MONASH University

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ACBD Australian Centre for Blood Diseases

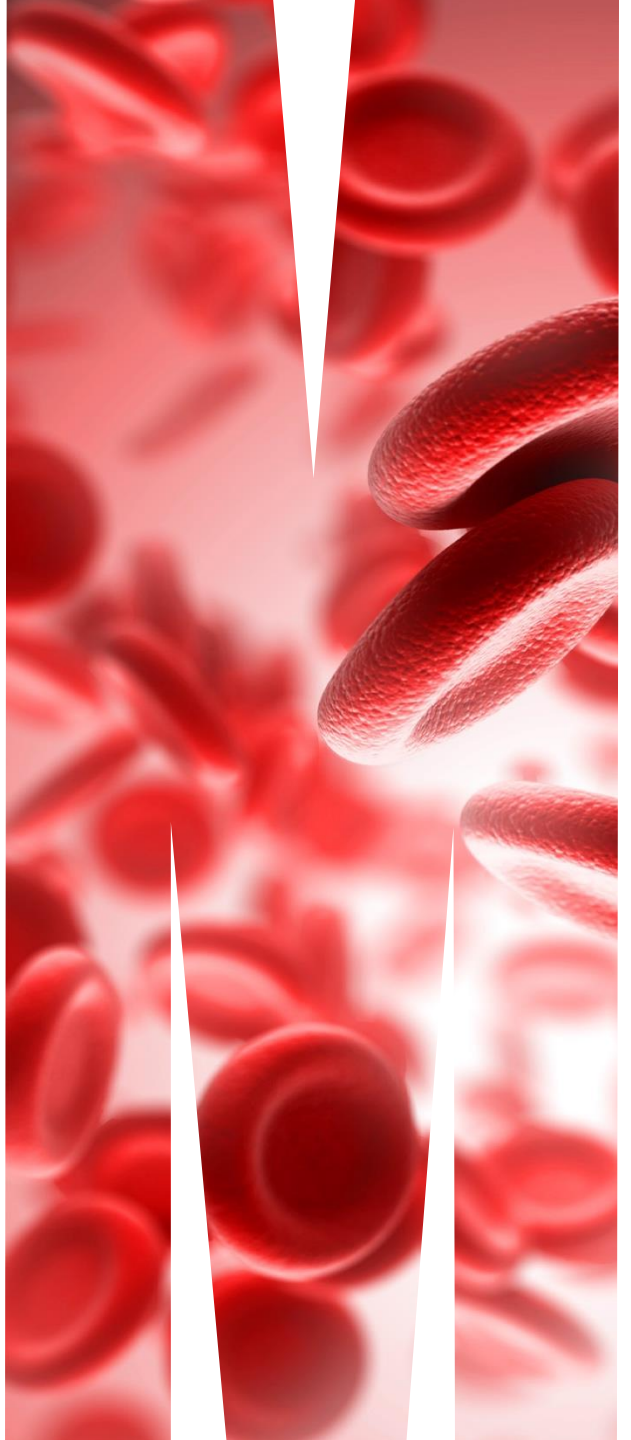
MPN Education Symposium

What is an MPN in 2025

Andrew Perkins

- Professor of Haematology, The Alfred Hospital
- Head of Blood Cancer Genomics, Australian Centre for Blood Diseases, Central Clinical School, Monash University
- Director of Molecular Pathology, Alfred Health

Perth, Oct 25th, 2025

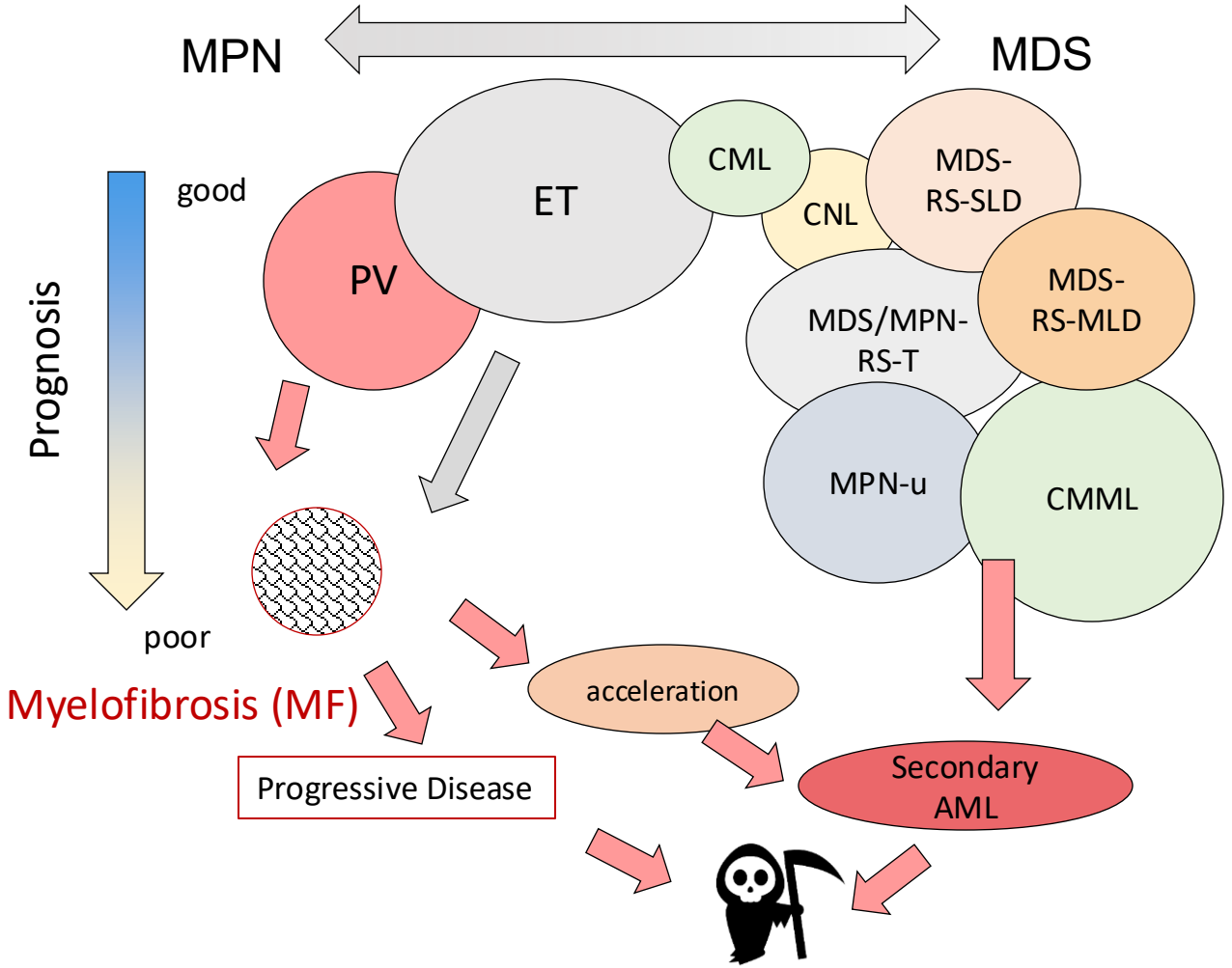


Talk Outline

- What are MPNs
- Haematopoietic Cytokine Signalling 101
- NGS in MPNs: Diagnosis, Prognosis and Discovery
- Germline risk of MPN
- Unmet needs in MPN – a couple of current and future clinical trials

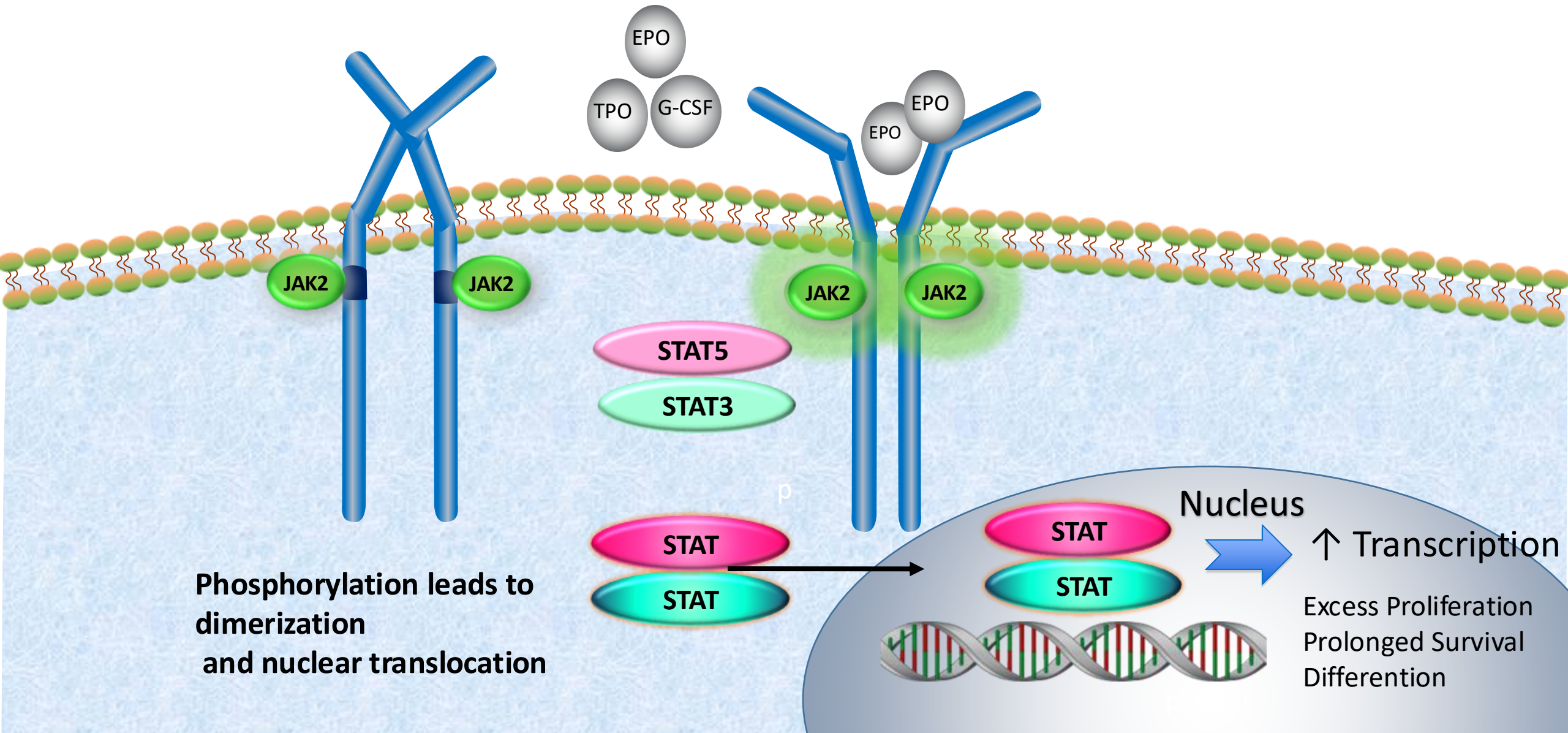
What are MPNs?

Excess Blood Production



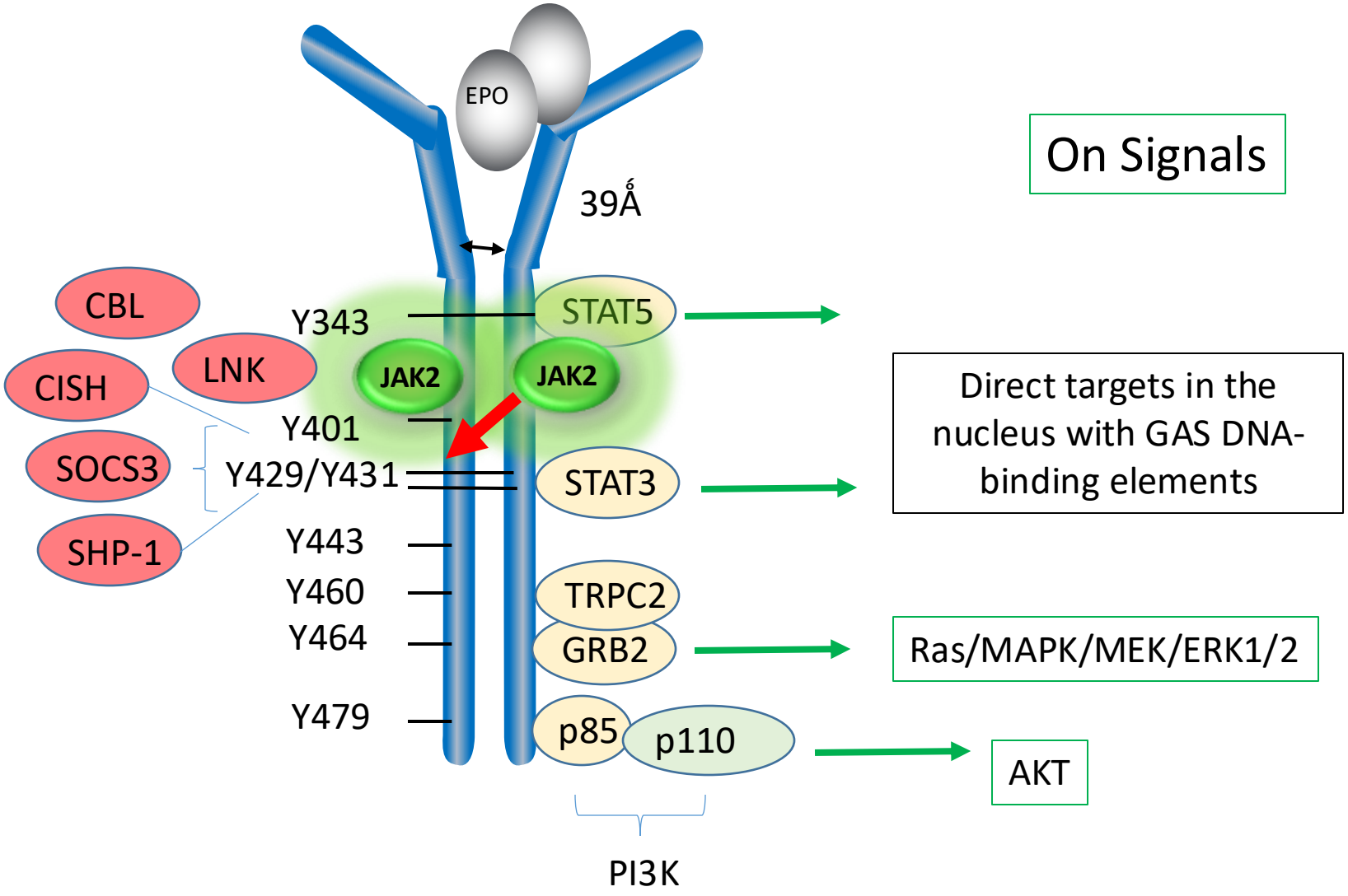
Impaired Blood Differentiation

JAK-STAT signalling from type 1 cytokine receptors



Designing a targeted NGS panel from the ground up: Consider EPO and MPL receptor Signalling

