

MPN – molecular biology

Jyoti Nangalia

Joyce Niblack MPN Patient Conference

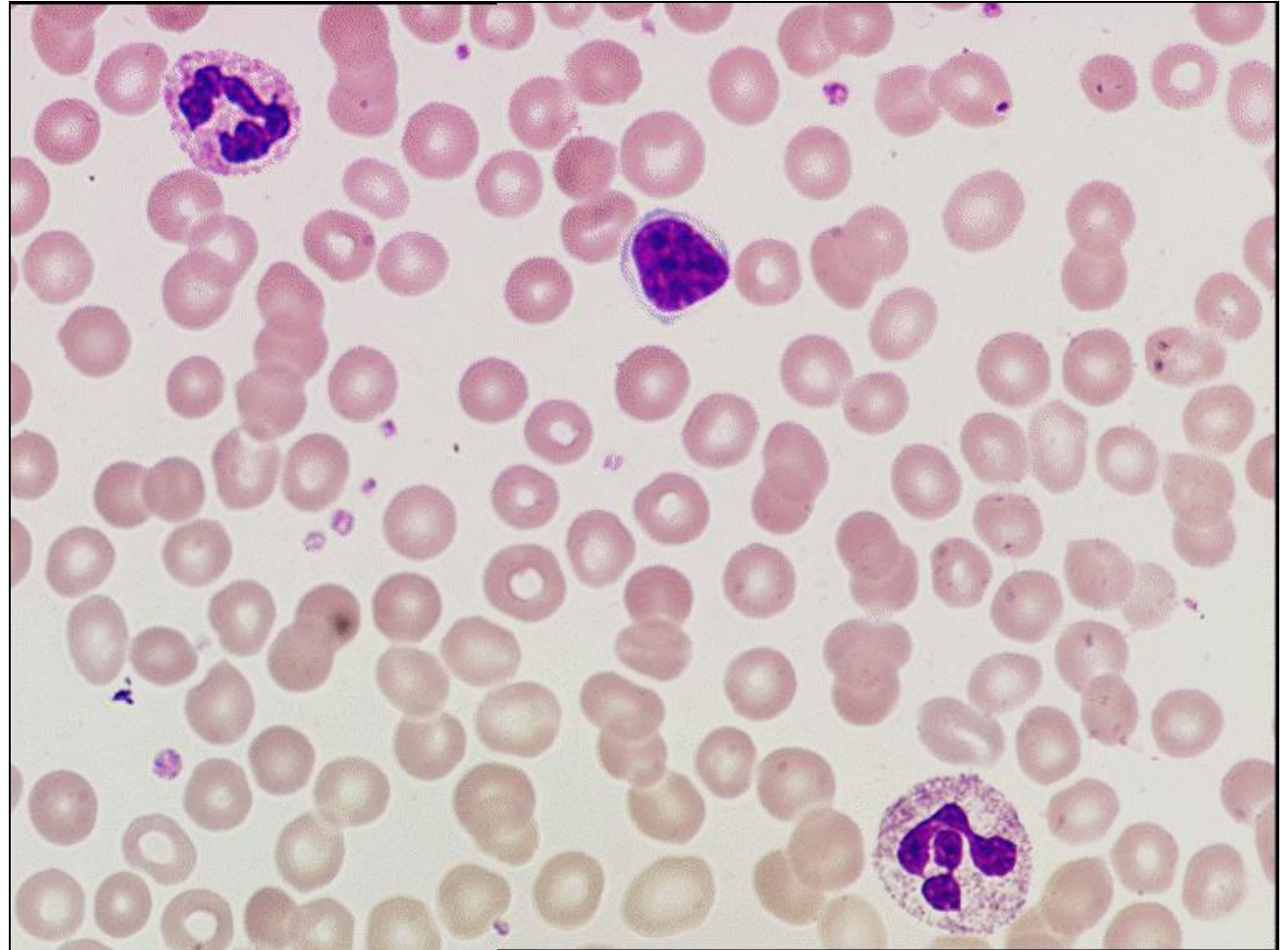


Wellcome - MRC Cambridge Stem Cell Institute

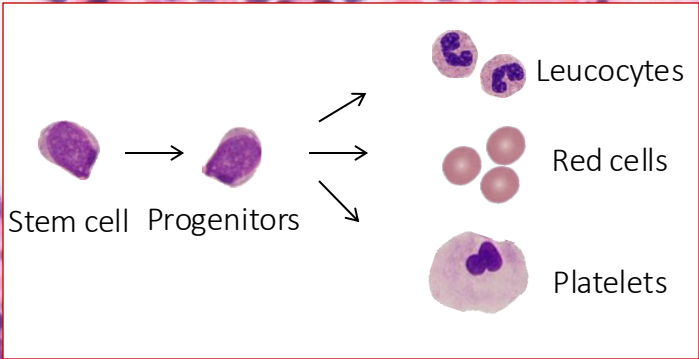
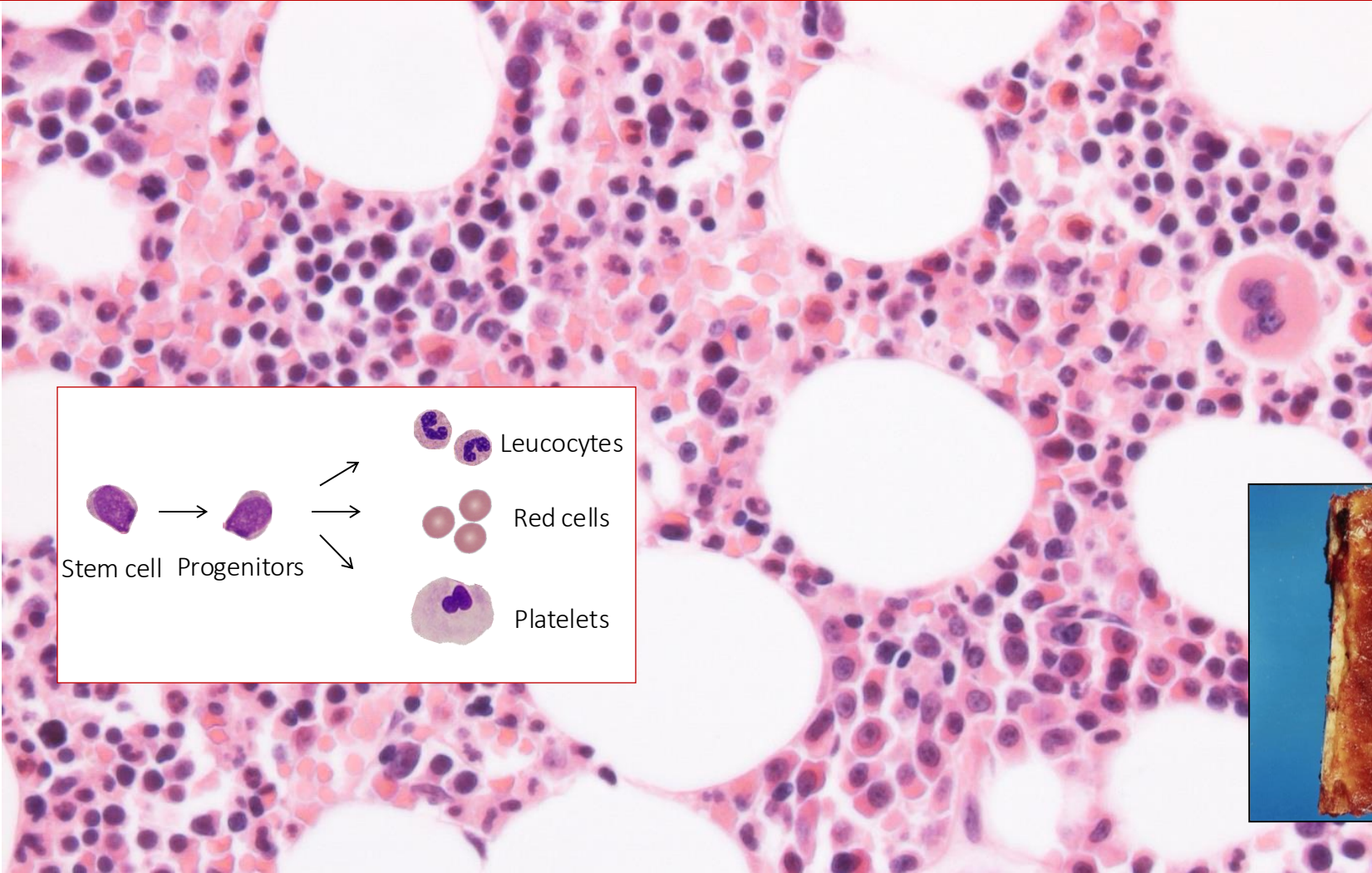


UNIVERSITY OF
CAMBRIDGE

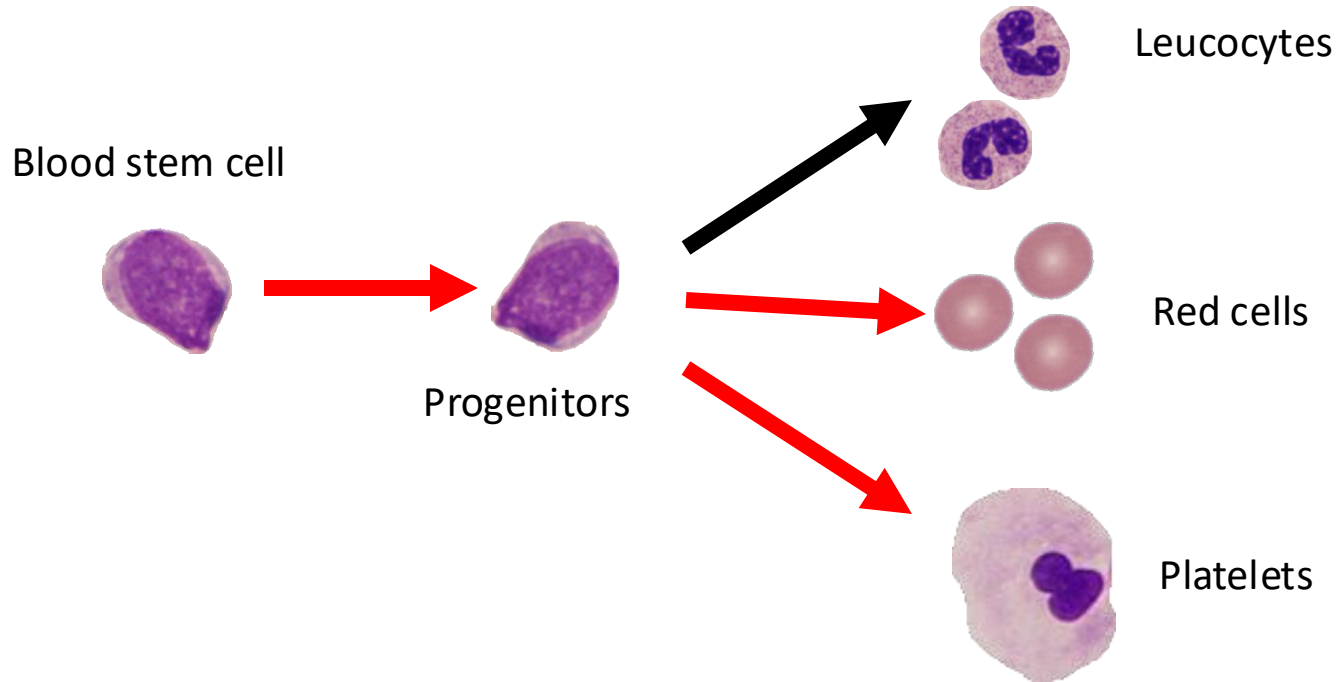
Blood



Bone marrow



Myeloproliferative neoplasms



Have been around for a long time....

SUR UNE FORME SPÉCIALE
DE CYANOSE S'ACCOMPAGNANT D'HYPERGLOBULIE EXCESSIVE ET
PERSISTANTE.

par M. H. VAQUEZ.

C R Soc Biol (Paris) 1892

Hämorrhagische Thrombocythämie bei vasculärer Schrumpfmilz.

Von

Privatdozent Dr. Emil Epstein und Privatdozent Dr. Alfred Goedel.

Mit 4 Abbildungen im Text.

(Eingegangen am 14. November 1933.)

Virchow's Archiv Abteilg; 293; 233-247. 1934

Have been around for an even longer time ...

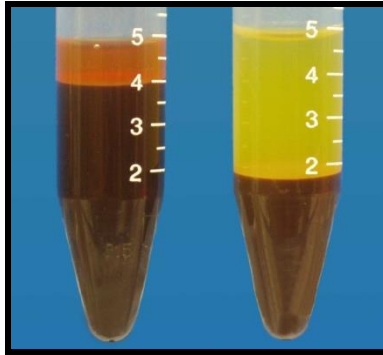


Identification of JAK2 mutations in canine primary polycythemia

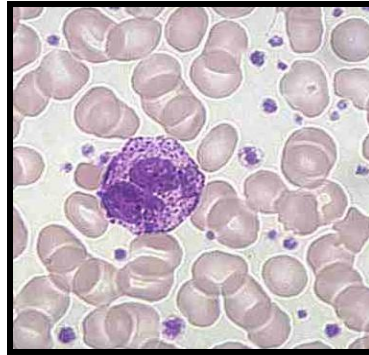
Stephanie Beurlet^{a,b,c}, Patricia Krief^{a,b}, Arnaud Sansonetti^{a,b}, Alexandra Briend-Marchal^d,
Jean-Jacques Kiladjian^e, Rose Ann Padua^{a,b}, Christine Chomienne^{a,b,f}, and Bruno Cassinat^{a,b,f}

Myeloproliferative neoplasms

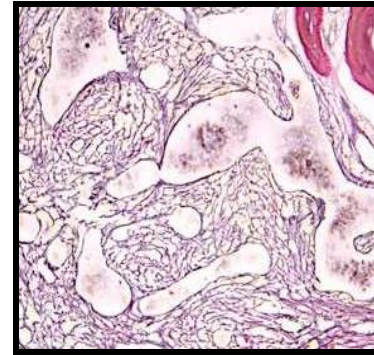
PV



ET



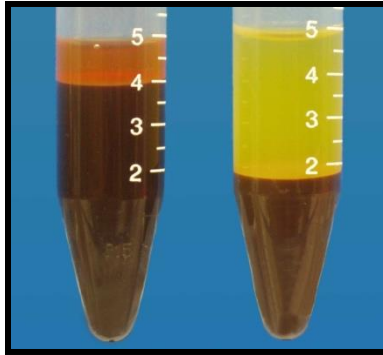
MF



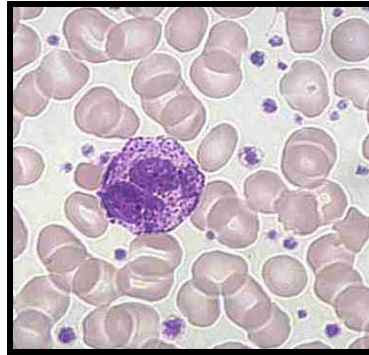
- *What causes it?*
- *Was it just bad luck?*
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Myeloproliferative neoplasms

