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### Myelodysplastic syndromes: recent advances

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**M**yelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders characterized by ineffective dysplastic hematopoiesis, peripheral cytopenias and substantial risk of progression to acute myeloid leukemia (AML). They typically occur in elderly people, with a median age at diagnosis ranging between 60 and 75 years in most series.<sup>1</sup> The natural history of these syndromes ranges from relatively benign clonal bone marrow disorders (refractory anemias with or without ring sideroblasts) to forms with a rapid evolution to AML. Although the full spectrum of leukemic progression has not yet been completely clarified, recent clinical and biological studies indicate that MDS and AML (especially those arising in older individuals) can be considered as part of the same continuous disease spectrum rather than as distinct disorders.<sup>2,3</sup>

MDS are *disorders characterized by step-wise genetic progression*. Cytogenetic and molecular data provide evidence for the existence of a clonal phase prior to the acquisition of the characteristic cytogenetic abnormalities associated with MDS.<sup>4,5</sup> The initiating genetic lesions in a clonal hematopoietic stem cell population may be inherited or acquired. The *primary* genetic abnormalities promote the acquisition of *secondary* genetic lesions. These latter are the cytogenetic abnormalities associated with MDS, characterized by stepwise gains and loss of specific chromosomal regions (e.g. 5q-, 7q-, 12p-, +8), and accompanied during disease progression by point mutations of members of the RAS family of proto-oncogenes and inactivation of the p53 and p15 tumor suppressor genes by point mutations and hypermethylation.<sup>3</sup>

#### Etiology of myelodysplastic syndromes

The models for the development of *sporadic* MDS suggest the role of *cumulative environmental exposures in genetically predisposed individuals*. There is increasing evidence for a complex genetic predisposition to MDS involving naturally occurring DNA polymorphisms in genes that mediate DNA repair and metabolize environmental carcinogens.<sup>3</sup>

Large epidemiologic studies link MDS to radiation, smoking, occupational exposure to pesticides, organic chemicals and heavy metals.<sup>6-8</sup> The mechanisms responsible for the initiation of MDS include nuclear and mitochondrial DNA mutations by carcinogen-DNA adducts and formation of oxygen-free radicals (OFRs), defective DNA repair resulting in genomic instability and dysregulation of immune surveillance. This last probably synergizes with genomic mutations to promote leukemogenesis.

An emerging model of carcinogenic effects mediated via both genotoxic and non-genotoxic mechanisms is furnished by exposure to benzene. Benzene metabolites form DNA adducts and generate mutagenic OFRs. Furthermore, benzene-induced OFRs induce apoptosis. The genotoxic effects include RAS oncogene mutations and chromosomal aberrations, such as deletions/translocations. Non-genotoxic benzene effects are activation of protein kinase C, enhanced granulocyte-macrophage colony-stimulating factor (GM-CSF)-dependent proliferation, and immunologic dysregulation.<sup>1,9-12</sup>

Genetically, individuals differ greatly in the level of many enzymes, including those involved in the activation or detoxification of carcinogens. It

was found that the level of enzymes involved in the metabolism of benzene (e.g. NAD(P)H:quinone oxidoreductase) greatly influenced the risk of MDS after exposure to benzene. Likewise, glutathione S transferase levels appear to be correlated with the risk of MDS in persons exposed to industrial compounds. Greater knowledge of the relationship between enzymatic profiles and the risk of MDS could possibly lead to preventive measures in occupational medicine.

A specific multistep sequence for the development of idiopathic MDS based on cell culture, molecular and clinical research has recently been proposed.<sup>2</sup> In this model four pathophysiologic phases can be recognized (Table 1). In the *pre-MDS phase* the process is initiated by environmental, occupational or toxic exposure in genetically susceptible individuals. The *early MDS phase* is characterized by accelerated apoptosis of hematopoietic stem cells. In this phase an important role is played by extrinsic immunologic and microenvironmental factors. Progenitor cells damaged by toxin exposure or spontaneous mutation evoke an immunologic response. As in aplastic anemia (AA), a clonally expanded T-cell population elicits an autoimmune myelosuppression contributing to the cytopenia of MDS.<sup>3</sup> The evidence for an immune-mediated myelosuppression in MDS has important therapeutic implications.<sup>13</sup> The restoration of marrow function in AA with immunosuppressive treatment has provided the rationale for using the same therapy in MDS.<sup>14,15</sup> The experimental basis in support of this approach has recently been furnished by studies showing that depletion of lymphocytes increases *in vitro* hematopoiesis in long-term marrow cultures of patients with MDS.<sup>16</sup>

The persisting autoimmune attack results in chronic overproduction of pro-apoptotic cytokines, produced by MDS mononuclear stem cells-tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) or by stromal cells-interferon  $\gamma$  (IFN- $\gamma$ ), interleukin (IL-1 $\beta$ ) and transforming growth factor (TGF- $\beta$ ). Elevation of TNF- $\alpha$  induces, in MDS cells, increased FAS, down-regulation of Fap-1, and an increase in caspases causing apoptosis. Other extrinsic factors contributing to accelerated apoptosis are altered adhesive interactions between clonogenic hematopoietic stem cells and the underlying marrow stroma or endothelium. Excess apoptosis might be the reason for ineffective hematopoiesis and marrow failure in MDS.

Another abnormality of marrow stroma is the increased angiogenesis due to the substantial production by MDS cells of vascular endothelial growth factor (VEGF). Increased density of blood

**Table 1. Multistep pathogenesis of MDS: pathophysiologic phases.<sup>2</sup>**

<i>Pre MDS phase</i>	MDS initiation: environmental, occupational or toxic exposure in genetically susceptible individuals
<i>Early MDS phase</i>	Immunologic response to damaged progenitor cells
<i>Late MDS phase</i>	Diminution of cell cycle control and genomic instability → development of secondary AML
<i>MDS-related AML</i>	

vessels could favor disease progression by supporting unregulated cell growth. This observation provides the rationale for testing anti-angiogenic agents (e.g. thalidomide) for their potential to retard MDS evolution into AML.

As MDS progresses to the *late MDS phase*, apoptotic signals decrease (FAS antigen, c-Myc oncoprotein) whereas anti-apoptotic signals increase (bcl-2 oncoprotein).<sup>17-19</sup> Progression to advanced MDS and AML has been linked to inactivation of the tumor suppressor genes p15<sup>INK4b</sup> (hypermethylation) and p53 (point or missense mutations).<sup>20,21</sup> In fact, the late MDS phase is characterized by diminution of cell cycle control and genomic instability, which lead to genetic evolution and development of *MDS-related AML*.

#### *Inherited genetic predisposition to MDS*

Genetic and family mapping studies have clearly demonstrated that mutations in a specific gene, such as AML1, NF1, or genes mediating DNA repair, can predispose to the acquisition of secondary cytogenetic abnormalities and MDS.<sup>22</sup> Fanconi's anemia and Bloom's syndrome are characterized by defects of DNA repair, while neurofibromatosis is associated with RAS activation via deletion of the tumor suppressor gene NF-1. Such activation has been shown to result, in animal models, in hypersensitivity of myeloid progenitors to GM-CSF, which is a hallmark of juvenile chronic myelomonocytic leukemia (JMML).

#### *Aplastic anemia, paroxysmal nocturnal hemoglobinuria and MDS*

Aplastic anemia (AA) and paroxysmal nocturnal hemoglobinuria (PNH) may evolve into MDS. In the series of Socié *et al.*,<sup>23</sup> 30% of PNH cases evolved from AA and 5% evolved further into MDS. Evolution of AA to PNH and MDS usually follows immunosuppression with antithymocyte globulin, with a 10-year cumulative incidence for MDS evo-

**Table 2. Relationship between MDS and aplastic anemia.**

	MDS	Aplastic anemia
↑ Hemopoietic inhibitors	Yes	Yes
↓ Progenitor cells	Yes	Yes
Apoptosis of marrow cells	Yes	Yes
Apoptosis-related cytokine	TNF- $\alpha$	IFN- $\gamma$
Telomere shortening	Yes	Yes
Cytogenetic abnormality	Common	Rare
Production of G-CSF and GM-CSF	↓	↑
Age	Older	Younger

**Table 3. Therapy-related myelodysplastic syndrome/acute myeloid leukemia.<sup>41</sup>**

	Peak latency	Preleukemia phase	Cytogenetic abnormalities
Alkylating agents	5-10 years	MDS	-5/del(5q) -7/del(7q) Complex
Topoisomerase II inhibitors	6 months-5 years	None	t(11q23) t(21q22)
Various agents	2-3 years	None	t(15:17)
	<3 years	None	inv(16)

lution of 9.6%.<sup>24</sup>

The similarities between early MDS and aplastic anemia (Table 2) may reflect a common marrow response to stem/progenitor cell injury and could explain why similar therapeutic strategies may be effective in both diseases. The higher frequency of AML in MDS may be due to the shorter telomere lengths (typical of older patients) that foster a heightened susceptibility to genomic instability.<sup>2</sup> One difference between MDS and AA is the milieu of hematopoietic inhibitory cytokines, with a tendency for IFN- $\gamma$ -mediated apoptosis in AA and TNF- $\alpha$ -driven apoptosis in MDS.<sup>25,26</sup>

#### *Ionizing radiation and chemotherapeutic agents*

Leukemogenic effects of radiation are dependent upon dose and duration of exposure. The mutagenic effects have been evaluated in animals, in atomic-bomb survivors, and in patients submitted to spinal irradiation for ankylosing spondylitis. Low-dose high linear energy transfer  $\alpha$ -particle ionizing radiation of human bone marrow *in vitro*

is associated with induction of chromosomal aberrations, while high-dose radiotherapy does not appear to be leukemogenic.<sup>27,28</sup>

Distinct clinical and biological forms of therapy-related MDS/acute myeloid leukemia (t-MDS/AML) have been recognized in relation to different groups of therapeutic agents (Table 3). The molecular mechanisms involved in the genetic damage are mostly represented by microsatellite instability and p53 mutations.<sup>29</sup> Exposure, however, to X-rays and alkylating agents may reveal those individuals who are *inherently* compromised in DNA damage repair, and deletions may be one of the consequences of the inability to repair excessive DNA damage.

Therapy-related MDS differs from *de novo* MDS in many respects: younger age of onset, more patients presenting with RA with excess blasts (RAEB) or RAEB in transformation (RAEB-t), bone marrow cellularity more often reduced, increased frequency of moderate fibrosis. Secondary MDS/AML following Hodgkin's disease (HD) therapy is directly proportional to the total dose of alkylating agents. Newer regimens (e.g. ABVD) with lower total exposure to these drugs, give a much lower cumulative incidence of t-MDS/AML.<sup>30</sup> Therapy-related MDS/AML following chemotherapy for childhood acute lymphoblastic leukemia is rare, except in association with twice-weekly epipodophyllotoxin-containing regimens.<sup>31</sup> Also the incidence of this type of secondary AML, characterized by t(11q23) cytogenetic abnormality (Table 3), might be substantially reduced by a modification of treatment schedules.

#### *Occupational and environmental carcinogens*

Several case control studies have identified an increased risk of MDS in subjects with jobs exposing them to industrial and agricultural compounds. In a case controlled study of 400 MDS patients in Wales,<sup>8</sup> a characteristic relationship between histories of occupational or environmental exposure and the presence of cytogenetic abnormalities was demonstrated (Table 4). Benzene, organic solvents, pesticides, and smoking (Table 5) have been etiologically implicated in several studies.<sup>6,7,12,32</sup> Among controversial risk factors are hair-dye use and alcohol.<sup>33,34</sup>

#### *Epidemiology of myelodysplastic syndromes*

Data from recent epidemiologic studies suggest that MDS are relatively common hematologic disorders. MDS are geriatric diseases with more than 80% of patients being over 60 years old at diagno-

**Table 4. Environmental or occupational risk factors for progenitor-cell damage.**

Benzene:	dose-related, constant exposure, recent exposure (<10 years) dose-related cytogenetic abnormalities: -5q, -7q, +8, +21, t(8;21)
Pesticides:	odds ratio 3.00
Organic solvents:	exposure marginally associated with the risk (OR:1.99)
Smoking:	risk increased with duration and intensity of smoking ↑ risk for "recent" smokers (within prior 20 yrs) ↑ risk for RA and RARS ↑ risk for chromosome 7 abnormalities

**Table 5. Cytogenetic abnormalities in MDS according to environmental or occupational exposure.<sup>8</sup>**

Odds ratio for all exposures higher among cytogenetically abnormal (2.0) than normal (1.0)	
Type of exposure	
Semi-metals (As)	
Inorganic dusts (asbestos, silica, formica)	
Metals (Cu, Ni, Sn, steel)	
Organics	
Radiation	
Relationship of type of exposure to specific cytogenetics	
Radiation, metals, organics	→ Chromosome 8
Inorganic fumes	→ Chromosome 5 and 7

sis. In elderly populations accumulated environmental exposure provides a cumulative probability of mutations that increases with time. Recently, a number of cancer registries have published data on the regional occurrence of MDS. In the Dusseldorf Bone Marrow Registry,<sup>1</sup> the crude incidence rate (IR) is 4.4/100,000/year. In a Northern Spanish area<sup>35</sup> the global IR was 8.1/100,000/year and the age-adjusted incidence rate 2.8/100,000/year (median age of MDS patients 74.1±10.6).

#### Age distribution and sex ratio

Considering the characteristic age distribution of MDS, it is more appropriate to determine age-specific incidence rates than crude incidences. Table 6 shows the crude and age-specific incidences of MDS in three different geographic areas. The incidence rates of 15-50/100,000/year in people over the age of 70 suggest that in older persons MDS are as common as chronic lymphocytic leukemia and multiple myeloma. Because, however, of the paucity of clinical symptoms in early-stage MDS, the true incidence of MDS is difficult to obtain and is probably underestimated.

