2nd POSTGRADUATE CLL Conference

CLL pathogenesis: microenviromental and genetics

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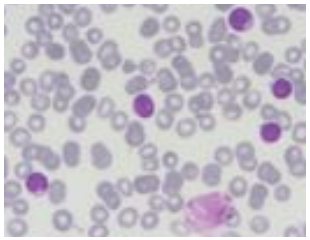
Universitat de Barcelona

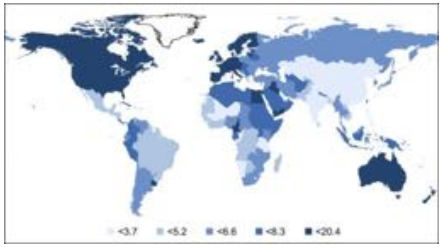
Clínic Barcelona Hospital Universitari

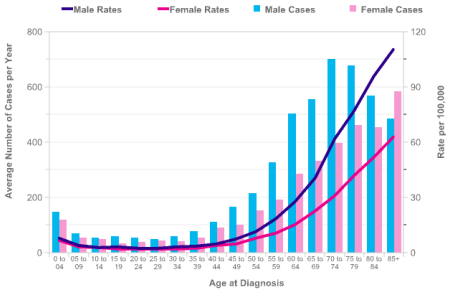
No conflict of interest

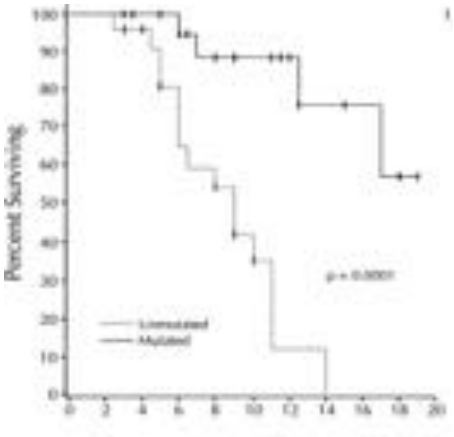
Chronic Lymphocytic Leukemia



- **Most frequent leukemia in adults in Western countries (5-7 cases /100,000 /year)**
- **Median age at diagnosis 70 years and increases with age**
- **Male predominance**
- **Incurable and heterogeneous disease with two major molecular subtypes**
- **Familiar risk**







Damle RN et al, Blood 1999;94(6):1840-7

CLL Pathogenesis

Integrating genetic and microenvironment interactions

Genetic susceptibility

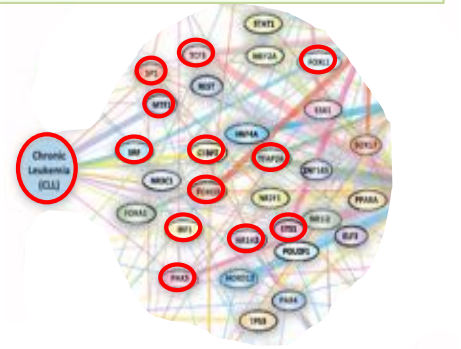
- Familial aggregation
- Susceptibility loci

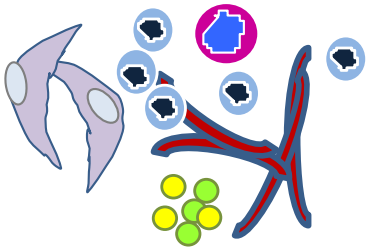
Microenvironment Interactions

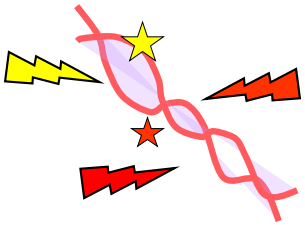
- Skewed IG gene repertoire
- Stereotyped BCR

