Blood and bone marrow

- Stem cell → Progenitors
- Leucocytes
- Red cells
- Platelets
Myeloproliferative neoplasms

- Blood stem cell
  - Progenitors
    - Red cells
    - Leucocytes
    - Platelets
Myeloproliferative neoplasms

- What causes it?
- How long have I had it for?
- How fast did it grow?
Myeloproliferative neoplasms

- What causes it?
- How long have I had it for?
- How fast did it grow?
The code of life – counting chromosomes

Tjio and Levan 1955
A change in the gene *JAK2*

V617F mutation
“It all starts to make sense”

EPO → JAK2 → STAT → nucleus → More red cells
JAK2 mutations in MPN

PV  
JAK2: 98%

ET  
50-60%

MF  
50-60%

2005
Identification of JAK2 mutation

2010
Therapeutic JAK2 inhibitors

Recognition of new disease subtypes
Molecular testing in hospitals

James et al, 2005; Baxter et al, 2005; Kralovics et al, 2005; Levine et al, 2005
The theme repeated... increased growth signal to cells

? Pathogenic mechanism of 50% of ET and MF

**CALR** mutations in majority of **JAK2**-unmutated MPNs

Nangalia, Massie et al NEJM 2013; Klampfl et al NEJM 2013
A new test for the clinic and patients...
Changes in the JAK2, CALR and MPL genes *drive* MPNs....

- >90% patients have mutations in *JAK/CALR/MPL*
Additional mutations in other genes also found....

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

Nangalia et al, NEJM 2013
Myeloproliferative neoplasms

- *What causes it?*
- *How long have I had it for?*
- *How fast did it grow?*
Patient cohort and experimental design
Patient cohort and experimental design

- In-vitro single cell expansion

- Whole-genome Sequencing (952 colonies)

- In-vitro single cell expansion

- Clonal DNA

- Capture

- Colonies

- ET
- PV
- MF

- Age at diagnosis
- Years post diagnosis
- Starting from the zygote, all cells are acquiring mutations
- Mutations in individual cells act as a natural barcodes
- 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
Rich mutational landscape at the clonal level
Using somatic mutations to build a phylogenetic “tree”
Using somatic mutations to build a phylogenetic "tree"
Using somatic mutations to build a phylogenetic “tree”
Putting **driver mutations** on “branches” of the “tree”
A molecular clock to *time* the ‘branches’ of the tree

~18 mutations per year
A molecular clock to *time* the ‘branches’ of the tree

~18 mutations per year
\textit{JAK2^{V617F}} is acquired in early life in MPN

![Graph showing the acquisition of JAK2 p.V617F mutation over time. The graph illustrates the transition from wildtype haematopoiesis to an MPN clone. The timeline indicates the progression from birth to the diagnosis of essential thrombocythaemia (ET) at 21 years of age. The JAK2 p.V617F mutation is acquired around 6.2 weeks of age. ]
JAK2^{V617F} is acquired in early life in MPN

PD7271 (ET diagnosed 21 yrs)

Wildtype haematopoiesis

MPN clone
JAK2V617F is acquired in early life in MPN

PD7271 (ET diagnosed 21yrs)

Age at Sample
23

MUTATIONS (n)

6.2 wks (pc)

1.3 yrs

Wildtype haematopoiesis
MPN clone
JAK2V617F is acquired in early life in MPN
**DNMT3A** – clonal haematopoiesis acquired early in life

---

**PD5163 (PV diagnosed 31 yrs)**

- **Age at Sample**
  - 38

- **DNMT3A:p.T275fs*41**
  - 4.6 wks (pc)

- **JAK2c.p.V617F**
  - 4.2 wks (pc)

- **chrX_DEL**
  - 8.6 yrs

---

**Clonal haematopoiesis**

**MPN clone**
JAK2 and DNMT3A mutations can be acquired in utero

- Rich driver mutation landscape
**JAK2** and **DNMT3A** mutations can be acquired *in utero*

- Rich driver mutation landscape
- **JAK2** and **DNMT3A** mutations acquired very early in life – including *in utero*
JAK2 and DNMT3A mutations can be acquired in utero

- Rich driver mutation landscape
- JAK2 and DNMT3A mutations acquired very early in life – including in utero
**JAK2** and **DNMT3A** mutations can be acquired *in utero*

- Rich driver mutation landscape
- **JAK2** and **DNMT3A** mutations acquired very early in life – including *in utero*
- Additional driver mutation acquisitions separated by decades
JAK2^{V617F} acquired "second"
JAK2^{V617F} acquired "second"
Myeloproliferative neoplasms

- *What causes it?*
- *How long have I had it for?*
- *How fast did it grow?*
Estimating growth rates of the clones

- Mutations re-sequenced in blood samples
- Pattern of branching in the tree + mutation fractions in blood inform growth rates of mutant clones
### Measures of growth rates (fitness) of clones

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clade</th>
<th>% additional growth per yr</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>PD5847</td>
<td>JAK2, 9pUPD, TET2</td>
<td>233</td>
<td>143-360</td>
</tr>
<tr>
<td>PD5182</td>
<td>JAK2, 9pUPD</td>
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<td>DNMT3A, JAK2</td>
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**Additional driver mutations promote rapid growth**

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### Patient specific factors influence consequences of JAK2

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Fitness influences both CHANCE and TIME to disease
Detection decades in advance of disease presentation

Slowest growing JAK2 clone
Detection decades in advance of disease presentation
Early detection **AND** measurement of growth rates
Summary

• **In utero** and childhood driver mutation acquisition for **JAK2** and **DNMT3A**.

• Variable rates of clonal expansion – likely combination of **DRIVER + PATIENT** effects.

• Sequential driver mutation acquisition separated by decades with rapid growth.

• Expansion rates determine latency to diagnosis.

• Mutation detection **AND** rates of expansion could enable preventative strategies.

https://www.biorxiv.org/content/10.1101/2020.11.09.374710v1  @jyoti_nangalia