Myelodysplastic syndrome - from bone marrow studies to new therapy

EAHP Uppsala 2010

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Pathobiology of MDS

Genetic alterations

Bone marrow microenvironment

Epigenetic alterations

Adapted from Reya et al. Nature (2001)
The Central Conundrum in MDS

- progenitor apoptosis
  - anemia
  - neutropenia
  - thrombocytopenia
  - other "penia"

Dysfunctional mature blood cells

Adapted from Reya et al. Nature (2001)
WHO classification 2008

- Refractory cytopenia with unilineage dysplasia
  - Refractory anemia (RA)
  - Refractory thrombocytopenia (RT)
  - Refractory neutropenia (RN)
- Refractory anemia with ring sideroblasts
  - Unilineage erythroid dysplasia
- Refractory cytopenia with multilineage dysplasia
  - ± ring sideroblasts
- Refractory cytopenia with excess of blasts
  - RAEB-1, <10% marrow blasts
  - RAEB-2, 10-19% marrow blasts
- MDS associated with isolated del(5q)
- MDS-unclassified

- 1-2 cytopenia, 1 dysplasia, <5% blasts
- ≥15% ring sideroblasts (RS), <5% blasts, erythroid dysplasia
- >2 cytopenias or ≥2 dysplasias, <5% blasts, ±RS
- 5-9% blasts, no Auer rods
- 10-19% blasts, ±Auer rods
- <5% blasts, isolated del(5q) abnormality
- Only cytogenetic abn, or non-interpretable morphology, eg in case of fibrosis
MDS and “adjacent” disorders

AA-PNH

PNH

Hypoplastic MDS

Mixed MDS-MPD

MDS

MPD

MDS-AML

AML
**Improved survival in EPO-G-CSF treated patients**

Cox regression curves adjusted for major prognostic variables

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**Survival**

- **HR**: 0.61
- **P**: 0.002

**AML evolution**

- **HR**: 0.89
- **P**: 0.66

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**Jädersten, JCO, 2008**

**Nordic EPO-G cohort**

- N=129 1990-99

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Eva Hellström Lindberg, 20107

Cumulative response, 129 GE treated MDS, 1990-99

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<th>Ery Response</th>
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<td>RA</td>
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<td>RARS</td>
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<td>RAEB</td>
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FAB subtype

RA

RARS

RAEB

Cumulative Response

Time (months)

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<th>Resp. duration, median (IQR), months</th>
<th>P (resp dur)</th>
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<td>RARS 28 (8-116+)</td>
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<td>RCMD-RS 25 (17-94)</td>
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WHO-RARS characteristics

- Clonal disease - limited progression
- Hyperplastic erythropoiesis - ringsideroblasts
- Hypochromic macrocytic red cells
- Accumulation of mitochondrial ferritin
- Mitochondrial cytochrome c release

What are the underlying genetic defect(s) causing sideroblast formation and clonal advantage in RARS?

Tehranchi et al., Blood 2003 & 2005
Cazzola et al., Blood 2003
Ljung et al., Haematologica 2004

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Mitochondrial dysfunction in MDS erythroblasts

- Marked cytochrome c release
- Erythroblasts express G-CSF R
- Early MtF accumulation (day 4)
- ROS production
- G-CSF inhibits cytochrome c release

Tehranchi et al., Blood, 2003 & 2005 CCR 2005
G-CSF inhibits intrinsic apoptosis - and anemia - in RARS

Clonality and JAK2 mutations in RARS-T

- >90% of neutrophils and CD34+ cells in RARS are clonal by HUMARA
- 50% of RARS-T have JAK2 mutations, impact on prognosis not clear
- The JAK2+ clone constitutes a subset of all cells
- RARS-T can also have TET2 mutations, impact on prognosis not clear
GEP analysis: *abcb7* may be a key gene in RARS
**ABCB7** expression during erythropoiesis

- Expression inversely correlated to % ring sideroblasts in MDS
- Not mutated or methylated in acquired RARS
- Expression decreases with erythroid differentiation in RARS
- Transfer of iron from mitochondria to cytosol
- Maturation of cytosolic Fe/S enzymes.
- Mutated in XLSA with telangiectasia

Pellagatti 2008, Nikpour, Br J Haem 2010
Modulation of Abcb7 in RARS and NBM CD34+ cells

Colony cultures
- Erythroid
- Myeloid
- Total

Overexpression RARS CD34+

- Overexpression in K562 enhances erythroid differentiation
- Overexpression in RARS decreases MtF expression and increases erythroid colonies
- Abcb7 -/- not consistent with hemopoiesis
Transcriptome analysis - RARS and NBM

- Four cDNA samples from erythroid culture
- SOLiD data

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5q- syndrome
Low-risk MDS with isolated del(5q)

- Clonal advantage of del(5q) HSC pool 90-100 %
- RPS14 haploinsufficiency may explain anemia
- Why clonal advantage of del(5q) HSC?
- Why progression / AML?
- Effects of lenalidomide

Effects of lenalidomide in low-risk MDS with del(5q)

- MDS-001 phase II (43 pts)
  - Isolated del 5q, 83% erythroid response
- MDS-003 randomized phase II (148 pts)
  - 67% TI
  - 45% complete cytogenetic response
- MDS-004 randomized phase II (not reported)
  - Same efficacy
- Licensed in US 2005
- Not approved by EMEA 2008
  - Worry that there might be an increased risk for leukemic evolution

*List AF, et al. NEJM 2005 and 2006*
Lenalidomide in del5q MDS
- response duration 001 and 003

No cure, median response duration 2 years

min, max = 0.2, 4.8+ years
73 TI response ≥ 1 years
47 TI response ≥ 2 years
50 ongoing responders

List, EHA Education program 2008

Eva Hellström Lindberg, 2010
Lenalidomide has limited effect on HSC

Tehranchi et al, (Jacobsen) NEJM 2010
How does lenalidomide work in 5q- syndrome?

