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A retrospective study of the clinical profile and outcome of adult patients with Hypoplastic Myelodysplastic syndrome (hMDS) in a tertiary centre in India.

CERTIFICATE

This is to certify that this thesis titled "A retrospective study of the clinical profile and outcome of adult patients with Hypoplastic Myelodysplastic syndrome (hMDS) in a tertiary centre in India**,**" is a bonafide work of the candidate, Dr. Fouzia.N.A, during the period from August 2010 to July 2013 in partial fulfillment, towards the award of degree of Doctorate of Medicine (higher specialty) in Clinical Haematology for the examinations to be conducted by the Dr.M.G.R Medical University in August 2013.

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(In the name of God, Most Gracious; Most Merciful)

CONTENTS

INTRODUCTION:

Myelodysplastic syndromes (MDS) are a very heterogeneous group of clonal hematopoietic stem cell disorders that represents a spectrum of diseases characterized by; ineffective erythropoiesis and marrow failure limited by acute leukemias, chronic leukemias and myeloproliferative disorders at one end of the spectrum, in which hypercellular marrow is typical, to aplastic anemia at the other end (1–3).

Hypoplastic Myelodysplastic syndromes (hMDS) refers to a morphological entity in which the bone marrow cellularity is low for the age (<30% cellularity if age is <60 years or <20% cellularity if age is >60 years) (2). It represents approximately 10-15% of all MDS cases (2,4–9). Hypoplastic MDS however, does not represent a defined MDS category according to the WHO classification, but it rather denotes the morphologic status of other MDS categories (2). It is difficult to distinguish hMDS from acquired aplastic anemia (AA), because of considerable clinical, histologic, and cytologic similarities between the two disorders (10).

Patients with hMDS tend to be younger, have more profound thrombocytopenia and neutropenia, lower percentage of blasts, lower probability to evolve to leukemia, and they are less likely to display abnormal karyotype, compared to patients with normocellular or hypercellular MDS (1,2,6,11). Compared to AA, hMDS have a poorer prognosis and have frequent karyotypic and FISH abnormalities and are prone to conversion to acute myeloid leukemia (12). The prognosis for hMDS falls between that of severe and very severe AA patients (12).

The pathophysiology of hMDS is not very well known. Evidence suggests that immune mediated mechanisms may play a role (5). This subtype is most likely to respond to treatment with immunosuppressive agents (13).

Other than a few case reports and small case series, there are no published data on the clinical profile and response to treatment in patients with hypoplastic MDS from India.

REVIEW OF LITERATURE

DEFINITION:

MYELODYSPLASTIC SYNDROMES: The myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell diseases characterized by cytopenia(s), dysplasia in one or more of the myeloid cell lines, ineffective haematopoiesis, and increased risk of development of acute myeloid leukemia (AML) (14). The enhanced degree of apoptosis in these disorders contribute to the cytopenia(s) (14). The thresholds for cytopenias recommended in the IPSS (International Prognostic Scoring System) for risk stratification in MDSs are as follows (14);

- Hemoglobin < 10g%

- Absolute neutrophil count < 1800/mm^3, and

- Platelets <100,000/mm^3.

If definite morphologic and / or cytogenetic findings are present, values above the thresholds are not exclusionary for a diagnosis of MDS (14). There may be increase in myeloblasts in the peripheral blood or bone marrow accompanying the dysplasia, but the number is <20% (7,14).

HYPOPLASTIC MYELODYSPLASTIC SYNDROMES: Usually, patients with MDS have a hypercellular bone marrow (5,14) . In a minority of the cases (~10 - 15% of cases), the cytopenia is associated with a hypoplastic bone marrow (2,4–9,14). Hypoplastic Myelodysplastic syndromes (hMDS) refers to a morphological enitity in which the bone marrow cellularity is low for the age (<30% cellularity if age is <60 years or <20% cellularity if age is >60 years) (2,5,14). In these cases distinguishing between hMDS and aplastic anemia may be difficult; the presence of a hypocellular marrow with features of dysplasia in one or more cell lines, increase in reticulin content on bone marrow trephine, increase in number of blasts/CD 34+ cells on bone marrow

trephine, or abnormal karyotype showing malignant clonal cells, all favor the diagnosis of hMDS (2,3,5,6,9,14–16).

EPIDEMIOLOGY:

According to the WHO classification, hMDS does not represent a defined MDS category, but rather denotes the morphologic status of other MDS categories. Patients with hMDS tend to be younger (2) than those with normo/hypercellular MDS. The median age of patients with hMDS have been reported between 39 and 58 years (1,12), where as in normo/hypercellular MDS the age ranges from 60 and 75 years (1,5,9,14). Maschek H et al reported a higher median age (72.6 years; range;33-88 years) in patients with hMDS (9). The non-age adjusted annual incidence of MDS (including hMDS) reported is 3-5/100,000 persons, rising to over 15-50 /100,000 in those over 70 years of age (5,9,14) in the US population. The reports suggest that the sex distribution is balanced, although there are studies reporting a male preponderance in the hMDS group (1,5,9,14). There is no published data on the exact prevalence or incidence of hMDS among Indian population. In a study of 30 cases of MDS (April 1998 to May 2006) reported from India by Shah NM et al, the mean age at presentation was 55 years (range 8-73 years) with a male preponderance (1,17). A recent initiative is the 'Indian MDS registry' aimed at analyzing the different aspects (epidemiology, clinico-pathology, diagnosis, therapeutic protocols and outcome) of MDSs among the Indian population.

ETIOLOGY AND PATHOGENESIS:

The exact pathophysiology of hMDS is not known; there are multiple, complex and poorly understood mechanisms involving abnormalities in the regulation of cellular proliferation, maturation, and survival (2,5,18). Association of hMDS with increasing age suggests a genetic damage caused by hazardous exposure or inherited susceptibility (14,18).

In hypoplastic MDS, marrow failure results not only from ineffective erythropoiesis of abnormal clones, but also due to the inhibition of normal progenitors (2). There is increasing experimental and clinical indication of an immune-mediated damage to the hematopoietic precursors and changes in the hematopoiesis-supporting microenvironment contributing to the development of the disease (2). Immunosuppressive therapy with anti-thymocyte globulin, cyclosporine, or alemtuzumab may alleviate cytopenias and induce cytogenetic remission in some instances (2). However, all patients do not respond to immunosuppression. Identification of relevant biomarkers for an immune mechanism may help in identifying those patients who may benefit from immunosuppressive therapy (5,18).

IMMUNOLOGICAL CHANGES IN hMDS (2)

As observed in aplastic anaemia, abnormalities indicative of an active immune process mediated by a Th1-cell response are observed in MDS, including hMDS. These include;

1. **Abnormal Cytokine Profile** (2)**:**

    a. High levels of tumor necrosis factor-α (TNF-α).

    b. Over-expression of TNF-related apoptosis-inducing ligand (TRAIL) - preferentially targets abnormal clonal cells with aberrant chromosomes, inducing apoptosis.

    c. Lower levels of FLIP, a cytoplasmic inhibitor of apoptosis; this explains the higher sensitivity to TRAIL in cytogenetically abnormal clones.

    d. Over-expression of Interferon-γ (INF-γ) by bone marrow mononuclear cells in MDS.

Both IFN-γ and TNF-α activate the expression of iNOS (induced nitric oxide synthase), which potentially mediate the dysregulation of haematopoiesis in MDS through Fas-mediated apoptosis. The cytokine expression profile may also have prognostic significance. In a recent study,

interleukin-4 (IL-4) and C-C motif chemokine 3 (CCL3) serum levels were consistently under-expressed in MDS and independently associated with survival (2) .

2. **T-Cell Mediated Attack** (2)**:**

There are strong laboratory evidence suggesting that the marrow failure is the result of an antigen-driven lymphocyte destruction of the haemopoietic tissue. An expansion of cytotoxic T cells expressing defined T-cell-receptor (TCR) Vβ chain, indicating the oligoclonality of the T-cell repertoire is observed in these patients as well as in MDS. These skewed T-cell populations are observed to reduce or disappear after response to immunosuppressive therapy. Conversely, the dominant T-cell clone persists after treatment in those who fail treatment. The antigens triggering the immune response in MDS are not known. Patients with trisomy 8 often respond to immunosuppression, indicating a strong immunological mechanism for the underlying marrow failure. In patients with trisomy 8, the CD8+ T cells are able to recognize WT1 peptides and engage INFγ expression in vitro, suggesting that this antigen may contribute to elicit an immune response. Whether WT1 antigenicity may be used therapeutically is still unknown (2).

3. **Genetic factors**:

As observed in several auto-immune disorders, HLA-DR15 antigen is overrepresented in patients with acquired AA, MDS with refractory anaemia, and in patients with MDS bearing a PNH clone These observations taken together further suggest that some MDS cases are immune-mediated (2).

Aplastic anaemia and hMDS also may share genetic defects. Gene mutations encoding the telomerase complex (responsible for maintaining the length of telomeres), resulting in excessive telomere shortening in hematopoietic progenitors, are found in some cases of apparently acquired aplastic anaemia (2,9). The observation that a small subset of patients with hMDS respond to androgenic steroids, support this theory. These patients also often evolve to hMDS and acute

myeloid leukemia. Acquired aplastic anaemia patients with shorter telomeres (lowest quartile for telomere length) are those with higher risk to evolve to MDS, especially monosomy 7 (2).

CLINICAL FEATURES:

Majority of patients (i.e.50%), are asymptomatic at the time of initial diagnosis (5,14), with the median age of presentation in the fourth to sixth decade (1,17). These patients present with signs and symptoms secondary to the cytopenia(s), while some are diagnosed on a routine blood count. The dominant findings in hMDS include anemia, thrombocytopenia, and leukopenia, either alone or in any combination, resulting from progressive hematopoietic failure (5,14). At the time of diagnosis, anemia is an almost universal characteristic finding, with > 80% of patients presenting with a hemoglobin level <10 g/dl and a reduced reticulocyte count (5,14). In 25-30% of patients, the blood leukocyte count is low, and the granulocytes may exhibit features of dysplasia (5,14). One third of the individuals have recurrent infections, due to granulocytopenia as well as the result of defects of neutrophil function (i.e. impaired chemotaxis & reduced phagocytic activity) (5,14). Thrombocytopenia and the concomitant platelet function defect results in bleeding manifestations which may include mainly petechiae, gum bleeding, or hematoma following trivial injuries; with <10% of patients presenting with serious bleeding (i.e. gastrointestinal bleed, macro hematuria, menorrhagia, or retinal or central nervous system hemorrhage) (5,14).

DIAGNOSTIC WORK-UP

Patients with hMDS have more profound thrombocytopenia and neutropenia, lower blast percentage, and one less likely to display an abnormal karyotype in comparison to patients with normo / hypercellular MDS. Diagnosis of low grade MDS is not always straight forward despite the

well-established diagnostic criteria and ever-expanding battery of molecular and cytogenetic diagnostic assays. The reasons for such difficulty are multiple, i.e. inter-observer disparity in documenting extent of dysplasia, (especially when dysplasia is mild to moderate) and the frequent lack of detectable chromosomal abnormalities (19). The most difficult part in the diagnosis of hMDS is differentiating it from acquired aplastic anemia.

The diagnostic work-up of hMDS includes morphologic evaluation of peripheral blood, bone marrow aspirate, and bone marrow biopsy specimens, interpreted in the context of an adequate clinical information and CBC results (18). Correlation with marrow cytogenetics is essential. A normal karyotype however does not exclude a diagnosis of an MDS (18). Recently multiparameter flow cytometry has proven to be an important diagnostic tool, especially when added to cytomorphology (CM) and cytogenetics (CG) in patients with suspected MDS (19,20).

Blood and bone marrow morphology and immunohistochemistry:

Although MDS can be suspected from the clinical history and the peripheral blood counts, the diagnosis is often made by morphologic inspection of the peripheral blood, bone marrow aspirate, and bone marrow biopsy specimen (18).

The current World Health Organization (WHO; 2008) system approach is a more comprehensive one, which stresses the importance of integrating other techniques; ie bone marrow biopsy histologic examination, molecular genetics, and cytogenetics in the light of relevant clinical information (18). Morphologic dysplasia is not necessarily synonymous with an MDS. To address the issue of "false-positive" myelodysplasia, the current WHO classification system recommends that, to declare a dysplasia of a particular lineage, at least 10% of cells in the lineage has to be morphologically dysplastic (14,18). Specific criteria for dysplasia in the three different cell lineages

are detailed in the table in Appendix 1 (*Table: 1 in Appendix 1*) below (14,21). The presence of dysgranulopoiesis and dysmegakaryopoiesis favor the diagnosis of hMDS over AA.

Blasts:

The percentage of blasts in the bone marrow is important for the diagnosis, classification and prognostication of MDS. It is also an integral component of the currently used prognostic scoring systems; ie International Prognostic Scoring System (IPSS), WHO classification–based prognostic scoring system and the more recent revised International Prognostic Scoring System (IPSS-R) (18). Immature cells to be included in the blast count include myeloblasts (with and without a few fine azurophilic granules), megakaryoblasts and monoblasts, promonocytes are considered as "blast equivalents" in the WHO classification scheme (14,18). It may be hard to appreciate blast in marrow biopsy specimen, particularly if there is marrow fibrosis (14,18). Immunohistochemical (IHC) stain for CD34 antigen may be very helpful in such a situation (14,18). Additional markers used to facilitate visibility of CD34– blasts include; CD117, lysozyme, and CD68 (14,18). The blasts seen in MDS, are often myeloperoxidase negative or only weak positive (18).

Flow cytometry may help in confirming the immunophenotype and assessing the frequency of blasts. In addition, side scatter abnormalities (due to granulocyte hypogranularity) and aberrant antigen expression have also been shown to correlate with the severity of the MDS (18). Aplastic anemia can be distinguished from hMDS by the presence of a decreased number of CD34+ cells and reduced expression of proliferating cell nuclear antigen (PCNA) in bone marrow (10). The presence of increased reticulin content on silver staining on trephine biopsy favors the diagnosis of hMDS over AA.

CLASSIFICATION OF MYELODYSPLASTIC SYNDROMES:

To classify MDSs, a number of morphological classifications are in place; the most recent one being the WHO classification (2008 Revision, 4th ed) (22). The current WHO classification of MDS is principally based on the blast percentage in the peripheral blood and bone marrow, and the type and degree of dysplasia (14,18). In particular, the extent of dysplasia, multilineage vs unilineage, and the presence of ring sideroblasts (assessed by iron staining) have important roles in the WHO sub-classification (14,18). The absence of monocytosis (monocytes <1,000/µL in the blood and <5% in bone marrow) is important to distinguish between CMML (chronic myelomonocytic leukemia), and MDS (18).

The WHO Classification of Myelodysplastic Syndromes (table below), recently published, distinguishes the following MDS subtypes (14,18):

(1) Refractory cytopenia with unilineage dysplasia (RCUD); subcategories- Refractory anemia (RA), Refractory neutropenia (RN), & Refractory thrombocytopenia (RT);

(2) Refractory anemia with ring sideroblasts (RARS);

(3) Refractory cytopenia with multilineage dysplasia (RCMD);

(4) Refractory anemia with excess blasts (RAEB), with subcategories RAEB-1 & 2.

(5) MDS, unclassifiable (MDS-U); and

(6) MDS with isolated del (5q) chromosomal abnormality.

The WHO 2008 classification of MDS is detailed in Appendix 1 (*Table:2 in Appendix 1*).

Hypoplastic MDS: In about 10-15 % of patients with myelodysplastic syndromes, the bone marrow is hypocellular, referred to as 'hypoplastic MDS' (5,14,18). This group do not have independent prognostic significance per se (14,18). The major problem is in the differential diagnosis with aplastic anemia. Toxic myelopathies and auto-immune disorders should be excluded when a diagnosis of hypoplastic MDS is considered (5,14,18). According to the WHO classification, Hypoplastic MDS however, does not represent a defined MDS category, but it rather denotes the morphologic status of other MDS categories (2,14,18).

MDS with fibrosis: In a small subset of patients (~10%) with MDS, significant degrees of myelofibrosis are observed (14). These are referred to as MDS with fibrosis. Most of these cases have excess blasts and an aggressive clinical course (14). In the fibrotic group, due to inadequate aspirate, most often blast determination requires immunohistochemical studies (for CD34 on the trephine biopsy) (14).

Multiparameter Flow Cytometry (MFC) in MDS: The current World Health Organization classification of MDSs is based on morphological evaluation of bone marrow dysplasia. The reproducibility of the recognition of dysplasia is poor in clinical practice, especially in cases where specific markers such as ring sideroblasts and clonal cytogenetic abnormalities are lacking (23,24). In patients with MDS, a recent complementary DNA microarray analyses on CD34+ hematopoietic progenitor cells have found that MDS, including the early-stage/low-grade MDS, is characterized by a B-cell progenitor defect (19). Many genes involved in B-lymphocyte development are down-regulated (19). This observation was validated by the flow cytometric finding that in a small number of MDS patients, the maturing B-lineage precursors (hematogones) are reduced (19,23).

Recently a multiparameter flow cytometric scoring system has been validated for the diagnosis of myelodysplastic syndrome. This encompass four reproducible parameters i.e. CD34+ myeloblast-related and B-progenitor- related cluster size (defined by CD45 expression and side scatter characteristics on CD34+marrow cells), myeloblast CD45 expression and granulocyte side scatter value (23). A flow cytometric score may help to establish the diagnosis of myelodysplastic syndrome, especially when morphology and cytogenetics are indeterminate (i.e. early-stage/low-grade MDS). The calculations of flow cytometric score for the diagnosis of low-risk MDS is detailed in Appendix 1 (*Table: 3 in Appendix 1*).

Cytogenetic and molecular studies: Molecular and cytogenetic studies have a major role in the evaluation of patients with myelodysplastic syndrome with regard to prognosis, determination of clonality and recognition of morphologic, cytogenetic and clinical correlates (5,14). Clonal cytogenetic abnormalities are usually observed in ~ 50% of cases of MDS (and upto 80% of cases with mutagen-related MDS) (5,14). A study by Vundinti BR et al (25) reported 54.48% chromosome abnormalities including novel chromosome aberrations in patients with and that these chromosome aberrations increased with advancing age. Cytogenetic changes observed in MDS are not unique to the disease; both numerical and structural cytogenetic changes may occur. Most frequent chromosomal abnormalities observed involve, deletions of chromosomes 5, 7, 11, 12, and 20 and/or trisomy 8 (5,14). The chromosomal aberrations and its frequencies are described in detail in table in Appendix 1 (*Table:4 in Appendix 1*)

Detection of certain chromosomal abnormalities, either by routine cytogenetic analysis or FISH, aids in the classification of MDS and determination of the prognostic risk group (5,14). With occasional exceptions (i.e. 5q- syndrome/MDS with isolated del(5q)), chromosomal abnormalities in MDS have not correlated with specific clinical or morphological subsets using the WHO

classification system (5,14). Deletion of the long arm of chromosome 5 (5q) is the most common

chromosomal abnormality seen in MDS (in 15% of cases). Cytogenetic and molecular analysis has

led to the identification of two small commonly deleted regions; i.e. del (5q33.1) which is most

commonly associated with the 5q minus syndrome with a relatively good prognosis, and del (5q31),

which is more commonly seen with therapy-related MDS and is associated with more aggressive

disease. MDS with 5q minus syndrome occur primarily in women, and is characterized by

refractory macrocytic anemia, normal or increased platelet count, megakaryocytes with non-lobated

or hypolobated nuclei, a favorable clinical course & good response to lenalidomide treatment (14).

Cytogenetics as a predictor of prognosis: Cytogenetic abnormalities have an impact on outcomes

in patients with MDS. The abnormalities are risk categorized into 3 different cytogenetic categories

in the international prognostic scoring system (IPSS) and WHO prognostic scoring system, and

into 5 groups in the recent revised IPSS (IPSS-R) (26) . The risk categories, their risk for

progression to AML and median survival for the different groups are as follows;

Cytogenetic subgroups in the IPSS & WPSS for adults with MDS.

Prognostic subgroups	Cytogenetic abnormality	25 % AML progression	Median survival
Good risk	Normal karyotype, isolated del(5q), del(20q), or –Y	5.6 years	3.8 years
Intermediate risk	Other abnormalities	1.6 years	2.4 years
Poor risk	-7/del(7q), or complex karyotypes(≥3 abn)	0.9 years	0.8 years

The 5 cytogenetic risk categories and their median OS in the revised IPSS (IPSS-R) are as follows;

Cytogenetic subgroups in the IPSS-R for adults with MDS

Prognostic Subgroups	Cytogenetic abnormality	Median OS

Very Good	del(11q),-Y	60.8 months
Good	Normal, del(20q), del(5q) alone and double, del(12p)	48.5 months
Intermediate	+8, 7q-, i(17q), +19, +21, any other single or double, independent clones	25.0 months
Poor	der(3)q21/q26-7, double including 7q-, complex (3 abnormalities	15.0 months
Very poor	Complex (>3 abnormalities)	5.7 months

GENE MUTATIONS:

Abnormalities in certain genes have been identified in patients with MDS and acute myeloid leukemia with or without the presence of chromosomal abnormalities. These include mutations in **TET2**, **ASXL1, TP53** tumor suppressor gene, **RUNX1** transcriptional core-binding factor gene(CBF), **IDH** gene, **FLT3** gene and **SF3B1** genes. These gene mutations also confer prognostic significance in adult patients with MDS, for instance, it has been reported that patients with TET2 mutations may have higher response rates to azacitidine than those without the mutations (26).

PROGNOSIS & RISK STRATIFICATION:

Myelodysplastic syndromes, in view of the progressive impairment in the ability of the myelodysplastic stem cells to differentiate, are clinically characterized by an increased risk of evolution into acute myeloid leukemia (AML) (27). The natural history of MDS ranges from indolent conditions spanning years to rapid progression to leukemia. The probability of leukemic evolution is lower in hMDS (1,6) than the normo-/hypercellular MDS (NH-MDS) (1,2,6), but compared to AA, hMDS have poorer prognosis with frequent karyotypic and FISH abnormalities and a higher probability of leukemic evolution.

As the prognosis of patients with MDS is very heterogeneous, development of a prognostic system that allow risk stratification and help in the timing and choice of therapy is essential (26). A number of prognostic scores are currently in use, and these include;

a. **IPSS (International Prognostics Scoring System):** This is the most commonly used score and has been in use since 1997 (26). Prognostic score includes, the number of cytopenias, percentage of blasts, and the type of cytogenetic abnormality (26). Based on this scoring system there are 4 prognostic risk categories; i.e. 'Low' (score:0), 'Intermediate-1' (score :0.5-1), 'Intermediate-2' (score:1.5-2) and 'High risk' (score:≥2.5). IPSS is highly reproducible and very simple to use, but has several limitations; i.e. it is not a very precise predictor of prognosis in those with lower risk disease and it attributes relatively little weight to cytogenetics (26). The IPSS scores, prognostic risk categories and their clinical outcomes (in terms of survival and risk of transformation to AML) are detailed in Appendix 1 (*Table:5 in Appendix 1*).

b. **Revised international score (IPSS-R) :** This score presented at the 2011 MDS Meeting in Edinburgh, incorporates a new cytogenetic score and includes different cut off for cytopenias (26). Prognostic score includes, the type of cytogenetic abnormality, percentage of blasts, hemoglobin level, platelet count and ANC (absolute neutrophil count) (26). Based on this systems, there are 5 prognostic risk categories i.e. 'Very low' (score:≤1.5), 'Low' (score:.1.5-3), 'Intermediate' (score:>3-4.5), 'High' (score: >4.5-6) and 'Very high' (score:>6) risk groups. The IPSS-R scores, prognostic risk categories along with their clinical outcomes (i.e. survival and risk of AML transformation) are outlined in Appendix 1 (*Table: 6 in Appendix 1*).

c. **WPSS (WHO prognostic scoring system):** This is another commonly used system that incorporates the transfusion dependency in addition to cytogenetics and WHO diagnostic

category (26). The main limitations of this system is that it requires, prior information of transfusion needs and WHO classification (26). A recent modification of the WPSS score included hemoglobin levels instead of transfusion needs(26). Prognostic score includes, the WHO MDS category, the type of cytogenetic abnormality and the transfusion requirement (26). Based on this system patients are categorized into 5 prognostic risk groups; i.e. 'Very low' (score=0), 'Low' (score=1), 'Intermediate' (score=2), 'High' (score=3-4) and 'Very high' (score=5-6). WHO prognostic scoring system with its clinical outcomes is detailed in Appendix 1 (*Table:7 in Appendix 1*).

d. **Global MDACC (MD Anderson Cancer Center) model:** A more recent one is the global MDACC model that allows evaluation of all patients considered to have MDS at any time during the course of their disease without needed WHO evaluation(26). The Global MDACC and MDACC MDS lower risk Prognostic Models are tabulated in Appendix 1 (*Tables:8a & 8b in Appendix 1*).

e. Recently, based on the study on a cohort of 253 patients with hypocellular MDS (diagnosed at The University of Texas MD Anderson Cancer Center between 1993 and 2007) and a cohort of 1725 patients with hyper-/normocellular MDS (diagnosed during the same time period), a new prognostic model was built that segregated patients into 3 distinct risk categories independent of International Prognostic Scoring System (IPSS) score (26). This model is independent from the IPSS, and further refines IPSS-based prognostication. It may be used to develop of risk-adapted therapeutic approaches for patients with hypocellular MDS (26,28). The details of the prognostic model of hypoplastic MDS is tabulated in Appendix 1 (*Table: 9 in Appendix 1*)

HYPOPLASTIC MDS AND APLASTIC ANAEMIA: Because of considerable clinical, histologic and cytologic similarities between these two disorders, it is sometimes difficult to distinguish Hypoplastic myelodysplasia from acquired aplastic anemia (10). The presence of dysmegakaryopoiesis, dysgranulopoiesis, increased percentage of blasts, increased bone marrow reticulin content and abnormal karyotype, favour the diagnosis of hMDS. In addition, an abnormal antigen expression pattern in marrow CD34+ cells indicating an aberrant clone, and the presence of elevated haemoglobin F-containing erythroblast production suggest the diagnosis of hMDS. However, findings compatible with an immune process (oligoclonal T-cell expansion, relative lymphocytosis in the marrow and increased cytokine levels) do not contribute to differential diagnosis, as these elements are present in both (2).

The distinction between hMDS and AA is of great prognostic and therapeutic importance. With modern therapies; ie bone marrow transplantation and immunosuppressive therapy, severe AA patients have long term survival (80% at 14 years), and those with moderate AA have a median survival of >174 months with androgen and supportive therapy; while for patients with hMDS, the median survival is not significantly different from hypercellular MDS (22 to 33 months). Some cases of hMDS may show a transient response to androgens and/or immunosuppressive therapy thus adding further diagnostic difficulty. Further, there is a higher risk of progression to acute leukemia in patients with hMDS compared with AA (29). Recent studies have suggested that in AA, bone marrow (BM) is characterized by a decreased number of CD34+ cells and reduced expression of proliferating cell nuclear antigen (PCNA), which is not a feature associated with MDS (10). A role for tumor necrosis factor-alpha (TNF-alpha) in the development of AA has been suggested by recent studies(29). Careful examination of peripheral blood, may also provide sufficient information to allow for the distinction between hMDS and AA early in the course of the disease (30). Certain morphologic findings i.e. hypochromic red cells, circulating blasts, left shift,

hypersegmentation with long filaments, Dohle bodies hypogranular, ring, and pelgeroid neutrophils, circulating micromegakaryocytes and megakaryocytic fragments are seen only in hMDS but not in AA (30). The table below summarizes the major differences between hMDS and AA. (3,6,15,16,31,32).

Distinction between hypoplastic MDS and Aplastic anemia

Characteristics	Hypoplastic MDS	AA
Dyserythropoiesis	Yes	Sometimes
Abnormal neutrophil	Yes	No
Dysplastic megakaryocytes	Yes	No
Fibrosis	Occasional	No
Increased blasts	Sometimes (ALIPS)	No
CD34+ cells in BM	Sometimes increased	< 1.0%
Clonality	Sometimes	Possible
Splenomegaly	Occasional	Absent

It is fortunate that the distinction is not critical since both aplastic anaemia and hypoplastic MDS respond to similar forms of treatment. Indeed there is close similarity between the two conditions and they are sometimes called 'overlap syndromes'.

DIFFERENTIAL DIAGNOSES:

Acquired aplastic anemia is the most important and difficult differential diagnosis of hMDS (discussed above). Other conditions that can present with cytopenia(s) and dysplastic changes include; Vitamin B_{12} & folic acid deficiencies, Copper deficiency & arsenic poisoning, medications & drugs, liver failure or hypothyroidism causing macrocytic anemia with a low reticulocyte count,

auto-immune and other hematopoietic neoplasms, e.g. lymphomas, and non-hematopoietic malignancies causing para-neoplastic myelodysplasia, Viral infections (e.g. HIV infection,, chronic parvovirus, Epstein-Barr virus, and cytomegalovirus infections) and rarely, hemophagocytosis can produce marrow changes that resemble MDS (18).

TREATMENT OF hMDS:

The treatment of hMDS is similar to aplastic anemia in view of similarity in its pathophysiology. The options include;

A. **Options for newly diagnosed patients:** In newly diagnosed lower risk group patients, therapy is based on the transfusion needs of the patients (26). Transfusion independent patients are usually observed until they become transfusion dependent (26). A new and important concept in the treatment of lower risk MDS is early intervention in patients with ''poor prognosis'' lower risk MDS (26). To improve on the natural history of the disease, the identification of these patients is going to be fundamental (26). The upcoming new prognostic scoring systems and development of new molecular informations for these patients will help in early identification and intervention (26). The list of agents currently available for treatment of patients with hMDS is as follows;

1. **Immune therapy:** Hypoplastic MDS is characterized by dysregulation of immunity, and it has been observed that patients with hMDS benefit from immunosuppressive therapy (26). The agents studied include cyclosporine-A (CSA), corticosteroids and antithymocyte globulin (ATG) (26,33). Standard IST is the combination of ATG & CSA (horse or rabbit ATG) with a short course steroid. The dose of ATG used is 40 mg/kg over 4 hours, daily for 4 days after premedication along with prednisolone (1 mg/kg from day 1 for 2 weeks) for serum sickness prophylaxis, and CSA (dose: 10 mg/kg/day from day 1 to target trough

level- 200 and 400 ng/ml. Cyclosporine monotherapy (6mg/Kg) is an easily available, and is a safe and cheap IST. The group at the NHLBI (National Heart, Lung and Blood Institute) has developed an algorithm to predict response to these agents, i.e. younger age, shorter duration of transfusion dependency and HLA-DR15 (34). Bone marrow hypocellularity is the most important predictor for response (35). Recently alemtuzumab (humanized monoclonal antibody that specifically kills CD52-bearing cells via both antibody-dependent cellular cytotoxicity & complement-mediated lysis; dose is 10 mg Alemtuzumab s/c daily x 5 days along with CSA (2 mg/kg Q12H) x 3 months, has also been reported to have significant activity in those patients with MDS predicted to respond to immune suppressive therapy (26,34). The most important predictor for response has been the presence of marrow hypocellularity (26,34). In younger patients with severe hypoplastic MDS, allogeneic stem cell transplantation (Allo SCT) should be considered as soon as possible. For those that are not candidates, a combination with equine ATG is recommended.

Response to immune therapy (IST) reported in literature is a haematologic recovery of 75- 90% after one or two courses of IST (36). A response rate of 68% is reported for ATG/CSA in AA, while following alemtuzumab therapy upto 57% response has been reported by some studies (26,33,36,37).

2. **<u>Allogeneic Peripheral blood stem cell transplant:</u>** Allogeneic hematopoietic stem cell transplantation is the treatment of choice in young patients with severe aplastic anemia or hypoplastic MDS. The main causes of failure after this procedure are graft versus host disease, infections and graft failure, often exacerbated by large numbers of transfusions and prolonged disease duration before transplant (38). A less toxic regimen comprising reduced cyclophosphamide (Cy), fludarabine, and anti-thymocyte globulin (ATG) (Cy-Flu-ATG)

was used to condition high-risk patients scheduled for allogeneic hematopoietic cell transplantation (allo HSCT) instead of standard Cy-ATG in patients with severe aplastic anemia (AA) and hMDS (39). Preconditioning with Cy-Flu-ATG was superior to that afforded by Cy-ATG in terms of reducing RRT levels without increasing engraftment failure (39). Recent study by Szczylik C. et al showed that transplantation of hematopoietic stem cell using alemtuzumab, fludarabine and melphalan as a conditioning therapy is safe, inexpensive and effective treatment for patients with severe aplastic anemia, including multi-transfused adults having their disease for a long time (38). Five year OS following matched related and unrelated Allogeneic PBSCT reported are 73% and 60% respectively (36,39).

3. **Androgenic steroids:** Androgenic steroids i.e. Danazol (derivative of synthetic steroid ethisterone-17 α ethinyl testosterone; 300mg daily), Oxymetholone, and Stanazolol (synthetic anabolic steroid derived from testosterone; 1mg/Kg/day) have been proved to be of benefit in hMDS as seen in Aplastic anemia (40). Androgens are enzymatically converted into estradiol (E2) via aromatase. E2 passively diffuses into cells and binds the α isoform of the estrogen receptor (ERα), which acts as a transcriptional activator by binding to estrogen response elements (ERE) in genomic DNA. The telomerase reverse transcriptase (TERT) promoter contains 2 putative EREs. Therefore, both androgens and estrogens increase TERT expression, ultimately resulting in increased telomerase activity in hematopoietic cells. Androgens also inhibit both interleukin-1 and TNF-a production. In patients unresponsive to IST, a response rate of 30-35% has been reported to androgens in some studies (41).

4. **Haematopoietic growth factor support:** Currently a number of erythroid stimulating agents (ESA) are available. Reported rates of response to these agents range from 30 to 60%

(26,42). In a retrospective observational study by Jadersten M et al (26,43), it was observed that addition of G-CSF to erythropoietin increased the response rates, and early introduction of this combination in patients with minimally transfusion-dependent and low risk disease may have an impact on survival. In patients with significant anemia and with no other cytopenias, a course of ESA with or without G-CSF is not contraindicated (26). Early incorporation of these agents has been found to be more effective than in patients with heavy transfusion burdens. Due to complications related to disease transformation and marrow fibrosis, the use of Romiplostin in lower risk MDS is questionable (26).

5. **Supportive care measures:** The supportive care measures in hMDS include; use of prophylactic antibiotics and iron chelation. No randomized data exists to make formal recommendation for any of these interventions (26).

B. **Options for patients with higher risk MDS:**

Treatment options for patients with higher risk MDS have significantly evolved over the last decade. Earlier most patients were treated with cytarabine based therapy as for AML. Recently the use of azanucleosides (Decitabine & 5-Azacytidine) has modified this practice (44,45). Two candidate biomarkers and a clinical model have been proposed recently. mutations on TET2 and levels of miR29b and have been reported to be associated with response to azacitidine and decitabine respectively (26,46).

AML-like chemotherapy: In higher risk MDS, AML-like protocols have generally used classical anthracycline-araC combinations, similar to that used in de novo AML. AML-like therapy results in lower CR rates (40–60%), and shorter CR duration (10–12 months) when used in MDS or AML post-MDS. They tend to be associated with more prolonged periods of aplasia, and in addition, due to the advanced median age of the patients, the feasibility of AML-like therapy is also reduced (26).

Allogeneic Stem cell transplant: Allogeneic SCT is the only curative treatment of higher-risk MDS. Selected studies report prolonged DFS in about 30% to 50% of the patients. However its use is restricted mainly to younger patients with an appropriate donor (26,47).

RESPONSE CRITERIA:

Definition of IWG response criteria in MDS: The IWG (International working group) criteria define 4 aspects of responses based on treatment goals: (1) altering the natural history of the disease, (2) cytogenetic response, (3) hematologic improvement (HI), and (4) QOL (Quality of life) (48). The responses assessed include CR (complete remission), PR (Partial remission), Stable disease, Failure, Relapse after CR or PR, Cytogenetic response and disease progression. The details of the proposed modified International working group response criteria are described in Appendix 1 (*Tables:10a & 10b in Appendix 1*).

SUMMARY:

Hypoplastic MDS is a distinct clinic-pathologic entity characterized by bone marrow hypoplasia, severe leucopenia and thrombocytopenia, macrocytosis, low incidence of progression to acute leukemia, and unresponsiveness to conventional therapy (6,8). It represents approximately 10 - 15% of all MDS cases (2,4–9,14). It is difficult to distinguish hMDS from acquired aplastic anemia (AA), because of considerable clinical, histologic, and cytologic similarities between the two disorders (1,6,10,14). However, compared to AA, hMDS have poorer prognosis and frequent karyotypic and FISH abnormalities (prone to leukemic conversion) (12). The Pathophysiology of hMDS is not very well known; auto-reactive and clonal-involved T-cells are believed to suppress the normal hematopoietic cells by secretion of inhibitory cytokines (5). This subtype is most likely to respond to treatment with immunosuppressive agents; therapy with antithymocyte globulin (ATG), cyclosporine (CSA) or both has been shown good response in patients with hMDS (13).

Aims and objectives:

1. To analyze the clinical profile of adult patients with Hypoplastic Myelodysplastic syndrome (hMDS).

2. To assess the response to different drug therapies in patients with hMDS.

3. To identify the demographic, clinical, and laboratory parameters that can predict prognosis in hMDS.

Patients and Methods

This study protocol was approved by our Institutional Review Board (IRB). This is a retrospective analysis of patients diagnosed to have hMDS from January 1998 to June 2012.

Duration of the study: October 2012 to December 2012.

Settings of the study: Department of Clinical Haematology.

Diagnostic criteria: Hypoplastic MDS was diagnosed in patients presenting with cytopenia(s) (defined as per the recommendation in the IPSS for risk stratification in MDSs (i.e. Hemoglobin <10g%, Absolute neutrophil count <1800/mm^3, and Platelets <100,000/mm^3) associated with a hypoplastic bone marrow for the age (2,5,14), and with features of dysplasia in one or more cell lines, with or without increase in number of blasts/CD 34+ cells on bone marrow, or increase in reticulin content on bone marrow trephine, or abnormal karyotype showing malignant clonal cells (all favoring diagnosis of hMDS) (2,3,5,6,9,14–16).

Patients:

    Inclusion Criteria:

    1. All adult patients (age≥18yrs) diagnosed to have hypoplastic myelodysplastic syndrome from January 1998 to June 2012

    Exclusion Criteria:

    1. Patients with other types of Myelodysplastic syndromes.

    2. Patients with hMDS whose data are not retrievable.

    3. Patients on drugs that can cause dysplasia (e.g. post renal transplant patients)

4. Patients with hypoplastic cytopenia(s) and positive test for PNH, or positive stress cytogenetic test (clastogenic stress-induced chromosomal breakage).

