UNMET CHALLENGES IN HIGH RISK HEMATOLOGICAL MALIGNANCIES:
FROM BENCHSIDE TO CLINICAL PRACTICE – 2nd EDITION

GENETICS AND BIOLOGY OF CLASSSIC HODGKIN LYMPHOMA (cHL): CLINICAL IMPLICATIONS

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2nd edition

Unmet challenges in high risk hematological malignancies: from benchside to clinical practice

Turin, September 13-14, 2021

Starhotels Majestic

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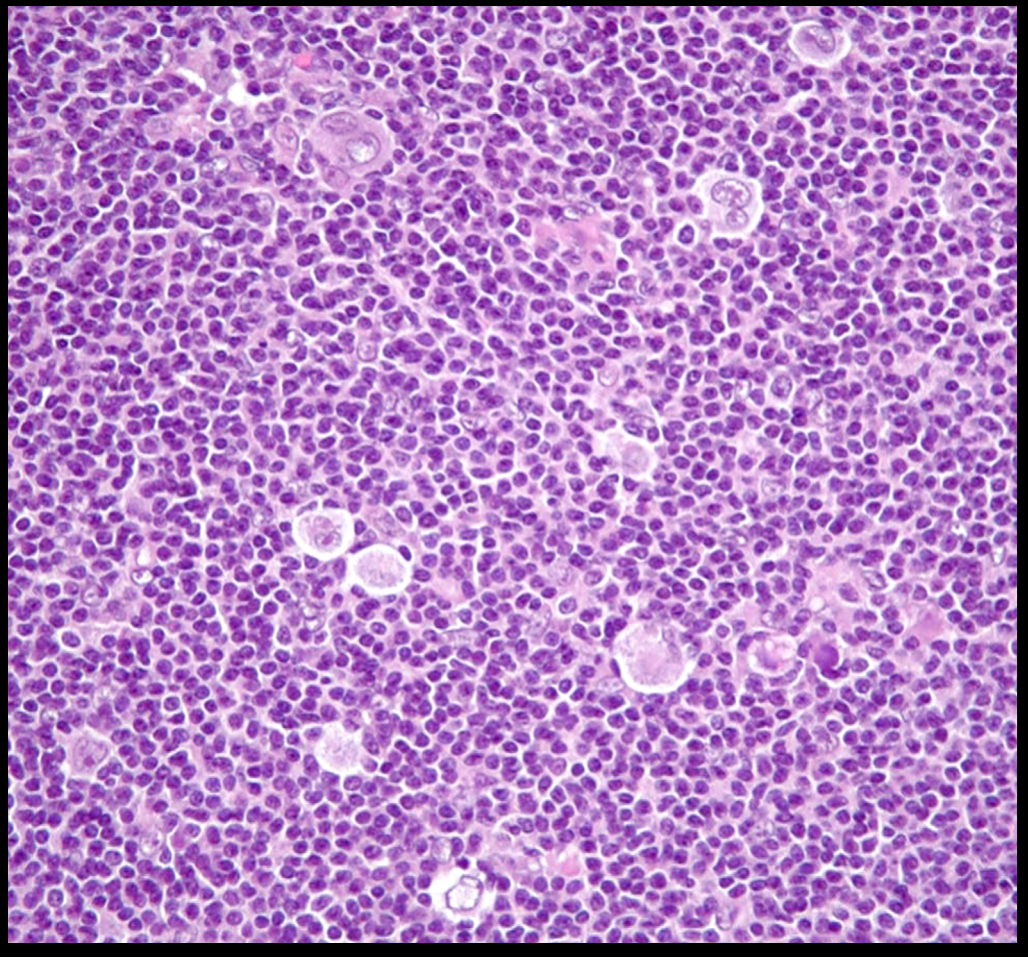
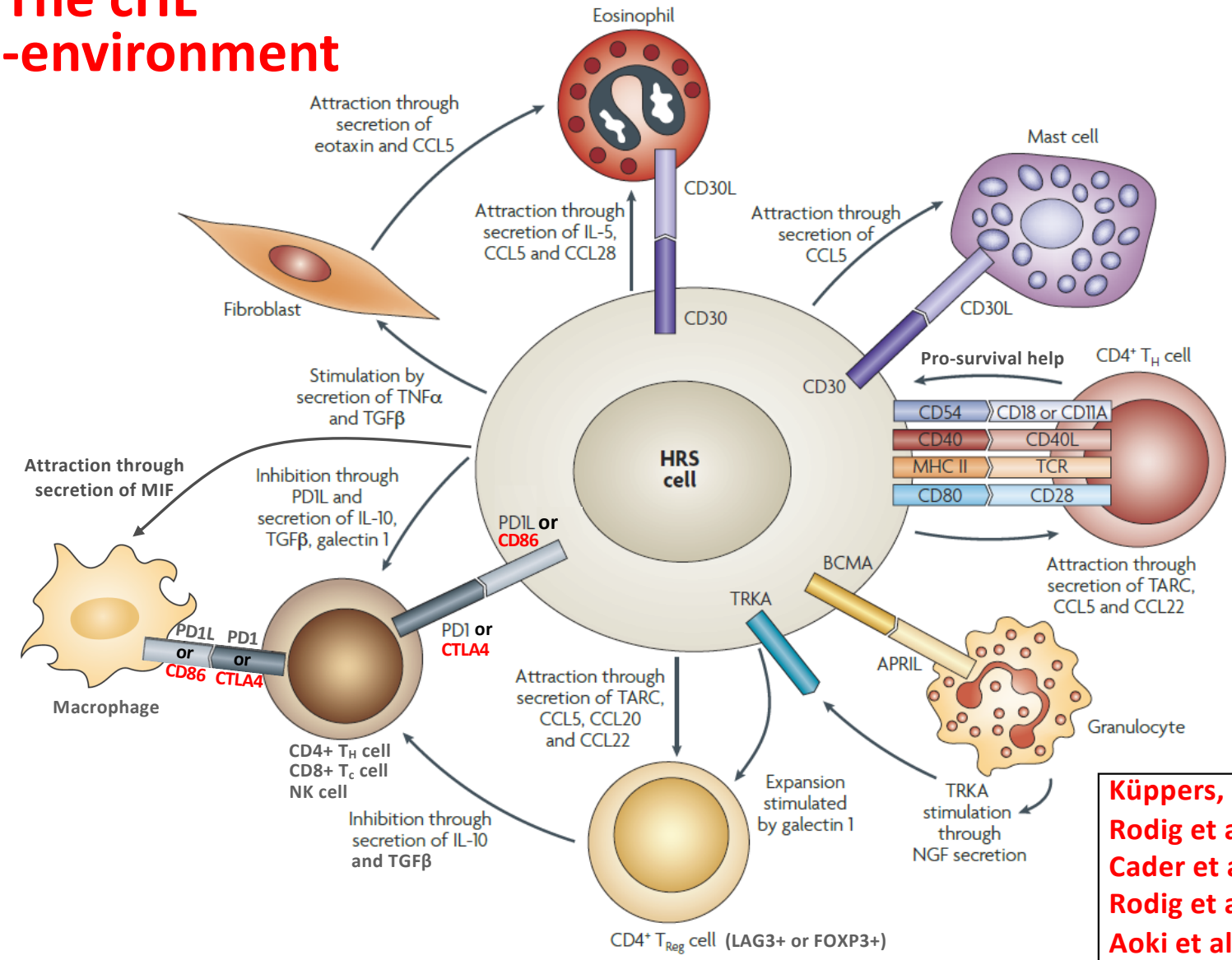
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Innate Pharma			X				

Hodgkin/Reed-Sternberg (HRS) cells

HRS cells are rare (usually <5% of lymph node cellularity) and dispersed in a prominent but immune-suppressive reactive background largely of hematopoietic origin



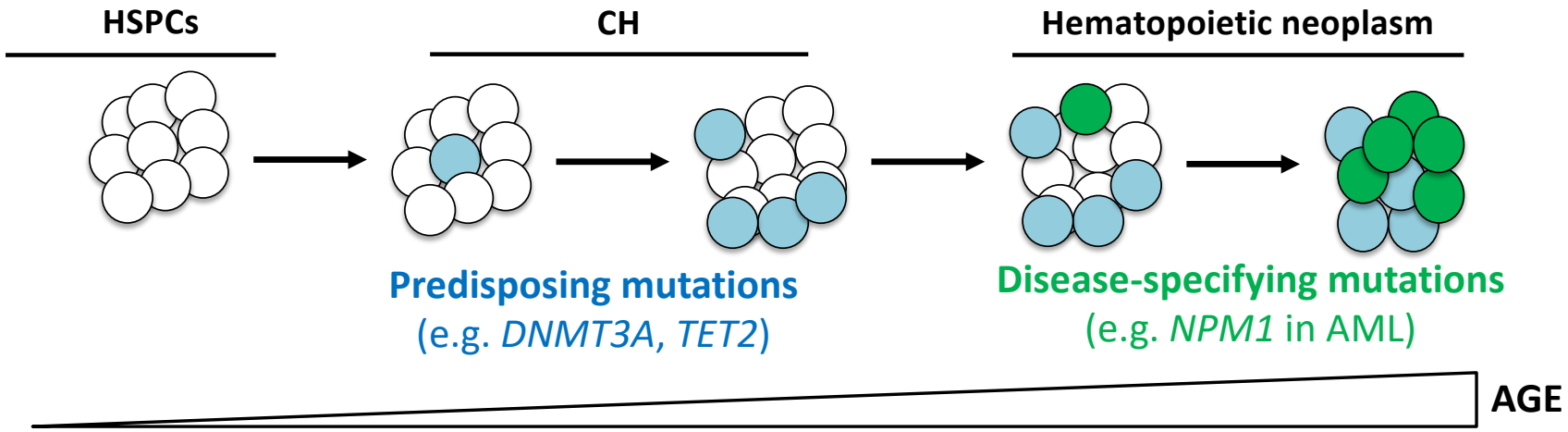
The cHL micro-environment



Küppers, Nat Rev Cancer 2009 [modif.]
Rodig et al, Blood 2017
Cader et al, Blood 2018
Rodig et al, Blood 2019
Aoki et al, Cancer Discov 20200

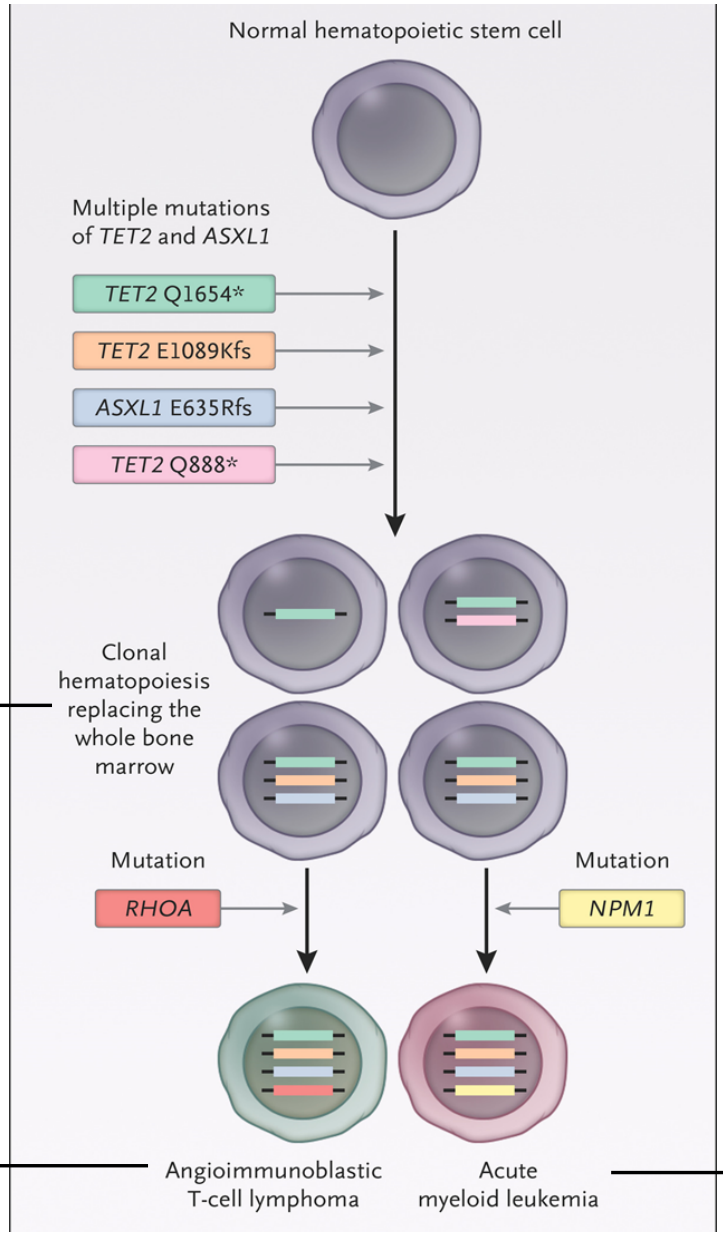
CLONAL HEMATOPOIESIS (CH)

- CH is promoted by the **age-related stochastic occurrence** in hematopoietic stem/progenitor cells (**HSPCs**) of gene **mutations conferring a fitness advantage**
- CH is **frequent in the elderly**; it **predisposes** to atheroscelosis and **to hematopoietic neoplasms mostly of myeloid or T-cell origin**
- CH with indeterminate potential (CHIP): CH with a mutant allele frequency ≥ 2% in the blood
  The **risk** of **tumor** development **depends on** the number and type of **mutated gene(s)** and on **clone size**



Quivoron et al., Cancer Cell 2011
Jaiswal et al, NEJM 2014 & 2017
Genovese et al, NEJM 2014
Tiacci et al, NEJM 2018

Tiacci et al, NEJM 2018



Massive CH (~90% of bone marrow cells), but normal blood counts and no dysplasia

45-year old patient
CR after CHOEP + autotransplant

1 year after lymphoma diagnosis

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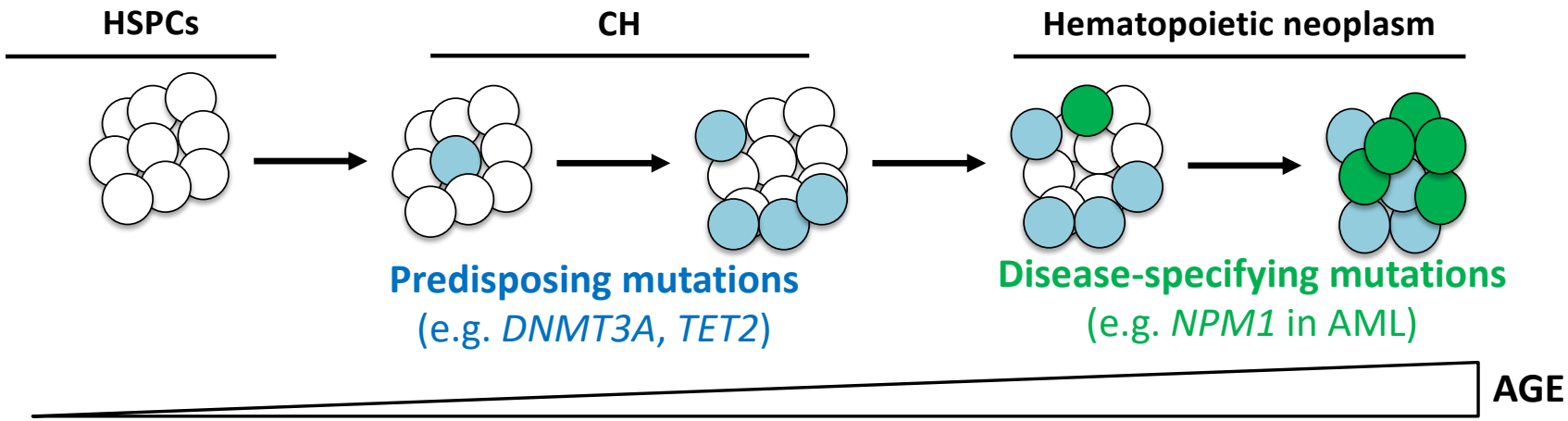
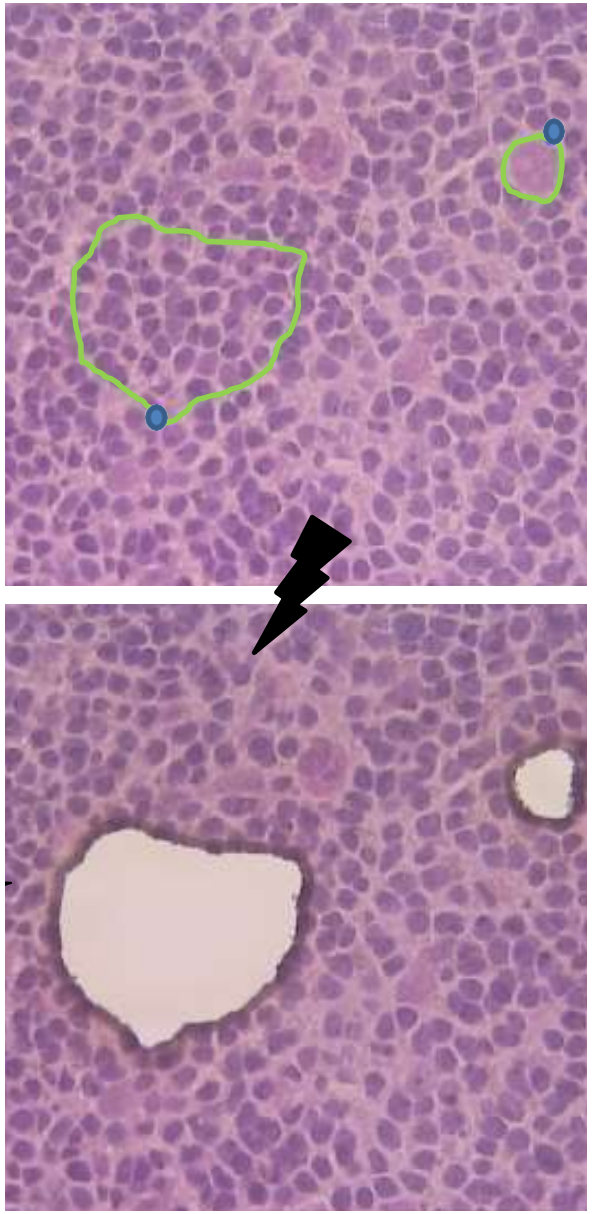


CH found in HSPCs from 9/64 (14%) relapsed cHL cases undergoing autotransplant

But: no data on the presence of **CH in the microenvironmental and/or neoplastic tissue components of cHL**, a germinal center B-cell lymphoma most frequent in the young and with the most **abundant microenvironment** (largely **hematopoietic**)

Quivoron et al., Cancer Cell 2011
Jaiswal et al, NEJM 2014 & 2017
Genovese et al, NEJM 2014
Tiacci et al, NEJM 2018
Husby et al, Leukemia 2020



Clonal hematopoiesis (CH) in cHL - AIMS AND METHODS

- Characterization of **CH frequency and tissue distribution** in **40 cHL cases,** largely studied at disease onset (n=33; at relapse, n=7)

- **Laser microdissection**, from frozen lymph node sections stained with hematoxylin and eosin, of 1200-1800 **HRS cells** and as may **reactive cells** (mostly of lymphoid morphology), followed by whole-genome amplification in duplicate

- **Whole-exome sequencing** (n=34 cases*; mean coverage ~150X) **and/or targeted sequencing** of **35 genes driving CH°** (n=9 cases; mean coverage ~3500X) on **HRS and reactive tissue cells** (n=40 cases), as well as matched **blood leukocytes** (n=27 cases)

