Discovering driver mutations in chronic lymphocytic leukemia

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BRIGHAM AND WOMEN'S HOSPITAL

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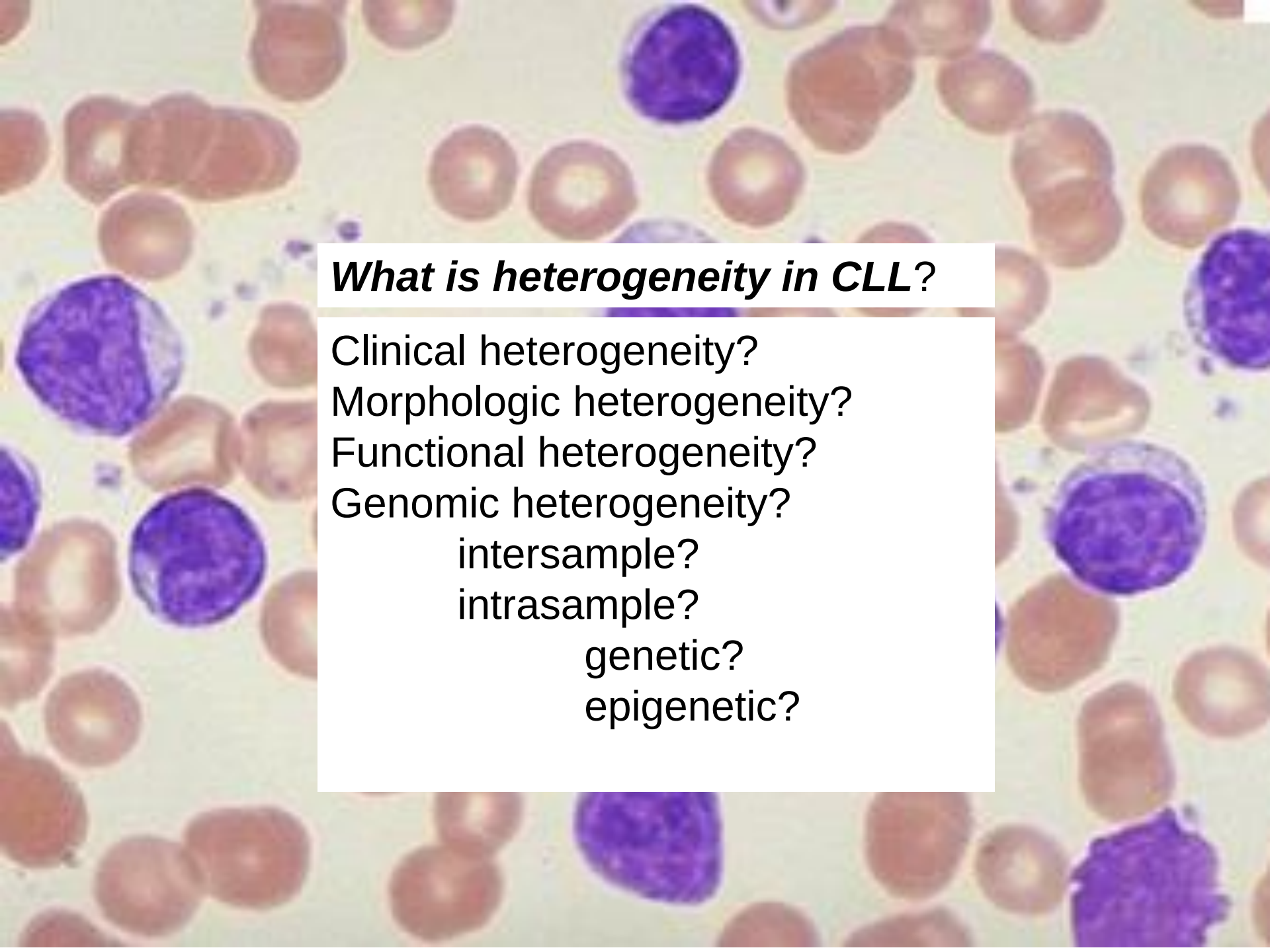
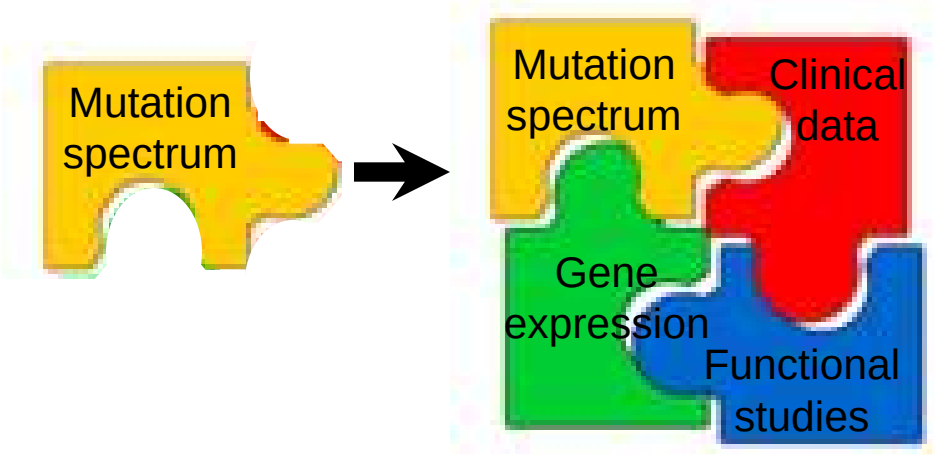
BROAD INSTITUTE



What is heterogeneity in CLL?

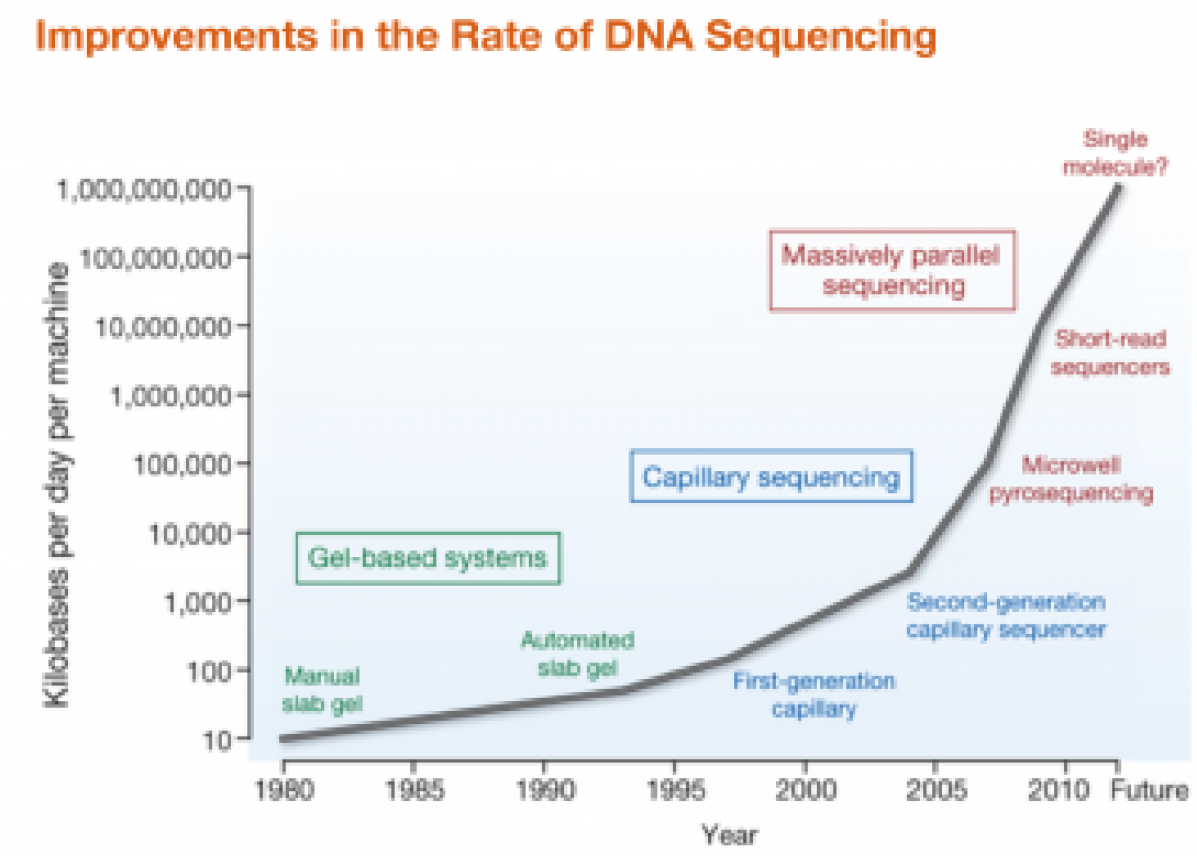
Clinical heterogeneity?
Morphologic heterogeneity?
Functional heterogeneity?
Genomic heterogeneity?
- intersample?
- intrasample?
  - genetic?
  - epigenetic?

A path to understanding CLL biology



How do we identify drivers?

- Functional characterization
- Statistical inference



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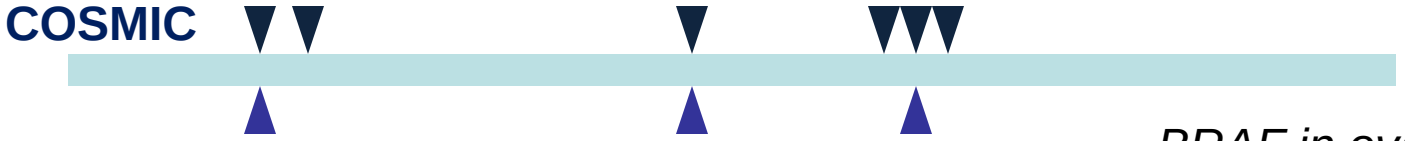
Identification of candidate drivers based on statistical frequency

1. Based on *positional configuration* of mutations

DIS3 in multiple myeloma
Chapman et al. *Nature* (2011)

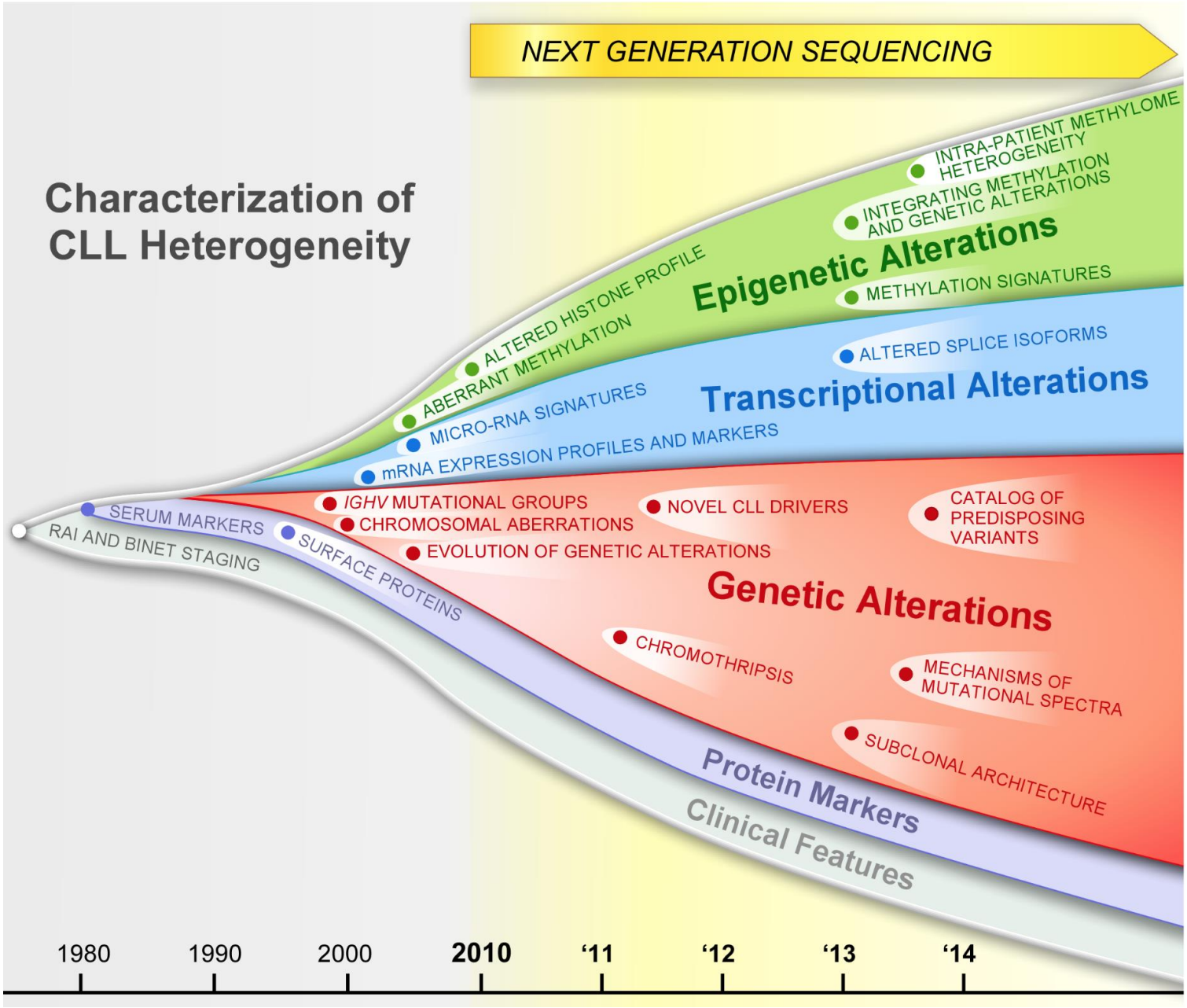
2. Based on *prior knowledge* on sites in gene (COSMIC, predicted fxn, conservation)



BRAF in ovarian cancer
TCGA Network. *Nature* (2011)

3. Based on *gene sets / pathways* (different genes mutated/tumor in a pathway)

Coagulation pathway in MM
Chapman et al. *Nature* (2011)

