2017 Society for Hematopathology Workshop

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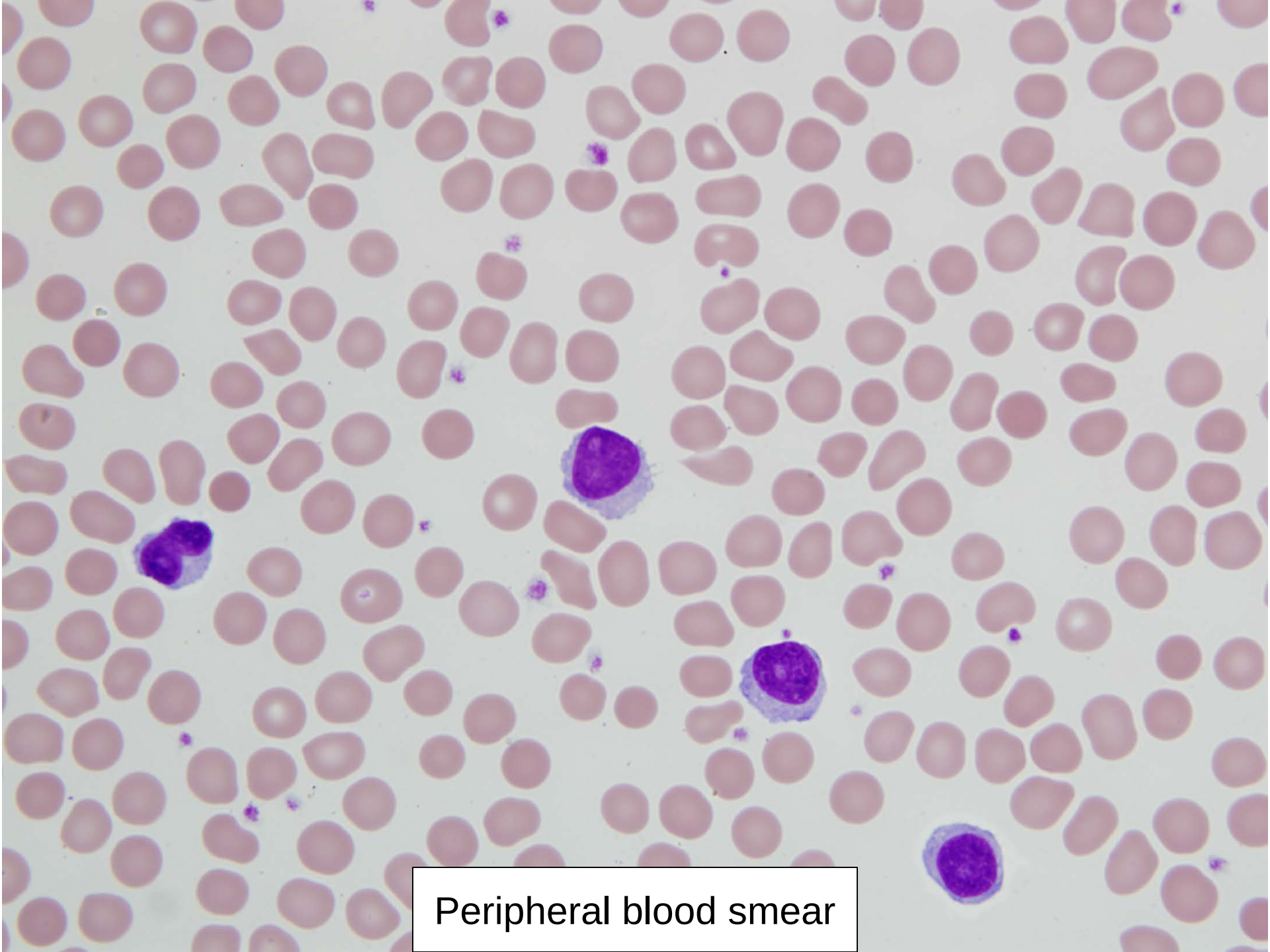
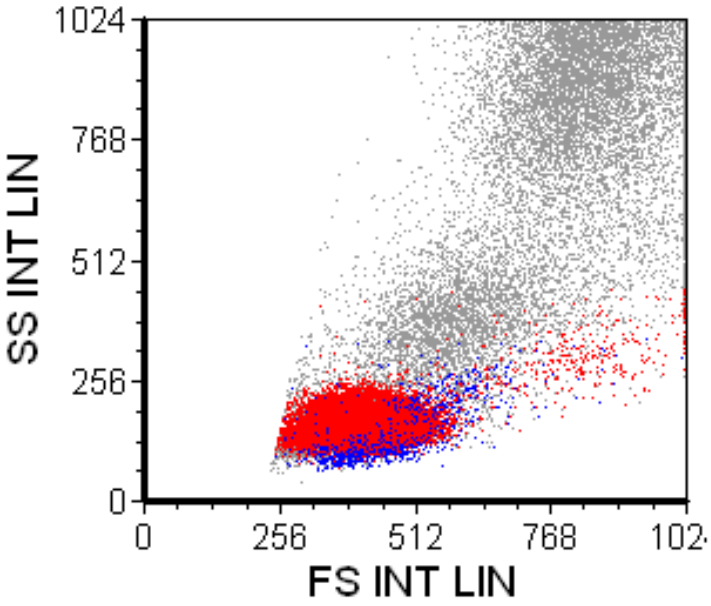
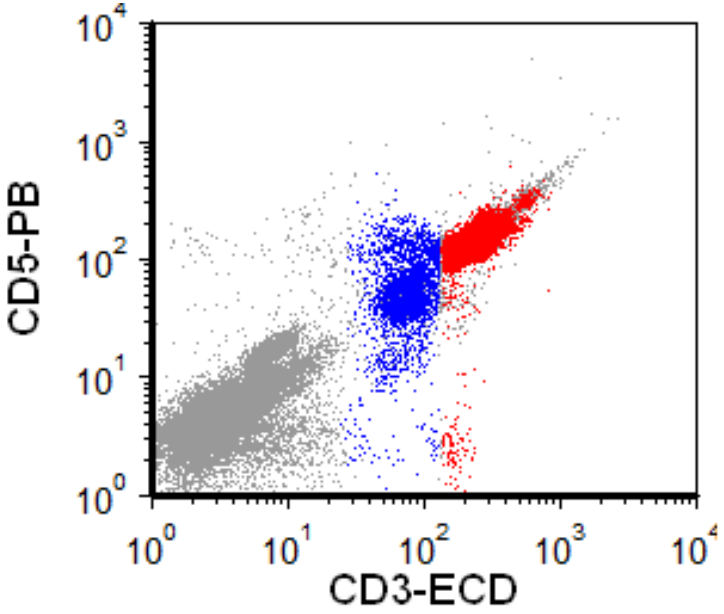
Clinical History