- **CH** defined as the presence of a **mutation in a CH driver gene** at a variant allele frequency (**VAF**) **≥ 2%** in non-neoplastic blood and/or tissue cells

°As defined by Tuval & Shlush, Haematologica 2019

*From Tiacci et al., Blood 2018

Clonal hematopoiesis (CH) in cHL - RESULTS

- ***Case cohort:***
  40 patients (pts.)
  Median age: 35 years (range 15-83)
  5 pts. had >70 years
  13 pts had >55 years

- ***CH in 5/40 pts. (12.5%)***: more often in pts. >70 years (3/5, 60%) than in pts. <70 years (2/35, 6%; p<0.05)

- ***Extensive tissue CH in 3/5 pts.:***
  - aged 30-45-73 years
  - CH in up to 92%-60%-33% of reactive cells

Venanzi, …, Tiacci
Blood Cancer Discov 2021

CH present	Years of age
NO (*n* = 35)	Median 35 Range 15-75
YES (*n* = 5)	30
	83
	81
	73
	45

CH, clonal hematopoiesis; VAF, variant allele frequency; NA, not available; ND, Not detected

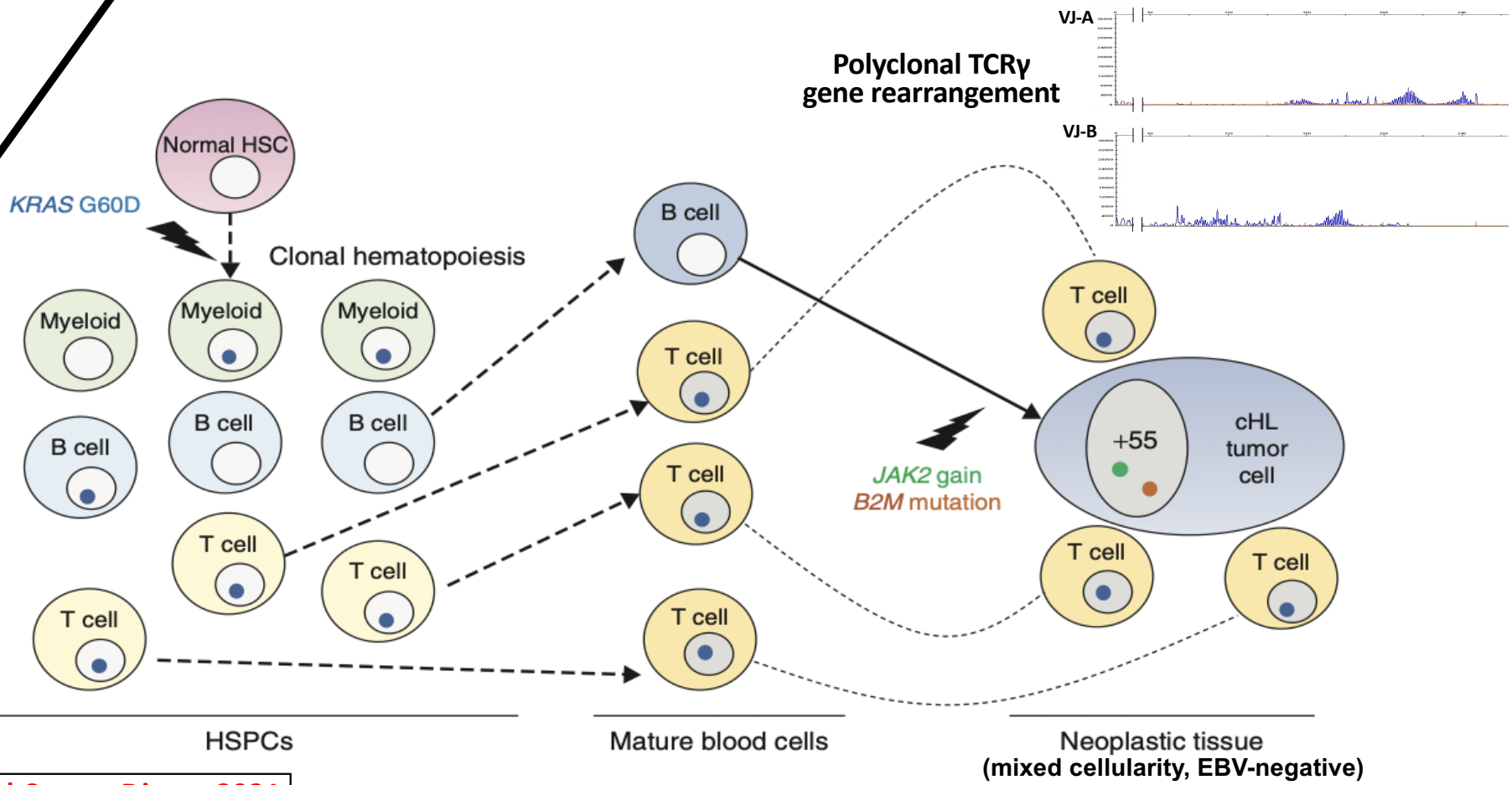
Pt.#	Time of sampling	Progressed after first-line chemotherapy	Gene mutation	VAF in whole blood	VAF in microdissected		VAF in whole tissue section
					Reactive cells	HRS cells	
Case 2	2nd relapse	YES	*KRAS* G60D	NA	45.9%	ND	22.9%
Case 3	Onset	Not evaluable	*CBL* G375S	NA	2.5%	ND	NA
Case 4	Onset	NO	*TET2* N1487Ifs84	3.2%	ND	ND	NA
Case 5	Onset	YES	*DNMT3A* R882H	NA	NA	NA	32.4%
			TET2 Q1274[a]				22.3%
	1st relapse		*DNMT3A* R882H	NA	30%	43%	37.9%
			TET2 Q1274[a]		8.4%	31.1%	26.9%
Case 1	Onset	YES	*DNMT3A* R882H	47%	16.4%	ND	12.2%
			NPM1 W288CfsTer12	ND			
			PTPN11 E76K	ND			
			FLT3 ITD	NA			
			FLT3 ITD	NA			
			STAT6 N417Y	NA	ND	36.9%	NA
			STAT6 D419H			35.7%	
			SOCS1 P83Afs*25			98.6%	

CASE 2 – Age 30
(EBV-, Mixed Cellularity)

cHL case cohort (n=40)	
CH PRESENT	AGE (years)
YES (n=5)	45
	30
	83
	81
	73
NO (n=35)	Median 35 Range 15-75

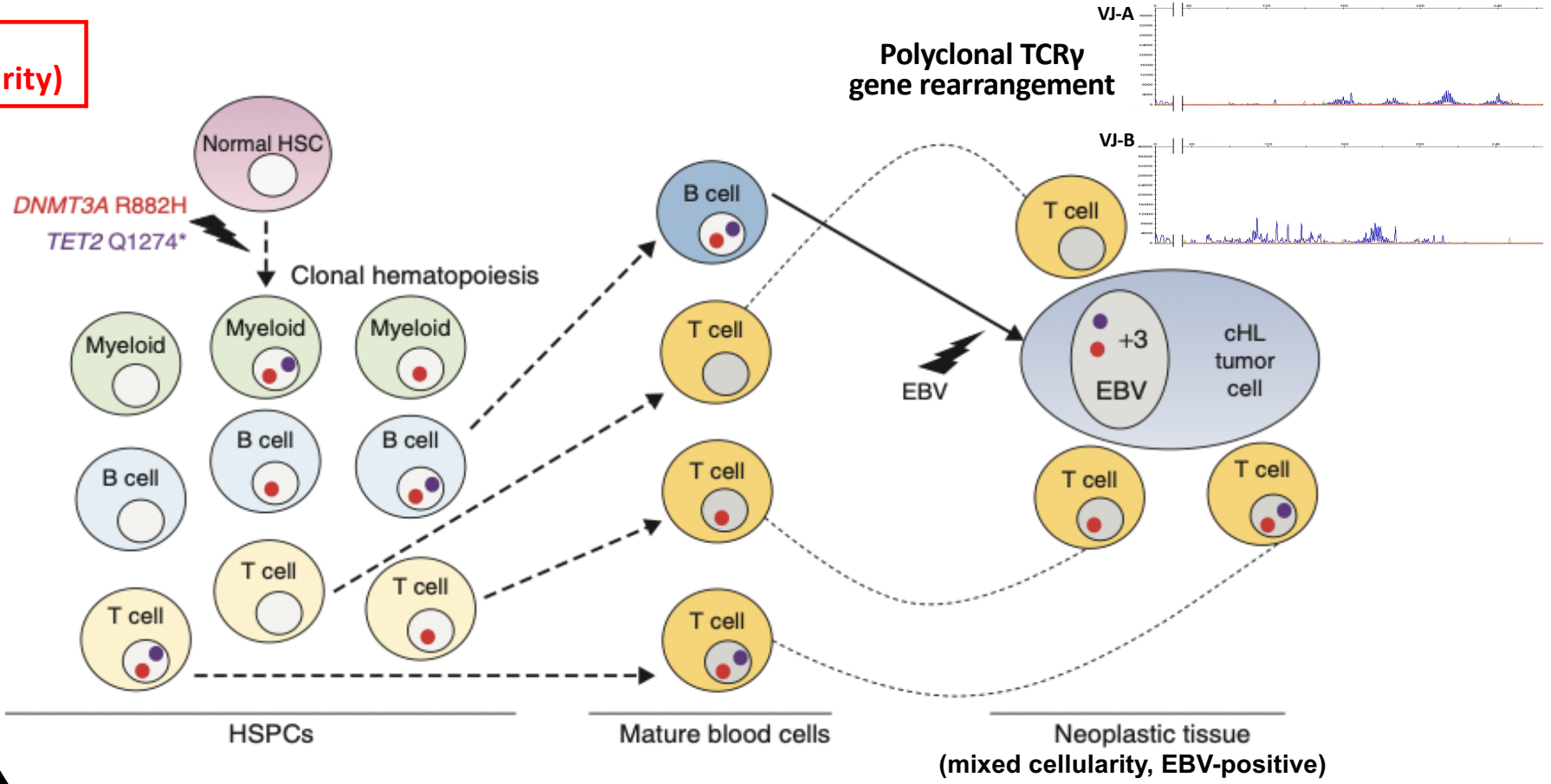
Pt. #	Time of sampling	Progressed after first-line chemotherapy	Gene mutation	VAF in microdissected		VAF in whole tissue section
				Reactive cells	HRS cells	
Case 2	2nd relapse	**YES**	***KRAS* G60D**	**45.9%**	Not detected	22.9%

VAF, variant allele frequency



Venanzi, …, Tiacci Blood Cancer Discov 2021

CASE 5 – Age 73
(EBV+, Mixed Cellularity)



cHL case cohort (n=40)	
CH PRESENT	AGE (years)
YES (n=5)	45
	30
	83
	81
	73
NO (n=35)	Median 35 Range 15-75

VAF, variant allele frequency

Pt. #	Time of sampling	Progressed after first-line chemotherapy	Gene mutation	VAF in microdissected		VAF in whole tissue section
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Case 5	I st relapse	YES	*DNMT3A* R882H	30%	43%	37.9%
			TET2 Q1274*	8.4%	31.1%	26.9%

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  CH in 92%-60%-33% reactive cells

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	73	Case 5	Onset	YES	*DNMT3A* R882H	NA	NA	NA	32.4%
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					STAT6 N417Y	NA	ND	36.9%	NA
					STAT6 D419H			35.7%	
					SOCS1 P83Afs*25			98.6%	

Venanzi, …, Tiacci
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CASE 1 – Age 45
(EBV-, Nodular Sclerosis)

cHL case cohort (n=40)	
CH PRESENT	AGE (years)
YES (n=5)	45
	30
	83
	81
	73
NO (n=35)	Median 35 Range 15-75

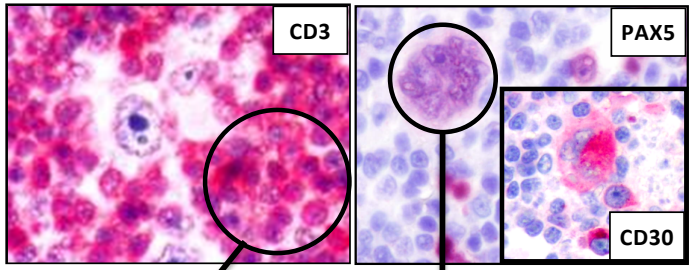
Whole blood

DNMT3A-R882H 47%

T cells: **DNMT3A-R882H 7%**

B cells: **DNMT3A-R882H 48%**

Classical Hodgkin Lymphoma (lymph node biopsy)

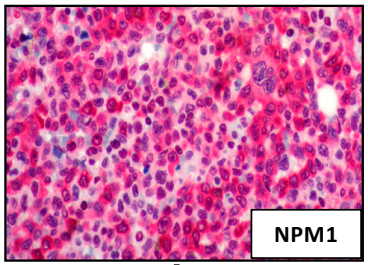


DNMT3A-R882H 16%
TCRγ genes: polyclonal

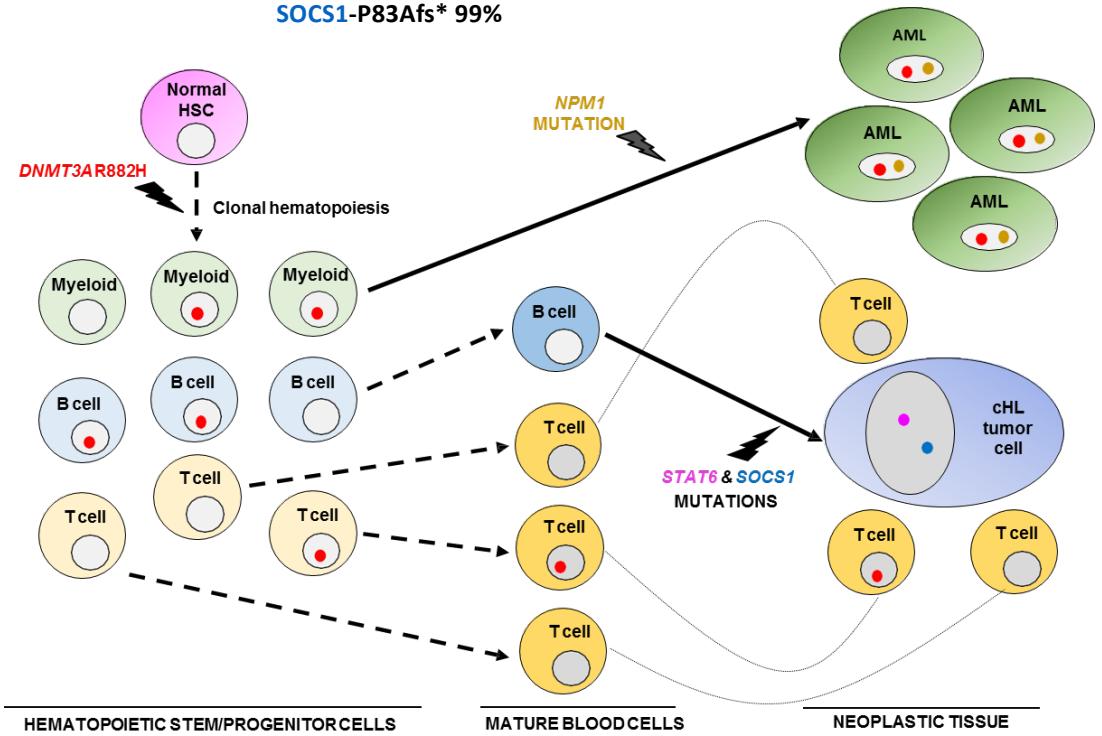
DNMT3A wild-type
STAT6-N417Y 37%
SOCS1-P83Afs* 99%

6 years after therapy for cHL
ABVD, IGEV, ABMT, RT

[De novo ?] Acute Myeloid Leukemia (bone marrow biopsy)



DNMT3A-R882H 45%, NPM1-W288Cfs* 41%;
TP53 and PPM1D wild-type, normal karyotype



Venanzi, …, Tiacci
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Clonal hematopoiesis (CH) in cHL: clinico-pathological correlations

N=40 patients		CLONAL HEMATOPOIESIS IN cHL TISSUE		P-value[b]
		Extensive[a] (n=3)	Absent/non-extensive (n=37)	
AGE	>60 years	1 (33%)	9 (24%)	1
	<60 years	2 (67%)	28 (76%)	
EBV STATUS	EBV+	1 (33%)	8 (22%)	0.55
	EBV-	2 (67%)	29 (78%)	
HISTOTYPE	Nodular sclerosis	1 (33%)	21 (57%)	0.58[c]
	Mixed cellularity	2 (67%)	12 (32%)	
	Other	0 (0%)	4 (11%)	
CLINICAL STAGE	Early (≤ IIA)	1 (33%)	13 (37%)[d]	1
	Advanced (≥ IIB)	2 (67%)	22 (63%)[d]	
OUTCOME OF FIRST-LINE THERAPY[e]	No progression	0 (0%)	24 (69%)[f]	0.043
	Progression	3 (100%)	11 (31%)[f]	
	Follow-up in months[g]	0-6-35	64 (median)[g]	0.034
			0-149 (range)[g]	

Extensive clonal hematopoiesis (VAF >10%) in the cHL tissue correlated with poorer prognosis

[a] Extensive: variant allele frequency (VAF) ≥10%.
[b] By Fisher exact test, except for comparison of follow-up where T-test was used.
[c] Nodular sclerosis vs all other subtypes.
[d] Clinical stage at diagnosis was not available for two patients (UPN26; UPN40).
[e] ABVD in all cases (except COPP/ABV in pediatric patient UPN27 without clonal hematopoiesis not progressing after first-line therapy; and COPP without vincristine in elderly patient UPN41 (Case 3), whose outcome was not evaluable), with omission of bleomycin in 3/35 patients (UPN13, UPN25/Case 4, and UPN30, all without extensive clonal hematopoiesis in the cHL tissue and all not progressing after first-line therapy).
[f] Outcome was not available for one patient (UPN40) and was not evaluable in another patient (UPN41-Case 3) who died early due to acute chemotherapy toxicity.
[g] Follow-up was not available for one patient (UPN40) and not evaluable in another (UPN41-Case 3).

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Clonal hematopoiesis (CH) in cHL - CONCLUSIONS

- CH can be observed in cHL **(5/40 cases; 12%)** and displays **a diverse propagation pattern through the HRS-cell and microenvironmental tissue components**, with representation in both (n=1), or only in the microenvironment (n=3) or in none of them (n=1).

