TET2 in MPN and MDS:
What is the role?

The role of TET2 in MPN and MDS

1- TET2 mutations in myeloid neoplasms
2- TET enzymes and 5-hydroxymethylcytosines
3- Function of TET2
4- TET2 mutations in the development of myeloid malignancies
5- Diagnostic and prognostic relevance of TET2 mutations
The role of TET2 in MPN and MDS

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Discovery of TET2 mutations in myeloid malignancies

Common 4q24 deletion in four cases of hematopoietic malignancy: early stem cell involvement?

Discovery of TET2 mutations in myeloid malignancies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Stage</th>
<th>Material</th>
<th>Karyotype</th>
<th>Interphase % del (4q24)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>APL-M4 diagnosis</td>
<td>BM</td>
<td>46, X, 6q (x2), 12p (x2)</td>
<td>96.5</td>
</tr>
<tr>
<td></td>
<td>CML (d/b 60 post diagnosis)</td>
<td>PB +/- BM (T lymphocytes)</td>
<td>46, X, 6q (x2), 12p (x2), 8p (x2)</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>CML (d/b 130)</td>
<td>BM</td>
<td>46, X, 13q, 14q</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CML (d/b 130)</td>
<td>PB +/- BM (B lymphocytes)</td>
<td>46, X, 13q, 14q, 15q (x2)</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>CML (d/b 280)</td>
<td>Skin biopsy (fibroblasts)</td>
<td>46, X, 20q</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>MDS diagnosis</td>
<td>BM</td>
<td>46, X, 7q, 8p, 11p (x2), 13q</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Nodal T NHL, diagnosis dia 40 post MDS diagnosis</td>
<td>PB +/- BM (T lymphocytes)</td>
<td>46, X, 7q, 8p, 11p, 13q, 15q</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>T NHL</td>
<td>Right cervical lymph node biopsy</td>
<td>46, X, 7q, 8p, 11p, 13q, 15q</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Corticosteroids, RMT (dia 300 post diagnosis)</td>
<td>BM</td>
<td>46, X, 7q, 8p, 11p, 13q</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Lymph node resection (dia 400 post MDS diagnosis)</td>
<td>PB +/- BM (T lymphocytes)</td>
<td>46, X, 7q, 8p, 11p, 13q, 15q</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>MDS (dia 450 post MDS diagnosis)</td>
<td>BM</td>
<td>46, X, 7q, 8p, 11p, 13q</td>
<td>ND</td>
</tr>
</tbody>
</table>
|         | Early clonal dominance in some patients with JAK2 mutant MPNs

Common 4q24 deletion in four cases of hematopoietic malignancy: early stem cell involvement?

Early clonal dominance in some patients with JAK2 mutant MPNs

![Diagram showing early clonal dominance in some patients with JAK2 mutant MPNs](Lisbon_MDS/MPN_2012)
Early clonal dominance in some patients with JAK2 mutant MPNs

- Early progenitors
- Committed progenitors
- Mature cells

JAK2 wild-type cells
- JAK2 V617F clone (majority of PV patients)
- JAK2 V617F clone (minority of MPN patients)

New mutations?

Discovery of TET2 mutations in myeloid malignancies

Chromosome 4 q24
- Minimal 4q24 region
- 500 kb

MDS
- RP11-1061M21
- RP11-356L5
- RP11-16G16

AML

MPN
- SNP-array
- LOH 4q
- Patient #1
- Patient #5

WT
- Myeloid cells

Acquired mutations in TET2

### TET2 mutations in myeloid malignancies

<table>
<thead>
<tr>
<th>Disease</th>
<th>TET2 mutant</th>
<th>total</th>
<th>%</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>All myeloid malignancies</td>
<td>725</td>
<td>3247</td>
<td>22,3</td>
<td>A meta-analysis of TET2 mutations shows a distinct distribution pattern in de novo acute myeloid leukemia and chronic myelomonocytic leukemia.</td>
</tr>
</tbody>
</table>

