

# INDIAN COUNCIL OF MEDICAL RESEARCH



## CONSENSUS DOCUMENT FOR MANAGEMENT OF MYELOYDYSPLASTIC SYNDROME (MDS)

*Prepared as an outcome of  
ICMR Subcommittee on Myelodysplastic Syndrome (MDS)*



Division of Non Communicable Diseases  
Indian Council of Medical Research  
2019



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Division of Non Communicable Diseases  
Indian Council of Medical Research  
Ansari Nagar, New Delhi – 110029  
2019

### **Disclaimer**

This consensus document represents the current thinking of experts on the topic based on available evidence. This has been developed by national experts in the field and does not in any way bind a clinician to follow this guideline. One can use an alternate mode of therapy based on discussions with the patient and institution, national or international guidelines. The mention of pharmaceutical drugs for therapy does not constitute endorsement or recommendation for use but will act only as a guidance for clinicians in complex decision –making.

Prof. Balram Bhargava  
Secretary,  
Department of Health Research  
and Director General, ICMR

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Dr. Neeraj Tandon, Head (Publication & Information)

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## Foreword

I am glad to write this foreword for Consensus document for Management of Myelodysplastic Syndrome (MDS). The ICMR had constituted sub-committees to prepare consensus document for management of various cancer sites. The various subcommittees constituted under Task Force project on Review of Cancer Management Guidelines worked tirelessly in formulating site-specific guidelines. The purpose of consensus document is to provide clear, consistent, succinct, evidence-based guidance for management of various cancers. I appreciate and acknowledge support extended by each member of the subcommittees for their contribution towards drafting of the document.



This document consolidates the modalities of treatment including the diagnosis and classification of MDS, cytogenetics and novel therapeutics in MDS. It also summarizes risk stratification and management aspects. Hope that it would provide guidance to practicing doctors and researchers for the management of patients suffering from Myelodysplastic Syndrome and also focusing their research efforts in Indian context.

It is understood that this document represents the current thinking of national experts on the subject based on available evidence. Mention of drugs and clinical tests for therapy do not imply endorsement or recommendation for their use, these are examples to guide clinicians in complex decision making. We are confident that this Consensus Document for Management of Myelodysplastic Syndrome would serve desired purpose.

*Balram Bhargava*

(Dr. Balram Bhargava)  
Secretary, Department of Health Research  
and Director-General, ICMR

## Message

I take this opportunity to thank Indian Council of Medical Research and all the expert members of the subcommittees for having faith and considering me as chairperson of ICMR Task Force project on guidelines for management of cancer.

The Task Force on management of cancers has been constituted to plan various research projects. Two sub-committees were constituted initially to review the literature on management practices. Subsequently, it was expanded to include more sub-committees to review the literature related to guidelines for management of various sites of cancer. The selected cancer sites are lung, breast, oesophagus, cervix, uterus, stomach, gall bladder, soft tissue sarcoma and osteo-sarcoma, tongue, acute myeloid leukemia, acute lymphoblastic leukaemia, CLL, Non Hodgkin's Lymphoma-high grade, Non Hodgkin's Lymphoma-low grade, Hodgkin's Disease, Multiple Myeloma, Myelodysplastic Syndrome, Pediatric Lymphoma, Pancreatic Cancer, Hepatocellular Carcinoma and Neuroendocrine Tumours. All aspects related to management were considered including, specific anti-cancer treatment, supportive care, palliative care, molecular markers, epidemiological and clinical aspects. The published literature till October 2015 was reviewed while formulating consensus document and accordingly recommendations are made.

Now, that I have spent over a quarter of a century devoting my career to the fight against cancer, I have witnessed how this disease drastically alters the lives of patients and their families. The theme behind designing of the consensus document for management of cancers associated with various sites of body is to encourage all the eminent scientists and clinicians to actively participate in the diagnosis and treatment of cancers and provide educational information and support services to the patients and researchers. The assessment of the public-health importance of the disease has been hampered by the lack of common methods to investigate the overall worldwide burden. ICMR's National Cancer Registry Programme (NCRP) routinely collects data on cancer incidence, mortality and morbidity in India through its co-ordinating activities across the country since 1982 by Population Based and Hospital Based Cancer Registries and witnessed the rise in cancer cases. Based upon NCRP's three year report of PBCR's (2012-2014) and time trends on Cancer Incidence rates report, the burden of cancer in the country has increased many fold.

In summary, the Consensus Document for management of various cancer sites integrates diagnostic and prognostic criteria with supportive and palliative care that serve our three part mission of clinical service, education and research. Widespread use of the consensus documents will further help us to improve the document in future and thus overall optimizing the outcome of patients. I thank all the eminent faculties and scientists for the excellent work and urge all the practicing oncologists to use the document and give us valuable inputs.



(Dr. G.K. Rath)  
Chairperson  
ICMR Task Force Project

# Preface

Myelodysplastic Syndromes (MDS) comprise of a heterogeneous group of clonal hematopoietic stem cell malignancies with significant morbidity and high mortality. The incidence of MDS increases markedly with age. The SEER–Medicare database suggests that the incidence of MDS is as high as 75 per 100,000 persons aged 65 years. Even though accurate data from India is not available, the disease is important from a treatment standpoint, because it affects patients in the prime of their life ( a decade or a decade and half earlier than the west ) and with appropriate management ; good quality life for several years is possible in the majority of cases.



For optimal outcome, the disease requires careful initial work up, diagnosis, prognostic scoring and specialized treatment at a center where all the investigative and treatment modalities are available, in addition to experts who are experienced and well versed in the management of the disease and its progression.

There have been major advancements in the treatment of MDS in the past two decades and new novel drugs have been discovered, tested and launched at regular intervals. Progress in this disease has been so rapid that the ICMR MDS subcommittee had a difficult time in finalizing this consensus document – as some new major development would happen and the team would feel them to be included in the final guideline. Rapid advances in the field of MDS will continue to happen and this document may have periodic reviews and up gradations at regular intervals.

In the run up to this consensus document, the team has highlighted the minimum workup and treatment in MDS which should be available to all patients, including patients with limited resources. Another important aspect of this report are the areas of future research in the field of MDS as relevant to our country.

As the Chair of this ICMR Sub-Committee on MDS, I would like to thank each and every member of the team for their timely and highly skilled contributions. I would also like to place on record my most sincere thanks to Dr. Balram Bhargava, Secretary, Department of Health Research and Director General of the ICMR for far-sightedness in conceptualizing and supporting this important project. Prof G.K Rath, Chair of this Task Force Project; Dr. RS. Dhaliwal and Dr. Tanvir Kaur of the Non-Communicable Diseases (NCD) of the ICMR; deserve a special mention for their constant support and guidance to the team at every stage of making this report. My sincere thanks to Col Rajan Kapoor for his valuable inputs.

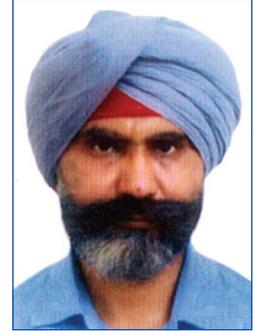
Last but not the least; I, on behalf of the MDS team hope and wish that this document will be found useful for the practising clinicians in their day-to-day workup and management of patients with MDS.

A handwritten signature in blue ink, appearing to read 'Deepak Mishra', with a horizontal line underneath.

Dr. (Col) Deepak Kumar Mishra  
Chairperson,  
Sub-committee on Myelodysplastic Syndromes (MDS)

# Preface

Cancer is a leading cause of death worldwide. Globally cancer of various types affect millions of population and leads to loss of lives. According to the available data through our comprehensive nationwide registries on cancer incidence, prevalence and mortality in India among males cancers of lung, mouth, oesophagus and stomach are leading sites of cancer and among females cancer of breast, cervix are leading sites. Literature on management and treatment of various cancers in west is widely available but data in Indian context is sparse. Cancer of gallbladder and oesophagus followed by cancer of breast marks as leading site in North-Eastern states. Therefore, cancer research and management practices become one of the crucial tasks of importance for effective management and clinical care for patient in any country. Hence, the need to develop a nationwide consensus for clinical management and treatment for various cancers was felt.



The consensus document is based on review of available evidence about effective management and treatment of cancers in Indian setting by an expert multidisciplinary team of oncologists whose endless efforts, comments, reviews and discussions helped in shaping this document to its current form. This document also represents as first leading step towards development of guidelines for various other cancer specific sites in future ahead. Development of these guidelines will ensure significant contribution in successful management and treatment of cancer and best care made available to patients.

I hope this document would help practicing doctors, clinicians, researchers and patients in complex decision making process in management of the disease. However, constant revision of the document forms another crucial task in future. With this, I would like to acknowledge the valuable contributions of all members of the Expert Committee in formulating, drafting and finalizing these national comprehensive guidelines which would bring uniformity in management and treatment of disease across the length and breadth of our country.



(Dr. R.S. Dhaliwal)  
Head, NCD Division

# Acknowledgement

The Consensus Document on Management of Myelodysplastic Syndromes (MDS) is a concerted outcome of effort made by experts of varied disciplines of oncology across the nation. The Indian Council of Medical Research has constituted various sub committees to formulate the document for management of different cancer sites. The Task Force on Management of Cancers has been constituted to formulate the guidelines for management of cancer sites. The sub-committees were constituted to review the literature related to management and treatment practices being adopted nationally and internationally of different cancer sites. The selected cancer sites are that of lung, breast, oesophagus, cervix, uterus, stomach, gallbladder, soft tissue sarcoma and osteo-sarcoma, tongue, acute myeloid leukaemia, ALL, CLL, NHL-high grade, NHL-low grade, HD, MM, MDS, and pediatric lymphoma. All aspects related to treatment were considered including, specific anti-cancer treatment, supportive care, palliative care, molecular markers, epidemiological and clinical aspects.



This document represents a joint effort of large number of individuals and it is my pleasure to acknowledge the dedication and determination of each member who worked tirelessly in completion of the document.

I would like to take this opportunity to thank Dr. GK Rath, chairperson, ICMR Task Force on Guidelines for Management of Cancer for his constant guidance and review in drafting the consensus document. The chairperson of subcommittee Dr. Deepak K. Mishra, is specially acknowledged in getting the members together, organizing the meetings and drafting the document.

I would like to express gratitude to Dr. Balram Bhargava, Secretary, Department of Health Research and Director General, Indian Council of Medical Research, for taking his special interest and understanding the need of formulating the guidelines which are expected to benefits the cancer patients.

I would like to thank Dr. R.S. Dhaliwal, head, Division of Non Communicable Diseases for his support and coordination in finalizing this document. I would like to acknowledge the assistance provided by administrative staff. This document is the result of the deliberations by subcommittees constituted for this purpose. The guidelines were further ratified by circulation to extended group of researchers and practitioners drawn from all over the country. It is hoped that these guidelines will help the practicing doctors to treat cancer patients effectively and thus help them to lead a normal and healthy life.

The ICMR appreciatively acknowledges the valuable contribution of the members for extending their support in formulating these guidelines. The data inputs provided by National Cancer Registry Programme are gratefully acknowledged.

A handwritten signature in blue ink that reads "Tanvir Kaur".

(Dr. Tanvir Kaur)  
Programme Officer & Coordinator

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## Members of the Sub-Committee

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### *Chairperson*

**Dr. (Col) Deepak Kumar Mishra**  
Senior Consultant & Professor  
(Laboratory Haematology & Molecular Genetics)  
Director, Department of Laboratory Sciences,  
Tata Medical Centre, Kolkata, West Bengal

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- |  |   |
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# Categories of Evidence and Consensus

## *Levels of Evidence*

**Level 1:** High quality randomized controlled trials (RCTs) showing (a) a statistically significant difference or (b) no statistically significant difference with narrow confidence intervals; systematic reviews of Level I RCTs

**Level 2:** Lesser quality RCTs (e.g. <80% follow-up, no blinding, or improper randomization); prospective comparative studies; systematic reviews of Level II studies or of Level I studies with inconsistent results

**Level 3:** Case control studies; retrospective comparative studies; systematic reviews of Level III studies; retrospective studies

**Level 4:** Case series

**Level 5:** Expert opinions

Grading A to C has been done by the sub-committee. Grade A is to be assigned to a treatment or regimen that is easy to administer, has the highest level of evidence, and is cost effective as evaluated by the National Institute for Health and Clinical Excellence or as deemed so by the task force experts on the particular cancer.

On consideration of peripheral oncology centres, regional cancer centres, and tertiary cancer centres in major cities, the set of recommendations can be divided into 2 categories:

**Desirable/Ideal:** Tests and treatments that may not be available at all centres but the centres should aspire to have them in the near future.

**Essential:** Bare minimum that should be offered to all patients by all centres treating patients with cancer.



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The Myelodysplastic Syndrome (MDS) are a group of clonal bone marrow (BM) neoplasms characterized by ineffective hematopoiesis, manifesting as morphologic dysplasia in hematopoietic cells and by peripheral cytopenia(s). The morphologic features and the spectrum of genomic alterations in MDS overlap with the closely related MDS/myeloproliferative neoplasms (MDS/MPN) and secondary AML (sAML).

The revised updated 2016 WHO classification has introduced refinements in morphologic interpretation and cytopenia assessment in MDS. It also addresses the influence of rapidly accumulating genetic information in MDS diagnosis and classification. In the revised updated 2016 WHO classification, terms such as “refractory anemia” and “refractory cytopenia” are replaced with “myelodysplastic syndrome” followed by the appropriate modifiers: single versus multilineage dysplasia, ring sideroblasts, excess blasts, or the del (5q) cytogenetic abnormality.

The risk stratification in untreated MDS cases is primarily done as per the Revised International Prognostic Scoring System (IPSS-R). IPSS-R is based on prognostic factors including bone marrow blasts, cytogenetics and cytopenias (anaemia, thrombocytopenia and neutropenia) is considered the most preferred prognostic system. The cytogenetic abnormalities which indicate good risk prognosis in MDS are Normal karyotype, del(5q), del(12p), del(20q), -Y, del(11q) whereas those indicating poor risk are -7, inv (3)/t(3q)/del(3q), double including -7/del(7q), Complex karyotype (> 3 cytogenetic abnormalities). These abnormalities should be demonstrated by conventional karyotyping rather than by fluorescence in situ hybridization (FISH) or sequencing technologies.

Recent advances and application of high-throughput molecular technologies mainly Whole Exome Sequencing (WES) and Next Generation Sequencing (NGS) has led to generation of large data on recurring mutations in MDS. Recurrent mutations identified by WES in MDS, affect a number of essential cellular processes like RNA splicing, epigenetic regulation of gene expression, transcription factors, tumour suppressor genes like *Tp53* and signalling pathways. Targeted Next Generation sequencing approach is preferred for combined analysis of hot spot gene mutations in one go rather than performing individual gene mutation analysis by Sanger sequencing or other molecular techniques which can be tedious and time consuming.

Recurrent mutations can be detected in 80-90% of MDS patients including a majority of those with a normal karyotype. The most commonly mutated genes in MDS are *Tp53*, *SF3B1*, *TET2*, *SRSF2*, *ASXL1*, *DNMT3A*, *RUNX1*, *U2AF1* and *EZH2*. Recently published literature on large cohorts of MDS patients highlight the adverse prognostic impact of the mutations in genes like *TP53*, *CBL*, *EZH2*, *RUNX1*, *U2AF1*, *ASXL1* while mutations in *SF3B1* gene suggest favourable risk in the MDS cases with ring sideroblasts and multilineage dysplasia.

An example of how mutational landscape is changing the risk stratification in MDS is the evaluation of *Tp53* gene mutation in cases of MDS with isolated del(5q). *Tp53* gene mutation helps to identify an adverse

prognostic subgroup and predicts poor response to lenalidomide in the generally favourable prognostic group of MDS with isolated del(5q). Tp53 gene mutation in general is associated with aggressive disease in MDS. Another example is recurrent mutation in the spliceosome gene SF3B1 which is associated with 40-60% cases of MDS with the presence of ring sideroblasts(MDS-RS). A diagnosis of MDS-RS may be made in presence of *SF3B1* mutation even when ring sideroblasts comprise as few as 5% of nucleated erythroid cells, while at least 15% ring sideroblasts are still required for diagnosis of MDS-RS in cases lacking a demonstrable *SF3B1* mutation. Similarly, published literature also suggests that mutations in the gene like *DNMT3A*, *TP53*, *WT1*, *SRSF2*, *IDH1/2*, *STAG2* are associated with risk of progression to AML in MDS and are paving a way to a new AML predicting model.