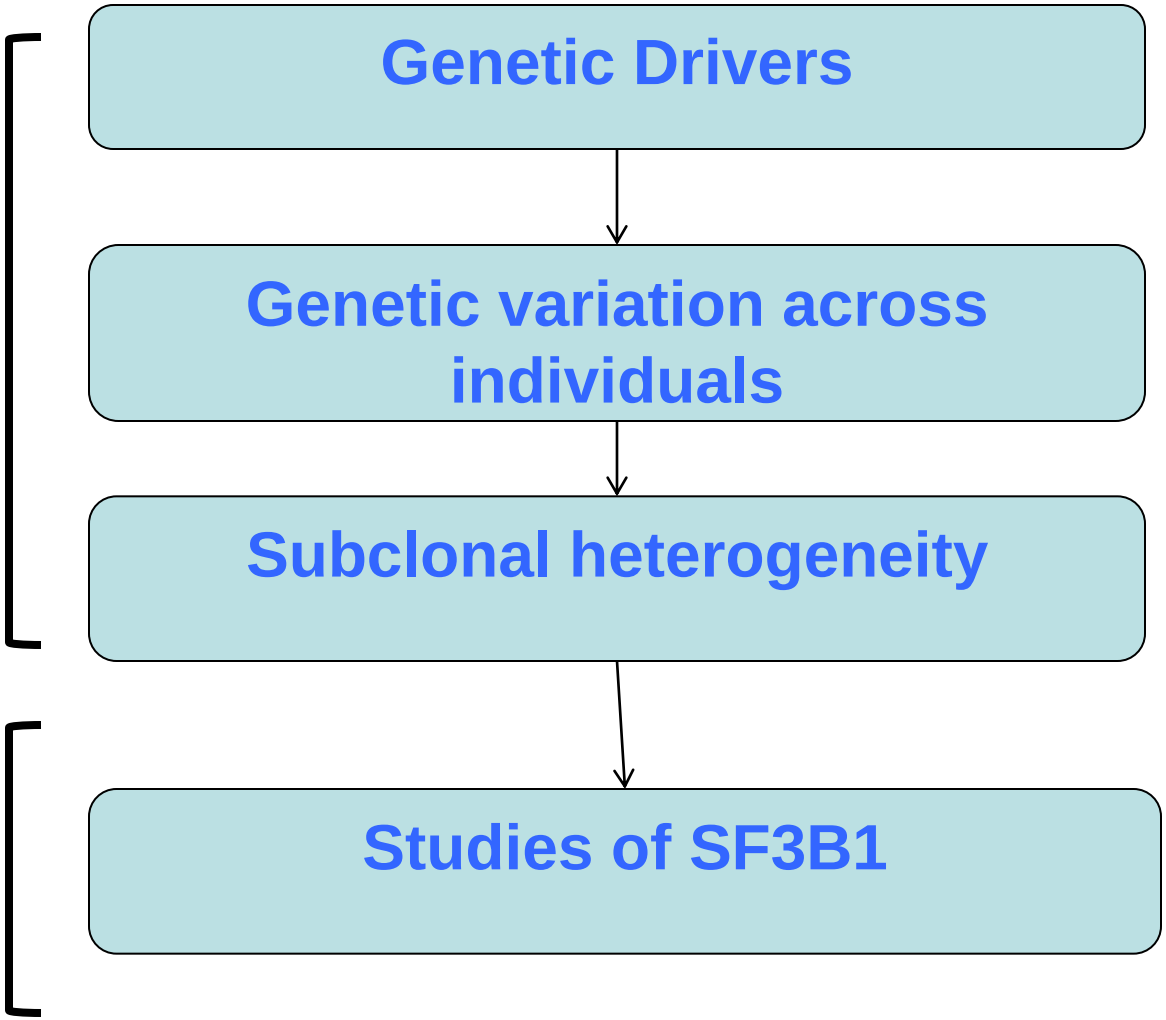


Gruber & Wu 2014

Genomic analysis of CLL

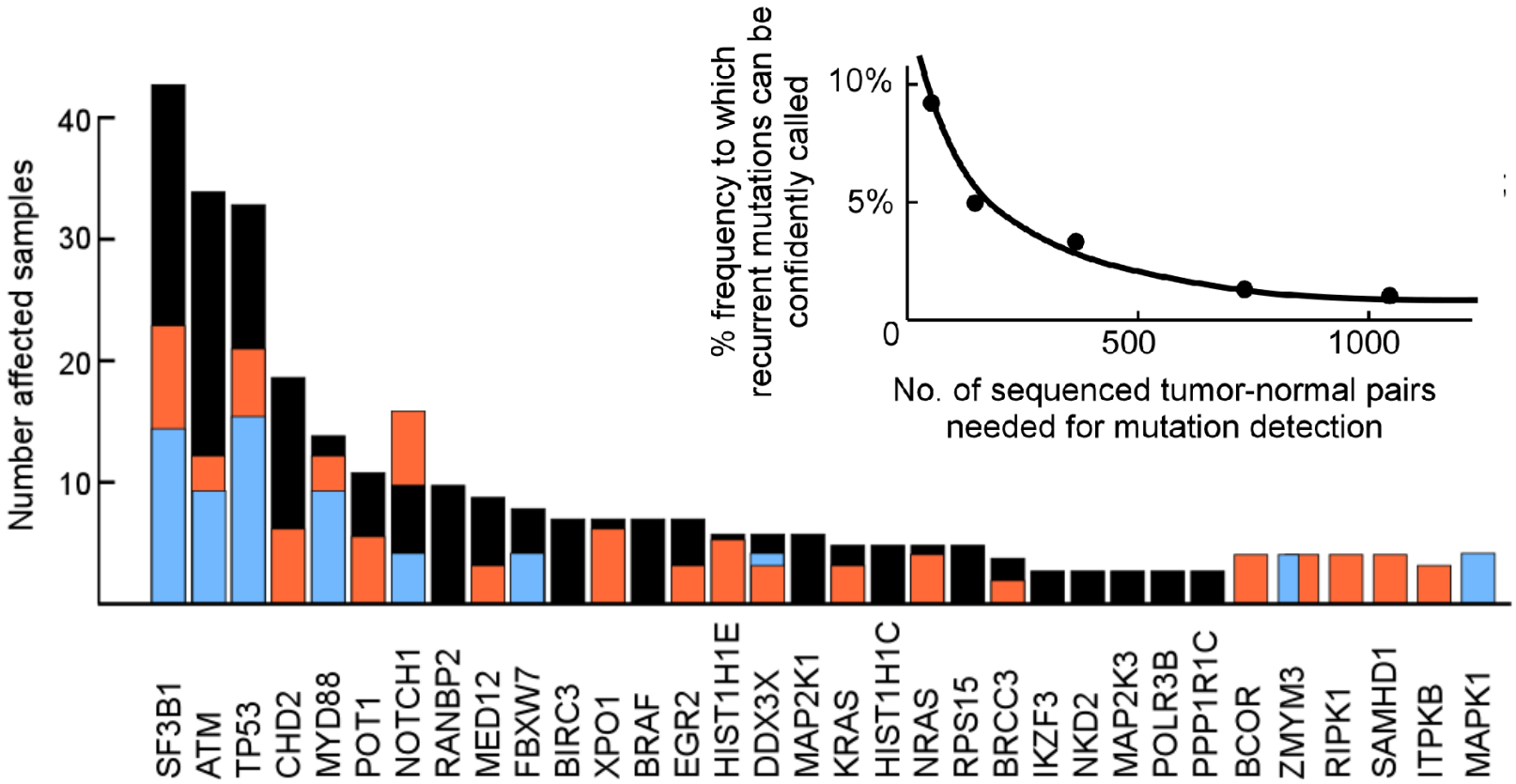
1. What can we learn from unbiased analyses of somatic mutations?

2. How are putative drivers related to functional cancer driving

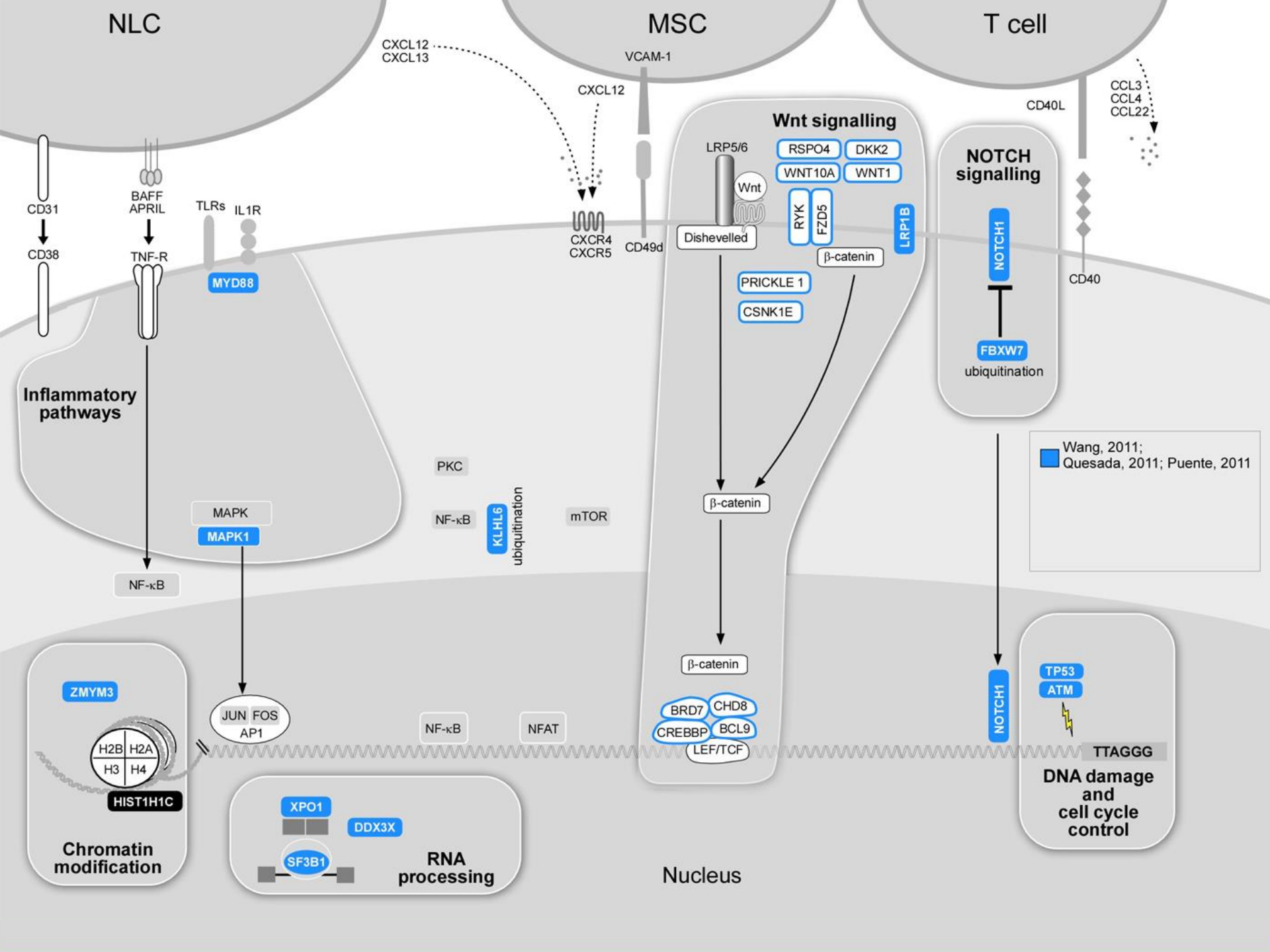


What can we learn from unbiased analyses of somatic mutations?

Discovery of drivers in CLL: impact of cohort size



Lawrence *Nature* 2014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T cell
CXCL12
CXCL13
CXCL12
VCAM-1
CD40L
CCL3
CCL4
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CD31
CD38
BAFF
APRIL
TNF-R
TLRs
IL1R
MYD88
CXCR4
CXCR5
CD49d
Wnt signalling
LRP5/6
Wnt
Dishevelled
RSPO4
DKK2
WNT10A
WNT1
RYK
FZD5
LRP1B
β-catenin
PRICKLE 1
CSNK1E
NOTCH signalling
NOTCH1
FBXW7
ubiquitination
CD40
Inflammatory pathways
MAPK
MAPK1
NF-κB
PKC
NF-κB
KLHL6
ubiquitination
mTOR
β-catenin
β-catenin
BRD7
CHD8
CREBBP
BCL9
LEF/TCF
Wang, 2011;
Quesada, 2011; Puente, 2011
ZMYM3
H2B
H2A
H3
H4
HIST1H1C
Chromatin modification
JUN
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AP1
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TP53
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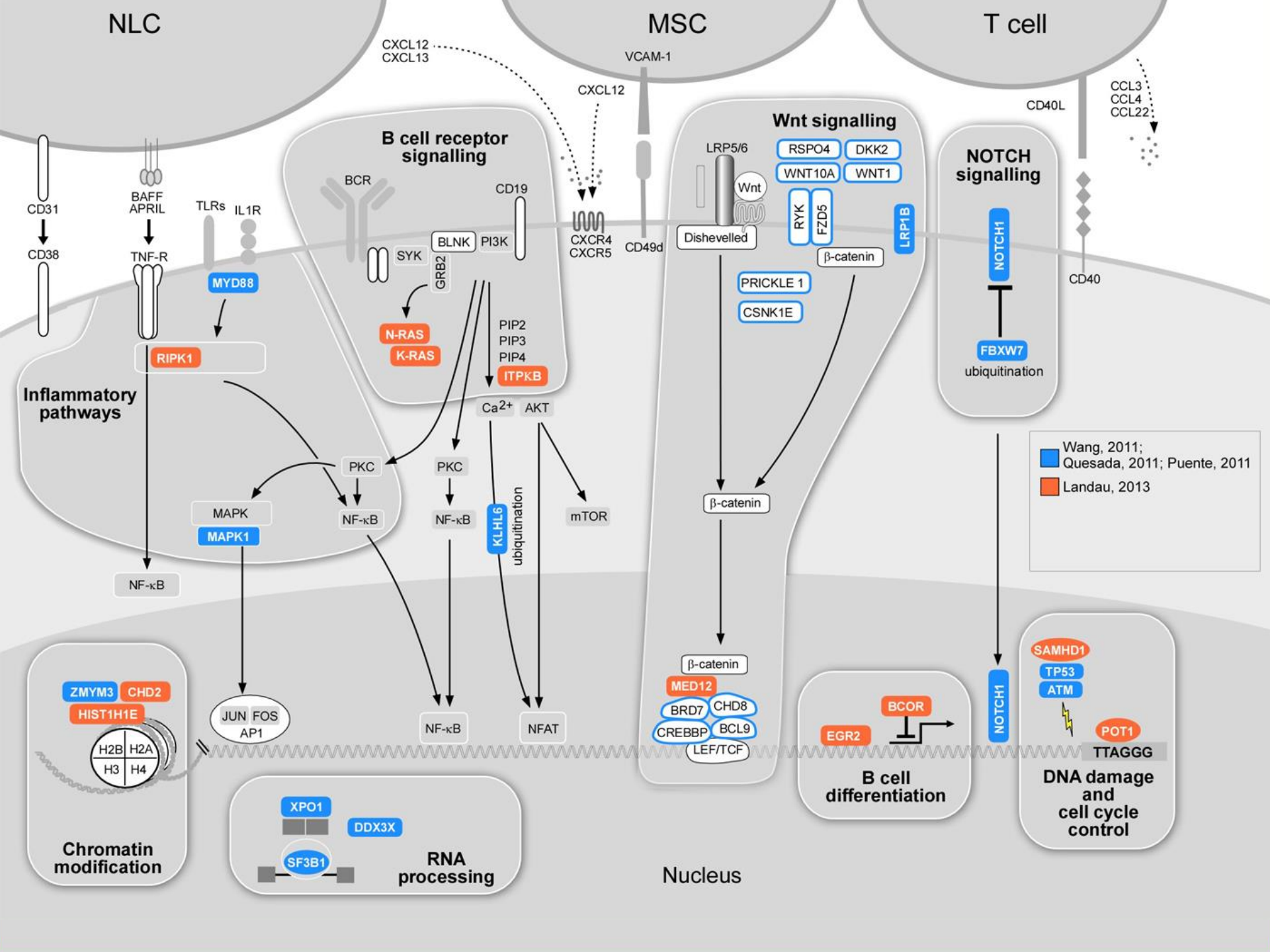
TTAGGG
DNA damage and cell cycle control
Nucleus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Inflammatory pathways
B cell receptor signalling
BCR
CD19
SYK
BLNK
PI3K
GRB2
N-RAS
K-RAS
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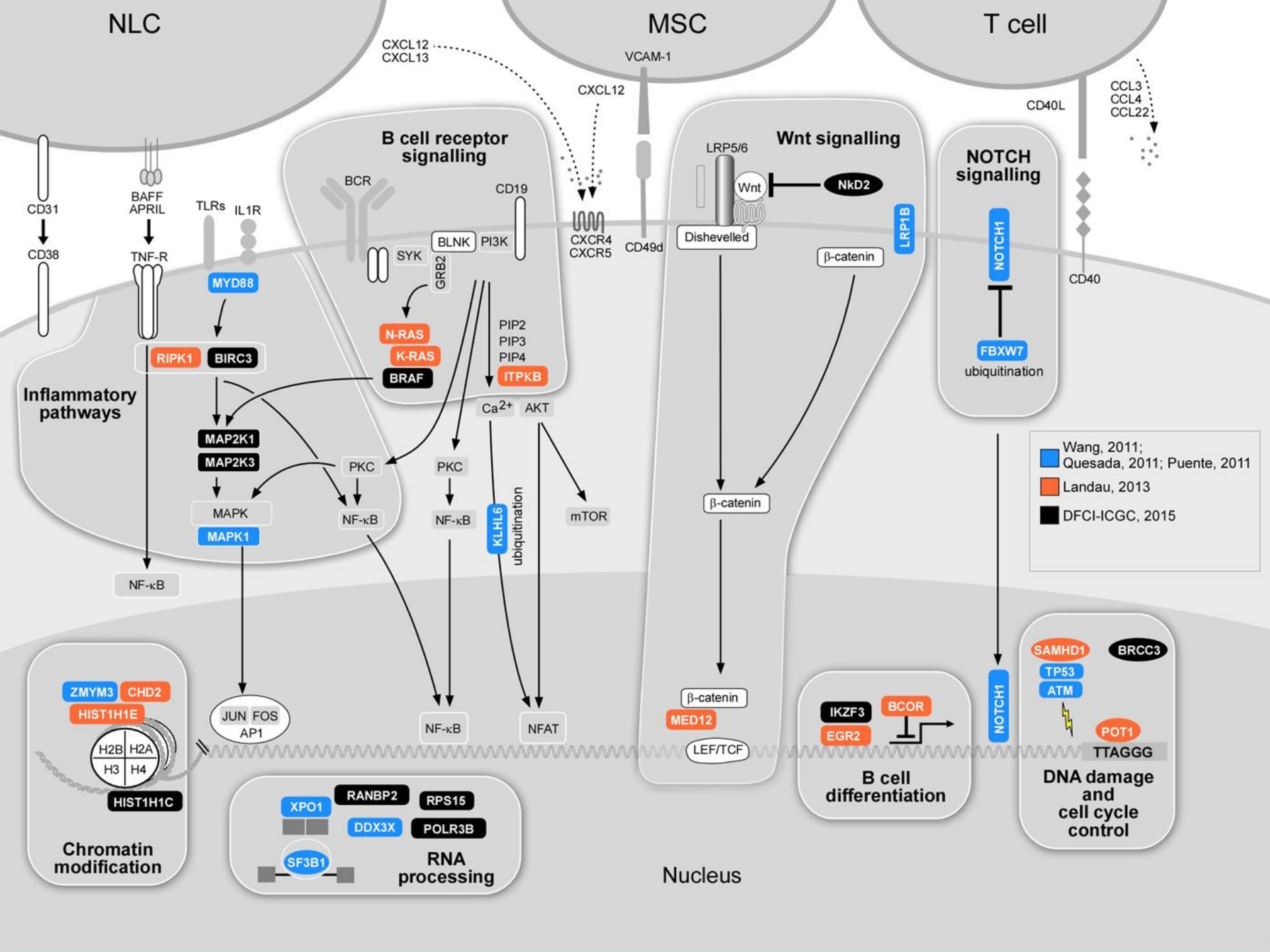
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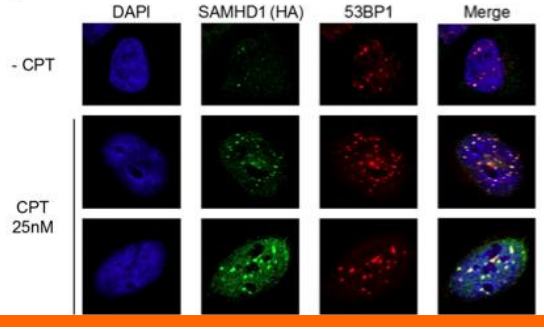
FUNCTIONAL ANALYSIS-I