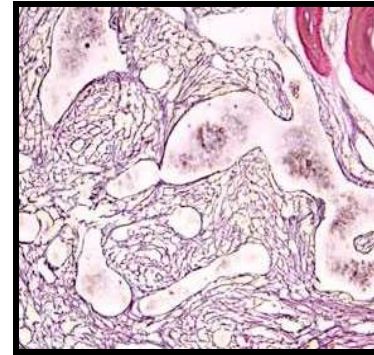
PV



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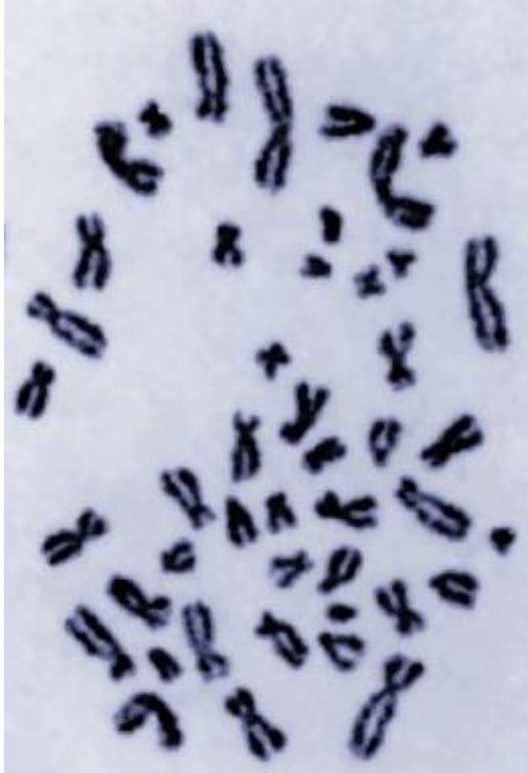


MF

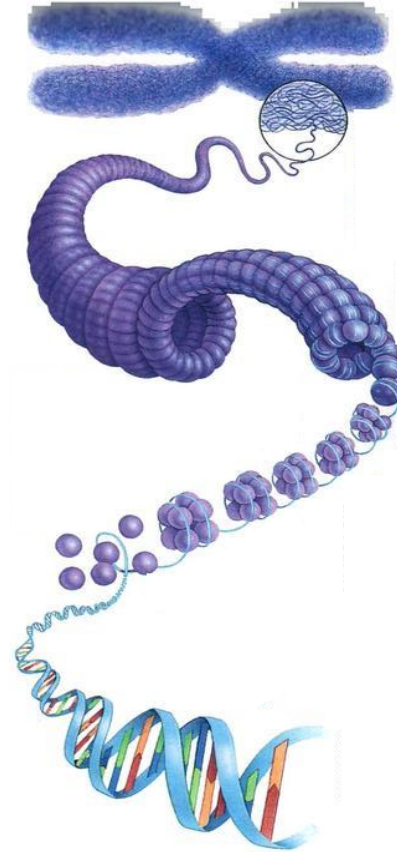


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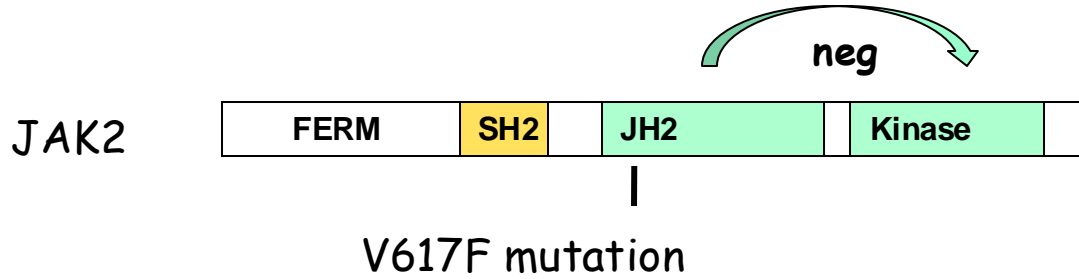
The code of life – counting chromosomes



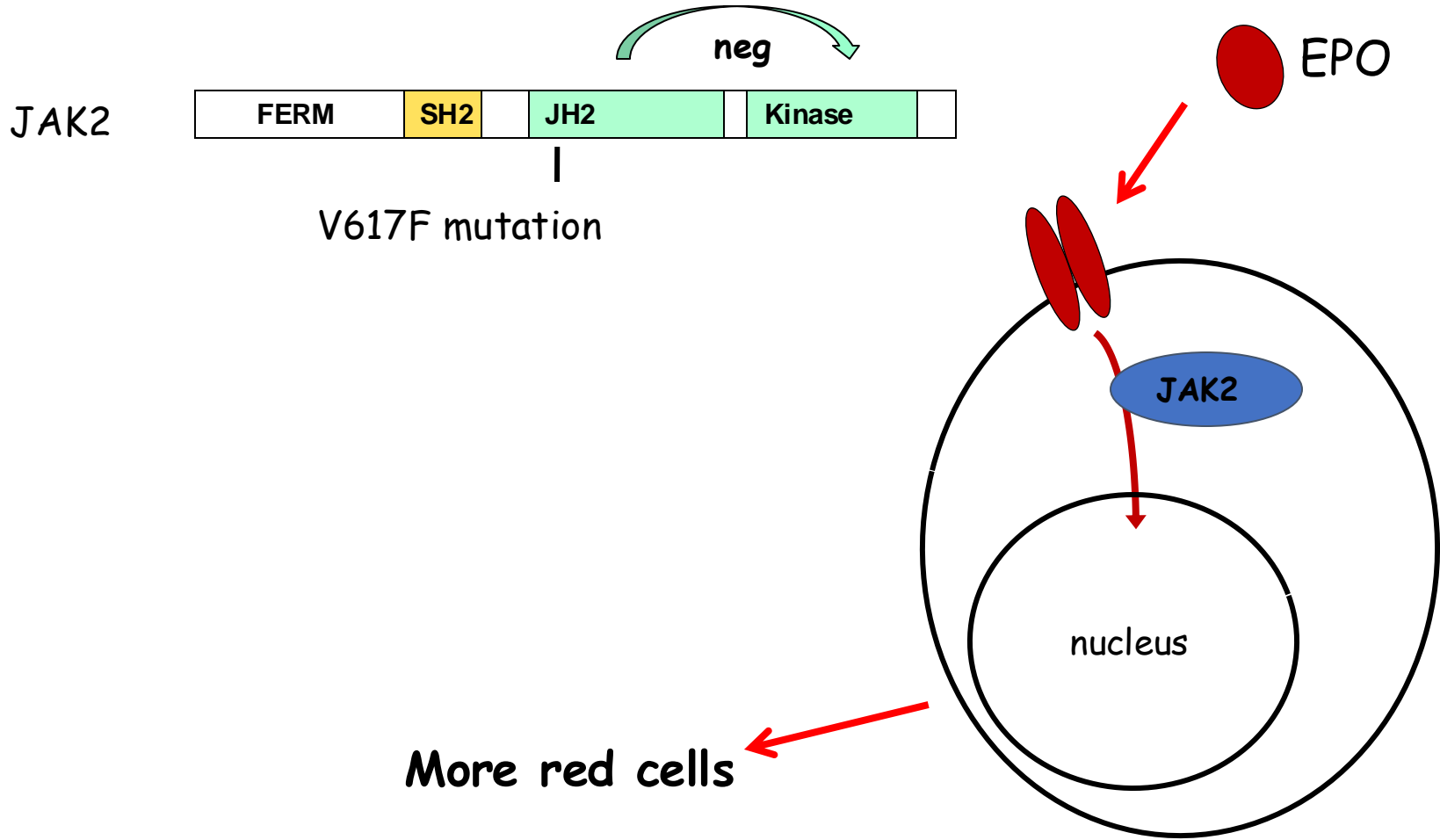
Tjio and Levan 1955



A change in the 'gene' *JAK2*

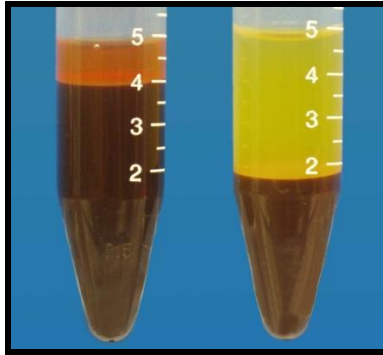


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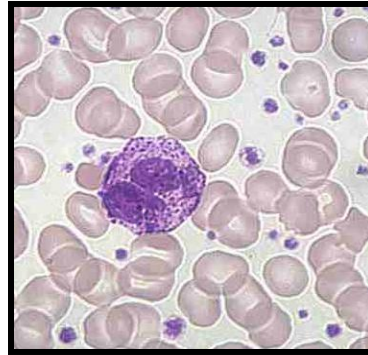
JAK2 mutations in MPN

PV



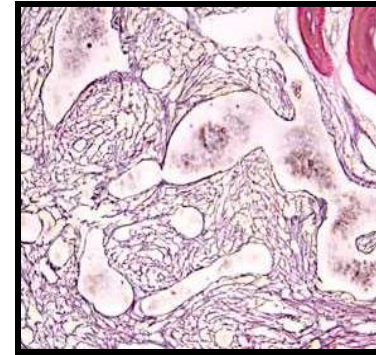
JAK2: 98%

ET

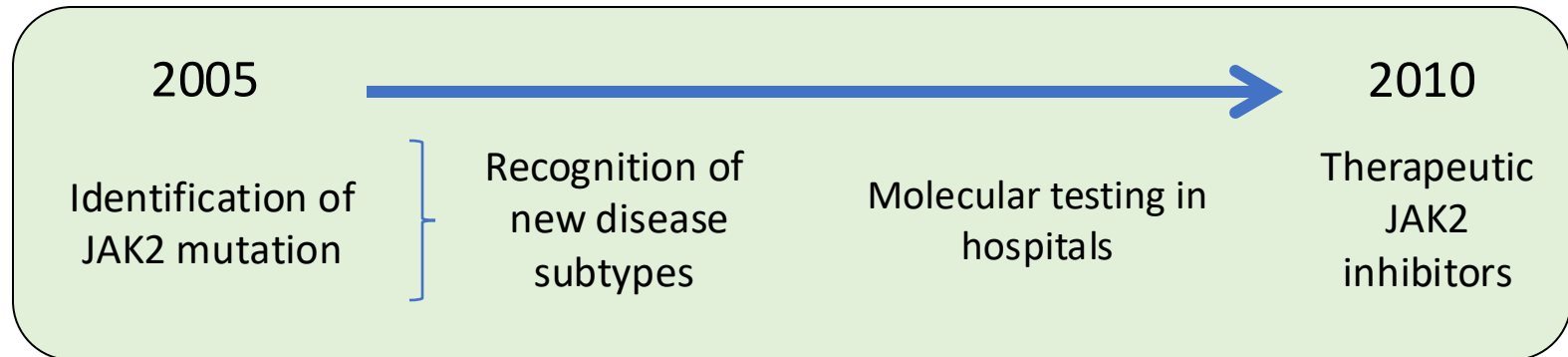


50-60%

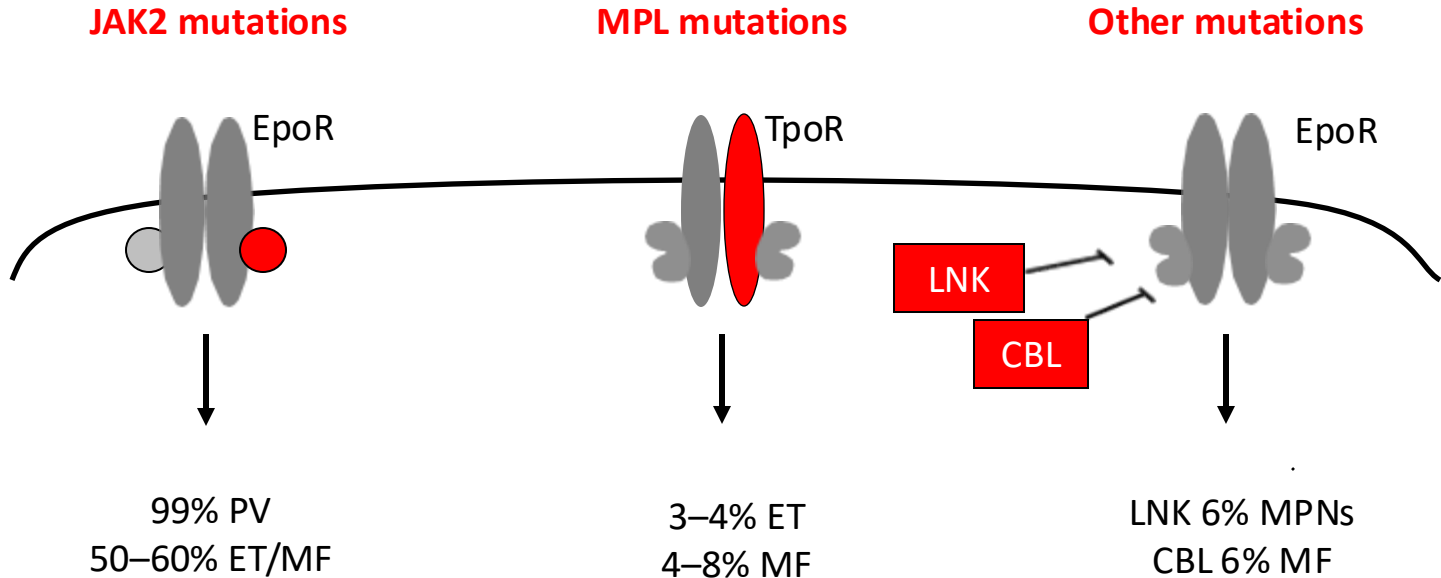
MF



50-60%



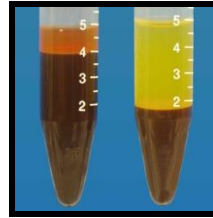
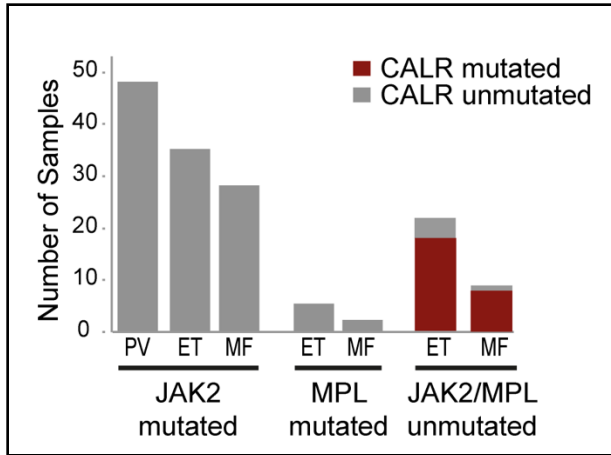
The theme repeated... increased growth signal to cells



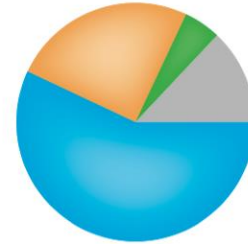
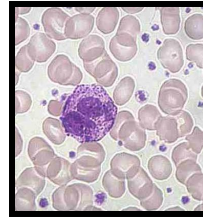
? Pathogenic mechanism of 50% of ET and MF

CALR mutations in majority of *JAK2*-unmutated MPNs

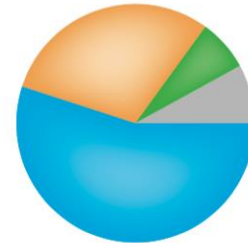
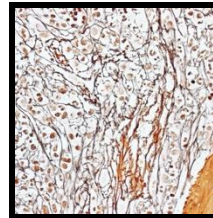
MPNs



PV



ET



MF

■ CALR

■ JAK2 exon 12

■ JAK2^{V617F}

■ JAK2/MPL/CALR unmutated

■ MPL

Re: What we've been looking for?

