

Functional Implications of Genomic Lesions

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Disclosures

I am co-founder of Neon Therapeutics, Inc; and receive research funding from Pharmacyclics

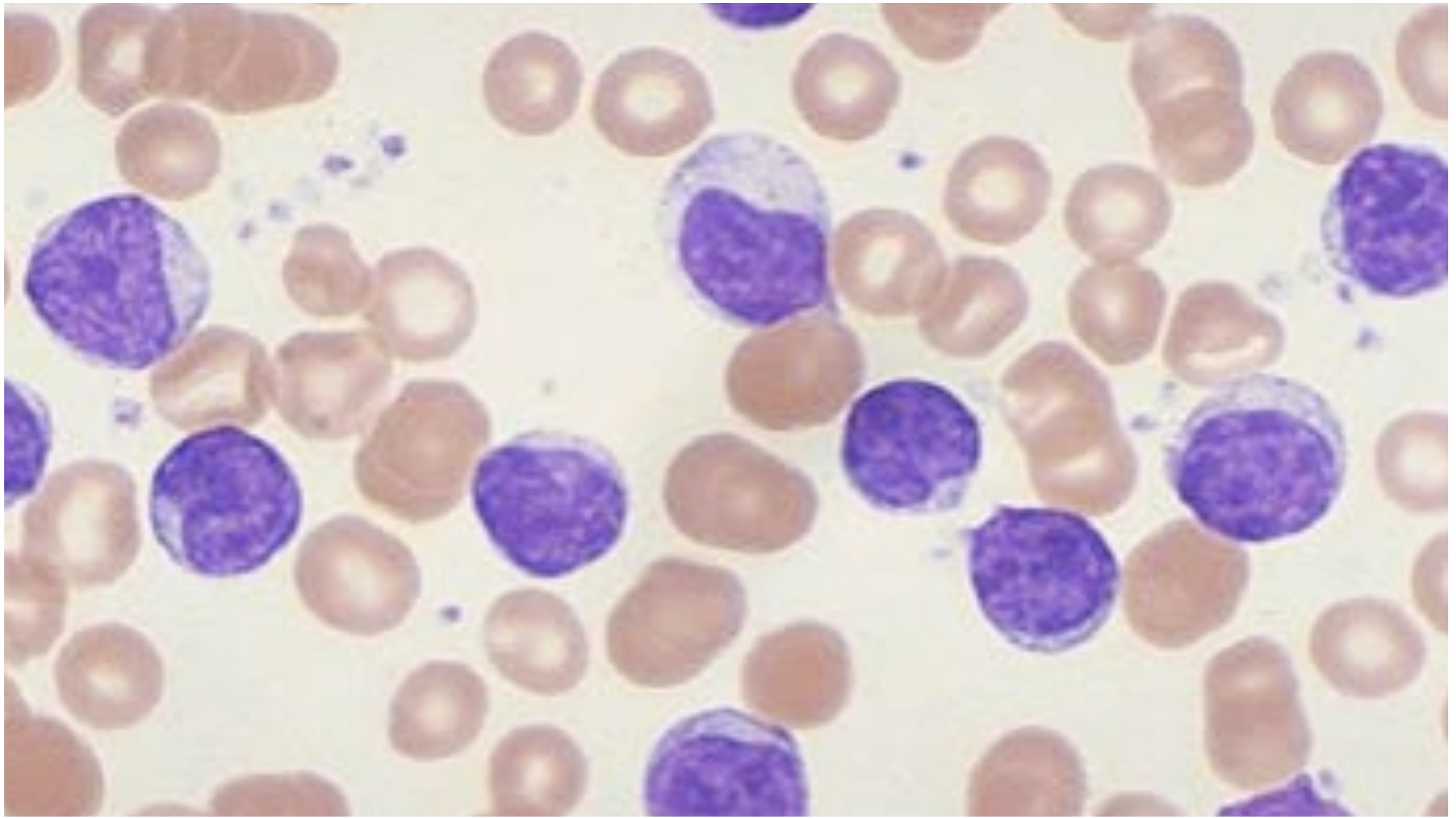


The Meadows,
Edinburgh

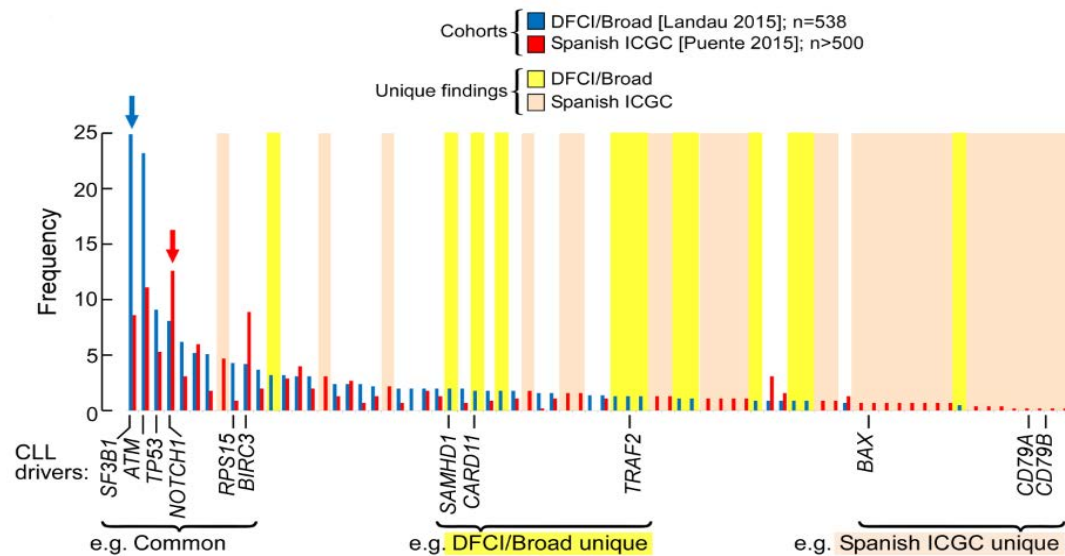
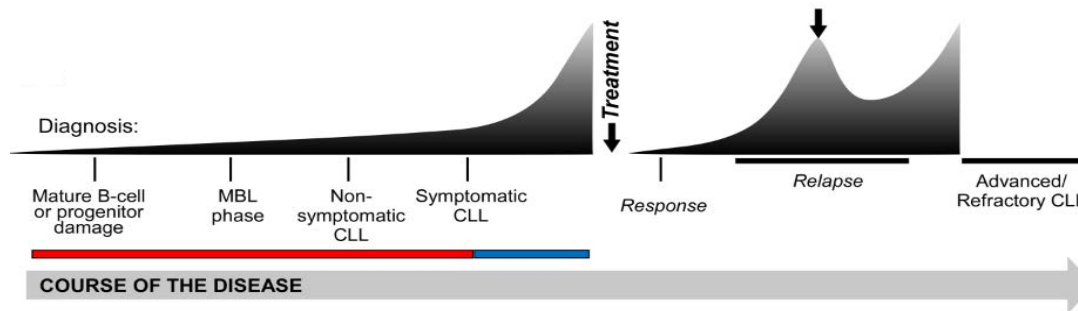


