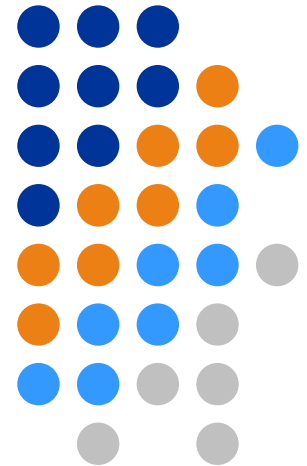


Introduction to diagnostics in MDS

Arjan van de Loosdrecht 
Amsterdam

Masterclass Flow Cytometry in MDS

September 5, 2012



The Myelodysplastic Syndromes



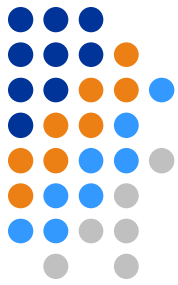
- MDS; a heterogeneous spectrum of myeloid disorders characterized by:
 - Ineffective hematopoiesis
 - Dysplastic cells in peripheral blood and BM
 - Low blood cell counts ([pan-] cytopenia)
 - progression to acute myeloid leukemia (AML)

Epidemiology of the myelodysplastic syndromes



- Epidemiology of MDS
 - Peak incidence 60–90 years of age;
 - median age: 74 y (EU-MDS registry)
 - 3.6-12.6 per 100,000: > 20 per 100,000 at 70 years
- Typical MDS patient
 - Elderly
 - Has shortened life expectancy, even with low-risk MDS → ineffective hematopoiesis
 - male predominance

Diagnostic approach to MDS 2012



Diagnostic tool	Diagnostic value	Priority
Peripheral blood smear	<ul style="list-style-type: none"> • Evaluation of dysplasia in one or more cell lines • Enumeration of blasts 	Mandatory
Bone marrow aspirate	<ul style="list-style-type: none"> • Evaluation of dysplasia in one or more myeloid cell lines • Enumeration of blasts • Enumeration of ring sideroblasts 	Mandatory
Bone marrow biopsy	<ul style="list-style-type: none"> • Assessment of cellularity, CD34+ cells, and fibrosis 	Mandatory
Cytogenetic analysis	<ul style="list-style-type: none"> • Detection of acquired clonal chromosomal abnormalities that can allow a conclusive diagnosis and also prognostic assessment 	Mandatory
FISH	<ul style="list-style-type: none"> • Detection of targeted chromosomal abnormalities in interphase nuclei following failure of standard G-banding 	Recommended
Flow cytometry immunophenotyping	<ul style="list-style-type: none"> • Detection of abnormalities in erythroid, immature myeloid, maturing granulocytes, monocytes, immature and mature lymphoid compartments 	Recommended
SNP-array	<ul style="list-style-type: none"> • Detection of chromosomal defects at a high resolution in combination with metaphase cytogenetics 	Suggested (likely to become a diagnostic tool in the near future)
Mutation analysis of candidate genes*	<ul style="list-style-type: none"> • Detection of somatic mutations that can allow a conclusive diagnosis and also reliable prognostic evaluation 	Suggested (likely to become a diagnostic tool in the near future)

Minimal diagnostic criteria in MDS

consensus (Vienna 2006)



- **A. Prerequisite Criteria**

- constant cytopenia in one or more cell lineages
- exclusion of all other hematopoietic or non-hematopoietic disorders

- **B. MDS-related (Decisive) Criteria**

- dysplasia in $> 10\%$ of all cells in one of the lineages, or $> 15\%$ ring sideroblasts (iron stain)
- 5–19% blast cells in bone marrow smears
- typical chromosomal abnormality (karyotyping or FISH)

Minimal diagnostic criteria in MDS

consensus (Vienna 2006)



- **C. Co-criteria**

- **abnormal phenotype of bone marrow cells by flow cytometry**
- molecular signs of a monoclonal cell population
 - HUMARA assay, **gene chip profiling, point mutation analysis** (e.g. *RAS* mutations), SNP-array
- markedly and persistently reduced colony-formation (CFU-assay)