Impairment in the DNA damage response

SAMHD1

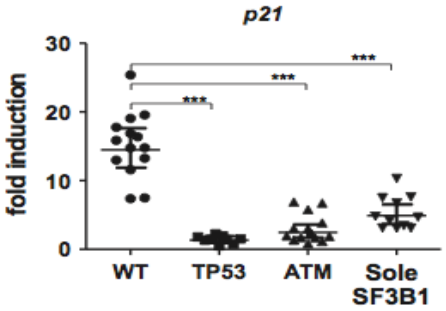
involved in the response to DNA DSBs and engages in specific protein interactions on DNA damage



Clifford *Blood* 2014

SF3B1

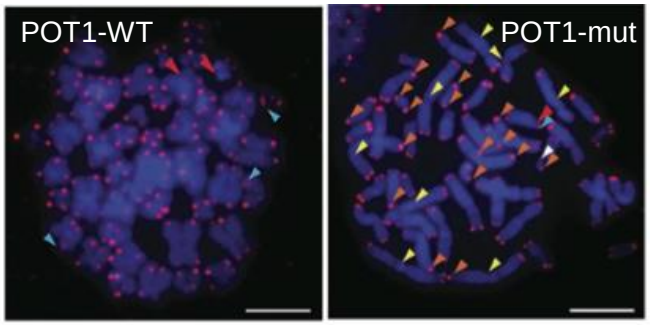
Mutations associated with increased DNA damage and/or an aberrant response to DNA damage



Te Raa *Leukemia* 2014

POT1

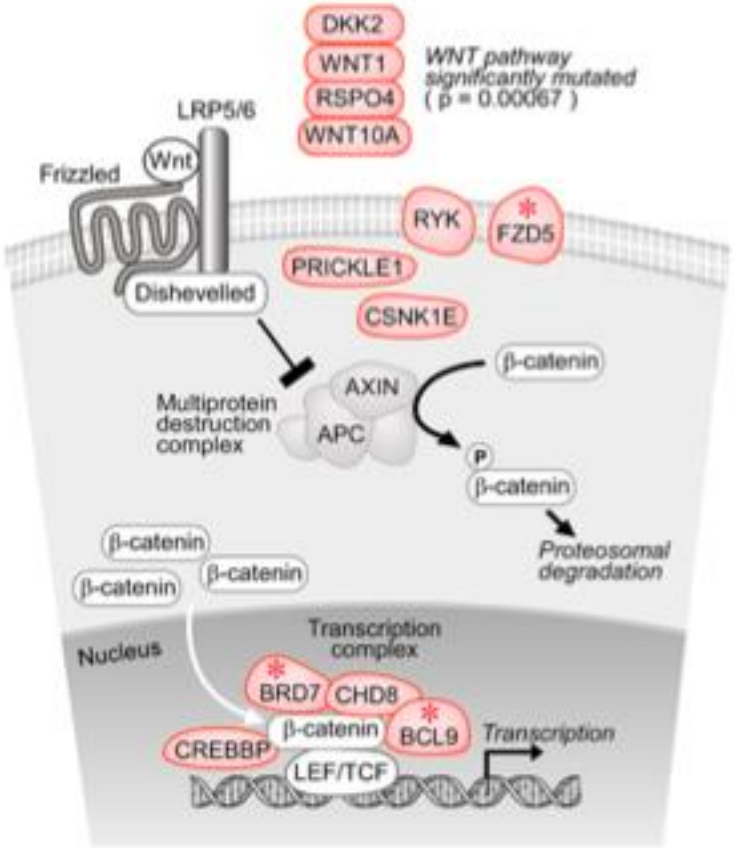
Mutations affect key residues for binding of telomeric DNA→ telomeric and chromosomal abnormalities



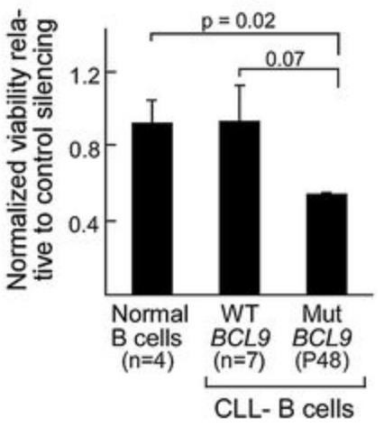
Ramsay *Nat Genet* 2013

FUNCTIONAL ANALYSIS-II

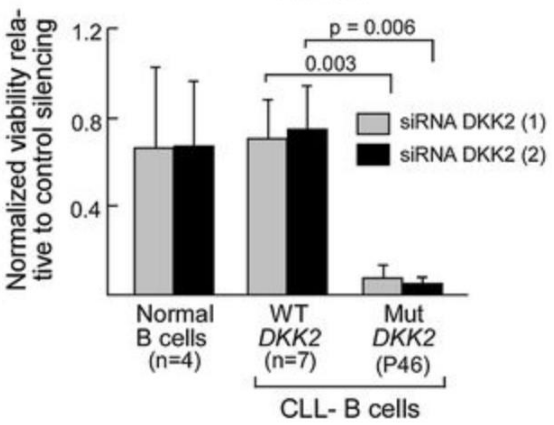
Low frequency mutated genes may affect key CLL nodes



BCL9

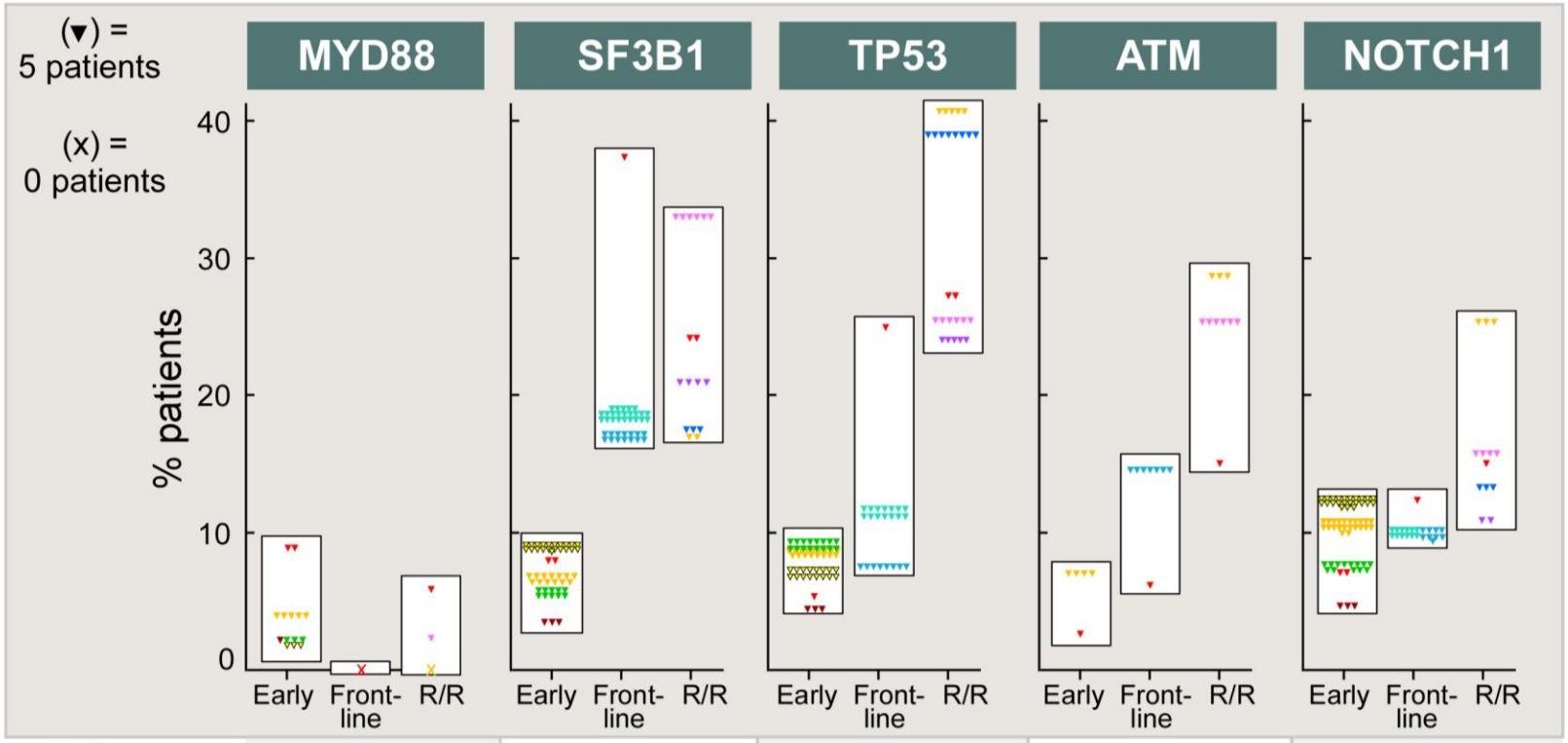


DKK2



Wang *Blood* 2014

Discovery of drivers in CLL: impact of cohort composition



	MYD88			SF3B1			TP53			ATM			NOTCH1		
	Early	Front-line	R/R	Early	Front-line	R/R	Early	Front-line	R/R	Early	Front-line	R/R	Early	Front-line	R/R
DFCI Broad Institute	10/111	0/16	2/33	9/111	6/16	8/33	6/111	4/16	9/33	3/111	1/16	5/33	8/111	2/16	5/33
Italy/Columbia	26/637		0/58	67/1007		10/59	87/1007		24/59	21/294		17/59	108/1007		15/59
ERIC	15/628			51/889			80/889						67/889		
MLL	15/969			104/1160			82/1151						112/908		
SCALE	6/263			13/358			16/362						17/358		
GCLLSG CLL8					114/621			72/628						62/622	
UK LRF CLL4					73/437			40/529			33/224			46/466	
GCLLSG CLL2H						17/97			39/100						13/97
GCLLSG CLL3X						21/100			24/100						11/100
French Intergroup UK NCRN			3/114			33/114			29/114			29/114			18/114

Guieze R Blood 2015

How can we better understand the genetic heterogeneity of CLL in 2016?

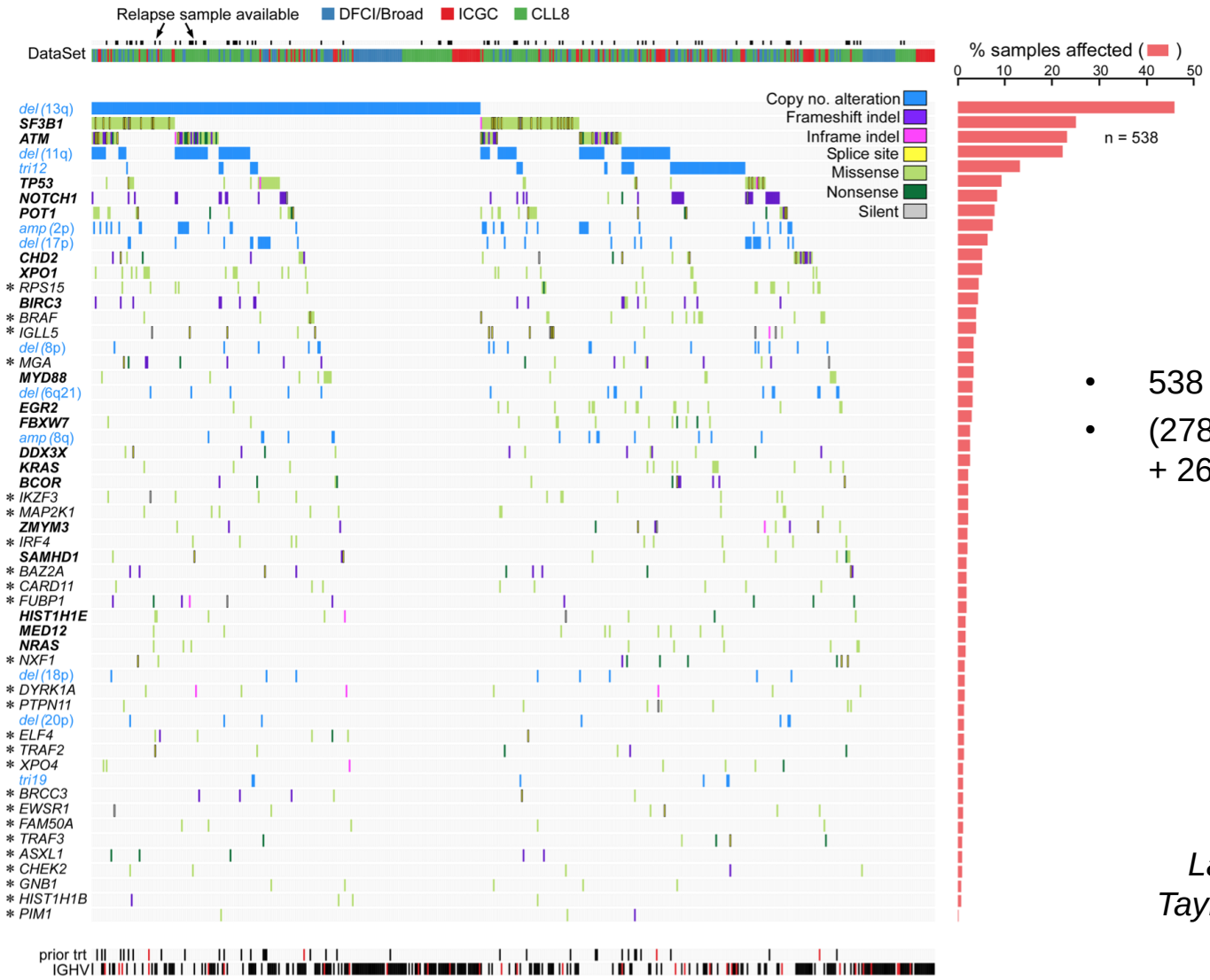
- Cohort composition: WES of uniform cohort (278 cases from GCLLSG-CLL8)
  - Hallek Lancet 2010:phase III study that established std of care chemotherapy
- Cohort size: Increased sensitivity by combining cohorts→ 538 cases (278 GCLLSG-CLL8 + 260 DFCI-ICGC)
  - Expected to saturate genes mutated in 5% pts
    - 94% power to detect genes mutated in 3%
    - 61% power to detect genes mutated in 2%

Can we find new drivers? Pathways?

Can we better reconstruct CLL phylogeny?

Can we better characterize clonal evolution in relationship to therapy?