Methods:

Collection of data: After approval by the IRB, the patient data base at our institution was reviewed to identify all patients diagnosed to have hypoplastic MDS at our institute between January 1998 to June 2012. Medical information regarding the clinical/laboratory details at diagnosis, post treatment response and adverse events were obtained from the hospital records (laboratory reports/ physician documentation in hospital charts/hospital discharge summaries). Attempts were made to contact all patients by post or e-mail to collect details on any missing data as well as the recent clinical status. Only patients who had at least 8 weeks follow up (including those who died within 8 weeks) after initiating therapy were categorized as 'evaluable' for assessment of response and survival.

Treatment: Various treatment modalities that the patients received were reviewed. This included; Cyclosporine, anti-thymocyte globulin + CSA, androgenic steroids, corticosteroids, supportive measures, and allogeneic peripheral blood stem cell transplant, and a few had received haematopoietic growth factors or lenalidomide. Data was collected with regard to type of treatment, duration of treatment, side effects and overall outcome with respect to the treatment given.

Data analysis: Results are analyzed in terms of the clinical characteristics and laboratory parameters at diagnosis, response to the different treatment regimens [drug(s)], the survival patterns and the prognostic effects of patient characteristics on overall survival. The response to treatment is assessed in terms of Complete Remission (CR), Stable disease, Relapse, Progression of disease, No response, and failure/death. CR was defined as the absence of any clinical sign of disease and attainment of the following haematological parameters i.e. pperipheral blood Hb $\geq$ 11 g/dL, Platelets $\geq$100 x10^9/L, Neutrophils $\geq$1.0 x10^9/L, Blasts - 0%. Patients with failure to achieve at

least CR, but with no evidence of progression for >8 weeks were considered to have 'Stable disease'.   Relapse after CR was defined as reduction   of values by ≥50% from maximum remission/response levels in granulocytes or platelets, and or reduction in Hb concentration by ≥1.5 g/dL or transfusion dependence. Progression of disease was defined as any one of the following i.e. (1) At least 50% decrement from maximum remission/response in granulocytes or platelets, (2) Reduction in Hb by ≥ 2 g/dL, (3) Transfusion dependence (Tables:10a & 10b in Appendix 1).

All patients started on treatment and with a minimum follow up of 8 weeks were considered evaluable for response and outcome. Overall survival (OS) was measured from the start of therapy until death (from any cause) or last follow-up (49–51). Event-free survival (EFS) was calculated from the start of therapy until the first adverse event, i.e. relapse or progression, secondary malignancy, death from any cause, or last follow- up (49–51). Progression-free survival (PFS) for all patients was taken from the start of therapy until disease progression or death from hypoplastic myelodysplastic syndrome (49–51). Disease-free survival (DFS) for patients in CR was measured from the first recording of response (CR or Stable disease) to the date of progression or relapse (49–51). The closing date for analysis was December 31, 2012.

<u>Statistics:</u> Descriptive statistics were calculated for all variables. Differences in proportions were assessed using the chi-square statistic or Fisher exact test. Differences in means were tested using a t-test or Mann-Whitney-U test as appropriate. Survival curves were drawn by the Kaplan-Meier method and compared by the log-rank test. The relationships of clinical features to the outcome of the procedure were analyzed by univariate Cox proportional Hazard model. For all tests, a 2-sided P-value of 0.05 or less was considered statistically significant. SPSS 16.0 software was used for the analysis.

RESULTS:

Between January 1998 and June, 30, 2012, a total of 54413 out patients were seen in the Haematology department, of which 1225 (2.3%) were diagnosed to have primary MDS. Of this, 173 (14.1% of MDS; 0.32% of total patients) were diagnosed to have hypoplastic MDS. The year wise distribution of total MDS and hMDS is depicted in Figure:1.

All patients (n=173) were included for the analysis of baseline characteristics. Out of the total 173 patients, only 111 (64.2%) who had a follow up of >8 weeks after initiation of treatment were considered 'evaluable' for assessment of response to treatment and for survival analysis.

Certain data are available on all patients, while certain data are available only on a portion of the patients. For each result category, the numbers of patients involved are mentioned.

DEMOGRAPHY & CLINICAL FEATURES AT DIAGNOSIS: (Table:1)

The median age of the 173 patients was 41 years (range: 18-64). Seventy two (41.6%) patients belonged to the age group of 18-40 years, 51 (29.5%) to 41-55years and 50 (28.9%) were above 55 years of age. Males were predominantly represented in the study group i.e. 112 (64.7%) males and 61 (35.3%) females. The male female ratio was 1.8:1.

Pallor and bleeding manifestations were the common presenting symptoms; i.e. in 94.2% (n=163) and 40.5% (n=70) respectively. Sixty six (38.2%) patients had history of infections (mainly recurrent febrile episodes; a few had lower respiratory tract and skin infections). The median duration of symptoms was 3 months (range: 1-120). Clinical examination showed mild (<2cms) splenomegaly in 6 patients (3.5%) and mild hepatomegaly (<2cms) in 2 (1.2%) patients.

Forty eight patients (27.7%) had received previous treatment. This included,. CSA (n=8), Azathioprine (n=2), Anabolic steroids (n=8), Prednisolone (n=11), EPO (n=2), GCSF (n=1) or Lenalidomide (n=1) for a median duration of 60 days (range: 7-720). Among the 173 patients, 139

had received transfusions (packed red cells and or platelet rich concentrates) before presenting to our Institution. The median number of transfusions per month was 2 (range;1-20) units.

Fourteen patients (8.1%) had past history of treatment for cytopenias; 11 patients were diagnosed and treated for Aplastic anemia before the diagnosis of hMDS was made. The median time from diagnosis of AA to diagnosis of hMDS was 38 months [range:4-149]. Among them, 2 each had attained CR or PR and were off therapy for a mean period of 53 months (range: 28-91). The remaining 7 remained symptomatic and were diagnosed to have hMDS on re-evaluation. The twelfth patient was diagnosed to have B12 deficiency with bicytopenia 6 years back and had been lost to follow up while on B12 & folate supplementation; a second patient (male; 23yrs) was diagnosed to have Chloramphenicol induced pancytopenia 12 years back which had resolved. The last patient (female; 61yrs) was diagnosed to have MDS with fibrosis 3 years back (hypercellular marrow with fibrosis, normal cytogenetics), was treated with thalidomide & prednisolone for one year followed by danazol for 2 years before she was diagnosed to have hMDS (with cytogenetics-del5q & WHO group RAEB-1). She was subsequently treated with Lenalidomide with no response and expired within 3 months of diagnosis of hMDS.

LABORATORY PARAMETERS AT DIAGNOSIS: (Table: 2)

At presentation, majority of the patients (65.3%; n=113) were pancytopenic, while bicytopenia was seen in 53 (30.6%) and only 7 (4.1%) had cytopenia involving a single lineage. The median hemoglobin for the entire cohort was 5.8g% (range:1.2-13.2); most of these patients (54.4%; n=94) had hemoglobin level <6g% while in 41% (n=71) the level was between 6.1-10g% and only 4.6% (n=8) had hemoglobin >10g% at presentation. Neutropenia (an absolute neutrophil count <1800/mm^3) was observed in 135 (78%) patients at presentation, while 38 (22%) had normal neutrophil count. ANC below 200/mm^3 was observed in 8.7% (n=15) while ANC between 201-

500/mm^3, 501-1000/mm^3, 1000-1500/mm^3, and 1501-1800mm^3 was found in 16.2% (n=28), 22.5% (n=39), 20.8% (n=36) and 9.8% (n=17) respectively. Thrombocytopenia (platelet <100 x10^9/L) was documented in 161 (93%) patients; 61.3% (n=106) had a count < 20 x10^9/L, while a count of 21-50 and 51-100 x10^9/L was observed in 39 (22.5%) and 16 (9.2%) patients respectively.

The median reticulocyte percentage at diagnosis (documented in 152 patients) was 1.8% (range: 0.05-7.06). Out of the 99 patients in whom the absolute reticulocyte count was available, the median absolute reticulocyte count was 37200/mm^3, with 18 (18.2%) patients having an absolute reticulocyte count <20 x10^9 /L). None of the patients had monocytosis or eosinophilia. The median absolute eosinophil (AEC), monocyte (AMC) and lymphocyte (ALC) counts were 0/mm^3 (range: 0-966), 36/mm^3 (range: 0-690) and 1656/mm^3 (range: 175-8800) respectively.

Data on auto-immune markers i.e. direct coomb's and antinuclear antibody tests were available only in 39 and 23 patients respectively, with 14 (35.9%) being coomb's positive and 8 (34.8%) positive for ANA. Serum LDH was documented in 160 patients, and majority (81.9%; n=131) had level <600 mg/dl. Serum ferritin was available in 24 patients; the median level was 825ng/ml (range: 138-46775). Report on serum B12 level was available in only 10 patients and the median value was 970pg/ml (range: 248-2000). Out of the 137 patients in whom blood borne virus screen was done at diagnosis, 3 patients were positive for HBV, while 1 each positive for HIV and HCV respectively.

BONE MARROW FEATURES & WHO CLASSIFICATION AT DIAGNOSIS: (Table:3 & Figures:2a-2h]

The bone marrow was aplastic, uniformly hypocellular or varyingly hypocellular in 5.2% (n=9), 83.2% (n=144) and 11.6% (n=20) of patients respectively (Fig: 2f). The data on BM blast count was available in 171 patients, out of which majority (46.8%; n=80) had no blasts on the aspirate and no increase in CD 34+ cells on the trephine biopsy. In 77 (45%) patients, the blast

percentage was 1-2%, while 10 (5.8%) had 3-4% blasts and 4 (2.4%) had ≥ 5% blasts on the marrow (Fig: 2h). Scattered ring sideroblasts (Fig: 2e) were documented in 47 (39.2% of the 120 cases where data was available) patients and one had >15% ring sideroblasts.

Trilineage dysplasia (Figs: 2a-d, 2f) was observed in 57 (33%) patients, while 36 (20.8%) patients had unilineage and 80 (46.2%) had bilineage dysplasia respectively. Data on bone marrow reticulin was available in 169 patients. Out of this 135 (79.9%) patients showed increased reticulin content (Fig: 2g) on silver stain. Most of these patients (47.3%; n=80) showed mild increase in reticulin, while 49 (29%) and 6 (3.6%) patients showed moderate and marked increase in reticulin respectively.

On categorizing the patients according to the WHO classification, majority (77.5%; n=134) belonged to the RCMD group, with 4 (2.3%; n=4) belonging to RAEB-1 and 35 (20.2%) belonging to the MDS unclassified (MDS-U) group.

CYTOGENETIC ABNORMALITIES AT DIAGNOSIS: (Table: 4)

Cytogenetic data was available on 116 (67%) patients. Majority (66.4%; n=77) had normal karyotype. Five patients had polyploidy out of which only 2 had associated structural chromosomal anomalies while 3 had no anomalies. Among those with chromosomal aberrations, 11.2% (n=13) had a sinlge abnormality, while 8 (6.9%) patients had 2 abnormalities, 3 (2.6%) had three abnormalities and 15 (12.9%) had more than 3abnormalities/associated monosomy 7.

Among the 39 patients with chromosomal abnormalities, 55 numerical abnormalities were observed. Most of the numerical anomalies were monosomies (n=37), while 18 were trisomies. There were 13 patients (11.2%) with monosomy 7, 7 patients (6%) with trisomy 8, and 8 patients (6.9%) with trisomy 21. Other abnormalities noted included translocations (n=5), deletions (n=15),

and other rare anomalies i.e. additions (n=5), derivatives (n=4) and duplications (n=1). Deletion 5q was observed in 7 (6%) cases. The details of each anomaly are detailed in table 4.

CYTOGNETIC & PROGNOSTIC RISK GROUPS AT DIAGNOSIS: (Tables: 5 & 6)

Cytogenetic risk groups: (Table 5)

One hundred and sixteen patients in whom the cytogenetic reports were available, were risk categorized into three cytogenetic risk groups (good, intermediate and poor) as per the 'IPSS & WPSS', and into 5 risk groups (very good, good, intermediate, poor and very poor) according to the revised IPSS (IPSS-R) systems. Majority of the patients belonged to the 'good' cytogenetic risk group, in either systems (69% [n=80] each in both), while 16.4% (n=19) and 14.6% (n=17) belonged to the intermediate and poor risk cytogenetic groups respectively in the 'IPSS&WPSS' group. Whereas in the IPSS-R group, 3 patients (2.6%) each belonged to 'very good' & 'very poor' risk groups, while 15 (12.9%) each belonged to the 'intermediate' & 'poor risk' groups respectively.

Prognostic risk groups: (Table: 6)

The 116 patients were categorized into different prognostic risk groups using three different prognostic scoring systems ie; IPSS, IPSS-R and WPSS scoring systems. Using the IPSS system, majority (82.7%; n=96) belonged to the 'intermediate-1' group, while 17 (14.7%) and 3 (2.6%) belonged to the 'intermediate-2' and 'low' risk groups respectively. There were no patients in the high risk group. Using the WHO classification based prognostic scoring system (WPSS), one patient (0.9%) was categorized into the 'very low' risk group, 68.1% (n=79) into 'low' risk group, 16.4% (n=19) into 'intermediate' risk and 14.6% (n=17) into high risk groups respectively. In the IPSS-R category, 2 (1.7%) patients were in the 'very low' risk group, 22 (19%) in the 'low' risk, 68

(58.6%) in the 'intermediate' risk, 18 (15.5%) in the 'high' risk and 6 (5.2%) were in 'very high' risk groups respectively.

TREATMENT AND RESPONSE: (Table: 7)

Patients who had a minimum of 8 weeks follow up after starting treatment (including those who expired within 2 months of treatment) were considered evaluable for assessment of response to treatment. Among the total 173 patients; 111 patients (109 with >2 months follow up + 2 who died within 2 months of starting treatment) were considered evaluable, and the remaining 62 were considered non-evaluable for assessment of response to therapy. Out of the 111 evaluable patients, cytogenetic data was available only in 79 patients.

Of the 111 evaluable patients, 99 received treatment with (a) Cyclosporine (CSA) or (b) Antithymocyte globulin+CSA (ATG+CSA) or (c) Androgenic steroids (Danazol/stanazolol) or (d) Prednisolone, or (e) Allogeneic PBSCT (Allo PBSCT) and 12 patients received 'Other' therapies (i.e. Lenalidomide or EPO/GCSF or supportive measures).

(a)   CSA   (Table:7):   Overall 81 patients received treatment with CSA; out of these only 59 were evaluable for response. Among the 59 evaluable patients, 41 showed a response (6 [10.2%] achieved CR while 35 [59.3%] achieved 'stable disease'). Six (102%) patients expired without attaining any response and the remaining 12 showed no response to CSA. Among the 6 patients who expired, one had progressed to AML prior to expiry. Of the non-responders, 8 were started on second line treatment with Androgenic steroids and 4 patients who had matched sibling donors were taken up for allogeneic PBSCT (detailed in the section on Allogeneic PBSCT). Among the responders, the 6 patients in CR continued to be in CR at last follow up. In the 35 patients with 'stable disease' 24 (68.6%) continued to be in 'stable disease' at last follow up (6 are

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stable & off treatment, while 16 patients are stable but remain on treatment, and 2 were lost to follow up while on treatment). Of the 35 patients with 'stable disease', 11 progressed while on follow up. One patient progressed to PNH and was started on Danazol. Another patient was started on danazol followed by ALG+CSA with no response (subsequently succumbed to disease progression and treatment failure). Of the remaining 9 patients, one expired following disease progression and 8 were restarted on CSA (2 were lost to follow up and 6 continue to be on follow up). While on treatment with CSA, 5 out of the total patients developed CSA related nephrotoxicity elevated serum creatinine, these were non-responders and was changed to Androgens) and 2 had gum hyperplasia. The mortality was 13.6% (n=8) among those treated with CSA. .Overall the response rate to CSA was 69% (41 out of 59), with mean time to response of 5.9months (range: 3-36months), and median duration of treatment of 19 months (range: 3-126). At a mean follow up duration of 103 months (range: 8-110), the 5 year OS of the responders (n=41) was 96.9% ± 3.1%.

(b) **Antithymocyte globulin+CSA (ATG+CSA) (Table:7):** A total of 10 patients were treated with ATG+CSA. Of these 7 showed response to treatment (2 CR and 5 'stable disease'), one patient expired without response and 2 showed no response. The 2 non-responders were changed over to Androgens. Among the responders, 2 patients who achieved CR continued to remain in CR (on CSA) at last follow up. At last follow up, 3 out of the 5 patients with' stable disease' continued to be so (2 were off treatment and one was on CSA) and 2 patients progressed (out of which one expired and one was continued on treatment). The mortality was 20% (n=2) among those treated with ATG+ CSA. Overall the response rate to ATG+CSA was 70% (7 out of 10), with mean time to response of 1.8 months (range: 1-3 months), and median duration of treatment of 12 months (range: 8-72). At a mean follow up duration of 58 months (range: 16-73), the 5 year OS of the responders (n=7) was 75.0% ± 21.7% .

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(c) <u>**Androgenic steroids (Danazol/stanazolol) (Table:7)**</u>: Out of the 74 patients who received treatment with androgenic steroids (29 received danazol while 45 received stanazolol), 51 were evaluable. Twenty four patients (47.1%) showed response (2 CR & 22 'stable disease'), while 25 (49%) showed no response to androgens. Two patients (3.9%) expired without any response (out of which one expired following progression to AML). Of the non-responders, 4 patients were changed over to ATG+ CSA, 17 patients to CSA and 4 were lost to follow up. One out of the 2 patients who attained CR continued to be in CR at last follow up, while the other patient relapsed and was restarted on treatment. Out of the patients who had stable disease, 17 continued to be stable, while 5 patients showed evidence of progression of disease. Two of the patients with progression of disease were restarted on treatment but were subsequently lost to follow up; while 3 patients expired following progression of disease (one patient expired following progression to AML, second one following progression of disease along with metastatic adenocarcinoma and the third due to disease progression). The mortality was 9.8% (n=5) among those treated with Androgenic steroids. Overall the response rate to Androgenic steroids was 47% (24 out of 51), with mean time to response of 5.3months (range: 1-27 months), and median duration of treatment of 11 months (range: 2-92). At a mean follow up duration of 68 months (range: 20-92), the 5 year OS of the responders (n=24) was 51.40% ± 23.1%.

(d) <u>**Prednisolone (Table:7)**</u>: Twenty four patients received treatment with steroids, but only 17 were evaluable. Only four (23.5%) patients showed response ('stable disease') to prednisolone. One patient (5.9%) expired, while 12 (70.6%) showed no response. Among the 12 non-responders, treatment was changed to CSA in 7, to androgens in 4 and one patient was taken up for allogeneic PBSCT. Among the 4 patients who attained response to prednisolone, 3 were lost to follow up after a mean follow up of 11 (7-13) months, and the fourth patient relapsed after

44 months and was then lost to follow up. The median duration of steroid treatment was 4 months (range: 2-36) and the mean time to response was 1.9 months (range: 1.3-2.7).

(e) **Allogeneic PBSCT (Allo PBSCT) (Table:7):** There were a total of 5 patients who underwent allogeneic PBSCT. All were males; with age between 19 to 56 years. One patient was diagnosed to have Aplastic anemia and had received treatment for 10 months prior to diagnosing hMDS. The treatment prior to allogeneic PBSCT included; Cyclosporine-A (CSA) for 2 months in 3 patients, CSA for 12 months in one patient and Prednisolone for 2 months in one patient. All except one patient had normal cytogenetics; the fifth patient had trisomy21 (+21). Four patients belonged to 'Intermediate-1' IPSS risk group, 'Low' WPSS risk group and 'Intermediate ' IPSS-R risk group; and the one patient with +21 belonged to intermediate-1 (IPSS), intermediate (WPSS) and intermediate (IPSS-R) risk groups respectively. The donors were related for all 5 patients (brother was the donor in 4 of them and sister in one). The conditioning regimen was Fludarabine/Cyclophosphamide in 4 patients and Fludarabine/Melphalan in one patient. One patient underwent second PBSCT following relapse and received Fludarabine/Melphalan/TBI for the second PBSCT.

Two patients died before engraftment by day 9 and 10 post PBSCT, due to sepsis with VOD and fungal pneumonia, and sepsis with actinomycosis of lung respectively. Three patients engrafted (by days 11, 12 & 13 respectively). Of the 3 patients who engrafted, one lost response by day 52 post Allo PBSCT, and died of grade IV acute liver GVHD on day 77 post PBSCT. The second patient who responded (age-19yrs) relapsed by day 70 post PBSCT, and underwent DLI followed by a second PBSCT (using the same donor). He engrafted by day 11 of second PBSCT, and continued to be in CR for next one year. After a year post second PBSCT, he relapsed with progression to AML, and succumbed to his illness during the post chemotherapy

(Cytosine/Idarubicin) neutropenic period due to sepsis and massive GI bleed. The third patient (age: 30 years) who responded is the one who was treated for Aplastic anemia for 10 months prior to diagnosing hMDS. Post PBSCT, he developed chronic skin GVHD which responded to treatment. Presently he is 7 years post PBSCT, on follow up and continues to be in CR off drugs (except for warfarin).

(f) '**Others':** This included 12 patients (not mentioned in table: 7), who had received treatment with Lenalidomide (n=3) or EPO/GCSF (n=1), or only supportive measures (n=4), or in whom the drug was not known due to unavailable/missing data (n=4). In this group 2 cases attained 'CR', 6 attained 'stable disease', 4 expired without response and 2 expired following progression of disease after initial response. Among those who received Lenalidomide, 2 attained 'Stable disease', and one expired without attaining any response. The one patient who received EPO/GCSF progressed and expired after a short period of response (stable disease). Two out of the 4 patients who opted for supportive measures expired following disease progression and the other 2 showed spontaneous recovery of blood counts (CR) within 2 months and were subsequently lost to follow up. There were 4 cases where data on the drug were not available. One patient expired without response and 3 had shown initial response to treatment (stable disease). Of the 3 who showed response, one was lost to follow up, one patient subsequently progressed and expired, and the third patient who had been diagnosed and treated in 1998 was found to be stable and off drug on last follow up (the information was obtained by mail, documents on previous drug treatment could not be retrieved).

TREATMENT RELATED MORBIDITY:

Sixty three (36.4%) patients developed morbidities during treatment. Drug induced nephropathy was observed in 6 (3.5%) of the patients. Of these, 5 (83.3%) patients had CSA

induced and one (16.7%) had analgesic induced nephropathy. Two patients had Cyclosporine related gum hyperplasia. Drug related hepatitis (transaminitis) was documented in 7 (4.0%) patients (stanazolol induced in 5 (71%) patients and Danazol induced in 2(29%)). Three (1.7%) patients were HBV positive at diagnosis, while an additional one patient (0.6%) was documented to be positive for hepatitis B virus during follow up. Three (1.7%) patients developed lower limb Deep vein thrombosis; one among them developed pulmonary embolism. Nine (5.2%) developed steroid induced diabetes mellitus, of which one had Cushing's habitus. Two patients (1.2%) developed tuberculosis while on treatment for hMDS (one had pulmonary tuberculosis and one had lymph node tuberculosis). Fourteen (8%) patients developed infectious complications during treatment (i.e. febrile neutropenia with/without sepsis in 10 cases, and Gram negative bacilli sepsis, peri-anal abscess, cellulitis and osteomyelitis in one patient each respectively. On follow up, 2 patients were found to have vitamin D deficiency while evaluating for back ache, and one of them presented with vertebral fracture. In Allo PBSCT patients, one had acute grade IV liver GVHD and the other had chronic skin GVHD.

MORTALITY: Table: 8

Twenty six (15%) patients expired on follow up. Of these, 16 (61.6%) patients died because of poor response to treatment, while 10 patients died following disease progression/relapse after attaining response ('CR' or 'stable disease'). Of the former 16 patients, 2 were of post Allo PBSCT status, while 2 had progressed to AML without attaining any response, and the remaining 14 patients died of primary treatment failure. Among the 10 patients who died following disease progression after CR or 'stable disease', one patient was of post Allo PBSCT status and had progressed to AML (following relapse after second Allo PBSCT), while a second patient was the one who had responded to androgens and then progressed to AML, the third patient was an initial

responder to androgens who subsequently progressed with associated metastatic adenocarcinoma. The remaining 7 patients died of progressive disease.

Sepsis with multi-organ failure was the immediate cause of death in 10 patients, while 2 patients died following massive intracranial bleed, 3 following severe pneumonia and one following grade IV GVHD. All the remaining 10 patients died of progressive disease related events (details of the exact events not available). Among those patients who died of sepsis, one had disseminated mucor ycosis, 4 had fungal pneumonia, one had pulmonary actinomycosis, and another one patient had associated massive GI bleed.

SURVIVAL ANALYSIS: (Figs: 4-11; Table: 9)

Survival analysis was done for the 111 patients who were evaluable for treatment response. Among these, only 79 patients had cytogenetic data.

Overall (OS), Event free (EFS), progression free (PFS) and disease free survivals (DFS) (Figs: 4-7): The mean follow up was 110 months (range: 1-178). There were a total of 26 deaths, all deaths were due to progressive disease and related complications. The 5 year and 10 year overall survivals for the whole cohort (n=173) was 61.9% ± 7.2%, and 53.1% ± 10.3% respectively.

With a mean follow up period of 70 months (range: 1-178), the 5 year EFS for the entire cohort was 37.9% ± 7.8%. The 5 year progression free survival with a mean follow up period of 86 months (range:1-78) for the entire cohort was 49.5% ± 9.3% and the 5 year disease free survival with a median follow up period of 69 months (range:1-166) was 46.6 ± 9.5%.

OS of the different IPSS risk groups (Table: 9 and Fig:8): Among the 79 evaluable patients with cytogenetic data, with a mean follow up period of 125 months (range:1-178) and 34 months (range:1-78) respectively, the 5 year OS in the lower risk groups (Low+Int-1) versus higher risk groups (Int-2+high) was 65.9% ± 9.7% versus 38.1% ± 20.4% respectively (*P= 0.056*).

OS of different WPSS risk groups (Table: 9 and Fig: 9): In the WPSS risk groups, the mean follow up period was 83 months (range:2-110), 92 months (range:1-178) and 34 months (range:1-78) for the lower risk (very low + low risk), intermediate risk and high risk groups respectively. The 5 year OS was noted to be significantly higher in the lower risk groups than the higher risk groups (Int and high risk groups); ie Lower versus Int (5yr OS= 66.6% ± 12.1% versus 50.5%±15.8%; *P=0.026*), Lower versus High (66.6%±12.1% vs 38.1%±20.4%; *P=0.017*). However there was no significant survival advantage for the intermediate risk group over high risk group (*P=0.973*).

OS of different IPSS-R risk groups (Table: 9 and Fig: 10): The mean follow up period of the IPSS-R risk groups were as follows; 83 months (range: 2-110) for the lower risk group (very low + low + Int) and 67 months (1-178) for the higher risk group (high + very high). The 5 year OS of the lower risk groups (ie very low + Low + Int) was again significantly better than the higher risk groups (High + very high), ie; 68.0% ± 10.8% *vs* 35.0% ± 15.7% (*P=0.002).*

OS of different WHO classification groups (Fig: 11): Using the WHO criteria, the out of the 111 evaluable patients, 82 belonged to RCMD, 26 to MDS unclassified and 3 to RAEB-1. With a mean follow up period of 109 (range:1-82), 61(2-78) and 8.5 (range:3-14) months, the 5 year OS was 61.2% ± 8.4%, 74.4 % ± 11.8% and 0 % ± 0% for RCMD, MDS-U and RAEB-1 respectively (*P=0.000*).

UNIVARIATE ANALYSIS OF ADVERSE EFFECTS OF PATIENT CHARACTERISTICS ON OVERALL SURVIVAL (Table: 10)

Univariate Cox proportional hazard model was used to find out significant prognostic factors for adverse effects on survival among overall hMDS patients. Variables with significant

adverse effects on overall survival included age, gender, blood counts (Haemoglobin, ANC, platelet count), number of cytopenias, serum LDH, bone marrow cellularity, bone marrow blast percentage, bone marrow dysplasia, bone marrow reticulin, WHO classification groups, chromosome changes and prognostic risk categories (IPSS, WPSS & IPSS-R). Parameters of independent significance associated with poor overall survival were; ANC < 0.2 x10^9/L at diagnosis (*RR=4.2; 95%CI=1.32-13.5;P= 0.015*), Bone marrow blast at diagnosis > 5% (*RR=11.0; 95%CI=2.27-53.77; P= 0.003*), WHO category RAEB-1, (*RR=7.5; 95%CI=1.68-34.12; P= 0.008*), Moderate/marked increase in BM reticulin, (*RR=4.0; 95%CI=1.13-14.17; P= 0.031*), >3 cytogenetic anomalies, (*RR=3.5; 95%CI=1.09-11.82; P= 0.035*), Monosomy 7, (*RR=5.1; 95%CI=1.54-17.03; P= 0.008*), Trisomy 21, (*RR=14.4; 95%CI=2.88-72.7; P= 0.001*),WPSS intermediate risk group, (*RR=3.3; 95%CI=1.11-10.00; P= 0.031*), WPSS high risk group , (*RR=3.6; 95%CI=1.11-12.00; P= 0.033*), and IPSS-R very high risk group (*RR=13.5; 95%CI=2.64-69.90; P= 0.002*). However on multivariate analysis, none of the above parameters retained its statistical significance.

RESULTS - TABLES:

Table 1: DEMOGRAPHY & CLINICAL FEATURES AT DIAGNOSIS:

n =173

Variables	n (%) / Median(Range)	
Age at diagnosis in years		
18-40	72	(41.6)
41-55	51	(29.5)
>55	50	(28.9)
Gender		
Male	112	(64.7)
Female	61	(35.3)
Presenting symptoms		
Pallor	163	(94.2)
Bleeding	70	(40.5)
Infection	66	(38.2)
Duration of symptoms (months)	3	(1-120)
Organomegaly		
Splenomegaly	6	(3.5)
Hepatomegaly	2	(1.2)
Patients who received prior treatment	48	(27.7)
Median duration of previous treatment (days)	60	(7-720)
Past history treatment for cytopenia (s)	14	(8.1)
Median transfusions per month (n=139)	2.0	(1-20)

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Table 2: LABORATORY PARAMETERS AT DIAGNOSIS

n=173

Variables	n (%) / Median (Range)	
Hemoglobin [g%]		
<6	94	(54.4)
6.1-10	71	(41.0)
>10.0	8	(4.6)
Absolute neutrophil count[x10^9/L]		
<0.2	15	(8.7)
0.21-0.5	28	(16.2)
0.51-1.0	39	(22.5)
1.1-1.5	36	(20.8)
1.51-1.8	17	(9.8)
>1.8	38	(22.0)
Platelet count [x10^9/L]		
<20	106	(61.3)
21-50	39	(22.5)
51-100	16	(9.2)
>100	12	(7.0)
Reticulocyte count		
Median Percentage of reticulocytes **(n=152)**	1.8	(0.05-7.06)
Median Absolute reticulocytes [x10^9/L] **(n=99)**	37200	(6175-133472)
No. of cytopenia(s)		
Single cytopenia	7	(4.1)
Bicytopenia	53	(30.6)
Pancytopenia	113	(65.3)
Direct coomb's test (n=39)		
Positive	14	(35.9)
Negative	25	(64.1)
Antinuclear antibody (n=23)		
Positive	8	(34.8)
Negative	15	(65.2)
Serum LDH [mg/dl] **(n=160)**		
<600	131	(81.9)
>600	29	(18.1)
Serum ferritin [ng/ml] **(n=24)**	825	138-46775)
Serum Vitamin B12 [pg/ml] **(n=10)**	970	(248-2000)
Blood borne virus screen (n=137)		
Positive	5	(3.6)
Negative	132	(96.4)

Table 3: BONE MARROW FEATURES & WHO CALSSIFICATION AT DIAGNOSIS:

n = 173

Variables	n	(%)
Bone marrow cellularity		
Aplastic	9	(5.2)
Uniformly hypocellular	144	(83.2)
Varyingly hypocellular	20	(11.6)
BM blasts[%] (n=171)		
0	80	(46.8)
1-2	77	(45.0)
3-4	10	(5.8)
≥5	4	(2.4)
BM ring sideroblasts (n=120)		
>15%	1	(0.8)
Scattered/occasional	47	(39.2)
Absent	72	(60.0)
BM dysplasia		
Unilineage	36	(20.8)
Bilineage	80	(46.2)
Trilineage	57	(33.0)
BM Reticulin (n=169)		
Normal	34	(20.1)
Mild increase	80	(47.3)
Moderate increase	49	(29.0)
Marked increase	6	(3.6)
WHO classification		
MDS-Unclassified	35	(20.2)
RCMD	134	(77.5)
RAEB-1	4	(2.3)

Abbreviations: MDS: Myelodysplastic syndrome, RCMD: Refractory

cytopenia with multilineage dysplasia, RAEB: Refractory anemia with

excess blast.

Table 4: CYTOGENETIC ABNORMALITIES AT DIAGNOSIS

n =116

Variables	n	(%)
Cytogenetic abnormalities		
No abnormality	77	(66.4)
One abnormality	13	(11.2)
Two abnormalities	8	(6.9)
Three abnormalities	3	(2.6)
>3 / chromosome 7 abn	15	(12.9)
Individual chromosomal aberrations		
Monosomies:		
-7	13	(11.2)
-5	2	(1.7)
-Y	3	(2.6)
-X	2	(1.7)
Other monosomies[@]	17	(14.7)
Trisomies:		
+8	7	(6.0)
+21	8	(6.9)
Other trisomies[@]	3	(2.6)
Translocations		
t(18;21)	1	(0.9)
t(20;21)	1	(0.9)
t(11;14)	1	(0.9)
t(7;13;16)	1	(0.9)
t(7;?)	1	(0.9)
Deletions:		
del 5q	7	(6.0)
del 11q	2	(1.7)
del 12p	1	(0.9)
del 20q	1	(0.9)
Other deletions[@]	4	(3.4)
Other rare anomalies[@]		
Additions	5	(4.3)
Derivatives	4	(3.4)
Duplications	1	(0.9)

[@]   Other anomalies included; monosomies (-1, -2,-3,-9,-10,-13,-14,-15,-16, & -19 seen in one patient each and -11, -18, &-20 seen in two patients each), trisomies (+1, +2,+4 seen in one patient each), deletions (del 11q in 2 patients and 1p, del 5p, del 6q, del 9q, del 12p, & del 20q seen in one patient each), additions (+11p,+11q,+14q,+1p, & +7q seen in one patient each), derivatives [der(7), der(12), der(14), & der(16 seen in one patient each) and duplication (dup1), seen in one patient.

Table 5: CYTOGNETIC RISK GROUPS AT DIAGNOSIS

n =116

	IPSS&WPSS cytogenetic risk group		IPSS-R cytogenetic risk group	
	n	(%)	n	(%)
Very good	-	-	3	(2.6)
Good	80	(69.0)	80	(69.0)
Intermediate	19	(16.4)	15	(12.9)
Poor	17	(14.6)	15	(12.9)
Very poor	-	-	3	(2.6)

Abbreviations: IPSS: International prognostic scoring system, WPSS: WHO prognostic scoring system, IPSSS-R: Revised IPSS.

Table 6: PROGNOSTIC RISK GROUPS AT DIAGNOSIS:

n = 116

Risk group	IPSS		WPSS		IPSS-R	
	n	(%)	n	(%)	n	(%)
Very low	-	-	1	(0.9)	2	(1.7)
Low	3	(2.6)	79	(68.1)	22	(19.0)
Intermediate-1/	96	(82.7)	-	-	-	-
Intermediate	-	-	19	(16.4)	68	(58.6)
Intermediate-2	17	(14.7)	-	-	-	-
High	0	(0.0)	17	(14.6)	18	(15.5)
Very high	-	-	-	-	6	(5.2)

Table 7: TREATMENT AND RESPONSE:

	DRUG GROUPS				
	CSA	**ATG +CSA**	**Androgens**	**Prednisolone**	**Allo-PBSCT**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Total patients (n=173)	81 (46.8)	10 (5.8)	74 (42.8)	24 (13.9)	5 (2.9)
Non-evaluable	22 (27.0)	0 (0.0)	23 (31.0)	7 (29.0)	0 (0.0)
Evaluable	59 (73.0)	10 (100.0)	51 (69.0)	17 (71.0)	5 (100.0)
Response:					
CR	6 (10.2)	2 (20.0)	2 (3.9)	0 (0.0)	2 (40.0)
Stable disease	35 (59.3)	5 (50.0)	22 (43.2)	4 (23.5)	1 (20.0)
No response (LFU/drug changed)	12 (20.3)	2 (20.0)	25 (49.0)	12 (70.6)	0 (0.0)
Failure (expired with no response)	6 (10.2)	1 (10.0)	2 (3.9)	1 (5.9)	2 (40.0)
On follow up:					
CR:					
Continued in CR	6 (100.0)	2 (100.0)	1(50.0)	0 (0.0)	1(50.0)
Relapse & alive/LFU	0 (0.0)	0 (0.0)	1(50.0)	0 (0.0)	0 (0.0)
Relapse & expired	0 (0.0)	0 (0.0)	0(0.0)	0 (0.0)	1(50.0)
Stable disease:					
Continued in Stable	24 (68.6)	3 (60.0)	17(77.3)	3(75.0)	0 (0.0)
Progression & alive/LFU	9 (25.7)	1 (20.0)	2 (9.1)	1(25.0)	0 (0.0)
Progression & expired	2 (5.7)	1 (20.0)	3(13.6)	0 (0.0)	1(100.0)
Duration of treatment-months; median(range)	19 (3-126)	12 (8-72)	11 (2-92)	4 (2-36)	--
Response rate	69%	70%	47%	23.5%	60%
Time to response - months; mean (range)	5.9 (3-36)	1.8 (1-3)	5.3 (1-27)	1.9 (1.3-2.7)	0.5 (0.4-0.5)
Follow up duration –months; mean(range)	103 (8-110)	58 (16-73)	68 (20-92)	19 (7-44)	39 (15-81)
Mortality in each drug group	8 (13.6)	2 (20.0)	5 (9.8)	1 (5.9)	4 (80.0)
5 year OS of responders	96.9% ± 3.1%	75.0% ± 21.7%	51.4% ± 23.1%	100%**	33.3% ± 27.2%

Table 8: MORTALITY:

Variables	n	(%)
Total number of death	26	(15.0)
Disease status at death (n=26)		
Failure without any response	16	(61.6)
Progression/Relapse after CR or 'stable disease'	10	(38.4)
Cause of death		
Disease failure related events (exact event unknown)	10	(38.5)
Sepsis with MODS	10	(38.5)
ICH	2	(7.7)
Liver GVHD	1	(3.8)
Pneumonia	3	(11.5)

Table 8: OVERALL SURVIVAL OF DIFFERENT PROGNOSTIC RISK GROUPS.