**Table 6. Crude and age-specific incidences of MDS (incidence figures per 100,000 population per year).**

Authors Area	Aul et al. <sup>42</sup> Germany	Radlund et al. <sup>43</sup> Sweden	Williamson et al. <sup>44</sup> England
Age group			
≤ 49 years	0.4	0.7	0.5
50-59 years	4.7	1.6	5.3
60-69 years			15.0
70-79 years	24.5	15.0	49.0
≥80 years			89.0
All ages	4.4	3.5	12.6

In a well-defined French population of 0.5 million inhabitants<sup>36</sup> the age-standardized rates were 2.6 for men and 1.3 for women. MDS were rare before the age of 60 (~10%). After 60, the incidence rose rapidly with age, more steeply in men than in women. MDS appeared to be more frequent in urban than in rural areas. This was especially true for men (3.5 vs 1.4 respectively,  $p < 10^{-5}$ ) while the incidence was quite stable in women.<sup>36</sup>

#### Real or apparent increase of MDS?

Much of the rising incidence of MDS reflects more accurate diagnosis and case registration: better laboratory facilities, expansion of diagnostic procedures, agreement on diagnostic criteria of MDS, improvement in geriatric medical care. Otherwise, the increase of MDS cannot be explained by an increased use of cytotoxic agents and other myelosuppressive drugs. In fact, the proportion of t-MDS in the Dusseldorf registry was 7% between 1976 and 1980 and 5.8% between 1986 and 1990.<sup>1,37</sup>

#### Classification and prognostic assessment of myelodysplastic syndromes

In 1982 the French-American-British (FAB) Co-operative Group proposed a classification for MDS based on easily obtainable laboratory data (Table 7).<sup>38</sup> The importance of the percentage of blasts and of the presence of more than 15% ringed sideroblasts for marrow with less than 5% blasts has been outlined and confirmed by many investigators. The FAB proposal was characterized by a strong emphasis on the neoplastic nature of MDS, because the Cooperative Group was initially involved in classifying acute leukemias. The greatest merit of this classification was that of having

provided a common language for physicians. Furthermore, it has served as the initial step for an expanding series of prognostic factors in the evaluation of patients with MDS. The limitations of the FAB classification include the wide range of marrow blast percentages for patients in the RAEB and CMML categories (5-20% and 1-20%, respectively), the absence of critical biological determinants such as marrow cytogenetics, and of the degree and number of cytopenias.

In 1999 the World Health Organization (WHO) proposed a new classification of neoplastic diseases of the hematopoietic and lymphoid tissues.<sup>39</sup> Among myeloid neoplasms the pathologists, using a combination of morphologic, immunophenotypic, genetic and clinical features, recognized four chief categories: myeloproliferative diseases, myelodysplastic/myeloproliferative diseases, myelodysplastic syndromes and acute myeloid leukemias. The myelodysplastic syndromes and the myelodysplastic/myeloproliferative diseases are presented in Table 8. The WHO classification differs from the FAB classification in several aspects. RAEB in transformation and CMML disappear from MDS. The former because the limit of 30% of blasts for diagnosis of acute myeloid leukemia has been lowered to 20%. The latter because CMML has long been recognized as a disorder with both myelodysplastic and myeloproliferative characteristics, some patients showing clinical and morphologic features resembling RAEB with monocytosis and others marked neutrophilia, monocytosis, and splenomegaly. The two types of the disease, however, show no differences in cytogenetic abnormalities, oncogene mutations, *in vitro* colony growth patterns and clinical outcome, so the consensus at the Meeting of the Clinical Advisory Committee was that CMML is one disease to be included in a separate category, along with juvenile myelomonocytic leukemia.<sup>39</sup> Refractory cytopenia with multilineage dysplasia is defined by the presence of dysplastic features in two or more lines, but with fewer than 5% of blasts in bone marrow. The cytogenetic abnormalities and clinical course are similar to those found in RAEB. Because of the distinctive morphologic and clinical features of the 5q-syndrome, this has been defined as a separate category within MDS. RA with and without ring sideroblasts will continue to be defined as a disorder involving the erythroid lineage only.

Several problems have also arisen with the WHO classification. The elimination of RAEB-t as a distinct clinical stage may be problematic for comparing the results of clinical trials in AML/MDS with historical controls. In fact, in several studies

RAEB-t patients show a worse response to chemotherapy than AML ones with similar biological and cytogenetic features. The categories *refractory cytopenia with multilineage dysplasia* and *MDS, unclassifiable* are vague and have no biological, clinical, or genetic basis. Furthermore, the WHO classification scheme generally lacks clinical and prognostic relevance.

In order to facilitate clinical decision-making, other authors have developed risk-based classification systems for MDS. The *International Prognostic Scoring System* (IPSS), which has achieved international acceptance, assigns scores according to marrow blast cell percentage, karyotype, and degree of cytopenia, providing a useful method for evaluating prognosis in MDS patients and for designing clinical trials.<sup>40</sup>

A few methods have been developed for evaluating the clinical outcome of patients with MDS. After the initial FAB Cooperative Group classification in 1982,<sup>38</sup> several additional risk classification systems have been used regarding prognostic clas-

**Table 7. The FAB classification of myelodysplastic syndromes.**

	% Marrow blasts	% Peripheral blasts	Others	% AML transform.
RA	< 5	≤1	–	10-20
RARS	< 5	≤1	>15% ringed sideroblasts	10-35
RAEB	5-20	<5	–	50+
CMML	1-20	<5	Monocytosis >1000/μL	40+
RAEB-t	21-29	≥5	Auer rods	60-100

**Table 8. WHO classification of neoplastic diseases of the hematopoietic and lymphoid tissue: myelodysplastic and myelodysplastic/myeloproliferative diseases.<sup>39</sup>**

Myelodysplastic syndromes
Refractory anemia
- with ringed sideroblasts
- without ringed sideroblasts
Refractory cytopenia with multilineage dysplasia
Refractory anemia with excess of blasts
5q- syndrome
MDS, unclassifiable
Myelodysplastic/myeloproliferative diseases
Chronic myelomonocytic leukemia (CMML)
Atypical chronic myelogenous leukemia (aCML)
Juvenile myelomonocytic leukemia (JMML)

sification of MDS and their potential for survival and evolution to AML.<sup>41-50</sup> These classification methods included both morphologic criteria and clinical variables such as bone marrow (BM) blast percentage, bone biopsy, cytopenias, age, lactate dehydrogenase level and cytogenetics.<sup>51,52</sup>

The morphologic criteria included in the FAB classification have been relatively effective for categorizing MDS patients: the median survival of patients with refractory anemia (RA) and RA with ringed sideroblasts (RARS) is 27-32 and 42-45 months, respectively, whereas the median survival of patients with CMML is 13-15 months and the median survival of patients with RAEB and RAEB-t is 9-19 and 5-11 months, respectively<sup>53,54</sup> (Table 9). Unfortunately the limitations of the FAB classification as a prognostic index for MDS have become evident. As previously mentioned, these limitations include the wide range of marrow blast percentages for patients in the RAEB and CMML categories, the lack of inclusion of critical biological determinants such as marrow cytogenetics, and the degree and number of associated cytopenias.

Increased marrow blast count is associated with poorer prognosis regarding survival and leukemic transformation in MDS patients.<sup>46,55,56</sup> The Spanish group showed that the addition of an extra cut-point of 10%, in addition to the generally accepted 5% and 20% FAB criteria clearly improves the prognostic value of this variable.<sup>46</sup>

One of the most important classifications of the prognostic factors detected at diagnosis of MDS is the IPSS; according to this scoring system, karyotype detected at diagnosis is crucial in influencing the outcome of MDS patients. An abnormal karyotype is found in 30% to 50% of patients with primary MDS.<sup>57</sup> Normal karyotype, 5q- syndrome and del(20q) seem to be related to a good progno-

sis.<sup>40,58,59</sup> On the other hand, single chromosomal abnormalities with an unfavorable prognosis include iso(17q), del(12p), -7, del(7q) and complex karyotype.<sup>59</sup> Whereas chromosomal stability does not preclude the development of AML, the appearance of chromosomal abnormalities in a patient with a previously normal karyotype, or the emergence of additional aberrations is associated with progression to a more aggressive subtype or evolution to AML and short survival.<sup>57</sup>

Another valid prognostic index is the peripheral blood count, with platelets and hemoglobin having a greater prognostic weight than neutrophil levels, and the number of cytopenias having a greater impact on the survival and the risk of leukemic evolution.<sup>46,47,53</sup> The prognostic value of age in MDS has been investigated too. Greenberg *et al.* showed that the prognosis of patients in the high-risk or intermediate 2 (INT-2) risk groups did not differ substantially whether patients were older or younger than 60 years of age; this was not true for low-risk or intermediate 1 (INT-1) risk groups, in which shorter survival times occur in patients > 60 years of age (Table 10).

Other prognostic factors have been considered: sex, with males carrying a worse prognosis in some series,<sup>46</sup> the number or proportion of blasts in peripheral blood, with patients having blasts in peripheral blood performing similarly to untreated AML patients,<sup>46</sup> the presence of immature myeloid precursors and nucleated RBC in peripheral blood, the presence of dysthrombocytopoiesis and dysgranulocytopenia. A German study highlighted the impact of lactate dehydrogenase (LDH) level on survival, as this can represent a measure of ineffective hematopoiesis and leukemic burden by reflecting increased cell turn-over.<sup>47</sup> Moreover, some BM biopsy findings, such as abnormal localizations of immature precursors (ALIP), hypercellularity and fibrosis are related to poor outcome in MDS.<sup>52</sup>

Finally, RAS and/or FMS mutations and reduced telomere stability, which correlates with genomic instability, methylation of the p15 gene, which correlates with BM blasts > 10% and risk of progression to AML, an increased FAS ligand expression, which correlate with FAB, Hb level, and overall survival, and shortened terminal restriction fragments, which correlates with Hb level, BM blasts and poor cytogenetic abnormalities were all the described as related to prognosis.<sup>60</sup>

As a result of wide research on prognostic factors, several scoring systems have been developed. The most important ones are shown in Table 11.

In conclusion, we consider that the most significant

**Table 9. The prognostic impact of the FAB classification.**

FAB subtype	Goasguen <sup>53</sup>	Mascheck <sup>54</sup>
	1990 (503 patients)	1994 (569 patients)
	Median survival (months)/% of leukemic progression	
RA	32/8	27/16
RARS	45/3	42/4
RAEB	19/20	9/42
RAEB-t	11/53	5/59
CMML	15/23	13/49

**Table 10. International Prognostic Scoring System for MDS (Greenberg et al.).<sup>40</sup>**

Risk group	Score	Median survival (years)		Time to 25% risk of AML evolution (years)	
		All patients	≤60 years patients	All patients	≤60 years patients
Low	0	5.7	11.8	9.4	> 9.4
Intermediate-1 (INT-1)	0.5-1.0	3.5	5.2	3.3	6.9
Intermediate-2 (INT-2)	1.5-2.0	1.2	1.8	1.1	0.7
High	≥ 2.5	0.4	0.3	0.2	0.2

prognostic factors for MDS are:

- a. leukemic burden; this is well indicated by the percentage of blasts in the peripheral blood and in the bone marrow (and, of course, by the FAB subtype). The presence of ALIP, immature precursors displaced intertrabecularly or into clusters (3-5 myeloid precursors) or as aggregates, instead of displaced paratrabecularly, is similarly related to the leukemic burden. The expression of CD34 antigen in the bone marrow, as well as the number of CD34+ cells in the

peripheral blood is another factor which correlates with the tumor burden. It is the same when considering the *in vitro* growth of myeloid progenitors: the absence (or few colonies) and the production of micro-macroclusters is associated with faster leukemic transformation and a shorter survival;

- b. cytogenetic abnormalities; with the exception of isolated del(5q) and del(20q), which are associated with longer survival, all the other more common abnormalities, such as -7, del(7q), iso(17q), del(12p), and complex karyotype, confer an aggressive clinical course;
- c. number of cytopenias; degree of dyshematopoiesis, especially the presence of dysthrombocytopoiesis and dysgranulocytopoiesis;
- d. patients' age; patients aged over 60 years perform worse than younger ones (especially in the low-risk and INT-1 risk groups according to the IPSS);
- e. primary or secondary MDS; with a poorer prognosis in patients with the latter; MDS following exposure to alkylating agents or radiotherapy (unfavorable karyotype abnormalities and older age) are often associated with a poorer prognosis than MDS following topoisomerase II inhibitors (favorable karyotype abnormalities and younger age);<sup>61</sup>

**Table 11. Main scoring systems.**

Points	0	0.5	1	1.5	2	Risk group	Score
<b>(IPSS)</b>							
Marrow blasts (%)	<5	5-10		11-20		Low	0
Karyotype*	Good	Intermediate	Poor			Intermediate 1	0.5-1
Cytopenias°	0 or 1	2 or 3				Intermediate 2	1.5-2
						High	2.5-3
<b>Bournemouth</b>							
Haemoglobin (g/dL)	>10	<10				Low	0 or 1
Neutrophils (×10 <sup>9</sup> /L)	>2.5 and <16	*2.5 or >16				Intermediate	2 or 3
Platelets (×10 <sup>9</sup> /L)	≥ 100	>100				High	4
Marrow blasts (%)	<5	*5					
<b>Spanish</b>							
Marrow blasts (%)	<5	5-10			11-30	Low (A)	0 or 1
Platelets (×10 <sup>9</sup> /L)	≥ 100	51-100			≤50	Intermediate (B)	2 or 3
Age (yrs)	≤60	>60				High (C)	4 or 5
<b>Goasguen</b>							
Hemoglobin (g/dL)	>10	*10				Low	0
Platelets (×10 <sup>9</sup> /L)	>100	*100				Intermediate	1 or 2
Marrow blasts (%)	<5	*5				High	3

\*Good: normal, del(5q) only, del(20q) only, -y only. Poor: complex (>2 abn), abn 7. Intermediate: other abnormalities. °Cytopenias: Hb <10 g/dL, PLT < 100×10<sup>9</sup>/L, PMN < 1.8×10<sup>9</sup>.