Intertumoral heterogeneity in CLL: independent evolutionary events



- 538 cases, WES
- (278 GCLLSG-CLL8 + 260 DFCI-ICGC)

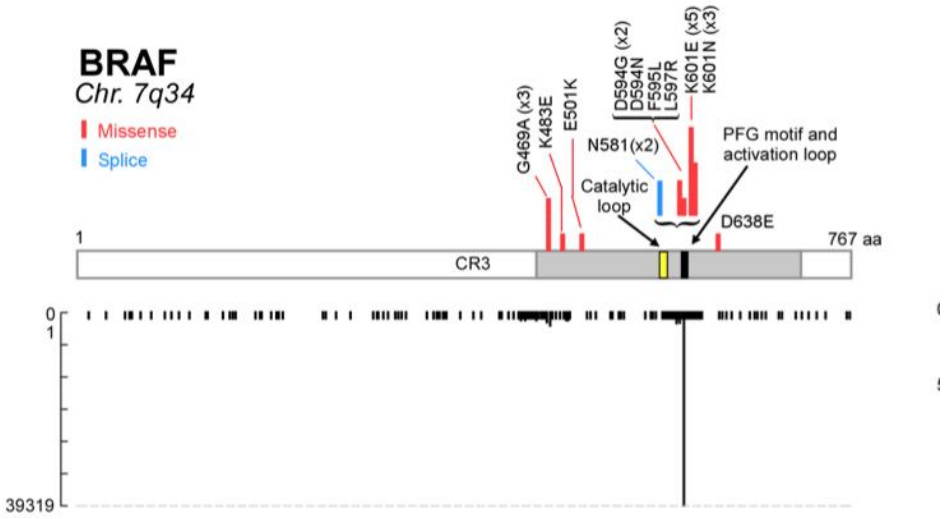
Landau Tausch & Taylor Weiner, Nature 2015

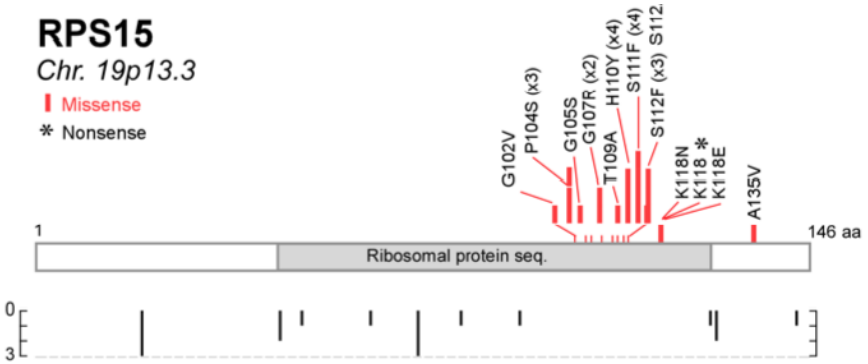
Quick numbers

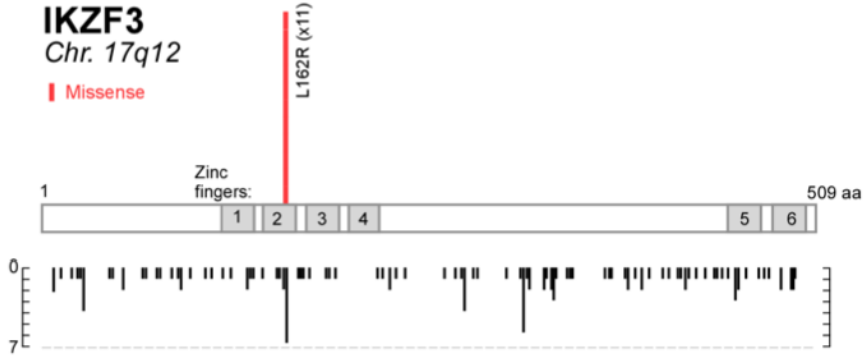
- 55 driver events: 44 sSNVs, 11 sCNVs
  - 26 additional candidate CLL genes
- Median of 2 drivers per sample
  - 91% with at least one driver
  - 65% with at least 2 drivers (56% if without the new 26)
  - 44% with at least 3 drivers (32%)

What are the new genes? Pathways?

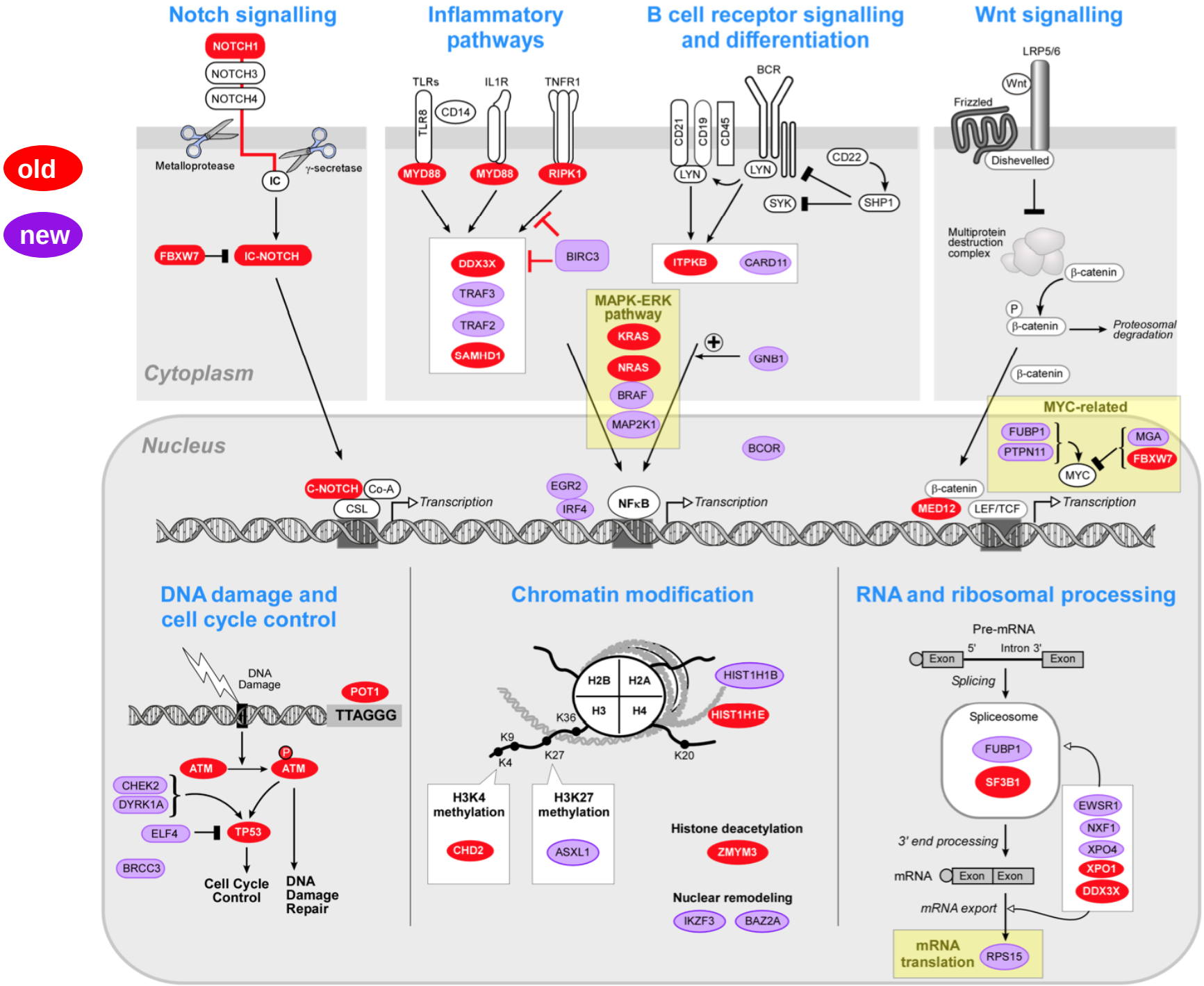
- Previously suggested
  - *IRF4, MGA*
- Noted in B cell malignancies
  - *CARD11, GNB1, PTPN11, TRAF2 and TRAF3*
- New pathways/cellular processes
  - MYC related proteins: *MGA, PTPN11, FUBP1*
  - MAPK-ERK pathway (5.6% patients)
    - NRAS, n=9; KRAS n=14, BRAF, n=20; MAP2K1 n=11)
- Novel genes
  - *RPS15* – previously identified as candidate tumor suppressor
  - *IKZF3* – transcription factor in B cell development







Ljungstrom
Blood 2015


old
new
Notch signalling
NOTCH1
NOTCH3
NOTCH4
Metalloprotease
γ-secretase
IC
FBXW7
IC-NOTCH
Inflammatory pathways
TLRs
IL1R
TNFR1
TLR8
CD14
MYD88
MYD88
RIPK1
BIRC3
DDX3X
TRAF3
TRAF2
SAMHD1
B cell receptor signalling and differentiation
BCR
CD21
CD19
CD45
LYN
LYN
CD22
SYK
SHP1
ITPKB
CARD11
Wnt signalling
LRP5/6
Wnt
Frizzled
Dishevelled
Multiprotein destruction complex
β-catenin
P
β-catenin
Proteosomal degradation
β-catenin
Cytoplasm
MAPK-ERK pathway
KRAS
NRAS
BRAF
MAP2K1
GNB1
MYC-related
FUBP1
PTPN11
MGA
FBXW7
MYC
Nucleus
BCOR
C-NOTCH
Co-A
CSL
Transcription
EGR2
IRF4
NFκB
Transcription
β-catenin
MED12
LEF/TCF
Transcription
DNA damage and cell cycle control
DNA Damage
POT1
TTAGGG
ATM
P
ATM
CHEK2
DYRK1A
ELF4
TP53
BRCC3
Cell Cycle Control
DNA Damage Repair
Chromatin modification
H2B
H2A
H3
H4
HIST1H1B
HIST1H1E
K36
K9
K4
K27
K20
H3K4 methylation
CHD2
H3K27 methylation
ASXL1
Histone deacetylation
ZMYM3
Nuclear remodeling
IKZF3
BAZ2A
RNA and ribosomal processing
Pre-mRNA
Exon
5'
Intron 3'
Exon
Splicing
Spliceosome
FUBP1
SF3B1
EWSR1
NXF1
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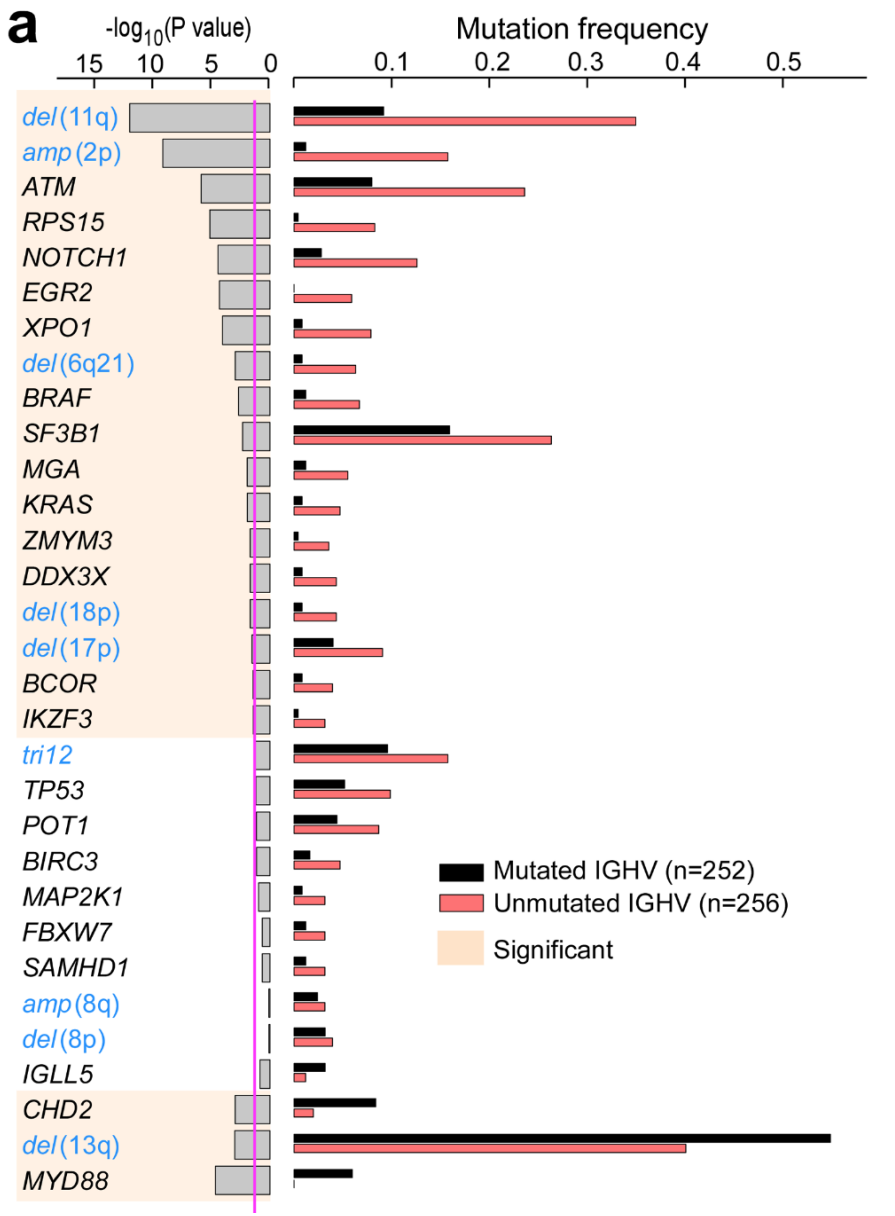
RPS15

Unmutated vs mutated CLL

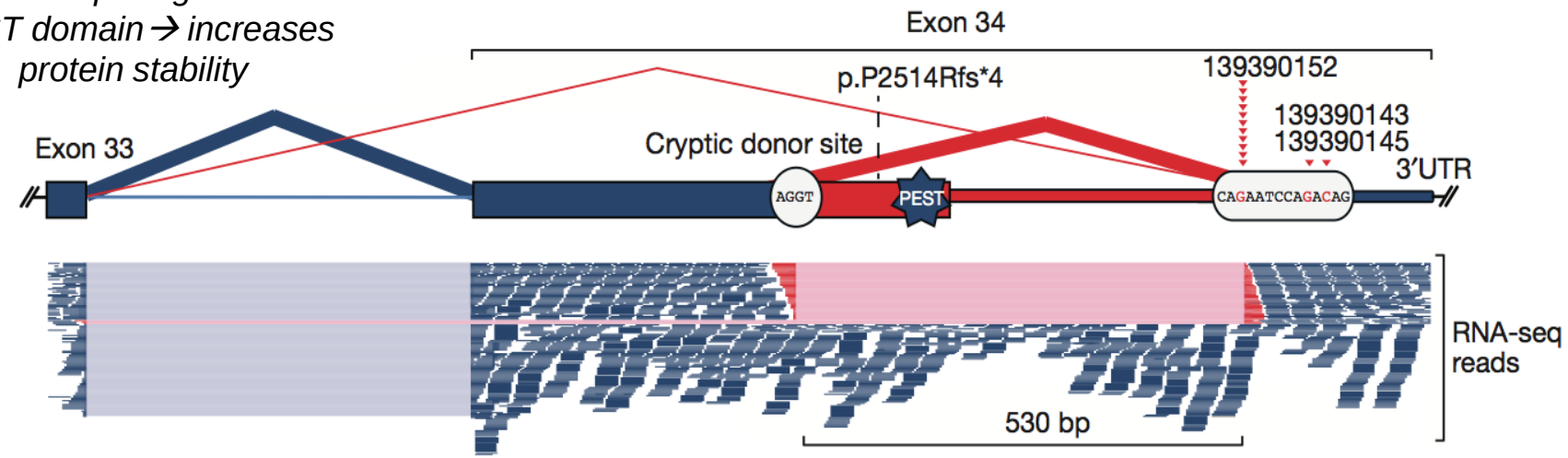


Unmut > mut CLL

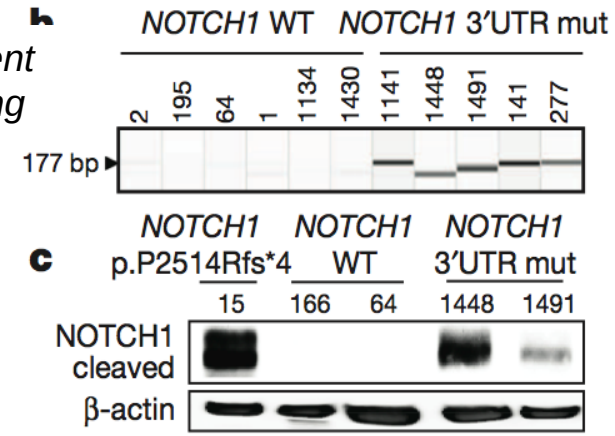
Mut > unmut CLL

Activating non-coding recurrent mutations in NOTCH1

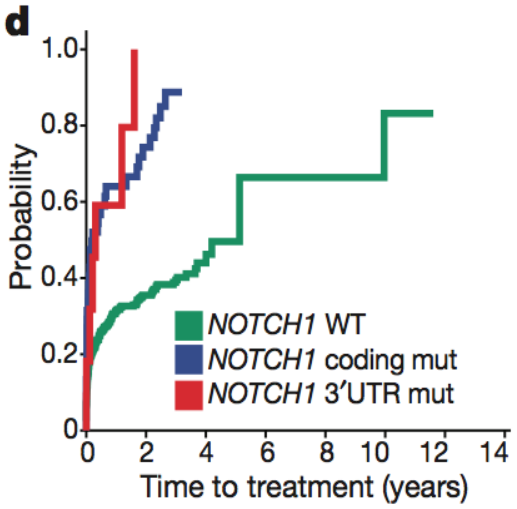
Intraexonic splicing → removes PEST domain → increases protein stability