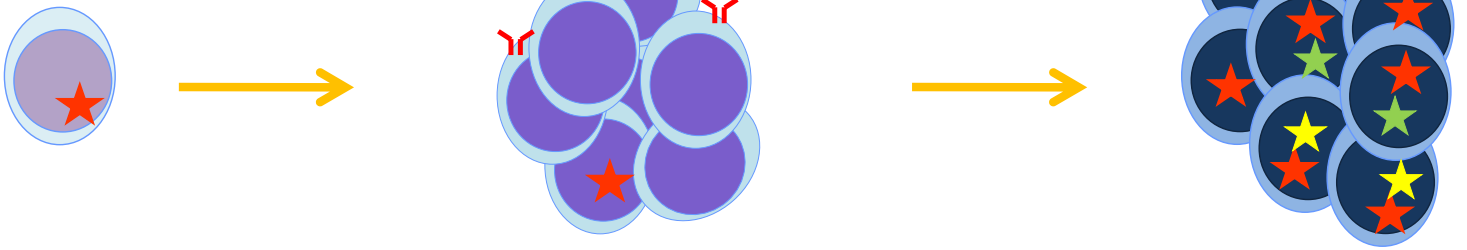
Somatic Genetic Alterations

- Chromosomal abnomalities
- Recurrent somatic mutations





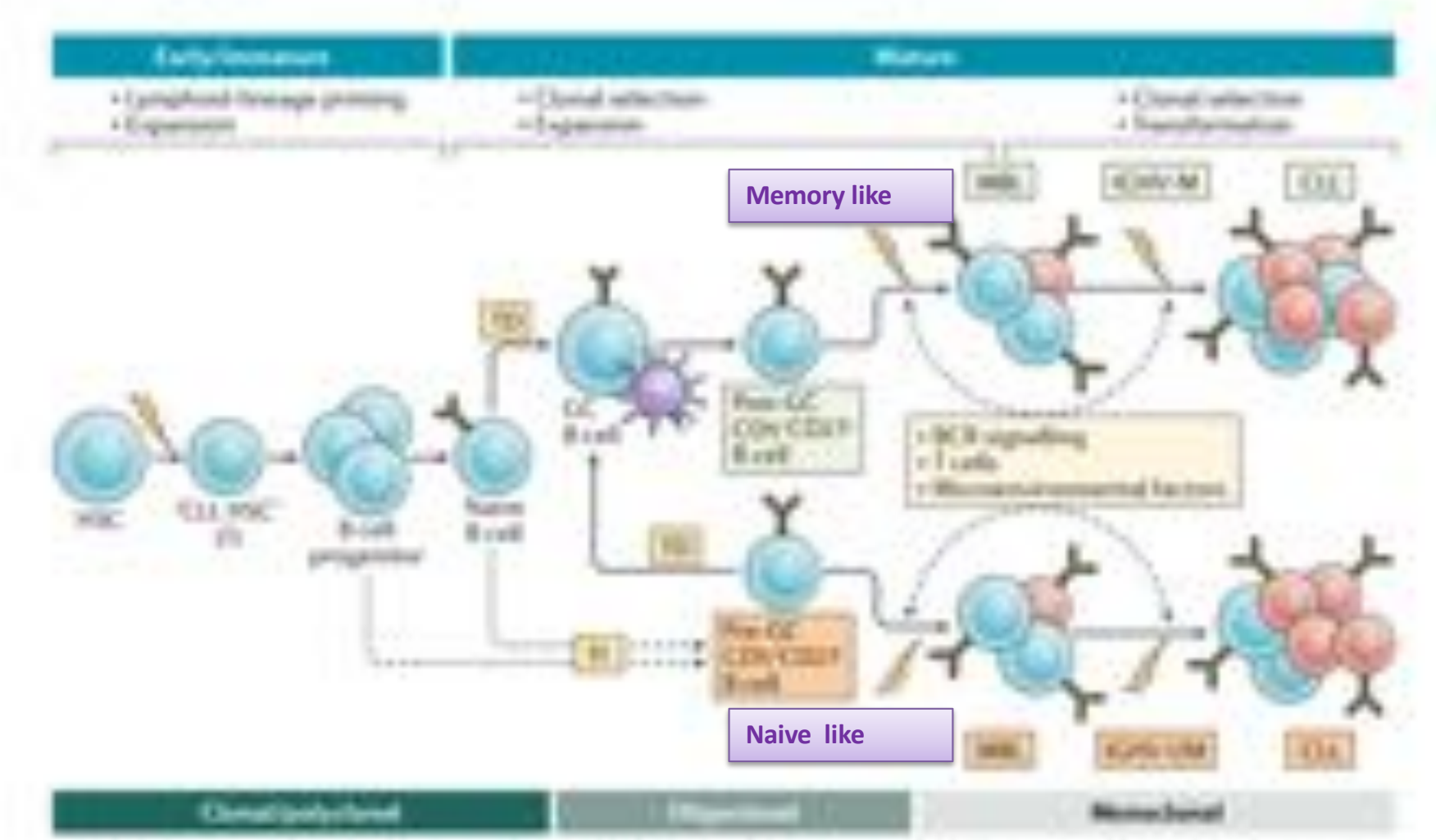




Microenvironment → **Genetic alterations**

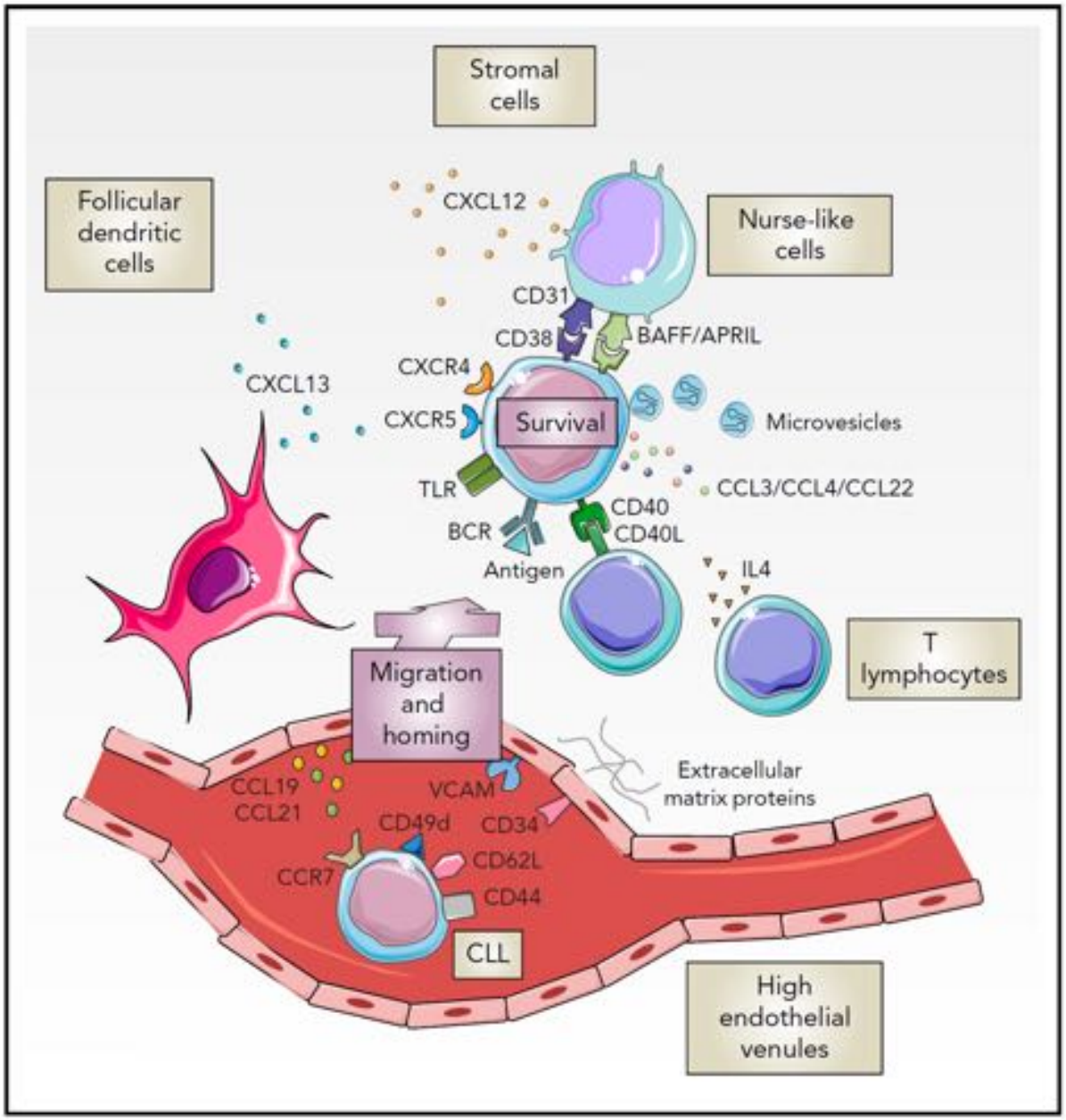
Modified from Puente XS, Jares P, Campo E. Blood. 2018 May 24;131(21):2283-2296.

The cellular origin of CLL



Modified from Bosch F, Dalla-Favera R.. Nat Rev Clin Oncol. 2019 Jul 5. doi: 10.1038/s41571-019-0239-8.

CLL MICROENVIRONMENT



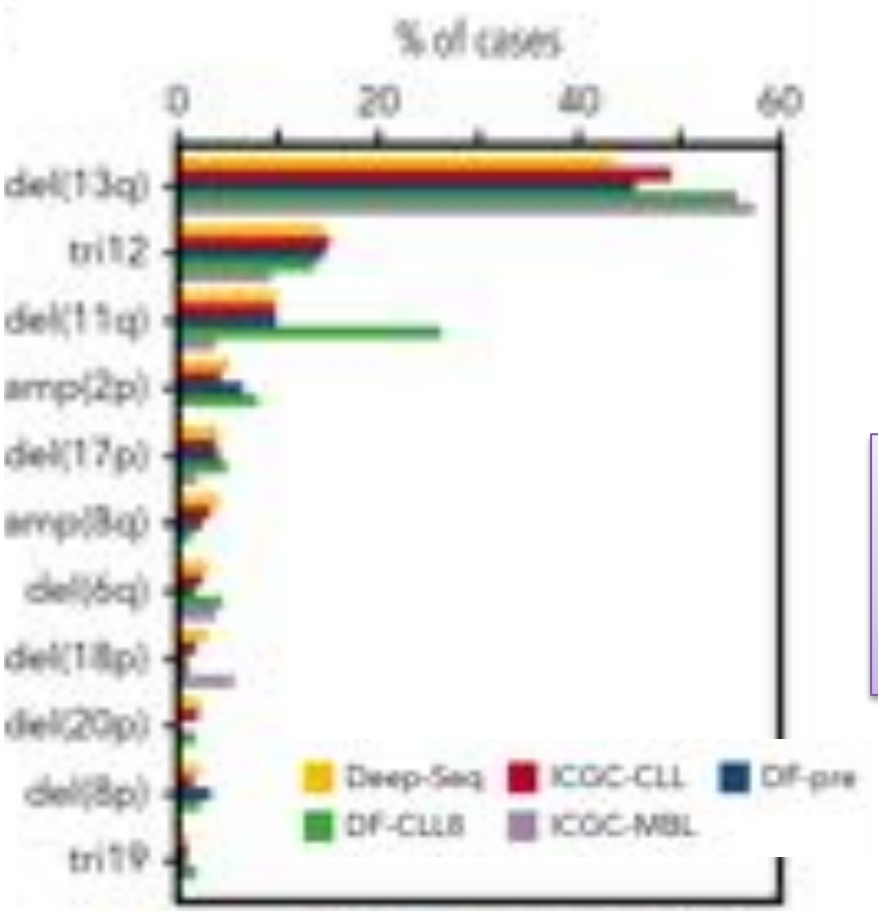
✓CLL cells recirculate between peripheral blood and tissues, mainly lymph nodes and bone marrow, where they receive survival and proliferation signals. These crucial interactions mainly occur in the proliferation centers of lymph nodes infiltrated by CLL that contain a loose meshwork of follicular dendritic cells (FDCs), a variable number of T cells, and other stromal cells

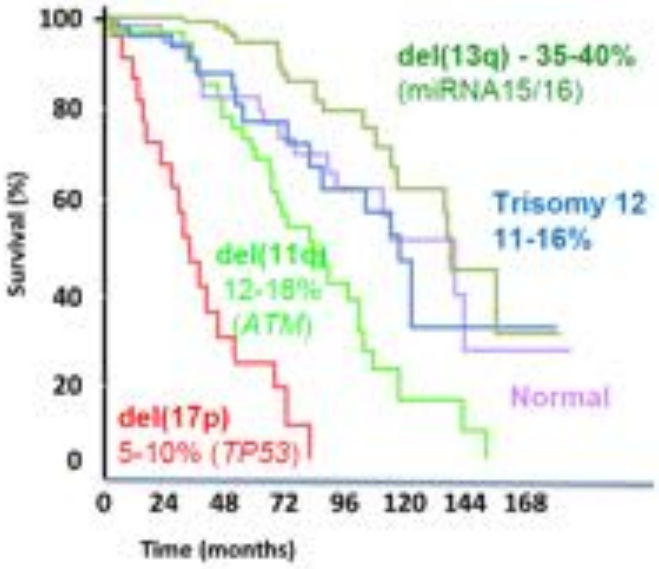
Puente XS, Jares P, Campo E.. Blood. 2018 May 24;131(21):2283-2296.

CLL: Genetics

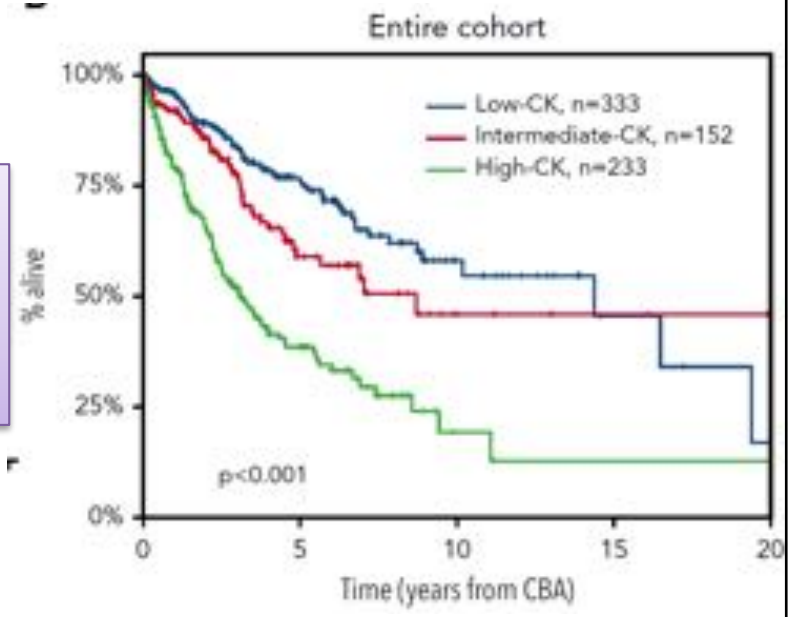
Cytogenetics (abnormal>80% patients)

- ✓Gains and loses (-11q, +12, -13q, -17p)
- ✓Not specific, prognostic information
- ✓Complex karyotype





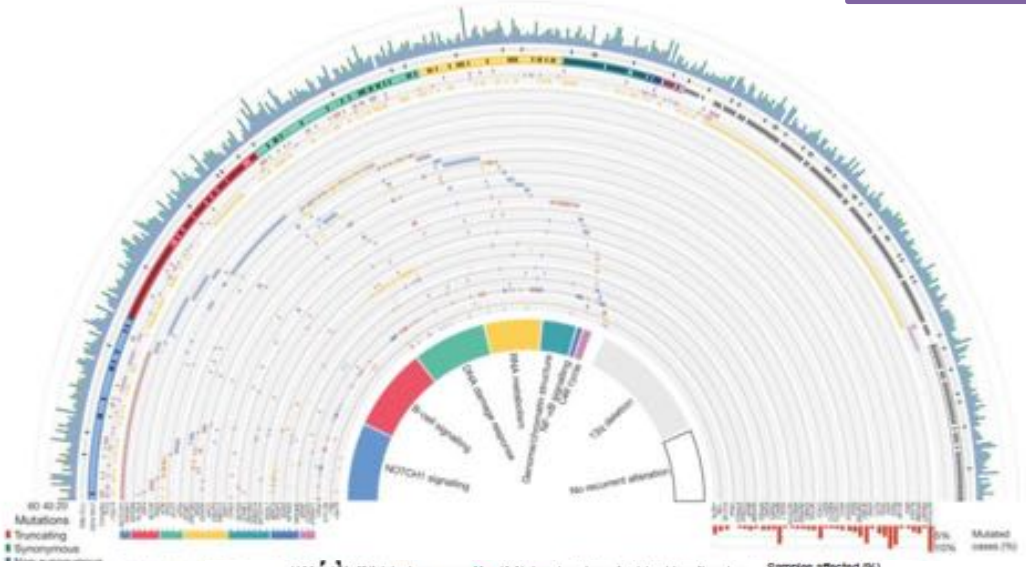
NUMBER ABERRATIONS
Low CK : 3
Intermediate CK: 4
High CK ≥ 5