Risk group	IPSS		WPSS		IPSS-R	
	n (%)	5 yr OS	n (%)	5 yr OS	n (%)	5 yr OS
Very low	-	-	1 (0.9)	0% ± 0%	2 (1.7)	0% ± 0%
Low	3 (2.6)	0% ± 0%	79 (68.1)	66.6% ±12.1%	22 (19.0)	73%±14%
Intermediate-1/	96 (82.8)	68.3%±9.7%	-	-	-	-
Intermediate	-	-	19 (16.4)	50.9%±15.8%	68 (58.6)	68.4%±12.2
Intermediate-2	17 (14.6)	39.3%±2%	-	-	-	-
High	0 (0.0)	0 (0)	17 (14.6)	39.3%±20.8%	18 (15.5)	48.8±19.3
Very high	-	-	-	-	6 (5.2)	0% ± 0%

TABLE 9: UNIVARIATE ANALYSIS OF ADVERSE EFFECTS OF PATIENT CHARACTERISTICS ON OVERALL SURVIVAL.

n =111

Variables	Alive n (%)	Dead n (%)	RR	95% CI	P-value
Age at diagnosis in years:					
18-40	38(44.7)	9(34.6)	1.0	-	-
41-55	28(32.9)	7(26.9)	1.0	0.38-2.80	0.933
>55	19(22.4)	10(38.5)	1.8	0.74-4.50	0.189
Sex					
Female	33(38.8)	5(19.2)	1.0	-	-
Male	52(61.2)	21(80.8)	2.3	0.87-6.13	0.092
Hb at diagnosis					
>10.1	4(4.7)	1(3.8)	1.0	-	-
6.1 – 10	39(45.9)	9(34.6)	2.1	0.26-17.91	0.466
<6	42(49.4)	16(61.5)	3.4	0.44-27.02	0.235
ANC [x10^9/L]					
>1.5	30(35.3)	7(26.9)	1.0	-	-
1.0-1.5	21(24.7)	3(11.5)	0.5	0.13-2.09	0.372
0.5-1.0	21(24.7)	8(30.8)	1.7	0.63-4.86	0.282
0.2-0.5	11(12.9)	3(11.5)	1.7	0.44-6.97	0.418
<0.2	2(2.4)	5(19.2)	4.2	1.32-13.50	**0.015**
Platelet [x10^9/L]					
>100	7(8.2)	2(7.7)	1.0	-	-
51-100	9(10.6)	4(15.4)	1.3	0.24-7.30	0.742
21-50	19(22.4)	8(30.8)	1.0	0.22-5.19	0.922
<20	50(58.8)	12(46.2)	0.6	0.15-3.14	0.637
No. of cytopenia(s)					
Single cytopenia	3 (3.8)	1(3.8)	1.0	-	-
Bicytopenia	30(35.3)	8(30.8)	0.8	1.00-6.48	0.837
Pancytopenia	52(61.2)	17(65.4)	0.9	0.12-7.30	0.974
Serum LDH (n=101)					
<600	65(83.3)	18(78.3)	1.0	-	-
>600	13(16.7)	5(21.7)	1.5	0.55-4.10	0.420
Bone marrow cellularity					
Varyingly hypocellular	11(12.9)	2(7.7)	1.0	-	-
Uniformly hypocellular	73(85.9)	23(88.5)	1.8	0.15-20.91	0.631
Aplastic	1(1.2)	1(3.8)	1.2	0.29-5.42	0.745
BM blasts [%; n=109]					
0	42(50.0)	9(36.0)	1.0	-	-
1-2	37(44.0)	11(44.0)	1.2	0.52-3.04	0.610
3-4	4(4.8)	3(12.0)	2.9	0.77-10.87	0.113
≥5	1(1.2)	2(8.0)	11.0	2.27-53.77	**0.003**

TABLE 10: UNIVARIATE ANALYSIS OF ADVERSE EFFECTS OF PATIENT CHARACTERISTICS ON OVERALL SURVIVAL contd…

Variables	Alive N (%)	Dead N (%)	RR	95% CI	P- value
BM dysplasia					
Unilineage	22(25.9)	5(19.2)	1.0	-	-
Bilineage	37(43.5)	10(38.5)	1.0	0.36-3.18	0.888
Trilineage	26(30.5)	11(42.3)	1.2	0.44-3.69	0.652
BM Reticulin (n=108)					
Normal	20(23.8)	3(12.5)	1.0	-	-
Mild increase	42(50.0)	8(33.3)	1.6	0.43-6.28	0.456
Moderate/Marked increase	22(26.2)	13(54.2)	4.0	1.13-14.17	**0.031**
WHO SUB-CLASS					
MDS-Unclassified	22(25.9)	4(15.4)	1.0	-	-
RCMD	62(72.9)	20(76.9)	0.7	0.24-2.15	0.569
RAEB1	1(1.2)	2(7.7)	7.5	1.68-34.12	0.008
Cytogenetic anomalies (n=79)					
No anomaly	47(77.0)	9(50.0)	1.0	-	-
One anomaly	5(8.2)	3(16.7)	2.4	0.65-9.00	0.182
2-3 anomalies	4(6.6)	2(11.1)	2.3	0.50-10.92	0.274
>3 anomalies or -7	5(8.2)	4(22.2)	3.5	1.09-11.82	**0.035**
Individual chromosomal abn (n=75)	47(82.4)	9(50.0)	1.0	-	-
Normal	4(7.0)	1(5.6)	1.0	0.13-8.55	0.947
del 5q	4(7.0)	4(22.2)	5.1	1.54-17.03	**0.008**
Monosomy 7	0(0.0)	2(11.0)	14.4	2.88-72.7	**0.001**
Trisomy 21	1(1.8)	1(5.6)	3.4	0.43-27.78	0.241
Trisomy 8	1(1.8)	1(5.6)	4.2	0.51-34.24	0.178
Monosomy Y					
IPSS Risk groups: (n=79)					
Low	2(3.3)	1(5.6)	1.0	-	-
Intermediate-1	54(88.5)	13(72.2)	0.6	0.08-4.88	0.664
Intermediate-2	5(8.2)	4(22.2)	1.8	0.20-16.84	0.583
WPSS Risk groups (n=79)					
Very low	1(1.6)	-	-	-	-
Low	49(80.3)	9(50.0)	1.0	-	-
Intermediate	6(9.8)	5(27.8)	3.3	1.11-9.95	**0.032**
High	5(8.2)	4(22.2)	3.7	1.13-12.24	**0.030**
IPSS-R Risk groups (n=79)					
Very low	1(1.6)	-	-	-	-
Low	16(26.2)	3(16.7)	1.0	-	-
Intermediate	36(59.0)	8(44.4)	1.1	0.30-4.30	0.847
High	8(13.1)	4(22.2)	2.9	0.65-13.66	0.157
Very high	0(0.0)	3(16.7)	14.1	2.75-72.19	**0.001**

RESULTS - FIGURES:
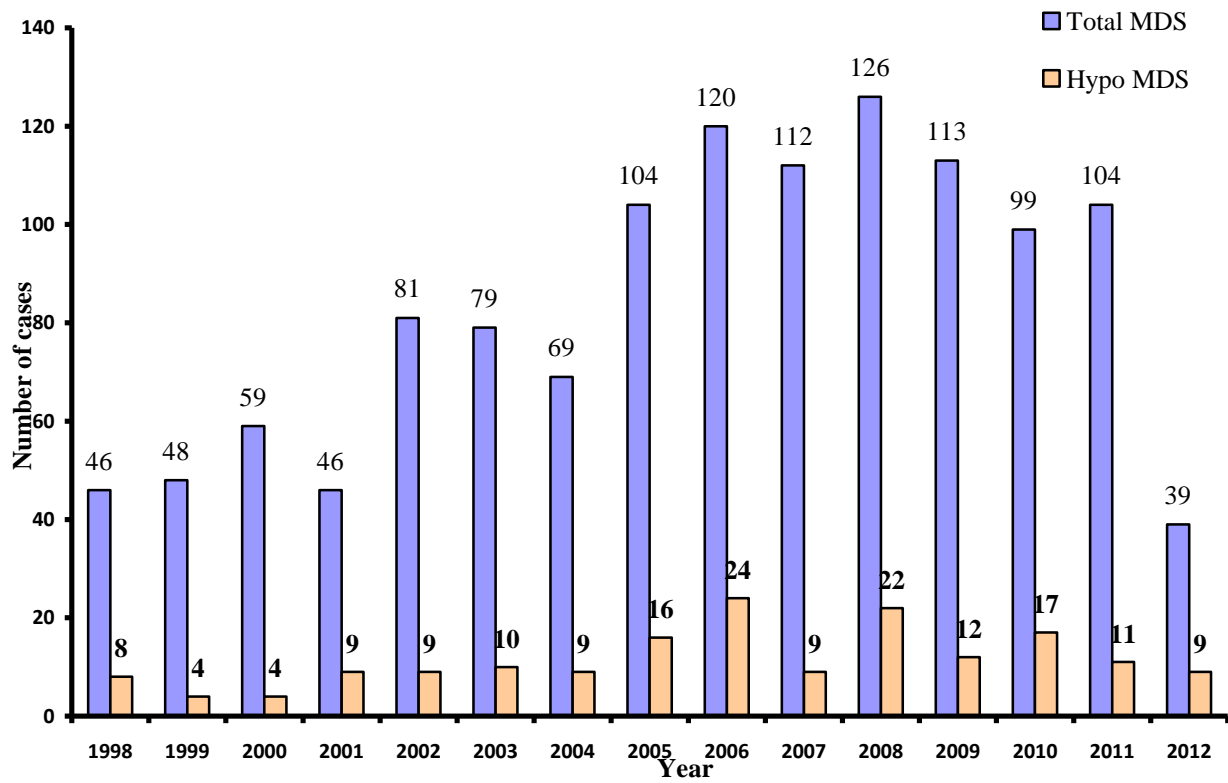
Figure 1: Year wise distribution of total MDS vs hMDS

Figure 1: Year wise distribution: Total MDS versus hypoplastic MDS cases from January 1998 to June 2012.

Figure 2: Peripheral blood and Bone marrow findings:

Figure 2: Peripheral blood and bone marrow findings. **Figures 2a & 2b**: PB smear showing granulocyte dysplasia - pelger heut anomaly and hypogranularity, **2c**: BM aspirate smear showing dyserythropoiesis, **2d**: BM aspirate smear showing micro-megakaryocytes, **2e:** BM aspirate smear showing ring sideroblasts. **2f:** BM trephine biopsy showing hypocellular marrow & dysplastic megakaryocytes. **2g:** BM trephine biopsy silver stain showing increased rericulin and **2h:** BM trephine biopsy showing CD34+ cells.

Figure 3: Response rate to different drugs

Figure 3: On comparing the response rate to different drugs, response rate to CSA was found to be significantly (69% vs 47%; *P=0.017*) superior to Androgenic steroids. The number of patients who received other drugs was few to compare enough number of patients to compare.

SURVIVALS:

Figure 4: Overall survival of total evaluable patients (n=111)
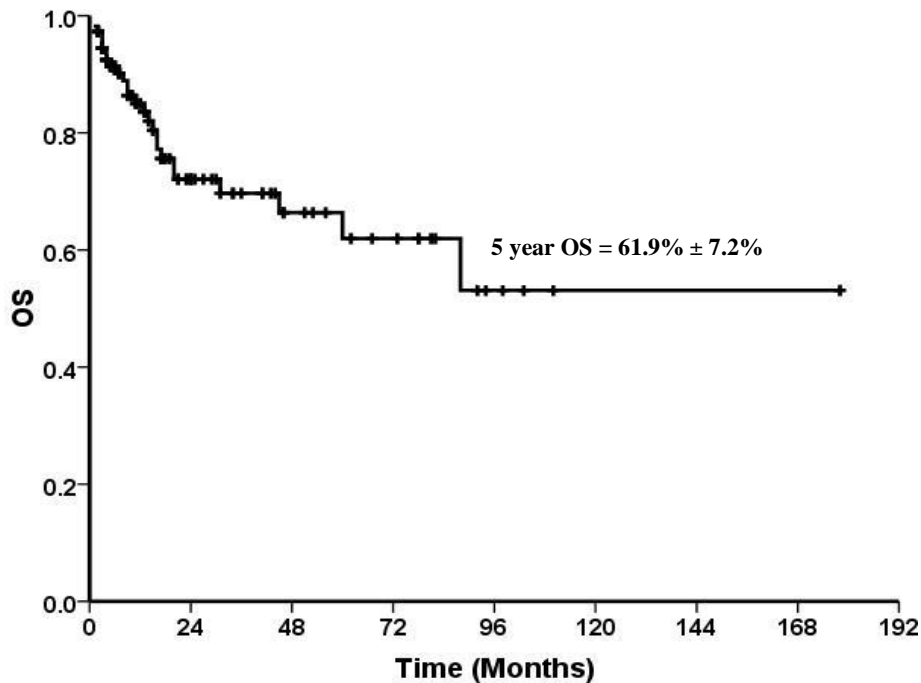
Figure 4: Kaplan Meier curve for overall survival of the entire cohort of evaluable patients (n=111). With a median follow up duration of 110 months (Range: 1-178), the 5 year and 10 year OS were 61.9% ± 7.2% & 53.1% ±10.3% respectively.

Figure 5: Event free survival of total evaluable patients (n=111)
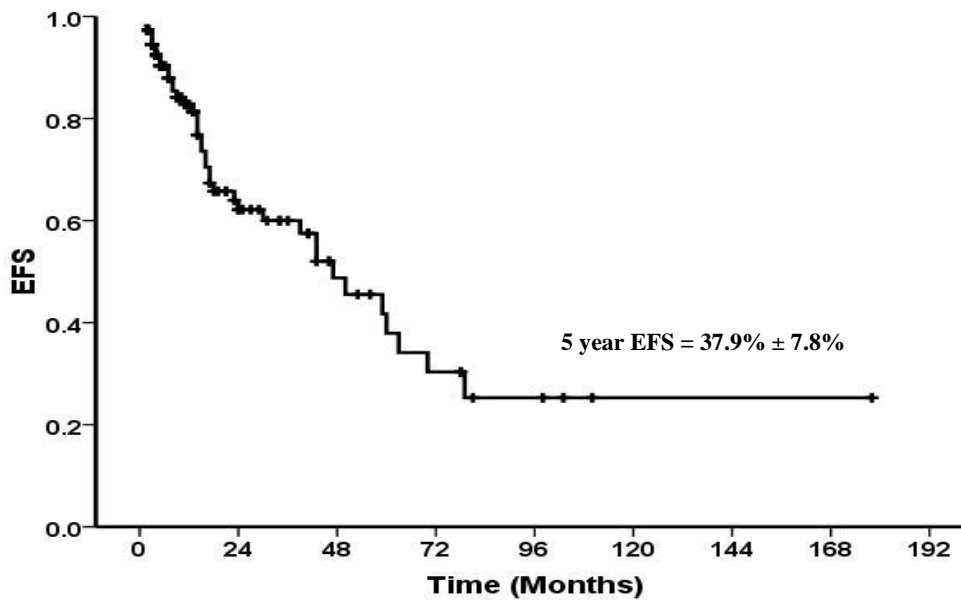
Figure 5: Kaplan Meier curve for Event free survival of the entire cohort of evaluable patients (n=111). With a mean follow up duration of 70 months (Range: 1-178), the 5 year & 10 year EFS were 37.9% ± 7.8% & 25.3% ±8.0% respectively.

Figure 6: Progression free survival of total evaluable patients (n=111)
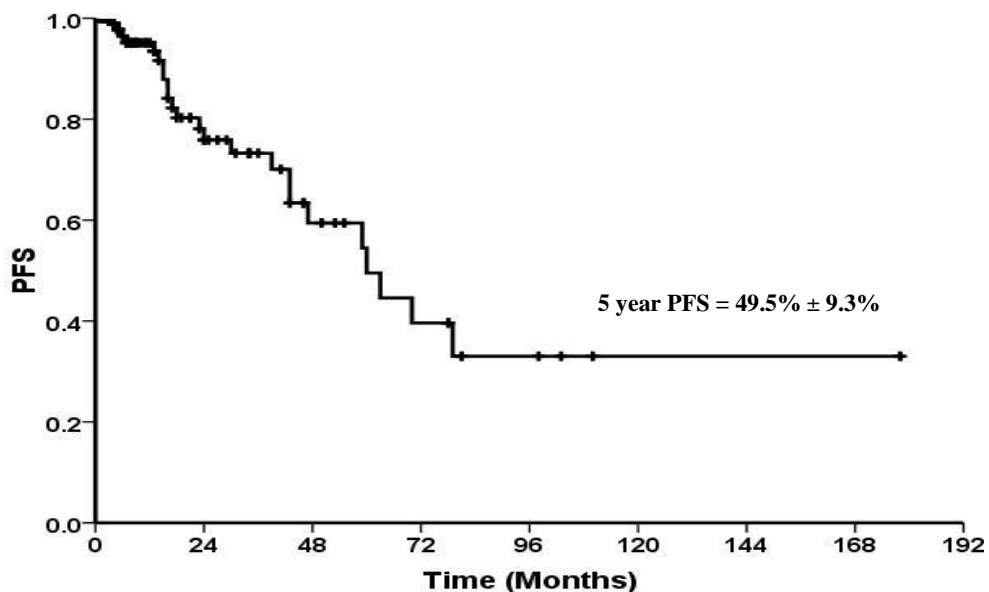
Figure 6: Kaplan Meier curve for Progression free survival of the entire cohort of evaluable patients (n=111). With a mean follow up duration of 86 months (Range: 1-78), the 5 year & 10 year PFS was 49.5% ± 9.3% & 33.0% ±10.1% respectively.

Figure 7: Disease free survival of total evaluable patients (n=111)
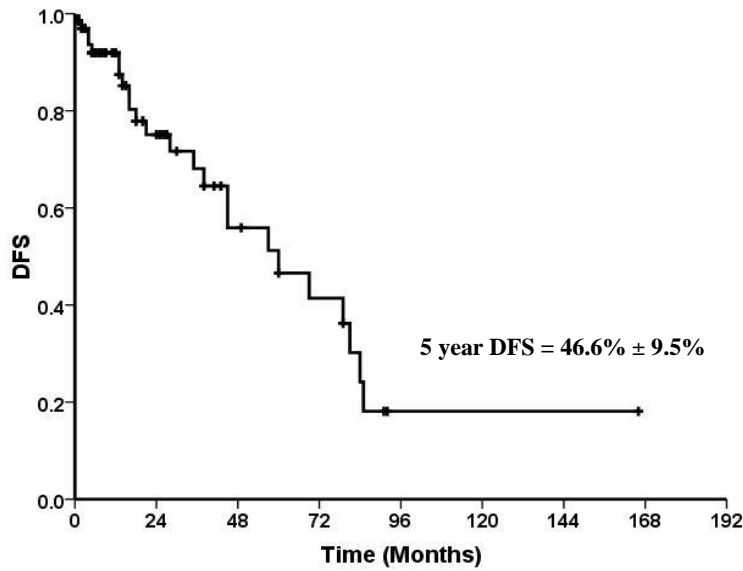
Figure 7: Kaplan Meier curve for Disease free survival of the entire cohort of evaluable patients (n=111). With a mean follow up duration of 69 months (Range: 1-166), the 5 year & 10 year DFS was 46.6% ± 9.5% & 18.1%±8.9% respectively.

SURVIVAL BY PROGNOSTIC RISK GROUPS:

Figure 8: OS of IPSS risk groups (n=79)
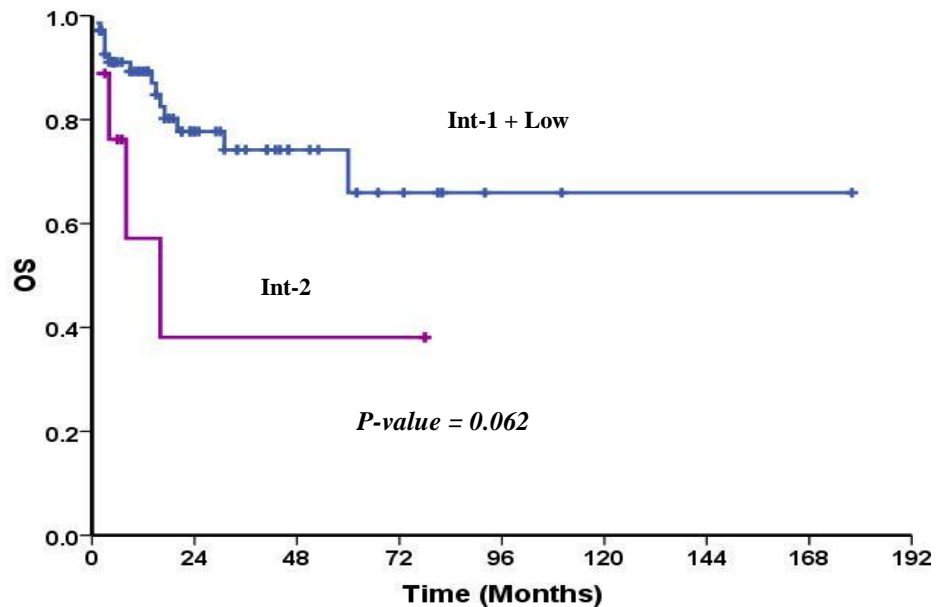
Figure 8: Kaplan Meier curve for OS IPSS risk groups. Among the 111 evaluable patients, cytogenetic data was available only in 79. With a mean follow up period of 125 months (range:1-178) and 34 months (range:1-78) the 5 year OS in the lower risk groups (Low+Int-1) versus higher risk groups (Int-2+high) was 65.9% ± 9.7% versus 38.1% ± 20.4% respectively.. The higher survival noted in the lower risk group was statistically near significant (p= 0.056).

Figure 9: OS of WPSS risk groups (n=79)
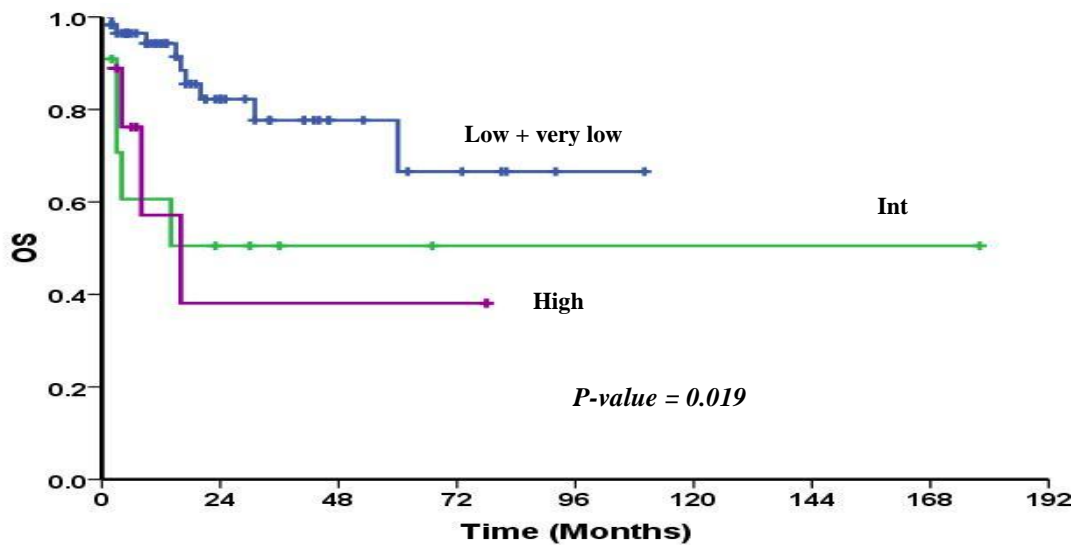
Figure 9: Kaplan Meier curve for OS of WPSS risk groups. With a mean follow up period was 83 (range:2-110), 92 (range:1-178) and 34 months (range:1-78) for the lower risk (very low + low risk), intermediate risk and high risk groups respectively, the 5 year OS were, Lower versus Int (66.6% ± 12.1% vs 50.5% ± 15.8%; *P value=0.026*), Lower vs High (66.6% ±12.1% vs 38.1%±20.4%; *P value=0.017*). OS was significantly higher in the lower risk groups than the higher risk groups (Int and high risk groups), but there was no significant survival advantage for the intermediate risk group over high risk group (*P value=0.973*).

Figure 10: OS of IPSS-R risk groups (n=79)
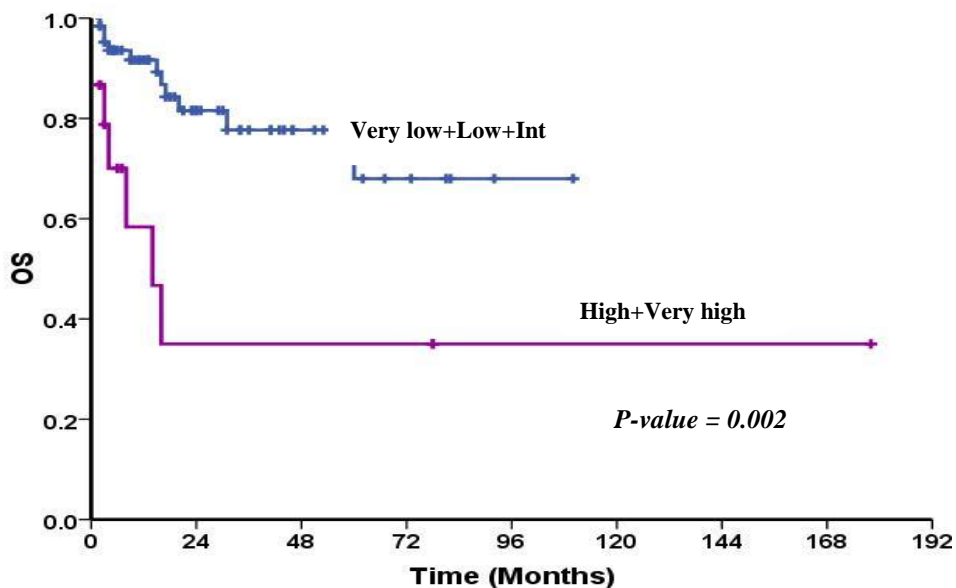
Figure 10: Kaplan Meier curve for OS of IPSS-R risk groups. With a mean follow up period of 83 months (range:2-110) for the lower risk group (very low+low+Int) and 67 months (1-178) for the higher risk group (high+very high), the 5 year OS was noted to be significantly higher in lower risk group than higher risk groups (High + very high), ie; 68.0% ± 10.8% *vs* 35.0% ± 15.7% (*P=0.002*).

Figure 11: OS by WHO classification for the entire evaluable patients (n=111)

Figure 11: Kaplan Meier curve for OS of WHO classification groups Using the WHO criteria, the out of the 111 evaluable patients, 82 belonged to RCMD, 26 to MDS unclassified and 3 to RAEB-1. With a mean follow up period of 109 (range:1-82), 61(2-78) and 8.5 (range:3-14) months, the 5 year OS were 61.2% ± 8.4%, 74.4 % ± 11.8% and 0 % ± 0% for RCMD, MDS-U and RAEB-1 respectively. MDS-U and RCMD showed significantly better survival than RAEB-1 (*P=0.000*).

Figure 12: Overall survival of responders to different drugs

Figure 12: **Kaplan Meier curve for OS of responders to different drugs.** The OS of the responders to different drugs showed no significant difference. The 5 yr OS of CSA vs Androgens was 96.9% ±3.1 % vs 51.4% ± 23.1% (*P=0.090*); ATG vs Androgens was 75.0 % ±21.7 % vs 51.4% ± 23.1% (*P=0.972*) and CSA vs ATG+CSA was 75.0 % ±21.7 % vs 96.9% ±3.1 % (*P=0.180*).

DISCUSSION:

Between January 1998 and June 2012, there were a total of 173 adult patients (age ≥18 years) diagnosed to have hypoplastic myelodysplastic syndrome from January 1998 to June, 30, 2012. The total number of patients seen in the outpatient department of Clinical Haematology during this period was 54413, out of which 1225 (2.3%) adult patients were diagnosed to have primary MDS. In this study, hMDS constituted 14.1% of MDS cases diagnosed during this period. This is similar to that reported in literature ; i.e. 10-15% of all MDS cases (2,4,7–9,14) [Fig:1].

In the present study the majority (41.6%; n=72) of the patients belonged to the age group 18-40 years, with a median age of 41 years (range: 18-64). In a study by Koh Y and colleagues, based on a medical record review at Seoul National University Hospital, 51 patients were diagnosed to have hMDS, and the median age reported was 39 years (12), similar to the observation in the present study. This is much lower than the median age of patients with normo/hypercellular MDS ie; 60–75 years (5). However in a comparative study of hypoplastic myelodysplastic syndrome (MDS) with normo-/hypercellular MDS by Huang et al, the median age reported was similar in both groups ie; 58 years (range: 26–86) in hMDS and 55 years in normo-/hypercellular MDS (1). In the present study, 112 were males (64.7%) and 61 females (35.3%), with a male: female ration of 1.8:1. A similar observation of male preponderance was reported by Huang et al (29:8) (1).

The median duration of symptoms before diagnosing hMDS was 3 months. The duration of symptoms before diagnosis is made ranges from 6-12 months as per reports by Hoffman and Koeffler (5). Majority of the patients in the present study (94.2%; n=163) were diagnosed following evaluation for pallor. Diagnosis was made following a routine medical check-up in 5.8% (n=10) of the patients. In MDS as a whole, it is reported that 50% of the patients are asymptomatic and are

diagnosed following routine check-up (5). The presence of occasional splenomegaly has been reported by some authors (3,16); in the present study 3.5% (n=6) of patients had mild splenomegaly [Table:1].

Haemogram showed that 95.4% (n=165) had a hemoglobin level below 10g%, with a median hemoglobin of 5.8g% (range: 1.2- 13.2). In the study by Huang et al (1) the median hemoglobin reported was 7.8g% (range: 4.0–14.0), a little higher than that observed in the present study. The median WBC count and ANC at diagnosis were 3.3×10^9/L (range: 0.2-13.8) and 1.05 $\times 10^9$/L (0.0-5.32) respectively. This is slightly higher than that reported by Huang et al (1) i.e. 2.37 $\times 10^9$/L (range:1.00–15.70) of WBC count and 0.98 $\times 10^9$/L (range: 0.196–10.360) of ANC. In the present study, majority of the patients had a severe thrombocytopenia with 61.3% (n=106) of patients presenting with platelet count <20 $\times 10^9$/L. The median platelet count was 14 $\times 10^9$/L (range:1-307), much lower than that observed in the study by Huang et al ie; 54 $\times 10^9$/L (range: 3–433) (1). However, only 70 patients presented with bleeding manifestations [Table:2].

The diagnosis of hMDS was based on the presence of a hypocellular marrow with features of dysplasia in one or more cell lines, increase in reticulin content, increase in the number of blasts/CD34+ cells on the bone marrow trephine, or abnormal karyotype, all favoring the diagnosis of hMDS (3,6,15,16). Out of the 169 cases where data on reticulin content was available, increase in reticulin content was seen in 79.9% (n=135) [Table3]. The median bone marrow blast percentage was 1 (range: 0-5), as compared to 2.6% (0–26.0) in the Huang et al study (1). All cases with bone marrow blasts greater than 5%, and/or those with a diagnosis of RAEB-2 or acute leukemia at presentation, irrespective of the presence of hypocellular marrow were excluded from the study. This may be the reason for the lower range of BM blasts observed in this study. Ring sideroblasts were observed in 48 cases (27.8%), of which only one (0.6%) had >15% ring

sideroblasts (RCMD-RS). This is similar to the study published by Huang et al (1), where only 2 (5.4%) cases were diagnosed to have RARS.

WHO classification of the cases in the present study showed that 77.5% (n=134) befitted the RCMD (Refractory cytopenia with multilineage dysplasia) group, while 20.2% (n=35) were MDS-U (MDS unclassified) and 4 (2.3%) were RAEB-1 (Refractory anemia with excess blasts-1) [Table:3]. In the studies by Nand S et al (8) and Huang et al (1), the FAB classification was followed. In these studies the FAB subgroups included RA (n=7), RARS (n=1) and RAEB (n=3) in the former and RA (n=21), RARS (n=2), RAEB (n=9) and RAEB-T (n=5) in the latter study respectively.

Cytogenetic data was available in 116 (67%) patients. A normal karyotype was found in 77 (66.4%). Earlier reports by Toyoma K et al have shown that patients with hMD frequently had complex aberrations (chromosome changes at three or more regions) (53). In the present study among the 39 (33.6%) patients with abnormal karyotype, 2.6% (n=3) had three aberrations and 12.9% (n=15) had more than 3 aberrations / monosomy 7. Among the numerical abnormalities observed in 55 (47.4%), majority had monosomies (n= 37). Monosomy 5 (-5)/del 5q (5q-) was observed in 9 (7.8%) cases, and monosomy 7 (-7) was found in 13 (11.2%) patients [Table: 4]. The reports on chromosomal aberrations in hypoplastic MDS are limited (1,8,9). In the report by Nand and Gonwinz (1,8), none of the nine h-MDS patients harboured monosomy 7/7q-, while only one out of 23 hMDS patients reported by Tuzuner et al (1,54) showed this abnormality. On the contrary, Maschek et al (1,9) demonstrated monosomy 7 in two out of the six h-MDS patients. The karyotype profile in the Huang et al (1) study showed that 57.6% (n=19) had normal karyotype, and among those with abnormal karyotype, 3% (n=1) had -5/5q-, none had chromosome7 abnormalities, 12.1% (n=4) had trisomy 8, 24.2% (n=8) had single aberration, 9.1% (n=3) had double aberrations and 9.1% (n=3) had complex aberrations. This observation is similar to the observation in the

present study except that chromosome7 abnormalities were observed in 11.2% of patients in this study. Further studies on more patients may be needed to clarify whether the variations in the frequency of cytogenetic abnormalities, especially involving chromosome7, in h-MDS observed among these reports may represent the difference of the pathogenesis of h-MDS in different geographical areas.

As per the IPSS/WPSS and IPSS-R cytogenetic risk categorization, in the present study, majority belonged to the Intermediate cytogenetic risk group (69% each in either category) [Table:5]. While applying various MDS prognostic scoring systems, majority fell into the intermediate -1 (82.8%; n=96) by IPSS, intermediate category (58.6%; n= 68) by IPSS-R, and low risk (68.1% (n= 79) by WPSS [Table: 6]. A similar observation was noted in the comparative study by Huang et al (1), where 57.6% (n=19) of patients were scored into the intermediate -1 risk category of IPSS. In our study none of the cases belonged to the IPSS high risk group, while 9.1% (n=3) cases belonged to high risk group in the study by Huang et al (1). This may be due to the fact that patients with RAEB-2 and Acute leukemia were excluded in the present study.

One hundred and eleven (64.2%) patients who had atleast 8 weeks follow up after initiation of therapy were evaluated for response. Eighty seven (78.4%) showed response, either 'Complete remission'[CR] (12.6%; n=14) or 'Stable disease" (65.8%; n=73) [Table:7]. On comparing the response rate to different drugs, the response rate to cyclosporine was found to be significantly higher than Androgens (69.5% vs 47.1%; *P=0.017*) . Although a 70% response rate was seen to ATG+CSA, this was not found to be significantly superior over other drugs [Fig:3]. The 5 year overall survival among the responders to the different drugs however showed no statistically significant difference [Fig;12]. The mean time to response were 5.9 months (range: 3-36) in CSA group, 1.8 months (range: 1-3) in ATG+CSA group and 5.3 months (1-27) in androgen group [Table:7]. In a prospective randomized multicenter phase III trial (where 9 out of total 45 cases

were hMDS) comparing Antithymocyte globulin + cyclosporine with best supportive care by Passweg JR et al (33), 29% of the total 45 patients showed response to ATG+CSA by 6 months. In a study on Cyclosporine therapy in hMDS by Jonasova A et al (55), out of the 9 patients of hMDS (of total 17 MDS cases), 8 showed response, and the time to response was observed to be between 3 to 9 months, similar to the observation in our study. However in another study by Catalano Let al (56), out of 9 patients with hypoplastic refractory anemia who were treated with cyclosporine, 3 showed response in a mean duration of 22 months (median 14.5 months); longer than that observed in this study.

Literature review shows reports suggesting better response to immunosuppressive therapies (CSA, ATG) in patients with hMDS by several authors (2,13,33,57,58). However data comparing the response to different drug groups in hMDS could not be found. In our study, the number of patients in other drug groups are very few (eg; ATG+CSA [n=10], Lenalidomide [n=3], Allogeneic PBSCT [n=5], Haematopoietic growth factors [n=1], and supportive treatment [n=4]), limiting significant correlative study between these groups.

For the entire cohort, with a median follow up duration of 110 months, the 5 year OS, EFS, PFS and DFS are 61.9%$\pm$7.2%, 37.9%$\pm$7.8%, 49.5%$\pm$9.3% and 46.6% $\pm$ 9.5% respectively [Figs: 4-7]. Bartl and colleagues, in a retrospective and prospective follow-up study of 495 patients with MDS between 1975 to 1991, reported a median survival of 29 months (n=95) in patients with hMDS (7). In the study by Huang et al (1), the OS was less than the present study (5b year OS: <60%; median survival = 58 months).

To identify different survival groups in h-MDS patients, we took advantage of the different risk scoring systems (IPSS, WPSS & IPSS-R). Obviously, in this study, h-MDS patients of lower risk groups had a significantly higher 5 year OS than those of higher risks groups in the WPSS (*P=0.018*) and IPSS-R (*P=0.002*) systems [Figs:9,10]. Risk categorization by the IPSS showed

only near significant survival benefit in the lower over the higher groups [Fig:8]. Our study demonstrate that in hypoplastic MDS, WPSS and IPSS-R prognostic scoring systems are better than the IPSS systems in predicting survival advantage. However in the comparative study by Huang et al (1), a significant survival difference was observed between these two groups in h-MDS patients (median survival 112 vs 16 months, *P=0.002*). The OS of IPSS lower risk groups reported by Huang et al (1), however is much lower than that observed in our study (5 year OS: <40%) versus 66.0% ± 9.7%) .