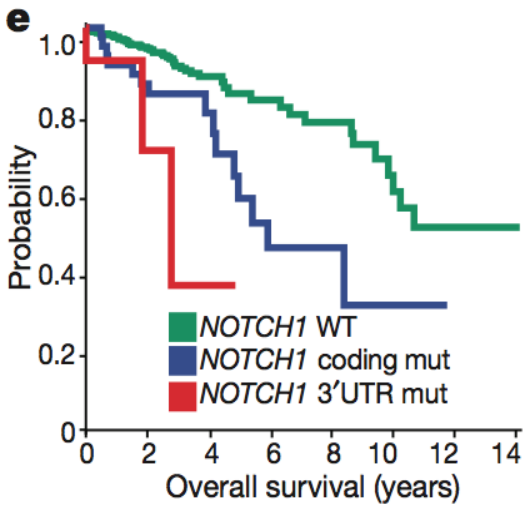


Aberrant splicing

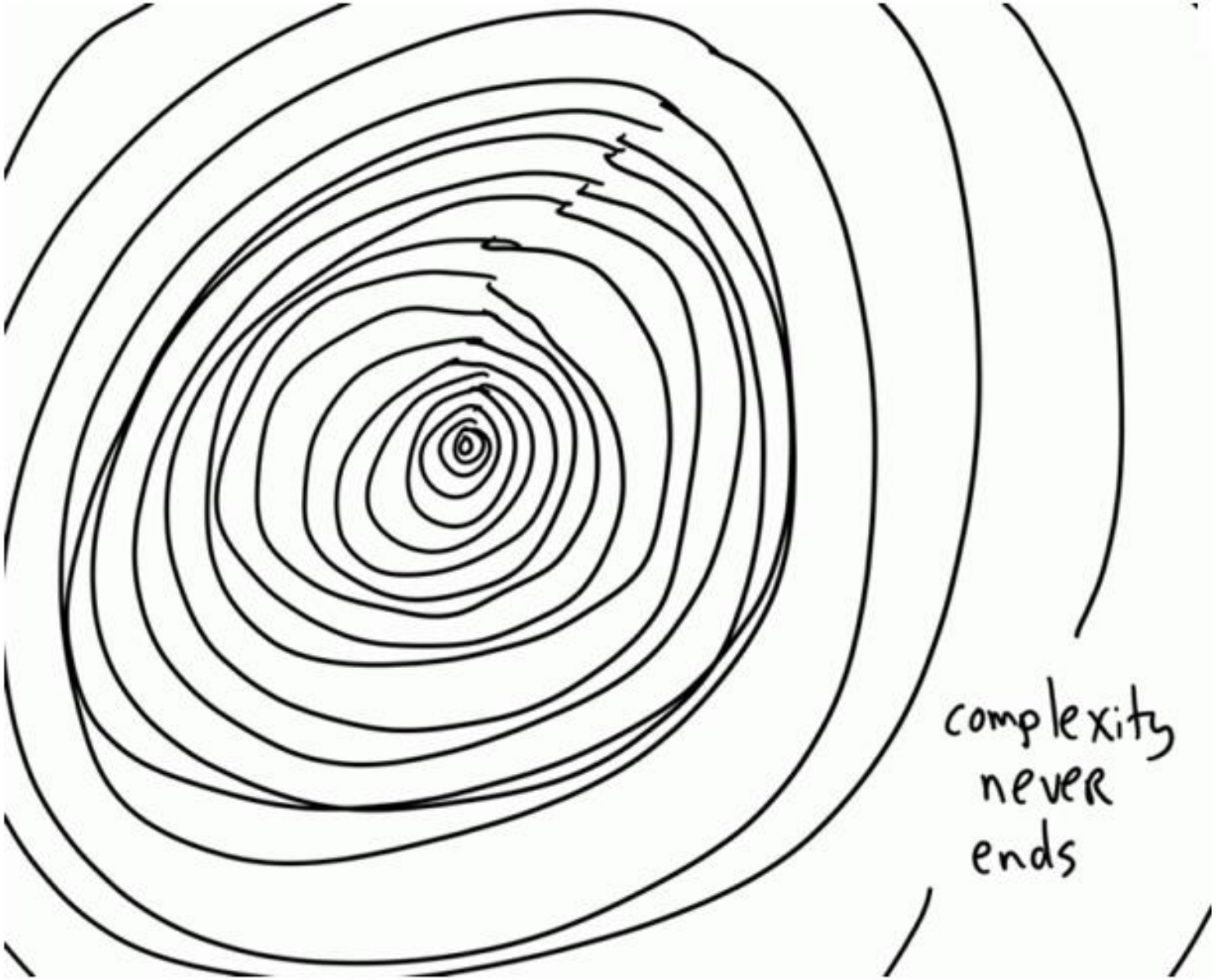


Presence of a smaller band

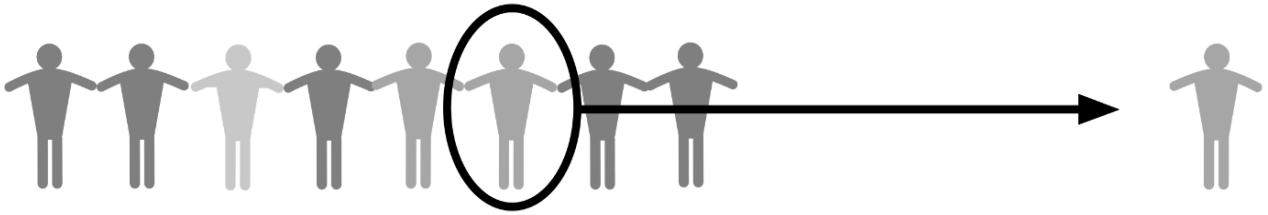


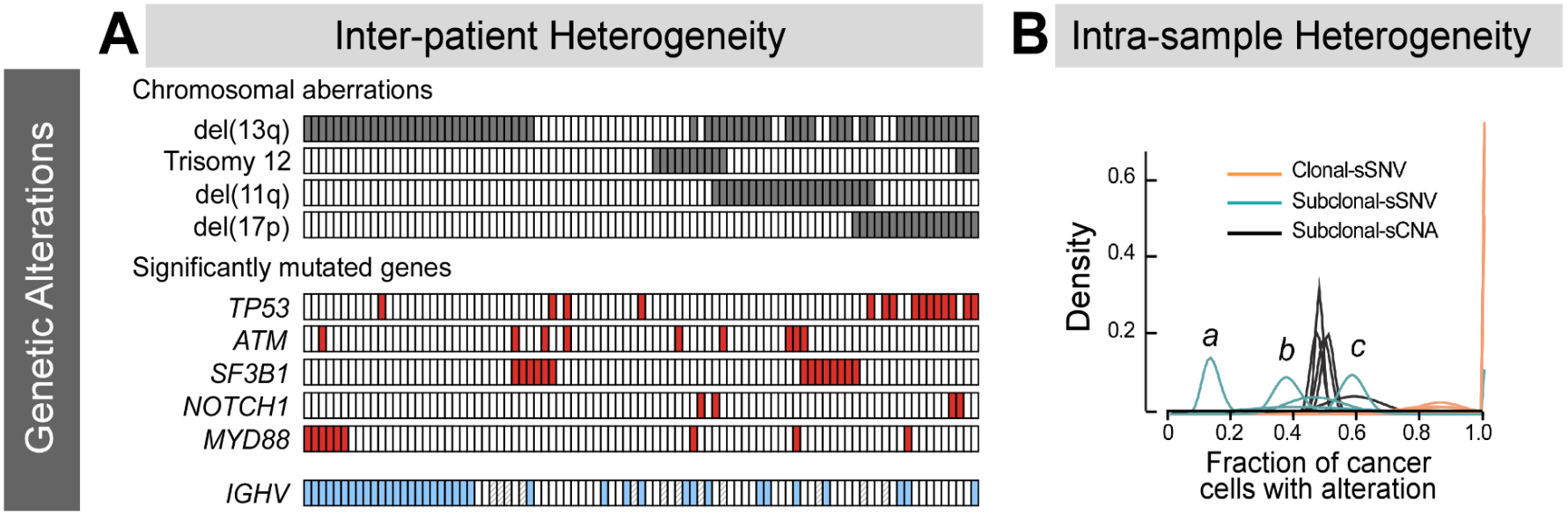


Puente, Nature 2015

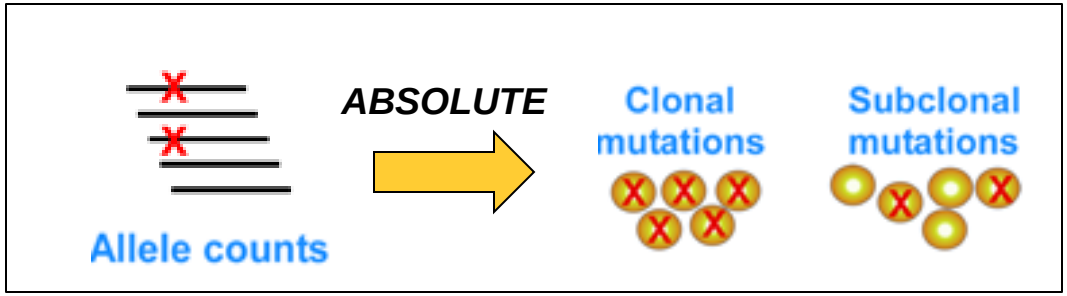

complexity
never
ends

Studying intratumoral heterogeneity in CLL

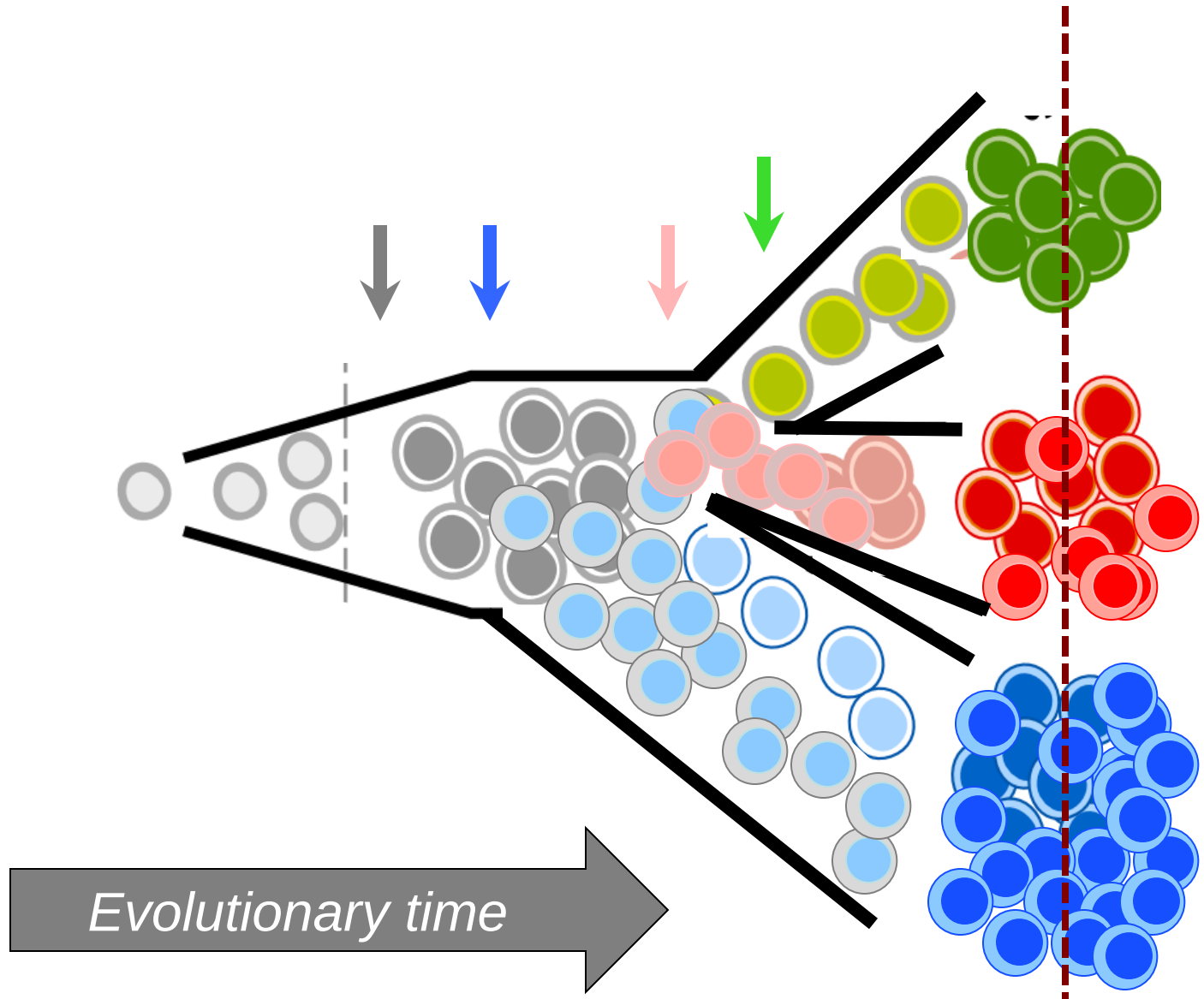




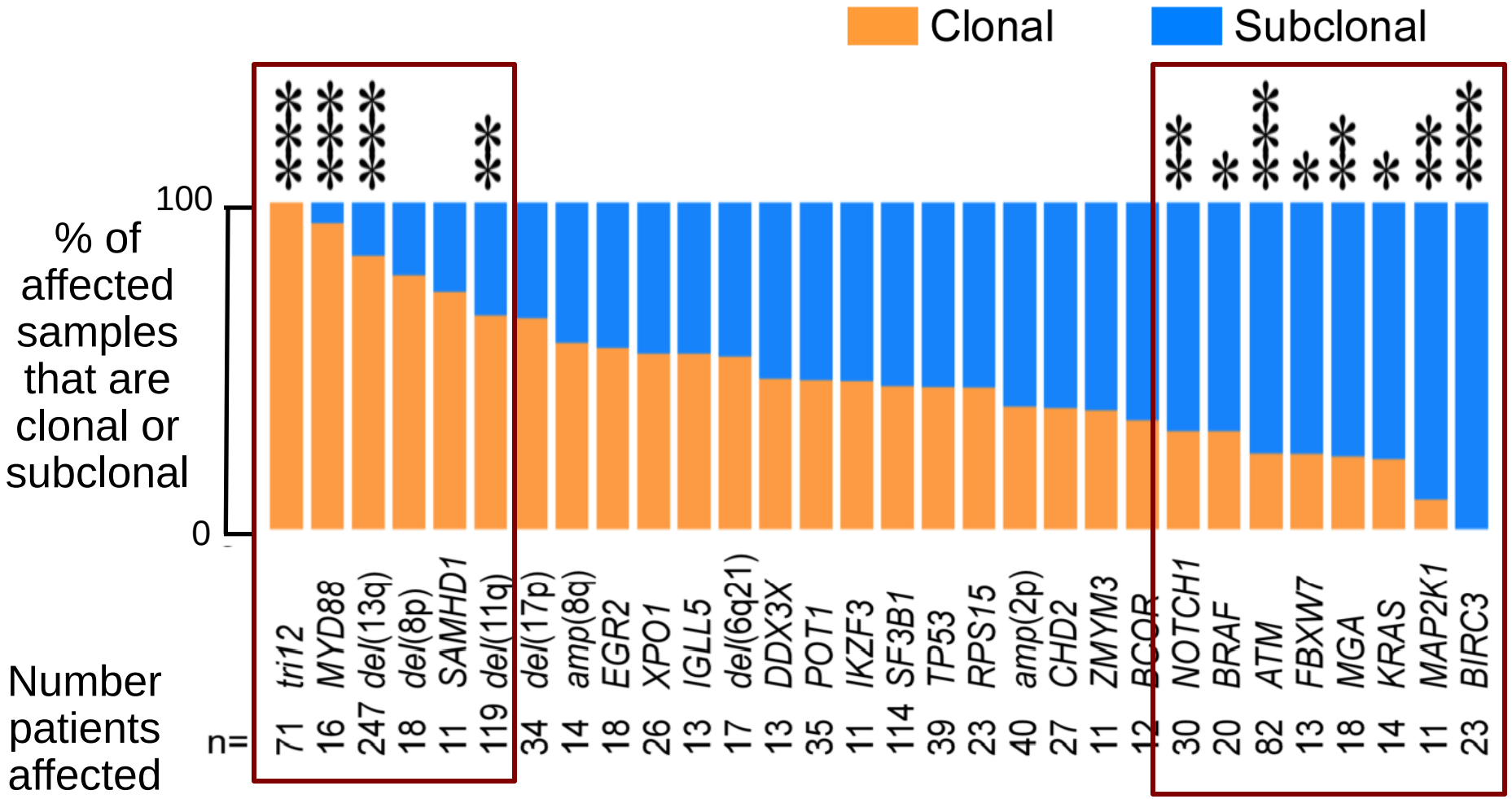
Gruber M & Wu 2014



Subclonal analysis as a temporal snapshot



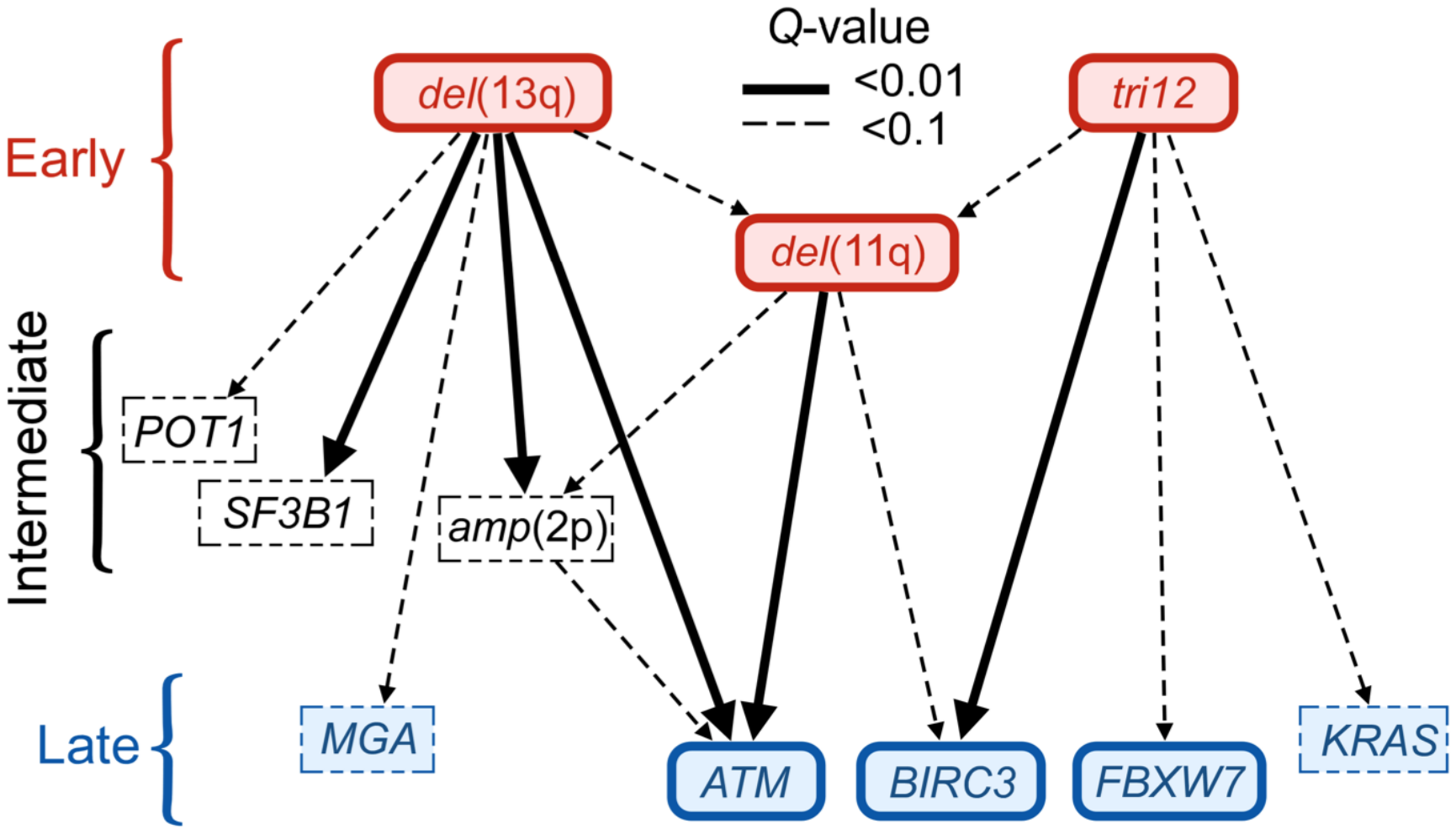
Inferring earlier and later CLL drivers from aggregate frequencies



Dan Landau, Eugen Tausch, Chip Stewart, Amaro Taylor-Weiner

Generating a network