The Common,
Boston





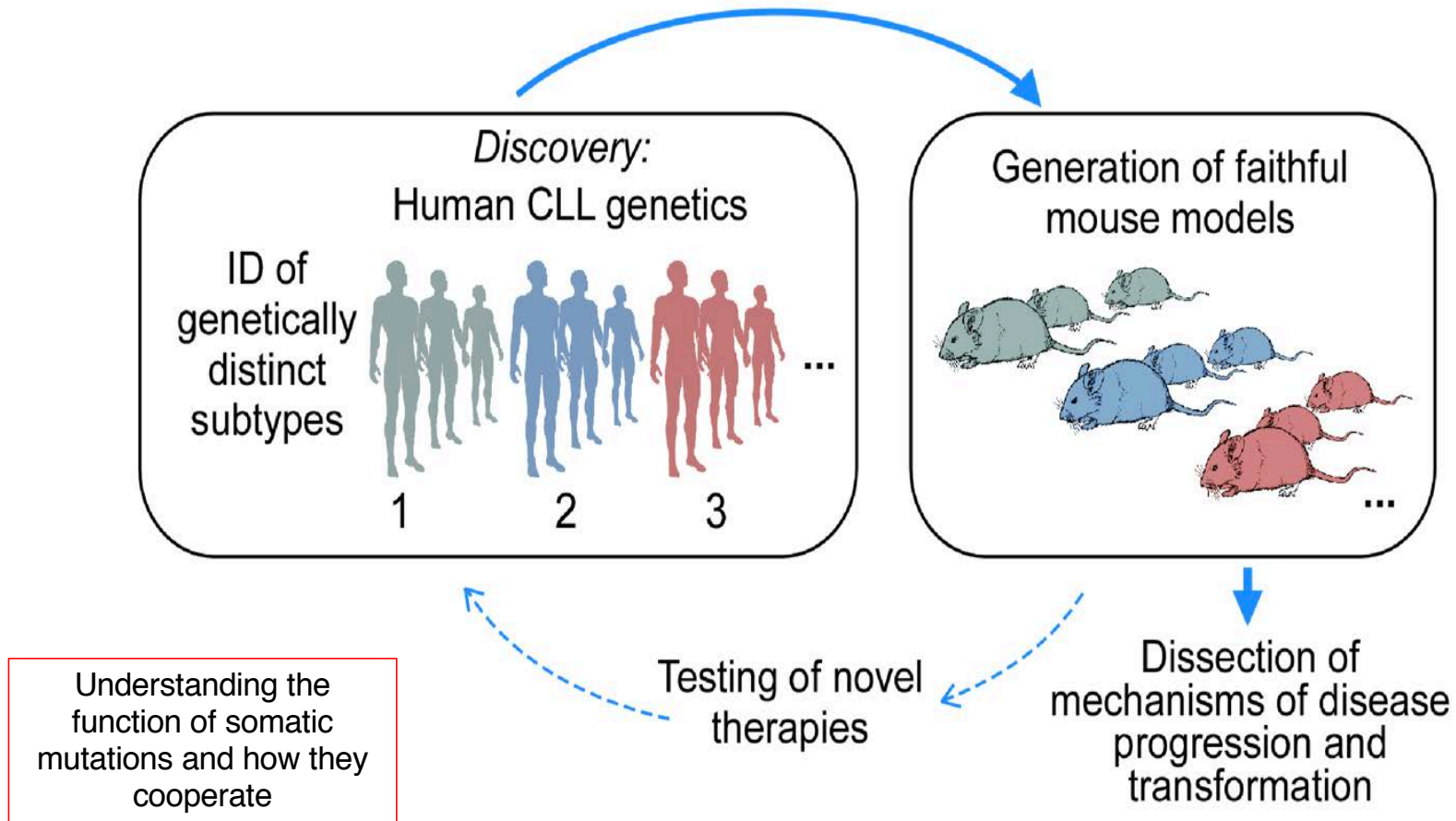
Intertumoral heterogeneity in CLL



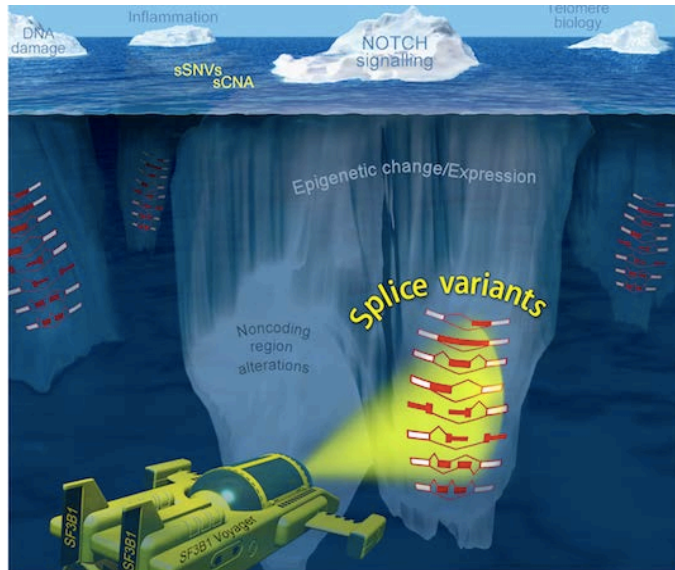
Purroy N. *Cold Spring Harb Perspect Med* 2017

- • ***What is the functional impact of these mutations?***
- ***What is the role of genomic changes in therapeutic resistance?***

Developing genetically faithful murine models of CLL

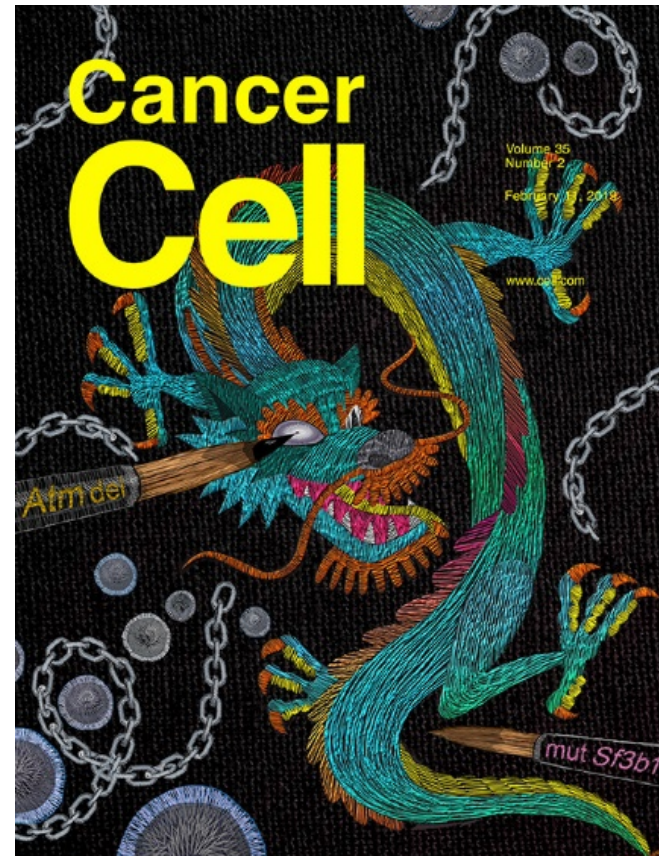


Analysis of *SF3B1* in man and mouse

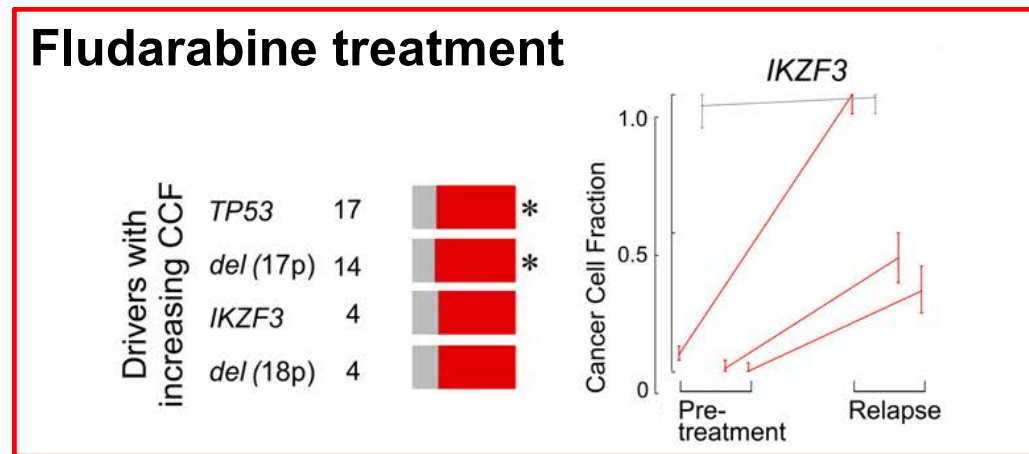
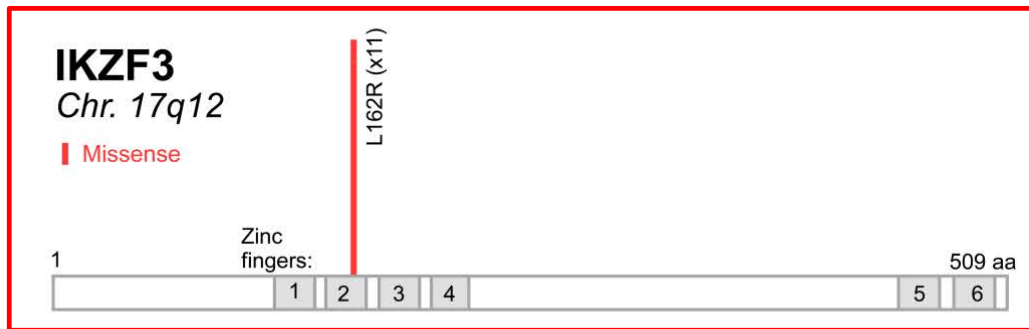


Wang Fan Brooks & Wan *Cancer Cell* 2016

Yin SY *Cancer Cell* 2019



Hot-spot mutation in *IKZF3* is an putative driver

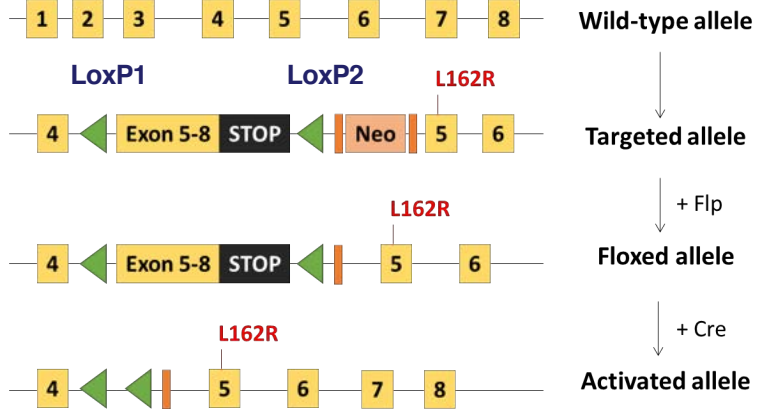


Landau DA. *Nature* 2015

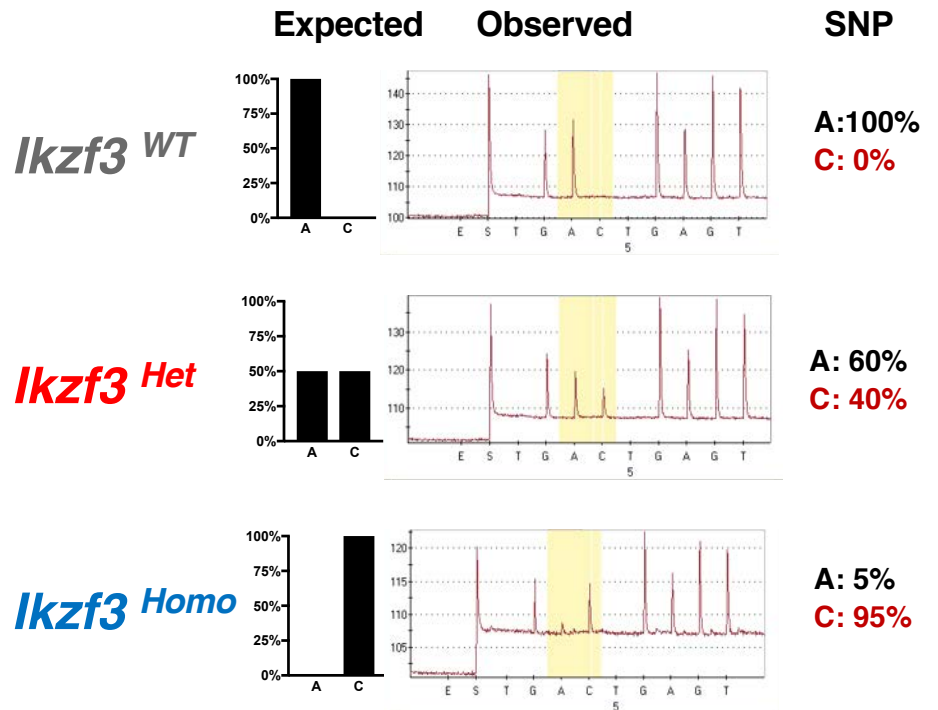
- *IKZF3* (*AIOLOS*) is a hematopoietic cell-specific transcription factor essential for B cell development
- Associated with fludarabine-resistance
- Mutation is present in a DNA binding domain → direct regulation of gene expression