On 13 Jul 2013, at 03:03, "Jyoti Nangalia"
<jn218@cam.ac.uk<<mailto:jn218@cam.ac.uk>>> wrote:

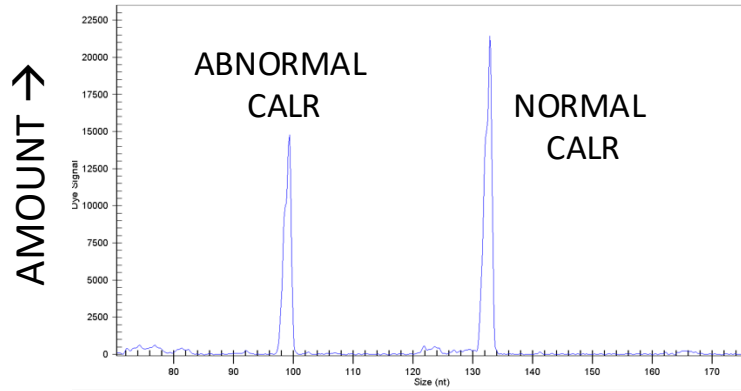
Dear Peter,

Thanks for the meeting this morning to chat exomes/follow up. Later in the day, I stumbled across something unusual. Having looked at it in further detail, I think it is really exciting and I now can't sleep. It may well be a recurrently mutated gene found in the majority of JAK-ve ETs+MFs (or a very cruel artefact).

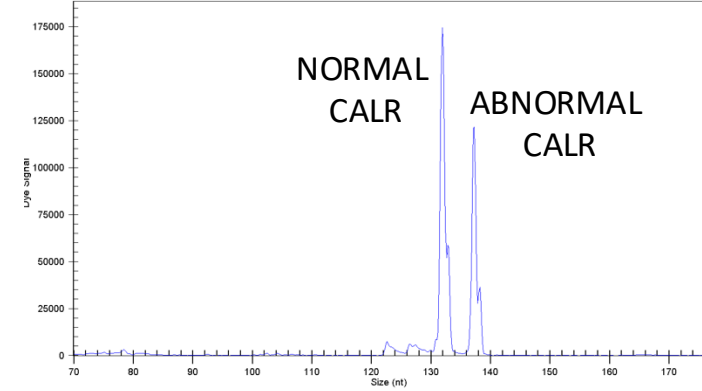
The gene is CALR (aka Calreticulin/CRT in the literature). Initially, there were two frameshift deletions found (two different patients), both towards the C-terminus of the protein and both quite close to each other so I investigated further.

I have now gone back to the original prefiltered outputs for the 170-ish exomes and have found a further 20 patients. Uncanningly, every case is ET and MF, and according to my clinical information, all are JAK/MPL mutation negative. That is over two-thirds of the JAK/MPL-ve cases we submitted. I have now gone to the 1000 follow ups and a quick scan of the PINDEL'all' file has revealed a further 50 cases - 47 ET or MF, 2 SM, 1 CMML. No PVs at all. All cases are reportedly JAK2/MPL mutation negative.

A new test for the clinic and patients....

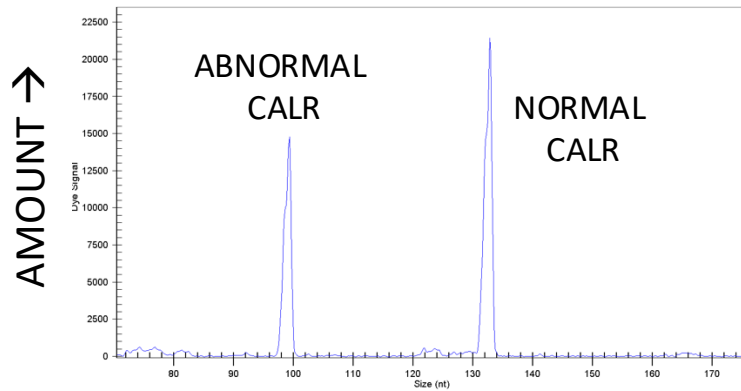


DNA SIZE →

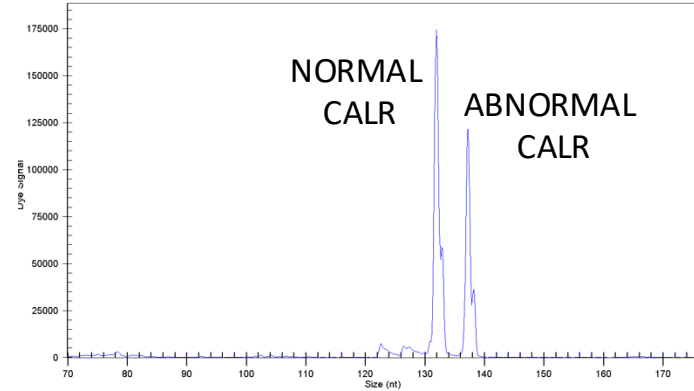


DNA SIZE →

A new test for the clinic and patients....



DNA SIZE →



DNA SIZE →

Several CALR antibody “first-in-human” trials ongoing...

11 years since *CALR*

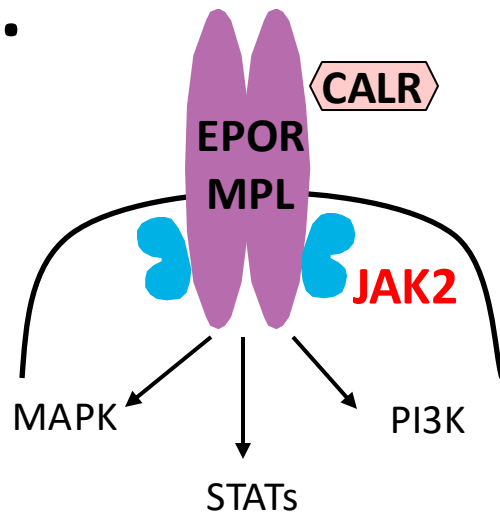


11 years since CALR

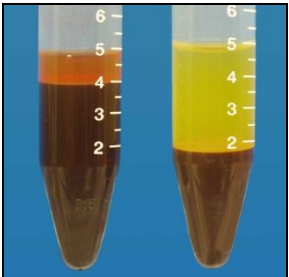


Changes in the JAK2, CALR and MPL genes *drive*

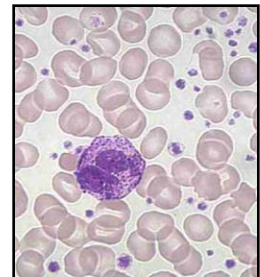
MPNs....



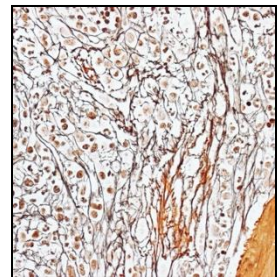
Polycythaemia Vera (PV)



Essential thrombocythaemia (ET)

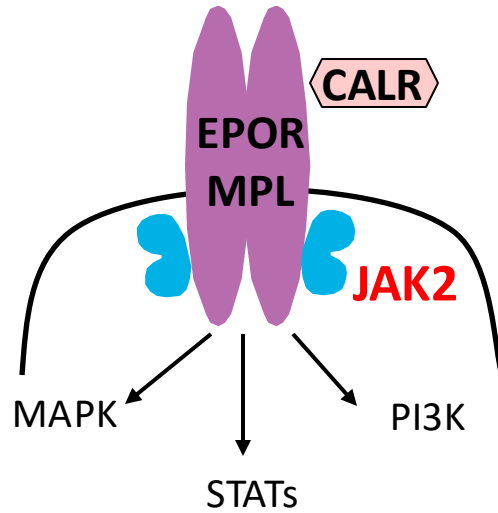


Myelofibrosis (MF)



- >90% patients have mutations in *JAK/CALR/MPL*

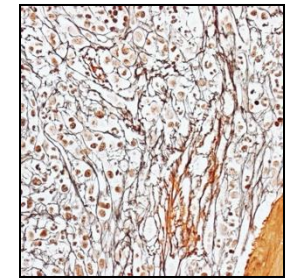
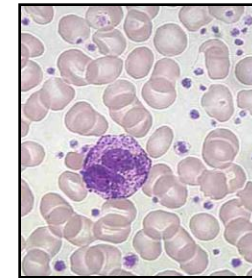
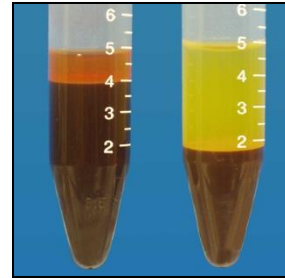
Additional mutations in other genes also found....



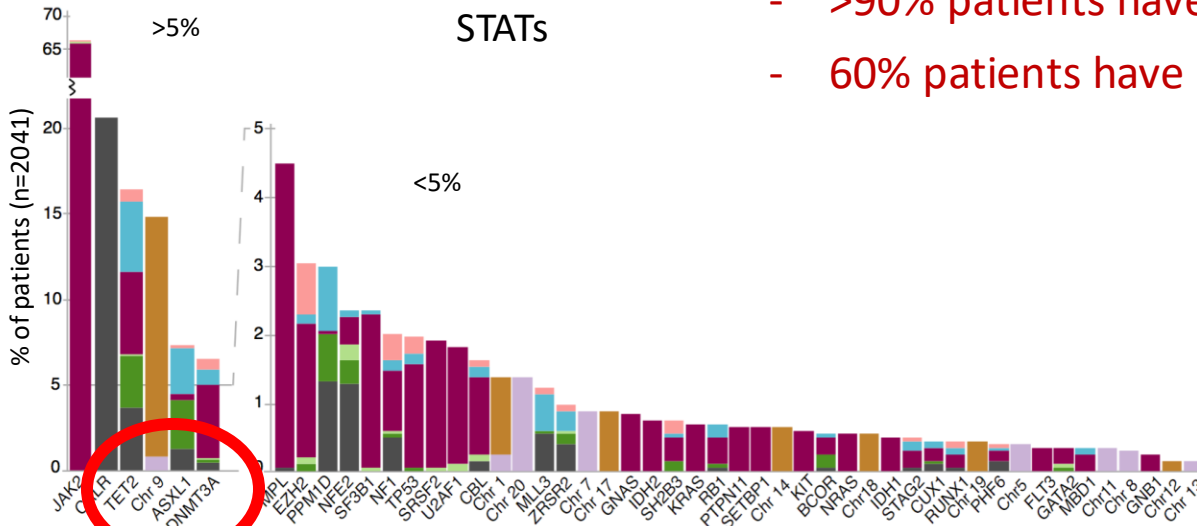
Polycythaemia
Vera (PV)

Essential
thrombocythaemia
(ET)

Myelofibrosis
(MF)

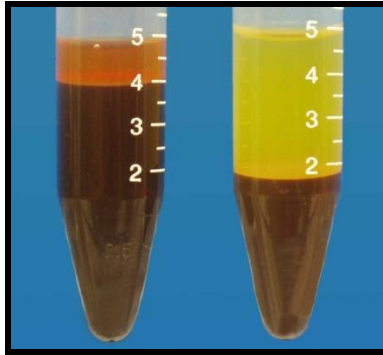


- >90% patients have mutations in *JAK/CALR/MPL*
- 60% patients have mutations in additional genes

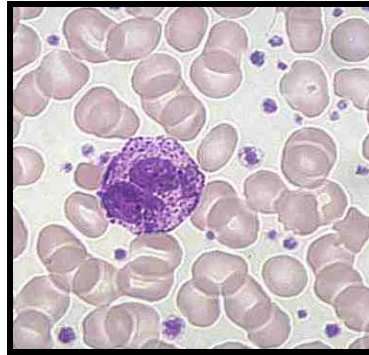


Myeloproliferative neoplasms

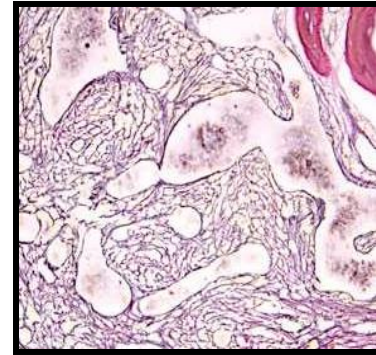
PV



ET



MF

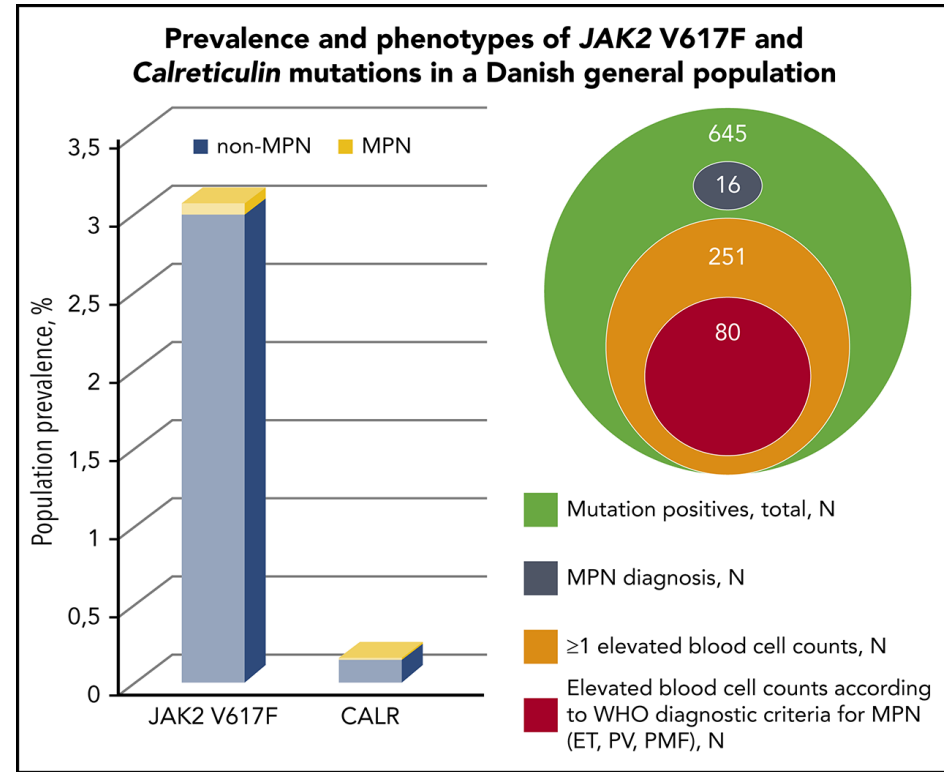


- *What caused it?*
- *Was it just bad luck?*
- *What causes the differences in disease between individuals?*
- *How long have I had it for?*
- *How fast did it grow?*