Off Signals



On Signals

Direct targets in the nucleus with GAS DNA-binding elements

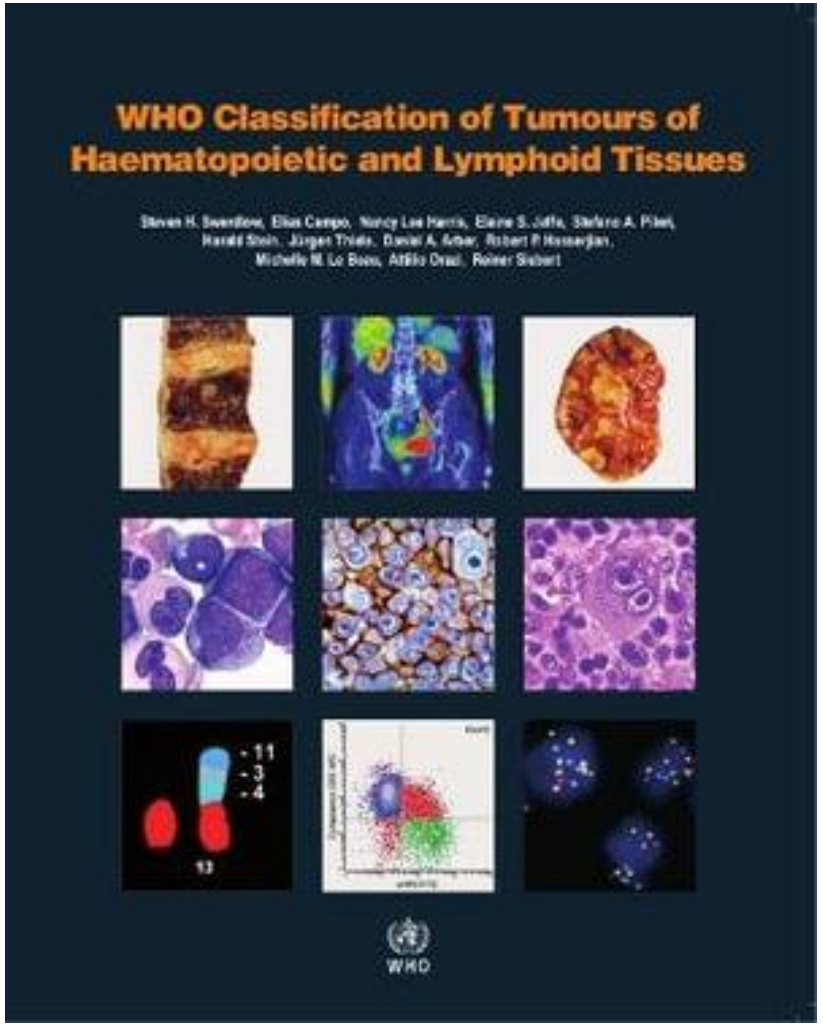
Ras/MAPK/MEK/ERK1/2

AKT

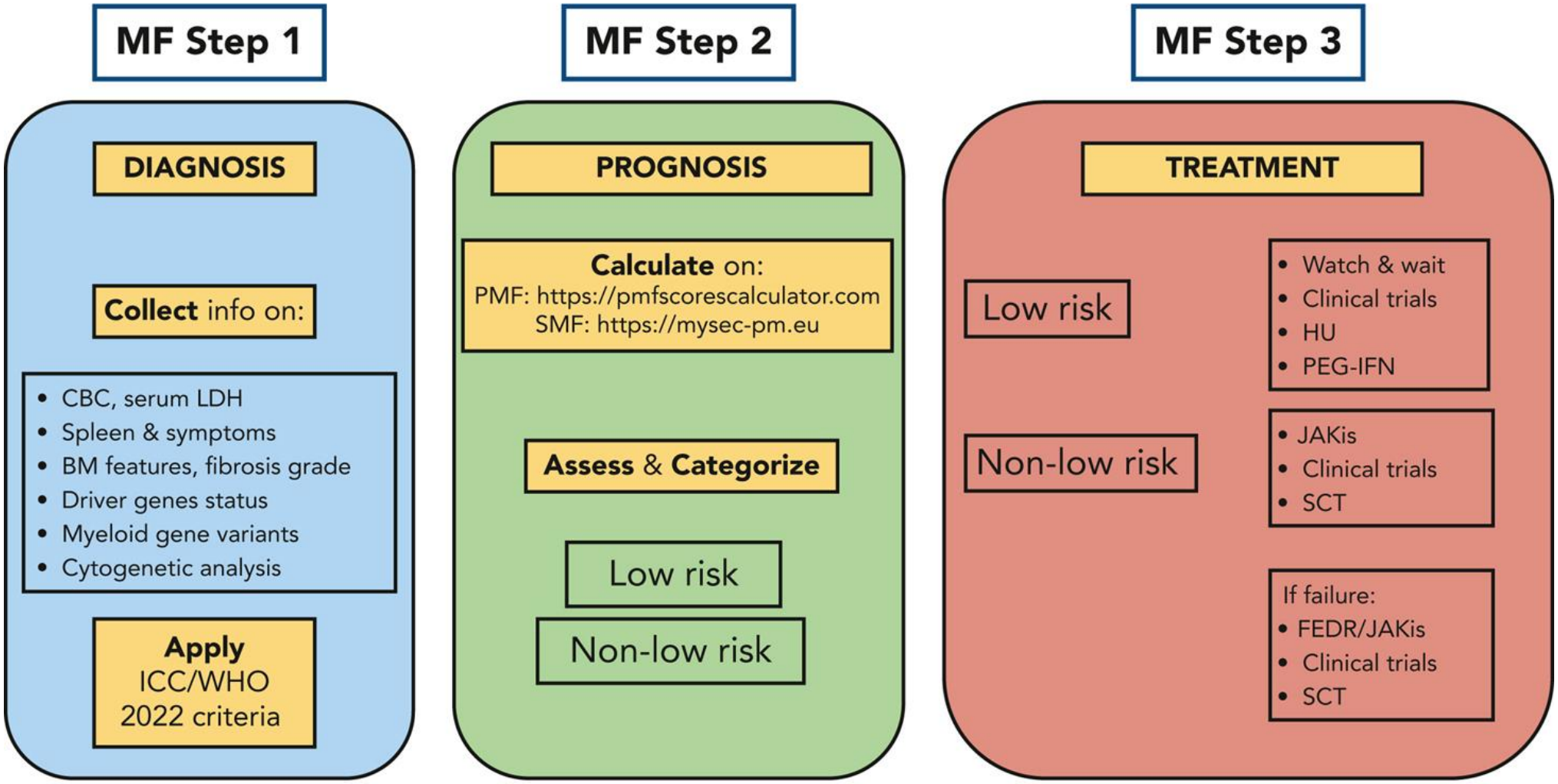
PLUS
Epigenetic Regulators
Splicing factors
A few other things

What might we want to use Next Generation Sequencing (NGS) for?

- Diagnosis – increasingly important for WHO classification of all myeloid malignancies
- Prognosis
- Personalised Treatment; e.g. trial options
- Clonal architecture and evolution/progression
- Minimal Residual Disease Monitoring
- Discovery



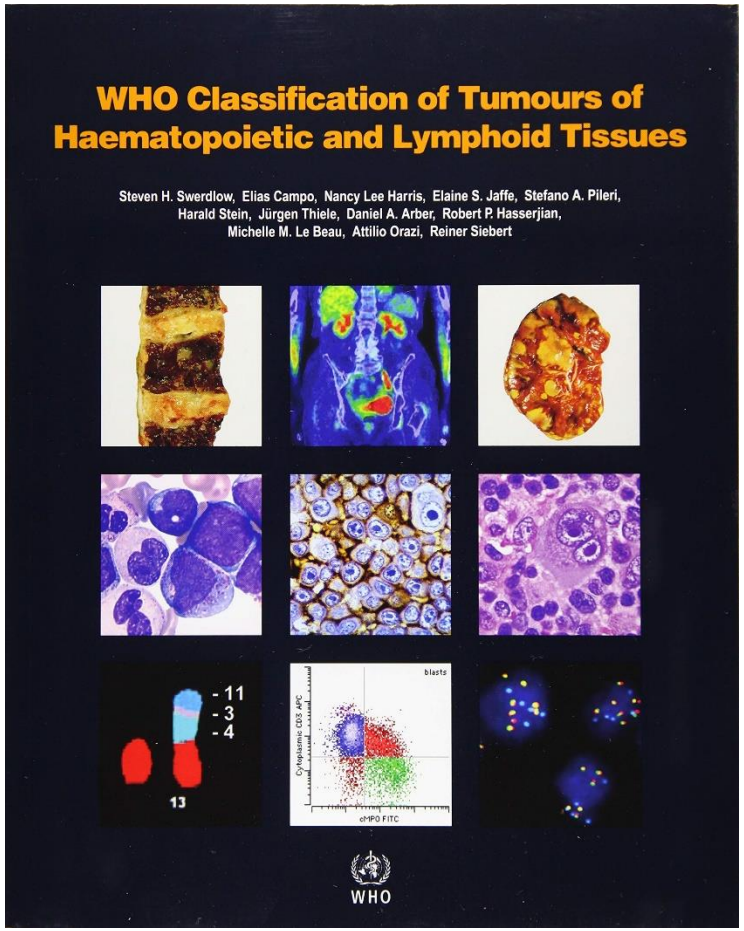
The 3 steps of MF management



Legend: BM= bone marrow; CBC= complete blood count; FEDR= fedratinib, HU= hydroxyurea; ICC= International Consensus Classification; JAKis= JAK inhibitors; LDH= lactate dehydrogenase; MF= myelofibrosis; PEG-IFN= PEGylated-Interferon; PMF= primary myelofibrosis; SCT= stem cells transplantation; SMF= secondary myelofibrosis; WHO= World Health Organization.

Francesco Passamonti, Barbara Mora, Myelofibrosis, Blood, 2023,

Diagnosis of MPNs



Histology

Imaging

Cytogenetics

FISH

FACS

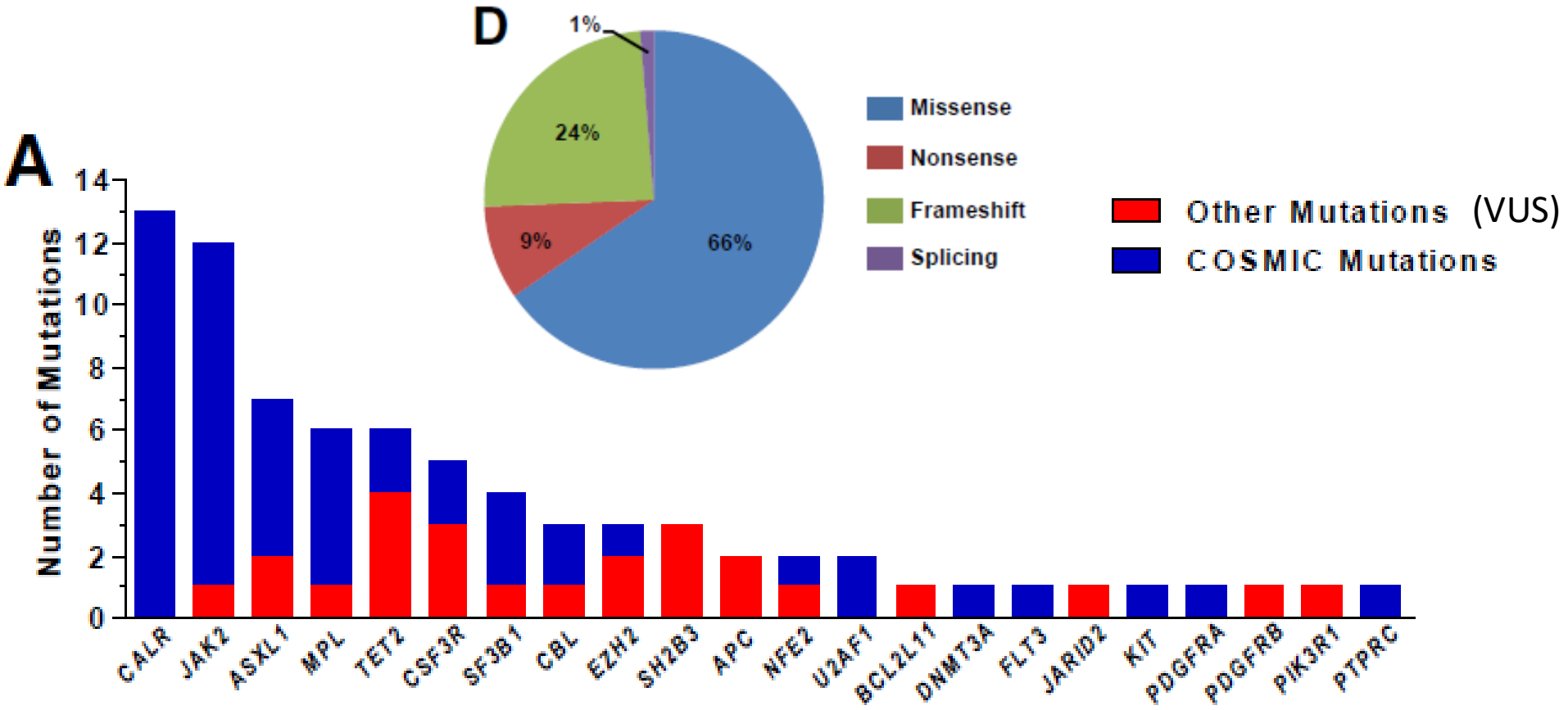
Molecular Testing

Pathologists love to put things in boxes

The Journal of Molecular Diagnostics, Vol. 18, No. 5, September 2016

Rapid Molecular Profiling of Myeloproliferative Neoplasms Using Targeted Exon Resequencing of 86 Genes Involved in JAK-STAT Signaling and Epigenetic Regulation

Graham W. Magor,* Michael R. Tallack,* Nathan M. Klose,† Debra Taylor,‡ Darren Korbie,§¶ Peter Mollee,†‖ Matt Trau,§¶** and Andrew C. Perkins*‡‖††



What Genes are important in MPNs?

- 2035 MPNs (ET, PV, MF [309], other [49])
- 69 genes including CNV
- >200 WES

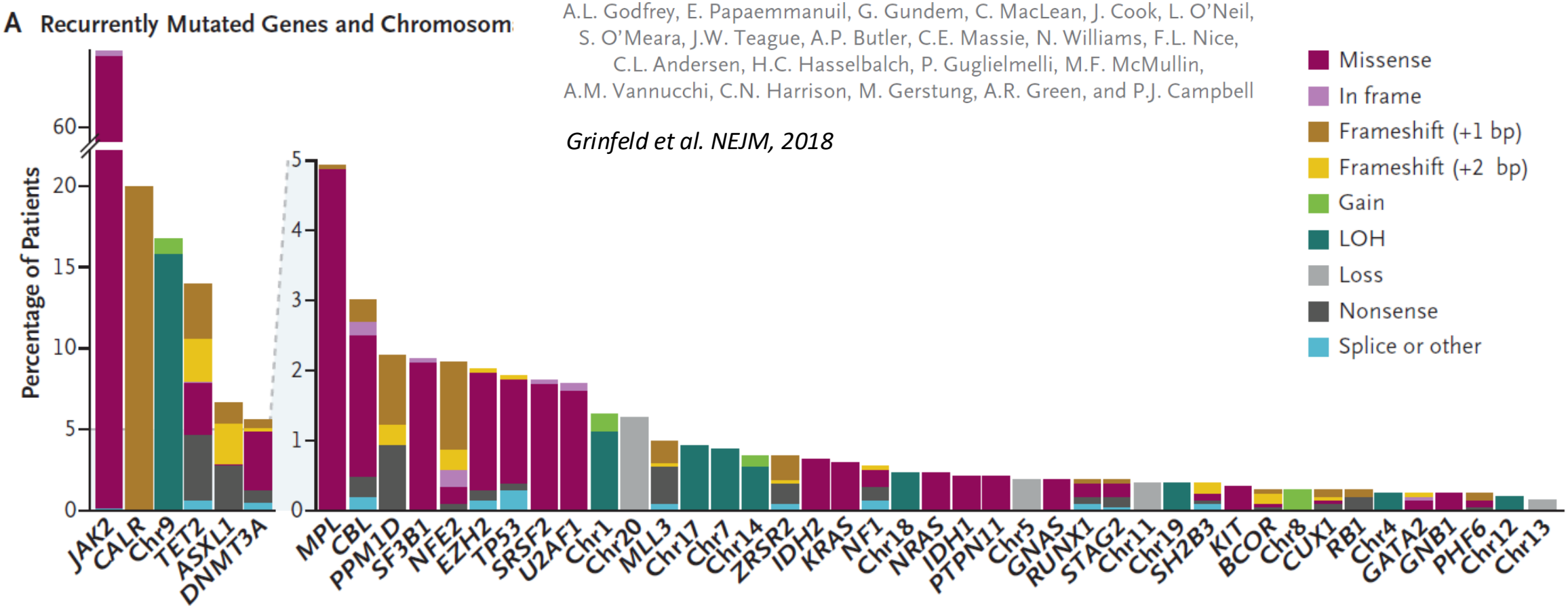
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Classification and Personalized Prognosis in Myeloproliferative Neoplasms

J. Grinfeld, J. Nangalia, E.J. Baxter, D.C. Wedge, N. Angelopoulos, R. Cantrill, A.L. Godfrey, E. Papaemmanuil, G. Gundem, C. MacLean, J. Cook, L. O'Neil, S. O'Meara, J.W. Teague, A.P. Butler, C.E. Massie, N. Williams, F.L. Nice, C.L. Andersen, H.C. Hasselbalch, P. Guglielmelli, M.F. McMullin, A.M. Vannucchi, C.N. Harrison, M. Gerstung, A.R. Green, and P.J. Campbell

Grinfeld et al. NEJM, 2018



Australian Recommendations for NGS in MPNs

Pathology (April 2021) 53(3), pp. 339–348

MYELOID NEOPLASMS

Myeloid somatic mutation panel testing in myeloproliferative neoplasms

David M. Ross[1,2,3,4], Candice Thomson[1,2], Nada Hamad[1,5], Steven W. Lane[1,6,7], Kate Manos[1,8], Andrew P. Grigg[1,8], Belinda Guo[1,9], Wendy N. Erber[1,9,10], Ashleigh Scott[1,6], Nick Viiala[1,11], Lynette Chee[1,12], Maya Latimer[1,13], Courtney Tate[1,14], Carolyn Grove[1,9,10,15], Andrew C. Perkins[1,16], Piers Blombery[1,12]

[1]*Myeloproliferative Neoplasms Working Party, Australasian Leukaemia and Lymphoma*