- Larger cohort size gives the opportunity to infer consistent temporal relationships between pairs of drivers
- 501 treatment-naive samples→ 681 pairs with both a clonal and subclonal driver in the same individual: to define a temporally directed ‘edge’
- We examined for:
  - Early drivers (enriched in outgoing edges)
  - Late drivers (enriched for ingoing edges)
  - Intermediary (no enrichment) drivers



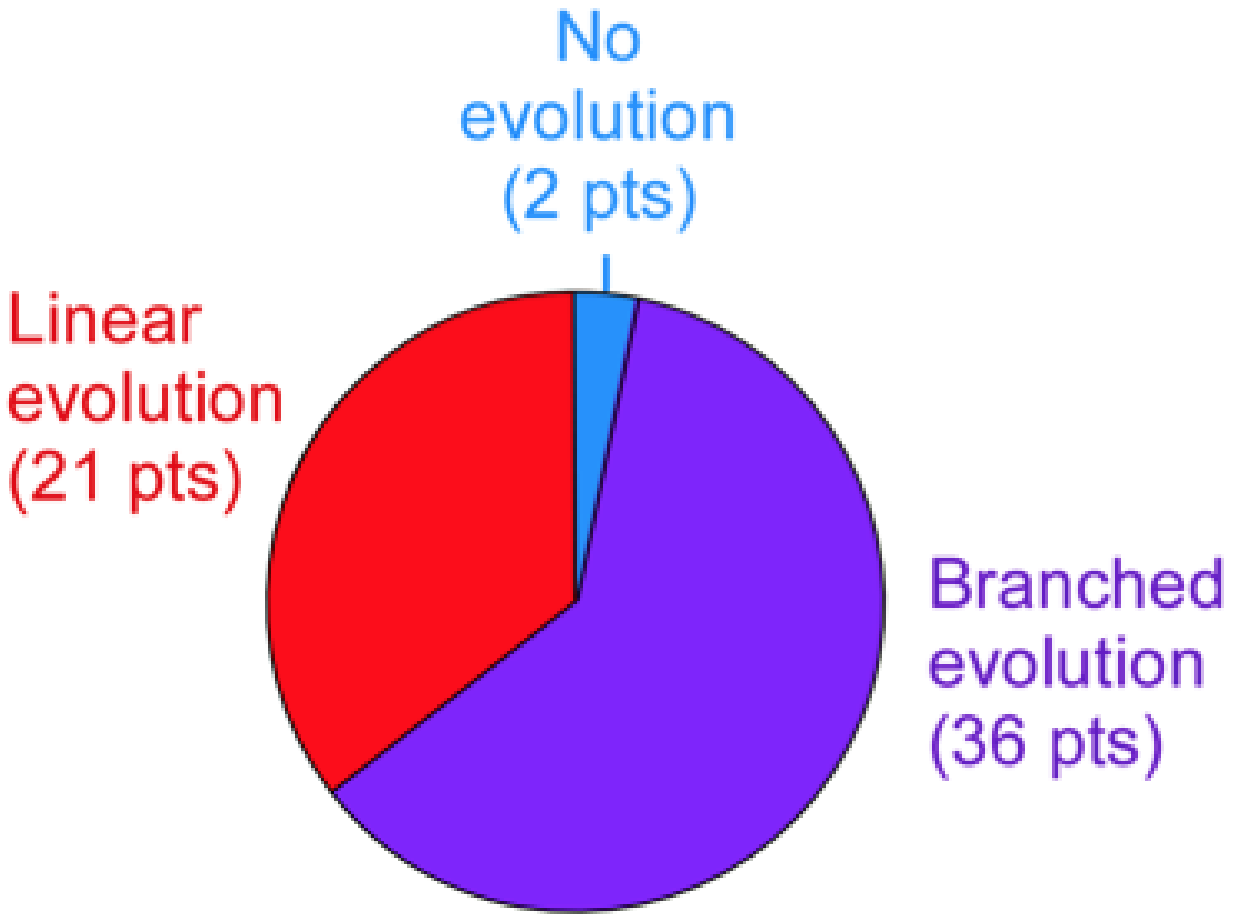
M. Nowak
Ivana Bozic
Johannes Reiter
Dan Landau

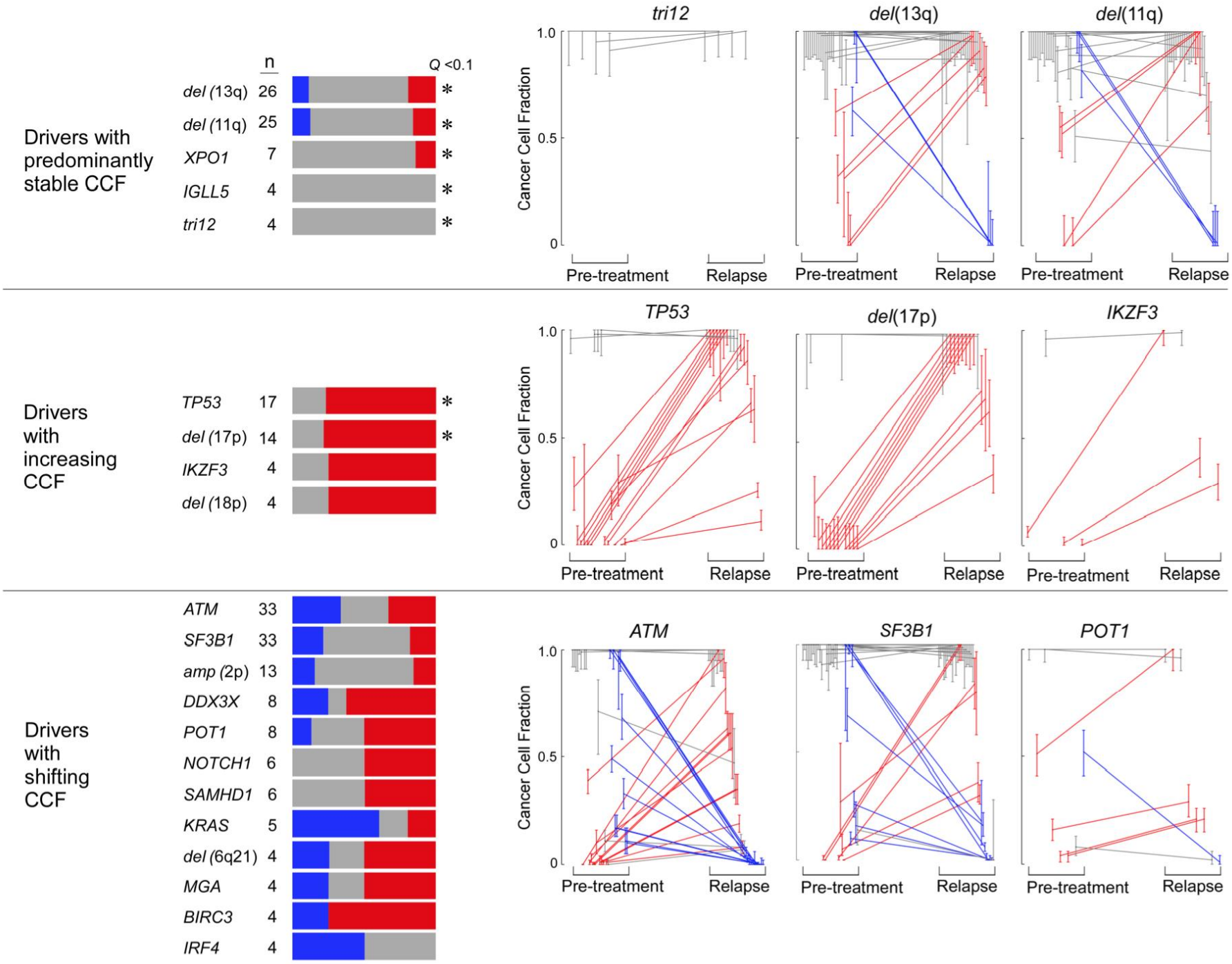
How can we confirm this network model?

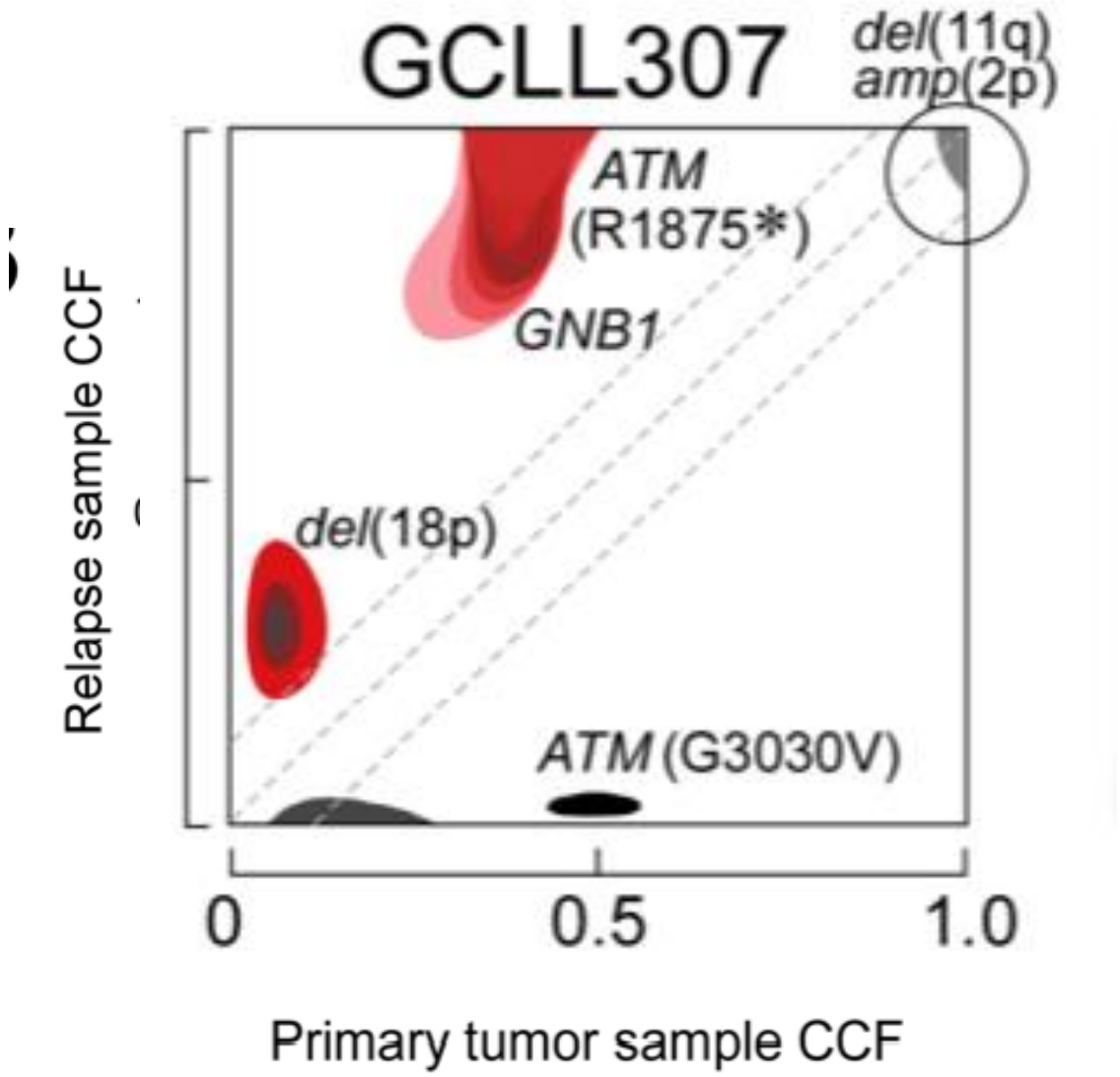
GCLLSG-CLL8: Clinical associations

- Median 6 years followup
- 278 pretreatment samples
- 59 samples at relapse (median 35.1 months)