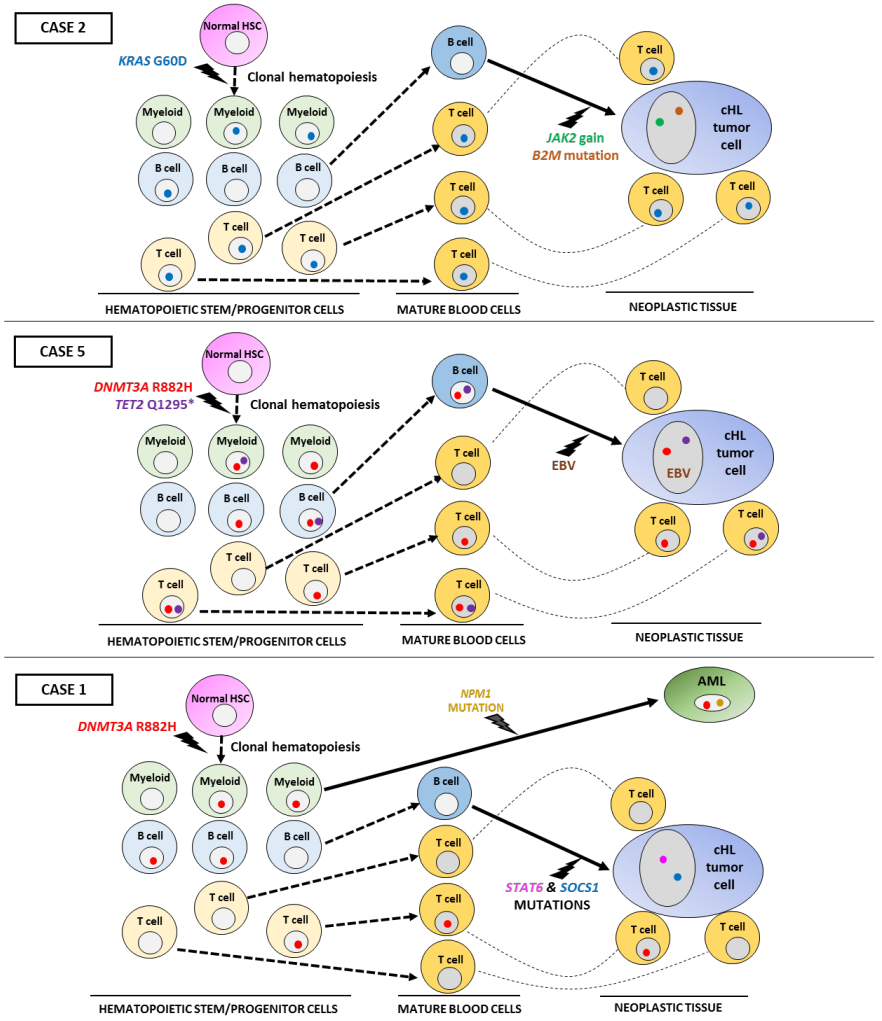
- **Extensive tissue CH** was present in 3/5 cases (involving up to 32%-94% of reactive cells), which **all progressed after first-line therapy** (vs 11/35 pts with absent/non-extensive CH; p-value 0.043).

- Multiple lymphoid and myeloid neoplasms in a patient with CH (even when massive) do not necessarily derive all from CH.

- CH might contribute to the **pathogenesis and prognosis of cHL**

	EXTENSIVE TISSUE CH (VAF >10%)	
PATIENTS (n=40)	**ABSENT**	**PRESENT**
Number of cases	37 (92.5%)	3 (7.5%)
Years of age	Median: 35 Range: 15-83	30 73 45
Progressed after 1st-line therapy	11/40 (31%)*	3/3 (100%)*

* p-value 0.043



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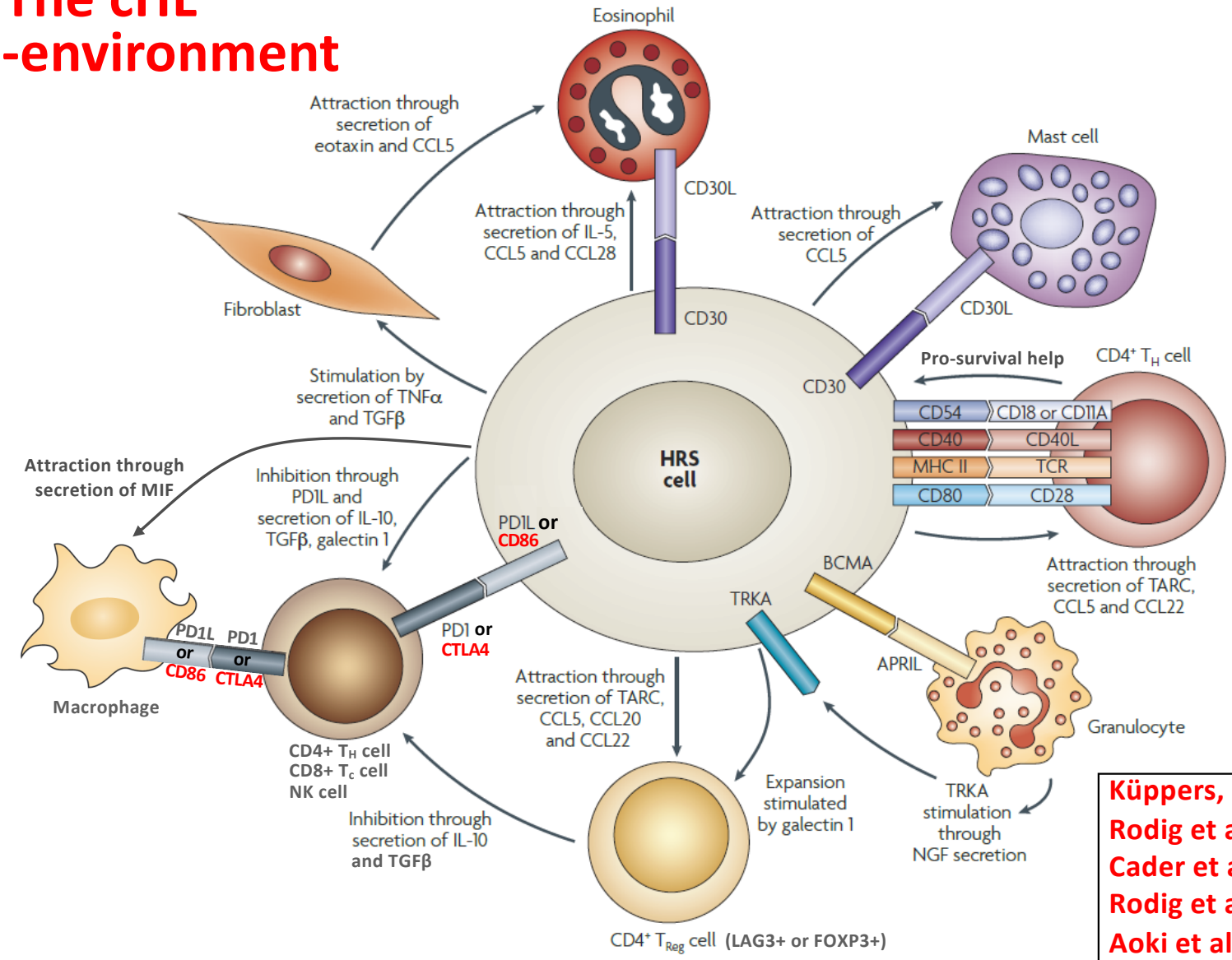

Küppers, Nat Rev Cancer 2009 [modif.]
Rodig et al, Blood 2017
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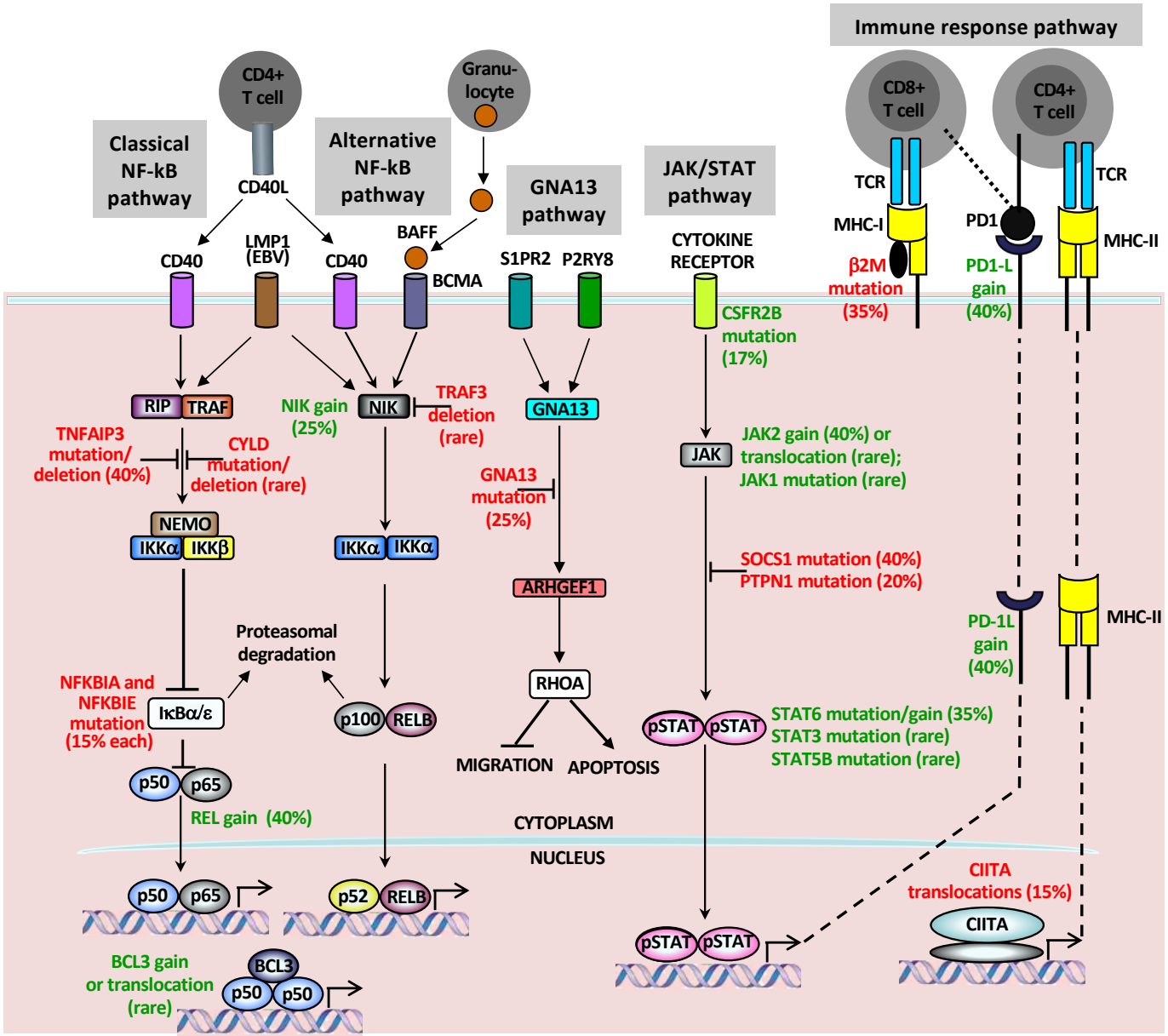
MAIN SOMATIC GENETIC LESIONS IN cHL (activating; inactivating)



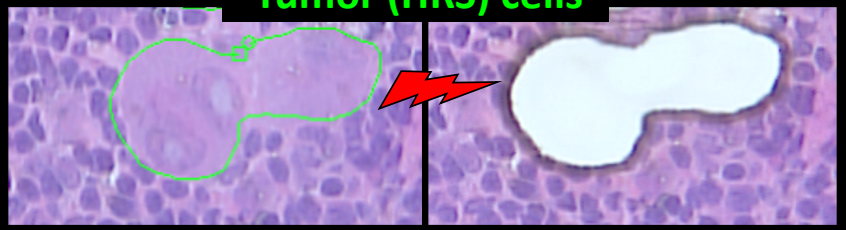
OTHER GENETIC LESIONS:

- TP53 mutation (10%)
- ITPKB mutation (15%)
- TNFRSF14 deletion (20%)
- ARID1A mutation (25%)
- XPO1 mutation/gain (20%)

Schiavoni and Tiacci, EHA 2018 Educational Book (updated)

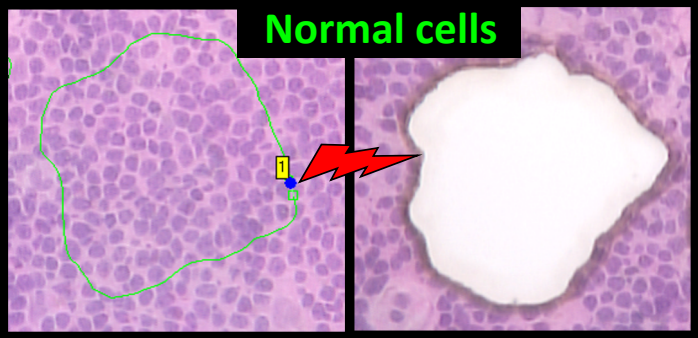
Laser microdissection from H&E-stained dried frozen sections (n=34 pts.)

Tumor (HRS) cells



1200-1800 cells per case

Normal cells



1200-1800 cells per case

WHOLE-GENOME AMPLIFICATION (WGA) IN DUPLICATE

WGA-1 WGA-2 WGA-1 WGA-2

WHOLE-EXOME SEQUENCING (WES)

WES-1 WES-2 WES-1 WES-2

Median 99X ····· COVERAGE DEPTH ····· Median 113X

Median 88% ····· BASES WITH ≥10X COVERAGE ····· Median 93%

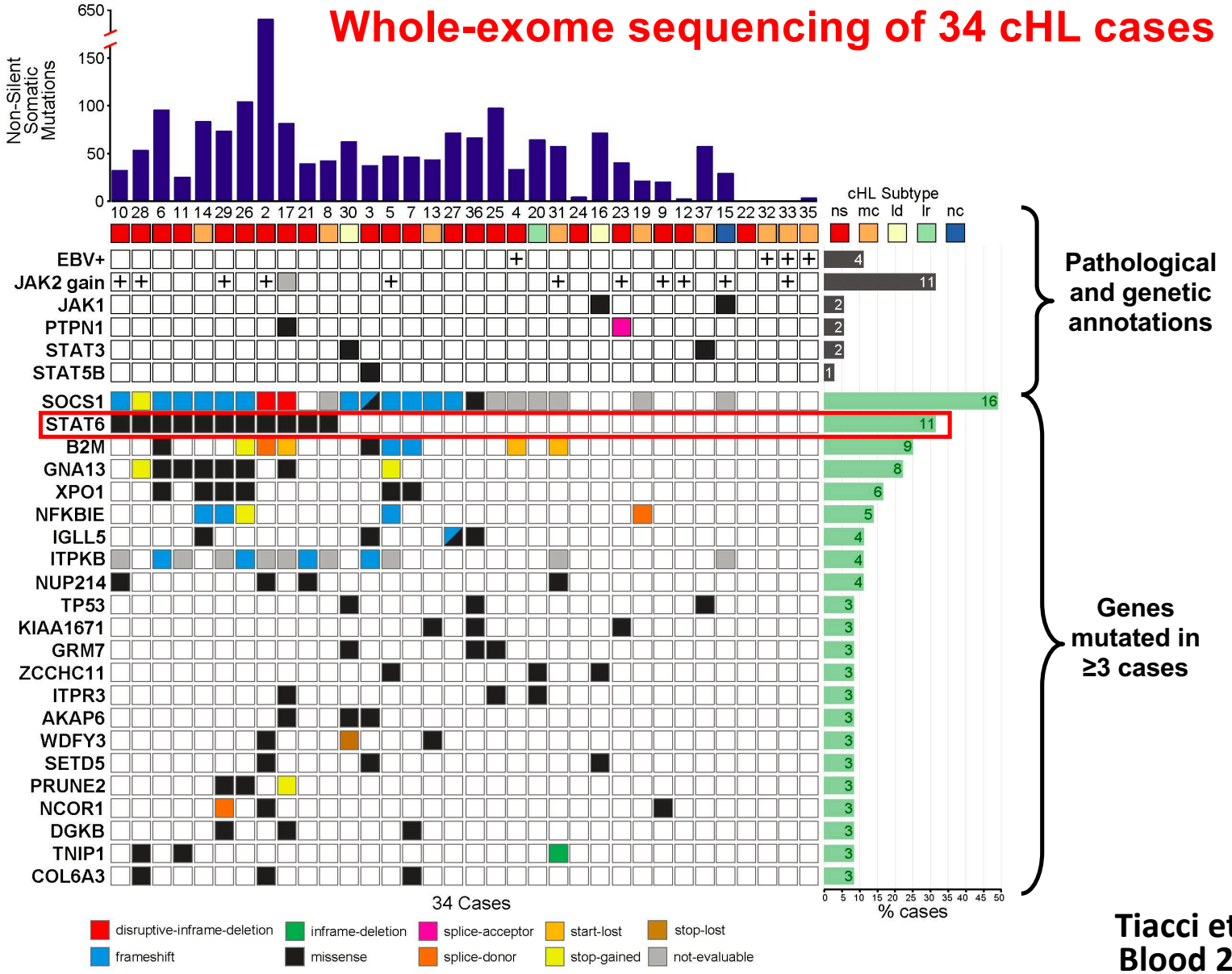
MUTATIONS CALLED BY SAVI BIOINFORMATIC ALGORITHM IF:

- somatic in all 4 comparisons of each tumor vs. each normal duplicate
- allele frequency ≥ 20%; protein-changing

92% validation rate (114/124 mutations across 26 patients) upon targeted deeper sequencing (~3000X) of tumor vs. normal WGA-DNA

Tiacci et al., Blood 2018

Whole-exome sequencing of 34 cHL cases

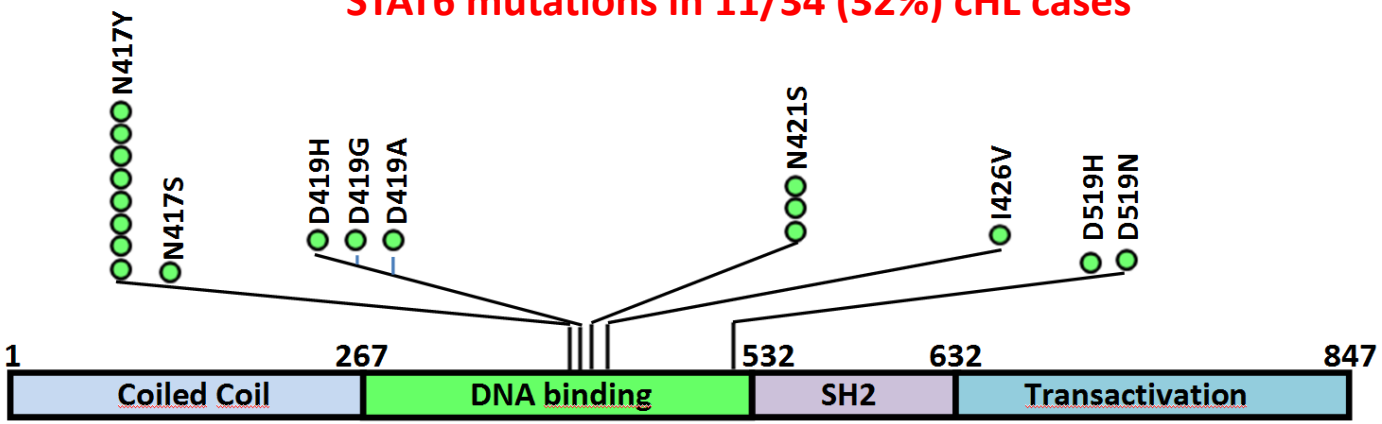


Pathological and genetic annotations

Genes mutated in ≥3 cases

Tiacci et al., Blood 2018

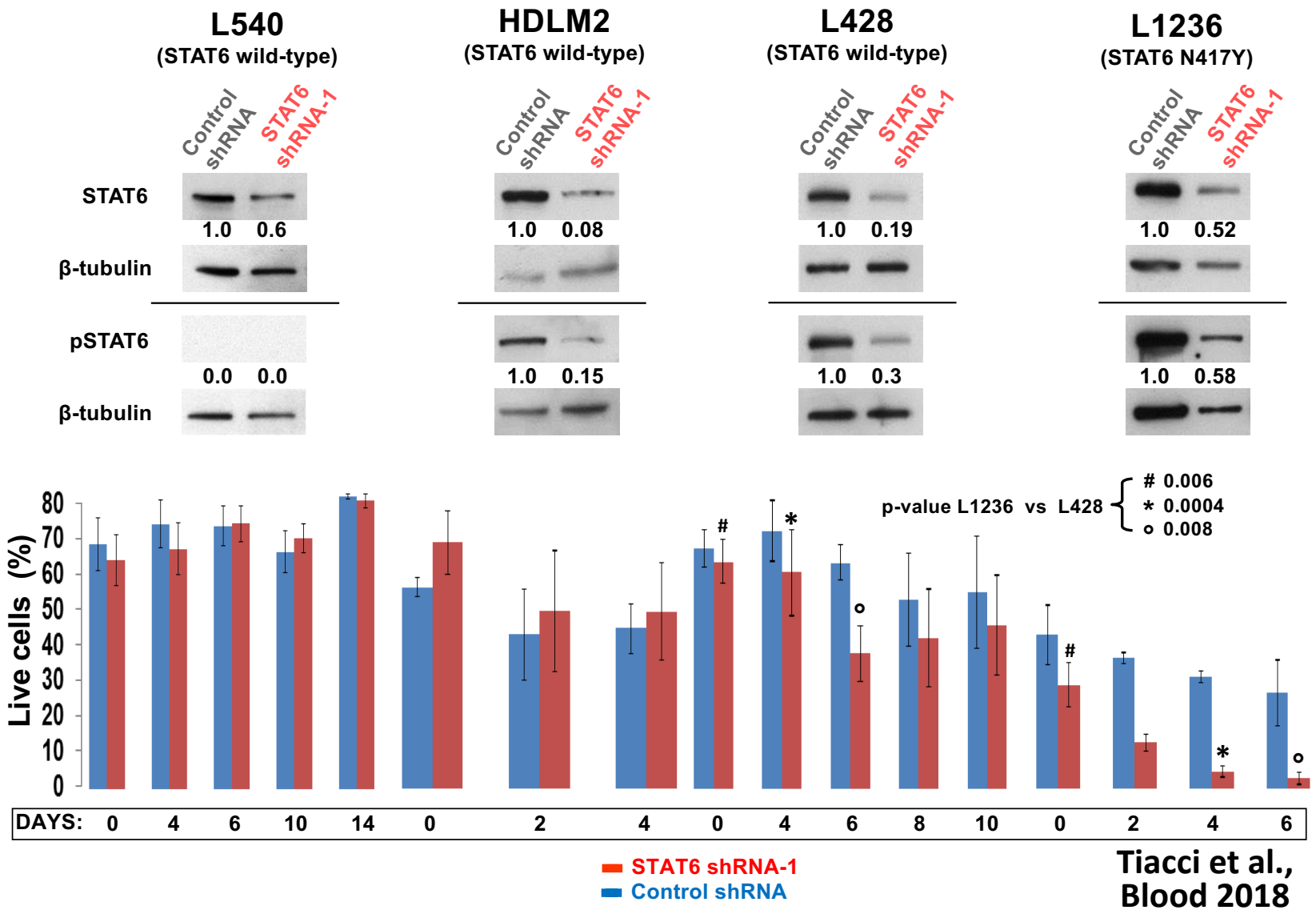
STAT6 mutations in 11/34 (32%) cHL cases



