4th POSTGRADUATE

CLL Conference

Bologna
November 13-14
2023

Royal Hotel Carlton

President:
Pier Luigi Zinzani

Disclosures of Name Surname

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

UNIVERSITY OF OXFORD

Genomic analysis of CLL

Dr Kate Ridout

University of Oxford

CLL in the clinic

CLL-IPI (Chronic Lymphocytic Leukemia-International Prognostic Index)

- Age
- Rai stage
- Beta-2 macroglobulin ,IgHV mutational status, TP53 disruption

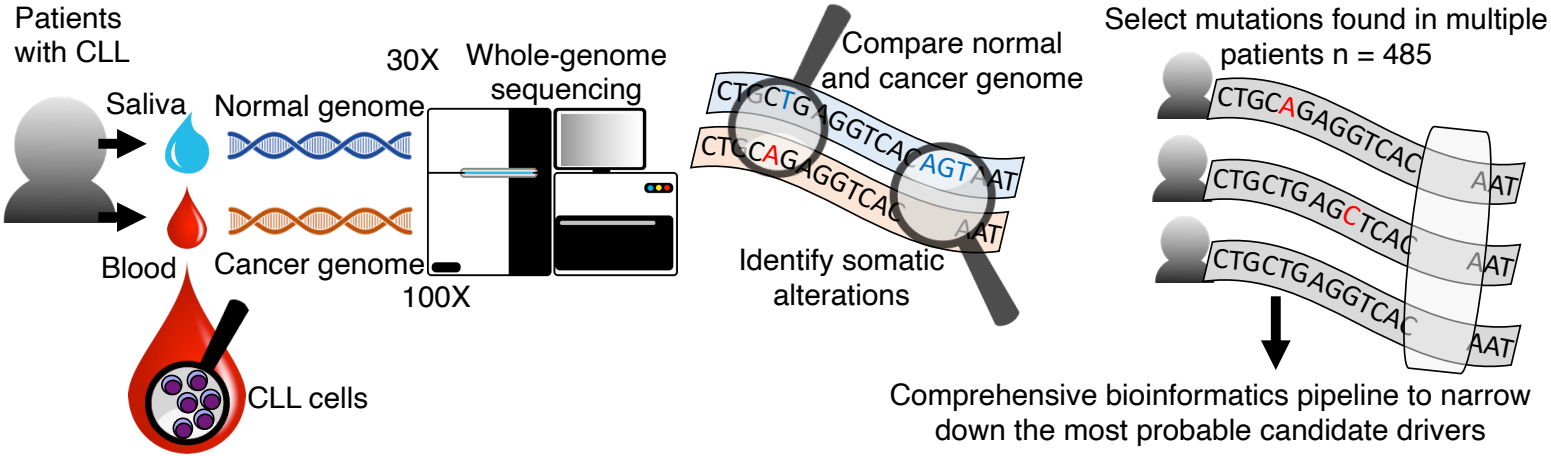
CLL4

- Relapsed disease
- LDH
- Beta-2 macroglobulin, TP53 disruption

Genomic risk stratification can combine hundreds of features.

Mutations with functional consequences in exons mutated across our CLL patient cohort

- CLL remains incurable and patients still relapse
- Patients may not tolerate a targeted therapy
- Combination therapies

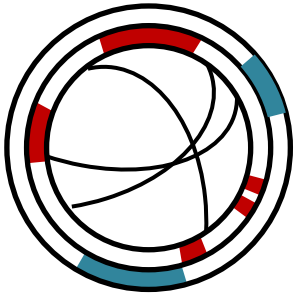


- Tumour DNA is taken from whole blood
- In pre-treatment CLL samples >90% of cells can be CLL cells
- Germline DNA from saliva
- Data are subtracted to leave only tumour DNA

