DISCLOSURE

- I have the following financial relationships:
  Contracted Research: Celgene
Ineffective Erythropoiesis in MDS

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MDS Foundation Symposium ASH 2019
• Which subtypes of MDS frequently show hyperplastic erythropoiesis?

• MDS with fibrosis
• 5q- syndrome
• MDS with ring sideroblasts
• CMML
Learning objectives

• The impact of anemia
• Erythropoiesis
• Mechanisms of anemia in MDS
• Modeling erythropoiesis in SF3B1 mutated MDS with ring sideroblasts
• Targeting ineffective erythropoiesis
Symptomatic anemia and chronic transfusion need

- 309 consecutive MDS and MDS-MPN patients
- 11,350 red cell and 1956 platelet units over 777 person-years of follow-up,
- Overall transfusion intensity of 14.6 and 2.5 units/person-year, respectively

Lower-risk MDS — RBC transfusions over time

EU MDS Registry data

QoL significantly impaired in anemic patients

ESA treatment before TD prolongs time to first transfusion

Erythropoiesis

Growth factors:
- SCF, IL-3, IL-6

Transcription Factors:
- GATA2
- GATA1

Receptors:
- CD34
- CD117
- CD45
- CD71
- CD36
- CD105
- CD235a and hemoglobin

Factors:
- HSC
- MEP
- BFU-E
- CFU-E
- PRO-EB
- BASO-EB
- POLY-EB
- ORTHO-EB
- RETIC
- RBC

Transcription:
- GATA2
- GATA1

Growth factors:
- EPO
- IRON

Factors:
- SCF, IL-3, IL-6
- EPO
- IRON

Transcription:
- GATA2
- GATA1
Bone marrow microenvironment
Erythroblastic islands

Young reticulocyte
Extruded nucleus
Enucleating erythroblast
Early erythroblast
Macrophage
Nuclear phagocytosis
Late erythroblast
Patterns of ineffective hematopoiesis in MDS

- Hyperplastic erythropoiesis
- Hyperplastic bone marrow
- Low BM blasts
- High BM blasts
- Hypoplastic bone marrow
- Cytogenetic aberrations
- Hypoplastic erythropoiesis

Ineffective hematopoiesis
Anemia and aberrant erythropoiesis
the most common feature in MDS

- Underrepresented erythropoiesis (few erythroblasts)
  - Hypoplastic MDS, low general cellularity,± immune-mediated cytopenia
    - As examplified by many germline conditions; DBA, GATA2 mut, etc
    - But also in immune-mediated aplastic anemia
  - Advanced MDS, increased blast percentage (MDS EB-2)
  - Mixed MDS-MPN, often maximal cellularity
  - MDS och MDS-MPD with fibrosis
  - 5q- syndrome, in particular in later stages

- Hyperplastic ineffective erythropoiesis
  - Normo / hyperplastic bone marrow with erythroid dysplasia / apoptosis
  - MDS-RS
  - Some forms of MDS-SLD and MLD, and MDS EB-1
  - Myelodysplastic syndrome with erythroid predominance
Low-risk MDS with isolated del(5q)

- Hyperplastic erythropoiesis
- Hyperplastic bone marrow
- Low BM blasts
- Hypoplastic bone marrow
- Normo/hypoplastic erythropoiesis
- High BM blasts
- Mutations
- Cytogenetic aberration del(5q)
The 5q- syndrome

- 3-4% of all MDS, female preponderance
- Hypolobated megakaryocytes, thrombocytosis
- Isolated deletion of 5q
  - involving 5q32-33
  - ~ 1.5Mb, 44 gene
  - Including RPS14, CSNK1A1, and miR-145
- Del(5q) alone causes macrocytic anemia
- No progression to higher-risk MDS / AML unless clonal evolution (mainly TP53, RUNX1, additional cytogenetic aberrations)

5q-syndrome: cellular and molecular characteristics

- Stem cell disorder
- Haploinsufficiency of RPS14 mediates erythroid apoptosis through p53 activation
- Haploinsufficiency of CSNK1A1 explains clonal dominance
- Combined haploinsufficiency of RPS14, CSNK1A1, and miR-145 recapitulate the clinical phenotype
- Lenalidomide (LEN) induce CR through inhibiting the 5q clone
- However, del(5q) HSC, megakaryocytes, and TP53 mut cells persist during LEN-induced CR