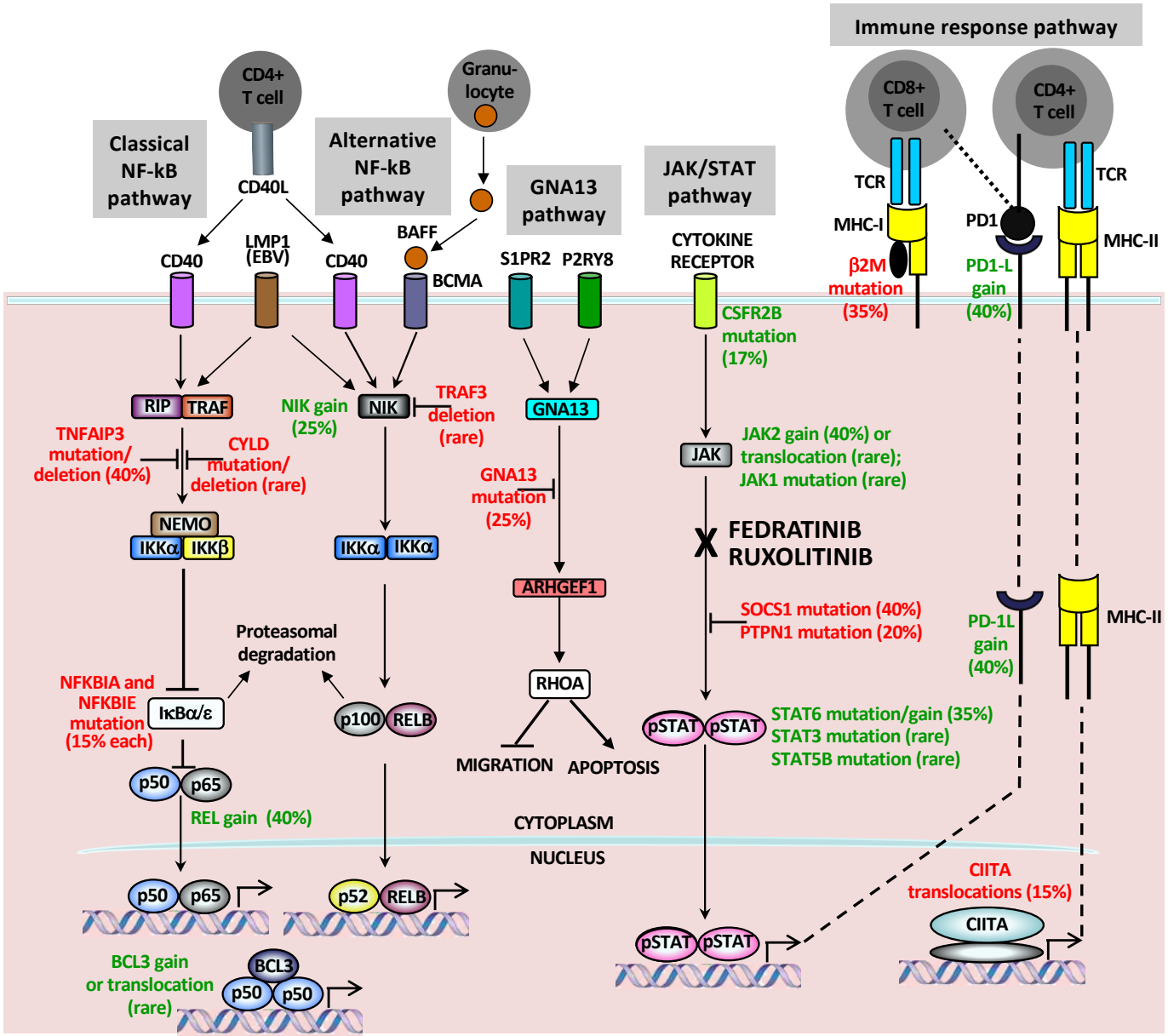
- All are missense mutations in the DNA binding domain, mostly (10/11 pts.) heterozygous

Tiacci et al, Blood 2018

DOWNREGULATION OF STAT6 TRIGGERS APOPTOSIS OF STAT6-MUTATED cHL CELLS



Tiacci et al., Blood 2018



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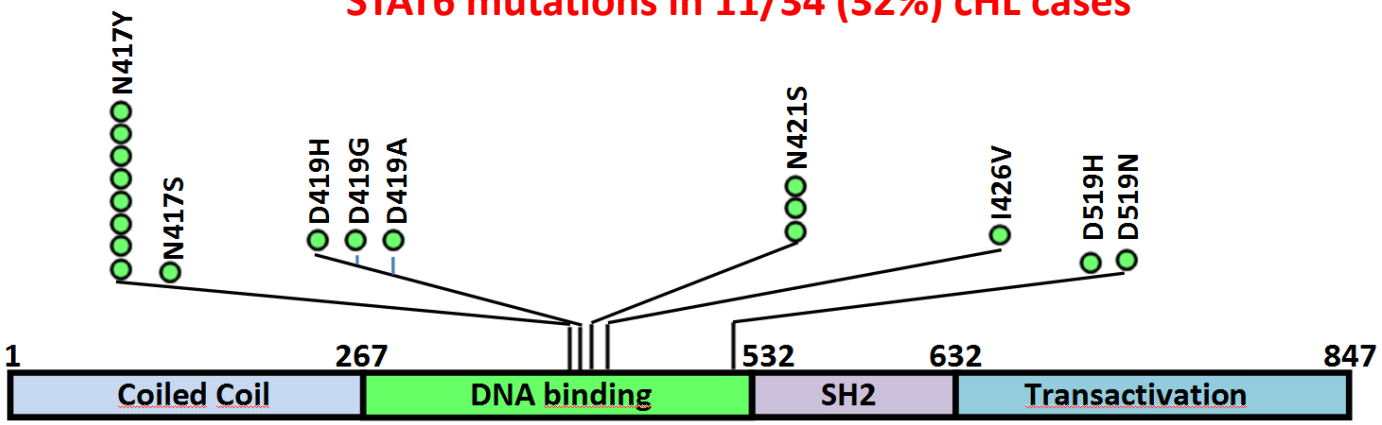

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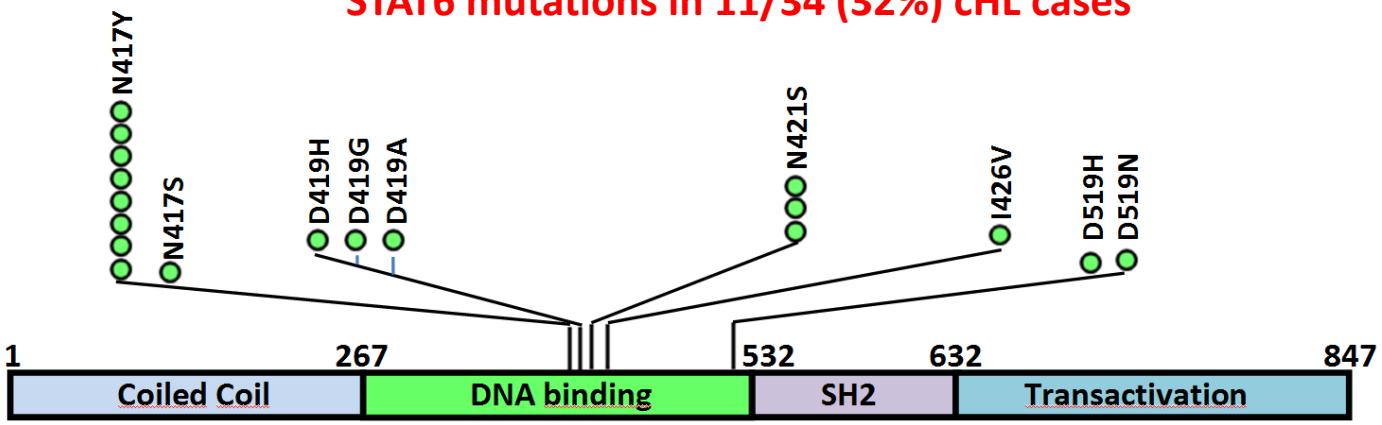

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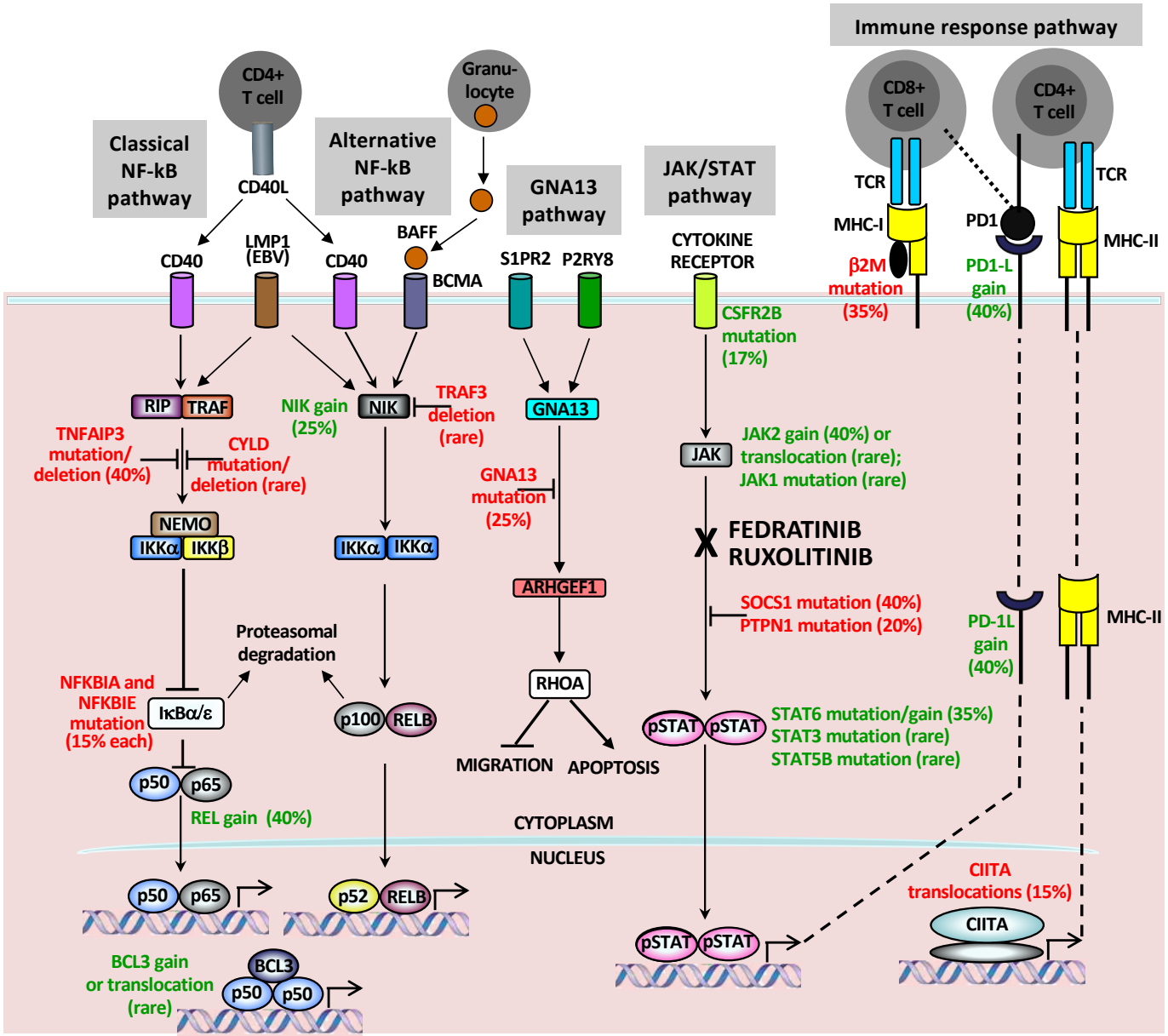
- STAT6 mutations in cHL confer a survival advantage distinct from (and beyond that of) STAT6 phosphorylation, perhaps due to aberrant DNA binding activity following pSTAT6-dependent entry into the nucleus

Tiacci et al, Blood 2018

MAIN SOMATIC GENETIC LESIONS IN cHL (activating; inactivating)



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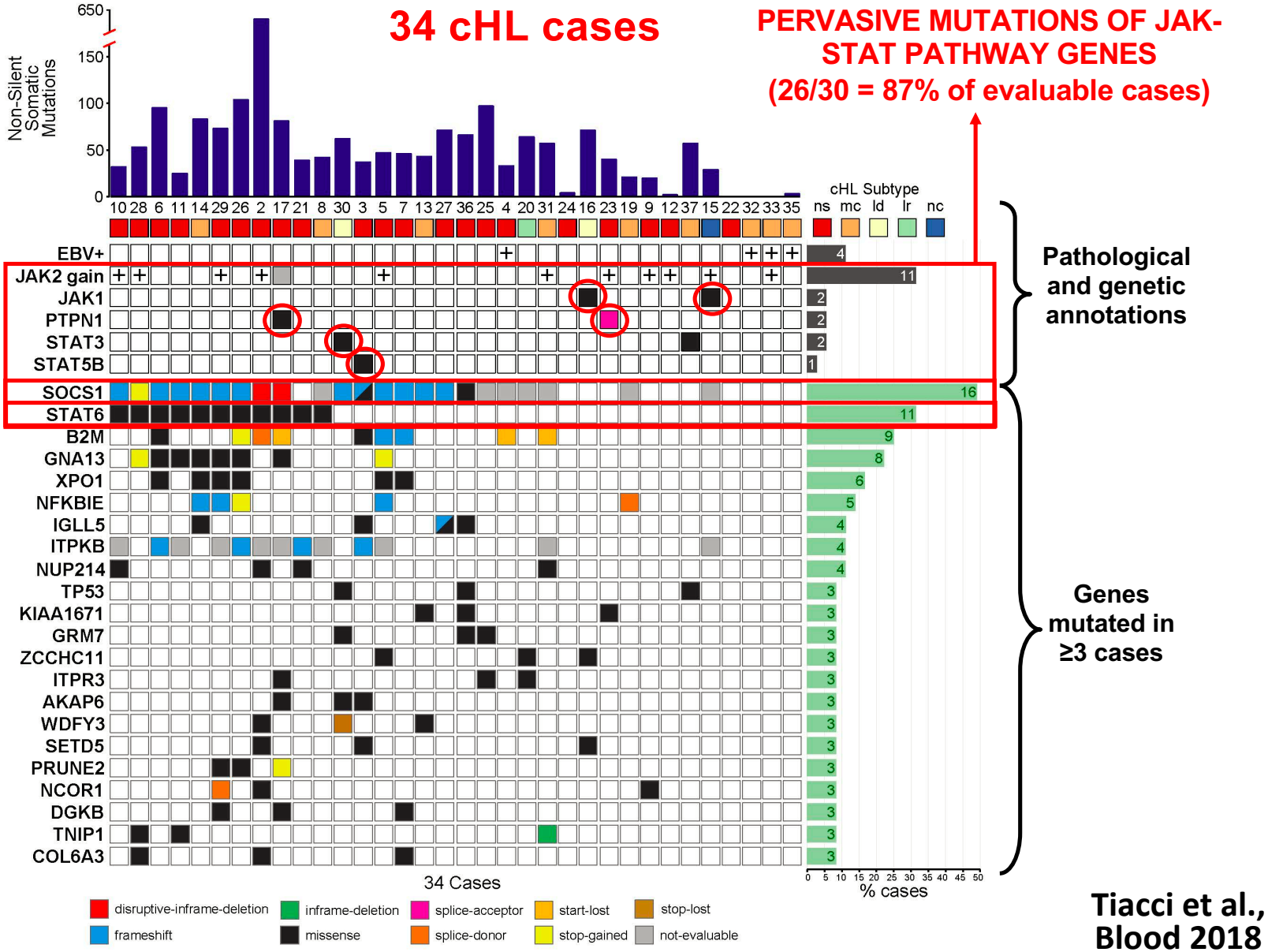
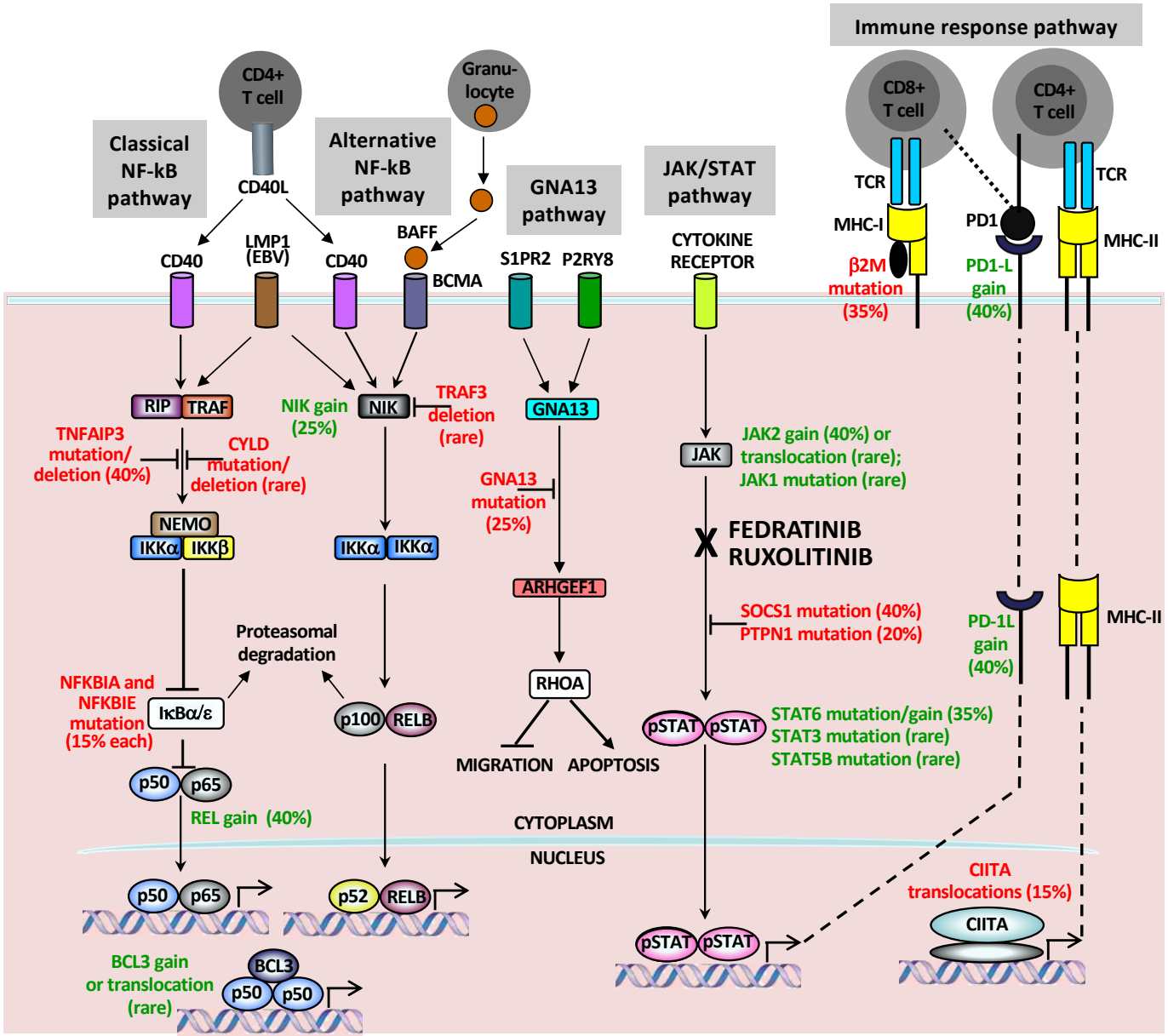
Schiavoni and Tiacci, EHA 2018 Educational Book (updated)