Primarily the IPSS-R based risk stratification does not take into consideration the genetic mutations that have been shown to be associated with disease prognosis in MDS. Thus a new comprehensive risk stratification system in MDS, Revised International Prognostic Scoring System “molecular”(IPSS-Rm) has been proposed as a modification of IPSS-R by including mutational data.

Advances in technology have provided significant insights into understanding the pathogenesis of MDS and has led to the identification of many new genetic lesions in MDS patients. Molecular analysis of specific mutations in MDS can help in disease subtyping and risk stratification and thus has now been integrated into diagnostic criteria and clinical management algorithms.

**Defining MDS:** MDS are a group of myeloid disorders characterised by cytopenias with variable degree of dyspoiesis and characterised by their variable rate of progression to Acute Myeloid Leukemia.

Diagnosis of MDS is a combined effort of a clinician and pathologist / haematopathologist .

**Patient Data to be recorded:**

**Age:** It is predominantly a disease of the older age group. But it has been observed in some studies that MDS occurs in younger age group in India (with an average age of < 50 yrs<sup>1, 2,3</sup>) than in western population.

**Sex:** Frequent association of 5q- syndrome in females is seen.

**Patient History:**

***Presentation:***

Many patients of MDS are asymptomatic. The clinical signs and symptoms are predominantly due to the underlying cytopenias and are not disease specific. Weakness, lethargy and fatigue, effort intolerance, dizziness etc are very common as anemia is the most common presentation along with recurrent infections or bleeding symptoms in the form of petechiae or other skin bleeds. Life threatening bleeds are uncommon. A typical history is that of patient presenting with refractory anemia, may be transfusion dependent, not responding to hematinics (iron, vitamin B12 & folic acid).

***Past History:***

It is important to get the following history:

H/O treatment with hematinics: To elicit refractory nature of the disease.

H/O blood transfusion: To assess the severity of anemia and transfusion dependency

H/O chronic disease: especially tuberculosis, HIV as these can lead to dysplasia, which reverts back to normal after treatment of the primary condition<sup>2</sup>.

H/O connective tissue disorder: As they can lead to anemia of chronic disease causing refractory anemia and treatment with hydroxychloroquine and other immunomodulators found to be associated with secondary MDS.

H/O any previous treatment especially by alkylating agents or topoisomerase inhibitors and ionising radiation for previous malignancies to rule out therapy related MDS.

**Investigations:** Aimed at establishing dysplasia and excluding out known causes of dysplasia.

The investigations can be divided into two parts

- Those which can be done at a routine hematology laboratory.
- Those which are to be done at a referral laboratory for hematology/ a specialised hematological laboratory.

#### **A. Investigation that can be done at a routine hematology laboratory**

**1. Routine Haemogram:** This may be done in an automated counter ensuring good quality control measures. In MDS this may show cytopenias which may be unilineage or multilineage. Cytopenias as defined in International Prognostic Scoring System (IPSS):

Hemoglobin 10 g/dl

Absolute Neutrophil Count <  $1.8 \times 10^9/L$

Platelets <  $100 \times 10^9/L$

Note: A diagnosis of MDS may be made in rare cases with milder levels of cytopenia, but at least 1 cytopenia must be present in order to make the diagnosis. Again, some ethnic groups may have a reference range for normal absolute neutrophil count that is lower than  $1.8 \times 10^9/L$ , and thus caution should be exercised in interpreting neutropenia if it is the only cytopenia.

#### **2. Peripheral Smear examination:**

- Smear should be made as soon as possible (not later than 2 hours of blood collection). Stored blood shows artefactual changes which can be confused with dysplasia.
- It should be a well spread peripheral smear.
- Smears should be stained with Giemsa stain/Jenner Giemsa
- Differential count should be done of at least 200 WBCs.
- Peripheral smear findings that may be seen in a case of MDS are

**RBC:** Generally normocytic or macrocytic.

**WBC:** Leukopenia due to neutropenia is a common finding. The characteristic features of dysplasia in the form of pseudo Pelger-Huet anomaly or hypogranulation may be seen. Occasionally it may show presence of myeloblast and myelocytes.

**Platelets:** Platelets are reduced in most of the patients. Presence of normal or raised platelets may be seen especially in association 5q-syndrome .

**Note:** A suspicion for the diagnosis of MDS should always be kept in mind in an elderly patient presenting with cytopenias (uni, bi or pancytopenia) with no other explanations for it, and a sample for cytogenetics should be sent for whenever a bone marrow is done.

**3. Bone Marrow examination:** Bone marrow examination by an experienced hematopathologist is a must for diagnosis of MDS.

Both bone marrow aspirate and trephine biopsy should be examined.

#### **Bone Marrow Aspirate:**

- Well spread bone marrow aspirate smears.
- Aspirate should be stained with May-Grunwald Giemsa or Jenner- Giemsa stain.

- At least 500 cells should be counted on the cell trails of marrow particles.
- Dysplasia: In at least > 10% of cells in any particular lineage.
- The following features are required to make diagnosis of MDS:

**Table 1: Features of dysplasia**

	<b>Peripheral Smear</b>	<b>Bone Marrow</b>
Erythroid: (Dyserythropoiesis)	Normocytic Macrocytic Microcytic (in Indian context due to iron deficiency) Nucleated RBC	Megaloblastoid appearance Nuclear budding Nuclear irregularity Mutinucleation Internuclear bridging Karyorrhexis Ringed sideroblast Cytoplasmic vacuolation PAS positivity
Myeloid : Dysmyelopoiesis	Hypogranulated Neutrophils Ringed neutrophils Pseudo-PelgerHuet Anomaly Irregular hypersegmentation Pseudo Cheediak-Higashi granules Auer rods	Defective granulation Hypogranulation Ringed neutrophils Pseudo-Pelger Huet Anomaly Irregular hypersegmentation Pseudo Cheediak-Higashi granules Auer rods
Megakaryocytes & Platelets : Dysmegakaryopoiesis	Reduced platelets Giant platelets Hypogranular platelets	Multinucleation Hypolobation Dysjointed nuclei micromegakaryocytes

Adapted from: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue. Geneva, Switzerland: World Health Organization; 2016.

**Note:** Giant myeloid forms are more common in megaloblastic anemia in contrast to megaloblastoid change seen in MDS

**4.Perls' Prussion Blue stain:** On bone marrow aspirate to look for ringed sideroblast.

**5. Bone Marrow Trepine Biopsy:**

- At least 1.5 cm long showing 10 intra-trabecular areas.
- It should be well fixed and thin sections 3-4 micron stained with Hematoxylin and Eosin (H&E).
- Look for cellularity and also fibrosis using reticulin stain or Masson trichrome stain.

**B. Investigations which are to be done at a Referral Laboratory for Haematology or a Specialised Hematological Laboratory:**

These Investigations are aimed at exclusion of other causative factors of dysplasia and cytopenias:

**1. Nutritional anemia:**

- Serum Iron studies ( S.Iron,TIBC,% saturation) and serum ferritin.
- Vitamin B 12/Folic acid levels.

2. **Infections:** R/O TB, Parvovirus B19, CMV, EBV, HCV, HIV.
3. **Connective tissue disorders** (SLE, RA): dsDNA, Rheumatoid Factor, ANA.
4. **Anemia of chronic disease:** Iron studies and S. Ferritin.
5. **PNH:** By Flowcytometry.(FLAER, CD15, CD33, CD14 and CD24)
6. **CDA:** HEMPAS, Electron microscopy.
7. **Serum Erythropoietin:** levels are important as low risk MDS patients with levels < 500 mU/ml may respond to erythropoietin treatment with improvement in hemoglobin levels.
8. **HLA-DR15:** To assess patient potential to respond to immunosuppressive therapies especially in relation to Hypoplastic MDS.
9. **JAK-2 mutation:** In patient with thrombocytosis to rule out Essential Thrombocytheamia.

#### 10. Conventional Cytogenetics

(8;21),t(16;16)(p13.1;q22)/inv(16)(p13.1q22) or t(15;17)(q22;q12) makes a diagnosis of AML even if blasts are <20%.] for prognostic score and MDS defining cytogenetic abnormality.

Cytogenetics to exclude AML [presence of

#### Classification of MDS:

Once a diagnosis is made it is important to subtype MDS.

**Table 2: Classification of De Novo MDS**

Name	Dysplastic lineages	Cytopenias	Ring sideroblasts (% of marrow erythroid elements)	BM and PB blasts	Cytogenetics (Conventional karyotyping)
<b>MDS with single lineage dysplasia (MDS-SLD)</b>	1	1 or 2	<15%/ <5%†	BM<5%, PB<1%, No Auer rods	Any, unless fulfils all criteria for MDS with isolated del (5q)
<b>MDS with multilineage dysplasia (MDS-MLD)</b>	2 or 3	1-3	<15%/ <5%†	BM<5%, PB<1%, No Auer rods	Any, unless fulfils all criteria for MDS with isolated del (5q)
<b>MDS with ring sideroblasts (MDS-RS)</b>					
• MDS-RS with single lineage dysplasia (MDS-RS-SLD)	1	1 or 2	≥15%/ ≥5%†	BM<5%, PB<1%, No Auer rods	Any, unless fulfils all criteria for MDS with isolated del (5q)
• MDS-RS with multilineage dysplasia (MDS-RS-MLD)	2 or 3	1-3	≥15%/ ≥5%†	BM<5%, PB<1%, No Auer rods	Any, unless fulfils all criteria for MDS with isolated del (5q)
<b>MDS with isolated del (5q)</b>	1-3	1-2	None or any	BM<5%, PB<1%, No Auer rods	del(5q) alone or with 1 additional abnormality except -7 or del (7q)

<b>MDS with excess blasts (MDS-EB)</b>					
• MDS-EB-1	0-3	1-3	None or any	BM 5-9% or PB 2-4%, No Auer rods	Any
• MDS-EB-2	0-3	1-3	None or any	BM 10-19% or PB 5-19% or Auer rods	Any
<b>MDS, unclassifiable (MDS-U)</b>					
• With 1% PB blasts	1-3	1-3	None or any	BM<5%, PB=1%, ‡ No Auer rods	Any
• With single lineage dysplasia and pancytopenia	1	3	None or any	BM<5%, PB<1%, No Auer rods	Any
	0	1-3	<15%§	BM<5%, PB<1%, No Auer rods	MDS-defining abnormality
• Based on defining cytogenetic abnormality					
<b>Refractory cytopenia of childhood</b>	1-3	1-3	None	BM<5%, PB<2%	Any

PB monocytes must be  $<1 \times 10^9/L$

†If SF3B1 mutation is present.

‡One percent PB blasts must be recorded on at least 2 separate occasions.

§Cases with >15% ring sideroblasts by definition have significant erythroid dysplasia, and are classified as MDS-RS-SLD.

“The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia”

### Hypoplastic MDS:

It is seen in a minor group of patients (<10%). Bone marrow is hypocellular in these cases. Aplastic anemia is a close differential diagnosis in this group. This group per se does not behave differently. It is important to rule out autoimmune disorders and toxic myelopathies before diagnosing hypoplastic MDS. Some studies support the group could be benefitted by immunosuppressive therapies similar to those used for aplastic anemia. Presence of the HLA-DR15 allele showed less association with disease progression and greater association with BM failure.

### Childhood MDS:

MDS is very uncommon in children, accounting less than 5%. Childhood MDS is a distinct entity from adult MDS. It differs from it in the following ways:

- Isolated anemia is very uncommon. However Indian data shows pallor to be present in all (100%) cases followed by fever and bleeding symptoms in >50% of cases.
- Neutropenia and thrombocytopenia are common.
- Hypocellularity is more common.
- RARS and Isolated del (5q) are very rare.

A distinct entity called Refractory Anemia of Childhood (RCC) is proposed by WHO. It is the most common type of MDS seen in children.

**Table 3 : Difference between RCC and Aplastic Anemia\***

	RCC		APLASTIC ANEMIA	
	Bone marrow aspirate	Bone biopsy	Bone marrow aspirate	Bone biopsy
Erythropoiesis	Nuclear lobation Multinuclearity Megaloblastic changes	Patchy cellularity Left shift Increased mitosis	Very few cells No dysplasia or megaloblastoid change	Lacking Single small focus with <10 cells with maturation
Granulopoiesis	Pseudo-Pelger-Heut anomaly Hypogranular/agranular cytoplasm Nuclear-cytoplasmic maturation defects	Marked decrease Left shift	Few maturing cells with no dysplasia	Lacking Few small focus with dysplasia
Megakaryopoiesis	Micromegakaryocytes Multiple nuclei Small round nuclei	Marked decrease Dysplastic changes Micromegakaryocytes	Very few No dysplastic megakaryocytes	Lacking or very few, No dysplasia in megakaryocytes
Lymphocytes	May be increased	May be increased (Focally or depressed)	May be increased	May be increased
CD34+ cells	No increase		No increase	

\* **Adapted from** Baumann I, Niemeyer CM, Bennett JM, Shannon K. Childhood myelodysplastic syndrome. In: Swerdlow S, Campo E, Harris NL, eds.; International Agency for Research on Cancer. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue*. Geneva, Switzerland: World Health Organization; 2008; 104-107.

Apart from RCC children with blasts between 2-19 % on PS and 5-19% on bone marrow are classified as Refractory Anemia with Excess of Blast (RAEB). Although studies have not been done to judge the difference between RAEB-1 and RAEB-2 in children. WHO recommendations for classifying it into RAEB-1 and RAEB-2 should be followed for further investigations.

Another recommendation is to keep the category of Refractory Anemia with Excess of Blast in transformation (RAEB-t) to categorize children with 20-29% blasts as they as shown to behave differently from AML.

### **MDS with Myelofibrosis (MDS-F)**

Significant marrow fibrosis is seen in 10-15% MDS .Most cases have excess blasts, aggressive course. It is unclear whether fibrosis has independent prognostic value. Blast count from aspirate smears alone may understage the disease as it may be diluted with peripheral blood due to marrow fibrosis. CD34 by IHC can be on bone marrow biopsy may help. Mutation for JAK-2 is negative.

**Karyotype abnormalities in MDS and gene haplo-insufficiency**

BM cytogenetic (CG) analysis is a key component in both International Prognostic Scoring System and WHO Classification-Based Scoring System. Fluorescence in-situ hybridization (FISH) analysis is useful in identification of sub-microscopic abnormalities and in the event of failure of metaphase yield. Detection of subcytogenetic copy number alterations using SNP array complements standard metaphase cytogenetics. Detection of uniparental disomies (UPD) in MDS bone marrow (BM) cells has facilitated identification of segments harbouring biallelic mutations in genes, including *EZH2*, *CBL*, *TET2*, & *TP53*. Common cytogenetic alterations observed in MDS are shown in Table 4.

**Table 4: Cytogenetic Alterations in MDS**

Alterations	Frequency
5q-	15%
-7/7q-	11%
+8	8%
-18/18q-; 20q-	4% each
-5; -Y	3% each
+21; -17/17q-; inv/t(3q); -13/13q-	2% each
+1/1q-; -21; +11; 12p-; t(5q); 11q-; 9q-; t(7q); -20	1% each

Table 5 shows the list of MDS defining cytogenetic abnormalities. These cytogenetic abnormalities remain MDS-defining in a cytopenic patient, even in the absence of diagnostic morphologic dysplasia. In such cases, the abnormality must be demonstrated by conventional karyotyping, not by FISH or sequencing technologies. The presence of +8, -Y, or del(20q) is not considered to be MDS defining in the absence of diagnostic morphologic features of MDS. del(5q) is the only cytogenetic or molecular genetic abnormality that defines a specific MDS subtype. The entity of 'MDS with isolated del(5q)' can have 1 additional cytogenetic abnormality besides the del(5q), other than -7 or del(7q). Although cytogenetic findings are not taken into account to define other specific subtypes of MDS, they have strong correlation with prognosis.

**Table 5 : MDS defining cytogenetic abnormalities**

Unbalanced abnormalities	Balanced abnormalities
-7/del(7q)	t(11;16)(q23.3;p13.3)
del(5q)/t(5q)	t(3;21)(q26.2;q22.1)
i(17q)/t(17p)	t(1;3)(p36.3;q21.2)
-13/del(13q)	t(2;11)(p21;q23.3)
del(11q)	t(5;12)(q32;p13.2)
del(12p)/t(12p)	t(5;7)(q32;q11.2)
idic(X)(q13)	t(5;17)(q32;p13.2)
	t(5;10)(q32;q21.2)
	t(3;5)(q25.3;q35.1)

mapped. An interstitial deletion of one copy of chromosome 5q (5q-), alone or in combination, is the most common cytogenetic change in de novo MDS. Multigene haploinsufficiency is the dominant genetic mechanism caused by 5q deletions. Several studies have mapped the commonly deleted regions (CDRs) on 5q. Deletions 5q in the proximal region on 5q31.2 are associated with high risk for AML transformation; those in the distal region on 5q33.1 are associated with '5q- syndrome' and a lower risk of AML transformation.