- f. histopathology; as previously said, ALIP;
- g. kind of chemotherapy employed; submitting MDS patients to allogeneic or autologous stem cell transplantation, or to other intensive chemotherapies, in complete remission (CR) or with active disease is strictly correlated to patients' age, to their performance status at diagnosis and to the adverse prognostic factors, such as unfavorable karyotype abnormalities, multidrug resistance expression and previous exposure to alkylating agents.

### Cytogenetic findings

Cytogenetic results have a critical role for both correct diagnosis and identification of prognostic subgroups of MDS.<sup>62</sup> Thus, among refractory anemias a typical clinical hematologic syndrome is characterized by an interstitial deletion on the long arm of chromosome 5 (5q-) as an isolated karyotypic aberration. Identification of a clonal cytogenetic anomaly is critical in the differential diagnosis between aplastic anemia and myelodysplastic syndrome with profound bone marrow hypoplasia. Complex karyotypes with multiple structural and/or numerical aberrations are a hallmark of secondary MDS due to iatrogenic or environmental genotoxics. In the IPSS (see later) cytogenetic subgroups have a significant impact on survival and disease progression. In cases evolving from MDS to overt acute myeloid leukemia a clonal karyotypic evolution, i.e. appearance of new chromosomal anomalies in addition to those present at diagnosis, may be observed. However, up to now, additional chromosomal changes appearing during the follow-up and predicting evolution to AML have not been definitively established.

Taking into account the so-called karyotypic changes, i.e. those chromosomal rearrangements

which occur as isolated aberrations and which are thought to play a role in the early pathogenetic events of the malignancy, we will discuss three main groups of cytogenetic rearrangements: typical deletions in MDS; changes common to MDS and AML; changes common to MDS and chronic myeloproliferative disease (CMPD).

The fact that the same change may occur in MDS as well as in AML or in CMPD suggests that, at least in these cases, the malignant clone originates from a genetic insult at the level of a pluripotent stem cell and that the clinico-hematologic findings of the malignant disorder are related to additional factors influencing the balance between cell growth, survival, and differentiation of bone marrow populations.

### Typical deletions in MDS

Simple deletions are more frequent than reciprocal translocations in MDS when compared to AML. Partial (usually interstitial) deletions are more frequent than full monosomies (Table 12). The incidence of detected deletions in MDS is increasing because of the application of molecular cytogenetics which is helpful for checking loss of chromosomal bands or of full chromosomes in interphase nuclei.<sup>63</sup> Moreover interphase fluorescent *in situ* hybridization (FISH) on intact cells identified by morphology or immunophenotype is an elegant tool for assigning a given chromosomal deletion to a specific cell lineage. From this approach it is emerging that the malignant clone bearing one of the most frequent MDS deletions, i.e., a del(20q), or a monosomy 7, or the 5q- chromosome, may include B-lymphocytic cells.<sup>64-66</sup>

### Partial or complete deletion of chromosome 7 (7q-/-7)

Chromosome 7 is often involved in different types of myelodysplastic syndromes because of either partial deletion of the long arm (7q-) or loss of one homolog (-7). Both the 7q- and the -7 changes are consistently associated with MDS or AML induced by radiotherapy and/or chemotherapy for a previous lymphoma or solid tumor.<sup>67,68</sup> Alkylating agents have been mostly implicated. Interestingly loss of 7q is also the result of a genomic unbalance due to the t(1;7)(q10;p10) translocation consistently found in secondary MDS.

Despite its frequency, the biological significance of monosomy 7 is still undefined. An intriguing observation is that a clone with monosomy 7 may emerge during evolution of a number of genetic conditions predisposing to MDS/AML, such as Fanconi's anemia, Schwachman's syndrome, familial

Table 12. Typical deletions in MDS.

del(3)(p14-21) <sup>s</sup>
del(5)(q13q33)/-5 <sup>s</sup>
del(6)(p21)
del(6)(q21)
del(7)(q22q32-q35)/-7 <sup>s</sup>
del(9)(q13q22)
del(11)(q14q23)
del(12)(p13)
del(17)(p13) / p53
del(18)(p11)
-Y
-7

<sup>s</sup>:secondary disorder.

myelodysplasia, and Kostman's syndrome.<sup>69</sup> In the last syndrome the emergence of a monosomy 7 is possibly favored by treatment with granulocyte colony-stimulating factor (G-CSF).<sup>70</sup> In the multi-step process of malignant transformation monosomy 7 in children may be preceded by mutations of RAS and/or NF1 genes (see below).<sup>71</sup> Monosomy 7 is a poor prognostic marker in adults and very serious infectious complications are related to a profound disturbance of chemotaxis.<sup>72</sup>

The target gene of the 7q deletion has not so far been identified and, in addition, conflicting results have been generated on its position. Whereas some authors<sup>73</sup> observed a loss of heterozygosity (LOH) in a region immediately telomeric to the EPO gene in 7q21.3-22, others,<sup>74</sup> studying a family of constitutional carriers of inv(7)(q22.1q34) including a MDS case, were able to map the proximal breakpoint within the asparagine-synthetase gene (ASNS) which is centromeric to the EPO gene.

More recent data<sup>75,76</sup> suggest that more than a single region on 7q could be involved by deletions in MDS: one mapping on 7q22 and a second more telomeric in 7q32-33. The segment of minimal common deletion in 7q22 has been restricted to only 2 MB (megabase=1,000 kb=1,000,000 bp) but, unfortunately, no candidate tumor-suppressor genes have so far been identified in this area (Figure 1).

#### The 5q- deletion

The 5q- is the chromosomal marker of a distinct clinico-hematologic disease significantly affecting

elderly women and characterized by macrocytic anemia, normal or elevated platelet count, trilineage bone marrow dysplasia with typical monolobulated micromegakaryocytes.<sup>77</sup> The WHO classification keeps this syndrome separate from all other refractory anemias as its prognosis is relatively good with a chronic course and very rare evolution to acute leukemia. However the need of intensive support with red cell transfusions may lead to serious complications from hemosiderosis. Attempts of treatments with different growth factors, including erythropoietin, GM-CSF, and G-CSF, have been successful in controlling anemia only in sporadic cases.<sup>78</sup>

The occurrence of a 5q- chromosome is not limited to the 5q- syndrome, since it can also be found in other MDS, such as refractory anemia with excess of blasts, and typical secondary MDS, especially after radiotherapy or alkylating agents for a previous neoplasia.<sup>79</sup> In those cases the anomaly may result not only from simple deletions, but also from unbalanced translocations, such as a t(5;17).<sup>80</sup> In secondary MDS loss of an entire homolog (monosomy 5) is also frequent. A 5q- in a complex karyotype is associated with bad prognosis.

The chromosomal rearrangement is always an interstitial deletion with available loss of material.<sup>81-83</sup> However, the molecular lesions associated with the 5q- deletion are still elusive. The most recent data suggest the existence of two different regions of minimal deletion: one in 5q31 (Figure 1) where a number of genes responsible for regulation of growth and/or differentiation of hematopoietic

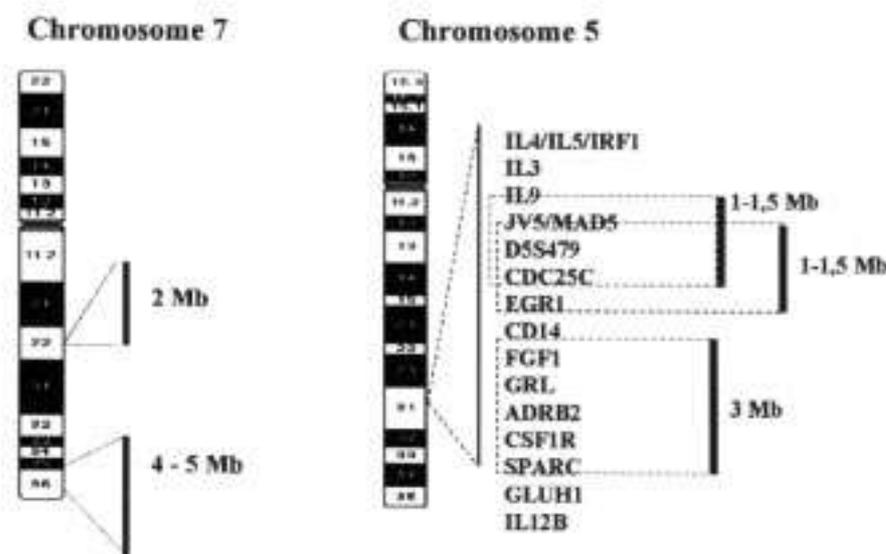


Figure 1. Maps of the minimal deleted regions on chromosome 5 and chromosome 7 with indications of some of the genes possibly involved (see text for details).

**Table 13. Changes common to MDS and AML.**

Chromosome rearrangements	Genes
t(6;9)(p23;q34)	DEK-CAN
t(3;5)(q25;q35)	MLF1-NPM
t(1;3)(p36;q21)	MEL1
t(3;21)(q26;q22)s	AML1-EVI1/MDS
del(9)(q)	
t(12;22)(p13;q11)	ETV6-MN1
inv(3)(q21q26)	EVI1
t(X;...)(q13;...)	
t(7;11)(p15;p15)	HOX9-NUP98
t(11;...)(q23;...)	MLL
t(8;21)(q22;q22)	AML1-ETO
t(15;17)(q22;q12)	PML-RAR $\alpha$
inv(16)(p13q22)	CBF $\beta$ -MYH11
Trisomy 4	KIT
Trisomy 11	MLL
Trisomy 13	
Trisomy 21	AML1

S: secondary disorder.

cells are located and another in 5q21, which was mapped in some cases with the 5q- syndrome.<sup>84</sup> In both cases the gene targets of the deletions remain unknown and their identification is a prerequisite to finally knowing whether the different clinical behaviors found to be associated with 5q- have a molecular basis.

Recently, however, an interesting association between p53 mutations and 5q-deletions has been described, suggesting that both these lesions can be part of the same molecular pathway, particularly favored by conditions determining genomic instability.<sup>85,86</sup>

Combined immunophenotypic and FISH studies showed that the cell of origin of the 5q- change is an early progenitor such as a CD34<sup>+</sup> CD19<sup>+</sup> lymphomyeloid stem cell. Similarly to the Philadelphia-negative chromosome in chronic myeloid leukemia, T-lymphocytes are not involved.<sup>4,5</sup>

#### The 17p- syndrome

A deletion on the short arm of chromosome 17 may result from different types of chromosomal rearrangements, i.e., unbalanced translocations, isochromosome of the long arm, simple deletions.<sup>87</sup> The anomaly is usually associated with other rearrangements. A common molecular event is the involvement of the p53 gene at 17p13, namely a p53 mutation is found in around 70% of patients with 17p deletion and MDS, so that both p53 alleles are abnormal, one is deleted and the second one is mutated. The typical hematologic stigmata

of the 17p- syndrome are represented by dysgranulocytopenia, with pseudo Pelger-Huet hypolobulated nuclei and small vacuoles in the cytoplasm of neutrophils.<sup>88</sup> Prognosis is usually poor. Chromosome 17p is also a hot site for rearrangements arising after radio-chemotherapy or exposure to toxic environmental agents.<sup>85</sup>

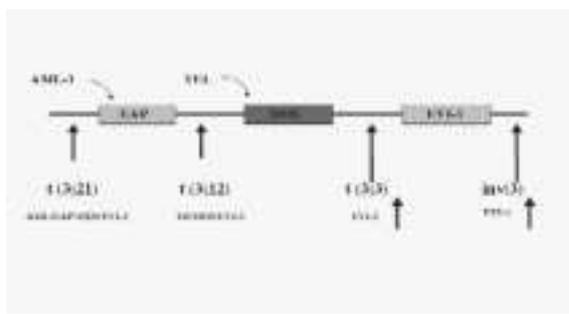
#### Changes common to MDS and AML

Table 13 summarizes the structural and numerical chromosomal changes which may be found in either MDS (especially refractory anemia with excess of blasts) or AML.

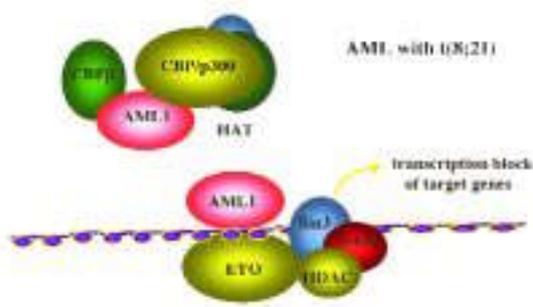
Structural changes such as inv(3); t(1,3); t(3;21) are often present in MDS or AML secondary to known genotoxics. These lesions have different breakpoints on chromosome 3 but they have a common target, the EVI 1 gene located on band 3q26 (Figure 2).<sup>89</sup> In some cases they determine the presence of hybrid transcripts, whereas in others they induce only a high and inappropriate expression of the EVI 1 gene, a transcriptional factor originally identified as a target of the insertion of murine retroviruses capable of inducing leukemias. Indeed, increased EVI 1 expression has been observed in a high percentage of MDS apparently lacking cytogenetic abnormalities of the 3q26 band.<sup>90</sup>

As in AML, also in MDS the abnormalities involving chromosome 11q23 affect the MLL gene and are characterized by marked heterogeneity in the partner gene/chromosome.<sup>91</sup> The 11q23 translocations are often secondary to exposure to topoisomerase inhibitors, suggesting that topo II sites could be involved in the genesis of these translocations.<sup>92</sup> It is possible to distinguish primary from secondary MDS based on the breakpoint position within the MLL gene. Although always located in the so called *BCR region* of the MLL, the secondary MDS have a breakpoint more 3' than the others, in a segment where six different topo II *consensus sites* are present.