### TET2 mutations in myeloproliferative neoplasms

<table>
<thead>
<tr>
<th>MPN</th>
<th>Frequency of TET2 mutations</th>
<th>Frequency of TET2 mutations (details)</th>
<th>patients with TET2 mutation/total number of patients</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycythemia Vera</td>
<td>13.8%</td>
<td>17%</td>
<td>14/89 (357 MPN)</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>NA</td>
<td>[2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11%</td>
<td>13/100</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8%</td>
<td>1/14</td>
<td>[4]</td>
</tr>
<tr>
<td>Essential Thrombocythemia</td>
<td>8.5%</td>
<td>5%</td>
<td>3/57 (357 MPN)</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11%</td>
<td>NA</td>
<td>[2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8%</td>
<td>9/84</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7%</td>
<td>1/12</td>
<td>[4]</td>
</tr>
<tr>
<td>Primary Myelofibrosis</td>
<td>20.5%</td>
<td>17%</td>
<td>10/60 (357 MPN)</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8%</td>
<td>NA</td>
<td>[2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11%</td>
<td>3/16</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13%</td>
<td>4/16</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4%</td>
<td>1/12</td>
<td>[1]</td>
</tr>
<tr>
<td>Post-PV/ ET Myelofibrosis</td>
<td>23.5%</td>
<td>17%</td>
<td>14/89 (357 MPN)</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13%</td>
<td>14/89 (357 MPN)</td>
<td>[2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32%</td>
<td>13/100</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43%</td>
<td>12/64</td>
<td>[4]</td>
</tr>
<tr>
<td>Post-MPN AML</td>
<td>22.7%</td>
<td>17%</td>
<td>2/12</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32%</td>
<td>7/36</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13%</td>
<td>6/19</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25%</td>
<td>2/16</td>
<td>[6]</td>
</tr>
<tr>
<td>Systemic Mastocytosis</td>
<td>29%</td>
<td>29%</td>
<td>12/42</td>
<td>[8]</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia, non-chronic phase</td>
<td>13.3%</td>
<td>15%</td>
<td>6/40</td>
<td>[9]</td>
</tr>
</tbody>
</table>

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5- Diagnostic and prognostic relevance of TET2 mutations
TET oncogene family

Mohr et al, exp hem, 2010

TET protein function

TET protein function


Mercher et al., Médecine Sciences 2011; 12;27 : 1064-1066

Cycle of cytosine / 5mC / 5hmC


Dawlaty et al. Tet1 is dispensable for maintaining pluripotency and its loss is compatible with embryonic and postnatal development. Cell Stem Cell 2011 ; 9 : 166-75.


The role of TET2 in MPN and MDS

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Expression of TET2 in human hematopoietic cells


TET2 knock-down in human leukemic cell lines


TET2 knock-down in human CD34+ cells

Quantification of 5hmC in primary cells according to TET2 status

MPN

5-hmC antibody

Methylene blue (MB)


Other myeloid malignancies

TET2 function: hydroxylation of 5-methylcytosine -> DNA demethylation in hematopoietic stem cells?

Consequences on HSC biology?

BER

DNMTs

Polycomb Complex

Transcriptional repression

Transcriptional activation

Activator

Repressor

TET2 knock-down in human CD34+ cells

Early progenitors (CD34+/CD38-)

lymphoid → myeloid

Impairs terminal erythroid differentiation in vitro
**TET2 knock-down in human CD34+ cells**

Early progenitors (CD34+/CD38-)

lymphoid → myeloid

**Impairs terminal erythroid differentiation in vitro**

- **shRNA scramble**
- **shRNA TET2**

<table>
<thead>
<tr>
<th>% of clones</th>
<th>Lymphoid</th>
<th>Lymphoid/Myeloid</th>
<th>Myeloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Disturbs in vitro granulomonocytic differentiation

**Tet2 knock-down in mouse hematopoietic cells**

- Increase in monocytic differentiation

**Ko et al., Nature 2010;468:839 – 843.**
Tet2 knock-down in mouse hematopoietic cells


increase in monocytic differentiation


increase in Lin-Sca1+C-kit+ %

Tet2 gene disruption models in the mouse


Shide et al. TET2 is essential for survival and hematopoietic stem cell homeostasis. Leukemia. 2012 Apr 3. doi: 10.1038/leu.2012.94.
**Tet2 gene disruption models in the mouse**

Competitive repopulation assays

**Moran-Crusio, Cancer Cell 2011;20:11-24.**

Myeloid malignancies with features reminiscent of myelomonocytic leukemia

+ Aberrant lymphoid maturation and lymphoid malignancies

**Quivoron et al, Cancer Cell 2011;20:25-38.**
The role of TET2 in MPN and MDS

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*TET2* mutations are present in hematopoietic stem cells in myeloid malignancies

- SRC

- lymphoid/myeloid progenitors

- committed progenitors

- mature myeloid cells

Are TET2 mutations early events in the development of myeloid malignancies?
Patients with TET2 and JAK2 mutations

Single cell level analysis
- early progenitors (CD34+CD38-) in B/M/NK long term cultures
- committed progenitors (CD34+CD38+) in methylcellulose assay
- Genotyping of individual colonies

Multi-step model

Common early TET2 mutations
- Increased apoptosis
- Abnormal differentiation
- Selective advantage clonality

Increased proliferation
Normal differentiation
Increased proliferation
Blocked differentiation

Effects of common early mutations
Effects of specific mutations

HSC
Early progenitors
Committed progenitors
Mature cells
Normal hematopoiesis
MDS
MPN
AML

Lisbon_MDS/MPN_2012
-> *TET2* mutations frequently precede *JAK2* mutations

But the opposite order of events also occurs....