ALLG
Australasian Leukaemia & Lymphoma Group

Just 17 genes that cover the majority of diagnostic and prognostic requirements

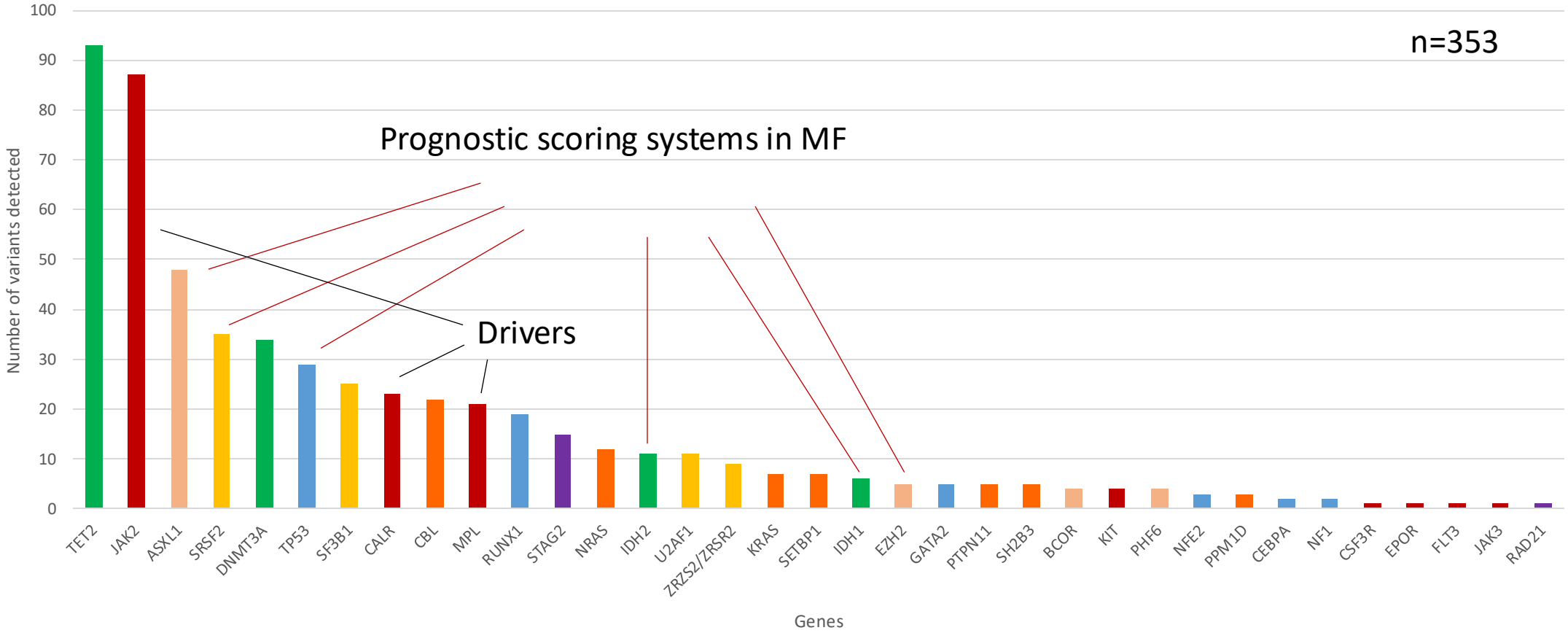
Categories of mutations in chronic myeloid malignancies

Pathways	Some Genes
Cytokine Receptor and Signaling	JAK2, CALR, MPL, CSF3R, KIT, EPOR, JAK3, NRAS, KRAS
Negative regulators of cytokine signalling	SH2B3, CBL, PTPN11
Epigenetics - DNA methylation	TET2, DNMT3A, IDH1, IDH2
Epigenetics - histone modification	EZH2, BCOR, IDH1, IDH2
Splicing	SF3B1, U2AF1, SRSF2, ZRSR2
Transcription	TP53, RUNX1, GATA2, NFE2, CEBPA
Cohesin Complex	STAG1, STAG2, RAD21

GoF mutations

LoF mutations

NGS results in chronic myeloid malignancy (2020-2021): Prognosis

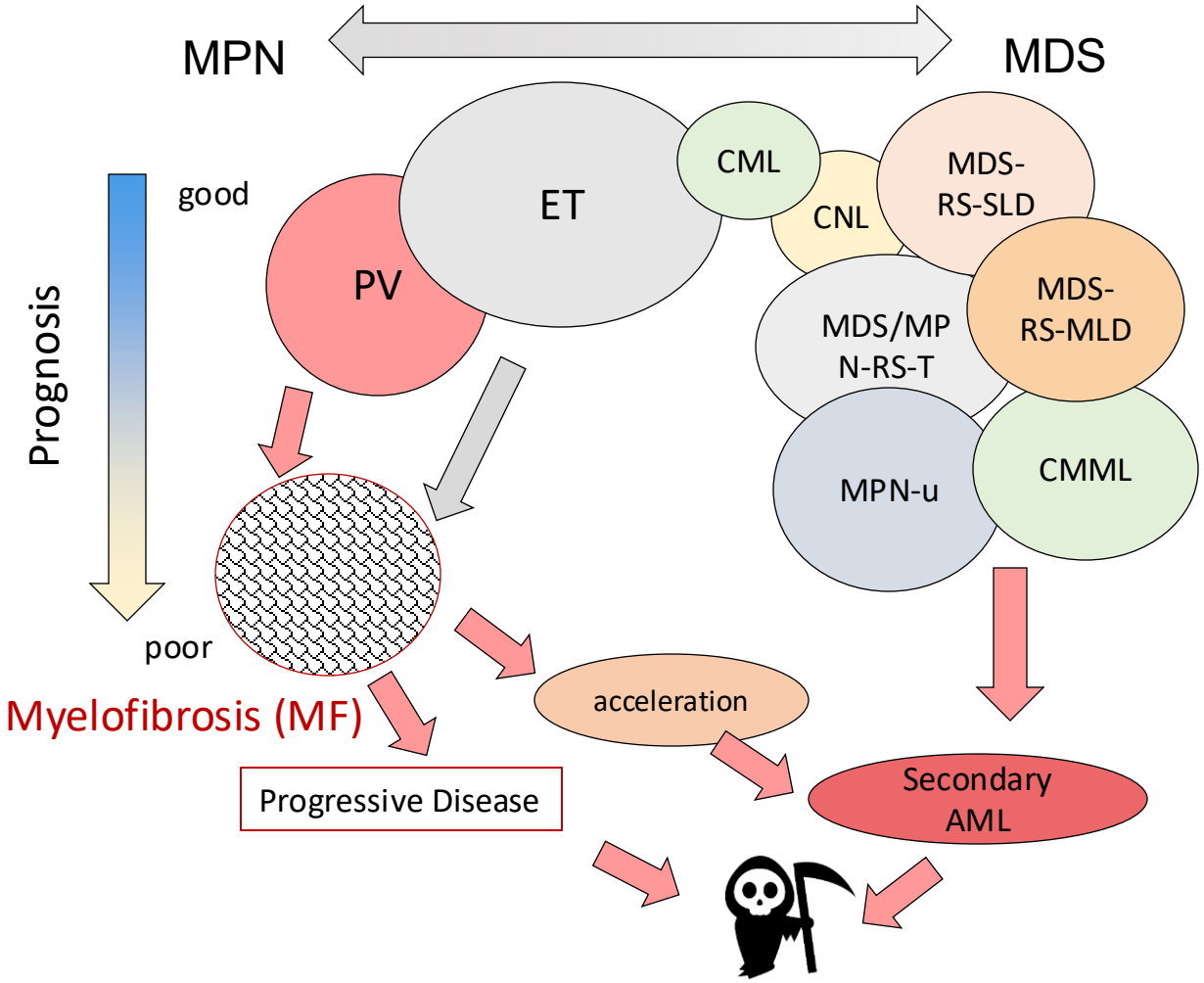


Justin Ng

NGS for Diagnosis

Drivers
(JAK-STAT signalling activation)

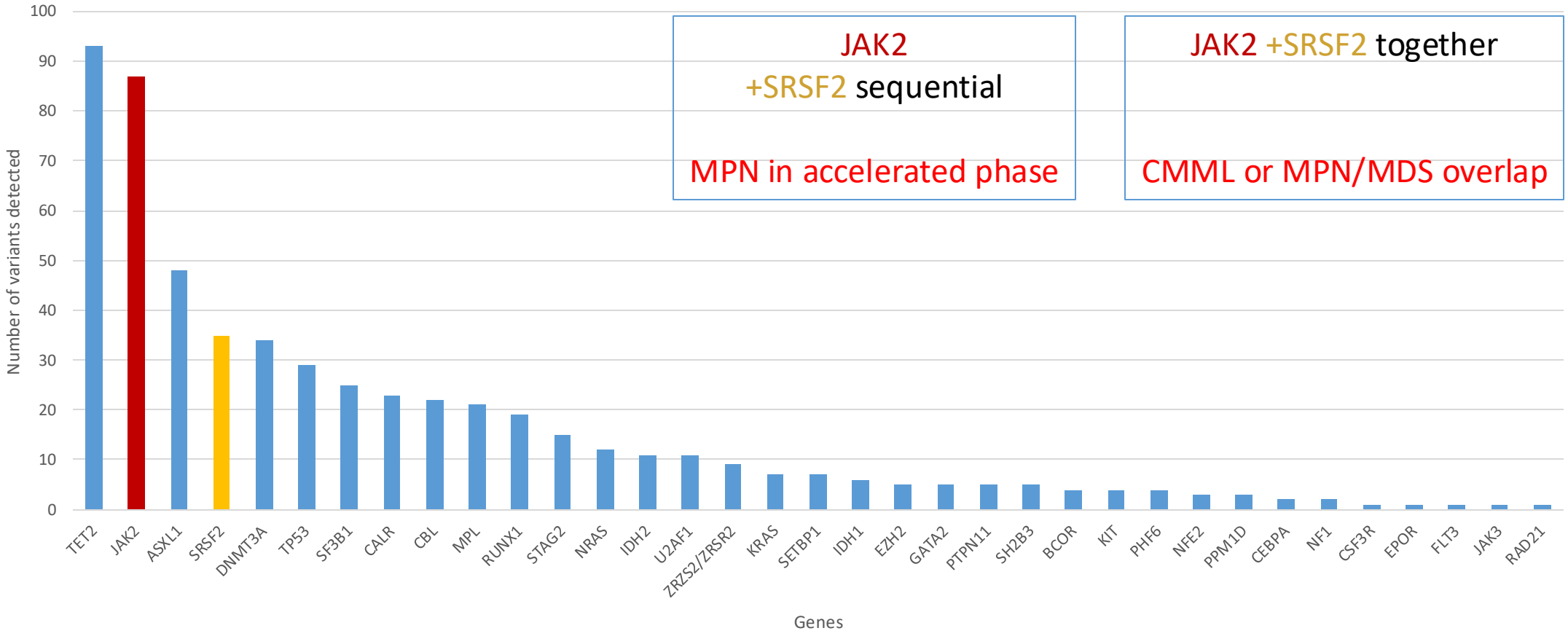
JAK2 V617F
CALR exon 9 Indels
MPL W515 missense



Other common diagnostic mutations

CSF3R - CNL
SF3B1 – ring sideroblasts
CBL - CMML
SRSF2 – CMML/MDS

Combinations of mutations result in specific disease entities

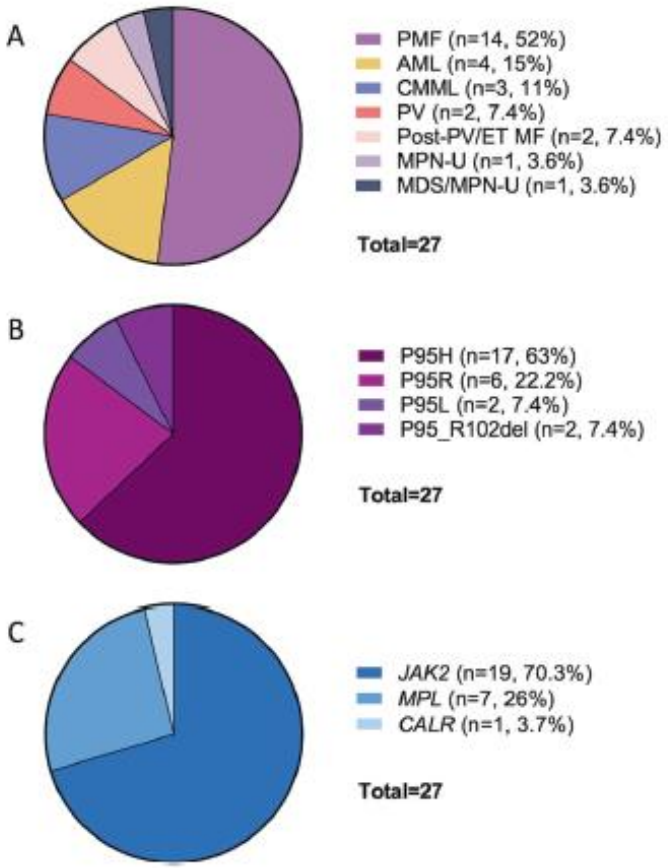


ARTICLE

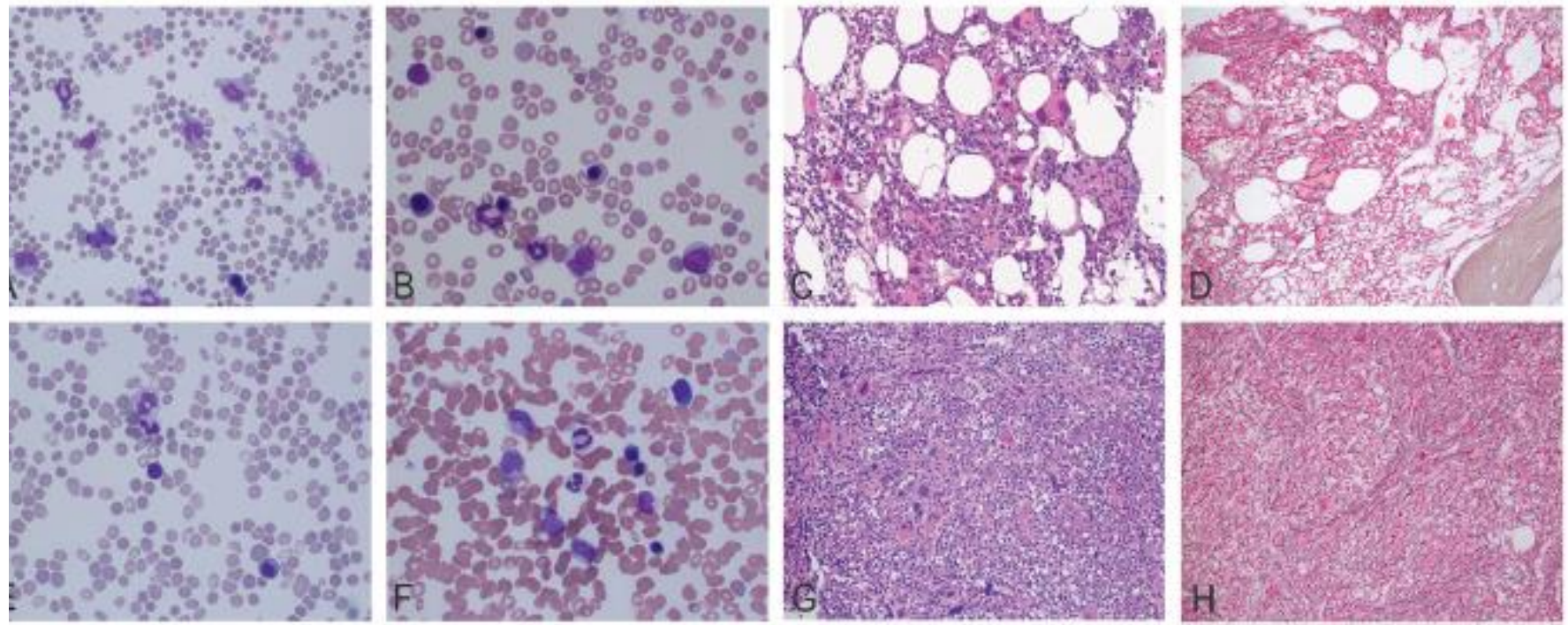
Check for updates

Clinicopathologic spectrum of myeloid neoplasms with concurrent myeloproliferative neoplasm driver mutations and *SRSF2* mutations

Mehrnoosh Tashakori[1], Joseph D. Khoury[1], Mark J. Routbort[1], Keyur P. Patel[1], Sa A. Wang[1], Chi Young OK[1], Siba El-Hussein[1], Rashmi Kanagal-Shamanna[1], Rajyalakshmi Luthra[1], Shimin Hu[1], Pei Lin[1], Naveen Pemmaraju[2], Prithviraj Bose[2], Srdan Verstovsek[2], Carlos E. Bueso-Ramos[1], L. Jeffrey Medeiros[1] and Sanam Loghavi[1]✉