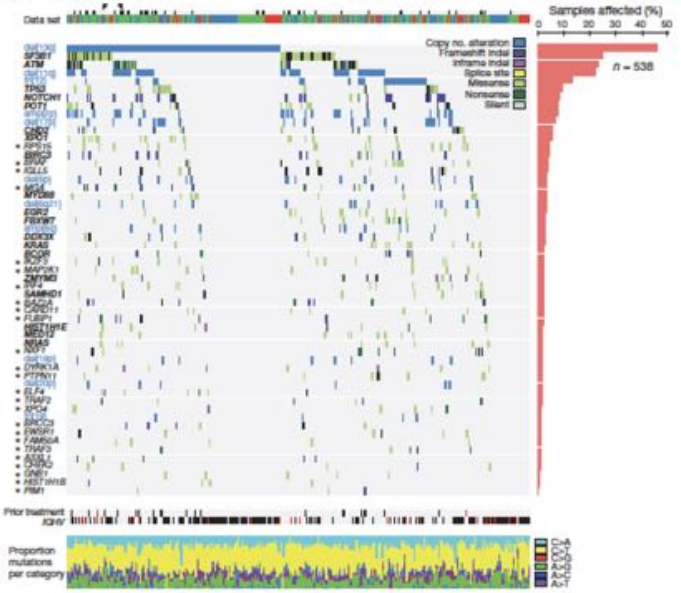


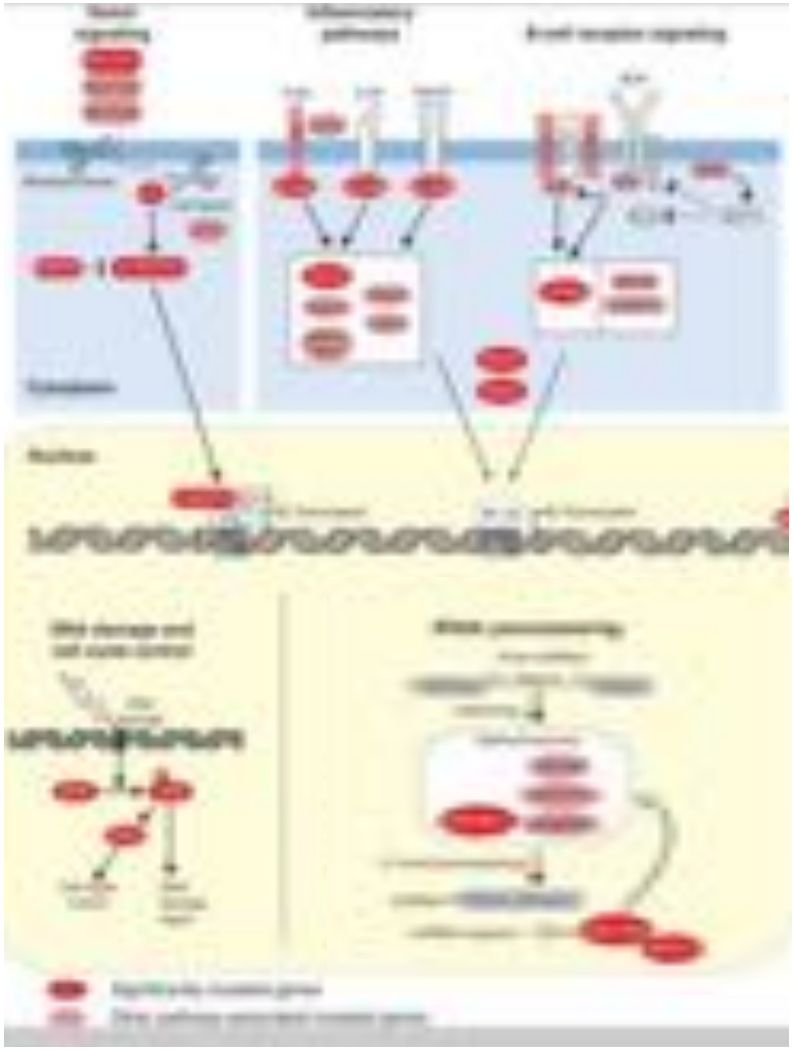
Modified Döhner H, N Engl J Med. 2000;343:1910-6; Puente XS, et al. Blood. 2018;131:2283-2296; Baliakas P, et al Blood. 2019;133(11):1205-1216.

Mutational landscape CLL

✓Mutational diversity

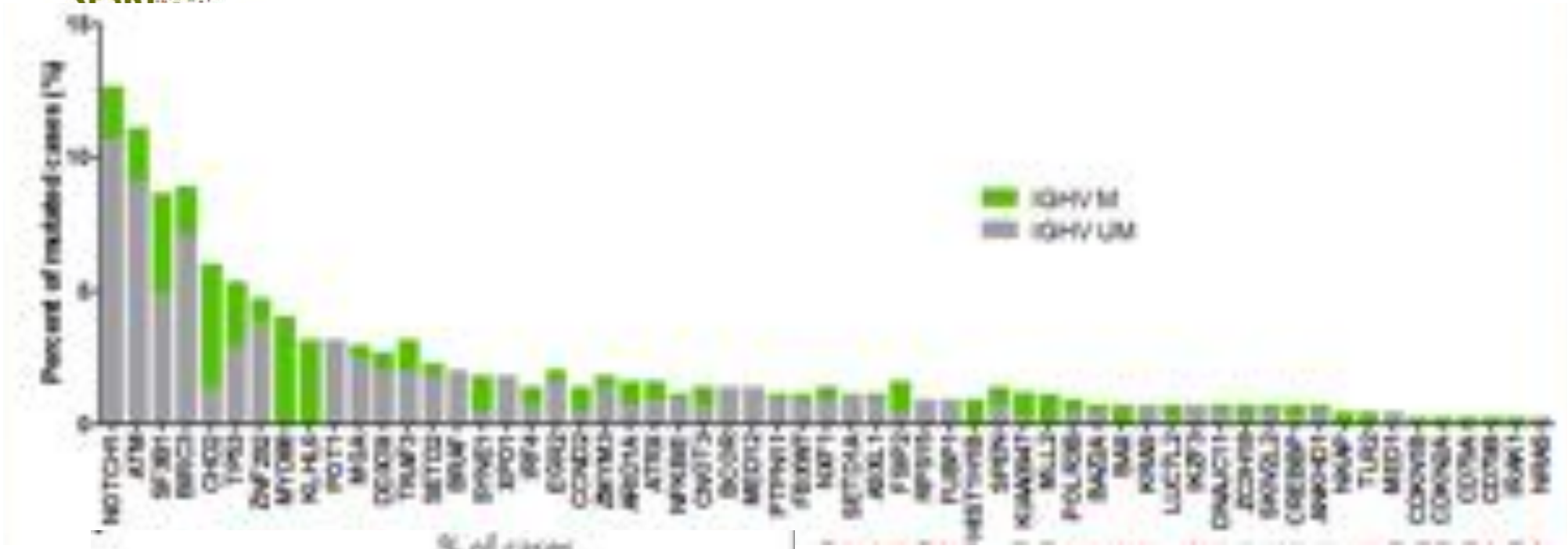


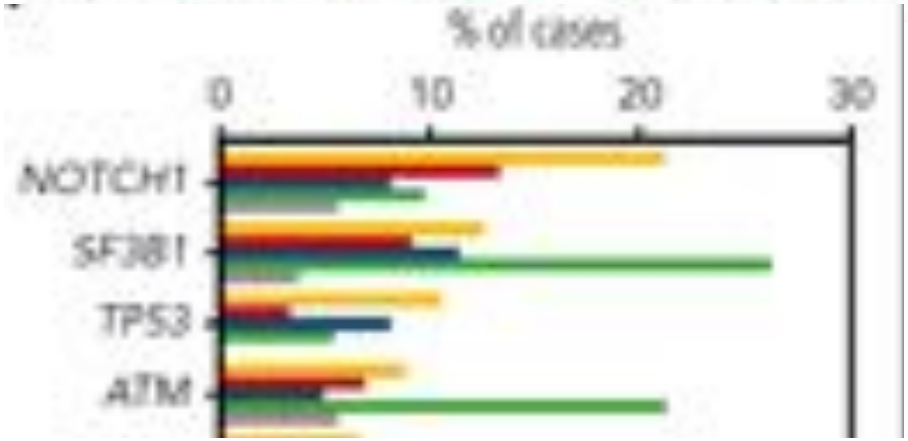




Puente XS,et al Nature. 2015;526:519-24; Landau DA, et al. Nature. 2015;526:525-30; Modified from Landau & Wu, Genome Med. 2013; 5(5): 47.