MDS with ring sideroblasts (MDS-RS)

- Hyperplastic erythropoiesis +RS
- Hyperplastic bone marrow
- Low BM blasts
- High BM blasts
- Hypoplastic bone marrow
- Hypoplastic erythropoiesis
- SF3B1 Mutation
- Cytogenetic aberrations

Björkman S, Blood 1956
- Hyperplastic erythropoiesis
- Ring sideroblasts (RS) in the bone marrow
- Severe anemia, transfusion dependency and low risk of transformation to AML
- $SF3B1$ mutations in >80% of patients

**MDS-RS**

- HSC
- MEP
- BFU-E
- CFU-E
- PRO-EB
- BASO-EB
- POLY-EB
- ORTHO-EB

Mitochondrial dysfunction in MDS erythroblasts

- Marked cytochrome c release
- Caspase-9 and 3 activation
- Erythroblasts express G-CCF R
- G-CSF inhibits cyt c release
Early Erythroblasts in RARS express an aberrant form of mitochondrial ferritin (MtF)

MtF$^+$ Cells (median)

- **RARS**
- **RA**
- **NBM**

Cazzola et al, Blood 2003, Tehranchi et al, Blood 2005
EPO+G-CSF act through unspecific but highly effective inhibition of erythroid apoptosis
- does not inhibit iron accumulation
- Increases release of aberrant RBC
Spliceasome mutations in MDS

Papaemmanuil, NEJM, 2011
Somatic SF3B1 Mutation in Myelodysplasia with Ring Sideroblasts

Frequent pathway mutations of splicing machinery in myelodysplasia
Kenichi Yoshida*, Masashi Sanada*, Yuichi Shiraki*, Daniel Nowak*, Yasunobu Nagata*, Aiko Sato-Otsubo†, Ayana Koni†, Masao Nagasaki*, George Chalkidis*, Yutaka Suzuki†, Masashi Tomoyuki Yamaguchi†, Makoto Otsu†, Naoshi Ohara†, Mamiiko Sakata†-Yanagimoto†, Ken Ishii†, Florian Nolte*, Wolf-Karsten Hofmann*, Shoichiro Miyawaki*, Sumio Sugano†, Claudia Haerfler, Lee-Yung Shih*, Torsten Haerfler†, Shigeru Chiba†, Hiromitsu Nakauchi†, and Satoru Miyano*
The consequences of *SF3B1* mutations affects erythropoiesis during terminal maturation

**ABC7**

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*Papaemmanuil NEJM 2011, Conte, Br J Haematol, 2015,*
The iron transporter *ABCB7* in RARS medates erythroid failure

Altered exon usage and nonsense-mediated decay

*ABCB7* mutated in XLSA-T, these patients have RS in BM

**CFU data**

- ShRNA in normal BM induces a RARS phenotype
- Overexpression in RARS BM restores a normal phenotype phenotype

Nikpour M, Br J Haematol. 2010, Nikpour Leukemia 2013
**SF3B1** mutations associated with lower-risk MDS with ring sideroblasts

*Overall Survival*

HR .35, P=.007

HR .32, P=.005

HR .26, P=.045

Malcovati, Blood, 2015
Experimental models of MDS-RS

- SF3B1 mutation conditional knock-in mice do not support RS formation
  - Murine orthologues of genes associated with RS in humans not mis-spliced
  - Poor conservation of splice sites between the species

- CD34+ suspension cultures do not mimic the mature MDS-RS phenotype
  - Proliferation of erythroblasts
  - Aberrant mitochondrial ferritin accumulation
  - Limited production of mature red blood cells or RS
What is the cell of origin in MDS-RS?

**In Vitro**
Long Term Co Cultures

| LTC-CFC | Functional assays | CFU |

**In Vivo**

| NSG | Engraftment and RS formation |
| 8 | 12 | 24 |

Targeted sequencing

Mortera Blanco, Blood, 2017
Recurrent *SF3B1* mutations are a part of the lymphoid lineage.
Only RARS HSC give rise to LTC-IC and engraft in NSG mice

<table>
<thead>
<tr>
<th>Patient n.</th>
<th>% of RS of total cells (NSG mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>19.1</td>
</tr>
<tr>
<td>11</td>
<td>4.3</td>
</tr>
<tr>
<td>13</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Mortera Blanco, Blood, 2017
Modelling of terminal erythropoiesis *in vitro*

