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An overview of the CLL genome

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No conflicts to disclose

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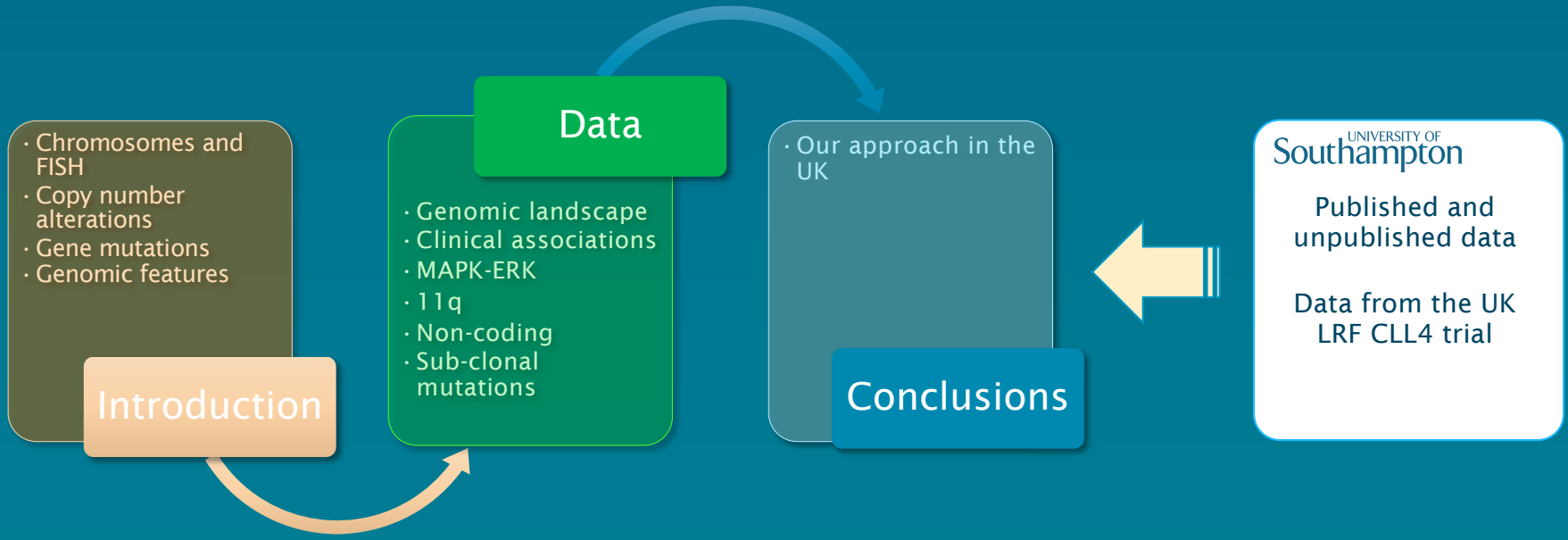
Content overview



The importance of CLL4

LRF UKCLL4 – data published in 30 papers


Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial

777 patients randomly assigned to F (n=194), FC (n=196) or C (n=387)

Feb 1, 1999 to Oct 31, 2004
Published in 2007

Clinical data
PFS (October 2010)
OS (September 2016)

Base-line biomarkers – FISH, IGHV Status, CD38, ZAP70

Long-term survivors Else et al BJH 2016
Quality of life Else et al Leuk Lymph 2012 Else et al BJH 2008
Risk Stratification Oscier et al Haematologica 2016

Copy number alterations

13q deletions	Parker et al Leukemia 2011
11q deletion	Rose-Zerilli et al Haematol 2014
3p deletion	Parker et al Leukemia 2016

Somatic Mutations

NOTCH1 / SF3B1	Oscier et al Blood 2013
NOTCH1 3'UTR	Larrayoz et al Leukemia 2017
EGR2	Young et al Leukemia 2017
RPS15	Ljungström et al Blood 2016
NFKBIE	Mansouri et al J Exp Med 2015
ATM	Skowronska et al JCO 2012
TP53	Gonzalez et al JCO 2011