Figure courtesy of P. Robbe

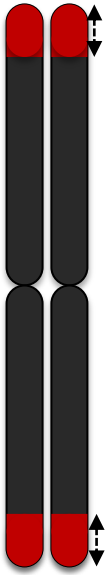
Whole genome methodologies

Genome complexity



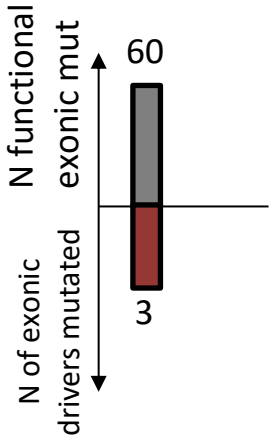
CN Gain
CN Loss

Telomere length

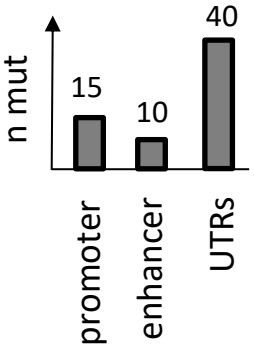


Telomere estimation in bp

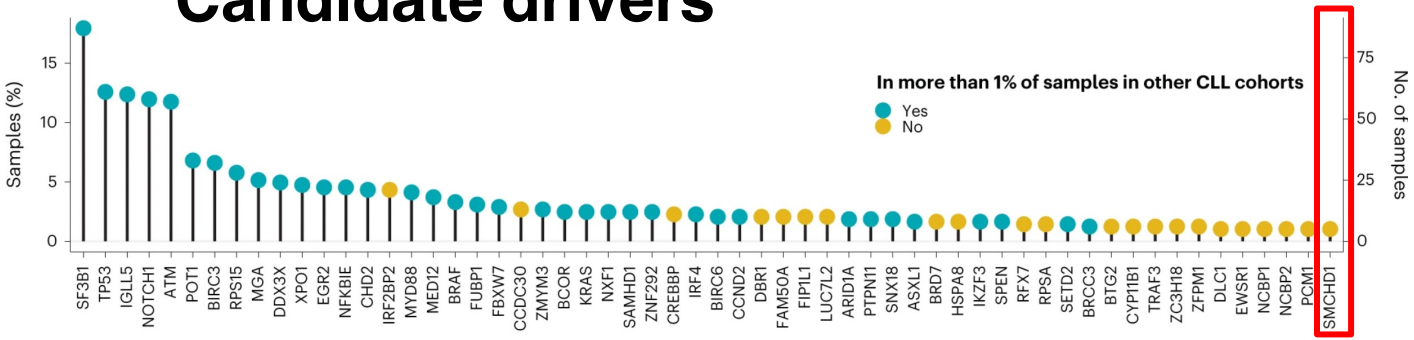
Coding mutation burden & Driver count

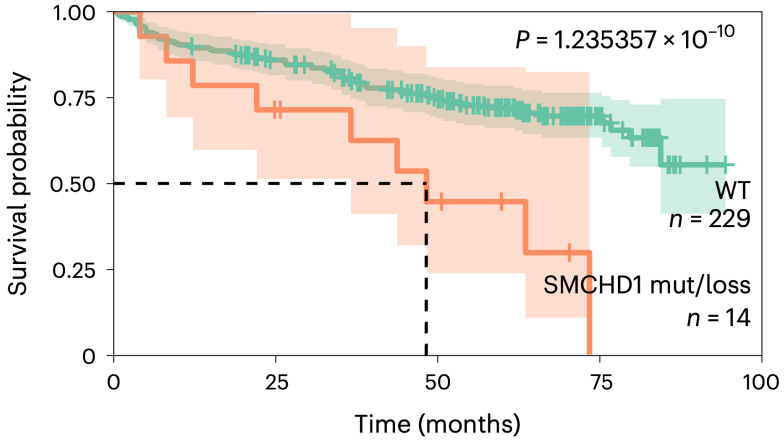


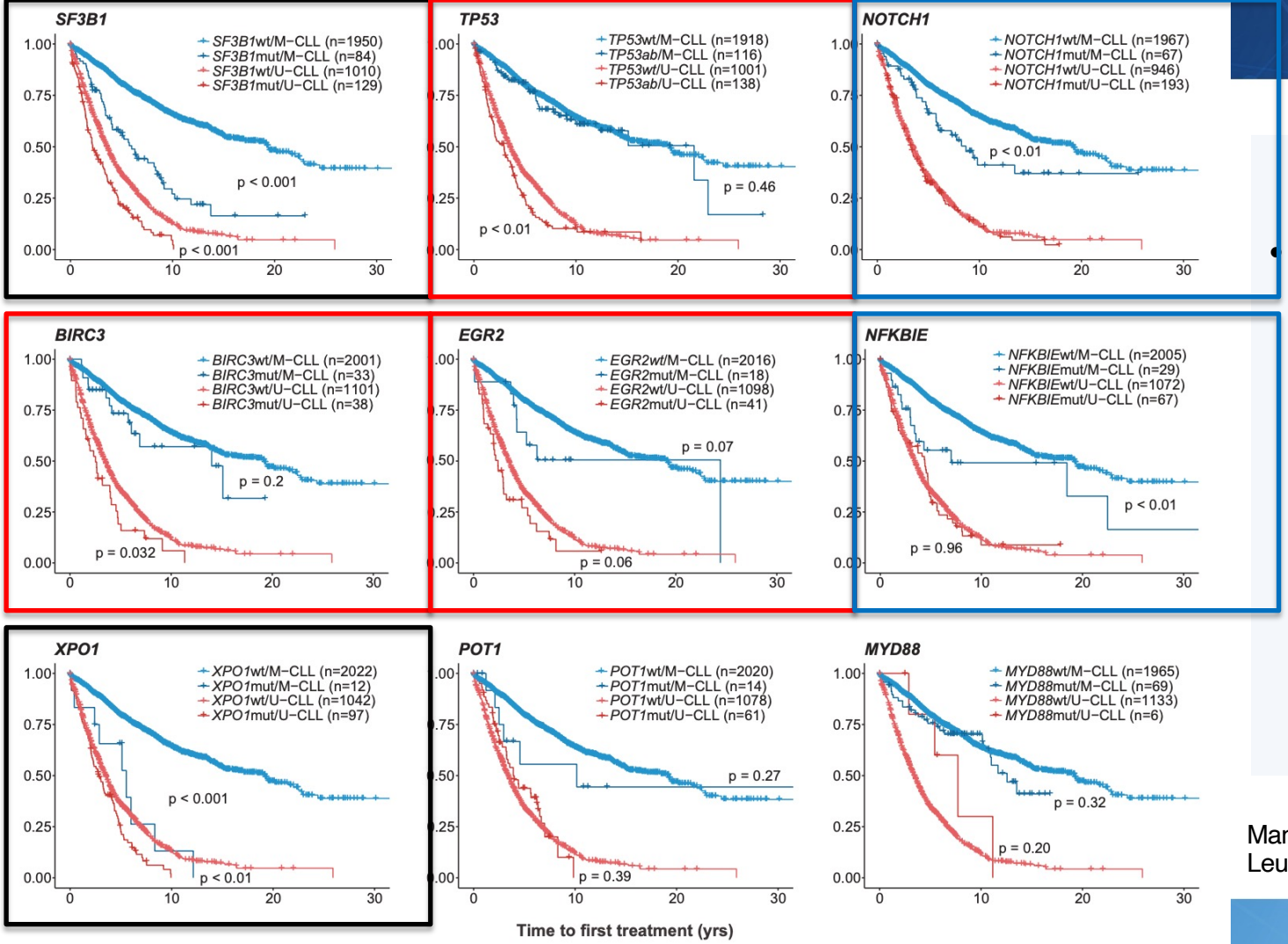
Non-coding mutational burden



Candidate drivers



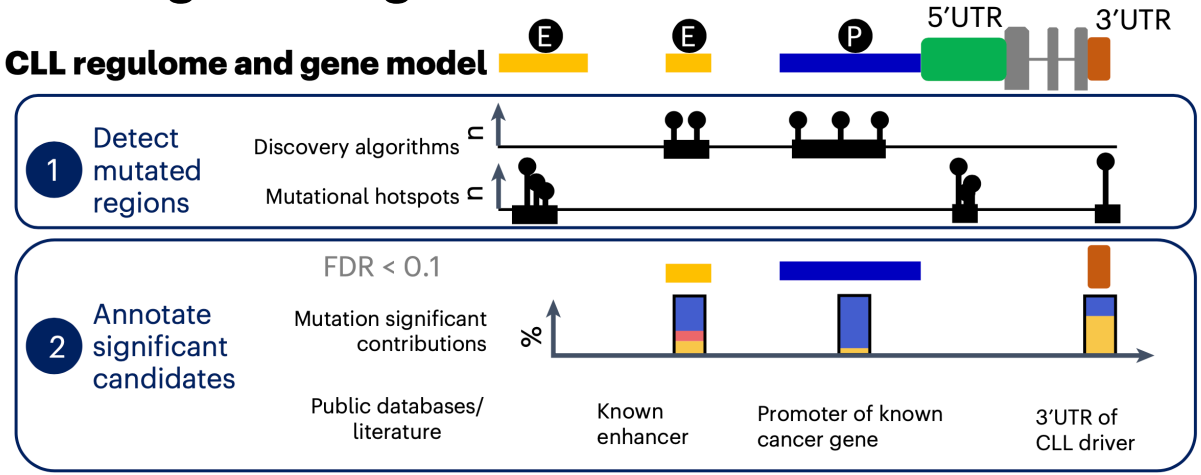




- Driver gene mutations have varying prognostic impacts depending on IgHV mutation status