The most typical changes in AML, such as t(8;21); t(15;17); and inv(16), are rarely seen in MDS and are mostly found in progressive disorders, including secondary MDS/AML, with more than 10% of blasts at presentation. A similar pathogenetic mechanism, involving the chromatin remodeling system which is fundamental to transcription, seems to be present in these types of leukemia.<sup>93</sup> The best understood example is acute promyelocytic leukemia (APL).<sup>94</sup> It has been shown that PML/RAR retains the ability of RAR to regulate transcription of ATRA-target genes and to recruit the N-CoR/histone-deacetylase complex, which leads to a repressive chromatin confor-



**Figure 2.** Different breakpoint positions on chromosome 3q23 caused by the structural changes associated with the presence of *inv(3)*, *t(1;3)* and *t(3;21)* chromosomal abnormalities present in secondary and treatment-related MDS and AML. For details see ref. #28.



**Figure 3.** Scheme showing the possible disruption that the AML1-ETO fusion product may produce in the transcriptional machinery of the myeloid cells (see text for details).

mation. High doses of ATRA release histone-deacetylase activity from PML/RAR, thus delineating the rationale for APL response to ATRA therapy. This model outlines the normal control of chromatin remodeling during gene-specific transcription. Disruption of these mechanisms gives rise to transcriptional chaos and leukemic transformation. Therefore, recruitment of the N-CoR/HD and regulation of ATRA-target genes are crucial to the transforming potential of RAR-fusion proteins. The AML1-ETO oncoprotein has recently been shown to alter gene expression through an analogous mechanism of aberrant recruitment of an N-CoR repressor complex (Figure 3).<sup>95</sup>

In an interesting recent example, Song *et al.*<sup>96</sup> demonstrated that haploinsufficiency of the AML1 gene is the genetic basis of a form of familial thrombocytopenia which predisposes the affected individuals to the development of acute myeloid leukemia. This example allows us to envisage an extremely interesting model of leukemogenesis. Indeed, hematopoiesis is the complex developmen-

tal process through which undifferentiated, pluripotent, hematopoietic stem cells generate mature, functional blood cells. This process is regulated by specific transcription factors. Leukemias represent one form of disruption of this normal developmental process. There are no significant molecular differences in the occurrence of the changes listed in Table 13 between MDS and AML.

Detailed immunophenotypic information in leukemias associated with trisomy 13 or *t(6;9)* (p23;q34) support the origin of the malignant transformation being at the level of an early myeloid progenitor in the bone marrow.<sup>97,98</sup>

#### *Changes common to MDS and CMPD*

The chronic myeloproliferative disorders with chromosomal anomalies overlapping those seen in MDS are Philadelphia-negative CML, polycythemia vera, and myelofibrosis (Table 14). Among MDS a number of changes, such as *iso(14q)*, trisomy 4, monosomy 7, and *t(5;12)* cluster in chronic myelomonocytic leukemia, following the FAB classification. A peculiar change is the *t(5;12)*, leading to a fusion gene between platelet-derived growth factor  $\beta$  receptor (PDGFR $\beta$ ) on 5q33 and ETV6 (TEL) gene on 12p13, which may also show some cytogenetic variants with either the PDGFR $\beta$  or ETV6 fusing to alternative partners.<sup>99</sup> Interestingly the hematologic disorders associated with the typical *t(5;12)*, but also with the so-called variants, have been classified alternatively among MDS with monocytosis and/or eosinophilia, chronic myelomonocytic leukemia, or Philadelphia-negative chronic myeloid leukemia. Thus, such different hematologic disorders could be different phenotypic expressions of a genetic event which involves the PDGFR $\beta$  and/or ETV6 gene.<sup>100</sup> This interpretation of biological results needs clinical confirmation in a large number of cases.

As in CMPD, so too in MDS, alterations of the *RAS signaling pathway* are frequently observed. Single nucleotide mutations at codons 12, 13 and 61, capable of fixing the corresponding RAS p21 proteins in the GTP-bound activated forms, have been described particularly in N-ras and K-ras with a variable incidence (3-40%) according to the MDS subgroup.<sup>101</sup> They are, however, particularly frequent in CMML (approximately 40%) and in juvenile myelomonocytic leukemia (JMML) (20-30%). Concerning the latter, it is well known that children affected by hereditary neurofibromatosis have a relative risk 200 times higher than normal of developing a JMML.<sup>102</sup> This is due to the fact that the NF1 gene, the tumor-suppressor gene responsible

for NF1 syndrome, normally acts as an activator of the GAP (GTPase activating) protein of RAS, whose function is to inhibit RAS activity. The complete inactivation of NF1, facilitated of course by the inheritance of an already inactivated allele, leads to unrestrained RAS activity in hematopoietic stem cells which could favor the onset of JMML.

A del(20q) is a recurrent change in polycythemia vera, while among MDS it is frequently associated with a relatively good prognosis, usually corresponding to the refractory anemia subgroup, according to the FAB classification.<sup>103</sup> A del(13q) is found in different types of chronic myeloproliferative disorders, e.g. polycythemia vera, myelofibrosis, essential thrombocythemia, and also atypical chronic myelogenous leukemia (CML), as well as in low risk MDS, as a primary karyotypic change.<sup>104</sup> Among all these malignancies a commonly deleted region could be identified which also overlaps with the smallest deleted genomic region in chronic lymphocytic leukemia.

#### *Other changes in MDS*

Trisomy 8 is a frequent, non-specific change in MDS. Indeed trisomy 8 is found in both myeloid and lymphoid acute leukemia, in Philadelphia-positive and -negative chronic myeloid leukemia as well as in all chronic myeloproliferative disorders.<sup>105</sup> Some authors claim that constitutional mosaicism underlies the selection of a bone marrow clone with trisomy 8. The prognostic significance, if any, of this numerical change in MDS has not been definitively established. Trisomy 8 in MDS is associated with a variable clinical course, from rapid evolution into acute leukemia to spontaneous disappearance of the abnormal clone.<sup>48</sup>

Partial trisomies of the 1q arm are also recurrent changes in MDS and other myeloid or lymphoid malignancies. However some unbalanced translocations containing the 1q trisomy, such as a t(1,15) or

a t(Y;1), seem to be specific to MDS.<sup>106,107</sup> The additional copy of 1q chromosome is thought to attribute a growth advantage to the malignant clone.

Finally, studies on the methylation pattern of genes involved in the control of the cell cycle as well as of a variety of other genes in MDS are raising increasing interest.<sup>108</sup> Although preliminary, these studies suggest that methylation, rather than deletion, could be a preferential mechanism for gene silencing in these disorders. This, of course, opens new perspectives that need to be addressed mainly by means of new methods of molecular screening such as the use of microarrays for gene expression profiling.<sup>109</sup> This process will probably end in a consistent contribution to a new classification of MDS that takes the genetic lesion responsible for the disease onset and progression as its consideration.

#### **Alternatives to conventional or myeloablative chemotherapy in myelodysplastic syndromes**

Two considerations are relevant to the treatment of MDS.<sup>40,101,110</sup> First, the normal hematopoietic stem cell reservoir declines with time, so that most patients with long-lasting, advanced disease have very few, if any, normal residual stem cells left. Second, although the clinical course is highly variable from patient to patient, the IPSS<sup>40</sup> provides an improved method for evaluating prognosis in individual MDS patients.<sup>110</sup>

Facing an individual patient with MDS and bearing the above considerations in mind, clinicians basically have three therapeutic choices:<sup>111</sup> 1) to avoid any manipulation of hematopoiesis and just rely upon supportive therapy; 2) to stimulate normal residual hematopoietic progenitors and/or improve the efficiency of the myelodysplastic hematopoiesis; 3) to eradicate the myelodysplastic clone and restore a normal hematopoiesis.

This chapter will examine the alternatives to chemotherapy or stem cell transplantation in the treatment of myelodysplastic syndromes. A detailed review article on this topic has been published recently in the *International Journal of Hematology*<sup>13</sup> and this chapter is essentially derived from it.

#### *Recombinant human erythropoietin*

Anemia is a major clinical problem in MDS with many patients being adversely affected by transfusion-dependency and secondary hemochromatosis. The phase I-II studies on the use of recombinant human erythropoietin (rHuEpo) in MDS have been previously reviewed.<sup>112,113</sup> Overall 15 to 20% of patients with MDS respond to rHuEpo treatment but the vast majority of responders are not transfu-

**Table 14. Changes common to MDS and CMPD.**

iso(17)(q)
iso(14)(q)/trisomy 14
del(13)(q14)
del(20)(q11)
t(9;22)(q34;q11)/BCR-ABL
t(5;12)(q33;p13)/PDGFR-ETV6
t(3;12)(q26;p13)/EVI1/MDS-ETV6

sion-dependent and the doses required to achieve response are > 450 IU/kg per week.<sup>114-116</sup> Factors predicting response include serum erythropoietin levels < 100 mU/mL, female gender and normal karyotype. MDS are typical stem cell disorders, so that the typical anemic MDS patient is expected to have a high serum Epo level, and appropriately increased endogenous Epo production. It is, therefore, unclear why some individuals show inappropriately low Epo levels, although it is now clear that the level of serum Epo reflects a balance between renal production and erythroid consumption.<sup>115</sup>

Recognizing potential responders to rHuEpo can be extremely important in individual cases of MDS.<sup>116</sup> In general, we favor a patient-oriented approach to the use of rHuEpo, such that the physician carefully evaluates the individual patient's needs and likelihood of response:<sup>113</sup> such an approach can be applied also to MDS patients.

#### *rHuEpo combined with cytokines*

Based on the hypothesis that the addition of other cytokines might improve the response to rHuEpo, several clinical trials have studied the combination of rHuEpo with either G-CSF, GM-CSF or IL-3.

The largest experience is with the combination of G-CSF and rHuEpo. The first two phase I-II pilot studies showed response rates of 38% and 42% respectively, suggesting that the response rate to this treatment was better than with rHuEpo alone.<sup>117,118</sup> Both study groups then proceeded with enlarged studies. Additional data from the American study showed that around 50% of the patients with a response to the combination lost their response when G-CSF was withdrawn and regained it when G-CSF was reintroduced.<sup>119</sup> In the other study, addition of G-CSF to unsuccessful rHuEpo-treatment induced erythroid responses in a substantial number of the patients.<sup>120</sup> These findings and the fact that the best response to G-CSF plus rHuEpo occurs in patients with RARS (who generally respond less well to rHuEpo alone) provide evidence of an *in vivo* synergy between the two drugs. Four additional studies have studied the effects of G-CSF plus rHuEpo.<sup>121-124</sup> In two of these, results were comparable with those of the larger studies while two failed to show a good response to treatment. The reason for this might have been the lower rHuEpo dose used in these negative studies. Data from the Scandinavian and American studies have recently been put together in a joint multivariate analysis, showing that the level of serum erythropoietin (< 100 mU/mL, 500-1000 mU/mL or > 500 mU/mL) and the pre-treatment transfusion need (< or  $\geq$  2 units per month) are good predictors of

erythroid response to treatment and may be combined in a predictive model.<sup>122</sup> The response rates in the good, intermediate and poor groups were 74%, 23% and 7%, respectively.

GM-CSF and rHuEpo have been combined in four smaller phase II studies.<sup>123-126</sup> In these studies, 5 out of 23 patients with a documented lack of response to rHuEpo alone responded to the combination. In a preliminary report of a randomized phase II study<sup>124</sup> it seemed that the two drugs had a synergistic effect in patients with serum Epo values < 500 mU/L. Interleukin-3 and rHuEpo show synergistic effects *in vitro* but results of two preliminary reported clinical studies have not met with the expectations.<sup>125,126</sup> Only minor hematologic improvements have been observed along with substantial adverse reactions including eosinophilia and induction of TNF- $\alpha$ .

#### *Differentiating agents*

The rationale for differentiation therapy in MDS is to overcome the phenotypic differentiation arrest and to induce a normalization of differentiation with normally functioning mature cells. Based on the findings with leukemic cell lines, clinical trials have been performed with retinoic acids, vitamin D3, interferons, hematopoietic growth factors, certain chemical differentiation inducers, e.g. hexamethylene bisacetamide, and combinations of these.<sup>127</sup> Although leading to some encouraging results *in vivo*,<sup>114</sup> the ways by which the differentiation-inducing agents actually work have remained largely unresolved. Such treatments should be performed only within prospective clinical trials and should concentrate on the low-risk groups of MDS. Further patient populations are the elderly not qualifying for the intensive chemotherapy and stem cell transplantation.

#### *Amifostine*

Amifostine, is an organic thiophosphate cytoprotective agent that has the unique ability to protect normal tissues but not tumor cells from radiation or chemotherapy through several mechanisms.<sup>128</sup> Based on the observation that amifostine promotes the *in vitro* formation and survival of primitive hematopoietic progenitors derived from myelodysplastic bone marrow specimens, List *et al.*<sup>129</sup> evaluated the hematologic effects of amifostine in 18 patients with myelodysplastic syndrome and one or more refractory cytopenias. Single- or multi-lineage hematologic responses occurred in 15 treated patients (83%). Fourteen patients had a 50% or greater increase in absolute neutrophil count.