PMF with monocytosis



CMML with MF3

PROGNOSIS

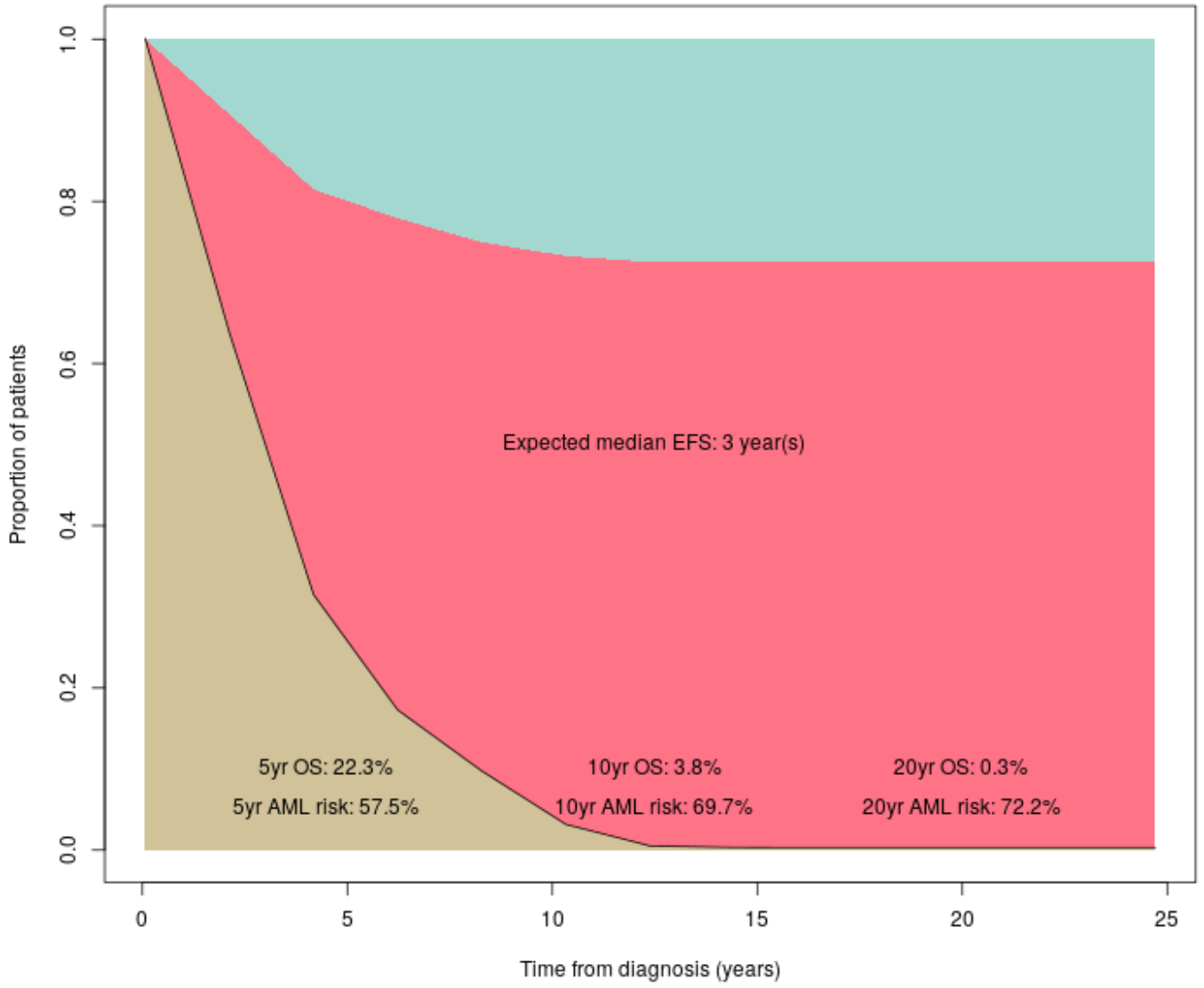
MIPSS70 score Home Credits

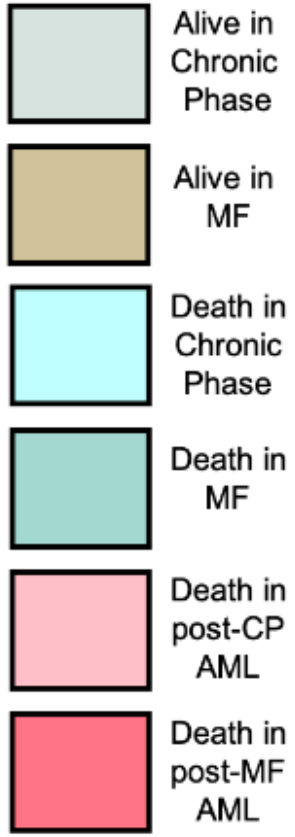
#	Question	Answer
1	Severe Anemia (hemoglobin <80g/L)	○ Yes ○ No
2	Moderate Anemia (hemoglobin 80-100g/L)	○ Yes ○ No
3	Leucocytosis >25x10^9/L	○ Yes ○ No
4	Thrombocytopenia (platelet count <100x10^9/L)	○ Yes ○ No
5	Peripheral blood blast count ≥2%	○ Yes ○ No
6	Bone marrow fibrosis grade ≥2	○ Yes ○ No
7	Constitutional symptoms	○ Yes ○ No
8	Absence of CALR type 1/like mutation	○ Yes ○ No
9	HMR[1] category	○ Yes ○ No } IHD1, IDH2, SRSF2, ASXL1, U2AF1
10	≥2 HMR mutated genes	○ Yes ○ No
11	Unfavorable karyotype[2]	○ Yes ○ No ○ Not available
12	Very High Risk karyotype[3]	○ Yes ○ No ○ Not available

Score	Result
MIPSS70	

MPN report patient survival diagram

https://www.sanger.ac.uk/science/tools/progmod/progmod/





Based on Grinfeld et al. NEJM 2018

Inherited Genetic Risk for Late Onset Development of MPNs

Article

Inherited myeloproliferative neoplasm risk affects haematopoietic stem cells

https://doi.org/10.1038/s41586-020-2786-7

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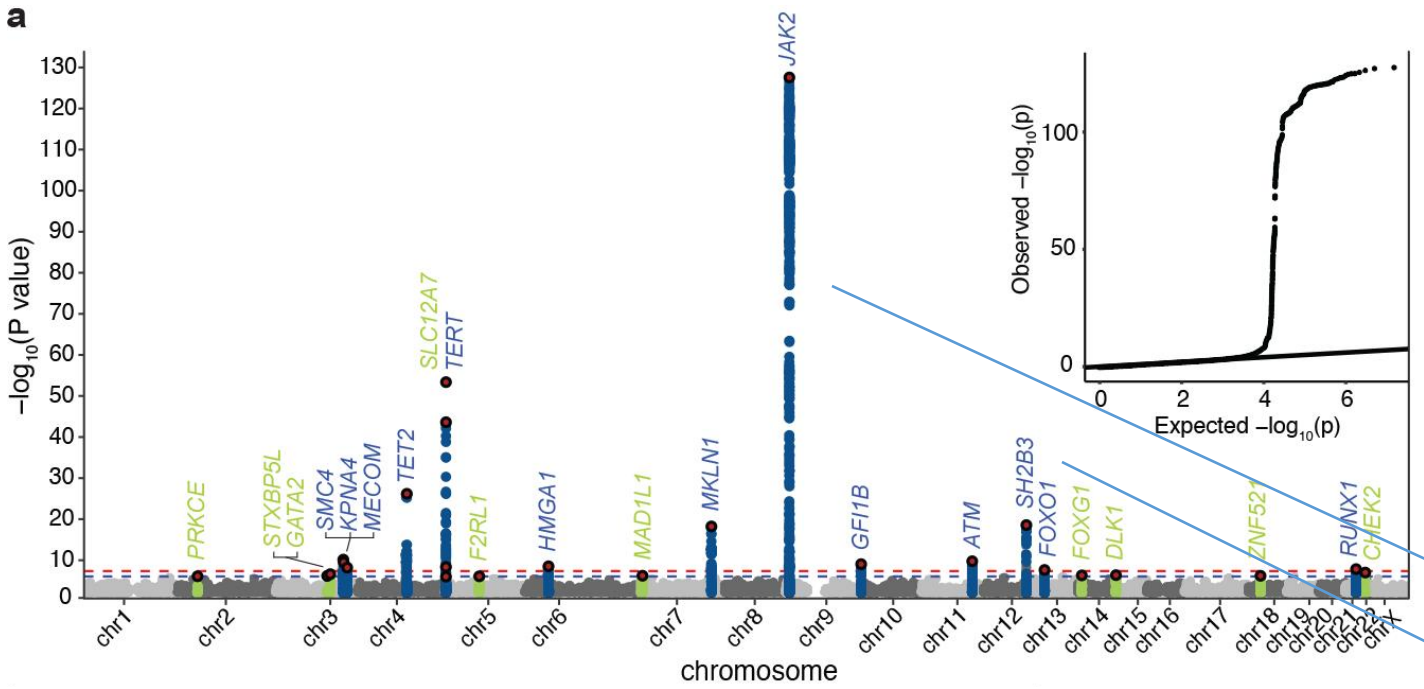
Accepted: 3 July 2020

Published online: 14 October 2020

Check for updates

Erik L. Bao[1,2,3,4,59], Satish K. Nandakumar[1,2,3,59], Xiaotian Liao[1,2,3,59], Alexander G. Bick[3,5,6,7,8], Juha Karjalainen[9], Marcin Tabaka[3], Olga I. Gan[10,11], Aki S. Havulinna[9], Tuomo T. J. Kiiskinen[9], Caleb A. Lareau[1,2,3,12], Aitzkoa L. de Lapuente Portilla[13], Bo Li[3,14], Connor Emdin[3,5], Veryan Codd[15,16], Christopher P. Nelson[15,16], Christopher J. Walker[17], Claire Churchhouse[3], Albert de la Chapelle[17], Daryl E. Klein[18], Björn Nilsson[3,13], Peter W. F. Wilson[19,20], Kelly Cho[21,22], Saiju Pyarajan[21], J. Michael Gaziano[21,22], Nilesh J. Samani[15,16], FinnGen*, 23andMe Research Team*, Aviv Regev[3,23,24], Aarno Palotie[3,9], Benjamin M. Neale[3], John E. Dick[10,11], Pradeep Natarajan[3,5,25], Christopher J. O'Donnell[7,22], Mark J. Daly[3,9], Michael Milyavsky[26], Sekar Kathiresan[3,5,27] & Vijay G. Sankaran[1,2,3,28]

Inherited MPN Risk



a. GWAS meta-analysis for MPNs (n = 2,949 cases and 835,554 controls). X axis is chromosomal position, and y axis is the $-\log_{10}(P)$ value of association (two-tailed, logistic regression). Association signals reaching genome-wide significance ($P < 5 \times 10^{-8}$) and suggestive significance ($P < 1 \times 10^{-6}$) are shown in blue and green, respectively. Red points represent conditionally independent lead variants within each locus.

JAK2 46/1 haplotype of four non-coding SNPs

SH2B3

Bao et al. Nature Genetics 2020

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SH2B3 In Myeloproliferative Neoplasms

Andrew Perkins Liesl Butler

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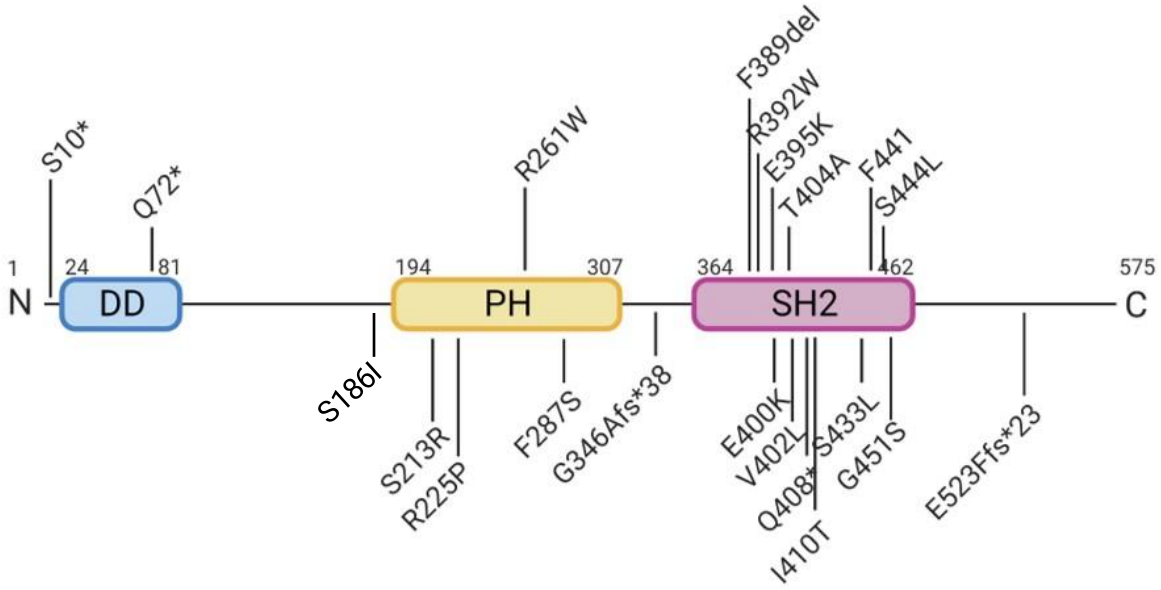
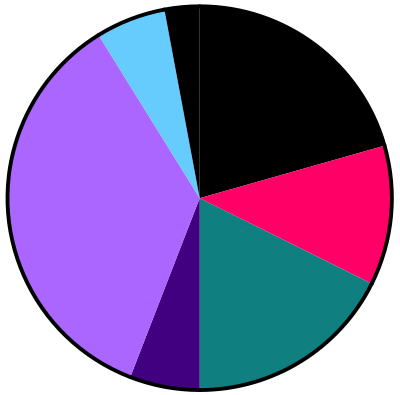
SH2B3 variants are frequently missense and in the SH2 domain. Clinical presentations are diverse.



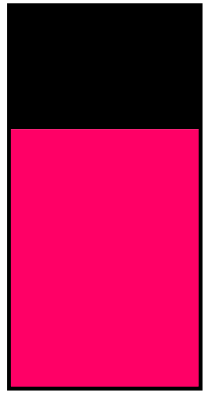
~2.5% of all Alfred Pathology myeloid next generation sequencing samples are found to have an *SH2B3* variant

Diagnosis



- 20.59% 7 MPN
- 11.76% 4 MDS
- 17.65% 6 MDS/MPN
- 5.88% 2 AML
- 35.29% 12 Cytoses
- 5.88% 2 Cytopenias
- 2.94% 1 Other

Other Mutations



- 32.35% 11 Sole Mutation
- 67.65% 23 Additional Mutation/s

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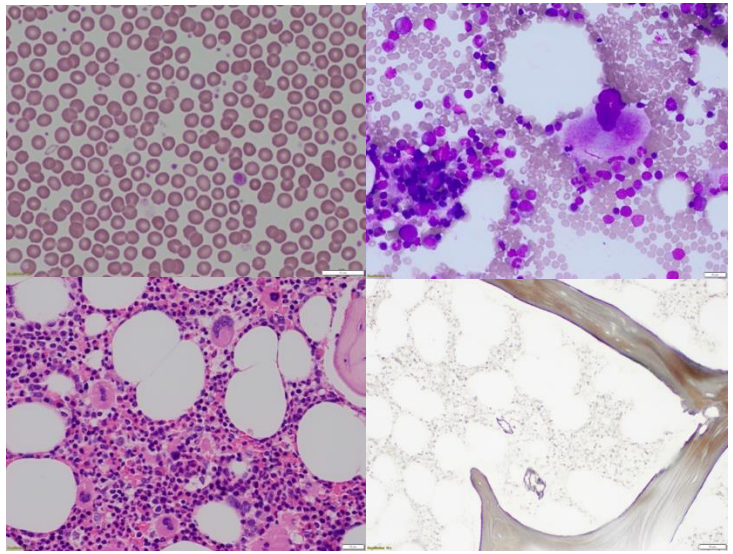
Germline missense *SH2B3* variants may lead to myeloproliferative disease

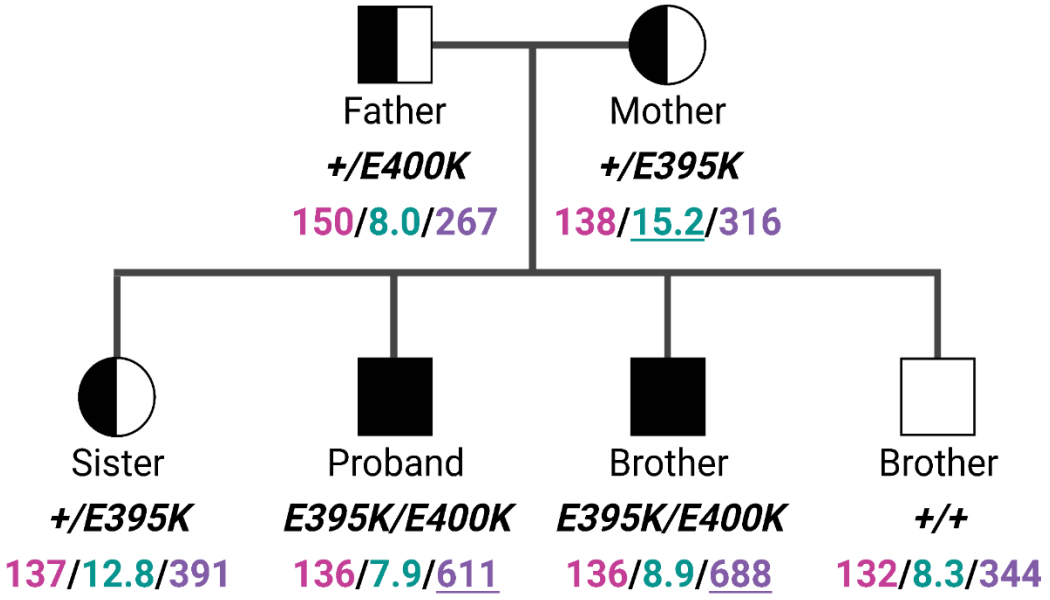
Family 1: E395K

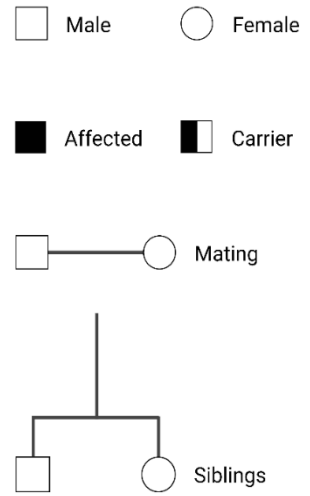
Family 2: E395K/E400K

Family 3: T404A



Proband's blood and bone marrow (aspirate, trephine and reticulin stain)