- Polyurethan foam, pore size of human bone marrow
- Coated with collagen type I
- Cut into scaffold cubes that could be aspirated and fixated

*Mortera-Blanco et al. Biomaterials 2010 and 2011, Elvarsdottir, Leukemia 2019*
3D culture facilitates expansion of CD34+ cells with self-renewal capacity.
CD34+ 3D facilitates erythropoiesis more effectively than MNC cultures

Elvarsdottir, Leukemia 2019
Erythroblastic islands detected after 4 weeks of MNC and CD34+ 3D cultures

Elvarsdottir, Leukemia 2019
Erythroblastic island inside fixed scaffold

Nucleus (Draq5)
Macrophage/monocyte (CD68)
Erythrocyte (CD235a)
Maintenance of SF3B1 VAF and RS formation
Scaffold cultures now used to study targeted drugs

Elvarsdottir, Leukemia 2019
New treatment options for MDS
TGF-β superfamily ligand traps

- Increased hemoglobin levels and RBC counts in
  - Post menopausal women
  - MDS murine model

- Phase 2/3 studies in MDS patients
  - Significantly increased hemoglobin and reduced transfusion burden
  - Greater response rate in MDS-RS patients

Cytokine measurements in extracted medium of scaffolds

<table>
<thead>
<tr>
<th>Category</th>
<th>Cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative regulation of erythropoiesis</td>
<td>IFNγ, IL-13, IL-1α, IL-6, MIP-1α, TNFα</td>
</tr>
<tr>
<td>Promote erythropoiesis</td>
<td>G-CSF, GM-CSF, IL-10, IL17A</td>
</tr>
<tr>
<td>Elevated in serum of MDS patients</td>
<td>GDF11, TGF-β1, VEGF</td>
</tr>
<tr>
<td>Related to other hematological malignancies</td>
<td>TGF-α, IL-9, IL-8</td>
</tr>
<tr>
<td>Other</td>
<td>Flt-3L, MCP-3, MCP-1, MIP-1β, TNFβ</td>
</tr>
</tbody>
</table>

**Graphs:**
- **GDF11**: Peaks in NBM MNCs and MDS-RS MNCs.
- **VEGF**: Peaks in NBM MNCs and MDS-RS MNCs.
- **MCP-1**: Peaks in MNCs NBM and CD34+ NBM.

*Note: PG/mL = picograms per milliliter.*
Research question

Which cells mediate the response to ESAs and other therapeutic compounds in SF3B1 MDS with ring sideroblasts?
Experimental workflow

MDS-RS patients

At diagnosis

During response

At relapse

FACS sorting of HSPCs

ddPCR for SF3B1 mt VAF

Group 1
Normal donors
n = 5

Group 2
Untreated
(Stable anemia without treatment)

n = 7

Group 3
Responders to EPO
n = 5

Group 4
Non-responders to EPO
n = 5

CD34+ CD38+

CD34-PE/Red
CD90-PE
CD123-PECy7

HSC
MPP/LMPP
CMP
GMP
MEP

Experimental workflow

MDS-RS patients

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CD123-PECy7

HSC
MPP/LMPP
CMP
GMP
MEP

Isabel Hofman poster # 2993, Sunday
MDS-RS with stable anemia have lower VAF% in CD34+ BM cells.

**VAF% in total BM**

- Untreated
- Treated

VAF% in CD34+ BM cells

- Untreated
- Treated

***

VAF% of SF3B1 %

- Untreated
- Treated

Unpublished data

Isabel Hofman poster # 2993, Sunday
Concluding remarks

- Severe anemia has an immense impact on the quality of life of elderly MDS patients
- Lower-risk MDS patients are high-consumers of packed RBC
- The mechanism of ineffective erythropoiesis differs largely between MDS subtypes and is related to response to various treatments
- *SF3B1* mutations arise at the primitive lymphomyeloid stem cell stage and lead to terminal erythroid apoptosis
- New *in vitro* and *in vivo* models of erythropoiesis in MDS can help to dissect cellular and molecular disease mechanisms as well as the effects of pro-erythroid therapies
- A key research question is whether pro-erythroid treatment mainly affects mutated or wild-type bone marrow cells

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Vetenskapsrådet
CME question

- Which subtypes of MDS frequently show hyperplastic erythropoiesis?
  
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  - 5q- syndrome
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  - CMML