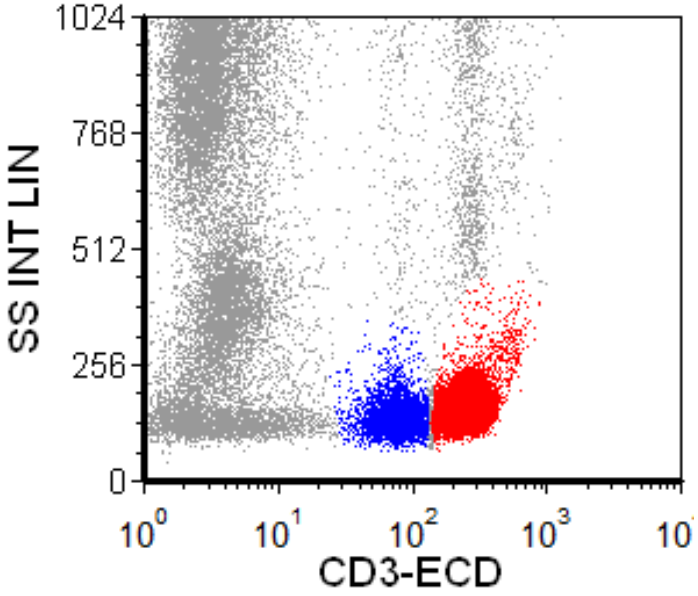
- 61 year-old asymptomatic male with no significant prior clinical history
- Slowly progressing lymphocytosis first diagnosed in 2014
- Current CBC:
  - WBC 16.1 k/ul with absolute lymphocyte count of 10.7 k/ul
  - hemoglobin 14.7 gm/dL
  - MCV 90 fL
  - platelet count 219 k/ul
  - Differential count: neutrophils 25%, lymphocytes 67%, monocyte 6%, eosinophils 1%, basophils 1%
- No lymphadenopathy or FDG avid lesions on skull base to mid-thigh PET/CT
- No skin lesions
- Peripheral blood smear review, flow cytometry and fluorescence in situ hybridization, and bone marrow exam with flow cytometry, cytogenetic and molecular genetic testing have been performed