The overall mortality was 26 patients (15% of the total 173 cases and 23.4% of the 111 evaluable patients). Of these 4 had shown progression to Acute myeloid leukemia, whereas one patient developed metastatic adenocarcinoma along with progression of disease. With a mean follow up duration of 110 months in 111 evaluable patients, the cumulated incidence of transformation to acute leukemia at 5 years was 3.6 % (n=4) [Table:8]. In the study by Huang et al (1), with a median follow-up duration of 98 months in 187 evaluable patients, the cumulated incidence of acute leukemic transformation at 7 years was 8.1% for h-MDS. This is higher than the observation in our study. This may be due to the fact that hypocellular RAEB 2 was excluded from our study.

Univariate Cox proportional hazard model was used to find out significant prognostic factors for survival among overall hMDS patients [Tables:10-12]. Parameters of independent significance for adverse effects on overall survival were ANC < 0.2 $\times 10^9$/L at diagnosis, bone marrow blast at diagnosis >5%, WHO category RAEB-1, moderate/marked increase in BM reticulin, >3 cytogenetic anomalies, monosomy7, trisomy 21, WPSS intermediate risk group, WPSS high risk group , and IPSS-R very high risk group. In the multivariate model by Huang et al (1), parameters of independent significance for overall survival were age, marrow hypocellularity, RA with excess of blast, RA with excess of blast-T, monosomy 5 or 5q deletion and monosomy 7

or 7q deletion. However on multivariate analysis, none of the above parameters retained its statistical significance.

LIMITATIONS OF THE STUDY:

1. Retrospective study: limited available data due to non-retrievable records, intractability of patients due to wrongly recorded/changed/non-availability of address.

2. Not all hypocellular MDS were recruited in this study; RAEB-2 and AML with hypocellular marrow were excluded. In the reported studies on hMDS; all categories of hypocellular MDS are included into the group hypoplastic MDS. This limits the comparison with these studies.

3. Karyotyping is not available for all patients- limited by the nature of the study.

4. Lesser number of patients in drug groups limiting comparison between drug groups.

CONCLUSION:

Hypoplastic MDS is a distinct subgroup of MDS of unknown etiology which needs to be distinguished from aplastic anemia. It is a disease associated with a relatively good prognosis, with significant response to immunosuppressive therapy and reasonable response to treatment with androgens, and a lower probability for leukemic transformation. Cytogenetic analysis at diagnosis is crucial in prognostic risk categorization of the patient. WHO classification based prognostic scoring system and revised IPSS appear to be better than IPSS in predicting survival.

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APPENDIX-1

(Table: 1)   **Criteria for dysplasia in the three different cell lineages**

Granulocytic dysplasia	Erythroid dysplasia	Megakaryocytic dysplasia
Hypogranularity	Anisopoikilocytosis	Small/large megakaryocytes
Abnormal nuclear segmentation	Nuclear lobulation, budding, karyorrhexis	Abnormal nuclear lobulation Hypo- or hyperlobulated
Hyposegmentation (most common)	Megaloblastoid changes	Large, hypogranular platelets
Hypersegmentation	Abnormal iron incorporation - Ring sideroblasts	Megakaryocytic clustering in core biopsy
Hypercondensed nuclear chromatin	Increased coarse iron granules	Platelet dysfunction
Megaloblastoid changes	Loss of colony formation in core biopsy	
Abnormal localization of immature precursors (ALIP) in core biopsy	Defective hemoglobinization	
Neutrophil dysfunction		

(Table:2)   **WHO Classification of Myelodysplastic Syndromes**

Disease	Blood findings	Bone marrow findings
RCUD (RA,RN,RT)	Unicytopenia or bicytopenia; blasts (<1%)	Dysplasia (≥10%) in 1 lineage only; <5% blasts; <15% ring sideroblasts
RARS	Anemia; no blasts	Erythroid dysplasia only;<5% blasts; ≥15% ring sideroblasts
RCMD	Cytopenia(s); blasts (<1%)	Dysplasia in >10% of the cells of ≥2 myeloid lineages, <5% blasts; ±15% ring sideroblasts; no Auer rods
RAEB-1	Cytopenias; <5% blasts, no Auer rods	Unilineage or multilineage dysplasia; 5%-9% blasts, no Auer rods
RAEB-2	Cytopenias; 5%-19% blasts; Auer rods +/–	Unilineage or multilineage dysplasia; 10%-19% blasts, Auer rods +/–
MDS- U	Cytopenia(s); <1% blasts	Uneqivocal dysplasia in <10% of the cells in one/ more myeloid cell lines when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS; <5% blasts
MDS with isolated del(5q)	Anemia; usually normal or slightly increased platelets; <5% blasts; no Auer rods	Normal to increased megakaryocytes with hypolobated nuclei; Unilineage erythroid dysplasia; <5% blasts; del (5q) is sole cytogenetic abnormality; no Auer rods

(Table: 3) Calculation of flow cytometric score (FCM-score) for diagnosis of low-risk MDS

Cytometric Parameter	Cut-off values	Regression coefficient	Variable weighted Score[#]
Myeloblast-related cluster size (%)*	≥ 2	2.59	1
B-progenitor-related cluster size (%)**	≤ 5	1.87	1
Lymphocyte to myeloblast CD45 ratio	≤ 4 or≥ 7.5	1.76	1
Granulocyte to lymphocyte SSC ratio	≤ 6	2.31	1

*In all nucleated cells; **in all CD34+ cells; # a diagnosis of MDS is formulated in the presence of a FCM-score value ≥ 2.*

(Table:4)   Most frequent chromosomal aberrations in MDS patients

Numerical (%)	Translocations (%)	Deletions (%)
+8* (19%)	inv 3 (7%)	del 5q (27%)
−7 (15%)	t(1;7) (2%)	del 11q (7%)
+21 (7%)	t(1;3) (1%)	del 12q (5%)
−5 (7%)	t(3;3) (1%)	del 20q (5%)
-Y* (5%)	t(6;9) (<1%)	del 7q (4%)
	t(5;12) (<1%)	del 13q (2%)

Symbols: −, loss of chromosome; +, additional chromosome; inv, inversion; t, translocation; del, deletion. *In the absence of morphologic criteria, the presence of these abnormalities as the sole cytogenetic abnormality is not considered as a definitive evidence for MDS. The other abnormalities if present in the setting of persistent cytopenia of undetermined origin, in the absence of definitive morphologic features, are considered as presumptive evidence of MDS.

(Table:5)   International Prognostic Scoring System (IPSS)

Prognostic variables	Score value				
	0	**0.5**	**1.0**	**1.5**	**2.0**
BM blasts (%)	<5	5-10	-	11-20	21-30
Karyotype	Good	Intermediate	Poor		
Cytopenias	0/1	2/3			

IPSS Prognostic Risk Category & Clinical Outcomes

Risk categories	Low	Intermediate-1	Intermediate -2	High
Score	0	0.5-1.0	1.5-2.0	≥2.5
Median survival (yrs)	5.7	3.5	1.2	0.4
25% AML Evolution(yrs)	9.4	3.3	1.1	0.2

(Table: 6)   Revised International Prognostic Scoring System (IPSS-R)

Variable	Score						
	0	**0.5**	**1**	**1.5**	**2**	**3**	**4**
Cytogenetic group	Very good	-	Good	-	Intermediate	Poor	Very poor
Blasts	≥2	-	>2-<5%	-	5-10%	>10%	-
Hemoglobin	≥10	-	8-<10	<8	-	-	-
Platelet	≥100	50-<100	<50	-	-	-	-
ANC	≥0.8	<0.8	-	-	-	-	-

IPSS-R Prognostic Risk Category & Clinical Outcomes

Risk groups	Very Low	Low	Intermediate	High	Very High
Score	≤1.5,	>1.5-3	>3 – 4.5	>4.5 – 6	>6
Survival	8.8	5.3	3.0	1.6	0.8
AML/25%	NR	10.8	3.2	1.4	0.7

(Table: 7)   WHO classification-based prognostic scoring system for MDS (WPSS)

Variables	Score			
	0	**1**	**2**	**3**
WHO Category	RA, RARS, 5q-	RCMD	RAEB-1	RAEB-2
Karyotype	Good	Intermediate	Poor	-
Transfusion requirement*	None	Regular	-	-

WPSS Prognostic Risk Category & Clinical Outcomes					
Risk category	**Very low**	**Low**	**Intermediate**	High	Very high
Score	0	1	2	3 - 4	5 – 6
Median OS (months)	141	66	48	26	9
2yr probability of progression to AML	3%	6%	21%	38%	80%

*Transfusion dependency is defined as at least one red transfusion every 8 weeks over a period of 4 months.

(Table: 8a)The Global MDACC MDS Prognostic Model

Prognostic factor	Points
PS ≥2	2
Age :	
60-64	1
64	2
Platelets x 109/L	
30	3
30 – 49	2
50 – 199	1
Hemoglobin <12g%	2
BM blasts	
5-10	1
11-19	2
WBC .20	2
Alteration of chromosome 7 or ≥3 alterations	3
Prior transfusion	1

PS, performance status; BM, bone marrow; WBC- white blood cell count. Patients with 0 to 4 points had a median survival of 54 months and a 3 year 63% survival. Patients with 5 and 6 points had a median survival of 23 to 30 months and 3-year survival of 30 to 40%. Patients with 7 to 8 points had a median survival of 13 months and a 3-year survival rate of 13 to 19%. Patients with 9 or more points had a median survival of 5 to 10 months and a 2% 3-year survival. Adapted from Ref. 16.

(Table: 8b)   MDACC MDS Lower Risk Prognostic Model

Characteristics	Points
Unfavorable cytogenetics	1
Age ≥60 years	2
Hemoglobin < 10 (g/dL)	1
Platelets 10^9/l <50l 50-200	 2 1
Bone Marrow Blasts >4%	1
Risk group assignments	
Category 1	0-2
Category 2	3-4
Category 3	5-7

(Table: 9)   A Prognostic Model of Hypoplastic MDS.

Prognostic factor	P-value
Hemoglobin <10 g/dl	0.00026
Performance status >2	0.00484
Unfavorable cytogenetics	0.00667
Bone marrow blast ≥5%	0.00765
Serum LDH >600 IU/l	0.00990
Estimated survival according to independent risk factors in the study group				

Risk group	No. of Risk factors	Patient n (%)	Median (months)	2-year/3-year survival, %
Low	**0**	17 (10)	Not reached	71/61
	1	49 (29)	27	59/38
Intermediate	**2**	44 (26)	19.4	43/20
High	**3**	39 (23)	9.3	14/7
	4	17 (10)	4.7	12/6
	5	3 (2)	2	0/0

(Table:10a) Modified International Working Group (IWG) response criteria for MDS

Proposed modified IWG response criteria for altering natural history of MDS	
Category	**Response criteria (responses must last at least 4 week)**
Complete remission	Bone marrow: <5% myeloblasts with normal maturation of all cell lines* Persistent dysplasia will be noted*† Peripheral blood‡ Hgb ≥ 11 g/dL Platelets ≥ 100 x10^9/L Neutrophils ≥1.0 x 10^9/L† Blasts - 0%
Partial remission	All CR criteria if abnormal before treatment except: Bone marrow blasts decreased by ≥ 50% over pretreatment but still >5% Cellularity and morphology not relevant
Marrow CR†	Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment† Peripheral blood: if HI responses, they will be noted in addition to marrow CR†
Stable disease	Failure to achieve at least PR, but no evidence of progression for > 8 weeks
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Relapse after CR or PR	At least 1 of the following: Return to pretreatment bone marrow blast percentage Decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets Reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence
Cytogenetic response	Complete: Disappearance of the chromosomal abnormality without appearance of new ones Partial: At least 50% reduction of the chromosomal abnormality
Disease progression	For patients with: Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts 5%-10% blasts: ≥ 50% increase to > 10% blasts 10%-20% blasts: ≥ 50% increase to > 20% blasts 20%-30% blasts: ≥ 50% increase to > 30% blasts **Any of the following:** At least 50% decrement from maximum remission/response in granulocytes or platelets Reduction in Hgb by ≥ 2 g/dL Transfusion dependence
Survival	Endpoints: Overall: death from any cause Event free: failure or death from any cause PFS: disease progression or death from MDS DFS: time to relapse Cause-specific death: death related to MDS

Deletions to IWG response criteria are not shown. To convert hemoglobin from grams per deciliter to grams per liter, multiply grams per deciliter by 10. MDS indicates myelodysplastic syndromes; Hgb, hemoglobin; CR, complete remission; HI, hematologic improvement; PR, partial remission; FAB, French-American-British; AML, acute myeloid leukemia; PFS, progression-free survival; DFS, disease-free survival. *Dysplastic changes should consider the normal range of dysplastic changes (modification).†Modification to IWG response criteria. ‡In some circumstances, protocol therapy may require the initiation of further treatment (eg, consolidation, maintenance) before the 4-week period. Such patients can be included in the response category into which they fit at the time the therapy is started. Transient cytopenias during repeated chemotherapy courses should not be considered as interrupting durability of response, as long as they recover to the improved counts of the previous course.

Table: 10b <u>Modified IWG response criteria for hematologic improvement</u>

Proposed modified International Working Group response criteria for hematologic improvement	
Erythroid response (pretreatment, < 11 g/dL)	Hgb increase by $\geq$ 1.5 g/dL Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of $\leq$ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation†
Platelet response (pretreatment,<100 x 10^9/L)	Absolute increase of $\geq$ 30 x 10^9/L for patients starting with > 20 x 10^9/L platelets Increase from < 20 x 10^9/L to > 20 x 10^9/L and by at least 100%†
Neutrophil response (pretreatment, < 1.0 x 10^9/L)	At least 100% increase and an absolute increase > 0.5 x 10^9/L
Progression or relapse after HI‡	**At least 1 of the following**: At least 50% decrement from maximum response levels in granulocytes or platelets Reduction in Hgb by $\geq$ 1.5 g/dL Transfusion dependence

Deletions to the IWG response criteria are not shown. To convert hemoglobin levels from grams per deciliter to grams per liter, multiply grams per deciliter by 10. Hgb indicates hemoglobin; RBC: red blood cell; HI: hematologic improvement. *Pretreatment counts averages of at least 2 measurements (not influenced by transfusions) > 1 week apart (modification). †Modification to IWG response criteria. ‡In the absence of another explanation, such as acute infection, repeated courses of chemotherapy (modification), gastrointestinal bleeding, hemolysis, and so forth. It is recommended that the 2 kinds of erythroid and platelet responses be reported overall as well as by the individual response pattern.

<u>PROFORMA:</u>

HYPOPLASTIC MDS DATA COLLECTION PROFORMA

Sl No: ………..

1. Name: 2. Hospital number:
3. Age at diagnosis: 4. Sex:
5. Diagnosis: 6. Date of diagnosis:
7. Symptomatic at diagnosis:
8. If yes, what symptoms:

     8a. fever   (1. Yes   2. No)   8b. pallor   (1. Yes   2. No)
     8c. breathlessness   (1. Yes   2. No)   8d. skin bleed   (1. Yes   2. No)
     8e. gum bleed   (1. Yes   2. No)   8f. epistaxis   (1. Yes   2. No)
     8g. hematuria   (1. Yes   2. No)   8h. systemic infections (1. Yes   2. No)
     8i. skin infections   (1. Yes   2. No)   8j. Others (mention):

9. Transfusion requirement (1. Yes   2. No)
10. Duration of symptoms in months:
11. Previous treatment taken (1. Yes   2. No 3.NA) If yes, what treatment?

     11a. dexamethasone   (1. Yes   2. No)   11b. prednisolone   (1. Yes   2. No)
     11c danazol   (1. Yes   2. No)   11d. stanazolol   (1. Yes   2. No)
     11e. cyclosporine   (1. Yes   2. No)   11f. thalidomide   (1. Yes   2. No)
     11g. lenalidomide   (1. Yes   2. No)   11h. ATG   (1. Yes   2. No)
     11i. EPO   (1. Yes   2. No)   11j. PC transfusion (1. Yes   2. No)
     11k. if yes PC number: 11L. PRC transfusion: (1. Yes   2. No)
     11m. If yes PRC number: 11n. previous treatment duration in months:
     11o. Others (mention):

12. **Associated illness**:   (1. Yes   2. No)

     12a. If yes type of disease:
     12b. drug for associated illness:
     12c. treatment duration of associllness in months:

13. **History of malignancy**:   (1. Yes   2. No)

     13a. if yes type:
     13b. history of chemotherapy drug: (1.yes   2.No)
     13c. if yes types of drugs:
     13d. duration of chemotherapy in months:
     13e. last date of chemotherapy:

14. **Clinical examination findings**: (1.yes   2.No)

     14a. pallor: (1.yes   2.No)   14b. skin bleeds: (1.yes   2.No)
     14c. gum bleeds: (1.yes   2.No)   14d. haematuria: (1.yes   2.No)
     14e. LNE: (1.yes   2.No)   14f. hepatomegaly: (1.yes   2.No)
     14g. spleenomegaly: (1.yes   2.No)   14h. dysmorphic features:

LABORATORY FINDINGS AT DIAGNOSIS:

     15. **Peripheral blood**

          15a. Hb g%:   15b. TWBC per cumm:
          15c. Platelet per cumm: 15d. ANC per cumm:
          15e. Blasts %:   15f. ALC per cumm:

15g. AEC per cumm: 15h. ARC per cumm:

15i. LDH: 15j. EPO:

15k. Vit B12 (if done):   15L. folate 9if done):

15m. Monocytosis: (1.yes   2.No) 15n. DCT (if done):

15o. ANA (if done):   15p. PNH IPT (if done):

16. **Bone marrow study**:

16a.BM blasts%:   16b.BM Asp Celularity:

16c.BM biopsy Celularity: 16d.Erythroid dysplasia:

16e.Myeloid dysplasia: 16f.Megakaryocyte dysplasia:

16g.BM Ring sideroblasts: 16h.BM mast cells:

16i.BM plasma cells: 16j.BM Eosinophils:

16k.BM Monocytosis: 16L.BM Erythroids:

16m.BM Myeloids: 16n.BM Megakaryocytes:

16o.BM Reticulin: 16p.Cytogenetics:

17.**IPSS** score: 17a.IPSS risk categ:

18. **WPSS** score: 18a.WPSS risk categ:

19. **IPSS-R** score: 19a.   IPSS-R risk category:

20. First Treatment :

20a.type of first treatment: 20b. dose of first drug (mg):

20c .date of starting first treatment: 20d. duration of first treatment in months:

21. HI to first treatment: (1.Yes   2.No   3.NA)

21a. if yes what HI: (1. EI   2.NI   3.PI)   21b. date of HI:

22. Overall response to first treatment: (1.Yes   2.No   3.NA)

22a. if yes what response: 22b. date of response to first treatment:

23. Second Treatment:

23a.type of second treatment: 23b. dose of second drug (mg):

23c. date of starting second treatment: 23d. duration of second treatment in months:

24. HI to second treatment: (1.Yes   2.No   3.NA)

24a. if yes what HI: (1. EI   2.NI   3.PI)   24b. date of HI :

25. Overall response to second treatment: (1.yes   2.No)

25a.if yes what response: 25b. date of response to second treatment:

26. Third Treatment: (1.Yes   2.No   3.NA)

26a. type of third treatment: 26b. dose of third drug (mg):

26c. date of starting third treatment: 26d. duration of third treatment in months:

27. HI to third treatment: (1.Yes   2.No   3.NA)

27a.if yes what HI;   (1. EI   2.NI   3.PI)   27b. date of HI:

28. Overall response to third treatment: (1.yes   2.No)

28a.if yes what response: 28b.date of response to third treatment:

29. Relapse of disease: (1.Yes   2.No   3.NA)

29a. if yes date of relapse:

29b. treatment at relapse:

29c. response to relapse treatment: (1.Yes   2.No   3.NA)

29d.type of response:

30. CLINICAL STATUS & BLOOD COUNTS AT FOLLOW UP:

31.

Date	Symptoms	Transfusions	Hb	TWBC	ANC	Platelet	Response

Chelation: (1.Yes   2.No   3.NA)
        32a. Date of starting chelation:
        32b. Ferritin level:
        32c. Chelation drug:_
        32d.Duration of chelation in months:

32.   **PBSCT** :   (1.yes   2.No)
        33a. Donor;
        33b. HLA match with donor:
        33c. Date of PBSCT:
        33d. disease status of disease at PBSCT:
        33e. Transfusion requirement at PBSCT: (1.Yes   2.No)
        33f. PC transfusions since diagnosis to PBSCT :
        33g.PRC transfusions since diagnosis to PBSCT:
        33h. serum ferritin at PBSCT:
        33i. Response to PBSCT:
        33j. complications of PBSCT:

33.   **LAST FOLLOW UP DATE**:
        34a. Status at LFU: (1.Alive   2.Dead   3.Not known/lost to FU)
        34b. Symptoms at LFU:
        34c. Hb at LFU:
        34d.Platelets at LFU:
        34e. ANC at LFU:
        34f. Disease status at LFU:
        34g.Drug at LFU:

34.If dead, date of death:
        35a.Cause of death:

MASTER CHART:

sno	Age	Sex	DOD	WHO gr	Du Sym	Pallor	Bleed	Infection	Liver	Spleen	PC	PRC	h/o Cytopenia	Trt res	Hb	WBC	Blast %	ANC	PLT	Retic	ARC	LDH	FERITIN
1	66	2	1-2-2012	1	1	1	1	2	2	2	na	na	4	9	5	4300	0	2322	3000	na	na	308	na
2	47	2	2-1-2012	1	3	1	2	2	2	2	6	na	4	9	3	12300	0	1476	11000	2.03	65366	486	na
3	51	2	2-24-2012	1	7	1	2	2	2	2	12	na	4	9	6	4000	0	1520	11000	2.09	48070	321	na
4	58	1	3-16-2012	3	12	1	2	1	2	2	2	na	4	9	7	1200	0	144	95000	1.15	39100	409	na
5	42	1	6-18-2012	1	24	1	2	2	1	2	3	na	4	9	7	3600	0	1368	44000	na	na	456	na
6	19	2	2-16-2012	3	1	1	1	1	2	2	2	2	4	9	5	3700	0	2220	45000	5.74	13489	318	na
7	20	2	5-25-2012	1	2	1	1	2	2	2	3	3	4	9	6	3900	0	234	16000	0.55	9735	261	na
8	62	2	5-14-2012	3	10	1	1	1	2	2	5	2	4	9	9	5100	0	1683	8000	1.49	44700	na	na
9	19	1	12.31.2011	1	1	1	2	2	2	2	3	na	4	9	3	2400	0	456	12000	na	na	327	na
10	37	1	2-7-2011	3	1	1	1	1	2	2	na	na	4	9	4	1100	0	528	5000	1.26	12222	373	na
11	41	2	7-8-2011	3	120	1	2	2	2	2	60	na	4	9	6	2700	0	1053	56000	1.5	37200	947	46775
12	60	1	3-4-2011	1	1	1	2	2	2	2	2	na	4	9	7	7500	0	5175	21000	na	na	600	na
13	31	1	7-22-2011	3	2	1	2	1	2	2	7	na	4	9	5	1400	0	560	14000	2.57	34181	409	na
14	66	1	6-8-2011	1	3	1	2	1	2	2	10	na	4	9	10	900	0	180	13000	na	na	na	na
15	56	1	9-12-2011	1	7	1	2	2	2	2	4	na	4	9	5	3100	0	1054	13000	1.85	53095	599	na
16	26	1	10-5-2011	1	2	1	1	1	2	2	12	na	4	9	2	1800	0	486	8000	0.82	24272	392	na
17	45	1	8-3-2011	1	3	1	1	1	2	2	6	na	4	9	7	4900	0	1568	2000	1.01	20503	na	na
18	68	1	1-1-2011	1	12	1	2	2	1	2	16	na	4	9	3	2100	0	714	14000	na	na	na	na
19	33	2	2-14-2011	3	4	2	1	2	2	2	7	na	1	3	7	3300	0	1254	11000	3.31	61897	437	na
20	63	1	3-4-2011	3	1	1	1	1	2	2	1	na	4	9	6	3600	0	1800	5000	2.4	65750	498	na
21	57	1	5-17-2010	3	1	1	1	1	2	2	na	na	4	9	7	2900	0	116	5000	1.96	39984	319	na
22	36	1	8-10-2010	3	3	1	2	1	2	2	18	na	4	9	8	2200	0	1100	43000	na	na	380	na
23	37	1	1-4-2010	3	3	1	1	2	2	2	5	na	1	1	6	3300	0	1419	46000	1.83	49842	379	na
24	30	1	1-19-2010	1	1	1	1	2	2	2	na	na	1	3	7	2400	0	480	11000	na	na	316	na
25	58	2	3-1-2010	1	1	1	1	1	2	2	na	na	4	9	8	3000	0	1110	31000	1.98	59796	566	na
26	39	2	3-3-2010	1	7	1	2	1	2	2	14	na	4	9	6	4200	0	546	19000	0.92	21436	415	na
27	41	1	4-7-2010	1	1	1	1	1	2	2	na	na	1	3	5	1600	0	64	12000	2.59	35742	515	2595
28	61	1	6-29-2010	1	1	1	2	2	2	2	4	na	9	9	6	2300	0	943	247000	0.82	26896	na	883
29	59	1	7-30-2010	1	4	1	1	1	2	2	7	na	4	9	6	2200	0	660	7000	2.02	14342	288	na
30	30	2	9-18-2010	1	24	1	2	2	2	2	10	5	4	9	6	2100	0	1428	7000	na	na	832	na
31	31	1	11-19-2010	1	5	1	1	1	2	2	10	7	1	3	7	2500	0	500	5000	3.37	31341	335	na
32	45	1	10-9-2010	1	1	1	2	1	2	2	2	4	4	9	5	3600	0	972	17000	2.51	41415	385	na
33	25	1	12-27-2010	1	2	1	2	1	2	2	6	4	4	9	7	3700	0	1332	11000	na	na	629	na
34	35	1	12-2-2010	1	2	1	2	1	2	2	na	na	4	9	7	2000	0	680	44000	4	80400	395	na
35	28	2	11-12-2010	1	1	2	1	2	2	2	na	na	1	2	9	1800	0	1224	4000	na	na	na	na
36	31	1	12-28-2010	1	3	1	1	2	2	2	2	na	1	3	4	3800	0	1216	13000	2.04	51204	523	na
37	20	2	7-1-2010	3	8	1	2	2	2	2	10	na	4	9	3	3900	0	351	17000	1.44	43920	282	510
38	46	2	1-9-2009	1	5	1	1	1	2	2	6	na	4	9	4	3200	0	1280	8000	na	na	528	697
39	54	2	1-12-2009	2	1	1	2	1	2	2	3	na	4	9	6	2100	0	252	21000	0.19	6175	395	na
40	33	1	3-29-2009	1	3	1	1	1	2	2	4	6	4	9	8	4600	0	920	11000	0.44	16192	444	na

Sl no: serial number, DOD:date of diagnosis, Dur symp:duration of symptoms, Trt res: Treatment response.

Sl No	DCT	ANA	BBVS	BM Cellularity	Dysplasia	Blast %	RS	Megs	Mye	Ery	Reticulin	Cyt-IPSS/WPSS	Cyt IPSS-R	IPSS score	WPSS score	IPSS-R score	CSA	CSA-res	ATG	ATG-res	Andr	Andr-res
1	9	9	4	2	3	2	2	4	2	2	1	2	2	0.5	1	3.5	2	4	2	4	1	4
2	9	9	9	2	4	0	1	2	4	3	2	2	2	0.5	1	3.5	1	2	2	4	2	4
3	9	9	4	4	2	0	2	2	2	3	3	2	2	0.5	1	3.5	2	4	2	4	1	2
4	9	9	4	2	1	0	2	2	2	2	2	2	2	0.5	1	3	1	3	2	4	1	3
5	1	2	4	2	4	3	1	2	2	2	2	9	9	na	9	na	2	4	2	4	1	2
6	1	1	4	2	1	1	9	2	2	3	2	2	2	0.5	1	3.5	2	4	1	2	2	4
7	9	9	4	2	3	1	2	2	2	2	1	2	2	0.5	1	4	2	4	2	4	1	2
8	9	9	4	2	8	1	2	2	2	2	2	2	2	0.5	1	3	1	2	2	4	2	4
9	9	9	4	4	2	2	2	2	2	2	1	2	2	0.5	1	4	2	4	1	1	2	4
10	2	9	9	2	1	2	9	2	2	2	2	9	9	na	9	na	2	4	2	4	1	3
11	1	9	4	2	6	1	9	2	2	3	3	9	9	na	9	na	2	4	2	4	1	2
12	1	9	4	2	2	0	1	2	2	3	2	9	9	na	9	na	2	4	1	1	2	4
13	2	9	4	2	1	0	1	2	2	3	2	2	2	0.5	1	4	1	2	2	4	1	2
14	9	9	4	2	2	2	1	2	2	2	1	9	9	na	9	na	2	4	2	4	1	4
15	9	9	4	4	4	0	2	2	1	3	2	2	2	0.5	1	3.5	1	2	2	4	1	1
16	9	9	4	2	4	1	2	2	2	2	2	4	5	1.5	3	7	1	4	2	4	2	4
17	9	9	9	1	3	0	2	4	2	2	1	9	9	na	9	na	1	3	2	4	2	4
18	9	9	9	2	7	2	2	2	2	2	2	2	2	0.5	1	4	2	4	2	4	2	4
19	2	2	4	2	1	2	1	2	2	2	2	2	2	0.5	1	3.5	1	2	2	4	1	2
20	2	2	4	2	1	0	2	4	2	3	2	9	9	na	9	na	1	4	2	4	2	4
21	9	9	4	4	1	0	1	2	2	2	3	2	2	0.5	1	4	1	2	2	4	2	4
22	9	9	9	2	6	0	9	2	2	3	4	9	9	na	9	na	1	2	2	4	2	4
23	9	9	1	2	6	0	9	2	2	2	3	3	1	1	2	2.5	1	3	2	4	1	1
24	2	9	4	2	7	1	2	2	2	2	2	2	2	0.5	1	4	2	4	1	2	1	3
25	1	1	4	2	2	1	2	2	2	2	2	2	2	0.5	1	3.5	2	4	2	4	2	4
26	9	9	4	2	4	1	2	2	2	2	1	3	3	1	2	5	2	4	2	4	1	2
27	9	9	4	4	2	1	1	2	2	2	2	2	1	0.5	1	3	2	4	1	2	1	3
28	9	9	4	2	2	0	1	2	2	3	2	9	9	na	9	na	1	4	2	4	2	4
29	9	9	9	2	3	1	1	4	2	2	2	9	9	na	9	na	2	4	2	4	1	4
30	9	9	4	2	7	4	2	1	2	2	3	9	9	na	9	na	2	4	2	4	1	2
31	9	9	4	2	4	0	2	2	2	2	2	9	9	na	9	na	2	4	1	3	1	2
32	2	9	9	2	3	0	1	2	2	2	2	9	9	na	9	na	2	4	2	4	1	2
33	2	2	4	2	4	1	1	2	2	2	2	2	2	0.5	1	3.5	1	1	2	4	2	4
34	9	9	4	2	2	1	2	2	2	2	2	2	2	0.5	1	4	1	2	2	4	2	4
35	9	9	4	2	4	0	1	2	2	2	2	2	2	0.5	1	3	2	4	2	4	1	2
36	9	9	4	2	4	1	1	4	2	2	1	2	2	0.5	1	3.5	1	2	2	4	2	4
37	1	9	4	2	1	0	1	2	2	2	2	9	9	na	9	na	1	2	2	4	2	4
38	2	9	4	2	4	0	1	4	2	2	1	2	2	0.5	1	3.5	2	4	2	4	1	2
39	1	2	4	2	4	5	2	1	2	2	2	2	2	0.5	1	6	2	4	2	4	2	4
40	9	9	4	4	7	2	2	2	2	2	3	9	9	na	9	na	1	2	2	4	1	4

DCT: direct coomb's test, ANA:antinuclear antibody, BBVS: blood borne viral screen, RS: ring sideroblasts, Megs, Megakaryocytes, Mye: Myeloids, Ery:Erythroids, CSA: cyslosporine Treatment, CSA-res: Reposne to CSA, ATG-res: Response to ATG.

Sl No	Pred	Pred-res	Allo	Allo-res	Others	Others-res	Sup	Sup-res	Response	DO res	DO relapse	Trt rela	Do LFU	Alive/Dead	Drg LFU	Disease statusLFU	Cause death
1	2	4	2	4	1	3	2	4	4	na	na	18	1-21-2012	2	3	7	9
2	2	4	2	4	2	4	2	4	2	5-8-2012	na	18	12-15-2012	2	1	13	9
3	2	4	2	4	2	4	2	4	2	6-16-2012	na	18	12-1-2012	2	3	17	9
4	2	4	2	4	2	4	2	4	3	na	na	18	12-4-2012	1	15	4	2
5	2	4	2	4	2	4	2	4	2	10-16-2012	na	18	11-30-2012	2	2	6	9
6	2	4	2	4	2	4	2	4	2	6-26-2012	na	18	11-23-2012	2	1	12	9
7	2	4	2	4	2	4	2	4	2	11-5-2012	na	18	11-5-2012	2	3	14	9
8	2	4	2	4	2	4	2	4	2	8-28-2012	na	18	10-26-2012	2	1	14	9
9	2	4	2	4	2	4	2	4	1	5-25-2012	na	18	10-23-2012	2	1	1	9
10	2	4	2	4	2	4	2	4	3	na	na	18	3-16-2012	1	2	4	3
11	2	4	2	4	2	4	2	4	2	11-2-2011	na	18	12-4-2012	2	3	14	9
12	2	4	2	4	2	4	2	4	1	12-27-2011	na	18	12-27-2011	2	1	1	9
13	2	4	2	4	2	4	2	4	2	5-31-2012	9-3-2012	6	10-1-2012	2	6	8	9
14	2	4	2	4	2	4	2	4	4	na	na	18	6-16-2011	2	2	7	9
15	2	4	2	4	2	4	2	4	1	3-23-2012	na	18	9-28-2012	2	2	1	9
16	2	4	2	4	2	4	2	4	4	na	na	18	10-18-2011	2	1	7	9
17	2	4	2	4	2	4	2	4	3	na	na	18	1-5-2012	1	1	4	1
18	2	4	2	4	1	2	1	4	2	12-1-2011	na	18	12-23-2011	2	6	6	9
19	2	4	2	4	2	4	2	4	2	7-1-2011	na	18	8-31-2012	2	1	6	9
20	2	4	2	4	2	4	2	4	4	na	na	18	3-6-2011	2	1	7	9
21	2	4	2	4	2	4	2	4	2	2-1-2011	na	18	4-17-2012	2	15	14	9
22	1	2	2	4	1	4	2	4	2	3-4-2011	na	18	3-4-2011	2	9	14	9
23	2	4	2	4	2	4	2	4	1	12-14-2012	na	18	12-14-2012	2	3	1	9
24	2	4	2	4	2	4	2	4	2	8-24-2010	na	18	11-7-2012	2	15	14	9
25	2	4	2	4	2	4	1	1	1	3-23-2010	na	18	3-23-2010	2	15	1	9
26	2	4	2	4	2	4	2	4	2	4-9-2010	na	18	5-11-2010	2	3	18	9
27	2	4	2	4	2	4	2	4	2	6-24-2010	5-24-2011	6	7-18-2011	1	6	8	2
28	2	4	2	4	2	4	2	4	2	na	na	18	10-11-2010	2	1	7	9
29	2	4	2	4	2	4	2	4	4	na	na	18	8-10-2010	2	2	7	9
30	2	4	2	4	2	4	2	4	2	3-26-2011	1-19-2012	18	5-19-2012	1	3	8	1
31	2	4	2	4	2	4	2	4	2	6-21-2011	na	18	9-20-2011	2	2	16	9
32	2	4	2	4	2	4	2	4	2	2-15-2011	na	18	2-17-2011	2	2	16	9
33	2	4	2	4	2	4	2	4	1	4-10-2012	na	18	10-1-2012	2	1	1	9
34	2	4	2	4	2	4	2	4	2	4-29-2011	na	18	4-29-2011	2	1	11	9
35	2	4	2	4	2	4	2	4	2	11-12-2011	na	18	8-11-2012	2	3	14	9
36	2	4	2	4	2	4	2	4	2	12-5-2011	na	18	12-13-2012	2	1	12	9
37	2	4	2	4	2	4	2	4	2	10-13-2011	na	18	9-24-2012	2	1	14	9
38	2	4	2	4	2	4	2	4	2	5-25-2009	na	18	1-11-2011	2	3	14	9
39	2	4	2	4	2	4	1	1	1	3-17-2009	na	18	3-17-2009	2	15	1	9
40	2	4	2	4	2	4	2	4	2	5-3-2010	na	18	5-12-2010	2	2	14	9

Andr:Androgens, Andr-res: Reposne to androgens, Pred: Prednisoloen, Pred-res: Response to prednisolone, Allo:Allogenic PBSCT, ALLO-res: Response to ALLo PBSCT, Sup:Suppotove treatment,
Sup-res:Response to supportive treatment, DO res:Date of response, DO Relapse:Date of relapse, Trt relapse: Treatment of relapse, Do LFU:Date of last follow up, Drug LFU:Drug at last follow up,