Tiacci et al., Blood 2018



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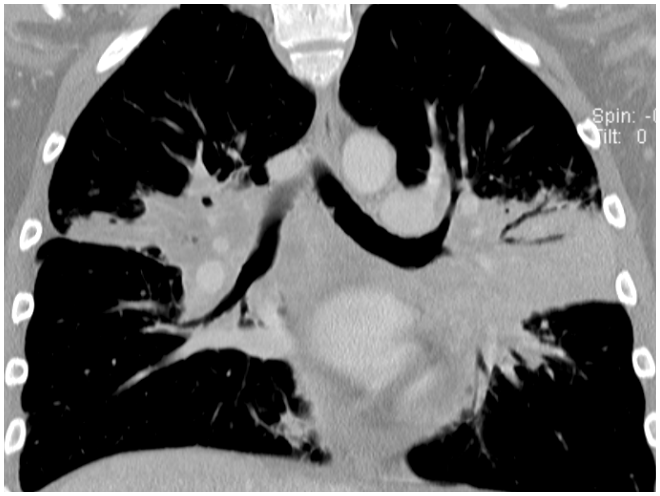
- CSF2RB mutation (17%)

Schiavoni and Tiacci, EHA 2018 Educational Book (updated)

- **24 year-old male with cHL refractory (never CR) to 9 lines of therapy:**
  - ABVD: SD
  - BEACOPP: PR
  - IGEV: SD
  - Brentuximab: PD
  - Nivolumab (24 doses): SD, then PD
  - Bendamustine: SD
  - Adriamycin: SD
  - BEGEV: PR
  - FEAM + autotransplant: PD

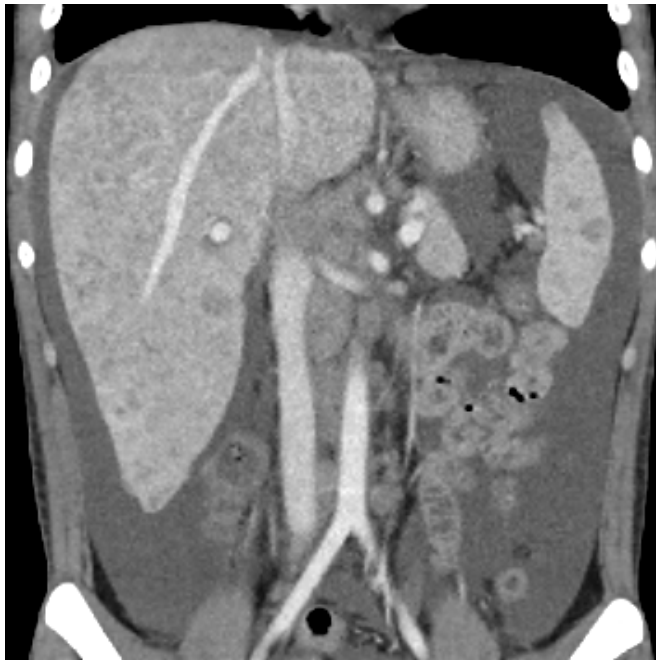


- **Presents at our center with stage IVB disease:**
  lymph nodes, lung, bones, liver, spleen, ascites, fever, sweats

  Liquid biopsy*: - somatic STAT6 E444K, 53.7% VAF
  - circulating tumor (ct) DNA 71.55 ng/ml

  Solid biopsy: - somatic STAT6 E444K, 13.2% VAF*
  (archival) - FISH 9p24 (JAK2, PDL1/2): no gain/amplification^

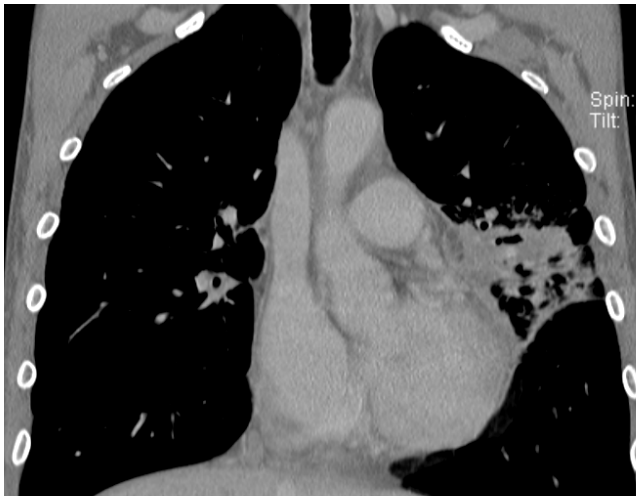
Fever
Sweats
74 kg
(ascites)



- **Starts ruxolitinib (JAK1/2 inhibitor) 10 mg BID + nivolumab 240 mg Q2W**

NGS for 6 JAK-STAT genes (SOCS1, STAT6, STAT3, STAT5B, JAK1, PTPN1) in plasma vs leukocyte DNA

^Polysomy in 80% tumor cells, disomy in 20% tumor cells

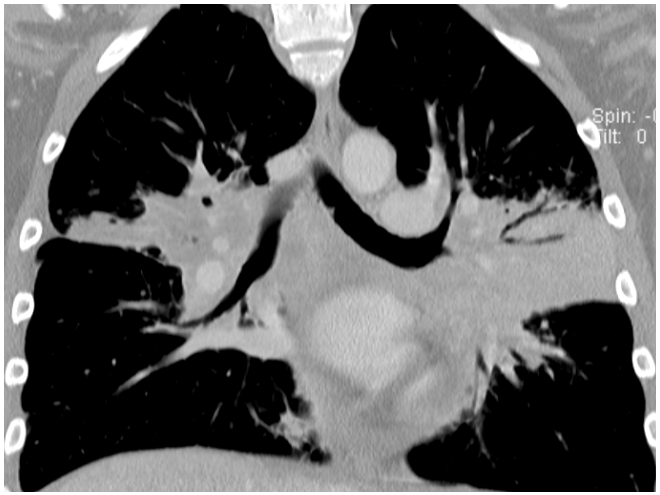


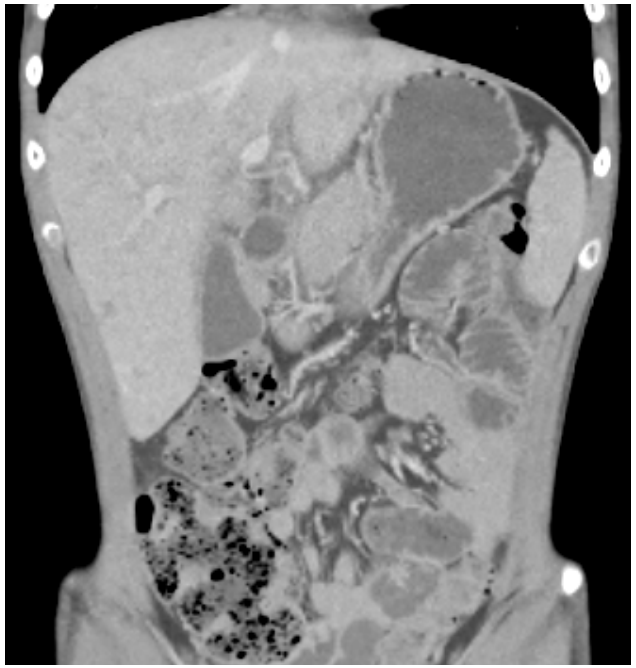
ctDNA
0.43
ng/ml

>2 log_{10} reduction

Ruxo 28 days + *Nivo 2 doses*

ctDNA
71.5
ng/ml





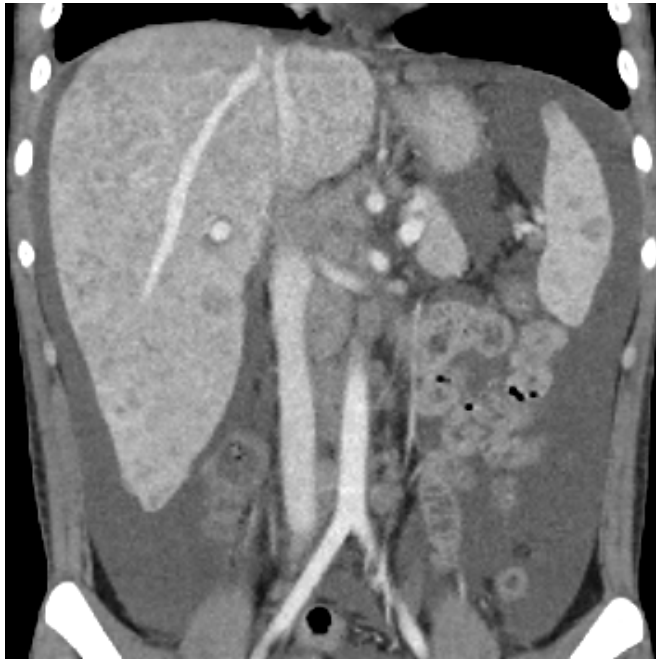
~~Fever~~
Sweats
60 kg
~~(ascites)~~

Ruxo 28 days (total) + Nivo 2 doses (total)

~~Fever~~
~~Sweats~~
60 kg
~~(ascites)~~

Ruxo 10 days + Nivo 1 dose

Fever
Sweats
74 kg
(ascites)



Academic multi-center phase-2 trial of the oral JAK1/2 inhibitor ruxolitinib combined with either brentuximab or pembrolizumab in rel./refr. cHL patients

- JAK-STAT pathway gene mutations almost ubiquituous in cHL
- JAK2 inhibition kills cHL cell lines with mutant *STAT6* or *STAT3* *in vitro*
  Adding brentuximab to ruxolitinib eradicates their xenograft in vivo

Tiacci et al, Blood 2018
Wienand et al, Blood Adv 2019
Hao et al, CCR 2014
Lee et al, Oncotarget 2018
Ju et al, PNAS 2016

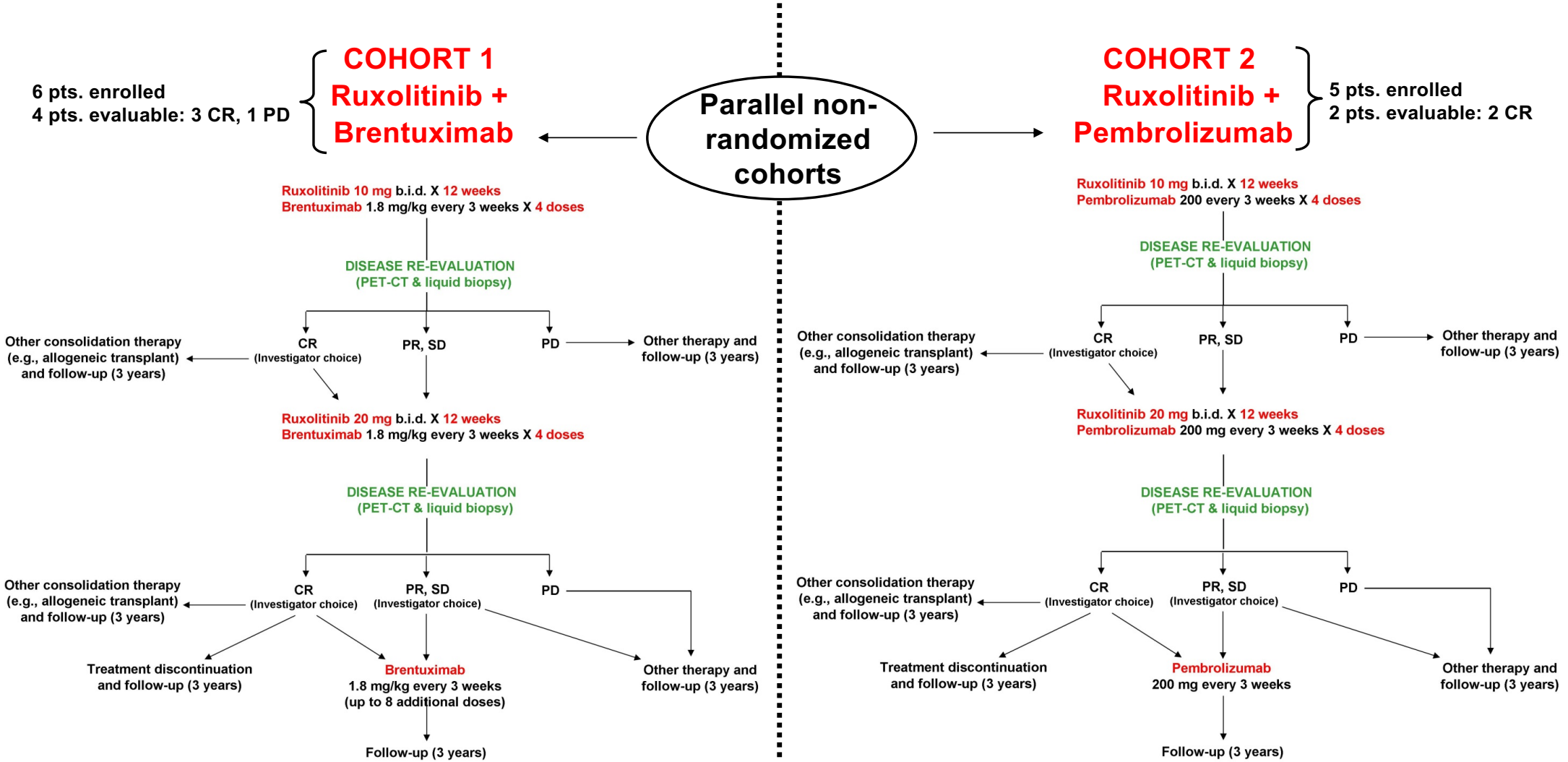
- Monotherapy with ruxolitinib is safe and has some efficacy in relapsed/refractory cHL patients

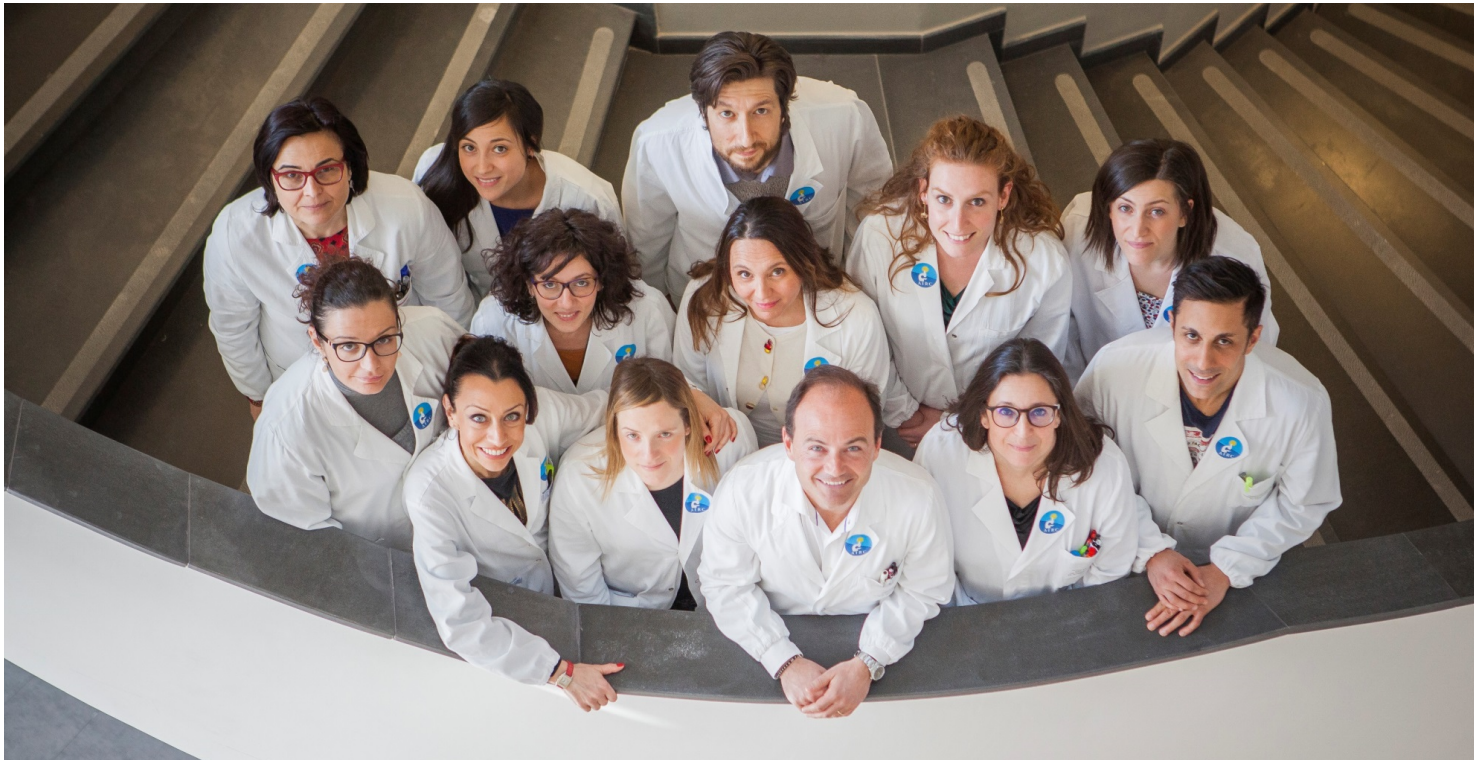
  South Korean study (BMC Cancer 2019):
  - 13 pts. with 4 median prior lines (84% refractory to last prior line)
  - 46% overall responses (1 CR, 5 PR), lasting a median of 5.6 months
  - Ruxolitinib given at 20 mg b.i.d. for a median of 20 weeks; largely grade 1-2 toxicities