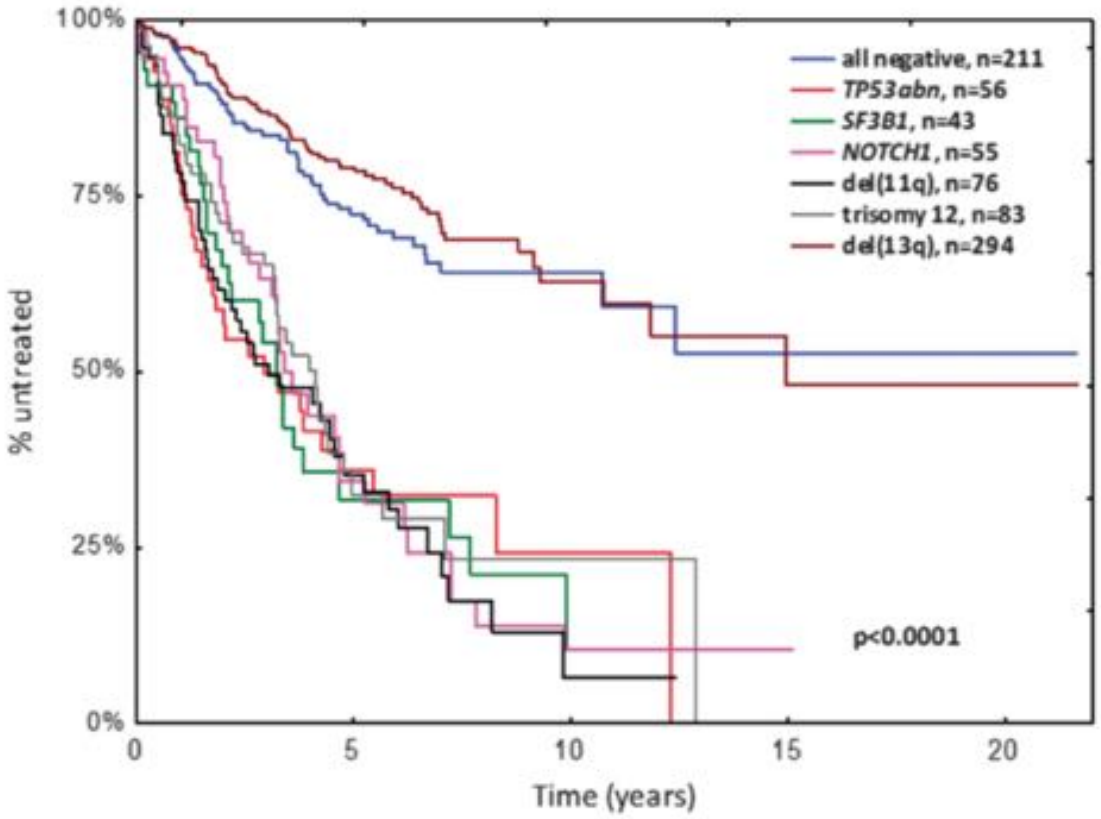
Mutational landscape CLL





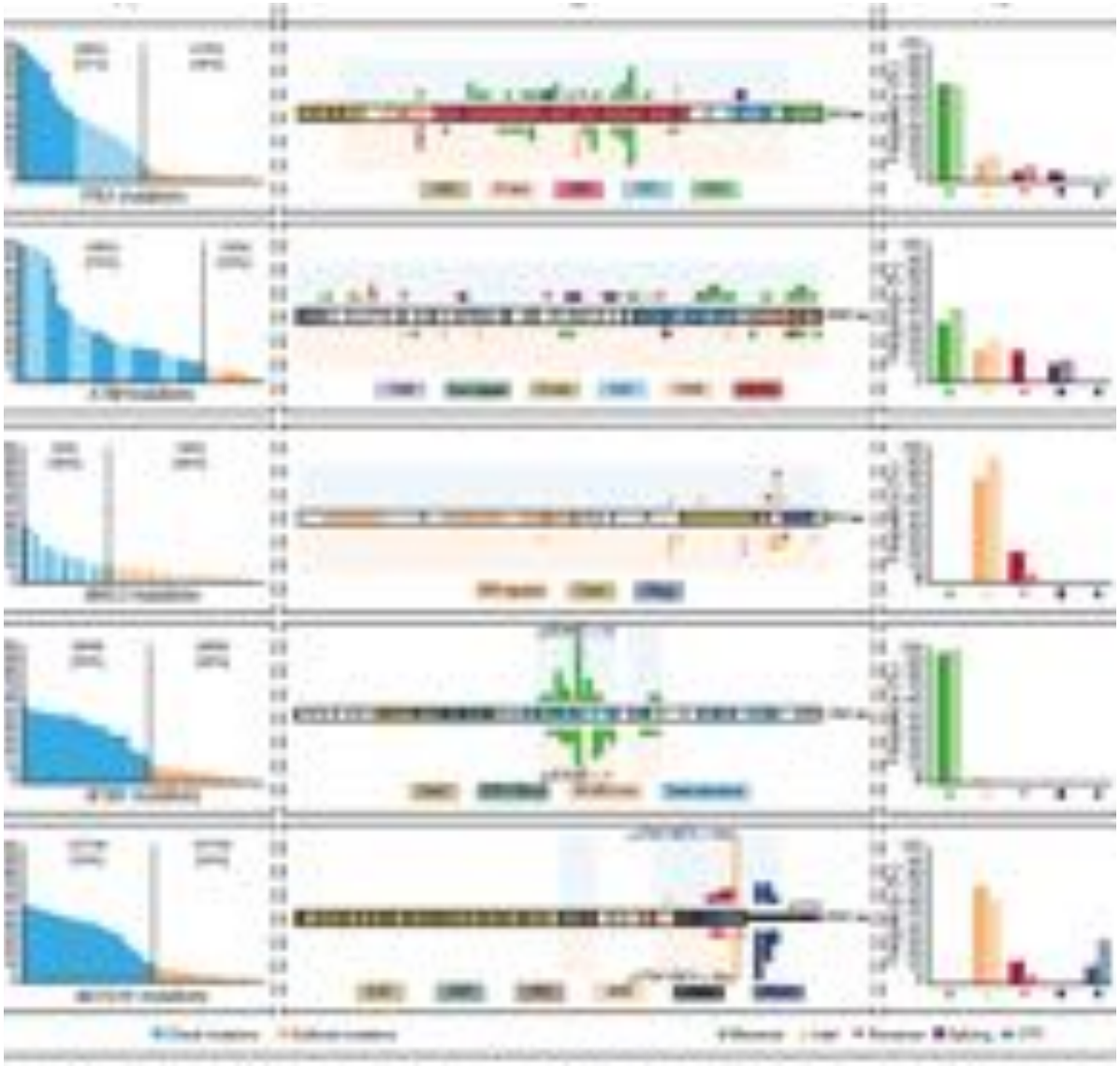
Dara taken from *Puente XS,et al Nature. 2015;526:519-24 and Puente XS et al, Blood. 2018 May 24;131(21):2283-2296*

Recurrent mutations and structural variants refine prognosis



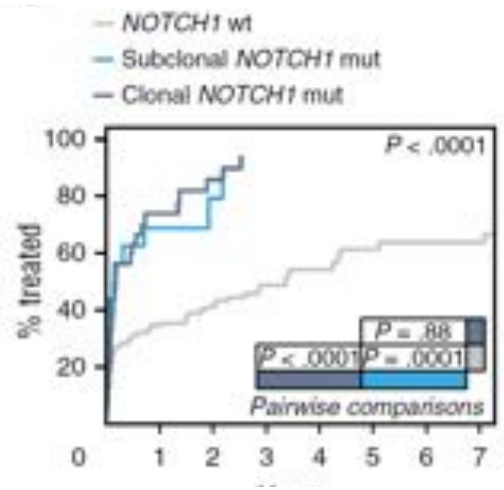
Baliakas P, et al. Leukemia. 2015;29(2):329-36

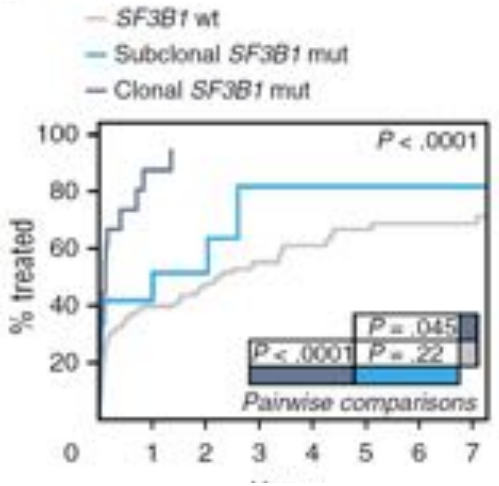
Most of the mutacions are subclonal

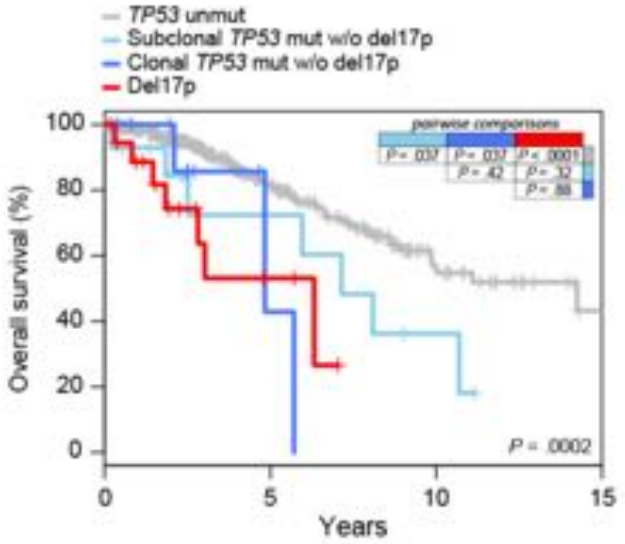


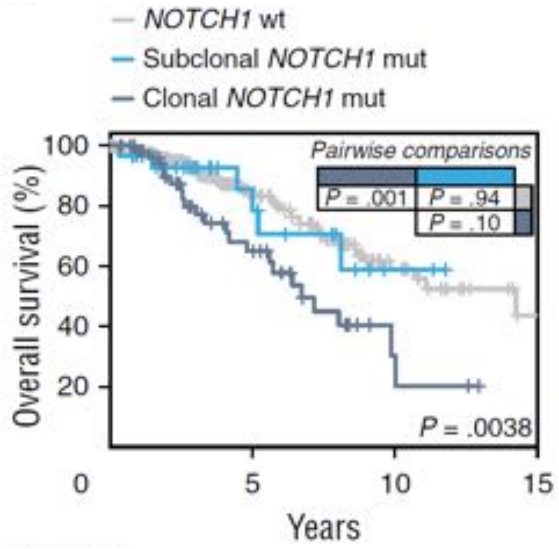
Nadeu F, et al.Blood. 2016;127(17):2122-30