Other biomarkers

Telomeres	Strefford et al Leukemia 2015
DNA methylation	Wojdacz et al Blood Adv 2019
β2-microglobulin	Pratt et al Leuk Lymph 2016
Cell morphology	Oscier et al BJH 2016
IGHV identify	Davis et al BJH 2016
CLLU1 mRNA	Gonzalez et al Haematol 2013
p53 pathway	Lin et al Clin Can Res 2012
Germline SNPs	Sellick et al Blood 2008 Johnson et al Blood 2013

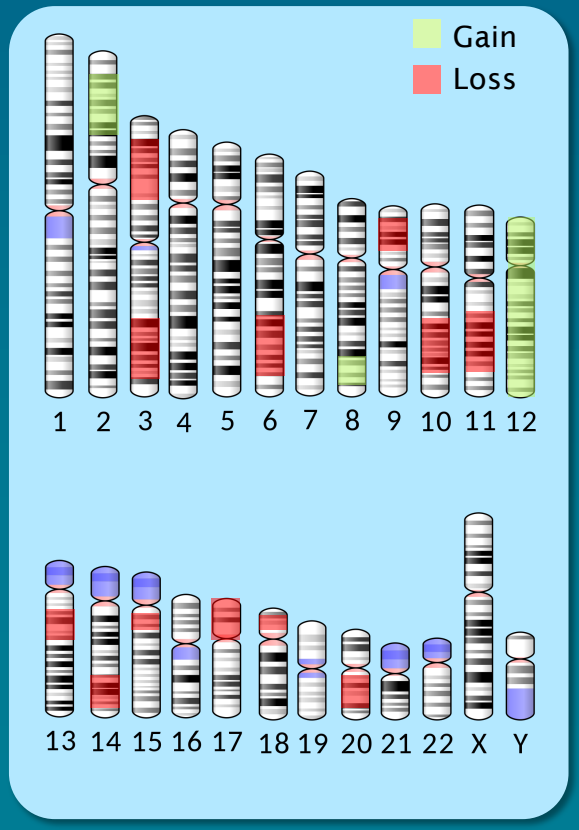
Unpublished study – 499 patients analyzed with deep-sequencing, 22 genes
Comprehensive analysis in context of long follow-up and expansive characterization
Extensive orthogonal confirmation

Why study a chemotherapy trial?

1. Chemotherapy is still widely used globally
2. Many similarities to data from FCR-based trials
3. Long follow-up is required to identify clinically relevant subgroups for study in trials of target agents

Cannot study novel resistance mechanisms

Other chromosomal aberrations

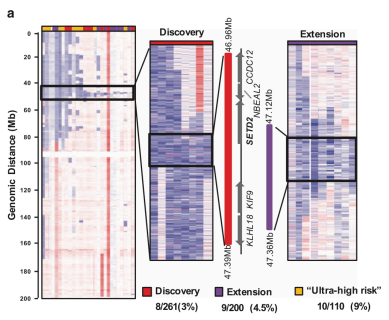


Deletion of 13q

- 50-70% (dependant on technology)
- **microRNA 15a/16-1**

Deletion of 11q

- 20-30% (dependant on disease stage)
- *ATM* (DDR), *BIRC3* (NFKB)



3p deletions (3-6%)
MDR including SETD2
Enriched for TP53
Complex genomes
Reduced expression