Major differences between FAB and WHO classification in MDS

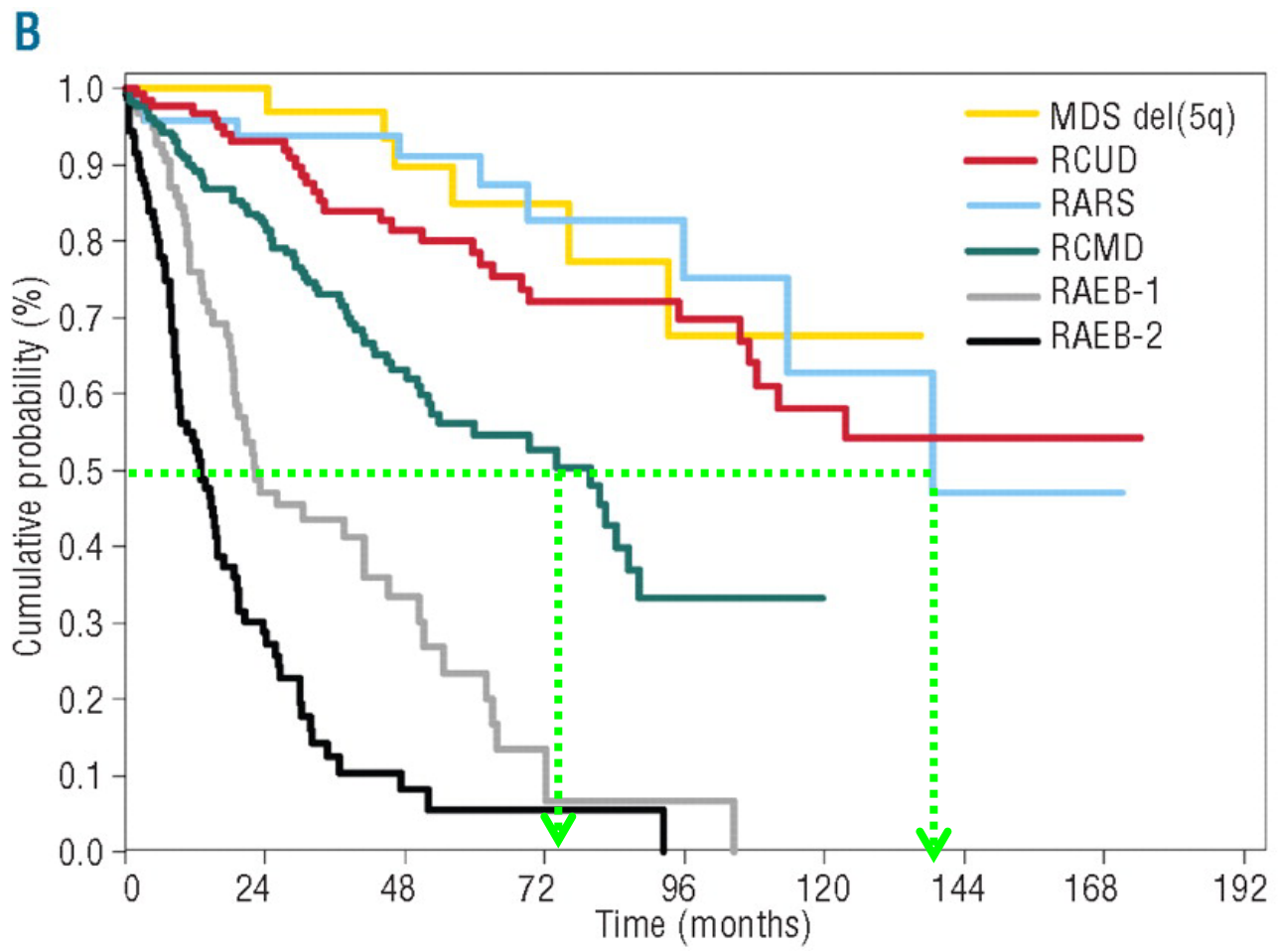


1. AML if bone marrow blasts > 20% (FAB: RAEB-t)
2. MDS and Auer rods with bone marrow blasts < 20% → RAEB-2
3. Lineage dysplasia defined if ≥ 10% of cells are dysplastic
4. **RCUD (RA, RN, RT)**
5. **Refractory anemia with ring sideroblasts**
6. **Refractory cytopenia with multilineage dysplasia**
7. **MDS-U**
8. **MDS associated with isolated del(5q)**
9. RAEB-1 and RAEB-2 according to blast % and uni- and/or multilineage dysplasia
10. separate category: myelodysplastic/myeloproliferative diseases
11. Specific cytogenetic abnormalities: t(8;21), inv(16), t(15;17), and < 20% blasts → AML
12. **ICUS [discussed]**