Germline genetics are important

Only some individuals with $JAK2^{V617F}$ get MPN

1 in 33 individuals



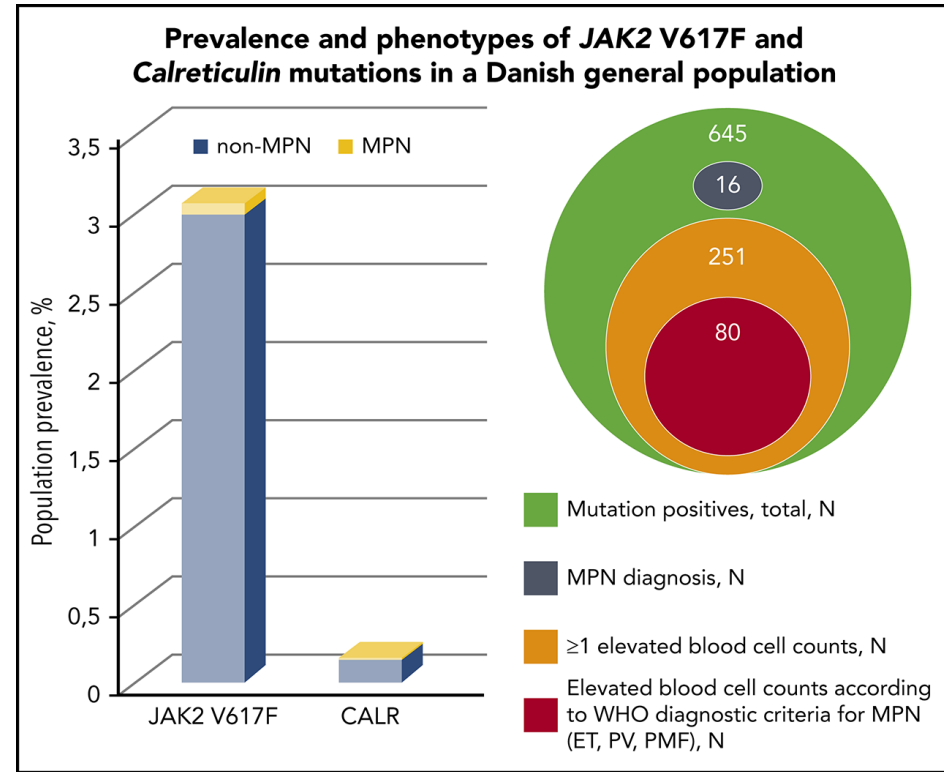
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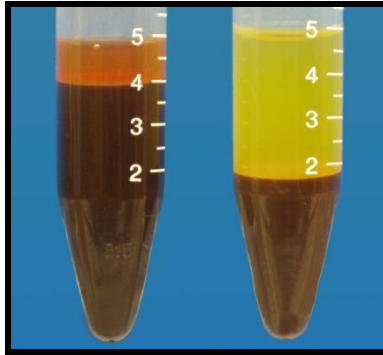
Inherited germline risk

- 46/1 haplotype
- MPN GWAS
- DNA sites influencing normal blood counts important

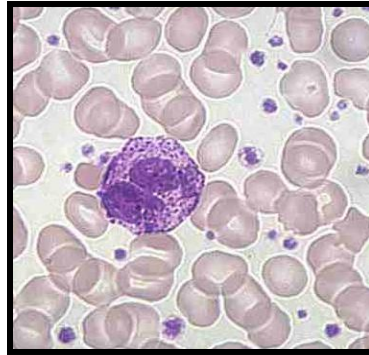


Myeloproliferative neoplasms

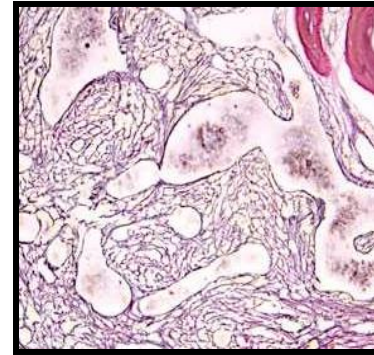
PV



ET

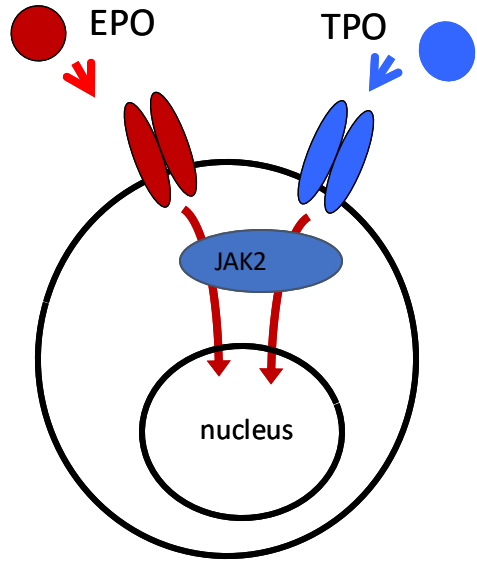


MF



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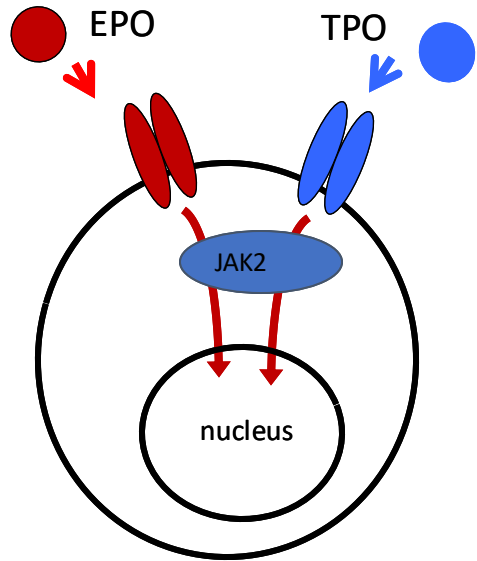
Why do some patients get ET and others PV with *JAK2*?



1. Higher levels of *JAK2* → PV

Dupont et al, Blood 2007; Vannucchi et al, Leukemia 2007; Passamonti et al, Leukemia 2010; Kittur et al, Cancer 2007

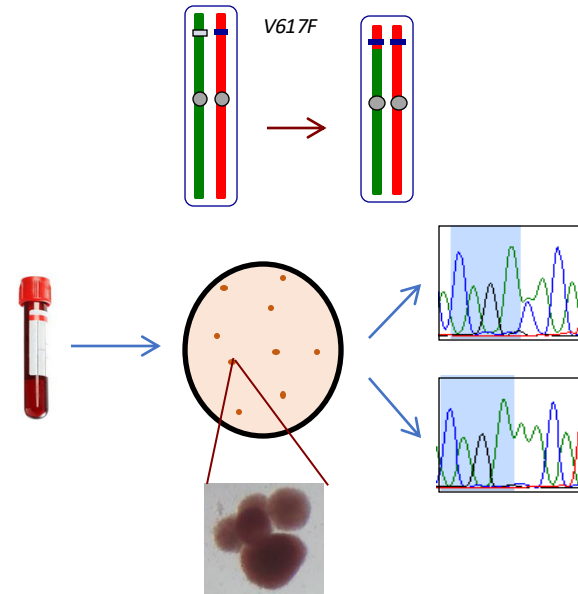
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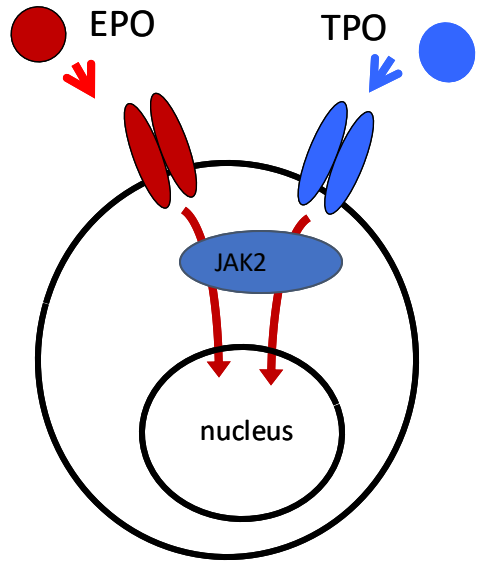
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2. Double copies of *JAK2* → PV



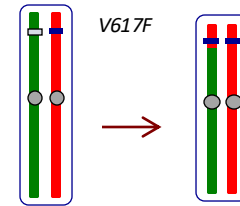
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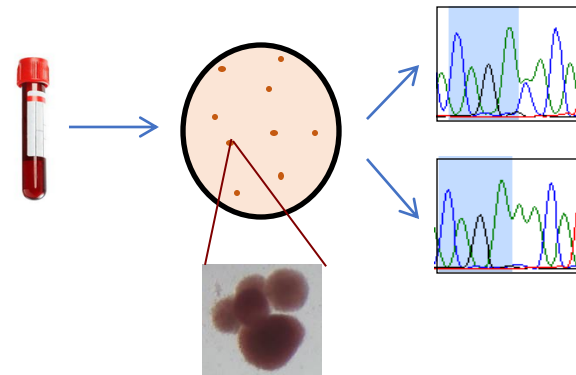
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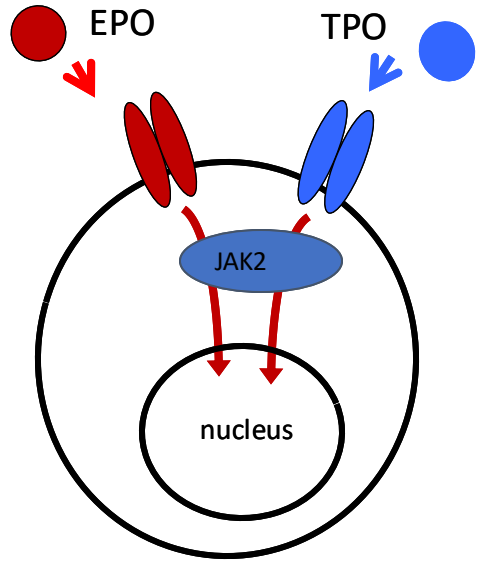
3. *JAK2* Exon 12 mutation → PV

Scott et al, 2007



Scott et al, Blood 2006

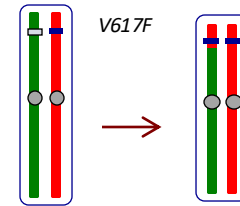
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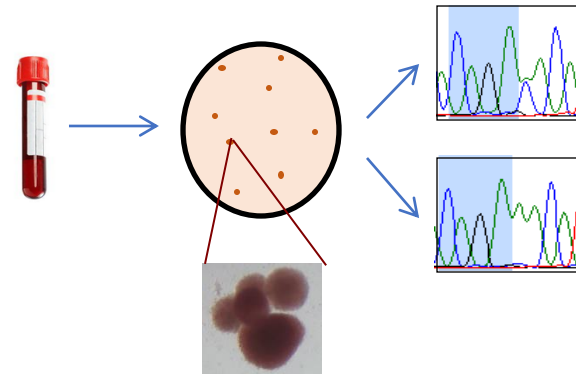
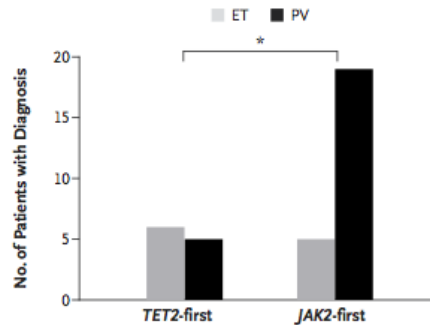


