Myeloproliferative neoplasms – from pathogenesis to personalized predictions
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Myeloproliferative neoplasms
– from pathogenesis to personalized predictions
No disclosures
Myeloproliferative neoplasms

- Arise in stem cell compartment
- Lineage-restricted increase in mature cells
- Window on earliest stages of tumorigenesis
- Clonal assays
Myeloproliferative neoplasms - from origins to outcomes

Molecular genetics

Pathogenesis
- Somatic mutations
- Mutation order

Management
- Classification
- Prognosis
2005 – Identification of JAK2 V617F mutation

Polycythaemia vera

95%

Essential thrombocythaemia

50-60%

Primary myelofibrosis

50-60%

JAK2

<table>
<thead>
<tr>
<th>FERM</th>
<th>SH2</th>
<th>JH2</th>
<th>Kinase</th>
</tr>
</thead>
</table>

V617F mutation

Levine et al Cancer Cell 2005; Kralovics et al NEJM 2005
Myeloproliferative neoplasms and JAK/STAT pathway

**JAK/STAT signaling**
- EPOR
- TPOR
- JAK2
- MAPK
- PI3K
- pSTAT5

**Illuminated MPN pathogenesis**
- FERM
- SH2
- JH2
- Kinase
- Exon 12
- PV variant
- V617F
- MPNs
- Exon 16
- ALL

**JAK2 V617F is causal**
- retroviral/transgenic models develop ET/PV
- het knock-in mice develop ET/PV
- hom mice develop HCT

**Rapid clinical impact**
- regional diagnostic service
- international guidelines
- therapeutic JAK2 and STAT inhibitors

What about JAK2 V617F negative MPNs?

Polycythaemia vera

- JAK2 V617F
- 95%
- JAK2 exon 12 mutations
- Scott et al NEJM 2007

Essential thrombocythaemia

- 50-60%
- ~5% - THPO receptor mutations
- Pikman et al PLoS Medicine 2006

Myelofibrosis

- 50-60%
JAK2-negative ET and PMF: exome sequencing of 151 patients

Somatic CALR Mutations in Myeloproliferative Neoplasms with Nonmutated JAK2

Nangalia, Massie et al NEJM 2013

WT CALR

Insertion

Deletion
Knock-in mouse – humanised mutant CALR

Heterozygous mice develop ET

Platelets

** ***

Time (mths)

Homozygous mice develop extreme ET with MF

Increased phenotypic HSCs but no functional advantage

Repopulating ability in PB (% test / test + comp)

Li et al Blood 2018

- genetic background
- unidentified somatic mutations
- selection by aged environment
- steady state vs stress haematopoiesis
Mutant CALR interacts with THPO receptor and activates JAK/STAT pathway

Model:
MPN phenotype determined by cytokine receptor activation

JAK2 V617F negative disease

EPOR
JAK2 Exon 12

THPOR
mutant

CALR

PV
ET/MF
Patient labelled as PV or ET depending on balance of EPOR and THPOR signaling – influenced by:

- inherited genotype, somatic mutations
- physiological/pathological factors influencing Hb and Pl
Phenotypic mutations
JAK2, CALR, MPL

Co-mutations

Normal → MPN → Co-mutations

Accel phase
MF
AML

Nangalia et al. NEJM 2013; Grinfeld et al. in preparation
Other somatic driver mutations

**DNA methylation**
- Cytosine
- 5-methylcytosine
- TET2
- 5-OHmethylcytosine
- Demethylation

**Chromatin biology**
- H3K27
- EZH2
- ASXL1
- IDH1/2

**Splicing machinery**
- SF3B1
- SRSF2

**Others**
- TP53 pathway
- Signaling
- Transcription
Cancer biology: Does mutation order matter?

Identified 24 MPN patients with JAK2 and TET2 mutations.

Determined mutation order by genotyping >7000 colonies.