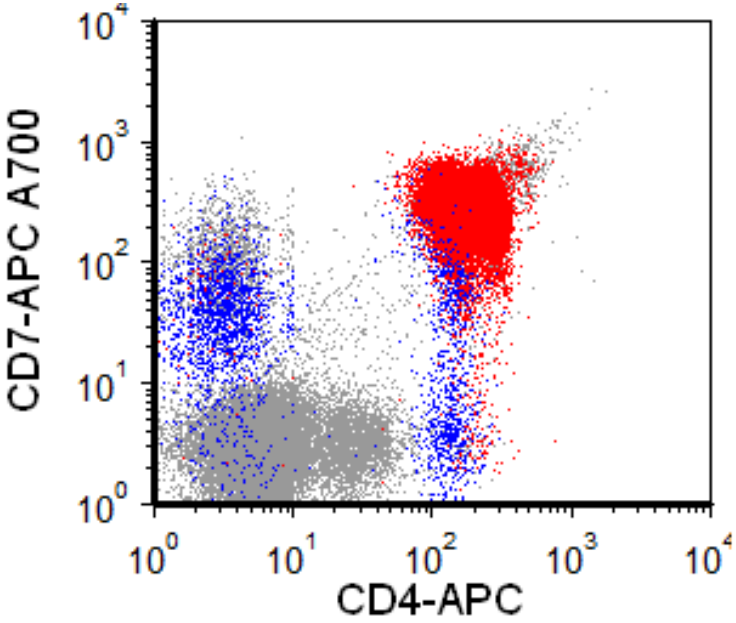

Peripheral blood smear

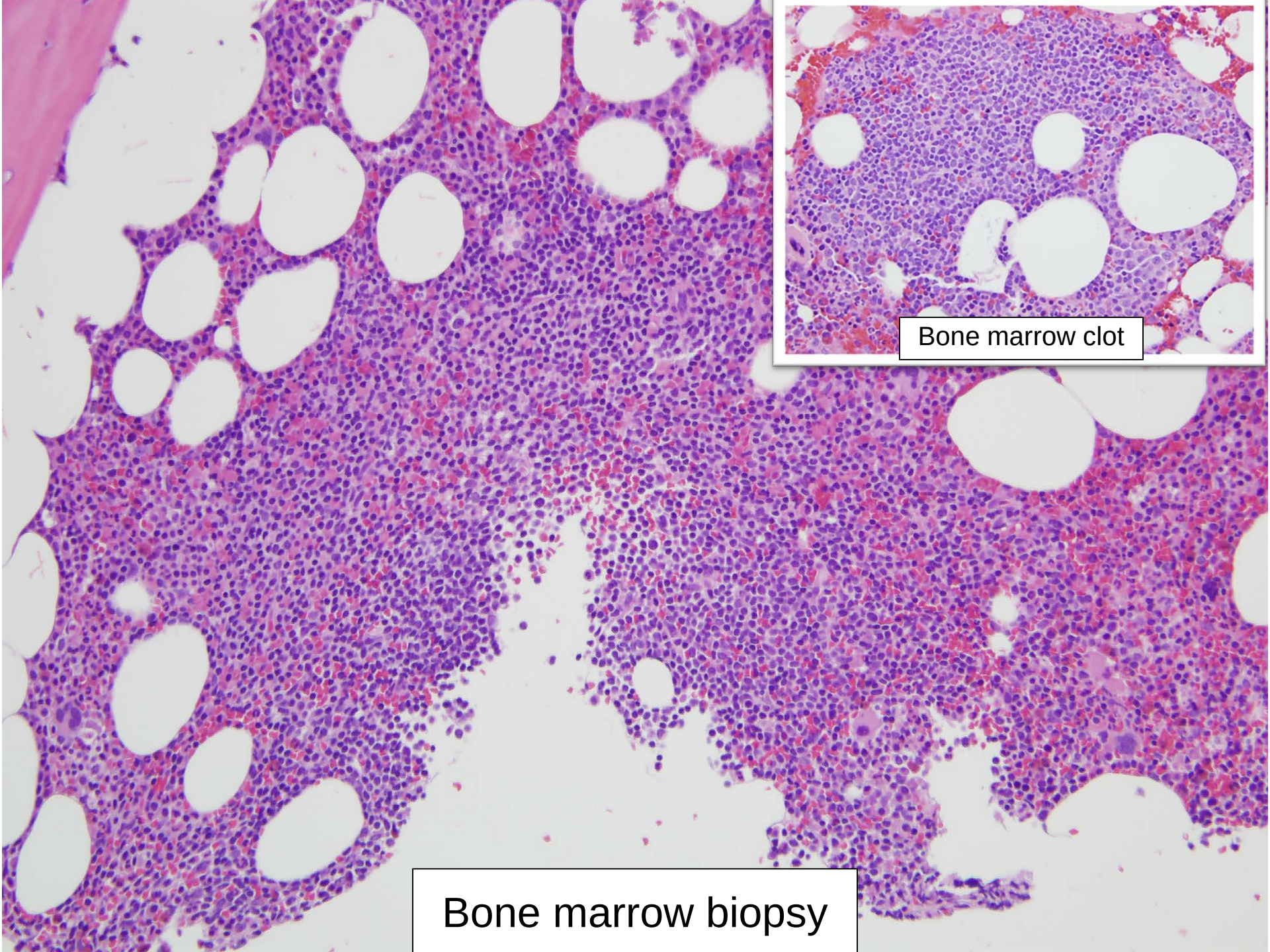
Peripheral Blood Flow Cytometry

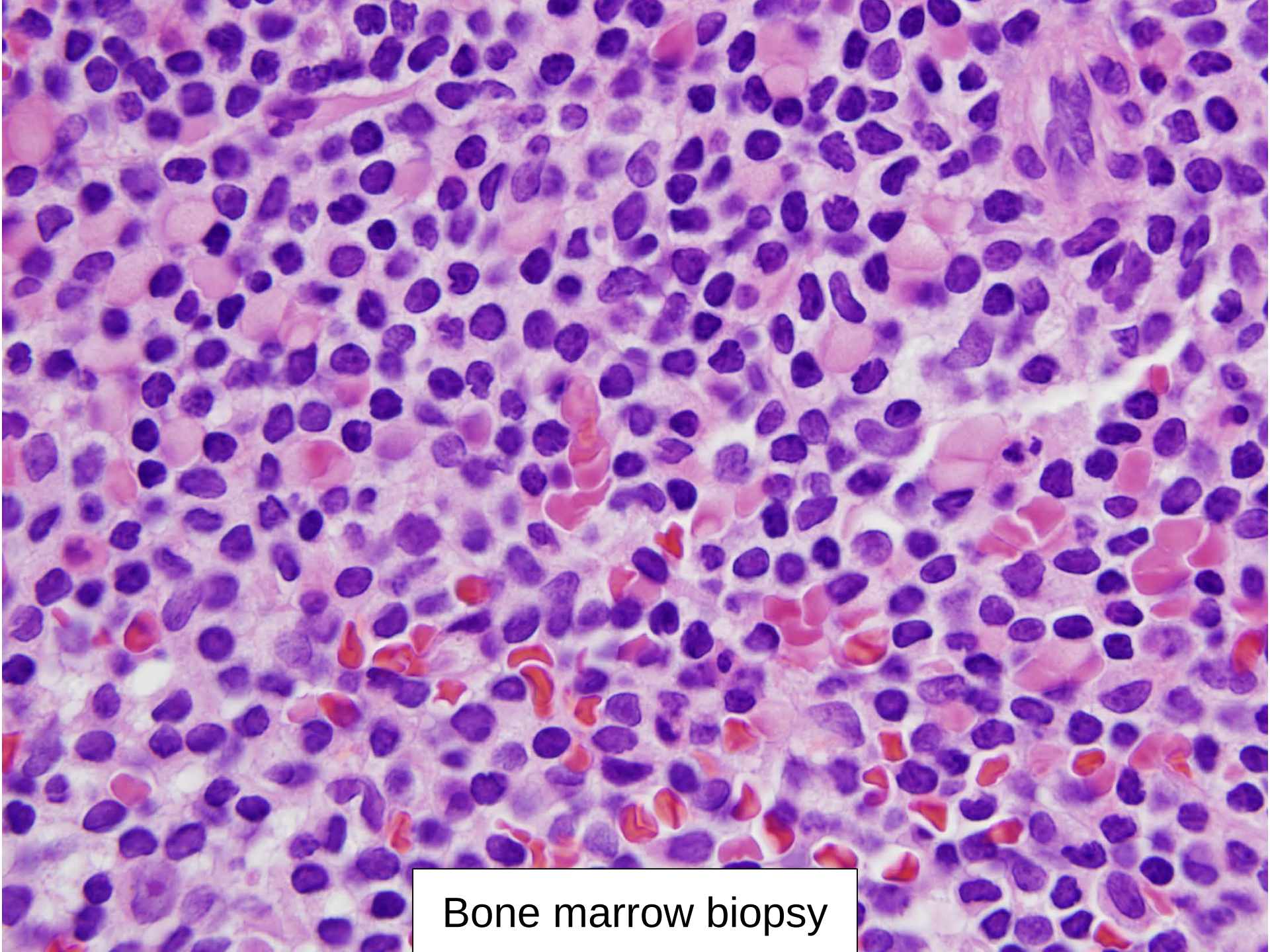


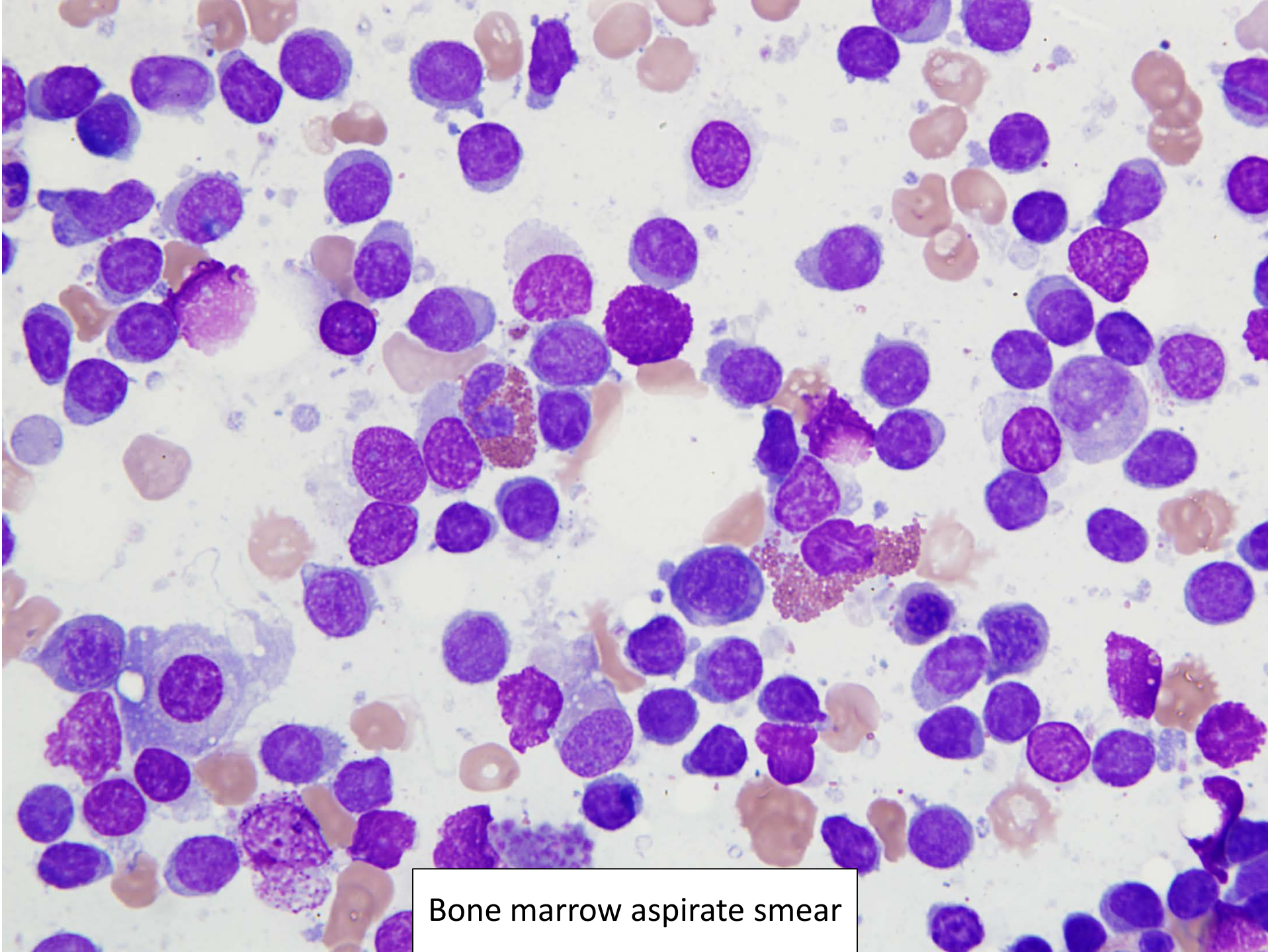







Bone marrow clot
Bone marrow biopsy


Bone marrow biopsy


Bone marrow aspirate smear

Cytogenetic and Molecular Genetic Studies

- 46,XY[20] (bone marrow)
- Fluorescence in situ hybridization performed on blood sample:
  - rearrangement involving *TCL1A* (14q32) in 29% nuclei
  - 17p13.1 deletion in 64% nuclei
  - deletion of chromosome 11 centromere in 60% nuclei
  - monosomy 13 in 60% of nuclei
  - 6q23 deletion in 21% of nuclei
- *TCRG@* – clonally rearranged
- Mutation *TP53* G334V with allelic frequency of 55% (blood and bone marrow)
  - Negative for mutations of *JAK-STAT* pathway, cell cycle and epigenetic regulators in T-PLL
- P53 and STAT5B (phosphorylated) are not expressed by immunohistochemistry