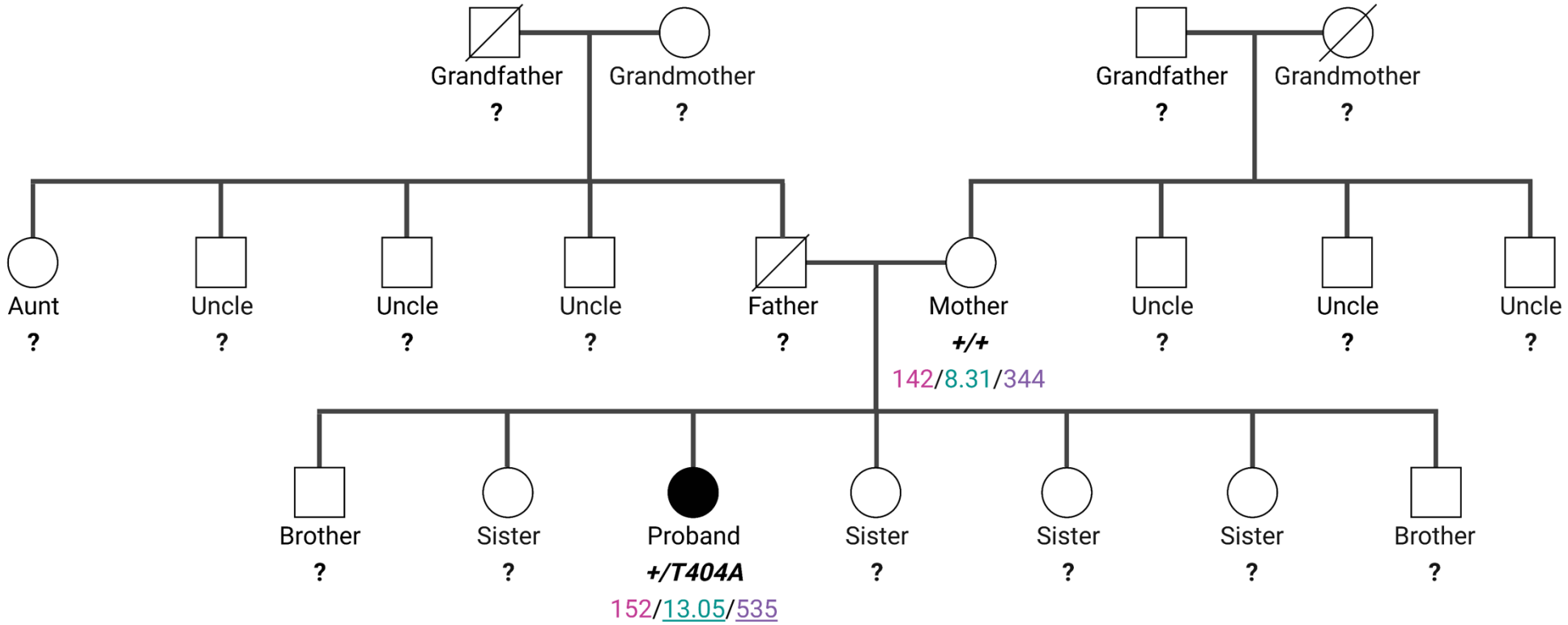


Normal Ranges

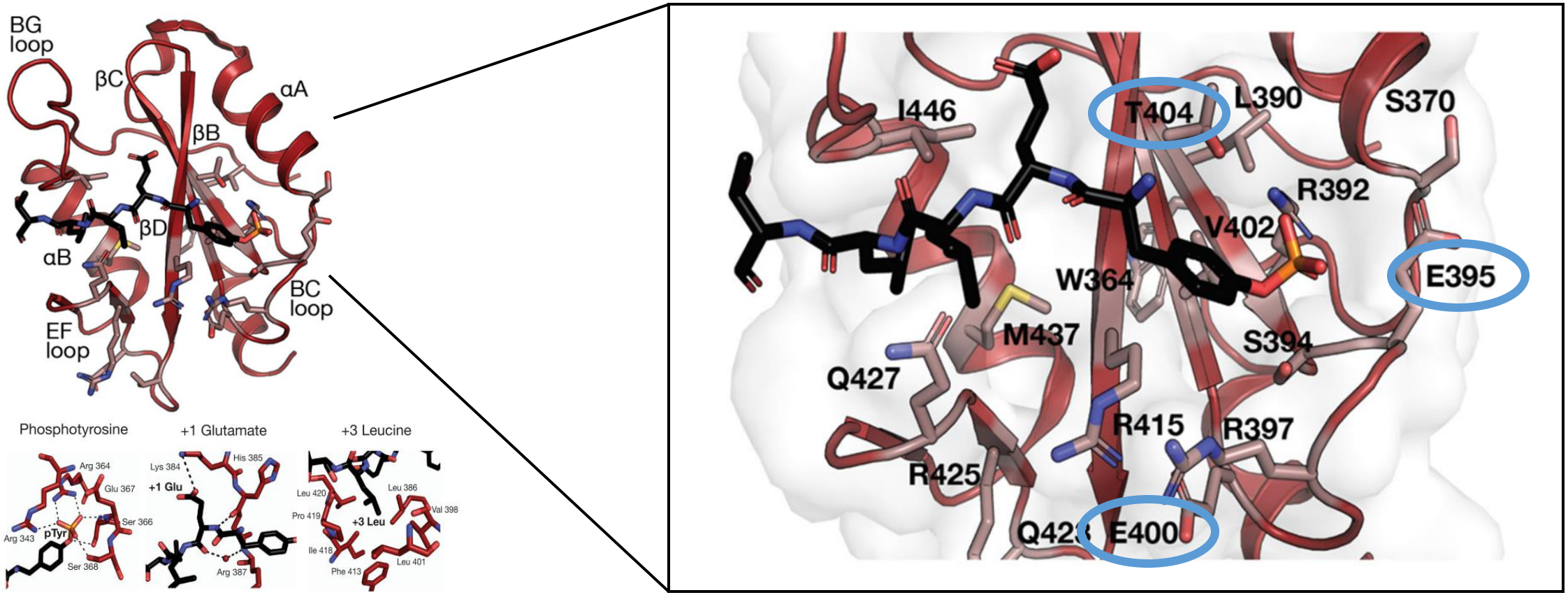
Haemoglobin	135 - 180 g/L (Adult Male) 115 - 160 g/L (Adult Female) 115 - 155 g/L (Child)
White Cell Count	4.0 - 11.0 x10^9/L (Adult) 4.5 - 13.0 x10^9/L (Child)
Platelets	150 - 450 x 10^9/L

SH2B3-T404A mutations may represent a germline cause for myeloproliferative disease

Family 2: *T404A*



Variants in the SH2 domain of SH2B3 affect interaction with phosphorylated JAK2



(Morris et al. 2021)
(Morris et al. 2022)

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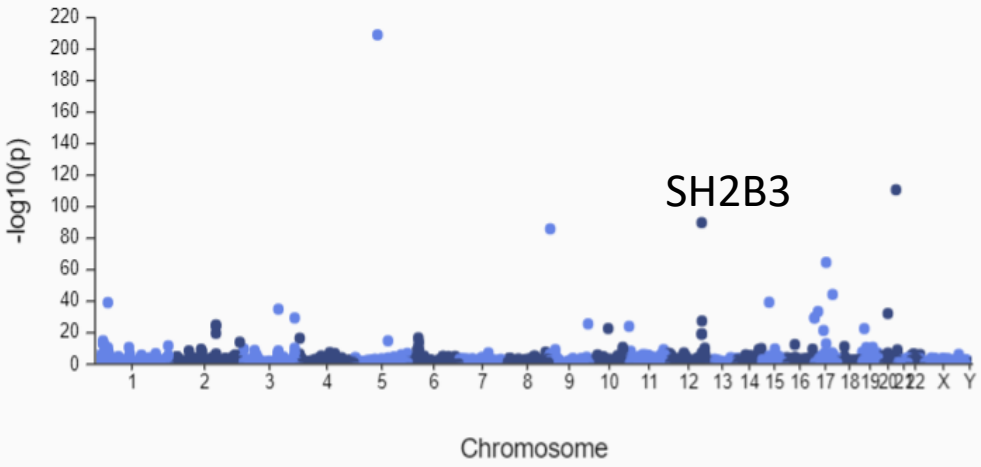
HOME | Result view: Top gene associations | Gene manhattan | Variant manhattan | Phenotype info | Gene PheWAS | Variant PheWAS | Result pane width: hide | 1/4 | 1/3 | 1/2 | 2/3 | full | Phewas options | Gene options | Search by gene or phenotype | v0.7.8-alpha

Gene: SH2B3 (ENSG00000111252) **Phenotype:** Platelet count **Burden set:** missense|LC

missense|LC gene burden associations with Platelet count



Burden set: pLoF | missense|LC | synonymous

Filter genes | Min P-value: -Infinity

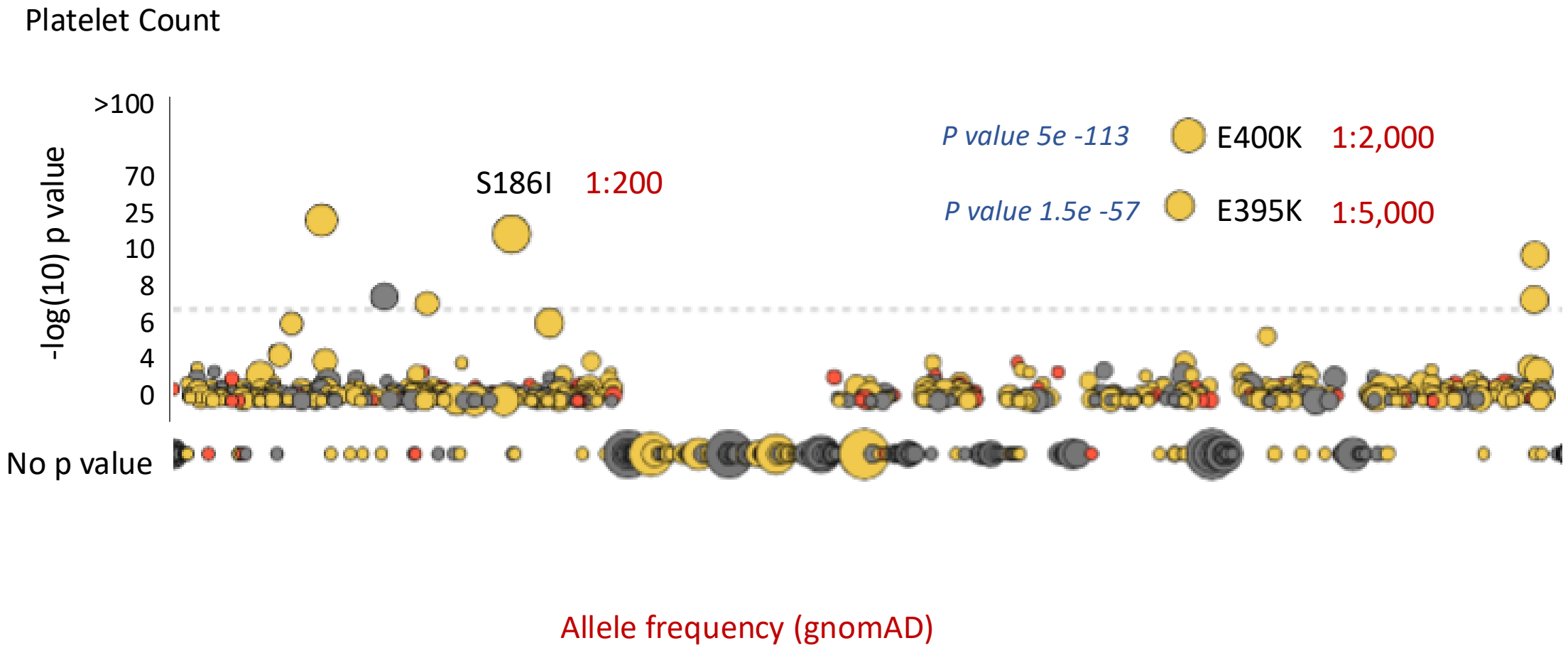
Gene Name	P-Value SKATO	P-Value Burden	P-Value SKAT	BETA Burden	Details
IQGAP2	8.41e-210	3.36e-74	1.24e-198	-5.13e-3	
TUBB1	1.94e-111	4.6e-53	2.81e-111	-4.76e-3	
SH2B3	1.36e-90	7.74e-36	2.66e-88	5.14e-3	
JAK2	1.24e-86	2.58e-65	6.12e-70	7.02e-3	
ITGA2B	2.36e-65	6.6e-60	1.78e-46	-6.85e-3	
APOH	5.42e-45	9e-6	7.74e-46	1.38e-3	

Blood Cancer Lab....xlsx | 2021-12-21-Grant-....csv | 02349 02342 807

Type here to search

8:05 AM

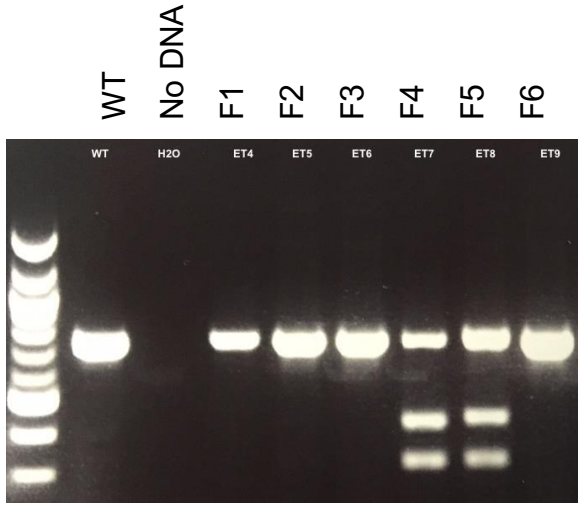
Inherited missense variants in SH2B3 cause thrombocytosis and leukocytosis

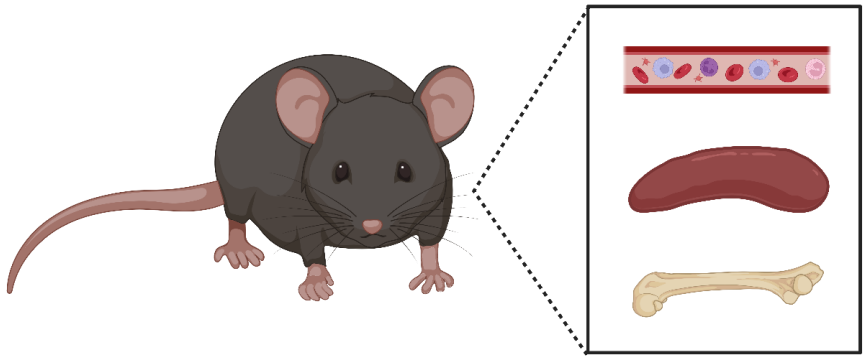


Functional Validation: Does *SH2B3-E395K* result in a myeloproliferative disease phenotype?