PV (4)  ET (5)  MF (3)

JAK2-first (n=12)

PV (7)  ET (2)  MF (3)

TET2-first (n=12)

Do malignant properties reflect sum of mutations acquired? OR Does order matter?

Ortmann, Kent et al NEJM 2015
JAK2-first patients present at a younger age

... and have increased risk of thrombosis

... and respond better to JAK2 inhibitor in vitro

Ortmann, Kent et al NEJM 2015
Knock-in mice

- JAK2V617F selectively increases downstream erythroid progenitor production but does not increase HSC self-renewal
  - eg Green, Mulally, Villeval

- TET2 mutation confers an HSC self-renewal advantage without increased production of megakaryocytic/erythroid cells
  - eg Levine, Rao, Bernard
Analysis of single HSCs

Mutation order influences:
- composition of HSC compartment
  - TET2-first: TET2 single-mut HSCs dominate
  - JAK2-first: double-mutant HSCs dominate

- proliferation of HSC progeny
  - JAK2 mutation has proliferative effect only if occurs first

- the intrinsic properties of individual double mutant HSPCs
  - e.g. TET2-first double mutant HSCs 10x less able to generate progenitors than JAK2-first equivalents
Mutation order influences clonal evolution

Single stem cell derived clone

HSC

Prog

Diff

Excess

Excess production of differentiated cells (i.e., above normal counts)

TET2-first

JAK2-first

wildtype

TET2 mutant

JAK2 het

JAK2 hom
Mutation order influences clonal evolution

Single stem cell derived clone

Excess production of differentiated cells (i.e., above normal counts)

HSC

Prog

Diff

Excess

wildtype

TET2 mutant

JAK2 het

JAK2 hom

1

2

3

TET2-first

JAK2-first

Acquisition order impacts clonal evolution

Mutation order influences clonal evolution
Mutation order influences clonal evolution

Single stem cell derived clone
Excess production of differentiated cells (i.e., above normal counts)

HSC

Prog
Diff
Excess

1
2
3

TET2-first

JAK2-first

wildtype

TET2 mutant

JAK2 het

JAK2 hom

Excess production of differentiated cells (i.e., above normal counts)

Mutation order influences clonal evolution
Mutation order influences clonal evolution

**TET2-first**
- Single mutant HSPCs dominate
- ET phenotype
- Present at older age
- Less thrombosis

**JAK2-first**
- Double mutant HSPCs dominate
- PV phenotype
- Present at younger age
- Increased thrombosis

- Single stem cell derived clone
- Excess production of differentiated cells (i.e., above normal counts)

- TET2 wildtype
- TET2 mutant
- JAK2 het
- JAK2 hom
Molecular mechanism
Mutation order influences transcriptional response to JAK2V617F

Prior TET2 mutation prevents JAK2V617F up-regulating a proliferative transcriptional program

- First demonstration in any cancer
- Extended to other mutations
- Implications for targeted therapies and patient stratification

Ortmann, Kent et al NEJM 2015
Nangalia et al Haematologica 2016
Grinfeld et al under review
Myeloproliferative neoplasms - from origins to outcomes

- Molecular genetics
  - Pathogenesis
  - Management

Classification

Prognosis
Current classification based on phenotype

Polycythaemia vera  
Essential thrombocythaemia  
Myelofibrosis

- JAK2, CALR and MPL embedded in diagnostic guidelines
- However we still map mutations onto conventional classification.
- Fundamental problems with phenotypic classification include:
  - diagnostic difficulties - where to draw the line
  - management anomalies
  - based on consequences and not on biological causes
Infectious diseases

Phenotypes
- Fever

Biological causes
- P falciparum
- S typhi

Rash
MPN classification based on biological causes
ie driver mutations

Comprehensive targeted gene sequencing
- 2041 patients
- 70 genes - myeloid drivers
- 1700 SNPs genome wide

Rich dataset - large numbers of patients

Grinfeld, Nangalia et al, under review
Informs pathogenesis – eg patterns of co-mutation