Proposed diagnosis

T-cell prolymphocytic leukemia in indolent phase

T-cell Prolymphocytic Leukemia (T-PLL)

Formulating a diagnosis

- Morphology
- Immunophenotype
- TCR gene rearrangement
- Genetic alterations
  - Cytogenetics
  - Molecular

T-PLL: Morphology

- Irregular nuclear membrane; mature, condensed chromatin, prominent nucleolus; cytoplasm- basophilic with protrusions
- Prolymphocytic morphology
- Small cell variant - chronic lymphocytic leukemia-like morphology (6%-19% with much higher frequency 38% in Japanese population)
- Sézary cell-like - cerebriform morphology (2%-7%)
- Multilobated morphology- Adult T-cell leukemia like (5%)

(Matutes *et al.* Blood 1991; Garand *et al.* Br J Haematol 1998; Kameoka *et al. Blood* 2012; Swerdlow (Eds.) 2008)

T-PLL: Immunophenotype

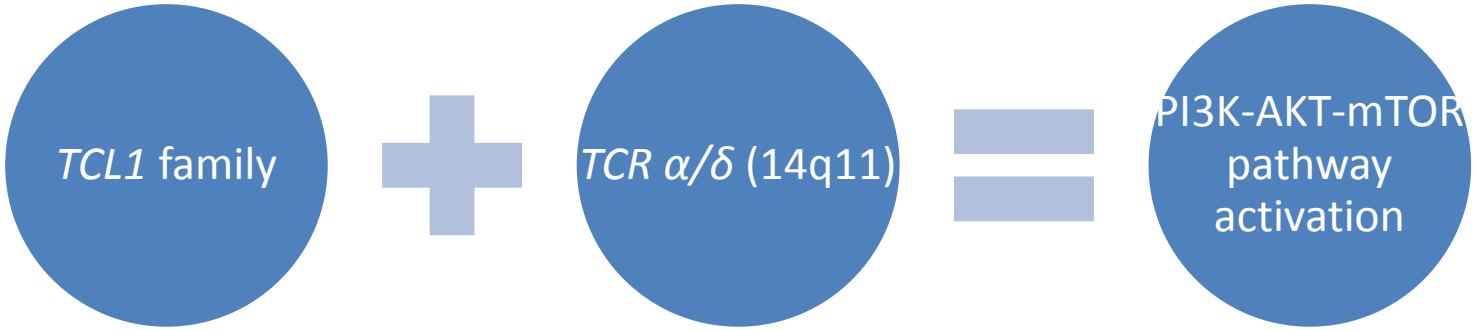
- Pan-T-cell antigens: CD2+, CD3+(dim, surface), CD5+, CD7+ (bright)
- CD4/CD8 variable
    - CD4+ CD8- (60-65%)
    - CD4+ CD8+ (17-21%)
    - CD4- CD8+ (13-15%)
    - CD4- CD8- (1-8%)
- Others:
    - CD52+, CD26+, TCL1 +
    - TdT - , CD1a- , CD25- (rare positive)
- Postulated normal counterpart : Post-thymic mature T-cell

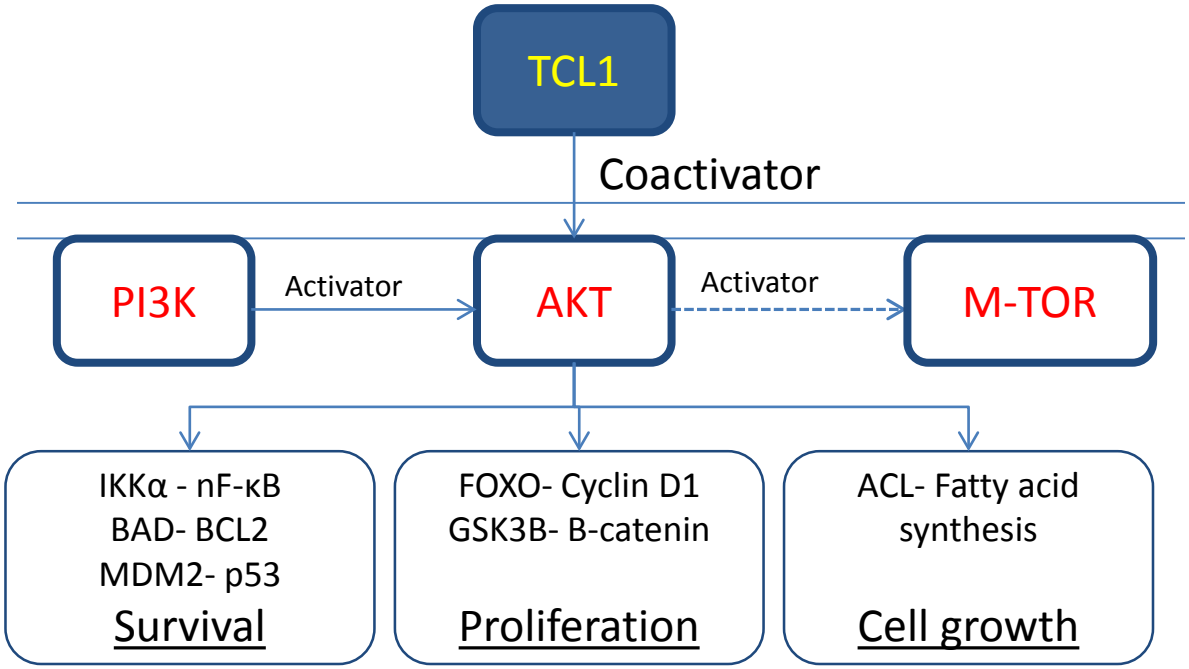
(Matutes *et al.* Blood 1991; Garand *et al.* Br J Haematol 1998; Saitou *et al.* 2000; Swerdlow et al(Eds.) 2008)

T-PLL: Cytogenetic abnormalities

-*TCL1A/TCL1B* (14q32.1) (80%)