Generation of a B-cell restricted model of *Ikzf3-L162R* mutation

Allele knock-in strategy

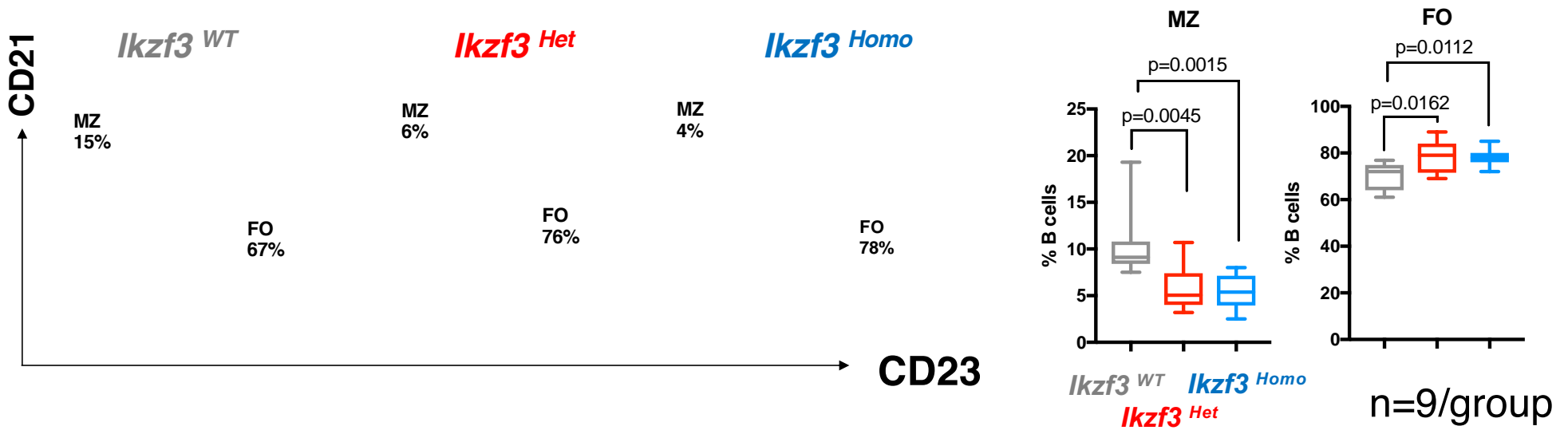


cDNA pyrosequencing



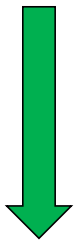
Gregory Lazarian, Shanye Yin, Elisa ten Hacken

Ikzf3-L162R alters B cell development and impairs MZ formation



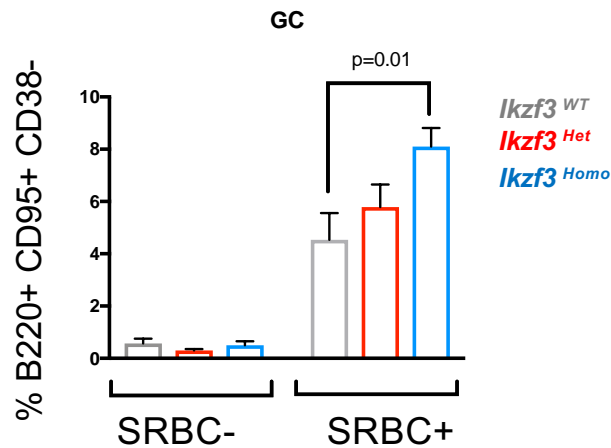
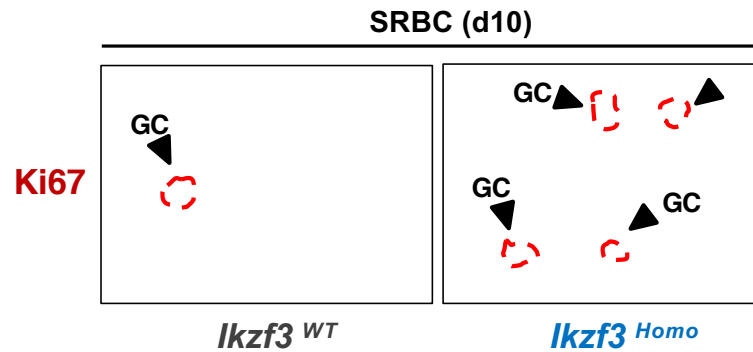
Ikzf3-L162R increases GC formation upon SRBC immunization

SRBC 1×10^9 cells/mL, I.P. injection



10 days
n=6/group

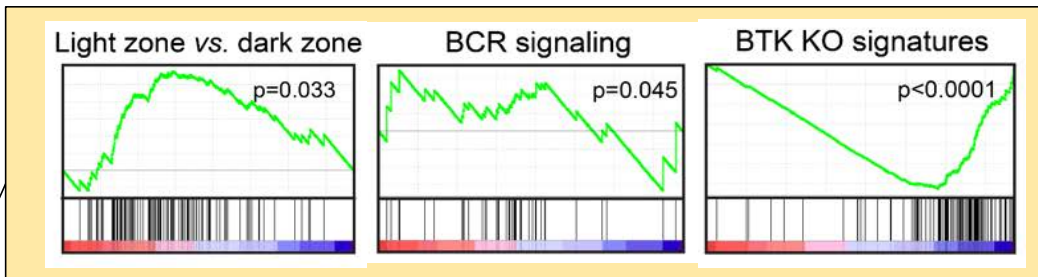
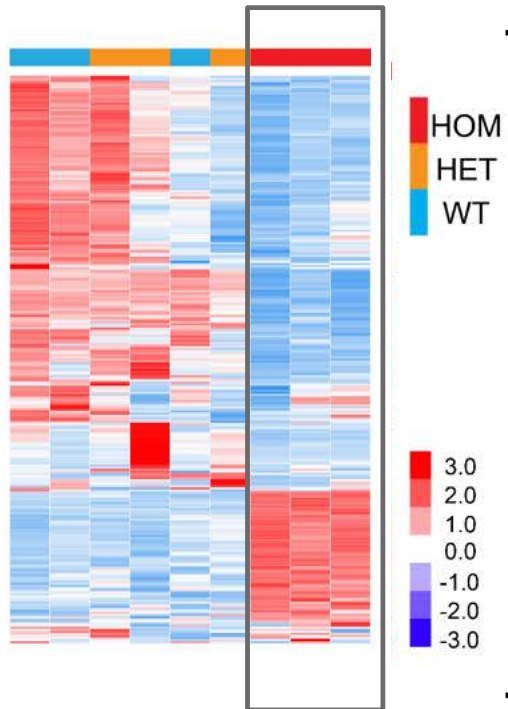
analysis



Mut-*Ikzf3* mice reveal an unique transcriptional signature

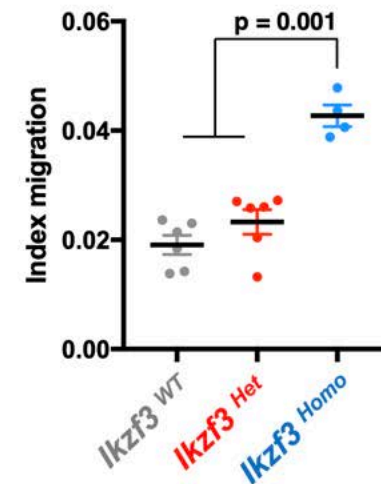
Young mice (3 month old)