SETD2 mutations (4%)
Reduced expression of mutant
Clonal, early events

Somatic SETD2 lesion linked to
Poor survival

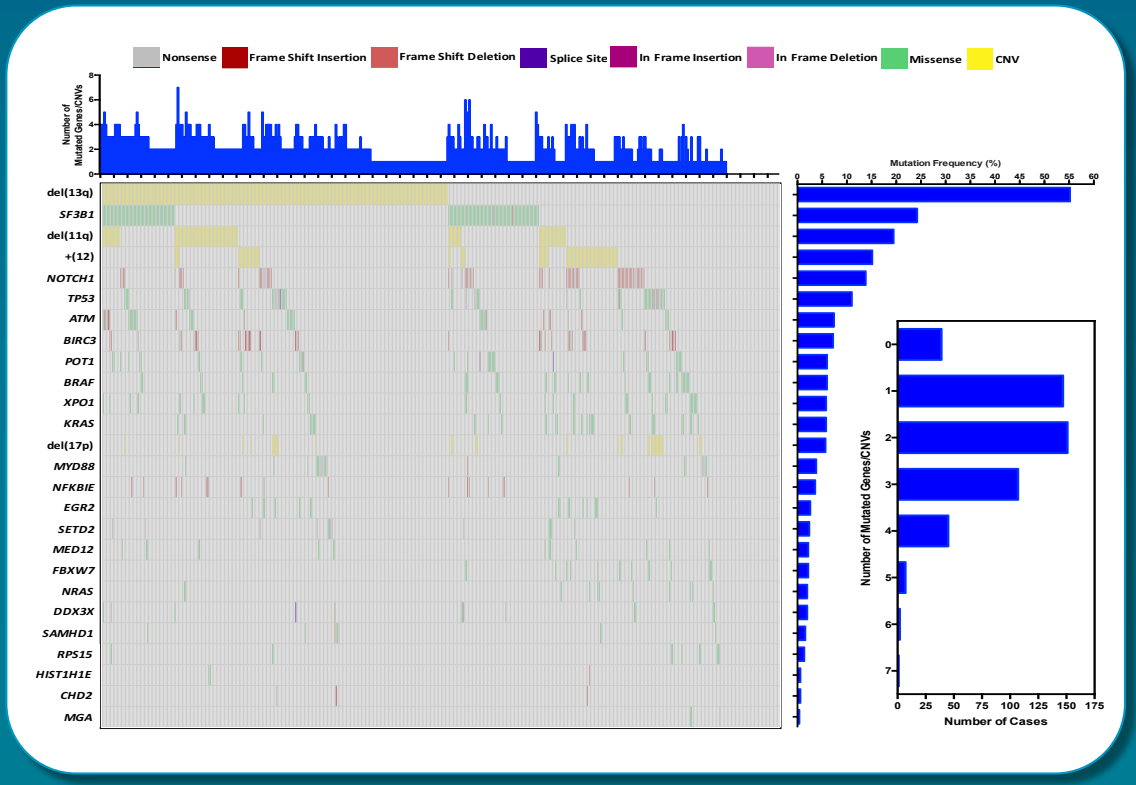
Parker et al (2016) Leukemia 30(11):2179-2186

Mutational landscape of CLL

- 0.87 mutations per Mb
- Complexity, chromothripsis, mutational signatures (Age, AID, others)
- 22 recurrently mutated genes across key studies (lack of concordance at low frequency, 3-5%) , cluster within biological pathways
- TP53, ATM, NOTCH1 and SF3B1 are mutated at >5% across cohorts
- Clonal versus sub-clonal (*SAMHD1, SETD2*) and temporal order
- Patterns of evolution (branching, linear), impact of therapy
- Definition of biological sub-groups based on genomics and clinico-biological characteristics (IGHV usage and stereotypes)
- Outcome correlations and refining clinical models