Sl No	Age	Sex	DOD	WHO gr	Du Sym	Pallor	Bleed	InfectIon	Liver	Spleen	PC	PRC	h/o cytopenia	Trt res	Hb	WBC	Blast %	ANC	PLT	Retic	ARC	LDH	FERITIN
41	49	1	5-19-2009	3	24	1	2	1	2	1	1	na	4	9	7	1700	0	918	46000	1.9	31730		332
42	41	1	6-11-2009	2	3	1	2	2	2	2	10	na	4	9	5	3100	0	1395	17000	2.73	75348	471	na
43	56	2	6-9-2009	2	3	1	2	1	2	2	na	na	4	9	8	1300	0	260	201000	3.26	65844	1062	na
44	35	1	6-18-2009	3	1	1	2	2	2	2	na	na	4	9	9	2100	0	252	40000	1.14	30096	621	na
45	59	2	9-14-2009	1	12	1	1	2	2	2	8	na	4	9	6	4100	0	2337	9000	1.44	18000	411	na
46	42	1	8-31-2009	1	2	1	2	1	2	2	5	4	4	9	4	5400	0	1620	6000	2.21	62543	822	na
47	61	2	10-26-2009	2	4	1	2	2	2	2	8	na	3	3	5	3800	0	1710	22000	0.1	na	348	5448
48	38	1	7-15-2008	1	1	1	2	2	2	2	na	na	1	2	7	2300	0	115	29000	na	na	291	
49	63	1	11-21-2008	1	1	1	2	1	2	2	1	7	4	9	9	3100	0	1643	65000	2.16	62208	764	408
50	43	1	2-29-2008	1	3	1	2	2	2	2	na	na	4	9	10	3800	0	1292	26000	1.85	51060	343	138
51	58	1	7-1-2008	1	1	1	2	2	2	2	5	na	4	9	5	2800	0	1512	136000	0.79	12877	371	na
52	43	1	1-23-2008	1	3	1	2	2	2	2	3	0	4	9	6	3800	0	1976	307000	na		412	1910
53	74	1	2-20-2008	3	1	1	1	2	2	2	2	4	4	9	6	1900	0	304	9000	2.48	36952	355	na
54	24	1	2-28-2008	1	3	1	2	1	2	2	4	8	4	9	4	1800	0	144	9000	0.94	25662	325	na
55	24	1	5-17-2008	1		1	1	1	2	2	na	na	4	9	9	7300	0	1752	5000	2.48	68200	595	942
56	49	2	5-12-2008	1	4	1	2	2	2	2	2	na	4	9	5	5700	0	3363	15000	2.09	41862	342	na
57	35	2	7-25-2008	1	6	1	1	1	2	2	40	na	4	9	7	3100	0	682	5000	1.07	22791	360	na
58	59	2	7-18-2008	1	1	1	1	2	2	2	20	20	4	9	10	1400	0	476	15000	2.62	80696	533	2000
59	75	2	5-2-2008	1	3	1	2	1	2	2	8	na	4	9	6	3300	0	792	6000	1.85	34595	441	na
60	22	1	8-20-2008	1		9	9	9	9	9	na	na	9	9	4	2000	0	560	4000	0.94	10246	421	na
61	24	1	8-25-2008	1		9	9	9	9	9	na	na	9	9	4	2900	0	1450	61000	4.78	50190	1206	na
62	55	2	8-25-2008	1	1	1	1	1	2	2	na	na	2	9	6	5800	0	5220	5000	7.06	na	687	na
63	40	2	10-24-2008	1	5	1	2	1	2	2	5	na	9	9	8	2500	0	325	74000	2.08	60942	465	na
64	22	1	11-24-2008	3		9	9	9	9	9	na	na	9	9	6	1800	0	434	7000	1.03	21527	260	na
65	35	1	11-19-2008	1	12	1	1	1	2	2	na	na	4	9	3	1700	0	884	9000	2.71	26267	276	na
66	23	1	11-5-2008	3	3	1	1	2	2	2	5	2	4	9	4	3300	0	1089	10000	1.85	27935	409	na
67	54	2	11-7-2008	1	12	1	1	1	2	2	16	8	4	9	4	1200	0	552	3000	1.07	27941	459	3905
68	72	1	4-7-2008	1	6	1	2	2	2	2	na	na	4	9	7	3100	0	1178	246000	1.47	14259	449	768
69	19	1	4-28-2008	1		9	9	9	9	9	na	na	9	9	7	3500	0	3325	197000	2.3	62677	935	na
70	65	1	11-22-2008	3	36	1	2	1	2	2	6	na	4	9	8	2300	0	1012	46000	1	na	324	341
71	62	2	8-13-2008	3	24	1	2	2	2	2	9	na	4	9	4	2100	0	1008	99000	1.86	18972	440	na
72	56	1	4-28-2007	1	7	1	2	2	2	2	na	na	4	9	8	4700	0	1081	30000	1.88	49820	518	na
73	34	1	1-8-2007	1	3	1	2	1	1	1	na	na	4	9	3	1800	0	1260	15000	2.62	17816	380	na
74	64	2	1-31-2007	1	1	1	1	2	2	2	3	na	4	9	6	3400	0	1564	7000	3.2	78080	400	na
75	21	1	4-23-2007	1	1	1	1	1	2	2	1	2	4	9	6	600	0	60	17000	0.71	14839	2401	na
76	42	2	7-24-2007	1	12	1	1	2	2	2	6	na	4	9	10	4800	0	2496	8000	3.01	77658	427	406
77	20	2	8-4-2007	1	3	1	2	2	2	2	6	na	4	9	8	3600	0	1652	48000	0.51	13719	442	na
78	55	2	7-31-2007	1		1	2	2	2	2	7	2	4	9	3	6200	0	3410	8000	1.05	36225	616	na
79	53	1	9-1-2007	1	7	1	1	2	2	2	25	2	4	9	3	1300	0	260	9000	2.73	22113	326	na
80	52	2	12-22-2007	3	12	1	2	2	2	2	na	na	9	9	9	5000	0	2350	13000	2.39	58794	424	na

Sl No	DC T	AN A	BBV S	BM Celllarit y	Dysplasi a	Blast %	R S	Megs	My e	Ery	Reticuli n	Cyt-IPSS/WPS S	Cyt IPSS -R	IPSS scor e	WPS S score	IPSS -R score	CS A	CSA-res	ATG	ATG-res	And r	Andr -res
41	9	2	4	2	1	1	1	2	2	2	3	2	2	0.5	1	3.5	2	4	2	4	2	4
42	9	9	4	1	3	5	1	2	2	2	1	9	9	na	9	na	2	4	2	4	2	4
43	9	9	4	2	5	5	2	2	2	2	3	3	3	1	2	6	1	3	2	4	2	4
44	9	9	4	1	6	2	2	2	2	2	3	4	4	1.5	3	5.5	2	4	2	4	1	4
45	9	9	4	1	2	1	1	2	2	2	2	3	3	1	2	4.5	1	4	2	4	2	4
46	9	9	9	2	3	0	2	2	2	2	2	2	2	0.5	1	3.5	1	2	2	4	2	4
47	1	9	4	2	4	5	2	2	2	2	4	3	3	1	2	6.5	2	4	2	4	2	4
48	9	9	4	2	4	3	1	2	2	2	2	4	4	1.5	3	7	1	3	2	4	1	3
49	1	1	4	2	2	0	2	2	2	3	1	2	2	0.5	1	2.5	1	2	2	4	2	4
50	2	9	4	2	3	1	2	2	2	2	1	2	2	0.5	0	3	1	2	2	4	2	4
51	9	9	4	2	4	0	2	2	2	2	3	2	2	0	1	2.5	1	3	2	4	1	2
52	2	2	4	2	7	0	2	2	2	2	2	2	2	0	1	2.5	2	4	2	4	1	1
53	9	9	4	2	1	1	2	4	2	2	1	4	4	1.5	3	6	1	3	2	4	1	4
54	9	9	4	2	2	0	1	2	2	2	1	9	9	na	9	na	1	1	2	4	2	4
55	9	9	4	2	3	0	1	2	2	3	1	2	2	0.5	1	3	1	2	2	4	2	4
56	9	1	4	4	3	0	1	2	2	2	1	3	3	1	2	4.5	1	4	2	4	2	4
57	2	9	4	2	3	1	2	4	2	2	1	2	2	0.5	1	4	1	2	2	4	1	2
58	1	9	4	2	4	0	2	2	2	2	2	4	4	1.5	3	5.5	1	4	2	4	2	4
59	9	9	4	2	3	0	2	2	2	3	2	2	2	0.5	1	4	1	2	2	4	1	4
60	9	9	4	2	3	2	2	2	2	3	1	2	2	0.5	1	4	2	4	2	4	1	4
61	9	9	4	2	4	1	2	1	2	2	3	9	9	na	9	na	2	4	2	4	2	4
62	9	9	4	2	4	0	2	2	2	2	3	9	9	na	9	na	2	4	2	4	1	4
63	2	9	4	2	4	2	1	2	2	3	3	4	4	1.5	3	5.5	1	2	2	4	1	2
64	9	9	1	4	5	1	2	2	2	3	2	2	2	0.5	1	4	1	4	2	4	2	4
65	2	9	4	1	4	0	2	4	2	2	1	9	9	na	9	na	2	4	2	4	1	4
66	9	9	4	2	1	0	1	2	2	2	1	3	3	1	2	4.5	2	4	2	4	1	4
67	1	1	4	2	2	2	2	2	2	3	2	2	2	0.5	1	4	1	4	2	4	2	4
68	2	9	4	2	4	2	2	2	2	2	1	3	3	1	2	3.5	1	2	2	4	2	4
69	9	9	4	2	4	2	2	2	2	2	2	9	9	na	9	na	2	4	2	4	2	4
70	9	2	4	2	6	0	1	2	2	2	2	3	3	1	2	4	1	2	2	4	2	4
71	9	9	4	2	6	0	2	1	2	3	1	2	2	0.5	1	3	2	4	2	4	1	3
72	9	9	4	2	4	2	1	2	2	3	2	2	2	0.5	1	3.5	1	3	2	4	2	4
73	9	9	4	2	4	3	2	2	2	3	3	2	2	0.5	1	4.5	2	4	2	4	1	2
74	2	9	4	2	3	0	2	4	2	2	2	2	2	0.5	1	3.5	2	4	1	2	2	4
75	2	2	4	2	4	0	2	2	2	2	2	9	9	na	9	na	2	4	2	4	2	4
76	2	9	2	4	4	0	2	2	2	3	2	9	9	na	9	na	1	4	2	4	2	4
77	9	9	4	2	7	2	2	2	2	2	2	3	2	1	2	3	2	4	2	4	2	4
78	9	9	4	2	3	0	1	2	2	2	1	2	2	0.5	1	3.5	1	2	2	4	2	4
79	9	9	4	1	3	1	1	4	2	2	2	2	2	0.5	1	4	1	4	2	4	2	4
80	2	9	4	4	1	1	1	2	2	3	2	9	9	na	9	na	1	4	2	4	1	3

Sl No	Pred	Pred-res	Allo	Allo-res	Others	Others-res	Sup	Sup-res	Response	DO res	DO relapse	Trt rela	Do LFU	Alive/Dead	Drg LFU	Disease statusLFU	Cause death
41	1	2	2	4	2	4	2	4	2	4-5-2010	na	18	6-25-2010	2	15	12	9
42	2	4	2	4	2	4	2	4	4	na	na	18	6-19-2009	2	15	7	9
43	2	4	2	4	2	4	2	4	3	na	na	18	8-12-2010	1	1	4	2
44	2	4	2	4	2	4	2	4	4	na	na	18	6-19-2009	2	2	7	9
45	2	4	2	4	2	4	2	4	4	na	na	18	9-18-2009	2	1	7	9
46	1	3	2	4	2	4	2	4	2	2-4-2010	na	18	2-4-2010	2	1	18	9
47	2	4	2	4	1	3	2	4	3	na	na	18	1-24-2010	1	5	4	1
48	2	4	2	4	2	4	2	4	3	na	na	18	11-19-2009	1	2	4	9
49	2	4	2	4	2	4	2	4	2	3-22-2011	na	18	9-4-2012	2	15	12	9
50	1	3	2	4	2	4	2	4	2	8-15-2008	4-19-2012	4	4-27-2012	2	4	8	9
51	2	4	2	4	1	3	2	4	2	8-27-2010	12-21-2010	18	1-13-2011	1	15	8	9
52	1	3	2	4	2	4	2	4	1	4-28-2008	6-4-2009	1	6-5-2009	2	1	10	9
53	2	4	2	4	2	4	2	4	4	na	na	18	3-28-2008	2	3	7	9
54	2	4	2	4	2	4	2	4	1	9-1-2010	na	18	9-19-2012	2	15	1	9
55	2	4	2	4	2	4	2	4	2	9-4-2008	na	18	10-4-2011	2	15	14	9
56	2	4	2	4	2	4	2	4	4	na	na	18	5-12-2008	2	1	7	9
57	2	4	2	4	2	4	2	4	2	9-8-2010	na	18	12-3-2012	2	3	14	9
58	2	4	2	4	2	4	2	4	4	na	na	18	7-18-2008	2	1	7	9
59	2	4	2	4	2	4	2	4	2	8-25-2008	na	18	10-3-2008	2	3	16	9
60	2	4	2	4	2	4	2	4	4	na	na	18	8-26-2008	2	2	7	9
61	2	4	2	4	2	4	2	4	4	na	na	18	8-25-2008	2	18	7	9
62	2	4	2	4	2	4	2	4	4	na	na	18	9-4-2008	2	3	7	9
63	1	3	2	4	2	4	2	4	2	1-23-2009	5-11-2009	19	5-12-2009	2	3	8	9
64	2	4	2	4	2	4	2	4	4	na	na	18	11-28-2008	2	1	7	9
65	2	4	2	4	2	4	2	4	4	na	na	18	11-21-2008	2	2	7	9
66	2	4	2	4	2	4	2	4	4	na	na	18	11-14-2008	2	2	7	9
67	1	3	2	4	2	4	2	4	4	na	na	18	12-4-2008	2	1	7	9
68	2	4	2	4	2	4	2	4	2	7-1-2008	7-8-2009	1	10-4-2010	2	1	8	9
69	2	4	2	4	2	4	2	4	4	na	na	18	4-28-2008	2	15	7	9
70	2	4	2	4	2	4	2	4	2	1-19-2009	10-7-2010	1	10-7-2010	2	1	8	9
71	2	4	2	4	1	2	2	4	2	1-22-2009	na	18	9-3-2010	2	15	14	9
72	2	4	1	2	2	4	2	4	2	6-20-2008	7-7-2008	6	8-1-2008	1	6	8	4
73	1	3	2	4	2	4	2	4	2	12-24-2007	na	18	5-28-2010	2	2	14	9
74	2	4	2	4	2	4	2	4	2	4-3-2007	na	18	8-10-2010	2	15	14	9
75	2	4	2	4	2	4	2	4	4	na	na	18	4-25-2007	2	15	7	9
76	2	4	2	4	2	4	2	4	4	na	na	18	8-3-2007	2	1	7	9
77	1	4	2	4	2	4	2	4	4	na	na	18	8-4-2007	2	4	7	9
78	2	4	2	4	2	4	2	4	2	11-7-2007	na	18	5-20-2011	2	15	14	9
79	2	4	2	4	2	4	2	4	4	na	na	18	10-2-2007	2	1	7	9
80	2	4	2	4	2	4	2	4	3	na	na	18	3-8-2008	2	1	4	9

Sl No	Age	Sex	DOD	WHO gr	Du Sym	Pallor	Bleed	InfectIon	Liver	Spleen	PC	PRC	h/o cytopenia	Trt res	Hb	WBC	Blast %	ANC	PLT	Retic	ARC	LDH	FERITIN
81	19	1	2-25-2006	3	1	1	1	2	2	2	na	na	4	9	6	1800	0	360	16000	1.35	28755	272	na
82	61	1	1-20-2006	1	12	1	1	2	2	2	16	na	4	9	6	2200	0	616	11000	1.55	34100	254	na
83	46	1	6-23-2006	1	2	1	1	2	2	2	3	3	4	9	7	4200	0	2058	35000	1.99	36019	487	na
84	50	1	6-26-2006	1	8	1	2	2	2	2	5	na	4	9	5	2900	0	2639	60000	0.98	17150	357	na
85	32	1	1-12-2006	1	na	9	9	9	9	9	na	na	9	9	4	1200	0	96	22000	2.42	36784	301	na
86	56	1	2-28-2006	1	na	9	9	9	9	9	na	na	9	9	5	5000	0	3450	7000	2.79	54784	335	na
87	49	2	2-8-2006	3	3	1	1	2	2	2	2	na	4	9	7	5500	0	3025	16000	2.14	54998	632	na
88	45	2	1-4-2006	3	3	1	1	1	2	2	3	na	4	9	2	2600	0	858	6000	na	na	na	na
89	68	1	3-11-2006	1	6	1	2	2	2	2	na	na	4	9	6	1800	0	288	15000	0.39	7644	278	na
90	45	2	3-7-2006	1	10	1	2	2	2	2	10	na	4	9	5	600	0	60	4000	1.27	23241	252	na
91	25	1	5-22-2006	1	84	1	1	1	2	2	220	na	4	9	3	1100	0	308	3000	na	na	204	na
92	68	1	7-26-2006	1	2	1	2	2	2	2	na	na	4	9	5	4700	0	2256	13000	3.56	42364	237	na
93	73	1	8-26-2006	1	3	1	1	2	2	2	15	na	4	9	6	3100	0	1364	7000	1.29	24668	296	na
94	70	1	7-1-2006	3	3	1	2	2	2	2	na	na	4	9	4	7900	0	5372	9000	na	na	2610	na
95	63	1	8-11-2006	3	na	9	9	9	9	9	na	na	9	9	4	4600	0	1978	15000	3.58	45266	379	na
96	20	1	9-20-2006	1	1	1	1	1	2	2	3	na	4	9	6	2300	0	184	6000	1.84	36616	235	na
97	60	2	9-13-2006	3	2	1	2	2	2	2	6	na	4	9	5	3400	0	1020	10000	4.89	61125	517	na
98	45	2	9-6-2006	1	3	1	1	2	2	2	10	na	4	9	6	3300	0	990	26000	3.28	109224	388	2150
99	55	2	10-4-2006	1	48	1	1	1	2	2	46	na	1	9	5	4100	0	1353	6000	2.99	65481	429	9350
100	19	2	10-6-2006	1	3	1	2	2	2	2	3	na	4	9	9	3700	0	703	30100	na	na	na	na
101	45	2	11.21.2006	1	2	1	2	2	2	2	4	na	4	9	4	3400	0	1564	91000	1.89	51975	340	na
102	55	1	11.11.2006	1	na	9	9	9	9	9	na	na	9	9	12	6500	0	2990	144000	3.06	96696	562	na
103	47	1	11-4-2006	3	2	1	2	2	2	2	6	na	4	9	4	3800	0	912	17000	1.17	51363	345	na
104	61	2	5-10-2006	1	6	1	1	1	2	2	25	20	4	9	2	2500	0	1500	3000	1.04	14248	366	na
105	30	1	11.17.2005	1	4	1	2	2	2	2	3	na	4	9	5	13800	0	4968	64000	3.71	98686	1801	na
106	46	2	6-14-2005	3	6	1	2	2	2	2	3	na	4	9	8	5700	0	1311	13000	2.41	65311	492	na
107	40	1	10.28.2005	3	na	1	2	2	2	2	na	na	4	9	4	5300	0	742	14000	na	na	681	na
108	38	1	2-25-2005	1	4	1	2	2	2	2	30	na	4	9	9	3200	0	1056	4000	0.99	27225	245	na
109	48	1	11-1-2004	1	4	1	2	1	2	2	6	na	1	1	11	5300	0	2014	28000	1.61	46690	557	na
110	38	1	6-21-2005	1	24	1	2	2	2	2	31	na	4	9	5	9000	0	3330	42000	2.14	41088	531	333
111	40	1	4-1-2005	1	10	1	1	2	2	2	12	na	4	9	5	1300	0	780	7000	1.44	27360	454	na
112	72	1	5-31-2005	1	1	1	2	2	2	2	2	na	4	9	6	3000	0	1110	13000	1.88	na	345	na
113	40	2	2-22-2005	1	4	1	2	1	2	2	14	na	4	9	3	1400	0	224	21000	1.01	24543	541	na
114	23	1	3-22-2005	1	1	1	2	1	2	2	1	na	1	1	5	2300	0	414	7000	2.21	37791	610	na
115	18	2	5-17-2005	3	24	1	2	2	2	2	12	na	4	9	7	5400	0	2106	30000	3.57	74970	833	428
116	50	1	8-19-2005	3	1	1	1	1	2	2	5	9	4	9	4	6800	0	2312	14000	3.44	133472	342	na
117	68	1	1-22-2005	1	3	1	2	2	2	2	5	na	4	9	4	7800	0	1638	225000	0.8	14400	348	na
118	41	2	11-26-2005	1	7	1	2	2	2	2	5	na	4	9	3	6200	0	2542	73000	3.63	90387	899	270
119	56	2	7-27-2005	1	3	1	1	2	2	2	2	5	4	9	7	4800	0	4224	11000	2	72200	367	na
120	30	1	10-26-2005	1	11	1	2	2	2	2	na	na	1	3	6	3900	0	858	11000	3.51	56511	478	na

Sl no	DCT	ANA	BBVS	BM Cellularity	Dysplasia	Blast %	RS	Megs	Mye	Ery	Reticulin	Cyt-IPSS/WPSS	Cyt IPSS-R	IPSS score	WPSS score	IPSS-R score	CSA	CSA-res	ATG	ATG-res	Andr	Andr-res
81	9	9	4	2	6	2	2	2	2	2	3	2	2	0.5	1	4	1	3	2	4	2	4
82	9	9	4	2	3	2	2	2	2	2	2	2	2	0.5	1	4	2	4	2	4	1	2
83	9	9	9	2	3	1	2	2	2	2	2	2	2	0.5	1	3.5	1	4	2	4	2	4
84	9	9	4	2	4	0	2	2	1	1	3	3	3	1	2	4	2	4	2	4	1	2
85	9	9	4	2	2	1	1	2	2	3	3	2	2	0.5	1	4	2	4	2	4	1	4
86	9	9	9	2	4	4	2	2	2	2	2	9	9	na	9	na	2	4	2	4	2	4
87	9	9	9	2	5	2	2	4	2	2	2	2	2	0.5	1	3.5	1	2	2	4	2	4
88	9	9	4	2	1	0	1	2	2	2	2	9	9	na	9	na	2	4	2	4	1	4
89	9	9	9	2	4	1	2	2	2	2	2	4	4	1.5	3	6	1	4	2	4	2	4
90	9	9	3	2	3	0	1	2	2	2	2	3	3	1	2	5	2	4	2	4	1	4
91	9	9	4	2	4	0	1	2	2	3	2	4	4	1.5	3	6	1	4	2	4	2	4
92	2	9	4	2	4	0	1	2	2	3	1	2	2	0.5	1	3.5	2	4	2	4	2	4
93	9	9	4	1	3	0	1	4	2	2	2	9	9	na	9	na	2	4	2	4	1	4
94	9	9	9	2	1	0	2	4	3	2	2	2	2	0.5	1	3.5	2	4	2	4	1	4
95	9	9	4	1	5	0	2	2	2	2	1	9	9	na	9	na	2	4	2	4	1	4
96	9	9	4	2	4	1	2	2	2	2	2	2	2	0.5	1	4	2	4	2	4	1	4
97	9	9	4	2	1	0	2	2	2	3	2	9	9	na	9	na	2	4	2	4	1	2
98	2	1	4	2	4	4	1	2	2	2	1	2	2	0.5	1	4.5	1	2	2	4	2	4
99	9	2	4	2	7	0	9	2	2	2	2	2	2	0.5	1	3.5	1	4	2	4	2	4
100	9	9	4	2	4	1	2	1	2	2	2	2	2	0.5	1	2.5	1	1	2	4	2	4
101	9	9	4	4	7	2	2	2	2	2	3	3	3	1	2	4	2	4	2	4	2	4
102	9	9	4	4	4	0	2	2	2	2	2	2	2	0.5	1	1	2	4	2	4	2	4
103	9	9	4	2	1	0	1	4	2	3	3	2	2	0.5	1	3.5	2	4	2	4	2	4
104	9	9	4	2	3	0	2	4	2	2	1	2	2	0.5	1	3.5	1	4	2	4	2	4
105	2	9	4	2	3	2	1	2	2	2	1	2	2	0.5	1	3	2	4	2	4	1	2
106	9	9	4	2	1	1	2	2	2	2	1	4	5	1.5	3	6	1	1	2	4	2	4
107	9	9	4	2	9	na	9	2	2	2	9	2	2	0.5	1	4	1	3	2	4	2	4
108	9	9	9	2	2	0	1	2	2	3	2	2	2	0.5	1	3	1	2	2	4	1	2
109	2	9	4	2	4	1	2	2	2	2	2	9	9	na	9	na	1	2	2	4	2	4
110	9	9	4	2	4	1	2	2	2	3	3	2	2	0.5	1	3.5	1	2	2	4	2	4
111	9	9	4	2	3	0	9	2	2	2	2	2	2	0.5	1	4	1	3	2	4	2	4
112	9	9	4	2	4	2	9	2	2	2	3	2	2	0.5	1	3.5	2	4	2	4	1	4
113	9	9	4	2	4	1	2	2	2	2	3	4	5	1.5	3	7	2	4	2	4	1	4
114	9	9	1	2	4	2	2	2	2	2	2	2	2	0.5	1	4	1	4	2	4	2	4
115	1	2	4	4	1	1	9	2	2	2	2	4	4	1.5	3	5.5	1	2	2	4	2	4
116	9	9	9	4	1	0	2	2	2	2	2	4	4	1.5	3	5.5	1	1	2	4	2	4
117	9	9	4	2	4	0	2	2	2	3	1	2	1	0	1	1.5	1	2	2	4	1	3
118	2	9	4	2	2	0	2	2	1	3	1	2	2	0.5	1	3	1	2	2	4	1	2
119	9	9	9	4	4	0	1	2	2	3	3	2	2	0.5	1	3.5	2	4	2	4	2	4
120	9	9	4	2	4	na	9	2	2	2	9	2	2	0.5	1	3.5	1	3	2	4	2	4

Sl no:	Pred	Pred-res	Allo	Allo-res	Others	Others-res	Sup	Sup-res	Response	DO res	DO relapse	Trt rela	Do LFU	Alive/Dead	Drg LFU	Disease statusLFU	Cause death
81	2	4	1	1	2	4	2	4	1	6-22-2006	8-3-2006	22	10-24-2007	1	23	10	2
82	2	4	2	4	2	4	2	4	2	2-14-2007	na	18	8-13-2008	2	15	14	9
83	2	4	2	4	2	4	2	4	4	na	na	18	6-29-2006	2	1	7	9
84	1	3	2	4	2	4	2	4	2	4-9-2007	4-26-2010	21	12-15-2011	2	2	14	9
85	2	4	2	4	2	4	2	4	4	na	na	18	1-12-2006	2	2	7	9
86	2	4	2	4	2	4	1	4	4	na	na	18	3-1-2006	2	15	7	9
87	2	4	2	4	2	4	2	4	2	4-11-2006	8-14-2009	1	3-15-2011	2	1	8	9
88	1	3	2	4	2	4	2	4	4	na	na	18	2-7-2006	2	3	7	9
89	2	4	2	4	2	4	2	4	4	na	na	18	4-20-2006	2	1	7	9
90	2	4	2	4	2	4	2	4	4	na	na	18	3-12-2006	2	3	7	9
91	2	4	2	4	2	4	2	4	4	na	na	18	5-26-2006	2	1	7	9
92	1	2	2	4	2	4	2	4	2	1-23-2007	10-1-2009	4	3-2-2010	2	4	6	9
93	2	4	2	4	2	4	2	4	4	na	na	18	8-29-2006	2	3	7	9
94	2	4	2	4	2	4	2	4	4	na	na	18	8-25-2006	2	2	7	9
95	2	4	2	4	2	4	2	4	4	na	na	18	9-18-2006	2	2	7	9
96	2	4	2	4	2	4	2	4	4	na	na	18	9-22-2006	2	2	7	9
97	2	4	2	4	2	4	2	4	2	1-12-2007	na	18	1-12-2007	2	3	6	9
98	2	4	2	4	2	4	2	4	2	12-7-2006	na	18	1-27-2009	2	1	14	9
99	2	4	2	4	2	4	2	4	4	na	na	18	10-6-2006	2	1	7	9
100	2	4	2	4	2	4	2	4	1	3-8-2007	na	18	3-8-2007	2	1	1	9
101	1	4	2	4	2	4	2	4	4	na	na	18	12-27-2006	2	4	7	9
102	2	4	2	4	2	4	1	4	4	na	na	18	11-21-2006	2	15	7	9
103	1	3	2	4	2	4	1	4	4	na	na	18	12-5-2006	2	6	7	9
104	2	4	2	4	2	4	2	4	4	na	na	18	6-16-2006	2	1	7	9
105	2	4	2	4	2	4	2	4	2	2-28-2006	na	18	3-2-2006	2	2	12	9
106	2	4	2	4	2	4	2	4	1	12-12-2006	na	18	11-8-2011	2	1	1	9
107	2	4	1	3	2	4	2	4	3	na	na	18	12-17-2005	1	8	4	2
108	1	3	2	4	2	4	2	4	2	11-25-2005	na	18	8-25-2006	2	2	6	9
109	2	4	2	4	2	4	2	4	2	4-26-2005	10-24-2006	1	10-24-2006	2	1	8	9
110	2	4	2	4	2	4	2	4	2	9-1-2005	12-6-2011	1	3-5-2012	2	1	6	9
111	2	4	2	4	2	4	2	4	3	na	na	18	6-25-2005	1	1	3	9
112	2	4	2	4	2	4	2	4	4	na	na	18	6-14-2005	2	2	7	9
113	2	4	2	4	2	4	2	4	4	na	na	18	3-10-2005	2	3	7	9
114	2	4	2	4	2	4	2	4	4	na	na	18	4-6-2005	2	1	7	9
115	1	3	2	4	2	4	2	4	2	10-11-2005	na	18	11-8-2005	2	1	17	9
116	2	4	2	4	2	4	2	4	1	12-28-2007	na	18	1-17-2012	2	15	1	9
117	2	4	2	4	2	4	2	4	2	6-1-2005	na	18	2-17-2006	2	1	14	9
118	2	4	2	4	2	4	2	4	2	10-31-2007	na	18	9-19-2008	2	3	14	9
119	1	3	2	4	2	4	2	4	3	na	na	18	9-6-2005	2	4	3	9
120	2	4	1	1	2	4	2	4	1	1-20-2006	na	18	7-3-2012	2	15	1	9

Sl No	Age	Sex	DOD	WHO gr	Du Sym	Pallor	Bleed	InfectIon	Liver	Spleen	PC	PRC	h/o cytopenia	Trtres	Hb	WBC	Blast%	ANC	PLT	Retic	ARC	LDH	FERITIN
121	27	1	6-15-2004	1	8	1	1	1	1	2	25	na	9	9	13	4400	0	660	1000	0.3	na	553	na
122	63	1	6-16-2004	1	4	1	2	2	2	2	12	na	4	9	10	6400	0	3584	21000	0.6	na	757	na
123	72	1	1-4-2004	1	2	1	2	1	1	2	4	na	4	9	6	1300	0	702	45000	0.4	na	354	na
124	56	1	7-27-2004	1	6	1	2	1	2	2	2	na	4	9	5	1900	0	912	54000	1.2	na	558	1079
125	44	2	12-17-2004	1	12	1	2	2	2	2	24	na	4	9	6	2200	0	572	54000	0.69	11520	718	na
126	18	1	7-10-2004	1	3	1	1	1	2	2	15	na	4	9	3	2400	0	600	6000	0.1	na	254	na
127	50	2	7-10-2004	1	6	1	1	2	2	2	12	na	9	9	10	4500	0	1485	10000	0.05	na	432	na
128	24	2	3-9-2004	1	4	1	1	2	2	2	19	na	4	9	10	4600	0	1500	21000	3.5	na	477	na
129	45	1	8-31-2004	1	4	1	1	1	2	2	10	na	4	9	6	2700	0	540	12000	2	na	275	na
130	73	2	4-25-2003	1	1	1	2	2	2	2	9	9	4	9	6	3100	0	1085	32000	1	na	349	na
131	23	1	6-13-2003	1	12	1	1	2	2	2	9	na	4	9	6	3400	0	884	11000	1.5	na	247	na
132	40	2	6-24-2003	1	6	1	1	2	2	2	28	na	4	9	5	4700	0	1551	11000	1.3	na	427	na
133	55	1	9-26-2003	1	2	1	2	1	2	2	4	na	4	9	9	3300	0	1352	40000	3	na	580	na
134	69	1	11-14-2003	1	48	1	2	2	2	2	11	na	4	9	11	6400	0	2048	39000	0.6	na	535	na
135	26	1	7-1-2003	1	3	1	2	1	2	2	4	na	4	9	7	1400	0	280	140000	2.5	na	788	na
136	36	2	8-18-2003	1	6	1	2	1	2	2	1	na	4	9	7	200	0	0	69000	2	na	1191	na
137	46	2	11-5-2003	1	1	1	1	1	2	2	2	2	4	9	6	1500	0	30	11000	0.1	na	680	na
138	38	1	10-31-2003	1	6	1	2	1	2	2	20	na	4	9	5	3500	0	735	63000	1	na	267	na
139	43	1	4-25-2003	3	1	1	2	2	2	2	3	na	4	9	7	4700	0	1457	11000	0.4	na	680	na
140	63	1	11-10-2001	1	6	1	2	2	2	2	5	na	4	9	4	900	0	108	2000	0.3	na	346	na
141	62	1	8-13-2002	1	6	1	1	1	2	2	9	na	4	9	8	5200	0	3900	8000	0.7	na	534	na
142	30	1	9-11-2002	1	6	1	1	2	2	2	9	na	4	9	5	6400	0	3456	9000	3.4	na	438	na
143	58	1	10-26-2002	1	3	1	2	2	2	2	3	na	4	9	6	1300	0	728	20000	1.2	na	306	na
144	26	2	11-12-2002	1	12	1	1	1	2	2	10	na	4	9	6	2800	0	1344	31000	1.3	34060	332	na
145	44	2	7-19-2002	3	12	1	2	2	2	2	4	na	4	9	6	4500	0	2925	103000	6	na	298	na
146	49	1	9-20-2002	1	2	1	1	2	2	2	2	na	4	9	7	8200	0	4018	10000	2	na	326	na
147	35	1	7-1-2002	1	6	1	1	2	2	2	6	na	4	9	6	3700	0	925	14000	1.2	na	367	na
148	24	1	10-22-2002	1	7	1	1	2	2	2	3	na	4	9	6	2400	0	1968	50000	1.2	na	284	na
149	22	1	5-15-2001	1	3	1	1	2	2	2	13	na	4	9	5	3700	0	444	3000	2.3	na	650	na
150	38	1	6-5-2001	1	4	1	2	2	2	2	3	na	4	9	8	4100	0	738	16000	0.6	na	420	na
151	21	1	9-10-2001	1	6	1	1	1	2	2	6	na	4	9	2	1500	0	360	5000	0.2	na	280	na
152	38	1	12-4-2001	1	3	1	2	2	2	2	5	na	9	9	8	1600	0	480	7000	2.41	na	312	259
153	57	1	3-23-2001	1	1	1	1	1	2	2	4	na	4	9	10	1100	0	240	21000	1.8	na	459	na
154	44	1	10-17-2001	1	3	1	2	2	2	2	7	na	4	9	9	6100	0	1586	48000	4.4	na	1189	3302
155	71	2	7-26-2001	1	6	1	2	2	2	2	5	na	4	9	4	4600	0	1702	207000	1.44	na	479	na
156	41	1	8-24-2001	1	4	1	1	2	2	2	4	na	4	9	3	2000	0	400	13000	3.04	na	518	na
157	22	1	9-11-2001	1	6	1	2	2	2	2	8	na	4	9	9	6300	0	2898	19000	3.16	na	411	na
158	58	1	9-30-2000	1	4	1	2	2	2	2	4	na	4	9	6	4000	0	1600	50000	0.2	na	457	na
159	46	1	4-21-2000	1	18	1	2	2	2	2	6	na	9	9	3	1800	0	468	2000	4	na	na	Na
160	52	1	4-25-2000	1	5	1	2	1	1	2	3	na	9	9	6	3700	0	1850	24000	1.74	na	na	na

Sl no:	DCT	ANA	BBVS	BM Celllarity	Dysplasia	Blast%	RS	Megs	Mye	Ery	Reticulin	Cyt-IPSS/WPSS	Cyt IPSS-R	IPSS score	WPSS score	IPSS-R score	CSA	CSA-res	ATG	ATG-res	Andr	Andr-res
121	1	9	4	2	7	2	2	2	2	2	2	9	9	na	9	na	1	2	2	4	1	4
122	9	9	4	2	4	3	9	2	2	2	3	9	9	na	9	na	1	4	2	4	2	4
123	9	9	4	2	7	2	9	2	2	2	3	3	3	1	2	5	2	4	2	4	2	4
124	9	9	4	2	4	1	9	2	2	2	2	3	3	1	2	4	2	4	2	4	2	4
125	9	9	4	2	2	1	9	2	2	2	3	9	9	na	9	na	1	3	2	4	2	4
126	9	9	9	2	2	3	9	2	2	3	3	4	4	1.5	3	7	1	4	2	4	2	4
127	9	9	9	1	4	1	9	2	2	2	1	9	9	na	9	na	1	2	2	4	2	4
128	9	9	4	2	2	1	1	2	2	2	2	2	2	0.5	1	3.5	1	4	2	4	2	4
129	9	9	4	2	2	0	9	2	2	2	2	9	9	na	9	na	1	2	2	4	1	2
130	9	9	4	2	3	1	1	2	2	2	3	2	2	0.5	1	3	1	4	2	4	2	4
131	9	9	9	2	2	0	9	4	2	2	3	9	9	na	9	na	2	4	2	4	1	4
132	9	9	9	2	2	1	9	2	2	2	2	2	2	0.5	1	3.5	1	3	2	4	1	2
133	9	9	4	2	2	1	9	2	2	2	2	2	2	0.5	1	3	1	2	2	4	2	4
134	9	9	4	2	2	1	1	2	2	2	3	2	2	0.5	1	2	1	2	2	4	2	4
135	2	2	4	2	4	0	9	2	2	2	2	2	2	0.5	1	3	1	4	2	4	1	3
136	1	2	4	2	2	0	9	2	2	2	3	9	9	na	9	na	2	4	2	4	2	4
137	9	9	4	2	2	0	9	2	2	2	3	9	9	na	9	na	2	4	2	4	1	4
138	2	2	4	2	2	0	9	2	2	2	3	9	9	na	9	na	2	4	2	4	1	2
139	9	9	9	2	6	0	9	2	2	2	2	2	2	0.5	1	3.5	1	4	2	4	2	4
140	9	9	4	2	4	0	9	2	2	2	3	4	4	1.5	3	6	2	4	1	4	2	4
141	9	2	4	2	4	0	2	2	2	3	2	2	2	0.5	1	3.5	2	4	1	2	1	3
142	9	9	4	2	2	2	1	2	2	3	9	9	9	na	9	na	2	4	2	4	1	4
143	9	9	4	2	4	0	1	2	2	2	3	4	4	1.5	3	6	1	2	1	3	1	3
144	9	9	4	2	4	0	9	2	2	3	2	2	2	0.5	1	3.5	2	4	2	4	1	2
145	9	9	9	2	6	0	9	2	2	2	3	9	9	na	9	na	2	4	2	4	2	4
146	9	9	9	2	2	1	2	2	2	2	2	9	9	na	9	na	1	4	2	4	2	4
147	9	9	4	4	2	2	9	2	2	3	2	9	9	na	9	na	2	4	2	4	1	4
148	9	1	4	2	2	1	2	2	2	3	3	2	2	0.5	1	3.5	2	4	2	4	1	3
149	9	9	4	2	2	1	9	2	2	2	2	9	9	na	9	na	2	4	2	4	1	2
150	9	9	4	2	2	2	9	1	2	2	3	4	4	1.5	3	6	2	4	2	4	1	2
151	9	9	4	2	4	0	9	2	2	2	2	9	9	na	9	na	2	4	2	4	1	4
152	9	9	4	2	4	1	9	2	2	3	3	2	2	0.5	1	4	1	3	2	4	1	2
153	9	9	4	2	2	0	9	2	2	2	3	2	2	0.5	1	3.5	2	4	2	4	2	4
154	9	9	4	2	3	0	9	2	2	2	3	2	2	0.5	1	3	2	4	2	4	1	4
155	9	9	4	2	2	0	9	2	2	2	2	3	2	1	2	2.5	2	4	2	4	1	4
156	9	9	4	2	4	0	9	2	2	2	3	9	9	na	9	na	2	4	2	4	2	4
157	2	2	4	2	2	0	9	2	2	2	3	9	9	na	9	na	2	4	2	4	1	3
158	9	1	4	2	4	0	1	2	2	2	9	9	9	na	9	na	1	2	2	4	2	4
159	9	9	9	2	4	3	9	2	2	2	4	9	9	na	9	na	1	4	2	4	2	4
160	9	9	9	2	2	4	9	2	2	2	4	4	4	1.5	3	6.5	2	4	2	4	2	4