3. *JAK2* Exon 12 mutation → PV

Scott et al, 2007

4. *JAK2* acquired 1st → PV

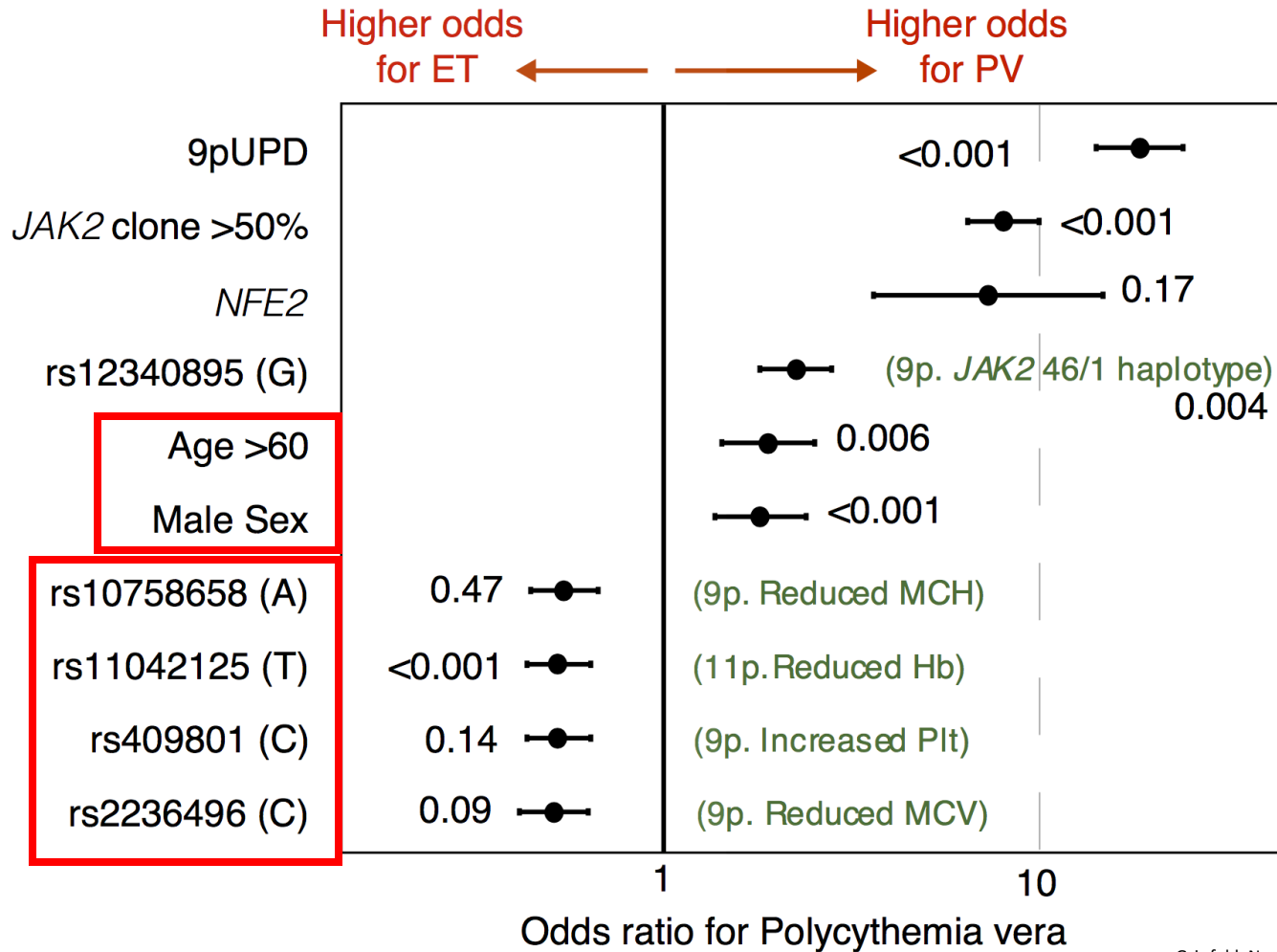
Ortmann, Kent et al 2015



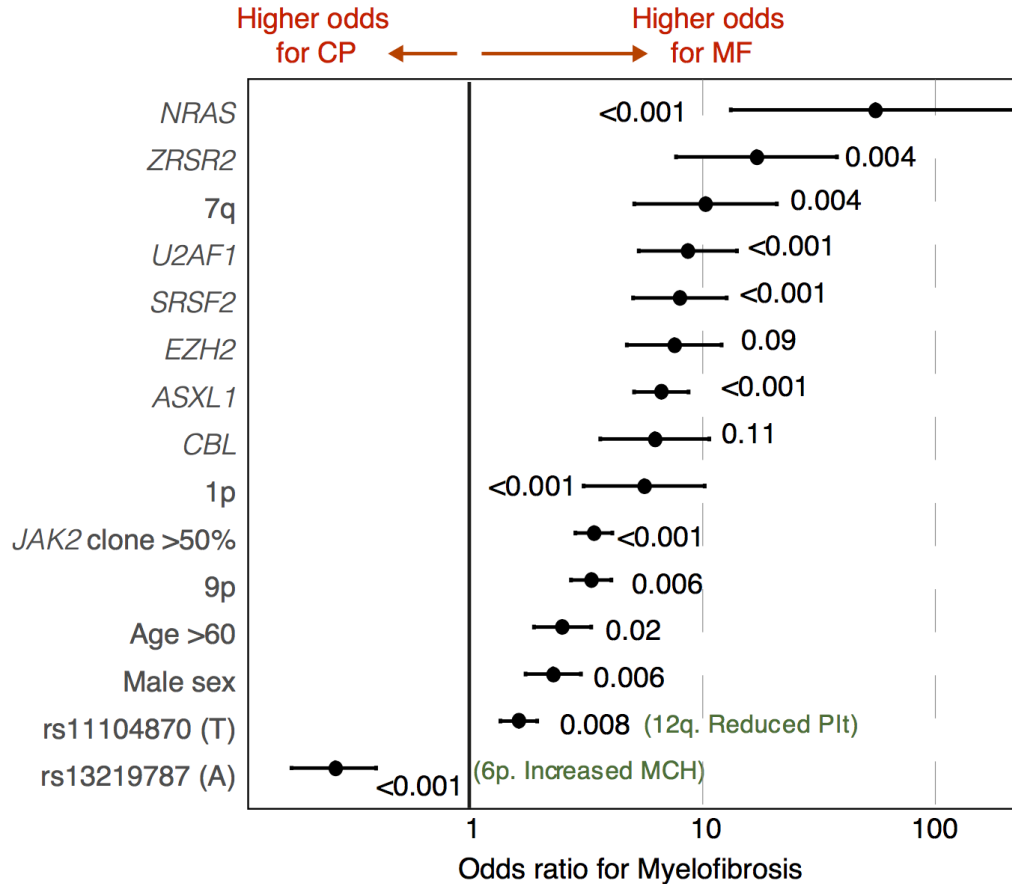
Scott et al, Blood 2006

Each person is unique in terms of age, gender, their own

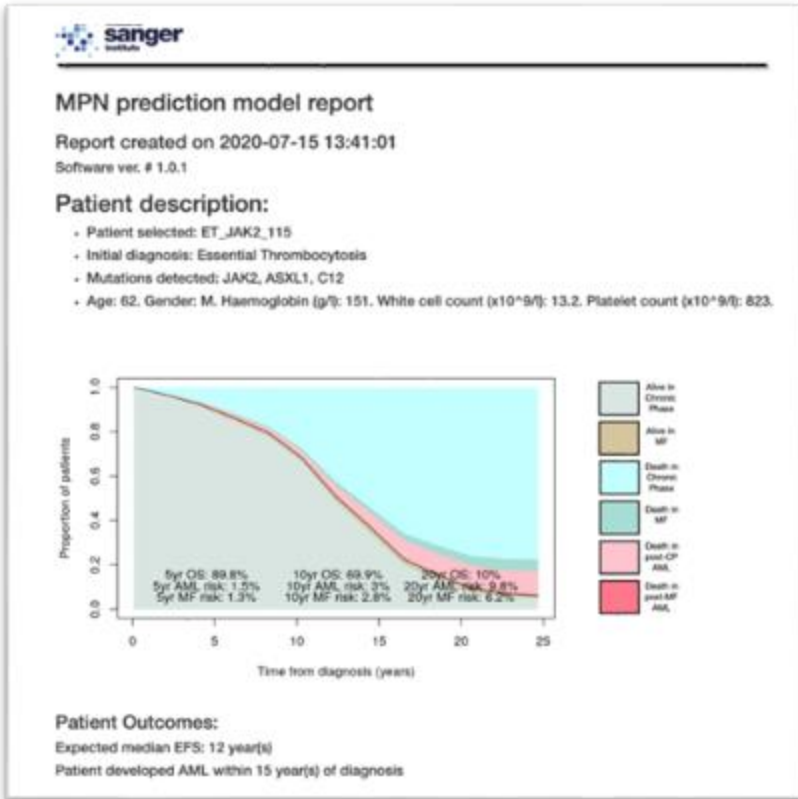
DN^A



Same applies to MF, although additional mutations are more common



Can take into account some of these factors to personalize an individual's MPN



predict blood

Home About Predict Blood PredictBloodTool Gene Panel Cohort Data Cohort Mutations Contact Legal

Predict Blood

Predict is an online research tool which produces personalised predictions for patients with Myeloproliferative Neoplasms

[Start Predict Blood](#)

What does Predict do?
Predict asks for some details about the patient and the blood cancer. It then uses data about the progression of disease and

Who is Predict for?
Predict is for clinicians, patients and their families.
Patients should use it in consultation

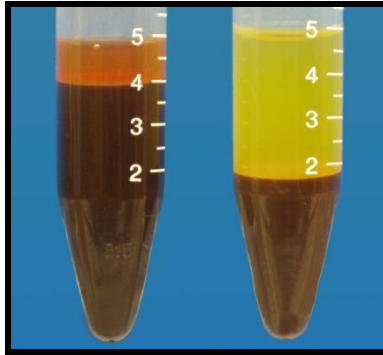
Where can I find out more?
To read more go to [About Predict Blood](#).

<https://blood.predict.nhs.uk>

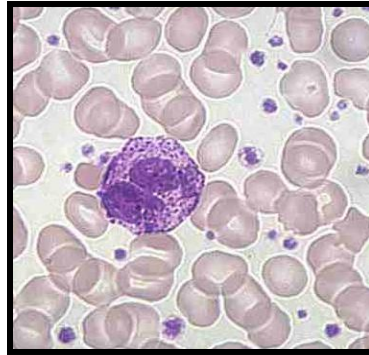
Grinfeld, Nangalia et al *NEJM* 2018
New website UKCA software 2023

Myeloproliferative neoplasms

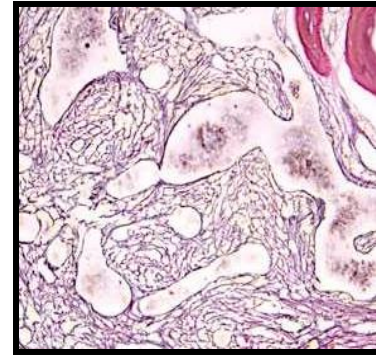
PV



ET

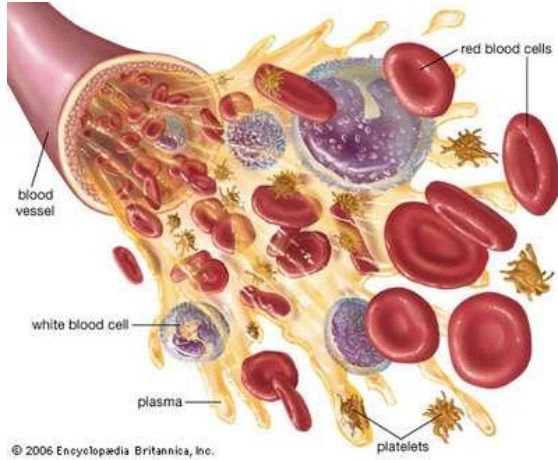


MF



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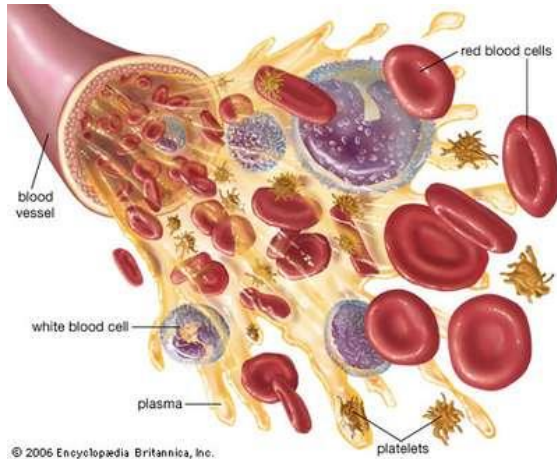
The challenge of making blood



The bone marrow makes 200 billion red cells and 10 billion white cells every single day.

Every time one cell is made, the parent cell has to divide into two cells.

The challenge of making blood



The bone marrow makes 200 billion red cells and 10 billion white cells every single day.

Every time one cell is made, the parent cell has to divide into two cells.

Every cell division requires a copy of the entire DNA to be made by the cell.

3 BILLION nucleotides of DNA need to be copied each time.



Mistakes happen.....

Mutations

17 mutations in every stem cell each year

100,000 stem cells in an adult

2% of our DNA codes for PROTEINS

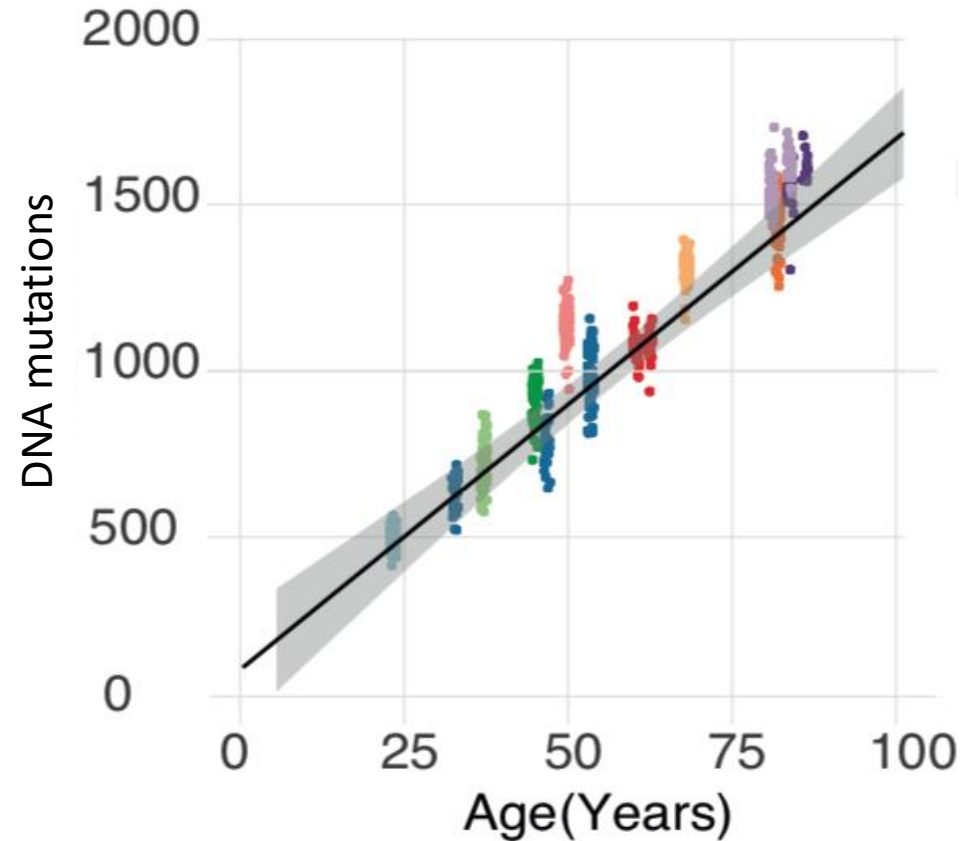
One protein mutation every 15 minutes!

Mistakes happen.....