Platelet count increased in 6 (43%) of 14 patients with thrombocytopenia and 5 of 15 red blood cell transfusion-dependent patients had a 50% or greater reduction in transfusion needs.

Subsequent studies on the use of amifostine in MDS have provided conflicting results.<sup>150,151</sup> Phase III clinical trials are needed to establish whether amifostine can really be effective in MDS patients, particularly in improving their quality of life and/or survival. At present, these treatments are to be considered strictly experimental and should be reserved exclusively to patients enrolled in clinical trials.

#### *Immunosuppressive therapy*

T-cell-mediated myelosuppression may be found in subgroups of MDS patients<sup>132</sup> so that these individuals may respond favorably to cyclosporin A (CyA) or antithymocyte globulin (ATG).

In a phase II study 25 transfusion-dependent MDS patients (with < 20% blasts) were treated with ATG 40 mg/kg/d for four doses.<sup>14</sup> Eleven subjects responded and became transfusion-independent after ATG; the median response duration was 10 months (range 3-38 months). These results have been confirmed in a recent meeting report.<sup>13</sup> Factors predicting response to immunosuppressive therapy include younger age, shorter duration of RBC transfusion duration, and positivity for HLA DRB1 15.<sup>3</sup> Biesma *et al.*<sup>133</sup> reported similar responses in two patients with hypoplastic MDS treated with ATG and CyA.

Favorable responses have been reported also with the use of CyA alone in cytopenic patients with MDS.<sup>15,194</sup> Randomized studies are now required to establish the clinical usefulness of immunosuppressive therapy in MDS patients while simple tools for revealing T-cell-mediated myelosuppression in the individual patients would be extremely useful in decision-making.

#### **Intensive chemotherapy for myelodysplastic syndromes**

Current indications for therapy in patients with MDS are based on the combined assessment of IPSS, age and performance status. At the present time, a consensus on treatment strategy has been reached for only two categories of patients.<sup>111</sup> For elderly individuals (> 60-65 years old) with low-risk MDS supportive care remains the mainstay of treatment. For high-risk patients up to 55-60 years of age initial evaluation aims at allogeneic stem cell transplantation (SCT), the only curative treatment available to date, which results in a long-term disease-free survival (DFS) rate of approxi-

mately 40%.<sup>135</sup> For all other patients no standard management has been acknowledged and such patients are usually considered for investigative clinical trials with novel therapeutic interventions. Among these, intensive chemotherapy with or without autologous SCT has emerged as an effective treatment strategy in a low but significant fraction of MDS patients.<sup>136</sup> In published studies, patients who are offered intensive chemotherapy are usually those aged 60-65 years or less with good performance status and MDS likely to experience a short-term unfavorable evolution (intermediate-2 to high-risk IPSS score).

#### *Rationale of intensive chemotherapy*

The goal of intensive chemotherapy in patients with MDS is suppression of the malignant dysplastic clone and restoration of normal polyclonal hematopoiesis. The results of several studies support the actual feasibility of this objective. First of all, the majority of patients who reach a morphologic remission after intensive chemotherapy appear to achieve a cytogenetic remission as well.<sup>137</sup> In addition, Delforge *et al.*<sup>138</sup> have analyzed the clonal pattern of highly purified hematopoietic progenitors in mobilized peripheral blood collections obtained from five female patients with high-risk MDS in complete hematologic remission after intensive induction and consolidation chemotherapy. X-chromosome inactivation patterns of flow-sorted immature (CD34<sup>+</sup>38<sup>-</sup>) and committed (CD34<sup>+</sup>38<sup>+</sup>) progenitors were studied with the polymerase chain reaction-based HUMARA assay. In four patients, a polyclonal remission was shown in all stem cell subpopulations whereas one patient was found to remain skewed in all fractions, except T-lymphocytes. In another study, peripheral blood progenitor cells were harvested during the recovery phase following induction chemotherapy in nine patients with MDS or secondary AML (sAML).<sup>139</sup> All patients had a clonal cytogenetic marker at diagnosis, and in six of them the apheresis product was found to be karyotypically normal. These studies provide strong evidence that a polyclonal, putatively normal hematopoiesis can actually be restored in patients with high-risk MDS after treatment with intensive chemotherapy.

#### *Single agent chemotherapy*

A treatment strategy based on the use of single cytotoxic agents, administered at low doses for shorter or longer periods of time, is widely employed for the palliative treatment of unfavorable MDS in patients considered unable to withstand the rigors of

myelosuppressive treatments because of advanced age and/or co-morbidities. Anecdotal reports and numerous small series suggested that cytarabine, administered either subcutaneously or by continuous intravenous infusion at 10-20% of the conventional dose used in AML, could be effective in MDS possibly by inducing cellular differentiation, although other reports did not support the activity of this approach. In a comprehensive literature review,<sup>140</sup> the overall CR rate after low-dose cytarabine was only 17% with 19% of patients achieving partial remission (PR). Myelosuppression was documented in 88% of patients, with a 15% treatment-related mortality. Low-dose oral melphalan (2 mg/day until progression/toxicity or response) was administered to 21 patients with high-risk MDS, resulting in a 38% overall response rate (7 CR, 1 PR) with minimal toxicity.<sup>141</sup> Factors predictive of response included a normal or favorable karyotype and an hypocellular bone marrow. Recently, these results were reproduced by a German group who reported a response rate of 40% (CR 30% + PR 10%) in a cohort of 21 elderly patients with high-risk MDS or sAML, reconfirming the predictive value of favorable cytogenetics and marrow hypocellularity.<sup>142</sup> Oral idarubicin has shown activity in advanced MDS, but only when clearly myelosuppressive doses were used.<sup>143</sup>

In contrast, reports on intensive single-agent chemotherapy for the treatment of MDS are rather scant in the literature. There are only two published series dealing with the use of high-dose cytarabine (HiDAC) as a single agent in MDS. In the study by Preisler *et al.*,<sup>144</sup> 15 patients were treated with 3 g/m<sup>2</sup> (2 g/m<sup>2</sup> for patients > 70 years of age) of cytarabine every 12 hours for 6 days. The CR rate was only 13%, with more than 40% toxic deaths. Larson *et al.*<sup>145</sup> treated 17 patients with MDS or sAML with cytarabine at 1-3 g/m<sup>2</sup> every 12 hours for 12 doses. Fifteen of the sixteen patients with an abnormal karyotype had anomalies involving chromosomes 5 and/or 7. Hematologic remissions were achieved in 8 patients (47%) after one (6 patients) or two (2 patients) induction courses and were confirmed by recovery of a 100% normal marrow karyotype in six of the seven patients who were retested. Patients in remission received one to four consolidation courses with HiDAC alternating with cytarabine/doxorubicin, but seven relapsed within 8 months (median remission duration, 5 months).

Recent trials with topoisomerase I-reactive agents have shown promising activity in high-risk MDS<sup>146,147</sup> (Table 15). Investigators at the MD Anderson Cancer Center have reported that topote-

can (given at 2 mg/m<sup>2</sup> by continuous i.v. infusion daily for 5 days every 4 to 6 weeks until remission) is able to induce CR in roughly a third of patients with advanced MDS and CMML, but the toxicity associated with myelosuppression is considerable, leading to a death rate of 20%. Interestingly, conversion to a normal karyotype was documented in all eight patients with clonal abnormalities who entered CR.

Decitabine (5-aza-2'-deoxycytidine) is a hypomethylating agent that has recently been evaluated in patients with MDS. By reverting the aberrant methylation pattern of specific regulatory sequences, the drug induces reactivation of silenced genes involved in the control of cell growth and differentiation. An overview of three European trials has recently been presented by Wijermans *et al.*<sup>148</sup> (Table 16). The drug was administered either as a continuous i.v. infusion (at 40-50 mg/m<sup>2</sup>/day) or as an intermittent 4-hour i.v. infusion repeated three times daily (45 mg/m<sup>2</sup>/day), for three days every 6 weeks. The results indicate that decitabine has significant activity in MDS, especially in high-risk patients, with an acceptable toxic profile. Cytogenetic responses were noted even in the high-risk patients. The high degree of myelosuppression observed in these trials suggests a cytotoxic rather than a gene demethylation-associated differentiative mechanism of action exerted by the drug.

#### *Combination chemotherapy*

Because of close similarities to AML, it comes as no surprise that intensive chemotherapy for MDS has relied mostly on the use of AML-type regimens. Since the early 1980s, the most commonly employed induction programs have included an anthracycline or mitoxantrone and either conventional or high-dose cytarabine, with or without 6-thioguanine. More recently, 3-drug combinations inclusive of etoposide and fludarabine/cytarabine or topotecan/cytarabine-based investigational regimens have drawn considerable interest based on promising early results.

Overall, the data indicate that the CR rate varies widely (range 15% to 64%) in high-risk MDS, not only as a consequence of the quality and dose intensity of the applied chemotherapy but also in relation to differences in numbers and presenting features of the patients selected for treatment.<sup>137,149</sup> In this view, it must be pointed out that selection bias is a common feature of most published series as evidenced by the median age of treated patients which is generally lower than that

**Table 15. Trials of topotecan in high-risk MDS.**

<i>Beran, 1996</i> <sup>146</sup>	<i>Beran, 1998</i> <sup>147</sup>
47 patients (median age 66 years)	60 patients (median age 66 years)
22 MDS, 25 CMML	30 MDS, 30 CMML
CR rate: 27% MDS, 28% CMML	CR rate: 37% MDS, 27% CMML
Toxic death rate: 19%	Toxic death rate: 20%
Median CR duration: 7.5 months	Median CR duration: 7.5 months
Median survival: 10.5 months	Median survival: 10.5 months

**Table 16. Trials of decitabine in MDS.**

<i>Wijermans, 1999</i> <sup>148</sup>	
125 patients (median age 70 years)	
Overall response rate (ORR): 49%	
CR:	20%
PR:	10%
Improv:	19%
ORR by IPSS	
INT-1:	39%
INT-2:	45%
HR:	58%
Toxic death rate: 8%	
Median response duration: 38 weeks	
Median survival: 15 months	

reported in unselected series of MDS.

In general, the CR rates of patients with MDS are lower than those achieved in patients with *de novo* AML treated with similar induction regimens. The reference study supporting this concept was that published by Mertelsmann *et al.*,<sup>150</sup> who performed a retrospective analysis of 263 cases of AML treated with cytarabine, daunorubicin and 6-thioguanine: 45 patients were reclassified as having MDS, and 16 as having AML that had evolved from MDS. In this group of 61 patients, the CR rate of 48% was comparable to that observed in patients with less differentiated AML subtypes (50%), but lower than the 59% CR rate associated with a more differentiated phenotype. Likewise, a retrospective study of 20 children with MDS found a significantly lower CR rate (35% vs 74%) after intensive induction chemotherapy than in 31 controls with *de novo* AML.<sup>151</sup>

Factors accounting for the lower CR rate in MDS

include: a) prolonged therapy-induced pancytopenia leading to higher early death rate, especially in older patients; b) elevated incidence of unfavorable karyotypes and frequent expression of a multidrug resistant (MDR) phenotype by the MDS clone resulting in a high degree of chemoresistance.

More recent studies, however, do not validate the assumption that patients with MDS have a worse response to intensive chemotherapy than do patients with *de novo* AML. In fact, using an identical chemotherapy regimen, De Witte *et al.*<sup>149</sup> achieved a similar CR rate in patients younger than 45 years of age irrespective of whether the patients had *de novo* AML or MDS (75% versus 71%). This finding has been confirmed in a more recent study published by the CALGB.<sup>152</sup> In this retrospective analysis, the CR rate (68% vs 79%) and the median duration of response (11 vs 15 months) did not differ significantly between younger patients with MDS or *de novo* AML when they were treated with the same protocols. Furthermore, a recent analysis by Estey *et al.*<sup>153</sup> clearly indicates that patients with RAEB and RAEB-t have the same chances of responding to intensive chemotherapy as patients with *de novo* AML presenting with comparable prognostic features including age, performance status, cytogenetics and history of cytopenias.

Analysis of the published data indicates that variables such as younger age, RAEB-t subtype, primary rather than secondary MDS, shorter interval between diagnosis and treatment and normal or favorable karyotype are all predictive of a higher CR rate after intensive chemotherapy. In particular, the combination of RAEB-t and younger age<sup>154</sup> or RAEB-t and normal karyotype<sup>155</sup> defines subsets of MDS patients highly responsive to standard AML-like chemotherapy, with CR rates in the 80% range.

Newer investigational regimens, including either fludarabine or topotecan in association with cytarabine, have recently been tested in MDS yielding encouraging results also in patients presenting with poor-risk features (Table 17). In a phase II study of 19 patients with high-risk MDS/sAML treated with the FLAG-IDA (fludarabine/cytarabine/idarubicin and G-CSF) regimen, 63% of patients entered CR with 7/12 complete responders remaining alive in CR after a median follow up of 10 months.<sup>156</sup> Response was associated with age < 50 years, shorter disease duration and cytogenetics other than abnormalities of chromosome 7. In a similar study the FLAG (fludarabine/cytarabine and G-CSF) regimen yielded an impressive CR rate of 74% in a group of 42 patients with high-risk MDS, with a toxic mortality of 9%.<sup>157</sup>

Patients with favorable cytogenetics had a significantly better treatment outcome compared with those presenting with an adverse karyotype. The efficacy and safety of topotecan (1.25 mg/m<sup>2</sup>/day by continuous i.v. infusion for 5 days) combined with high-dose cytarabine (1 g/m<sup>2</sup>/day i.v. over 2 hours for 5 days) was evaluated in 59 patients with advanced MDS and 27 with CMML.<sup>158</sup> CR was achieved more frequently in MDS than CMML (61% vs 44%), and the regimen proved to be particularly effective in patients with unfavorable karyotypes and secondary MDS, producing CR rates of 71% and 72%, respectively. This antileukemic activity in patients presenting with poor-risk cytogenetics or sAML, associated with a low induction mortality (7%), is particularly noteworthy.