Ikzf3 *Homo*

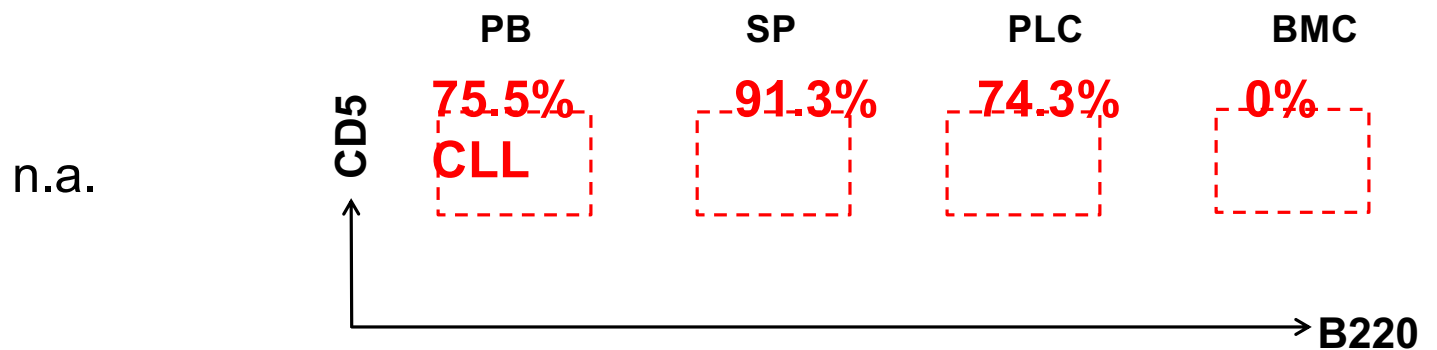


BCR Signalling

Chemotaxis

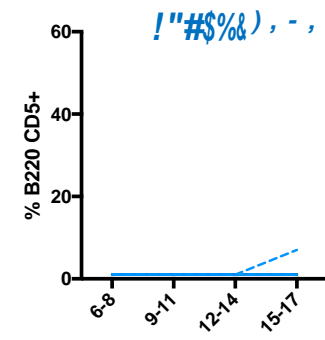
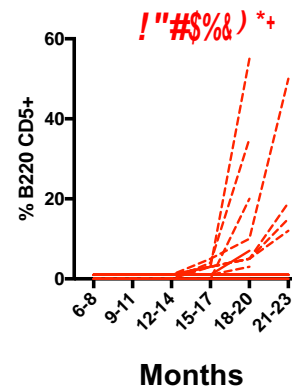
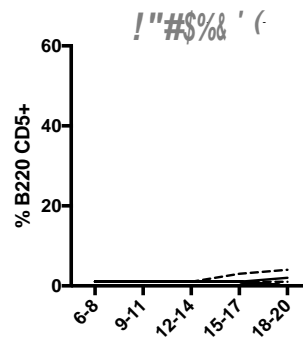


Ikzf3 mutation can induce CLL development in mice



23% (7/30) CLL
6% (2/30) lymphoma

4% (1/21) CLL

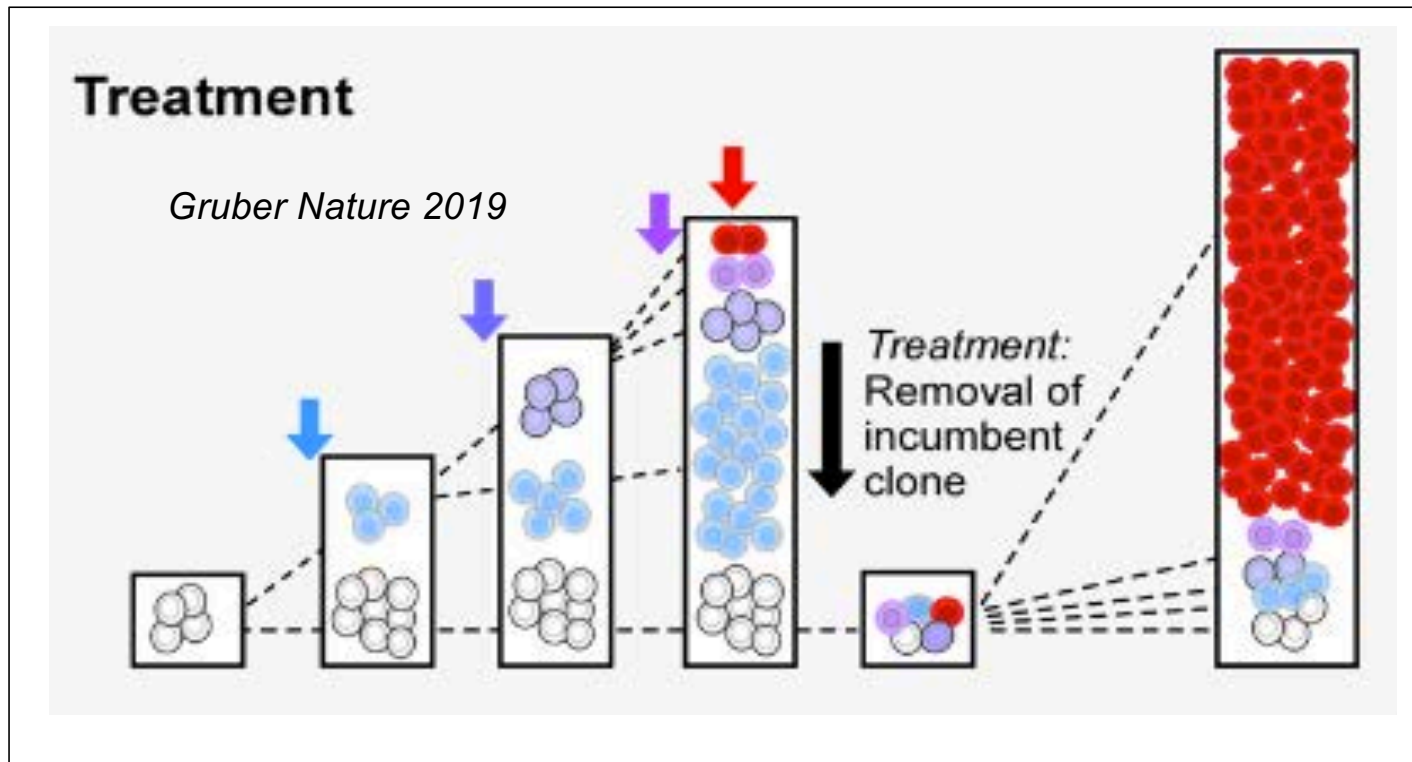


Summary

- *Ikzf3*-mutant cells show a unique transcriptional signature, associated with BCR signaling activation
- Marked functional changes in BCR signaling and migratory capacity are associated to *Ikzf3* mutation
- *Ikzf3* mutation can induce CLL in mice
- Distinct functional effects compared to mut-*SF3B1*

- ***What is the functional impact of these mutations?***
- • ***What is the role of genomic changes in resistance?***

Clonal evolution: Selection of fitter subclones



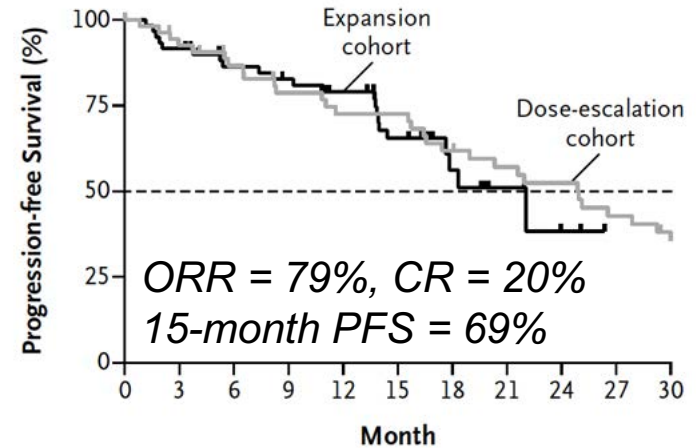
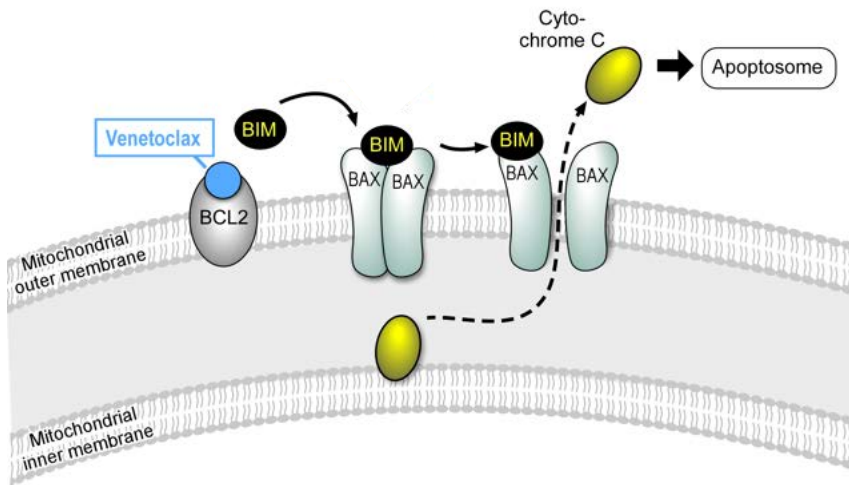
Treatment

- Chemotherapy
- Targeted therapy
- Immunotherapy

*Landau Nature 2015;
Burger Nat Comm 2016;
Landau Nat Comm 2017
Bachireddy ASH 2018*