Mutations

17 mutations in every stem cell each year
100,000 stem cells in an adult
2% of our DNA codes for PROTEINS
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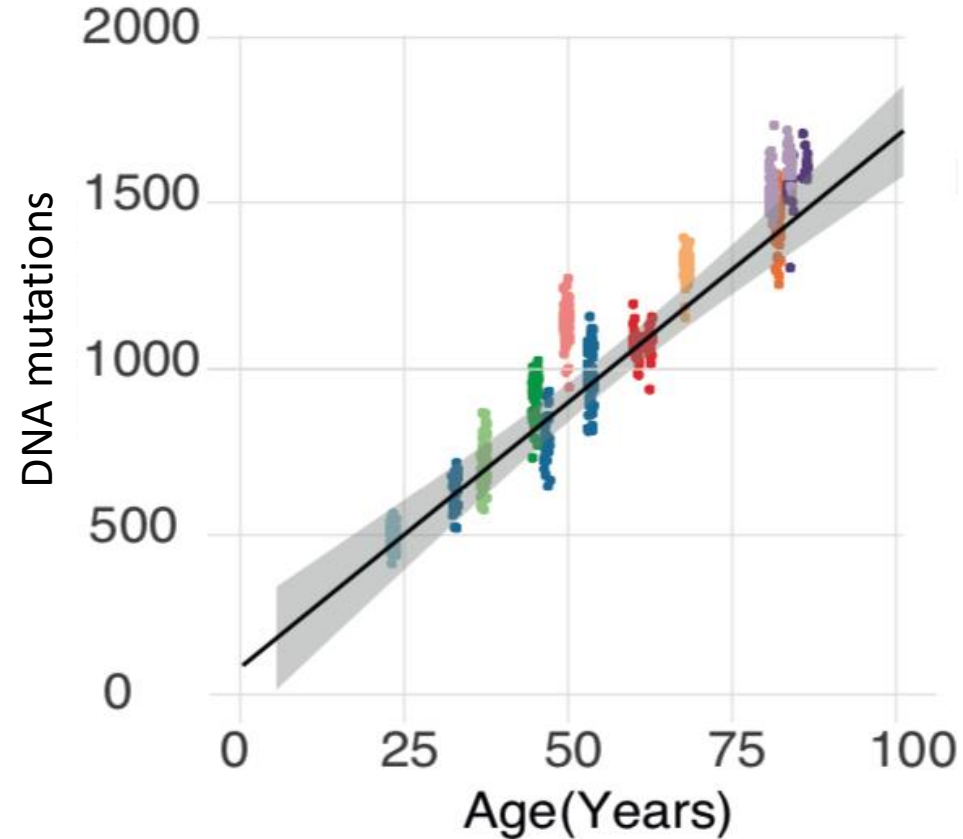
***DNA mutation numbers
give away your age!***



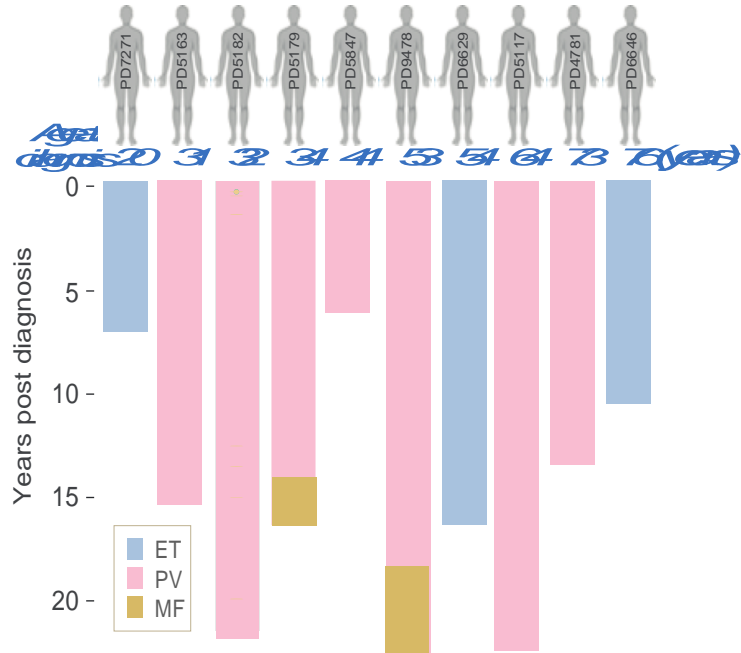
But we use this to our advantage to understand MPN



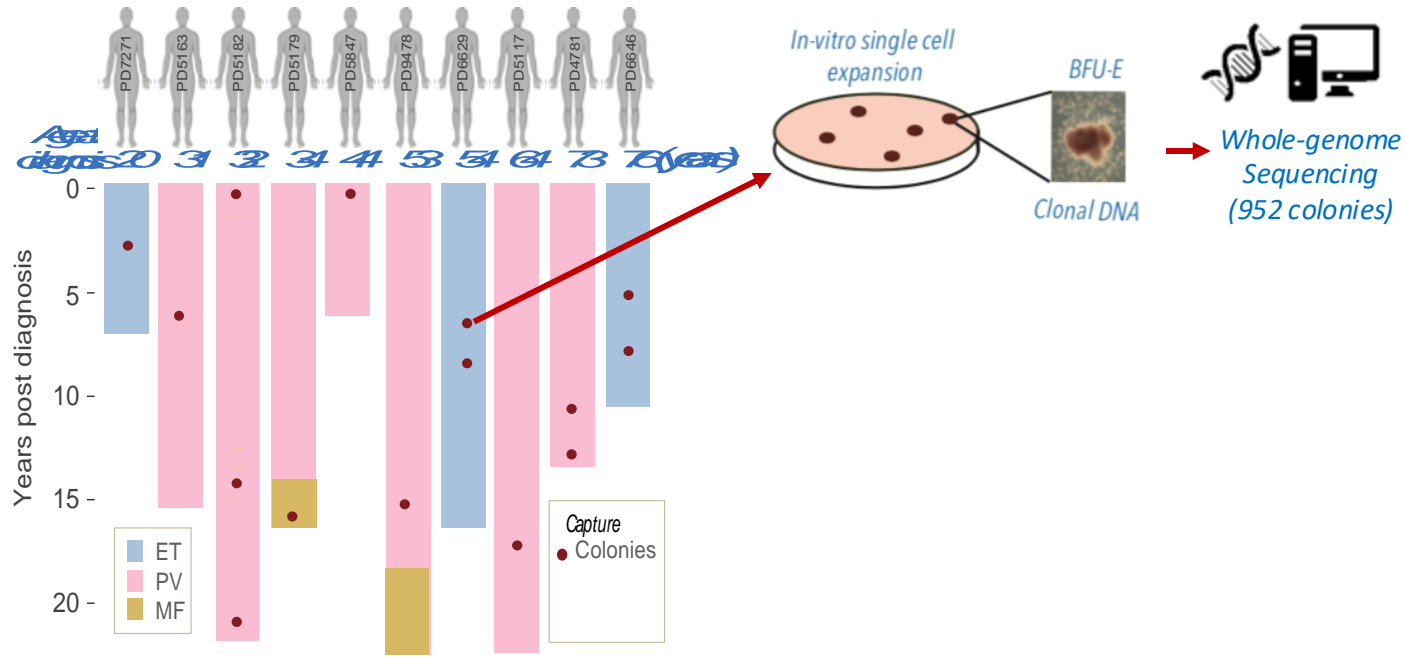
**A DNA CLOCK
to time events**



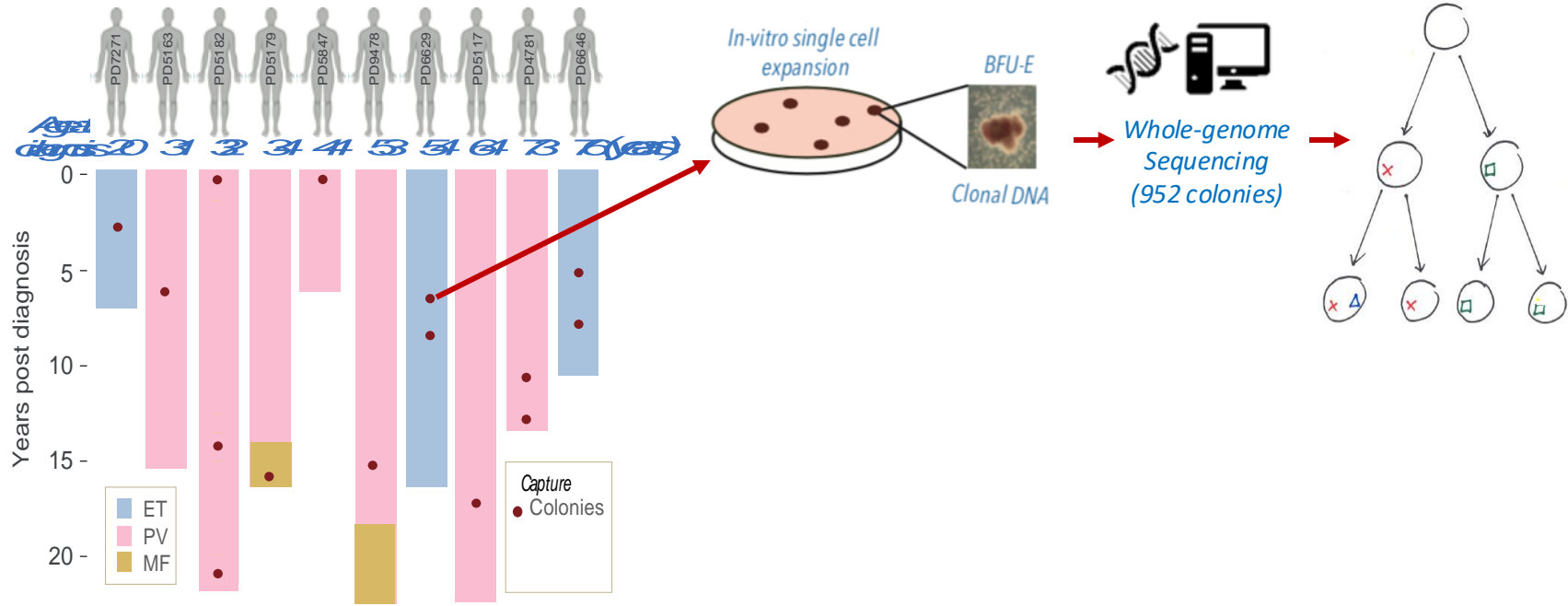
Patient cohort and experimental design



Patient cohort and experimental design

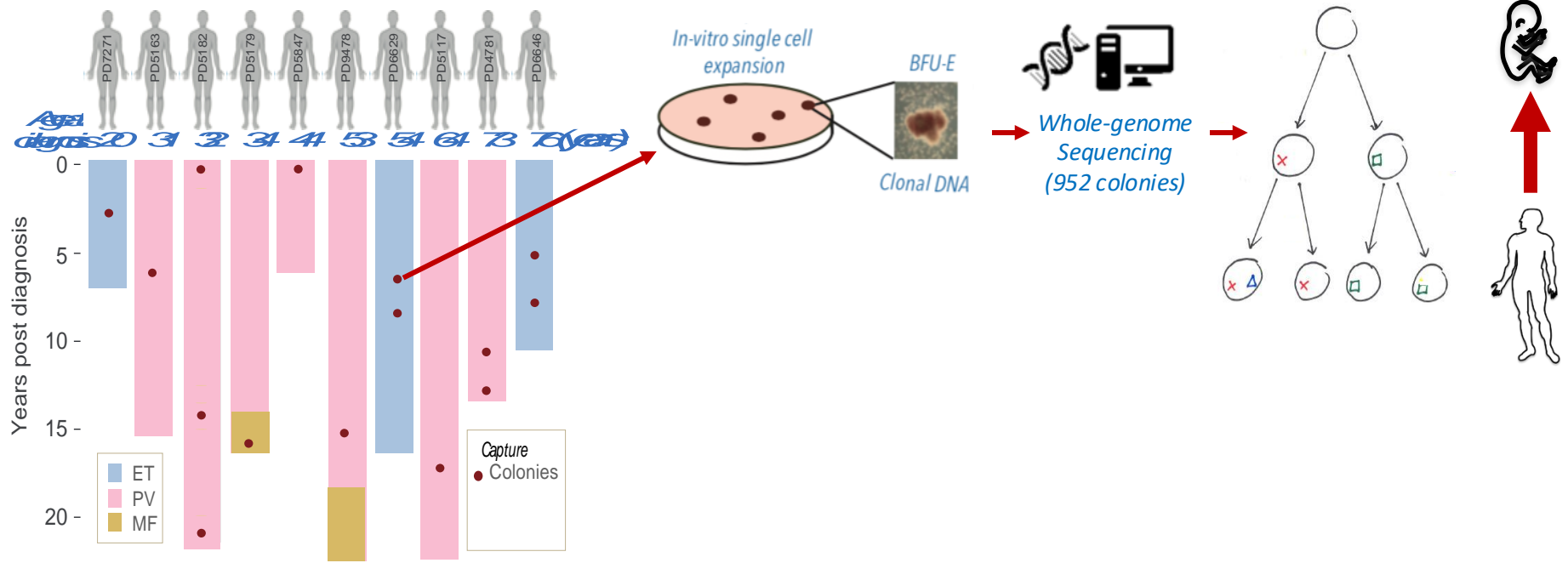


Patient cohort and experimental design



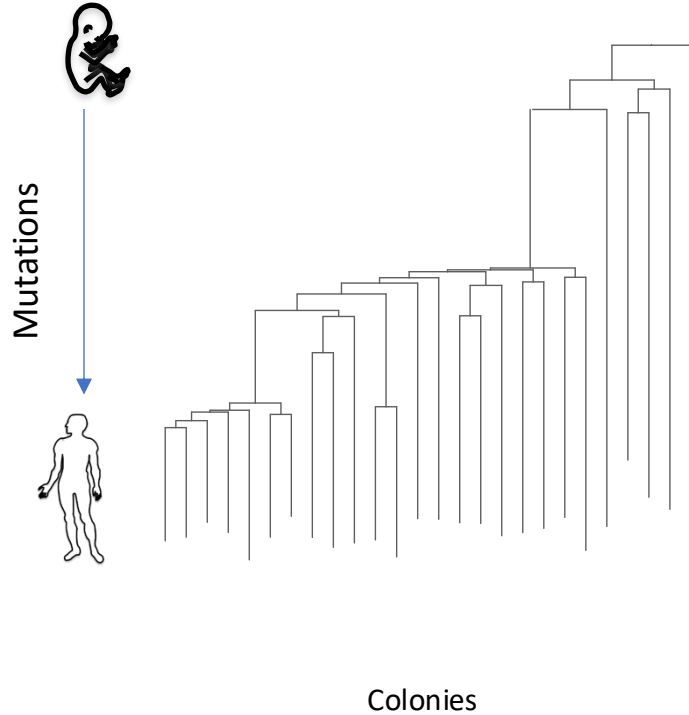
- Starting from the zygote, all cells are acquiring mutations
- Mutations in individual cells act as a natural barcodes