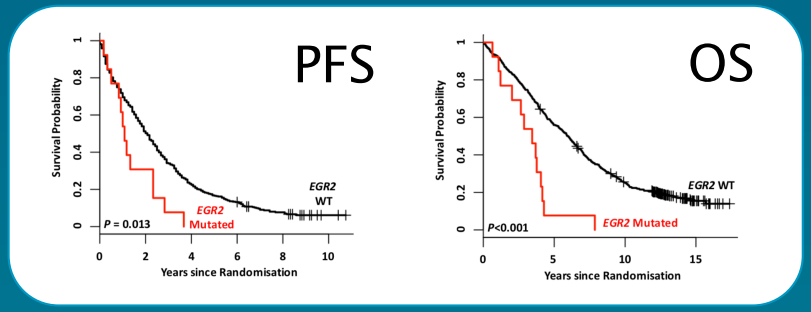
Sequencing analysis of the CLL4 trial

- 623 mutations (mean 1.25, range 0-7)
- 95.5% of cohort harbored at least one CNA or mutation
- **>10%** - *SF3B1*, *NOTCH1*, *TP53*
- **>5%** - *ATM*, *BIRC3*, *POT1*, *BRAF*, *XPO1*, *KRAS*
- **<5%** - *MYD88*, *EGR2*, *NFKBIE*, *NRAS*, *RPS15*, *SETD2*, *MED11*, *FBXW7*, *DDX3X*, *SAMHD1*, *HIST1H1E*, *CHD2*, *MGA*



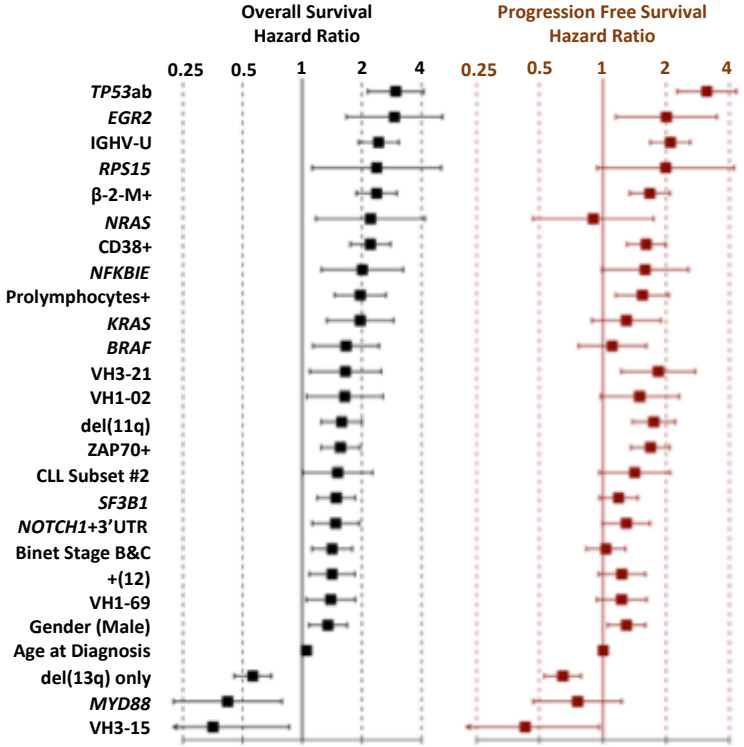
Blakemore et al [unpublished]

Clinical importance of molecular biomarkers

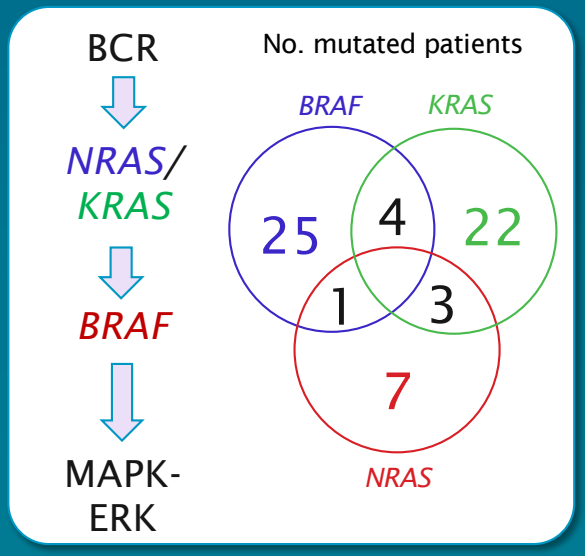


For PFS, *TP53* and *EGR2* were associated with reduced survival.