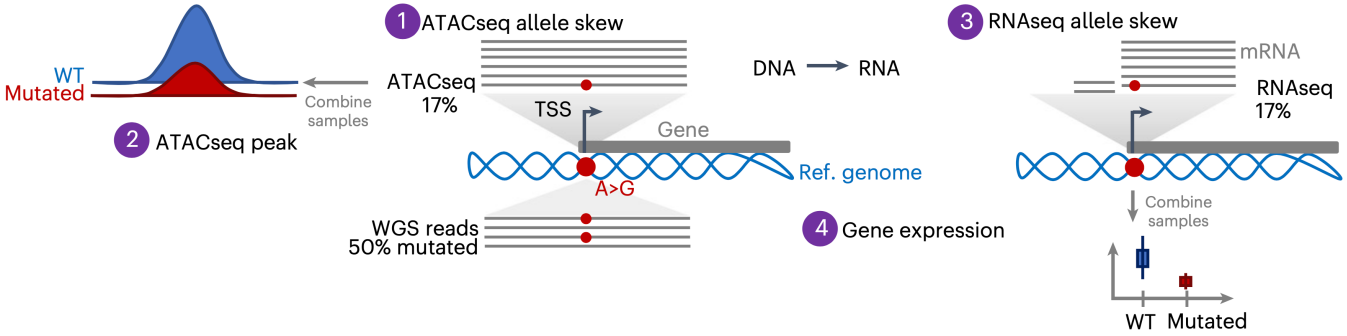
Mansouri *et al.* (2023) Leukemia

Non-coding Driver gene identification



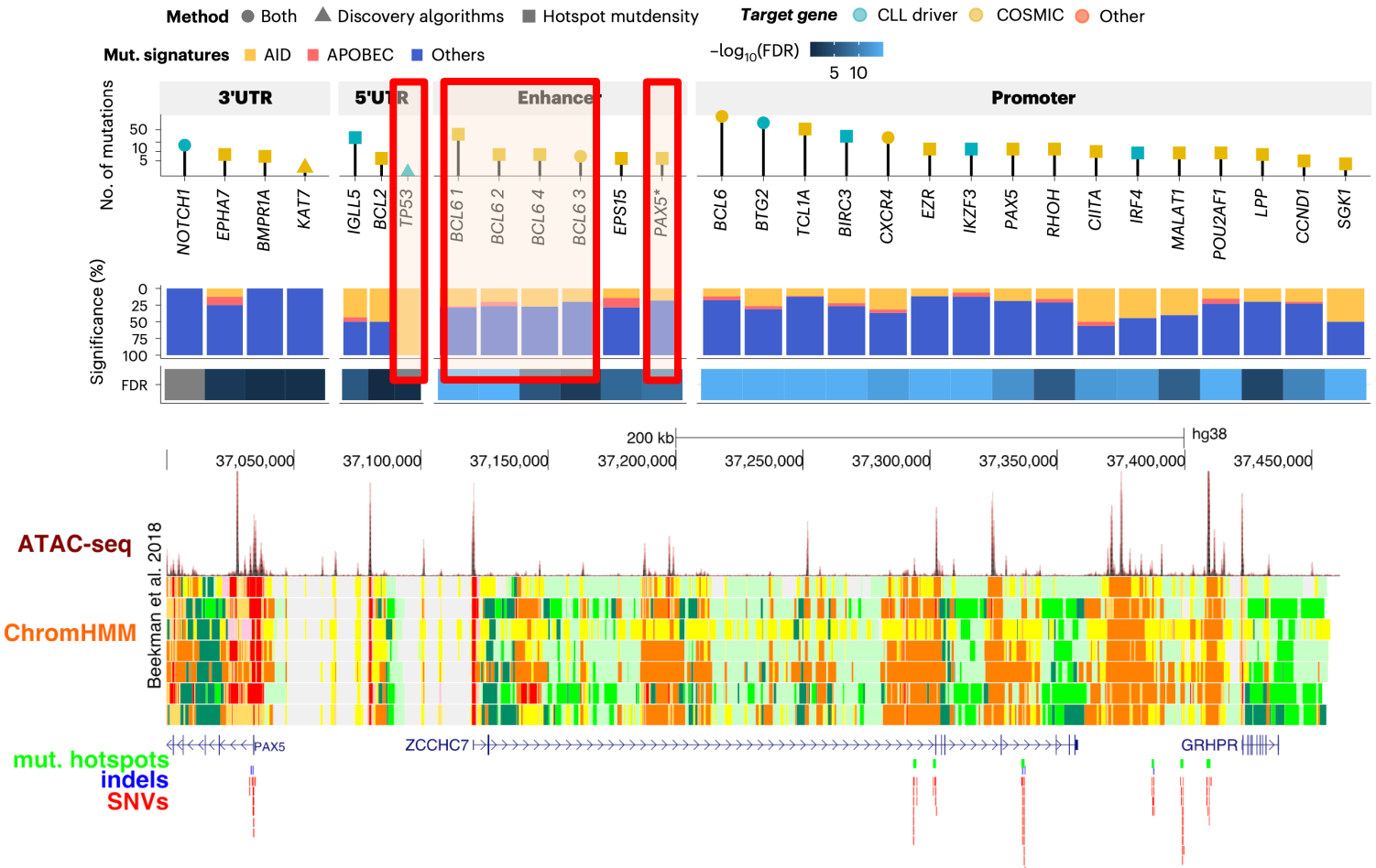
Exclude Ig regions and common sequencing/mapping artefacts