The expression of the multidrug resistance (MDR) phenotype by the leukemic clone is a factor which might adversely affect treatment outcome after intensive chemotherapy. In a recent study by Lepelletier *et al.*,<sup>159</sup> expression of the P-glycoprotein was documented in 25 of 60 patients with high-risk MDS and in 7 of 10 patients with AML secondary to MDS. Response to AML-like induction chemotherapy was found to be significantly inferior in the cohort of patients expressing the MDR phenotype (CR rate 14% vs 69%). Addition of agents capable of modulating multidrug resistance, such as quinine or derivatives of cyclosporin A, may result in an improved response rate and duration of CR as recently reported by French investigators.<sup>160</sup>

The use of myeloid growth factors (G-CSF, GM-CSF) as an adjunct to the treatment program is generally associated with a faster recovery of granulocytes post-chemotherapy, but this favorable effect did not convincingly translate into an improved treatment outcome.<sup>161-162</sup> Furthermore, attempts at boosting the proliferative activity of the MDS clone by administering the growth factors before/during chemotherapy in order to render the blast cells more susceptible to the cytotoxic effects of antileukemic drugs, have yielded disappointing results both in the short-term (CR rate) and in the long-term (DFS, overall survival).<sup>163</sup>

Once achieved, CR tends to be short lived in MDS due to a very high rate of disease recurrence.<sup>137,155</sup> In most series the median duration of CR in high-risk MDS is less than 12 months, and a long-term DFS in excess of 10-15% is exceptional. However, as recently reported by the Dusseldorf group in a large series of high-risk MDS patients, intensification of post-remission chemotherapy might be of value in improving long-term results (DFS 25% at

**Table 17. Investigational chemotherapy regimens for high-risk MDS.**

Author	No. pts	Median age (yrs)	Regimen	CR%	Outcome
Parker, 1997 <sup>156</sup>	19	44	FLAG-IDA	63	7/12 in CCR (median FUP 10 months)
Ferrara, 1999 <sup>157</sup>	42	61	FLAG	74	Median DFS: 18 months Median OS: 13 months
Beran, 1999 <sup>158</sup>	86 (MDS 59) (CMML 27)	64	Topotecan + Cytarabine	61 (MDS) 44 (CMML)	MDS Median CR: 50 weeks Median OS: 60 weeks CMML Median CR: 33 weeks Median OS: 44 weeks

5 years), thus supporting the concept that a treatment strategy based on intensive induction/consolidation chemotherapy is potentially curative for a fraction of patients with MDS.<sup>164</sup> Karyotype is the most powerful indicator of DFS in high-risk MDS. In a prospective, pilot study of intensive chemotherapy for high-risk MDS and sAML conducted by the EORTC Leukemia Group, the presence of chromosomal abnormalities was predictive both for a lower CR rate and a significantly inferior DFS (8% vs 33% at 2 years).<sup>137</sup>

The generally advanced age of patients, the notion that adverse prognostic features such as MDR expression and unfavorable cytogenetics increase with age, and the indication that in patients up to the age of 60-65 years CR rates in the range of 50% can be achieved with intensive chemotherapy, have certainly made the matter of optimal treatment of high-risk MDS in the elderly a point of intense debate. The management of these patients requires careful evaluation of which treatment is the most appropriate: palliative care, intensive chemotherapy, or investigational therapies. Good performance status, preserved organ function and a relatively young age (60-70 years) may identify a subgroup of patients likely to benefit from an intensive chemotherapy approach; alternatively, investigational treatments can be offered. Among these, antibody-targeted chemotherapy is of particular interest, especially in the light of the promising results

recently reported in elderly patients with relapsed AML after treatment with gemtuzumab ozogamicin (Mylotarg), an immunotoxin consisting of an anti-CD33 monoclonal antibody conjugated with the antitumor antibiotic calicheamicin.<sup>165</sup> The drug offers the advantage of effective myelosuppression associated with a favorable toxic profile characterized by minimal extrahematologic toxicity and acceptable rates of infectious morbidity and mortality. These encouraging, albeit preliminary, results in AML may represent the basis to extend the use of Mylotarg to the treatment of elderly patients with high-risk MDS.

### Autologous stem cell transplantation

Treatment of high-risk MDS with intensive chemotherapy results in few long-term remissions as a consequence of a very high risk of disease recurrence. This has prompted, in recent years, trials of more intensive post-remission cytoreduction followed by autologous stem cell rescue in patients not eligible for allogeneic transplantation. Although restrictions based primarily on age and performance status limit the application of autologous stem cell transplantation (autoSCT) to a minority of patients with MDS, the potential for cure has encouraged extensive investigation of this treatment modality.

The rationale behind the use of autoSCT in MDS is based on the feasibility of collecting normal polyclonal stem cells at the time of chemotherapy-induced remission, a concept that has been definitively proved only in recent years.<sup>137-138</sup> Initial experience with autoSCT in MDS and sAML focused on the bone marrow as a source of hematopoietic stem cells. A recent analysis of 79 patients from the EBMT registry who received autologous marrow grafts in first CR showed 2-year DFS and overall survival respectively of 34% and 39%, with an actuarial relapse rate of 64%.<sup>136</sup> Age < 40 years was associated with a significantly superior DFS (39% vs 25%) mainly as a consequence of an increased risk of relapse in the older age group (72% vs 59%). When compared to an age-matched group of 110 patients autografted for *de novo* AML in first CR, the results obtained in a cohort of 55 patients with MDS/sAML, for whom the duration of first CR was known, were found to be significantly inferior both in terms of DFS (28% vs 51%) and overall survival (31% vs 54%). The inferior outcome was largely due to a significantly higher rate of relapse (69% vs 40%) in the MDS/sAML group since the treatment-related mortality was comparably low (around 10%) in both cohorts. In order to

speed up the somewhat delayed repopulation kinetics associated with the autografting of marrow stem cells, several groups have explored the feasibility of harvesting and transplanting mobilized peripheral blood stem cells (PBSC) in patients with high-risk MDS. Demuyneck *et al.*<sup>166</sup> treated 11 patients in CR after induction chemotherapy with one course of intensive consolidation followed by G-CSF administration. Seven patients yielded sufficient numbers ( $>1 \times 10^6/\text{kg}$ ) of CD34<sup>+</sup> cells. Five of these patients subsequently underwent autologous PBSC transplantation. All patients had rapid neutrophil recovery (median 14 days), whilst platelet recovery was somewhat delayed (median 41 days, in 4 patients). In the study by Carella *et al.*<sup>139</sup> PBSC were collected during the G-CSF-supported recovery phase of remission induction therapy in 9 patients with MDS or sAML, all presenting with clonal chromosomal abnormalities. In 6 patients the stem cell product was found to be karyotypically normal. Three patients were autografted with relatively fast hematopoietic engraftment.

Having showed that mobilization and collection of polyclonal, presumably normal PBSC is indeed feasible in high-risk MDS, these studies set the stage for large scale clinical trials of AML-like chemotherapy intensified with autoSCT. In a prospective intergroup study (trial 06921) performed between 1992 and 1997 by the EORTC Leukemia Group (EORTC-LG) in collaboration with EBMT, SAKK and GIMEMA, patients aged < 61 years with high-risk MDS or sAML received intensive induction chemotherapy with idarubicin, cytarabine and etoposide (ICE). Post-remission therapy consisting of intermediate-dose cytarabine and mitoxantrone (NOVIA) was followed by either allogeneic or autologous SCT based on the availability of a matched sibling donor. The CR rate was 54% among the 184 evaluable patients and the 4-year DFS and overall survival rates were 29% and 26%, respectively (*de Witte, personal communication*). Thirty-five of 57 patients (61%) with no suitable donor underwent autoSCT in first CR (17 bone marrow cells, 13 G-CSF mobilized peripheral stem cells, 5 both). Three patients died of complications, 19 relapsed and 13 were alive in continuous CR at last follow-up. Analysis of the kinetics of engraftment indicated a more rapid hematopoietic recovery after reinfusion of PBSC resulting in a significantly shorter duration of total hospitalization. The study suggests that an intensive treatment strategy including autoSCT can be applied to patients with high-risk MDS and sAML and long-term DFS can be

achieved. The ongoing 06961 trial conducted by the EORTC-LG in collaboration with EBMT, HOVON, SAKK and GIMEMA is addressing the question of whether this strategy is better than a chemotherapy only approach. Careful analysis of the nature of the induced remissions (cytogenetic, clonal versus non-clonal) is an integral part of the study and will serve as a tool to predict prognosis in responding patients. After induction of remission with the ICE regimen and consolidation with intermediate-dose cytarabine and idarubicin, patients without a matched sibling donor receive G-CSF during the recovery phase post-consolidation to mobilize peripheral stem cells. Patients are then randomized between autologous PBSC transplantation or a second consolidation course with high-dose cytarabine. Between December 1996 and June 2000, 246 patients (194 MDS, 52 sAML) were enrolled and had a CR rate of 58%, and a DFS at 1.5 years of 33% (*de Witte, personal communication*). Ninety-four patients received the first consolidation and PBSC were successfully harvested in 22 of 54 patients (41%) without a donor, suggesting that insufficient stem cell collection may represent a major limit to the applicability of the autografting procedure in MDS. Preliminary risk factor analysis confirms the role of adverse cytogenetics as a major determinant of treatment outcome after intensive antileukemic therapy, resulting in significantly poorer rates of CR, DFS and overall survival.

#### Remarks

Advanced age of the average patient associated with extreme heterogeneity in terms of clinical and biological features are the main reasons why the treatment of MDS continues to be problematic. The recently developed IPSS may be of assistance to the clinician in better defining the prognostic profile of any given patient, thus allowing for more individualized therapeutic options to be offered.

Selected patients with high-risk MDS or sAML may benefit from the application of intensive chemotherapy programs such as those currently employed for the treatment of *de novo* AML. However, although a substantial proportion of patients can be induced into CR, the response is generally less durable than in patients with *de novo* AML because of a higher rate of disease recurrence. The relatively high failure rate of intensive chemotherapy in MDS can be explained partly by the drug resistant profile of the leukemic clone, as suggested by the higher incidence of unfavorable cytogenetics and increased expression of the MDR phenotype compared to primary AML.

Newer investigational regimens incorporating agents with promising activity in MDS/sAML (decitabine, topotecan, fludarabine, MDR modulators) may be useful for improving not only the rate but also the quality of induced remission, but their contribution requires further testing in larger prospective clinical trials.

In an attempt to improve long-term outcome, autoSCT has been proposed as a post-remission strategy to reduce the risk of relapse in patients not eligible for allografting. The feasibility of harvesting normal hematopoietic stem cells in patients with MDS has been challenged until recently. However, it has now been established that the majority of patients entering CR after intensive chemotherapy do achieve a cytogenetic remission as well. Furthermore, peripheral stem cell collections from patients induced into CR with intensive chemotherapy are frequently polyclonal, when tested with X-linked polymorphic genes, leading to a faster hematopoietic recovery after myeloablative therapy compared to marrow-derived stem cells. Preliminary data indicate that this approach is indeed feasible in roughly 50% of complete responders and may lead to prolonged disease control in a substantial fraction of them.

The long-term benefit of an intensive treatment strategy in which autoSCT is given post-remission in alternative to consolidation chemotherapy awaits the results of ongoing, randomized clinical trials.

#### Allogeneic hemopoietic stem cell transplantation in MDS

Allogeneic bone marrow or peripheral blood stem cell transplantation is the only curative therapy for patients with myelodysplastic syndrome. The best results have been seen in young patients with less advanced disease, while in patients with RAEB, RAEB-t or CMML, the post-transplant outcome is poor mainly because of high transplant-related mortality and relapse.<sup>167-171</sup> (Table 18). Studies of feasibility of transplantation began in the 1980s: most transplants were performed in young patients with advanced disease using bone marrow as the source of hematopoietic stem cells.<sup>172-177</sup>

Recently, the age limit has been raised to 66 years,<sup>178</sup> while for young patients who lack a suitable family donor, the use of bone marrow from an unrelated donor is now a feasible alternative:<sup>179-181</sup> cord blood cells have also been successfully used.

#### *Less advanced diseases: refractory anemia or refractory anemia with sideroblasts*

In a recent paper the *European Bone Marrow Transplant Group* reported an actuarial DFS of 55%

for patients with refractory anemia or refractory anemia with sideroblasts, which is better than that observed in patients with more advanced disease; the probability of relapse was 13%.<sup>167</sup> Anderson has reported a relapse-free survival of 60% and a relapse rate of 5% in patients who received a transplant from an HLA-identical related donor.<sup>182</sup> Other authors reported a long-term survival ranging from 49 to 73% (Table 18).

Despite the low incidence of relapse, some authors in the early 1980s recommended the use of intensive preparative regimens.<sup>172</sup> In a study comparing busulphan-cyclophosphamide (BU-CY) and total body irradiation-cyclophosphamide (TBI-CY) the 3-year actuarial probability of survival was similar in the two groups: only one of 38 patients treated with TBI-CY relapsed.<sup>182</sup> Because of the low relapse rate, patients with less advanced disease could be good candidates for light intensive conditioning regimens.