  Lysa study (Haematologica 2018):
  - 33 pts. with 5 median prior lines (82% refractory to last prior line)
  - 18% best overall responses (1 CR, 5 PR), lasting a median of 7.7 months;
  - Transient disease stabilization in 33% (11/33) pts.
  - Ruxolitinib given at 20 mg b.i.d. for a median of 16 weeks; largely grade 1-2 toxicities

- Brentuximab and PD1 inhibitors effective in relapsed/refractory cHL, but CR rate still relatively low (12-34%)
- Aim: to increase the CR rate in patients eligible (and naive) to brentuximab or pembrolizumab per EMA label by combining ruxolitinib (up to 24 weeks) with brentuximab (up to 8 doses) or with pembrolizumab (up to 8 doses), respectively, in two parallel non-randomized cohorts
- Biomarkers of response (e.g., gene mutations of JAK-STAT and other pathways) in solid and liquid biopies

Academic multi-center phase-2 trial of the oral JAK1/2 inhibitor ruxolitinib combined with either brentuximab or pembrolizumab in rel./refr. cHL patients





Alessandra Venanzi, Andrea Marra, Gianluca Schiavoni, Elisabetta Fortini, Valentina Pettirossi
Sara. G. Milner, Alessandra Pucciarini, Luisa Tasselli, Marta Naccari,
Alessia Tabarrini, Barbara Bigerna, Mara Merluzzi

AIRC

CLINICAL TRIAL:

B. FALINI, L. Flenghi, E. Bonifacio, A. D'Arpino, M. Ricci (Perugia)

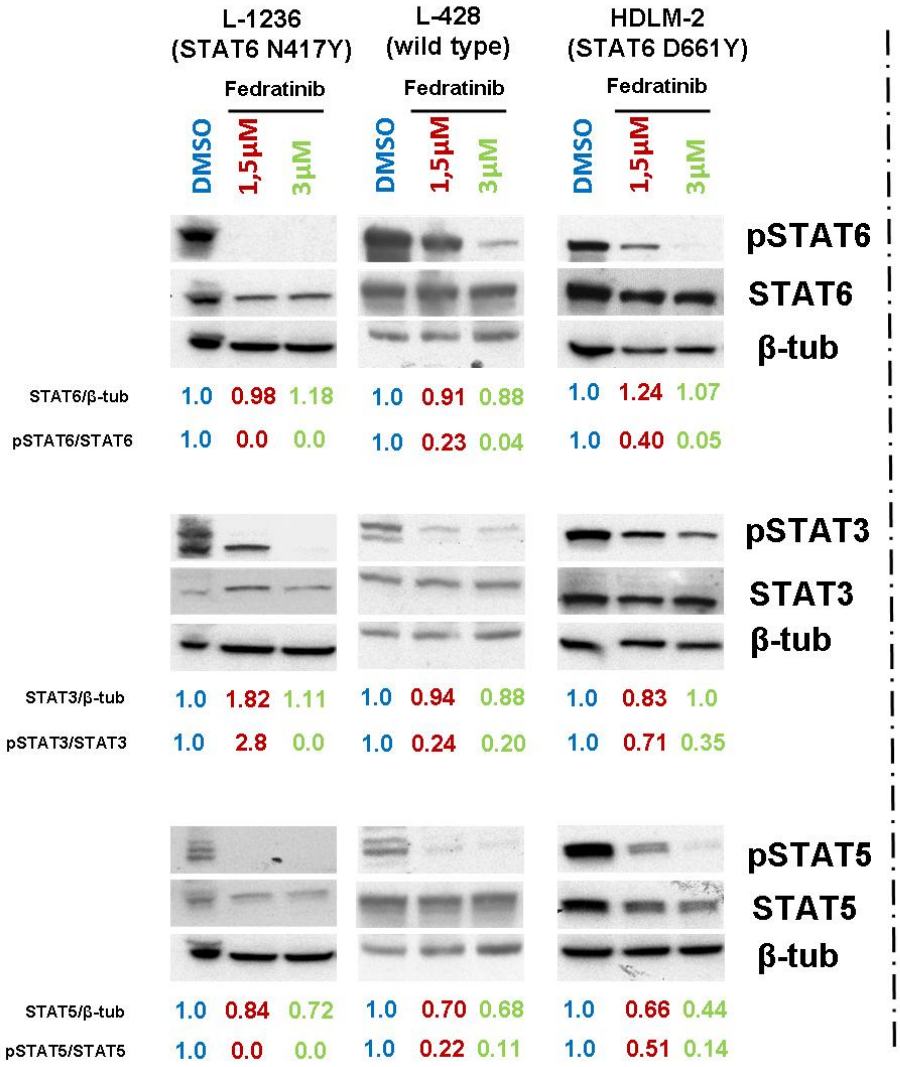
A. Pulsoni, G. D'Elia (Roma)

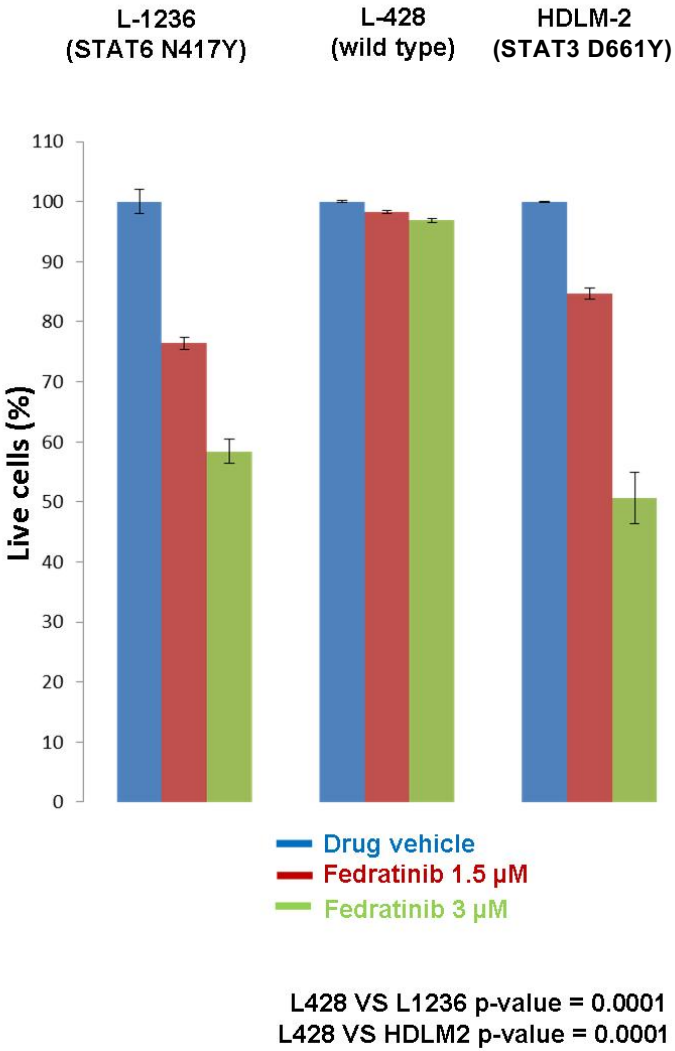
F. Zaja (Trieste)

V. Fraticelli (Campobasso)

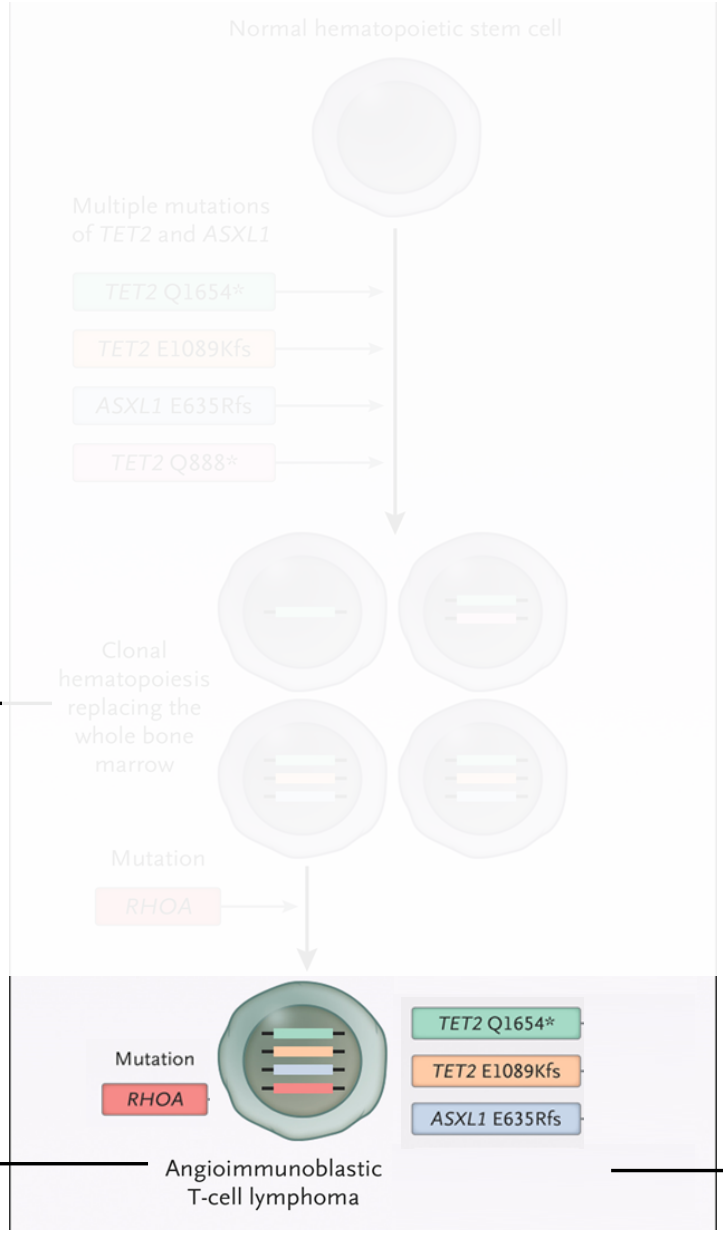
Ministero della Salute

PHARMACOLOGICAL JAK2 INHIBITION CAUSES APOPTOSIS OF cHL CELLS WITH *STAT* GENE MUTATIONS





Tiacci et al, NEJM 2018



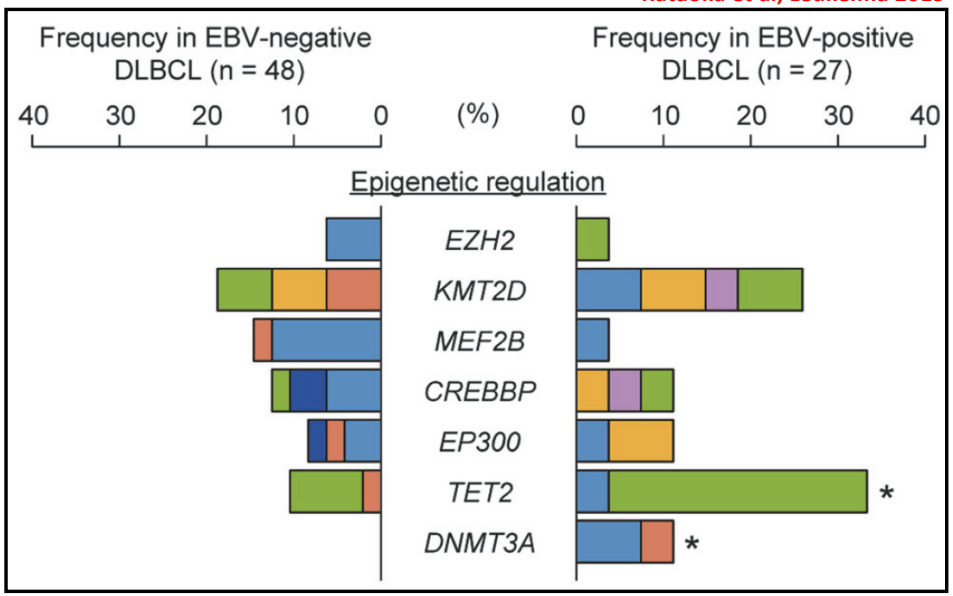
Massive CH (~90% of bone marrow cells), but normal blood counts and no dysplasia

45-year old patient
CR after CHOEP + autotransplant

1 year after lymphoma diagnosis

CASE 5
(EBV+, Mixed Cellularity)

Kataoka et al, Leukemia 2019



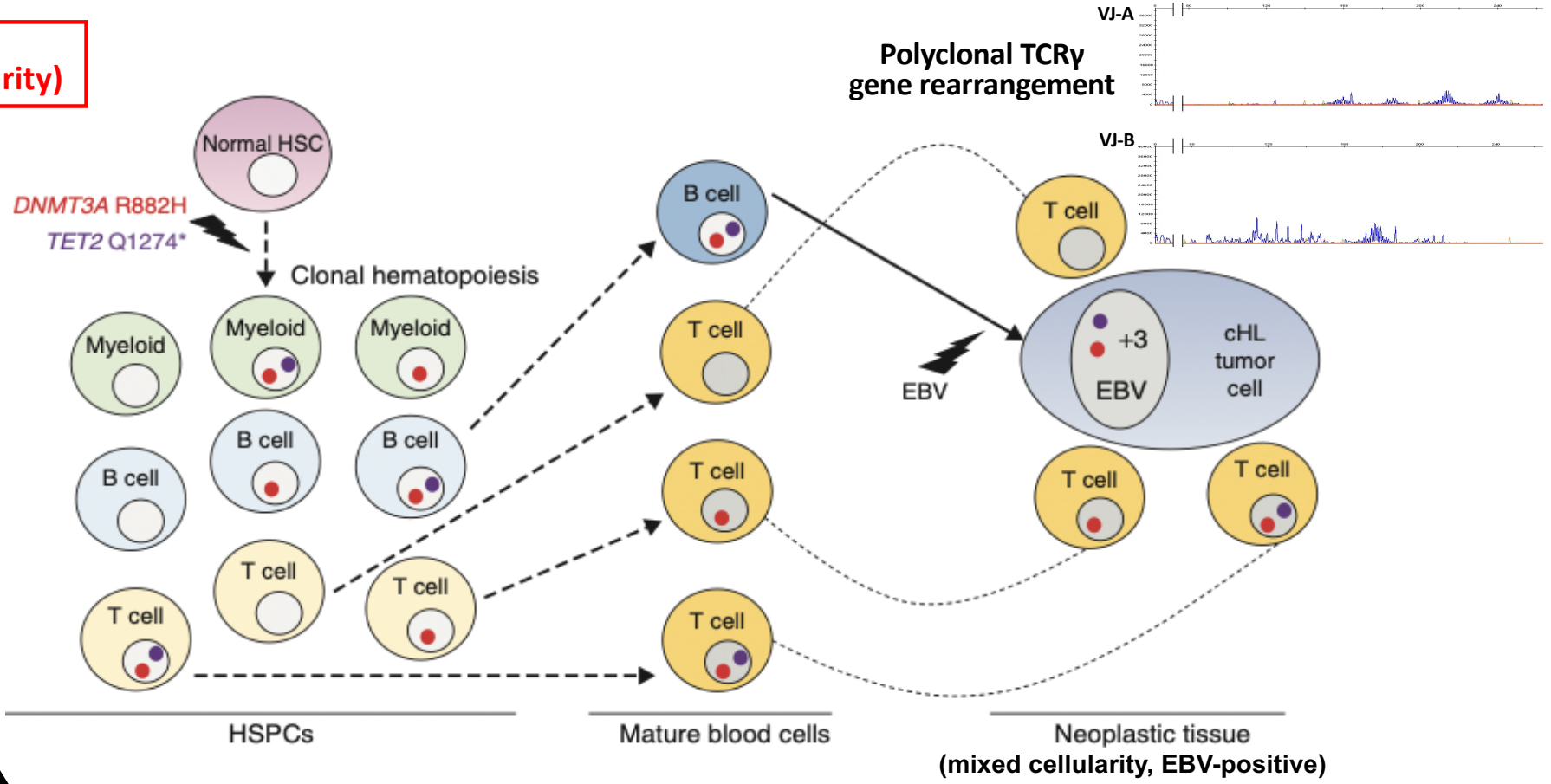
- First description of mutations in the epigenetic DNA modifiers *DNMT3A* and *TET2* in tumor cells of cHL[1]
- **TET2** functions as a **tumor suppressor gene in germinal centre (GC) B-cell lymphomagenesis**[2], and is **mutated in** diffuse large B-cell lymphomas (**DLBCL**) at a frequency of 10% overall[3] and 48% within the genetic subtype ST2 (largely of GC B-cell phenotype)[4]
- In **DLBCL: TET2 and DNMT3A mutations** are **more frequent in** tumor cells of **EBV+** vs EBV- cases (TET2: 33% vs 10%; DNMT3A 11% vs 0%; $p < 0.05$); and ***DNMT3A* mutations** were found **only in EBV+ cases comutated for TET2**[5]

This **genetic configuration is similar to cHL Case 5**, which featured an **EBV⁺** lymphoma cell clone carrying **DNMT3A and TET2 comutations** (in addition to an extensive clonal hematopoiesis of the tumor microenvironment)[1]

Potential role for DNMT3A and TET2 comutations in the pathogenesis of some EBV+ Hodgkin and non-Hodgkin GC B-cell derived lymphomas?