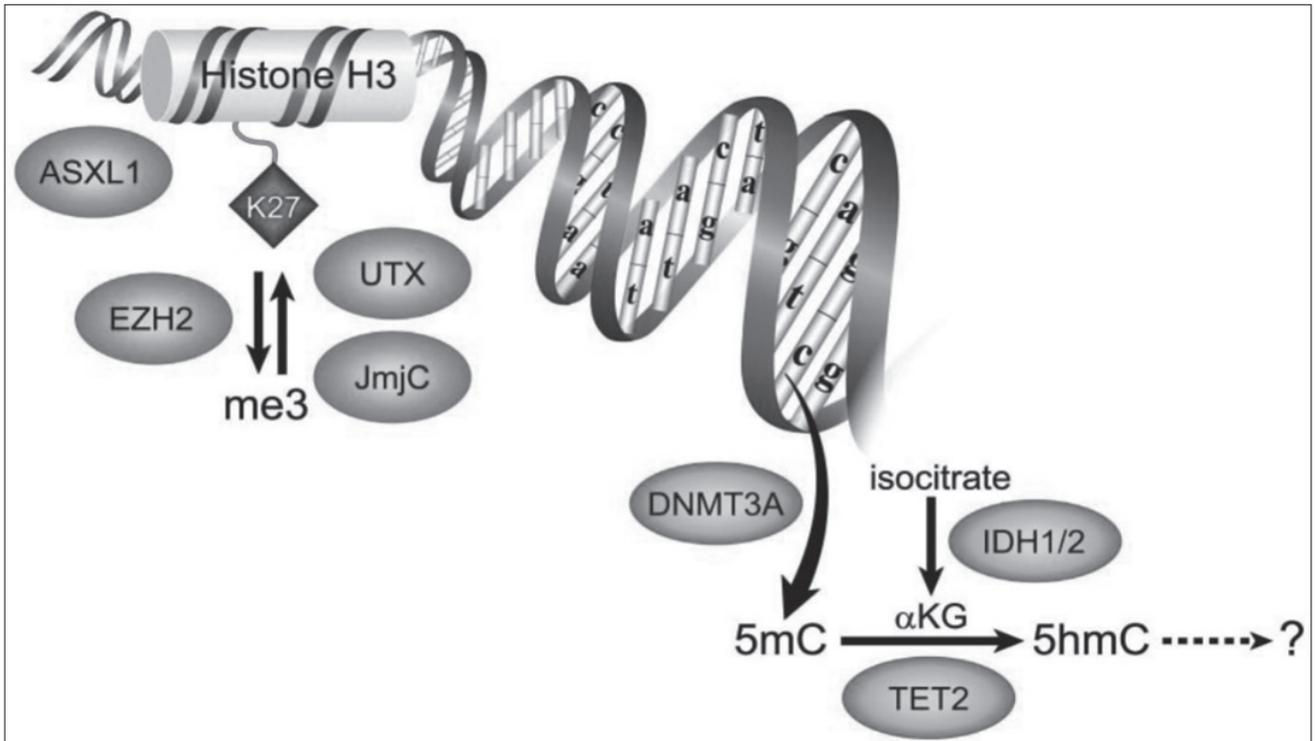
Haploinsufficiency of *RPS14* on the distal 5q33 region was found to contribute to the abnormal erythroid differentiation and apoptosis that is commonly observed in patients with the 5q- syndrome. Haploinsufficiency of miR-145 and miR-146 (both located on the 5q33 CDR), leads to thrombocytosis in vivo. Several CDRs have been for chromosome 7q deletions (7q21, 7q22, 7q32-33, and 7q35-36). Identification of the critical genes in these intervals has been lacking until recently with the identification of mutations in *EZH2* located on 7q36 in patients with MDS. No definitive causal gene has been identified for chromosome 20q deletions (although *ASXL1* is located on 20q) or trisomy 8.

## Genetic alterations in MDS

### I. Genetic alterations of epigenetic pathways in MDS

#### A. Mutations in regulators of DNA methylation

Global methylation pattern of cytosine residues in CpG dinucleotide sequences is different between normal and MDS BM cells. Cytosine methylation status influences gene transcription, which may contribute to the altered growth and differentiation of MDS cells. Several genes that regulate cytosine methylation are somatically mutated in MDS genomes (*DNMT3A*, *TET2*, and *IDH1/IDH2*) (Figure 1). *IDH1/IDH2* and *TET2* mutations are mutually exclusive. There is clinical benefit to patients with MDS when treated with cytosine analog drugs that interfere with methylation (5-azacytidine and 5-aza-2'-deoxycytidine). Methylation at the 5' position of cytosine in CpG dinucleotides is mediated by DNA methyltransferases (DNMTs). DNMT3A and DNMT3B are the dominant DNMTs involved in de novo methylation and DNMT1 maintains hemimethylated DNA during replication. 5'-methylcytosine (5mC) can be further modified by a group of 3 paralogous Ten Eleven Translocation (TET) proteins (TET1, TET2, and TET3), that are alpha-ketoglutarate (KG) and Fe(II)-dependent oxygenases that catalyze the conversion of 5mC to 5'-hydroxymethylcytosine (5hmC). Mutations in *DNMT3A* were first discovered in AML and were subsequently identified in up to 8% of de novo MDS samples. Sequencing of *TET2* (located in a 4q24 microdeletion) identified missense, frame shift, and nonsense somatic mutations in myeloid cancers, including 11%-26% of patients with MDS. No consistent impact of *TET2* mutations has been observed on survival of MDS patients.



**Figure 1: Genetic alterations of epigenetic pathways in MDS.** The normal function of selected factors important for histone modification and DNA methylation is depicted. Left panel: Both ASXL1 and EZH2 (enhancer of zeste homolog 2, encoding an H3K27 methyltransferase) are frequently mutated in MDS. Rare mutations (deletions) have also been identified in MDS samples in H3K27 demethylase enzymes, including UTX and other JmjC domain-containing proteins. Right panel: DNMT3A is a de novo DNA methyltransferase that converts unmethylated cytosine to 5'-methylcytosine (5mC). 5mC is converted to 5'-hydroxy-methylcytosine (5hmC) by the TET proteins in the presence of alpha-ketoglutarate (αKG) generated by the IDH enzymes. DNMT3A, TET2, IDH1, and IDH2 are all recurrently mutated in MDS samples.

### Mutations affecting Histone function

Histone proteins organize DNA into zones of active ("open") and inactive ("closed") chromatin. This process is regulated in part by a complex series of posttranslational modifications (including acetylation, methylation, and others) to histone tails. These modifications affect the recruitment of transcriptional regulators (eg, transcription factors, corepressors, and coactivators) and the histone-modifying enzymes themselves (eg, histone acetyltransferases, deacetylases, methyltransferases, and demethylases). Trimethylation of the lysine at position 27 in histone H3 (H3K27) typically results in reduced gene expression; mutations that decrease H3K27 methylation activate transcription. Recently, recurrent mutations in several genes encoding histone regulators have been identified in MDS patients (Figure 1). The first described involve EZH2 (enhancer of zeste homolog 2), a polycomb group protein that methylates histones H3 (at K27) and H1 (at K26), generally resulting in transcriptional repression. *EZH2* point mutations (including missense, nonsense, frame shift, splice site, and deletions) were detected in 2%-6% of MDS patients.

Table 6 shows the recurrent gene mutations described in MDS. Recently discovered mutations have completely changed the genomic landscape of MDS. Genes involved in RNA splicing (including *SF3B1*, *SRSF2*, *U2AF1* and *ZRSR2*) and epigenetic modification (including *TET2*, *ASXL1* and *DNMT3A*) are most commonly mutated in MDS. Till recently abnormalities of mRNA splicing were not known to be involved in pathogenesis of MDS. Now nearly 50% MDS patients reveal mutations in spliceosome. Mutations are also identified in many regulators of signal transduction and transcription factors. New biological and therapeutic insights have been obtained from better understanding of molecular pathogenesis of MDS.

**Table 6 : Commonly mutated genes in MDS patients**

Genes	Chromosomal Location	Frequency in MDS	Protein function
<b>Splicing factor genes</b>			
<i>SF3B1</i>	2q33.1	20-28% in MDS >70% in MDS with ring sideroblasts	Spliceosome component important for assembly of the spliceosome complex
<i>SRSF2</i>	17q25.1	12-15%	
<i>U2AF1</i>	21q22.3	7-9 %	
<i>ZRSR2</i>	Xp22.1	3-11%	
<i>PRPF8</i>	17p13.3	1-4%	Spliceosome component essential for the catalytic step II in pre-mRNA splicing process
<b>DNA methylation</b>			
<i>TET2</i>	4q24	20%	Dioxygenase that catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine; required for myelopoiesis
<i>IDH1</i> <i>IDH2</i>	2q33.3 15q26.1	5%	Dehydrogenase that catalyzes the conversion of isocitrate to $\alpha$ -ketoglutarate; regulates TET2 function
<i>DNMT3A</i>	2p23.3	10%	DNA methyltransferase involved in <i>de novo</i> DNA methylation and coordinated with histone methylation to repress transcription
<b>Histone modification</b>			
<i>ASXL1</i>	20q11.2	11-15%	Histone-binding protein that disrupts chromatin in localized areas enhancing or repressing gene transcription
<i>EZH2</i>	7q36.1	5%	Histone methyltransferase involved in transcriptional repression
<b>Signal transduction and transcription factors</b>			
<i>RUNX1</i>	21q22	10%	Transcription factor critical in hematopoiesis
<i>TP53</i>	17p13	5-10%	Tumor suppressor that regulates apoptosis, cell cycle, DNA repair, senescence and metabolism
<i>NRAS</i>	1p13.2	5%	GTPase with oncogenic function when mutated and constitutively active
<i>KRAS</i>	12p12.1	2%	
<i>ETV6</i>	12p13.2	2-5%	Transcription factor required for hematopoiesis
<i>EVII</i>	3q26	1-2%	Transcriptional regulator implicated in hematopoiesis, apoptosis, development, differentiation and proliferation
<i>JAK2</i>	9p24.1	5% in MDS 50% in RARS-T	Non-receptor tyrosine kinase involved in cell cycle, genomic instability, apoptosis and mitotic recombination
<i>FLT3</i>	13q12	1-4%	Class III receptor tyrosine kinase; regulates hematopoiesis

Cohesin complex			
<i>STAG2</i>	Xq25	7%	Subunit of the cohesin complex, which regulates the separation of sister chromatids during cell division
<i>RAD21</i>	8q24	1%	
<i>SMC3</i>	10q25	1%	
Others			
<i>CBL</i>	11q23.3	2-5%	E3 ubiquitin ligase involved in cell signaling and protein ubiquitination; functions as a negative regulator of signal transduction in hematopoietic cells
<i>SETBP1</i>	18q21.1	4%	Binds the SET nuclear oncogene which is involved in DNA replication and cell division
<i>BCOR</i>	Xp11.4	4%	Interacting corepressor of BCL6, a transcription repressor that may influence apoptosis

In nearly 80-90% of MDS patients, targeted sequencing of a limited number of genes can detect mutations; the most commonly mutated genes in MDS are *SF3B1*, *TET2*, *SRSF2*, *ASXL1*, *DNMT3A*, *RUNX1*, *U2AF1*, *TP53*, and *EZH2*. Importantly, acquired clonal mutations identical to those seen in MDS can occur in the hematopoietic cells of apparently healthy older individuals without MDS, so-called “clonal hematopoiesis of indeterminate potential” (CHIP). Although some patients with CHIP subsequently progress to MDS, the natural history of this condition is not yet fully understood; thus, the presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS in this classification, even in a patient with unexplained cytopenia. Further studies are required to determine the optimal management and monitoring of such patients and to investigate possible links between specific mutations, mutant allele fraction, or mutation combinations and subsequent development of bona fide MDS. The number and types of specific mutations are strongly associated with disease outcome in MDS, and the addition of mutation data improves the prognostic value of existing risk-stratification schemes in MDS.

With regard to MDS with ring sideroblasts (MDS-RS), recurrent mutations in the spliceosome gene *SF3B1* are frequently detected in MDS and are associated with the presence of ring sideroblasts. A change in the classification of MDS is the inclusion now of MDS cases with ring sideroblasts and multilineage dysplasia, lacking excess blasts or an isolated del(5q) abnormality, into the category of MDS-RS. This change is based largely on the link between ring sideroblasts and an *SF3B1* mutation, which appears to be an early event in MDS pathogenesis, manifests a distinct gene expression profile, and correlates with a favorable prognosis. Recent studies have shown that in cases of MDS with any ring sideroblasts, the exact percentage of ring sideroblasts is not prognostically relevant. Thus, in the revised classification, if an *SF3B1* mutation is identified, a diagnosis of MDS-RS may be made even if ring sideroblasts comprise as few as 5% of nucleated erythroid cells, whereas at least 15% ring sideroblasts are still required in cases lacking a detectable *SF3B1* mutation.

### Recommendations for cytogenetic and molecular genetic analysis

- **Cytogenetic analysis** should be performed using bone marrow (BM) sample, at the time of diagnosis, at a centre/ institute, having requisite expertise and resources. At least 20 G-banded metaphases should be studied.
  - MDS associated abnormalities (look for 5q-, -7/7q-, +8, -18/18q-, 20q-, -5, -Y and others)
  - Recurrent cytogenetic abnormalities in AML: t(8;21)(q22;q22), inv(16)(p13.1q22), t(16;16)(p13.1;q22) and t(15;17)(q22;q12). Case should be diagnosed as AML, even when blasts are <20%.

- **Fluorescence in-situ Hybridization (FISH) analysis** can be performed on metaphase as well as inter-phase nuclei, and is useful in identification of sub-microscopic abnormalities. FISH is the only available option for obtaining cytogenetic/genetic information in the event of i) patients' refusal to undergo BM examination, ii) dry tap and iii) failure of metaphase yield (due to a particulate aspirate etc). BM smears should be preferred (results are reported to be approximately 6% better) over peripheral blood smears (TLC  $>3 \times 10^9/l$ ). A minimum of 200 inter-phase nuclei need to be analyzed.
  - FISH should be performed at a centre/ institute, having requisite expertise and resources.
  - FISH panel should include at least 5 probes to target 5q-, -7/7q-, +8, -18/18q-, 20q-

#### **Recommendations for Molecular Genetic analysis**

- In view of the available literature, at present molecular genetic analyses have research applications only.
- Molecular genetic analysis might find a wider role in future, for investigations of MDS cases:
  - TP53;17p; (10-18%): FISH or PCR
  - TET2; 4q; (11-26%): PCR
  - ASXL1; 20q; (11-15%): PCR
  - DNMT3A; 2p; (8%): PCR
  - IDH1/IDH2; 2q/15q; (4-11%): PCR

## 4

## RISK STRATIFICATION AND MANAGEMENT OF PATIENTS WITH LOW RISK MDS

Once the patient has been clinically evaluated and diagnostic and prognostic investigations have been done, line of management is to be decided. Various prognostic models have been developed for risk stratification of MDS. At one end in low risk group survival is in years and on the other hand it high risk group it is in months. Treatment decisions depends on the risk stratifications. Two commonly used risk stratification systems are International Prognostic Scoring System (IPSS) and revised International prognostic scoring system (IPSS-R)

IPSS takes into consideration the marrow blast percentage, cytogenetics and cytopenias. IPSS-R includes individual cytopenias and more detailed cytogenetics in the classification.

**IPSS\***

Prognostic category	IPSS Prognostic score value				
	0	0.5	1	1.5	2.0
Cytogenetics	Good	Intermediate	Poor		
Bone marrow blast (%)	<5	5-10		11-20	21-30
Cytopenias	0/1	2/3			

\*Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood*. 1997 Mar 15. 89(6):2079-88.