Sl No:	Pred	Pred-res	Allo	Allo-res	Others	Others-res	Sup	Sup-res	Response	DO res	DO relapse	Trt rela	Do LFU	Alive/Dead	Drg LFU	Disease statusLFU	Cause death
121	2	4	2	4	2	4	2	4	2	4-1-2005	12-9-2005	21	2-22-2012	2	4	8	9
122	2	4	2	4	2	4	2	4	4	na	na	18	6-18-2004	2	1	7	9
123	2	4	2	4	2	4	1	4	3	na	na	18	1-24-2004	1	15	4	2
124	2	4	2	4	1	2	2	4	2	9-10-2004	11-13-2004	14	11-22-2004	1	24	8	2
125	2	4	2	4	2	4	2	4	3	na	8-5-2005	20	9-2-2005	1	20	4	9
126	2	4	2	4	2	4	2	4	4	na	na	18	7-12-2004	2	1	7	9
127	2	4	2	4	2	4	2	4	2	5-17-2005	na	18	12-12-2012	2	1	12	2
128	2	4	2	4	2	4	2	4	4	na	na	18	3-12-2004	2	1	7	9
129	2	4	2	4	2	4	2	4	2	4-26-2005	na	18	10-2-2012	2	15	14	9
130	2	4	2	4	2	4	2	4	4	na	na	18	4-25-2003	2	1	7	9
131	2	4	2	4	2	4	2	4	4	na	na	18	6-14-2003	2	2	7	9
132	2	4	2	4	2	4	2	4	2	5-13-2004	na	18	5-13-2004	2	3	17	9
133	2	4	2	4	2	4	2	4	2	1-2-2004	na	18	1-2-2004	2	1	17	9
134	2	4	2	4	2	4	2	4	2	1-1-2011	na	18	12-10-2012	2	15	5	9
135	1	2	2	4	2	4	2	4	2	11-20-2003	na	18	7-23-2004	2	1	15	9
136	2	4	2	4	2	4	1	4	4	na	na	18	8-24-2003	2	6	7	9
137	2	4	2	4	2	4	2	4	4	na	na	18	11-13-2003	2	3	7	9
138	2	4	2	4	2	4	2	4	2	2-28-2004	na	18	2-28-2004	2	2	11	9
139	2	4	2	4	2	4	2	4	4	na	na	18	4-29-2003	2	1	7	9
140	2	4	2	4	2	4	1	4	3	na	na	18	3-4-2002	1	1	4	2
141	2	4	2	4	2	4	2	4	2	11-16-2002	6-11-2007	1	7-29-2008	2	1	8	9
142	2	4	2	4	2	4	2	4	4	na	na	18	9-11-2002	2	2	7	9
143	2	4	2	4	2	4	2	4	3	na	na	18	6-19-2003	1	13	4	3
144	2	4	2	4	2	4	2	4	2	9-10-2003	8-12-2008	19	5-19-2010	2	1	8	9
145	1	2	2	4	2	4	2	4	2	9-1-2002	na	18	2-6-2003	2	15	14	9
146	2	4	2	4	2	4	2	4	4	na	na	18	9-25-2002	2	1	7	9
147	2	4	2	4	2	4	2	4	3	na	na	18	10-5-2002	2	2	3	9
148	2	4	2	4	2	4	2	4	3	na	3-5-2004	6	3-5-2004	1	6	8	9
149	2	4	2	4	2	4	2	4	2	9-7-2001	na	18	9-7-2001	2	2	12	9
150	2	4	2	4	2	4	2	4	2	9-10-2001	na	18	9-10-2001	2	2	16	9
151	2	4	2	4	2	4	2	4	4	na	na	18	9-17-2001	2	2	7	9
152	2	4	2	4	2	4	2	4	2	2-11-2003	10-31-2006	20	11-1-2006	1	20	8	9
153	1	4	2	4	2	4	2	4	4	na	na	18	3-29-2001	2	4	7	9
154	1	3	2	4	2	4	2	4	3	na	na	18	12-28-2001	2	2	3	9
155	2	4	2	4	2	4	2	4	4	na	na	18	7-27-2001	2	3	7	9
156	1	4	2	4	2	4	2	4	3	na	na	18	7-20-2002	1	18	4	1
157	2	4	2	4	2	4	2	4	3	na	na	18	11-1-2001	2	2	3	9
158	2	4	2	4	2	4	2	4	2	12-15-2000	12-12-2005	21	12-18-2007	1	24	8	9
159	1	3	2	4	2	4	2	4	3	na	na	18	7-26-2000	2	1	3	9
160	2	4	2	4	2	4	1	3	3	na	na	18	5-16-2000	1	18	4	2

Sl No	Age	Sex	DOD	WHO gr	Du Sym	Pallor	Bleed	Infection	Liver	Spleen	PC	PRC	h/o cytopenia	Trt res	Hb	WBC	Blast %	ANC	PLT	Retic	ARC	LDH	FERITIN
161	50	2	7-28-2000	3	12	1	2	1	2	2	1	na	9	9	4	4100	0	2870	4000	3.9	na	321	na
162	30	1	11-14-2000	1	2	1	1	2	2	2	na	na	4	9	9	4100	0	2829	22000	2	na	511	na
163	24	2	5-25-1999	3	24	1	2	2	2	2	3	na	9	9	9	2900	0	899	52000	na	na	390	na
164	35	1	9-28-1999	1	2	1	1	1	2	2	0	na	9	9	8	2300	0	276	3000	2.2	na	na	na
165	49	2	7-6-1999	1	9	1	1	2	2	2	6	na	9	9	11	4800	0	1920	17000	na	na	605	na
166	38	1	10-1-1999	1	4	1	1	2	2	2	12	na	9	9	10	3300	0	792	3000	1.25	na	520	na
167	46	1	9-22-1998	1	1	1	2	1	2	2	0	na	9	9	4	2400	0	384	9000	0.5	na	512	na
168	34	2	4-29-1998	1	2	1	2	1	2	2	4	na	9	9	9	2200	0	880	25000	0.1	na	469	na
169	38	1	6-26-1998	1	1	1	2	2	2	2	3	na	9	9	5	2200	0	660	21000	2.2	na	1020	na
170	56	2	7-7-1998	1	3	1	2	2	2	2	4	na	9	9	5	2300	0	598	5000	0.3	na	466	na
171	26	2	9-22-1998	1	60	1	2	2	2	2	15	na	9	9	5	10000	0	1000	25000	1.5	na	297	na
172	50	1	1-27-1998	1	6	1	2	1	2	2	20	na	9	9	6	1100	0	44	72000	0.8	na	na	na
173	70	1	11-13-1998	1	6	1	1	2	2	2	7	na	9	9	9	3400	0	1224	1000	0.2	na	501	Na

CODING

SEX:	WHO group	Pallor, Bleeding, Infection, Liver, Spleen:	h/o cytopenia	Treatment response	Hb in g%
1=male 2=Female	1=RCMD 2=RAEB1 3=MDS-U	1=yes 2=No 9=NA	1=AA 2=Bicytopenia 3=MDS fibrosis 4=Nil 9=NA	1=CR 2=PR 3=NR 9=NA	ANC,WBC,ARC & Platelet: /cumm, LDH - mg% Ferritin- ng/ml

Sl No:	DCT	ANA	BBVS	BM Cellarity	Dysplasia	Blast%	RS	Megs	Mye	Ery	Reticulin	Cyt-IPSS/WPSS	Cyt IPSS-R	IPSS score	WPSS score	IPSS-R score	CSA	CSA-res	ATG	ATG-res	Andr	Andr-res
161	9	9	4	4	1	0	9	2	2	2	4	2	2	0.5	1	3.5	2	4	2	4	2	4
162	9	9	4	2	4	1	1	2	2	2	1	3	3	1	2	4.5	2	4	2	4	2	4
163	9	9	9	2	1	0	9	2	2	2	4	9	9	na	9	na	2	4	2	4	2	4
164	9	9	9	2	2	0	9	2	2	2	3	9	9	na	9	na	2	4	2	4	2	4
165	9	9	9	4	2	1	9	2	2	2	2	9	9	na	9	na	2	4	2	4	2	4
166	9	9	9	2	4	0	9	2	2	2	2	2	2	0.5	1	3.5	2	4	2	4	2	4
167	9	9	9	4	2	1	9	2	2	2	3	9	9	na	9	na	2	4	2	4	2	4
168	9	9	9	2	2	1	9	2	2	2	3	3	4	1	2	5	2	4	2	4	2	4
169	9	9	9	2	2	1	9	2	2	2	3	9	9	na	9	na	2	4	2	4	2	4
170	9	9	9	4	2	0	9	2	2	2	1	9	9	na	9	na	2	4	2	4	2	4
171	9	9	9	2	2	1	9	2	2	2	3	3	3	1	2	4.5	2	4	2	4	2	4
172	9	9	9	2	2	0	9	2	2	2	1	9	9	na	9	na	2	4	2	4	2	4
173	9	9	9	2	2	0	9	2	2	2	3	9	9	na	9	na	2	4	2	4	1	4

CODING

DCT & ANA:	BBVS:	BM cellularity:	Dysplsia:	Reticulin:	Drugs:	Response to drug:
1=postive 2=Negative 3=nil 9=NA	1=HBV+ 2=HCV+ 3=HIV+ 4=Negative 9=NA	1=aplasticc 2=Hypocellular 3,4,5,6=Varyingly hypo	1=one lineage 2=bilineage 3=Trilineage	1=Normal 2=Mild increase 3=mod increase 4=marked increase 9=NA	1= yes 2=No	1=CR 2=Stable 3=NR 4=NA

Si No:	Pred	Pred-res	Allo	Allo-res	Others	Others-res	Sup	Sup-res	Response	DO res	DO relapse	Trt rela	Do LFU	Alive/Dead	Drg LFU	Disease statusLFU	Cause death
161	2	4	2	4	2	4	1	2	2	2-9-2001	na	18	2-9-2001	2	18	17	9
162	1	3	1	3	2	4	2	4	3	na	na	18	2-24-2001	1	8	4	2
163	2	4	2	4	2	4	2	4	4	na	na	18	10-1-1999	2	18	7	9
164	2	4	2	4	2	4	2	4	4	na	na	18	10-1-1999	2	18	7	9
165	2	4	2	4	2	4	1	2	2	5-1-2000	1-30-2003	18	3-30-2003	1	18	8	1
166	2	4	2	4	2	4	2	4	4	na	na	18	10-1-1999	2	18	7	9
167	2	4	2	4	2	4	2	4	4	na	na	18	3-1-1999	2	18	7	9
168	2	4	2	4	2	4	1	2	2	5-1-1999	na	18	12-10-2012	2	15	5	9
169	2	4	2	4	2	4	2	4	4	na	na	18	6-26-1998	2	18	7	9
170	2	4	2	4	2	4	2	4	4	na	na	18	8-1-1998	2	18	7	9
171	2	4	2	4	2	4	2	4	4	na	na	18	9-22-1998	2	18	7	9
172	2	4	2	4	2	4	2	4	3	na	na	18	9-1-1998	1	18	4	2
173	2	4	2	4	2	4	2	4	4	na	na	18	11-13-1998	2	2	7	9
CODING																	

Drug:
1=yes
2=No

Response to drug:
1=CR
2=Stable
3=NR
4=NA

Over all response
1=CR
2=Stable
3=NR
4=NA

1= Dead
2=Alive

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A retrospective study of the clinical profile 21**and outcome of** adult **patients with** Hypoplastic Myelodysplastic **syndrome** (hMDS) 21**in a tertiary centre in India.

64 INTRODUCTION: Myelodysplastic syndromes (MDS) are a very heterogeneous group of clonal hematopoietic stem cell disorders that represents a spectrum of diseases characterized by; ineffective erythropoiesis and marrow failure limited by acute leukemias, chronic leukemias and myeloproliferative disorders at one end of the spectrum, in which hypercellular marrow is typical, to aplastic anemia at the other end (1–3). Hypoplastic Myelodysplastic syndromes (hMDS) refers to a morphological entity in which the bone marrow cellularity is low for the age (<30% cellularity if age is <60 years or <20% cellularity if age is >60 years) (2). It represents approximately 10-15% of all MDS cases (2,4–9). Hypoplastic MDS however, does not represent a defined MDS category according to the WHO classification, but it rather denotes the morphologic status of other MDS categories (2). It is difficult to distinguish hMDS from acquired aplastic anemia (AA), 13 because of considerable clinical, histologic, and cytologic similarities between the two disorders (10). Patients with hMDS tend to be younger, have more profound thrombocytopenia and neutropenia, lower percentage of blasts, lower probability to evolve to leukemia, and they are less likely to display abnormal karyotype, compared to patients with normocellular or hypercellular MDS (1,2,6,11). Compared to AA, hMDS have a 23 poorer prognosis and have frequent karyotypic and FISH abnormalities and are prone to conversion to acute myeloid leukemia (12). The prognosis for hMDS 23 falls between that of severe and very severe AA patients (12). The pathophysiology of hMDS is not very well known. Evidence suggests that immune mediated mechanisms may play a role (5). 4 This subtype is most likely to respond to treatment with immunosuppressive agents (13). Other than a few case reports and small case series, there are no published data on the clinical profile and 29 response to treatment in patients with hypoplastic MDS from India. DEFINITION: MYELODYSPLASTIC SYNDROMES: The

myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell diseases characterized by cytopenia(s), dysplasia in one or more of the myeloid cell lines, ineffective haematopoiesis, and increased risk of development of acute myeloid leukemia (AML) (14). The enhanced degree of apoptosis in these disorders contribute to the cytopenia(s) (14). The thresholds for cytopenias recommended in the IPSS (International Prognostic Scoring System) for risk stratification in MDSs are as follows (14); - Hemoglobin < 10g% - Absolute neutrophil count < 1800/mm3, and - Platelets <100,000/mm3. If definite morphologic and / or cytogenetic findings are present, values above the thresholds are not exclusionary for a diagnosis of MDS (14). There may be increase in myeloblasts in the peripheral blood or bone marrow accompanying the dysplasia, but the number is <20% (7,14).

HYPOPLASTIC MYELODYSPLASTIC SYNDROMES: Usually, patients with MDS have a hypercellular bone marrow (5,14) . In a minority of the cases (~10 - 15% of cases), the cytopenia is associated with a hypoplastic bone marrow (2,4–9,14). Hypoplastic Myelodysplastic syndromes (hMDS) refers to a morphological enitity in which the bone marrow cellularity is low for the age (<30% cellularity if age is <60 years or <20% cellularity if age is >60 years) (2,5,14). In these cases distinguishing between hMDS and aplastic anemia may be difficult; the presence of a hypocellular marrow with features of dysplasia in one or more

cell lines, increase in reticulin content on bone marrow trephine, increase in number of blasts/CD 34+ cells on bone marrow trephine, or abnormal karyotype showing malignant clonal cells, all favor the diagnosis of hMDS (2,3,5,6,9,14–16). EPIDEMIOLOGY: According to the WHO classification, hMDS does not represent a defined MDS category, but rather denotes the morphologic status of other MDS categories. Patients with hMDS tend to be younger (2) than those with normo/hypercellular MDS. The median age of patients with hMDS have been reported between 39 and 58 years (1,12), where as in normo/hypercellular MDS the age ranges from 60 and 75 years (1,5,9,14). Maschek H et al reported a higher median age (72.6 years; range;33-88 years) in patients with hMDS (9). The non-age adjusted annual incidence of MDS (including hMDS) reported is 3-5/100,000 persons, rising to over 15-50 /100,000 in those over 70 years of age (5,9,14) in the US population. The reports suggest that the sex distribution is balanced, although there are studies reporting a male preponderance in the hMDS group (1,5,9,14). There is no published data on the exact prevalence or incidence of hMDS among Indian population. In a study of 30 cases of MDS (April 1998 to May 2006) reported from India by Shah NM et al, the mean age at presentation was 55 years (range 8-73 years) with a male preponderance (1,17). A recent initiative is the 'Indian MDS registry' aimed at analyzing the different aspects (epidemiology, clinico-pathology, diagnosis, therapeutic protocols and outcome) of MDSs among the Indian population. ETIOLOGY AND PATHOGENESIS: The exact pathophysiology of hMDS is not known; there are multiple, complex and poorly understood mechanisms involving abnormalities in the regulation of cellular proliferation, maturation, and survival (2,5,18). 63**Association of** hMDS **with increasing age suggests** a **genetic damage caused by hazardous exposure or inherited susceptibility** (14,18). In hypoplastic MDS, marrow failure results not only from 16**ineffective erythropoiesis of abnormal clones, but also due to** the **inhibition of normal progenitors** (2). 16**There is increasing experimental and clinical indication** of **an immune-mediated damage to** the **hematopoietic precursors and changes in the hematopoiesis-supporting microenvironment** contributing **to** the

development of the disease (2). [16]**Immunosuppressive therapy with anti-thymocyte globulin,** cyclosporine, **or alemtuzumab may alleviate cytopenias and** induce cytogenetic remission **in some instances** (2). However, all patients do not [16]**respond to immunosuppression. Identification of relevant biomarkers for an immune mechanism** may help in identifying those patients who may benefit from immunosuppressive therapy (5,18). IMMUNOLOGICAL CHANGES IN hMDS (2) As observed in aplastic anaemia, abnormalities indicative of an active immune process mediated by a Th1-cell response are observed in MDS, including hMDS. These include; 1. Abnormal Cytokine Profile (2): a. High [67]**levels of** tumor **necrosis** factor **-α (TNF-α).** b. Over-expression of TNF-related apoptosis-inducing ligand (TRAIL) - preferentially targets abnormal clonal cells with aberrant chromosomes, inducing apoptosis. c. Lower levels of FLIP, a cytoplasmic inhibitor of apoptosis; this explains the higher sensitivity to TRAIL in cytogenetically abnormal clones. d. Over-expression of Interferon-γ (INF-γ) by bone marrow mononuclear cells in MDS. Both IFN-γ and TNF-α activate the expression of iNOS (induced nitric oxide synthase), which potentially mediate the dysregulation of haematopoiesis in MDS through Fas-mediated apoptosis. The cytokine expression profile may also have prognostic significance. In a recent study, interleukin-4 (IL-4) and C-C motif chemokine 3 (CCL3) serum levels were consistently under- expressed in MDS and independently associated with survival (2) . 2. T-Cell Mediated Attack (2): There are strong laboratory evidence suggesting that the marrow failure is the result of an antigen-driven lymphocyte destruction of the haemopoietic tissue. An expansion of cytotoxic T cells expressing defined T-cell-receptor (TCR) Vβ chain, indicating the oligoclonality of the T-cell repertoire is observed in these patients as well as in MDS. These skewed T-cell populations are observed to reduce or disappear after response to immunosuppressive therapy. Conversely, the dominant T-cell clone persists after treatment in those who fail treatment. The antigens triggering the immune response in MDS are not known. Patients with trisomy 8 often respond to immunosuppression, indicating a strong immunological mechanism for the underlying marrow failure. In patients with trisomy 8, the CD8+ T cells are able to recognize WT1 peptides and engage INFγ expression in vitro, suggesting that this antigen may contribute to elicit an immune response. Whether WT1 antigenicity may be used therapeutically is still unknown (2). 3. Genetic factors: As observed in several auto-immune disorders, HLA-DR15 antigen is overrepresented in

patients with acquired AA, MDS with refractory anaemia, and in patients with MDS bearing a PNH clone These observations taken together further suggest that some MDS cases are immune-mediated (2). Aplastic anaemia and hMDS also may share genetic defects. Gene mutations encoding the telomerase complex (responsible for maintaining the length of telomeres), resulting in excessive telomere shortening in hematopoietic progenitors, are found in some cases of apparently acquired aplastic anaemia (2,9). The observation that a small subset of patients with hMDS respond to androgenic steroids, support this theory. These patients also often evolve to hMDS and acute myeloid leukemia. Acquired aplastic anaemia patients with shorter telomeres (lowest quartile for telomere length) are those with higher risk to evolve to MDS, especially monosomy 7 (2). CLINICAL FEATURES: Majority of patients (i.e.50%), **are asymptomatic at the time of initial diagnosis** (5,14), with **the** median age of presentation in the fourth to sixth decade (1,17).

These patients **present with signs and symptoms** secondary **to** the cytopenia(s), while some **are diagnosed** on **a routine blood** count. **The** dominant findings in hMDS include **anemia, thrombocytopenia, and leukopenia, either alone or in any combination,** resulting from progressive hematopoietic failure (5,14). At the time of diagnosis, **anemia is an almost universal characteristic** finding, with > **80% of patients presenting with a hemoglobin** level **<10 g/dl** and a reduced **reticulocyte count** (5,14). In 25-30% of patients, the blood leukocyte count is low, and the granulocytes may exhibit features of dysplasia (5,14). **One third of the individuals have recurrent infections,** due to granulocytopenia as well as the **result of defects of neutrophil function** (i.e. **impaired chemotaxis & reduced phagocytic activity)** (5,14). Thrombocytopenia and the concomitant platelet function defect results in bleeding manifestations which may include

mainly petechiae, gum **bleeding, or hematoma following trivial injuries;** with <10% of patients presenting with serious bleeding (i.e. gastrointestinal bleed, macro hematuria, menorrhagia, **or retinal or central nervous system hemorrhage)** (5,14).

DIAGNOSTIC WORK-UP Patients with hMDS have more profound thrombocytopenia and neutropenia, lower blast percentage, and one less likely to display an abnormal karyotype in comparison to patients with normo / hypercellular MDS. Diagnosis of low grade MDS is not always straight forward despite the well-established diagnostic criteria and ever-expanding battery of molecular and cytogenetic diagnostic assays. The reasons for such difficulty are multiple, i.e. inter-observer disparity in documenting extent of dysplasia, (especially when dysplasia is mild to moderate) and the frequent lack of detectable chromosomal abnormalities (19). The most difficult part in the diagnosis of hMDS is differentiating it from acquired aplastic anemia. The diagnostic work-up of hMDS includes morphologic evaluation of peripheral blood, bone marrow aspirate, and bone marrow biopsy specimens, interpreted in the context of an adequate clinical information and CBC results (18). Correlation with marrow cytogenetics is essential. A normal karyotype however does not exclude a diagnosis of an MDS (18). Recently multiparameter flow cytometry has proven to be an important diagnostic tool, especially when added to cytomorphology (CM) and cytogenetics (CG) in patients with suspected MDS (19,20). Blood and bone marrow morphology and immunohistochemistry: Although MDS can be suspected from the clinical history and the peripheral blood counts, the **diagnosis is** often **made by** morphologic **inspection of the peripheral blood,** bone marrow aspirate, **and bone marrow** biopsy specimen (18). The current World Health Organization (WHO; 2008) system approach is a more comprehensive one, which stresses the importance of integrating other techniques; ie bone marrow biopsy histologic examination, molecular genetics, and cytogenetics in the light of relevant clinical information (18). Morphologic dysplasia is not necessarily synonymous with an MDS. To address the issue of "false-positive" myelodysplasia, the current WHO classification system recommends that, to declare a dysplasia of a particular lineage, at least 10% of cells in the lineage has to be morphologically dysplastic (14,18). Specific criteria for dysplasia in the three different cell lineages are detailed in the table in Appendix 1 (Table: 1 in Appendix 1) below (14,21). The presence of dysgranulopoiesis and dysmegakaryopoiesis favor the diagnosis of hMDS over AA. Blasts: The **percentage of**

blasts in the bone marrow is important for **the** diagnosis, classification **and** **prognostication of** MDS. It is also an integral component of the currently used prognostic scoring systems; ie [49]**International Prognostic Scoring System (IPSS), WHO** **classification–based prognostic scoring system** and **the** more recent revised International Prognostic Scoring System (IPSS-R) (18). Immature cells to be included in the blast count include myeloblasts (with and without a few fine azurophilic granules), megakaryoblasts and monoblasts, promonocytes are considered as "blast equivalents" in the WHO classification scheme (14,18). It may be hard to appreciate blast in marrow biopsy specimen, particularly if there is marrow fibrosis (14,18). Immunohistochemical (IHC) stain for CD34 antigen may be very helpful in such a situation (14,18). Additional markers used to facilitate visibility of CD34– blasts include; CD117, lysozyme, and CD68 (14,18). The blasts seen in MDS, are often myeloperoxidase negative or only weak positive (18). Flow cytometry may help in confirming the immunophenotype and assessing the frequency of blasts. In addition, side scatter abnormalities (due to granulocyte hypogranularity) and aberrant antigen expression have also been shown to correlate with the severity of the MDS (18). Aplastic anemia can be distinguished from hMDS by the presence of a decreased number of CD34+ cells and reduced [13]**expression of proliferating cell nuclear antigen** (PCNA) **in bone** **marrow** (10). The presence of increased reticulin content on silver staining on trephine biopsy favors the diagnosis of hMDS over AA. CLASSIFICATION OF MYELODYSPLASTIC SYNDROMES: To classify MDSs, a number of morphological classifications are in place; the most recent one being the WHO classification (2008 Revision, 4th ed) (22). The current WHO [3]**classification of** **MDS is principally based on the** blast percentage **in the** peripheral blood and **bone** **marrow, and the type and degree of dysplasia** (14,18). In particular, the extent of dysplasia, multilineage vs unilineage, [56]**and the presence of ring sideroblasts** (assessed by iron staining) have important roles in the WHO sub-classification (14,18). The absence

of monocytosis (monocytes <1,000/μL in the blood and <5% in bone marrow) is important to distinguish between CMML (chronic myelomonocytic leukemia), and MDS (18). **The WHO Classification of Myelodysplastic Syndromes** (table below), recently published, distinguishes the following MDS subtypes (14,18): **(1) Refractory cytopenia with unilineage dysplasia** (RCUD); subcategories- **Refractory anemia (RA), Refractory neutropenia (RN), & Refractory thrombocytopenia (RT); (2) Refractory anemia with ring sideroblasts (RARS); (3) Refractory cytopenia with multilineage dysplasia (RCMD); (4) Refractory anemia with excess blasts (RAEB), with** subcategories RAEB-1 & 2. (5) MDS, unclassifiable (MDS-U); and (6) MDS with isolated del (5q) chromosomal abnormality. The WHO 2008 classification of MDS is detailed in Appendix 1 (Table:2 in Appendix 1). Hypoplastic MDS: In about 10-15 % of patients with myelodysplastic syndromes, the bone marrow is hypocellular, referred to as 'hypoplastic MDS' (5,14,18). This group do not have independent prognostic significance per se (14,18). The major problem is in the differential diagnosis with aplastic anemia. Toxic myelopathies and auto-immune disorders should be excluded when a diagnosis of hypoplastic MDS is considered (5,14,18). According to the WHO classification, Hypoplastic MDS however, does not represent a defined MDS category, but it rather denotes the morphologic status of other MDS categories (2,14,18). MDS with fibrosis: In **a small subset of patients** (~10%) **with** MDS, **significant degrees of myelofibrosis are observed** (14). These are **referred to as MDS with fibrosis. Most of these cases have excess blasts and an aggressive clinical course** (14). In the fibrotic group, due to inadequate aspirate, most often blast determination requires immunohistochemical studies (for CD34 on the trephine biopsy) (14). Multiparameter Flow Cytometry (MFC) in MDS: The

current World Health Organization classification of MDSs is based on morphological evaluation of bone marrow dysplasia. The reproducibility of the recognition of dysplasia is poor in clinical practice, especially in cases where specific markers such as ring sideroblasts and clonal cytogenetic abnormalities are lacking (23,24). In patients with MDS, a recent complementary DNA microarray analyses on CD34+ hematopoietic progenitor cells have found that MDS, including the early-stage/low-grade MDS, is characterized by a B-cell progenitor defect (19). Many genes involved in B-lymphocyte development are down- regulated (19). This observation was validated by the flow cytometric finding that in a small number of MDS patients, the maturing B-lineage precursors (hematogones) are reduced (19,23). Recently a multiparameter flow cytometric scoring system has been validated for the diagnosis of myelodysplastic syndrome. This encompass four reproducible parameters i.e. CD34+ myeloblast-related and B-progenitor- related cluster size (defined by CD45 expression and side scatter characteristics on CD34+marrow cells), myeloblast CD45 expression and granulocyte side scatter value (23). A flow cytometric score may help to establish the diagnosis of myelodysplastic syndrome, especially when morphology and cytogenetics are indeterminate (i.e. early-stage/low- grade MDS). The calculations of flow cytometric score for the

diagnosis of low-risk MDS is detailed in Appendix 1 (Table: 3 in Appendix 1). Cytogenetic and molecular studies: Molecular and cytogenetic **studies have a major role in the evaluation of patients with** myelodysplastic syndrome with **regard to prognosis, determination of clonality and recognition of** morphologic, **cytogenetic and clinical correlates** (5,14). **Clonal cytogenetic abnormalities are** usually **observed in** ~ 50% **of cases** of MDS (and upto 80% of cases with mutagen-related MDS) (5,14). A study by Vundinti BR et al (25) reported 54.48% **chromosome abnormalities including novel chromosome aberrations in patients** with **and** that **these chromosome aberrations increased with advancing age.** 27 **Cytogenetic changes** observed **in MDS are not unique to the disease; both** numerical **and** structural cytogenetic **changes may occur. Most frequent chromosomal abnormalities** observed **involve, deletions of chromosomes 5, 7, 11, 12, and 20 and/or trisomy 8** (5,14). The chromosomal aberrations and its frequencies are described in detail in table in Appendix 1 (Table:4 in Appendix 1) Detection of certain chromosomal abnormalities, either by routine cytogenetic analysis or FISH, aids in the classification of MDS and determination of the prognostic risk group (5,14). With occasional exceptions (i.e. 5q- syndrome/MDS with isolated del(5q)), chromosomal abnormalities in MDS have not correlated with specific clinical or morphological subsets using the WHO classification system (5,14). **Deletion of the long arm of chromosome 5** (5q) **is the most** common chromosomal **abnormality** seen **in MDS (in** 15% of cases). Cytogenetic and molecular

analysis has led to the identification of two small commonly deleted regions; i.e. del (5q33.1) which is most commonly associated with the 5q minus syndrome with a relatively good prognosis, and del (5q31), which is more commonly seen with therapy-related MDS and is associated with more aggressive disease. MDS with 5q minus syndrome occur primarily in women, and is characterized by refractory macrocytic anemia, normal or increased platelet count, megakaryocytes with non-lobated or hypolobated nuclei, a favorable clinical course & good response to lenalidomide treatment (14). Cytogenetics as a predictor of prognosis: Cytogenetic abnormalities have an impact on outcomes in patients with MDS. The abnormalities are risk categorized into 3 different cytogenetic categories 34**in the international prognostic scoring system (IPSS) and WHO prognostic scoring system,** and into 5 groups in the recent revised IPSS (IPSS-R) (26) .

The risk categories, their risk for progression to AML and median survival for the different groups are as follows; Cytogenetic subgroups in the IPSS & WPSS for adults with MDS. Prognostic subgroups Cytogenetic abnormality 25 % AML progression Median survival 3**Good risk Normal karyotype, isolated del(5q), del(20q),** or **–Y** 5.6 years 3.8 years Intermediate risk Other abnormalities 1.6 years 2.4 years Poor risk -7/del(7q), or complex 0.9 years karyotypes(≥3 abn) 0.8 years The 5 cytogenetic risk categories and their median OS in the revised IPSS (IPSS-R) are as follows; Cytogenetic subgroups in the IPSS-R for adults with MDS Prognostic Subgroups Cytogenetic abnormality Median OS 19**Very Good del(11q),-Y** 60.8 months **Good** Normal, **del(20q), del(5q) alone and double, del(12p)** 48.5 months **Intermediate +8, 7q-, i(17q), +19, +21, any other single or double, independent clones** 25.0 months **Poor der(3)q21/q26-7, double including 7q-,** 15.0 months **complex (3 abnormalities Very poor Complex (>3 abnormalities)** 5.7 months GENE MUTATIONS: Abnormalities in certain genes have been identified in patients with MDS and acute myeloid leukemia with or without the presence of chromosomal abnormalities. These include mutations in TET2, ASXL1, TP53 tumor suppressor gene, RUNX1 transcriptional core-binding factor gene(CBF), IDH gene, FLT3 gene and

SF3B1 genes. These gene mutations also confer prognostic significance in adult patients with MDS, for instance, it has been reported that patients with TET2 mutations may have higher response rates to azacitidine than those without the mutations (26). PROGNOSIS & RISK STRATIFICATION: Myelodysplastic syndromes, in view of the 50**progressive impairment in the ability of** the **myelodysplastic stem cells to differentiate,** are clinically characterized by an increased 44**risk of evolution into acute myeloid leukemia (AML)** (27). **The natural history of MDS** ranges **from indolent conditions spanning years to** rapid progression **to leukemia.** The probability of leukemic evolution is lower in hMDS (1,6) than the normo-/hypercellular MDS (NH-MDS) (1,2,6), but compared to AA, hMDS have poorer prognosis with frequent karyotypic and FISH abnormalities and a higher probability of leukemic evolution. As the prognosis of patients with MDS is very heterogeneous, development of a prognostic system 1**that allow risk stratification and help in the timing and choice of therapy** is essential (26). 1**A number of prognostic scores are currently in use,** and these include; a. IPSS (International Prognostics Scoring System): This is the most commonly used score and has been in use since 1997 (26). Prognostic score includes, the number of cytopenias, percentage of blasts, and the type of cytogenetic abnormality (26). Based on this scoring system there are 4 prognostic risk categories; i.e. 45**'Low' (score:0), 'Intermediate-1' (score :0.5-1), 'Intermediate-2' (score:1.5-2) and 'High risk' (score:≥2.5).** IPSS is highly reproducible 1**and very simple to use,** but **has several limitations;** i.e. 1**it is not a very precise predictor of prognosis in** those **with lower risk disease and it attributes relatively little weight to cytogenetics** (26). The IPSS scores, prognostic risk categories and their clinical outcomes (in terms of survival and risk of transformation to AML) are

detailed in Appendix 1 (Table:5 in Appendix 1). b. Revised international score (IPSS-R) : This score presented at the 2011 MDS Meeting in Edinburgh, incorporates a new cytogenetic score and includes different cut off for cytopenias (26). Prognostic score includes, the type of cytogenetic abnormality, percentage of blasts, hemoglobin level, platelet count and ANC (absolute neutrophil count) (26). Based on this systems, there are 5 prognostic risk categories i.e. 70**'Very low'** **(score:≤** 1.5), **'Low' (score:.1.** 5-3), **'Intermediate' (score:>3-4.** 5), **'High' (score:** >4.5-6) and 'Very high' (score:>6) risk groups. The IPSS-R scores, prognostic risk categories along with their clinical outcomes (i.e. survival and risk of AML transformation) are outlined in Appendix 1 (Table: 6 in Appendix 1). c. WPSS (WHO prognostic scoring system): This is another commonly used system that incorporates the transfusion dependency in addition to cytogenetics and WHO diagnostic category (26). The main limitations of this system is that it requires, prior information of transfusion needs and WHO classification (26). A recent modification of the WPSS score included hemoglobin levels instead of transfusion needs(26). Prognostic score includes, the WHO MDS category, the type of cytogenetic abnormality and the transfusion requirement (26). Based on this system 34**patients are categorized into** 5 prognostic **risk groups; i.e.** 40**'Very low'** **(score=0), 'Low' (score=1), 'Intermediate' (score=2), 'High' (score=3-4)** and **'Very high' (score=5-6).** WHO **prognostic scoring system** with its clinical outcomes is detailed in Appendix 1 (Table:7 in Appendix 1). d. Global MDACC (MD Anderson Cancer Center) model: A more recent one is the 1**global MDACC model that allows evaluation of all patients** considered to have 1**MDS at any time during** the **course of their disease without** **needed WHO evaluation(** 26). The Global MDACC and MDACC MDS lower risk Prognostic Models are tabulated in Appendix 1 (Tables:8a & 8b in Appendix 1). e. Recently, based on the study on 9**a cohort of 253 patients with hypocellular MDS (diagnosed at The University** **of Texas MD Anderson Cancer Center between 1993 and 2007) and a cohort of**

1725 patients with hyper-/normocellular MDS (diagnosed during the same time period), a new prognostic model [9]**was built that segregated patients into 3 distinct risk categories independent of International Prognostic Scoring System (IPSS) score** (26). **This model is independent from the IPSS,** and **further refines IPSS-based prognostication.** It **may be used to develop of risk-adapted therapeutic approaches for patients with hypocellular MDS** (26,28). The details of the prognostic model of hypoplastic MDS is tabulated in Appendix 1 (Table: 9 in Appendix 1)

HYPOPLASTIC MDS AND APLASTIC ANAEMIA: [13]**Because of considerable clinical,** histologic **and** cytologic **similarities between these two disorders,** it is sometimes difficult to distinguish Hypoplastic myelodysplasia from acquired aplastic anemia (10). The presence of dysmegakaryopoiesis, dysgranulopoiesis, increased percentage of blasts, increased bone marrow reticulin content and abnormal karyotype, favour the diagnosis of hMDS. In addition, an abnormal antigen expression pattern in marrow CD34+ cells indicating an aberrant clone, and the presence of elevated haemoglobin F-containing erythroblast production suggest the diagnosis of hMDS. However, findings compatible with an immune process (oligoclonal T-cell expansion, relative lymphocytosis in the marrow and increased cytokine levels) do not contribute to differential diagnosis, as these elements are present in both (2). The distinction between hMDS [7]**and AA is of great** prognostic **and** therapeutic **importance. With modern** therapies; ie **bone marrow transplantation and immunosuppressive therapy,**[13]**severe AA** patients **have long term survival**[7]**(80% at 14 years),** and those **with moderate AA have** a **median survival** of **>174 months with androgen and supportive therapy;** while

[13]for patients with hMDS, the median survival is not significantly different from hypercellular MDS (22 to 33 months). [7]Some cases of hMDS may show a transient response to androgens and/or immunosuppressive therapy thus adding further diagnostic difficulty. Further, [13]there is a higher risk of progression to acute leukemia in patients with hMDS compared with AA (29). Recent studies have suggested that in AA, bone marrow (BM) is characterized by a decreased number of CD34+ cells and reduced expression of [6]proliferating cell nuclear antigen (PCNA), which is not a feature associated with MDS (10). A role for tumor necrosis factor-alpha (TNF-alpha) in the development of AA has been suggested by recent studies(29). [7]Careful examination of peripheral blood, may also provide sufficient information to allow for the distinction between hMDS and AA early in the course of the disease (30). Certain morphologic findings i.e. [7]hypochromic red cells, circulating blasts, left shift, hypersegmentation with long filaments, Dohle bodies hypogranular, ring, and pelgeroid neutrophils, circulating micromegakaryocytes and megakaryocytic fragments are seen only in hMDS but not in AA (30). The table below summarizes the major differences between hMDS and AA. (3,6,15,16,31,32). Distinction between hypoplastic MDS and Aplastic anemia Characteristics Dyserythropoiesis Abnormal neutrophil Dysplastic megakaryocytes Fibrosis Increased blasts CD34+ cells in BM Clonality Splenomegaly Hypoplastic MDS Yes Yes Yes Occasional Sometimes (ALIPS) Sometimes increased

Sometimes Occasional AA Sometimes No No No No < 1.0% Possible Absent

24It is fortunate that the distinction is not critical since both aplastic anaemia and hypoplastic MDS respond to similar forms of treatment. Indeed there is close similarity between the two conditions and they are sometimes called 'overlap syndromes'.