-*MTCP1* (Xq28)





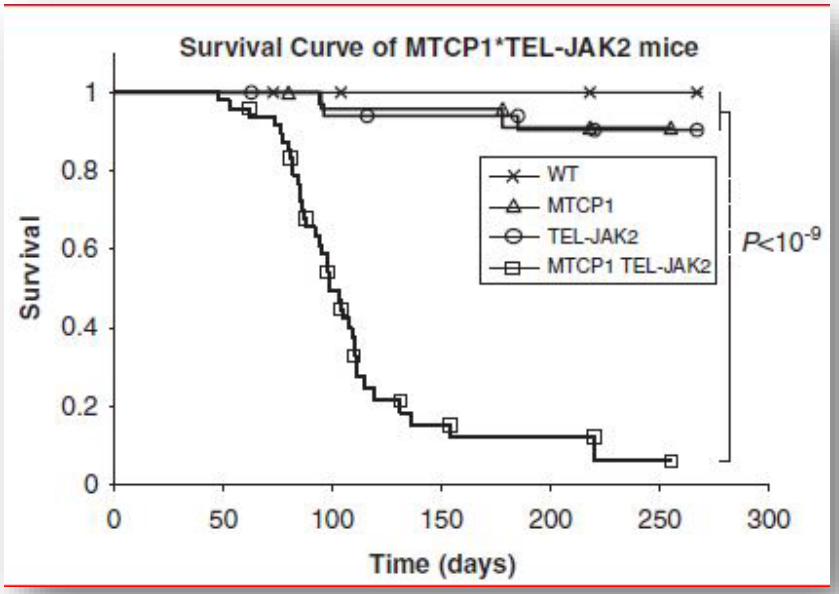
From Wagner et al. (Eds.). Cancer Signalling

(Maljaei *et al.* Cancer Genet Cytogenet 1998; Swerdlow (Eds.) 2008; Madani *et al. Blood* 1996; Laine *et al.* Mol Cell 2000; Narducci *et al. Blood* 1995; Hashimoto *et al.* Oncogenesis 2013)

Are rearrangements of *TCL1* family genes sufficient for leukemogenesis?

- *TCL1* or *MTCP1* transgenic mice develop mature T-PLL like leukemia (15-20 months):
  - *TCRβ@* monoclonal
  - CD3+/CD8+ immunophenotype predominant in most mouse models
  - Both prolymphocytic and small cell morphology were identified
- "Pre-leukemic phase" characterized by:
  - Lymphocytosis (75-90% blood lymphocytes vs. normal ~60%)
  - TCL1 positive T cell aggregates in spleen – which may be polyclonal by *TCRβ@*

- p13^{MTCP1} oncoprotein showed a dose - response relation with leukemia incidence
- Early and aggressive presentation in a transgenic line with intermediate level p13^{MTCP1} expression- potential role of other genetic factors
- Double transgenic mice *MTCP1 + TEL-JAK2* die significantly faster



(Bellanger *et al.* Leukemia 2014; Virgilio *et al.* Proc Natl Acad Sci 1998; Gritti *et al. Blood* 1998)

T-PLL: Cytogenetic abnormalities

- *ATM* (11q22.3): deletions, loss of heterozygosity/biallelic deletions
  - Ataxia telangiectasia patients may have circulating T lymphocytes with chromosome 14 abnormalities without clonal *TCR* gene rearrangements
  - Narducci *et al.* described ataxia telangiectasia patient with a persistent T-cell population with inv14 and expansion of this population from 4% to 60% over nearly 8 years without leukemic manifestations

- Other anomalies
  - Gains of 8q and losses of 8p (70-80%)
  - Deletions of 12p13 (43%)
  - Chromosome 6 (33%)
  - Chromosome 17 alterations (33%)
    - P53 over-expressed in all cases with allele deletion or any chromosome 17 anomaly
  - 22q alterations (6%)

(Vorechovský *et al.* Nat Genet. 1997; Stilgenbauer *et al.* Nat Med. 1997; Maljaei *et al.* Cancer Genet Cytogenet 1998; Swerdlow (Eds.) 2008; : Brito-Babapulle *et al.* Br J Haematol 2000; Le Toriellec *et al.* Blood 2008*;* Narducci *et al.* Blood 1995; Taylor *et al.* Blood 1996)