**Cytogenetics:**

Good : -Y, del (20q), del(5q)

Intermediate : Any abnormality which is not considered good and poor risk

Poor : Chromosome 7 abnormalities, complex cytogenetics ( 3 abnormalities)

**Cytopenias:**

Hemoglobin <10 gm/dl

WBC < 1.8X10<sup>9</sup>/L

Platelets <100x10<sup>9</sup>/L

## Prognosis according to IPSS:

Risk category	Risk score	Overall survival	
		≤70 years	>70 years
Low	0-1	9.0	3.9
Intermediate-1	1.5-2.0	4.4	2.4
Intermediate-2	2.0-2.5	1.3	1.2
High	>2.5	0.4	0.4

## IPSS-R

Prognostic factors	IPSS-R Prognostic score values						
	0	0.5	1.0	1.5	2	3	4
Cytogenetics	Very Good		Good		Intermediate	Poor	Very poor
BM Blast%	<2		2-5		5-10	>10	
Hemoglobin	>10		8-<10	<8			
Plateletsx10 <sup>9</sup>	>100		50- <100	<50			
ANCx10 <sup>9</sup>	0.8	<0.8					

## Cytogenetic groups

Very good : -Y, del(11q)

Good : Normal, del(5q), del(20q), del(12p),del(5q)+1additional abnormality

Intermediate : del7q,+8,+19, i(17q) other abnormalities not in other groups

Poor : -7, inv(3)/t(3q)/del(3q), -7/del7q+ 1 additional, complex (3 abnormalities)

Very poor : Complex (> 3 abnormalities)

## Risk Category and risk scores and Survival:

Risk category	Risk score (for 70 years)	Survival* (years)
Very low	<1.5	8.8
Low	>1.5-3.0	5.3
Intermediate	>3.0-4.5	3.0
High	>4.5-6.0	1.6
Very High	>6.0	0.8

\*Greenberg, Tuechler, Schanz et al, Revised International Prognostic Scoring System (IPSS-R) for Myelodysplastic Syndrome, Blood 120: 2454, 2012.

Low risk MDS patients that includes very low ( 1.5 points) and low (>1.5-3.0 points) risk (IPSS-R scores) or low risk and intermediate- 1 risk (IPSS scores) now have better chance of survival than ever before. These patients have adverse outcomes due to marrow failure rather than transformation to AML. The goals of treatment of lower risk MDS patient are mainly a) to minimize blood transfusion, b) to restore effective blood cell production, and c) to maximize quality of life. There are many therapeutic options available. But there is a need for evidence based consensus guidelines, suitable and customized for Indian patients. These goals can now be achieved to varied extent with the help of different modalities. The treatment options are chosen according to various clinical and laboratory parameters. Patients with lower

risk MDS having asymptomatic/ minimal symptoms need not to be treated with available agents as it is not clear whether early treatment is better than late treatment in such patients. Immediate treatment is indicated for patients with symptomatic anemia, symptomatic thrombocytopenia and recurrent infections in the setting of severe neutropenia.

Following is a short description of treatment modalities available for patients with lower risk MDS. An algorithm is given at the end to guide in making therapeutic decisions in an individual patient.

### **Management of Asymptomatic Low risk MDS:**

Asymptomatic patients may be followed expectantly with periodic clinical examination and routine laboratory tests. This helps in assessing the rate of disease progression and exclude reversible causes of cytopenias. During this follow up, patient can be vaccinated against pneumococcus, hemophilus influenza B and influenza infections. Initially patients may be followed every month for several months followed by every 3 months if the disease is stable. Once patient develops recurrent infections or bleeding or transfusion requirement, it is time to go for treatment

### **Management of Symptomatic Lower risk MDS:**

There is no consensus regarding optimal treatment of MDS and still clinical trials are the best to enroll a new patient whenever available. In clinical practice individualized care is more important for patients of MDS.

### **Supportive Care:**

Supportive care includes packed cell transfusion for symptomatic anemia, platelet transfusion for severe thrombocytopenia and thrombocytopenic bleeding and antibiotics for infections. About 90% of all MDS patients with permanent anemia become dependent on blood transfusions to maintain their quality of life and to survive. Transfusion dependency has been shown to be associated with a poorer prognosis. Targeted haemoglobin should be individualized. Overall it would be appropriate to maintain Hb of 10gm/dl. Chelation should be considered for all patients with lower risk MDS patients and patients with 5q del who receive regular red cell transfusions therapy. Chelation therapy should be started after patient had received 20 packed red blood cell units (i.e. 4 g of iron) or serum ferritin is >1000 µg/L. Deferasirox is recommended as first line therapy in MDS patient with clinically significant iron overload. A multicenter study by the GFM (Groupe Francophone des Myelodysplasies) showed that the overall survival was significantly better for patients who received iron chelation therapy. These results were consistent across all subgroups analyzed (IPSS low and intermediate-1, sex, age). However, not all agree with the idea of iron chelation and substantial disagreements persist on this topic.

### **Erythropoiesis stimulating agents (ESA):**

In lower risk MDS patients having cytopenias or transfusion dependency, treatment with growth factor may be initiated. Growth factors include ESA's and colony stimulating factors at varying schedules and dosage in combinations. At least 3 retrospective studies suggest survival advantage for ESAs possibly through role in minimizing transfusion needs and also decreasing iron load. In Nordic trial and GFM study, those receiving ESAs based therapy had significant survival advantage ( $p=.002$ ). ESAs group did not have any difference in the rate of AML transformation. In addition, a systemic review of 162 trials (Cleveland study) had also shown significantly better survival in ESAs group. Hellstrom-Lindberg et al developed decision tools to help predict which patients are more likely to benefit from ESAs group. Patients with low transfusion need (<2 PRBC unit per month) and low base line serum EPO level (< 500 U/L) had 74% chances of responding to ESAs as compared to those with high transfusion needs (> 2 PRBC unit per

month) and high EPO level (> 500 U/L) who had only 7% chance of responding. Hence patients with lower risk MDS falling into a good ESAs predictive group should initially be treated with ESAs while those falling into intermediate and poor ESAs predictive group should initially be treated with other therapies. Only few data exist to support the advantage of the combination therapy ie ESAs plus GCSF. During treatment with ESAs, hemoglobin should be not allowed to go beyond 12 gm/dl. Dose adjustments should be made and ESA should be continued till its efficacy starts decreasing. Patients with lower risk MDS with thrombocytopenia necessitating platelet transfusion or resulting in bleeding episodes can be given thrombopoietin mimetic agents such as romiplostim or eltrombopag. A single arm study had shown durable platelet response with romiplostim in 46 % patients although there was increased risk of AML.

#### **Immuno-suppressive therapy:**

A combination of anti-thymocyte globulin (ATG) 40 mg/kg/d for 4 days with cyclosporine is generally used as immunosuppressive therapy (IST). Their role in MDS is not very clear. Most studies are single center studies. A study from National Heart Lung and Blood Institute (1971-2003) reported an improvement in survival in lower risk MDS patients. The response rate was 24 % (ATG alone), 48 % (ATG + Cys), and 8 % (Cys alone). There is increase in overall survival and the response is generally durable ( 5 year 76 %). Also there is decreased risk of AML transformation. The pretreatment variables affecting response to IST in a univariate analysis were found to be; age < 60 yrs, short duration of RBC transfusion ( <6 months), hypocellular bone marrow, PNH clones, and HLA DR 15 phenotype. (ASH 2007). A multivariate analysis (NIH) revealed following factors as pretreatment variables; age < 60 yrs, duration of transfusion need, and HLA DR 15 phenotype. There is a concern regarding short and long term toxicities. Hence IST should be considered in previously untreated younger patients with hypoplastic MDS or HLA DR 15 phenotype.

#### **Lenalidomide:**

It has antiangiogenic, antiproliferative, anti cytoadhesive and immunomodulatory properties. In a non-randomised study (MDS 003) on lower risk MDS patient with 5 q del, 67 % patients achieved response within 1 month of taking the medication with a transfusion independence (TI) in 62 % of responders at one year. A randomised control trial in lower risk MDS patients with 5 q del (MDS 004) revealed TI response in 61 % patients and complete remission in 24 % for 10 mg dose of Len while 8 % TI for placebo. It did not increase the risk of AML.<sup>15</sup> The most common adverse effect is cytopenia which correlates with response only in 5 q del patients. Venous thromboembolism (VTE) was reported in 3 % of 5 q del and in 1 % of non- 5 q del patients. The dose needs to be adjusted in renal compromised patients.

The only known clinical predictor for the response to Lenalidomide is del 5 q. Len is superior to other treatments in patients of lower risk MDS with 5 q del, while comparable to others in non 5 q del patients.<sup>16</sup> The role of Len plus EPO in patients not responding to lenalidomide is still to be confirmed in trials. However lenalidomide is FDA approved only for lower risk MDS patients with 5 q del with transfusion dependent anemia if a trial with EPO has failed or there is poor EPO response profile.

#### **Hypomethylating Agents:**

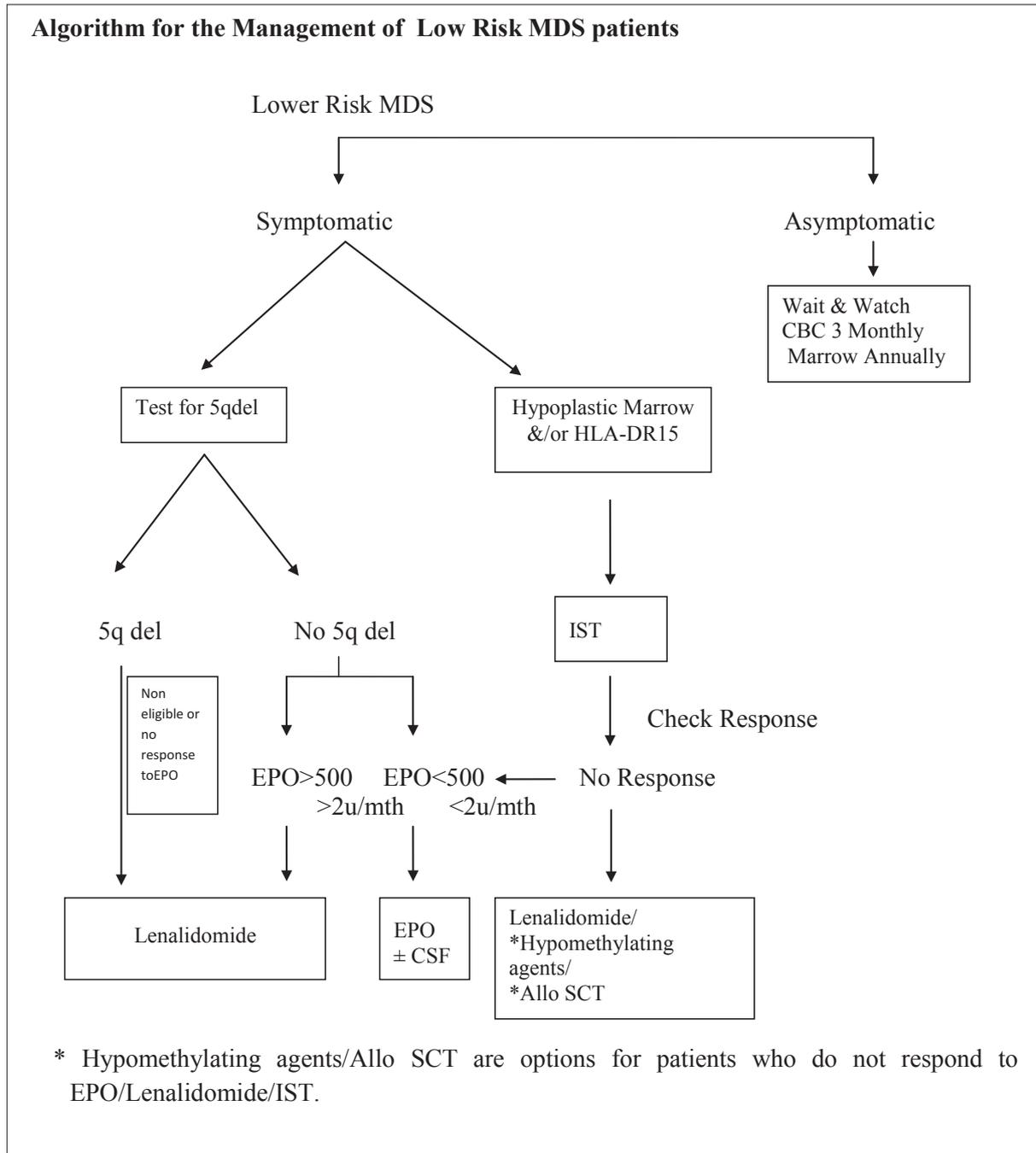
The CALGB 9221 azacytidine trial or D-0007 Decitabine registration study have shown an improvement in overall hematopoietic response rates as seen in higher risk patients, although this was not convincingly proved with decitabine. CALGB 9221 trial or D-0007 trial have shown similar overall hematopoietic response rates. It is not known whether a similar survival benefit as obtained in higher risk MDS will be obtained for lower risk patients who received azacytidine or decitabine.

Given the potential risk associated with hypomethylating agents, the compelling indication in lower risk MDS for hypomethylating agents are patients who are transfusion dependent and where recombinant EPO or IST has failed.

**STEM CELL TRANSPLANT:**

The role of allogeneic transplant in low risk MDS is controversial, although it is the only modality which has curative potential in MDS. As survival in lower risk MDS is good with non transplant modalities, it should be delayed till disease progression.

**Algorithm for the management of Low Risk MDS patients**



\* Hypomethylating agents/Allo SCT are options for patients who do not respond to EPO/Lenalidomide/IST.

The International prognostic scoring system (IPSS) and the WHO classification have been used in prognostication and dividing patients into specific risk categories and in making therapeutic decisions.

The Intermediate -1 risk MDS is generally categorised along with low risk MDS as lower risk MDS while the Intermediate - 2 risk MDS is classified along with High risk MDS as Higher risk MDS.

The definitive management of intermediate -1 risk MDS consists of treatment with the following categories of drugs

1. Immunomodulatory drugs
2. Immunosuppressants
3. Erythropoietic stimulating agents
4. Hypo-methylating agents
5. Stem cell transplantation

#### **Immunomodulatory agents:**

Immunomodulatory drugs (IMiDs) have been the mainstay of treatment of MDS based upon their anti-angiogenic and cytokine-modulating features, and their inherent ability to alter the bone marrow microenvironment. In addition to their ability to suppress tumor necrosis factor-alpha (TNF- ) elaboration, IMiDs affect the generation of a cascade of pro-inflammatory cytokines that activate cytotoxic T cells even in the absence of costimulatory signals. Moreover, IMiDs suppress endothelial responsiveness to angiogenic stimuli as well as the elaboration of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), thereby antagonizing neoangiogenesis within the bone marrow stroma.

The immunomodulatory agents used include Thalidomide and Lenalidomide.

**Lenalidomide** (lenalidomide; Revlimid®), is a 4-amino-glutarimide analog with more potent inhibition of TNF- and other inflammatory cytokines, and greater capacity to promote T-cell activation and suppress angiogenesis. Lenalidomide lacks the neurotoxicity of thalidomide but displays intrinsic myelosuppressive properties

**Lenalidomide** in 5q- deletion: List et al [MDS 003 trial] published their data on 148 patients who received treatment with Lenalidomide (10 mg daily days every 4 weeks or daily). Among them, 112 (76%) had a reduced need for transfusions and 99 (67%) no longer required transfusions, regardless of the karyotype complexity. Response to lenalidomide was rapid (median time to response, 4.6 weeks) and sustained; the median duration of transfusion independence had not been reached after a median of 104 weeks of follow-up. Among 85 patients who could be evaluated, 62 had cytogenetic improvement,

and 38 of the 62 had a complete cytogenetic remission. There was complete resolution of cytologic abnormalities in 38 of 106 patients whose serial bone marrow samples could be evaluated. Moderate-to-severe neutropenia (in 55% of patients) and thrombocytopenia (in 44%) were the most common reasons for interrupting treatment or adjusting the dose of lenalidomide.

The MDS 003 trial was followed by the MDS 004 trial, a randomized placebo-controlled study of LEN in patients with lower intermediate-1 risk MDS with del5q in which patients were randomized to receive LEN 10 mg/d (3 weeks on/1 week off), LEN 5 mg/d, or placebo (although after 4 months of treatment, most placebo patients were switched to LEN treatment). RBC-TI was achieved in 56% versus 41% versus 6% of the patients, and CyR in 41% versus 17% versus 0% of the patients, respectively. Median RBC-TI duration was not achieved with either LEN dosage, with a median follow-up of 1.55 years.

Lenalidomide in patients with abnormalities other than 5Q deletion: Raza et al<sup>6</sup> studied the role of lenalidomide given at 10 mg daily or for 21 days every month in patients with MDS without 5q deletion. 214 patients were analysed. 4% had Int-2 or high risk IPSS and in 18%, an IPSS category could not be assigned. 93 patients (43%) responded to lenalidomide treatment according to the modified IWG 2000 criteria. 56 (26%) patients achieved RBC TI with a concurrent 10 g/L or higher peak rise in hemoglobin; 37 (17%) patients had a 50% or greater reduction in transfusions. The median interval to the beginning of the RBC transfusion-independent period was 4.8 weeks and ranged from 1 to 39 weeks. The median duration of transfusion independence was 41 weeks. RBC TI continued for at least 6 months in 35 (63%) of 56 responders, and in 20 (36%) responders, the duration of RBC TI exceeded 1 year.