WHO-2008 and Overall Survival in MDS

Classification based on morphology!



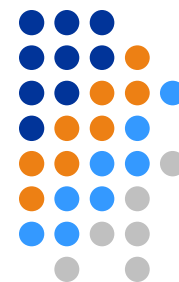
Questions in Diagnostic FC and Classification of MDS



- MDS *vs* non clonal diseases
- MDS RA *vs* RCMD *vs* others (MDS-U?)
- MDS subgroups based on FCM e.g. del(5q)+/-FCM

- ICUS *vs* MDS
- IDUS *vs* MDS
- [inconclusive *vs* MDS]

International Prognostic Scoring System (IPSS) in myelodysplastic syndromes



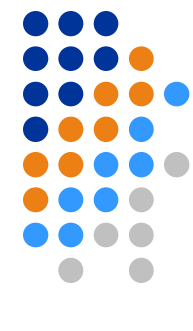
Prognostic variable	Score				
	0	0.5	1.0	1.5	2.0
Bone marrow blasts (%)	< 5	5–10		11–20	
Cytogenetics	Good	Intermediate	Poor		
Cytopenias*	0–1	2–3			

*Hb < 10 g/dL; 6.2 mmol/L;
platelets < 100 x 10⁹/L;
ANC < 1.8 x 10⁹/L

ANC = absolute neutrophil count.