- **TET2** mutations frequently precede **JAK2** mutations

But the opposite order of events also occurs….

….. and complex multiclonal diseases may exist

---

**MPN transformation**

- **TET2 mutation**
- **Selective advantage clonality**
- **Progression / Transformation**
- **Increased proliferation**
- **Blocked differentiation**

---

Clonal evolution at the time of progression to AML


**PV phase**

- WT 17%
- V617F 66%
- TET2 550X 17%

JAK2^{V617F} -> TET2^{550X}

**Acute Leukemia**

- JAK2^{V617F} TET2^{550X} TET2 857 fs 77%
- 9pUPD
- JAK2 WT 16%
- JAK2 WT TET2 WT
- JAK2 WT TET2 857 fs 6%

JAK2^{V617F} -> TET2^{550X} -> TET2^{857 fs}
Molecular analysis of post MPN-AML


Additionnal mutations in NRAS, RUNX1, TP53, CBL, TET2, FLT3
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Diagnostic and prognostic relevance of TET2 mutations in myeloid malignancies

Many myeloid malignancies with TET2 mutations
Many other acquired genetic alterations in myeloid neoplasms → role of TET2 mutations?

- MPN

Polycythemia vera

Primary myelofibrosis

Diagnostic and prognostic relevance of *TET2* mutations in myeloid malignancies

- **MPN**

  ![Graph showing Kaplan-Meier plots for ASXL1 and TET2 mutations in MPN](Image)


Some patients with polycythemia vera are treated with pegylated interferon α-2a (IFNa)

High rate of hematologic response

High rate of molecular response

![Graph showing hematologic and molecular response to IFNa treatment](Image)

Some patients have both JAK2 and TET2 mutations


JAK2 exon 14

c.1849G>T, p.Val617Phe

Prior IFNa therapy

Month 24

Month 36

Good JAK2 molecular response

Wild type JAK2

Wild type TET2

Poor TET2 molecular response

Diagnostic and prognostic relevance of TET2 mutations in myeloid malignancies

Many myeloid malignancies with TET2 mutations

Many other acquired genetic alterations in myeloid neoplasms

→ role of TET2 mutations?

- MDS

Overall survival (Months)

Kosmider et al, Blood. 2009;114:3285-3291


Lisbon_MDS/MPN_2012
Diagnostic and prognostic relevance of TET2 mutations in myeloid malignancies

- CMML

Kosmider et al, haematologica. 2009; 94(12):1676-1681

Survival

Overall survival

Time from diagnosis (months)

CMML 1 and 2

Non-mutated TET2

Mutated TET2

n=25

p=0.95

n=18

Mutated TET2

n=21

p=0.01

n=10

CMML 1

Diagnostic and prognostic relevance of TET2 mutations

- AML

TET2 mutations : adverse prognostic factor

Metzeler et al J Clin Oncol 2011 29:1373-1381

Chou et al, Blood. 2011;118(14): 3803-3810

Weissmann et al, Leukemia doi:10.1038/leu.2011.326
Diagnostic and prognostic relevance of TET2 mutations

- AML

A Revised Risk Stratification

Cytogenetic Classification | Mutations | Overall Risk Profile
--- | --- | ---
Favorable | Any | Favorable
Normal karyotype or intermediate-risk cytogenetic lesions
FLI1/TET2-negative | Mutant NPM1 and DNMT3A or IDH1 | Intermediate
FLI1/TET2-negative | Wild-type ASXL1, MLL-PTD, SHH, and TET2 | Intermediate
FLI1/TET2-positive | Mutant CEBPA | Intermediate
FLI1/TET2-positive | Wild-type MLL-PTD, TET2 and DNMT3A and KDM1A-negative | Intermediate
FLI1/TET2-negative | Mutant TET2, MLL-PTD, DNMT3A or KDM1A-negative | Unfavorable
FLI1/TET2-positive | Mutant TET2, MLL-PTD, DNMT3A or KDM1A-negative, or bcr/abl | Unfavorable
Unfavorable | Any | Unfavorable


B Test Cohort

C Validation Cohort

Conclusion

Mutations in TET2 → low 5-hmC content in primary cells from patients with myeloid malignancies

Function of TET2 not fully elucidated: differences between the types of mutations? Link with other enzymes / mutations (IDH1/2)? Consequences on DNA methylation?

TET2 mutations in other cancers: lymphoid malignancies: role in hematopoietic stem cell biology?

Prognostic value to be integrated in a comprehensive genetic analysis of myeloid malignancies