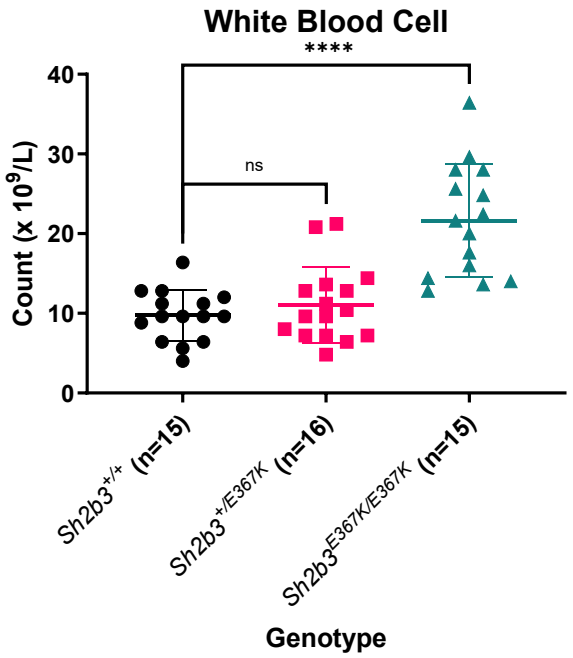
```
1  GCA CAG CTG GTC CAG CTC CAG GGC CCT GAT GCC CAC GGC GTG TTC
1▶  A   Q   L   V   Q   L   Q   G   P   D   A   H   G   V   F
                            XbaI
                          E367K
46 CTG GTG CGG CAG AGT AAG TCT AGA AGA GGA GAG TAT GTA CTC ACA
16▶ L   V   R   Q   S   K   S   R   R   G   E   Y   V   L   T
91 TTC AAC TTA CAG GGC AGA GCC AAG GTAGGGGCAG GAACT
31▶ F   N   L   Q   G   R   A   K
```

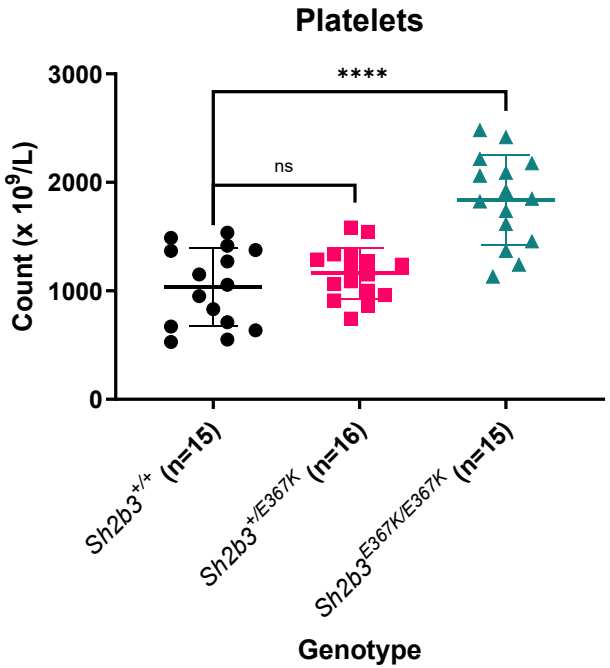
Monash Genome Editing Platform

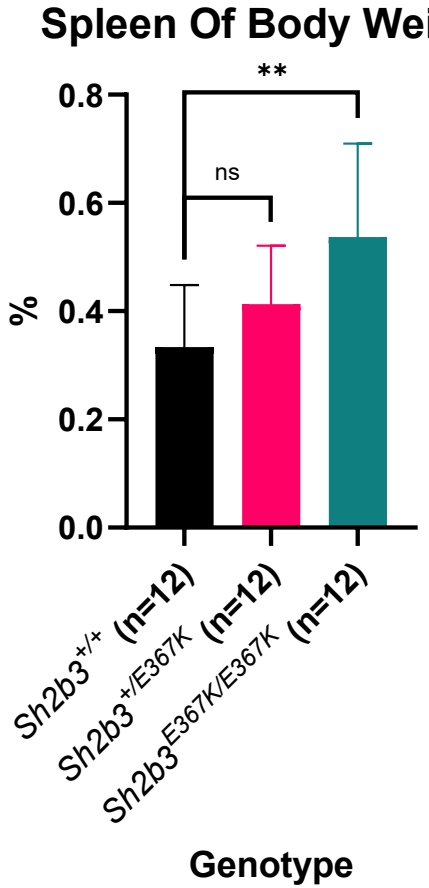


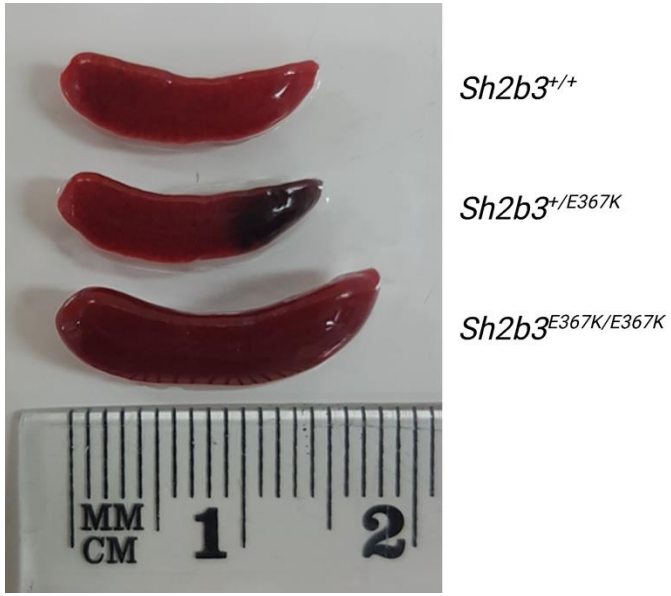


Sh2b3-E367K mutant mice demonstrate peripheral blood cytoses and splenomegaly



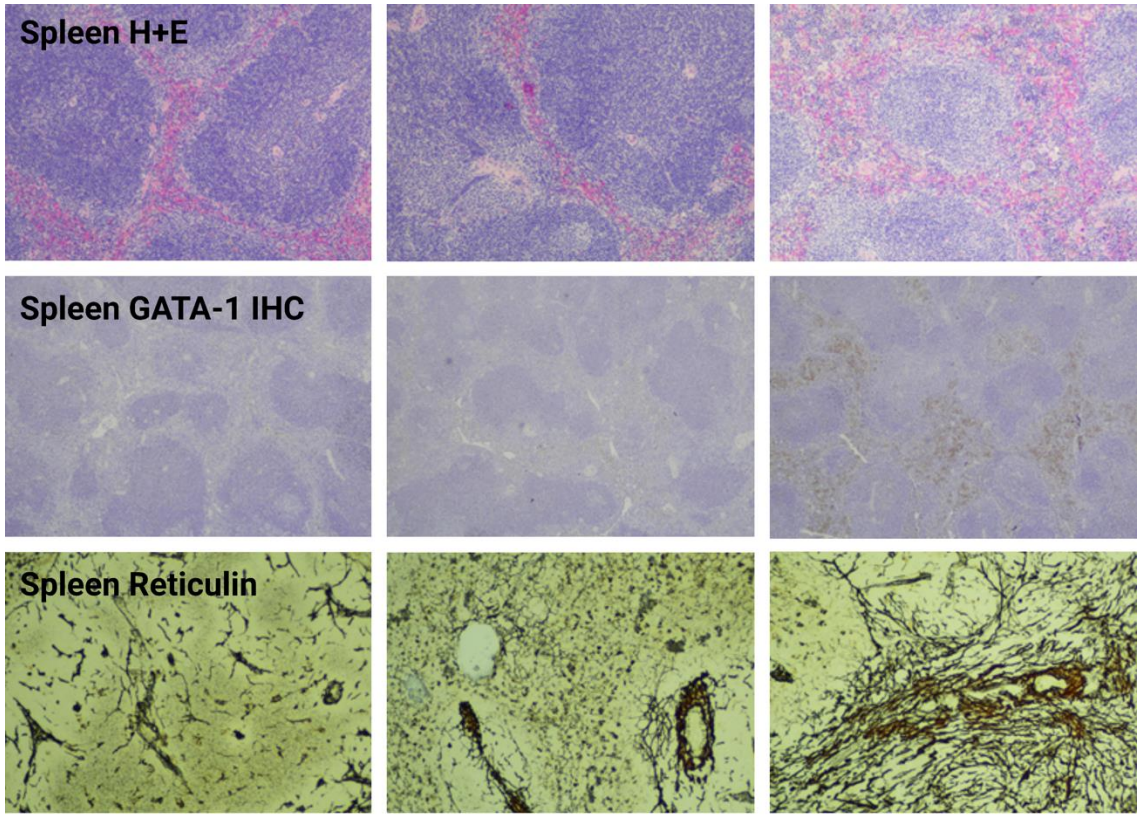


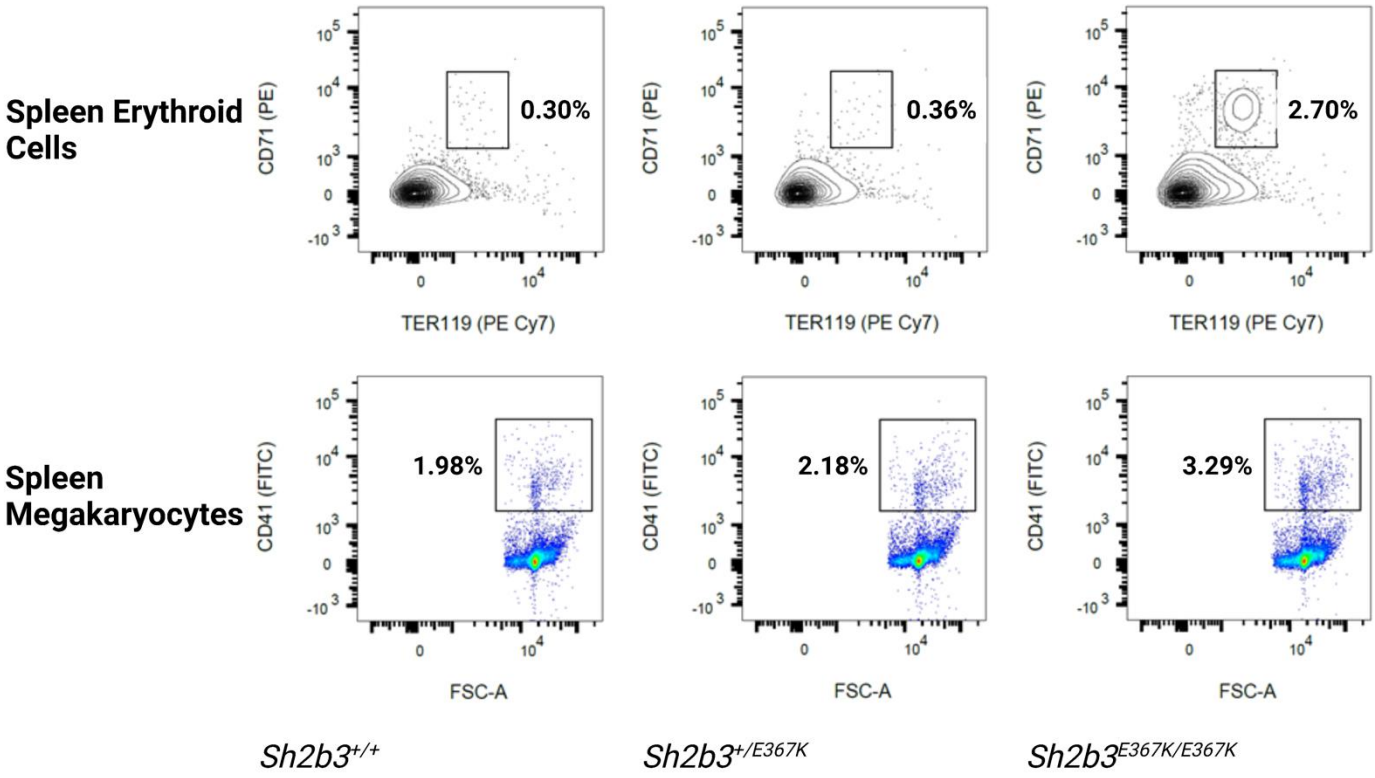




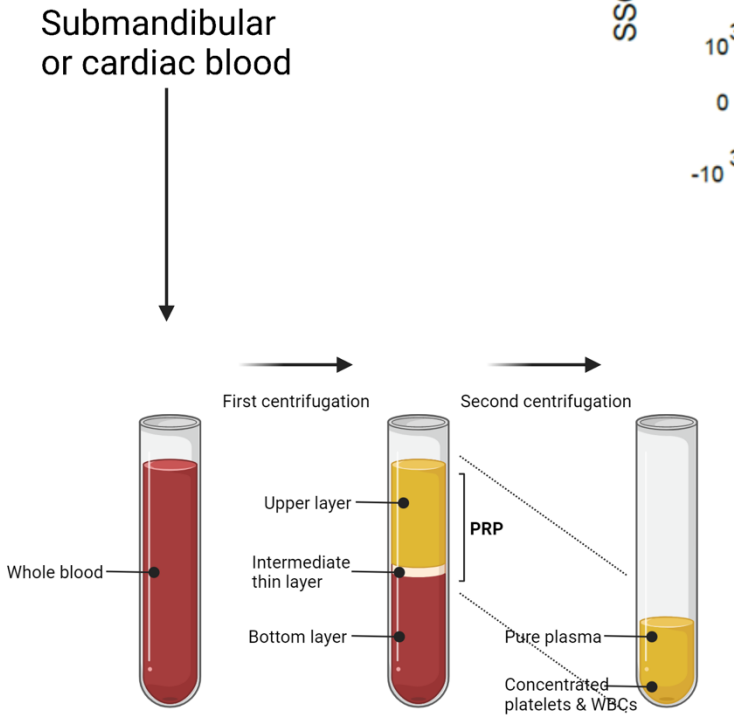
Statistical significance determined using the Mann Whitney test

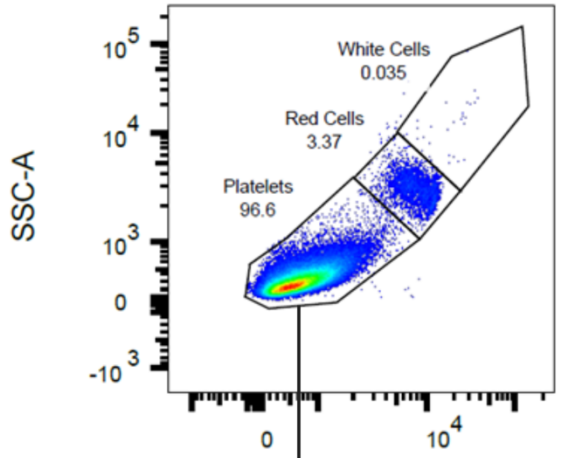
Sh2b3-E367K mutated mice exhibit reticulin fibrosis

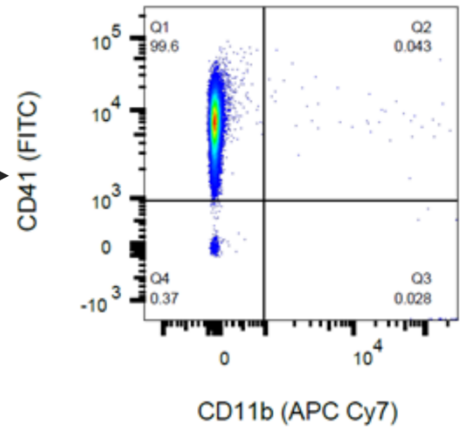




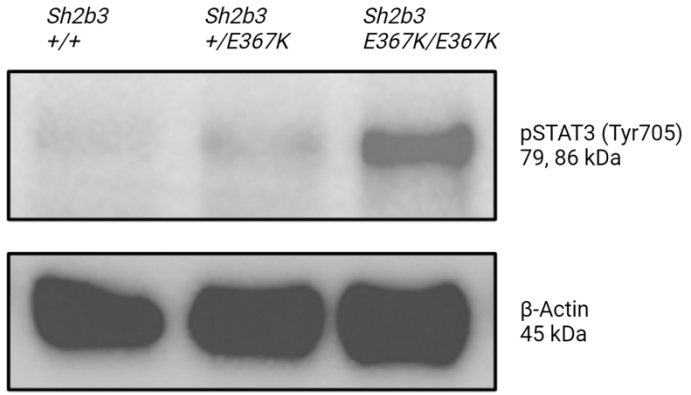
Sh2b3-E367K homozygous mouse platelets have heightened JAK-STAT activity







Unstimulated Platelets

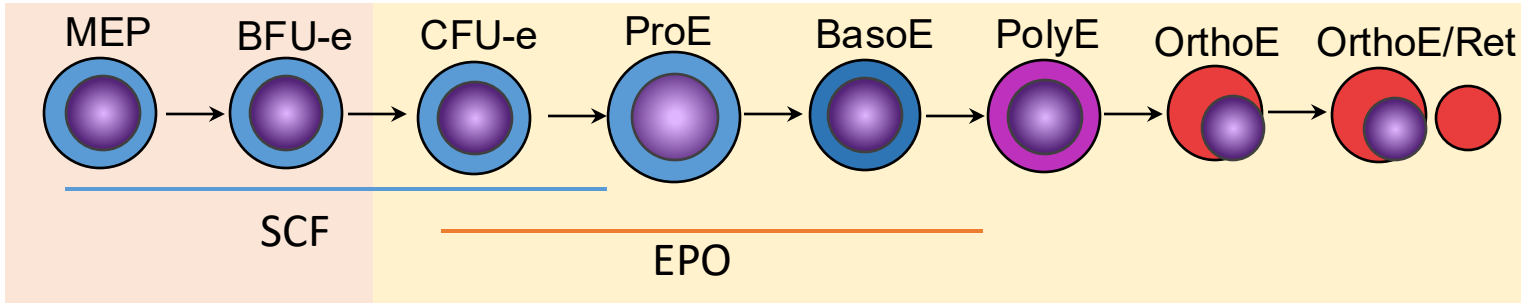


Some Research Questions worth addressing

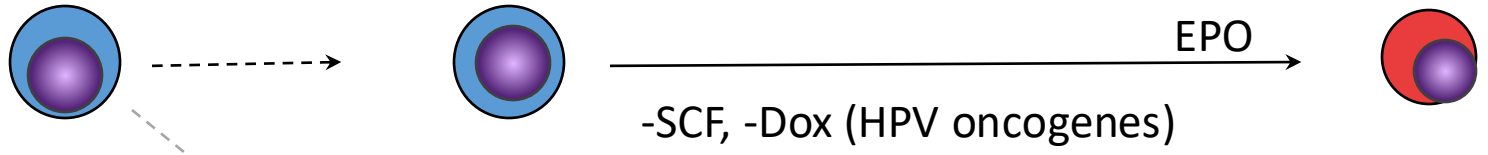
- What is the full repertoire of STAT targets in the nucleus?
- Are there new EPOR-directed or MPL-directed biological processes to be found?
- Are the STAT targets in humans the same as in mice?
- Are there any STAT-independent transcriptional programs? How can we find them?
- Does the JAK2-V617F driver mutation induce any different transcriptional outcomes to normal signalling?