Software:
OncodriveFML
OncodriveCLUSTL

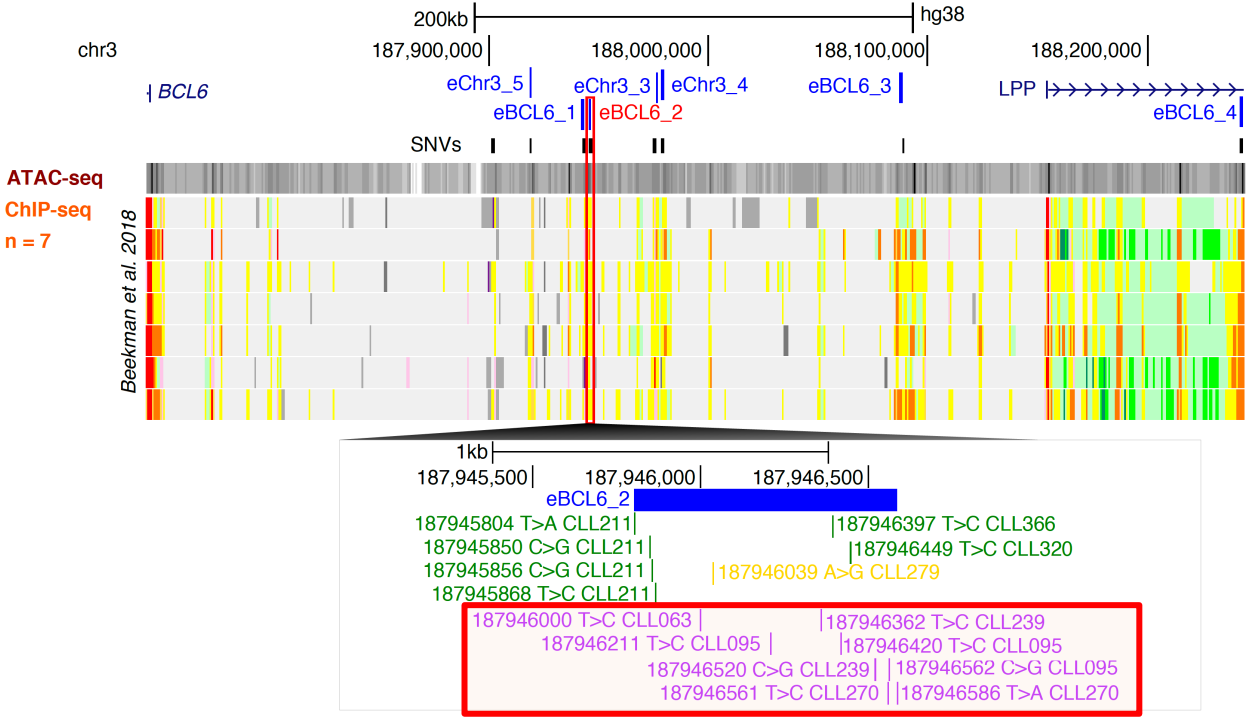


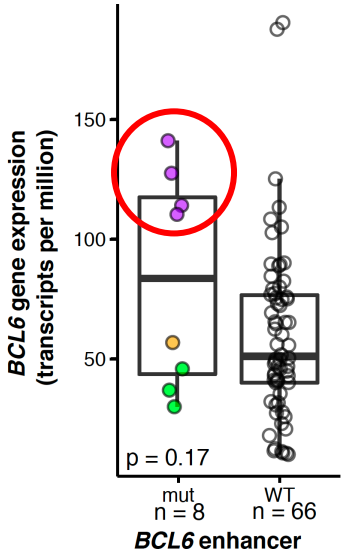
Cross validation

Non-coding Drivers

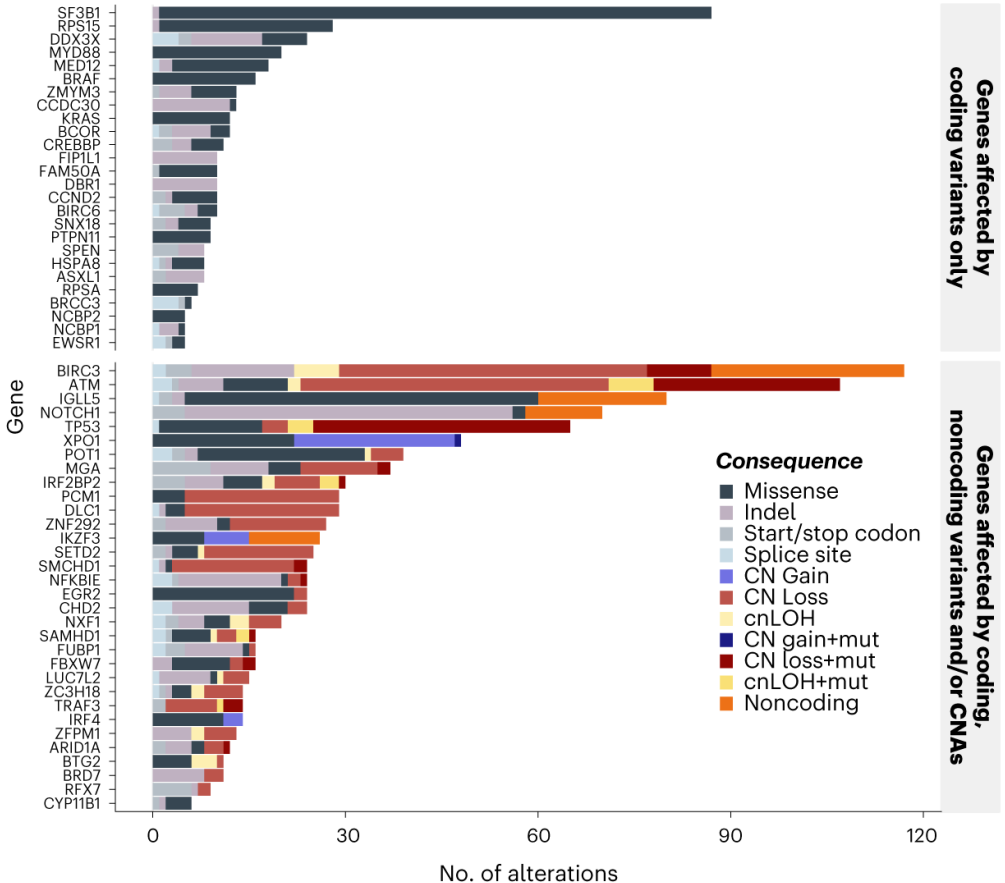


BCL6 enhancer mutations





Integration of coding, non-coding and CNAs

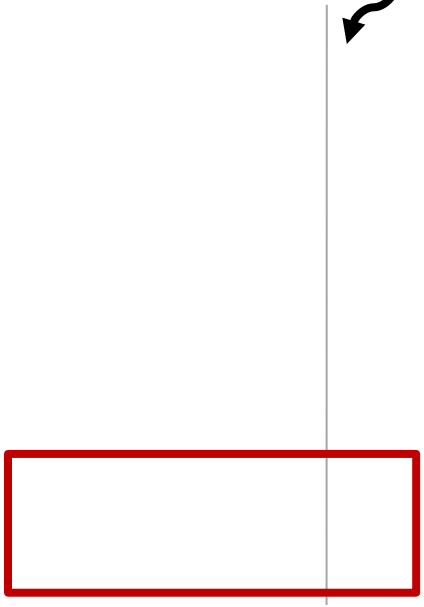


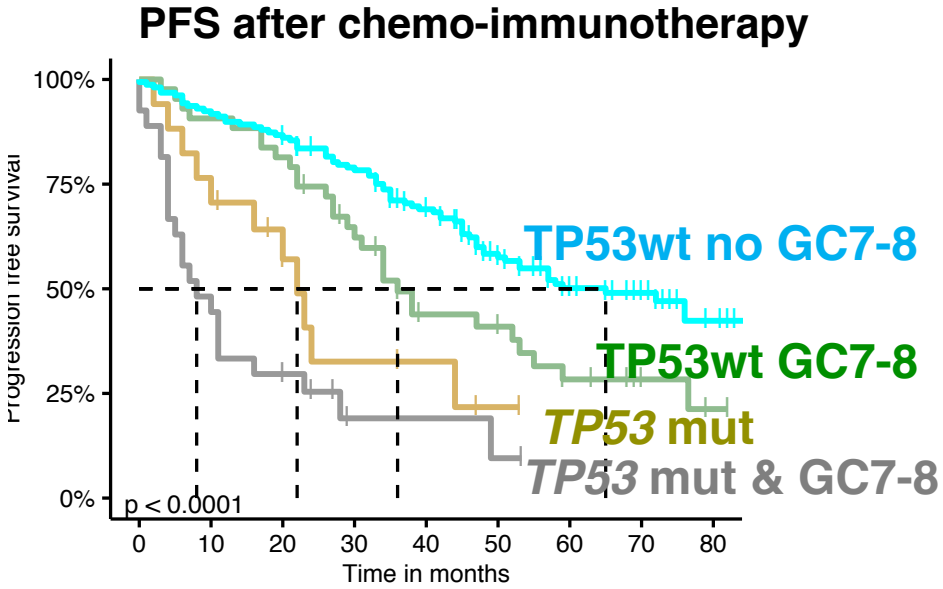