T-PLL: JAK-STAT pathway

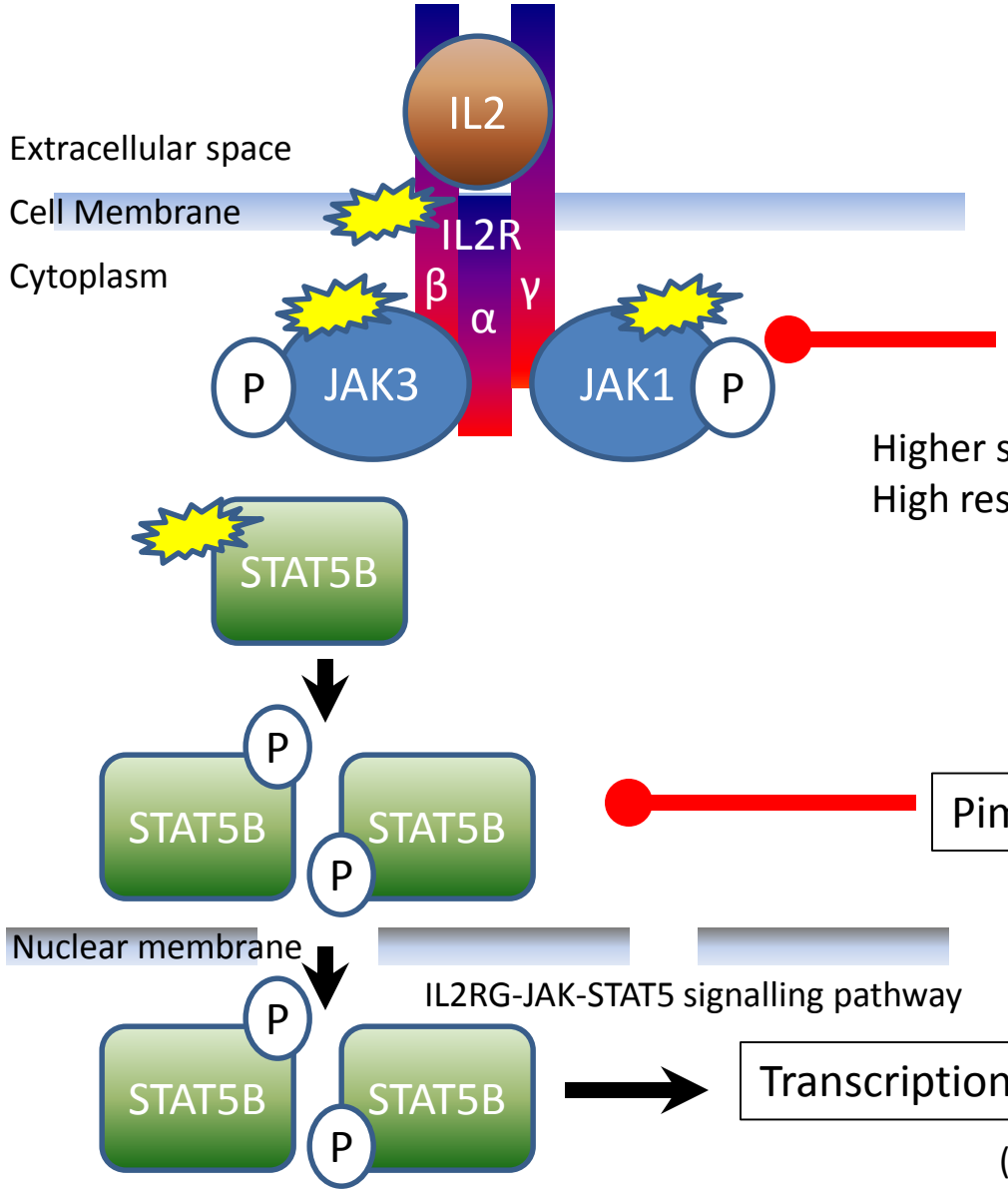
JAK-STAT pathway mutations involve 76% T-PLL cases

Gene	Chrom-osome	Cases involved	Molecular alterations	Protein Domain Involved
IL2RG	X	2%	G268_M270del*, K315E*	Transmembrane domain, other regions
JAK1	1	8%	R629_D630del*, V658F, S703I, T901R*	Pseudokinase domain
JAK3	19p	30%-42%	M511I, A573V, R657W*, Q507P, K563_C565del*	SH2- Pseudokinase junction, Pseudokinase domain
STAT5B	17	7%-36%	T628S*, N642H, R659C*, Y665H, Q706L*	SH2 domain

* Novel mutation

(Kiel *et al.* Blood 2014; Bellanger *et al.* Leukemia 2014; Bergmann *et al.* Genes Chromosomes Cancer 2014; López *et al.* Br J Haematol 2016, Andersson *et al.* Leukemia 2017)

Targeting JAK-STAT pathway



Ruxolitinib (JAK1/2)
Tofacitinib (JAK1/3)

Higher sensitivity to ruxolitinib than to tofacitinib
High resistance of *STAT5B* (p.N642H) to JAK inhibitors

Pimozide

Reduce phosphorylated STAT5 levels
Reduce STAT5 transcriptional activity
Induce apoptosis in T-PLL cells

IL2RG-JAK-STAT5 signalling pathway

Transcriptional activation

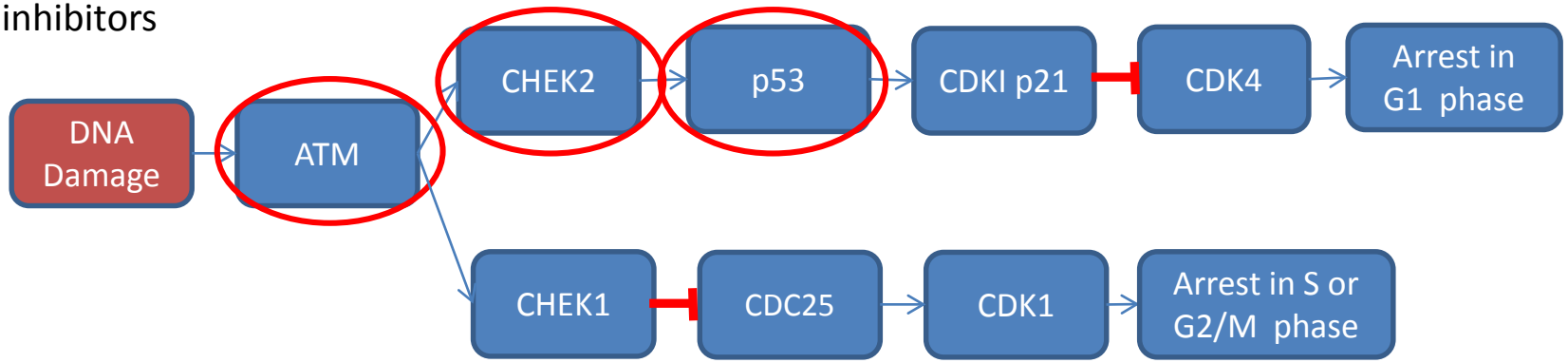
(Kiel *et al.* Blood 2014, Anderson *et al.* Leukemia 2017)