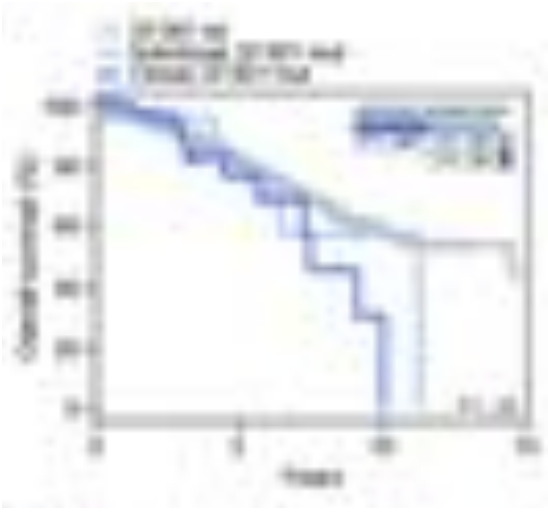
Impact of subclonal mutations

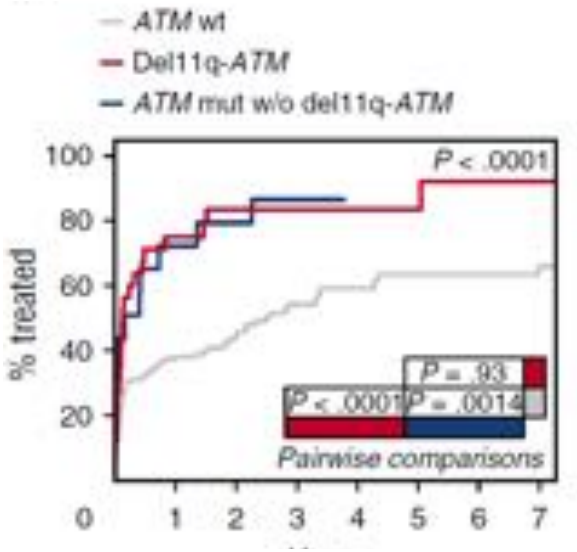






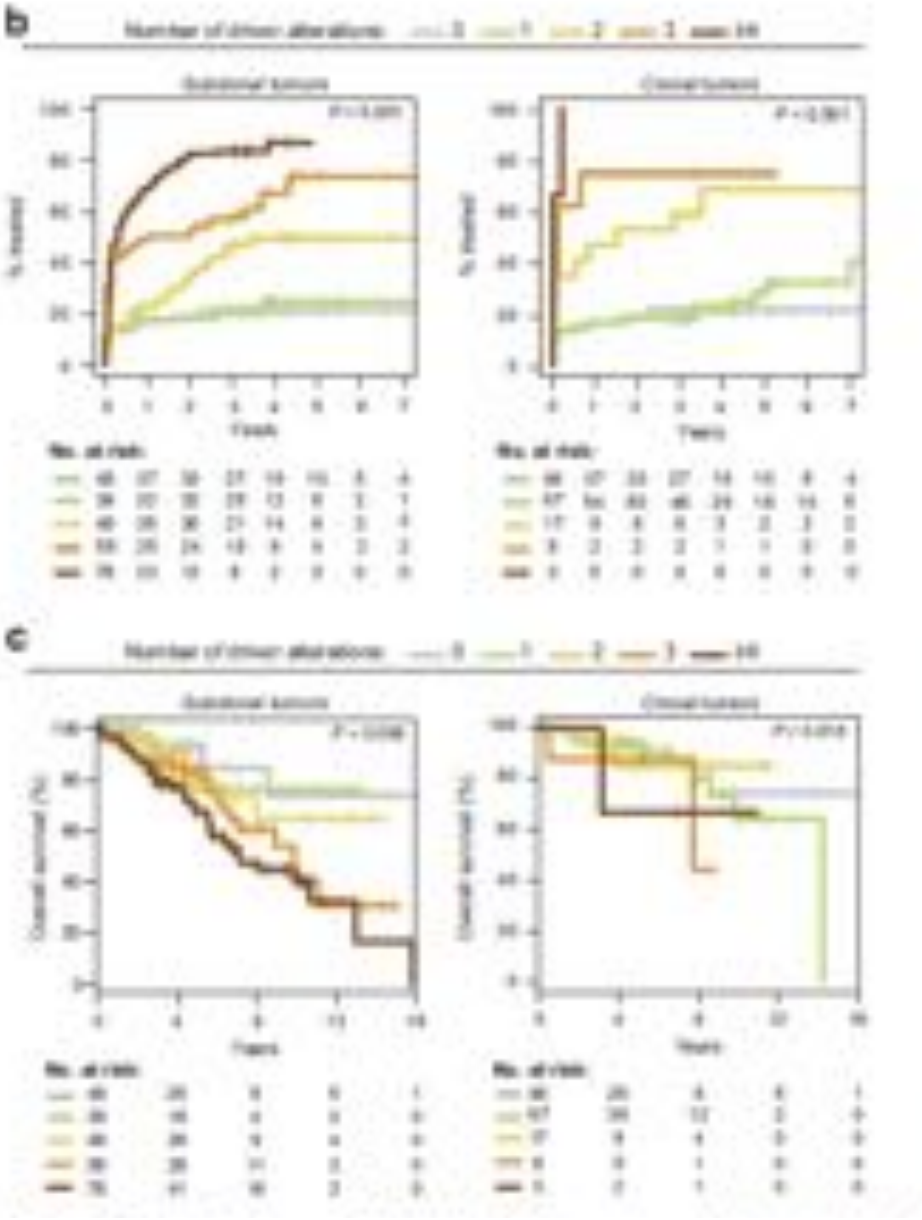






Nadeu F, et al.Blood. 2016;127(17):2122-30

Role of the subclonal architecture and mutational complexity in CLL evolution



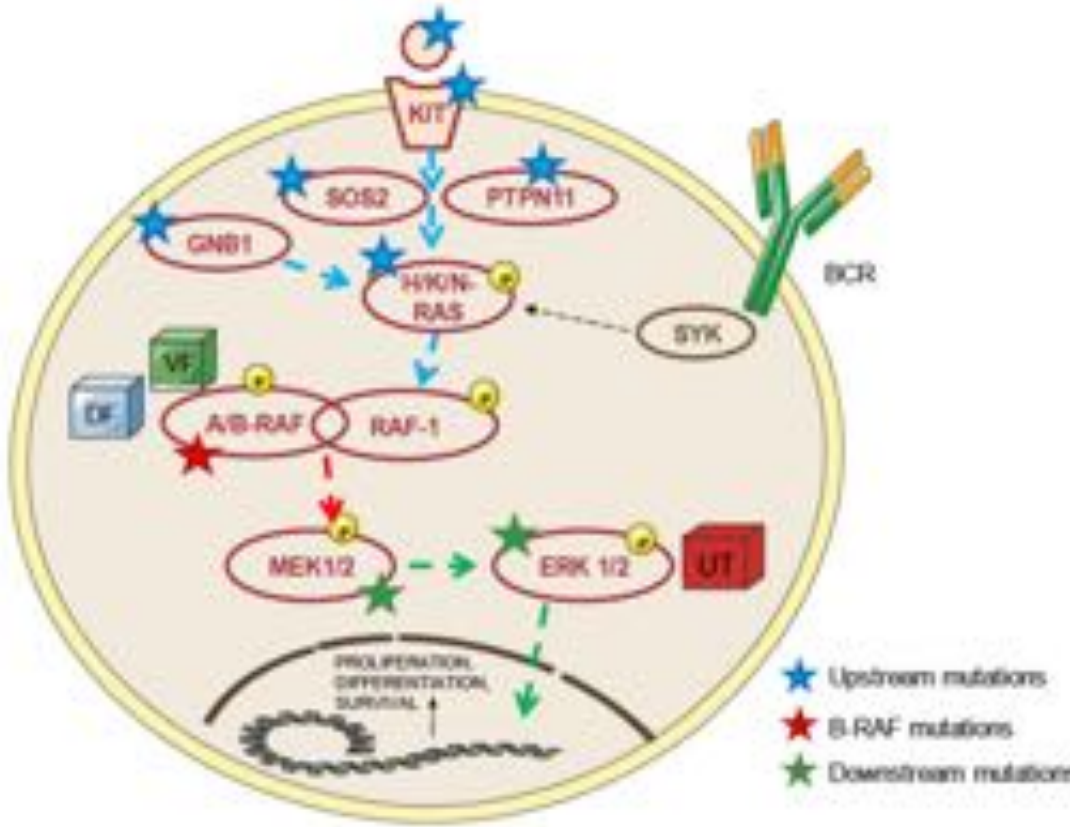
✓The number of drivers in both clonal and subclonal tumors gradually shortened the TTFT of the patients with a similar prognostic value

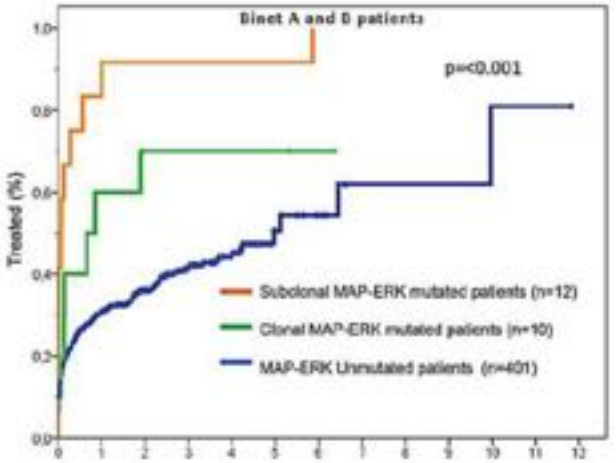
✓the number of drivers, rather than their clonal/subclonal representation, is the main predictor for short TTFT

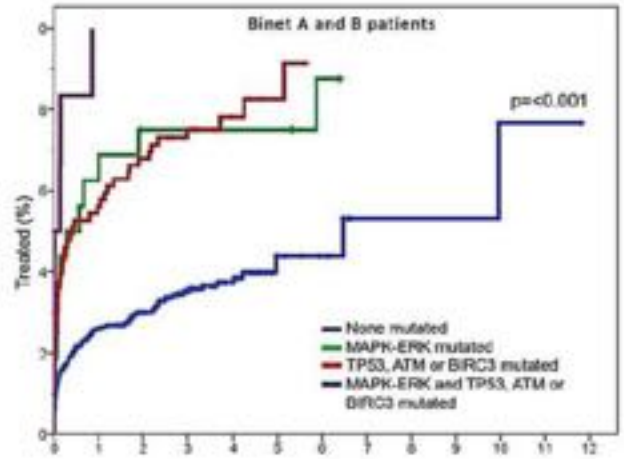
✓the number of drivers in subclonal tumors, but not in clonal tumors, was steadily associated with a worse outcome