- LEN has a direct growth inhibitory effect on 5q- progenitors while leaving normal progenitors unaffected.
- LEN exposure of CD34+ del(5q) cultures upregulates one of the genes within del CDR on 5q31; SPARC.
- SPARC is a tumor suppressor gene with anti-adhesive function.

_Pellagatti & Jädersten, 2007. PNAS 104_
Hypotheses for the pathogenesis of 5q- MDS

MDS stem cells

BM stromal cell

Chemokines and hematopoietic growth factors

Adhesion molecules

SPARC? Other genes?

Erythrocyte

10-20%

80-90%

Apogetic progenitors

RPS14?

Scharenberg and Jädersten, ongoing work

Eva Hellström Lindberg, 2010
Expression of adhesion markers in HSC

- CD11a
- CD18
- alpha-4-integrin
- alpha-5-integrin

Colors:
- NBM
- 5q During LEN response
- 5q Pre LEN
- FMO control
Hypothesis for the action of LEN in 5q- syndrome

- decreased expression of SPARC leads to increased adhesion of del(5q) HSC to the niche
  - increased self-renewal (p21? BMI1?)
  - may explain the clonal advantage

- lenalidomide abrogates this clonal advantage via its increase in SPARC expression
  - decrease in adhesion
  - increase in apoptosis
Working model for immune dysregulation in MDS

CD8 activation clonal expansion

T cell

MDS clones

T cell-mediated cytotoxicity

TNF

Apoptosis

pancytopenia

Barrett and Sloand 2008
Immunosuppressive therapy in MDS

• ATG +/- cyclosporin A
  – 30% responses, median duration around 3 years
  » Lower RR in unselected patient cohorts
  – May give tri-lineage responses
  – A small subgroup will remain in CR

• Indications
  – RA or RCMD
  – Transfusion dependent anemia / trc-penia, or severe neutropenia
  – Below 70 years – high toxicity and poor response in elderly**

*Sloand, JCO 2008  **Broliden, Haematologica 2006
Alemtuzumab (Campath-1H)

- Anti-CD52 Antibody, more effective than ATG

- Murine hypervariable regions fused into human IgG1

- CD52 expressed:
  - Normal, malignant B and T cells
  - NK cells, dendritic cells
  - Monocytes, macrophages
  - Plasma cells, Eos

- No CD52 expression on:
  - RBCs, platelets
  - Hematopoietic stem cells

Ravandi and O’Brien, Cancer Invest. 2007 24: 718-725
Hernández-Campo PM, Cytometry B Clin Cytom. 2006 70:71
Alemtuzumab responses in INT-1/2 risk MDS

31 patients treated

10 mg IV/day for 10 days

INT-1

INT-2

15/20 (75%)

3 mo

6 mo

1 yr

17/23 (74%) 2/7 (29%)

8 of 8 (100%) 4/8 (50%) normal counts

7/8 transfusion independent

15/20 (75%) 1/5 (20%)

2/4 (50%)
Probability of responses to Campath-1H

Sloand, JCO 2010
Epigenetic control of gene expression

• Meiotically and mitotically heritable changes in gene expression
  – DNA methylation
  – Histone deacetylation

• Changes in gene expression by translational repression by non-coding micro RNAs

• Potent tumor mechanisms affecting cell cycle control genes and tumor suppressor genes
Frequently methylated tumor suppressor genes in MDS

- Cell cycle regulation
  - P15\(^{\text{ink4b}}\)
- Cell adhesion
  - CDH1
  - Alpha cathepin
- Apoptosis
  - HIC1
  - DAP Kinase
- Inhibitors of growth signaling
  - RASSF1A, SOCS-1
- Transcriptional repressors
  - FHIT
Promoter DNA hypermethylation of more than one analysed gene is associated with a poor response to induction CT

No difference in methylation status between MNC and CD34+ cells

Grövdal et al, CCR, 2007
Azacitidine Treatment Prolongs Overall Survival in Higher-Risk MDS Patients Compared with Conventional Care Regimens: Results of the AZA-001 Phase III Study (ASH 2007)

AZA 75 mg/m²/d x 7 d q28 d

Median no of cycles: 9

Screening/Central Pathology Review

Investigator CCR Tx Selection

Randomization

CCR

• Best Supportive Care (BSC) only
• Low Dose Ara-C (LDAC, 20 mg/m²/d x 14 d q28-42 d)
• Std Chemo (7 + 3)

BSC was included with each arm
Tx continued until unacceptable toxicity or AML transformation or disease progression

Fenaux et al, Lancet Oncology 2009
Overall Survival: Azacitidine vs CCR
ITT Population

Log-Rank  \( p=0.0001 \)
HR = 0.58 [95% CI: 0.43, 0.77]
Deaths: AZA = 82, CCR = 113
Difference: 9.4 months

Fenaux et al, Lancet Oncology 2009
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Acknowledgements

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Alf Grandien
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Maryam Nikpour
Michael Grövdal
Christian Scharenberg
Lalla Forsblom
Monika Jansson
Ramin Tehranchi
Jan Schmidt-Mende

The Nordic MDS Group
Martin Jädersten
Michael Grövdal
John Radcliff, Oxford Jackie
Jackie Boulwood
Andrea Pellagatti
University Pavia
Mario Cazzola
Luca Malcovati
King´s College Hospital
Ghulam Mufti
University Hannover
Brigitte Schegelberger
Gudrun Göhring