For OS, recurrent mutations in nine genes; *TP53, SF3B1, NOTCH1*+3'UTR, *EGR2, RPS15, NFKBIE, BRAF, KRAS,* and *NRAS* were associated with reduced survival.



Mutations in *BRAF*, *KRAS* and *NRAS*

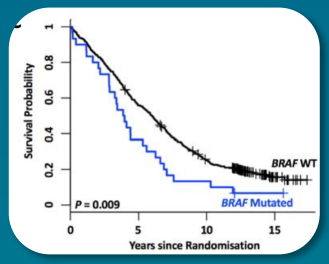


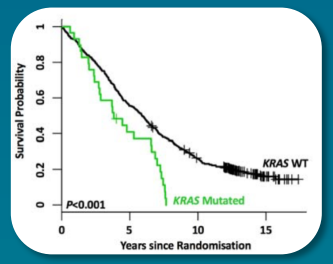
12% prevalence in CLL4

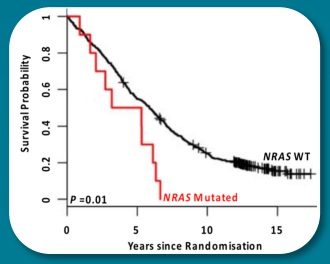
- Overall survival
- BRAF (OS median: 3.92yrs vs. 6yrs, P = 0.009)
- KRAS (OS median: 3.83yrs vs. 5.89yrs, P<0.001)
- NRAS (OS median: 275 4.24yrs vs. 5.88yrs, P = 0.01)

- All genes (OS median: 3.83yrs vs. 6.10yrs, P<0.001)
- Negatively associated with long-term survival (Odds Ratio = 0.19, P<.001)

Blakemore (2019) unpublished



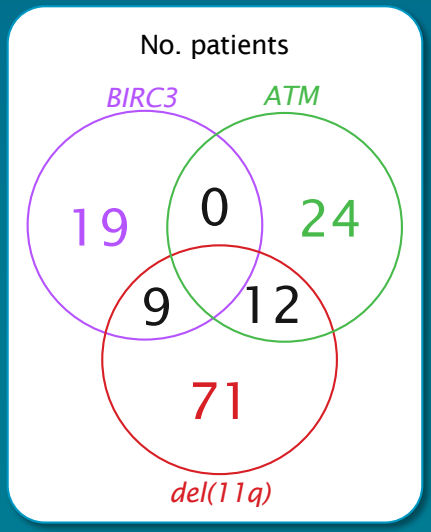




- Independently associated with reduced OS in MVA
- HR: 1.683 (P=0.002)

Sub-grouping del(11q) CLL by *ATM* and *BIRC3*

- 11q deletion but not *ATM* / *BIRC3* mutation associated with reduced PFS and OS
- Performed a stratified analysis based on 11q deletion (Diop et al, 2019, Raponi et al, 2019, Skowronska et al 2012)



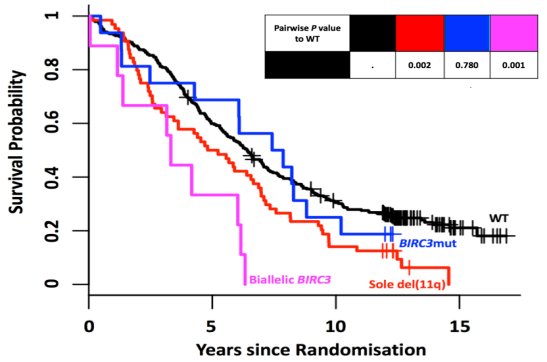
ATM and *BIRC3* mutations were mutually exclusive

Quantified *BIRC3* and *ATM* CNA from SNP6 and sWGS

Define 5 groups
1) del(11q) only
2) Biallelic *ATM*
3) Biallelic *BIRC3*
4) ATM mutation only
5) BIRC3 mutation only