Higher rates of response seen with:

- a) Transfusion burden less than 4 units RBC (OR - 4.00)
- b) Baseline platelet count of 150 000 mm<sup>3</sup> or higher (OR - 2.66),
- c) Shorter duration of MDS (OR - 3.03), and
- d) Serum Lactate dehydrogenase less than or equal to the ULN (OR - 3.29)

There were no significant differences in TI response rate with respect to age, sex, FAB type, IPSS category. Thalidomide has shown some efficacy in the treatment of anemia in lower-risk MDS, with erythroid responses observed in approximately 35% of patients who could receive the drug for at least 8-12 weeks<sup>7</sup>. However, in a published series, 20%-50% of the patients discontinued the drug rapidly, mainly due to side effects including fatigue, sleepiness, or constipation. Increasing the daily dose to > 200 mg did not appear to increase the response rate, but did increase the side effects. Conversely, lowering the daily dose to 100 or even 50 mg was associated with fewer side effects, but somewhat lower response rates. Responders generally required drug discontinuation after several months due to persistence of the same side effects or to the occurrence of peripheral neuropathy. Side effects of thalidomide may be more important in MDS than in myeloma, possibly due to the somewhat older median age of MDS patients compared with myeloma patients.

**Erythropoietic stimulating agents:** Recombinant human erythropoietin (rhu-EPO) was one of the mainstay of therapy for lower-risk, transfusion-dependent patients with MDS until the appearance of immunomodulatory agents. Among unselected patients with MDS, 15% to 30% will experience an erythroid response to rhu-EPO, with considerably higher rates, 40% to 70% expected for the minority of selected patients with a favourable response profile.

Features predictive for response to erythropoiesis-stimulating agents (ESAs) include:

- a) Low endogenous erythropoietin concentration (<500 U/mL)
- b) Low transfusion requirements (<2 U/month) and
- c) Bone marrow blasts fewer than 10%

Presence of deletion 5q, with or without an additional cytogenetic abnormality, did not affect response rate in patients treated with ESA, but did significantly shorten response duration (mean 12 months vs 24 months,  $P = .019$ ).

With responses of approximately 40% in lower-risk patients using International Working Group (IWG) criteria for response, it is recommended that, in those patients responding to growth factors, hemoglobin levels be maintained no higher than 11 to 12 g/dL on erythropoietin. Long-term outcome of treatment of anemia in MDS with erythropoietin and G-CSF.

Which patients are most likely to benefit from ESAs? Two decision tools assist in defining appropriate MDS populations.

The first, developed by Hellström-Lindberg and colleagues, included 94 patients across three ESA studies to determine predictors of response to a combination of ESAs and G-CSF. Patients with low transfusion needs (< 2 units packed red blood cell transfusions [pRBC] monthly) and a low baseline serum erythropoietin level (less than 500 IU—the “good” ESA predictive group) had a 74% chance of responding to ESAs, while those with high transfusion needs (> 2 units pRBC per month) and a high serum erythropoietin level (> 500 IU—the “poor” ESA predictive group) had only a 7% chance of responding. Patients who had a mixed picture (low transfusion needs and high erythropoietin level, or high transfusion needs and low erythropoietin level—the “intermediate” ESA predictive group) had a 23% chance of responding to ESAs.

A second decision tool incorporated individual patient data, including response rates, OS, and quality of life, on 799 patients treated with either ESAs (394 patients) or nongrowth factor (405 patients) approaches from 90 articles published over a 20-year period and applied these data to the Hellström-Lindberg model to determine the most appropriate up-front therapy for patients with low-risk MDS. This treatment algorithm suggests that lower-risk patients with MDS falling into a good ESA predictive group should initially be treated with ESAs, while other low-risk patients with MDS (those falling into intermediate or poor ESA predictive groups) should probably be treated initially with other, non-growth factor therapies.

#### MDS Patients Who Are Likely to Benefit Most From Management Iron Overload

Characteristic	NCCN <sup>1</sup>	MDS Foundation <sup>2</sup>
Transfusion status	<ul style="list-style-type: none"> <li>• Received 20-30 packed RBC units</li> <li>• Continuing transfusions</li> </ul>	<ul style="list-style-type: none"> <li>• Transfusion dependent, requiring 2 units/month for &gt; 1 year</li> </ul>
Serum ferritin level	<ul style="list-style-type: none"> <li>• &gt; 2500 µg/L</li> </ul>	<ul style="list-style-type: none"> <li>• 1000 µg/L</li> </ul>
MDS risk	<ul style="list-style-type: none"> <li>• IPSS: Low- or Int-1</li> </ul>	<ul style="list-style-type: none"> <li>• IPSS: Low- or Int-1</li> <li>• WHO: RA, RARS and 5q-</li> </ul>
Patient profile	<ul style="list-style-type: none"> <li>• Candidates for allografts</li> </ul>	<ul style="list-style-type: none"> <li>• Life expectancy &gt; 1 year and no comorbidities that limit progress</li> <li>• A need to preserve organ function</li> <li>• Candidates for allografts</li> </ul>

**Refer :** NCCN Clinical Practice Guidelines in Oncology.

Bennett JM. J Hematol. 2008;83:858-861.

## Summary on Hypomethylation in Low Risk- Intermediate Risk

- Lower risk patients - transfusion dependent or have severe cytopenias, although goals of therapy and “acceptable” toxicity must be appreciated.
- Critical to its success - repetitive cycles of therapy and selection of appropriate patients who are willing and able to undergo prolonged therapy.
- Counseling- potential treatment benefit of these agents without CR
- Patient expectation of immediate disease response, and disappointment at the lack thereof, is likely an important contributor to the “treatment fatigue”
- Minimum # of cycles 4-6 repetitions.

Up to 15% of MDS patients exhibit a del(5q) karyotype. This group is comprised of 3 karyotypically defined subsets: isolated del(5q), which includes patients with the “5q-syndrome”; del(5q) with 1 additional chromosome abnormality; and del(5q) with 2 or more cytogenetic abnormalities (ie, complex karyotype). Preliminary work using RNA interference screening has identified haploinsufficiency of the ribosomal protein encoding the RSP14 gene as being necessary for the characteristic 5q- syndrome phenotype.

The primary mechanism of action of lenalidomide in MDS involves direct anti-proliferative effects on the del(5q) malignant clone by inducing G1 arrest via a p21-dependent pathway. Another primary mechanism of lenalidomide in MDS is its pro-erythropoietic effects involving an increase in red blood cells and hemoglobin levels. Secondary mechanisms include anti-angiogenesis and immunomodulatory effects. The drug also enhances erythropoietin receptor signaling. In del(5q), lenalidomide suppresses the malignant clone, but in non-del(5q) it appears to promote erythropoiesis.

The National Comprehensive Cancer Network (NCCN) guidelines in accord with the Italian Society of Hematology (SIE) recommend the use of the immunomodulatory agent lenalidomide as first line therapy within a clinical register or a clinical trial in patients IPSS low-intermediate-1 risk, transfusion dependent with 5q- cytogenetic abnormality.

The best cohort of patients to respond to Lenalidomide are as follows:

- IPSS diagnosed low/int-1 MDS w/o del(5q) abnormality
- 2 U RBC/8 wks
- Platelets > 50,000/ $\mu$ L
- ANC > 500/ $\mu$ L

Medications that lower the immune system (immunosuppressants- ISTs) improve blood counts in some patients with MDS. These drugs include anti-thymocyte globulin (ATG) and cyclosporine.

### Anti -Thymocyte Globulins

IST in the form of cyclosporine A or Antithymocyte globulin (ATG) should be considered if there is a good probability of response: IPSS low/int-1, WPSS very low, low or intermediate, age less than 60, hypocellular marrow, HLA-DR15 or PNH clone positivity. However, if the patient lacks the above predictors to ESA and IST, hypomethylating agents should be considered early in the treatment plan.

ATG and CsA in MDS remain among the most durable (and perplexing) of all available therapies in the disease. ATG is given at 40 mg/kg intravenously for four consecutive days, in conjunction with methylprednisone and CsA. However, this therapy is hampered by toxicity.

In some patients with MDS, T-lymphocytes interfere with normal blood cell production. Cyclosporine is another drug given orally that works by suppressing the immune system. It was first used to block immune responses in people who have had organ or bone marrow transplants, but, it has helped some patients with MDS.

**Chemotherapy** – Because MDS can progress to acute leukemia, patients are often treated with regimens used in that disease. These treatments usually require patients to stay in the hospital for several weeks. Chemotherapy reduces the abnormal stem cells and allows healthy ones to grow back. These effects are temporary unless followed by a bone marrow transplant. Side effects can depend on which drugs were given and how much, but include fatigue, hair loss, poor appetite, nausea or vomiting, diarrhea, or infertility.

AML-like Chemotherapy treatment remains an option only for selected cases (bone marrow blast percentage >10% and aged less than 65 years) refractory to hypo-methylating agents.

**The guidelines for the treatment of low risk MDS in our country should cover all the following aspects:**

- Provide clinical practice recommendations - support the appropriate choice of therapeutic interventions in adult patients with MDS both in rural and urban India.
- Implementation will depend upon expertise, finances, availability, insurance, etc
- As of now all recommendations are based on western data, based on proceedings from ASH, ASCO, EHA, NCCN, MDS international meetings data.
- What we need are Indian Data, for which we should have our own trials.

Role of a haematologist or physician practicing haematology would be to counsel the patient for better acceptance of the disease, improve patient compliance, provide reading material, assess the age and co morbidities in the patient to help optimize treatment schedules based on what can be affordable to the patient.

In lower-risk patients, decision tools can be used to determine the likelihood of response to erythropoiesis stimulating agents (ESAs), which have demonstrated survival advantages in retrospective studies in patients with MDS, and whether these patients should be treated initially with ESAs or non-growth factor (“active”) therapies.

#### **Algorithm approach to management of Low Risk and Int-1 Risk MDS**

- Suspected MDS – Watch and Wait – Serial haemograms - Counseling
- Rule out other causes of chronic anaemias (nutritional, haemoglobinopathy, renal, autoimmune, liver disease, early aplasia, HIV, etc)
- Correct any of the above if detected by appropriate means
- Refer to higher centre for molecular markers or any of the above if not available locally (to prevent re testing of marrow)
- Bone marrow examination with both aspirate and biopsy along with cytogenetics
- IPSS or WPSS scoring for stratification
- Check co-morbidities

- S. Erythropoietin level. If level < 500 mU/ml and hemoglobin is <10 g/dl, then consider erythropoietin injections upto 60-80,000 units weekly to get a 1.5 gm/dl rise in one month.
- If 5q minus- Lenolidamide would be the drug of choice with monitoring of the CBC at regular intervals
- If cytopenias, consider steroids, cyclosporine, TPO mimetics
- If the choice is to give transfusion, then keep Hb >10 gm/dl with judicious transfusions from quality blood transfusion labs only.
- Monitor iron status at regular intervals (every 3 months) and start iron chelation where appropriate (after 20 units of packed red cells)
- If non- responsive, then Hypomethylating agents (Azacytidine or Decitabine) or Immunosuppressive therapy.
- The International prognostic scoring system (IPSS) and the WHO classification have been used in prognostication and dividing patients into specific risk categories and in making therapeutic decisions.

**Patients with intermediate – 2 risk MDS are classified along with High risk MDS as Higher risk MDS.**

The definitive management of intermediate -2 / High Risk MDS consists of the following treatment options –

1. Stem cell transplantation [for transplant eligible patients]
2. Hypo-methylating agents
3. Immunomodulatory agents

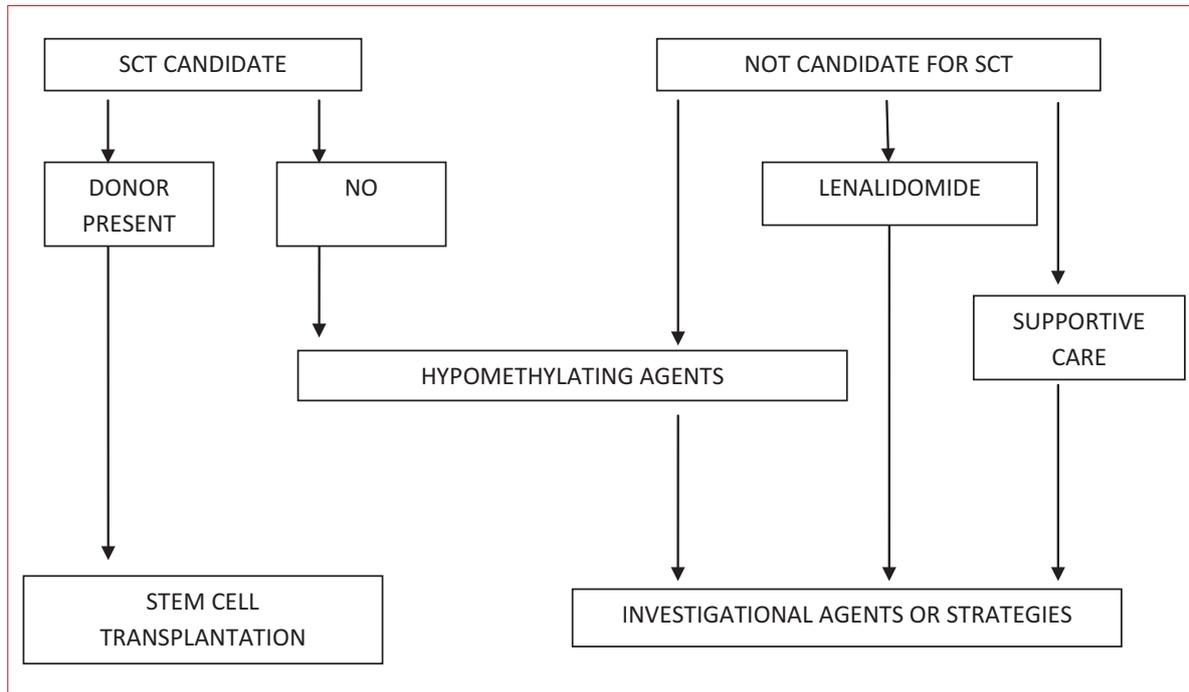
**Stem Cell Transplantation** – The role of allogeneic stem cell transplantation has been extensively evaluated in MDS and is based on whether the use of SCT is associated with a survival advantage compared to supportive therapy<sup>1</sup>. Data from Cutler et al seems to suggest that is preferable to perform early transplants in patients with intermediate -2 and high risk MDS while it may be prudent to consider SCT only at progression in patients with low or intermediate risk -1 MDS.

**Table : Approximate life expectancy (yrs) for myeloablative allogeneic SCT**

Risk Status	SCT at diagnosis	SCT in 2 yrs	SCT at progression
Low risk	6.51	6.86	7.21
Intermediate – 1	4.61	4.74	5.16
Intermediate – 2	4.93	3.21	2.84
High risk	3.20	2.75	2.75

Ref: Cutler C et al; Blood 2004; 104:579-85.

## Algorithmic Management of Intermediate Risk -2 / High Risk MDS



## Background

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only curative option for MDS, despite new therapeutic interventions. Patients with an intermediate, high or very high risk International Prognostic Scoring System (IPSS) score should be assessed for eligibility for HSCT which is in line with recommendations from the European LeukemiaNet. Utility of HSCT has been limited by donor availability and the advanced age of most patients with this disorder. However, the advent of reduced intensity HSCT, which relies on a graft-versus-leukemia (GVL) effect and the use of matched unrelated donors has extended the applicability of HSCT to patients in their 60s and even early 70s. It is important to carry out risk stratification of patients at diagnosis as specific cytogenetic abnormalities in MDS have considerable prognostic significance and affect treatment planning. These cytogenetic abnormalities have also demonstrated prognostic value in patients undergoing allogeneic hematopoietic cell transplantation (allo-HSCT). It is recommended that revised International Prognostic Scoring System (IPSS-R) rather than original IPSS be used to guide management decisions.

## Indications of HSCT

1. Patients with intermediate, high or very high risk groups as per IPSS-R risk stratification are considered candidates for early HSCT.
2. HSCT is delayed in other risk categories (very low and low risk groups).