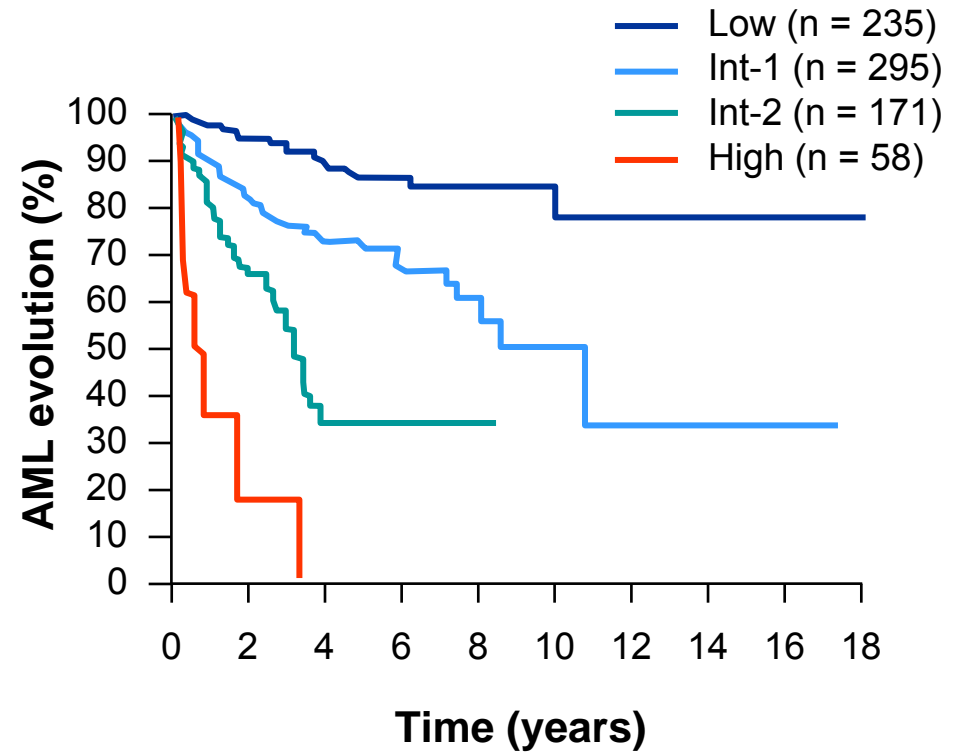
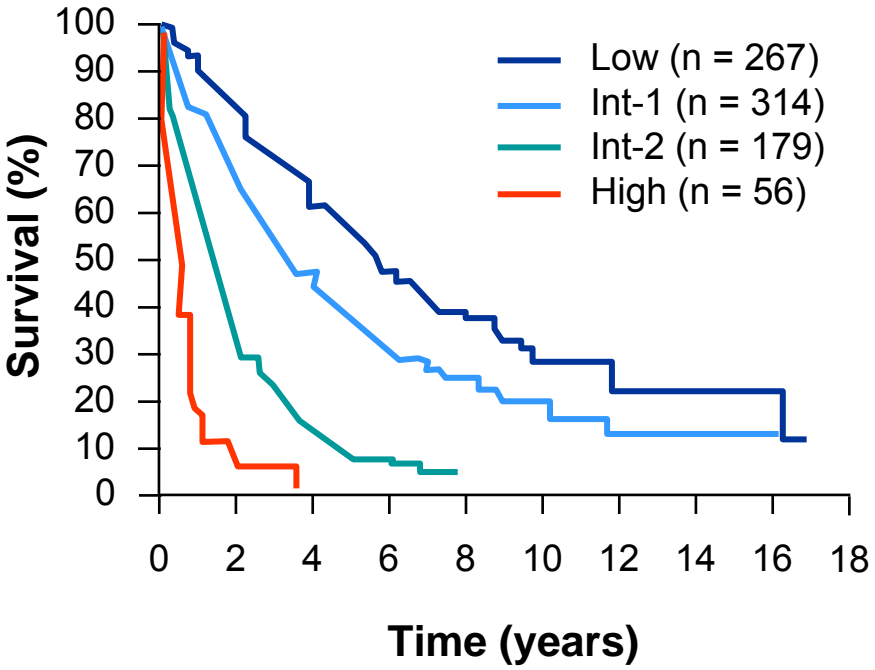
Greenberg P, et al. Blood. 1997;89:2079-88.