Performed survival analysis

- All 11q groups inferior compared to WT
- *BIRC3* biallelic exhibited worst survival



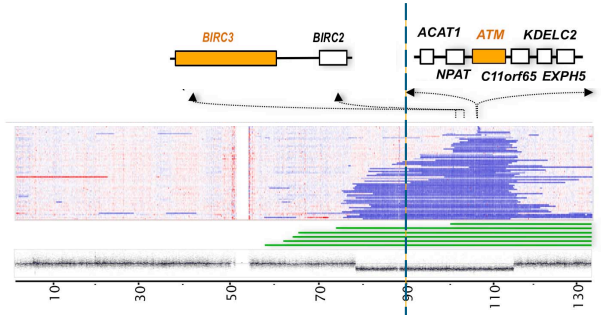
- Independent marker of inferior OS and PFS in MVA
- 2nd highest HR after *TP53*

Blakemore (2019) unpublished

11q deleted CLL sub-groups

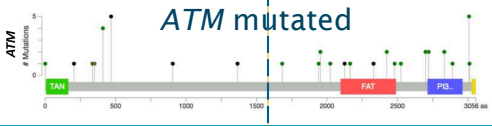
11q deleted

Rose-Zerilli *et al* (2014) Haematologica 99(4):736-42



83% delete one copy *BIRC3*

ATM mutated



Biallelic loss

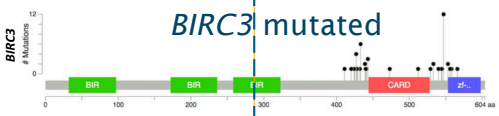
ATM dysfunction

Poor survival

Same survival as WT

Intermediate survival

BIRC3 mutated



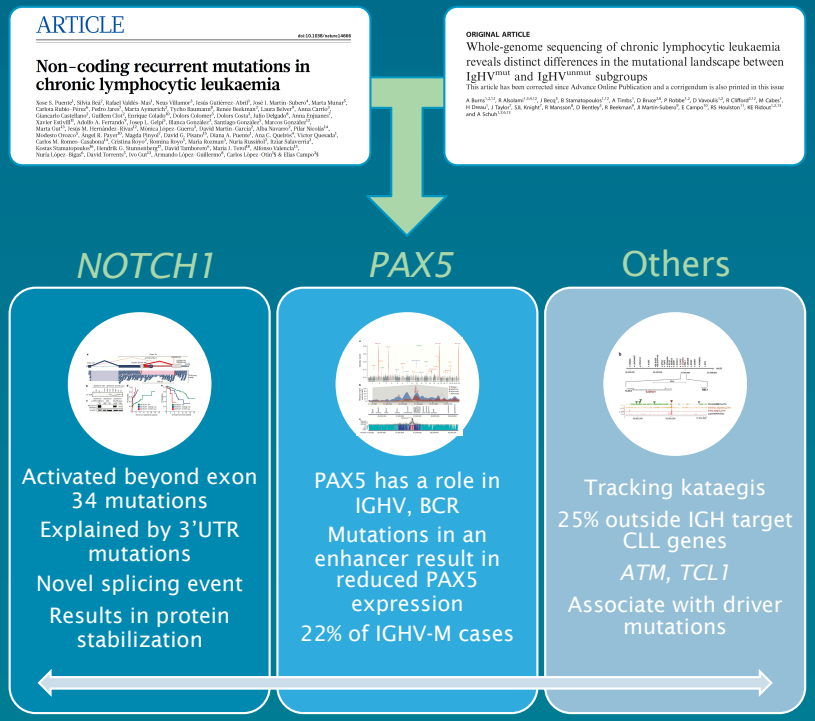
Biallelic loss

NF-KB activation

Poor survival

Same survival as WT

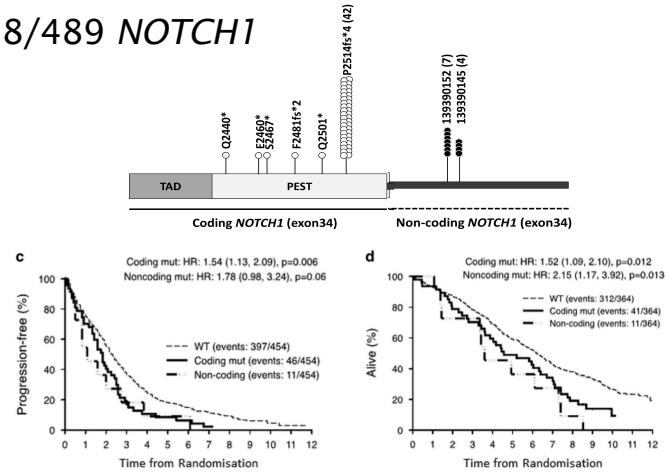
NOTCH1 as an important example of non-coding mutations



Puente et al (2015) Nature 526:519-524
Burns et al (2018) Leukemia 32;573

- Validate in a clinical and assess improved prognostication

58/489 *NOTCH1*



10% of *NOTCH1* mutations are non-coding

Power to predict outcome events is improved

Larrayoz et al (2016) Leukemia 31(2):510-514

Clinical importance of genomic lesions

All significant variables in univariate, with backward selection to generate final model

OS - (391 patients, 323 events)
PFS – (225 patients, 210 events)

TP53 del/mut remains the strongest marker of reduced PFS and OS

Supports importance of *EGR2*, *SF3B1*