Patient cohort and experimental design



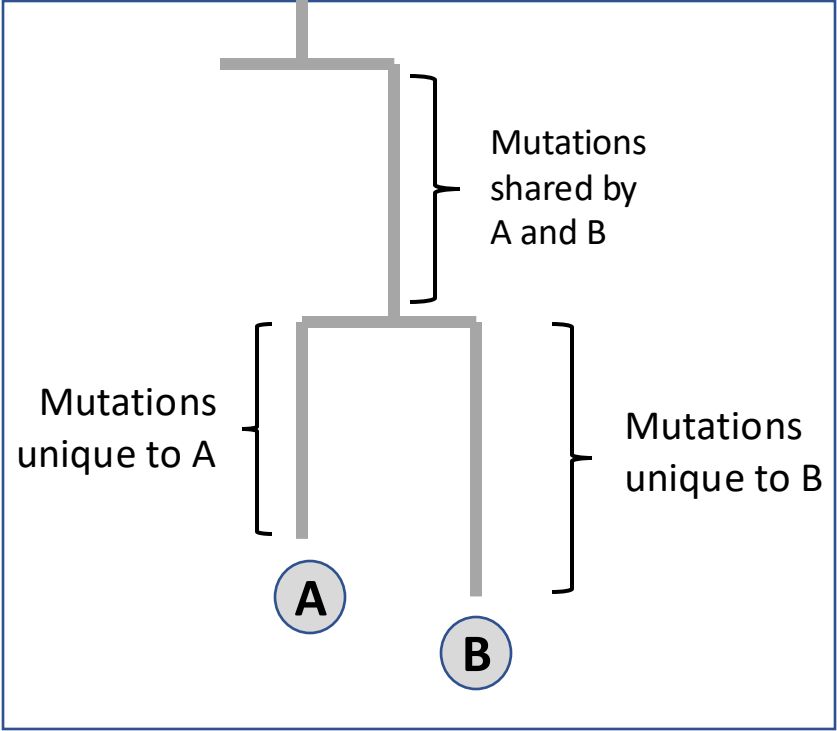
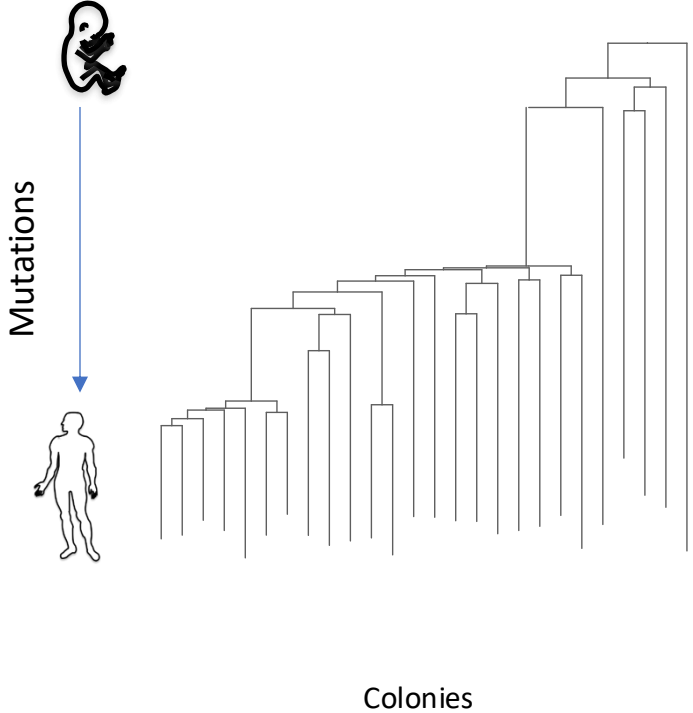
- Starting from the zygote, all cells are acquiring mutations
- Mutations in individual cells act as a natural barcodes
- **Mutations can trace family relationships back to start of life**

Using somatic mutations to build a phylogenetic “tree”



Using somatic mutations to build a phylogenetic

“tree”

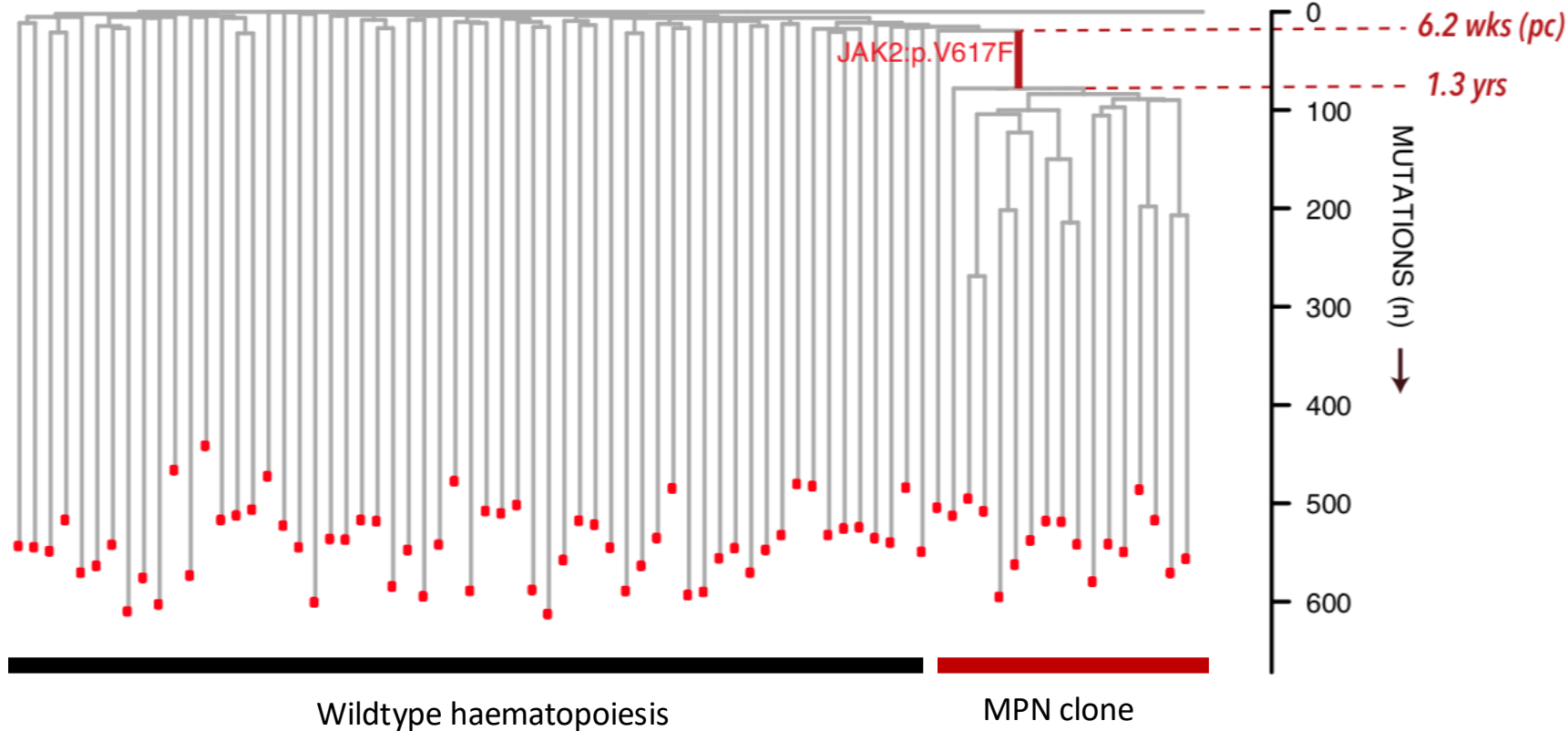


$JAK2^{V617F}$ is acquired in early life in MPN

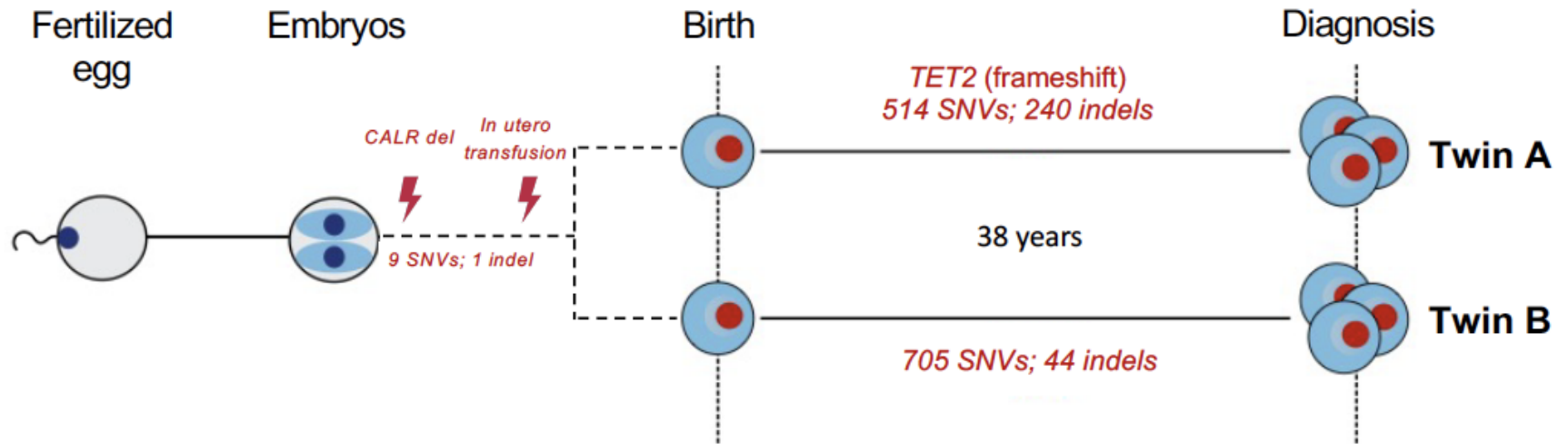
PD7271 (ET diagnosed 21yrs)

Age at Sample

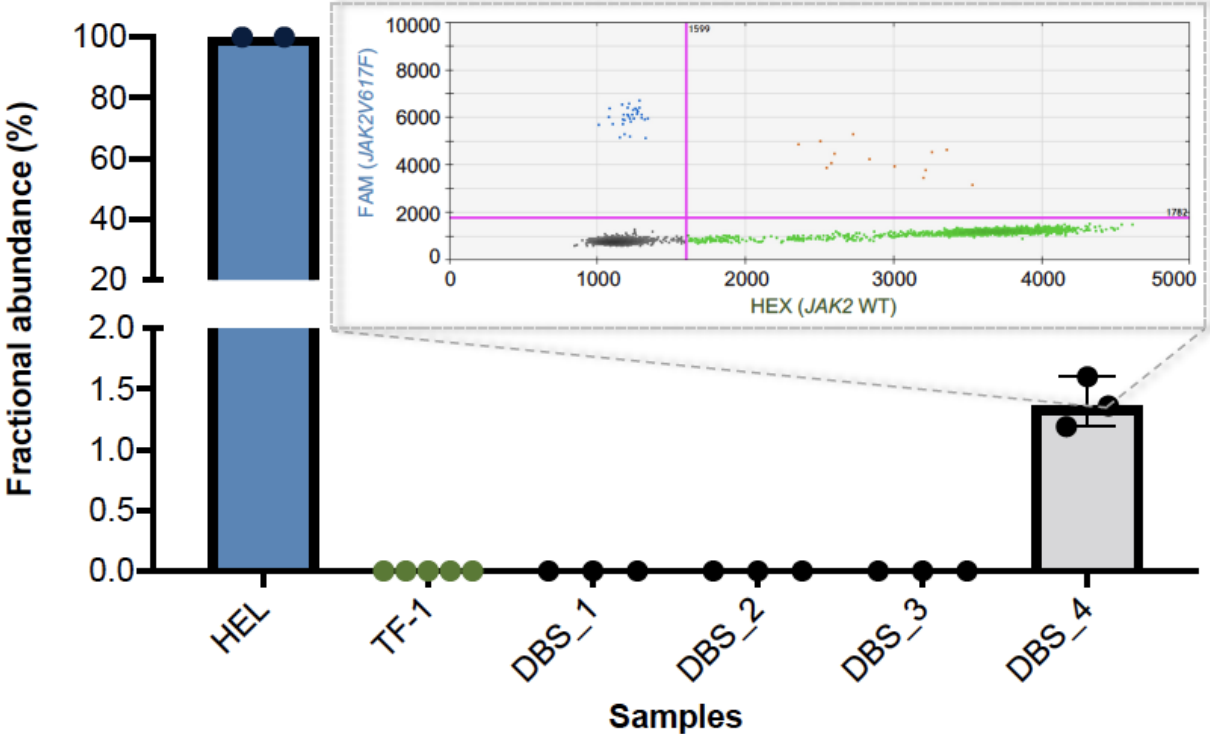
■ 23



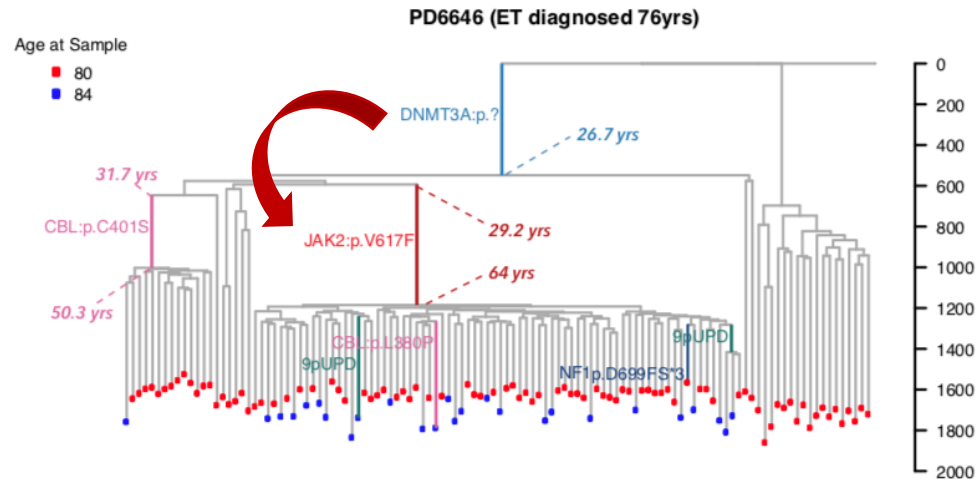
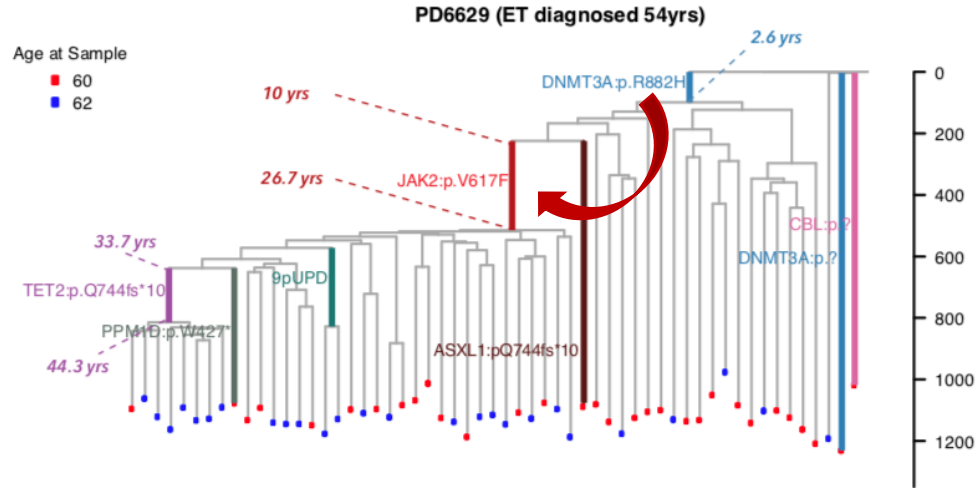
In utero acquisition of *CALR* in monozygotic twins