DIFFERENTIAL DIAGNOSES: Acquired aplastic anemia is the most important and difficult differential diagnosis of hMDS (discussed above). Other conditions that can present with cytopenia(s) and dysplastic changes include; Vitamin B12 & folic acid deficiencies, Copper deficiency & arsenic poisoning, medications & drugs, liver failure or hypothyroidism causing macrocytic anemia with a low reticulocyte count, auto-immune and other hematopoietic neoplasms, e.g. lymphomas, and non-hematopoietic malignancies causing para-neoplastic myelodysplasia, Viral infections (e.g. HIV infection,, chronic parvovirus, Epstein-Barr virus, and cytomegalovirus infections) and rarely, hemophagocytosis can produce marrow changes that resemble MDS (18). TREATMENT OF hMDS: The treatment of hMDS is similar to aplastic anemia in view of similarity in its pathophysiology. The options include; A. 1Options for newly diagnosed patients: In newly diagnosed lower risk group patients, therapy1is based on the transfusion needs of the patients (26). Transfusion independent patients are usually observed until they become transfusion dependent (26). A new and important concept 34in the treatment of lower risk MDS is early intervention in patients with "poor prognosis" 18lower risk MDS (26). To improve on the natural history of the disease, the identification of these patients is going to be fundamental (26). The upcoming new prognostic scoring systems and development of new molecular informations for these patients will help in early identification and intervention (26). The list of agents currently available for treatment of patients with hMDS is as follows; 1. Immune therapy: Hypoplastic MDS is characterized by dysregulation of immunity, and it

has been observed that patients with hMDS benefit from immunosuppressive therapy (26). The agents studied include cyclosporine-A (CSA), corticosteroids and antithymocyte globulin (ATG) (26,33). Standard IST is the combination of ATG & CSA (horse or rabbit ATG) with a short course steroid. The dose of ATG used is 40 mg/kg over 4 hours, daily for 4 days after premedication along with prednisolone (1 mg/kg from day 1 for 2 weeks) for serum sickness prophylaxis, and CSA (dose: 10 mg/kg/day from day 1 to target trough level- 200 and 400 ng/ml. Cyclosporine monotherapy (6mg/Kg) is an easily available, and is a safe and cheap IST. The group 36**at the** NHLBI **(National Heart, Lung and Blood Institute)** has developed an algorithm 1**to predict response to these agents,** i.e. **younger age,** shorter duration of transfusion dependency and HLA-DR15 (34). Bone marrow hypocellularity is the most important predictor for response (35). Recently alemtuzumab (humanized 51**monoclonal antibody that specifically kills CD52-bearing cells via both antibody-dependent cellular cytotoxicity & complement-mediated lysis;** dose **is** 10 mg Alemtuzumab s/c daily x 5 days along with CSA (2 mg/kg Q12H) x 3 months, has also been reported to have 1**significant activity in** those **patients with MDS predicted to** respond **to immune suppressive therapy** (26,34). **The**1**most important predictor for response has been the** presence **of marrow hypocellularity** (26,34). In 1**younger patients with severe hypoplastic MDS, allogeneic stem cell transplantation** (Allo SCT) **should be considered as soon as possible. For those that are not** candidates, **a combination with equine ATG is recommended.** Response to immune therapy (IST) reported in literature is a haematologic recovery of 75- 90% after one or two courses of IST (36). A response rate of 68% is reported for ATG/CSA in AA, while following alemtuzumab therapy upto 57%

response has been reported by some studies (26,33,36,37). 2. Allogeneic Peripheral blood stem cell transplant: 8Allogeneic hematopoietic stem cell transplantation is the treatment of choice in young patients with severe aplastic anemia or hypoplastic MDS. The main causes of failure after this procedure are graft versus host disease, infections and graft failure, often exacerbated by large numbers of transfusions and prolonged disease duration before transplant (38). 15A less toxic regimen comprising reduced cyclophosphamide (Cy), fludarabine, and anti-thymocyte globulin (ATG) (Cy-Flu-ATG) was used to condition high-risk patients scheduled for allogeneic hematopoietic cell transplantation (allo HSCT) instead of standard Cy-ATG in patients with severe aplastic anemia (AA) and hMDS (39). 15Preconditioning with Cy-Flu-ATG was superior to that afforded by Cy-ATG in terms of reducing RRT levels without increasing engraftment failure (39). Recent study by Szczylik C. et al showed 8that transplantation of hematopoietic stem cell using alemtuzumab, fludarabine and melphalan as a conditioning therapy is safe, inexpensive and effective treatment for patients with severe aplastic anemia, including multi-transfused adults having their disease for a long time (38). Five year OS following matched related and unrelated Allogeneic PBSCT reported are 73% and 60% respectively (36,39). 3. Androgenic steroids: Androgenic steroids i.e. Danazol (derivative of

synthetic steroid ethisterone-17 α ethinyl testosterone; 300mg daily), Oxymetholone, and Stanazolol (synthetic anabolic steroid derived from testosterone; 1mg/Kg/day) have been proved to be of benefit in hMDS as seen in Aplastic anemia (40). **14Androgens are enzymatically converted into estradiol (E2) via aromatase. E2 passively diffuses into cells and binds theα isoform of the estrogen receptor** (ERα), **which acts as a transcriptional activator by binding to estrogen response elements (ERE) in genomic DNA. The telomerase reverse transcriptase (TERT) promoter contains 2 putative EREs. Therefore, both androgens and estrogens increase TERT expression, ultimately resulting in increased telomerase activity in hematopoietic cells.** Androgens also inhibit both interleukin-1 and TNF-a production. In patients unresponsive to IST, a response rate of 30-35% has been reported to androgens in some studies (41). 4. Haematopoietic growth factor support: Currently a number of erythroid stimulating agents (ESA) are available. Reported rates of response to these agents range from 30 to 60% (26,42). In a retrospective observational study by Jadersten M et al (26,43), it was observed that addition of G-CSF to erythropoietin increased the response rates, and **1early introduction of this combination in patients with** minimally transfusion-dependent **and** low risk disease may have an impact on survival. In patients with significant anemia and with no other cytopenias, **1a course of ESA with or without G-CSF is not contraindicated** (26). Early incorporation of these agents has been found to be **1more effective than in patients with heavy transfusion burdens.** Due to complications related to disease transformation and marrow fibrosis, the use of Romiplostin in lower risk MDS is questionable (26). 5. Supportive care measures: The supportive care measures in

hMDS include; use of prophylactic antibiotics and iron chelation. No randomized data exists to make formal recommendation for any of these interventions (26). B. Options for patients with higher risk MDS: Treatment options for patients with higher risk MDS have significantly evolved over the last decade. Earlier most patients were treated with cytarabine based therapy as for AML. Recently the use of azanucleosides (Decitabine & 5-Azacytidine) has modified this practice (44,45). Two candidate biomarkers and a clinical model have been proposed recently. mutations on TET2 and levels of miR29b and have been reported to be associated with response to azacitidine and decitabine respectively (26,46). AML-like chemotherapy: In higher risk MDS, AML-like protocols have generally used classical anthracycline-araC combinations, similar to that used in de novo AML. AML-like therapy results in lower CR rates (40–60%), and shorter CR duration (10–12 months) when used in MDS or AML post-MDS. They tend to be associated with more prolonged periods of aplasia, and in addition, due to the advanced median age of the patients, the feasibility of AML-like therapy is also reduced (26). Allogeneic Stem cell transplant: Allogeneic SCT is the only curative treatment of

higher-risk MDS. Selected studies report prolonged DFS in about 30% to 50% of the patients. However its use is restricted mainly to younger patients with an appropriate donor (26,47). RESPONSE CRITERIA: Definition of IWG response criteria in MDS: The IWG (International working group) criteria define 4 aspects of responses based on treatment goals: (1) altering the natural history of the disease, (2) cytogenetic response, (3) hematologic improvement (HI), and (4) QOL (Quality of life) (48). The responses assessed include CR (complete remission), PR (Partial remission), Stable disease, Failure, Relapse after CR or PR, Cytogenetic response and disease progression. The details of the proposed modified International working group response criteria are described in Appendix 1 (Tables:10a & 10b in Appendix 1). SUMMARY: Hypoplastic MDS is a distinct clinic-pathologic entity characterized by bone marrow hypoplasia, severe leucopenia and thrombocytopenia, macrocytosis, low incidence of progression to acute leukemia, and unresponsiveness to conventional therapy (6,8). It represents approximately 10 - 15% of all MDS cases (2,4–9,14). It is difficult to distinguish hMDS from acquired aplastic anemia (AA), because of considerable clinical, histologic, and cytologic similarities between the two disorders (1,6,10,14). However, compared to AA, hMDS have

23**poorer prognosis and frequent karyotypic and FISH abnormalities** (prone **to** leukemic conversion) (12). The Pathophysiology of hMDS is not very well known; auto-reactive and 4**clonal-involved T-cells are believed to suppress** the **normal hematopoietic cells by secretion of inhibitory cytokines** (5). **This subtype is most likely to respond to** treatment with **immunosuppressive agents;** therapy with antithymocyte 31**globulin (ATG), cyclosporine (CSA) or both** has been shown good response in patients with hMDS (13). Aims and objectives: 1. To analyze the clinical profile of adult patients with Hypoplastic Myelodysplastic syndrome (hMDS). 2. To assess the response to different drug therapies in patients with hMDS. 3. To identify the demographic, clinical, and laboratory parameters that can predict prognosis in hMDS. Patients and 20**Methods This study** protocol **was approved by** our **Institutional Review Board** (IRB). 20**This is a** retrospective **analysis of patients diagnosed** to have hMDS from January 1998 to June 2012. Duration of the study: October 2012 to December 2012. Settings of the study: Department of Clinical Haematology. Diagnostic criteria: Hypoplastic MDS was diagnosed in patients presenting with cytopenia(s) (defined as per the recommendation in the IPSS for risk stratification in MDSs (i.e. Hemoglobin <10g%, Absolute neutrophil count <1800/mm3, and Platelets <100,000/mm3) associated with a hypoplastic bone marrow for the age (2,5,14), and with features of dysplasia in one or more cell lines, with or without increase in number of blasts/CD 34+ cells on bone marrow, or increase in reticulin content on bone marrow trephine, or abnormal karyotype showing malignant clonal cells (all favoring diagnosis of hMDS) (2,3,5,6,9,14–16). Patients: Inclusion Criteria: 1. All adult patients (age≥18yrs) diagnosed to have hypoplastic myelodysplastic syndrome from January 1998 to June 2012 Exclusion Criteria: 1. Patients with other types of Myelodysplastic syndromes. 2. Patients with hMDS whose data are not retrievable. 3. Patients on drugs that can cause dysplasia (e.g. post renal transplant patients) 4. Patients with hypoplastic cytopenia(s) and positive test for PNH, or positive stress cytogenetic test (clastogenic stress-induced chromosomal breakage). Methods: Collection of data: After approval by the IRB, the patient data base at our institution was reviewed to identify all patients diagnosed to

have hypoplastic MDS at our institute between January 1998 to June 2012. Medical information regarding the clinical/laboratory details at diagnosis, post treatment response and adverse events were obtained from the hospital records (laboratory reports/ physician documentation in hospital charts/hospital discharge summaries). Attempts were made to contact all patients by post or e-mail to collect details on any missing data as well as the recent clinical status. Only patients who had at least 8 weeks follow up (including those who died within 8 weeks) after initiating therapy were categorized as 'evaluable' for assessment of response and survival. Treatment: Various treatment modalities that the patients received were reviewed. This included; Cyclosporine, anti-thymocyte globulin + CSA, androgenic steroids, corticosteroids, supportive measures, and 30**allogeneic peripheral blood stem cell** transplant, and **a** few had received haematopoietic growth factors or lenalidomide. Data was collected with regard to type of treatment, duration of treatment, side effects and overall outcome with respect to the treatment given. Data analysis: Results are analyzed in terms of the clinical characteristics and laboratory parameters at diagnosis, response to the different treatment regimens [drug(s)], the survival patterns and the prognostic effects of patient characteristics on overall survival. The response to treatment is assessed in terms of Complete Remission (CR), Stable disease, Relapse, Progression of disease, No response, and failure/death. 17**CR was defined** as **the absence of any clinical sign of disease** and attainment of the following haematological parameters i.e. pperipheral blood Hb ≥ 38**11 g/dL, Platelets ≥100** x109 **/L, Neutrophils ≥1.0** x109 **/L, Blasts - 0%.** Patients with 38**failure to achieve at least** CR, **but** with **no evidence of progression for >8** weeks were considered to have 'Stable disease'. Relapse after CR was defined as reduction of values by 31**≥50% from maximum remission/response levels in granulocytes or platelets,** and or **reduction in** Hb **concentration by ≥1.5 g/dL or transfusion dependence.** Progression **of** disease was defined as any 39**one of the following** i.e. (1) **At least**

50% decrement from maximum remission /response in granulocytes or platelets, (2) Reduction in Hb by≥ 2 g/dL, (3) Transfusion dependence (Tables:10a & 10b in Appendix 1). All patients started on treatment and with a minimum follow up of 8 weeks were considered evaluable for response and outcome. Overall survival (OS) was measured from the start of therapy until death (from any cause) or last follow-up (49–51). Event-free survival (EFS) was calculated from the start of therapy until the first adverse event, i.e. relapse or progression, secondary malignancy, death from any cause, or last follow- up (49–51). Progression-free survival (PFS) for all patients was taken from the start of therapy until disease progression or death from hypoplastic myelodysplastic syndrome (49–51). Disease-free survival (DFS) for patients in CR was measured from the first recording of response (CR or Stable disease) to the date of progression or relapse (49–51). The closing date for analysis was December 31, 2012. Statistics: Descriptive statistics were calculated for all variables. Differences in proportions were assessed using the chi-square statistic or Fisher exact test. Differences in means were tested using a t-test or Mann-Whitney-U test as appropriate. Survival curves were drawn by the Kaplan-Meier method and compared by the log-rank test. The relationships of clinical features to the outcome of the procedure were analyzed by univariate Cox proportional Hazard

model.[57] For all tests, a 2-sided P-value of 0.05 or less was considered statistically significant. SPSS 16.0 software [6] was used for the analysis.

RESULTS: Between January 1998 and June, 30, 2012, [11] a total of 54413 out patients were seen in the Haematology department, of which 1225 (2.3%) were diagnosed to have primary MDS. Of this, 173 (14.1% of MDS; 0.32% of total patients) were diagnosed to have hypoplastic MDS. The year wise distribution of total MDS and hMDS is depicted in Figure:1. All patients (n=173) were included for the analysis of baseline characteristics. Out of the total 173 patients, only 111 (64.2%) who had a follow up of >8 weeks after initiation of treatment [26] were considered 'evaluable' for assessment of response to treatment and for survival analysis. Certain data are available on all patients, while certain data are available only on a portion of the patients. For each result category, the numbers of patients involved are mentioned. DEMOGRAPHY & CLINICAL FEATURES AT DIAGNOSIS: (Table:1) The median age of the 173 patients was 41 years (range: 18-64). Seventy two (41.6%) [10] patients belonged to the age group of 18-40 years, 51 (29.5%) to 41-55years and 50 (28.9%) were above 55 [21] years of age. Males were predominantly represented in the study group i.e. 112 [35] (64.7%) males and 61 (35.3%) females. The male female ratio was 1.8:1. Pallor and bleeding manifestations were the common presenting symptoms; i.e. in 94.2% (n=163) and 40.5% (n=70) respectively. Sixty six (38.2%) patients had history of infections (mainly recurrent febrile episodes; a few had lower respiratory tract and skin infections). [33] The median duration of symptoms was 3 months (range: 1-120). Clinical examination showed mild (<2cms) splenomegaly in 6 patients (3.5%) and mild hepatomegaly (<2cms) in 2 (1.2%) patients. Forty eight patients (27.7%) had received previous treatment. This included,. CSA (n=8), Azathioprine (n=2),

Anabolic steroids (n=8), Prednisolone (n=11), EPO (n=2), GCSF (n=1) or Lenalidomide (n=1) for a median duration of 60 days (range: 7-720). Among the 173 patients, 139 had received transfusions (packed red cells and or platelet rich concentrates) before presenting to our Institution. The median number of transfusions per month was 2 (range;1-20) units. Fourteen patients (8.1%) had past history of treatment for cytopenias; 11 patients were diagnosed and treated for Aplastic anemia before the diagnosis of hMDS was made. The median time from diagnosis of AA to diagnosis of hMDS was 38 months [range:4-149]. Among them, 2 each had attained CR or PR and were off therapy for a mean period of 53 months (range: 28-91). The remaining 7 remained symptomatic and were diagnosed to have hMDS on re-evaluation. The twelfth patient was diagnosed to have B12 deficiency with bicytopenia 6 years back and had been lost to follow up while on B12 & folate supplementation; a second patient (male; 23yrs) was diagnosed to have Chloramphenicol induced pancytopenia 12 years back which had resolved. The last patient (female; 61yrs) was diagnosed to have MDS with fibrosis 3 years back (hypercellular marrow with fibrosis, normal cytogenetics), was treated with thalidomide & prednisolone for one year followed by danazol for 2 years before she was diagnosed to have hMDS (with cytogenetics- del5q & WHO group RAEB-1). She was subsequently treated with Lenalidomide with no response and expired within 3 months of diagnosis of hMDS. LABORATORY PARAMETERS AT DIAGNOSIS: (Table: 2) At presentation, majority of the patients (65.3%; n=113) were pancytopenic, while bicytopenia was seen in 53 (30.6%) and only 7 (4.1%) had cytopenia involving a single lineage. The median hemoglobin for the entire cohort was 5.8g% (range:1.2-13.2); most of these patients (54.4%; n=94) had hemoglobin level <6g% while in 41% (n=71) the level was between 6.1-10g% and only 4.6% (n=8) had hemoglobin >10g% at presentation. Neutropenia (an absolute neutrophil count <1800/mm3) was observed in 135 (78%) patients at presentation, while 38 (22%) had normal neutrophil count. ANC below 200/mm3 was observed in 8.7% (n=15) while ANC between 201- 500/mm3, 501-1000/mm3, 1000-1500/mm3, and 1501-1800mm3 was found in 16.2% (n=28), 22.5% (n=39), 20.8% (n=36) and 9.8% (n=17) respectively. Thrombocytopenia (platelet <100 x109/L) was documented in 161 (93%) patients; 61.3% (n=106) had a count < 20 x109/L, while a count of 21-50 and 51-100 x109/L was observed in 39 (22.5%) and 16 (9.2%) patients respectively. The median reticulocyte percentage at diagnosis (documented in 152 patients) was 1.8% (range: 0.05-7.06). Out of the 99 patients in whom the absolute reticulocyte count was available, the median

absolute reticulocyte count was 37200/mm3, with 18 (18.2%) patients having an absolute reticulocyte count <20 x109 /L). None of the patients had monocytosis or eosinophilia. The median absolute eosinophil (AEC), monocyte (AMC) and lymphocyte (ALC) counts were 0/mm3 (range: 0-966), 36/mm3 (range: 0-690) and 1656/mm3 (range: 175-8800) respectively. Data on auto-immune markers i.e. direct coomb's and antinuclear antibody tests were available only in 39 and 23 patients respectively, with 14 (35.9%) being coomb's positive and 8 (34.8%) positive for ANA. Serum LDH was documented in 160 patients, and majority (81.9%; n=131) had level <600 mg/dl. Serum ferritin was available in 24 patients; the median level was 825ng/ml (range: 138-46775). Report on serum B12 level was available in only 10 patients and the median value was 970pg/ml (range: 248-2000). Out of the 137 patients in whom blood borne virus screen was done at diagnosis, 3 patients were positive for HBV, while 1 each positive for HIV and HCV respectively. BONE MARROW FEATURES & WHO CLASSIFICATION AT DIAGNOSIS: (Table:3 & Figures:2a-2h] The bone marrow was aplastic, uniformly hypocellular or varyingly hypocellular in 5.2% (n=9), 83.2% (n=144) and 11.6% (n=20) of patients respectively (Fig: 2f). The data on BM blast count was available in 171 patients, out of which majority (46.8%; n=80) had no blasts on the aspirate and no increase in CD 34+ cells on the trephine biopsy. In 77 (45%) patients, the blast percentage was 1-2%, while 10 (5.8%) had 3-4% blasts and 4 (2.4%) had ≥ 5% blasts on the marrow (Fig: 2h). Scattered ring sideroblasts (Fig: 2e) were documented in 47 (39.2% of the 120 cases where data was available) patients and one had >15% ring sideroblasts. Trilineage dysplasia (Figs: 2a-d, 2f) was observed in 57 (33%) patients, while 36 (20.8%) patients had unilineage and 80 (46.2%) had bilineage dysplasia respectively. Data on bone marrow reticulin was available in 169 patients. Out of this 135 (79.9%) patients showed increased reticulin content (Fig: 2g) on silver stain. Most of these patients (47.3%; n=80) showed mild increase in reticulin, while 49 (29%) and 6 (3.6%) patients showed moderate and marked increase in reticulin respectively. On categorizing the patients according to the WHO classification, majority (77.5%; n=134) belonged to the RCMD group, with 4 (2.3%; n=4) belonging to RAEB-1 and 35 (20.2%) belonging to the MDS unclassified (MDS-U) group. CYTOGENETIC ABNORMALITIES AT DIAGNOSIS: (Table: 4) Cytogenetic data was available on 116 (67%) patients. Majority (66.4%; n=77) had normal karyotype. Five patients had polyploidy out of which only 2 had associated structural chromosomal anomalies while 3 had no anomalies. Among those with chromosomal aberrations, 11.2% (n=13) had a sinlge abnormality, while 8 (6.9%) patients had 2 abnormalities, 3 (2.6%) had three abnormalities and 15 (12.9%) had more than 3abnormalities/associated monosomy 7. Among the 39 patients with chromosomal abnormalities, 55 numerical abnormalities were observed. Most of the numerical anomalies were monosomies (n=37), while 18 were trisomies. There were 13 patients (11.2%) with monosomy 7, 7 patients (6%) with trisomy 8, and 8 patients (6.9%) with trisomy 21. Other abnormalities noted included translocations (n=5), deletions (n=15), and other rare anomalies i.e. additions (n=5), derivatives (n=4) and duplications (n=1). Deletion 5q

was observed in 7 (6%) cases. The details of each anomaly are detailed in table 4. CYTOGNETIC & PROGNOSTIC RISK GROUPS AT DIAGNOSIS: (Tables: 5 & 6) Cytogenetic risk groups: (Table 5) One hundred and sixteen patients in whom the cytogenetic reports were available, were risk categorized into three cytogenetic risk groups (good, intermediate and poor) as per the 'IPSS & WPSS', and into 5 risk groups (very good, good, intermediate, poor and very poor) according to the revised IPSS (IPSS-R) systems. 21**Majority of the patients belonged to the 'good' cytogenetic risk** group, in either systems (69% [n=80] each in both), while 16.4% (n=19) and 14.6% (n=17) 61**belonged to the intermediate and poor risk cytogenetic groups** respectively in **the 'IPSS&** WPSS' group. Whereas in the IPSS-R group, 3 patients (2.6%) each belonged to 'very good' &'very poor' risk groups, while 15 (12.9%) each belonged to the 'intermediate' &'poor risk' groups respectively. Prognostic risk groups: (Table: 6) The 116 patients were categorized into different prognostic risk groups using three different prognostic scoring systems ie; IPSS, IPSS-R and WPSS scoring systems. Using the IPSS system, majority (82.7%; n=96) belonged to the 'intermediate-1' group, while 17 (14.7%) and 3 (2.6%) belonged to the 'intermediate-2' and 'low' risk groups respectively. There were no patients in the high risk group. Using the WHO classification based prognostic scoring system (WPSS), one patient (0.9%) was 61**categorized into the**'very **low' risk group,** 68.1% (n=79) into 'low' risk group, 16.4% (n=19) into 'intermediate' risk and 14.6% (n=17) into high risk groups respectively. In the IPSS-R category, 2 (1.7%) patients were 32**in the 'very** low' **risk group,** 22 (19%) **in the 'low' risk,** 68 (58.6%) in the 'intermediate' risk, 18 (15.5%) in the 'high' risk and 6 (5.2%) were in 'very high' risk groups respectively. TREATMENT AND RESPONSE: (Table: 7) Patients who had a minimum of 8 weeks follow up after starting treatment (including those who expired within 2 months of treatment) 26**were considered evaluable for assessment of response to treatment.** Among the total 173 patients; 111 patients (109 with >2 months follow up + 2 who died within 2 months of starting treatment) were considered evaluable, and the remaining 62

26 **were considered** non **-evaluable for assessment of response to** therapy. Out of the 111 evaluable patients, cytogenetic data was available only in 79 patients. Of the 111 evaluable patients, 99 received treatment with (a) Cyclosporine (CSA) or (b) Antithymocyte globulin+CSA (ATG+CSA) or (c) Androgenic steroids (Danazol/stanazolol) or (d) Prednisolone, or (e) Allogeneic PBSCT (Allo PBSCT) and 12 patients received 'Other' therapies (i.e. Lenalidomide or EPO/GCSF or supportive measures). (a) CSA (Table:7): Overall 81 patients received treatment with CSA; out of these only 59 were evaluable for response. Among the 59 evaluable patients, 41 showed a response (6 [10.2%] achieved CR while 35 [59.3%] achieved 'stable disease'). Six (102%) patients expired without attaining any response and the remaining 12 showed no response to CSA. Among the 6 patients who expired, one had progressed to AML prior to expiry. Of the non-responders, 8 were started on second line treatment with Androgenic steroids and 4 patients who had matched sibling donors were taken up for allogeneic PBSCT (detailed in the section on Allogeneic PBSCT). Among the responders, the 6 patients in CR continued to be in CR at last follow up. In the 35 patients with 'stable disease' 24 (68.6%) continued to be in 'stable disease' at last follow up (6 are stable & off treatment, while 16 patients are stable but remain on treatment, 6 **and 2 were lost to follow up** while on treatment). **Of** the 35 **patients** with 'stable disease', 11 progressed while on follow up. One patient progressed to PNH and was started on Danazol. Another patient was started on danazol followed by ALG+CSA with no response (subsequently succumbed to disease progression and treatment failure). Of the remaining 9 patients, one expired following disease progression and 8 were restarted on CSA 6 **(2 were lost to follow up and** 6 continue to be on follow up). While on treatment with CSA, 5 out of the total patients developed CSA related nephrotoxicity elevated serum creatinine, these were non-responders and was changed to Androgens) and 2 had gum hyperplasia. The mortality was 13.6% (n=8) among those treated with CSA. .Overall the response rate to CSA was 69% (41 out of 59), with mean time to response of 5.9months (range: 3-36months), and median 11 **duration of treatment of 19** months **(range:** 3-126). 11 **At a** mean **follow up** duration **of** 103 **months (range:** 8-110), **the 5 year** OS of the responders (n=41) was 96.9% ± 3.1%. (b) Antithymocyte globulin+CSA

(ATG+CSA) (Table:7): 12**A total of 10 patients were treated with** ATG+CSA. Of these 7 showed response to treatment (2 CR and 5 'stable disease'), one patient expired without response and 2 showed no response. The 2 non-responders were changed over to Androgens. Among the responders, 2 patients who achieved CR continued to remain in CR (on CSA) at last follow up. At last follow up, 3 out of the 5 patients with' stable disease' continued to be so (2 were off treatment and one was on CSA) and 2 patients progressed (out of which one expired and one was continued on treatment). The mortality was 20% (n=2) among those treated with ATG+ CSA. Overall the response rate to ATG+CSA was 70% (7 out of 10), with mean time to response of 1.8 11**months (range: 1-** 3 **months), and median duration of** treatment of 12 months (range: 8-72). 11**At a** mean **follow up** duration **of** 58 **months (range:** 16-73), **the 5 year** OS of the responders (n=7) was 75.0% ± 21.7% . (c) Androgenic steroids (Danazol/stanazolol) (Table:7): Out of the 74 patients who received treatment with androgenic steroids (29 received danazol while 45 received stanazolol), 51 were evaluable. Twenty four patients (47.1%) showed response (2 CR & 22 'stable disease'), while 25 (49%) showed no response to androgens. Two patients (3.9%) expired without any response (out of which one expired following progression to AML). Of the non-responders, 4 patients were changed over to ATG+ CSA, 17 patients to CSA and 4 52**were lost to follow up.** One out **of the** 2 **patients** who attained CR continued to be in CR at last follow up, while the other patient relapsed and was restarted on treatment. Out of the patients who had stable disease, 17 continued to be stable, while 5 patients showed evidence of progression of disease. Two of the patients with progression of disease were restarted on treatment but were subsequently lost to follow up; while 3 patients expired following progression of disease (one patient expired following progression to AML, second one following progression of disease along with metastatic adenocarcinoma and the third due to disease progression). The mortality was 9.8% (n=5) among those treated with Androgenic steroids. Overall the response rate to Androgenic steroids was 47% (24 out of 51), with mean time to response of 5.3months 11**(range: 1-** 27 **months), and median duration of** treatment of 11 months (range: 2-92). 11**At a** mean **follow up** duration **of** 68 **months (range:** 20-92), **the 5 year** OS of the responders

(n=24) was 51.40% ± 23.1%. (d) Prednisolone (Table:7): Twenty four patients received treatment with steroids, but only 17 were evaluable. Only four (23.5%) patients showed response ('stable disease') to prednisolone. One patient (5.9%) expired, while 12 (70.6%) showed no response. Among the 12 non-responders, treatment was changed to CSA in 7, to androgens in 4 and one patient was taken up for allogeneic PBSCT. Among the 4 patients who attained response to prednisolone, 3 were lost to follow up after a mean follow up of 11 (7- 13) months, and the fourth patient relapsed after 44 months and was then lost to follow up. The median duration of steroid treatment was 4 months (range: 2-36) and the mean time to response was 1.9 months (range: 1.3- 2.7). (e) Allogeneic PBSCT (Allo PBSCT) (Table:7): There were a total of 5 patients who underwent allogeneic PBSCT. All were males; with age between 19 to 56 years. One patient was diagnosed to have Aplastic anemia and had received treatment for 10 months prior to diagnosing hMDS. The treatment prior to allogeneic PBSCT included; Cyclosporine-A (CSA) for 2 months in 3 patients, CSA for 12 months in one patient and Prednisolone for 2 months in one patient. All except one patient had normal cytogenetics; the fifth patient had trisomy21 (+21). Four patients belonged to 'Intermediate-1' IPSS risk group, 'Low' WPSS risk group and 'Intermediate ' IPSS-R risk group; and the one patient with +21 belonged to intermediate-1 (IPSS), intermediate (WPSS) and intermediate (IPSS-R) risk groups respectively. The donors were related for all 5 patients (brother was the donor in 4 of them and sister in one). The conditioning regimen was Fludarabine/Cyclophosphamide in 4 patients and Fludarabine/Melphalan in one patient. One patient underwent second PBSCT following relapse and received Fludarabine/Melphalan/TBI for the second PBSCT. Two patients died before engraftment by day 9 and 10 post PBSCT, due to sepsis with VOD and fungal pneumonia, and sepsis with actinomycosis of lung respectively. Three patients engrafted (by days 11, 12 & 13 respectively). Of the 3 patients who engrafted, one lost response by day 52 post Allo PBSCT, and died of grade IV acute liver GVHD on day 77 post PBSCT. The second patient who responded (age-19yrs) relapsed by day 70 post PBSCT, and underwent DLI followed by a second PBSCT (using the same donor). He engrafted by day 11 of second PBSCT, and continued to be in CR for next one year. After a year post second PBSCT, he relapsed with progression to AML, and succumbed to his illness during the post chemotherapy (Cytosine/Idarubicin) neutropenic period due to sepsis and massive GI bleed.