Landau Cell 2013

Venetoclax is a FDA-approved BCL-2 inhibitor



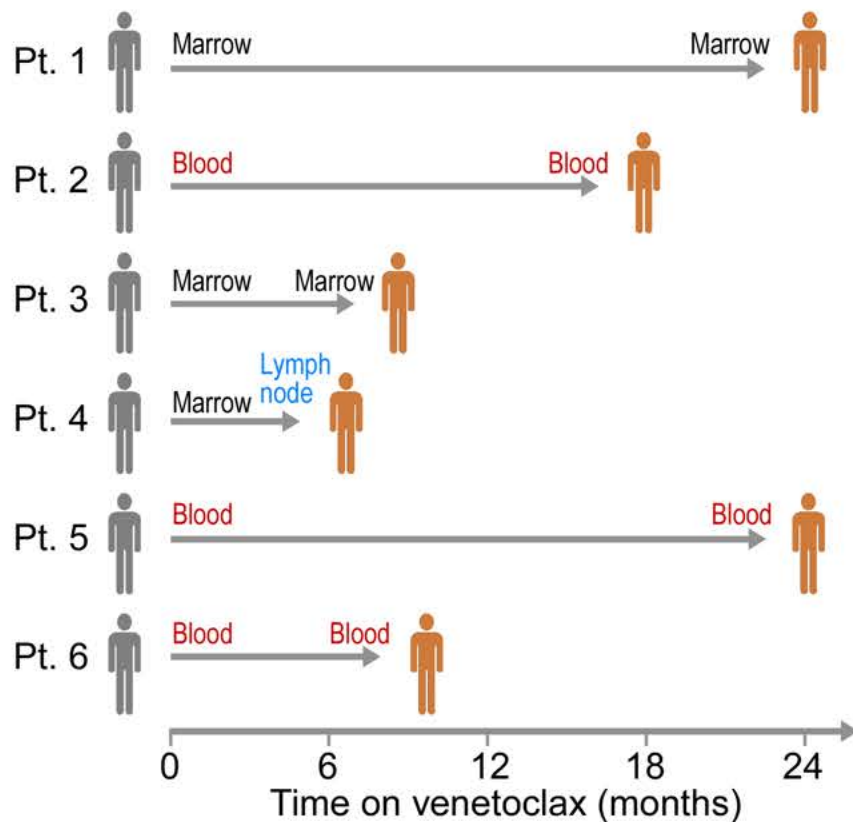
ORR = 79%, CR = 20%
15-month PFS = 69%

Roberts AW, *NEJM* 2016

Can we identify mechanisms of resistance to BCL-2 inhibition in lymphoid malignancies ?

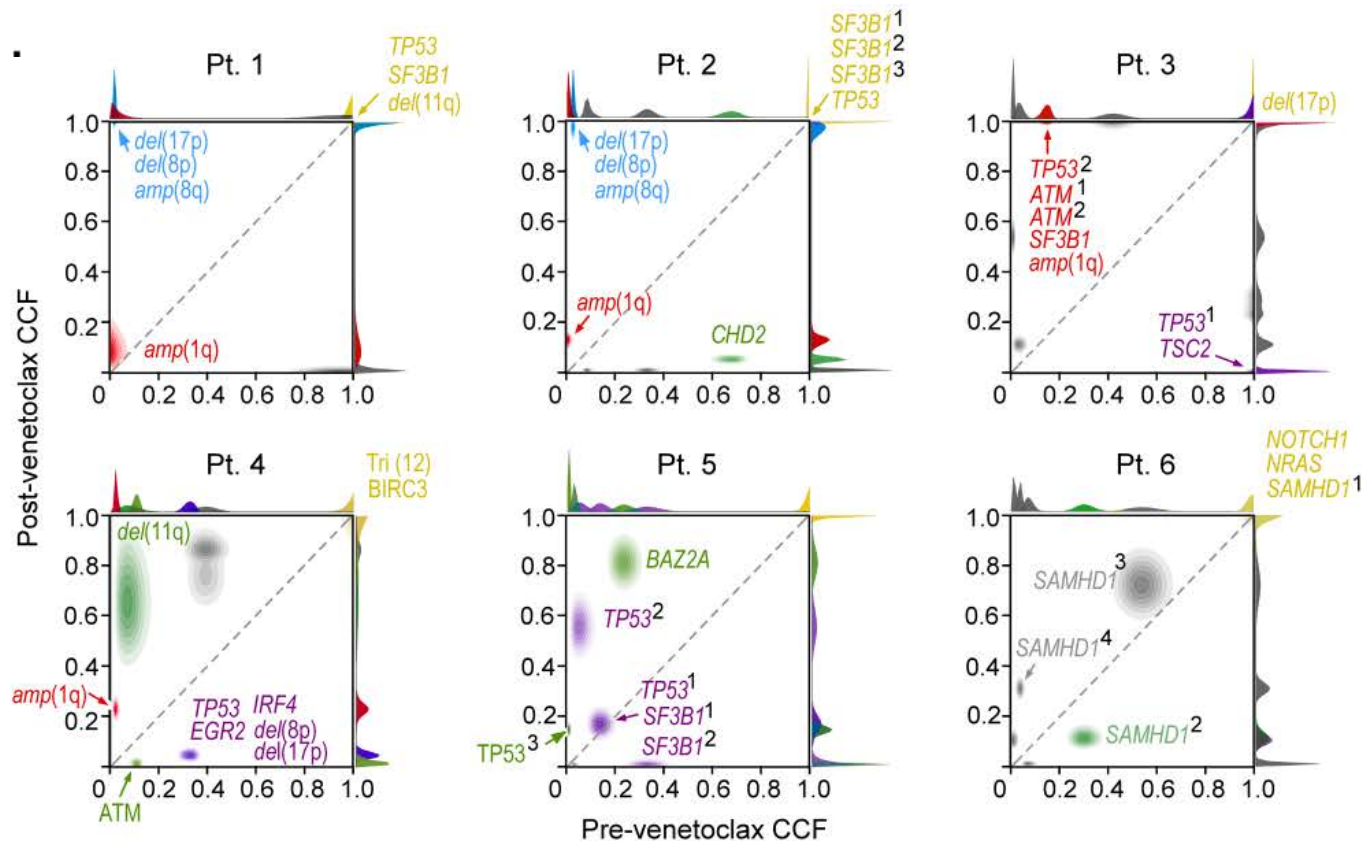
Guieze R & Liu V. *Cancer Cell* 2019

Approach #1 | Clonal evolution in patients with CLL developing resistance to venetoclax



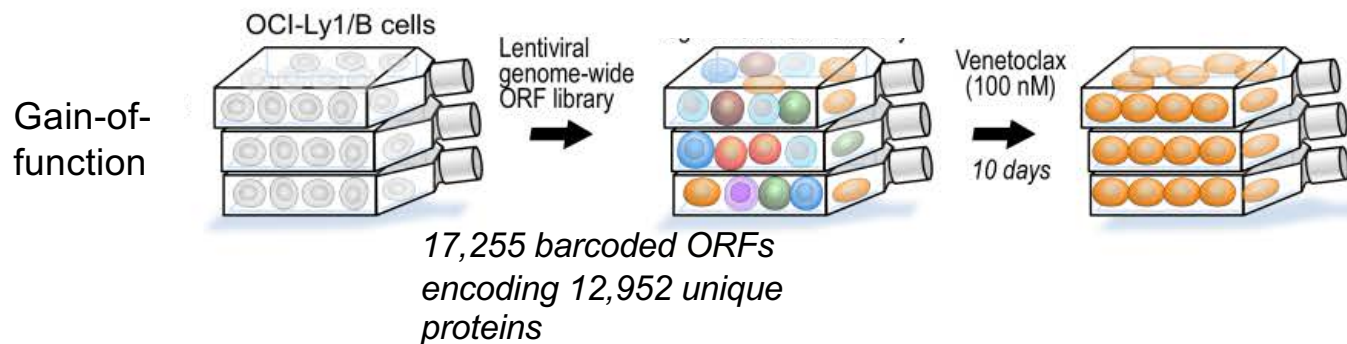
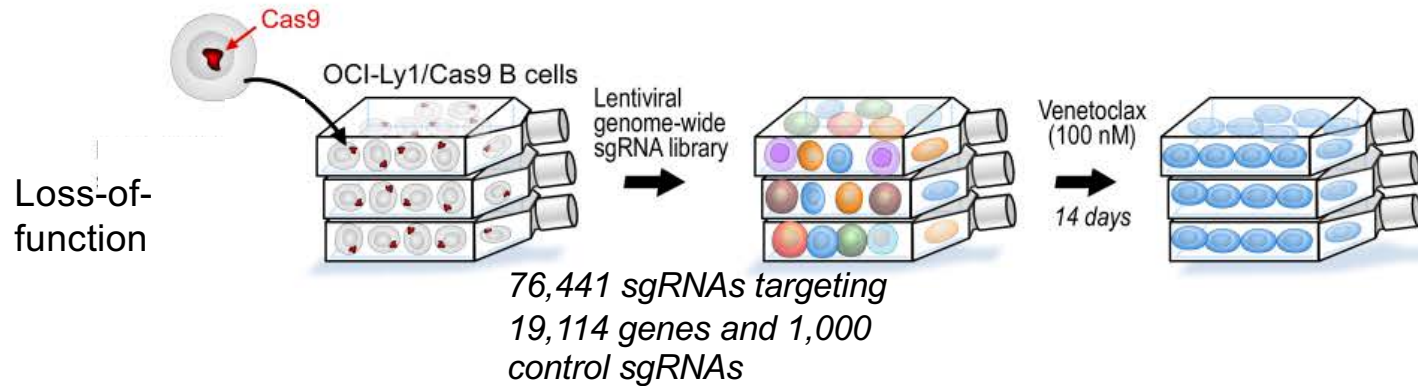
- 6 patients with R/R CLL (DFCI, MDACC)
Relapse after a median of 16 months
- WES of DNA from paired CLL samples
(before and at relapse on venetoclax)
- ASBOLUTE to estimate cancer cell fraction
(CCF) of individual somatic alterations
- CCF clustering to delineate distinct
subclonal populations

Approach #1 | Clonal evolution in patients with CLL developing resistance to venetoclax



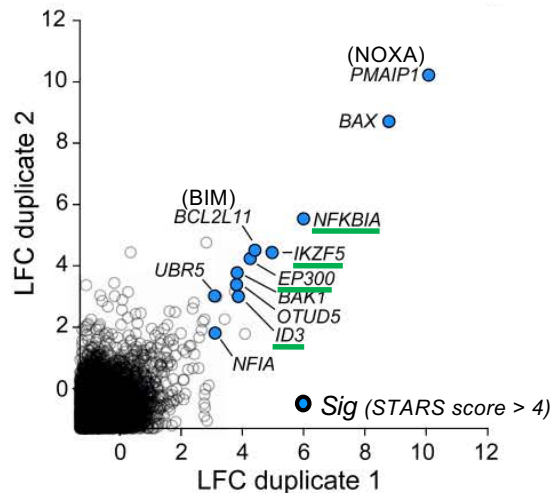
- Marked clonal shifts
- No *BCL2* mutation
- No CLL driver mutation consistently selected with resistance
- Common evolutionary trajectory : *TP53/SF3B1* → *del(17p)/del(8p)/amp(8q)*

