What can we learn from hemato-oncology?

Myeloproliferative neoplasms: Interferon-alpha to eliminate leukemia propagating cells?
Nothing to disclose
The hierarchical organisation of the hematopoietic tissue might protect from somatic evolution.

More susceptible to somatic evolution.
Chronic myelomonocytic leukemia (CMML)
Chronic myelomonocytic leukemia
Single cell analyses in bone marrow cell compartments

Stem cells and progenitors sorted at the unicellular level
Grown in colonies
Analyzed by mutation specific PCR

Itzykson et al, Blood 2013
Itzykson et al, J Clin Oncol 2013
Clonal architecture in chronic myelomonocytic leukemia

Early clonal dominance

<table>
<thead>
<tr>
<th></th>
<th>HSC</th>
<th>CMP</th>
<th>GMP</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>56</td>
<td>77</td>
<td>14</td>
</tr>
<tr>
<td>TET2</td>
<td>2%</td>
<td>2%</td>
<td>3%</td>
</tr>
<tr>
<td>SRSF2</td>
<td>2%</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>NRAS</td>
<td>94%</td>
<td>97%</td>
<td>100%</td>
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</table>

TET2 N1156I; SRSF2 P95H; NRAS A59G

Linear acquisition of mutations in the HSCs

Clonal sweep of the most mutated cells with differentiation
Clonal architecture in chronic myelomonocytic leukemia

Some branching events due to mitotic recombination

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Sample Size</th>
<th>SRSF2 Events</th>
<th>CBL Events</th>
<th>TET2 Events</th>
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<td>2%</td>
</tr>
<tr>
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<td>n=116</td>
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<td>2%</td>
<td>2%</td>
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<td>3%</td>
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<tr>
<td></td>
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<td></td>
<td>8%</td>
<td>2%</td>
</tr>
<tr>
<td>CMP</td>
<td>n=56</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
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<tr>
<td></td>
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<td>2%</td>
<td>3%</td>
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<tr>
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<td></td>
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<td>94%</td>
</tr>
<tr>
<td>GMP</td>
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<td></td>
<td></td>
<td>42%</td>
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<td>58%</td>
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</table>
Current therapies reduce the size and fitness of the most mutated clone w/o eradication.

Number of studied cells: 56, 72, 14, 88, 55, 57

Percentage of cells:
- MPP: IC
- CMP: HMA
- GMP: HSCT/Relapse

Red: 3 mutations
Orange: 2 mutations
Yellow: 1 mutation
White: no mutation

Itzykson R et al, 2013
A non-cytotoxic treatment reduces the fitness of the most mutated clone w/o eradication

Number of studied cells: 55, 110, 124, 222, 56, 24

Percentage of cells

- CD34+/CD38-
- CMP
- GMP

EPO

Red: 3 mutations
Orange: 2 mutations
Yellow: 1 mutation
White: no mutation

TET2
SRSF2
CBL
Chronic myeloid leukemia (CML)
Chronic myeloid leukemia (CP-CML)
TKI cessation & curability are important issues

Treatment-free survival
at 60 months
40%

Treatment-free survival
at 42 months
42%

STIM study
Mahon FX et al, The Lancet Oncology 2010
Mahon FX et al, update ASH 2013

TWISTER study
Ross DM et al, Blood. 2013

Relapses are sensitive to imatinib
Treatment-free remission
Loss of Major (MMR) versus Complete (CMR) molecular response?

MMR loss could be used as a criterion for discontinuation studies

Could we predict the stable MMR pot-discontinuation?
Potential savings in France: 9 million €/y

Combination of IFN-α with Imatinib improves the molecular response

Cumulative incidences of a superior molecular response

BRC-ABL monitoring in treatment-free remission


Also

Palandri F et al, Haematologica 2010
Simonsson B et al, Blood 2011

Interferon-alpha combination with imatinib and maintenance improves relapse-free survival.

Burchert A et al. JCO 2010
A minority of CP-CML patients concerned by TKI monotherapy discontinuation

**Quiescence-dependent persistence of BCR-ABL HSCs?**

Breaking dormancy of slowly cycling subclones of CML stem cells?