Nadeu F, et al. Leukemia. 2018;32(3):645-653

RAS-BRAF-MAPK-ERK pathway mutations define a clinically aggressive subgroup



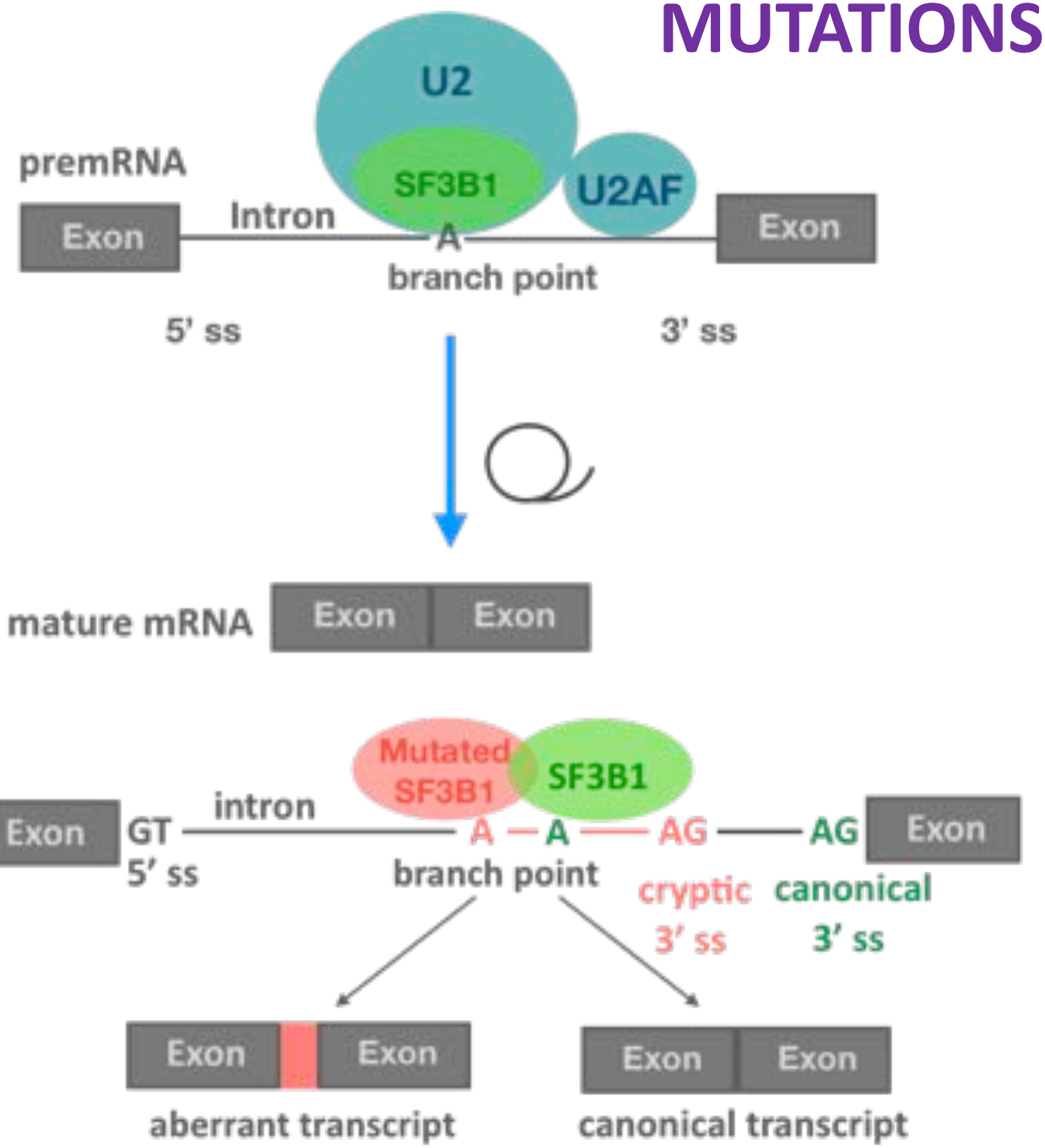




- ✓5.5 % of CLL cases
- ✓These mutations are missense, subclonal and mutually exclusive
- ✓Predominantly U-IGHV
- ✓High expression of ZAP-70, CD49d, CD38 and trisomy 12
- ✓Worse 5-year time to first treatment

Giménez N,et al. Haematologica. 2019;104(3):576-586.

MUTATIONS IN SPLICING GENES



✓SF3B1 mutated is mutated in >10% of patients with CLL with aggressive clinical and biological features

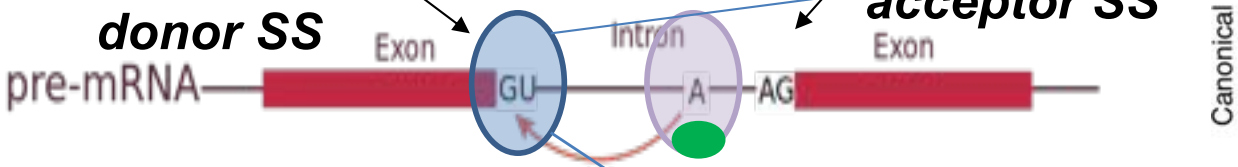
✓SF3B1 is a critical component of the splicing machinery (U2 component) in which the introns from precursor messenger RNAs are removed.

✓When SF3B1 is mutated change the branch point and new cryptic 3' sites is generated with the expression of aberrant transcripts

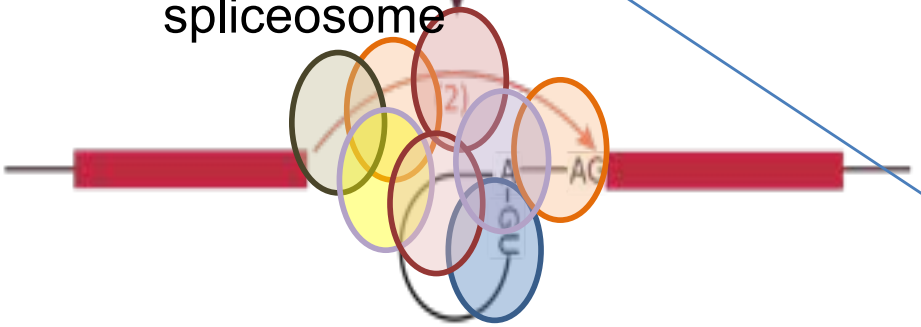
U1 a non-coding component of the spliceosome (snRNA)

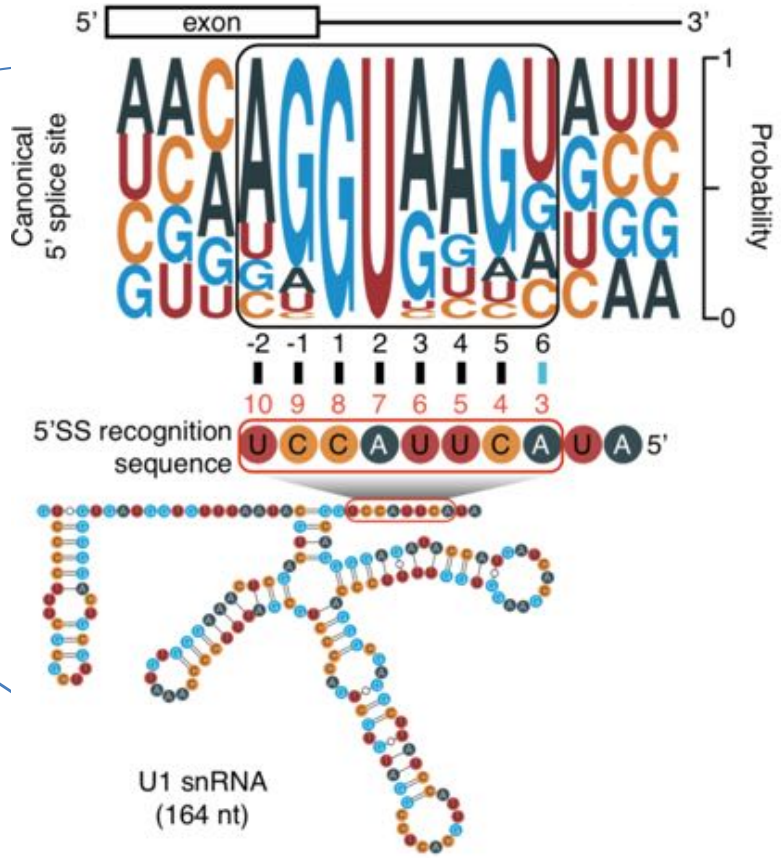
5' splice site (SS) or donor SS

3' splice site (SS) or acceptor SS



U1 snRNA U2 snRNA (SF3B1)





Normal Function: To recognize the 5'SS via base-pairing.

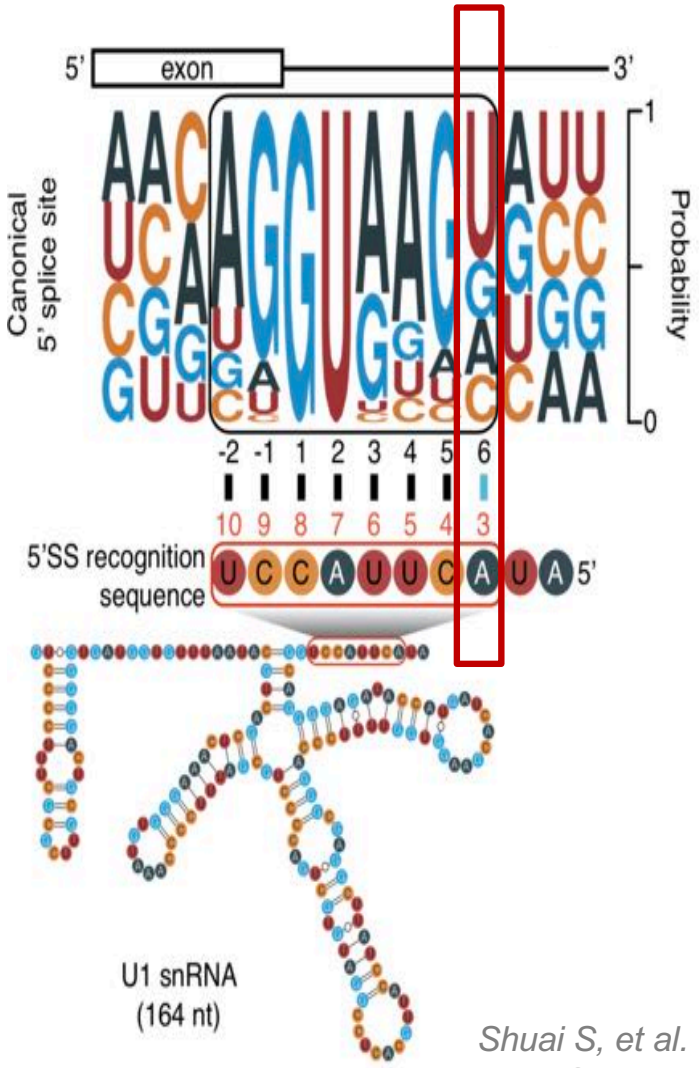
Spliced intron

+ 130 pseudogenes with variant U1 sequences

7 isoforms

RNU1-1
RNU1-2
RNU1-3
RNU1-4
RNVU1-18
RNU1-27P
RNU1-28P

Mutations in U1 a non-coding component of the spliceosome (snRNA)