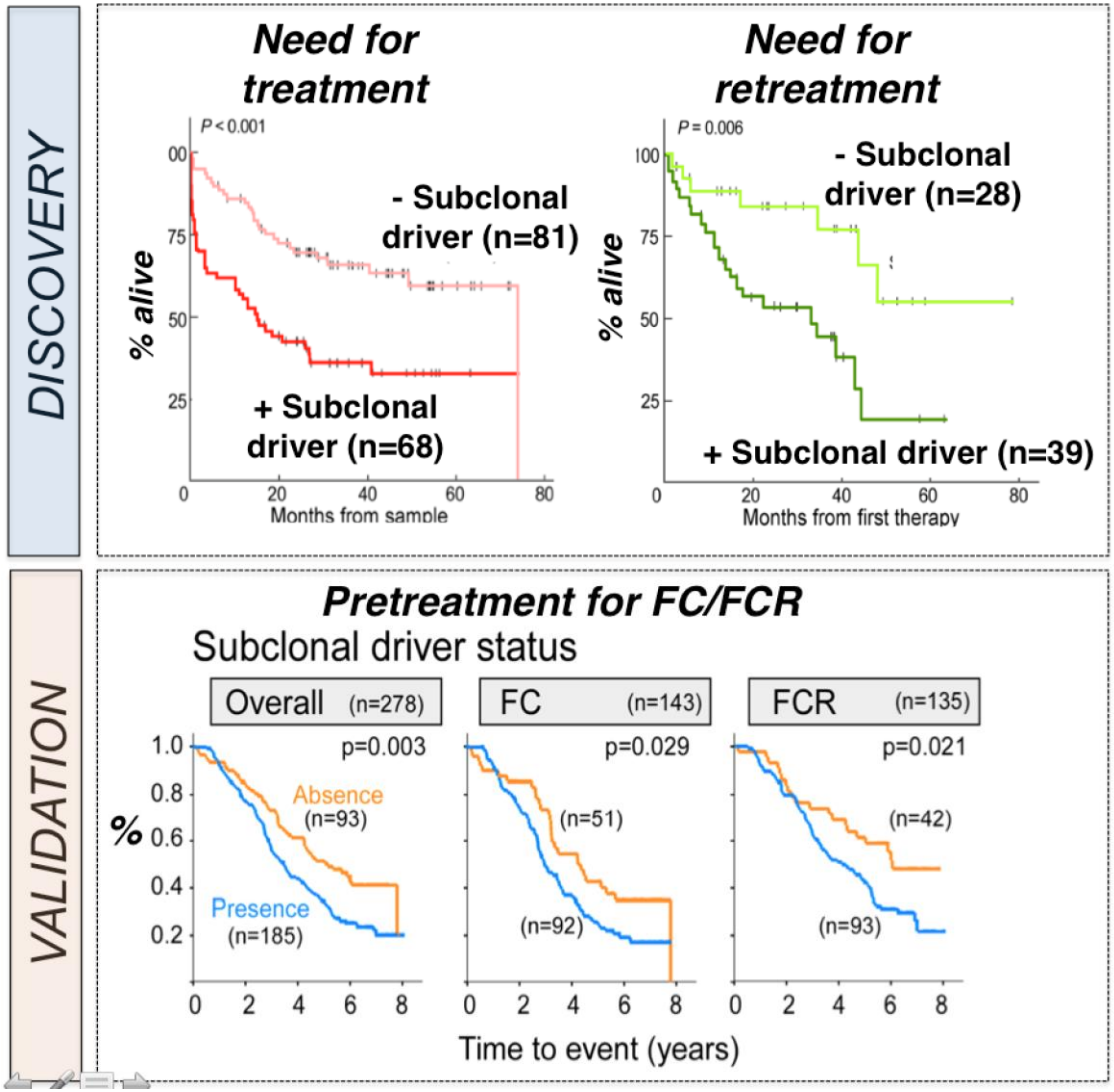
Marked clonal evolution following combination chemotherapy




Drivers with predominantly stable CCF
n
Q <0.1
del (13q) 26 *
del (11q) 25 *
XPO1 7 *
IGLL5 4 *
tri12 4 *
tri12
del(13q)
del(11q)
Cancer Cell Fraction
1.0
0.5
0
Pre-treatment
Relapse
Drivers with increasing CCF
TP53 17 *
del (17p) 14 *
IKZF3 4
del (18p) 4
TP53
del(17p)
IKZF3
Drivers with shifting CCF
ATM 33
SF3B1 33
amp (2p) 13
DDX3X 8
POT1 8
NOTCH1 6
SAMHD1 6
KRAS 5
del (6q21) 4
MGA 4
BIRC3 4
IRF4 4
ATM
SF3B1
POT1


GCLL307
del(11q)
amp(2p)
ATM
(R1875*)
GNB1
del(18p)
ATM (G3030V)
Relapse sample CCF
0
0.5
1.0
Primary tumor sample CCF

Subclonal driver status as a marker of active evolution – associated with poorer outcome



Landau Cell 2013

Summary

WES can be used to define clonal heterogeneity in cancer

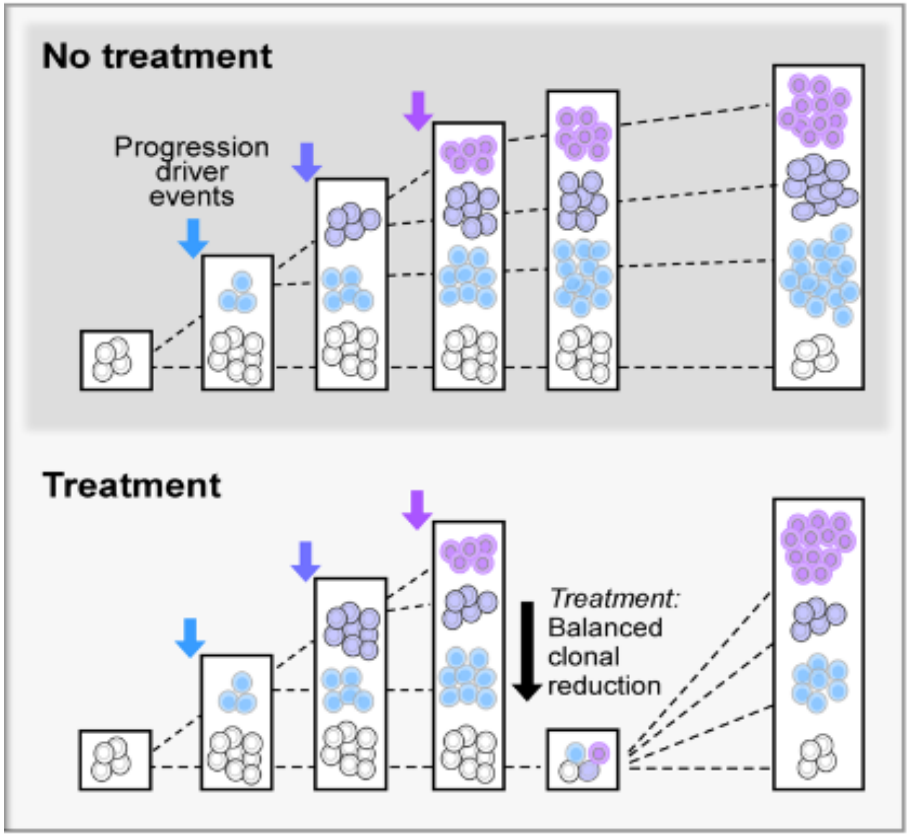
Higher sample power enables us to better explore the disease subclasses, evolutionary relationships

Subclonal drivers are linked to adverse clinical outcome, and are the engine and fuel of resistance and relapse

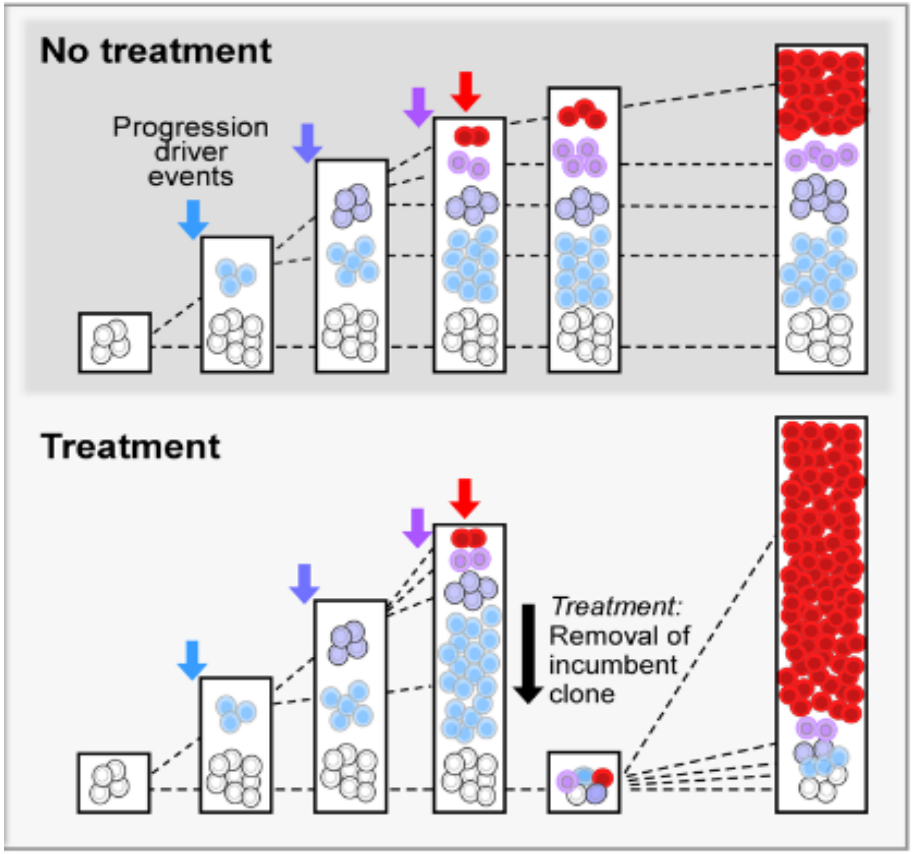
Multiple genetic escape trajectories following combination chemo(immuno) therapy

- Mut TP53 is bad
- Other surprises

Stable: clonal equilibrium

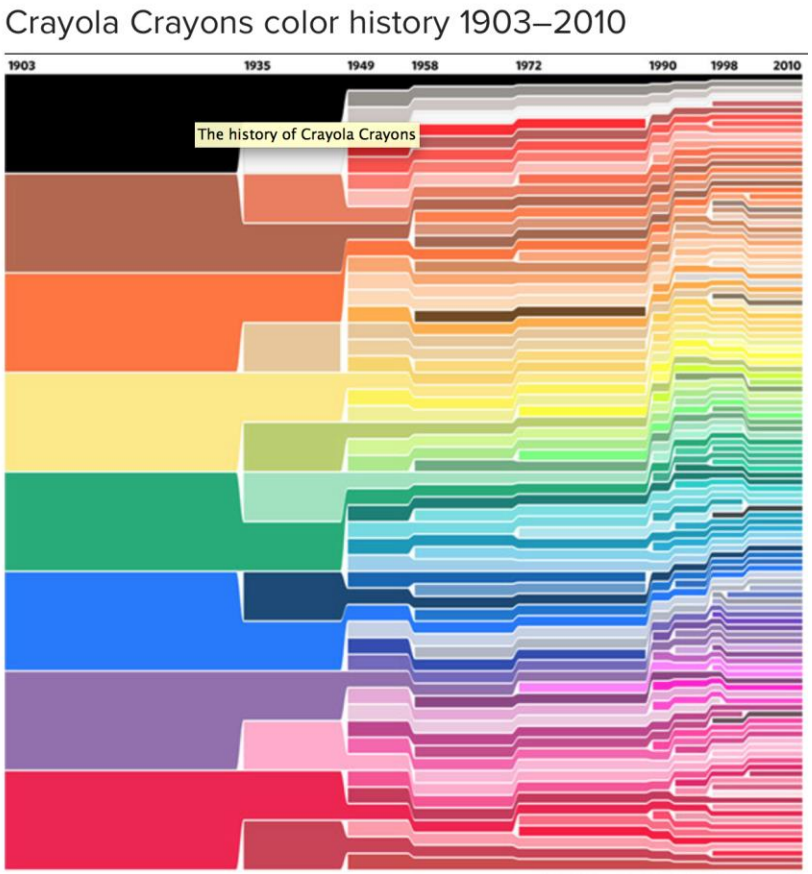


Evolving: fitter subclones emerge with therapy



Drivers?

Not just one thing but different kinds of drivers



It is not so monolithic

--there are initiating drivers vs progression drivers

--drivers that are discoverable by large scale studies

Vs –

Drivers that are private to the individual

What's next?

1. Better understanding of the subclasses of CLL
   - Assembly of large, well-annotated cohorts
   - We have not saturated discovery yet

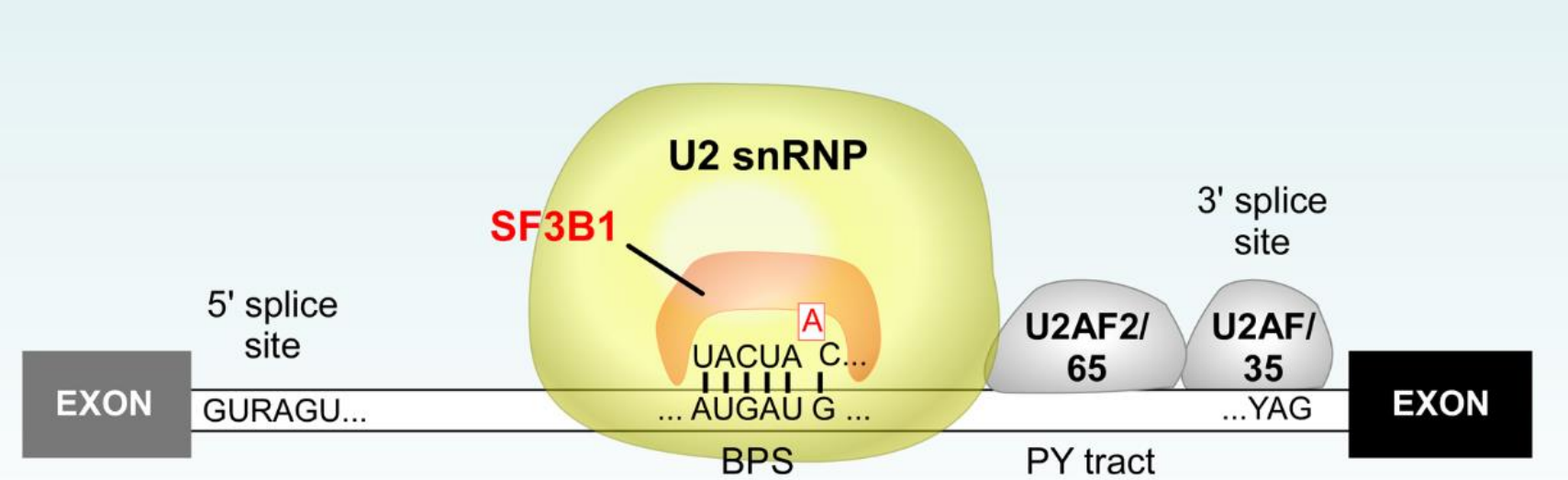
2. What selective pressure do specific therapies impose on CLL?
   - What is the basis of resistance and transformation?
   - What is the impact of novel agents?

3. How do genomics and functional behaviour relate?

Relating genetics to function:

SF3B1 mutation in CLL--studies in human and mice

SF3B1 is the catalytic core of the spliceosome



Wan & Wu., *Blood* 2013

MDS/AML

Patnaik *et al.* 2011
Yoshida *et al.* 2011
Papaemmanuil *et al.* 2011
Graubert *et al.* 2011