Genes with only coding variants

More than half of genes are affected by more than just exonic mutations

Patients with both *TP53* mutations and GC#7/8 changes have ultra-high-risk disease

Presence / absence



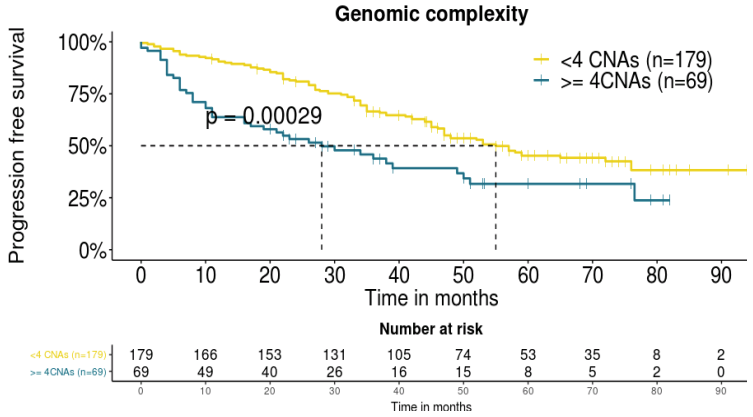


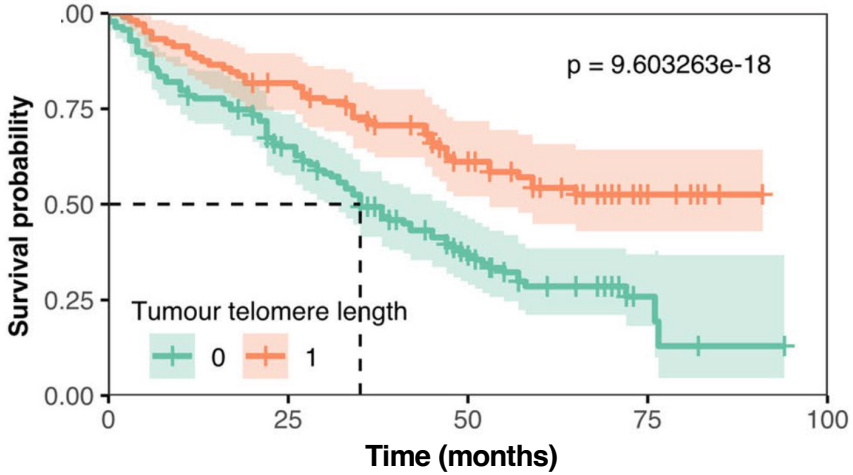
CN gain / loss
Aneuploidy / trisomy
Translocation / Inversion