T-PLL: Additional Molecular Abnormalities

Gene	Chromosome	Cases involved	Alteration	Domain/Function
ATM	11q22	70%	Frameshift, nonsense and missense	FAT and PI3K domains
EZH2	7	13%	Frameshift, nonsense and missense	Transcriptional repressor
CHEK2	22	5%	Frameshift, Missense	Protein kinase -DNA repair
FBWX10	17	7.5%	Frameshift, nonsense and missense	Ubiquitin ligase
TET2	4	17%	Missense mutations	DNA methylation
BCOR	X	8%-9%	Missense mutations	Histone deacetylation
TP53	17p	14%	Missense mutation	DNA repair

High selective drug sensitivity scores:

- CDK inhibitor
- P53 activator
- MDM2 inhibitors



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(Kiel *et al.* Blood 2014; López *et al.* Br J Haematol 2016; Stengel *et al.* Genes Chrom Cancer 2015; Andersson *et al.* Leukemia 2017)

T-PLL: Clinical - Molecular correlation

TCL1 family Cytogenetic anomaly	JAK-STAT pathway mutation	Case% (number)
Absent	Present or absent	20% (10)
Present or absent	Absent	24% (12)
Absent	Absent	6% (3)

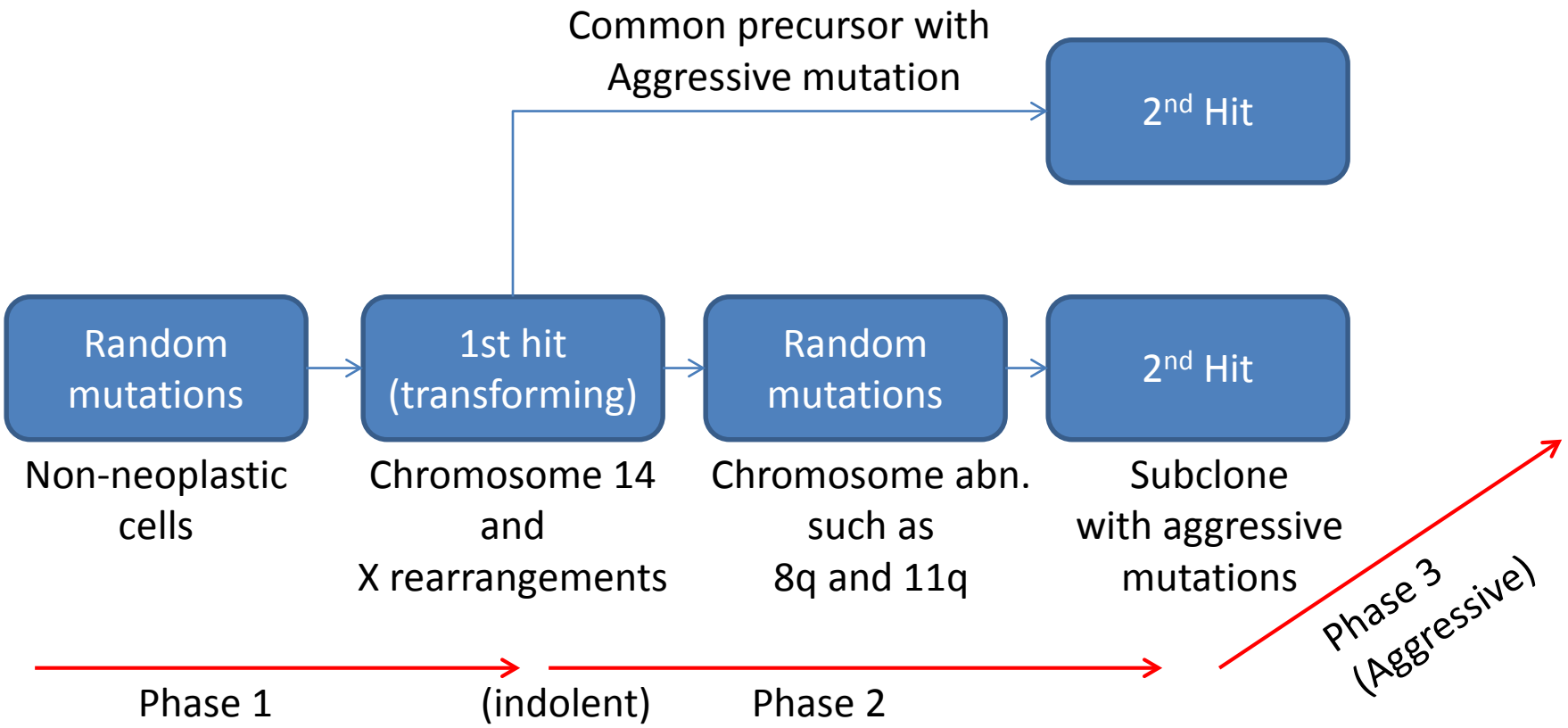
(Kiel *et al.* 2014)

- In general, *JAK-STAT* pathway mutation status did not correlate with overall survival

- However, *JAK3* mutation carry a poor prognosis on median overall survival (OS)
  - $JAK3^{mut}$ vs. $JAK3^{wt}$ OS: 11 months (n=7) vs. 37 months (n=33); P=0.018 (Stengel *et al.* 2015)
  - $JAK3^{mut}$ vs. $JAK3^{wt}$ OS: 15 months (n=14) vs. 48 months (n=17); P=0.008 (Andersson *et al.* 2017)
  - $JAK3^{p.M511Imut}$ vs. all patients median OS: 15.1 months vs. 27.1 months, trend noted (Kiel *et al.* 2014)

(Kiel *et al.* Blood 2014; Bergmann *et al.* Genes Chromosomes Cancer 2014; Stengel *et al.* Genes Chromosomes Cancer 2015)

Molecular evolution in T-PLL



(Rashidi *et al.* Eur J Haematol. 2015)

Final Panel Diagnosis

T-cell prolymphocytic leukemia