[1] Venanzi et al, Blood Cancer Discov 2021
[2] Dominguez et al, Cancer Discov 2018
[3] Reddy et al, Cell 2017
[4] Wright et al, Cancer cell 2020
[5] Kataoka et al, Leukemia 2019

CASE 5
(EBV+, Mixed Cellularity)

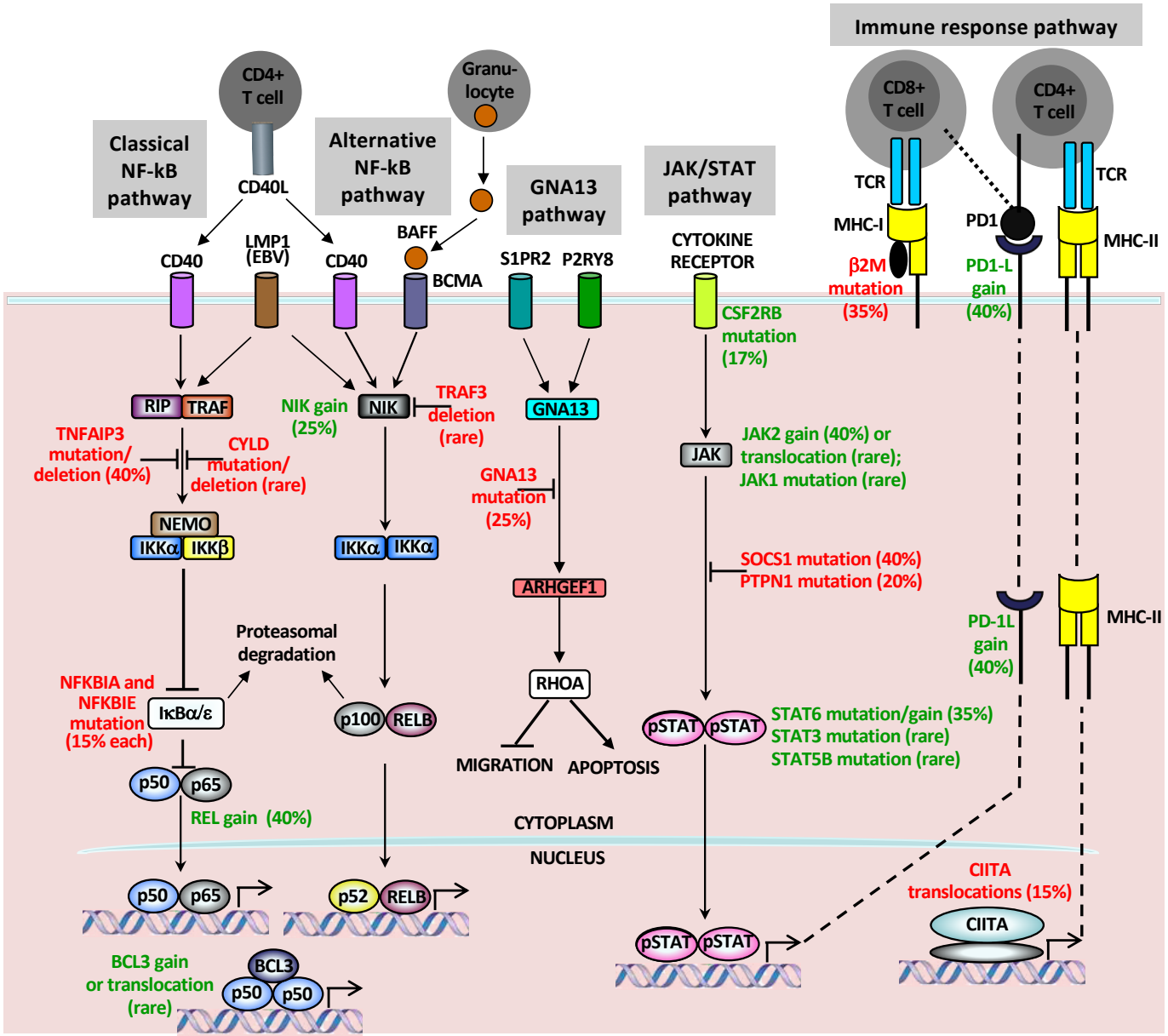


cHL case cohort (n=40)	
CH PRESENT	AGE (years)
YES (n=5)	45
	30
	83
	81
	73
NO (n=35)	Median 35 Range 15-75

VAF, variant allele frequency

Pt. #	Time of sampling	Progressed after first-line chemotherapy	Gene mutation	VAF in microdissected		VAF in whole tissue section
				Reactive lymphoid cells	HRS cells	
Case 5	I° relapse	YES	*DNMT3A* R882H	30%	43%	37.9%
			TET2 Q1274*	8.4%	31.1%	26.9%

Venanzi, …, Tiacci
Blood Cancer Discov 2021



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- ARID1A mutation (25%)
- XPO1 mutation/gain (20%)

Schiavoni and Tiacci, EHA 2018 Educational Book (updated)

GENETICS OF CLASSICAL HODGKIN LYMPHOMA

- Landscape of frequently mutated genes in cHL largely defined
  - *NF-KB signaling*: TNFAIP3, REL, NFKBIA, NFKBIE, NIK
  - *JAK-STAT* signaling: SOCS1, JAK2, STAT6, PTPN1
  - PI3K-AKT signaling?: GNA13, ITPKB
  - Immune evasion: PDL1/2, B2M, CIITA
  - Other genes: XPO1, TP53, KMT2D/MLL2, TNFRSF14/HVEM
- Better understanding of cHL pathogenesis
- New therapeutic targets:
  - actual, i.e. PD1 inhibitors
  - potential, e.g. JAK and XPO1 inhibitors
- New liquid-biospy targets for non-invasive:
  - cHL genotyping
  - monitoring of clonal evolution
  - monitoring of response to therapy
- All cHL-mutated genes also found in other lymphomas, and likely not explaining the unique phenotype and histo-morphology of cHL:
  - particular sets of mutations must occurr in a certain order during specific GC and/or post-GC B-cell stages to produce cHL vs NHL?
  - mutations in the non-coding genome?

CASE 1
(EBV-, Nodular Sclerosis)

cHL case cohort (n=40)	
CH PRESENT	AGE (years)
YES (n=5)	45
	30
	83
	81
	73
NO (n=35)	Median 35 Range 15-75

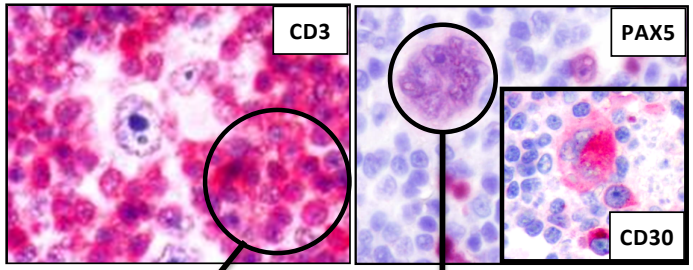
Whole blood

T cells: DNMT3A-R882H 47%
B cells: DNMT3A-R882H 48%

D

DNMT3A-R882H 7%

Classical Hodgkin Lymphoma
(lymph node biopsy)

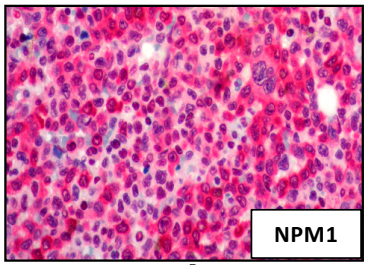


DNMT3A-R882H 16%

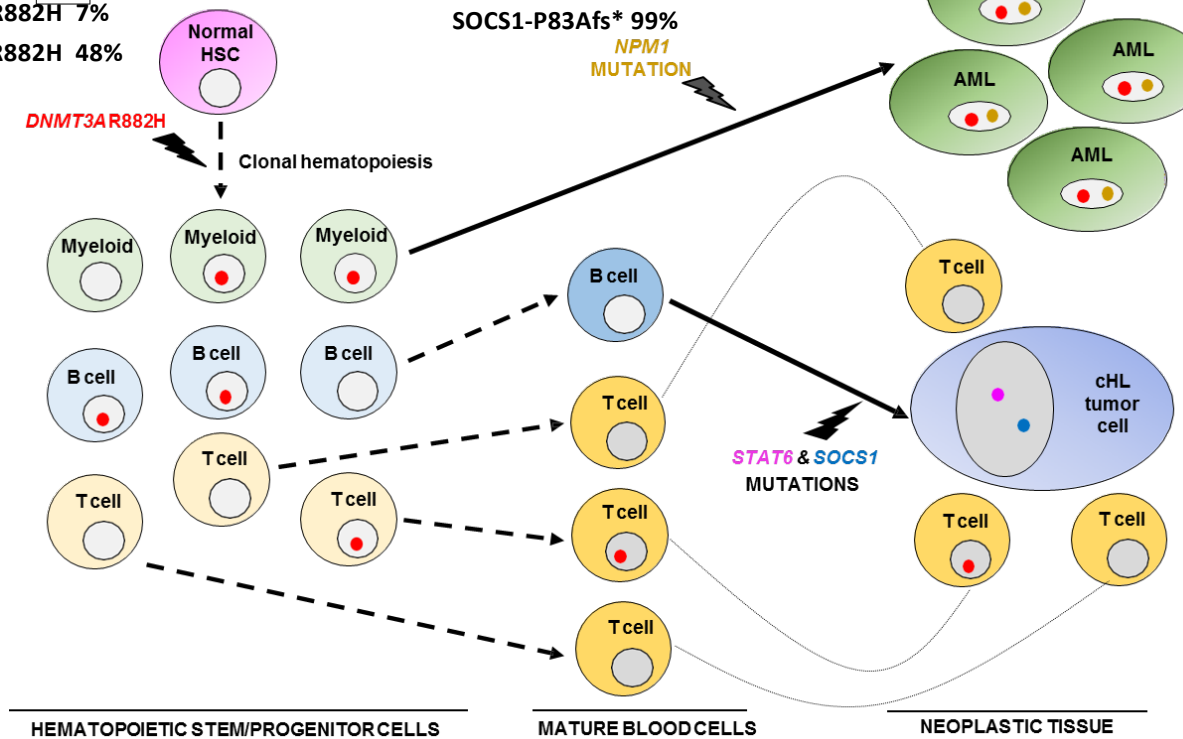
DNMT3A wild-type
STAT6-N417Y 37%
SOCS1-P83Afs* 99%

6 years after therapy for cHL
ABVD, IGEV, ABMT, RT

[De novo ?] Acute Myeloid Leukemia
(bone marrow biopsy)

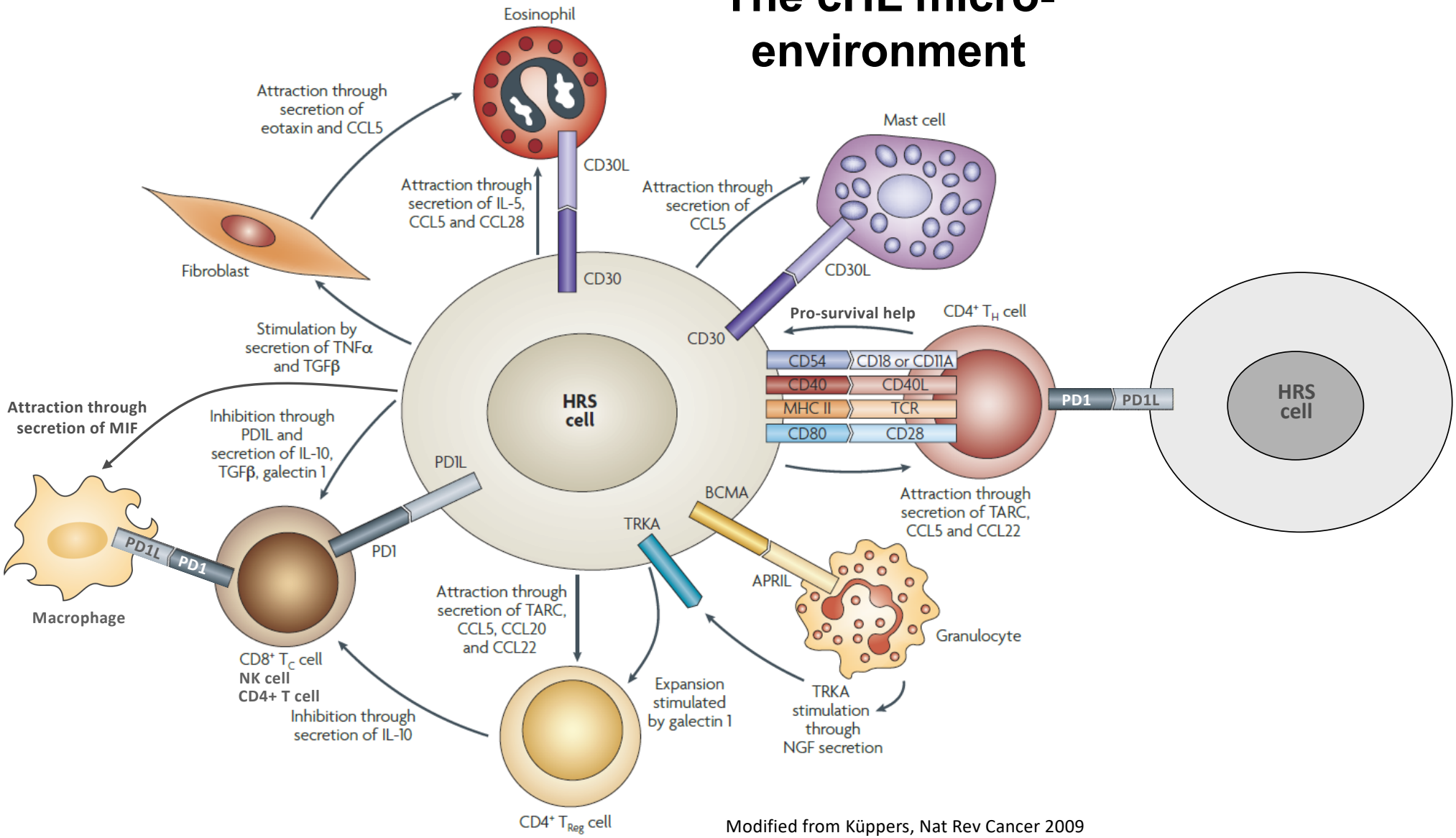


DNMT3A-R882H 45%
NPM1-W288Cfs* 41%
Normal karyotype
TP53 and PPM1D wild-type



Venanzi, …, Tiacci
Blood Cancer Discov 2021

The cHL micro-environment



Modified from Küppers, Nat Rev Cancer 2009

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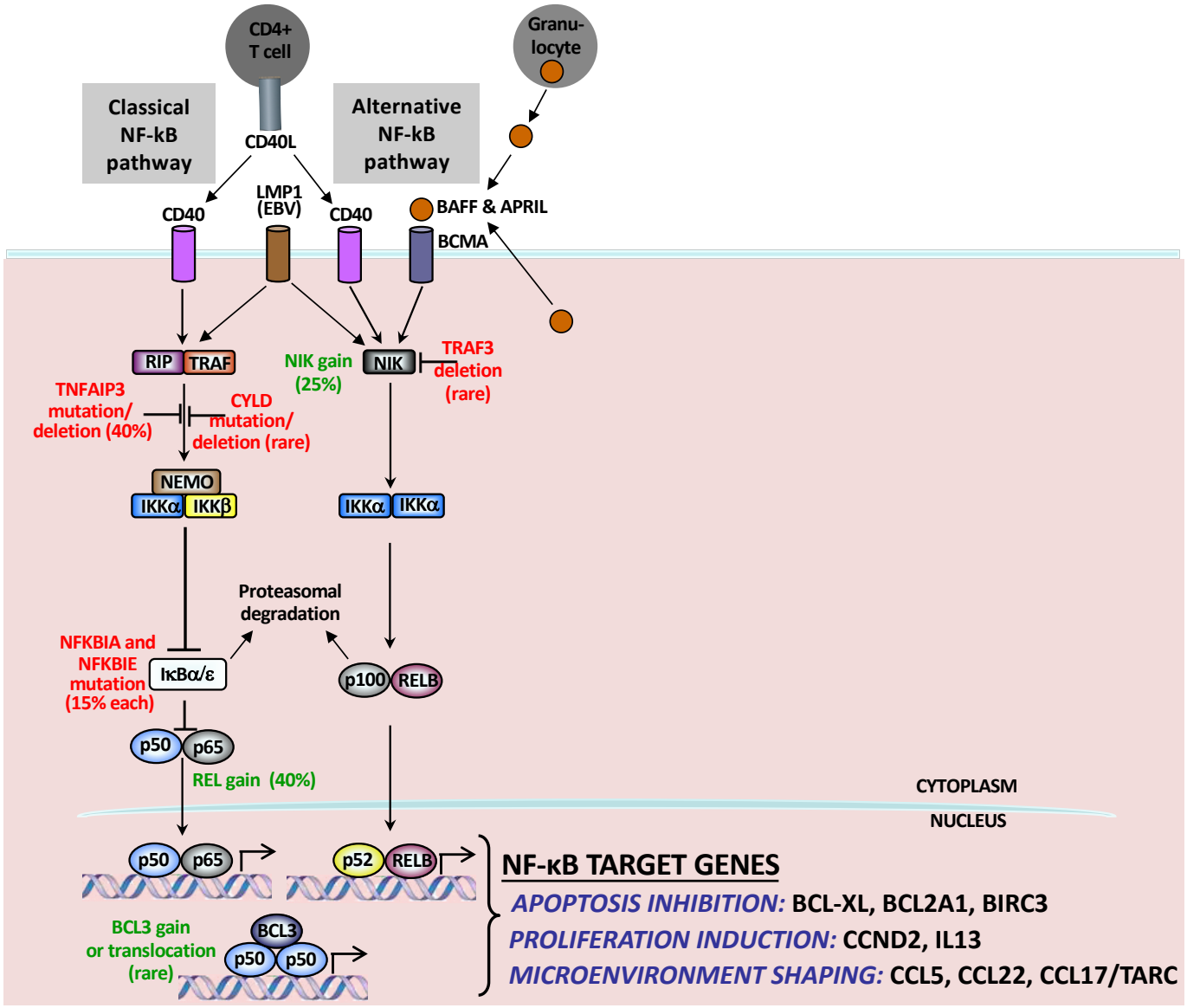

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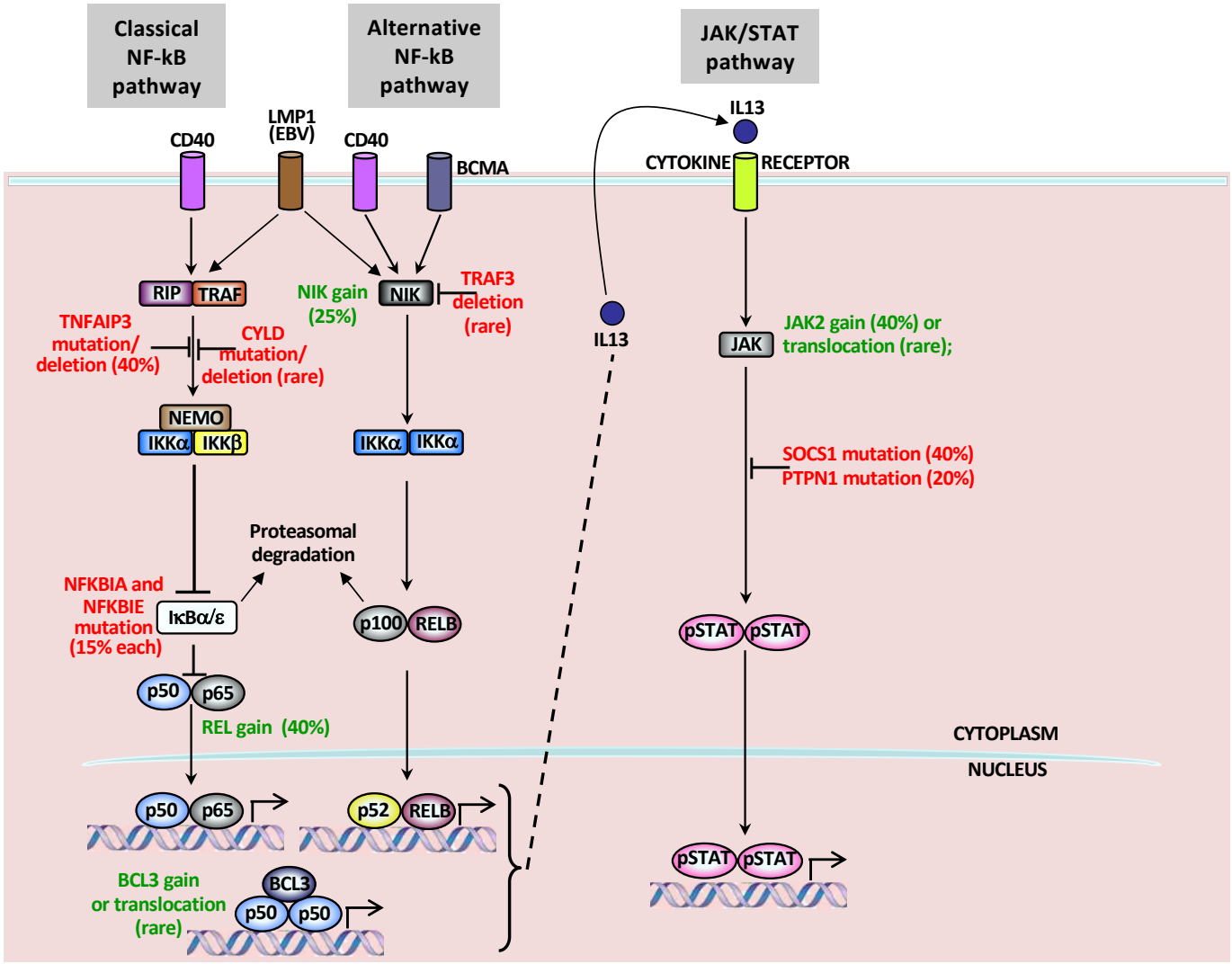