**BCR-ABL kinase-independent persistence?**

Targeting BCR-ABL kinase independent properties

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**Potential targets**

- PML
- Smo / Hedgehog
- Autophagy inhibitors
- HDACi
- Bortezomub
- PP2A phosphatase
- PTH

Essers MAG & Trupp A, Mol Oncol 2010

Corbin AS et al, JCI 2011

Ito K et al, Nature 2008
Dierks C et al, Cancer Cell 2008
Bellodi C et al, JCI 2009
Zhang B et al, Cancer Cell 2010
Heaney NB et al, Blood 2010
Nevani P et al, JCI 2013
Enforced proliferation in the presence of IFN-1s depletes HSCs

Steady state
Quiescence
HSC
Survival
Relaxation of quiescence
IFN-1s
Transient Proliferation
HSC
Apoptosis
Acute phase
Restoration of quiescence
IFN-1s
Relapse into quiescence
HSC
Survival
Chronic phase
Culture Transplant 5-FU
Forced Proliferation
HSC
Apoptosis

BCR-ABL-negative myeloproliferative neoplasms
Cell signaling activation in BCR-ABL negative myeloproliferative neoplasms

TK activation

BCR-ABL  PDGFR  KIT

CML  MLN-Eo  Mastocytosis

JAK2 activation

JAK2 exon 12  JAK2 V617F  MPL W515  CALR Exon 9  CSF3R T618I

ET  ET  ET

PV  PV

MF  MF  MF  MF  CNL
JAK2 kinase inhibitors: current situation

The JAK1/2 inhibitor, ruxolitinib is approved for the treatment of patients with myelofibrosis

JAK inhibitors effectively reduce splenomegaly and high cytokine levels in patients, leading to improvements in quality of life.

JAK inhibitors have not been successful in eliminating the mutant clone in a majority of patients: no impact on mutated allele burden

Combination therapies that target JAK2 and other components of the JAK-STAT pathway along with JAK inhibitors have to be tested
**PEG-INFα-2 in PV and ET patients**

IFNα is an efficient treatment for PV and ET patients, whose development has been limited by its adverse events and cost.

Pegylated-IFNα-2a has shown promising results in pilot studies:
- Quintas Cardama et al, *JCO*, 2009

To explore the safety and long-term benefits of Peg-IFNα, a multicenter, open label, phase 2 study of peg-IFNα-2a was launched in PV:
  - Primary end-point: RR
  - 40 patients, follow up 75 months
PEG-IFNα2 decreases JAK2V617F allele burden

![Box plot showing decrease in V617F allele burden over time](image)

<table>
<thead>
<tr>
<th>%V617F</th>
<th>M0</th>
<th>M12</th>
<th>M24</th>
<th>M36</th>
<th>M48</th>
<th>M60</th>
<th>M72</th>
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<tr>
<td>Median</td>
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<td>25%</td>
<td>5%</td>
<td>5%</td>
<td>10%</td>
<td>6%</td>
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<td>0 - 50</td>
<td>0 - 40</td>
<td>0 - 40</td>
<td>0 - 45</td>
<td>0 - 45</td>
</tr>
</tbody>
</table>
With a median follow-up of 75 months

Complete hematological responses 78%

Complete molecular responses 30%

Treatment discontinuation with stable MR 27% (median 31 months)
**INF-α impacts $JAK2^{V617F}$ PV clonal architecture**

Homozygous clones outcompete heterozygous clones during myeloid differentiation in progenitors.

IFN-α preferentially eliminates homozygous cells.

Additionnal mutations might not affect the response to IFN-α.

Hasan et al., Leukemia 2013
See also Quintas-Cardama et al, Blood 2013
IFN-α eliminates

TET2 mutated / JAK2 mutated clones, not TET2 mutated / JAK2 wildtype clones

Clonal hematopoiesis
Pre-neoplastic step?

PV, ET
Does IFN-α eliminate JAK2 V617F disease propagating cells?

JAK2+/V617F KI mice that faithfully model human PV evolving into secondary myelofibrosis

murine rIFNa (30K IU), SID, IP, 6 weeks post BMT during 13 weeks

Interferon α

30% JAK2V617F KI

30% JAK2V617F KI

Vehicle

Hasan et al., Blood 2013
Does IFN-α eliminate disease propagating cells?

Interferon-α normalized the spleen size
induced hematological and molecular response
including in immature bone marrow cells

Hasan et al., Blood 2013
IFN-α eradicates the disease initiating cells

Hasan et al., Blood 2013

Secondary recipients transplanted with the IFNα-treated BM do not develop the disease
Combine JAK inhibitors and IFNα?  
TPO and IFNα signaling share similarities

Are HSCs addicted to JAK2 signalling that IFN blunts?

Does JAK2 mutation prime HSCs to IFN-α?
In chronic myeloid neoplasms

1 – Most of the current therapies do not eradicate mutated stem cells
   - Decrease the fitness of the most mutated cells
   - Or eliminate mutated progenitors and mature cells

2 – CML stem cells might not be addicted to BCR-ABL kinase activity
   - Combination of Peg-IFN-α2 improves the efficacy of TKI in CML.

3 – JAK2 V617F provides a competitive advantage to progenitors
   - IFN-α, the only strategy to eradicate JAK2 mutated stem cells

4 – Combination of PEG-IFN-α2
   - with JAK2 inhibitors (Synergistic or antagonistic?)
   - with other drugs (e.g., nutlin-3)
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