Approach #2 | Genome-scale screens



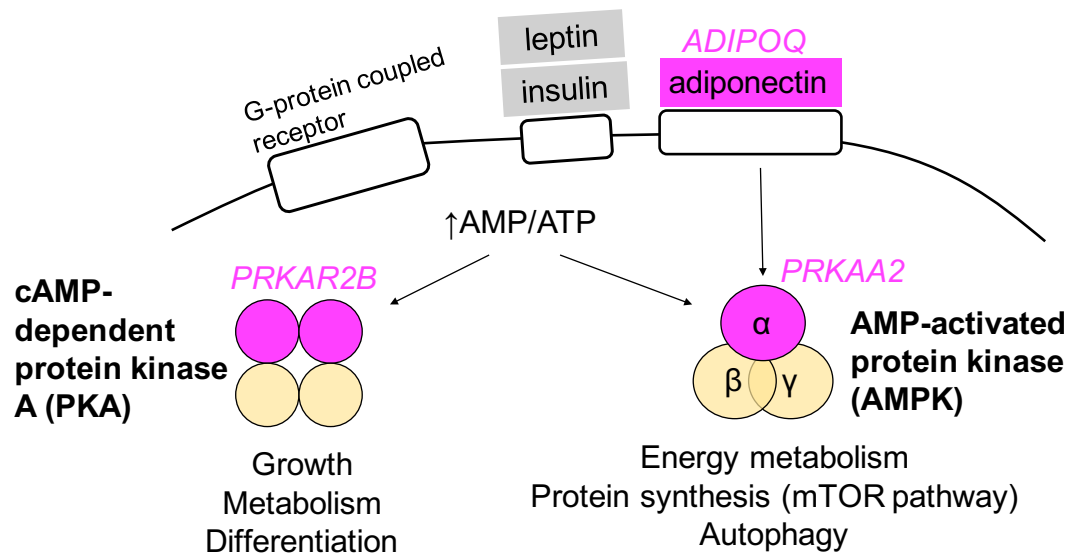
Venetoclax promotes changes in lymphoid transcription regulators

CRISPR screen

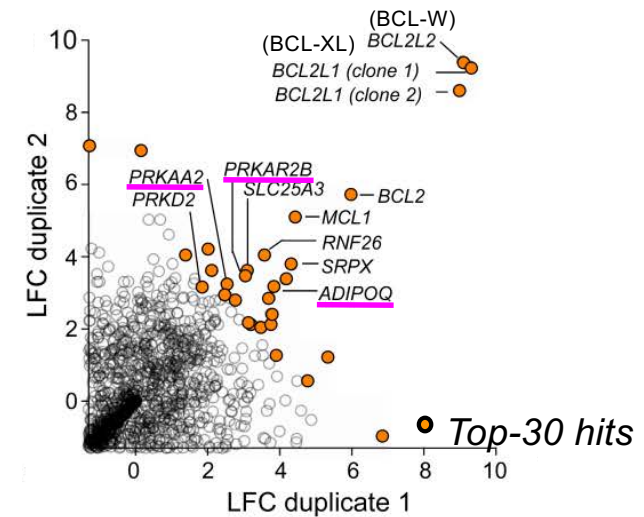


	Full name/protein	Cancer driver
NFKBIA	NFKB inhibitor alpha (IκBα)	Hodgkin lymphoma (20%)
EP300	Histone acetyltransferase p300	Follicular lymphoma (10-20%)
ID3	Inhibitor of DNA binding 3, HLH protein	Burkitt lymphoma (70%)
UBR5	E3 ubiquitin protein ligase	Mantle cell lymphoma (20%)
IKZF5	IKAROS family zinc finger 5 (PEGASUS)	-

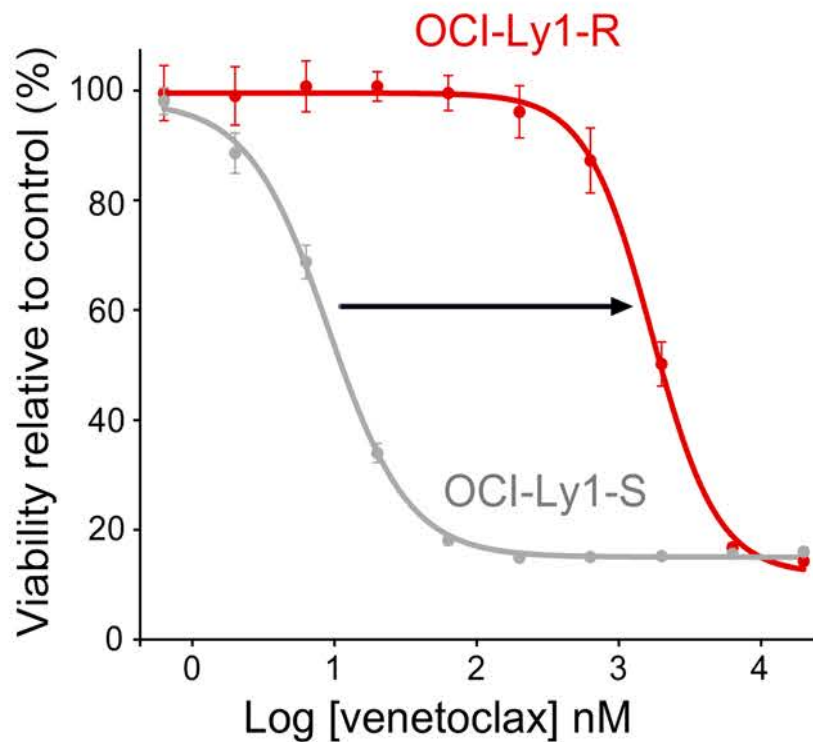
Venetoclax promotes changes in the cAMP network (PKA, AMPK)



ORF screen

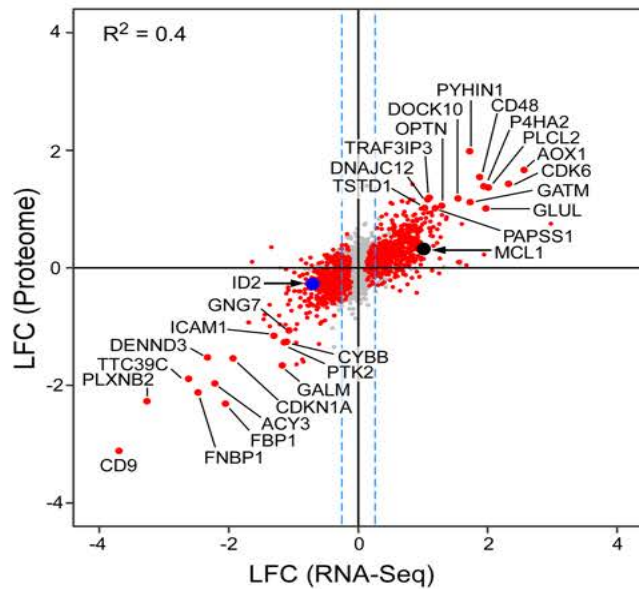


Approach #3 | Characterization of a resistant OCI-Ly1 cell line

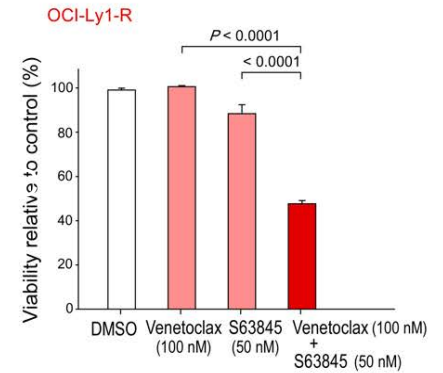
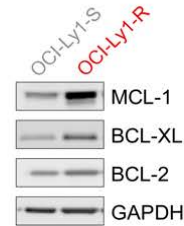


- Transcriptome (RNA-sequencing)
- Proteome (mass spectrometry)
- Functional investigations
- Testing of relevant drug combinations

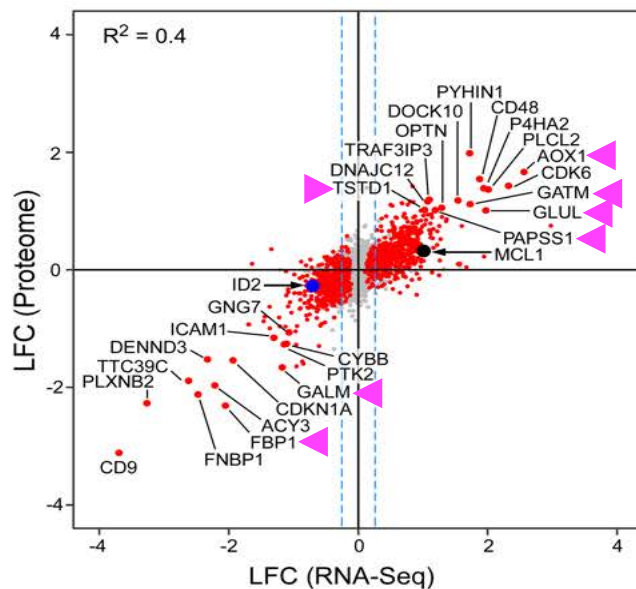
Expression changes related to venetoclax resistance