CLL

Wang *et al.* 2011
Rossi *et al.* 2011
Quesada *et al.* 2011

Breast Cancer

TCGA 2012

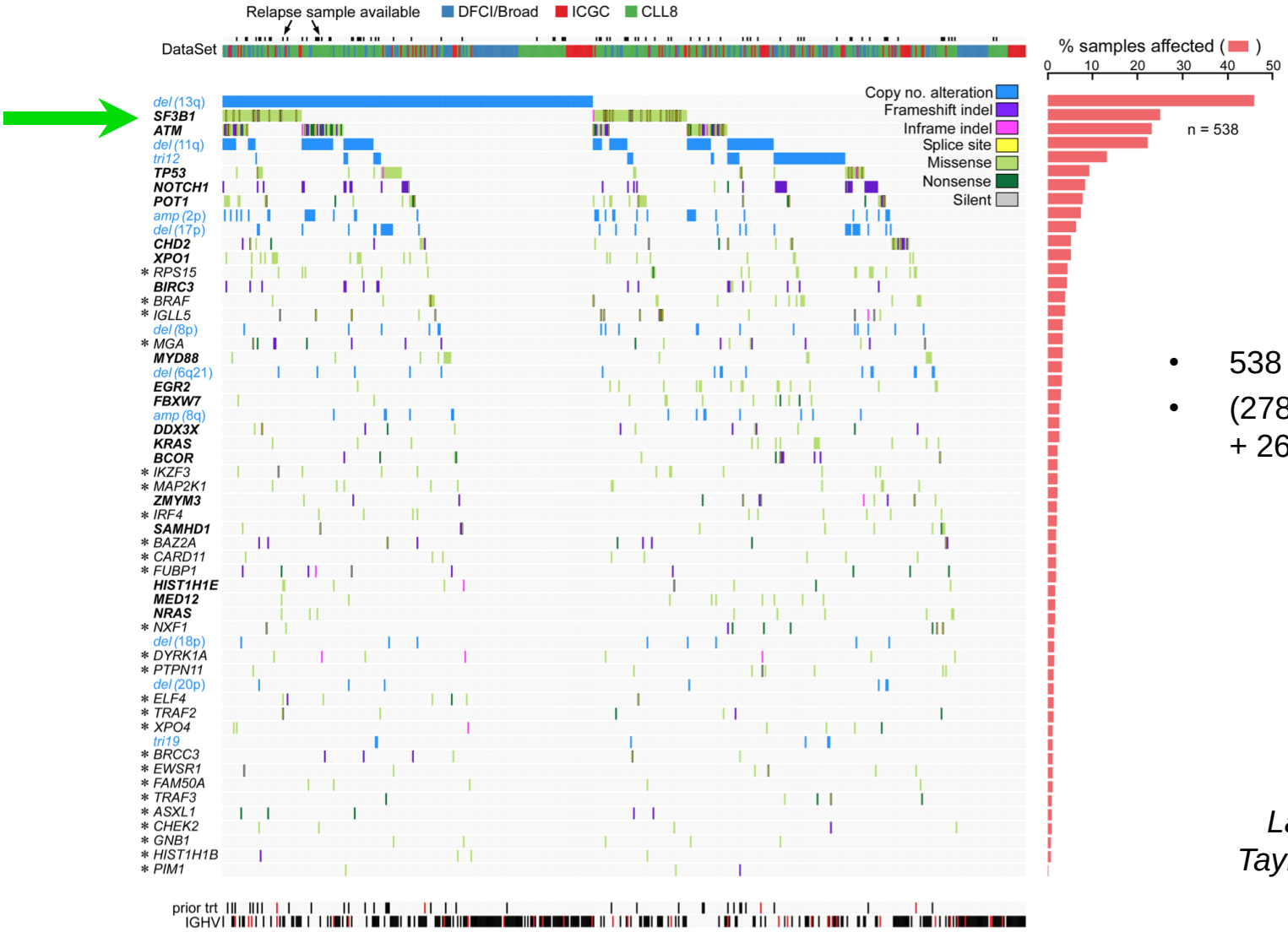
Uveal Melanoma

Harbour *et al.* 2013

Pancreatic ductal adenocarcinoma

Biankin *et al.* 2012

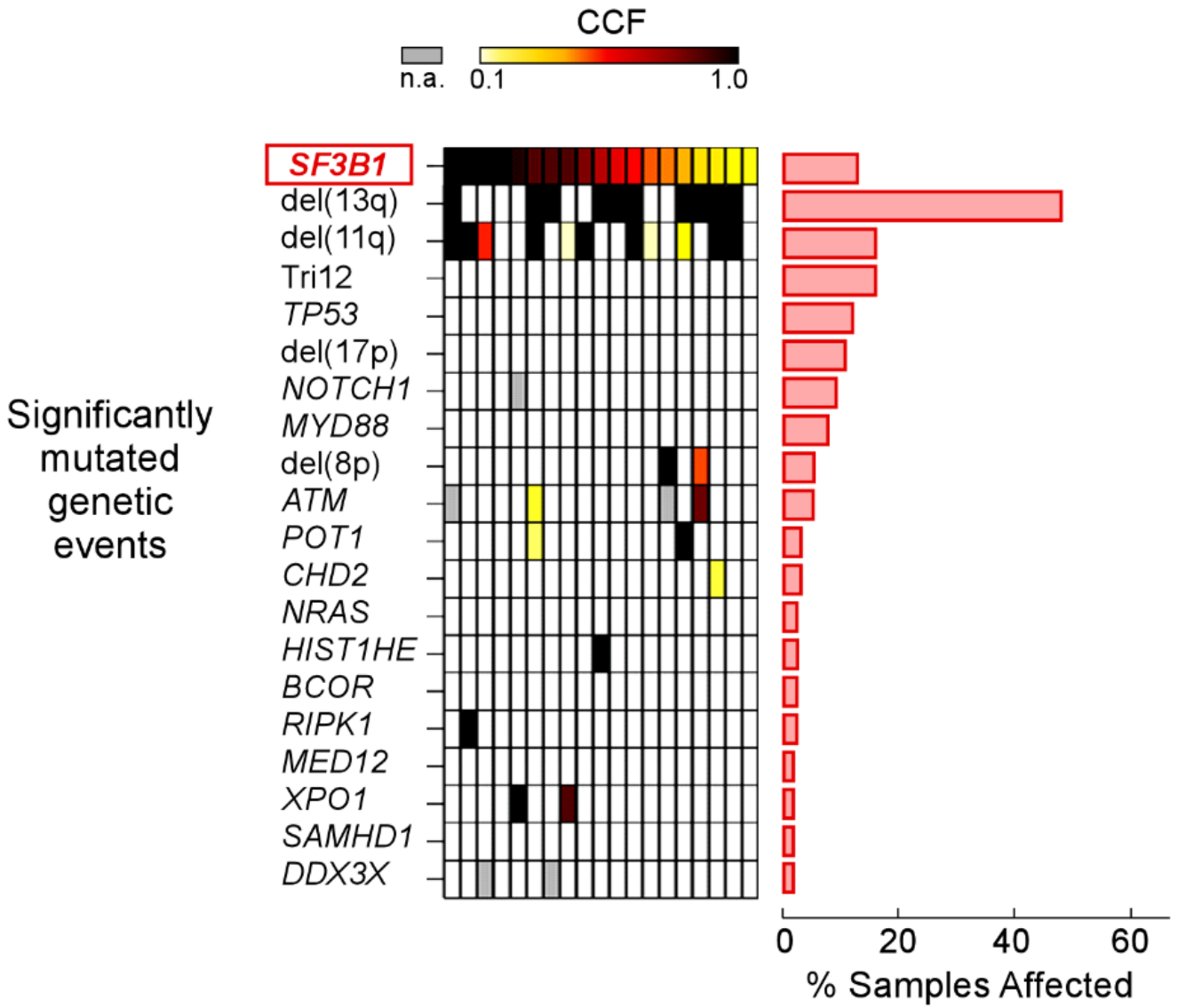
Intertumoral heterogeneity in CLL: independent evolutionary events



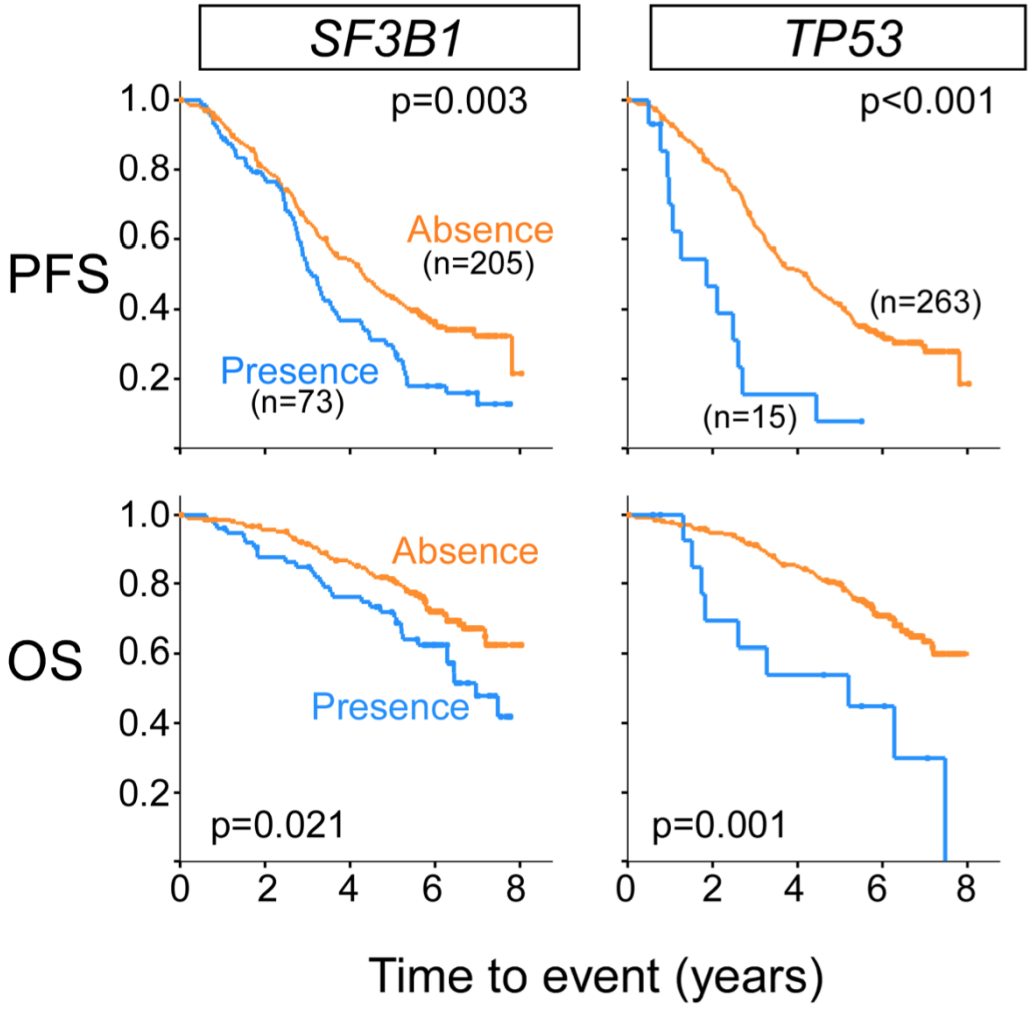
- 538 cases, WES
- (278 GCLLSG-CLL8 + 260 DFCI-ICGC)

Landau Tausch & Taylor Weiner, Nature 2015

Mutated *SF3B1* is a predominantly subclonal event



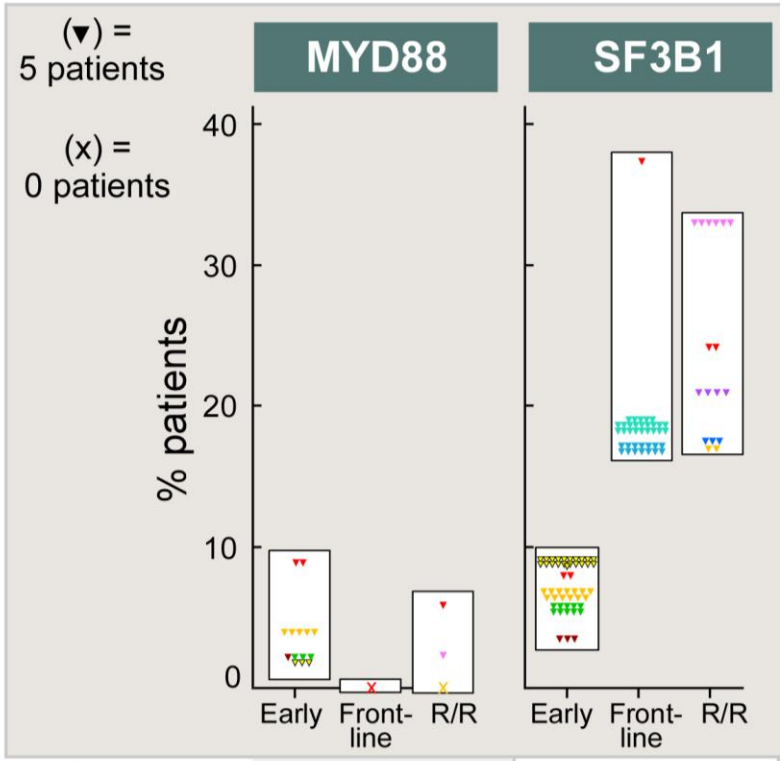
SF3B1 mutation independently predicts poor prognosis



- Observed in earlier studies
  - Wang *NEJM* 2011; Quesada *Nat Gen* 2011
  - Jeromin, *Leukemia* 2014
- GCLLSG-CLL8 cohort:
  - Median 6 years followup
  - 278 pretreatment samples

Landau *Nature* 2015

Rising frequency of SF3B1 with progression

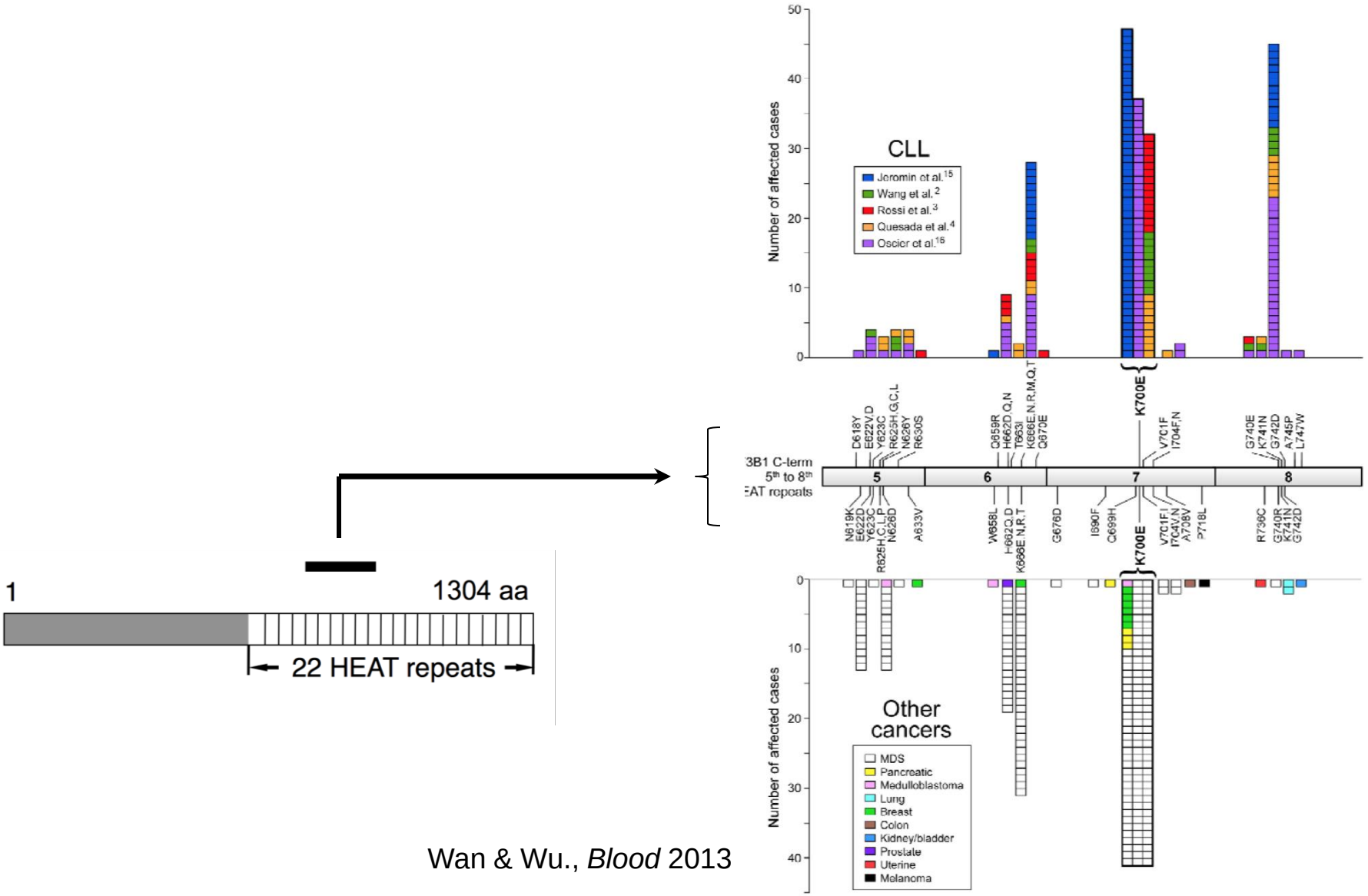


	MYD88			SF3B1		
DFCI Broad Institute	10/111	0/16	2/33	9/111	6/16	8/33
Italy/Columbia	26/637		0/58	67/1007		10/59
ERIC	15/628			51/889		
MLL	15/969			104/1160		
SCALE	6/263			13/358		
GCLLSG CLL8					114/621	
UK LRF CLL4					73/437	
GCLLSG CLL2H						17/97
GCLLSG CLL3X						21/100
French Intergroup UK NCRN			3/114			33/114

Guieze R Blood 2015

How does mutation in SF3B1 impact CLL?

SF3B1 mutations localize to a restricted region



Wan & Wu., *Blood* 2013

2016: What do we know about mut-*SF3B1* in CLL?

Known

- Associated with splicing alterations (DeBoever PLOS Compbio 2015; Ferreira Genome Res 201; Darman Cell Reports 2015)
  - enriched for 3' splice site alterations
- Aberrant 3'ss selection
- Implicated in impaired DNA damage response (te Raa Leukemia 2015)

Unknown

- Does *SF3B1* mutation *cause* the alterations?
  - Technical barrier: overexpressing full-length construct
- Can single cell analysis yield novel insights?
  - Frequently subclonal
- Do splice variants mediate functional activities of *SF3B1* mutation: are there 'driver' vs 'passenger' splice variants?

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National Heart Lung and Blood Institute People Science Health
National Human Genome Research Institute
Leukemia & Lymphoma Society fighting blood cancers
ST2C
Blavatnik Family Foundation