## Timing of HSCT

Given that chemotherapy is generally ineffective in MDS, it is natural to consider a treatment with curative potential, such as HSCT. However, there are several competing problems that have made this approach less than ideal. The average age of patients in the original IPSS study was 69 years, with 75 and 45 percent of patients being older than 60 and 70 years, respectively. Most patients are therefore too old for myeloablative allo-HSCT. Patients under age 60 with the most favorable IPSS or IPSS-R scores have median survivals of 5 to 12 years with standard care. They also have the highest success rate following HSCT but also have a 20 to 40 percent risk of HSCT-related death (i.e., treatment-related mortality, TRM). Overall five-year survival post-HSCT is 40 to 60 percent for this group. In a single center, retrospective study of 1007 patients with MDS or acute leukemia evolving from MDS who underwent related (547 patients) or unrelated (460 patients) allo-HCT, the estimated rates of relapse, non-relapse mortality, overall survival, and relapse-free survival at five years were 25, 40, 38, and 35 percent, respectively. The estimated cumulative incidence of relapse at five years ranged from <20 percent for patients with very good cytogenetics to approximately 50 percent for patients with very poor cytogenetics. Thus, selection of which patients are appropriate for allo-HSCT, and whether to use myeloablative or reduced intensity regimens continues to be an area of ongoing discussion. A comprehensive geriatric assessment may be

useful in assessing co-morbidity and functional status in the elderly patient with MDS, thus permitting the formulation of an appropriate, individualized treatment plan.

Using decision analysis and prospectively collected registry data, and in the absence of data from randomized trials, the following recommendations have been made concerning the timing of allo-HSCT in patients with MDS:

1. For patients in the very low and low IPSS-R risk groups, it is recommended to delay allo-HSCT. The optimal timing for transplantation in this group is at the time of the development of a new cytogenetic abnormality, appearance of a clinically important cytopenia, or the progression to a higher risk group.
2. For patients with intermediate, high and very high IPSS-R risk scores, who have poor survival irrespective of their age, allo-HSCT at the time of diagnosis is associated with improved survival for the entire cohort of patients.

Given the effectiveness of allo-HSCT in younger patients with MDS, attempts have been made both to increase the safety of the procedure and to find donors other than HLA-matched siblings. The use of reduced-intensity allo-HSCT relies on a graft-versus-leukemia effect and permits HSCT to be performed in patients up to 70 to 75 years of age, or in younger patients with co-morbid conditions.

### **Choice of conditioning regimens**

The choice of conditioning regimen type and intensity is strongly associated with investigator preference, experience, and even geographic location. In the absence of large prospective, randomized studies, recommendations often are subject to personal or institutional biases. In our perception, TBI-based conventional conditioning regimens are being used with decreasing frequency in patients with MDS. Busulfan (Bu) either, with oral dosing or intravenously with pharmacokinetic guidance in combination with cyclophosphamide (Cy) or Fludarabine (Flu) is a widely used alternative to TBI. The development of less toxic and better tolerated non-myeloablative or reduced intensity Conditioning (RIC) regimens allows allo-HSCT to be performed in patients with MDS and advanced age or co-morbidity. However the flip side of such regimens is increased risk of relapse with a reasonable balance between GVHD and the GVL.

The balance between treatment-related mortality (TRM) relapse-free survival (RFS) and overall survival (OS), using RIC or myeloablative conditioning was evaluated in a retrospective analysis that compared these three outcomes following HLA-identical sibling donor allo-HSCT in 836 patients with MDS. Of these, 215 patients received RIC and 621 myeloablative conditioning the following outcomes were observed:

- Treatment-related mortality at three years was significantly lower in those receiving RIC (22 versus 32 percent; hazard ratio 0.61; 95% CI 0.41-0.91)
- The cumulative incidence of relapse at three years was significantly higher in those receiving RIC (45 versus 27 percent; hazard ratio 1.64; 95% CI 1.2-2.2).
- The increase in relapse following RIC is offset due to reduced TRM, such that three-year progression-free and overall survival were not significantly different between the two conditioning approaches.
- Most of the RIC regimens contain fludarabine and have shown comparable results in terms of OS and disease free survival (DFS). A novel regimen including photopheresis, pentostatin, and TBI (600 cGy) was associated with one year OS of 65 percent.

These studies demonstrate that non- myeloablative transplantation is feasible for patients with MDS who are generally beyond ages typically considered for myeloablative conditioning.

### **Should all elderly patients receive only reduced- intensity conditioning?**

The exploration of reduced- intensity conditioning regimen(RIC) resulted in less toxicity and has broadened the application of allo-HSCT especially in the cohort of elderly patients with haematological malignancies. The EBMT performed a retrospective study comparing RIC with standard myeloablative conditioning in 836 patients with MDS who received stem cell graft from HLA identical siblings. The EBMT found a lower risk for TRM ( $p=0.015$ ) but a significantly higher risk for relapse ( $p=0.001$ ) for the RIC transplanted patients, resulting in similar survival rates in both groups.<sup>23</sup> Similar results were found in the CIBMTR study. Other single center retrospective studies have shown a lower intensity of the conditioning regimen results in higher relapse rate and inferior outcome, especially in patients with more advanced disease.

However, in discussing intensity of conditioning regimen, it has to be pointed out that there is no clear cut-off between reduced-intensity conditioning and the myeloablative conditioning regimen. The major achievement of the new conditioning regimen is the reduced organ toxicity that broadens the application of allo-HSCT in elderly patients. Despite, reducing organ toxicity GVL can still be preserved. For instance, targeting drug levels while using intravenous formulation of “Bu” as part of the conditioning regimen has substantially reduced its toxicity, allowing the use of this drug at myeloablative doses safely. Furthermore, “Cy” with only limited anti-leukemic activity can be substituted by fludarabine, with less organ toxicity without compromising anti-leukemic activity. Newer alkylating drugs, such as treosulfan, can also be a part of myeloablative conditioning regimen with low toxicity and TRM in MDS patients.

### **Reducing the number of blasts prior to HSCT: the issue of cytoreductive pretransplant therapy**

The EBMT study of allo-HSCT in elderly MDS patients suggests that in advanced stage, relapse is the most frequent cause of treatment failure. The number of blasts and not being in complete remission at time of transplantation are the most significant factors for inferior outcome, especially after RIC transplantation in MDS patients. Therefore, the issue of performing induction chemotherapy before transplantation has been a matter of debate since starting allogeneic transplantation in MDS patients, the only randomized study from EBMT had to be stopped due to slow recruitment, and no valid data are available. Smaller retrospective single center studies showed no conclusive results, To overcome the limitation of long lasting myelosuppression but gain the anti-leukemic effect of pre-transplant induction chemotherapy, some centers have evaluated AML-like induction chemotherapies, such as anthracycline/ cytosine,arabioside/fludarabine, or clofarabine/cytosine arabioside based chemotherapy, followed by only 3 days rest before performing reduced- intensity conditioning and subsequent allo-HSCT. With those conditioning regimens and matched related or unrelated donors, 2 year overall survival rates of 69% to 56% can be achieved.

Newer agents, such as 5-azacytidine or decitabine which have been shown to be active in MDS, may also be used as pre-transplant cytoreductive therapy. However, the CR rate of about 10% is much lower than after conventional induction chemotherapy, and reported trials confirmed the feasibility without significant survival benefit. On the basis of the available evidence, intensive chemotherapy should be administered to those patients with 10% or more bone marrow blasts who are candidates for allo-HSCT.

## Matched unrelated donor

There is increasing use of matched unrelated donor (MUD) transplants, which may be as effective as HLA matched sibling transplants. However, use of more restrictive matching criteria means that fewer patients will find donors. As with all reports of new treatments, attention must be given to the possibility that patients receiving these treatments represent only a small, unrepresentative subset of patients with MDS. In a large series of MUD transplants for MDS, estimated rates of disease-free survival, nonrelapse mortality, and relapse at three years were 25, 58, and 41 percent, respectively.

## Relapse after HSCT

Relapse remains a major problem after HSCT for MDS. Effective post HSCT monitoring needs to be applied for early detection of relapse. Bone marrow cyto- and histomorphology, cytogenetic monitoring, PCR for molecular markers, assessment of donor-host chimerism and immunophenotyping have all been applied for detection of minimal residual disease (MRD). In a large study MRD was found to significantly influence after HSCT. Once relapse occurs, outcomes have been dismal. Preemptive donor lymphocyte infusions (DLI) have been used with equivocal results. Chemotherapy has generally been disappointing and second HSCT is associated with low success rates.

## Recommendations

- For patients with intermediate, high or very high risk scores who are eligible for allo-HSCT and have an available related or unrelated matched donor, we recommend allo-HSCT (Grade 1B). If a donor is not available, these patients may be treated with intensive induction therapy alone such as that used for acute myeloid leukemia (AML) or with a DNA hypomethylating agents with plans to treat with intensive induction therapy at the time of AML transformation.
- Patients with therapy related MDS are treated in a similar fashion to those with therapy related acute myeloid leukemia.
- Patients with IPSS-R very low or low risk should be managed conservatively and offered HSCT only i) at the time of progression to higher risk IPSS group, ii) appearance of clinically important cytopenia (s), or iii) development of new cytogenetic abnormality.
- The preferred donor for patients undergoing HSCT for MDS is an HLA-matched sibling. For those who do not have an HLA-matched sibling, a fully matched (10 of 10) unrelated donor will result in comparable survival rates but higher treatment-related mortality. Transplantation with a partially matched (9 of 10) unrelated donor will result in lower survival rates and higher treatment-related mortality, but is an acceptable alternative in selected few high risk cases.
- Non myeloablative or reduced intensity conditioning (RIC) allo-HSCT can be considered for patients with MDS who are not candidates for myeloablative allo-HSCT.
- “In the recent years improvement of haploidentical HSCT especially with use of post transplant cyclophosphamide which acts as GVHD prophylaxis and helps in engraftment, has offered many patients the opportunity to undergo this curative approach. However there are only small scale studies or case reports which have reported use of haploidentical HSCT in high risk MDS. Hence at present haploidentical HSCT should be offered only in clinical trial setting and not as a first line treatment approach in cases of high risk MDS.”

This chapter is attempting to describe the progress in understanding the pathophysiology of MDS and the next chapter describing the various novel therapeutic agents. These two chapters are not mutually exclusive. A better understanding of the underlying pathogenic mechanisms and information gathered from gene expression profiling studies helps us understand why some patients are at increased risk of progression to high risk MDS. It has perhaps also helped us in defining different therapeutic approaches for the various subtypes.

### TRANSLATIONAL STUDIES IN MDS

Myelodysplastic syndrome is characterised by the clonal proliferation of hematopoietic cells with leukemic potential. It comprises a spectrum of disorders which have varying risk of progressing to acute myeloid leukemia.

#### Molecular basis of MDS:

##### *The role of inflammatory cytokines and their correlation with underlying genetic changes*

An unknown genetic event triggers the initial process involving increased apoptosis of normal hematopoietic precursors and impaired differentiation and maturation of hematopoietic stem cell precursors. This results from abnormal function of genes which encode inflammatory cytokines such as TNF- $\alpha$ , TGF- $\beta$ , IL-1 $\beta$ , INF- $\gamma$ , INF- $\gamma$  receptor, VEGF and Fas/Fas ligand<sup>1</sup>. TNF- $\alpha$  mediates apoptotic death. Excessive levels of TNF- $\alpha$  are seen in MDS. It is also up-regulated in macrophages, fibroblasts and stroma of MDS.

The initial suppression of the normal hemopoiesis enables the emergence of a neoplastic clone characterised by dysplastic features and a proliferative advantage. As time goes by, the neoplastic clone becomes more and more unstable and accumulates various genetic defects. This might ultimately transform into the more advanced forms of MDS and thereon to leukemia. Disease progression is thus characterised by decreased pro-apoptotic signalling as neoplastic cell clones proliferate in the marrow at the expense of normal cells. These neoplastic cells are less susceptible to proapoptotic signals. There also occurs an upregulation of antiapoptotic signals such as Bcl-2 with progression of disease subtypes. The c-myc gene, having proapoptotic properties is upregulated in RA and RARS forms of MDS as compared to Bcl2 (which has anti apoptotic effect) which was upregulated in the advanced forms of MDS.

#### Genetic aberrations in MDS

Chromosomal anomalies are noted in 30-50% of primary MDS patients using conventional karyotyping techniques. Karyotyping forms an essential component of IPSS scoring into prognostic subgroups. Fluorescent In Situ Hybridization (FISH) techniques have the added advantage of not being dependent on dividing cells. However FISH has not been found to be superior to conventional karyotyping and might be useful in cases with none or insufficient number of detectable metaphases. High-resolution techniques such as single nucleotide polymorphism (SNP) arrays, comparative genome hybridization (CGH) array

and next generation sequencing (NGS) detect additional abnormalities in up-to 75-80% of cases of MDS and are expected to play a complementary role to conventional karyotyping. It is important to note that neither SNP array nor CGH can detect balanced translocations and might miss minor clones in case of SNP array. Hence neither test can replace conventional karyotyping.

An essential difference vis a vis acute myeloid leukemia (AML) is the preponderance of unbalanced chromosomal changes such as del(5q), del(11q), del(20q), del(7q), del(17p) and trisomies involving chromosomes 8 and 21. These chromosomal anomalies are seen more frequently in the progressive forms of MDS possibly representing the genomic instability of the more advanced forms of MDS rather than causative events. The affected region of chromosome 5 is the site of several genes which encode hematopoietic cytokines such as colony stimulating factor (CSF), IL3, IL4, IL5, as also hormone and growth receptors such as glucocorticoid receptor1 and proteins involved in transcription and signal transduction such as EGF1 and IRF1. Similarly the affected regions of chromosome 7 on the long arm encode for several tumor suppressor genes.

Lenalidomide was found to be useful in patients with the del(5q) resulting in transfusion independence and in several cases, even a cytogenetic response. The success of lenalidomide underlined the need to identify cytogenetic abnormalities in MDS. The characteristic presentation and clinical features of 5q- syndrome was known for some time before one of the possible genes on the 5q was identified. Ebert et al used a RNA mediated interference technique to identify RPS14 as a candidate gene. Loss of RPS14 resulted in a 5q- type of defect in normal hematopoietic cells and addition of RPS14 rescued patient derived bone marrow cells. RPS14 is a component of the 40S ribosomal unit and appears to be responsible for erythroid maturation. This defect was also seen in patients with Diamond Blackfan anemia (DBA). However patients with DBA are not associated with thrombocytosis and therefore some additional defects, such as recently identified haplo-insufficiencies for microRNAs on chromosome 5q33, might be responsible for the complete 5q- phenotype .

The MDS/EVI1 gene is involved in several balanced anomalies involving the chromosome 3 in patients with MDS, AML and CML. The MDS/EVI1 gene gives rise to two variant products, EVI1 and MDS/EVI1. The AML1/MDS/EVI1 gene product and EVI1 protein promote proliferation and are antiapoptotic through inhibition of TGF $\beta$  whereas the MDS/EVI1 protein has been reported to demonstrate opposite effects<sup>5</sup>. The TEL-PDGFRB tyrosine kinase is derived from t (5;12) and has been seen in patients with CMML. It presents a potential target for the use of Imatinib in patients who have this defect.

### **Somatic point mutations in MDS**

Point mutations involve genes responsible for downstream signaling such as KIT, G-CSFR, PDGFRB, FLT3, FMS, N-RAS, K-RAS, JAK2 or genes involved in signal transcription such as RUNX1, CEBPA, GATA-1, p53, MLL. The FMS gene encodes for cell surface receptors of CSF-1 which promotes proliferation and differentiation of monocytes and macrophages.

RUNX1 has been frequently identified in patients with MDS. The mutated RUNX1 resulted in MDS phenotype in mouse models. RUNX1 also known as AML1, CBFA2 encodes for a protein which functions as a subunit of the core binding factor complex. It functions as a transcription factor and mutations in RUNX1 lead to block in differentiation resulting in the cytopenias typical of MDS. Germline mutations in the RUNX1 are associated with the Familial platelet disorder which has an increased risk of progressing to MDS or AML.