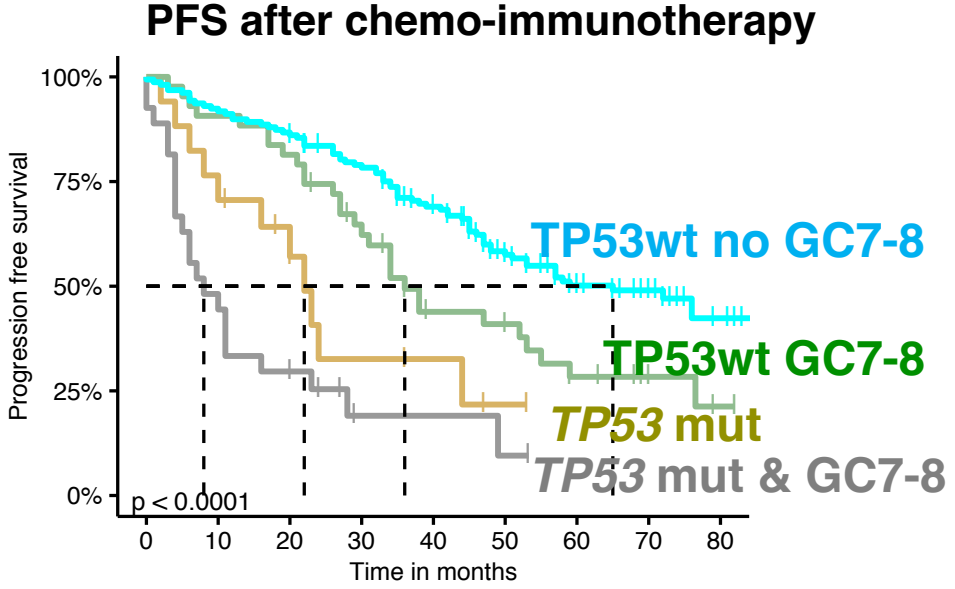
Genomic complexity

Slide courtesy of P. Robbe

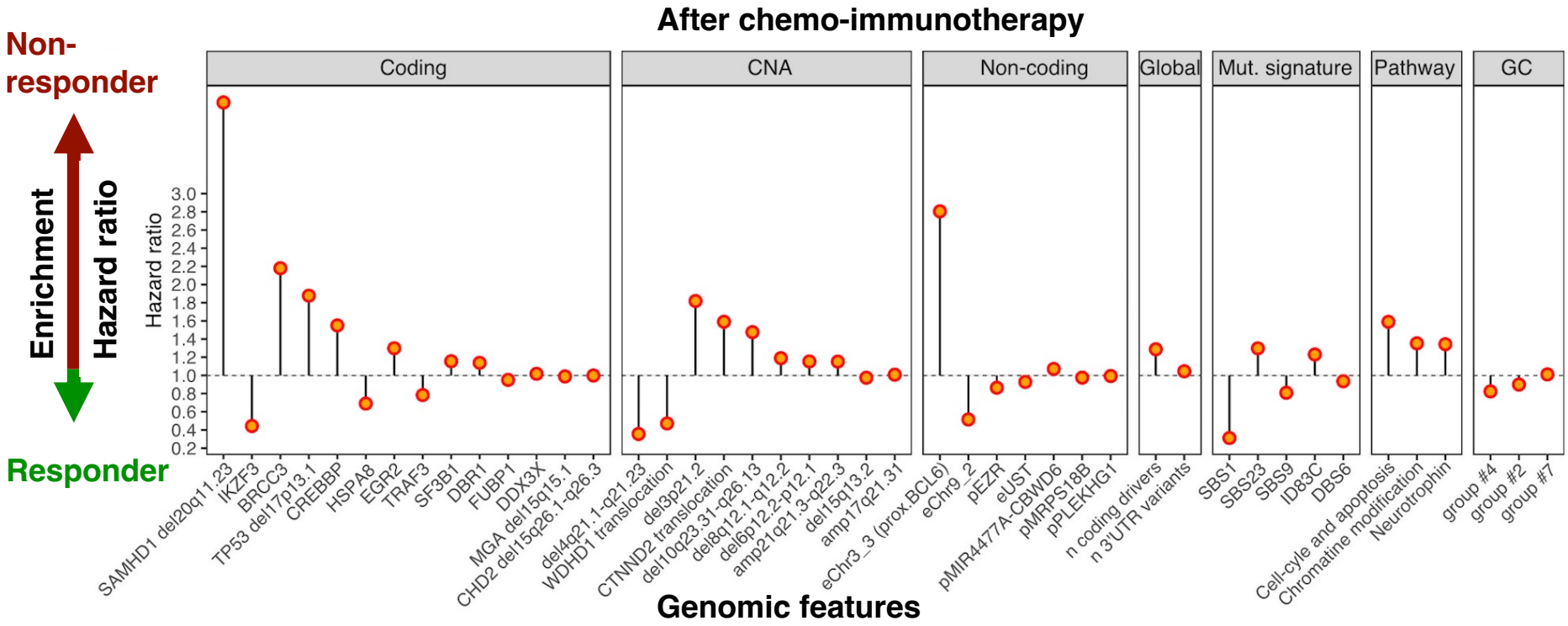
Whole genome features







Identification of independent predictors of progression using multivariate analysis

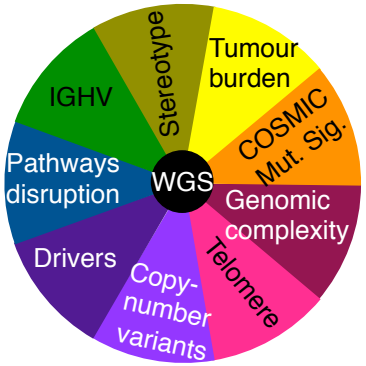


- Each individual alteration is rare: prevents efficient stratification of patients

Slide courtesy of P. Robbe and Dr Dimitris Vavoulis

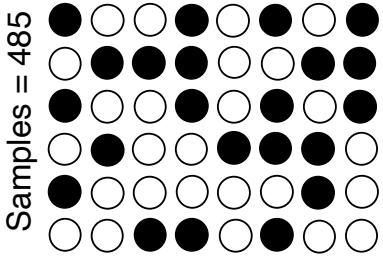
Statistical clustering of 186 genomic features

