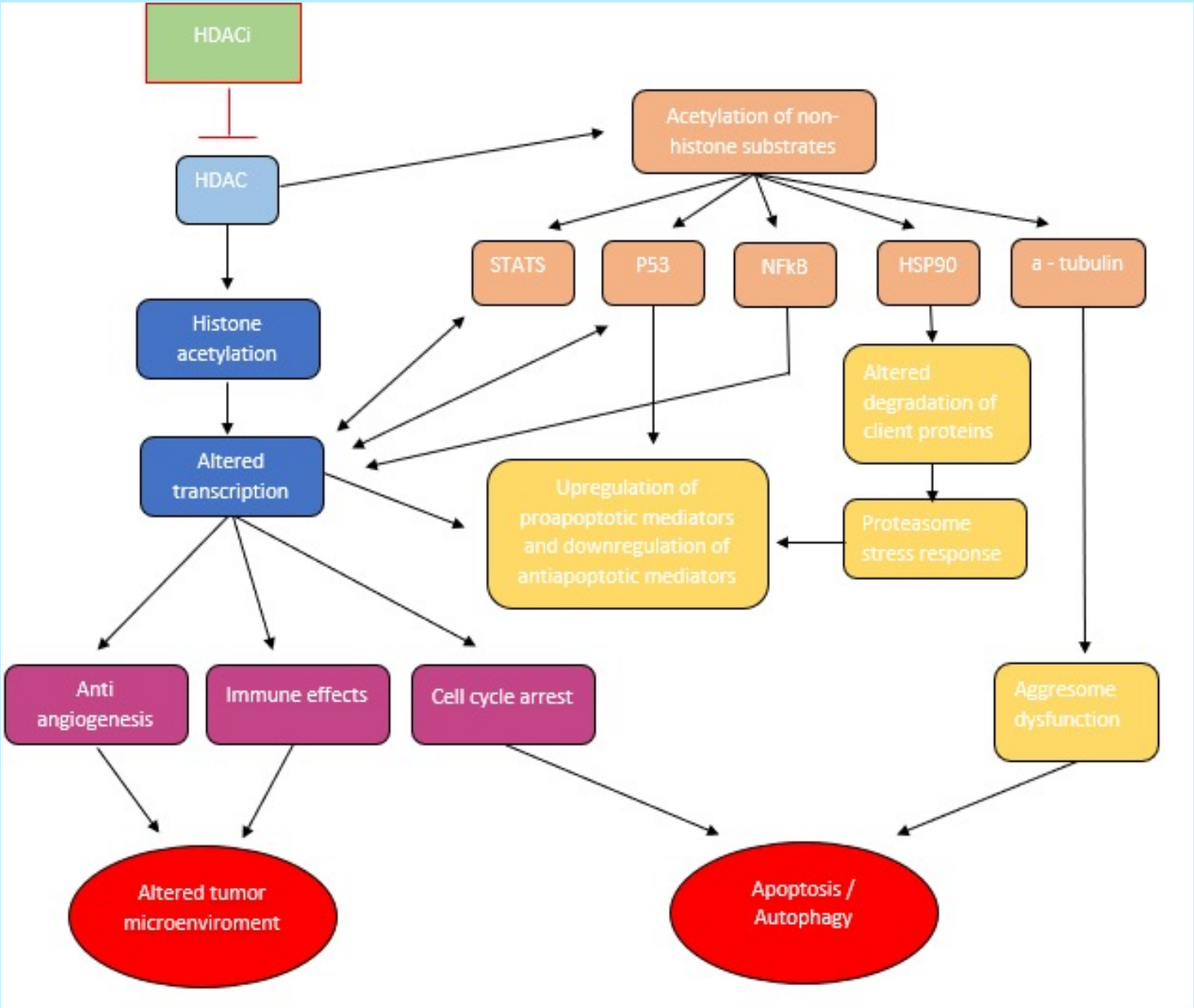
HDACi acetylate more that just histones



What we need to consider when considering targeting molecular (genomic/epigenetic) pathways in PTCL

- What are the key pathogenic pathways in PTCL?
- Which are the most important pathways in the various ‘cells of origin’ in the different PTCL entities
  e.g. AITL vs. ALCL etc.
- Are epigenetic-targeting drugs always targeting the epigenome?

Thankyou