Landscape of genetic alterations in myeloproliferative neoplasms

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Solid and “liquid” tumors

Solid tumor

Leukemia
Blood production - hematopoiesis

Bone marrow

Peripheral blood

Tissue
Clinical presentations of hematological cancers

<table>
<thead>
<tr>
<th>Disease type</th>
<th>Number of subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myeloid leukemia (AML)</td>
<td>16</td>
</tr>
<tr>
<td>Myelodysplastic syndromes (MDS)</td>
<td>7</td>
</tr>
<tr>
<td>Myeloproliferative neoplasms (MPN)</td>
<td>8</td>
</tr>
<tr>
<td>Mixed MPN/MDS neoplasms</td>
<td>5</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Disease type</th>
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<tbody>
<tr>
<td>B lymphoblastic leukemia</td>
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<tr>
<td>T lymphoblastic leukemia</td>
</tr>
<tr>
<td>Mature B-cell neoplasms</td>
</tr>
<tr>
<td>Mature T-cell and NK-cell neoplasms</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
</tr>
<tr>
<td>Histiocytic and dendritic cell neoplasms</td>
</tr>
<tr>
<td>Post-transplant lymphoproliferative disorders</td>
</tr>
</tbody>
</table>
Hematopoiesis

Progenitor cells

Stem cells

Bone marrow

Blood

proliferation

differentiation
Progenitor cells

Bone marrow

Blood

Monoclonal hematopoiesis

Dominant stem cell clone

Progenitor cells

Healthy stem cells

BCR-ABL

Chronic myeloid leukemia
Monoclonal hematopoiesis

Progenitor cells

Dominant stem cell clone

Healthy stem cells

Bone marrow

Blood

Polycythemia vera

JAK2-V617F

Progenitor cells

Healthy stem cells

Bone marrow

Blood

Polycythemia vera

JAK2-V617F
Monoclonal hematopoiesis

- Progenitor cells
- Dominant stem cell clone
- Healthy stem cells
- Bone marrow
- Blood

RUNX1-ETO

Acute myeloid leukemia
Myeloid malignancies

Myeloproliferative neoplasms

- Chronic myelogenous leukemia – BCR-ABL
- Polycythemias
  - Polycythemia vera
  - Essential thrombocytopenia
- Primary myelofibrosis
- Chronic neutrophilic leukemia
- Chronic eosinophilic leukemia
- Hypereosinophilic syndrome
- Mast cell disease
- MPNs, unclassifiable

Acute myeloid leukemia

- de novo
- secondary

Myelodysplastic syndromes

Polycythemia vera

- BCR-ABL negative

Essential thrombocytopenia

Primary myelofibrosis
Myeloid Malignancies

- Polycythemia vera
- Primary myelofibrosis
- Essential trombocythemia

Leukemic transformation: 7%

MPN: Myeloproliferative neoplasms

Secondary Acute myeloid leukemia
General mutational landscape in MPN

Disease phase

Acute leukemia
Myelodysplastic
Normal
Myeloproliferative

Germline factors  Clonal drivers  Disease initiators  Adaptation to therapy  Disease progression
Oncogenic mutation of the Jak2 kinase

Kralovics et al., N Engl J Med, 2005
Exome sequencing identifies somatic CALR mutations in JAK2 and MPL nonmutated MPN

**Genes affected by somatic mutations**

<table>
<thead>
<tr>
<th>ID</th>
<th>Genes affected by somatic mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>CALR, COL11A1, MYBPC1, RNF10, C15orf2, LEPREL4, EIF4G1, TATDN2, DNMT1, SHROOM3, KIF12, BBS9, UBAC2</td>
</tr>
<tr>
<td>191</td>
<td>CALR, GAB2, PHF16, METTL11B</td>
</tr>
<tr>
<td>202</td>
<td>CALR, FGF6, ASAP1, ZNF521, DNMT3A, SLC17A9, KLHDC10, PIM2</td>
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<tr>
<td>296</td>
<td>CALR, C10orf71, PIK3R1, SNAPC4</td>
</tr>
<tr>
<td>333</td>
<td>CALR, BAI2, MYO16, WWOX, UNC13D, SMYD1, COL4A3BP, GPC2, ZNF462, LARP1, SF3B1</td>
</tr>
<tr>
<td>386</td>
<td>CALR, RYR3, UNK</td>
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</tbody>
</table>

Klapmfl et al. NEJM 2013
All mutations are generating frameshifts to the same alternative frame.
Somatic mutations in MPN

PV: n = 382

ET: n = 311

PMF: n = 203

- JAK2 mutant
- MPL mutant
- CALR mutant
- JAK2, MPL, CALR wild type

Genes: NF1, SUZ12, TET2, DNMT3A, FOXP1, IKZF1, ETV6, MDM4, ETS2, ERG, MYC, TP53, CBL, CALR, MPL, TET2, CBL
Clinical significance of genetic lesions

- Database of clinical phenotypes
- Sample bank
- Deep genotyping
- Validated mutations
- Clonal hierarchy
- Functional impact
- Drug sensitivity
- SNP array data = 1150
  - Exome-seq = 105
  - RNA-seq = 35
  - Whole-genome seq = 3

CeMM/MUW = 950
Univ. Pavia = 750
Univ. Florence = 850
Brno/Prague = 450
INSERM-Paris = 400
Univ. Basel = 250

N = 3650

Optimize therapy

Association with phenotype

Validation

Diagnosis
Prognosis

Database of somatic genetic variation

SNP array data = 1150
Exome-seq = 105
RNA-seq = 35
Whole-genome seq = 3
Genetic causes of disease progression in MPN

Chronic phase  ➔  Accelerated phase  ➔  Leukemic phase

SNP array genotyping

Deep sequencing

Whole genome, exome – somatic variants

Whole genome – structural variants
Cytogenetic lesions in MPN disease progression

Klampfl et al., Blood, 2011
MPN Tumor Suppressor Gene Network (deletion map from 1040 SNP arrays)
Does the list of somatically mutated genes overlap with chromosomal aberration hotspots?

79 genes ≥5 aberrations
Chromosome-centered genome sequencing
Models of leukemic transformation in MPN

**Mono-clonal**
- JAK2-V617F positive AML
- TP53
- del7q

**Bi-clonal**
- JAK2-V617F negative AML
- FLT3/NPM1/NRAS
- JAK2-V617F
  - *de novo* like
Exome sequencing in post-MPN AML

Chronic phase MPN

Accelerated phase MPN

T cells as the control tissue

Chronic phase sample

Mutations causing MPN

Mutations causing disease progression

Accelerated phase sample

Mutations causing leukemic transformation

Leukemic sample

Technical equipment

RNA sequencing for fusion detection

Secondary AML
Clonal reconstruction in post-MPN AML
Genetic changes in MPN

phenotype

Acute leukemia
Myelodysplastic
Normal
Myeloproliferative

Germline factors
Clonal drivers
del20q
del13q
TET2 mutations

Disease causing
JAK2
MPL
CALR

Progression associated
Adaptation to therapy

therapy
Mechanisms and pathways targeted by mutations in MPN

- MPL mutations
- JAK2 mutations
- CALR mutations
- SH2B3 mutations
- SH2B2 mutations
- LNK
- CBL defects (del11q)
- TET2 defects (del4q)
- SOCS deletions
- NRAS mutations
- NRAS deletions
- SOCS methylation
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