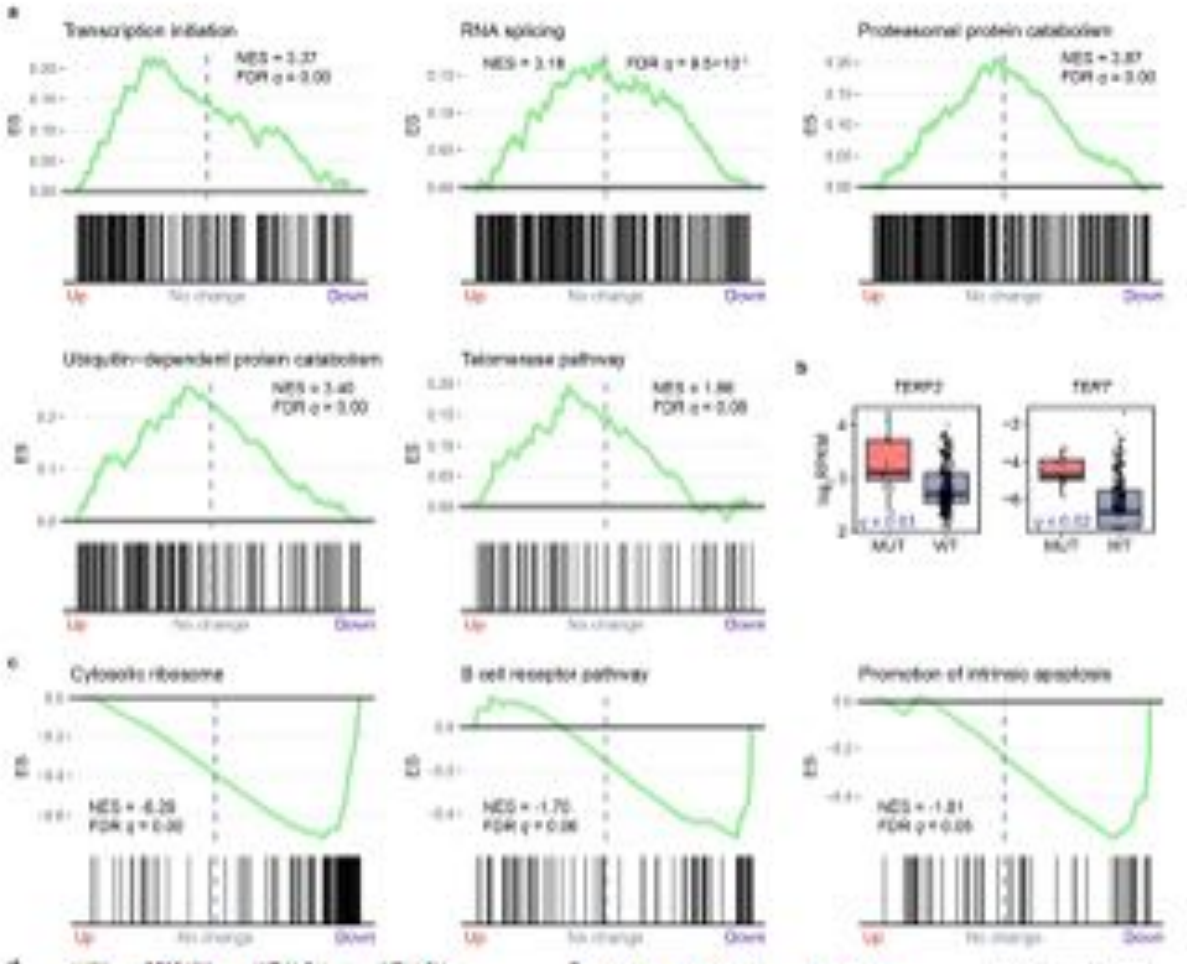


The mutation changes the preferential A-U base paring between U1 and 5`SS to C-G base pairing, creating a novel splice junctions and altering the splicing pattern of multiple genes

A>G mutation:
26/135 (19.3%) medulloblastomas
1/230 (0.4 %) pancreatic adenocarcinomas
A>C mutation (g.3A>C):
12/318 (3.8%) CLL
130/510 (5.9 %) hepatocellular carcinoma

Shuai S, et al. The U1 spliceosomal RNA is recurrently mutated in multiple cancers. Nature. 2019 Oct 9. doi: 10.1038/s41586-019-1651-z.

Functional effect of U1 mutation

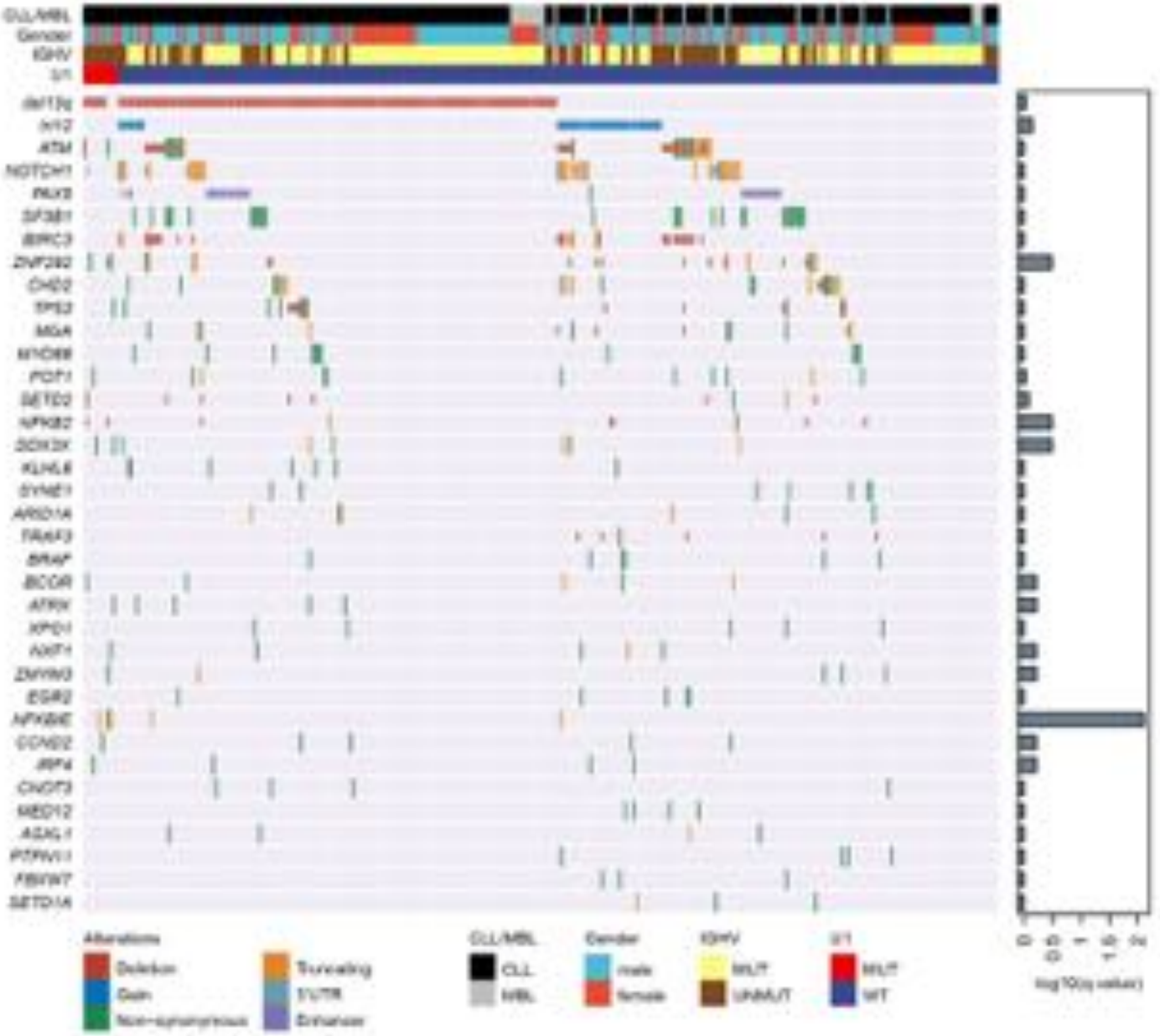


Up-regulated
genes related to:
- ✓ mRNA transcription
- ✓RNA splicing
- ✓protein ubiquitination
- ✓telomere maintenance

Down-regulated
genes related to:
- ✓apoptosis
- ✓B cell receptor signaling
- ✓cytoplasmic ribosomes