JAK2 V617F is present in neonatal dried blood spots

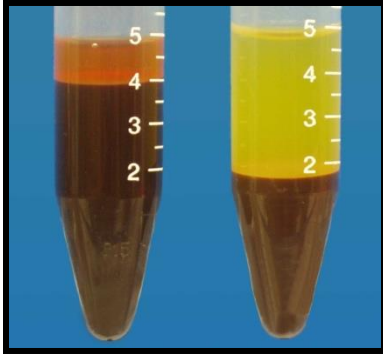


JAK2^{V617F} acquired “second”

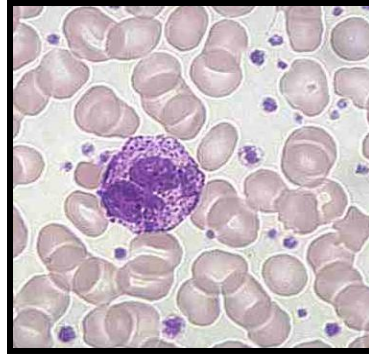


Myeloproliferative neoplasms

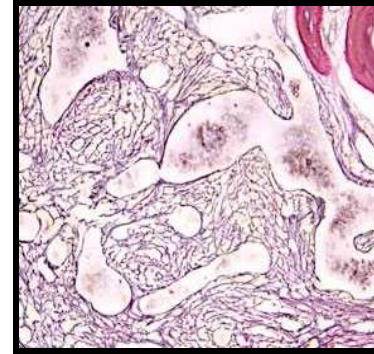
PV



ET

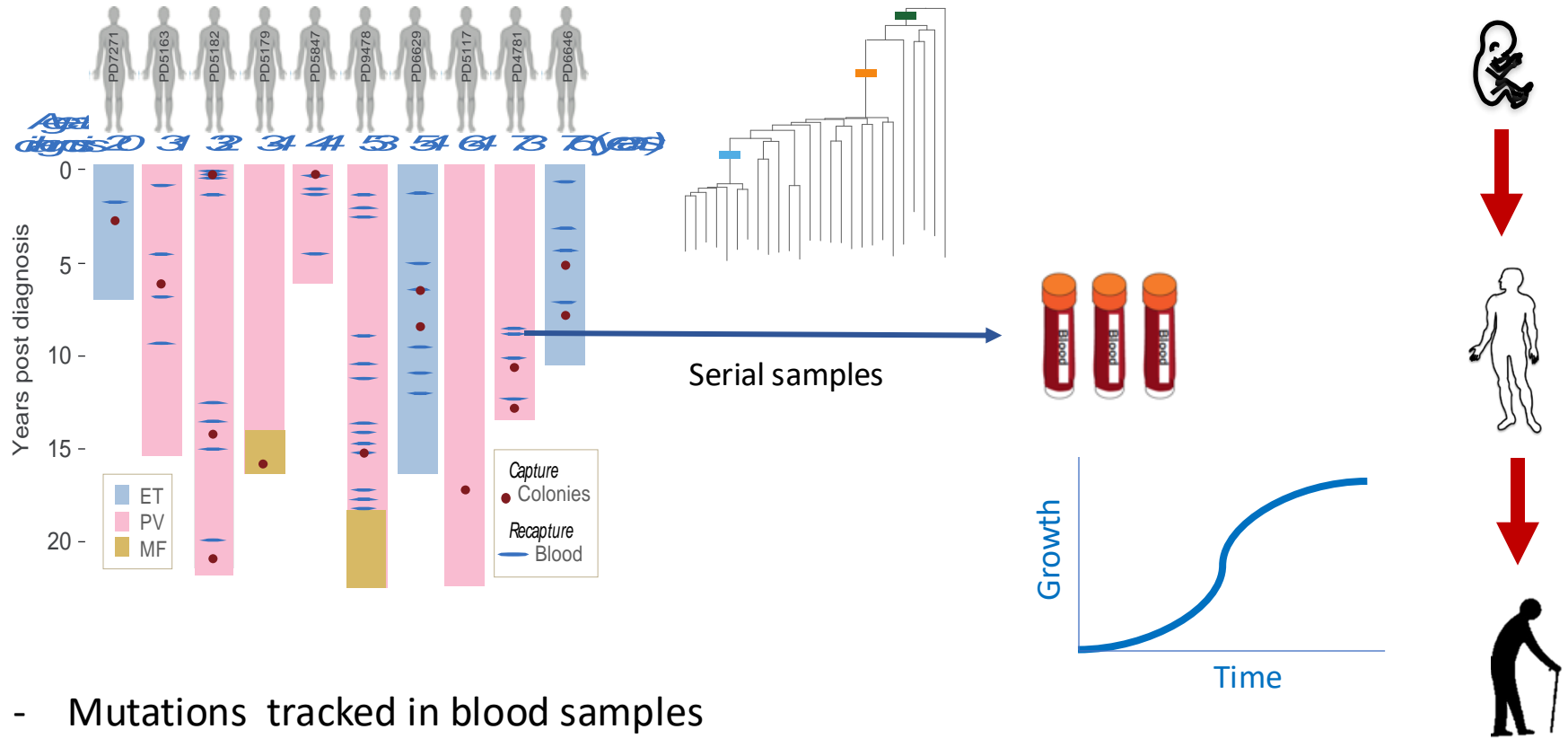


MF



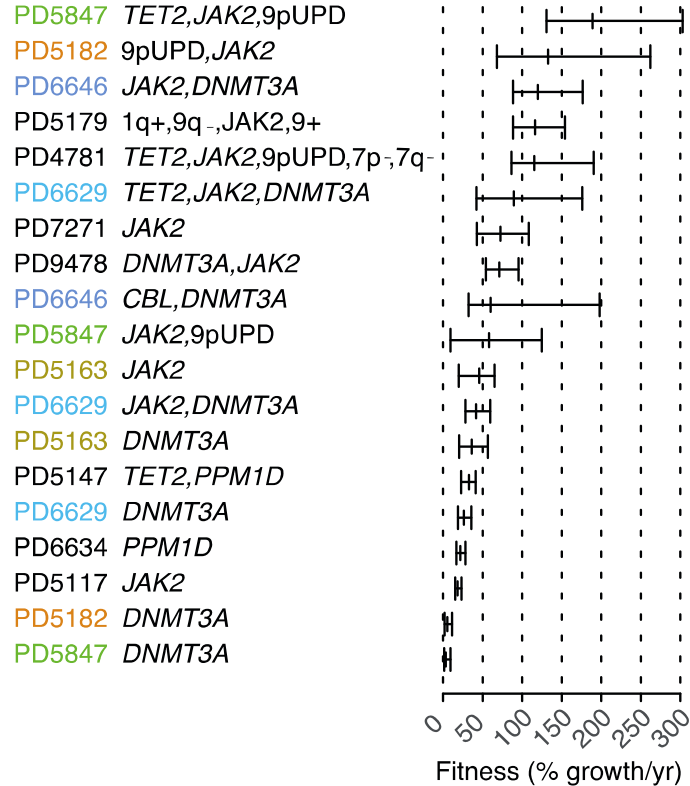
- *What caused it?*
- *Was it just bad luck?*
- *What causes the differences in disease between individuals?*
- *How long have I had it for?*
- *How fast did it grow?*

Estimating growth rates of the clones

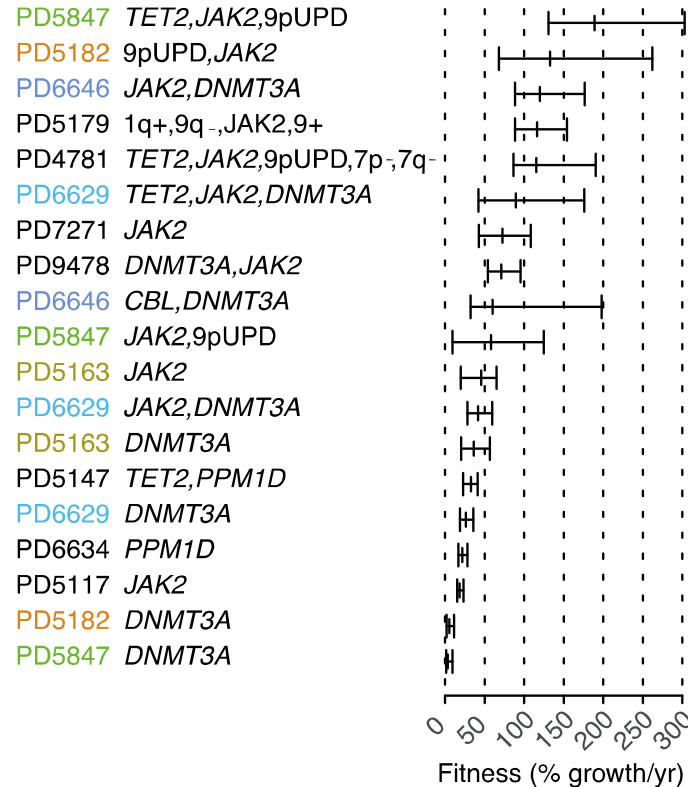


- Mutations tracked in blood samples
- Pattern of branching in the tree also gave clues

Clone growth rates across MPN patients



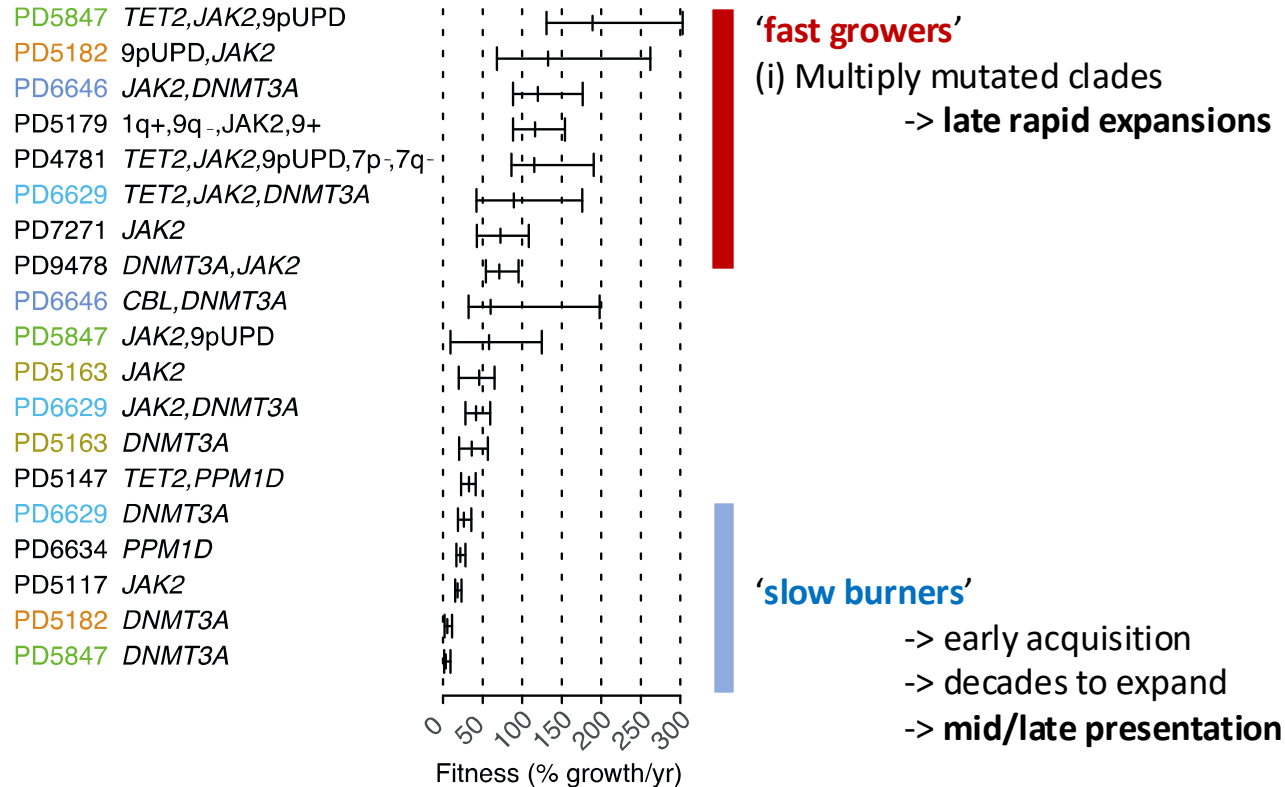
Clone growth rates across MPN patients



'slow burners'

- > early acquisition
- > decades to expand
- > **mid/late presentation**

Clone growth rates across MPN patients



Summary

- Blood cancers are driven by acquired DNA mutations in blood stem cells
- Variable speeds of growth of these abnormal clones – slow to fast – different cancers
- Mutations can be acquired very early indeed.
- Mutation acquisition is a normal part of our lives and occurs in all cells in all humans.
- Understanding what influences the growth rates and slowing them down could enable preventative strategies in the future.



THE *KAY KENDALL* LEUKAEMIA FUND



**Thank you
and
Questions**



Bridge of Sighs, St John's College, Cambridge, UK