BRAF, *NRAS* and *KRAS* predict reduced OS

Biallelic *BIRC3* has the second highest HR, after *TP53*

Survival	Variable	HR	LCI	UCI	*P*
Overall	***TP53*ab**	4.247	2.932	6.151	<0.0001
	Biallelic *BIRC3*	2.756	1.397	5.438	0.003
	***EGR2* mutated**	2.188	1.167	4.099	0.015
	IGHV-U	1.831	1.417	2.364	<0.0001
	***MAPK-ERK* mutated**	1.683	1.202	2.356	0.002
	***SF3B1* mutated**	1.544	1.191	2.002	0.001
	Binet Stage B & C	1.454	1.102	1.918	0.008
	11q deletion	1.431	1.081	1.895	0.012
Progression-Free	***TP53*ab**	4.975	3.049	8.118	<0.001
	Short Telomeres	1.964	1.466	2.629	<0.001
	11q deletion	1.816	1.226	2.688	0.003
	Biallelic *BIRC3*	3.833	1.537	9.557	0.004
	Prolymphocytes	1.508	1.034	2.198	0.033

The OS model: MAPK-ERKmut, TP53ab (after removal of <12% TP53 mutations), EGR2mut, RPS15mut, NFKBIEmut, MYD88mut, SF3B1mut, NOTCH1+3'UTRmut, Binet Stage B&C, 11q deletion, biallelic ATM, biallelic BIRC3, sole 13q deletion, trisomy 12, IGHV-U. The final model for OS consisted of 391 patients and 323 events.

The PFS model : TP53ab, EGR2mut, biallelic ATM, biallelic BIRC3, 11q deletion without ATM or BIRC3 mutations, sole 13q deletion, Short Telomeres, Prolymphocytes+, and IGHV-U. The final model for PFS consisted of 225 patients and 210 events.

Blakemore (2019) unpublished

Sub-clonal mutations

- *TP53* mutations below the resolution of Sanger sequencing confer inferior survival in retrospective cohorts (Rossi et al, Nadau et al).