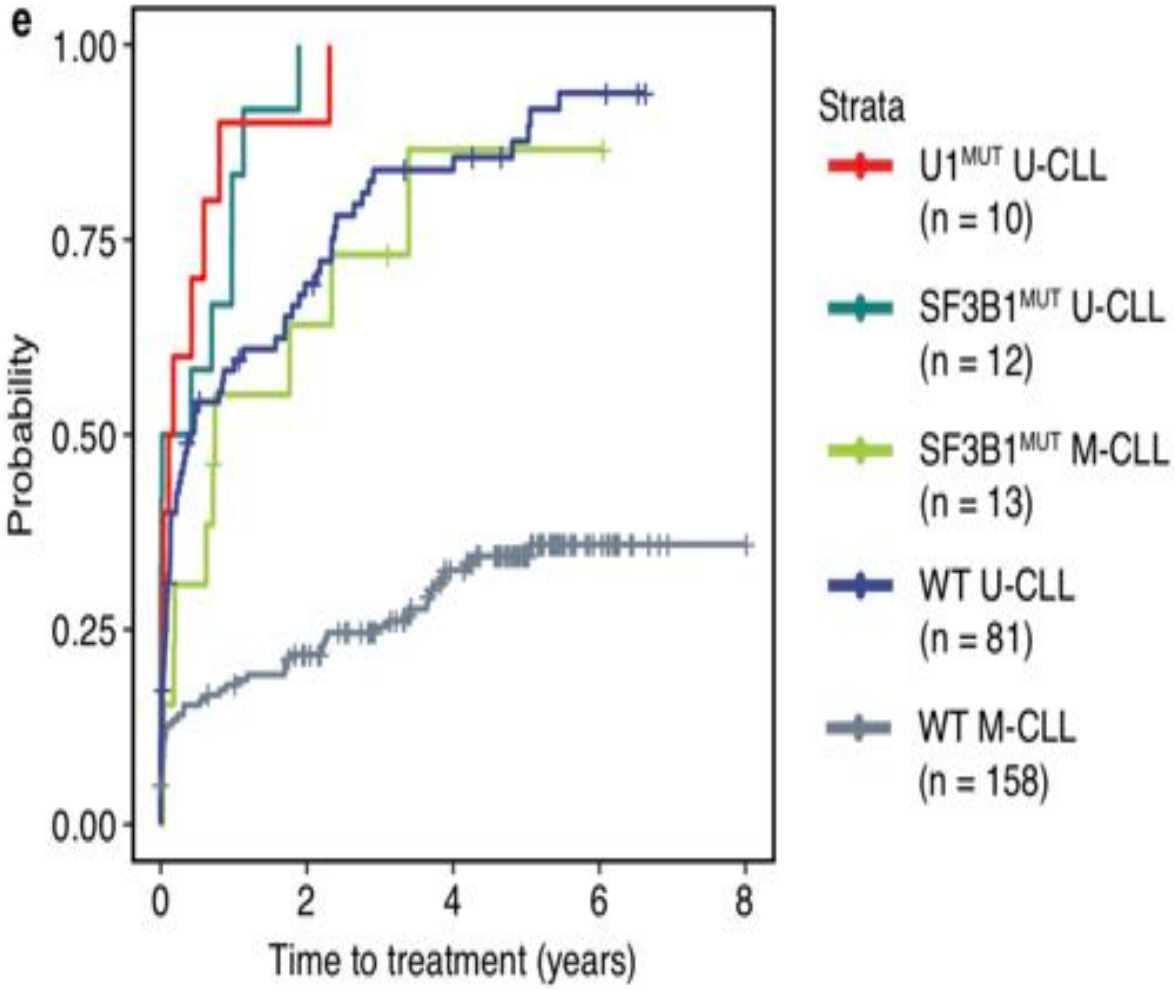
Shuai S, et al. The U1 spliceosomal RNA is recurrently mutated in multiple cancers. Nature. 2019 Oct 9. doi: 10.1038/s41586-019-1651-z.

Relationship to other drivers



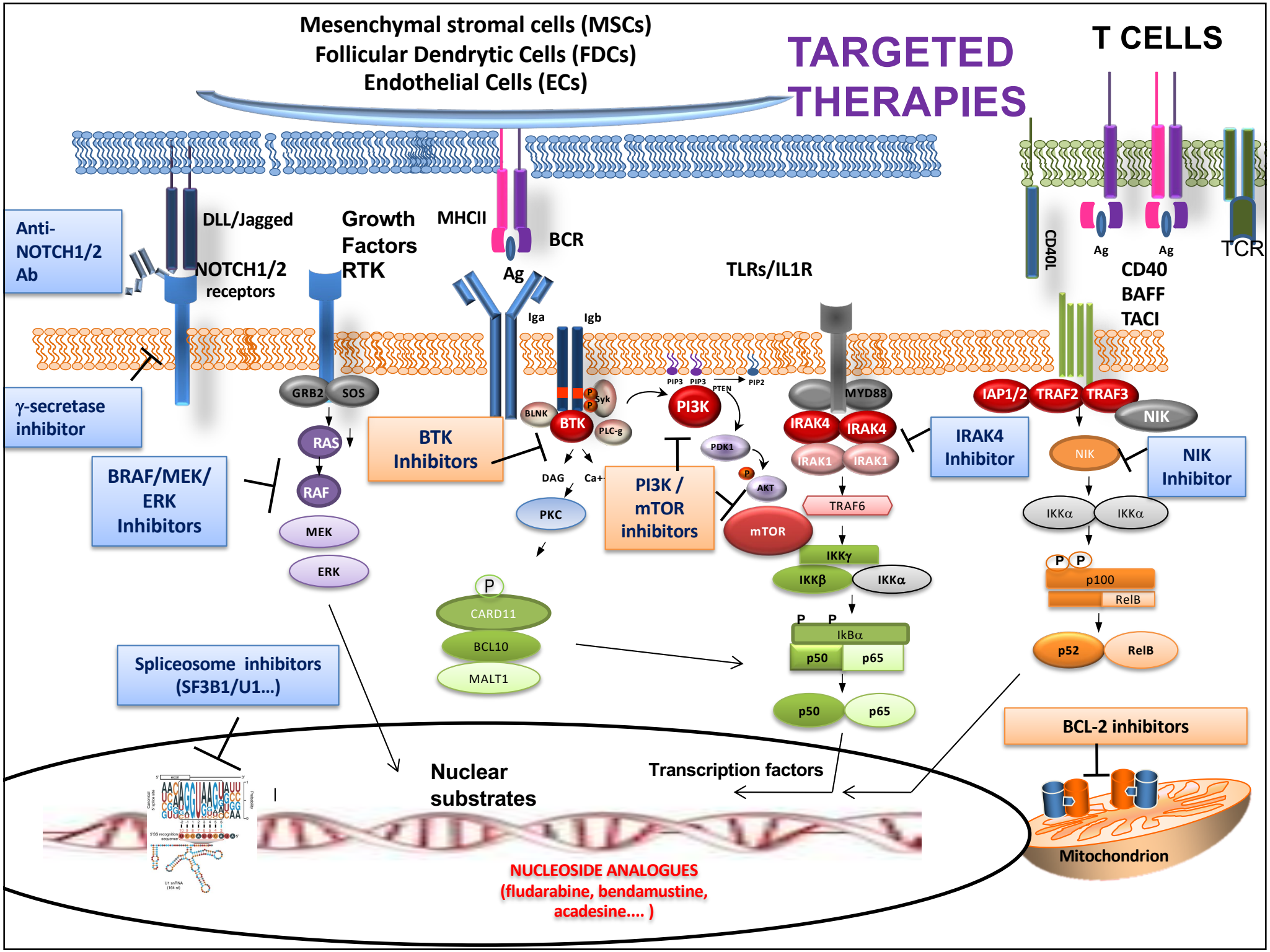
Shuai S, et al. The U1 spliceosomal RNA is recurrently mutated in multiple cancers. Nature 2019 Oct 9. doi: 10.1038/s41586-019-1651-z.

U1 mutation is associated with poor prognosis

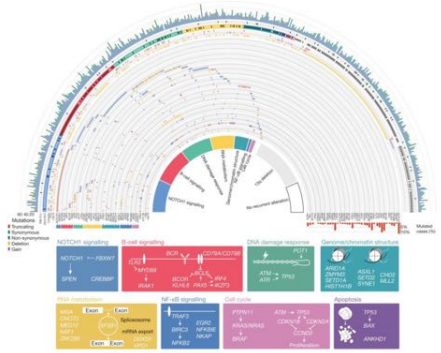


Variable	HR (95% CI)	*P*
U1 (MUT vs. WT)	1.999 (1.021-3.911)	0.043
SF3B1 (MUT vs. WT)	2.723 (1.691-4.383)	3.73×10^{-5}
Binet stage (B/C vs. A)	3.071 (1.958-4.817)	1.02×10^{-6}
IGHV (UNMUT vs. MUT)	4.164 (2.926-5.926)	2.32×10^{-15}

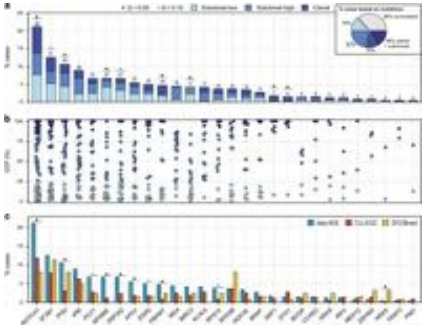
Shuai S, et al. The U1 spliceosomal RNA is recurrently mutated in multiple cancers. Nature. 2019 Oct 9. doi: 10.1038/s41586-019-1651-z.


Mesenchymal stromal cells (MSCs)
Follicular Dendrytic Cells (FDCs)
Endothelial Cells (ECs)
TARGETED THERAPIES
T CELLS
Anti-NOTCH1/2 Ab
DLL/Jagged
NOTCH1/2 receptors
Growth Factors RTK
MHCII
BCR
Ag
Iga
Igb
TLRs/IL1R
CD40L
Ag
Ag
TCR
CD40
BAFF
TACI
γ-secretase inhibitor
GRB2
SOS
RAS
RAF
MEK
ERK
BRAF/MEK/ERK Inhibitors
BTK Inhibitors
BLNK
BTK
PLC-g
Syk
PIP3
PIP2
PTEN
PI3K
PDK1
AKT
mTOR
PI3K / mTOR inhibitors
DAG
Ca+
PKC
CARD11
BCL10
MALT1
MYD88
IRAK4
IRAK1
TRAF6
IKKγ
IKKβ
IKKα
IkBα
p50
p65
IRAK4 Inhibitor
IAP1/2
TRAF2
TRAF3
NIK
NIK Inhibitor
IKKα
p100
RelB
p52
Spliceosome inhibitors (SF3B1/U1...)
Nuclear substrates
Transcription factors
BCL-2 inhibitors
Mitochondrion
NUCLEOSIDE ANALOGUES
(fludarabine, bendamustine, acadesine....)

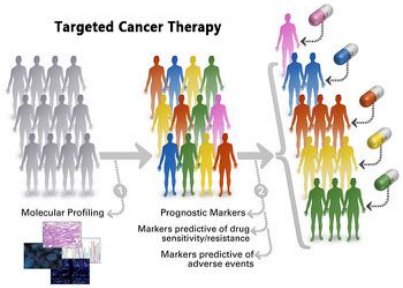
Summary



- **CLL is genetically highly heterogeneous**
  - **Most genes mutated at low frequency**
  - **Clustering in functional pathways differentially represented in subsets patients**



- **The subclonal architecture of the disease may modulate the evolution of the patients**



- **The integration of the genomic profile in the clinics may open new perspectives for the management of the patients**

IDIBAPS
Institut D'Investigacions Biomèdiques August Pi i Sunyer

CLÍNIC BARCELONA
Hospital Universitari

CONSORTIUM FOR THE STUDY OF THE CLL GENOME
CLL
CHRONIC LYMPHOCYTIC LEUKEMIA GENOME PROJECT

Molecular pathology in limphoid malignancies
Nadeu F
Clot G
Bea S
Pinyol M
Jares P
Navarro A
Martin-Garcia D
Salaverria I
Enjaunes A
Campo E

Biomedical Epigenomics
Kulis M
Beekman R
Chapaprieta V
Duran M
Vilarrasa-Blasi R
Martin-Subero I

Experimental Therapeutics in lymphoid malignancies
Giménez N
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