The interest in the feasibility of transplantation from HLA identical unrelated donors is high because 30-40% of patients lack an available matched related donor. In a series of 40 patients with refractory anemia who received a marrow unrelated transplant, the survival rate at three years from transplantation was 56%. A better survival rate up to 66% was observed in a subgroup of patients who received transplant from a donor serologically matched for HLA and B and molecularly matched for HLA-DRB1 and HLA-DQB1.<sup>183</sup>

*Advanced diseases: refractory anemia with excess of blasts, refractory anemia with excess of blasts in transformation, chronic myelomonocytic leukemia*

The results were significantly less favorable for patients with more advanced disease. The actuarial probability of DFS survival range from 19% to 40%. A recently published update of the EBMT experience reported a DFS and a relapse rate of 28% and 43%, respectively.<sup>167</sup> Data from the GITMO group on 36 adult patients with advanced myelodysplastic syndromes showed a DFS of 40% at five years after transplantation.<sup>184</sup> There is evidence that the increasing marrow blast count gradually impairs the post-transplant outcome: Anderson reported results on 41 patients conditioned by standard TBI and CY; twenty-one patients were affected with RAEB, twenty with RAEB-t: DFS was, respectively, 38% and 19% and the Kaplan Meier estimate of relapse was 42% and 61%, respectively.<sup>185</sup> O'Donnell reported on 18 patients with RAEB or RAEB-t: the actuarial probability of survival was 56% but the follow-up was short (24

**Table 18. Allogeneic bone marrow transplantation in MDS according to FAB classification: cumulative data from published reports on allogeneic bone marrow transplantation for MDS.**

Diagnoses	No. of cases	DFS	% relapse
RA/RARS	254	49-73	0-13
RAEB	143	31-40	45
RAEBt	128	19-25	25-61
CMML/JMML	43	28-31	58

from: Appelbaum F.R. et al.<sup>168,173</sup>; De Witte T et al.<sup>169,175</sup>; O'Donnell M., et al.<sup>186</sup>; Anderson J.E. et al.,<sup>181,182,189</sup>; Sierra J. et al.<sup>187</sup>; Alessandrino E.P. et al.<sup>194</sup>; Locatelli F et al.<sup>171</sup>

months).<sup>186</sup> In another study presented in 1997, DFS was 31% in RAEB patients, 25% in RAEB-t and 28% in CMML.<sup>187</sup>

Although intensive chemotherapy may induce remission in about 60% of patients with RAEB or RAEB-t,<sup>188</sup> the role of pretransplant chemotherapy is debatable: patients treated by chemotherapy who do not achieve a response will face an eventual transplant in a bad performance status which could increase their post-transplant mortality.

*Acute leukemia from myelodysplastic syndrome*

It is rather difficult to establish the true limit between RAEB-t and acute leukemia from myelodysplastic syndromes (AL-MDS). Patients with a borderline bone marrow blast count of 30-40% may be classified as having RAEB-t or AL-MDS: the lack of a clear distinction and the status of disease at transplant may, in part, explain the wide variability in reported DFS and relapse rates.

The DFS after allogeneic transplantation in AL-MDS seems to be approximately 20% when patients receive their transplant as front-line therapy while for patients in CR or PR DFS is better (44% at three years).<sup>167</sup> A report from the Seattle Group, however, failed to demonstrate that the use of intensive chemotherapy before transplantation ameliorates the results.<sup>189</sup> Moreover some authors suggest that patients with a long history of MDS, hypocellular marrow, and multiple chromosomal aberrations who are uncertain of achieving CR after chemotherapy should be addressed to transplant without any attempt to achieve remission.<sup>167,190</sup> Because of the lack of large prospective studies, it is still questionable whether patients with RAEB-t or AL-MDS should receive induction chemotherapy before

transplantation: in patients with a donor and a slight chance of cure by first-line transplantation, there is a risk of pre-transplant death during induction chemotherapy if this is chosen.

#### *Prognostic factors*

There are several data showing that the proportion of blast cells in the marrow, advanced patient's age and multiple cytogenetic aberrations have a negative impact on the post-transplant outcome).<sup>173,191-2</sup> Runde, for the *Chronic Leukaemia Working Party of the EBMTG*, analyzed 131 patients with MDS who received a transplant as first-line therapy; in this study younger age, short disease duration, and absence of excess of blasts were associated with better post-transplant outcome.<sup>170</sup> Similarly, the Seattle team showed that age, time from diagnosis to transplant, advanced versus less advanced MDS, and poor versus good cytogenetic risk are of statistical significance for risk of relapse.<sup>168,173</sup> Other studies have confirmed that cytogenetic aberrations are independent predictors of post transplant outcome.<sup>191</sup> Considering which pre-transplant variables foretell a good post-transplant outcome, the EBMTG demonstrated that age and stage of disease had independent prognostic significance for DFS and transplant-related mortality (TRM); in addition patients transplanted in early disease had a lower risk of relapse than patients transplanted in advanced phase.<sup>167</sup> The IPSS score was developed to evaluate prognosis in MDS more carefully;<sup>14</sup> it considers the percentage of blast cells in the marrow, the karyotype, and the number of peripheral blood cytopenias. On the basis of these variables, patients are classified as having low risk, intermediate 1, intermediate 2, or high risk disease. Data from Seattle suggest that the IPSS score may also be used to predict survival after hematopoietic stem cell transplantation: in a series of 251 patients, the 5-year DFS rates were 60% for low and intermediate-1 risk patients, 36% for intermediate--2 risk ones, and 28% for high risk patients.<sup>168</sup>

#### *Older patients*

The post-transplant outcome is generally poor in patients older than 55 years of age because of a high incidence of transplant-related mortality; it is questionable whether old patients are candidates for transplant. Recently, Deeg reported on a cohort of 50 patients with MDS who were 55-66 years old (median age 59 years) and who received a transplant from an HLA identical related donor (36 cases), an HLA non-identical family member (4 cases) or from an unrelated identical donor (6 cases); four patients received a transplant from an identi-

cal twin. Forty-five patients were classified according to IPSS score: two had low risk, 14 intermediate-1 risk, 19 intermediate-2 risk, and 10 high risk disease. The Kaplan-Meier estimate of relapse-free survival was 39% at three years. Survival was high among patients who received cyclophosphamide and busulphan (busulphan plasma level target, 900 µg/mL).<sup>178</sup> Future studies will, however, need to optimize conditioning regimens in this subset of patients. The recent use of non-myeloablative conditioning regimens could find in MDS a more appropriate application, particularly in patients with less advanced disease. Slavin reported on one patient with MDS who entered CR.<sup>193</sup> Long-lasting complete remission and acceptable toxicity have been observed after thiotepa and fludarabine given in association in three old patients with RAEB.

#### *Transplant from unrelated donors*

Suitable related donors are available to one third of patients with MDS. However, thanks to the increasing size of the worldwide registries of volunteer donors, the use of unrelated donor transplants is becoming more widespread. Following the first data reported in the literature<sup>179,194,195</sup> an increasing number of patients receive a transplant from an unrelated donor.<sup>167,180,181</sup> Disease-free survival ranges from 18 to 38%. The TRM is higher among older patients and in those with longer disease duration, ranging from 48 to 58%. Results from Seattle on 52 patients with MDS or AL-MDS show a 2-year actuarial DFS, risk of relapse and transplant-related mortality of 38%, 28% and 48%, respectively.<sup>181</sup> The EBMTG reported on 198 patients: DFS was 25%, transplant-related mortality 58% and risk of relapse 41%. In patients over 40 years old, DFS was 11% with a high TRM.<sup>167</sup> Although the results from allogeneic transplantation seems to improve over time, the high incidence of TRM in the elderly suggests that, at present, recourse to unrelated donors should be reconsidered, at least in patients over 50 years old.

#### *Remarks*

All patients with MDS aged less than 50 years with a related or unrelated histocompatible donor are candidates for bone marrow transplantation.

The IPSS may help us to define the timing of transplantation: in MDS patients at high, intermediate-1 or intermediate-2 risk the procedure should be performed as early as possible. In low risk patients, weighing the median survival against the high TRM, caution is necessary. In this subset of patients, transplantation may be delayed without fear.

## Myelodysplastic syndromes in childhood

### *Peculiarities of childhood myelodysplastic syndromes*

Among the clonal disorders, MDS are relatively unusual in childhood, representing only 5-7% of pediatric hematologic malignancies,<sup>196,197</sup> although it has been suggested that up to 17% of cases of pediatric acute myeloid leukemia (AML) may have had a previous myelodysplastic phase.<sup>198</sup> A non-significant trend toward an increase in childhood MDS in recent years has been reported.<sup>199</sup>

Childhood MDS may be primary or secondary disorders. Secondary MDS occur in children with constitutional/genetic disease, in patients with severe aplastic anemia given immunosuppressive treatment, or in patients exposed to myelotoxic agents.<sup>196</sup> An increasing number of secondary AML and MDS, most frequently in children previously treated for HD, but also after other hematologic and non-hematologic malignancies, have been reported.<sup>200-202</sup>

Characteristically, some genetic conditions such as Fanconi's anemia, Shwachman's syndrome and Down's syndrome predispose to the development of MDS in childhood. Recent studies indicate that up to 30% of children with MDS have an inherited constitutional genetic disorder.<sup>21,199</sup> A list of genetic conditions associated with the development of MDS in childhood is reported in Table 19. Some of these disorders deserve particular consideration and special comments.

The familial occurrence of complete or partial monosomy of chromosome 7 in association with MDS, AML and not otherwise specified myeloproliferative disorders has been documented in 13 pedigrees.<sup>203-205</sup> Most of the reported cases with familial monosomy 7 share a young age of onset of MDS/AML (22 out of 26 cases were below 18 years of age), and, noteworthy, in each family the range of onset age of the affected members was narrow, being 4 years or less in 11 out of the 13 families. A different parental origin of the lost chromosome 7 was demonstrated in 3 of these 13 families.<sup>203,204</sup> This finding strongly argues against the hypothesis of a germ-line mutation of a possible tumor-suppressor gene located on chromosome 7. An inherited gene mutation displaying a mutagenic effect has been hypothesized to exist in familial monosomy 7. According to this theory, the gene mutation is responsible for a form of chromosomal instability leading to marrow chromosome 7 anomalies and, in turn, to development of MDS/AML. Support to this speculation is provided by the find-

**Table 19. Chromosomal and genetic disorders predisposing to development of myelodysplastic syndromes in childhood.**

<i>Chromosomal disorders</i>	<i>Genetic disorders</i>
Trisomy 21	Fanconi's anemia
Mosaics for trisomy 8	Neurofibromatosis-type I
Klinefelter's syndrome	Schwachman-Diamond's syndrome
	Noonan's syndrome
	Kostmann's syndrome
	Bloom's syndrome
	Familial platelet disorders with acute myeloid leukemia
	Familial monosomy 7 syndrome

ings in autosomal dominant, familial platelet disorder with leukemia (FPD/AML), a disease characterized by thrombocytopenia and anomalies of platelet aggregation.<sup>206</sup> Affected individuals have a high propensity to develop MDS and AML, with abnormalities of chromosome 5q and 7q regions. The FPD/AML predisposition locus has been mapped on chromosome 21q22 and the causative gene for this disorder is CBFA2 (AML1),<sup>96</sup> the function of which is frequently disrupted in acute leukemia by various reciprocal translocations, such as t(8;21), t(3;21) and t(12;21). Heterogeneous point mutations and small deletions of a single AML1 gene have been documented in different FPD/AML pedigrees<sup>96</sup> and it has, therefore, been hypothesized that AML1 may act as a tumor suppressor gene, the loss of one allele (hemizygous loss) being sufficient to initiate tumorigenesis. The loss of function of a single AML1 gene would confer a susceptibility to acquire secondary mutations and/or the loss of chromosome regions frequently associated with development of MDS and AML.

MDS have a particular relevance for patients with Fanconi's anemia. In fact, the actuarial probability of developing MDS or AML in this disease increases over time, approaching a value of 50% in the rare patients who reach the fourth decade of life.<sup>207</sup> Moreover, the exquisite sensitivity of patients with Fanconi's anemia to DNA cross-linking agents and the markedly reduced reserve of hematopoietic progenitors make treatment of these patients extremely difficult. Not surprisingly, the risk of developing MDS or AML has been shown to be higher in Fanconi's anemia patients with a prior clonal cytogenetic abnormality than in those without such abnormalities.<sup>207</sup>

The FAB classification has been widely employed for pediatric patients as well for adults. Several studies highlighted that the less aggressive subtypes, RA and RARS, are rare in children, since the majority of children with MDS fall into the bad risk categories (RAEB and RAEB-t).<sup>197,208,209</sup> However, the applicability of the FAB classification to childhood MDS is neither completely satisfactory nor comprehensive of all disorders classically included in the group of pediatric myelodysplasias. In fact, the FAB classification does not include the commonest variants of pediatric MDS, namely juvenile myelomonocytic leukemia (JMML, in the past also known as juvenile chronic myelogenous leukemia), a disorder that shares clinical and biological features common to both MDS and myeloproliferative disorders (MPD). JMML has now been recognized as a distinct entity by the new WHO classification of MDS.<sup>39</sup>

#### *Juvenile myelomonocytic leukemia*

JMML is a malignant disorder of the multipotent hematopoietic stem cell, accounting for 2-3% of all cases of childhood leukemia.<sup>210,211</sup> A higher incidence of the disease in males and in patients with type 1 neurofibromatosis (NF-1) has been reported. In particular, in a recent large series of children with JMML, 14% were found to have NF-1.<sup>210</sup> At diagnosis, most of the patients are aged less than 2 years and approximately 90% are younger than 4 years of age.<sup>210</sup> Patients are often difficult to diagnose because of the clinical heterogeneity. Massive splenomegaly, hepatomegaly, generalized lymphadenopathy and skin manifestations (eczematous rash, xanthomata) are common clinical features.<sup>196,210-212</sup> Leukemia infiltration of the lungs can determine a clinical picture characterized by cough, tachypnea and bronchospasm, with a radiological interstitial pattern.