Cumulative survival of MDS patients by IPSS



Survival

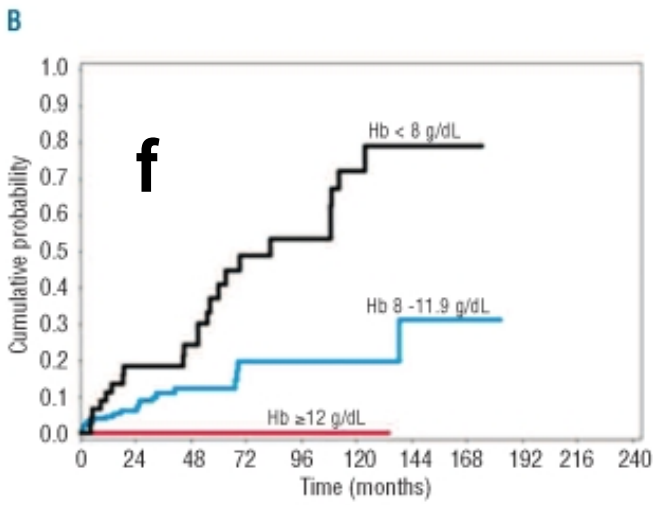
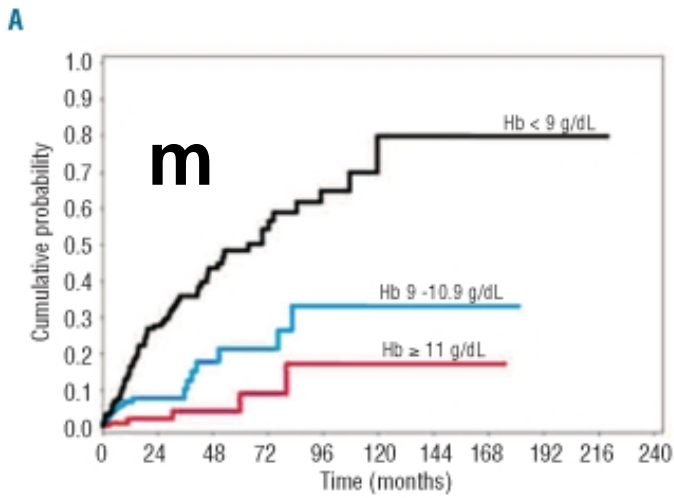
AML evolution





WHO-classification-based Prognostic Scoring System (WPSS and refined-WPSS)

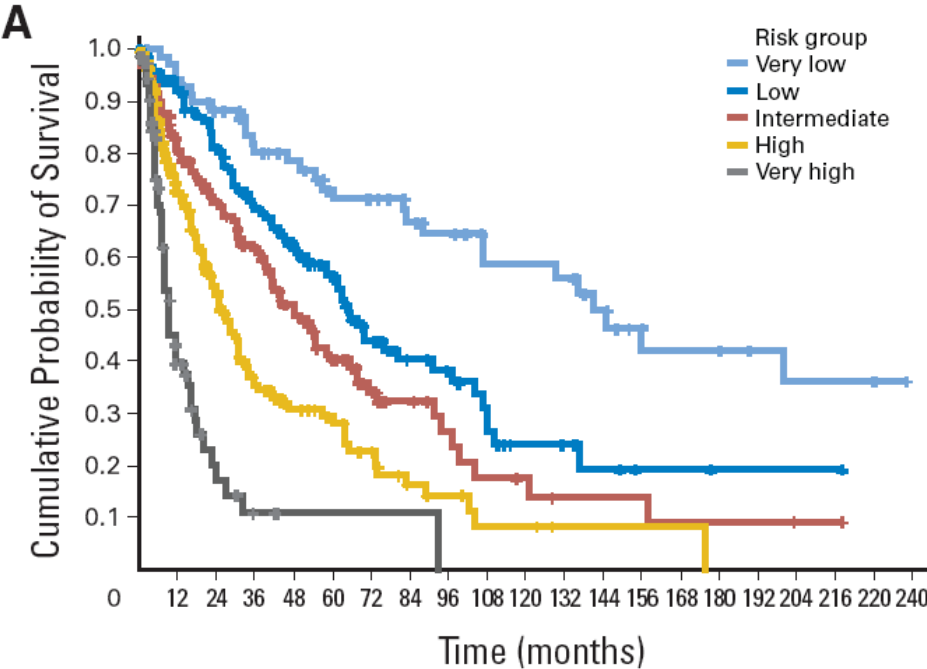
Variable	0	1	2	3
WHO	RA, RARS, 5q-	RCMD+/-RS	RAEB-1	RAEB-2
Karyotype	good	intermediate	poor	--
Trf/Hb*	no/(Hb>8/9 g/dl)	regular (Hb<8/9 g/dl)		