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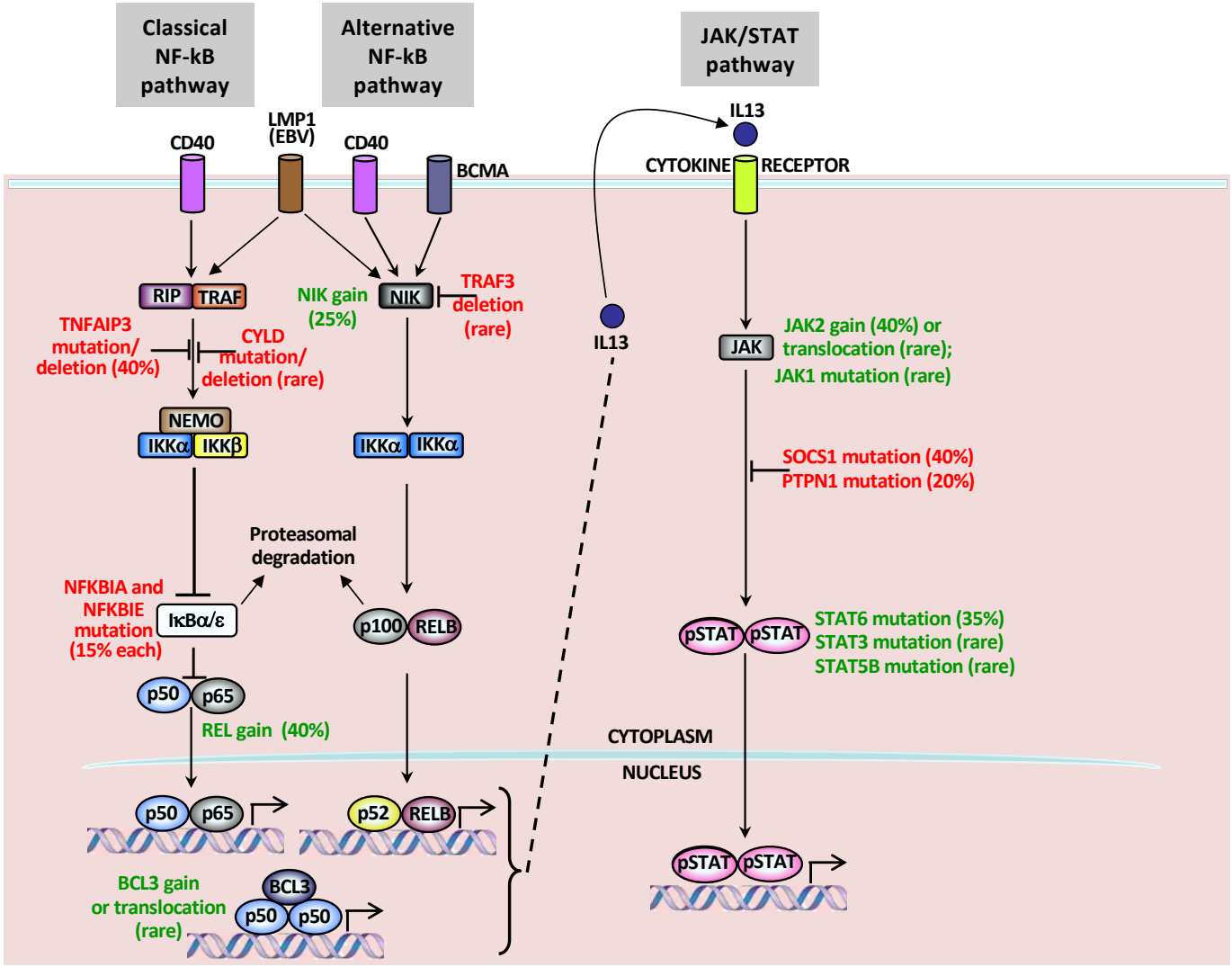

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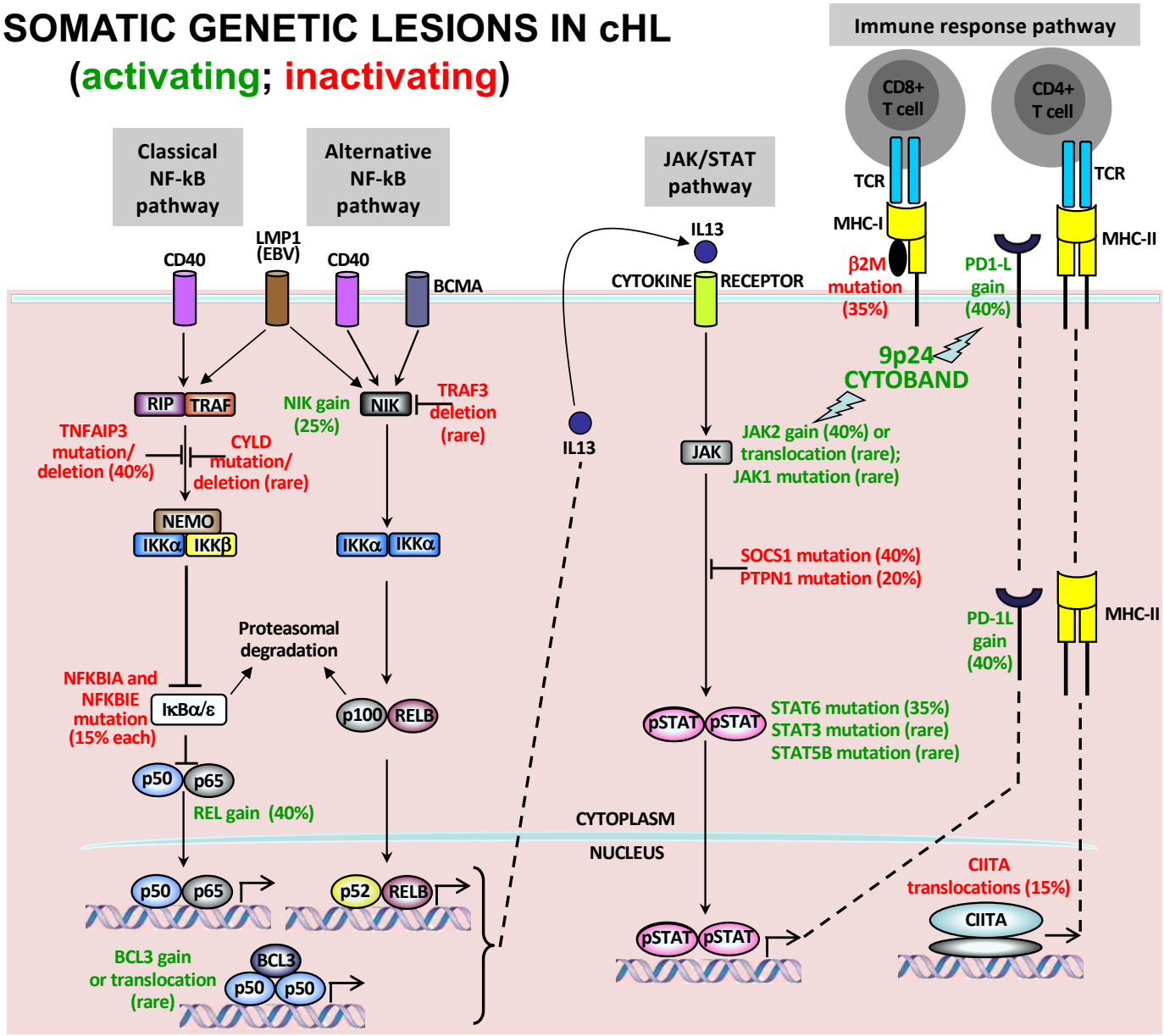

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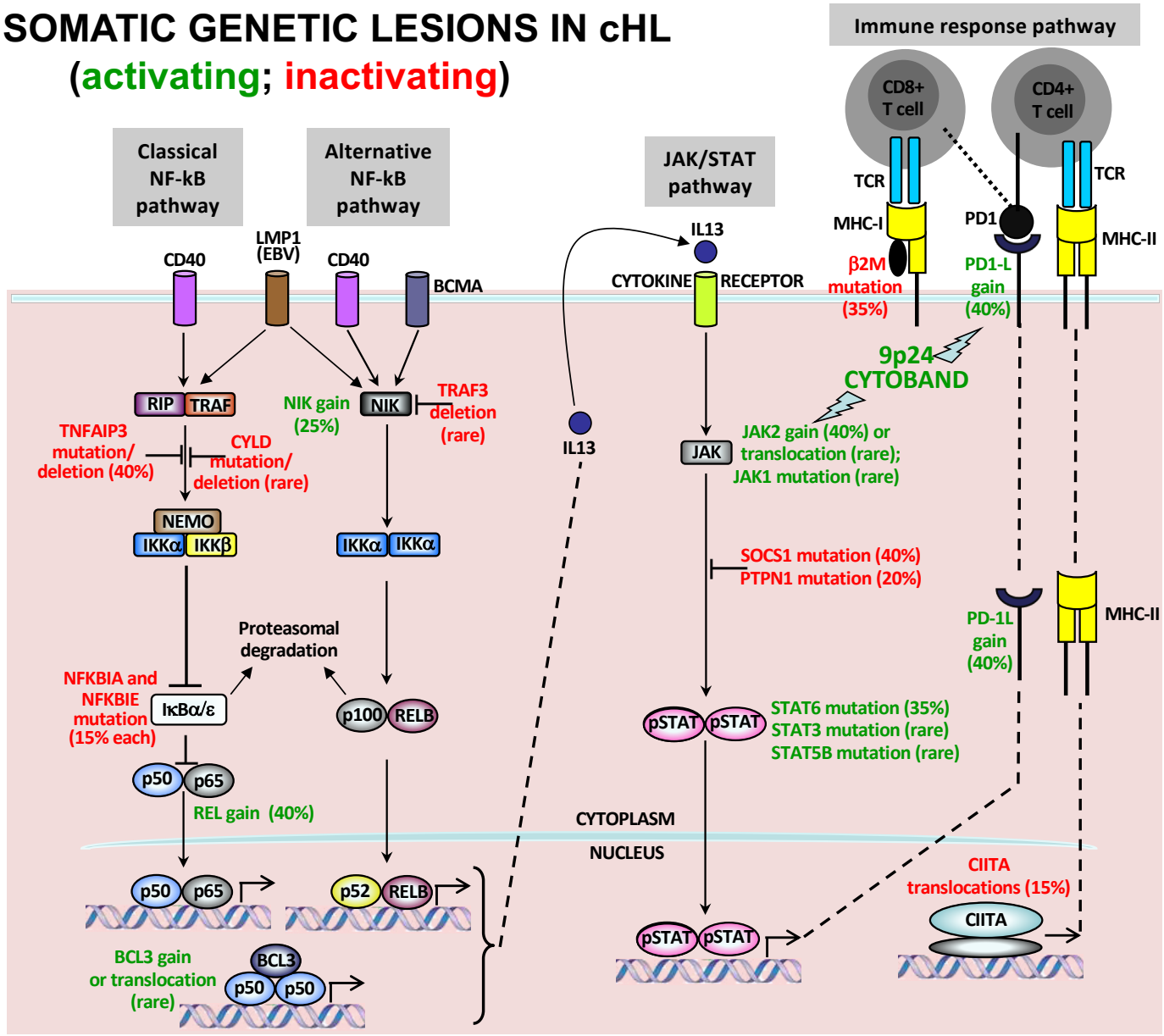

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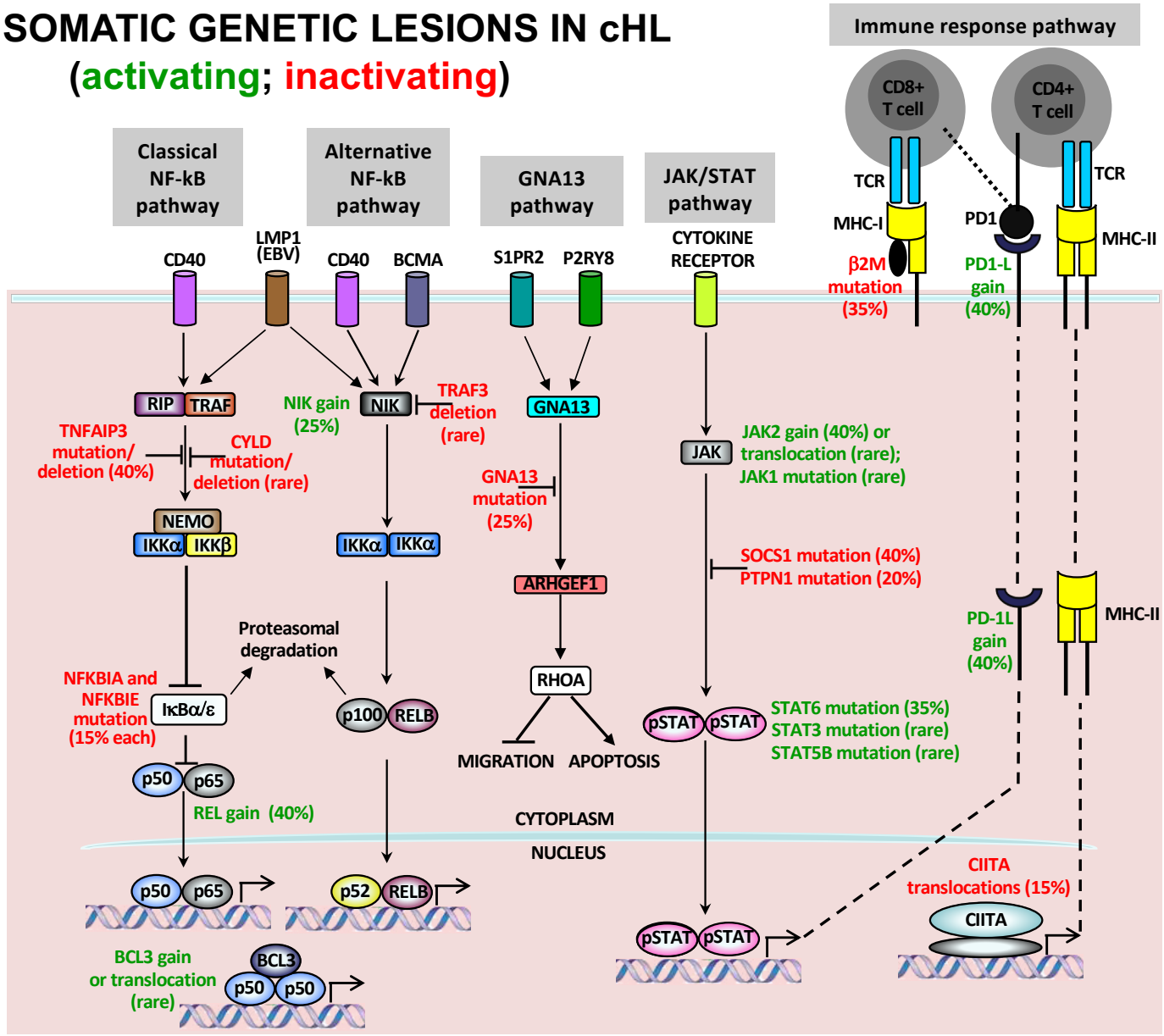

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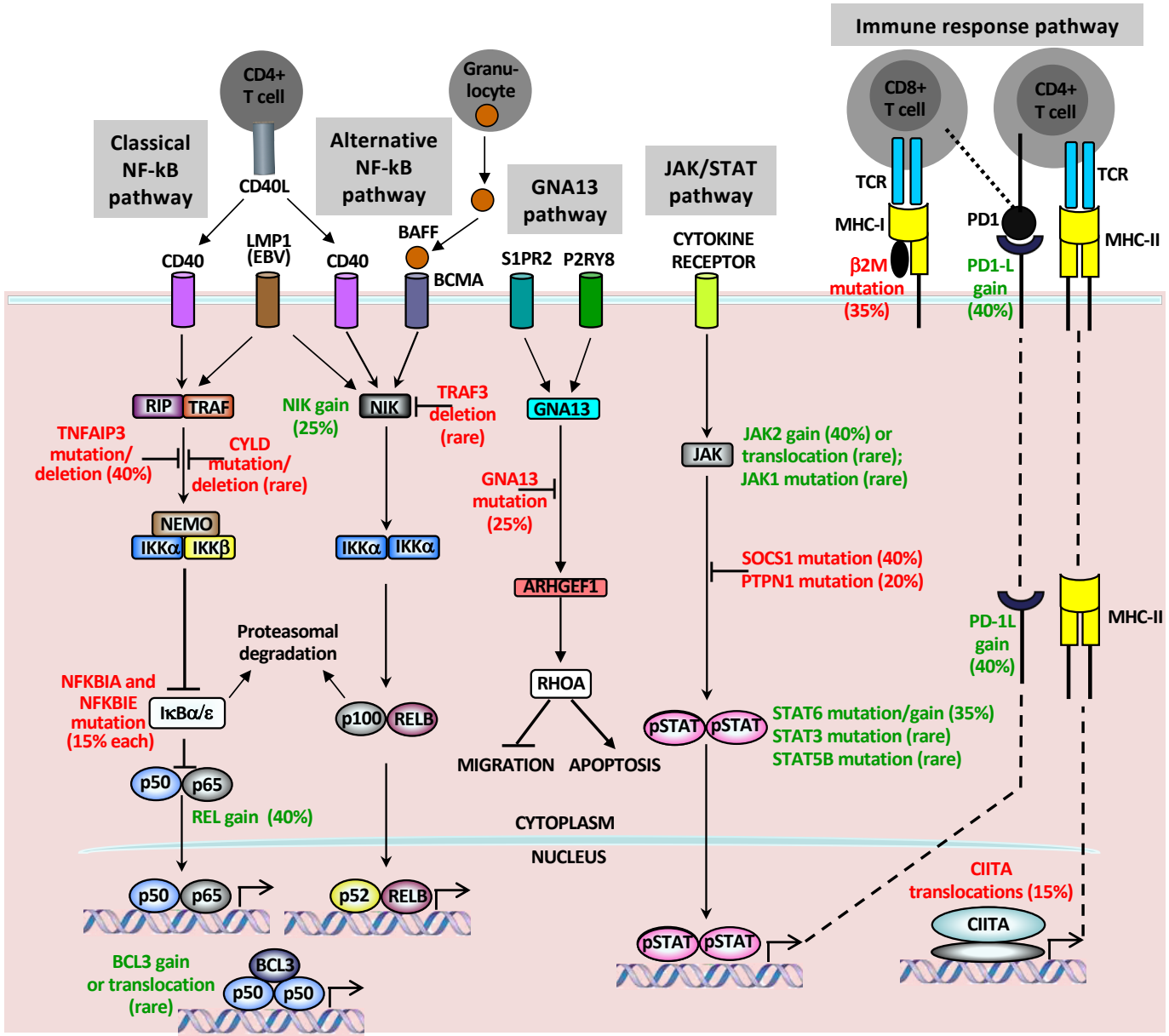

Schiavoni and Tiacci, EHA 2018 Educational Book
Weniger and Küppers, Leukemia 2021;35:968

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