Leukocytosis (usually below  $100 \times 10^9/L$ ), absolute monocytosis ( $>1 \times 10^9/L$ ), anemia, variable normoblastemia and thrombocytopenia are the hallmarks of the peripheral blood picture frequently reported at the onset of the disease.<sup>210-212</sup> Other laboratory findings include increased synthesis of hemoglobin F (associated with reversion to a true pattern of fetal hematopoiesis) and elevated serum levels of muramidase, vitamin B<sub>12</sub> and IgG, IgA and IgM. The presence of autoantibodies is also common. Leukocyte alkaline phosphatase cannot be regarded as a specific marker of the disease, since 60% of patients have a normal or even increased score. Philadelphia chromosome is always absent, even though other chromosomal abnormalities

(mainly monosomy of chromosome 7) have been reported in 30-40% of the described cases.<sup>196,210,212</sup>

JMML is characterized by an aggressive clinical course and, even though the disease rarely undergoes transformation to a frank blast crisis, the median survival is less than 10 months from the diagnosis.<sup>210,212,213</sup> Response to single agent chemotherapy is poor and even intensive combination treatment has been demonstrated to produce only suppression, but not eradication of the malignant clone.<sup>214,215</sup> Variables documented in several studies to be associated with shorter survival are: age older than 2 years at presentation, thrombocytopenia ( $>40 \times 10^9/L$ ), and increased levels of HbF ( $>10\%$ ).<sup>210-213</sup> Other variables documented in one study to affect patients' survival unfavorably include hepatomegaly, bleeding, high counts of normoblasts and blast cells in the peripheral blood.<sup>212</sup> Chromosomal abnormalities influence neither the natural course of the disease nor the patients' clinical outcome with the various treatment options.

Spontaneous growth of CFU-GM and inhibition of normal hematopoietic progenitors have been documented to be the main pathogenic mechanism of JMML. A number of *in vitro* studies have tried to elucidate the biological behavior of hematopoietic progenitors of JMML patients, demonstrating that: i) peripheral blood CFU-GM can proliferate in semi-solid cultures in the absence of added growth factors;<sup>216</sup> ii) spontaneous growth of peripheral blood CFU-GM depends on the presence of monocyte-macrophages, since it can be suppressed by depletion of adherent cells;<sup>217</sup> iii) the spontaneous CFU-GM growth is promoted by GM-CSF, which induces this proliferation as an autocrine-paracrine growth factor;<sup>218</sup> iv) CFU-GM growth is ascribable to an exquisite hypersensitivity of JMML CFU-GM to GM-CSF and not to cytokine overproduction.<sup>219</sup> In fact, growth of peripheral blood CFU-GM in patients with JMML reaches maximal values at very low concentrations of this cytokine and the hypersensitivity of these hematopoietic progenitors is not expressed with other growth factors. Besides providing fundamental insights for the comprehension of the pathogenesis of the disease, both spontaneous growth of peripheral blood CFU-GM and hypersensitivity to GM-CSF represent diagnostic confirmatory tests of paramount importance.

The primary pathogenic mechanism of JMML also seems to involve autocrine production and release of TNF- $\alpha$ . In fact, TNF- $\alpha$  plays a central role in inhibiting normal hematopoiesis and directly pro-

motes proliferation of malignant monocytes-macrophages and GM-CSF production. This, in turn, further favors replication of GM-CSF *hypersensitive* cells, with IL-1 representing an important accessory factor which further augments the effect of the other cytokines.<sup>220</sup> TNF- $\alpha$  significantly contributes to the striking cachexia shown by a relevant proportion of these patients at diagnosis or during the course of the disease. The crucial role played by GM-CSF and TNF- $\alpha$  in the pathogenesis of JMML makes these cytokines and their receptors attractive targets for treatment with receptor-specific monoclonal antibodies, growth factor analogs, diphtheria toxin fused with GM-CSF or catalytic RNA molecule.<sup>221,222</sup>

As mentioned above, children with NF-1 are particularly prone to developing JMML. Neurofibromin, encoded by the NF-1 gene, is a GTPase-activating protein that binds to RAS and accelerates hydrolysis of GTP to GDP.<sup>223,224</sup> Since GTPase-activating proteins regulate the process of signal transduction involving RAS genes, loss or inactivation of a GTPase-activating protein, such as NF-1, could lead to elevated levels of RAS-GTP and this, in turn, might be an essential step in malignant transformation. Shannon *et al.*<sup>225</sup> demonstrated loss of heterozygosity for the NF-1 gene in bone marrow samples of 5 out of 11 children with NF-1 in whom malignant myeloid disorders developed, thus providing further evidence that the NF-1 gene acts *in vivo* as a tumor suppressor in myeloid cells. The role of NF-1 gene in the pathogenesis of MDS in children who do not have NF-1 disease is still unclear, even though the same authors, studying 25 children with myeloid disorders and monosomy 7, found that all bone marrow samples retained parental alleles. More recently, Side *et al.* found mutations of the NF-1 gene in bone marrow cells of 3 out of 20 children with JMML, without clinical evidence of NF-1.<sup>226</sup> Since other studies suggested that 10-15% of patients with JMML have a clinical diagnosis of NF-1,<sup>210,211</sup> it can be estimated that mutations of the NF-1 gene exist in approximately 30% of JMML cases. Considering that an additional 20-30% of cases have been reported to be associated with somatic RAS mutations,<sup>227</sup> altered RAS pathway signaling can be present in up to 60% of patients with JMML. This latter finding provides the rationale for investigating inhibitors of RAS pathway in the treatment of patients with JMML. Farnesyltransferase inhibitors are compounds capable of blocking the prenylation of RAS. They were demonstrated to have

significant growth inhibitory effects *in vitro* on cells of JMML,<sup>228</sup> suggesting a potential role in the treatment of this disorder. Clinical studies are needed to evaluate the efficacy of these RAS pathway-signaling inhibitors.

Monosomy and partial deletion of chromosome 7 have been described in primary AML and MDS and, particularly, in secondary or therapy-related AML and MDS. In the past, monosomy 7 has been considered to characterize a distinct variant of hematologic malignancy in early childhood, namely monosomy 7 syndrome.<sup>197,209,229</sup> Monosomy 7 syndrome most often affects boys less than 2 years of age and its clinical presentation resembles that observed in some myeloproliferative disorders, particularly JMML. Compared to children with JMML, patients with monosomy 7 syndrome usually have lower WBC count, lower fetal hemoglobin, higher percentage of monocytes and conspicuous bone marrow erythroid hyperplasia.<sup>210</sup> As mentioned above, familial forms of monosomy 7 have also been described, with some subjects carrying the cytogenetic lesion without any clearly evident clinical or hematologic abnormality.<sup>203-205</sup> A practical consideration for this condition is that children with monosomy 7 syndrome should be given marrow transplantation from an HLA-identical sibling only after a clear and unequivocal demonstration that the potential donor does not have the cytogenetic abnormality. However, in view of the clinical and biological similarities between JMML and monosomy 7 syndrome, the latter disorder is no longer considered to be a separate entity, but, reasonably, represents a variant of the former.

#### *Treatment of childhood MDS*

As mentioned above, myelodysplasia in children is often characterized by an aggressive clinical course, by the virtual absence of some subgroups (i.e. RARS) and by the presence of peculiar variants (i.e. JMML). These facts must be held in due consideration in the process of deciding the optimal therapeutic strategy. Moreover, on the basis of the longer life-expectancy of children as compared to adults, data available on the different options of treatment mainly referring to adult patients are only partially applicable to the management of childhood MDS. In fact, the primary aim of the pediatric hematologist must be a definitive cure, achievable with the eradication or alternatively the differentiation of the malignant clone, leading to reconstitution of normal hematopoiesis.

Strategies based on supportive treatment, use of

differentiating agents or hematopoietic growth factors are of limited utility in children with MDS.<sup>196</sup> The observation that most pediatric MDS have an aggressive clinical course,<sup>196,208,209</sup> has justified the use of intensive treatment, aimed at eradicating the malignant clone and reconstituting normal hematopoiesis. Even though chemotherapy has been found to induce hematologic remission in a percentage of young MDS patients similar to that observed in subjects with primary AML,<sup>149,230</sup> the response to chemotherapeutic agents is complicated by prolonged periods of aplasia.<sup>196,230</sup> Moreover, the duration of hematologic remission in patients with MDS has been reported to be generally short. A study published by the Nordic Pediatric Haematology group comparing the outcome of children with *de novo* MDS and children with *de novo* AML documented that patients belonging to the former group had a lower rate of CR and a higher risk of death of treatment-related complications.<sup>151</sup> More recently, the role of intensive chemotherapy before allogeneic hematopoietic stem cell transplantation (HSCT) has been evaluated in children with MDS other than JMML.<sup>231</sup> The outcome of patients given intensive chemotherapy prior to the allograft was found to be absolutely comparable to that of children who were transplanted directly. Moreover, the probability of survival was not influenced by the marrow blast percentage at time of transplantation. Thus, it remains to be proven whether treatment with intensive chemotherapy can be helpful and can increase the rate of patients cured with an allogeneic HSCT. Only prospective studies will resolve the issue of whether patients with MDS other than JMML should receive remission-induction chemotherapy prior to an allograft. For the time being, some patient categories with a low likelihood of entering CR after intensive chemotherapy may be identified. These patients are characterized by a prolonged history of MDS, hypocellular marrow or multiple chromosomal abnormalities.<sup>153</sup> In these cases, allogeneic HSCT may be considered as first-line therapy.

The use of autologous HSCT in childhood MDS other than JMML, theoretically questionable in a disorder of the multipotent hematopoietic stem cell, has been proposed by the Children's Cancer Group<sup>232</sup> after a conditioning regimen consisting of busulfan and cyclophosphamide. In this trial, including patients with both AML and MDS, children lacking an HLA-identical sibling received intensively timed induction therapy, which was followed by 4-

hydroperoxycyclophosphamide-purged autologous marrow transplantation. The reported results are encouraging, but they were obtained in only one study, which enrolled a limited number of children. Therefore, they should be considered preliminary and need to be confirmed in a larger, randomized study.

Hydroxyurea and IFN- $\alpha$  with or without splenectomy, while effective in Philadelphia chromosome positive CML, has not proved to be particularly useful in children with JMML. Contrariwise, these patients have been reported to have some benefit in terms of disease control from treatment with oral 6-mercaptopurine alone or combined with subcutaneous Ara-C.<sup>196,233</sup> Nonetheless, Castro-Malaspina *et al.*<sup>212</sup> reported that none of 33 patients affected by JMML and given chemotherapy achieved complete remission. Partial or even complete responses to 13-cis-retinoic acid have been reported in 5 out of 10 children with JMML.<sup>234</sup> However, responding patients were usually below the age of 2 and, as mentioned above, younger children have less aggressive disease. Moreover, the results of this study have not been further confirmed in larger cohorts of patients.

Currently, allogeneic HSCT represents the only therapy definitively proved to be able to cure a significant proportion of children with MDS. However, few studies,<sup>235-238</sup> the majority of which enrolled a limited number of patients, have specifically addressed the issue of the role of allografting in children with MDS and several crucial questions are still unsolved. In particular, it has still not been precisely defined what percentage of children with MDS are cured by an allograft and the optimal preparative regimen to be employed. Previously published studies have suggested that patients given a busulfan-based preparative regimen have an outcome comparable to or even better than that observed in patients given radiotherapy.<sup>237,238</sup> Furthermore, since several reports documented the long-term morbidity of total body irradiation (TBI), avoiding radiotherapy could have the advantage of reducing the risk of radiation-induced growth retardation,<sup>239</sup> hypothyroidism and neuropsychological sequelae, all factors that have a deleterious impact on the quality of life, particularly that of young children.

A previously published study of the European Working Group on Childhood MDS (EWOG-MDS) registry on 43 patients with JMML given allogeneic HSCT documented that in this cohort of patients the 5-year Kaplan-Meier event-free survival (EFS) was 31%, the actuarial probabilities of EFS for children

transplanted from either HLA-identical siblings or mismatched family or unrelated donors being 38 and 22%, respectively.<sup>49</sup> Patients given transplantation from a compatible relative after a busulfan-based preparative regimen enjoyed a better EFS than those treated with TBI (62 vs 11%, respectively). The impact of other claimed disease-related prognostic factors, such as hematologic findings at diagnosis, is rendered null by the transplant procedure. The role of splenectomy before HSCT in patients with JMML is uncertain, the potential advantages having to be weighed against the risks related to the procedure or to post-splenectomy infections. In the group of patients mentioned above, as well as in patients with CML, splenectomy prior to HSCT does not seem to influence patients' outcome. However, this treatment should be considered in children with massive splenomegaly or evidence of hypersplenism in order to reduce the tumor burden, to hasten hematologic recovery or to increase platelet count at the time of transplantation with a consequent lower risk of hemorrhagic complications. In view of the available results, no particular chemotherapy treatment can be recommended before HSCT in children with JMML.

#### Manuscript processing

*This manuscript was peer-reviewed by the Editor-in-Chief. A list of papers on myelodysplastic syndromes published in the last two years in this journal appears at the end of the references.<sup>239</sup> Manuscript received June 7, 2001; accepted October 1, 2001.*

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