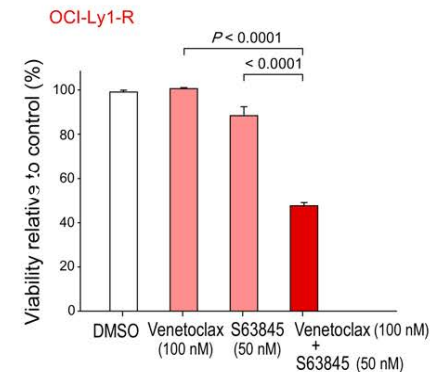
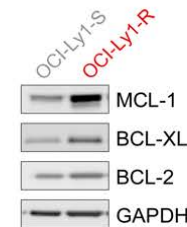
MCL-1 overexpression



Expression changes related to venetoclax resistance



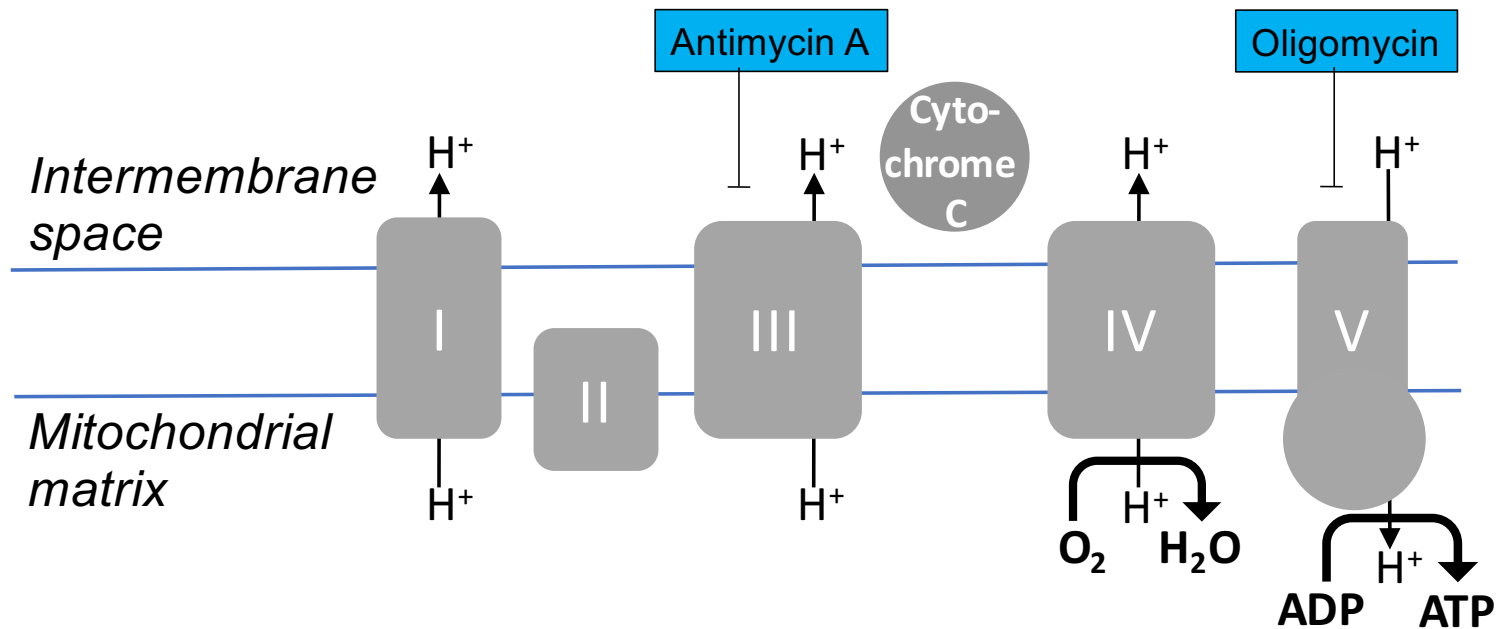
MCL-1 overexpression



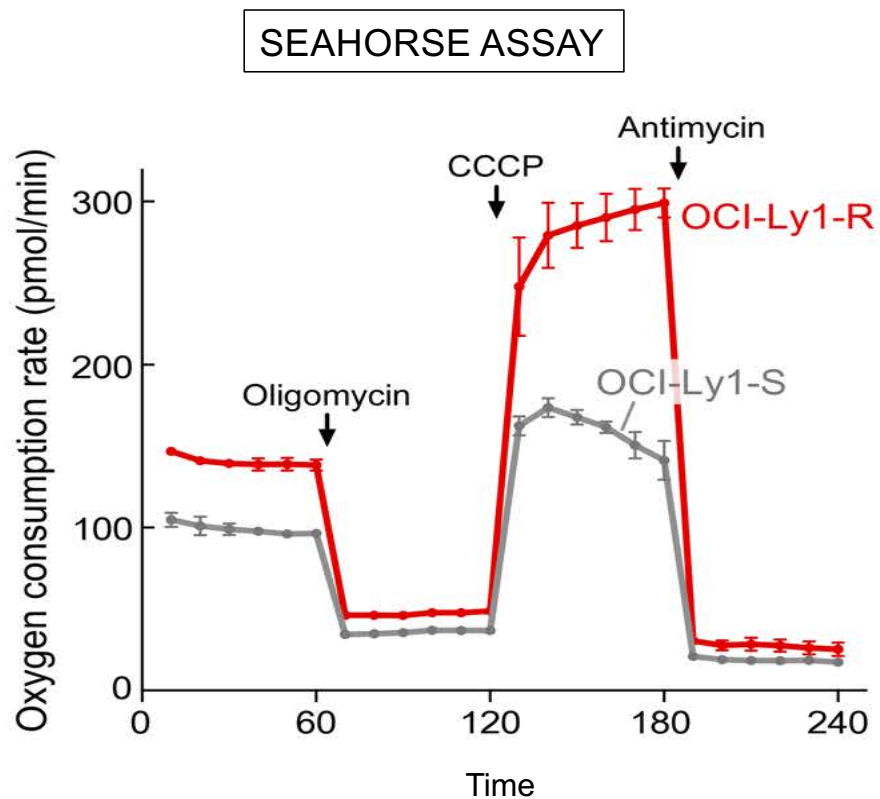
Cellular energy metabolism

- Dysregulation of metabolic actors
 - Correlation with the genome-scale screens
- Lymphoid transcription factors are metabolic gatekeepers ([Chan Nature 2017](#))
- AMPK is a key regulator of cellular energy metabolism and OXPHOS ([Arroyo Cell Metabolism 2016](#))

Energy metabolism & oxidative phosphorylation (OXPHOS) changes as a venetoclax resistance mechanism?



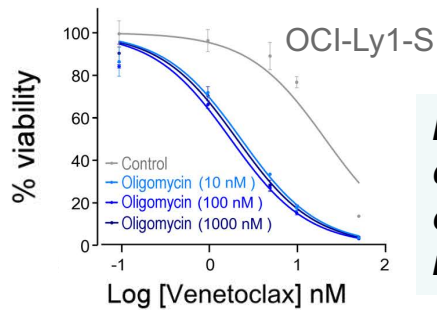
Venetoclax resistant OCI-Ly1 cells display high levels of OXPHOS



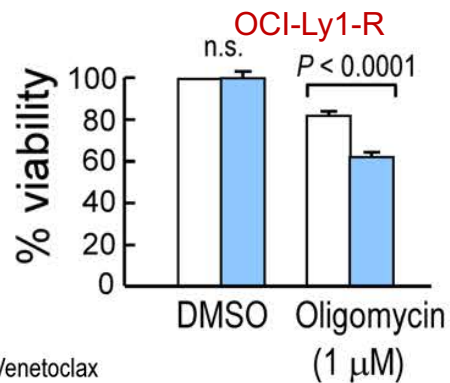
- Increased (basal and maximal) mitochondrial respiration
- In multiple cell lines models