Are we just re-inventing the wheel?

Discovery: New targets of EPOR signalling in Erythropoiesis



Human Cell Line Models

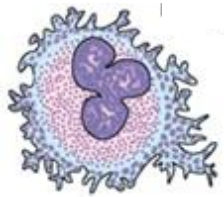


12 copies HEL

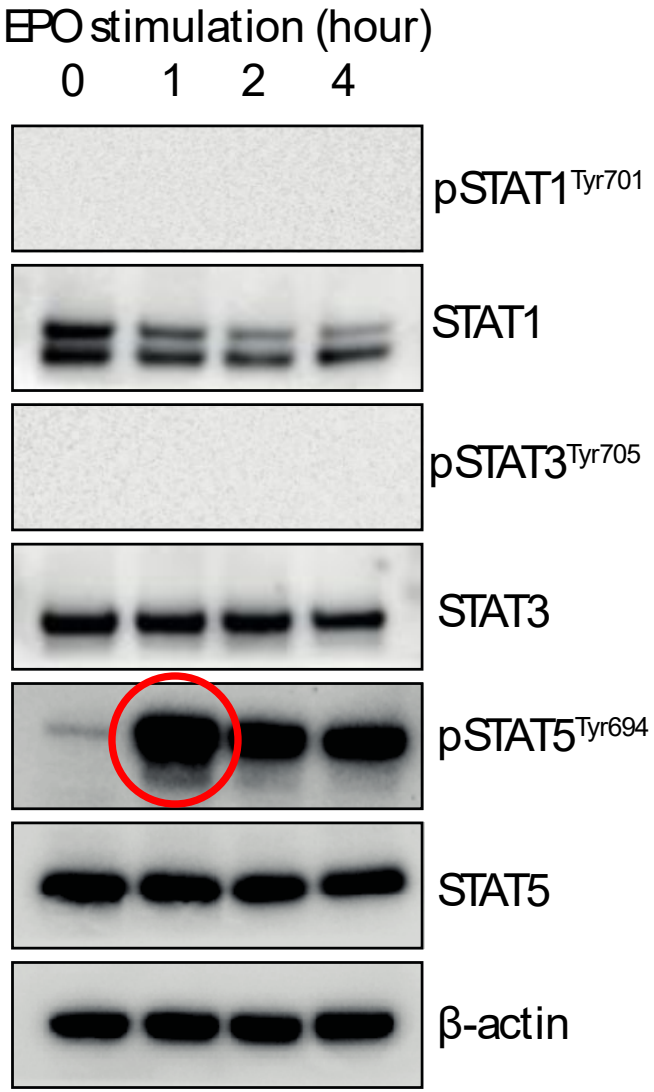
JAK2-V617F +

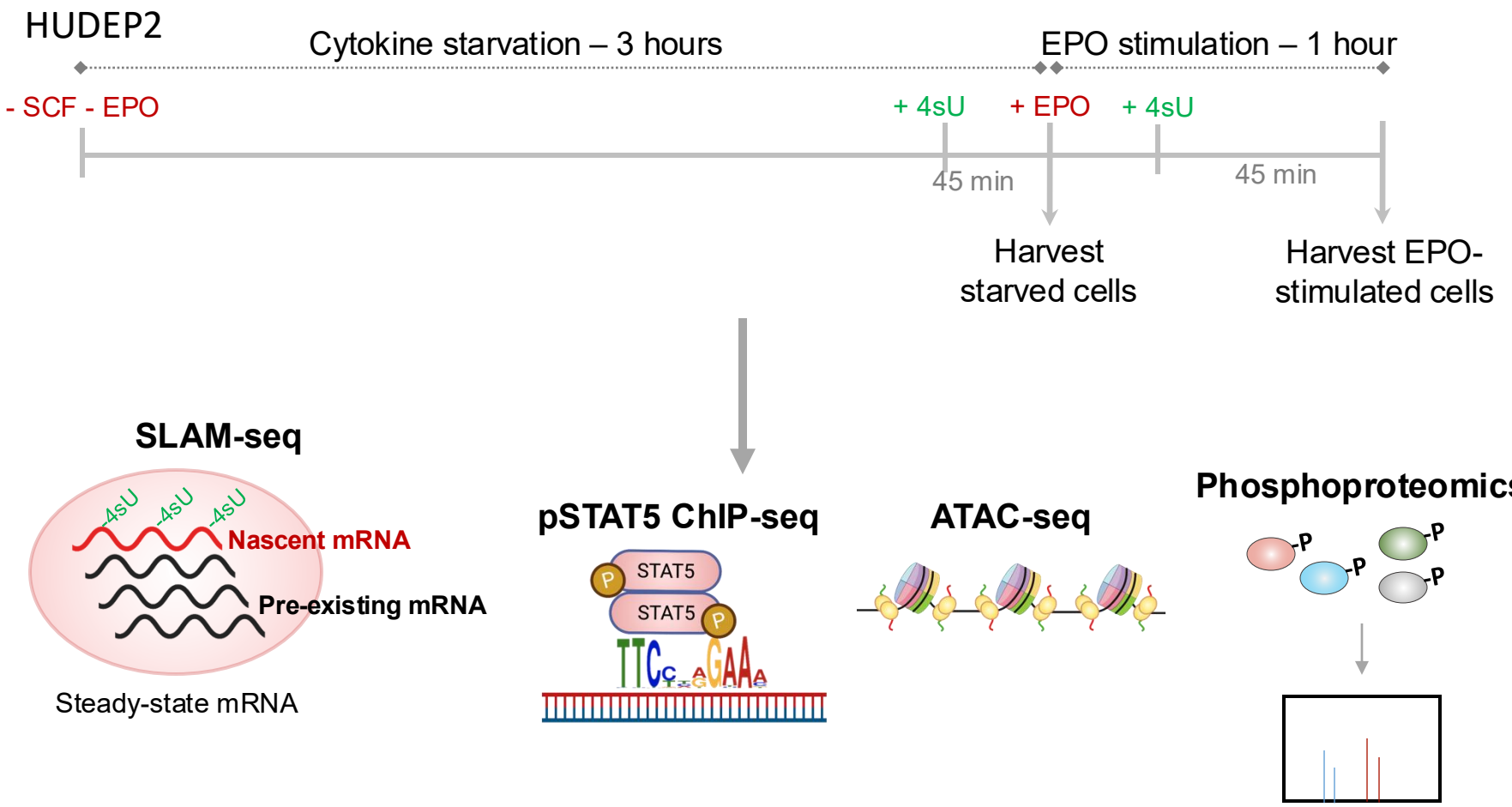
HUDEP2

5 copies SET2



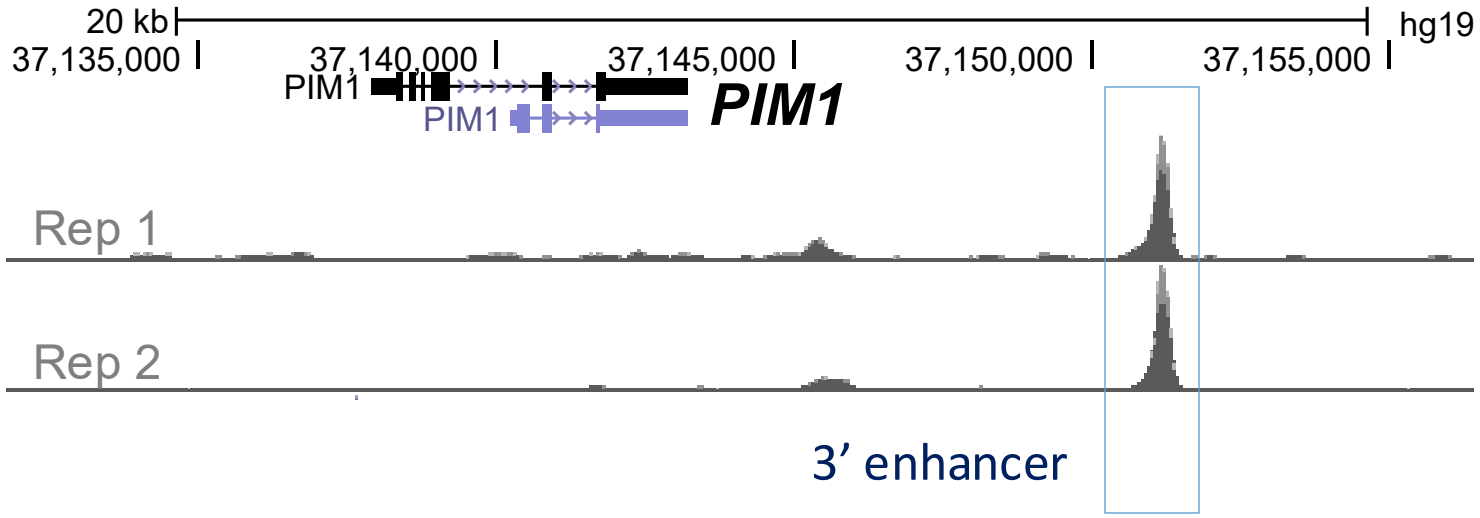
Experimental Design

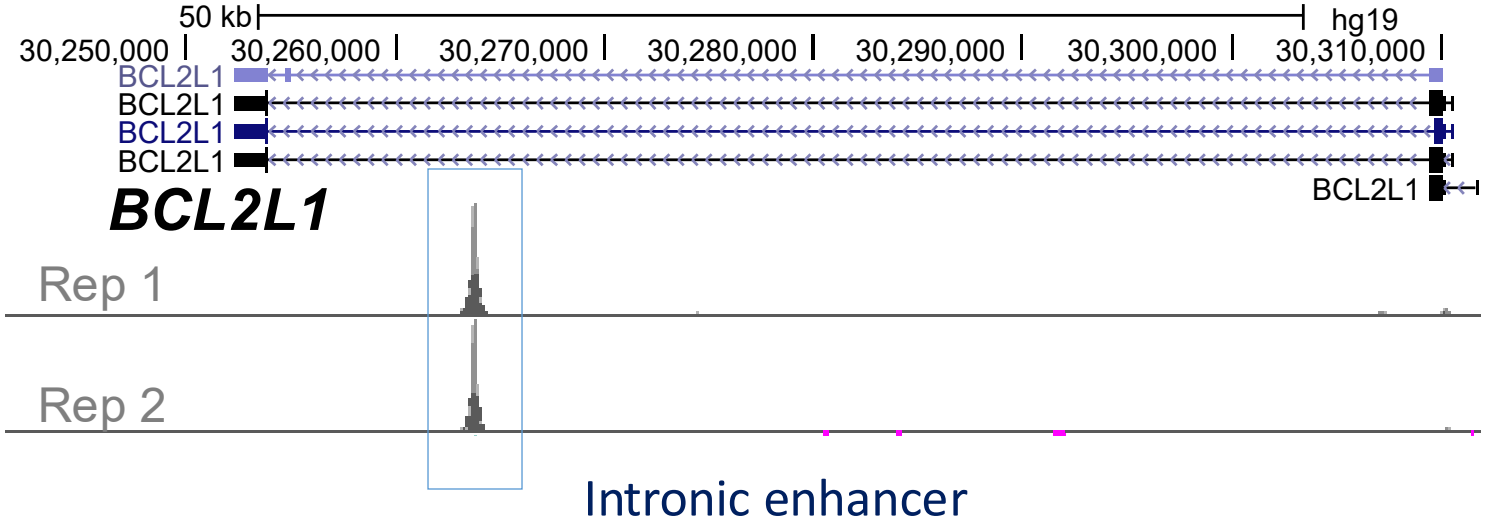


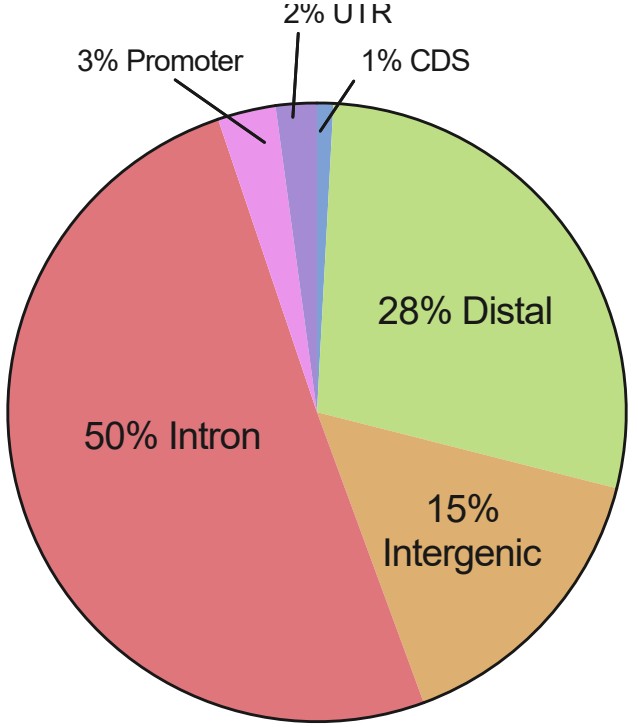


EPO – 5U/ml; 4sU – 400 uM

pSTAT5 binds enhancers regions at the canonical STAT-binding motif







Total =3128

MEME
Multiple Em for Motif Elicitation

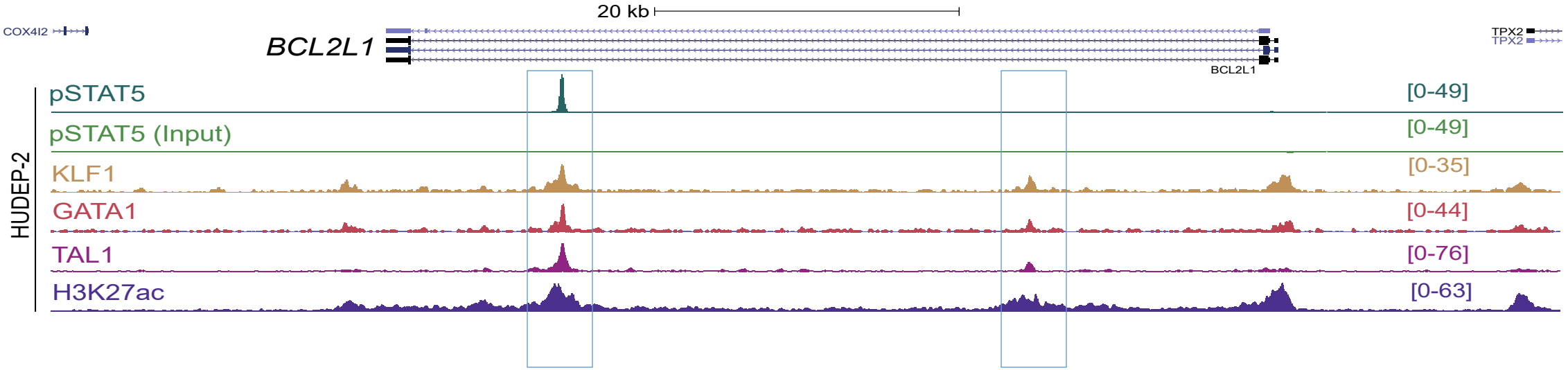
E-value = $1.2e^{-853}$

HOMER

P-value = $1e^{-1347}$

Motif present in >80% of peaks

Integration of signals from EPOR and master transcription factors at important enhancers for MPNs