Different type of "candidate drivers" / Genomic features



Genomic features = 186

Samples = 485



Extract meaningful sets of features to cluster patients' genome

Statistical clustering using non-negative matrix factorization

Genomic subgroup 1

Genomic subgroup 2

Genomic subgroup 3

Clinical outcome metric : PFS after treatment

Good responder

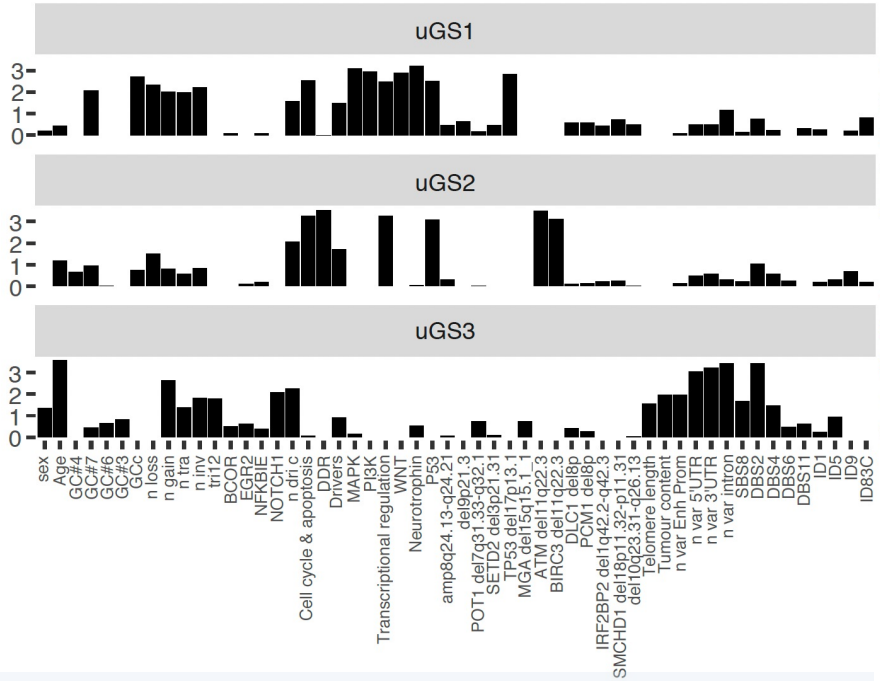
Intermediate responder

Poor responder

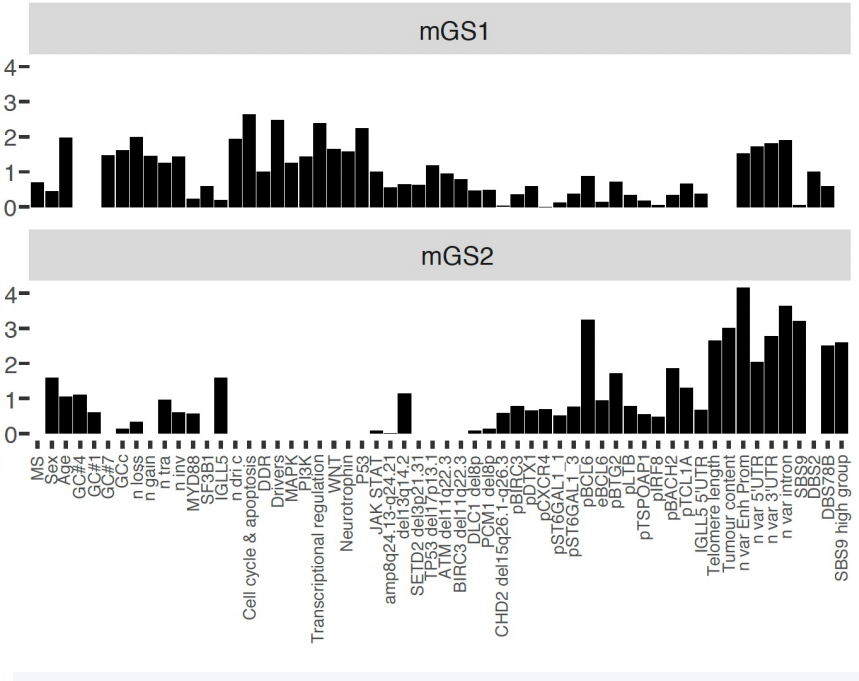
Slide courtesy of P. Robbe

Genomic subgroups

IGHV unmutated samples



IGHV hyper-mutated samples

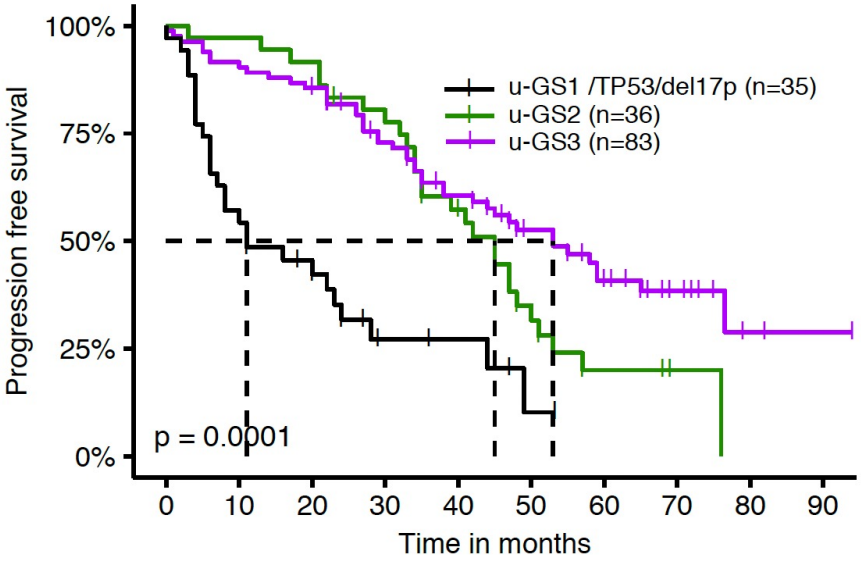


uGS1: TP53/del17p, high genomic complexity
uGS2: ATM, BIRC3, DDR and driver mutaitons
uGS3: High mutation burden in the non-coding space but without specific known drivers

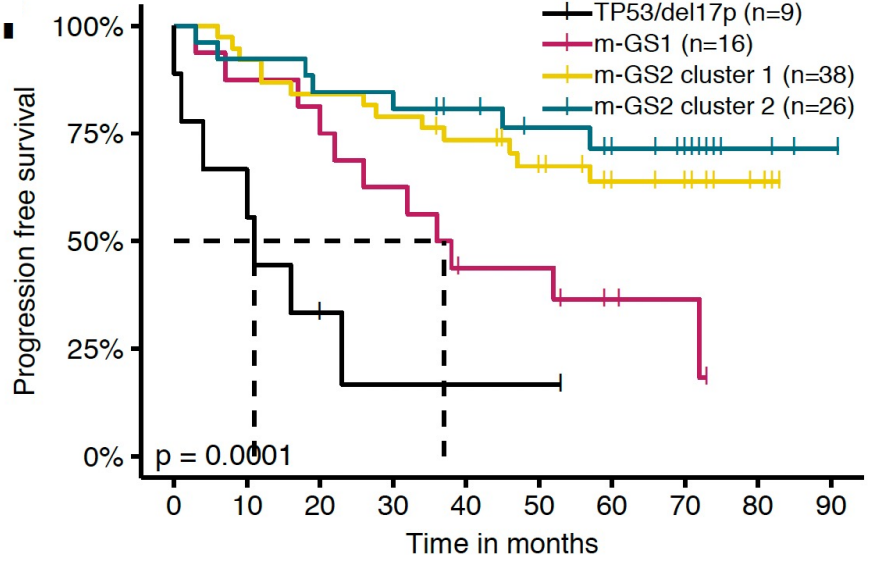
mGS1: TP53/del17p, high genomic complexity, mutations in key pathways
mGS2: High mutation burden in the non-coding space but without specific known drivers

Survival analysis of genomic subgroups

IGHV unmutated samples



IGHV hyper-mutated samples



In both cases TP53/del17p were manually separated to demonstrate the meaningfulness of the grouping without it

Whole genome classification of pre-malignant B-cells disorder of the OXPLORED study

Pauline Robbe, Kate E Ridout, Niamh Appleby, Georgeta Ciuban, Gagandeep Batth, Hélène Dréau, Thomas Wenban-Smith, Melinda Kormandy, Grigore-Aristide Gafencu, Piero Carninci, Clare Freestone, Daniel McAleese, Dimitrios V Vavoulis, Anna Schuh

OXPLORED clinical trial (Oxford Pre-cancerous Lymphoproliferative Disorders: Analysis and Interception study)

WGS in 400 samples from 200 individuals

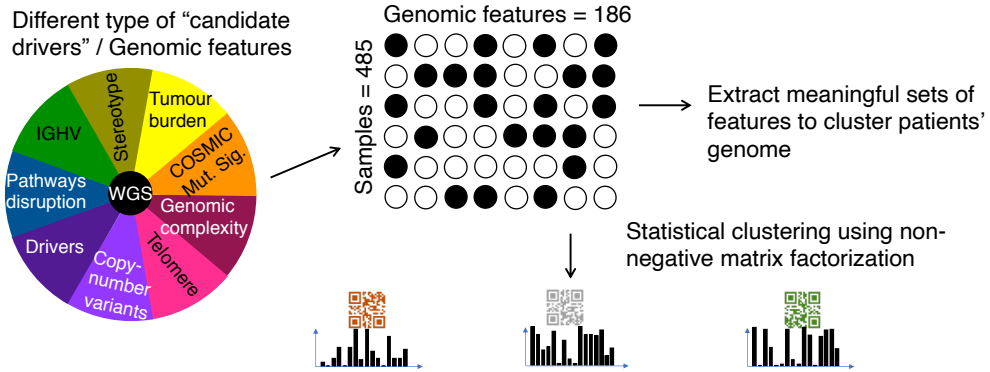
sorted CD19+ B-cells as tumour

matched salivary DNA as germline

How do groupings predict clinical outcome in patients treated with targeted therapies

Analysis:

186 genomic features from Robbe*, Ridout* et al.



Slide adapted from P.