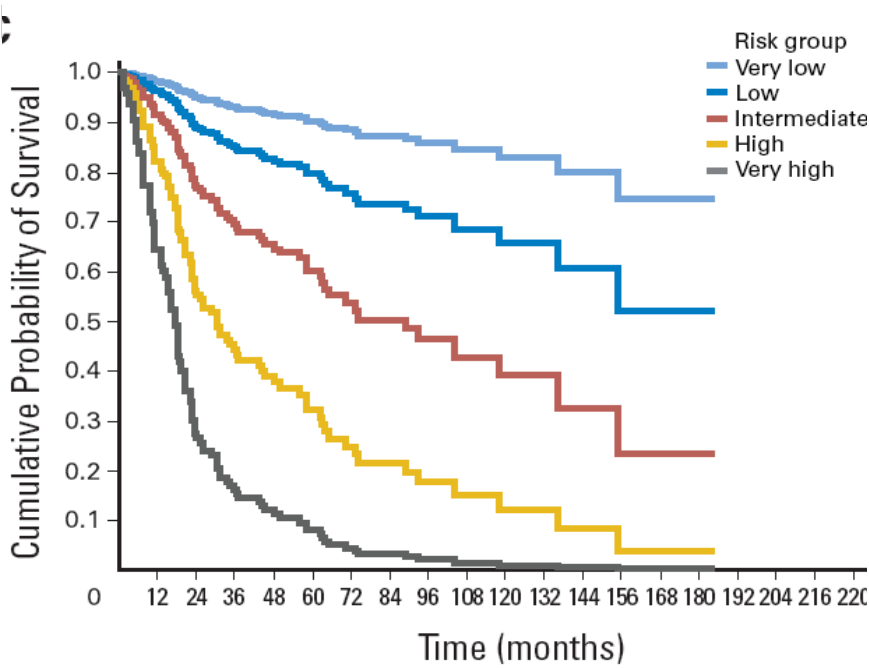
Time-dependent prognostic scoring system: validation cohort WPSS



WPSS at diagnosis



WPSS time-dependent

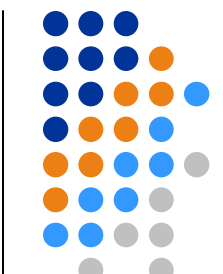


International Prognostic Scoring System (IPSS-revised) in myelodysplastic syndromes