Several such point mutations might accumulate over time. For example the N-RAS mutations are seen in association with RUNX1 mutation. Mutations in RAS oncogenes result in ligand independent

proliferation and prolonged growth signals. The farnesyl transferase inhibitor, Tipifarnib (Zarnestra/R115777) indirectly inhibits RAS and presents an opportunity to study the utility of identifying targeted therapy against point mutations involved in MDS. Tipifarnib was evaluated in a phase 2 multicenter trial in patients with poor-risk myelodysplastic syndrome. A previous dose finding study by the same group had identified 300 mg orally twice daily as the appropriate dose. Tipifarnib 300 mg orally was administered twice daily for the first 21 days of each 28-day cycle. Twenty-six patients (32%) responded to tipifarnib of whom 12 (15%) had complete responses (CRs) and 14 (17%) hematologic improvements; 37 patients (45%) had stable disease. The median response duration among the patients with CR was 11.5 months (range, 2.0-21.9 months), the median time to progression was 12.4 months (range, 3.9-23.8 months), and 7 were still alive at time of analysis (all > 3 years). Median overall survival was 11.7 months (95% CI, 9.4-15.0). Grade 3-4 neutropenia (18%) and thrombocytopenia (32%) were the most common treatment-related adverse events. This study was published in 2009 after azacitidine was approved for this group of patients. However the patients included in this study were recruited between 2002 and 2003. The results appeared comparable to those achieved with azacitidine and decitabine. There have been no trials comparing hypomethylating agents with Tipifarnib. In a phase III randomized trial comparing azacitidine with best supportive care in elderly patients with low blast count acute myeloid leukemia (blast count between 20-30%), median OS for azacitidine-treated patients at a median follow-up of 20.1 months was 24.5 months compared with 16.0 months for patients on best supportive care which included low dose cytarabine (hazard ratio = 0.47; 95% CI, 0.28 to 0.79;  $P = .005$ ), and 2-year OS rates were 50% and 16%, respectively ( $P = .001$ ). A phase III trial comparing Tipifarnib in a similar subgroup of patients failed to show any advantage over best supportive care.

Mutations involving genes responsible for signal transduction have also been seen in JAK2 and CBL. JAK2 mutations are typically noted in myeloproliferative disorders. However they are also seen in 5% cases of MDS. They are present in nearly 50% cases of MDS cases having ring sideroblasts and thrombocytosis (RARS-T).

### **Epigenetic changes in MDS**

Epigenetic changes are being recognised as one of the mechanisms underlying the pathogenesis of MDS. Hypermethylation of CpG islands in the promoter regions of several genes is thought to be responsible for silencing of tumor suppressor genes in MDS and also several other malignancies. The p15<sup>INK4B</sup> was observed to be hypermethylated in advanced MDS subtypes and was associated with increased progression to acute leukemia. The proof for epigenetic phenomena underlying MDS comes from the success of azacitidine and decitabine which were approved for use in MDS. These drugs and the clinical trials which formed the basis for their approval are described earlier on in this review. High-resolution SNP array analysis identified mutations involving TET2, ASXL1 and EZH2 which also have epigenetic activity. These genes code for enzymes responsible for the DNA and histone methylation. Tet proteins catalyse the conversion of 5 methylcytosine of DNA to 5-hydroxy-methylcytosine and this has important role in embryonic stem cell maintenance. TET2 mutations (Ten Eleven Translocation; TET) are seen in 14-26% of MDS and is associated with a normal karyotype and favorable overall survival (76.8% 5 year overall survival vs 18.3% in wild type). The ASXL1 (Additional Sex Comb Like-1) is a tumor suppressor gene, which also behaves as a co-activator for the retinoic acid receptor, and is found to be mutated in 6-11% cases of MDS. Most ASXL1 mutations are of the frameshift variety and are associated with a shorter overall survival and faster progression to AML. EZH2 (Enhancer of Zeste Homologue) gene codes for a protein which transfers methyl groups to Histone H3 lysine 27. It is also a tumor suppressor gene and mutations involving EZH2 have been seen in 6% cases of MDS, where they are most commonly

frameshift/nonsense mutations. Studies suggest that TET2, EZH2 and ASXL1 mutations are particularly susceptible to the effects of azacitidine.

### **Prognostic and therapeutic utility of detecting genetic defects in MDS**

All patients of MDS with the 5q- do not respond to lenalidomide, with 45% response among intermediate-1 risk MDS and 67% response among patients with low risk MDS with del(5q). Patients who failed to respond had an increased risk of developing AML. Such patients were found to have del(17p) which led to loss of TP53. Additionally patients who progressed to AML had shorter telomeres. A response to lenalidomide is associated with telomere elongation.

Previously described cytogenetic abnormalities which were thought to have an adverse outcome might not remain important adverse markers with improvements in treatment.. Two recent papers showed that while poor risk cytogenetics are important prognostic factors in the outcome of transplant in MDS, the FAB classification plays an independent role . Onida et al showed that patients with poor risk cytogenetics in RA/RARS had similar outcome as compared to those with standard risk cytogenetics. Patients with standard cytogenetics in untreated RA/RARS had a 5 year relapse free survival of 48% compared to 47% for those untreated RA/RARS with poor risk cytogenetics. The small number of monosomal karyotype patients did not allow them to study them separately. However transplant early on in the disease before progression towards AML might provide outcomes similar to those with good risk cytogenetics.

Complex karyotypes ( 3 chromosome aberrations) have been traditionally associated with a uniformly poor outcome. MDS patients with a monosomal karyotype, which is defined by the presence of at least two autosomal monosomes or one monosomy with at least one structural abnormality, were found to have an outcome similar to other poor risk cytogenetics. Patients with complex cytogenetics were more likely to have a worse outcome if they also had a monosomal karyotype. Patients who have at least one structural abnormality (deletion of part of a chromosome, inversion within a chromosome, translocation between chromosomes, or addition of chromosomal material), probably fare worse as compared to those without. In a EWOG study on childhood MDS treated with hematopoietic stem cell transplant, patients with a complex karyotype without a structural anomaly fared as well as those with a normal karyotype. However, patients who had a structural anomaly as part of the complex karyotype had a 2 year overall survival of only 14%. Bejar et al described the presence of point mutations in MDS including nearly half the cases of normal cytogenetic MDS. In addition to the known somatic mutations in NRAS, KRAS, BRAF, JAK2 and PTPN11 they also identified a novel mutation in GNAS. They also identified focal deletion involving ETV6 which was not previously described in MDS. Mutations involving ASXL1, RUNX1, TP53, EZH2, CBL and ETV6 were independently associated with a poor overall survival with hazard ratios for death ranging from 1.38 for ASXL1 to 2.48 for presence of TP53 mutation. Thus lower risk MDS with these mutations might require a more aggressive therapy. Currently used therapies with hypomethylating agents generally have not been found to alter the treatment response to patients with these mutations, except for CBL mutations, which are associated with a lower response.

The patients with TET2 mutation were mostly present in patients with normal karyotype whereas patients with TP53 mutation were more commonly associated with complex karyotype. Mutations of RUNX1, TP53 and NRAS were associated with severe thrombocytopenia and increased blast percentage.

The TET2 mutation is also seen in patients with myeloproliferative disorders. Therefore it might not be directly associated with the dysplasia. The TET2 mutation might simply enable the clonal predominance of the diseased cell. Even so, TET2 mutations are consistently associated with an improved response to hypomethylating agents. TET2 is responsible for DNA demethylation. Small populations of TET2

mutated clones can be detected by sensitive techniques as compared to traditional Sanger sequencing. It was found that the prognostic significance of TET2 in predicting response to hypomethylating agents exists only for large clone sizes. Thus the volume of mutated clones is perhaps more important than mere detection of a mutation.

DNAMT3A (DNA methyltransferase 3 alpha) mediates the addition of methyl groups to CpG islands. Mutated DNAMT3A (3-13% of MDS is responsible for poor outcome; reduced overall survival and early leukemic transformation. However unlike with TET2, hypomethylating agents have not been able to change outcome in patients with DNAMT3A mutations.

Another gene, besides n-RAS, which has been found to be mutated in association with RUNX1 is the gene which codes for isocitrate dehydrogenase (IDH), an important part of the Krebs cycle. IDH1/IDH2 gene mutations result in a protein which blocks the enzymatic function of TET proteins among others and has been associated with high platelet counts and poor overall survival. Owing to their relative rarity in MDS (5-10%), studies evaluating the effect of hypomethylating agents on these mutations have not been consistent. Some mutations such as NPM1, CEBPA, WT1, FLT3, CKIT are more common with AML and rarely seen in MDS where they might be associated with a poor prognosis and faster progression to AML. Patients with trisomy 8 are at intermediate risk in the IPSS-R. However trisomy 8 in younger patients with HLADR15 and refractory anemia of short duration are associated with a good response to immunosuppressive therapy. The various gene mutations commonly associated with MDS are summarised in table 1`.

### **Spliceosomal machinery in MDS**

RNA splicing is a process whereby non coding sequences or introns are removed from the pre-mRNA by small nuclear ribonucleoproteins (snRNPs) and small nuclear RNAs. These are also known as spliceosomes. Mutations in spliceosomal genes such as splicing factor 3 subunit b1 (SF3b1) was first seen in MDS. Thereafter several other genes were also identified such as U2AF1, SRSF2, ZRSR2, SF3a1, PRPF40B, U2AF65 and SF1. These mutations are mutually exclusive but can coexist with other somatic mutations described in the previous section. Mutations in SF3b1 were more likely to be seen in RARS and RARS-T. The clinical significance of these mutations is still not clear. The SF3b1 mutation has been associated with a better prognosis and lower risk of AML transformation. This gene is important for polycomb mediated repression of HOX genes in mice. ASXL1 and EZH2 are some of the genes in the polycomb complex. However mutations of ASXL1 and EZH2 have been associated with more aggressive varieties of MDS. The SF3b1 mutation has also been seen in chronic lymphocytic leukemia where it is associated with a worse phenotype. The serine arginine-rich splicing factor 2 or SRSF2 is presumed to be a component of the acetylation/phosphorylation network which regulates DNA stability. Depletion of SRSF2 might thus lead to genetic instability and a poorer outcome. However it is most common in CMML where it is also associated with a better outcome if there are concomitant RUNX1 mutations. Hypomethylating agents have not been found to alter the prognosis in patients with spliceosome mutations.

Thus even though the spliceosomal mechanism has some role in MDS, the exact nature of the defect and its mechanism is still not clear.

### **Utility of next generation sequencing in MDS**

The next generation sequencing techniques demonstrate the presence of mutations in most case with MDS. However the clinical significance of many of the mutations, which occur at low frequencies, remains to be determined. Routine clinical practice might instead, consider incorporating a small cohort of genes, already known for their association with MDS as done by Bejar et al. They investigated 213 patients

with MDS for the presence of mutations in a panel of 40 genes, which had previously demonstrated association with MDS<sup>23</sup>. They were able to detect mutations involving 39 genes in 94% of the cases. Other than ASXL1 which was mutated in 46% of their cases unlike other studies with less frequency, all other mutations had similar frequencies as described in Table 1.

5q- MDS stem cells are associated with upregulation of several genes (BMI1, ID1, DNMT3A, MYC, CEBPA) and haploinsufficiency of tumor suppressor genes like RPS14 and SPARC. Similar gene expression is also seen in Diamond Blackfan anemia. Early MDS stages showed a different gene expression profile as compared to the more advanced forms of MDS. Patients with CMML are more likely to have mutations in TET2, ASXL1, NRAS, EZH2, and SRSF2. Spliceosome mutations in RARS and RARS-T have been described earlier. Mutations in TP53, IDH1, IDH2 are less likely in low risk MDS.

Gene expression profiling could also be used to predict the response to drugs. As described earlier, presence of ASXL1, EZH2 and TET2 mutations predicted for response to azacitidine. This has also been used to predict response to lenalidomide in non 5q- MDS patients. Genes responsible for erythroid differentiation were downregulated in responders as compared to non-responders. A phase II multicentre study also confirmed the immunological basis for lenalidomide's action in MDS. Gene expression profiling of selected genes evaluated at baseline and after 3 and 6 months of treatment showed that RPS-14, miR-145, and miR-146 were downregulated at baseline and significantly increased during treatment<sup>21</sup>. Genes in the apoptotic pathways (TNF, IL-1B, and IL-10) were upregulated at baseline and significantly downregulated during lenalidomide treatment.

**Epigenetic basis for use of hypomethylating agents in myelodysplastic syndrome:**

Chromatin forms the packaging material of DNA. Changes in the chromatin alter the way genes interact with transcription signals and thereby affect gene expression without actual changes in the gene sequence. A study of such changes is known as Epigenomics. These changes are inheritable thereby meaning that these are passed on to daughter cells when the cells divide. Such changes result in gene silencing which is an essential process during development of normal cells. This is mediated through processes such as histone modification in chromatin, DNA cytosine methylation and RNA interference. Disordered silencing can potentially lead to cancer through silencing of tumour suppressor genes.

The four histone proteins of the nucleosome form the building blocks of the chromatin. Acetylation of histone proteins stimulates transcription and histone deacetylation inhibits transcription. The same histone modification has the potential to either suppress or activate a gene. For example H3K36 methylation has a positive effect when this occurs in the coding region but represses transcription when it occurs in the promoter region. DNA methylation typically occurs at cytosine residues within CpG dinucleotides (where CpG stands for cytosine and guanine bases bound by phosphodiester bonds) and this opposes the transcription process. The CpG clusters in promoter regions are generally resistant to DNA hyper methylation. However myelodysplastic syndrome and several other cancer processes are associated with aberrant and excessive methylation of CpG islands in promoter regions of tumour suppressor genes.

Once these CpG islands are methylated, they bind to methylated-DNA-binding proteins like MeCp2. This in turn recruit histone deacetylase (HDAC) protein complexes which finally results in the chromatin forming a closed structure. It no longer allows the binding of transcription factors to the gene which becomes “silenced”. HDAC is just one of several chromatin remodelling proteins which are attracted to the methylated CpG.

Drugs targeting one or the other processes which alter the chromatin structure have been studied for myelodysplastic syndromes. The FDA has approved azacitidine(VIDAZA) in 2004 and decitabine (DACOGEN) in 2006 for patients with all MDS subtypes. Lower risk MDS subtypes were also approved for hypomethylating agents provided they were transfusion dependent for either blood or platelets. These drugs have been described earlier under the appropriate sections. Optimised delivery of these two drugs and predictors of response and resistance to hypomethylating agents would be dealt with in the appropriate sections. This part will deal with the histone deacetylating agents and newer therapies for MDS.

**Current status of histone deacetylase inhibitors (HDACi)**

Acetylation of histone proteins is maintained by the opposing effects of histone acetyltransferases and histone deacetylases (HDAC). Histone deacetylases have been found to have several non histone targets

which are also involved in cell proliferation and apoptosis. These non histone targets include transcription factors (such as NF- $\kappa$ B, GATA1), estrogen receptors and tumor suppressors (such as p53 which also functions as a transcription factor). Several haematological malignancies associated with fusion proteins (PML-RARA, AML-ETO) and BCL6 in non-hodgkins lymphoma have aberrant transcription repression mediated through HDAC activity. It is thought that HDAC inhibitors could overcome the transcription repression characteristic of neoplastic cells and thereby encourage the growth of normal cells.

Sodium butyrate was the first compound identified to inhibit histone deacetylase followed by trichostatin A, a fungal antibiotic. Valproic acid used in the treatment of epileptic diseases has been found to have HDAC inhibitor activity. The other compounds with HDAC inhibitor activity (HDACi) include suberoylanilide hydroxamic acids (SAHAs); cyclic peptides (e.g., romidespin); benzamides (e.g., MS-275/entinostat and panobinostat/LBH589), aliphatic or short chain fatty acids (e.g., butyrate and valproate); and hydroxamates (vorinostat). The inhibition of histone deacetylation by these compounds generally up-regulates the expression of tumor suppressors such as p53 which had been repressed aberrantly.

There have been several trials involving HDACi which aim to demonstrate clinical activity similar to those of the hypomethylating agents.

Entinostat is a synthetic benzamide derivative which has activity against human tumor cells in vitro, including leukemia cell lines and primary leukemia blasts. It activates antiproliferative genes like p21 and transforming growth factor beta-type II receptor. It also induces maturation marker gelsolin. Entinostat (SNDX-275/MS-275) and another benzamide, MGCD0103 selectively inhibit only class I HDACs whereas other agents, such as panobinostat (NVP-LBH589) inhibit HDACs more broadly. It is not yet known if this selective action has any clinical significance.

A phase I trial with oral entinostat demonstrated in vivo histone acetylation activity as demonstrated by western blotting. This acetylation increased with time and persisted beyond the last dose of entinostat for 2-3 weeks. Nevertheless, the drug did not demonstrate any significant clinical activity in the form of either PR or CR. This group of patients included, besides high risk MDS, acute myeloid leukemia patients who had progressed from MDS and acute promyelocytic leukemia refractory to ATRA and arsenic. Another orally active benzamide, MGCD0103 was tested in a phase I study which enrolled 29 patients of whom 3 had demonstrated marrow response. This response lasted for 1-3 cycles of therapy and necessitated stoppage of therapy even in responders on account of disease progression or failure to correct counts. The FDA had put a hold on further drug studies with MGCD0103 on account of hemodynamically significant pericardial effusion. Similarly belinostat, a hydroxamic acid derivative, was tested in a phase II trial sponsored by the NCI where only one patient out of 22 had a documented haematological improvement in neutrophils. The study was stopped as there were no responses. A phase II trial on vorinostat in patients with low and intermediate-risk MDS patients was also terminated as there were no clinical responses.