Allows genomic classification – Bayesian clustering identifies 7 genomic groups

- TP53 mutation/aneupoidy
- Chromatin/spliceosome mut^n
- CALR mutation
- MPL mutation
- Homozyg JAK2 or NFE2 mut^n
- Heterozyg JAK2 mutation
- No driver mutation

- Simple rules
- Each group spans >1 conventional diagnostic category
- Each group adds prognostic info to conventional MPN categories
- Validated using independent cohort
Several studies have developed scoring systems for PMF

But - molecular data not comprehensive
- little available for chronic phase patients
- convert continuous variables into categories
- no personalized predictions
By combining genomic and clinical data can use multi-state modelling to assess what contributes to outcomes.

**Chronic Phase → Death**

- Genomic
- Cohort
- Clinical (other)

**Chronic Phase → MF**

- Age
- Genetic (PHF6, ZRSR2)
- CBL
- NFE2
- IDH2
- SF3B1
- MPL
- ASXL1
- TET2
- SRSF2
- CALR
- Blood count (↑Plts)
- Clinical (Splen, etc)

Substantial contribution of genomics to Transformation (but not to Death in Chronic Phase)
Allows individualised patient predictions: ET example

Patient Description:
ET, female, 70 at diagnosis
Diagnostic counts: Hb 104, WBC 8.4, PI 2300
Mutations : CALR, SRSF2, IDH2, 18qUPD
Individualised patient predictions: ET example

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Model Predictions:
5yr OS 65%, AML risk 10%, MF risk 38%
Individualised patient predictions: ET example

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Mutations: CALR, SRSF2, IDH2, 18qUPD

Model Predictions:
5yr OS 65%, AML risk 10%, MF risk 38%
10yr OS 27%, AML risk 16%, MF risk 53%
Individualised patient predictions: ET example

Patient Description:
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Mutations: CALR, SRSF2, IDH2, 18qUPD

Model Predictions:
5yr OS 65%, AML risk 10%, MF risk 38%
10yr OS 27%, AML risk 16%, MF risk 53%

Outcome:
Patient transformed to myelofibrosis and died within 5 years
Additional validation of model performance: chronic phase patients

Leave-one-out cross-validation

Concordant EFS ranking in 80%

Accuracy: (Brier score) 0.06-0.1

External cohort
(n=325, 47% genomic data available)

Concordant EFS ranking in 68%

Accuracy (Brier score) 0.08-0.16
**Online calculator**

**Please select initial Diagnosis:**
- Essential Thrombocytosis (n=1251)

**Use existing or new patient data**
- Use existing patient data

**Select Patient:**
- ET_JAK2_1209

**Patient Description**
Patient Selected: ET_JAK2_1209
Mutations detected: JAK2, ASXL1, SRSF2, IDH2

**Expected median EFS: 11 year(s)**

- 5yr OS: 86.9%
- 5yr AML risk: 10.3%
- 5yr MF risk: 10.2%
- 10yr OS: 70%
- 10yr AML risk: 20%
- 10yr MF risk: 20.8%
- 20yr OS: 17%
- 20yr AML risk: 54.2%
- 20yr MF risk: 37.9%

**Time from diagnosis (years)**

**Patient Outcomes**
Patient developed AML within 2 year(s) of diagnosis
We are on the cusp of a new era

- Molecular analysis (JAK2, CALR, MPL) has already revolutionized diagnosis

- Comprehensive genomic data:
  - provides classification based on biological causes
  - allows personalised predictions
  - will improve management
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Funding

Welcome

Cancer Research UK

Bloodwise

The Kay Kendall Leukaemia Fund
Constitutional genotype

Normal

Phenotypic mutations
eg JAK2, CALR

MPN

Co-mutations

Co-mutations

MPN

Co-mutations

Accel phase

MF

AML
Inherited predisposition

Common but weak effects (GWAS studies)