The third patient (age: 30 years) who responded is the one who was treated for Aplastic anemia for 10 months prior to diagnosing hMDS. Post PBSCT, he developed chronic skin GVHD which responded to treatment. Presently he is 7 years post PBSCT, on follow up and continues to be in CR off drugs (except for warfarin). (f) 'Others': This included 12 patients (not mentioned in table: 7), who had received treatment with Lenalidomide (n=3) or EPO/GCSF (n=1), or only supportive measures (n=4), or in whom the drug was not known due to unavailable/missing data (n=4). In this group 2 cases attained 'CR', 6 attained 'stable disease', 4 expired without response and 2 expired following progression of disease after initial response. Among those who received Lenalidomide, 2 attained 'Stable disease', and one expired without attaining any response. The one patient who received EPO/GCSF progressed and expired after a short period of response (stable disease). Two out of the 4 patients who opted for supportive measures expired following disease progression and the other 2 showed spontaneous recovery of blood counts (CR) within 2 months and were subsequently lost to follow up. There were 4 cases where data on the drug were not available. One patient expired without response and 3 had shown initial response to treatment (stable disease). Of the 3 who showed response, one was lost to follow up, one patient subsequently progressed and expired, and the third patient who had been diagnosed and treated in 1998 was found to be stable and off drug on last follow up (the information was obtained by mail, documents on previous drug treatment could not be retrieved). TREATMENT RELATED MORBIDITY: Sixty three (36.4%) patients developed morbidities during treatment. Drug induced nephropathy was observed in 6 (3.5%) of the patients. Of these, 5 (83.3%) patients had CSA induced and one (16.7%) had analgesic induced nephropathy. Two patients had Cyclosporine related gum hyperplasia. Drug related hepatitis (transaminitis) was documented in 7 (4.0%) patients (stanazolol induced in 5 (71%) patients and Danazol induced in 2(29%)). Three (1.7%) patients were HBV positive at diagnosis, while an additional one patient (0.6%) was documented to be positive for hepatitis B virus during follow up. Three (1.7%) patients developed lower limb Deep vein thrombosis; one among them developed pulmonary embolism. Nine (5.2%) developed steroid induced diabetes mellitus, of which one had Cushing's habitus. Two patients (1.2%) developed tuberculosis while on treatment for hMDS (one had pulmonary tuberculosis and one had lymph node tuberculosis). Fourteen (8%) patients developed infectious complications during treatment (i.e. febrile neutropenia with/without sepsis in 10 cases, and Gram negative bacilli sepsis, peri-anal abscess, cellulitis and osteomyelitis in one patient each respectively. On follow up, 2 patients were found to have vitamin D deficiency while evaluating for back ache, and one of them presented with vertebral fracture. In Allo PBSCT patients, one had acute grade IV liver GVHD and the other had chronic skin GVHD. MORTALITY: Table: 8 Twenty six (15%) patients expired on follow up. Of these, 16 (61.6%) patients died because of poor response to treatment, while 10 patients died following disease progression/relapse after attaining response ('CR' or 'stable disease'). Of the former 16 patients, 2 were of post Allo PBSCT status, while 2 had

progressed to AML without attaining any response, and the remaining 14 patients died of primary treatment failure. Among the 10 patients who died following disease progression after CR or 'stable disease', one patient was of post Allo PBSCT status and had progressed to AML (following relapse after second Allo PBSCT), while a second patient was the one who had responded to androgens and then progressed to AML, the third patient was an initial responder to androgens who subsequently progressed with associated metastatic adenocarcinoma. The remaining 7 patients died of progressive disease. Sepsis with multi-organ failure was the immediate cause of death in 10 patients, while 2 patients died following massive intracranial bleed, 3 following severe pneumonia and one following grade IV GVHD. All the remaining 10 patients died of progressive disease related events (details of the exact events not available). Among those patients who died of sepsis, one had disseminated mucor ycosis, 4 had fungal pneumonia, one had pulmonary actinomycosis, and another one patient had associated massive GI bleed. SURVIVAL ANALYSIS: (Figs: 4-11; Table: 9) Survival analysis was done for the 111 patients who were evaluable for treatment response. Among these, only 79 patients had cytogenetic data. 26**Overall (OS), Event free (EFS), progression free (PFS)** and disease free survivals (DFS) (Figs: 4-7): The 6**mean follow up was** 110 **months (range: 1-** 178). There were a total of 26 deaths, all deaths were due to progressive disease and related complications. The 5 year and 10 year overall survivals for the whole cohort (n=173) was 61.9% ± 7.2%, and 53.1% ± 10.3% respectively. With a mean follow up period of 70 months (range: 1-178), the 5 year EFS for the entire cohort was 37.9% ± 7.8%. The 5 year progression free survival 6**with a mean follow up** period **of** 86 **months (range:1-** 78) for **the** entire cohort was 49.5% ± 9.3% and the 65**5 year disease free survival with**33**a median follow up** period **of** 69 **months (range:1-** 166) **was** 46 .6 ± 9.5%. OS of the different IPSS risk groups (Table: 9 and Fig:8): Among the 79 evaluable patients with cytogenetic data, 10**with a** mean **follow up** period **of** 125 **months (range:** 1-178) **and** 34 **months (range:** 1-78) respectively, **the** 5 year OS in the lower risk groups (Low+Int-1) versus higher risk groups (Int-2+high) was 65.9% ± 9.7% versus 38.1% ± 20.4% respectively (P=

0.056). OS of different WPSS risk groups (Table: 9 and Fig: 9): In the WPSS risk groups, the mean follow up period was 83 months (range:2-110), 92 months (range:1-178) and 34 months (range:1-78) for the lower risk (very low + 46low risk), intermediate risk and high risk groups respectively. The 5 year OS was noted to be significantly higher 55in the lower risk groups than the higher risk groups (Int and high risk groups); ie Lower versus Int (5yr OS= 66.6% ± 12.1% versus 50.5%±15.8%; P=0.026), Lower versus High (66.6%±12.1% vs 38.1%±20.4%; 55P=0. 017). However there was no significant survival advantage for the intermediate risk group over high risk group (P=0.973). OS of different IPSS-R risk groups (Table: 9 and Fig: 10): The mean follow up period of the IPSS-R risk groups were as follows; 83 months (range: 2-110) for the lower risk group (very low + low + Int) and 67 months (1-178) for the higher risk group (high + very high). The 5 year OS of the lower risk groups (ie very low + Low + Int) was again significantly better than the higher risk groups (High + very high), ie; 68.0% ± 10.8% vs 35.0% ± 15.7% (P=0.002). OS of different WHO classification groups (Fig: 11): Using the WHO criteria, the out of the 111 evaluable patients, 82 belonged to RCMD, 26 to MDS unclassified and 3 to RAEB-1. 6With a mean follow up period of 109 (range:1- 82), 61(2-78) and 8.5 (range:3-14) months, the 5 year OS was 61.2% ± 8.4%, 74.4 % ± 11.8% and 0 % ± 0% for RCMD, MDS-U and RAEB-1 respectively (P=0.000). UNIVARIATE ANALYSIS OF ADVERSE EFFECTS OF PATIENT CHARACTERISTICS ON OVERALL SURVIVAL (Table: 10) Univariate Cox proportional hazard 2model was used to find out significant prognostic factors for adverse effects on survival among overall hMDS patients. Variables with significant adverse effects on overall survival included age, gender, blood counts (Haemoglobin, ANC, 48platelet count), number of cytopenias, serum LDH, bone marrow cellularity, bone marrow blast percentage, bone marrow dysplasia, bone marrow reticulin, WHO classification groups,

chromosome changes and prognostic risk categories (IPSS, WPSS & IPSS-R). Parameters of independent significance associated with poor overall survival were; ANC < 0.2 x10 /L at diagnosis (RR=4.2; 95%CI=1.32-13.5; P= 0.015), Bone marrow blast at diagnosis > 5% (RR=11.0; 95%CI=2.27-53.77; P= 0.003), WHO category RAEB-1, (RR=7.5; 95%CI=1.68-34.12; P= 0.008), Moderate/marked increase in BM reticulin, (RR=4.0; 95%CI=1.13-14.17; P= 0.031), >3 cytogenetic anomalies, (RR=3.5; 95%CI=1.09-11.82; P= 0.035), Monosomy 7, (RR=5.1; 95%CI=1.54-17.03; P= 0.008), Trisomy 21, (RR=14.58.4; 95%CI=2.88-72.7; P= 0.001), WPSS intermediate risk group,(RR=3.3; 95%CI=1.11-10.00; P= 0.031), WPSS high risk group , (RR=3.6; 95%CI=1.11 -12.00; P= 0.033), and IPSS-R very high risk group (RR=13.5; 95%CI=2.64-69.90; P= 0.002). However on multivariate analysis, none of the above parameters retained its statistical significance. RESULTS - TABLES: Table 1: DEMOGRAPHY & CLINICAL FEATURES AT DIAGNOSIS: n =173 Variables n (%) / Median(Range) Age at diagnosis in years 18-40 72 (41.6) 41-55 51 (29.5) >55 50 (28.9) Gender Male 112 (64.7) Female 61 (35.3) Presenting symptoms Pallor 163 (94.2) Bleeding 70 (40.5) Infection 66 (38.2) Duration of symptoms (months) 3 (1-120) Organomegaly Splenomegaly 6 (3.5) Hepatomegaly 2 (1.2) Patients who received prior treatment 48 (27.7) Median duration of previous treatment (days) 60 (7-720) Past history treatment for cytopenia (s) 14 (8.1) Median transfusions per month (n=139) 2.0 (1-20) . Table 2: LABORATORY PARAMETERS AT DIAGNOSIS n=173 Variables n (%) / Median (Range) Hemoglobin [g%] <6 6.1-10 >10.0 Absolute neutrophil count[x109/L] 94 71 8 (54.4) (41.0) (4.6) <0.2 0.21-0.5 0.51-1.0 1.1-1.5 1.51-1.8 >1.8 Platelet count [x109/L] 15 28 39 36 17 38 (8.7) (16.2) (22.5) (20.8) (9.8) (22.0) <20 21-50 51-100 >100 106 39 16 12 (61.3) (22.5) (9.2) (7.0) Reticulocyte count Median Percentage of reticulocytes (n=152) Median Absolute reticulocytes [x109/L] (n=99) 1.8 37200 (0.05-7.06) (6175-133472) No. of cytopenia(s) Single cytopenia Bicytopenia Pancytopenia 7 53 113 (4.1) (30.6) (65.3) Direct coomb's test (n=39) Positive Negative 14 25 (35.9) (64.1) Antinuclear antibody (n=23)

Positive Negative 8 15 (34.8) (65.2) Serum LDH [mg/dl] (n=160) <600 >600 131 29 (81.9) (18.1) Serum ferritin [ng/ml] (n=24) 825 138-46775) Serum Vitamin B12 [pg/ml] (n=10) 970 (248-2000) Blood borne virus screen (n=137) Positive Negative 5 132 (3.6) (96.4) Table 3: BONE MARROW FEATURES & WHO CALSSIFICATION AT DIAGNOSIS: n = 173 Variables n (%) Bone marrow cellularity Aplastic 9 (5.2) Uniformly hypocellular 144 (83.2) Varyingly hypocellular 20 (11.6) BM blasts[%] (n=171) 0 80 (46.8) 1-2 77 (45.0) 3-4 10 (5.8) ≥5 4 (2.4) BM ring sideroblasts (n=120) >15% 1 (0.8) Scattered/occasional 47 (39.2) Absent 72 (60.0) BM dysplasia Unilineage 36 (20.8) Bilineage 80 (46.2) Trilineage 57 (33.0) BM Reticulin (n=169) Normal 34 (20.1) Mild increase 80 (47.3) Moderate increase 49 (29.0) Marked increase 6 (3.6) WHO classification MDS-Unclassified 35 (20.2) RCMD 134 (77.5) RAEB-1 4 (2.3)

59**Abbreviations: MDS: Myelodysplastic syndrome, RCMD: Refractory cytopenia with multilineage dysplasia, RAEB: Refractory anemia with excess blast.**

Table 4: CYTOGENETIC ABNORMALITIES AT DIAGNOSIS n =116 Variables n (%) Cytogenetic abnormalities No abnormality One abnormality Two abnormalities Three abnormalities >3 / chromosome 7 abn 77 (66.4) 13 (11.2) 8 (6.9) 3 (2.6) 15 (12.9) Individual chromosomal aberrations Monosomies: -7 -5 -Y -X Other monosomies@ 13 (11.2) 2 (1.7) 3 (2.6) 2 (1.7) 17 (14.7) Trisomies: +8 +21 Other trisomies@ 7 (6.0) 8 (6.9) 3 (2.6) Translocations t(18;21) t(20;21) t(11;14) t(7;13;16) t(7;?) 1 (0.9) 1 (0.9) 1 (0.9) 1 (0.9) 1 (0.9) Deletions: del 5q del 11q del 12p del 20q Other deletions@ Other rare anomalies@ 7 (6.0) 2 (1.7) 1 (0.9) 1 (0.9) 4 (3.4) @ Other anomalies included; monosomies (Additions Derivatives Duplications 5 (4.3) 4 (3.4) 1 (0.9) -1, -2,-3,-9,-10,-13,-14,-15,-16, & -19 seen in one patient each and -11, -18, &-20 seen in two patients each), trisomies (+1, +2,+4 seen in one patient each), deletions (del 11q in 2 patients and 1p, del 5p, del 6q, del 9q, del 12p, & del 20q seen in one patient each), additions (+11p,+11q,+14q,+1p, & +7q seen in one patient each), derivatives [der(7), der(12), der(14), & der(16 seen in one patient each) and duplication (dup1), seen in one patient. Table 5: CYTOGNETIC RISK GROUPS AT DIAGNOSIS n =116 IPSS&WPSS cytogenetic risk group IPSS-R cytogenetic risk group n (%) n (%) Very good - - 3 (2.6) Good 80 (69.0) 80 (69.0) Intermediate 19 (16.4) 15 (12.9) Poor 17 (14.6) 15 (12.9) Very poor - - 3 (2.6) Abbreviations:

32**IPSS: International prognostic scoring system, WPSS: WHO prognostic scoring system,**

IPSSS-R: Revised IPSS. Table 6: PROGNOSTIC RISK GROUPS AT DIAGNOSIS: n

= 116

Risk group

Risk group	IPSS n (%)	WPSS n (%)	IPSS-R n (%)
Very low	-	1 (0.9)	2 (1.7)
Low	3 (2.6)	79 (68.1)	22 (19.0)
Intermediate-1/	96 (82.7)	-	-
Intermediate	-	19 (16.4)	68 (58.6)
Intermediate-2	17 (14.7)	-	-
High	0 (0.0)	17 (14.6)	18 (15.5)
Very high	-	-	6 (5.2)

Table 7: TREATMENT AND RESPONSE:

	CSA n (%)	ATG +CSA n (%)	Androgens n (%)	Prednisolone n (%)	Allo-PBSCT n (%)
Total patients (n=173)	81 (46.8)	10 (5.8)	74 (42.8)	24 (13.9)	5 (2.9)
Non-evaluable	22 (27.0)	0 (0.0)	23 (31.0)	7 (29.0)	0 (0.0)
Evaluable	59 (73.0)	10 (100.0)	51 (69.0)	17 (71.0)	5 (100.0)
Response:					
CR	6 (10.2)	2 (20.0)	2 (3.9)	0 (0.0)	2 (40.0)
Stable disease	35 (59.3)	5 (50.0)	22 (43.2)	4 (23.5)	1 (20.0)
No response (LFU/drug changed)	12 (20.3)	2 (20.0)	25 (49.0)	12 (70.6)	0 (0.0)
Failure (expired with no response)	6 (10.2)	1 (10.0)	2 (3.9)	1 (5.9)	2 (40.0)
On follow up: CR:					
Continued in CR	6 (100.0)	2 (100.0)	1 (50.0)	0 (0.0)	1 (50.0)
Relapse & alive/LFU	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)
Relapse & expired	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)
Stable disease:					
Continued in Stable	24 (68.6)	3 (60.0)	17 (77.3)	3 (75.0)	0 (0.0)
Progression & alive/LFU	9 (25.7)	1 (20.0)	2 (9.1)	1 (25.0)	0 (0.0)
Progression & expired	2 (5.7)	1 (20.0)	3 (13.6)	0 (0.0)	1 (100.0)
Duration of treatment-months; median(range)	19 (3-126)	12 (8-72)	11 (2-92)	4 (2-36)	--
Response rate	69%	70%	47%	23.5%	60%
Time to response - months; mean (range)	5.9 (3-36)	1.8 (1-3)	5.3 (1-27)	1.9 (1.3-2.7)	0.5 (0.4-0.5)
Follow up duration –months; mean(range)	103 (8-110)	58 (16-73)	68 (20-92)	19 (7-44)	39 (15-81)
Mortality in each drug group	8 (13.6)	2 (20.0)	5 (9.8)	1 (5.9)	4 (80.0)
5 year OS of responders	96.9% ± 3.1%	75.0% ± 21.7%	51.4% ± 23.1%	100%**	33.3% ± 27.2%

Table 8: MORTALITY:

Variables	n (%)
Total number of death	26 (15.0)
Disease status at death (n=26)	
Failure without any response	16 (61.6)
Progression/Relapse after CR or 'stable disease'	10 (38.4)
Cause of death	
Disease failure related events (exact event unknown)	10 (38.5)
Sepsis with MODS	10 (38.5)
ICH	2 (7.7)
Liver GVHD	1 (3.8)
Pneumonia	3 (11.5)

Table 8: OVERALL SURVIVAL OF DIFFERENT PROGNOSTIC RISK GROUPS.

Risk group	IPSS n (%)	5 yr OS	WPSS n (%)	5 yr OS	IPSS-R n (%)	5 yr OS
Very low	-		1 (0.9)	0% ± 0%	2 (1.7)	0% ± 0%
Low	3 (2.6)	0% ± 0%	79 (68.1)	66.6% ±12.1%	22 (19.0)	73%±14%
Intermediate-1/	96 (82.8)	68.3%±9.7%	-		-	
Intermediate	-		19 (16.4)	50.9%±15.8%	68 (58.6)	68.4%±12.2
Intermediate-2	17 (14.6)	39.3%±2%	-		-	
High	0 (0.0)	0 (0)	17 (14.6)	39.3%±20.8%	18 (15.5)	48.8±19.3
Very high	-		-		6 (5.2)	0% ± 0%

TABLE 9: UNIVARIATE ANALYSIS OF ADVERSE EFFECTS OF PATIENT CHARACTERISTICS ON OVERALL SURVIVAL. n =111

Variables	Alive n (%)	Dead n (%)	RR	95% CI	P-value
Age at diagnosis in years:					
18-40	38 (44.7)	9 (34.6)	1.0	-	
41-55	28 (32.9)	7 (26.9)	1.0	0.38-2.80	0.933
>55	19 (22.4)	10 (38.5)	1.8	0.74-4.50	0.189
Sex					
Female	33 (38.8)	5 (19.2)	1.0	-	
Male	52 (61.2)	21 (80.8)	2.3	0.87-6.13	0.092
Hb at diagnosis					
>10.1	4 (4.7)	1 (3.8)	1.0	-	
6.1 – 10	39 (45.9)	9 (34.6)	2.1	0.26-17.91	0.466
<6	42 (49.4)	16 (61.5)	3.4	0.44-27.02	0.235
ANC [x10^9/L]					
>1.5	30 (35.3)	7 (26.9)	1.0	-	
1.0-1.5	21 (24.7)	3 (11.5)	0.5	0.13-2.09	0.372
0.5-1.0	21 (24.7)	8 (30.8)	1.7	0.63-4.86	0.282
0.2-0.5	11 (12.9)	3 (11.5)	1.7	0.44-6.97	0.418
<0.2	2 (2.4)	5 (19.2)	4.2	1.32-13.50	0.015
Platelet [x10^9/L]					
>100	7 (8.2)	2 (7.7)	1.0	-	
51-100	9 (10.6)	4 (15.4)	1.3	0.24-	
21-50	19 (22.4)	8 (30.8)	1.0		
<20	50 (58.8)	12 (46.2)	0.6		

7.30 0.22-5.19 0.15-3.14 - 0.742 0.922 0.637 No. of cytopenia(s) Single cytopenia Bicytopenia Pancytopenia 3 (3.8) 30(35.3) 52(61.2) 1(3.8) 8(30.8) 17(65.4) 1.0 0.8 0.9 - 1.00-6.48 0.12-7.30 - 0.837 0.974 Serum LDH (n=101) <600 >600 65(83.3) 13(16.7) 18(78.3) 5(21.7) 1.0 1.5 - 0.55-4.10 - 0.420 Bone marrow cellularity Varyingly hypocellular Uniformly hypocellular Aplastic 11(12.9) 73(85.9) 1(1.2) 2(7.7) 23(88.5) 1(3.8) 1.0 1.8 1.2 - 0.15-20.91 0.29-5.42 - 0.631 0.745 BM blasts [%; n=109] 0 1-2 3-4 ≥5 42(50.0) 37(44.0) 4(4.8) 1(1.2) 9(36.0) 11(44.0) 3(12.0) 2(8.0) 1.0 1.2 2.9 11.0 - 0.52-3.04 0.77-10.87 2.27-53.77 - 0.610 0.113 0.003 TABLE 10: UNIVARIATE ANALYSIS OF ADVERSE EFFECTS OF PATIENT CHARACTERISTICS ON OVERALL SURVIVAL contd… Variables Alive N (%) Dead N (%) RR 95% CI P- value BM dysplasia Unilineage Bilineage Trilineage 22(25.9) 37(43.5) 26(30.5) 5(19.2) 10(38.5) 11(42.3) 1.0 1.0 1.2 - 0.36-3.18 0.44-3.69 - 0.888 0.652 BM Reticulin (n=108) Normal Mild increase Moderate/Marked increase 20(23.8) 42(50.0) 22(26.2) 3(12.5) 8(33.3) 13(54.2) 1.0 1.6 4.0 - 0.43-6.28 1.13-14.17 - 0.456 0.031 WHO SUB-CLASS MDS-Unclassified RCMD RAEB1 22(25.9) 62(72.9) 1(1.2) 4(15.4) 20(76.9) 2(7.7) 1.0 0.7 7.5 - 0.24-2.15 1.68-34.12 - 0.569 0.008 Cytogenetic anomalies (n=79) No anomaly One anomaly 2-3 anomalies >3 anomalies or -7 47(77.0) 5(8.2) 4(6.6) 5(8.2) 9(50.0) 3(16.7) 2(11.1) 4(22.2) 1.0 2.4 2.3 3.5 - 0.65-9.00 0.50-10.92 1.09-11.82 - 0.182 0.274 0.035 Individual chromosomal abn (n=75) Normal del 5q Monosomy 7 Trisomy 21 Trisomy 8 Monosomy Y 47(82.4) 4(7.0) 4(7.0) 0(0.0) 1(1.8) 1(1.8) 9(50.0) 1(5.6) 4(22.2) 2(11.0) 1(5.6) 1(5.6) 1.0 1.0 5.1 14.4 3.4 4.2 - 0.13-8.55 1.54-17.03 2.88-72.7 0.43-27.78 0.51-34.24 - 0.947 0.008 0.001 0.241 0.178 IPSS Risk groups: (n=79) Low Intermediate-1 Intermediate-2 2(3.3) 54(88.5) 5(8.2) 1(5.6) 13(72.2) 4(22.2) 1.0 0.6 1.8 - 0.08-4.88 0.20-16.84 - 0.664 0.583 50**WPSS Risk groups** (n=79) **Very low Low Intermediate High** 1(1.6) 49(80.3) 6(9.8) 5(8.2) - 9(50.0) 5(27.8) 4(22.2) - 1.0 3.3 3.7 - - 1.11-9.95 1.13-12.24 - - 0.032 0.030 IPSS-R Risk groups (n=79) 54**Very low Low Intermediate High Very high 1(** 1.6) 16(26.2) 36(59.0) 8(13.1) 0(0.0) - 3(16.7) 8(44.4) 4(22.2) 3(16.7) - 1.0 1.1 2.9 14.1 - - 0.30-4.30 0.65-13.66 2.75-72.19 - - 0.847 0.157 0.001 RESULTS - FIGURES: Figure 1: Year wise distribution of total MDS vs hMDS 140 Total MDS 126 120 Hypo MDS 120 112 113 104 100 99 104 Number of cases 80 81 79 69 59 60 46 48 46 39 40 24 22 20 16 17 8 4 4 9 9 10 9 9 12 11 9 0 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 Year Figure 1: Year wise distribution: Total MDS versus hypoplastic MDS cases from January 1998 to June 2012. Figure 2: Peripheral blood and Bone marrow findings: 2a 2b 2c 2d 2e 2f 2g 2h Figure 2: Peripheral blood and bone marrow findings. Figures 2a & 2b: PB smear showing granulocyte dysplasia - pelger heut anomaly and hypogranularity, 2c: BM aspirate smear showing dyserythropoiesis, 2d: BM aspirate smear showing micro-megakaryocytes, 2e: BM aspirate smear showing ring sideroblasts. 2f: BM

trephine biopsy showing hypocellular marrow & dysplastic megakaryocytes. 2g: BM trephine biopsy silver stain showing increased rericulin and 2h: BM trephine biopsy showing CD34+ cells. Figure 3: Response rate to different drugs 80 70 CSA vs ATG+CSA (P= 0.974) Response rate (%) 60 50 69 70 40 30 47 20 P= 0.017 P= 0.185 10 0 CSA (n=59) ANDROGENS (n=51) ATG+CSA (n=10) Figure 3: On comparing the response rate to different drugs, response rate to CSA was found to be significantly (69% vs 47%; P=0.017) superior to Androgenic steroids. The number of patients who received other drugs was few to compare enough number of patients to compare. SURVIVALS: Figure 4: Overall survival of total evaluable patients (n=111) 5 year OS = 61.9% ± 7.2% Figure 4: Kaplan Meier curve for 20**overall survival** of **the entire** cohort **of** evaluable **patients** (n=111). With a median follow up duration of 110 months (Range: 1-178), the 5 year and 10 year OS were 61.9% ± 7.2% & 53.1% ±10.3% respectively. Figure 5: Event free survival of total evaluable patients (n=111) 5 year EFS = 37.9% ± 7.8% Figure 5: Kaplan Meier curve for Event free survival of the entire cohort of evaluable patients (n=111). 6**With a mean follow up** duration **of** 70 **months (Range: 1-** 178), **the** 5 year & 10 year EFS were 37.9% ± 7.8% & 25.3% ±8.0% respectively. Figure 6: Progression free survival of total evaluable patients (n=111) 5 year PFS = 49.5% ± 9.3% Figure 6: 47**Kaplan Meier curve** for **Progression free survival** of **the entire cohort of** evaluable patients (n=111). 6**With a mean follow up** duration **of** 86 **months (Range: 1-** 78), **the** 5 year & 10 year PFS was 49.5% ± 9.3% & 33.0% ±10.1% respectively. Figure 7: Disease free survival of total evaluable patients (n=111) 5 year DFS = 46.6% ± 9.5% Figure 7: 47**Kaplan Meier curve** for Disease **free survival** of **the entire cohort of** evaluable patients (n=111). 6**With a mean follow up** duration **of** 69 **months (Range: 1-** 166), **the** 5 year & 10 year DFS was 46.6% ± 9.5% & 18.1%±8.9% respectively.

SURVIVAL BY PROGNOSTIC RISK GROUPS: Figure 8: OS of IPSS risk groups (n=79) Int-1 + Low Int-2 P-value = 0.062 5 year DFS = 46.6% ± 9.5% Figure 8: Kaplan Meier curve for OS IPSS risk

groups. Among the 111 evaluable patients, cytogenetic data was available only in 79. 10**With a** mean **follow up** period **of** 125 **months (range:** 1-178) **and** 34 **months (range:** 1-78) **the** 5 year OS in 2**the lower risk** groups **(Low+Int-1)** versus **higher risk** groups **(Int-2+high) was** 65.9% ± 9.7% versus 38.1% ± 20.4% respectively.. The higher survival noted in the lower risk group was statistically near significant (p= 0.056). Figure 9: OS of WPSS risk groups (n=79) Low + very low Int High P-value = 0.019 Figure 9: Kaplan Meier curve for OS of WPSS risk groups. With a mean follow up period was 83 (range:2-110), 92 (range:1-178) and 34 months (range:1-78) for the lower risk (very low + 46**low risk), intermediate risk and high risk groups respectively, the** 5 year OS were, Lower versus Int (66.6% ± 12.1% vs 50.5% ± 15.8%; P value=0.026), Lower vs High (66.6% ±12.1% vs 38.1%±20.4%; P value=0.017). OS 32**was significantly higher in the** lower **risk groups** than **the** higher risk groups (Int and high risk groups), but 10**there was no significant survival** advantage **for the** intermediate risk **group** over high risk group (P value=0.973). Figure 10: OS of IPSS-R risk groups (n=79) Very low+Low+Int High+Very high P-value = 0.002 Figure 10: Kaplan Meier curve for OS of IPSS-R risk groups. 48**With a** mean **follow up** period **of** 83 **months (range:2-** 110) for **the** lower **risk** group (very low+low+Int) and 67 months (1-178) for the higher risk group (high+very high), the 5 year OS was noted 67**to be significantly higher in** lower risk **group than** higher risk groups (High + very high), ie; 68.0% ± 10.8% vs 35.0% ± 15.7% (P=0.002). Figure 11: OS by WHO classification for the entire evaluable patients (n=111) MDS Unclassified RCMD RAEB-1 P-value = 0.000 Figure 11: Kaplan Meier curve for OS of WHO classification groups Using the WHO criteria, the out of the 111 evaluable patients, 82 belonged to RCMD, 26 to MDS unclassified

and 3 to RAEB-1. 6With a mean follow up period of 109 (range:1- 82), 61(2- 78) and 8.5 (range:3-14) months, the 5 year OS were 61.2% ± 8.4%, 74.4 % ± 11.8% and 0 % ± 0% for RCMD, MDS-U and RAEB-1 respectively. MDS-U and RCMD showed significantly better survival than RAEB-1 (P=0.000). Figure 12: Overall survival of responders to different drugs CSA ATG+CSA Androgens P-value =0.213 Figure 12: Kaplan Meier curve for OS of responders to different drugs. The OS of the responders to different drugs showed no significant difference. The 5 yr OS of CSA vs Androgens was 96.9% ±3.1 % vs 51.4% ± 23.1% (P=0.090); ATG vs Androgens was 75.0 % ±21.7 % vs 51.4% ± 23.1% (P=0.972) and CSA vs ATG+CSA was 75.0 % ±21.7 % vs 96.9% ±3.1 % (P=0.180). DISCUSSION: Between January 1998 and June 2012, there were a total of 173 adult patients (age ≥18 years) diagnosed to have hypoplastic myelodysplastic syndrome from January 1998 to June, 30, 2012. The total number of patients seen in the outpatient department of Clinical Haematology during this period was 54413, out of which 1225 (2.3%) adult patients were diagnosed to have primary MDS. In this study, hMDS constituted 14.1% of MDS cases diagnosed during this period. This is similar to that reported in literature ; i.e. 10-15% of all MDS cases (2,4,7–9,14) [Fig 6:1]. In the present study the majority (41.6%; n=72) of the 10patients belonged to the age group 18-40 years, 11with a median age of 41 years (range: 18-64). In a study by Koh Y and colleagues, 23based on a medical record review at Seoul National University Hospital, 51 patients were diagnosed to have hMDS, 20and the median age reported was 39 years (12), similar to the observation in the present study. This is much lower than the median age of patients with normo/hypercellular MDS ie; 60–75 years (5). However in a comparative study 2of hypoplastic myelodysplastic syndrome (MDS) with normo-/hypercellular MDS by Huang et al, the median age reported was similar in both groups ie; 58 years (range: 26–86) in hMDS and 55 years in normo-/hypercellular MDS (1). In the present study, 112 were males (64.7%) and 61 females 35(35.3%), with a male: female

ration **of 1.8:1.** A similar observation **of** male preponderance was **reported** by Huang et al (

29:8) (1). The median duration of symptoms before diagnosing hMDS was 3 months. The duration of symptoms before diagnosis is made ranges from 6-12 months as per reports by Hoffman and Koeffler (5). Majority of the patients in the present study (94.2%; n=163) were diagnosed following evaluation for pallor. Diagnosis was made following a routine medical check-up in 5.8% (n=10) of the patients. In MDS as a whole, it is reported that 50% of the patients are asymptomatic and are diagnosed following routine check-up (5). The presence of occasional splenomegaly has been reported by some authors (3,16); in the present study 3.5% (n=6) of patients had mild splenomegaly [Table:1]. Haemogram showed that 95.4% (n=165) had a hemoglobin level below 10g%, with a median hemoglobin of 5.8g% (range: 1.2- 13.2). In the study by Huang et al (1) the median hemoglobin reported was 7.8g% (range: 4.0–14.0), a little higher than that observed in the present study. The median WBC count and ANC at diagnosis were 3.3x109/L (range: 0.2-13.8) and 1.05 x109/L (0.0-5.32) respectively. This is slightly higher than that reported by Huang et al (1) i.e. 2.37 x109/L (range:1.00–15.70) of WBC count and 0.98 x109/L (range: 0.196–10.360) of ANC. 58In

the present study, majority **of the patients had** a severe thrombocytopenia with 61.3%

(n=106) of patients presenting with platelet count <20 x109/L. The 10median platelet count

was 14 **x 109/L (range:1-** 307), much lower than that observed in the study by Huang et al ie;

54 x109/L (range: 3–433) (1). However, only 70 patients presented with bleeding manifestations

[Table:2]. 36The diagnosis of hMDS was **based on the presence of** a hypocellular

marrow with features of dysplasia in one or more cell lines, increase in reticulin content,

30increase in the number of blasts/CD34+ **cells** on **the** bone marrow trephine, or

abnormal karyotype, all favoring the diagnosis of hMDS (3,6,15,16). Out of the 169 cases where data on reticulin content was available, increase in reticulin content was seen in 79.9% (n=135) [Table3]. The median bone marrow blast percentage was 1 (range: 0-5), as compared to 2.6% (0– 26.0) in the Huang et al study (1). All cases with bone marrow blasts greater than 5%, and/or those with a diagnosis of RAEB-2 or acute leukemia at presentation, irrespective of the presence of hypocellular marrow were excluded from the study. This may be the reason for the lower range of

BM blasts observed in this study. Ring sideroblasts were observed in 48 cases (27.8%), of which only one (0.6%) had >15% ring sideroblasts (RCMD-RS). This is similar to the study published by Huang et al (1), where only 2 (5.4%) cases were diagnosed to have RARS. WHO classification of the cases in the present study showed that 77.5% (n=134) befitted the RCMD (Refractory cytopenia with multilineage dysplasia) group, while 20.2% (n=35) were MDS-U (MDS unclassified) and 4 (2.3%) were 54**RAEB-1 (Refractory anemia with excess blasts-1)** [Table:3]. In the studies by Nand S et al (8) and Huang et al (1), the FAB classification was followed. In these studies the FAB subgroups included RA (n=7), RARS (n=1) and RAEB (n=3) in the former and 29**RA (n=** 21), **RARS (n=2), RAEB (n=** 9) and **RAEB-T (n=** 5) in the latter study respectively.

Cytogenetic data was available in 116 (67%) patients. A normal karyotype was found in 77 (66.4%). Earlier reports by Toyoma K et al have shown that patients with hMD frequently had complex aberrations (chromosome changes at three or more regions) (53). In the present study among the 39 (33.6%) patients with abnormal karyotype, 2.6% (n=3) had three aberrations and 12.9% (n=15) had more than 3 aberrations / monosomy 7. Among the numerical abnormalities observed in 55 (47.4%), majority had monosomies (n= 37). Monosomy 5 (-5)/del 5q (5q-) was observed in 9 (7.8%) cases, and monosomy 7 (-7) was found in 13 (11.2%) patients [Table: 4]. The reports on 2**chromosomal aberrations in** hypoplastic **MDS are limited** (1,8,9). In the report by Nand and Gonwinz (1,8), 2**none of the nine h-MDS patients** harboured monosomy 2**7/7q-,** while **only one out of 23** hMDS **patients reported by Tuzuner et al** (1,54) **showed this abnormality. On the contrary, Maschek et al** (1,9) **demonstrated** monosomy 7 **in two out of the six h-MDS patients.** The karyotype profile in the Huang et al (1) study showed that 57.6% (n=19) had normal karyotype, and among those with abnormal karyotype, 3% (n=1) had -5/5q-, none had chromosome7 abnormalities, 12.1% (n=4) had trisomy 8, 24.2% (n=8) had single aberration, 9.1% (n=3) had double aberrations and 9.1% (n=3) had complex aberrations. This observation is similar to the observation in the present study except that

chromosome7 abnormalities were observed in 11.2% of patients in this study. 2**Further studies on more patients** may be **needed to clarify whether the** variations **in the frequency of** cytogenetic abnormalities, especially involving chromosome7, in 2**h-MDS** observed **among these reports may represent the difference of the pathogenesis of h-MDS in different geographical areas.** As per the IPSS/WPSS and IPSS-R cytogenetic risk categorization, in the present study, majority belonged to the Intermediate cytogenetic risk group (69% each in either category) [Table:5]. While applying various MDS prognostic scoring systems, majority fell into the intermediate -1 (82.8%; n=96) by IPSS, intermediate category (58.6%; n= 68) by IPSS-R, and low risk (68.1% (n= 79) by WPSS [Table: 6]. A similar observation was noted in the comparative study by Huang et al (1), where 57.6% (n=19) of patients were scored into the intermediate -1 risk category of IPSS. In our study none of the cases belonged to the IPSS high risk group, while 9.1% (n=3) cases 21**belonged to high risk group** in **the** study by Huang et al (1). This may be due to the fact that patients with RAEB-2 and Acute leukemia were excluded in the present study. One hundred and eleven (64.2%) patients who had atleast 8 weeks follow up after initiation of therapy were evaluated for response. Eighty seven (78.4%) showed response, either 'Complete remission'[CR] (12.6%; n=14) or 'Stable disease" (65.8%; n=73) [Table:7]. On comparing the response rate to different drugs, the response rate to cyclosporine was found to be significantly higher than Androgens (69.5% vs 47.1%; P=0.017) . Although a 70% response rate was seen to ATG+CSA, this was not found to be significantly superior over other drugs [Fig:3]. The 5 year overall survival among the responders to the different drugs however showed no statistically significant difference [Fig;12]. The mean time to response were 5.9 months (range: 3-36) in CSA group, 1.8 months (range: 1-3) in ATG+CSA group and 5.3 months (1-27) in androgen group [Table:7]. In 49**a prospective randomized multicenter phase III trial** (where 9 out of total 45 cases were hMDS) comparing Antithymocyte globulin + cyclosporine with best supportive care by Passweg JR et al (33), 29% of the total 45 patients

showed response to ATG+CSA by 6 months. In a study on Cyclosporine therapy in hMDS by Jonasova A et al (55), out of the 9 patients of hMDS (of total 17 MDS cases), 8 showed response, and the time to response was observed to be between 3 to 9 months, similar to the observation in our study. However in another study by Catalano Let al (56), out of 9 patients with hypoplastic refractory anemia who were treated with cyclosporine, 3 showed response in a mean duration of 22 months (median 14.5 months); longer than that observed in this study. Literature review shows reports suggesting better response to immunosuppressive therapies (CSA, ATG) in patients with hMDS by several authors (2,13,33,57,58). However data comparing the response to different drug groups in hMDS could not be found. In our study, the number of patients in other drug groups are very few (eg; ATG+CSA [n=10], Lenalidomide [n=3], Allogeneic PBSCT [n=5], Haematopoietic growth factors [n=1], and supportive treatment [n=4]), limiting significant correlative study between these groups. For the entire cohort, with 28a median follow up duration of 110 months, the 5 year OS, EFS, PFS and DFS are 61.9%+7.2%, 37.9%+7.8%, 49.5%+9.3% and 46.6% ± 9.5% respectively [Figs: 4-7]. Bartl and colleagues, in a retrospective and prospective 10follow-up study of 495 patients with MDS between 1975 to 1991, reported 10a median survival of 29 months (n=95) in patients with hMDS (7). In the study by Huang et al (1), the OS was less than the present study (5b year OS: <60%; median survival = 58 months). 2To identify different survival groups in h-MDS patients, we took advantage of the different risk scoring systems (IPSS, WPSS & IPSS-R). Obviously, in this study, h-MDS patients of lower risk groups 12had a significantly higher 5 year OS than those of higher risks groups in the WPSS 12(P=0.018) and IPSS-R (P=0. 002) systems [Figs:9,10]. Risk categorization by the IPSS showed only near significant survival benefit in the lower over the higher groups [Fig:8]. Our study demonstrate that in hypoplastic MDS, WPSS and IPSS-R prognostic scoring systems are better than the IPSS systems in predicting survival advantage. However in the comparative study by Huang et al (1), 2a significant survival difference was observed

between these two groups in h-MDS patients (median survival 112 vs 16 months, P=0.002). The OS of IPSS lower risk groups reported by Huang et al (1), however is much lower than that observed in our study (5 year OS: <40%) versus 66.0% ± 9.7%) . The overall mortality was 26 patients (15% of the total 173 cases and 23.4% of the 111 evaluable patients). Of these 4 had shown progression to Acute myeloid leukemia, whereas one patient developed metastatic adenocarcinoma along with progression of disease. **With a mean follow up duration of 110 months in 111 evaluable patients, the cumulated incidence of transformation to acute leukemia at 5 years was** 3.6 % (n=4) [Table:8]. In the study by Huang et al (1), **with a median follow-up duration of 98 months in 187 evaluable patients, the cumulated incidence of acute leukemic transformation at 7 years was 8.1% for h-MDS.** This is higher than the observation in our study. This may be due to the fact that hypocellular RAEB 2 was excluded from our study. Univariate Cox proportional hazard **model was used to find out significant prognostic factors for survival among overall hMDS patients** [Tables:10-12]. **Parameters of independent significance for adverse effects on overall survival were** ANC < 0.2 x10 /L at diagnosis, bone marrow blast at diagnosis >5%, WHO category RAEB-1, moderate/marked increase in BM reticulin, >3 cytogenetic anomalies, monosomy7, trisomy 21, WPSS intermediate risk group, WPSS high **risk group , and IPSS- R very high risk group. However on multivariate analysis** none of the parameters were In the multivariate model by Huang et al (1),

parameters of independent significance for overall survival were age, marrow hypocellularity, RA with excess of blast, RA with excess of blast-T, monosomy 5 or 5q deletion and monosomy 7 or 7q deletion. However on multivariate analysis, none of the above parameters retained its statistical significance. LIMITATIONS OF THE STUDY: 1. Retrospective study: limited available data due to non-retrievable records, intractability of patients due to wrongly recorded/changed/non-availability of address. 2. Not all hypocellular MDS were recruited in this study; RAEB-2 and AML with hypocellular marrow were excluded. In the reported studies on hMDS; all categories of hypocellular MDS are included into the group hypoplastic MDS. This limits the comparison with these studies. 3. Karyotyping is not available for all patients- limited by the nature of the study. 4. Lesser number of patients in drug groups limiting comparison between drug groups. CONCLUSION: **Hypoplastic MDS is a distinct subgroup of MDS** of unknown etiology which needs to be distinguished from aplastic anemia. It is a disease associated with a relatively good prognosis, with significant response to immunosuppressive therapy and reasonable response to treatment with androgens, and a lower probability for leukemic transformation. Cytogenetic analysis at diagnosis is crucial in prognostic risk categorization of the patient. WHO classification based prognostic scoring system and revised IPSS appear to be better than IPSS in predicting survival.