OXPHOS inhibition synergizes with venetoclax

In vitro

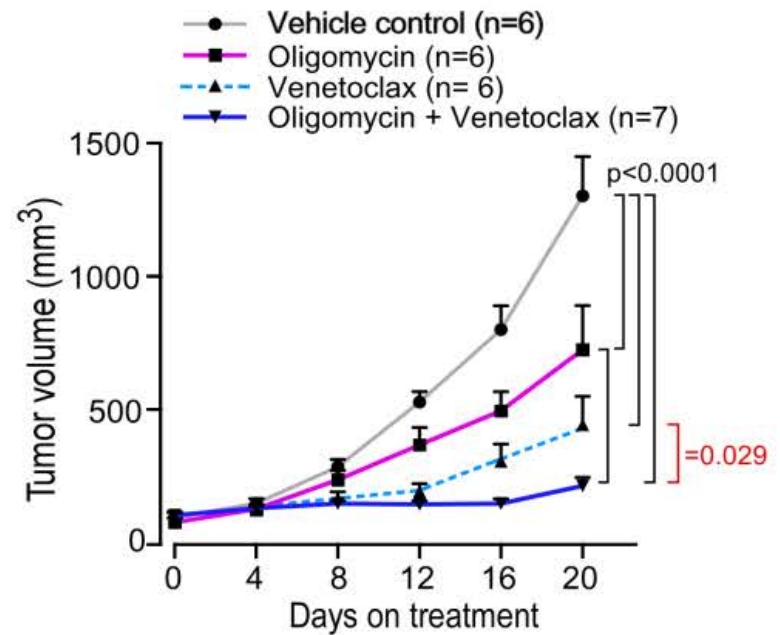


**In 4
different
cell lines
models**



In vivo

3



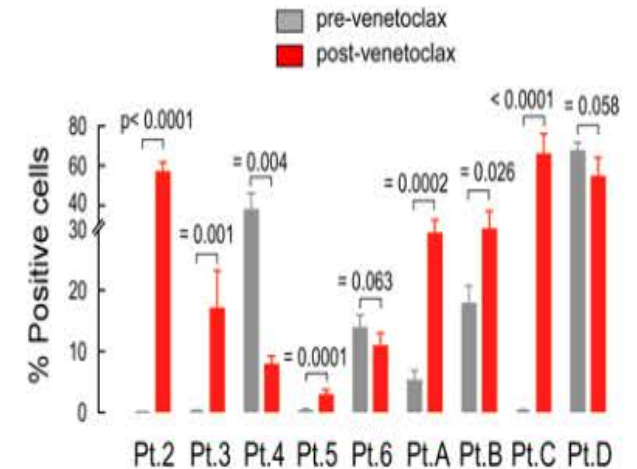
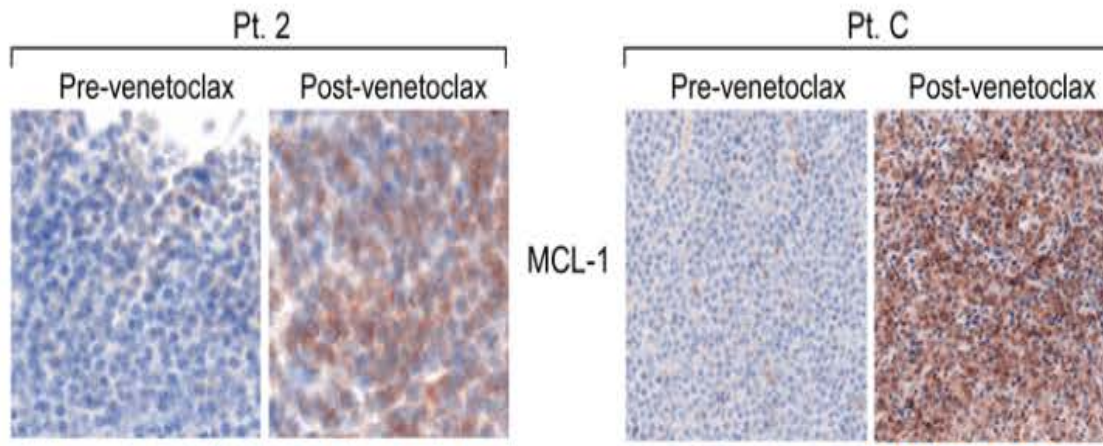
Elisa Ten Hacken

***Could we confirmed these
findings in patients ?***

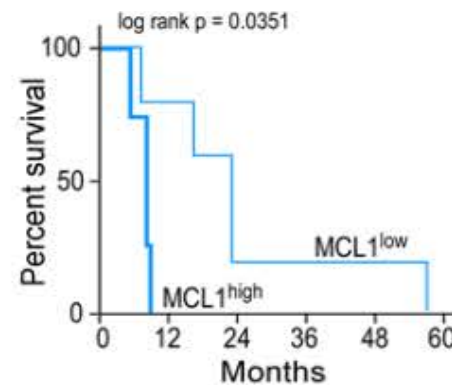
Venetoclax resistance is associated with amplification 1q in both cell lines and patients



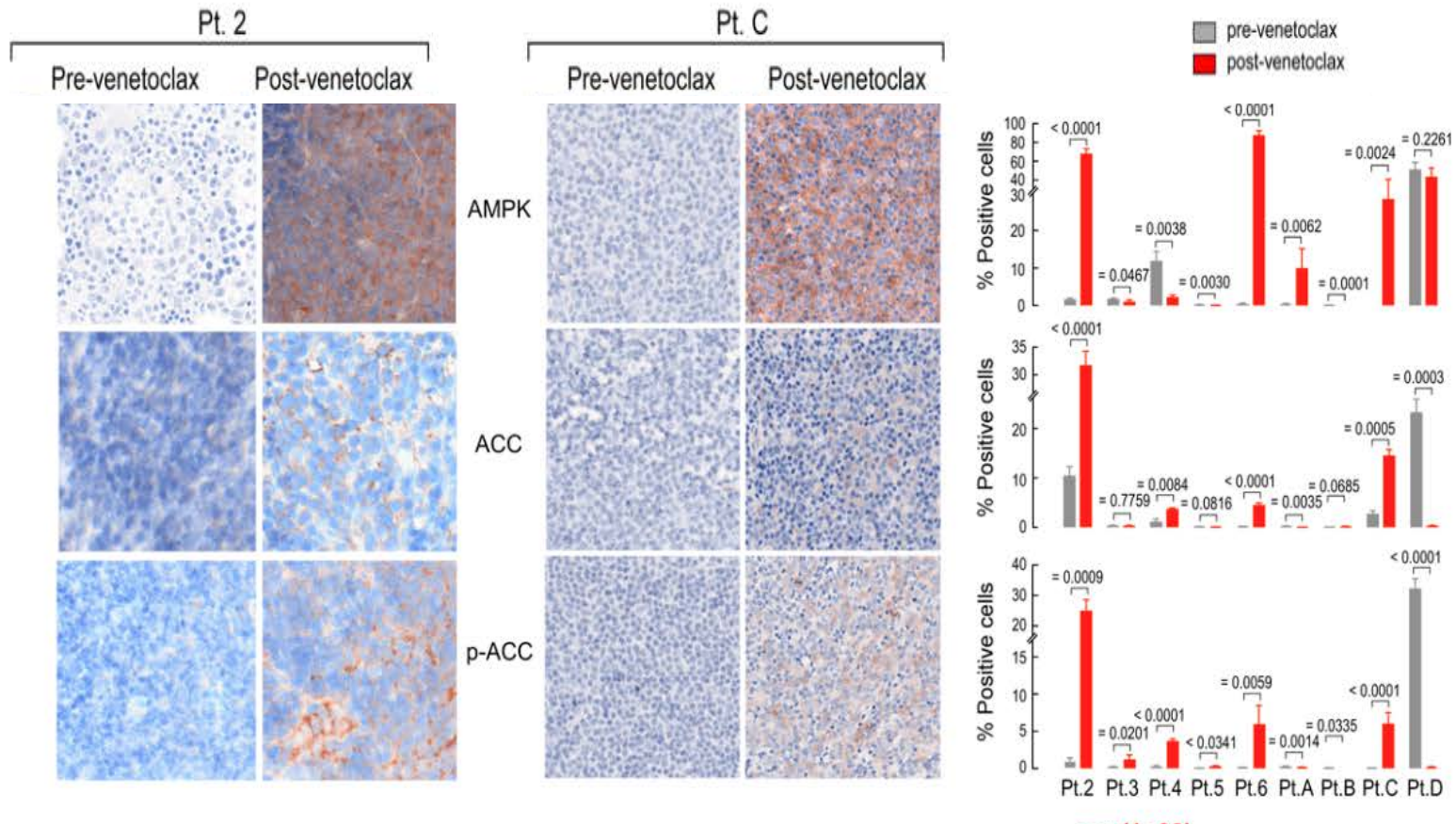
Relapse on venetoclax is associated with increased expression of MCL-1



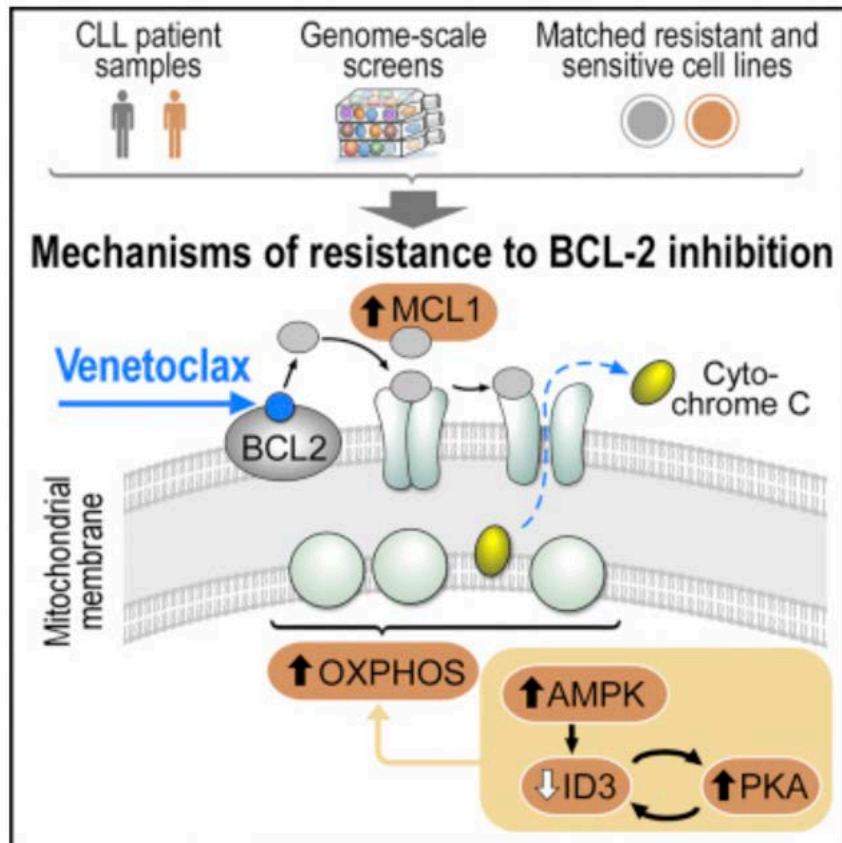
Baseline MCL-1 expression predicts response duration



Relapse on venetoclax is associated with increased AMPK expression and signaling



Summary



- Integrative analysis reveals novel mechanisms of therapeutic resistance
- Suggest novel therapeutic combinations

Acknowledgements

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