46/1 haplotype
SH2B3
GFI1B
MYB
MECOM
TET2
TERT
ATM
CHEK2
PINT

\{ JAK/STAT signaling \}
\{ Erythroid/Mega differnt \}
\{ Epigenetic regulation \}
\{ Cellular aging \}
\{ DNA repair response/tumor suppressor \}

Increased mutation rate
Niche/clone survival
Modulate resultant phenotype
Inherited predisposition

**Common but weak effect**

46/1 haplotype
SH2B3
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MECOM
TET2
TERT
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CHEK2
PINT

**JAK/STAT signaling**
**Erythroid/Mega different**
**Epigenetic regulation**
**Cellular aging**
**DNA repair response/tumor suppressor**

**Rare but stronger effect**

“Familial MPNs”

ATG2B/GSKIP duplication
*(Saliba et al Nat Genet 2015)*

RBBP6
*(Harutyunyan et al Blood 2016)*

SH2B3 (LNK)
*(Rumi et al Blood 2016)*
Rare but strong predisposition

5 families from West Indies – predisposed to MPNs (and MDS/AML) at young age

Germline tandem duplication 700 kb 6 genes

Functional studies (iPS and primary cells) show ATG2B and GSKIP can:

- cause mild megakaryocytic phenotype
- cooperate with JAK2 and TET2 mutations

With thanks to Isabelle Plo

Saliba et al., Nature Genet, 2015
Cellular mechanism
Mutation order influences:
- composition of stem, progenitor and mature cell compartments
- the intrinsic properties of single HSPCs

Molecular mechanism
Mutation order influences transcriptional response to JAK2V617F

Prior TET2 mutation prevents JAK2V617F up-regulating a proliferative transcriptional program
Comparison of JAK2 and CALR knock-in mutations

In both HSCs lack functional advantage in transplant studies

- genetic background
- unidentified somatic mutations
- selection by aged environment
- steady state vs stress haematopoiesis

- and yet clonal expansion in patients

Differences suggest JAK2- and CALR-mutant ET are distinct

<table>
<thead>
<tr>
<th></th>
<th>Hom mutant JAK2</th>
<th>Hom mutant CALR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counts</td>
<td>extreme↑ Hb</td>
<td>extreme↑ Pl</td>
</tr>
<tr>
<td>Platelet function</td>
<td>abnormal</td>
<td>normal</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>none</td>
<td>present</td>
</tr>
<tr>
<td>HSC numbers</td>
<td>reduced</td>
<td>increased</td>
</tr>
</tbody>
</table>
Clonal Haematopoiesis

Prevalence increases with age

- VAF≥0.1%
- VAF≥0.8%
- VAF≥2-10%

Age

- 20s
- 30s
- 40s
- 50s
- 60s
- 70s
- 80s
- 90s

% with clonal haemopoiesis

- Acuna-Hidalgo et al AJHG 2017
- McKerrell et al Cell Rep 2015
- Genovese et al NEJM 2014
- Jaiswal et al NEJM 2014
- Xie et al Nat Med Dec 2014

Present in all of us by 60!

Can evolve into MPNs over 5-15 yrs

Associated with:

- Increased coronary heart disease and atherosclerosis – inflamm macrophages
  Jaiswal et al NEJM 2017; Fuster et al Science 2017

- Increased haem malignancies
  DNMT3A, TET2, ASXL1 – 20-40 fold risk
  Jaiswal ASH 2017

Young et al, Nature Communications 2016

McKerrell et al, Blood Adv 2017
Mutant CALR is causal

Retroviral and transgenic mice develop ET
- Marty et al Blood 2016
- Elf et al Cancer Discov 2016
- Shide et al Leukemia 2016

Het knock-in mice develop ET

Homozygous knock-in mice develop extreme ET with MF

Increased phenotypic HSCs but no functional advantage

Li et al Blood 2018
Mission

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