Conclusions

- Multiple genomic features are linked to patient outcome on CIT and investigations are ongoing for targeted therapy
- Using WGS allows us to capture rare variants and variant combinations in order to understand the full range of heterogenetiy in CLL
- Patient stratification can be performed using multiple genomic features which could also be used to inform treatment decisions

Development of a single pipeline for this process is ongoing

nextflow

The patients and their families

Oxford team
Anna Schuh
Dimitris Vavoulis
Niamh Appleby
Hélène Dréau

International Collaborators
José I Martín-Subero
Richard S Houlston
Jon Strefford
Stephen Devereux
Robert Månsson
Ruth Clifford
Pete Hillmen
Basile Stamatapoulos
Daniel Chubb
Alex J Cornish
Jim Hughes
James Davies
Ben Kinnersley
Martí Duran-Ferrer
Nicholas Denny

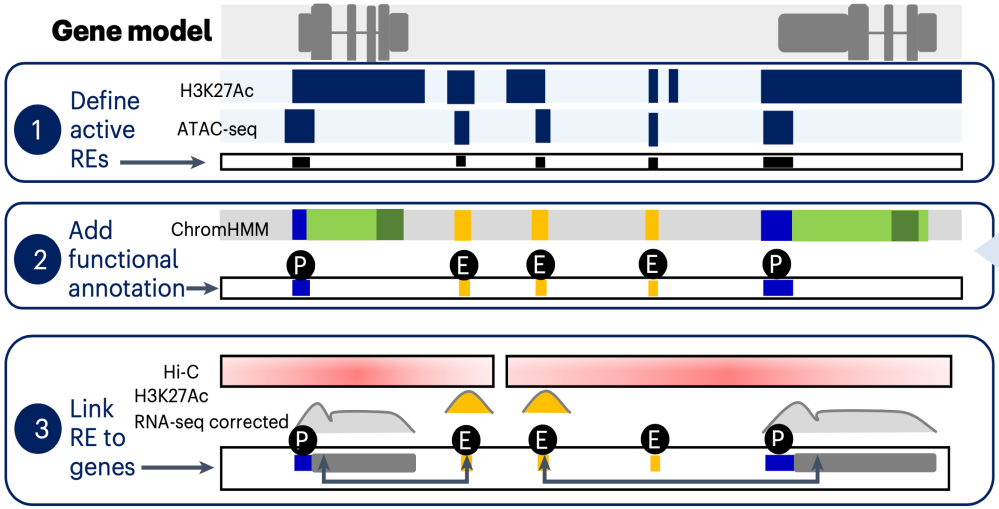
RIKEN IMS
Pauline Robbe
Piero Carninci

Illumina
David Bentley
Mark Ross

UKCLL BioBANK
Andy Pettitt
Melanie Oates

Genomics England
Angela Hamblin
Alona Sosinsky
Mark Caulfield

Non-coding Annotation



Non coding annotations are complex and multi-layered

Is the region active?

Methylation data, ATAC-seq

Is the region functional?

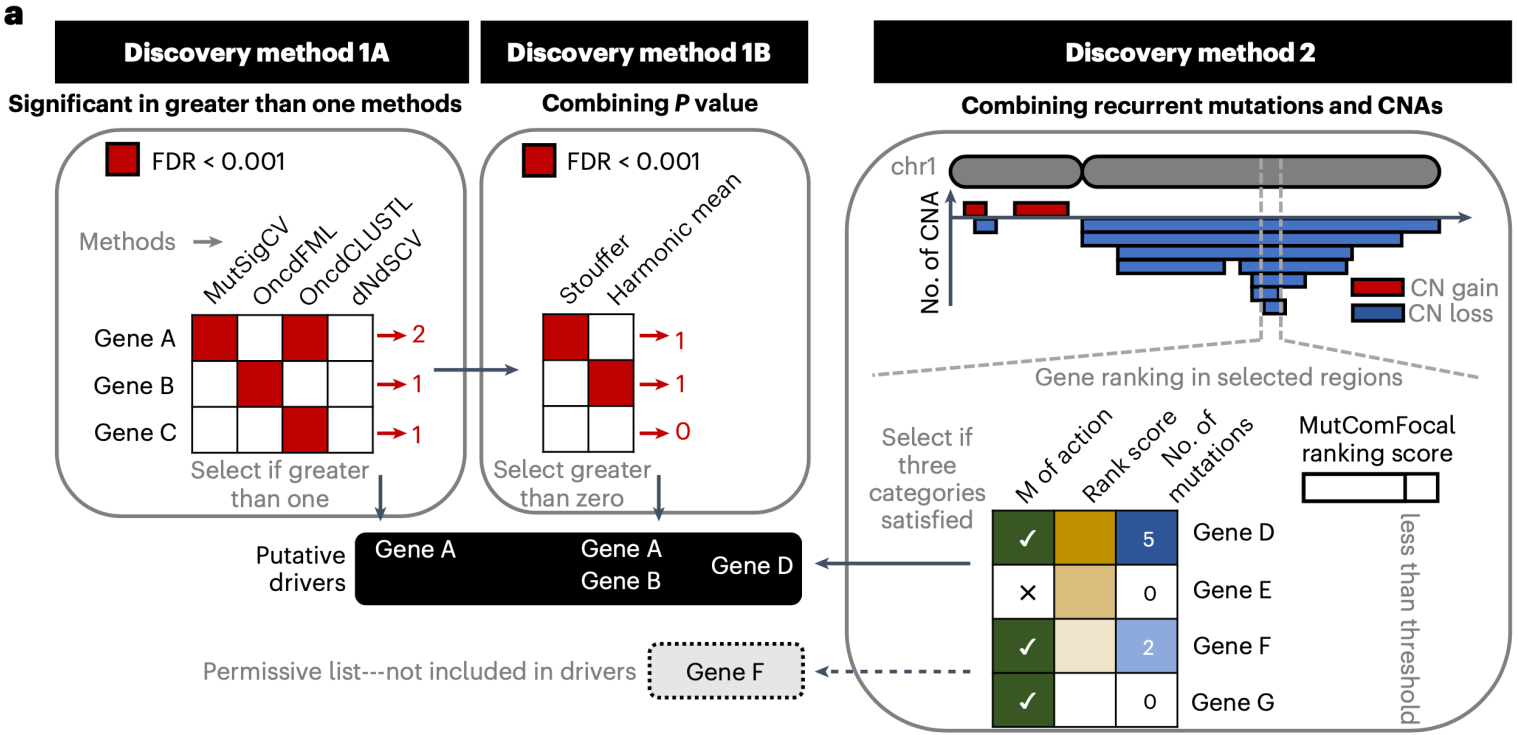
6 different methylation marks

Identification of promotor, enhancer, heterochromatin etc…

What does the region do?

What genes does it regulate?

Driver gene identification



Robbe*, Ridout* et al. Nat Gen 2022