Depsipeptide / Romidepsin is a cyclic peptide derived from *chromobacterium violaceum*. It was studied in twelve patients (nine AML, three MDS) who received one to five cycles of depsipeptide. Only one complete remission was achieved in a patient with AML, with either stable disease or progression of disease in the remaining. The invitro data on acetylation was not very encouraging either. Romiplostin continues to be studied in lymphomas and in combination therapies in multiple myeloma. However there have been no recent studies on Romiplostin during the last 4 years in patients with myelodysplastic syndrome.

**Table - : Molecular abnormalities in MDS**

Genetic abnormality	Locus	Frequency (%)	Pathogenic mechanism	Clinical effect
TET2	4q24	20	Uniparental disomy. Epigenetic modification ?mutation enables clonal predominance	Associated with a better prognosis and better response to azacitine
RUNX1/AML1	21q22	15-20	Mutation alters DNA binding domain	More common in t-MDS. Increased progression to AML
TP53	17p13	5-10	Loss of p53 tumor suppressor activity. Increased chromosomal instability	Relative resistance to therapy
ASXL1	20q11	10-15	Frameshift mutations. Epigenetic regulator. Coactivator for retinoic acid receptor	Poorer overall survival and increased progression to AML
NRAS	1p13	10	Loss of GTPase activity leads to constitutive serine/threonine kinase	Increased progression to AML
EZH2	7q36	6	Loss of histone methylation. Epigenetic activity	Poor prognosis.
JAK2	9p24	5% in MDS, 50% of RARS-T	Constitutive tyrosine kinase	Unknown
RPS14, SPARC, CTNNA1	5q31	15	Haploinsufficiency of the affected gene	Better prognosis. Response to lenalidomide
CBL/CBL B	11q23	Rare	Loss of ubiquitin ligase activity	Increased progression to leukemia in CMML
EVI1	3q26	5% overexpressed	Signal transduction	Presumptive evidence of MDS in patients with otherwise unexplained refractory anemia and no dysplasia
IDH1/IDH2	Codon 132 of IGH1	3.1-7.4% IDH1	The mutation blocks enzymatic function of other proteins	More advanced disease and progression to AML

### Combination therapy with HDACi and DNA hypomethylating agents

Histone deacetylase inhibitors (HDACi) have failed to show significant single agent activity despite invitro demonstration of histone deacetylase activity. On the other hand, HDACi have been shown to have synergistic invitro activity with DNA hypomethylating agents to re-express tumor suppressor genes. Combination therapy with Histone deacetylase inhibitors have been tried in an attempt to increase the CR rates achieved with hypomethylating agents alone . Entinostat was combined with azacitidine in a phase II trial (US leukemia intergroup trial E1905). However entinostat failed to add to the benefit achieved by azacitidine alone.

Other HDACi that have been combined are panobinostat,vorinostat, tenfinostat, mocetinostat and pracinostat. Gastrointestinal upset and fatigue are common side-effects seen with these drug combinations. Panobinostat has been tried in a phase I dose escalation trial in combination with a fixed dose of azacitidine. Preliminary efficacy data for 8 pts with AML and 8 with MDS/CMML showed 4 pts (2 at 30 mg, 2 at 40 mg) achieving CR or morphological CR (CRi) and 6 pts (4 at 30 mg, 2 at 40 mg) showing stable disease (SD). Hematologic improvement was seen in 4 pts, 2 of whom relapsed.

Vorinostat has been studied in combination with azacytidine in patients with renal and hepatic dysfunction, concomitant HIV infections and malignancies and patients with a poor performance status. Most studies on novel agents would exclude these patients. Global and gene specific hypomethylation, induction of histone acetylation, TET2 mutations and miRNA 29b levels were evaluated as pharmacological endpoints. Thirty patients were treated of whom 16 patients (53%) had MDS, 2 CMML and the rest AML. Most patients tolerated the therapy despite significant comorbidity with 1 patient (3%) developing severe nausea, vomiting) non-heme related toxicity. One (5%) patient died during induction therapy. Survival greater than 60 days was seen in 24 (80%) patients. Complete remission was seen in 8 patients and 1 had a complete marrow response (overall response rate of 30%). Of the 16 non-responding patients, 8 had stable disease for more than 8 weeks. At the last follow-up, 9 of the 30 patients were alive.

A phase II combination study of vorinostat with decitabine reported a complete response in 22% patients with MDS and hematologic improvement in another 22%. 63 patients were randomized to treatment of whom 35 patients discontinued therapy due to various reasons such as lack of efficacy or progression of disease (n=19), withdrawal of consent (n=8), adverse events (AEs) (n=6), physician decision (n=1), and protocol deviation (n=1). Complete response (CR) was achieved by 22% pts with MDS, 26% with untreated AML, and 8% with relapsed/refractory AML. Hematologic improvement was reported in 4% and 22% of patients with untreated AML and MDS, respectively. A similar proportion of patients achieved stable disease (SD) in all disease groups (range 30–46%). The authors concluded that toxicity due to the combination was manageable and showed promise. Phase I trials combining valproic acid to azacytidine have demonstrated some activity at cost of reversible neurological toxicity (usually somnolence). A phase II study which also added ATRA to a combination of valproic acid and azacytidine failed to show any evidence that ATRA contributed in any way to the response as differentiating activity could not be demonstrated.

Thus there is currently no evidence that these agents add to the benefit already seen with the hypomethylating agents alone. Data on combination therapy of azacytidine (75mg/m<sup>2</sup>/d x 5 days) and lenalidomide (10mg/d x 21 days (28-day cycle) was initially promising as there appeared to be an improvement in terms of complete remission, with an improved survival for patients who achieve CR.

However a subsequent phase II data from the North American Intergroup Study SWOG S1117 failed to demonstrate any advantage of the combination therapy with either Lenalidomide or Vorinostat over Azacytidine alone; patients in the combination arm suffered more toxicity.

**Other novel agents :** Studies into mechanisms of resistance to hypomethylating agents have introduced several new targets :

1. **Upregulation of cytidine deaminase (CDA)** has been noted in non responders and males, who have lower response rates as compared to females: CDA inhibitors (ASTX727; NCT02103478)
2. **Tumor mediated upregulation of immune checkpoint mediators** such as programmed death ligands (PDL1 and PDL2) and cytotoxic T lymphocyte associated antigen 4 inhibit the immune mediated destruction of malignant cells in the bone marrow environment: immune checkpoint inhibitors ( Nivolumab/PDL1 inhibitor; NCT02464657/NCT01822509, Ipilimumab/CTLA4 inhibitor; MDX101; NCT01757639/01822509, Divalumab/MED14736; NCT02117219)
3. **New epigenetic targets:** Evaluation of mutation status in a given individual provides an avenue for a more personalized therapy. TET2 mutations have been shown to provide a response advantage to conventional hypomethylating agents. Demonstrating presence of IDH mutations and EVI1 mutations

might provide rationale for IDH1/2 inhibitors (AG120/AG221; NCT 02074839/NCT01915498) or BET inhibitors (CPI-610, OTX015; NCT02158858) respectively.

**4. ON 01910.Na - Cyclin D1 inhibitor (Rigosertib): Targeted therapy in MDS to inhibit signal transduction.**

This small molecule is a styryl sulfonyl compound which interferes with the cell cycle at the G2/M phase and promotes apoptosis. Leukemic cells appear to be more sensitive as compared to normal cells thereby simulating a degree of selectivity. It also reduces the cyclin D1 and c-myc protein levels in the cancer cells. It reduces the amount of cyclin D1 by inhibiting the translation of cyclin D1 mRNA without any actual reduction of the mRNA levels. The effects of the cyclin D1 inhibitor are most apparent on cells with high levels of cyclin D1 and spares normal cells. Thus this represents a form of targeted therapy for high risk MDS which if effective could be one of the successful bedside applications of laboratory advances.

Cyclin D1 is one of several proteins such as retinoblastoma, c-myc, p27kip1 which are involved in cell growth and proliferation. The levels of cyclin D1 are increased in patients with high risk MDS as compared to normal cells and low risk MDS. While some studies found an increased level in MDS cells bearing trisomy 8 and monosomy 7, other authors have not found any particular cytogenetic association. Cyclin D1 upregulation is associated with increased levels of survivin which inhibits apoptosis of caspase 8, thereby preventing apoptosis of MDS cells.

The increased levels of cyclin D1 in high risk MDS is the result of excessive and aberrant activation of the PI3K-akt pathway which mediates its effects through the mammalian target of rapamycin or mTOR. The PI3K-akt pathway in turn is activated by various mitogenic stimuli which bind to receptors (PDGFR, IGF, Her, EGFR) on the cell surface. This pathway can also be directly activated by oncogenic ras. mTOR is either directly activated by PI3K-akt or indirectly via inhibition of the TSC1/TSC2 proteins (tuberous sclerosis complex). Activated mTOR phosphorylates the Eukaryotic translation initiation factor 4E binding protein complex (eIF4EBP1) and releases the translation initiating factor-4 component (eIF4E). This protein mediates the translation of proteins such as cyclin D1 and c-myc which initiate proliferation and antiapoptotic signals. In its unphosphorylated state, 4EBP1 tightly binds to eIF4E and prevents its actions on the target mRNA.

A phase I/II trial enrolled 13 patients with both trisomy 8 MDS or patients who had failed hypomethylating agents. The median survival of this group which received rigosertib was 10 months (range 3-17 months) and compared favourably to historical patients with high risk MDS who have progressed on therapy (median survival 4-5 months). The study achieved 4 marrow complete responses without significant hematological improvement, one of whom progressed on therapy. In the last ASH meeting of 2012, Navada et al presented their data on high risk MDS patients who had failed hypomethylating agents. They identified a maximum tolerated dose of 1375 mg/m<sup>2</sup> for a continuous 72 hours intravenous infusion of rigosertib. Half of the evaluable patients had a marrow/peripheral blood response or stable disease and this was achieved after 2-4 cycles of therapy. Patients with marrow response had a better survival as compared to those without (10.1 months versus 2 months). Patients who had already progressed to AML were less likely to respond. Responders had a higher incidence of unexplained cystitis.

The drug, however, did not show any benefit over the best supportive care in the ONTIME phase III trial. A subset analyses within the study population of the ONTIME trial showed a survival benefit for patients who had received less than 9 months of standard hypomethylating agent therapy without any benefit (not those with secondary failure after an initial response). This is the cohort under study in the INSPIRE trial.

## Vaccine therapy against myelodysplastic syndrome

PR-1 and WT-1 are selectively expressed on surface of CD34 positive malignant myeloid cells. Thus immune mediated attacks against these antigens could stimulate the graft versus leukemia effect seen with transplantation of the myeloid malignancies. WT1 codes for transcription factors which are vital for cell proliferation and survival. Injection of WT1 peptide elicited cytotoxic T lymphocytes directed against WT1 expressing leukemic cells in mice. A study from Japan administered a WT1 peptide (HLA-A\*2402-restricted natural or modified 9-mer WT1 peptide emulsified with MontanideISA51 adjuvant) in patients with AML and MDS<sup>15</sup>. The patients with AML appear to have been in remission after chemotherapy and prior to starting the vaccine. Three of the patients with AML out of the 26 patients on the trial continue to have normal levels of WT1 (normal level is < 50copies/mg RNA) even seven years after start of therapy.

However in another study, the antileukemic activity of these vaccines was transient even when they were administered repeatedly. These responses were often lost even before completing the scheduled number of courses. The antileukemic activity of PR-1 specific peptide vaccines are mediated by high avidity PR-1 specific T cells. Leukemia cells are known to secrete proteinases which selectively cause the destruction of high avidity T cells. This could explain the lack of sustained responses to vaccines directed against PR 1 antigen. The data on vaccine therapy is limited, but going by the premise that this would essentially behave like immunotherapy, its greatest benefit would probably be seen in patients who are already in remission, akin to DLI.

## Telintra/ezatiostat: Role of molecular profiling in directing therapy

Telintra (Ezatiostat HCl, TLK199) inhibits the enzyme, glutathione S-transferase P1-1 (GSTP1-1) leading to JNK activation by phosphorylation which in turn leads to phosphorylation of c-JUN. c-JUN stimulates the proliferation and maturation of hematopoietic progenitors of all hematopoietic cell lineages. In addition the molecule also promotes apoptosis of cancer cells by reactive oxygen species through activation of caspase dependent apoptotic pathway.

A phase 2 study of ezatiostat in patients with lower-risk MDS demonstrated some response in patients with trilineage cytopenias. Hematologic Improvement-Erythroid (HI-E) was reported in 29% cases (11 of 38 patients), HI-Neutrophil (HI-N) in 11 of 26 patients (42%), and HI-Platelet (HI-P) in 12 of 24 patients (50%). Hematological improvement HI-E was also observed in few patients who were red blood transfusion dependent and had never received prior therapy with hypomethylating agents. Three patients in the group achieved complete RBC-transfusion independence, and 3 of 9 (33%) reported multilineage responses. The same group subsequently tested an oral formulation of the same drug which was found as effective. In a subsequent analysis using gene expression profiling, the authors demonstrated that patients who had a lesser expression of genes comprising the jun-N terminal kinase/c-Jun molecular pathway were more likely to respond. Specifically responders were more likely to underexpress miR-129 and over-express miR-155. The miRNAs bind to the 3' untranslated region of mRNAs resulting in mRNA degradation or interference with translation. This study shows how molecular profiling of cancers could direct future therapy.

## Other novel agents under study

Clofarabine is a newer nucleoside analog which inhibits DNA synthesis and disrupts mitochondrial membrane. It also inhibits ribonucleotide reductase resulting in increased uptake of clofarabine into nucleoside depleted cells, a process described as self potentiation. There was a recent study which showed that a lower dose of 15 mg/m<sup>2</sup> clofarabine is as effective as 30 mg/m<sup>2</sup> with lesser toxicity (ORR 36%,

CR 26%), and a median overall survival of 7.4 months (13.4 months for responders). Oral preparations might be better tolerated but the current information is mostly with the intravenous preparation.

Sapacitabine is an orally active deoxycytidine nucleoside analog which causes G2 phase arrest after inducing irreversible single strand DNA breaks. It is still in phase II trials and data were presented in both ASCO 2011 and 2012 as abstract forms where 3 different dose schedules had a similar response (overall response rates of 13% in patients previously managed with first line therapy).

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# 10

## ABBREVIATIONS

MDS :	Myelodysplastic Syndrome	dsDNA :	Double Stranded Deoxy Ribo Nucleic Acid
SEER :	Surveillance, Epidemiology and End Results	FLAER :	Flourescent Aero Lysin
ICMR :	Indian Council of Medical Research	RCC :	Refractory Cytopenia of Childhood
NCD :	Non Communicable Diseases	RPS14 :	Ribosomal Protein Sequence
MPN :	Myelo Proliferative Neoplasm	miR :	Micro RNA
AML :	Acute Myeloid Leukaemia	PRBC :	Packed Red Blood Cell
WHO :	World Health Organisation	NIH :	National Institutes of Health
IPSS-R:	International Prognostic Scoring System - Revised	LEN :	Lenalidomide
FISH :	Flourescent In-Situ Hybridisation	ASH :	American Society of Haematology
WES :	Whole Exome Sequencing	EHA :	European Haematology Association
RNA :	Ribo Nucleic Acid	ASCO :	American Society of Clinical Oncology
NGS :	Next Generation Sequencing	WPSS :	WHO Prognostic Scoring System
MDS-RS :	Myelodysplastic Syndrome – Ring Sideroblasts	TPO :	Thrombopoietin
PAS :	Per-iodic Acid Schiff	HSCT :	Haematopoietic Stem Cell Transplantation
RBC :	Red Blood Cell	EBMT :	European Bone Marrow Transplant Registry
TIBC :	Total Iron Binding Capacity	WT1 :	Wilm's Tumour1
CMV :	Cytomegalovirus	CR :	Complete Response
EBV :	Epstein Barr Virus	GVHD :	Graft versus Host Disease
HCV :	Hepatitis C Virus	TNF :	Tumour Necrosis Factor
HIV :	Human Immuno-Deficiency Virus	IL :	Interleukin
SLE :	Systemic Lupus Erythematosus	INF :	Interferon
RA :	Rheumatoid Arthritis	VEGF :	Vascular Endothelial Growth Factor
ANA :	Anti Nuclear Factor	CGH :	Comparative Genomic Hybridisation





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