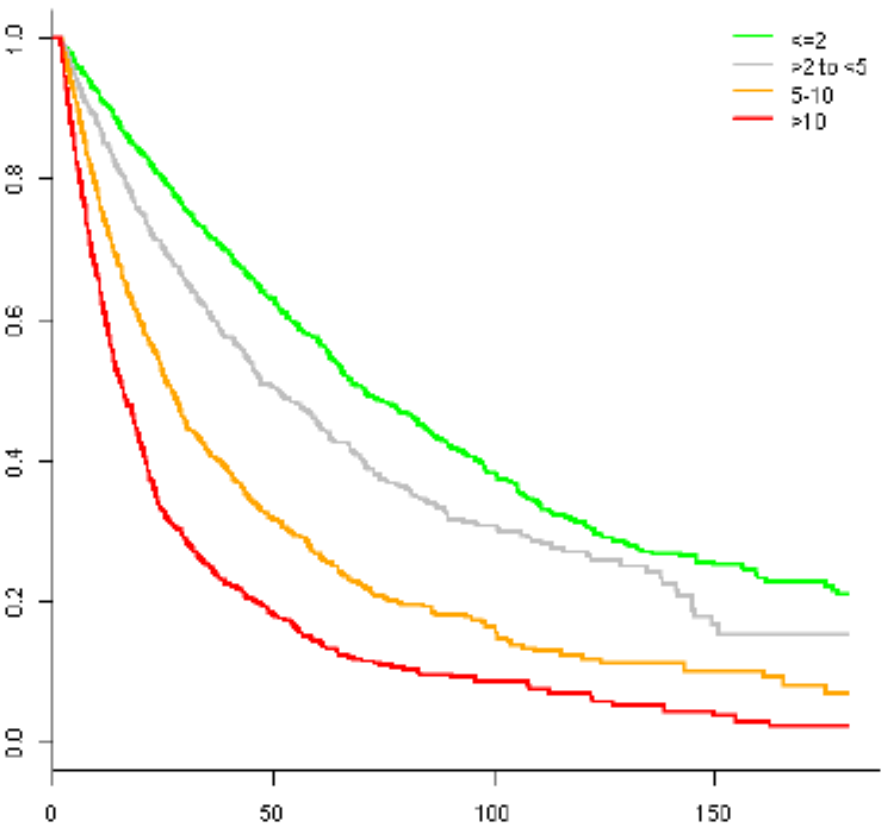


Prognostic variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very Good		Good		Inter	Poor	Very Poor
BM Blast %	≤2%		>2-<5%		5-10%	>10%	
Hemoglobin g/dl; mmol/l	≥10 ≥6.2		8-<10 5-<6.2	<8 <5			
Platelets	≥100	50-<100	<50				
ANC	≥0.8	<0.8					

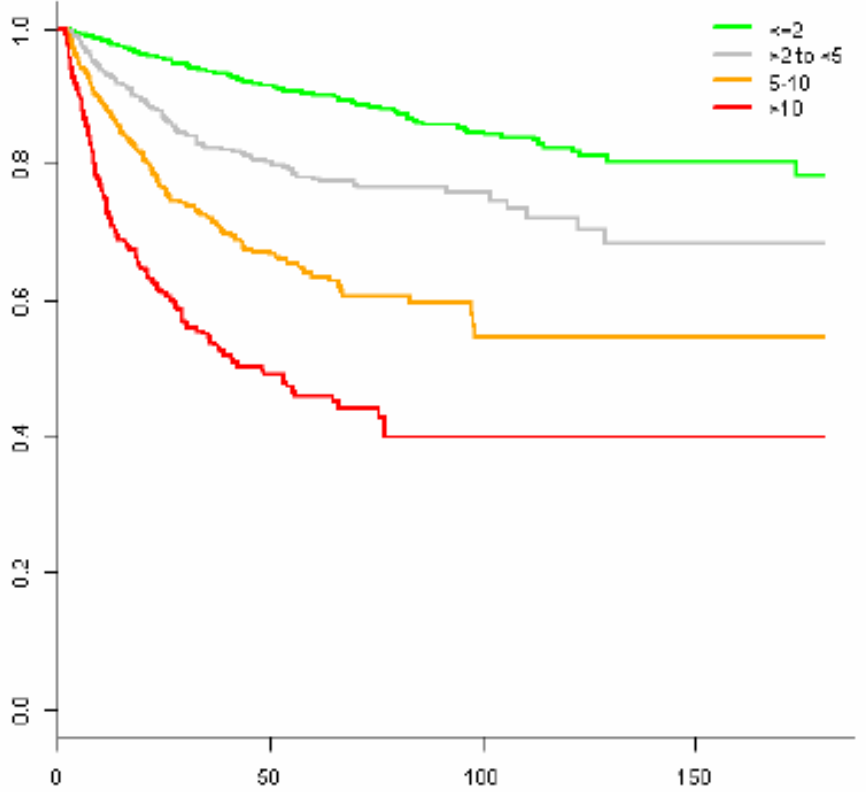
Survival and leukemic free survival: IPSS-revised; BM blasts



survival (months) by bone marrow blasts (categ <=2, <5, <=10, >10)



preleukemic time (mo) by bone marrow blasts (categ <=2, <5, <=10, >10)





Questions in Prognostic FC and Classification of MDS

- MDS prognostic subgroups
- MDS Flow Scores
- Addition of Flow Scores to IPSS, revised-IPSS, rWPSS
- Addition of Flow Scores to cytogenetic subgroups
- Addition of Flow Scores to molecular subgroups
- Addition of Flow Scores to predict response on R/



Outline of the Master Class

- NVC guidelines
- Flow analysis in laboratory practice
- Workshops
- Diagnostic flow scoring system
- Prognostic flow score system
- General discussion