En2

Stress-responsive enhancer

En1

Basal enhancer

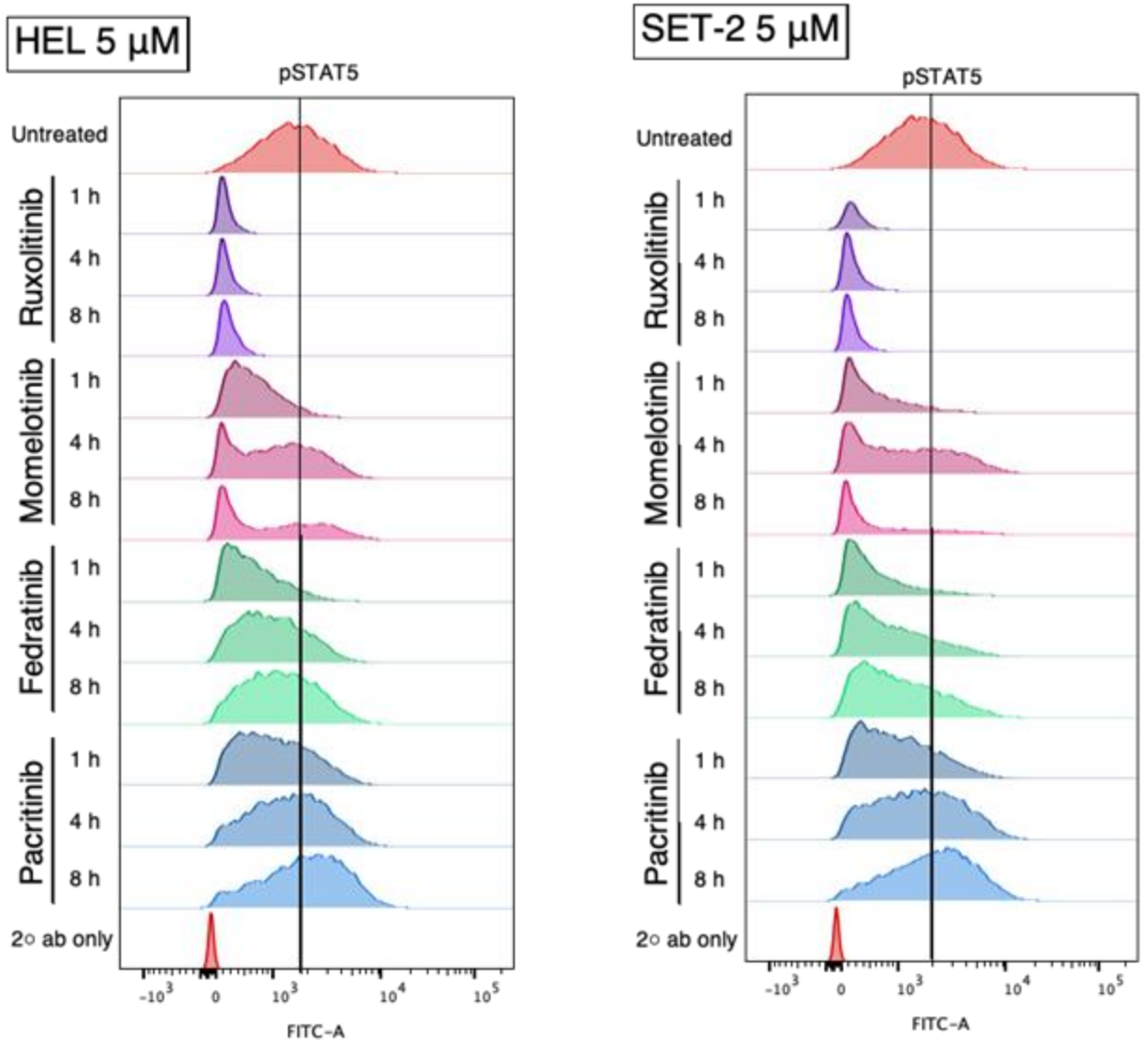
JAK inhibitors have a variety of targets

	Ruxolitinib[1]	Fedratinib[2]	Pacritinib[2,3]	Momelotinib[2]
JAK1	✓			✓
JAK2	✓	✓	✓	✓
ACVR1			✓	✓
FLT3			✓	
IRAK1			✓	
CSF1R			✓	

	IC50 (nM)			
Kinase	**Fedratinib**	**Ruxolitinib**	**Pacritinib**	**Momelotinib**
JAK1	105	3.3	1280	11
JAK2	3	2.8	23	18
JAK3	1002	428	520	155
TYK2	405	19	50	17

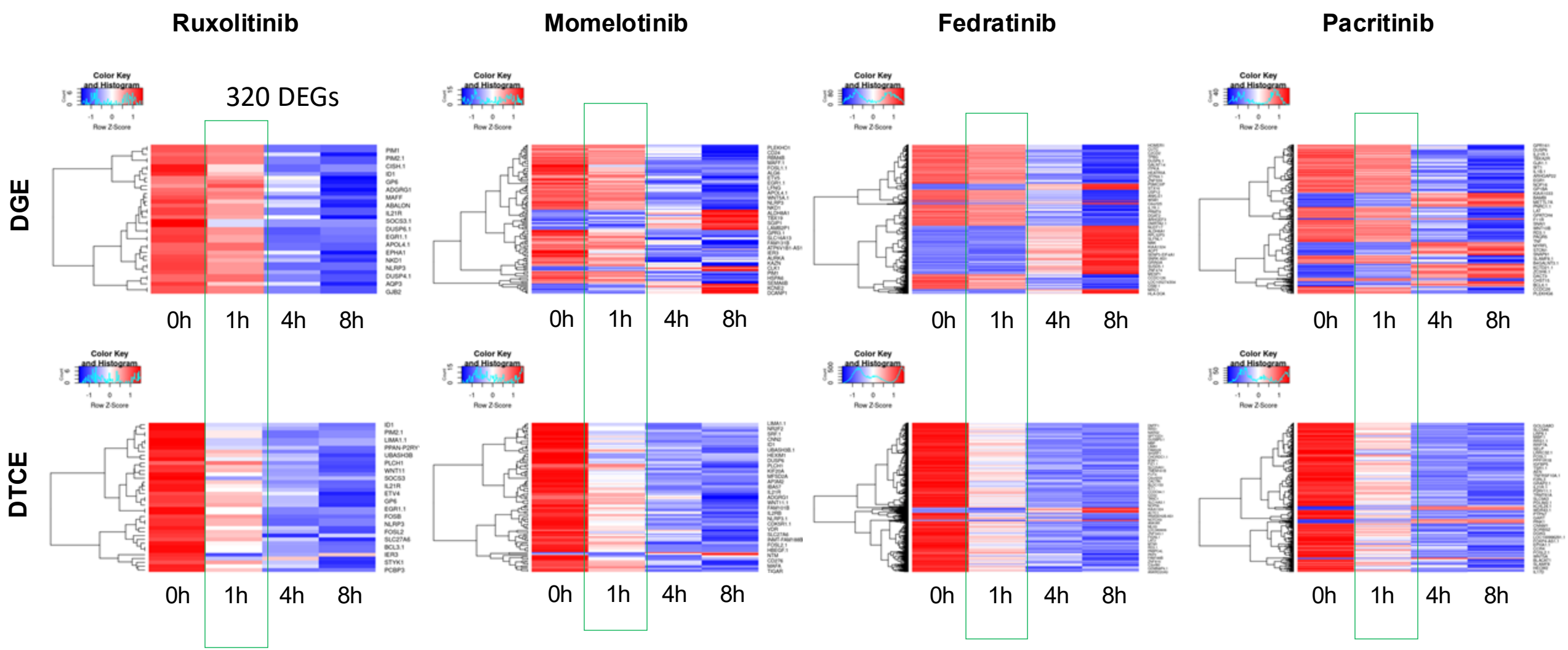
1. Harrison C, *et al. N Engl J Med* 2012; 366:787–798; 2. Kuykendall AT & Komrokji RS. *J Immunother Precis Oncol* 2021; **4**:129–141; 3.Oh S *et al.* ASH 2022. Abstract 628 (Oral).

Temporal dynamics of pSTAT5 inhibition by JAK inhibitors



Lam and Perkins, unpublished data

JAK2 inhibitors repress target gene expression in a time-dependent manner



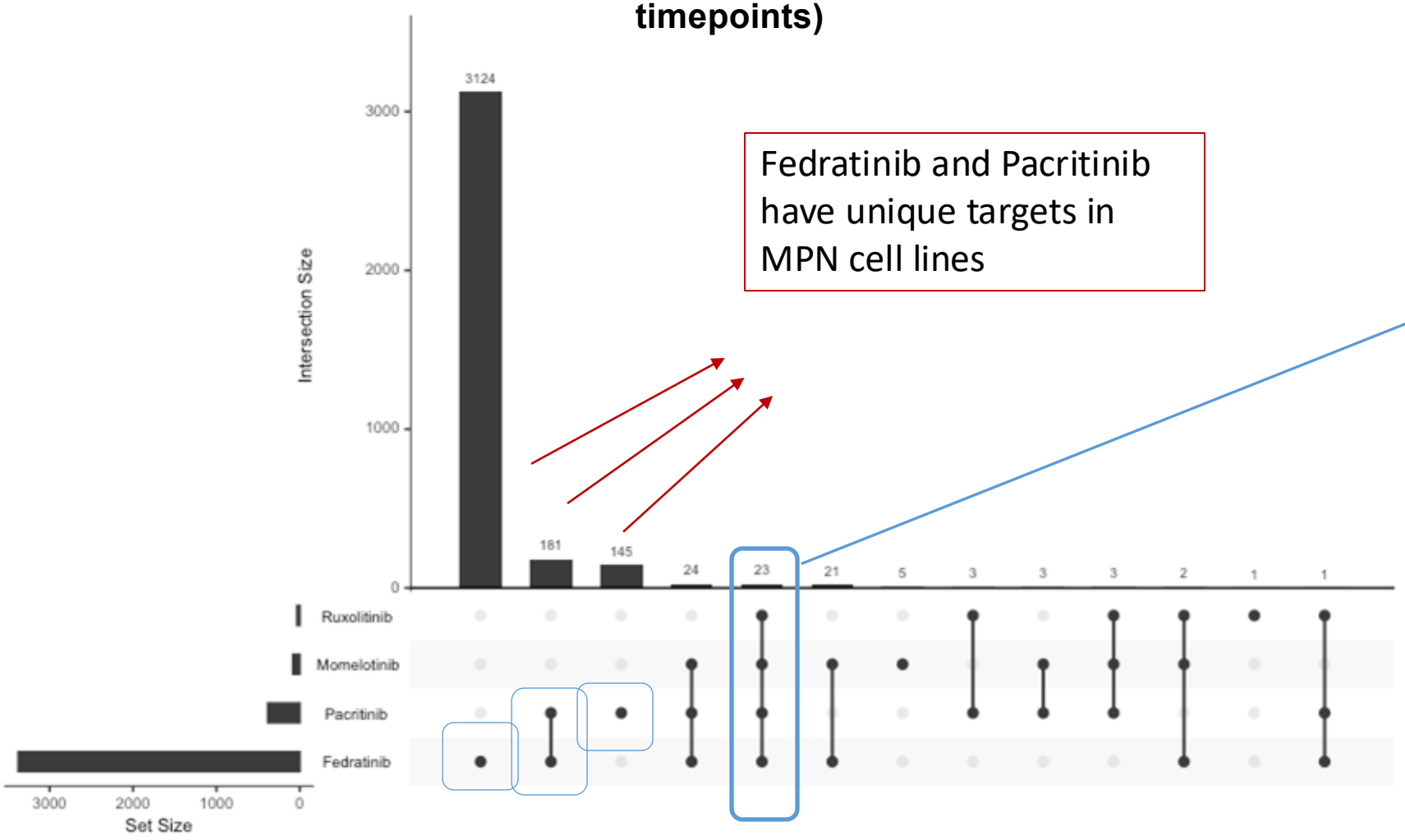
1610 DTGs – five fold increased sensitivity

SLAM-seq far more sensitive at early time points – 1 hour

Lam and Perkins, unpublished data

DRG analysis identified 23 common target genes of JAK2 inhibitors

Intersections of DTCEs of each JAK2 inhibitor in HEL cells (combined timepoints)



Fedratinib and Pacritinib have unique targets in MPN cell lines

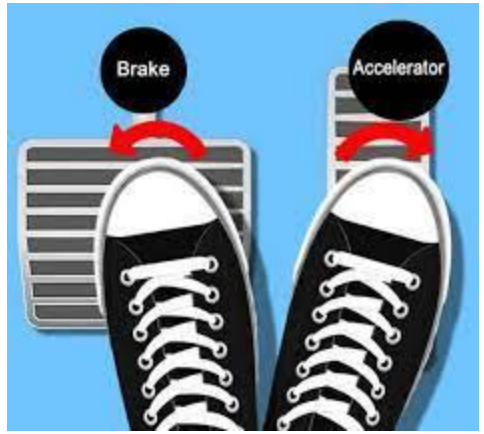
EGR1
PIM2
BCL3
APOL4
NLRP3
GP6
LIMA1
GPR3
FOSL2
FOSB
ETV5
WNT11
SLC27A6
FOSL1
ID1
SOCS3
PPP1R16B
VDR
IL21R
MAFF
UBASH3B
PLCH1
AP3M2
ETV4
PPAN-P2RY11
CNN2

Lam and Perkins, unpublished data

Pathways:

- Inflammation
- Proliferation
- Survival

- Negative regulation of JAK-STAT signalling



Conclusions

- pSTAT5 binds to ~3,000 putative enhancers in human erythroid cells (some likely signalling centres for GATA1 and KLF1)
- EPO signalling induces new DNA binding activities via non-STAT TFs (e.g. EGR1)
- There are clues to new biological processes induced by EPOR signalling
- There is a quantitatively increased transcriptional response in JAK2-V617F mutant cells but no new genes were identified

Can we improve survival in MF?

- Allogeneic BMT remains the only curative therapy in MF
- Ruxolitinib improves survival, but does not cure MF
- Potential disease modifying agents in trials (need well controlled up front trials with long end points (OS) or surrogate end points for OS (allele burden)
    - Interferon-α
    - BET inhibitors
    - Telomerase inhibitors
    - Combination therapies: PI3K inhibitors, PIM inhibitors, ERK inhibitors
    - Back to the drawing board….finding new targets

- Have a look at the draft recommendations for New Guidelines on Myelofibrosis

Myelofibrosis

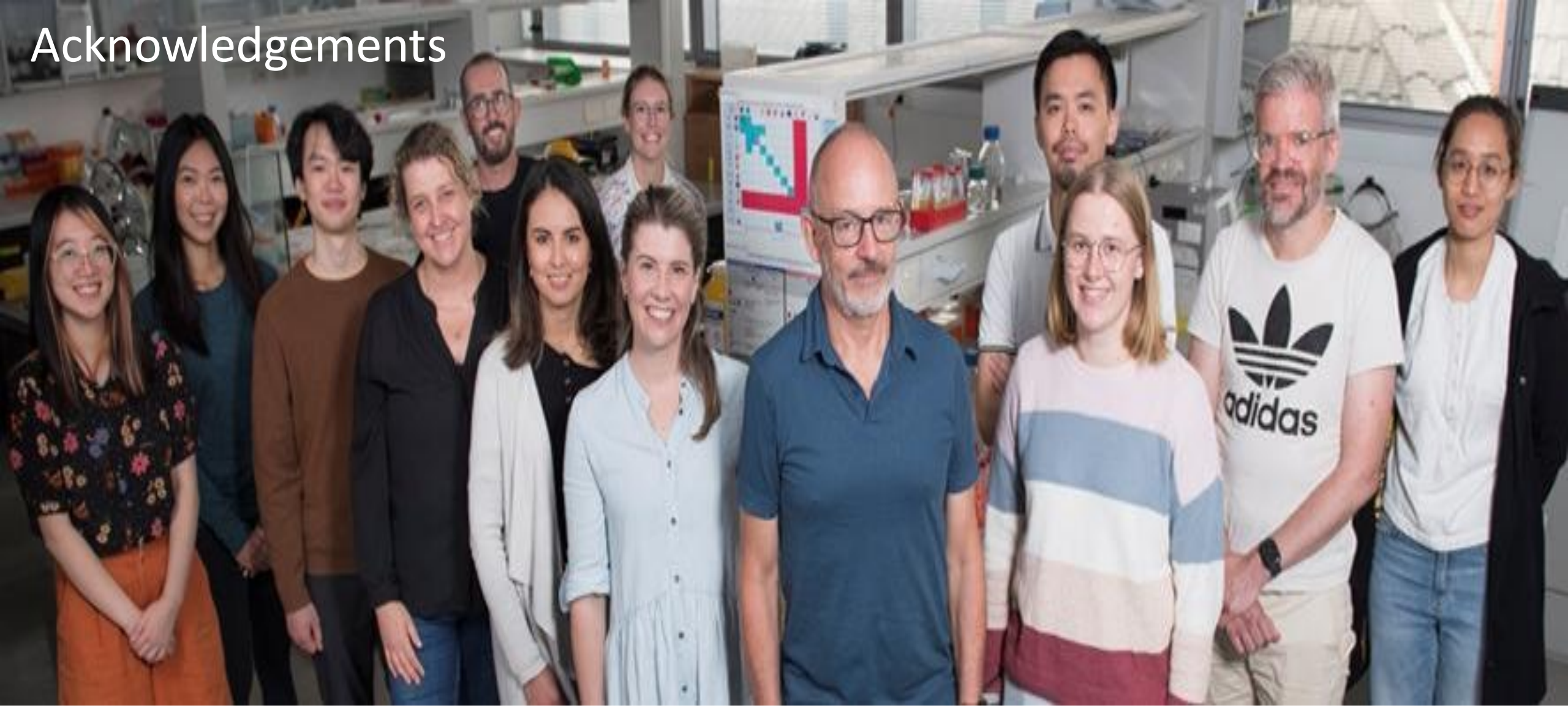
ASH Seeks Public Comment on Draft Recommendations for New Guidelines on Myelofibrosis

ASH is now accepting public comments on draft recommendations on myelofibrosis. Comments will be accepted until November 24, 2025. We encourage all interested healthcare professionals, stakeholder organizations, and individuals to share their feedback on these draft recommendations. All comments will be reviewed by the guideline panel before the guidelines are finalized.

MYELOFIBROSIS DRAFT RECOMMENDATIONS

Please submit all comments to guidelines@hematology.org.

Acknowledgements



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