All confirmed with orthogonal techniques (Ion Torrent, Hyd-based panels)

55 mutated patients identified

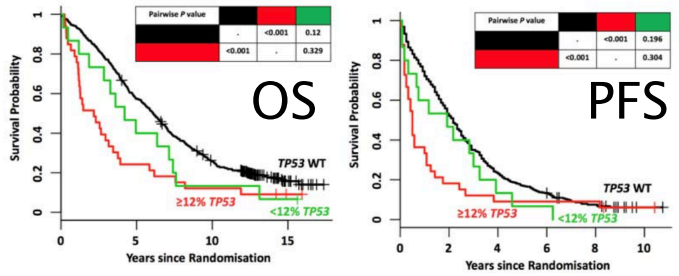
All curated in IACR

Check for purity adjustment of VAF

No bias in clinic-biological features, complexity, clonal drivers

>12% and <12% VAF

Stratified by FISH



- >12% mutations associated with reduced PFS (P<0.001) and OS (P<0.001)
- <12% mutations not significantly different from WT or >12% patients
- <12% cases with del(17p) may do worse, but limited numbers

Blakemore (2019) [unpublished]

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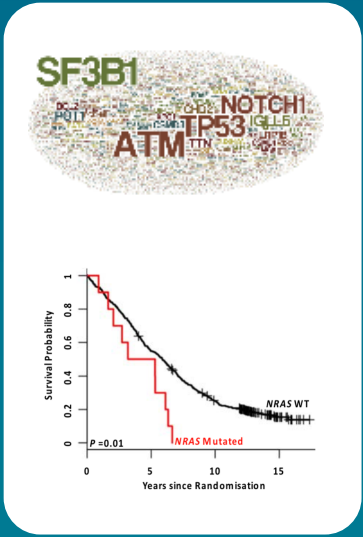
Conclusions



The CLL genome

- The CLL genome harbors less mutations that solid tumours and acute leukaemias
- Mutations do cluster within pathways of importance to B-cell biology and leukemogenesis
- Our analysis of the CLL4 trial has contributed to understanding the clinical importance of genomic lesions.
- Including our new analysis of 22 genes in 499 CLL4 patients

CLL4 has also helped characterize novel genomic subgroups

Confirm and extend observations in trials of novel agents

What are we doing in the UK?

Whole genome sequencing (Through NHS Genomic Hubs)

Patients requiring therapy

Observations from CLL4

Iterative computational and statistical analysis

Multiple prognostic genomic predictors

Mutation burden	Functional non-coding mutations	*TP53* mutation	Copy Number Alterations
SNVs in Ig region	Chromothripsis	CNA burden	AID signature
Driver gene mutations	Germ-line hits		Telomere length

Novel genomic features, or combinations of prognostic or predictive significance

National Research Strategy

Continues analysis of WGS dataset

Ongoing studies of early disease

National Biobanking for research

1) Single cell analysis of MRD populations
2) T-cell repertoire studies
3) Microenvironment and signaling analysis
4) Implementation of liquid biopsy approaches

Acknowledgements

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Dr Matthew Rose-Zerilli
Dr Helen Parker
Dr Harindra Amarashinghe
Dr Marta Larrayoz
Dr Dean Bryant
Dr Mersad Ghorbni
Dr Chantal Hargreaves
Dr Stuart Blakemore
Dr Jade Forster
Dr Jaya Thomas
Ms Kate Latham
Ms Lara Makewita
Ms Sarah Frampton

THE KAY KENDALL LEUKAEMIA FUND

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UK CLL researchers and clinicians
Prof Daniel Catovsky
Prof Peter Hillmen
Prof Andy Pettitt
Dr Melanie Oates
Dr Anna Schuh
Prof Tanja Stankovic
Prof Martin Dyer
Prof Duncan Baird

WRGL Salisbury
Prof Nick Cross

Bournemouth
Prof David Oscier
Dr Helen McCarthy
Dr Renata Walewksa
Dr Zadie Davies

International collaborators
Prof Richard Rosenquist
Prof Kostas Stamatopolous
Prof Davide Rossi
Prof Stephan Stilgenbauer
Prof Paolo Ghia
Prof Thorsten Zenz
Prof Michael Hallek
Dr Jennifer Edelmann
Prof Arnon Kater
Prof Jennifer Brown
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Prof Christoph Plass
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Prof Stephen Mulligan
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