The megakaryocyte defect in myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPN)

• Clonal haematopoietic stem cell disorders → over-production of blood cells
  – polycythemia vera (PV)
  – essential thrombocythemia (ET)
  – primary myelofibrosis (PMF)

• Characterised by driver mutations in JAK2, CALR and MPL

• Complications:
  – 30-40% have bleeding and haemorrhagic complications
  – 25% progress to develop bone marrow fibrosis (bone marrow failure)
  – 10% transform to acute myeloid leukaemia
Myelofibrosis (bone marrow fibrosis)

- Bone marrow failure:
  - bone pain, anaemia, bleeding and infections
  - enlarged and painful spleen
  - 20% chance of transforming to AML
  - treatment is largely palliative
  - significantly reduced life expectancy (~2 years)

- Marked morphological abnormalities in platelets and megakaryocytes

- Underlying cause is unknown and no way to predict who will progress
Megakaryocytes and fibrosis

• Produce platelets and regulate matrix deposition (Malara et al., Blood. 2013)

• Markedly abnormal MK with atypical clustering in fibrotic cases (WHO)

• $JAK2^{V617F}$ mice develop fibrosis, along with marked increase of abnormal MK (Li et al., Blood. 2010)

• Dysregulated megakaryopoiesis (Malherbe et al., J Clin Path. 2016)

• Aberrant release of pro-fibrogenic factors
  – MK from MF patients secrete increased levels of TGF-β (Ciurea et al., Blood. 2007)
  – GPS mice leak cytokines and chemokines → fibrosis (Guerrero et al., Blood. 2014)
Hypothesis and aim

**Hypothesis:** Genetic abnormalities in megakaryocytes underlies the development of bone marrow fibrosis in patients with MPN.

**Aim:** To profile genomic abnormalities present in MKs from patients with MPN using next generation sequencing.
Overview of the study

Patient recruitment → Cell separation → LCM → DNA extraction and WGA → DNA QC

Library construction → Library QC → Automated templating → NGS and variant calling → Data analysis

Guo B et al., Am J Pathol. 2017
Sample collection

- Fresh bone marrow aspirate:
  - pure megakaryocytes (MK)
  - MK-depleted marrow cells (BM)

- Studies of primary human MKs have been historically hampered by:
  - rare (<1%) microdissection from bone marrow smears
  - giant size isolate by FACS or Fluidigm C1

→ insufficient number and purity of MKs

- ex vivo cultures of human MKs ≠ in vivo bone marrow milieu
Laser capture microdissection (LCM)

- MKs are further enriched by LCM capture using Arcturus™ system.
## Study cohort

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<th>PV (n=6)</th>
<th>ET (n=12)</th>
<th>MF (n=8)</th>
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<td>Age (yrs)</td>
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<tr>
<td>Mutations status</td>
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<tr>
<td>(JAK2^{V617F}) positive</td>
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<tr>
<td>(CALR) positive</td>
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<td>(MPL) positive</td>
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<td>Triple negative</td>
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Megakaryocyte capture

- All megakaryocytes are captured
- 90-100% successful rate of capture by LCM
- Captured MK undergo whole genome amplification and subsequent NGS

Guo B et al., Am J Pathol. 2017 & unpublished data
Variant calling

- Total variants called
- Remove common polymorphisms
- maf ≥ 0.05
- maf < 0.05
- Remove variants that are less likely to affect function
- intronic
- Remove systematic errors
  - MK variants
  - BM variants
  - Somatic variants unique to MK

Guo B et al., Am J Pathol. 2017
Megakaryocytes contain somatic mutations

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<td>No. MK-unique variants (ave)</td>
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<td>Range</td>
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- Megakaryocytes in MPN contain somatic mutations that are not present in other cells in the bone marrow

Guo B et al., Am J Pathol. 2017 & unpublished data
Measuring extent of fibrosis

• Reticulin deposition is a direct measure of bone marrow fibrosis

• There is a spread of reticulin grades in our cohort when grouped into MPN subtypes

Guo B et al., Am J Pathol. 2017 & unpublished data
Megakaryocytes contain somatic mutations

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<th>Grade ≤1 reticulin (n=13)</th>
<th>Grade ≥2 reticulin (n=13)</th>
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- Increased mutation burden in megakaryocytes with increased reticulin deposition

Guo B et al., Am J Pathol. 2017 & unpublished data
MK mutation spectrum

- Most frequently mutated genes
- Possible “unstable” or “error-prone” genome for megakaryocytes in MPN?

Guo B et al., Am J Pathol. 2017 & unpublished data
MK-unique variant allelic burden

• Cases with increased allelic burden have increased reticulin deposition

• High frequency mutations are in genes encoding:
  – transcription regulators
  – Cytokine receptor

Guo B et al., Am J Pathol. 2017 & unpublished data
Summary

➢ Megakaryocytes in MPN contain unique somatic mutations not present in other cells in the bone marrow

➢ The mutation and allelic burden is higher in megakaryocytes from patients with increased reticulin deposition

➢ The mutation spectrum of megakaryocytes from patients with grade ≥2 reticulin is different to those with grade ≤1 reticulin

➢ The most frequently mutated genes regulate chromatin remodelling, chromosome alignment and stability → “error prone” genome
# Acknowledgements

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